EUROPEAN COMMISSION
Directorate-General for Communications Networks, Content and Technology

Artificial Intelligence and Digital Industry
Photonics

GRANT AGREEMENT

NUMBER 101017180 — NanoVIB

This **Agreement** ('the Agreement') is **between** the following parties:

on the one part,

the **European Union** ('the EU'), represented by the European Commission ('the Commission'), represented for the purposes of signature of this Agreement by Deputy Head of Unit, Authorised representative of the Director General, Directorate-General for Communications Networks, Content and Technology, Artificial Intelligence and Digital Industry, Administration and Finance, Paolo GARELLO,

and

on the other part,

1. 'the coordinator':

KUNGLIGA TEKNISKA HOEGSKOLAN (KTH), established in BRINELLVAGEN 8, STOCKHOLM 100 44, Sweden, VAT number: SE202100305401, represented for the purposes of signing the Agreement by Head of Research Support Office, Maria GUSTAFSON

and the following other beneficiaries, if they sign their 'Accession Form' (see Annex 3 and Article 56):

2. **KAROLINSKA INSTITUTET (KI)**, established in Nobels Vag 5, STOCKHOLM 17177, Sweden, VAT number: SE202100297301,

3. **ABBERIOR INSTRUMENTS GMBH (AI)**, established in HANS ADOLF KREBS WEG 1, GOTTINGEN 37077, Germany, VAT number: DE283588727,

4. **LASER-LABORATORIUM GOTTINGEN EV (LLG)**, established in HANS-ADOLF-KREBS WEG 1, GOTTINGEN 37077, Germany, VAT number: DE115312817,

5. **APE ANGEWANDTE PHYSIK UND ELEKTRONIK GMBH (APE)**, established in PLAUENER STRASSE 163 165, BERLIN 13053, Germany, VAT number: DE155557053,

6. **PI IMAGING TECHNOLOGY SA (PII)**, established in RUE DE LA PIERRE A MAZEL 39, NEUCHATEL 2000, Switzerland, VAT number: CHE496873037TVA,

Unless otherwise specified, references to 'beneficiary' or 'beneficiaries' include the coordinator.

The parties referred to above have agreed to enter into the Agreement under the terms and conditions below.

By signing the Agreement or the Accession Form, the beneficiaries accept the grant and agree to implement it under their own responsibility and in accordance with the Agreement, with all the obligations and conditions it sets out.

The Agreement is composed of:

Terms and Conditions

Annex 1 Description of the action

Annex 2 Estimated budget for the action

2a Additional information on the estimated budget

Annex 3 Accession Forms

Annex 4 Model for the financial statements

Annex 5 Model for the certificate on the financial statements

Annex 6 Model for the certificate on the methodology

TERMS AND CONDITIONS

TABLE OF CONTENTS

CHAPTER 1 GENERAL

ARTICLE 1 — SUBJECT OF THE AGREEMENT

This Agreement sets out the rights and obligations and the terms and conditions applicable to the grant awarded to the beneficiaries for implementing the action set out in Chapter 2.

CHAPTER 2 ACTION

ARTICLE 2 — ACTION TO BE IMPLEMENTED

The grant is awarded for the action entitled '**NANO-scale VIsualization to understand Bacterial virulence and invasiveness - based on fluorescence NANOscopy and VIBrational microscopy**' — '**NanoVIB**' **('action')**, as described in Annex 1.

ARTICLE 3 — DURATION AND STARTING DATE OF THE ACTION

The duration of the action will be **48 months** as of 1 January 2021 ('**starting date of the action**').

ARTICLE 4 — ESTIMATED BUDGET AND BUDGET TRANSFERS

4.1 Estimated budget

The '**estimated budget**' for the action is set out in Annex 2.

It contains the estimated eligible costs and the forms of costs, broken down by beneficiary and budget category (see Articles 5, 6).

4.2 Budget transfers

The estimated budget breakdown indicated in Annex 2 may be adjusted — without an amendment (see Article 55) — by transfers of amounts between beneficiaries, budget categories and/or forms of costs set out in Annex 2, if the action is implemented as described in Annex 1.

However, the beneficiaries may not add costs relating to subcontracts not provided for in Annex 1, unless such additional subcontracts are approved by an amendment or in accordance with Article 13.

CHAPTER 3 GRANT

ARTICLE 5 — GRANT AMOUNT, FORM OF GRANT, REIMBURSEMENT RATES AND FORMS OF COSTS

5.1 Maximum grant amount

The '**maximum grant amount**' is **EUR 5 635 529.00** (five million six hundred and thirty five thousand five hundred and twenty nine EURO).

5.2 Form of grant, reimbursement rates and forms of costs

The grant reimburses **100% of the action's eligible costs** (see Article 6) ('**reimbursement of eligible costs grant**') (see Annex 2).

The estimated eligible costs of the action are EUR **5 635 530.00** (five million six hundred and thirty five thousand five hundred and thirty EURO).

Eligible costs (see Article 6) must be declared under the following forms ('**forms of costs**'):

(a) for **direct personnel costs**:

- as actually incurred costs ('**actual costs**') or
- on the basis of an amount per unit calculated by the beneficiary in accordance with its usual cost accounting practices ('**unit costs**').

Personnel **costs for SME owners** or **beneficiaries that are natural persons** not receiving a salary (see Article 6.2, Points A.4 and A.5) must be declared on the basis of the amount per unit set out in Annex 2a (**unit costs**);

(b) for **direct costs for subcontracting**: as actually incurred costs (**actual costs**);

(c) for **direct costs of providing financial support to third parties**: not applicable;

(d) for **other direct costs**:

- for costs of internally invoiced goods and services: on the basis of an amount per unit calculated by the beneficiary in accordance with its usual cost accounting practices ('**unit costs**');
- for all other costs: as actually incurred costs (**actual costs**);

(e) for **indirect costs**: on the basis of a flat-rate applied as set out in Article 6.2, Point E ('**flat-rate costs**');

(f) **specific cost category(ies):** not applicable.

5.3 Final grant amount — Calculation

The '**final grant amount**' depends on the actual extent to which the action is implemented in accordance with the Agreement's terms and conditions.

This amount is calculated by the Commission — when the payment of the balance is made (see Article 21.4) — in the following steps:

Step 1 — Application of the reimbursement rates to the eligible costs

Step 2 — Limit to the maximum grant amount

Step 3 — Reduction due to the no-profit rule

Step 4 — Reduction due to substantial errors, irregularities or fraud or serious breach of obligations

5.3.1 Step 1 — Application of the reimbursement rates to the eligible costs

The reimbursement rate(s) (see Article 5.2) are applied to the eligible costs (actual costs, unit costs and flat-rate costs; see Article 6) declared by the beneficiaries (see Article 20) and approved by the Commission (see Article 21).

5.3.2 Step 2 — Limit to the maximum grant amount

If the amount obtained following Step 1 is higher than the maximum grant amount set out in Article 5.1, it will be limited to the latter.

5.3.3 Step 3 — Reduction due to the no-profit rule

The grant must not produce a profit.

'**Profit**' means the surplus of the amount obtained following Steps 1 and 2 plus the action's total receipts, over the action's total eligible costs.

The '**action's total eligible costs**' are the consolidated total eligible costs approved by the Commission.

The '**action's total receipts**' are the consolidated total receipts generated during its duration (see Article 3).

The following are considered **receipts**:

(a) income generated by the action; if the income is generated from selling equipment or other assets purchased under the Agreement, the receipt is up to the amount declared as eligible under the Agreement;

(b) financial contributions given by third parties to the beneficiary specifically to be used for the action, and

(c) in-kind contributions provided by third parties free of charge and specifically to be used for the action, if they have been declared as eligible costs.

The following are however not considered receipts:

(a) income generated by exploiting the action's results (see Article 28);

(b) financial contributions by third parties, if they may be used to cover costs other than the eligible costs (see Article 6);

(c) financial contributions by third parties with no obligation to repay any amount unused at the end of the period set out in Article 3.

If there is a profit, it will be deducted from the amount obtained following Steps 1 and 2.

5.3.4 Step 4 — Reduction due to substantial errors, irregularities or fraud or serious breach of obligations — Reduced grant amount — Calculation

If the grant is reduced (see Article 43), the Commission will calculate the reduced grant amount by deducting the amount of the reduction (calculated in proportion to the seriousness of the errors,

irregularities or fraud or breach of obligations, in accordance with Article 43.2) from the maximum grant amount set out in Article 5.1.

The final grant amount will be the lower of the following two:

- the amount obtained following Steps 1 to 3 or
- the reduced grant amount following Step 4.

5.4 Revised final grant amount — Calculation

If — after the payment of the balance (in particular, after checks, reviews, audits or investigations; see Article 22) — the Commission rejects costs (see Article 42) or reduces the grant (see Article 43), it will calculate the '**revised final grant amount**' for the beneficiary concerned by the findings.

This amount is calculated by the Commission on the basis of the findings, as follows:

- in case of **rejection of costs**: by applying the reimbursement rate to the revised eligible costs approved by the Commission for the beneficiary concerned;
- in case of **reduction of the grant**: by calculating the concerned beneficiary's share in the grant amount reduced in proportion to the seriousness of the errors, irregularities or fraud or breach of obligations (see Article 43.2).

In case of **rejection of costs and reduction of the grant**, the revised final grant amount for the beneficiary concerned will be the lower of the two amounts above.

ARTICLE 6 — ELIGIBLE AND INELIGIBLE COSTS

6.1 General conditions for costs to be eligible

'**Eligible costs**' are costs that meet the following criteria:

(a) for **actual costs**:

(i) they must be actually incurred by the beneficiary;

(ii) they must be incurred in the period set out in Article 3, with the exception of costs relating to the submission of the periodic report for the last reporting period and the final report (see Article 20);

(iii) they must be indicated in the estimated budget set out in Annex 2;

(iv) they must be incurred in connection with the action as described in Annex 1 and necessary for its implementation;

(v) they must be identifiable and verifiable, in particular recorded in the beneficiary's accounts in accordance with the accounting standards applicable in the country where the beneficiary is established and with the beneficiary's usual cost accounting practices;

(vi) they must comply with the applicable national law on taxes, labour and social security, and

(vii) they must be reasonable, justified and must comply with the principle of sound financial management, in particular regarding economy and efficiency;

(b) for **unit costs**:

(i) they must be calculated as follows:

{amounts per unit set out in Annex 2a or calculated by the beneficiary in accordance with its usual cost accounting practices (see Article 6.2, Point A and Article 6.2.D.5)

multiplied by

the number of actual units};

(ii) the number of actual units must comply with the following conditions:

- the units must be actually used or produced in the period set out in Article 3;
- the units must be necessary for implementing the action or produced by it, and
- the number of units must be identifiable and verifiable, in particular supported by records and documentation (see Article 18);

(c) for **flat-rate costs**:

(i) they must be calculated by applying the flat-rate set out in Annex 2, and

(ii) the costs (actual costs or unit costs) to which the flat-rate is applied must comply with the conditions for eligibility set out in this Article.

6.2 Specific conditions for costs to be eligible

Costs are eligible if they comply with the general conditions (see above) and the specific conditions set out below for each of the following budget categories:

A. direct personnel costs;
B. direct costs of subcontracting;
C. not applicable;
D. other direct costs;
E. indirect costs;
F. not applicable.

'Direct costs' are costs that are directly linked to the action implementation and can therefore be attributed to it directly. They must not include any indirect costs (see Point E below).

'Indirect costs' are costs that are not directly linked to the action implementation and therefore cannot be attributed directly to it.

A. Direct personnel costs

<u>Types of eligible personnel costs</u>

A.1 Personnel costs are eligible, if they are related to personnel working for the beneficiary under an employment contract (or equivalent appointing act) and assigned to the action ('**costs for employees (or equivalent)**'). They must be limited to salaries (including during parental leave), social security contributions, taxes and other costs included in the **remuneration**, if they arise from national law or the employment contract (or equivalent appointing act).

Beneficiaries that are non-profit legal entities[1] may also declare as personnel costs **additional remuneration** for personnel assigned to the action (including payments on the basis of supplementary contracts regardless of their nature), if:

(a) it is part of the beneficiary's usual remuneration practices and is paid in a consistent manner whenever the same kind of work or expertise is required;

(b) the criteria used to calculate the supplementary payments are objective and generally applied by the beneficiary, regardless of the source of funding used.

'Additional remuneration' means any part of the remuneration which exceeds what the person would be paid for time worked in projects funded by national schemes.

Additional remuneration for personnel assigned to the action is eligible up to the following amount:

(a) if the person works full time and exclusively on the action during the full year: up to EUR 8 000;

(b) if the person works exclusively on the action but not full-time or not for the full year: up to the corresponding pro-rata amount of EUR 8 000, or

(c) if the person does not work exclusively on the action: up to a pro-rata amount calculated as follows:

{{EUR 8 000

divided by

the number of annual productive hours (see below)},

multiplied by

the number of hours that the person has worked on the action during the year}.

A.2 The **costs for natural persons working under a direct contract** with the beneficiary other than an employment contract are eligible personnel costs, if:

(a) the person works under conditions similar to those of an employee (in particular regarding the way the work is organised, the tasks that are performed and the premises where they are performed);

(b) the result of the work carried out belongs to the beneficiary (unless exceptionally agreed otherwise), and

[1] For the definition, see Article 2.1(14) of the Rules for Participation Regulation No 1290/2013: '**non-profit legal entity**' means a legal entity which by its legal form is non-profit-making or which has a legal or statutory obligation not to distribute profits to its shareholders or individual members.

(c) the costs are not significantly different from those for personnel performing similar tasks under an employment contract with the beneficiary.

A.3 The **costs of personnel seconded by a third party against payment** are eligible personnel costs, if the conditions in Article 11.1 are met.

A.4 **Costs of owners** of beneficiaries that are small and medium-sized enterprises ('**SME owners**') who are working on the action and who do not receive a salary are eligible personnel costs, if they correspond to the amount per unit set out in Annex 2a multiplied by the number of actual hours worked on the action.

A.5 **Costs of 'beneficiaries that are natural persons'** not receiving a salary are eligible personnel costs, if they correspond to the amount per unit set out in Annex 2a multiplied by the number of actual hours worked on the action.

Calculation

Personnel costs must be calculated by the beneficiaries as follows:

{{hourly rate

multiplied by

the number of actual hours worked on the action},

plus

for non-profit legal entities: additional remuneration to personnel assigned to the action under the conditions set out above (Point A.1)}.

The number of actual hours declared for a person must be identifiable and verifiable (see Article 18).

The total number of hours declared in EU or Euratom grants, for a person for a year, cannot be higher than the annual productive hours used for the calculations of the hourly rate. Therefore, the maximum number of hours that can be declared for the grant are:

{number of annual productive hours for the year (see below)

minus

total number of hours declared by the beneficiary, for that person in that year, for other EU or Euratom grants}.

The '**hourly rate**' is one of the following:

(a) for personnel costs declared as **actual costs** (i.e. budget categories A.1, A.2, A.3): the hourly rate is calculated *per full financial year*, as follows:

{actual annual personnel costs (excluding additional remuneration) for the person

divided by

number of annual productive hours}.

using the personnel costs and the number of productive hours for each full financial year covered by the reporting period concerned. If a financial year is not closed at the end of the

reporting period, the beneficiaries must use the hourly rate of the last closed financial year available.

For the 'number of annual productive hours', the beneficiaries may choose one of the following:

(i) 'fixed number of hours': 1 720 hours for persons working full time (or corresponding pro-rata for persons not working full time);

(ii) 'individual annual productive hours': the total number of hours worked by the person in the year for the beneficiary, calculated as follows:

{annual workable hours of the person (according to the employment contract, applicable collective labour agreement or national law)

plus

overtime worked

minus

absences (such as sick leave and special leave)}.

'Annual workable hours' means the period during which the personnel must be working, at the employer's disposal and carrying out his/her activity or duties under the employment contract, applicable collective labour agreement or national working time legislation.

If the contract (or applicable collective labour agreement or national working time legislation) does not allow to determine the annual workable hours, this option cannot be used;

(iii) 'standard annual productive hours': the 'standard number of annual hours' generally applied by the beneficiary for its personnel in accordance with its usual cost accounting practices. This number must be at least 90% of the 'standard annual workable hours'.

If there is no applicable reference for the standard annual workable hours, this option cannot be used.

For all options, the actual time spent on **parental leave** by a person assigned to the action may be deducted from the number of annual productive hours.

As an alternative, beneficiaries may calculate the hourly rate *per month*, as follows:

{actual monthly personnel cost (excluding additional remuneration) for the person

divided by

{number of annual productive hours / 12}}

using the personnel costs for each month and (one twelfth of) the annual productive hours calculated according to either option (i) or (iii) above, i.e.:

- fixed number of hours or
- standard annual productive hours.

Time spent on **parental leave** may not be deducted when calculating the hourly rate per month. However, beneficiaries may declare personnel costs incurred in periods of parental leave in proportion to the time the person worked on the action in that financial year.

If parts of a basic remuneration are generated over a period longer than a month, the beneficiaries may include only the share which is generated in the month (irrespective of the amount actually paid for that month).

Each beneficiary must use only one option (per full financial year or per month) for each full financial year;

(b) for personnel costs declared on the basis of **unit costs** (i.e. budget categories A.1, A.2, A.4, A.5): the hourly rate is one of the following:

(i) for SME owners or beneficiaries that are natural persons: the hourly rate set out in Annex 2a (see Points A.4 and A.5 above), or

(ii) for personnel costs declared on the basis of the beneficiary's usual cost accounting practices: the hourly rate calculated by the beneficiary in accordance with its usual cost accounting practices, if:

- the cost accounting practices used are applied in a consistent manner, based on objective criteria, regardless of the source of funding;

- the hourly rate is calculated using the actual personnel costs recorded in the beneficiary's accounts, excluding any ineligible cost or costs included in other budget categories.

  The actual personnel costs may be adjusted by the beneficiary on the basis of budgeted or estimated elements. Those elements must be relevant for calculating the personnel costs, reasonable and correspond to objective and verifiable information;

and

- the hourly rate is calculated using the number of annual productive hours (see above).

B. Direct costs of subcontracting (including related duties, taxes and charges such as non-deductible value added tax (VAT) paid by the beneficiary) are eligible if the conditions in Article 13.1.1 are met.

C. Direct costs of providing financial support to third parties

Not applicable

D. Other direct costs

D.1 **Travel costs and related subsistence allowances** (including related duties, taxes and charges such as non-deductible value added tax (VAT) paid by the beneficiary) are eligible if they are in line with the beneficiary's usual practices on travel.

D.2 The **depreciation costs of equipment, infrastructure or other assets** (new or second-hand) as recorded in the beneficiary's accounts are eligible, if they were purchased in accordance with

Article 10.1.1 and written off in accordance with international accounting standards and the beneficiary's usual accounting practices.

The **costs of renting or leasing** equipment, infrastructure or other assets (including related duties, taxes and charges such as non-deductible value added tax (VAT) paid by the beneficiary) are also eligible, if they do not exceed the depreciation costs of similar equipment, infrastructure or assets and do not include any financing fees.

The costs of equipment, infrastructure or other assets **contributed in-kind against payment** are eligible, if they do not exceed the depreciation costs of similar equipment, infrastructure or assets, do not include any financing fees and if the conditions in Article 11.1 are met.

The only portion of the costs that will be taken into account is that which corresponds to the duration of the action and rate of actual use for the purposes of the action.

D.3 **Costs of other goods and services** (including related duties, taxes and charges such as non-deductible value added tax (VAT) paid by the beneficiary) are eligible, if they are:

(a) purchased specifically for the action and in accordance with Article 10.1.1 or

(b) contributed in kind against payment and in accordance with Article 11.1.

Such goods and services include, for instance, consumables and supplies, dissemination (including open access), protection of results, certificates on the financial statements (if they are required by the Agreement), certificates on the methodology, translations and publications.

D.4 **Capitalised and operating costs of 'large research infrastructure'**[2] directly used for the action are eligible, if:

(a) the value of the large research infrastructure represents at least 75% of the total fixed assets (at historical value in its last closed balance sheet before the date of the signature of the Agreement or as determined on the basis of the rental and leasing costs of the research infrastructure[3]);

(b) the beneficiary's methodology for declaring the costs for large research infrastructure has been positively assessed by the Commission ('**ex-ante assessment**');

(c) the beneficiary declares as direct eligible costs only the portion which corresponds to the duration of the action and the rate of actual use for the purposes of the action, and

(d) they comply with the conditions as further detailed in the annotations to the H2020 grant agreements.

[2] '**Large research infrastructure**' means research infrastructure of a total value of at least EUR 20 million, for a beneficiary, calculated as the sum of historical asset values of each individual research infrastructure of that beneficiary, as they appear in its last closed balance sheet before the date of the signature of the Agreement or as determined on the basis of the rental and leasing costs of the research infrastructure.

[3] For the definition, see Article 2(6) of the H2020 Framework Programme Regulation No 1291/2013: '**Research infrastructure**' are facilities, resources and services that are used by the research communities to conduct research and foster innovation in their fields. Where relevant, they may be used beyond research, e.g. for education or public services. They include: major scientific equipment (or sets of instruments); knowledge-based resources such as collections, archives or scientific data; e-infrastructures such as data and computing systems and communication networks; and any other infrastructure of a unique nature essential to achieve excellence in research and innovation. Such infrastructures may be 'single-sited', 'virtual' or 'distributed'.

D.5 **Costs of internally invoiced goods and services** directly used for the action are eligible, if:

(a) they are declared on the basis of a unit cost calculated in accordance with the beneficiary's usual cost accounting practices;

(b) the cost accounting practices used are applied in a consistent manner, based on objective criteria, regardless of the source of funding;

(c) the unit cost is calculated using the actual costs for the good or service recorded in the beneficiary's accounts, excluding any ineligible cost or costs included in other budget categories.

The actual costs may be adjusted by the beneficiary on the basis of budgeted or estimated elements. Those elements must be relevant for calculating the costs, reasonable and correspond to objective and verifiable information;

(d) the unit cost excludes any costs of items which are not directly linked to the production of the invoiced goods or service.

'Internally invoiced goods and services' means goods or services which are provided by the beneficiary directly for the action and which the beneficiary values on the basis of its usual cost accounting practices.

E. Indirect costs

Indirect costs are eligible if they are declared on the basis of the flat-rate of 25% of the eligible direct costs (see Article 5.2 and Points A to D above), from which are excluded:

(a) costs of subcontracting and

(b) costs of in-kind contributions provided by third parties which are not used on the beneficiary's premises;

(c) not applicable;

(d) not applicable.

Beneficiaries receiving an operating grant[4] financed by the EU or Euratom budget cannot declare indirect costs for the period covered by the operating grant, unless they can demonstrate that the operating grant does not cover any costs of the action.

F. Specific cost category(ies)

Not applicable

6.3 Conditions for costs of linked third parties to be eligible

[4] For the definition, see Article 121(1)(b) of Regulation (EU, Euratom) No 966/2012 of the European Parliament and of the Council of 25 October 2012 on the financial rules applicable to the general budget of the Union and repealing Council Regulation (EC, Euratom) No 1605/2002 ('**Financial Regulation No 966/2012**')(OJ L 218, 26.10.2012, p.1): '**operating grant**' means direct financial contribution, by way of donation, from the budget in order to finance the functioning of a body which pursues an aim of general EU interest or has an objective forming part of and supporting an EU policy.

Not applicable

6.4 Conditions for in-kind contributions provided by third parties free of charge to be eligible

In-kind contributions provided free of charge are eligible direct costs (for the beneficiary), if the costs incurred by the third party fulfil — *mutatis mutandis* — the general and specific conditions for eligibility set out in this Article (Article 6.1 and 6.2) and Article 12.1.

6.5 Ineligible costs

'**Ineligible costs**' are:

(a) costs that do not comply with the conditions set out above (Article 6.1 to 6.4), in particular:

(i) costs related to return on capital;

(ii) debt and debt service charges;

(iii) provisions for future losses or debts;

(iv) interest owed;

(v) doubtful debts;

(vi) currency exchange losses;

(vii) bank costs charged by the beneficiary's bank for transfers from the Commission;

(viii) excessive or reckless expenditure;

(ix) deductible VAT;

(x) costs incurred during suspension of the implementation of the action (see Article 49);

(b) costs declared under another EU or Euratom grant (including grants awarded by a Member State and financed by the EU or Euratom budget and grants awarded by bodies other than the Commission for the purpose of implementing the EU or Euratom budget); in particular, indirect costs if the beneficiary is already receiving an operating grant financed by the EU or Euratom budget in the same period, unless it can demonstrate that the operating grant does not cover any costs of the action.

6.6 Consequences of declaration of ineligible costs

Declared costs that are ineligible will be rejected (see Article 42).

This may also lead to any of the other measures described in Chapter 6.

CHAPTER 4 RIGHTS AND OBLIGATIONS OF THE PARTIES

SECTION 1 RIGHTS AND OBLIGATIONS RELATED TO IMPLEMENTING THE ACTION

ARTICLE 7 — GENERAL OBLIGATION TO PROPERLY IMPLEMENT THE ACTION

7.1 General obligation to properly implement the action

The beneficiaries must implement the action as described in Annex 1 and in compliance with the provisions of the Agreement and all legal obligations under applicable EU, international and national law.

7.2 Consequences of non-compliance

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such breaches may also lead to any of the other measures described in Chapter 6.

ARTICLE 8 — RESOURCES TO IMPLEMENT THE ACTION — THIRD PARTIES INVOLVED IN THE ACTION

The beneficiaries must have the appropriate resources to implement the action.

If it is necessary to implement the action, the beneficiaries may:

- purchase goods, works and services (see Article 10);
- use in-kind contributions provided by third parties against payment (see Article 11);
- use in-kind contributions provided by third parties free of charge (see Article 12);
- call upon subcontractors to implement action tasks described in Annex 1 (see Article 13);
- call upon linked third parties to implement action tasks described in Annex 1 (see Article 14);
- call upon international partners to implement action tasks described in Annex 1 (see Article 14a).

In these cases, the beneficiaries retain sole responsibility towards the Commission and the other beneficiaries for implementing the action.

ARTICLE 9 — IMPLEMENTATION OF ACTION TASKS BY BENEFICIARIES NOT RECEIVING EU FUNDING

Not applicable

ARTICLE 10 — PURCHASE OF GOODS, WORKS OR SERVICES

10.1 Rules for purchasing goods, works or services

10.1.1 If necessary to implement the action, the beneficiaries may purchase goods, works or services.

The beneficiaries must make such purchases ensuring the best value for money or, if appropriate, the lowest price. In doing so, they must avoid any conflict of interests (see Article 35).

The beneficiaries must ensure that the Commission, the European Court of Auditors (ECA) and the European Anti-Fraud Office (OLAF) can exercise their rights under Articles 22 and 23 also towards their contractors.

10.1.2 Beneficiaries that are 'contracting authorities' within the meaning of Directive 2004/18/EC[5] (or 2014/24/EU[6]) or 'contracting entities' within the meaning of Directive 2004/17/EC[7] (or 2014/25/EU[8]) must comply with the applicable national law on public procurement.

10.2 Consequences of non-compliance

If a beneficiary breaches any of its obligations under Article 10.1.1, the costs related to the contract concerned will be ineligible (see Article 6) and will be rejected (see Article 42).

If a beneficiary breaches any of its obligations under Article 10.1.2, the grant may be reduced (see Article 43).

Such breaches may also lead to any of the other measures described in Chapter 6.

ARTICLE 11 — USE OF IN-KIND CONTRIBUTIONS PROVIDED BY THIRD PARTIES AGAINST PAYMENT

11.1 Rules for the use of in-kind contributions against payment

If necessary to implement the action, the beneficiaries may use in-kind contributions provided by third parties against payment.

The beneficiaries may declare costs related to the payment of in-kind contributions as eligible (see Article 6.1 and 6.2), up to the third parties' costs for the seconded persons, contributed equipment, infrastructure or other assets or other contributed goods and services.

The third parties and their contributions must be set out in Annex 1. The Commission may however approve in-kind contributions not set out in Annex 1 without amendment (see Article 55), if:

- they are specifically justified in the periodic technical report and
- their use does not entail changes to the Agreement which would call into question the decision awarding the grant or breach the principle of equal treatment of applicants.

The beneficiaries must ensure that the Commission, the European Court of Auditors (ECA) and the

[5] Directive 2004/18/EC of the European Parliament and of the Council of 31 March 2004 on the coordination of procedures for the award of public work contracts, public supply contracts and public service contracts (OJ L 134, 30.04.2004, p. 114).

[6] Directive 2014/24/EU of the European Parliament and of the Council of 26 February 2014 on public procurement and repealing Directive 2004/18/EC. (OJ L 94, 28.03.2014, p. 65).

[7] Directive 2004/17/EC of the European Parliament and of the Council of 31 March 2004 coordinating the procurement procedures of entities operating in the water, energy, transport and postal services sectors (OJ L 134, 30.04.2004, p. 1)

[8] Directive 2014/25/EU of the European Parliament and of the Council of 26 February 2014 on procurement by entities operating in the water, energy, transport and postal services sectors and repealing Directive 2004/17/EC (OJ L 94, 28.03.2014, p. 243).

European Anti-Fraud Office (OLAF) can exercise their rights under Articles 22 and 23 also towards the third parties.

11.2 Consequences of non-compliance

If a beneficiary breaches any of its obligations under this Article, the costs related to the payment of the in-kind contribution will be ineligible (see Article 6) and will be rejected (see Article 42).

Such breaches may also lead to any of the other measures described in Chapter 6.

ARTICLE 12 — USE OF IN-KIND CONTRIBUTIONS PROVIDED BY THIRD PARTIES FREE OF CHARGE

12.1 Rules for the use of in-kind contributions free of charge

If necessary to implement the action, the beneficiaries may use in-kind contributions provided by third parties free of charge.

The beneficiaries may declare costs incurred by the third parties for the seconded persons, contributed equipment, infrastructure or other assets or other contributed goods and services as eligible in accordance with Article 6.4.

The third parties and their contributions must be set out in Annex 1. The Commission may however approve in-kind contributions not set out in Annex 1 without amendment (see Article 55), if:

- they are specifically justified in the periodic technical report and
- their use does not entail changes to the Agreement which would call into question the decision awarding the grant or breach the principle of equal treatment of applicants.

The beneficiaries must ensure that the Commission, the European Court of Auditors (ECA) and the European Anti-Fraud Office (OLAF) can exercise their rights under Articles 22 and 23 also towards the third parties.

12.2 Consequences of non-compliance

If a beneficiary breaches any of its obligations under this Article, the costs incurred by the third parties related to the in-kind contribution will be ineligible (see Article 6) and will be rejected (see Article 42).

Such breaches may also lead to any of the other measures described in Chapter 6.

ARTICLE 13 — IMPLEMENTATION OF ACTION TASKS BY SUBCONTRACTORS

13.1 Rules for subcontracting action tasks

13.1.1 If necessary to implement the action, the beneficiaries may award subcontracts covering the implementation of certain action tasks described in Annex 1.

Subcontracting may cover only a limited part of the action.

The beneficiaries must award the subcontracts ensuring the best value for money or, if appropriate, the lowest price. In doing so, they must avoid any conflict of interests (see Article 35).

The tasks to be implemented and the estimated cost for each subcontract must be set out in Annex 1 and the total estimated costs of subcontracting per beneficiary must be set out in Annex 2. The Commission may however approve subcontracts not set out in Annex 1 and 2 without amendment (see Article 55), if:

- they are specifically justified in the periodic technical report and
- they do not entail changes to the Agreement which would call into question the decision awarding the grant or breach the principle of equal treatment of applicants.

The beneficiaries must ensure that the Commission, the European Court of Auditors (ECA) and the European Anti-Fraud Office (OLAF) can exercise their rights under Articles 22 and 23 also towards their subcontractors.

13.1.2 The beneficiaries must ensure that their obligations under Articles 35, 36, 38 and 46 also apply to the subcontractors.

Beneficiaries that are 'contracting authorities' within the meaning of Directive 2004/18/EC (or 2014/24/EU) or 'contracting entities' within the meaning of Directive 2004/17/EC (or 2014/25/EU) must comply with the applicable national law on public procurement.

13.2 Consequences of non-compliance

If a beneficiary breaches any of its obligations under Article 13.1.1, the costs related to the subcontract concerned will be ineligible (see Article 6) and will be rejected (see Article 42).

If a beneficiary breaches any of its obligations under Article 13.1.2, the grant may be reduced (see Article 43).

Such breaches may also lead to any of the other measures described in Chapter 6.

ARTICLE 14 — IMPLEMENTATION OF ACTION TASKS BY LINKED THIRD PARTIES

Not applicable

ARTICLE 14a — IMPLEMENTATION OF ACTION TASKS BY INTERNATIONAL PARTNERS

Not applicable

ARTICLE 15 — FINANCIAL SUPPORT TO THIRD PARTIES

15.1 Rules for providing financial support to third parties

Not applicable

15.2 Financial support in the form of prizes

Not applicable

15.3 Consequences of non-compliance

Not applicable

ARTICLE 16 — PROVISION OF TRANS-NATIONAL OR VIRTUAL ACCESS TO RESEARCH INFRASTRUCTURE

16.1 Rules for providing trans-national access to research infrastructure

Not applicable

16.2 Rules for providing virtual access to research infrastructure

Not applicable

16.3 Consequences of non-compliance

Not applicable

SECTION 2 RIGHTS AND OBLIGATIONS RELATED TO THE GRANT ADMINISTRATION

ARTICLE 17 — GENERAL OBLIGATION TO INFORM

17.1 General obligation to provide information upon request

The beneficiaries must provide — during implementation of the action or afterwards and in accordance with Article 41.2 — any information requested in order to verify eligibility of the costs, proper implementation of the action and compliance with any other obligation under the Agreement.

17.2 Obligation to keep information up to date and to inform about events and circumstances likely to affect the Agreement

Each beneficiary must keep information stored in the Participant Portal Beneficiary Register (via the electronic exchange system; see Article 52) up to date, in particular, its name, address, legal representatives, legal form and organisation type.

Each beneficiary must immediately inform the coordinator — which must immediately inform the Commission and the other beneficiaries — of any of the following:

(a) **events** which are likely to affect significantly or delay the implementation of the action or the EU's financial interests, in particular:

  (i) changes in its legal, financial, technical, organisational or ownership situation

(b) **circumstances** affecting:

  (i) the decision to award the grant or

  (ii) compliance with requirements under the Agreement.

17.3 Consequences of non-compliance

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such breaches may also lead to any of the other measures described in Chapter 6.

ARTICLE 18 — KEEPING RECORDS — SUPPORTING DOCUMENTATION

18.1 Obligation to keep records and other supporting documentation

The beneficiaries must — for a period of five years after the payment of the balance — keep records and other supporting documentation in order to prove the proper implementation of the action and the costs they declare as eligible.

They must make them available upon request (see Article 17) or in the context of checks, reviews, audits or investigations (see Article 22).

If there are on-going checks, reviews, audits, investigations, litigation or other pursuits of claims under the Agreement (including the extension of findings; see Article 22), the beneficiaries must keep the records and other supporting documentation until the end of these procedures.

The beneficiaries must keep the original documents. Digital and digitalised documents are considered originals if they are authorised by the applicable national law. The Commission may accept non-original documents if it considers that they offer a comparable level of assurance.

18.1.1 Records and other supporting documentation on the scientific and technical implementation

The beneficiaries must keep records and other supporting documentation on scientific and technical implementation of the action in line with the accepted standards in the respective field.

18.1.2 Records and other documentation to support the costs declared

The beneficiaries must keep the records and documentation supporting the costs declared, in particular the following:

(a) for **actual costs**: adequate records and other supporting documentation to prove the costs declared, such as contracts, subcontracts, invoices and accounting records. In addition, the beneficiaries' usual cost accounting practices and internal control procedures must enable direct reconciliation between the amounts declared, the amounts recorded in their accounts and the amounts stated in the supporting documentation;

(b) for **unit costs**: adequate records and other supporting documentation to prove the number of units declared. Beneficiaries do not need to identify the actual eligible costs covered or to keep or provide supporting documentation (such as accounting statements) to prove the amount per unit.

In addition, **for unit costs calculated in accordance with the beneficiary's usual cost accounting practices**, the beneficiaries must keep adequate records and documentation to prove that the cost accounting practices used comply with the conditions set out in Article 6.2.

The beneficiaries may submit to the Commission, for approval, a certificate (drawn up in accordance with Annex 6) stating that their usual cost accounting practices comply with these

conditions ('**certificate on the methodology**'). If the certificate is approved, costs declared in line with this methodology will not be challenged subsequently, unless the beneficiaries have concealed information for the purpose of the approval.

(c) for **flat-rate costs**: adequate records and other supporting documentation to prove the eligibility of the costs to which the flat-rate is applied. The beneficiaries do not need to identify the costs covered or provide supporting documentation (such as accounting statements) to prove the amount declared at a flat-rate.

In addition, for **personnel costs** (declared as actual costs or on the basis of unit costs), the beneficiaries must keep **time records** for the number of hours declared. The time records must be in writing and approved by the persons working on the action and their supervisors, at least monthly. In the absence of reliable time records of the hours worked on the action, the Commission may accept alternative evidence supporting the number of hours declared, if it considers that it offers an adequate level of assurance.

As an exception, for **persons working exclusively on the action**, there is no need to keep time records, if the beneficiary signs a **declaration** confirming that the persons concerned have worked exclusively on the action.

18.2 Consequences of non-compliance

If a beneficiary breaches any of its obligations under this Article, costs insufficiently substantiated will be ineligible (see Article 6) and will be rejected (see Article 42), and the grant may be reduced (see Article 43).

Such breaches may also lead to any of the other measures described in Chapter 6.

ARTICLE 19 — SUBMISSION OF DELIVERABLES

19.1 Obligation to submit deliverables

The coordinator must submit the '**deliverables**' identified in Annex 1, in accordance with the timing and conditions set out in it.

19.2 Consequences of non-compliance

If the coordinator breaches any of its obligations under this Article, the Commission may apply any of the measures described in Chapter 6.

ARTICLE 20 — REPORTING — PAYMENT REQUESTS

20.1 Obligation to submit reports

The coordinator must submit to the Commission (see Article 52) the technical and financial reports set out in this Article. These reports include requests for payment and must be drawn up using the forms and templates provided in the electronic exchange system (see Article 52).

20.2 Reporting periods

The action is divided into the following '**reporting periods**':

- RP1: from month 1 to month 18
- RP2: from month 19 to month 36
- RP3: from month 37 to month 48

20.3 Periodic reports — Requests for interim payments

The coordinator must submit a periodic report within 60 days following the end of each reporting period.

The **periodic report** must include the following:

(a) a '**periodic technical report**' containing:

(i) an **explanation of the work carried out** by the beneficiaries;

(ii) an **overview of the progress** towards the objectives of the action, including milestones and deliverables identified in Annex 1.

This report must include explanations justifying the differences between work expected to be carried out in accordance with Annex 1 and that actually carried out.

The report must detail the exploitation and dissemination of the results and — if required in Annex 1 — an updated '**plan for the exploitation and dissemination of the results**'.

The report must indicate the communication activities;

(iii) a **summary** for publication by the Commission;

(iv) the answers to the '**questionnaire**', covering issues related to the action implementation and the economic and societal impact, notably in the context of the Horizon 2020 key performance indicators and the Horizon 2020 monitoring requirements;

(b) a '**periodic financial report**' containing:

(i) an '**individual financial statement**' (see Annex 4) from each beneficiary, for the reporting period concerned.

The individual financial statement must detail the eligible costs (actual costs, unit costs and flat-rate costs; see Article 6) for each budget category (see Annex 2).

The beneficiaries must declare all eligible costs, even if — for actual costs, unit costs and flat-rate costs — they exceed the amounts indicated in the estimated budget (see Annex 2). Amounts which are not declared in the individual financial statement will not be taken into account by the Commission.

If an individual financial statement is not submitted for a reporting period, it may be included in the periodic financial report for the next reporting period.

The individual financial statements of the last reporting period must also detail the **receipts of the action** (see Article 5.3.3).

Each beneficiary must **certify** that:

- the information provided is full, reliable and true;
- the costs declared are eligible (see Article 6);
- the costs can be substantiated by adequate records and supporting documentation (see Article 18) that will be produced upon request (see Article 17) or in the context of checks, reviews, audits and investigations (see Article 22), and
- for the last reporting period: that all the receipts have been declared (see Article 5.3.3);

(ii) an **explanation of the use of resources** and the information on subcontracting (see Article 13) and in-kind contributions provided by third parties (see Articles 11 and 12) from each beneficiary, for the reporting period concerned;

(iii) not applicable;

(iv) a '**periodic summary financial statement**', created automatically by the electronic exchange system, consolidating the individual financial statements for the reporting period concerned and including — except for the last reporting period — the **request for interim payment**.

20.4 Final report — Request for payment of the balance

In addition to the periodic report for the last reporting period, the coordinator must submit the final report within 60 days following the end of the last reporting period.

The **final report** must include the following:

(a) a '**final technical report**' with a **summary** for publication containing:

(i) an overview of the results and their exploitation and dissemination;

(ii) the conclusions on the action, and

(iii) the socio-economic impact of the action;

(b) a '**final financial report**' containing:

(i) a '**final summary financial statement**', created automatically by the electronic exchange system, consolidating the individual financial statements for all reporting periods and including the **request for payment of the balance** and

(ii) a '**certificate on the financial statements**' (drawn up in accordance with Annex 5) for each beneficiary, if it requests a total contribution of EUR 325 000 or more, as reimbursement of actual costs and unit costs calculated on the basis of its usual cost accounting practices (see Article 5.2 and Article 6.2).

20.5 Information on cumulative expenditure incurred

Not applicable

20.6 Currency for financial statements and conversion into euro

Financial statements must be drafted in euro.

Beneficiaries with accounting established in a currency other than the euro must convert the costs recorded in their accounts into euro, at the average of the daily exchange rates published in the C series of the *Official Journal of the European Union*, calculated over the corresponding reporting period.

If no daily euro exchange rate is published in the *Official Journal of the European Union* for the currency in question, they must be converted at the average of the monthly accounting rates published on the Commission's website, calculated over the corresponding reporting period.

Beneficiaries with accounting established in euro must convert costs incurred in another currency into euro according to their usual accounting practices.

20.7 Language of reports

All reports (technical and financial reports, including financial statements) must be submitted in the language of the Agreement.

20.8 Consequences of non-compliance

If the reports submitted do not comply with this Article, the Commission may suspend the payment deadline (see Article 47) and apply any of the other measures described in Chapter 6.

If the coordinator breaches its obligation to submit the reports and if it fails to comply with this obligation within 30 days following a written reminder, the Commission may terminate the Agreement (see Article 50) or apply any of the other measures described in Chapter 6.

ARTICLE 21 — PAYMENTS AND PAYMENT ARRANGEMENTS

21.1 Payments to be made

The following payments will be made to the coordinator:

- one **pre-financing payment**;
- one or more **interim payments**, on the basis of the request(s) for interim payment (see Article 20), and
- one **payment of the balance**, on the basis of the request for payment of the balance (see Article 20).

21.2 Pre-financing payment — Amount — Amount retained for the Guarantee Fund

The aim of the pre-financing is to provide the beneficiaries with a float.

It remains the property of the EU until the payment of the balance.

The amount of the pre-financing payment will be EUR **3 005 615.47** (three million five thousand six hundred and fifteen EURO and forty seven eurocents).

The Commission will — except if Article 48 applies — make the pre-financing payment to the coordinator within 30 days, either from the entry into force of the Agreement (see Article 58) or from 10 days before the starting date of the action (see Article 3), whichever is the latest.

An amount of EUR **281 776.45** (two hundred and eighty one thousand seven hundred and seventy six EURO and forty five eurocents), corresponding to 5% of the maximum grant amount (see Article 5.1), is retained by the Commission from the pre-financing payment and transferred into the '**Guarantee Fund**'.

21.3 Interim payments — Amount — Calculation

Interim payments reimburse the eligible costs incurred for the implementation of the action during the corresponding reporting periods.

The Commission will pay to the coordinator the amount due as interim payment within 90 days from receiving the periodic report (see Article 20.3), except if Articles 47 or 48 apply.

Payment is subject to the approval of the periodic report. Its approval does not imply recognition of the compliance, authenticity, completeness or correctness of its content.

The **amount due as interim payment** is calculated by the Commission in the following steps:

Step 1 — Application of the reimbursement rates

Step 2 — Limit to 90% of the maximum grant amount

21.3.1 Step 1 — Application of the reimbursement rates

The reimbursement rate(s) (see Article 5.2) are applied to the eligible costs (actual costs, unit costs and flat-rate costs; see Article 6) declared by the beneficiaries (see Article 20) and approved by the Commission (see above) for the concerned reporting period.

21.3.2 Step 2 — Limit to 90% of the maximum grant amount

The total amount of pre-financing and interim payments must not exceed 90% of the maximum grant amount set out in Article 5.1. The maximum amount for the interim payment will be calculated as follows:

{90% of the maximum grant amount (see Article 5.1)

minus

{pre-financing and previous interim payments}}.

21.4 Payment of the balance — Amount — Calculation — Release of the amount retained for the Guarantee Fund

The payment of the balance reimburses the remaining part of the eligible costs incurred by the beneficiaries for the implementation of the action.

If the total amount of earlier payments is greater than the final grant amount (see Article 5.3), the payment of the balance takes the form of a recovery (see Article 44).

If the total amount of earlier payments is lower than the final grant amount, the Commission will pay the balance within 90 days from receiving the final report (see Article 20.4), except if Articles 47 or 48 apply.

Payment is subject to the approval of the final report. Its approval does not imply recognition of the compliance, authenticity, completeness or correctness of its content.

The **amount due as the balance** is calculated by the Commission by deducting the total amount of pre-financing and interim payments (if any) already made, from the final grant amount determined in accordance with Article 5.3:

{final grant amount (see Article 5.3)

minus

{pre-financing and interim payments (if any) made}.

At the payment of the balance, the amount retained for the Guarantee Fund (see above) will be released and:

- if the balance is positive: the amount released will be paid in full to the coordinator together with the amount due as the balance;
- if the balance is negative (payment of the balance taking the form of recovery): it will be deducted from the amount released (see Article 44.1.2). If the resulting amount:
  - is positive, it will be paid to the coordinator
  - is negative, it will be recovered.

The amount to be paid may however be offset — without the beneficiaries' consent — against any other amount owed by a beneficiary to the Commission or an executive agency (under the EU or Euratom budget), up to the maximum EU contribution indicated, for that beneficiary, in the estimated budget (see Annex 2).

21.5 Notification of amounts due

When making payments, the Commission will formally notify to the coordinator the amount due, specifying whether it concerns an interim payment or the payment of the balance.

For the payment of the balance, the notification will also specify the final grant amount.

In the case of reduction of the grant or recovery of undue amounts, the notification will be preceded by the contradictory procedure set out in Articles 43 and 44.

21.6 Currency for payments

The Commission will make all payments in euro.

21.7 Payments to the coordinator — Distribution to the beneficiaries

Payments will be made to the coordinator.

Payments to the coordinator will discharge the Commission from its payment obligation.

The coordinator must distribute the payments between the beneficiaries without unjustified delay.

Pre-financing may however be distributed only:

(a) if the minimum number of beneficiaries set out in the call for proposals has acceded to the Agreement (see Article 56) and

(b) to beneficiaries that have acceded to the Agreement (see Article 56).

21.8 Bank account for payments

All payments will be made to the following bank account:

Name of bank: DANSKE BANK
Full name of the account holder: KUNGLIGA TEKNISKA HOEGSKOLAN
IBAN code: SE5212000000012810118175

21.9 Costs of payment transfers

The cost of the payment transfers is borne as follows:

- the Commission bears the cost of transfers charged by its bank;
- the beneficiary bears the cost of transfers charged by its bank;
- the party causing a repetition of a transfer bears all costs of the repeated transfer.

21.10 Date of payment

Payments by the Commission are considered to have been carried out on the date when they are debited to its account.

21.11 Consequences of non-compliance

21.11.1 If the Commission does not pay within the payment deadlines (see above), the beneficiaries are entitled to **late-payment interest** at the rate applied by the European Central Bank (ECB) for its main refinancing operations in euros ('reference rate'), plus three and a half points. The reference rate is the rate in force on the first day of the month in which the payment deadline expires, as published in the C series of the *Official Journal of the European Union*.

If the late-payment interest is lower than or equal to EUR 200, it will be paid to the coordinator only upon request submitted within two months of receiving the late payment.

Late-payment interest is not due if all beneficiaries are EU Member States (including regional and local government authorities or other public bodies acting on behalf of a Member State for the purpose of this Agreement).

Suspension of the payment deadline or payments (see Articles 47 and 48) will not be considered as late payment.

Late-payment interest covers the period running from the day following the due date for payment (see above), up to and including the date of payment.

Late-payment interest is not considered for the purposes of calculating the final grant amount.

21.11.2 If the coordinator breaches any of its obligations under this Article, the grant may be reduced (see Article 43) and the Agreement or the participation of the coordinator may be terminated (see Article 50).

Such breaches may also lead to any of the other measures described in Chapter 6.

ARTICLE 22 — CHECKS, REVIEWS, AUDITS AND INVESTIGATIONS — EXTENSION OF FINDINGS

22.1 Checks, reviews and audits by the Commission

22.1.1 Right to carry out checks

The Commission will — during the implementation of the action or afterwards — check the proper implementation of the action and compliance with the obligations under the Agreement, including assessing deliverables and reports.

For this purpose the Commission may be assisted by external persons or bodies.

The Commission may also request additional information in accordance with Article 17. The Commission may request beneficiaries to provide such information to it directly.

Information provided must be accurate, precise and complete and in the format requested, including electronic format.

22.1.2 Right to carry out reviews

The Commission may — during the implementation of the action or afterwards — carry out reviews on the proper implementation of the action (including assessment of deliverables and reports), compliance with the obligations under the Agreement and continued scientific or technological relevance of the action.

Reviews may be started up to two years after the payment of the balance. They will be formally notified to the coordinator or beneficiary concerned and will be considered to have started on the date of the formal notification.

If the review is carried out on a third party (see Articles 10 to 16), the beneficiary concerned must inform the third party.

The Commission may carry out reviews directly (using its own staff) or indirectly (using external persons or bodies appointed to do so). It will inform the coordinator or beneficiary concerned of the identity of the external persons or bodies. They have the right to object to the appointment on grounds of commercial confidentiality.

The coordinator or beneficiary concerned must provide — within the deadline requested — any information and data in addition to deliverables and reports already submitted (including information

on the use of resources). The Commission may request beneficiaries to provide such information to it directly.

The coordinator or beneficiary concerned may be requested to participate in meetings, including with external experts.

For **on-the-spot** reviews, the beneficiaries must allow access to their sites and premises, including to external persons or bodies, and must ensure that information requested is readily available.

Information provided must be accurate, precise and complete and in the format requested, including electronic format.

On the basis of the review findings, a '**review report**' will be drawn up.

The Commission will formally notify the review report to the coordinator or beneficiary concerned, which has 30 days to formally notify observations ('**contradictory review procedure**').

Reviews (including review reports) are in the language of the Agreement.

22.1.3 Right to carry out audits

The Commission may — during the implementation of the action or afterwards — carry out audits on the proper implementation of the action and compliance with the obligations under the Agreement.

Audits may be started up to two years after the payment of the balance. They will be formally notified to the coordinator or beneficiary concerned and will be considered to have started on the date of the formal notification.

If the audit is carried out on a third party (see Articles 10 to 16), the beneficiary concerned must inform the third party.

The Commission may carry out audits directly (using its own staff) or indirectly (using external persons or bodies appointed to do so). It will inform the coordinator or beneficiary concerned of the identity of the external persons or bodies. They have the right to object to the appointment on grounds of commercial confidentiality.

The coordinator or beneficiary concerned must provide — within the deadline requested — any information (including complete accounts, individual salary statements or other personal data) to verify compliance with the Agreement. The Commission may request beneficiaries to provide such information to it directly.

For **on-the-spot** audits, the beneficiaries must allow access to their sites and premises, including to external persons or bodies, and must ensure that information requested is readily available.

Information provided must be accurate, precise and complete and in the format requested, including electronic format.

On the basis of the audit findings, a '**draft audit report**' will be drawn up.

The Commission will formally notify the draft audit report to the coordinator or beneficiary concerned, which has 30 days to formally notify observations ('**contradictory audit procedure**'). This period may be extended by the Commission in justified cases.

The '**final audit report**' will take into account observations by the coordinator or beneficiary concerned. The report will be formally notified to it.

Audits (including audit reports) are in the language of the Agreement.

The Commission may also access the beneficiaries' statutory records for the periodical assessment of unit costs or flat-rate amounts.

22.2 Investigations by the European Anti-Fraud Office (OLAF)

Under Regulations No 883/2013[16] and No 2185/96[17] (and in accordance with their provisions and procedures), the European Anti-Fraud Office (OLAF) may — at any moment during implementation of the action or afterwards — carry out investigations, including on-the-spot checks and inspections, to establish whether there has been fraud, corruption or any other illegal activity affecting the financial interests of the EU.

22.3 Checks and audits by the European Court of Auditors (ECA)

Under Article 287 of the Treaty on the Functioning of the European Union (TFEU) and Article 161 of the Financial Regulation No 966/2012[18], the European Court of Auditors (ECA) may — at any moment during implementation of the action or afterwards — carry out audits.

The ECA has the right of access for the purpose of checks and audits.

22.4 Checks, reviews, audits and investigations for international organisations

Not applicable

22.5 Consequences of findings in checks, reviews, audits and investigations — Extension of findings

22.5.1 Findings in this grant

Findings in checks, reviews, audits or investigations carried out in the context of this grant may lead to the rejection of ineligible costs (see Article 42), reduction of the grant (see Article 43), recovery of undue amounts (see Article 44) or to any of the other measures described in Chapter 6.

Rejection of costs or reduction of the grant after the payment of the balance will lead to a revised final grant amount (see Article 5.4).

Findings in checks, reviews, audits or investigations may lead to a request for amendment for the modification of Annex 1 (see Article 55).

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[16] Regulation (EU, Euratom) No 883/2013 of the European Parliament and of the Council of 11 September 2013 concerning investigations conducted by the European Anti-Fraud Office (OLAF) and repealing Regulation (EC) No 1073/1999 of the European Parliament and of the Council and Council Regulation (Euratom) No 1074/1999 (OJ L 248, 18.09.2013, p. 1).

[17] Council Regulation (Euratom, EC) No 2185/1996 of 11 November 1996 concerning on-the-spot checks and inspections carried out by the Commission in order to protect the European Communities' financial interests against fraud and other irregularities (OJ L 292, 15.11.1996, p. 2).

[18] Regulation (EU, Euratom) No 966/2012 of the European Parliament and of the Council of 25 October 2012 on the financial rules applicable to the general budget of the Union and repealing Council Regulation (EC, Euratom) No 1605/2002 (OJ L 298, 26.10.2012, p. 1).

Checks, reviews, audits or investigations that find systemic or recurrent errors, irregularities, fraud or breach of obligations may also lead to consequences in other EU or Euratom grants awarded under similar conditions ('**extension of findings from this grant to other grants**').

Moreover, findings arising from an OLAF investigation may lead to criminal prosecution under national law.

22.5.2 Findings in other grants

The Commission may extend findings from other grants to this grant ('**extension of findings from other grants to this grant**'), if:

(a) the beneficiary concerned is found, in other EU or Euratom grants awarded under similar conditions, to have committed systemic or recurrent errors, irregularities, fraud or breach of obligations that have a material impact on this grant and

(b) those findings are formally notified to the beneficiary concerned — together with the list of grants affected by the findings — no later than two years after the payment of the balance of this grant.

The extension of findings may lead to the rejection of costs (see Article 42), reduction of the grant (see Article 43), recovery of undue amounts (see Article 44), suspension of payments (see Article 48), suspension of the action implementation (see Article 49) or termination (see Article 50).

22.5.3 Procedure

The Commission will formally notify the beneficiary concerned the systemic or recurrent errors and its intention to extend these audit findings, together with the list of grants affected.

22.5.3.1 If the findings concern **eligibility of costs**: the formal notification will include:

(a) an invitation to submit observations on the list of grants affected by the findings;

(b) the request to submit **revised financial statements** for all grants affected;

(c) the **correction rate for extrapolation** established by the Commission on the basis of the systemic or recurrent errors, to calculate the amounts to be rejected if the beneficiary concerned:

(i) considers that the submission of revised financial statements is not possible or practicable or

(ii) does not submit revised financial statements.

The beneficiary concerned has 90 days from receiving notification to submit observations, revised financial statements or to propose a duly substantiated **alternative correction method**. This period may be extended by the Commission in justified cases.

The Commission may then start a rejection procedure in accordance with Article 42, on the basis of:

- the revised financial statements, if approved;

- the proposed alternative correction method, if accepted

or

- the initially notified correction rate for extrapolation, if it does not receive any observations or revised financial statements, does not accept the observations or the proposed alternative correction method or does not approve the revised financial statements.

22.5.3.2 If the findings concern **substantial errors**, **irregularities or fraud** or **serious breach of obligations**: the formal notification will include:

(a) an invitation to submit observations on the list of grants affected by the findings and

(b) the flat-rate the Commission intends to apply according to the principle of proportionality.

The beneficiary concerned has 90 days from receiving notification to submit observations or to propose a duly substantiated alternative flat-rate.

The Commission may then start a reduction procedure in accordance with Article 43, on the basis of:

- the proposed alternative flat-rate, if accepted

or

- the initially notified flat-rate, if it does not receive any observations or does not accept the observations or the proposed alternative flat-rate.

22.6 Consequences of non-compliance

If a beneficiary breaches any of its obligations under this Article, any insufficiently substantiated costs will be ineligible (see Article 6) and will be rejected (see Article 42).

Such breaches may also lead to any of the other measures described in Chapter 6.

ARTICLE 23 — EVALUATION OF THE IMPACT OF THE ACTION

23.1 Right to evaluate the impact of the action

The Commission may carry out interim and final evaluations of the impact of the action measured against the objective of the EU programme.

Evaluations may be started during implementation of the action and up to five years after the payment of the balance. The evaluation is considered to start on the date of the formal notification to the coordinator or beneficiaries.

The Commission may make these evaluations directly (using its own staff) or indirectly (using external bodies or persons it has authorised to do so).

The coordinator or beneficiaries must provide any information relevant to evaluate the impact of the action, including information in electronic format.

23.2 Consequences of non-compliance

If a beneficiary breaches any of its obligations under this Article, the Commission may apply the measures described in Chapter 6.

SECTION 3 RIGHTS AND OBLIGATIONS RELATED TO BACKGROUND AND RESULTS

SUBSECTION 1 GENERAL

ARTICLE 23a — MANAGEMENT OF INTELLECTUAL PROPERTY

23a.1 Obligation to take measures to implement the Commission Recommendation on the management of intellectual property in knowledge transfer activities

Beneficiaries that are universities or other public research organisations must take measures to implement the principles set out in Points 1 and 2 of the Code of Practice annexed to the Commission Recommendation on the management of intellectual property in knowledge transfer activities[19].

This does not change the obligations set out in Subsections 2 and 3 of this Section.

The beneficiaries must ensure that researchers and third parties involved in the action are aware of them.

23a.2 Consequences of non-compliance

If a beneficiary breaches its obligations under this Article, the Commission may apply any of the measures described in Chapter 6.

SUBSECTION 2 RIGHTS AND OBLIGATIONS RELATED TO BACKGROUND

ARTICLE 24 — AGREEMENT ON BACKGROUND

24.1 Agreement on background

The beneficiaries must identify and agree (in writing) on the background for the action ('**agreement on background**').

'**Background**' means any data, know-how or information — whatever its form or nature (tangible or intangible), including any rights such as intellectual property rights — that:

(a) is held by the beneficiaries before they acceded to the Agreement, and

(b) is needed to implement the action or exploit the results.

24.2 Consequences of non-compliance

[19] Commission Recommendation C(2008) 1329 of 10.4.2008 on the management of intellectual property in knowledge transfer activities and the Code of Practice for universities and other public research institutions attached to this recommendation.

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such breaches may also lead to any of the other measures described in Chapter 6.

ARTICLE 25 — ACCESS RIGHTS TO BACKGROUND

25.1 Exercise of access rights — Waiving of access rights — No sub-licensing

To exercise access rights, this must first be requested in writing ('**request for access**').

'**Access rights**' means rights to use results or background under the terms and conditions laid down in this Agreement.

Waivers of access rights are not valid unless in writing.

Unless agreed otherwise, access rights do not include the right to sub-license.

25.2 Access rights for other beneficiaries, for implementing their own tasks under the action

The beneficiaries must give each other access — on a royalty-free basis — to background needed to implement their own tasks under the action, unless the beneficiary that holds the background has — before acceding to the Agreement —:

(a) informed the other beneficiaries that access to its background is subject to legal restrictions or limits, including those imposed by the rights of third parties (including personnel), or

(b) agreed with the other beneficiaries that access would not be on a royalty-free basis.

25.3 Access rights for other beneficiaries, for exploiting their own results

The beneficiaries must give each other access — under fair and reasonable conditions — to background needed for exploiting their own results, unless the beneficiary that holds the background has — before acceding to the Agreement — informed the other beneficiaries that access to its background is subject to legal restrictions or limits, including those imposed by the rights of third parties (including personnel).

'**Fair and reasonable conditions**' means appropriate conditions, including possible financial terms or royalty-free conditions, taking into account the specific circumstances of the request for access, for example the actual or potential value of the results or background to which access is requested and/or the scope, duration or other characteristics of the exploitation envisaged.

Requests for access may be made — unless agreed otherwise — up to one year after the period set out in Article 3.

25.4 Access rights for affiliated entities

Unless otherwise agreed in the consortium agreement, access to background must also be given — under fair and reasonable conditions (see above; Article 25.3) and unless it is subject to legal restrictions or limits, including those imposed by the rights of third parties (including personnel) —

to affiliated entities[20] established in an EU Member State or '**associated country**'[21], if this is needed to exploit the results generated by the beneficiaries to which they are affiliated.

Unless agreed otherwise (see above; Article 25.1), the affiliated entity concerned must make the request directly to the beneficiary that holds the background.

Requests for access may be made — unless agreed otherwise — up to one year after the period set out in Article 3.

25.5 Access rights for third parties

Not applicable

25.6 Consequences of non-compliance

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such breaches may also lead to any of the other measures described in Chapter 6.

SUBSECTION 3 RIGHTS AND OBLIGATIONS RELATED TO RESULTS

ARTICLE 26 — OWNERSHIP OF RESULTS

26.1 Ownership by the beneficiary that generates the results

Results are owned by the beneficiary that generates them.

'**Results**' means any (tangible or intangible) output of the action such as data, knowledge or information — whatever its form or nature, whether it can be protected or not — that is generated in the action, as well as any rights attached to it, including intellectual property rights.

26.2 Joint ownership by several beneficiaries

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[20] For the definition see Article 2.1(2) Rules for Participation Regulation No 1290/2013: '**affiliated entity**' means any legal entity that is:
- under the direct or indirect control of a participant, or
- under the same direct or indirect control as the participant, or
- directly or indirectly controlling a participant.

'Control' may take any of the following forms:
(a) the direct or indirect holding of more than 50% of the nominal value of the issued share capital in the legal entity concerned, or of a majority of the voting rights of the shareholders or associates of that entity;
(b) the direct or indirect holding, in fact or in law, of decision-making powers in the legal entity concerned.

However the following relationships between legal entities shall not in themselves be deemed to constitute controlling relationships:
(a) the same public investment corporation, institutional investor or venture-capital company has a direct or indirect holding of more than 50% of the nominal value of the issued share capital or a majority of voting rights of the shareholders or associates;
(b) the legal entities concerned are owned or supervised by the same public body.

[21] For the definition, see Article 2.1(3) of the Rules for Participation Regulation No 1290/2013: '**associated country**' means a third country which is party to an international agreement with the Union, as identified in Article 7 of Horizon 2020 Framework Programme Regulation No 1291/2013. Article 7 sets out the conditions for association of non-EU countries to Horizon 2020.

Two or more beneficiaries own results jointly if:

(a) they have jointly generated them and

(b) it is not possible to:

(i) establish the respective contribution of each beneficiary, or

(ii) separate them for the purpose of applying for, obtaining or maintaining their protection (see Article 27).

The joint owners must agree (in writing) on the allocation and terms of exercise of their joint ownership ('**joint ownership agreement**'), to ensure compliance with their obligations under this Agreement.

Unless otherwise agreed in the joint ownership agreement, each joint owner may grant non-exclusive licences to third parties to exploit jointly-owned results (without any right to sub-license), if the other joint owners are given:

(a) at least 45 days advance notice and

(b) fair and reasonable compensation.

Once the results have been generated, joint owners may agree (in writing) to apply another regime than joint ownership (such as, for instance, transfer to a single owner (see Article 30) with access rights for the others).

26.3 Rights of third parties (including personnel)

If third parties (including personnel) may claim rights to the results, the beneficiary concerned must ensure that it complies with its obligations under the Agreement.

If a third party generates results, the beneficiary concerned must obtain all necessary rights (transfer, licences or other) from the third party, in order to be able to respect its obligations as if those results were generated by the beneficiary itself.

If obtaining the rights is impossible, the beneficiary must refrain from using the third party to generate the results.

26.4 EU ownership, to protect results

26.4.1 The EU may — with the consent of the beneficiary concerned — assume ownership of results to protect them, if a beneficiary intends — up to four years after the period set out in Article 3 — to disseminate its results without protecting them, except in any of the following cases:

(a) the lack of protection is because protecting the results is not possible, reasonable or justified (given the circumstances);

(b) the lack of protection is because there is a lack of potential for commercial or industrial exploitation, or

(c) the beneficiary intends to transfer the results to another beneficiary or third party established in an EU Member State or associated country, which will protect them.

Before the results are disseminated and unless any of the cases above under Points (a), (b) or (c) applies, the beneficiary must formally notify the Commission and at the same time inform it of any reasons for refusing consent. The beneficiary may refuse consent only if it can show that its legitimate interests would suffer significant harm.

If the Commission decides to assume ownership, it will formally notify the beneficiary concerned within 45 days of receiving notification.

No dissemination relating to these results may take place before the end of this period or, if the Commission takes a positive decision, until it has taken the necessary steps to protect the results.

26.4.2 The EU may — with the consent of the beneficiary concerned — assume ownership of results to protect them, if a beneficiary intends — up to four years after the period set out in Article 3 — to stop protecting them or not to seek an extension of protection, except in any of the following cases:

(a) the protection is stopped because of a lack of potential for commercial or industrial exploitation;

(b) an extension would not be justified given the circumstances.

A beneficiary that intends to stop protecting results or not seek an extension must — unless any of the cases above under Points (a) or (b) applies — formally notify the Commission at least 60 days before the protection lapses or its extension is no longer possible and at the same time inform it of any reasons for refusing consent. The beneficiary may refuse consent only if it can show that its legitimate interests would suffer significant harm.

If the Commission decides to assume ownership, it will formally notify the beneficiary concerned within 45 days of receiving notification.

26.5 Consequences of non-compliance

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such breaches may also lead to the any of the other measures described in Chapter 6.

ARTICLE 27 — PROTECTION OF RESULTS — VISIBILITY OF EU FUNDING

27.1 Obligation to protect the results

Each beneficiary must examine the possibility of protecting its results and must adequately protect them — for an appropriate period and with appropriate territorial coverage — if:

(a) the results can reasonably be expected to be commercially or industrially exploited and

(b) protecting them is possible, reasonable and justified (given the circumstances).

When deciding on protection, the beneficiary must consider its own legitimate interests and the legitimate interests (especially commercial) of the other beneficiaries.

27.2 EU ownership, to protect the results

If a beneficiary intends not to protect its results, to stop protecting them or not seek an extension of

protection, the EU may — under certain conditions (see Article 26.4) — assume ownership to ensure their (continued) protection.

27.3 Information on EU funding

Applications for protection of results (including patent applications) filed by or on behalf of a beneficiary must — unless the Commission requests or agrees otherwise or unless it is impossible — include the following:

> "The project leading to this application has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101017180".

27.4 Consequences of non-compliance

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such a breach may also lead to any of the other measures described in Chapter 6.

ARTICLE 28 — EXPLOITATION OF RESULTS

28.1 Obligation to exploit the results

Each beneficiary must — up to four years after the period set out in Article 3 — take measures aiming to ensure '**exploitation**' of its results (either directly or indirectly, in particular through transfer or licensing; see Article 30) by:

(a) using them in further research activities (outside the action);

(b) developing, creating or marketing a product or process;

(c) creating and providing a service, or

(d) using them in standardisation activities.

This does not change the security obligations in Article 37, which still apply.

28.2 Results that could contribute to European or international standards — Information on EU funding

If results are incorporated in a standard, the beneficiary concerned must — unless the Commission requests or agrees otherwise or unless it is impossible — ask the standardisation body to include the following statement in (information related to) the standard:

> "Results incorporated in this standard received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101017180".

28.3 Consequences of non-compliance

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced in accordance with Article 43.

Such a breach may also lead to any of the other measures described in Chapter 6.

ARTICLE 29 — DISSEMINATION OF RESULTS — OPEN ACCESS — VISIBILITY OF EU FUNDING

29.1 Obligation to disseminate results

Unless it goes against their legitimate interests, each beneficiary must — as soon as possible — '**disseminate**' its results by disclosing them to the public by appropriate means (other than those resulting from protecting or exploiting the results), including in scientific publications (in any medium).

This does not change the obligation to protect results in Article 27, the confidentiality obligations in Article 36, the security obligations in Article 37 or the obligations to protect personal data in Article 39, all of which still apply.

A beneficiary that intends to disseminate its results must give advance notice to the other beneficiaries of — unless agreed otherwise — at least 45 days, together with sufficient information on the results it will disseminate.

Any other beneficiary may object within — unless agreed otherwise — 30 days of receiving notification, if it can show that its legitimate interests in relation to the results or background would be significantly harmed. In such cases, the dissemination may not take place unless appropriate steps are taken to safeguard these legitimate interests.

If a beneficiary intends not to protect its results, it may — under certain conditions (see Article 26.4.1) — need to formally notify the Commission before dissemination takes place.

29.2 Open access to scientific publications

Each beneficiary must ensure open access (free of charge online access for any user) to all peer-reviewed scientific publications relating to its results.

In particular, it must:

(a) as soon as possible and at the latest on publication, deposit a machine-readable electronic copy of the published version or final peer-reviewed manuscript accepted for publication in a repository for scientific publications;

Moreover, the beneficiary must aim to deposit at the same time the research data needed to validate the results presented in the deposited scientific publications.

(b) ensure open access to the deposited publication — via the repository — at the latest:

(i) on publication, if an electronic version is available for free via the publisher, or

(ii) within six months of publication (twelve months for publications in the social sciences and humanities) in any other case.

(c) ensure open access — via the repository — to the bibliographic metadata that identify the deposited publication.

The bibliographic metadata must be in a standard format and must include all of the following:

- the terms “European Union (EU)” and “Horizon 2020”;
- the name of the action, acronym and grant number;
- the publication date, and length of embargo period if applicable, and
- a persistent identifier.

29.3 Open access to research data

Not applicable;

29.4 Information on EU funding — Obligation and right to use the EU emblem

Unless the Commission requests or agrees otherwise or unless it is impossible, any dissemination of results (in any form, including electronic) must:

(a) display the EU emblem and

(b) include the following text:

> “This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 101017180”.

When displayed together with another logo, the EU emblem must have appropriate prominence.

For the purposes of their obligations under this Article, the beneficiaries may use the EU emblem without first obtaining approval from the Commission.

This does not however give them the right to exclusive use.

Moreover, they may not appropriate the EU emblem or any similar trademark or logo, either by registration or by any other means.

29.5 Disclaimer excluding Commission responsibility

Any dissemination of results must indicate that it reflects only the author's view and that the Commission is not responsible for any use that may be made of the information it contains.

29.6 Consequences of non-compliance

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such a breach may also lead to any of the other measures described in Chapter 6.

ARTICLE 30 — TRANSFER AND LICENSING OF RESULTS

30.1 Transfer of ownership

Each beneficiary may transfer ownership of its results.

It must however ensure that its obligations under Articles 26.2, 26.4, 27, 28, 29, 30 and 31 also apply to the new owner and that this owner has the obligation to pass them on in any subsequent transfer.

This does not change the security obligations in Article 37, which still apply.

Unless agreed otherwise (in writing) for specifically-identified third parties or unless impossible under applicable EU and national laws on mergers and acquisitions, a beneficiary that intends to transfer ownership of results must give at least 45 days advance notice (or less if agreed in writing) to the other beneficiaries that still have (or still may request) access rights to the results. This notification must include sufficient information on the new owner to enable any beneficiary concerned to assess the effects on its access rights.

Unless agreed otherwise (in writing) for specifically-identified third parties, any other beneficiary may object within 30 days of receiving notification (or less if agreed in writing), if it can show that the transfer would adversely affect its access rights. In this case, the transfer may not take place until agreement has been reached between the beneficiaries concerned.

30.2 Granting licenses

Each beneficiary may grant licences to its results (or otherwise give the right to exploit them), if:

(a) this does not impede the access rights under Article 31 and

(b) not applicable.

In addition to Points (a) and (b), exclusive licences for results may be granted only if all the other beneficiaries concerned have waived their access rights (see Article 31.1).

This does not change the dissemination obligations in Article 29 or security obligations in Article 37, which still apply.

30.3 Commission right to object to transfers or licensing

Not applicable

30.4 Consequences of non-compliance

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such a breach may also lead to any of the other measures described in Chapter 6.

ARTICLE 31 — ACCESS RIGHTS TO RESULTS

31.1 Exercise of access rights — Waiving of access rights — No sub-licensing

The conditions set out in Article 25.1 apply.

The obligations set out in this Article do not change the security obligations in Article 37, which still apply.

31.2 Access rights for other beneficiaries, for implementing their own tasks under the action

The beneficiaries must give each other access — on a royalty-free basis — to results needed for implementing their own tasks under the action.

31.3 Access rights for other beneficiaries, for exploiting their own results

The beneficiaries must give each other — under fair and reasonable conditions (see Article 25.3) — access to results needed for exploiting their own results.

Requests for access may be made — unless agreed otherwise — up to one year after the period set out in Article 3.

31.4 Access rights of affiliated entities

Unless agreed otherwise in the consortium agreement, access to results must also be given — under fair and reasonable conditions (Article 25.3) — to affiliated entities established in an EU Member State or associated country, if this is needed for those entities to exploit the results generated by the beneficiaries to which they are affiliated.

Unless agreed otherwise (see above; Article 31.1), the affiliated entity concerned must make any such request directly to the beneficiary that owns the results.

Requests for access may be made — unless agreed otherwise — up to one year after the period set out in Article 3.

31.5 Access rights for the EU institutions, bodies, offices or agencies and EU Member States

The beneficiaries must give access to their results — on a royalty-free basis — to EU institutions, bodies, offices or agencies, for developing, implementing or monitoring EU policies or programmes.

Such access rights are limited to non-commercial and non-competitive use.

This does not change the right to use any material, document or information received from the beneficiaries for communication and publicising activities (see Article 38.2).

31.6 Access rights for third parties

Not applicable

31.7 Consequences of non-compliance

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such breaches may also lead to any of the other measures described in Chapter 6.

SECTION 4 OTHER RIGHTS AND OBLIGATIONS

ARTICLE 32 — RECRUITMENT AND WORKING CONDITIONS FOR RESEARCHERS

32.1 Obligation to take measures to implement the European Charter for Researchers and Code of Conduct for the Recruitment of Researchers

The beneficiaries must take all measures to implement the principles set out in the Commission Recommendation on the European Charter for Researchers and the Code of Conduct for the Recruitment of Researchers[23], in particular regarding:

- working conditions;
- transparent recruitment processes based on merit, and
- career development.

The beneficiaries must ensure that researchers and third parties involved in the action are aware of them.

32.2 Consequences of non-compliance

If a beneficiary breaches its obligations under this Article, the Commission may apply any of the measures described in Chapter 6.

ARTICLE 33 — GENDER EQUALITY

33.1 Obligation to aim for gender equality

The beneficiaries must take all measures to promote equal opportunities between men and women in the implementation of the action. They must aim, to the extent possible, for a gender balance at all levels of personnel assigned to the action, including at supervisory and managerial level.

33.2 Consequences of non-compliance

If a beneficiary breaches its obligations under this Article, the Commission may apply any of the measures described in Chapter 6.

ARTICLE 34 — ETHICS AND RESEARCH INTEGRITY

34.1 Obligation to comply with ethical and research integrity principles

The beneficiaries must carry out the action in compliance with:

(a) ethical principles (including the highest standards of research integrity)

and

(b) applicable international, EU and national law.

Funding will not be granted for activities carried out outside the EU if they are prohibited in all Member States or for activities which destroy human embryos (for example, for obtaining stem cells).

The beneficiaries must ensure that the activities under the action have an exclusive focus on civil applications.

[23] Commission Recommendation 2005/251/EC of 11 March 2005 on the European Charter for Researchers and on a Code of Conduct for the Recruitment of Researchers (OJ L 75, 22.3.2005, p. 67).

The beneficiaries must ensure that the activities under the action do not:

(a) aim at human cloning for reproductive purposes;

(b) intend to modify the genetic heritage of human beings which could make such changes heritable (with the exception of research relating to cancer treatment of the gonads, which may be financed), or

(c) intend to create human embryos solely for the purpose of research or for the purpose of stem cell procurement, including by means of somatic cell nuclear transfer.

In addition, the beneficiaries must respect the fundamental principle of research integrity — as set out, for instance, in the European Code of Conduct for Research Integrity[24].

This implies compliance with the following fundamental principles:

- **reliability** in ensuring the quality of research reflected in the design, the methodology, the analysis and the use of resources;

- **honesty** in developing, undertaking, reviewing, reporting and communicating research in a transparent, fair and unbiased way;

- **respect** for colleagues, research participants, society, ecosystems, cultural heritage and the environment;

- **accountability** for the research from idea to publication, for its management and organisation, for training, supervision and mentoring, and for its wider impacts

and means that beneficiaries must ensure that persons carrying out research tasks follow the good research practices and refrain from the research integrity violations described in this Code.

This does not change the other obligations under this Agreement or obligations under applicable international, EU or national law, all of which still apply.

34.2 Activities raising ethical issues

Activities raising ethical issues must comply with the '**ethics requirements**' set out as deliverables in Annex 1.

Before the beginning of an activity raising an ethical issue, each beneficiary must have obtained:

(a) any ethics committee opinion required under national law and

(b) any notification or authorisation for activities raising ethical issues required under national and/or European law

needed for implementing the action tasks in question.

The documents must be kept on file and be submitted upon request by the coordinator to the Commission (see Article 52). If they are not in English, they must be submitted together with

[24] European Code of Conduct for Research Integrity of ALLEA (All European Academies) http://ec.europa.eu/research/participants/data/ref/h2020/other/hi/h2020-ethics_code-of-conduct_en.pdf

an English summary, which shows that the action tasks in question are covered and includes the conclusions of the committee or authority concerned (if available).

34.3 Activities involving human embryos or human embryonic stem cells

Activities involving research on human embryos or human embryonic stem cells may be carried out, in addition to Article 34.1, only if:

- they are set out in Annex 1 or
- the coordinator has obtained explicit approval (in writing) from the Commission (see Article 52).

34.4 Consequences of non-compliance

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43) and the Agreement or participation of the beneficiary may be terminated (see Article 50).

Such breaches may also lead to any of the other measures described in Chapter 6.

ARTICLE 35 — CONFLICT OF INTERESTS

35.1 Obligation to avoid a conflict of interests

The beneficiaries must take all measures to prevent any situation where the impartial and objective implementation of the action is compromised for reasons involving economic interest, political or national affinity, family or emotional ties or any other shared interest (‘**conflict of interests**’).

They must formally notify to the Commission without delay any situation constituting or likely to lead to a conflict of interests and immediately take all the necessary steps to rectify this situation.

The Commission may verify that the measures taken are appropriate and may require additional measures to be taken by a specified deadline.

35.2 Consequences of non-compliance

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43) and the Agreement or participation of the beneficiary may be terminated (see Article 50).

Such breaches may also lead to any of the other measures described in Chapter 6.

ARTICLE 36 — CONFIDENTIALITY

36.1 General obligation to maintain confidentiality

During implementation of the action and for four years after the period set out in Article 3, the parties must keep confidential any data, documents or other material (in any form) that is identified as confidential at the time it is disclosed (‘**confidential information**’).

If a beneficiary requests, the Commission may agree to keep such information confidential for an additional period beyond the initial four years.

If information has been identified as confidential only orally, it will be considered to be confidential only if this is confirmed in writing within 15 days of the oral disclosure.

Unless otherwise agreed between the parties, they may use confidential information only to implement the Agreement.

The beneficiaries may disclose confidential information to their personnel or third parties involved in the action only if they:

(a) need to know to implement the Agreement and

(b) are bound by an obligation of confidentiality.

This does not change the security obligations in Article 37, which still apply.

The Commission may disclose confidential information to its staff, other EU institutions and bodies. It may disclose confidential information to third parties, if:

(a) this is necessary to implement the Agreement or safeguard the EU's financial interests and

(b) the recipients of the information are bound by an obligation of confidentiality.

Under the conditions set out in Article 4 of the Rules for Participation Regulation No 1290/2013[25], the Commission must moreover make available information on the results to other EU institutions, bodies, offices or agencies as well as Member States or associated countries.

The confidentiality obligations no longer apply if:

(a) the disclosing party agrees to release the other party;

(b) the information was already known by the recipient or is given to him without obligation of confidentiality by a third party that was not bound by any obligation of confidentiality;

(c) the recipient proves that the information was developed without the use of confidential information;

(d) the information becomes generally and publicly available, without breaching any confidentiality obligation, or

(e) the disclosure of the information is required by EU or national law.

36.2 Consequences of non-compliance

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such breaches may also lead to any of the other measures described in Chapter 6.

ARTICLE 37 — SECURITY-RELATED OBLIGATIONS

[25] Regulation (EU) No 1290/2013 of the European Parliament and of the Council of 11 December 2013 laying down the rules for participation and dissemination in "Horizon 2020 - the Framework Programme for Research and Innovation (2014-2020)" (OJ L 347, 20.12.2013 p.81).

37.1 Results with a security recommendation

Not applicable

37.2 Classified information

Not applicable

37.3 Activities involving dual-use goods or dangerous materials and substances

Not applicable

37.4 Consequences of non-compliance

Not applicable

ARTICLE 38 — PROMOTING THE ACTION — VISIBILITY OF EU FUNDING

38.1 Communication activities by beneficiaries

38.1.1 Obligation to promote the action and its results

The beneficiaries must promote the action and its results, by providing targeted information to multiple audiences (including the media and the public) in a strategic and effective manner.

This does not change the dissemination obligations in Article 29, the confidentiality obligations in Article 36 or the security obligations in Article 37, all of which still apply.

Before engaging in a communication activity expected to have a major media impact, the beneficiaries must inform the Commission (see Article 52).

38.1.2 Information on EU funding — Obligation and right to use the EU emblem

Unless the Commission requests or agrees otherwise or unless it is impossible, any communication activity related to the action (including in electronic form, via social media, etc.) and any infrastructure, equipment and major results funded by the grant must:

(a) display the EU emblem and

(b) include the following text:

For communication activities:

"This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101017180".

For infrastructure, equipment and major results:

"This *[infrastructure][equipment][insert type of result]* is part of a project that has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101017180".

When displayed together with another logo, the EU emblem must have appropriate prominence.

For the purposes of their obligations under this Article, the beneficiaries may use the EU emblem without first obtaining approval from the Commission.

This does not, however, give them the right to exclusive use.

Moreover, they may not appropriate the EU emblem or any similar trademark or logo, either by registration or by any other means.

38.1.3 Disclaimer excluding Commission responsibility

Any communication activity related to the action must indicate that it reflects only the author's view and that the Commission is not responsible for any use that may be made of the information it contains.

38.2 Communication activities by the Commission

38.2.1 Right to use beneficiaries' materials, documents or information

The Commission may use, for its communication and publicising activities, information relating to the action, documents notably summaries for publication and public deliverables as well as any other material, such as pictures or audio-visual material received from any beneficiary (including in electronic form).

This does not change the confidentiality obligations in Article 36 and the security obligations in Article 37, all of which still apply.

If the Commission's use of these materials, documents or information would risk compromising legitimate interests, the beneficiary concerned may request the Commission not to use it (see Article 52).

The right to use a beneficiary's materials, documents and information includes:

(a) **use for its own purposes** (in particular, making them available to persons working for the Commission or any other EU institution, body, office or agency or body or institutions in EU Member States; and copying or reproducing them in whole or in part, in unlimited numbers);

(b) **distribution to the public** (in particular, publication as hard copies and in electronic or digital format, publication on the internet, as a downloadable or non-downloadable file, broadcasting by any channel, public display or presentation, communicating through press information services, or inclusion in widely accessible databases or indexes);

(c) **editing or redrafting** for communication and publicising activities (including shortening, summarising, inserting other elements (such as meta-data, legends, other graphic, visual, audio or text elements), extracting parts (e.g. audio or video files), dividing into parts, use in a compilation);

(d) translation;

(e) giving **access in response to individual requests** under Regulation No 1049/2001[27], without the right to reproduce or exploit;

[27] Regulation (EC) No 1049/2001 of the European Parliament and of the Council of 30 May 2001 regarding public access to European Parliament, Council and Commission documents, OJ L 145, 31.5.2001, p. 43.

(f) **storage** in paper, electronic or other form;

(g) **archiving**, in line with applicable document-management rules, and

(h) the right to authorise **third parties** to act on its behalf or sub-license the modes of use set out in Points (b), (c), (d) and (f) to third parties if needed for the communication and publicising activities of the Commission.

If the right of use is subject to rights of a third party (including personnel of the beneficiary), the beneficiary must ensure that it complies with its obligations under this Agreement (in particular, by obtaining the necessary approval from the third parties concerned).

Where applicable (and if provided by the beneficiaries), the Commission will insert the following information:

> "© – [year] – [name of the copyright owner]. All rights reserved. Licensed to the European Union (EU) under conditions."

38.3 Consequences of non-compliance

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such breaches may also lead to any of the other measures described in Chapter 6.

ARTICLE 39 — PROCESSING OF PERSONAL DATA

39.1 Processing of personal data by the Commission

Any personal data under the Agreement will be processed by the Commission under Regulation No 45/2001[28] and according to the 'notifications of the processing operations' to the Data Protection Officer (DPO) of the Commission (publicly accessible in the DPO register).

Such data will be processed by the '**data controller**' of the Commission for the purposes of implementing, managing and monitoring the Agreement or protecting the financial interests of the EU or Euratom (including checks, reviews, audits and investigations; see Article 22).

The persons whose personal data are processed have the right to access and correct their own personal data. For this purpose, they must send any queries about the processing of their personal data to the data controller, via the contact point indicated in the privacy statement(s) that are published on the Commission websites.

They also have the right to have recourse at any time to the European Data Protection Supervisor (EDPS).

39.2 Processing of personal data by the beneficiaries

[28] Regulation (EC) No 45/2001 of the European Parliament and of the Council of 18 December 2000 on the protection of individuals with regard to the processing of personal data by the Community institutions and bodies and on the free movement of such data (OJ L 8, 12.01.2001, p. 1).

The beneficiaries must process personal data under the Agreement in compliance with applicable EU and national law on data protection (including authorisations or notification requirements).

The beneficiaries may grant their personnel access only to data that is strictly necessary for implementing, managing and monitoring the Agreement.

The beneficiaries must inform the personnel whose personal data are collected and processed by the Commission. For this purpose, they must provide them with the privacy statement(s) (see above), before transmitting their data to the Commission.

39.3 Consequences of non-compliance

If a beneficiary breaches any of its obligations under Article 39.2, the Commission may apply any of the measures described in Chapter 6.

ARTICLE 40 — ASSIGNMENTS OF CLAIMS FOR PAYMENT AGAINST THE COMMISSION

The beneficiaries may not assign any of their claims for payment against the Commission to any third party, except if approved by the Commission on the basis of a reasoned, written request by the coordinator (on behalf of the beneficiary concerned).

If the Commission has not accepted the assignment or the terms of it are not observed, the assignment will have no effect on it.

In no circumstances will an assignment release the beneficiaries from their obligations towards the Commission.

CHAPTER 5 DIVISION OF BENEFICIARIES' ROLES AND RESPONSIBILITIES — RELATIONSHIP WITH COMPLEMENTARY BENEFICIARIES — RELATIONSHIP WITH PARTNERS OF A JOINT ACTION

ARTICLE 41 — DIVISION OF BENEFICIARIES' ROLES AND RESPONSIBILITIES — RELATIONSHIP WITH COMPLEMENTARY BENEFICIARIES — RELATIONSHIP WITH PARTNERS OF A JOINT ACTION

41.1 Roles and responsibility towards the Commission

The beneficiaries have full responsibility for implementing the action and complying with the Agreement.

The beneficiaries are jointly and severally liable for the **technical implementation** of the action as described in Annex 1. If a beneficiary fails to implement its part of the action, the other beneficiaries become responsible for implementing this part (without being entitled to any additional EU funding for doing so), unless the Commission expressly relieves them of this obligation.

The **financial responsibility** of each beneficiary is governed by Article 44.

41.2 Internal division of roles and responsibilities

The internal roles and responsibilities of the beneficiaries are divided as follows:

(a) Each **beneficiary** must:

(i) keep information stored in the Participant Portal Beneficiary Register (via the electronic exchange system) up to date (see Article 17);

(ii) inform the coordinator immediately of any events or circumstances likely to affect significantly or delay the implementation of the action (see Article 17);

(iii) submit to the coordinator in good time:

- individual financial statements for itself and, if required, certificates on the financial statements (see Article 20);
- the data needed to draw up the technical reports (see Article 20);
- ethics committee opinions and notifications or authorisations for activities raising ethical issues (see Article 34);
- any other documents or information required by the Commission under the Agreement, unless the Agreement requires the beneficiary to submit this information directly to the Commission.

(b) The **coordinator** must:

(i) monitor that the action is implemented properly (see Article 7);

(ii) act as the intermediary for all communications between the beneficiaries and the Commission (in particular, providing the Commission with the information described in Article 17), unless the Agreement specifies otherwise;

(iii) request and review any documents or information required by the Commission and verify their completeness and correctness before passing them on to the Commission;

(iv) submit the deliverables and reports to the Commission (see Articles 19 and 20);

(v) ensure that all payments are made to the other beneficiaries without unjustified delay (see Article 21);

(vi) inform the Commission of the amounts paid to each beneficiary, when required under the Agreement (see Articles 44 and 50) or requested by the Commission.

The coordinator may not delegate or subcontract the above-mentioned tasks to any other beneficiary or third party (including linked third parties).

41.3 Internal arrangements between beneficiaries — Consortium agreement

The beneficiaries must have internal arrangements regarding their operation and co-ordination to ensure that the action is implemented properly. These internal arrangements must be set out in a written '**consortium agreement**' between the beneficiaries, which may cover:

- internal organisation of the consortium;

- management of access to the electronic exchange system;
- distribution of EU funding;
- additional rules on rights and obligations related to background and results (including whether access rights remain or not, if a beneficiary is in breach of its obligations) (see Section 3 of Chapter 4);
- settlement of internal disputes;
- liability, indemnification and confidentiality arrangements between the beneficiaries.

The consortium agreement must not contain any provision contrary to the Agreement.

41.4 Relationship with complementary beneficiaries — Collaboration agreement

Not applicable

41.5 Relationship with partners of a joint action — Coordination agreement

Not applicable

CHAPTER 6 REJECTION OF COSTS — REDUCTION OF THE GRANT — RECOVERY — SANCTIONS — DAMAGES — SUSPENSION — TERMINATION — FORCE MAJEURE

SECTION 1 REJECTION OF COSTS — REDUCTION OF THE GRANT — RECOVERY — SANCTIONS

ARTICLE 42 — REJECTION OF INELIGIBLE COSTS

42.1 Conditions

The Commission will — after **termination of the participation of a beneficiary**, at the time of an **interim payment**, **at the payment of the balance** or **afterwards** — reject any costs which are ineligible (see Article 6), in particular following checks, reviews, audits or investigations (see Article 22).

The rejection may also be based on the **extension of findings from other grants to this grant** (see Article 22.5.2).

42.2 Ineligible costs to be rejected — Calculation — Procedure

Ineligible costs will be rejected in full.

If the rejection of costs does not lead to a recovery (see Article 44), the Commission will formally notify the coordinator or beneficiary concerned of the rejection of costs, the amounts and the reasons why (if applicable, together with the notification of amounts due; see Article 21.5). The coordinator or beneficiary concerned may — within 30 days of receiving notification — formally notify the Commission of its disagreement and the reasons why.

If the rejection of costs leads to a recovery, the Commission will follow the contradictory procedure with pre-information letter set out in Article 44.

42.3 Effects

If the Commission rejects costs at the time of an **interim payment** or **the payment of the balance**, it will deduct them from the total eligible costs declared, for the action, in the periodic or final summary financial statement (see Articles 20.3 and 20.4). It will then calculate the interim payment or payment of the balance as set out in Articles 21.3 or 21.4.

If the Commission rejects costs **after termination of the participation of a beneficiary**, it will deduct them from the costs declared by the beneficiary in the termination report and include the rejection in the calculation after termination (see Article 50.2 and 50.3).

If the Commission — **after an interim payment but before the payment of the balance** — rejects costs declared in a periodic summary financial statement, it will deduct them from the total eligible costs declared, for the action, in the next periodic summary financial statement or in the final summary financial statement. It will then calculate the interim payment or payment of the balance as set out in Articles 21.3 or 21.4.

If the Commission rejects costs **after the payment of the balance**, it will deduct the amount rejected from the total eligible costs declared, by the beneficiary, in the final summary financial statement. It will then calculate the revised final grant amount as set out in Article 5.4.

ARTICLE 43 — REDUCTION OF THE GRANT

43.1 Conditions

The Commission may — **after termination of the participation of a beneficiary**, **at the payment of the balance** or **afterwards** — reduce the grant amount (see Article 5.1), if :

(a) a beneficiary (or a natural person who has the power to represent or take decisions on its behalf) has committed:

  (i) substantial errors, irregularities or fraud or

  (ii) serious breach of obligations under the Agreement or during the award procedure (including improper implementation of the action, submission of false information, failure to provide required information, breach of ethical principles) or

(b) a beneficiary (or a natural person who has the power to represent or take decision on its behalf) has committed — in other EU or Euratom grants awarded to it under similar conditions — systemic or recurrent errors, irregularities, fraud or serious breach of obligations that have a material impact on this grant (**extension of findings from other grants to this grant**; see Article 22.5.2).

43.2 Amount to be reduced — Calculation — Procedure

The amount of the reduction will be proportionate to the seriousness of the errors, irregularities or fraud or breach of obligations.

Before reduction of the grant, the Commission will formally notify a '**pre-information letter**' to the coordinator or beneficiary concerned:

- informing it of its intention to reduce the grant, the amount it intends to reduce and the reasons why and

- inviting it to submit observations within 30 days of receiving notification.

If the Commission does not receive any observations or decides to pursue reduction despite the observations it has received, it will formally notify **confirmation** of the reduction (if applicable, together with the notification of amounts due; see Article 21).

43.3 Effects

If the Commission reduces the grant **after termination of the participation of a beneficiary**, it will calculate the reduced grant amount for that beneficiary and then determine the amount due to that beneficiary (see Article 50.2 and 50.3).

If the Commission reduces the grant **at the payment of the balance**, it will calculate the reduced grant amount for the action and then determine the amount due as payment of the balance (see Articles 5.3.4 and 21.4).

If the Commission reduces the grant **after the payment of the balance**, it will calculate the revised final grant amount for the beneficiary concerned (see Article 5.4). If the revised final grant amount for the beneficiary concerned is lower than its share of the final grant amount, the Commission will recover the difference (see Article 44).

ARTICLE 44 — RECOVERY OF UNDUE AMOUNTS

44.1 Amount to be recovered — Calculation — Procedure

The Commission will — after **termination of the participation of a beneficiary, at the payment of the balance** or **afterwards** — claim back any amount that was paid, but is not due under the Agreement.

Each beneficiary's financial responsibility in case of recovery is limited to its own debt, except for the amount retained for the Guarantee Fund (see Article 21.4).

44.1.1 Recovery after termination of a beneficiary's participation

If recovery takes place after termination of a beneficiary's participation (including the coordinator), the Commission will claim back the undue amount from the beneficiary concerned, by formally notifying it a debit note (see Article 50.2 and 50.3). This note will specify the amount to be recovered, the terms and the date for payment.

If payment is not made by the date specified in the debit note, the Commission will **recover** the amount:

(a) by '**offsetting**' it — without the beneficiary's consent — against any amounts owed to the beneficiary concerned by the Commission or an executive agency (from the EU or Euratom budget).

In exceptional circumstances, to safeguard the EU's financial interests, the Commission may offset before the payment date specified in the debit note;

(b) not applicable;

(c) by **taking legal action** (see Article 57) or by **adopting an enforceable decision** under Article 299 of the Treaty on the Functioning of the EU (TFEU) and Article 79(2) of the Financial Regulation No 966/2012.

If payment is not made by the date specified in the debit note, the amount to be recovered (see above) will be increased by **late-payment interest** at the rate set out in Article 21.11, from the day following the payment date in the debit note, up to and including the date the Commission receives full payment of the amount.

Partial payments will be first credited against expenses, charges and late-payment interest and then against the principal.

Bank charges incurred in the recovery process will be borne by the beneficiary, unless Directive 2007/64/EC[29] applies.

44.1.2 Recovery at payment of the balance

If the payment of the balance takes the form of a recovery (see Article 21.4), the Commission will formally notify a '**pre-information letter**' to the coordinator:

- informing it of its intention to recover, the amount due as the balance and the reasons why;
- specifying that it intends to deduct the amount to be recovered from the amount retained for the Guarantee Fund;
- requesting the coordinator to submit a report on the distribution of payments to the beneficiaries within 30 days of receiving notification, and
- inviting the coordinator to submit observations within 30 days of receiving notification.

If no observations are submitted or the Commission decides to pursue recovery despite the observations it has received, it will **confirm recovery** (together with the notification of amounts due; see Article 21.5) and:

- pay the difference between the amount to be recovered and the amount retained for the Guarantee Fund, **if the difference is positive** or
- formally notify to the coordinator a **debit note** for the difference between the amount to be recovered and the amount retained for the Guarantee Fund, **if the difference is negative**. This note will also specify the terms and the date for payment.

If the coordinator does not repay the Commission by the date in the debit note and has not submitted

[29] Directive 2007/64/EC of the European Parliament and of the Council of 13 November 2007 on payment services in the internal market amending Directives 97/7/EC, 2002/65/EC, 2005/60/EC and 2006/48/EC and repealing Directive 97/5/EC (OJ L 319, 05.12.2007, p. 1).

the report on the distribution of payments: the Commission will **recover** the amount set out in the debit note from the coordinator (see below).

If the coordinator does not repay the Commission by the date in the debit note, but has submitted the report on the distribution of payments: the Commission will:

(a) identify the beneficiaries for which the amount calculated as follows is negative:

{{{{beneficiary's costs declared in the final summary financial statement and approved by the Commission multiplied by the reimbursement rate set out in Article 5.2 for the beneficiary concerned}

divided by

the EU contribution for the action calculated according to Article 5.3.1}

multiplied by

the final grant amount (see Article 5.3)},

minus

{pre-financing and interim payments received by the beneficiary}.

(b) formally notify to each beneficiary identified according to point (a) a **debit note** specifying the terms and date for payment. The amount of the debit note is calculated as follows:

{{amount calculated according to point (a) for the beneficiary concerned

divided by

the sum of the amounts calculated according to point (a) for all the beneficiaries identified according to point (a)}

multiplied by

the amount set out in the debit note formally notified to the coordinator}.

If payment is not made by the date specified in the debit note, the Commission will **recover** the amount:

(a) by **offsetting** it — without the beneficiary's consent — against any amounts owed to the beneficiary concerned by the Commission or an executive agency (from the EU or Euratom budget).

In exceptional circumstances, to safeguard the EU's financial interests, the Commission may offset before the payment date specified in the debit note;

(b) by **drawing on the Guarantee Fund**. The Commission will formally notify the beneficiary concerned the debit note on behalf of the Guarantee Fund and recover the amount:

(i) not applicable;

(ii) by **taking legal action** (see Article 57) or by **adopting an enforceable decision** under Article 299 of the Treaty on the Functioning of the EU (TFEU) and Article 79(2) of the Financial Regulation No 966/2012.

If payment is not made by the date in the debit note, the amount to be recovered (see above) will be increased by **late-payment interest** at the rate set out in Article 21.11, from the day following the payment date in the debit note, up to and including the date the Commission receives full payment of the amount.

Partial payments will be first credited against expenses, charges and late-payment interest and then against the principal.

Bank charges incurred in the recovery process will be borne by the beneficiary, unless Directive 2007/64/EC applies.

44.1.3 Recovery of amounts after payment of the balance

If, for a beneficiary, the revised final grant amount (see Article 5.4) is lower than its share of the final grant amount, it must repay the difference to the Commission.

The beneficiary's share of the final grant amount is calculated as follows:

{{{beneficiary's costs declared in the final summary financial statement and approved by the Commission multiplied by the reimbursement rate set out in Article 5.2 for the beneficiary concerned}

divided by

the EU contribution for the action calculated according to Article 5.3.1}

multiplied by

the final grant amount (see Article 5.3)}.

If the coordinator has not distributed amounts received (see Article 21.7), the Commission will also recover these amounts.

The Commission will formally notify a **pre-information letter** to the beneficiary concerned:

- informing it of its intention to recover, the due amount and the reasons why and
- inviting it to submit observations within 30 days of receiving notification.

If no observations are submitted or the Commission decides to pursue recovery despite the observations it has received, it will **confirm** the amount to be recovered and formally notify to the beneficiary concerned a **debit note**. This note will also specify the terms and the date for payment.

If payment is not made by the date specified in the debit note, the Commission will **recover** the amount:

(a) by **offsetting** it — without the beneficiary's consent — against any amounts owed to the beneficiary concerned by the Commission or an executive agency (from the EU or Euratom budget).

In exceptional circumstances, to safeguard the EU's financial interests, the Commission may offset before the payment date specified in the debit note;

(b) by **drawing on the Guarantee Fund**. The Commission will formally notify the beneficiary concerned the debit note on behalf of the Guarantee Fund and recover the amount:

(i) not applicable;

(ii) by **taking legal action** (see Article 57) or by **adopting an enforceable decision** under Article 299 of the Treaty on the Functioning of the EU (TFEU) and Article 79(2) of the Financial Regulation No 966/2012.

If payment is not made by the date in the debit note, the amount to be recovered (see above) will be increased by **late-payment interest** at the rate set out in Article 21.11, from the day following the date for payment in the debit note, up to and including the date the Commission receives full payment of the amount.

Partial payments will be first credited against expenses, charges and late-payment interest and then against the principal.

Bank charges incurred in the recovery process will be borne by the beneficiary, unless Directive 2007/64/EC applies.

ARTICLE 45 — ADMINISTRATIVE SANCTIONS

In addition to contractual measures, the Commission may also adopt administrative sanctions under Articles 106 and 131(4) of the Financial Regulation No 966/2012 (i.e. exclusion from future procurement contracts, grants, prizes and expert contracts and/or financial penalties).

SECTION 2 LIABILITY FOR DAMAGES

ARTICLE 46 — LIABILITY FOR DAMAGES

46.1 Liability of the Commission

The Commission cannot be held liable for any damage caused to the beneficiaries or to third parties as a consequence of implementing the Agreement, including for gross negligence.

The Commission cannot be held liable for any damage caused by any of the beneficiaries or third parties involved in the action, as a consequence of implementing the Agreement.

46.2 Liability of the beneficiaries

Except in case of force majeure (see Article 51), the beneficiaries must compensate the Commission for any damage it sustains as a result of the implementation of the action or because the action was not implemented in full compliance with the Agreement.

SECTION 3 SUSPENSION AND TERMINATION

ARTICLE 47 — SUSPENSION OF PAYMENT DEADLINE

47.1 Conditions

The Commission may — at any moment — suspend the payment deadline (see Article 21.2 to 21.4) if a request for payment (see Article 20) cannot be approved because:

(a) it does not comply with the provisions of the Agreement (see Article 20);

(b) the technical or financial reports have not been submitted or are not complete or additional information is needed, or

(c) there is doubt about the eligibility of the costs declared in the financial statements and additional checks, reviews, audits or investigations are necessary.

47.2 Procedure

The Commission will formally notify the coordinator of the suspension and the reasons why.

The suspension will **take effect** the day notification is sent by the Commission (see Article 52).

If the conditions for suspending the payment deadline are no longer met, the suspension will be **lifted** — and the remaining period will resume.

If the suspension exceeds two months, the coordinator may request the Commission if the suspension will continue.

If the payment deadline has been suspended due to the non-compliance of the technical or financial reports (see Article 20) and the revised report or statement is not submitted or was submitted but is also rejected, the Commission may also terminate the Agreement or the participation of the beneficiary (see Article 50.3.1(l)).

ARTICLE 48 — SUSPENSION OF PAYMENTS

48.1 Conditions

The Commission may — at any moment — suspend payments, in whole or in part and interim payments or the payment of the balance for one or more beneficiaries, if:

(a) a beneficiary (or a natural person who has the power to represent or take decision on its behalf) has committed or is suspected of having committed:

  (i) substantial errors, irregularities or fraud or

  (ii) serious breach of obligations under the Agreement or during the award procedure (including improper implementation of the action, submission of false information, failure to provide required information, breach of ethical principles) or

(b) a beneficiary (or a natural person who has the power to represent or take decision on its behalf) has committed — in other EU or Euratom grants awarded to it under similar conditions — systemic or recurrent errors, irregularities, fraud or serious breach of obligations that have a material impact on this grant (**extension of findings from other grants to this grant**; see Article 22.5.2).

If payments are suspended for one or more beneficiaries, the Commission will make partial payment(s) for the part(s) not suspended. If suspension concerns the payment of the balance, — once suspension is lifted — the payment or the recovery of the amount(s) concerned will be considered the payment of the balance that closes the action.

48.2 Procedure

Before suspending payments, the Commission will formally notify the coordinator or beneficiary concerned:

- informing it of its intention to suspend payments and the reasons why and
- inviting it to submit observations within 30 days of receiving notification.

If the Commission does not receive observations or decides to pursue the procedure despite the observations it has received, it will formally notify **confirmation** of the suspension. Otherwise, it will formally notify that the suspension procedure is not continued.

The suspension will **take effect** the day the confirmation notification is sent by the Commission.

If the conditions for resuming payments are met, the suspension will be **lifted**. The Commission will formally notify the coordinator or beneficiary concerned.

During the suspension, the periodic report(s) for all reporting periods except the last one (see Article 20.3), must not contain any individual financial statements from the beneficiary concerned. The coordinator must include them in the next periodic report after the suspension is lifted or — if suspension is not lifted before the end of the action — in the last periodic report.

The beneficiaries may suspend implementation of the action (see Article 49.1) or terminate the Agreement or the participation of the beneficiary concerned (see Article 50.1 and 50.2).

ARTICLE 49 — SUSPENSION OF THE ACTION IMPLEMENTATION

49.1 Suspension of the action implementation, by the beneficiaries

49.1.1 Conditions

The beneficiaries may suspend implementation of the action or any part of it, if exceptional circumstances — in particular *force majeure* (see Article 51) — make implementation impossible or excessively difficult.

49.1.2 Procedure

The coordinator must immediately formally notify to the Commission the suspension (see Article 52), stating:

- the reasons why and
- the expected date of resumption.

The suspension will **take effect** the day this notification is received by the Commission.

Once circumstances allow for implementation to resume, the coordinator must immediately formally notify the Commission and request an **amendment** of the Agreement to set the date on which the action will be resumed, extend the duration of the action and make other changes necessary to adapt the action to the new situation (see Article 55) — unless the Agreement or the participation of a beneficiary has been terminated (see Article 50).

The suspension will be **lifted** with effect from the resumption date set out in the amendment. This date may be before the date on which the amendment enters into force.

Costs incurred during suspension of the action implementation are not eligible (see Article 6).

49.2 Suspension of the action implementation, by the Commission

49.2.1 Conditions

The Commission may suspend implementation of the action or any part of it, if:

(a) a beneficiary (or a natural person who has the power to represent or take decisions on its behalf) has committed or is suspected of having committed:

  (i) substantial errors, irregularities or fraud or

  (ii) serious breach of obligations under the Agreement or during the award procedure (including improper implementation of the action, submission of false information, failure to provide required information, breach of ethical principles);

(b) a beneficiary (or a natural person who has the power to represent or take decisions on its behalf) has committed — in other EU or Euratom grants awarded to it under similar conditions — systemic or recurrent errors, irregularities, fraud or serious breach of obligations that have a material impact on this grant (**extension of findings from other grants to this grant**; see Article 22.5.2), or

(c) the action is suspected of having lost its scientific or technological relevance.

49.2.2 Procedure

Before suspending implementation of the action, the Commission will formally notify the coordinator or beneficiary concerned:

- informing it of its intention to suspend the implementation and the reasons why and

- inviting it to submit observations within 30 days of receiving notification.

If the Commission does not receive observations or decides to pursue the procedure despite the observations it has received, it will formally notify **confirmation** of the suspension. Otherwise, it will formally notify that the procedure is not continued.

The suspension will **take effect** five days after confirmation notification is received (or on a later date specified in the notification).

It will be **lifted** if the conditions for resuming implementation of the action are met.

The coordinator or beneficiary concerned will be formally notified of the lifting and the Agreement will be **amended** to set the date on which the action will be resumed, extend the duration of the action and make other changes necessary to adapt the action to the new situation (see Article 55) — unless the Agreement has already been terminated (see Article 50).

The suspension will be lifted with effect from the resumption date set out in the amendment. This date may be before the date on which the amendment enters into force.

Costs incurred during suspension are not eligible (see Article 6).

The beneficiaries may not claim damages due to suspension by the Commission (see Article 46).

Suspension of the action implementation does not affect the Commission's right to terminate the Agreement or participation of a beneficiary (see Article 50), reduce the grant or recover amounts unduly paid (see Articles 43 and 44).

ARTICLE 50 — TERMINATION OF THE AGREEMENT OR OF THE PARTICIPATION OF ONE OR MORE BENEFICIARIES

50.1 Termination of the Agreement, by the beneficiaries

50.1.1 Conditions and procedure

The beneficiaries may terminate the Agreement.

The coordinator must formally notify termination to the Commission (see Article 52), stating:

- the reasons why and
- the date the termination will take effect. This date must be after the notification.

If no reasons are given or if the Commission considers the reasons do not justify termination, the Agreement will be considered to have been '**terminated improperly**'.

The termination will **take effect** on the day specified in the notification.

50.1.2 Effects

The coordinator must — within 60 days from when termination takes effect — submit:

(i) a periodic report (for the open reporting period until termination; see Article 20.3) and

(ii) the final report (see Article 20.4).

If the Commission does not receive the reports within the deadline (see above), only costs which are included in an approved periodic report will be taken into account.

The Commission will **calculate** the final grant amount (see Article 5.3) and the balance (see Article 21.4) on the basis of the reports submitted. Only costs incurred until termination are eligible (see Article 6). Costs relating to contracts due for execution only after termination are not eligible.

Improper termination may lead to a reduction of the grant (see Article 43).

After termination, the beneficiaries' obligations (in particular Articles 20, 22, 23, Section 3 of Chapter 4, 36, 37, 38, 40, 42, 43 and 44) continue to apply.

50.2 Termination of the participation of one or more beneficiaries, by the beneficiaries

50.2.1 Conditions and procedure

The participation of one or more beneficiaries may be terminated by the coordinator, on request of the beneficiary concerned or on behalf of the other beneficiaries.

The coordinator must formally notify termination to the Commission (see Article 52) and inform the beneficiary concerned.

If the coordinator's participation is terminated without its agreement, the formal notification must be done by another beneficiary (acting on behalf of the other beneficiaries).

The notification must include:

- the reasons why;
- the opinion of the beneficiary concerned (or proof that this opinion has been requested in writing);
- the date the termination takes effect. This date must be after the notification, and
- a request for amendment (see Article 55), with a proposal for reallocation of the tasks and the estimated budget of the beneficiary concerned (see Annexes 1 and 2) and, if necessary, the addition of one or more new beneficiaries (see Article 56). If termination takes effect after the period set out in Article 3, no request for amendment must be included unless the beneficiary concerned is the coordinator. In this case, the request for amendment must propose a new coordinator.

If this information is not given or if the Commission considers that the reasons do not justify termination, the participation will be considered to have been **terminated improperly**.

The termination will **take effect** on the day specified in the notification.

50.2.2 Effects

The coordinator must — within 30 days from when termination takes effect — submit:

(i) a report on the distribution of payments to the beneficiary concerned and

(ii) if termination takes effect during the period set out in Article 3, a '**termination report**' from the beneficiary concerned, for the open reporting period until termination, containing an overview of the progress of the work, an overview of the use of resources, the individual financial statement and, if applicable, the certificate on the financial statement (see Articles 20.3 and 20.4).

The information in the termination report must also be included in the periodic report for the next reporting period (see Article 20.3).

If the request for amendment is rejected by the Commission (because it calls into question the decision awarding the grant or breaches the principle of equal treatment of applicants), the Agreement may be terminated according to Article 50.3.1(c).

If the request for amendment is accepted by the Commission, the Agreement is **amended** to introduce the necessary changes (see Article 55).

The Commission will — on the basis of the periodic reports, the termination report and the report

on the distribution of payments — **calculate** the amount which is due to the beneficiary and if the (pre-financing and interim) payments received by the beneficiary exceed this amount.

The **amount which is due** is calculated in the following steps:

Step 1 — Application of the reimbursement rate to the eligible costs

The grant amount for the beneficiary is calculated by applying the reimbursement rate(s) to the total eligible costs declared by the beneficiary in the termination report and approved by the Commission.

Only costs incurred by the beneficiary concerned until termination takes effect are eligible (see Article 6). Costs relating to contracts due for execution only after termination are not eligible.

Step 2 — Reduction due to substantial errors, irregularities or fraud or serious breach of obligations

In case of a reduction (see Article 43), the Commission will calculate the reduced grant amount for the beneficiary by deducting the amount of the reduction (calculated in proportion to the seriousness of the errors, irregularities or fraud or breach of obligations, in accordance with Article 43.2) from the grant amount for the beneficiary.

If the payments received **exceed the amounts due**:

- if termination takes effect during the period set out in Article 3 and the request for amendment is accepted, the beneficiary concerned must repay to the coordinator the amount unduly received. The Commission will formally notify the amount unduly received and request the beneficiary concerned to repay it to the coordinator within 30 days of receiving notification. If it does not repay the coordinator, the Commission will draw upon the Guarantee Fund to pay the coordinator and then notify a **debit note** on behalf of the Guarantee Fund to the beneficiary concerned (see Article 44);

- in all other cases, in particular if termination takes effect after the period set out in Article 3, the Commission will formally notify a **debit note** to the beneficiary concerned. If payment is not made by the date in the debit note, the Guarantee Fund will pay to the Commission the amount due and the Commission will notify a debit note on behalf of the Guarantee Fund to the beneficiary concerned (see Article 44);

- if the beneficiary concerned is the former coordinator, it must repay the new coordinator according to the procedure above, unless:

    - termination takes effect after an interim payment and

    - the former coordinator has not distributed amounts received as pre-financing or interim payments (see Article 21.7).

  In this case, the Commission will formally notify a **debit note** to the former coordinator. If payment is not made by the date in the debit note, the Guarantee Fund will pay to the Commission the amount due. The Commission will then pay the new coordinator and notify a debit note on behalf of the Guarantee Fund to the former coordinator (see Article 44).

If the payments received **do not exceed the amounts due**: amounts owed to the beneficiary concerned will be included in the next interim or final payment.

If the Commission does not receive the termination report within the deadline (see above), only costs included in an approved periodic report will be taken into account.

If the Commission does not receive the report on the distribution of payments within the deadline (see above), it will consider that:

- the coordinator did not distribute any payment to the beneficiary concerned and that
- the beneficiary concerned must not repay any amount to the coordinator.

Improper termination may lead to a reduction of the grant (see Article 43) or termination of the Agreement (see Article 50).

After termination, the concerned beneficiary's obligations (in particular Articles 20, 22, 23, Section 3 of Chapter 4, 36, 37, 38, 40, 42, 43 and 44) continue to apply.

50.3 Termination of the Agreement or the participation of one or more beneficiaries, by the Commission

50.3.1 Conditions

The Commission may terminate the Agreement or the participation of one or more beneficiaries, if:

(a) one or more beneficiaries do not accede to the Agreement (see Article 56);

(b) a change to their legal, financial, technical, organisational or ownership situation is likely to substantially affect or delay the implementation of the action or calls into question the decision to award the grant;

(c) following termination of participation for one or more beneficiaries (see above), the necessary changes to the Agreement would call into question the decision awarding the grant or breach the principle of equal treatment of applicants (see Article 55);

(d) implementation of the action is prevented by force majeure (see Article 51) or suspended by the coordinator (see Article 49.1) and either:

  (i) resumption is impossible, or

  (ii) the necessary changes to the Agreement would call into question the decision awarding the grant or breach the principle of equal treatment of applicants;

(e) a beneficiary is declared bankrupt, being wound up, having its affairs administered by the courts, has entered into an arrangement with creditors, has suspended business activities, or is subject to any other similar proceedings or procedures under national law;

(f) a beneficiary (or a natural person who has the power to represent or take decisions on its behalf) has been found guilty of professional misconduct, proven by any means;

(g) a beneficiary does not comply with the applicable national law on taxes and social security;

(h) the action has lost scientific or technological relevance;

(i) not applicable;

(j) not applicable;

(k) a beneficiary (or a natural person who has the power to represent or take decisions on its behalf) has committed fraud, corruption, or is involved in a criminal organisation, money laundering or any other illegal activity;

(l) a beneficiary (or a natural person who has the power to represent or take decisions on its behalf) has committed:

(i) substantial errors, irregularities or fraud or

(ii) serious breach of obligations under the Agreement or during the award procedure (including improper implementation of the action, submission of false information, failure to provide required information, breach of ethical principles);

(m) a beneficiary (or a natural person who has the power to represent or take decisions on its behalf) has committed — in other EU or Euratom grants awarded to it under similar conditions — systemic or recurrent errors, irregularities, fraud or serious breach of obligations that have a material impact on this grant (**extension of findings from other grants to this grant**; see Article 22.5.2);

(n) not applicable.

50.3.2 Procedure

Before terminating the Agreement or participation of one or more beneficiaries, the Commission will formally notify the coordinator or beneficiary concerned:

- informing it of its intention to terminate and the reasons why and
- inviting it, within 30 days of receiving notification, to submit observations and — in case of Point (l.ii) above — to inform the Commission of the measures to ensure compliance with the obligations under the Agreement.

If the Commission does not receive observations or decides to pursue the procedure despite the observations it has received, it will formally notify to the coordinator or beneficiary concerned **confirmation** of the termination and the date it will take effect. Otherwise, it will formally notify that the procedure is not continued.

The termination will **take effect**:

- for terminations under Points (b), (c), (e), (g), (h), (j), (l.ii) and (n) above: on the day specified in the notification of the confirmation (see above);
- for terminations under Points (a), (d), (f), (i), (k), (l.i) and (m) above: on the day after the notification of the confirmation is received.

50.3.3 Effects

(a) for **termination of the Agreement**:

The coordinator must — within 60 days from when termination takes effect — submit:

(i) a periodic report (for the last open reporting period until termination; see Article 20.3) and

(ii) a final report (see Article 20.4).

If the Agreement is terminated for breach of the obligation to submit reports (see Articles 20.8 and 50.3.1(l)), the coordinator may not submit any reports after termination.

If the Commission does not receive the reports within the deadline (see above), only costs which are included in an approved periodic report will be taken into account.

The Commission will **calculate** the final grant amount (see Article 5.3) and the balance (see Article 21.4) on the basis of the reports submitted. Only costs incurred until termination takes effect are eligible (see Article 6). Costs relating to contracts due for execution only after termination are not eligible.

This does not affect the Commission's right to reduce the grant (see Article 43) or to impose administrative sanctions (Article 45).

The beneficiaries may not claim damages due to termination by the Commission (see Article 46).

After termination, the beneficiaries' obligations (in particular Articles 20, 22, 23, Section 3 of Chapter 4, 36, 37, 38, 40, 42, 43 and 44) continue to apply.

(b) for **termination of the participation of one or more beneficiaries**:

The coordinator must — within 60 days from when termination takes effect — submit:

(i) a report on the distribution of payments to the beneficiary concerned;

(ii) a request for amendment (see Article 55), with a proposal for reallocation of the tasks and estimated budget of the beneficiary concerned (see Annexes 1 and 2) and, if necessary, the addition of one or more new beneficiaries (see Article 56). If termination is notified after the period set out in Article 3, no request for amendment must be submitted unless the beneficiary concerned is the coordinator. In this case the request for amendment must propose a new coordinator, and

(iii) if termination takes effect during the period set out in Article 3, a **termination report** from the beneficiary concerned, for the open reporting period until termination, containing an overview of the progress of the work, an overview of the use of resources, the individual financial statement and, if applicable, the certificate on the financial statement (see Article 20).

The information in the termination report must also be included in the periodic report for the next reporting period (see Article 20.3).

If the request for amendment is rejected by the Commission (because it calls into question the

decision awarding the grant or breaches the principle of equal treatment of applicants), the Agreement may be terminated according to Article 50.3.1(c).

If the request for amendment is accepted by the Commission, the Agreement is **amended** to introduce the necessary changes (see Article 55).

The Commission will — on the basis of the periodic reports, the termination report and the report on the distribution of payments — **calculate** the amount which is due to the beneficiary and if the (pre-financing and interim) payments received by the beneficiary exceed this amount.

The **amount which is due** is calculated in the following steps:

Step 1 — Application of the reimbursement rate to the eligible costs

The grant amount for the beneficiary is calculated by applying the reimbursement rate(s) to the total eligible costs declared by the beneficiary in the termination report and approved by the Commission.

Only costs incurred by the beneficiary concerned until termination takes effect are eligible (see Article 6). Costs relating to contracts due for execution only after termination are not eligible.

Step 2 — Reduction due to substantial errors, irregularities or fraud or serious breach of obligations

In case of a reduction (see Article 43), the Commission will calculate the reduced grant amount for the beneficiary by deducting the amount of the reduction (calculated in proportion to the seriousness of the errors, irregularities or fraud or breach of obligations, in accordance with Article 43.2) from the grant amount for the beneficiary.

If the payments received **exceed the amounts due**:

- if termination takes effect during the period set out in Article 3 and the request for amendment is accepted, the beneficiary concerned must repay to the coordinator the amount unduly received. The Commission will formally notify the amount unduly received and request the beneficiary concerned to repay it to the coordinator within 30 days of receiving notification. If it does not repay the coordinator, the Commission will draw upon the Guarantee Fund to pay the coordinator and then notify a **debit note** on behalf of the Guarantee Fund to the beneficiary concerned (see Article 44);

- in all other cases, in particular if termination takes effect after the period set out in Article 3, the Commission will formally notify a **debit note** to the beneficiary concerned. If payment is not made by the date in the debit note, the Guarantee Fund will pay to the Commission the amount due and the Commission will notify a debit note on behalf of the Guarantee Fund to the beneficiary concerned (see Article 44);

- if the beneficiary concerned is the former coordinator, it must repay the new coordinator according to the procedure above, unless:

    - termination takes effect after an interim payment and

- the former coordinator has not distributed amounts received as pre-financing or interim payments (see Article 21.7).

In this case, the Commission will formally notify a **debit note** to the former coordinator. If payment is not made by the date in the debit note, the Guarantee Fund will pay to the Commission the amount due. The Commission will then pay the new coordinator and notify a debit note on behalf of the Guarantee Fund to the former coordinator (see Article 44).

If the payments received **do not exceed the amounts due**: amounts owed to the beneficiary concerned will be included in the next interim or final payment.

If the Commission does not receive the termination report within the deadline (see above), only costs included in an approved periodic report will be taken into account.

If the Commission does not receive the report on the distribution of payments within the deadline (see above), it will consider that:

- the coordinator did not distribute any payment to the beneficiary concerned and that
- the beneficiary concerned must not repay any amount to the coordinator.

After termination, the concerned beneficiary's obligations (in particular Articles 20, 22, 23, Section 3 of Chapter 4, 36, 37, 38, 40, 42, 43 and 44) continue to apply.

SECTION 4 FORCE MAJEURE

ARTICLE 51 — FORCE MAJEURE

'Force majeure' means any situation or event that:

- prevents either party from fulfilling their obligations under the Agreement,
- was unforeseeable, exceptional situation and beyond the parties' control,
- was not due to error or negligence on their part (or on the part of third parties involved in the action), and
- proves to be inevitable in spite of exercising all due diligence.

The following cannot be invoked as force majeure:

- any default of a service, defect in equipment or material or delays in making them available, unless they stem directly from a relevant case of force majeure,
- labour disputes or strikes, or
- financial difficulties.

Any situation constituting force majeure must be formally notified to the other party without delay, stating the nature, likely duration and foreseeable effects.

The parties must immediately take all the necessary steps to limit any damage due to force majeure and do their best to resume implementation of the action as soon as possible.

The party prevented by force majeure from fulfilling its obligations under the Agreement cannot be considered in breach of them.

CHAPTER 7 FINAL PROVISIONS

ARTICLE 52 — COMMUNICATION BETWEEN THE PARTIES

52.1 Form and means of communication

Communication under the Agreement (information, requests, submissions, 'formal notifications', etc.) must:

- be made in writing and
- bear the number of the Agreement.

All communication must be made through the Participant Portal **electronic** exchange system and using the forms and templates provided there.

If — after the payment of the balance — the Commission finds that a formal notification was not accessed, a second formal notification will be made by registered post with proof of delivery ('formal notification on **paper**'). Deadlines will be calculated from the moment of the second notification.

Communications in the electronic exchange system must be made by persons authorised according to the Participant Portal Terms & Conditions. For naming the authorised persons, each beneficiary must have designated — before the signature of this Agreement — a 'legal entity appointed representative (LEAR)'. The role and tasks of the LEAR are stipulated in his/her appointment letter (see Participant Portal Terms & Conditions).

If the electronic exchange system is temporarily unavailable, instructions will be given on the Commission website.

52.2 Date of communication

Communications are considered to have been made when they are sent by the sending party (i.e. on the date and time they are sent through the electronic exchange system).

Formal notifications through the **electronic** exchange system are considered to have been made when they are received by the receiving party (i.e. on the date and time of acceptance by the receiving party, as indicated by the time stamp). A formal notification that has not been accepted within 10 days after sending is considered to have been accepted.

Formal notifications **on paper** sent by **registered post** with proof of delivery (only after the payment of the balance) are considered to have been made on either:

- the delivery date registered by the postal service or
- the deadline for collection at the post office.

If the electronic exchange system is temporarily unavailable, the sending party cannot be considered in breach of its obligation to send a communication within a specified deadline.

52.3 Addresses for communication

The **electronic** exchange system must be accessed via the following URL:

https://ec.europa.eu/info/funding-tenders/opportunities/portal/screen/myarea/projects

The Commission will formally notify the coordinator and beneficiaries in advance any changes to this URL.

Formal notifications on paper (only after the payment of the balance) addressed **to the Commission** must be sent to the official mailing address indicated on the Commission's website.

Formal notifications on paper (only after the payment of the balance) addressed **to the beneficiaries** must be sent to their legal address as specified in the Participant Portal Beneficiary Register.

ARTICLE 53 — INTERPRETATION OF THE AGREEMENT

53.1 Precedence of the Terms and Conditions over the Annexes

The provisions in the Terms and Conditions of the Agreement take precedence over its Annexes.

Annex 2 takes precedence over Annex 1.

53.2 Privileges and immunities

Not applicable

ARTICLE 54 — CALCULATION OF PERIODS, DATES AND DEADLINES

In accordance with Regulation No 1182/71[30], periods expressed in days, months or years are calculated from the moment the triggering event occurs.

The day during which that event occurs is not considered as falling within the period.

ARTICLE 55 — AMENDMENTS TO THE AGREEMENT

55.1 Conditions

The Agreement may be amended, unless the amendment entails changes to the Agreement which would call into question the decision awarding the grant or breach the principle of equal treatment of applicants.

Amendments may be requested by any of the parties.

55.2 Procedure

[30] Regulation (EEC, Euratom) No 1182/71 of the Council of 3 June 1971 determining the rules applicable to periods, dates and time-limits (OJ L 124, 8.6.1971, p. 1).

The party requesting an amendment must submit a request for amendment signed in the electronic exchange system (see Article 52).

The coordinator submits and receives requests for amendment on behalf of the beneficiaries (see Annex 3).

If a change of coordinator is requested without its agreement, the submission must be done by another beneficiary (acting on behalf of the other beneficiaries).

The request for amendment must include:

- the reasons why;
- the appropriate supporting documents, and
- for a change of coordinator without its agreement: the opinion of the coordinator (or proof that this opinion has been requested in writing).

The Commission may request additional information.

If the party receiving the request agrees, it must sign the amendment in the electronic exchange system within 45 days of receiving notification (or any additional information the Commission has requested). If it does not agree, it must formally notify its disagreement within the same deadline. The deadline may be extended, if necessary for the assessment of the request. If no notification is received within the deadline, the request is considered to have been rejected.

An amendment **enters into force** on the day of the signature of the receiving party.

An amendment **takes effect** on the date agreed by the parties or, in the absence of such an agreement, on the date on which the amendment enters into force.

ARTICLE 56 — ACCESSION TO THE AGREEMENT

56.1 Accession of the beneficiaries mentioned in the Preamble

The other beneficiaries must accede to the Agreement by signing the Accession Form (see Annex 3) in the electronic exchange system (see Article 52) within 30 days after its entry into force (see Article 58).

They will assume the rights and obligations under the Agreement with effect from the date of its entry into force (see Article 58).

If a beneficiary does not accede to the Agreement within the above deadline, the coordinator must — within 30 days — request an amendment to make any changes necessary to ensure proper implementation of the action. This does not affect the Commission's right to terminate the Agreement (see Article 50).

56.2 Addition of new beneficiaries

In justified cases, the beneficiaries may request the addition of a new beneficiary.

For this purpose, the coordinator must submit a request for amendment in accordance with Article 55.

It must include an Accession Form (see Annex 3) signed by the new beneficiary in the electronic exchange system (see Article 52).

New beneficiaries must assume the rights and obligations under the Agreement with effect from the date of their accession specified in the Accession Form (see Annex 3).

ARTICLE 57 — APPLICABLE LAW AND SETTLEMENT OF DISPUTES

57.1 Applicable law

The Agreement is governed by the applicable EU law, supplemented if necessary by the law of Belgium.

57.2 Dispute settlement

If a dispute concerning the interpretation, application or validity of the Agreement cannot be settled amicably, the General Court — or, on appeal, the Court of Justice of the European Union — has sole jurisdiction. Such actions must be brought under Article 272 of the Treaty on the Functioning of the EU (TFEU).

As an exception, if such a dispute is between the Commission and PI IMAGING TECHNOLOGY SA, the competent Belgian courts have sole jurisdiction.

If a dispute concerns administrative sanctions, offsetting or an enforceable decision under Article 299 TFEU (see Articles 44, 45 and 46), the beneficiaries must bring action before the General Court — or, on appeal, the Court of Justice of the European Union — under Article 263 TFEU.

ARTICLE 58 — ENTRY INTO FORCE OF THE AGREEMENT

The Agreement will enter into force on the day of signature by the Commission or the coordinator, depending on which is later.

SIGNATURES

For the coordinator

Maria GUSTAFSON with ECAS id ngstmari signed in the Participant Portal on 08/12/2020 at 10:06:07 (transaction id SigId-34068-whmF pgMSkpq8ecvJLjDci5D9XwFrthR64Vy427D45Aw8PHo7A53iZRKyqtq nzQI3hO4qASQL34FrNozKfiBZgb-rS0vSrmBGYCMvs3SGKskZa-I16Yx hzpQsR4uYUhmH7HptFqFFRmmRrSs3ZFivZ90icF9OEx6HnjzRVwOv dZ6lzmZzKDbFTZRHbZ1MIzL3SpNLp). Timestamp by third party at 2020.12.08 11:06:14 CET

For the Commission

Signed by Paolo GARELLO with ECAS id garelpa as an authorised representative on 08-12-2020 12:19:02 (transaction id SigId-38174-k Rk7mkPqowjNvGroJaW89B5A15iXAFeiXke5fZOw1wa5XFtzLPJVM6m5 xoAYfFpZruSWJWvdntJVsex4wiFQ2O-rS0vSrmBGYCMvs3SGKskZa-GT MMD1xnheDeGMtajZ0XCv0GhsHckcFJl2R74KB2eHztrizqWzS5kdxzzx pcu63zGGmv55NhOTpgN47D09f0uH00)
2020.12.08 12:19:06 CET

EUROPEAN COMMISSION
Directorate-General for Communications Networks, Content and Technology

CNECT.A – Artificial Intelligence and Digital Industry
A.4 – Photonics

ANNEX 1 (part A)

Research and Innovation action

NUMBER — 101017180 — NanoVIB

Table of Contents

1.1. The project summary

Project Number [1]	101017180	Project Acronym [2]	NanoVIB

One form per project

General information	
Project title [3]	NANO-scale VIsualization to understand Bacterial virulence and invasiveness - based on fluorescence NANOscopy and VIBrational microscopy
Starting date [4]	01/01/2021
Duration in months [5]	48
Call (part) identifier [6]	H2020-ICT-2020-2
Topic	ICT-36-2020 Disruptive photonics technologies
Fixed EC Keywords	Photonics
Free keywords	super-resolution microscopy, MINFLUX, fluorescence, Stimulated Raman scattering, pneumococci, virulence, invasiveness

Abstract [7]

In an interdisciplinary project, we will prototype a next-generation super-resolution microscope (SRM) and demonstrate its capability to bring about a major leap forward in our understanding of inter- and intracellular processes, and thus the cellular origin of diseases. Based on the recently invented MINFLUX concept, which pushes spatial resolution an order of magnitude beyond any other SRM technique, and by concerted development of detector technologies, lasers and image acquisition procedures, we will be able to retrieve information, not within reach by any other photonics-based technique. By extending operation to the near infrared, a hitherto un-accessible spectral range for SRM, we will strongly reduce phototoxicity and scattering, increase penetration depth and provide an additional spectral window for multiplexing. The developed prototype will allow nanometer-scale protein localization patterns to be resolved and to be placed in a cellular context by overlaid morphological, biochemical and metabolic images generated by label-free stimulated Raman scattering (SRS) and two-photon excitation (TPE).
In a lead application, we will use the unique capabilities of the to-be-developed technology to study the molecular mechanisms underlying pneumococcal disease, largely attributed to localization patterns of specific bacterial surface proteins, and their intricate interactions with immune and host target cells. Pneumococci are a major contributor to morbidity and mortality worldwide and we aim to provide vital information which can lead to new treatments and vaccines. We will also offer hands-on access to the technology to researchers from both academia and industry in an open demonstration facility. Together with the lead application, this will generate demand for microscopes, lasers and detectors, which the industrial partners will develop subsequent to this project based on the prototypes, further strengthening Europe´s industrial position in the microscopy field.

1.2. List of Beneficiaries

Project Number [1]	101017180	Project Acronym [2]	NanoVIB

List of Beneficiaries

No	Name	Short name	Country	Project entry month[8]	Project exit month
1	KUNGLIGA TEKNISKA HOEGSKOLAN	KTH	Sweden	1	48
2	KAROLINSKA INSTITUTET	KI	Sweden	1	48
3	ABBERIOR INSTRUMENTS GMBH	AI	Germany	1	48
4	LASER-LABORATORIUM GOTTINGEN EV	LLG	Germany	1	48
5	APE ANGEWANDTE PHYSIK UND ELEKTRONIK GMBH	APE	Germany	1	48
6	PI IMAGING TECHNOLOGY SA	PII	Switzerland	1	48

1.3. Workplan Tables - Detailed Implementation

1.3.1. WT1 List of work packages

WP Number[9]	WP Title	Lead beneficiary[10]	Person-months[11]	Start month[12]	End month[13]
WP1	Platform development	3 - AI	81.00	1	48
WP2	Optical Integration	4 - LLG	81.00	7	42
WP3	Detector development	6 - PII	43.00	1	36
WP4	Laser for MINFLUX and SRS operation	5 - APE	57.00	1	36
WP5	Labels, acquistion and protocols	1 - KTH	95.00	1	36
WP6	Lead application and dissemination	2 - KI	93.00	25	48
WP7	Project management and communication	1 - KTH	30.00	1	48
WP8	Ethics requirements	1 - KTH	N/A	1	48
		Total	480.00		

1.3.2. WT2 list of deliverables

Deliverable Number[14]	Deliverable Title	WP number[9]	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D1.1	Provide two extensible VIS-MINFLUX platforms to LLG for integration work	WP1	3 - AI	Demonstrator	Confidential, only for members of the consortium (including the Commission Services)	12
D1.2	Platforms can interface with Gen I SPAD array electronics output	WP1	3 - AI	Demonstrator	Confidential, only for members of the consortium (including the Commission Services)	15
D1.3	Replace acquisition electronics with Gen II SPAD array electronics compatible version	WP1	3 - AI	Demonstrator	Confidential, only for members of the consortium (including the Commission Services)	32
D1.4	Demo ready integrated SPAD array based VIS-NIR-MINFLUX	WP1	3 - AI	Demonstrator	Public	48
D2.1	One platform for VIS-NIR-MINFLUX imaging installed at KTH	WP2	4 - LLG	Demonstrator	Public	12
D2.2	SRS-MINFLUX modality integrated	WP2	4 - LLG	Demonstrator	Public	24
D2.3	Platforms equipped with Gen I SPAD arrays	WP2	4 - LLG	Demonstrator	Public	27
D2.4	Two photon activation and TPE TRAST modality integrated	WP2	4 - LLG	Demonstrator	Public	36
D2.5	Report on prototype design	WP2	4 - LLG	Report	Confidential, only for members of the consortium (including the Commission Services)	42
D3.1	Two SPAD arrays with Gen I detection electronics integrated, tested and delivered to AI for integration	WP3	6 - PII	Demonstrator	Public	6
D3.2	Upgrade to Gen II detection electronics	WP3	6 - PII	Demonstrator	Public	30

Deliverable Number[14]	Deliverable Title	WP number[9]	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
	with time-tagging and 20ps timing resolution					
D3.3	Replace SPAD arrays with newly designed CMOS SPAD array with enhanced red and NIR sensitivity	WP3	6 - PII	Demonstrator	Confidential, only for members of the consortium (including the Commission Services)	32
D3.4	Characterization of 10×10 CMOS SPAD array	WP3	6 - PII	Report	Confidential, only for members of the consortium (including the Commission Services)	36
D4.1	Loan picoEmerald to bridge the gap until the prototype is finished to LLG for Task 2.2	WP4	5 - APE	Demonstrator	Confidential, only for members of the consortium (including the Commission Services)	15
D4.2	Fast tunable SRS-light source to LLG	WP4	5 - APE	Demonstrator	Public	22
D4.3	Femto-pico conversion upgrade of SRS-light source to LLG	WP4	5 - APE	Demonstrator	Public	25
D4.4	Fast tunable femto-pico SRS-light source to KTH	WP4	5 - APE	Demonstrator	Public	31
D5.1	At least one fluorophore verified for NIR-MINFLUX imaging	WP5	1 - KTH	Demonstrator	Public	12
D5.2	Protein labeling and sample preparation for VIS-NIR-MINFLUX imaging for bacterial studies	WP5	2 - KI	Demonstrator	Public	24
D5.3	Excitation, photo-activation and illumination schemes allowing combined VIS-NIR-MINFLUX, SRS and/or TPE TRAST imaging established	WP5	1 - KTH	Demonstrator	Public	27
D5.4	SRS and TPE TRAST imaging on bacteria and host cells established	WP5	1 - KTH	Demonstrator	Public	30

Deliverable Number[14]	Deliverable Title	WP number[9]	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D5.5	Combined use of MINFLUX together with SRS and/or TPE TRAST on bacteria and host cells	WP5	1 - KTH	Demonstrator	Public	36
D6.1	End-user facility up and running	WP6	2 - KI	Demonstrator	Public	36
D6.2	Finding nanometer precision localization patterns	WP6	2 - KI	Demonstrator	Public	48
D7.1	Internal web page	WP7	1 - KTH	Websites, patents filling, etc.	Confidential, only for members of the consortium (including the Commission Services)	1
D7.2	Public web page	WP7	1 - KTH	Websites, patents filling, etc.	Public	6
D7.3	End-user group established	WP7	1 - KTH	Report	Public	12
D7.4	End-user workshops	WP7	1 - KTH	Websites, patents filling, etc.	Public	45
D7.5	Public lectures on the activities and outcome of the project	WP7	1 - KTH	Websites, patents filling, etc.	Public	48
D8.1	HCT - Requirement No. 1	WP8	1 - KTH	Ethics	Confidential, only for members of the consortium (including the Commission Services)	24
D8.2	A - Requirement No. 2	WP8	1 - KTH	Ethics	Confidential, only for members of the consortium (including the Commission Services)	24
D8.3	A - Requirement No. 3	WP8	1 - KTH	Ethics	Confidential, only for members of the consortium (including the Commission Services)	24

1.3.3. WT3 Work package descriptions

Work package number [9]	WP1	**Lead beneficiary** [10]	3 - AI
Work package title	Platform development		
Start month	1	**End month**	48

Objectives

Provide two modular MINFLUX platforms for fluorescence super-resolution imaging that are fully functional in the VIS range and easily extensible by additional light sources and detectors and new acquisition algorithms. (Objective I)
Modify acquisition electronics to allow preliminary integration of existing SPAD arrays with multiple counter outputs. Develop a new acquisition electronics platform that allows leveraging the full information from Gen II SPAD array electronics with fluorescence lifetime information. (Objective V)
Integrate hardware control of additional detectors/light sources and newly developed acquisition control algorithms in a ready-to-use application software for dissemination. (Objective VII)

Description of work and role of partners

WP1 - Platform development [Months: 1-48]
AI, KTH, LLG, APE, PII
Description of work
Using its existing infrastructure and experience, AI will build two fully functional MINFLUX microscopes for the VIS range. The platforms' optical layout will be modified to allow both the integration of additional light sources and detectors for NIR-MINFLUX imaging, SRS and TPE TRAST imaging and two photon activation in WP2. The platforms will be equipped with an open and modular version of our operating software that allows seamless integration of hardware control modules for the additional hardware. It also allows fine-grained control of image acquisition sequences through python scripts and modification of the FPGA based localization and tracking algorithms through the same python interface.
Closely cooperating with PII, AI will redesign the acquisition electronics platform to be able to handle a real-time data stream from their Gen II SPAD array detection electronics. This will provide the MINFLUX acquisition control with positional and lifetime information of each photon detected. The software will allow our partners to design algorithms to control acquisition sequences and localization algorithms based on this data in real time and test them on their platform. Integration of these new algorithms in our regular MINFLUX user software will be handled by AI's software department based on the feedback gained from WPs 2, 5 and 6.
Task 1.1: Construction of two modular MINFLUX platforms (AI, LLG).
Task 1.2: Plan modifications to existing Gen I SPAD array detection electronics with PII (Task 3.1) and interface the modified version with current AI acquisition electronics (AI, PII).
Task 1.3: Develop next generation acquisition electronics interfacing with Gen II SPAD array detection electronics from Task 3.2 (AI, PII).
Task 1.4: Based on feedback from user interaction in the demo centers established in Task 6.3, design and test an integrated VIS-NIR-MINFLUX microscope with array detection (AI, APE, LLG, KTH). Make correlated label-free imaging and two photon activation more user friendly.

Participation per Partner

Partner number and short name	WP1 effort
1 - KTH	4.00
3 - AI	64.00
4 - LLG	9.00
5 - APE	2.00
6 - PII	2.00

Partner number and short name	WP1 effort
Total	81.00

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D1.1	Provide two extensible VIS-MINFLUX platforms to LLG for integration work	3 - AI	Demonstrator	Confidential, only for members of the consortium (including the Commission Services)	12
D1.2	Platforms can interface with Gen I SPAD array electronics output	3 - AI	Demonstrator	Confidential, only for members of the consortium (including the Commission Services)	15
D1.3	Replace acquisition electronics with Gen II SPAD array electronics compatible version	3 - AI	Demonstrator	Confidential, only for members of the consortium (including the Commission Services)	32
D1.4	Demo ready integrated SPAD array based VIS-NIR-MINFLUX	3 - AI	Demonstrator	Public	48

Description of deliverables

D1.1: Provide two extensible VIS-MINFLUX platforms to LLG for integration work (M12);
D1.2: Platforms can interface with Gen I SPAD array electronics output (M15);
D1.3: Replace acquisition electronics with Gen II SPAD array electronics compatible version (M32);
D1.4: Demo ready integrated SPAD array based VIS-NIR-MINFLUX (M48)

D1.1 : Provide two extensible VIS-MINFLUX platforms to LLG for integration work [12]

Provide two extensible VIS-MINFLUX platforms to LLG for integration work. Outcome of Task 1.1, described on p. 50 in Part B of Annex 1 of the Grant Agreement.

D1.2 : Platforms can interface with Gen I SPAD array electronics output [15]

Platforms can interface with Gen I SPAD array electronics output (Outcome of Task 1.2, described on p.51 in Part B of Annex 1 of the Grant Agreement.)

D1.3 : Replace acquisition electronics with Gen II SPAD array electronics compatible version [32]

Replace acquisition electronics with Gen II SPAD array electronics compatible version (Outcome of Task 1.3, described on p. 51 in Part B of Annex 1 of the Grant Agreement.)

D1.4 : Demo ready integrated SPAD array based VIS-NIR-MINFLUX [48]

Demo ready integrated SPAD array based VIS-NIR-MINFLUX (Outcome of Task 1.4, described on p. 52 in Part B of Annex 1 of the Grant Agreement.)

Schedule of relevant Milestones

Milestone number[18]	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS2	Proof-of-principle of NIR-MINFLUX	4 - LLG	15	Generate super-resolved image of samples using NIR dye. Will be verified via a publication.
MS3	Proof-of-principle of SRS-MINFLUX	4 - LLG	24	Generation of correlative SRS-MINFLUX image of samples. Will be verified via a publication.
MS6	Operate Gen II SPAD arrays with new FPGA platform	3 - AI	32	Handling of real-time data stream from Gen II SPAD array. Will be verified via a prototype up and running.

Work package number [9]	WP2	Lead beneficiary [10]	4 - LLG
Work package title	Optical Integration		
Start month	7	End month	42

Objectives

Realization of a MINFLUX platform for fluorescence super-resolution imaging (Objective I)
- in the near infrared wavelength range (Objective II)
- with faster image acquisition and lower background (Objective V)
- correlated with SRS and TPE TRAST imaging (Objective V)

Development and implementation of advanced localization algorithms for SPAD array-based MINFLUX and acquisition strategies for NIR-MINFLUX and correlative imaging.

Description of work and role of partners

WP2 - Optical Integration [Months: 7-42]
LLG, KTH, KI, AI, APE, PII
Two modular MINFLUX platforms, which are provided by the partner AI, will be further developed into multimodal super-resolution platforms, one at the LLG for continuous improvement and the other being transferred to KTH and regularly upgraded. The focus lies on optical and mechanical integration of necessary components partly provided and newly developed by our partners (detectors (PII), lasers (APE) and electronics (AI)). Further, image acquisition strategies and algorithms will be designed and implemented in close collaboration with KTH (photophysical dye properties) and AI (electronics and acquisition control).
Task 2.1: Expand MINFLUX platform from WP1 to the NIR by integrating additional excitation laser and point detectors (LLG, AI, KTH).
Task 2.2: Integrate SRS light source from Task 4.1 and SRS detector and implement acquisition strategies for correlated SRS-MINFLUX imaging (LLG, APE).
Task 2.3: Integrate Gen I SPAD array and prototype background suppression and molecule finding algorithms based on spatial detection (LLG, PII, AI).
Task 2.4: Implement two photon activation for MINFLUX and establish acquisition strategies for label-free TPE TRAST metabolic imaging for correlative TRAST-MINFLUX imaging (LLG, APE, AI, KTH).
Task 2.5: Optimize and stabilize optical setup and provide critical feedback (LLG, AI, APE, PII, KI).

Participation per Partner

Partner number and short name	WP2 effort
1 - KTH	14.00
2 - KI	3.00
3 - AI	10.00
4 - LLG	49.00
5 - APE	3.00
6 - PII	2.00
Total	81.00

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D2.1	One platform for VIS-NIR-MINFLUX imaging installed at KTH	4 - LLG	Demonstrator	Public	12
D2.2	SRS-MINFLUX modality integrated	4 - LLG	Demonstrator	Public	24
D2.3	Platforms equipped with Gen I SPAD arrays	4 - LLG	Demonstrator	Public	27
D2.4	Two photon activation and TPE TRAST modality integrated	4 - LLG	Demonstrator	Public	36
D2.5	Report on prototype design	4 - LLG	Report	Confidential, only for members of the consortium (including the Commission Services)	42

Description of deliverables

D2.1: One platform for VIS-NIR-MINFLUX imaging installed at KTH (M12);
D2.2: SRS-MINFLUX modality integrated (M24);
D2.3: Platforms equipped with Gen I SPAD arrays (M27);
D2.4: Two photon activation and TPE TRAST modality integrated (M36);
D2.5: Report on prototype design (M42)

D2.1 : One platform for VIS-NIR-MINFLUX imaging installed at KTH [12]

One platform for VIS-NIR-MINFLUX imaging installed at KTH, an outcome of Task 2.1, described on p. 53 in Part B of Annex 1 of the Grant Agreement.

D2.2 : SRS-MINFLUX modality integrated [24]

SRS-MINFLUX modality integrated (From Task 2.2, described in p. 53 in Part B of Annex 1 of the Grant Agreement.)

D2.3 : Platforms equipped with Gen I SPAD arrays [27]

Platforms equipped with Gen I SPAD arrays (Result of Task 2.3, described on p. 53 in Part B of Annex 1 of the Grant Agreement.)

D2.4 : Two photon activation and TPE TRAST modality integrated [36]

Two photon activation and TPE TRAST modality integrated (Outcome of Task 2.4, described on p.54 in Part B of Annex 1 of the Grant Agreement.)

D2.5 : Report on prototype design [42]

Report on prototype design (From Task 2.5, described on p.54 in Part B of Annex 1 of the Grant Agreement.)

Schedule of relevant Milestones

Milestone number[18]	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS2	Proof-of-principle of NIR-MINFLUX	4 - LLG	15	Generate super-resolved image of samples using NIR

Schedule of relevant Milestones

Milestone number[18]	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
				dye. Will be verified via a publication.
MS3	Proof-of-principle of SRS-MINFLUX	4 - LLG	24	Generation of correlative SRS-MINFLUX image of samples. Will be verified via a publication.
MS7	First SRS-MINFLUX measurement on bacteria	1 - KTH	33	Generation of SRS-MINFLUX images of bacteria with 5 nm resolution in MINFLUX channel. Will be verified via a report.
MS8	Installation of SRS-MINFLUX platform in end-user facility	2 - KI	40	First workshop performed

Work package number [9]	WP3	Lead beneficiary [10]	6 - PII
Work package title	Detector development		
Start month	1	End month	36

Objectives

Develop single-photon avalanche detector (SPAD) arrays, with >10 detectors, with at least equal performance to individual state-of-the-art SPADs, and with enhanced sensitivity in the NIR (Objectives III and VII) by
- tailoring the existing SPAD array for MINFLUX microscopes
- developing novel SPAD arrays with enhanced red and NIR sensitivity
- developing a 10×10 SPAD array with time-gating

Description of work and role of partners

WP3 - Detector development [Months: 1-36]
PII, KTH, AI, LLG
We will modify existing detection electronics to integrate Gen I SPAD arrays in MINFLUX microscopes. Further, we will develop Gen II detection electronics with time-tagging and 20ps timing resolution in close collaboration with AI. Gen II detection electronics will be used to communicate the information of all 23 pixels to AI's MINFLUX platforms, which will use photon-counts for iterative positioning within the sample.
We will develop a new CMOS SPAD array with enhanced red and NIR sensitivity, a peak quantum efficiency (QE) above 50% at 640 nm and specially enhanced QE spectra between 600nm and 900nm. This will finally lead to larger SPAD arrays (small image sensor) with dynamically pre-selected relevant photon events by chip-level time-gating and a frame rate of more than 1Mfps.
Task 3.1: Gen I detection electronics: Hardware platform adaptation to MINFLUX platform (PII, AI, LLG, KTH).
Task 3.2: Gen II detection electronics: Develop a new hardware platform following a newly defined communication protocol that passes time-resolved information to AI's MINFLUX platform (PII, AI)
Task 3.3: Develop enhanced red and NIR sensitivity CMOS SPAD (PII).
Task 3.4: Develop 10×10 CMOS SPAD array with integrated time-gating (PII, AI, LLG, KTH).

Participation per Partner

Partner number and short name	WP3 effort
1 - KTH	1.00
3 - AI	4.00
4 - LLG	2.00
6 - PII	36.00
Total	43.00

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D3.1	Two SPAD arrays with Gen I detection electronics integrated, tested and delivered to AI for integration	6 - PII	Demonstrator	Public	6

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D3.2	Upgrade to Gen II detection electronics with time-tagging and 20ps timing resolution	6 - PII	Demonstrator	Public	30
D3.3	Replace SPAD arrays with newly designed CMOS SPAD array with enhanced red and NIR sensitivity	6 - PII	Demonstrator	Confidential, only for members of the consortium (including the Commission Services)	32
D3.4	Characterization of 10×10 CMOS SPAD array	6 - PII	Report	Confidential, only for members of the consortium (including the Commission Services)	36

Description of deliverables

D3.1: Two SPAD arrays with Gen I detection electronics integrated, tested and delivered to AI for integration (M6);
D3.2: Upgrade to Gen II detection electronics with time-tagging and 20ps timing resolution (M30);
D3.3: Replace SPAD arrays with newly designed CMOS SPAD array with enhanced red and NIR sensitivity (M32);
D3.4: Characterization of 10×10 CMOS SPAD array (M36)

D3.1 : Two SPAD arrays with Gen I detection electronics integrated, tested and delivered to AI for integration [6]

Two SPAD arrays with Gen I detection electronics integrated, tested and delivered to AI for integration, as a result of task 3.1, as described on p. 54 in Part B of Annex 1 of the Grant Agreement..

D3.2 : Upgrade to Gen II detection electronics with time-tagging and 20ps timing resolution [30]

Upgrade to Gen II detection electronics with time-tagging and 20ps timing resolution (From Task 3.2, described in p. 55 in Part B of Annex 1 of the Grant Agreement.)

D3.3 : Replace SPAD arrays with newly designed CMOS SPAD array with enhanced red and NIR sensitivity [32]

Replace SPAD arrays with newly designed CMOS SPAD array with enhanced red and NIR sensitivity (Outcome of Task 3.3, as described on p. 55 in Part B of Annex 1 of the Grant Agreement.)

D3.4 : Characterization of 10×10 CMOS SPAD array [36]

Characterization of 10×10 CMOS SPAD array (Outcome of Task 3.4, described on p. 55 in Part B of Annex 1 of the Grant Agreement.)

Schedule of relevant Milestones

Milestone number[18]	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS5	SPAD with optimized NIR sensitivity	6 - PII	30	Demonstration of improved NIR sensitivity and use Gen II electronics. Will be verified via a prototype up and running.
MS6	Operate Gen II SPAD arrays with new FPGA platform	3 - AI	32	Handling of real-time data stream from Gen II SPAD

Schedule of relevant Milestones

Milestone number[18]	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
				array. Will be verified via a prototype up and running.

Work package number [9]	WP4	Lead beneficiary [10]	5 - APE
Work package title	Laser for MINFLUX and SRS operation		
Start month	1	End month	36

Objectives

Development of new tuning concepts and implementation into a one-box laser source for SRS imaging to allow fast wavelength tuning to target several Raman lines per image and to allow pulse length tuning to the fs regime for two photon activation and TPE TRAST imaging (Objectives IV and VII).

Description of work and role of partners

WP4 - Laser for MINFLUX and SRS operation [Months: 1-36]
APE, KTH, LLG
Using our core technologies, synchronously pumped OPOs and nonlinear optics, we will develop a new, easy to use one-box light source for two photon activation and TPE TRAST and SRS imaging.
We will validate and verify new OPO tuning concepts, which will allow to improve the tuning time by more than a factor of 10 to less than 5 seconds. The spectral broadening of the pulses inside the OPO cavity will be evaluated and pulse compressor designs will be developed, in order to achieve pulse length shortening into the femtosecond regime (~300fs). A new mechanical design of the OPO cavity will be made in order to realize both ultra-fast tuning and pulse length switching in a one-box light source.
Further, we will implement modern and flexible electronic hardware and software and implement software tuning and stabilization routines. This will allow for complete computer control of the light source and integration into microscope setups.
The prototypes are to be delivered to LLG and KTH for integration into their MINFLUX platforms and for validation of the technology.
Task 4.1: Develop ultra-fast targeting of arbitrary wavelengths (< 5s) for picosecond SRS-lasers and building of prototype light source (APE, LLG).
Task 4.2: Development of pulse-length switching between ps and fs regimes for the new laser platform and building of prototype light source (APE, LLG, KTH).

Participation per Partner

Partner number and short name	WP4 effort
1 - KTH	1.00
4 - LLG	2.00
5 - APE	54.00
Total	57.00

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D4.1	Loan picoEmerald to bridge the gap until the prototype is finished to LLG for Task 2.2	5 - APE	Demonstrator	Confidential, only for members of the consortium (including the Commission Services)	15

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D4.2	Fast tunable SRS-light source to LLG	5 - APE	Demonstrator	Public	22
D4.3	Femto-pico conversion upgrade of SRS-light source to LLG	5 - APE	Demonstrator	Public	25
D4.4	Fast tunable femto-pico SRS-light source to KTH	5 - APE	Demonstrator	Public	31

Description of deliverables

D4.1: Loan picoEmerald to bridge the gap until the prototype is finished to LLG for Task 2.2 (M13);
D4.2: Fast tunable SRS-light source to LLG (M22);
D4.3: Femto-pico conversion upgrade of SRS-light source to LLG (M25);
D4.4: Fast tunable femto-pico SRS-light source to KTH (M31)

D4.1 : Loan picoEmerald to bridge the gap until the prototype is finished to LLG for Task 2.2 [15]

Loan picoEmerald to bridge the gap until the prototype is finished to LLG for Task 2.2 (see further Task 4.1 p. 56 in Part B of Annex 1 of the Grant Agreement.).

D4.2 : Fast tunable SRS-light source to LLG [22]

Fast tunable SRS-light source to LLG (Outcome of Task 4.2, described on p.57 in Part B of Annex 1 of the Grant Agreement.)

D4.3 : Femto-pico conversion upgrade of SRS-light source to LLG [25]

Femto-pico conversion upgrade of SRS-light source to LLG, described on p. 57 in Part B of Annex 1 of the Grant Agreement.

D4.4 : Fast tunable femto-pico SRS-light source to KTH [31]

Fast tunable femto-pico SRS-light source to KTH (described on p.57 in Part B of Annex 1 of the Grant Agreement.)

Schedule of relevant Milestones

Milestone number[18]	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS3	Proof-of-principle of SRS-MINFLUX	4 - LLG	24	Generation of correlative SRS-MINFLUX image of samples. Will be verified via a publication.
MS4	Perform pulse-length switching between ps and fs regimes	5 - APE	25	Measurement of pulse lengths of 2ps and of about 300fs over whole tuning range. Will be verified via a prototype up and running.
MS7	First SRS-MINFLUX measurement on bacteria	1 - KTH	33	Generation of SRS-MINFLUX images of bacteria with 5 nm resolution in MINFLUX channel. Will be verified via a report.

Work package number [9]	WP5	Lead beneficiary [10]	1 - KTH
Work package title	Labels, acquistion and protocols		
Start month	1	End month	36

Objectives

Identify NIR fluorophores for MINFLUX, as a means to take super-resolution microscopy into the NIR, for improved multiplexing, reduced background and better sample penetration (Objective II).
Establish and optimize acquisition and sample preparation protocols for VIS-NIR-MINFLUX, SRS and TPE TRAST imaging, as well as the combined use of these techniques, for bacteria and host cell studies (Objectives V and VI).

Description of work and role of partners

WP5 - Labels, acquistion and protocols [Months: 1-36]
KTH, KI, AI, LLG, APE
NIR fluorophores will be evaluated regarding their photo-switching abilities in order to identify dyes suitable for MINFLUX in the NIR and for combined readout with SRS and/or TPE TRAST imaging. Excitation and acquisition schemes, use of additives and sample preparation to optimize the above properties will be established and evaluated for compatibility with live cell imaging.
Based on the instrument integration established in WP2 and with lasers established in WP4, VIS-NIR-MINFLUX, SRS and TPE TRAST imaging, as well as their combined use for correlative imaging, will be established and optimized for bacterial and host cell studies. The best practices and protocols regarding labeling, sample preparation, and acquisition will provide the starting point for the bacterial studies in WP6.
Task 5.1: Identify at least one NIR fluorophore suitable for MINFLUX, allowing an extended spectral range and increased multiplexing capabilities. Define additives and sample preparation to optimize its fluorescence and switching properties in fixed and live cells (KTH, LLG, KI).
Task 5.2: Define excitation, photo-activation and illumination schemes for combined VIS-NIR-MINFLUX, SRS and/or TPE TRAST imaging of fixed and live cells (KTH, LLG, AI, APE).
Task 5.3: Establish protein labeling and sample preparation protocols for VIS-NIR-MINFLUX on fixed and live bacteria and/or host cells (KI, KTH).
Task 5.4: Verify SRS and TPE TRAST imaging on bacteria and host cells, providing relevant morphological, chemical and/or metabolic information about the cells (KTH, KI, LLG, APE).
Task 5.5: Within the constraints given by dye photophysics, acquisition procedures and sample preparation, establish combined use of MINFLUX together with SRS and/or TPE TRAST imaging (KTH, LLG, AI, KI).

Participation per Partner

Partner number and short name	WP5 effort
1 - KTH	72.00
2 - KI	9.00
3 - AI	2.00
4 - LLG	11.00
5 - APE	1.00
Total	95.00

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D5.1	At least one fluorophore verified for NIR-MINFLUX imaging	1 - KTH	Demonstrator	Public	12
D5.2	Protein labeling and sample preparation for VIS-NIR-MINFLUX imaging for bacterial studies	2 - KI	Demonstrator	Public	24
D5.3	Excitation, photo-activation and illumination schemes allowing combined VIS-NIR-MINFLUX, SRS and/or TPE TRAST imaging established	1 - KTH	Demonstrator	Public	27
D5.4	SRS and TPE TRAST imaging on bacteria and host cells established	1 - KTH	Demonstrator	Public	30
D5.5	Combined use of MINFLUX together with SRS and/or TPE TRAST on bacteria and host cells	1 - KTH	Demonstrator	Public	36

Description of deliverables

D5.1: At least one fluorophore verified for NIR-MINFLUX imaging (M12);
D5.2: Protein labeling and sample preparation for VIS-NIR MINFLUX imaging for bacterial studies (M24);
D5.3: Excitation, photo-activation and illumination schemes allowing combined VIS-NIR-MINFLUX, SRS and/or TPE TRAST imaging established (M27);
D5.4: SRS and TPE TRAST imaging on bacteria and host cells established (M30);
D5.5: Combined use of MINFLUX together with SRS and/or TPE TRAST on bacteria and host cells (M36)

D5.1 : At least one fluorophore verified for NIR-MINFLUX imaging [12]

At least one fluorophore verified for NIR-MINFLUX imaging. Outcome of Task 5.1, described in p. 58 in Part B of Annex 1 of the Grant Agreement..

D5.2 : Protein labeling and sample preparation for VIS-NIR-MINFLUX imaging for bacterial studies [24]

Protein labeling and sample preparation for VIS-NIR-MINFLUX imaging for bacterial studies (From Task 5.3, described in 58 in Part B of Annex 1 of the Grant Agreement.)

D5.3 : Excitation, photo-activation and illumination schemes allowing combined VIS-NIR-MINFLUX, SRS and/or TPE TRAST imaging established [27]

Excitation, photo-activation and illumination schemes allowing combined VIS-NIR-MINFLUX, SRS and/or TPE TRAST imaging established (Outcome of Task 5.2, described on p. 58 in Part B of Annex 1 of the Grant Agreement.)

D5.4 : SRS and TPE TRAST imaging on bacteria and host cells established [30]

SRS and TPE TRAST imaging on bacteria and host cells established (Outcome of Task 5.4, described p 58 in Part B of Annex 1 of the Grant Agreement.)

D5.5 : Combined use of MINFLUX together with SRS and/or TPE TRAST on bacteria and host cells [36]

Combined use of MINFLUX together with SRS and/or TPE TRAST on bacteria and host cells (Outcome of Task 5.5, described on p.59 in Part B of Annex 1 of the Grant Agreement.)

Schedule of relevant Milestones

Milestone number[18]	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS1	Identification NIR dye and suitable embedding medium	1 - KTH	12	INFLUX test sample with NIR dye and embedding is ready. Will be verified via a report.
MS2	Proof-of-principle of NIR-MINFLUX	4 - LLG	15	Generate super-resolved image of samples using NIR dye. Will be verified via a publication.
MS7	First SRS-MINFLUX measurement on bacteria	1 - KTH	33	Generation of SRS-MINFLUX images of bacteria with 5 nm resolution in MINFLUX channel. Will be verified via a report.
MS9	Localization patterns of bacterial surface proteins and their interaction partners on host cells demonstrated	2 - KI	48	Demonstration of the l ocalization patterns of bacterial surface proteins and their interaction partners on host cells. Will be verified via a presentation of scientific data on international conference

Work package number [9]	WP6	**Lead beneficiary** [10]	2 - KI
Work package title	Lead application and dissemination		
Start month	25	**End month**	48

Objectives

Provide key information about the role of pneumococcal surface proteins in pneumococcal disease using the MINFLUX microscope system developed in WP1 – WP4, and established for pneumococcal-host cell studies in WP5 (Objective VIII).
Promote dissemination and exploitation of the developed microscopy, laser and detector technologies in the project by a lead application and via an open end-user facility, and make them available to researchers and stakeholders outside of the project (Objective VII).

Description of work and role of partners

WP6 - Lead application and dissemination [Months: 25-48]
KI, KTH, AI, LLG
With the next generation super-resolution MINFLUX microscope system developed in this project, we will address several central aspects of pneumococcal proteins and their role in disease, specifically exploiting the unique nanometer resolution and possibilities of correlative morphological and environmental images by SRS and TPE TRAST imaging.
Task 6.1: Study pneumococcal surface proteins with central, but yet incompletely understood roles in pneumococcal virulence and host defense evasion, and how they are affected by additives having potential antibiotic or bacteriostatic effects (KI, KTH).
Task 6.2: Study possible co-localization of pneumococcal surface and pilus proteins with receptor proteins on epithelial cells of the BBB, in brain biopsies from patients who died from pneumococcal meningitis and from mice models. Identify protein interactions promoting adhesion and investigate if they can be prevented by competitive binding by antibodies (KI, KTH).
Task 6.3: Study nanoscale localization of protein virulence factors in exosomes, their role in vesicle formation and in subsequent host cell interactions (KI, KTH).
Task 6.4: Study distribution patterns of pneumococcal proteins coupled to sustained bacterial growth in lungs of pneumococcal-influenza virus co-infected mice, and how such patterns correlate with presence of anti-oxidants and with the ability of the bacteria to evade immunological attack (KI, KTH).
As a result of the lead application studies in Tasks 6.1 – 6.4, we will be able to demonstrate nanometer precision localization patterns of at least two specific bacterial surface proteins and their interaction partners on host cells, show their correlation to bacterial virulence and invasiveness, and identify possible interfering strategies.
Task 6.5: Establish a facility open to potential end-users of the MINFLUX microscope system developed in the project (LLG, KTH, KI).

Participation per Partner

Partner number and short name	WP6 effort
1 - KTH	16.00
2 - KI	70.00
3 - AI	1.00
4 - LLG	6.00
Total	93.00

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D6.1	End-user facility up and running	2 - KI	Demonstrator	Public	36
D6.2	Finding nanometer precision localization patterns	2 - KI	Demonstrator	Public	48

Description of deliverables

D6.1: End-user facility up and running (M36);
D6.2: Finding nanometer precision localization patterns of at least two specific pneumococcal surface proteins, their interaction partners on host cells, showing their correlation to bacterial virulence and invasiveness, and identifying possible interfering strategies (M48)

D6.1 : End-user facility up and running [36]

End-user facility up and running (From Task 6.5, described on p.61 in Part B of Annex 1 of the Grant Agreement.)

D6.2 : Finding nanometer precision localization patterns [48]

Finding nanometer precision localization patterns of at least two specific pneumococcal surface proteins, their interaction partners on host cells, showing their correlation to bacterial virulence and invasiveness, and identifying possible interfering strategies. (Outcome from Tasks 6.1-6.4, as described in p.59 in Part B of Annex 1 of the Grant Agreement.)

Schedule of relevant Milestones

Milestone number[18]	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS8	Installation of SRS-MINFLUX platform in end-user facility	2 - KI	40	First workshop performed
MS9	Localization patterns of bacterial surface proteins and their interaction partners on host cells demonstrated	2 - KI	48	Demonstration of the l ocalization patterns of bacterial surface proteins and their interaction partners on host cells. Will be verified via a presentation of scientific data on international conference

Work package number [9]	WP7	Lead beneficiary [10]	1 - KTH
Work package title	Project management and communication		
Start month	1	End month	48

Objectives

Ensure effective management of NanoVIB and cohesiveness between WPs; establish effective communication activities, distribute EC grant & ensure effective coordination of legal, financial and administrative work; ensure that contractual obligations, such as reports and deliverables, are delivered to EC on time; coordinate contact between the Commission and the consortium; management of IPR.

Description of work and role of partners

WP7 - Project management and communication [Months: 1-48]
KTH, KI, AI, LLG, APE, PII
The project will be led by the project coordinator (PC) and managed by the project management committee (PMC). Each work package will be led by a work package coordinator (WPC). The PC will be assisted in the daily administrative work by a Technical Project Assistant (TPA). Major support will be provided by the central administration of KTH. Before the start of the project all IPR matters will be discussed and agreed on and confirmed in a consortium agreement. During the project IPR matters will be a standing item on the agenda for meetings with PMC. Patentable results and the partners responsible for protecting them will be identified.
Project meetings are held every 6 months, always alternating between video conferences and face-to-face meetings, covering reports on work during the last period, monitoring progress in accordance with the grant agreement and planning for the following period.
Lectures and seminars will be organized to present the progress and outcomes of the project to consortium members as well as to a general audience.
Internal project communication on a daily basis will be through an internal web page with password access. An external web page will be set up for dissemination of project results and dialogue with civil society.
For further information see Section 3.2.
Task 7.1: Kick-off meeting. Formal establishment of project management committee (PMC), advisory board (AB) and Innovation, IPR and Exploitation Management Group (I2EMG). Preparation of gender action plan (KTH, All).
Task 7.2: Communication activities, to the public, to potential end-users and scientific community; Public web page, open public lectures, end-user workshops, formal establishment of end-user group (EUG), seminars, international scientific exchange (KTH, All).
Task 7.3: Monitor progress through supervision of deliverables & intermediate targets (milestones) and mitigation of risk. Scientific coordination is overseen by the coordinator and WP leaders within technical WPs. Plan & facilitate project meetings and research visits.
Task 7.4: Prepare EC interim & final project reports (KTH, All).

Participation per Partner

Partner number and short name	WP7 effort
1 - KTH	20.00
2 - KI	2.00
3 - AI	2.00
4 - LLG	2.00
5 - APE	2.00
6 - PII	2.00
Total	30.00

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D7.1	Internal web page	1 - KTH	Websites, patents filling, etc.	Confidential, only for members of the consortium (including the Commission Services)	1
D7.2	Public web page	1 - KTH	Websites, patents filling, etc.	Public	6
D7.3	End-user group established	1 - KTH	Report	Public	12
D7.4	End-user workshops	1 - KTH	Websites, patents filling, etc.	Public	45
D7.5	Public lectures on the activities and outcome of the project	1 - KTH	Websites, patents filling, etc.	Public	48

Description of deliverables

D7.1: Internal web page (M1);
D7.2: Data management plan (M6);
D7.3: Public web page (M6);
D7.4: End-user group established (M12);
D7.5: End-user workshops (M45);
D7.6: Public lectures on the activities and outcome of the project (M48)

D7.1 : Internal web page [1]

Internal web page, see p. 65 in Part B of Annex 1 of the Grant Agreement.

D7.2 : Public web page [6]

Public web page, see p.45 and p. 65 in Part B of Annex 1 of the Grant Agreement.

D7.3 : End-user group established [12]

End-user group established, see GA annex B, p.32, p.36 p.46, and p.68 in Part B of Annex 1 of the Grant Agreement..

D7.4 : End-user workshops [45]

End-user workshops, described on p.46 in Part B of Annex 1 of the Grant Agreement.

D7.5 : Public lectures on the activities and outcome of the project [48]

Public lectures on the activities and outcome of the project, see p.45 in Part B of Annex 1 of the Grant Agreement.

Schedule of relevant Milestones

Milestone number[18]	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS8	Installation of SRS-MINFLUX platform in end-user facility	2 - KI	40	First workshop performed

Work package number [9]	WP8	Lead beneficiary [10]	1 - KTH
Work package title	Ethics requirements		
Start month	1	End month	48

Objectives

The objective is to ensure compliance with the 'ethics requirements' set out in this work package.

Description of work and role of partners

WP8 - Ethics requirements [Months: 1-48]
KTH
This work package sets out the 'ethics requirements' that the project must comply with.

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D8.1	HCT - Requirement No. 1	1 - KTH	Ethics	Confidential, only for members of the consortium (including the Commission Services)	24
D8.2	A - Requirement No. 2	1 - KTH	Ethics	Confidential, only for members of the consortium (including the Commission Services)	24
D8.3	A - Requirement No. 3	1 - KTH	Ethics	Confidential, only for members of the consortium (including the Commission Services)	24

Description of deliverables

The 'ethics requirements' that the project must comply with are included as deliverables in this work package.

D8.1 : HCT - Requirement No. 1 [24]

3.5. Copies of relevant documents for using, producing or collecting human cells or tissues (e.g., ethics approval, import licence, accreditation/designation/authorisation/licensing) must be kept on file (to be specified in the grant agreement).

D8.2 : A - Requirement No. 2 [24]

5.1. Copies of relevant authorisations for animal experiments (covering also the work with genetically modified animals, if applicable) must be kept on file (to be specified in the grant agreement). 5.3. If applicable, copies of training certificates/personal licenses of the staff involved in animal experiments must be kept on file (to be specified in the grant agreement).

D8.3 : A - Requirement No. 3 [24]

5.2. General information on the procedures to ensure animal welfare and adherence to the Three Rs principle must be submitted as a deliverable. The number of animals that will be used, in the context of the 3-Rs principle, must be specified.

Schedule of relevant Milestones

Milestone number[18]	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification

1.3.4. WT4 List of milestones

Milestone number[18]	Milestone title	WP number[9]	Lead beneficiary	Due Date (in months)[17]	Means of verification
MS1	Identification NIR dye and suitable embedding medium	WP5	1 - KTH	12	INFLUX test sample with NIR dye and embedding is ready. Will be verified via a report.
MS2	Proof-of-principle of NIR-MINFLUX	WP1, WP2, WP5	4 - LLG	15	Generate super-resolved image of samples using NIR dye. Will be verified via a publication.
MS3	Proof-of-principle of SRS-MINFLUX	WP1, WP2, WP4	4 - LLG	24	Generation of correlative SRS-MINFLUX image of samples. Will be verified via a publication.
MS4	Perform pulse-length switching between ps and fs regimes	WP4	5 - APE	25	Measurement of pulse lengths of 2ps and of about 300fs over whole tuning range. Will be verified via a prototype up and running.
MS5	SPAD with optimized NIR sensitivity	WP3	6 - PII	30	Demonstration of improved NIR sensitivity and use Gen II electronics. Will be verified via a prototype up and running.
MS6	Operate Gen II SPAD arrays with new FPGA platform	WP1, WP3	3 - AI	32	Handling of real-time data stream from Gen II SPAD array. Will be verified via a prototype up and running.
MS7	First SRS-MINFLUX measurement on bacteria	WP2, WP4, WP5	1 - KTH	33	Generation of SRS-MINFLUX images of bacteria with 5 nm resolution in MINFLUX channel. Will be verified via a report.
MS8	Installation of SRS-MINFLUX platform in end-user facility	WP2, WP6, WP7	2 - KI	40	First workshop performed
MS9	Localization patterns of bacterial surface proteins and their interaction partners on host cells demonstrated	WP5, WP6	2 - KI	48	Demonstration of the l ocalization patterns of bacterial surface proteins and their interaction partners on host cells. Will be verified via a presentation of scientific data on international conference

1.3.5. WT5 Critical Implementation risks and mitigation actions

Risk number	Description of risk	WP Number	Proposed risk-mitigation measures
1	NIR-MINFLUX: best NIR dye shows significant background due to residual fluorescence from switched off fluorophores (low contrast between on- and off-state) (Low)	WP2, WP5, WP6	By testing a broad range of fluorophores, specifically for good contrast between on- and off-state this risk will be minimal; as an additional measure the imaging buffer can be adjusted.
2	Correlative SRS-MINFLUX imaging: pre-bleaching of fluorophore due to SRS imaging (Low)	WP2, WP5, WP6	Perform SRS imaging after MINFLUX imaging, alternatively use fluorophore that does not show spectral absorption at SRS wavelengths for MINFLUX imaging, or select laser beam wavelengths for SRS to avoid the absorption band of the fluorophores.
3	Two photon activation for MINFLUX imaging: two photon process does not activate fluorophore or leads to bleaching (Medium)	WP1, WP2, WP5	Specific screening of NIR fluorophores with respect to both MINFLUX and two photon activation suitability. Alternatively, if no activation, use another laser and a one-photon activation process.
4	SPAD array: long design and manufacturing cycles of 1 year (Medium)	WP1, WP2, WP3, WP5, WP6	Store chips without finished metallization for potential changes (metal fix procedure).
5	Laser: nonlinear interaction scheme for fast tuning is not working (Low)	WP2, WP4, WP5	Three different tuning schemes will be evaluated.
6	Laser: conversion to femtosecond pulses leads to longer pulses than expected / femtosecond pulse generation leads to lower than expected power (Medium to High)	WP2, WP4, WP5	Use of alternative optical components for compression scheme; use of higher power pump lasers.
7	Association of nanometer scale protein distribution patterns of pneumococcal surface proteins for pneumococcal disease cannot be verified (Low)	WP6	Several different proteins and phenomena will be studied, and it is unlikely that all hypotheses would miss out. If we do not find a correlation, the data will be important anyway since it will give us information on how these proteins are localized and will give us new hypotheses that need to be tested.
8	Completion of next generation MINFLUX acquisition electronics delayed significantly due to technical difficulties or manufacturing delays (Low to Medium)	WP1, WP3	Extend modifications to first generation electronics and use Gen I PII detection electronics to allow WPs 2, 5 and 6 to proceed independently.
9	A WP leader leaves the NanoVIB (Low)	WP1, WP2, WP3, WP4, WP5, WP6, WP7	Another supervisor within the partner organisation concerned, or within the consortium as a 2nd option, will be appointed.

1.3.6. WT6 Summary of project effort in person-months

	WP1	WP2	WP3	WP4	WP5	WP6	WP7	WP8	Total Person/Months per Participant
1 - KTH	4	14	1	1	72	16	20	✓	128
2 - KI	0	3	0	0	9	70	2		84
3 - AI	64	10	4	0	2	1	2		83
4 - LLG	9	49	2	2	11	6	2		81
5 - APE	2	3	0	54	1	0	2		62
6 - PII	2	2	36	0	0	0	2		42
Total Person/Months	81	81	43	57	95	93	30		480

1.3.7. WT7 Tentative schedule of project reviews

Review number [19]	Tentative timing	Planned venue of review	Comments, if any
RV1	20	to be confrim	interim review
RV2	38	to be confirm	interim review
RV3	48	To Be confirm	final review

1. Project number

The project number has been assigned by the Commission as the unique identifier for your project. It cannot be changed. The project number **should appear on each page of the grant agreement preparation documents (part A and part B)** to prevent errors during its handling.

2. Project acronym

Use the project acronym as given in the submitted proposal. It can generally not be changed. The same acronym **should appear on each page of the grant agreement preparation documents (part A and part B)** to prevent errors during its handling.

3. Project title

Use the title (preferably no longer than 200 characters) as indicated in the submitted proposal. Minor corrections are possible if agreed during the preparation of the grant agreement.

4. Starting date

Unless a specific (fixed) starting date is duly justified and agreed upon during the preparation of the Grant Agreement, the project will start on the first day of the month following the entry into force of the Grant Agreement (NB : entry into force = signature by the Commission). Please note that if a fixed starting date is used, you will be required to provide a written justification.

5. Duration

Insert the duration of the project in full months.

6. Call (part) identifier

The Call (part) identifier is the reference number given in the call or part of the call you were addressing, as indicated in the publication of the call in the Official Journal of the European Union. You have to use the identifier given by the Commission in the letter inviting to prepare the grant agreement.

7. Abstract

8. Project Entry Month

The month at which the participant joined the consortium, month 1 marking the start date of the project, and all other start dates being relative to this start date.

9. Work Package number

Work package number: WP1, WP2, WP3, ..., WPn

10. Lead beneficiary

This must be one of the beneficiaries in the grant (not a third party) - Number of the beneficiary leading the work in this work package

11. Person-months per work package

The total number of person-months allocated to each work package.

12. Start month

Relative start date for the work in the specific work packages, month 1 marking the start date of the project, and all other start dates being relative to this start date.

13. End month

Relative end date, month 1 marking the start date of the project, and all end dates being relative to this start date.

14. Deliverable number

Deliverable numbers: D1 - Dn

15. Type

Please indicate the type of the deliverable using one of the following codes:

R	Document, report
DEM	Demonstrator, pilot, prototype
DEC	Websites, patent fillings, videos, etc.
OTHER	
ETHICS	Ethics requirement
ORDP	Open Research Data Pilot
DATA	data sets, microdata, etc.

16. Dissemination level

Please indicate the dissemination level using one of the following codes:

PU	Public
CO	Confidential, only for members of the consortium (including the Commission Services)
EU-RES	Classified Information: RESTREINT UE (Commission Decision 2005/444/EC)
EU-CON	Classified Information: CONFIDENTIEL UE (Commission Decision 2005/444/EC)
EU-SEC	Classified Information: SECRET UE (Commission Decision 2005/444/EC)

17. Delivery date for Deliverable

Month in which the deliverables will be available, month 1 marking the start date of the project, and all delivery dates being relative to this start date.

18. Milestone number

Milestone number:MS1, MS2, ..., MSn

19. Review number

Review number: RV1, RV2, ..., RVn

20. Installation Number

Number progressively the installations of a same infrastructure. An installation is a part of an infrastructure that could be used independently from the rest.

21. Installation country

Code of the country where the installation is located or IO if the access provider (the beneficiary or linked third party) is an international organization, an ERIC or a similar legal entity.

22. Type of access

TA-uc	if trans-national access with access costs declared on the basis of unit cost,
TA-ac	if trans-national access with access costs declared as actual costs, and
TA-cb	if trans-national access with access costs declared as a combination of actual costs and costs on the basis of unit cost,
VA-uc	if virtual access with access costs declared on the basis of unit cost,
VA-ac	if virtual access with access costs declared as actual costs, and
VA-cb	if virtual access with access costs declared as a combination of actual costs and costs on the basis of unit cost.

23. Access costs

Cost of the access provided under the project. For virtual access fill only the second column. For trans-national access fill one of the two columns or both according to the way access costs are declared. Trans-national access costs on the basis of unit cost will result from the unit cost by the quantity of access to be provided.

History of changes

Version	Date	Changes	Page
1.0	13/10/2020	Clerical corrections were done to increase the readability of the text and avoid any misunderstandings.	44, 52, 58, 59, 60, 72, 73, 74, 77,
		Communications requirements from the EC were added.	46
		WP 8 added to the work plan.	49, 62
		Two platforms' locations were clarified.	51
		Reporting periods of the project were updated according to the EC's preference.	72
		The frequency of Project Management Committee meetings were updated.	72
		The details of consortium-wide meetings were clarified.	74
		Ethics requirements from the EC were added.	89
2.0	04/11/2020	Clarification that the equipment listed in table 3.4b will be declared as (full) direct costs for construction of the prototypes	74-76, Table 3.4b

Contents

1. Excellence

1.1 Objectives

Not without reason, fluorescence is the by far most widely used modality for cellular imaging, offering a unique combination of spatial and temporal resolution, sensitivity and specificity. Following the remarkable development of fluorescence-based super-resolution microscopy techniques in the last decade, now MINFLUX, a next generation super-resolution concept, has recently been demonstrated, offering yet another order of magnitude higher spatial resolution.

In a coherent and interdisciplinary project, we will prototype a next-generation super-resolution microscope system based on this concept, capable to reveal intricate, detailed molecular mechanisms underlying inter- and intracellular processes and disease. As a lead application, we will demonstrate its unique capabilities to understand virulence and invasiveness of pathogenic bacteria, more precisely pneumococci. By concerted development of laser and detector technologies, microscopy devices and image acquisition procedures, we will be able to retrieve information, which is not within reach by any other microscopic or photonics-based technique. We will demonstrate how cellular nanoscale protein localization patterns can be resolved, which will not only help us revealing mechanisms of bacterial disease, but which also are likely to be of large relevance for many other diseases. Thus, the microscope system prototype will prove its broad applicability in biomedical research, as a tool to understand intra- and intercellular processes and the cellular origin of diseases.

The socio-economic and societal health relevance of our lead application is huge. In general, bacterial infections and the emerging antibiotic resistance pose major threats to mankind. *Streptococcus pneumoniae* is one of the major contributors to morbidity and mortality due to infectious diseases worldwide, causing millions of deaths annually. Pneumococci are the major cause of otitis media, sinusitis, and community-acquired pneumonia, but also a major cause of sepsis and meningitis (invasive pneumococcal disease, IPD). More than 50.000 individuals in Europe, and about 1,6 million individuals in the world, acquire IPD annually. Pneumococcal meningitis is a severe disease with a mortality of about 30%, and with high risk for sequelae. Despite that pneumococci can be devastating pathogens, carriage studies have shown that as many as up to 60% of healthy children in some European countries may harbor pneumococci in their noses.

What makes some bacteria virulent and invasive and others not, is largely attributed to detailed localization patterns of specific bacterial surface proteins, and their intricate interactions with immune and host target cells. However, there are currently no techniques at hand, which can resolve these patterns with high enough resolution.

The overall objectives of this project are technological, but are mainly driven by biomedical needs:

On the biomedical side, the overall need is to significantly increase our understanding of diseases, on a cellular, as well as sub- and intra-cellular level. In this context, research on the inter- and intra-cellular processes behind pneumococcal virulence and invasiveness serves as

a lead application. However, the knowledge gained will also form a basis for pathogenesis studies of other microbial pathogens as well as other diseases. To reach this goal, we will develop a MINFLUX-based prototype, as a next-generation super-resolution imaging system, to localize bacterial surface proteins implicated in pneumococcal virulence and invasiveness, with a ten-fold higher resolution compared to current state-of-the-art super-resolution microscopes. To fully discern the role of the proteins and their spatial distribution patterns, we will also resolve their interactions with different host cells and their proteins, and develop imaging procedures for simultaneous stimulated Raman scattering (SRS) and two photon excitation (TPE) imaging, which allows to place the protein patterns in a cell morphological and micro-chemical context, and to follow the metabolic states of the cells. The aim is to get a better understanding of mechanisms that influence how pneumococci cause disease. Data gained in this project will provide a basis for novel diagnostic, preventive, and therapeutic approaches to curb the extensive morbidity and mortality caused by these bacteria.

On the technology side, the overall objective is the successful development of a prototype of such a combined SRS/TPE and MINFLUX imaging system. More generally, nanoscale protein localization patterns on cells are likely to be of large relevance for many diseases, including microbial diseases and cancer development. The aim of this project is therefore that the prototype to be developed is suitable for a broad range of biomedical applications, and as a means to do this we will extend the spectral range of the microscope system into the near infrared (NIR), for the benefits of a lower background and higher penetration depths than attainable in biological samples. The aim is a truly disruptive photonics technology, allowing cellular protein localization patterns to be resolved, allowing the disentanglement of intra- and inter-cellular processes underlying a broad range of diseases. The successful development of laser and single-photon detector technologies, and the demonstration of their integration into the prototype of a combined SRS and super-resolution imaging system with unparalleled resolution, will undoubtedly strengthen the market position of the partner companies, and Europe's position on the market for microscopes, lasers and detectors as a whole.

The detailed objectives of the proposal are:

I. To construct prototypes of a next-generation fluorescence super-resolution microscopy platform for biomedical research and development, offering one order of magnitude higher spatial resolution than current state-of-the-art super-resolution microscopes.

II. To broaden the wavelength range of super-resolution microscopy into the near-infrared (NIR), for improved multiplexing, reduced background and better sample penetration.

III. To develop single-photon avalanche detector (SPAD) arrays, with >10 detectors, with at least equal performance to individual state-of-the-art SPADs and with enhanced sensitivity in the NIR.

IV. To develop a pulsed, narrow-linewidth, multi-line laser for SRS, which is quickly wavelength tunable and with rapid pulse length switching from ps to fs for TPE operation

V. To integrate the developed lasers and SPAD arrays into the prototypes of the super-resolution microscopy platform, for faster image acquisition, lower background, and with demonstration of combined SRS/TPE imaging.

VI. To demonstrate that the prototypes of objective V can resolve nanometer scale localization patterns of specific proteins in bacteria and host cells, provide overlaid morphological and chemical images of the bacteria, and investigate correlations of these patterns with virulence and invasiveness of the bacteria.

VII. To make the prototypes of lasers, SPAD arrays and microscope platforms developed in the project attractive for researchers and stakeholders outside of the project.

VIII. To provide key information regarding specific pneumococcal surface proteins and their spatial distribution patterns correlated to biological relevance and disease outcome, leading to a better understanding of why some pneumococcal strains are more prone to cause IPD than other strains, in turn taking a decisive step towards better diagnostics, effective treatments and prevention of IPD.

1.2 Relation to the work programme

This project aims to develop a next generation super-resolution imaging system for studies of molecular mechanisms underlying the cellular origin of diseases. This proposal addresses the call “Disruptive photonics technologies” (ICT-36-2020), and the work proposed will be disruptive in several aspects.

First, the imaging system to be developed will be based on the MINFLUX principle, offering an order of magnitude higher resolution than any other super-resolution microscopy technique available today. For most bacteria with a virulent potential, increasing evidence suggests that certain surface proteins critically influence to what extent the bacteria are virulent and invasive and to what extent they can evade the immune defense of the host and cause disease. Critical is not only the amount of certain proteins present on the bacteria, but in particular the precise, nanometer-scale, localization of these proteins on the bacteria. With the next generation super-resolution imaging system to be developed in this project, we will be able to determine the localization patterns of fluorescently marked bacterial surface proteins down to such length scales, far beyond what is possible with current state-of-the-art super-resolution microscopy techniques.

Second, by extending the wavelength range of super-resolution imaging into the NIR and by enabling SRS/TPE imaging in parallel to super-resolution imaging, we will allow multiple specific proteins to be precisely localized on bacteria and host cells, and their (co)localizations to be overlaid on images of the cellular morphology and the local chemical environment. In general, functions of proteins strongly depend on their local environment, and their interaction with other proteins. The other way around, how specific bacterial surface proteins localize or co-localize on the nano-scale level influences how they can exert harmful

and inflammatory effects on host cells, changing local environmental and metabolic conditions in these cells. The hitherto unparalleled ability aimed for in this project to resolve the nano-scale distribution patterns of several specific proteins in parallel, and to correlate them to local environmental parameters on the bacteria or in infected host cells, can thus generate a major leap in our understanding of the mechanisms behind bacterial virulence, invasiveness, and pathogenesis.

Indeed, the super-resolution technology to be developed in the project has the potential to revolutionize bacterial diagnostics and to spur the development of new treatment and vaccination strategies. This will create a completely new market for super-resolution microscopy systems. Knowledge of the disease mechanisms reflected in the nano-scale distribution patterns of specific bacterial surface proteins, can likely guide the design of new treatment schemes and strategies for vaccinations. Similarly, being able to distinguish different bacterial mutants/clones from each other by their detailed surface protein distribution patterns can open up for new diagnostic approaches. In the project, we will also for this reason explore how to scale down and simplify our imaging approach, opening for the additional use of the technology in clinical diagnostics and drug development.

In the project, we will develop prototypes of new detector-array and laser technologies, which in turn will be implemented into the super-resolution microscope prototype. The single-photon detector arrays will have an increased sensitivity in the NIR and will allow significantly increased image acquisition speeds. To take full benefit of the new array detector technology, real time data handling will also be addressed in the project. Operation of the microscope in the NIR will allow reduced background, increased penetration depths, and will broaden the spectral detection window allowing for increased multiplexing with less spectral crosstalk and thus higher specificity. Implementation of new laser technology into the prototype of the microscope system will, for the first time, demonstrate correlative imaging of nano-scale protein patterns with morphological and chemical SRS images, as well as TPE images of auto fluorescent co-enzymes reflecting cellular metabolic states. This will decisively contribute to an increased specificity in the assessment of cellular originated diseases, which will be done in collaboration with medical doctors and their research laboratories. Apart from identifying an important application of the developed laser and detector array technologies in the super-resolution technology to be developed, these laser and detector technologies will also by their own merits be of interest to many other microscopy and biophotonics based applications.

Finally, from a biomedical application point of view, it is important to point out that the way specific proteins distribute themselves on or inside cells is not only of relevance for bacterial infections. For instance, the different mechanical, proliferative or adhesive properties of cancer cells can be reflected in the spatial distribution patterns of e.g. membrane, cytoskeletal and cell cycle regulating proteins, which are difficult to resolve by state-of-the-art fluorescence microscopy methods (see e.g. Blom and Widengren[1] for a review). Taking full

[1] Blom H and Widengren J "Stimulated Emission Depletion Microscopy" *Chem. Rev.* 117(11), 7377-7427, 2017

benefit of the diagnostic information contained in such protein localization patterns in cancer cells has the potential to bring about a paradigm shift in cancer diagnostics. The decisive demonstration of resolution improvement with the super-resolution microscope prototype to be developed in this project, together with the demonstration that the correlation of nano-scale protein distribution patterns with metabolic, morphological and environmental parameters in the cells is feasible, is thus likely to open up new possibilities in cellular biology and disease diagnostics in general. It can thus be further iterated, as one of the cross-cutting priorities of the call addressed by this proposal, that the socio-economic and societal health-related impact of such progress would indeed be tremendous.

With respect to the other two cross-cutting priorities of this call, photonics and public-private-partnerships (PPPs), we'd like to point out that the aims of our proposal, as well as the constellation of the consortium (itself in a true sense a PPP), are very well in line with the major objectives expressed in the roadmap for the European PPP Photonics21[2]: It is an application-oriented and market-needs-driven project, with effective translation of a disruptive technology (MINFLUX) offering breakthrough advances in nanophotonics. It brings this technology into prototypes and demonstrates photonics solutions based on this technology to a true key societal challenge (to curb the devastating effects of pneumococcal and other bacterial diseases). It provides new ways to detect, possibly treat and even prevent a major disease (IPD), improving patient survivability and drastically reducing care. It drives the technological development and innovation in a strategic application area, where Europe is strong; biophotonics for medical and biomedical applications. It encompasses a broad cooperation across the whole value chain, also including end-users. This project will be coordinated by KTH in Stockholm, a member of PhotonicSweden, a national technology platform closely linked to Photonics21, and we will advertise our activities via this platform, via their workshops and conferences, as well as via corresponding organizations on a European level.

1.3 Concept and methodology

(a) Concept

This is a highly interdisciplinary project, covering areas from fluorescence and vibrational spectroscopy/imaging, physical and organic chemistry, optics, photonics, semiconductor physics, computer algorithm development and data handling/processing over to clinical bacteriology, molecular microbiology and pathogenesis. As a consequence of the interdisciplinary width of the project and its application-driven character, the main ideas, models and assumptions involved emanate from both biomedical/bacteriological, as well as photonics and microscopy technology related starting points. Naturally, the ideas and assumptions for these two starting points are mutually influencing each other: With world-leading expertise in clinical bacteriology, we can formulate the starting point from the biomedical side, from the societal needs and current bottle-necks in bacteriology research. At

[2] https://www.photonics21.org/download/about-us/structure/workgroups/photonics_roadmap.pdf?m=1513613877&

the same time, with likewise world-leading competence in next generation super-resolution and related biophotonic technology we can clearly identify where there is potential for the necessary further development. We can then focus our efforts on the identified specific features of these technologies, where we see that a further development would make a significant difference in biomedical applications. Likewise, from the biomedical side, we can subsequently direct our focus towards major bottle-necks and knowledge gaps where these newly developed devices have the potential to make a significant difference. From the lead application in bacteriology, we can then also extrapolate the developed microscope technology and procedures to be used on a much broader scale for cellular research and diagnostics.

From the biomedical/bacteriological side, the project thus takes as a starting point that the virulence and invasiveness of bacteria in general strongly depend on the properties of certain proteins on their surface. Of particular importance is how these proteins distribute themselves on the surface, and how their localization patterns are related to e.g. the cell cycle and local environment of the proteins. Currently used technologies in microbiology have proven insufficient to resolve these issues. The project aims to overcome these methodological barriers and is based on the idea that if we can resolve these aspects, we can also significantly increase our understanding of the intra- and inter-cellular processes underlying bacterial virulence and invasiveness.

While bacterial surface proteins and their localization patterns are likely key parameters of the virulence and invasiveness of a range of different bacteria, we will in this project focus on the major pathogen *Streptococcus pneumoniae* (pneumococci). Following a successful project, we then foresee that the developed procedures and techniques can be applied to understand the disease mechanisms also of other bacteria. *Streptococcus pneumoniae* is a major contributor to morbidity and mortality due to infectious diseases worldwide, causing millions of deaths annually. Pneumococci are the major cause of otitis media, sinusitis, and community-acquired pneumonia, but also a major cause of sepsis and meningitis (invasive pneumococcal disease, IPD). Pneumococcal meningitis is a severe disease with 30% mortality and with a high risk for sequelae. More than 50.000 individuals in Europe, and about 1,6 million individuals globally acquire IPD annually. Risk groups for IPD include young children and the elderly, as well as individuals with underlying diseases. A prior influenza virus infection also sensitizes for a pneumococcal infection. Pneumococcal infections are treated with antibiotics, but resistance rates to common antibiotics are increasing. Pneumococcal conjugated vaccines (PCVs) have been introduced in childhood vaccination programs in most countries in Europe, and have led to a dramatic decrease of IPD in vaccinated children. But concomitantly there has been an increase of IPD caused by non-vaccine types especially in the elderly, hampering the effectiveness of the PCVs. Importantly, even though pneumococci are devastating pathogens, they also frequently colonize the nasopharynx of healthy children. Hence, in carriage studies in Europe it was found that up to 60% of children harbor pneumococci in the nose without symptoms (Portugal and the Netherlands), and in Sweden we have found carriage rates of ca 30% in children post PCV introduction. Why pneumococci usually only colonize healthy individuals, but sometimes

cause severe disease, remains to be clarified, and both bacterial and host factors contribute. Clearly, we need more knowledge on which factors, both on the bacterial and host side, contribute to disease development. Thus, in order to find better diagnostics, effective treatment and prevention (vaccines), we need to better understand why some pneumococcal strains are more prone to cause IPD than other strains, the interplay between the bacteria and the host, and which underlying mechanisms lead to disease development.

Bacterial surface proteins: The answers to what makes some bacteria virulent and invasive and others not, are largely to be found in the detailed display and dynamics of bacterial surface proteins, and their intricate interactions with immune and host target cells. In pneumococci several bacterial factors have been identified as virulence factors, such as

- the surface protein PspC, that interacts with factor H on host cells, an important negative regulator of the immune response (the alternative complement pathway),
- pili proteins, that build pili protruding from the bacterial surface, and are important for adhesion to host cells and carriage,
- the cytotoxin pneumolysin, suggested to be intracellular but recently indicated also to be surface exposed, and which can kill host cells,
- the autolysin amidase LytA, which can cleave the peptidoglycan cell wall of bacteria and is activated upon penicillin treatment.

In recent work by the research groups of partner 1 (KTH) and 2 (KI) it was demonstrated that by super-resolution STED microscopy it is possible to resolve specific spatial distribution patterns of bacterial surface proteins, and that important information about underlying mechanisms for disease and invasiveness of the bacteria could be revealed from these patterns. [3,4] As an example, the pneumococcal surface proteins PspC1 and PspC2 were studied with STED and found to localize differently (Fig. 1.3a), thereby rendering these proteins different immune-protective functions, something which would not have been possible to resolve with state-of-the-art confocal microscopy. However, while demonstrating the importance of localization patterns of specific proteins, for the virulence, invasiveness, as well as for the ability of the bacteria to evade immune responses of the host, these studies also clearly indicate that with another ten-fold increase in resolution (as previously between confocal and STED imaging) an additional major leap in the understanding of these mechanisms can be anticipated. This additional resolution increase, coming even closer to the actual spatial scale of the protein interactions, is going to be demonstrated, and exploited for bacterial studies in the NanoVIB project. This will allow us to study pneumococcal surface proteins and their localization and interactions with host factors and cells at a new level of detail, and relate this to their function and disease-causing capabilities:

[3] Pathak A et al "Factor H binding proteins protect division septa on encapsulated Streptococcus pneumoniae against complement C3b deposition and amplification" *Nature Comm.* 9, 3398, 2018

[4] Iovino F et al "pIgR and PECAM-1 bind to pneumococcal adhesins RrgA and PspC mediating bacterial brain invasion" *J. Exp. Med.*, 214(6), 1619-1630, 2017

On bacteria in different stages of division, we will study how different variants of the surface protein PspC distribute on the bacterial surfaces, to what extent they bind factor H, and if and how this can be affected by additives having potential antibiotic or bacteriostatic effects. The autolysin LytA will be studied in a similar way, and from how it distributes on and between bacteria, we aim to better understand the function of this protein, which still remains controversial.

In biopsies from mice and from humans who died of pneumococcal meningitis (available via external collaboration with clinical researchers in the Netherlands), we will perform co-localization studies between pili proteins (RrgA, RrgB and RrgC) and receptor proteins on endothelial cells being part of the blood-brain barrier (BBB), to elucidate how these proteins bind and mediate invasion into the brain, and how the binding may be inhibited by antibodies competing for binding to the same sites as the pili proteins.

Nano-scale protein patterns correlated to cellular morphology, environment and metabolism: To take full benefit of the information contained in the imaged nano-scale localization patterns of the proteins above, we aim in this project also to put them in their cellular context, by monitoring their morphological, chemical and metabolic microenvironment in parallel. For this purpose, we will implement two imaging modalities. First, we will explore the possibility to correlate the nano-scale distribution patterns of specific bacterial proteins with the inflammatory response of host cells, as monitored by label-free two photon excitation (TPE) imaging of auto fluorescent NAD(P)H and flavins in the cells (described below). Second, we will use the chemical selectivity of stimulated Raman scattering (SRS) imaging[5], and then correlate the protein localization patterns to cytoskeletal structures as well as to lipid membranes, and even to specific lipid species in the membranes. [6] In this way, morphological images, e.g. from biopsies and cells as mentioned above, can be overlaid onto the protein distribution patterns, can put these patterns into context, and thereby enhance the biological significance of these patterns.

[5] Prince RC et al "Stimulated Raman Scattering: From Bulk to Nano" *Chem. Rev.* 117, 5070-5094, 2017

[6] Cao C et al "Label-free digital quantification of lipid droplets in single cells by stimulated Raman microscopy on a microfluidic platform" *Anal. Chem.* 88, 4931-4939, 2016

Bacterial exosomes, their possible role in disease and as a basis for vaccines: Exosomes are produced from the plasma-membrane of most eukaryotic cells and are currently intensively studied for practical use in medicine. However, also bacteria produce membrane vesicles. Most studies this far have been focused on outer membrane vesicles (OMVs) isolated from Gram-negative bacteria. Such OMVs contain most components that are expressed in the outer membrane, including endotoxin (lipopolysaccharide), and a cargo that primarily includes constituents of the periplasmic space. OMVs have attracted much interest in the vaccine industry as they may induce protective immune responses and are currently being used as antigens in a vaccine to prevent invasive meningococcal infections. Gram-positive bacteria such as pneumococci lack an outer membrane and have a thick peptidoglycan cell wall often decorated by capsular polysaccharides outside its plasma membrane. Therefore, formation of vesicles from Gram-positive bacteria was for long not expected. However, it was recently discovered that also Gram-positive bacteria indeed form extracellular vesicles. They are likely derived from the plasma membrane and seem similar to eukaryotic exosomes. Partner 2 (KI) has shown that membrane vesicles released by pneumococci can be internalized into host cells and potentially deliver the cytotoxin pneumolysin into the cells.[7] This will in turn affect their survival and lead to cytokine induction. Importantly, preliminary unpublished data from partner KI also suggest that immunization with membrane vesicles from pneumococci shows serotype-independent protection in mice. KI has profound expertise in microbial pathogenesis, focusing on Gram-positive bacteria, and has purified and characterized pneumococcal vesicles.[7] However, further studies are needed to understand how they are formed, as well as to understand the role of pneumococcal vesicles in disease development, and as potential candidates for new vaccines.

Using mass spectrometry, we know that these vesicles carry many pneumococcal surface proteins. However, we do not know whether these surface proteins are inside or on the membrane surface, how the proteins are distributed within and among the vesicles, and how

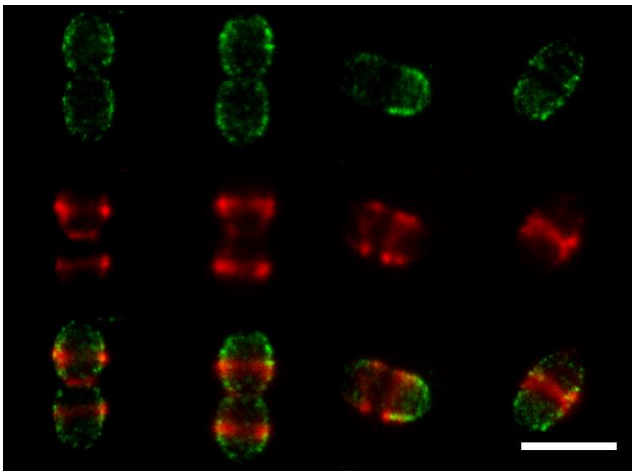

Figure 1.3a: Super-resolution STED microscopy images of different pneumococci in different growth phases, showing the distribution patterns for two variants of PspC, PspC1 (green) and PspC2 (red), during cell division and growth. Scale bar: 1 micrometer (from Pathak et al.[3]).

[7] Codemo M et al "Immuno-modulatory effects of pneumococcal extracellular vesicles on cellular and humoral host defences." *MBIO* 9(2), e00559-18, 2018

this may depend on which strain they come from and on the local lipid composition in the vesicles. Furthermore, we need more knowledge on mechanisms for how vesicles interact with host cells and consequences of such interactions.

In this project, we will make use of the ability to resolve the localizations of specific proteins by nano-meter scale resolution to address these questions. We will also overlay the protein localizations onto SRS images showing morphological cytoskeleton and lipid membrane maps of bacteria, host cells and vesicles, as a way to capture the role of the proteins in vesicle formation and in the subsequent vesicle interactions. By use of deuterium and other isotope labeling, we will use SRS imaging to track the lipogenesis from e.g. deuterated glucose added to the bacteria, and this will be specifically exploited to follow the generation and fate of membrane vesicles from specific bacteria.

When membrane vesicles are released by pneumococci and then internalized into host cells, they may deliver the cytotoxin pneumolysin into the host cells. This can result in metabolic activity changes and inflammatory reactions in the host cells. This is also a tightly coupled outcome to bacterial infections in general. To study this further, we will monitor the metabolic and inflammatory status of host cells by use of TPE transient state (TRAST) imaging of auto fluorescent co-enzymes.[8] Applying TPE TRAST in parallel with next generation super-resolution imaging will allow us to correlate local redox status and oxygenation in the cells to nano-scale localization patters of central bacterial and host cell proteins, as outlined above. We will study such correlations for bacteria-host cell interactions, adding whole bacteria, pneumococcal vesicles, or specific bacterial antigens (e.g. pneumolysin). Given that the inflammatory response of the host is a major component in the defense as well as a major reason for the detrimental effects of bacterial infections, it will be highly relevant to investigate these correlations.

Pneumococcal-viral-coinfection: A major risk factor for development of IPD is preceding infections with influenza A virus (IAV), and a significant part of the disease burden attributed to IAV is the result of superinfections with bacteria. Thus, during influenza pandemics, such as the Spanish flu during 1918 –1919, many of those that died, died due to superinfections with bacteria, in particular pneumococci. Why influenza A virus infections sensitize for pneumococcal infections is largely unknown. Our recent unpublished findings in mice suggest that influenza infections lead to capillary leakage and a nutrient rich milieu in the lower respiratory tract, promoting pneumococcal growth in the lower airways and pneumonia. Supply of antioxidants via this leakage to the lower airways was found to sustain bacterial growth, but pneumococcal adaptation to this oxidative environment also seems to entail induction of surface proteins, in particular the pneumococcal protease HtrA. By using the next generation super-resolution microscopy of this proposal, we envision that we can unravel in detail the distribution patterns of bacterial surface and membrane bound proteins including HtrA, how such patterns correlate with the microenvironment of the host, the presence of antioxidants, and the ability of the bacteria to evade immunological attack by

[8] Tornmalm J et al "Local redox conditions in cells imaged via non-fluorescent transient states of NAD(P)H" *Sci. Rep*. 9:15070, 2019

imaging the degree of complement deposition on their surface. In this way, we expect to unravel important metabolic aspects in bacterial-viral co-infections, which seem to be a major driving force in such infections, and thereby also find better strategies to curb these infections.

From the photonics and microscopy technology side, this project will develop and implement a next generation super-resolution imaging system, to meet the specific needs for understanding the cellular origin of diseases, e.g. bacterial infections as lead application. This project will also drive the development of laser and SPAD array technologies to meet these needs, which are then implemented into the imaging system:

The next generation super-resolution concept on which the technology development in this project is based uses fluorescence as the main readout modality, by far the most widely used modality for cellular imaging, in biomedical research, drug development and diagnostics. This development builds further on the remarkable development of fluorescence-based imaging technology in the last decade, allowing detection and characterization of single molecules, and offering a resolution far beyond the diffraction resolution limit (Nobel Prize in 2014)[9]. It also builds further on two previous EU projects (described further below), where we have successfully developed concepts and procedures for super-resolution imaging and their application for biomedical research, diagnostics and disease monitoring. In particular however, the development in this project takes as a starting point the very recent demonstration of a next generation super-resolution microscopy (SRM) concept launched by the Nobel prize winner Stefan W. Hell and his research group, called MINFLUX (Fig. 1.3b).

MINFLUX enables imaging of specific proteins or even epitopes within proteins with a resolution of up to 1-3 nm, in 3D, over large fields-of-view (FOV).[10] Thus, MINFLUX realizes the ultimate goal of biomedical imaging: to provide three-dimensional resolution in live cells, at length scales comparable to that of individual functional units within biological macro-molecules. In this sense, the MINFLUX concept has the potential to meet highly set demands from the biomedical field, to resolve the localization patterns of specific proteins down to the scale of nanometers, as we aim for in this project.

Apart from offering almost an order of magnitude higher spatial resolution than current fluorescence-based state-of-the-art super-resolution imaging techniques, such as stimulated emission depletion (STED) microscopy or single molecule localization based techniques using cameras, such as photo-activation light microscopy (PALM) and stochastic optical reconstruction microscopy (STORM), MINFLUX offers additional advantages from an application point of view, which we will specifically take advantage of in this project:

i) It does not require high excitation and/or depletion irradiances and is thus inherently live cell compatible and does also not rely on exceptional photo stability of the dye molecules.

[9] Ehrenberg M "Scientific background on the Nobel Prize in Chemistry 2014." *Royal Swedish Acad Sci*, 2014

[10] Gwosch KC et al "MINFLUX nanoscopy delivers 3D multicolor nanometer resolution in cells" *Nature Meth.* 17, 217-224, 2020

ii) It relies on far fewer detected photons than standard localization based super-resolution techniques and is thus not limited to the use of extraordinarily bright fluorophores.

Hence, with a resolution one order of magnitude beyond existing fluorescence based imaging techniques and with important limiting factors of other SRM techniques overcome, the MINFLUX concept is poised to open a new chapter in biomedical imaging within fixed as well as living samples:

Extend MINFLUX into the near infrared (NIR) – a hitherto unexplored spectral range in super-resolution fluorescence microscopy: In general, taking fluorescence-based cellular *microscopy* into the near-infrared (NIR) spectral range (700nm-850nm) promises several distinct advantages:

a) strongly reduced scattering of both the excitation and fluorescence light by the surrounding tissue,
b) much lower absorption of the signal and significantly reduced autofluorescence from the sample,
c) lower phototoxicity,
d) deeper penetration depths,
e) provision of an additional spectral window, allowing for the simultaneous imaging over multiple, spectrally separated color channels.

Together, these advantages provide a good basis for multiplexed cellular imaging with higher specificity/lower spectral crosstalk and improved signal-to-background ratio. However, despite the potential merits, there are some bottlenecks which to-date have limited the exploitation of fluorescence-based cellular microscopy into the NIR spectral range. In particular, NIR organic fluorophores essentially all show lower fluorescence quantum yields (a few %), shorter fluorescence lifetimes (~0,5ns or less), and limited photostability. Specifically, single-molecule and super-resolution microscopy techniques critically rely on fluorophores capable to generate high numbers of detected photons per marker molecule and time. Therefore, the implementation of such techniques into the NIR range has been especially hampered. Now, since MINFLUX neither relies on the use of fluorophores with very high molecular brightness, nor on a very high photostability of the fluorophores, the use of NIR dyes is no longer excluded. Taking MINFLUX into the NIR can also give specific advantages as compared to the visible range. In particular, a minimized background level is critical for the performance of MINFLUX and can be far better achieved in the NIR.

In this project, the idea is thus to take advantage of the attractive features of NIR fluorescence in MINFLUX. While the general features of NIR dyes do not exclude them from use with MINFLUX, there is the requirement on dyes used in MINFLUX that they must be photo-switchable (Fig. 1.3b). The red-emitting cyanine dye Alexa 647 has been found to be very suitable for MINFLUX.[10] From this point of view, we will in the first place focus on the use of NIR cyanine dyes in MINFLUX, with likely similar, but red-shifted, switching properties, and characterize them photo-physically with respect to their switching properties as well as for multicolor readout approaches.

Developments in single photon detector array technology to further improve MINFLUX performance: By replacing single-photon avalanche photo diodes (APDs), the current gold standard in single-molecule detection and super-resolution imaging, with new SPAD array detector technology in MINFLUX we expect to improve the MINFLUX system significantly, to overcome current application barriers, and to further adapt the technique to meet the requirements for the bacterial studies in this project. While we aim to develop SPAD arrays offering improved quantum efficiency and dark count rates, which are comparable to or better than single detectors, we also expect the additional spatial information in the SPAD arrays to

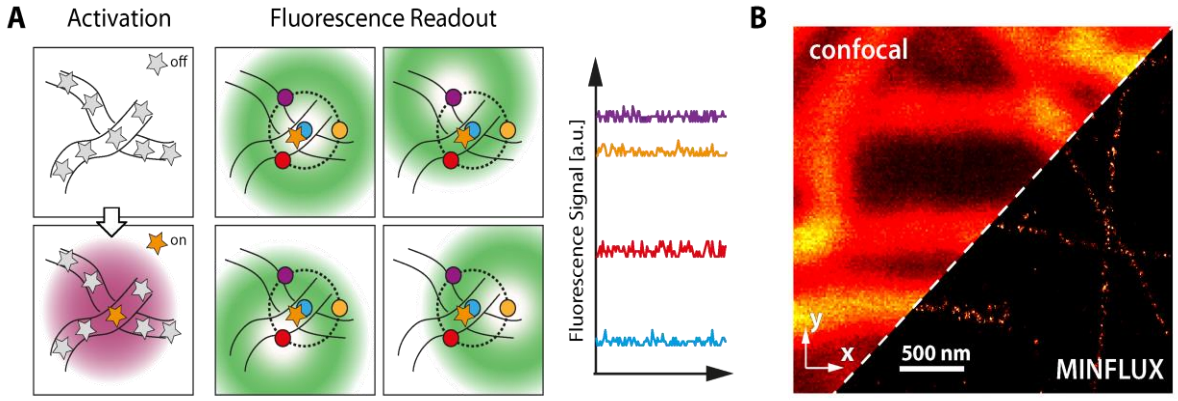

Figure 1.3b: Basic concept of MINFLUX microscopy. (A) Fluorescent molecules are sparsely driven from their non-fluorescent off-state into their on-state by a low intensity activation beam. Afterwards, fluorescence is probed with a low-intensity doughnut-shaped excitation laser. While the activated molecule resides within the center of the doughnut, no fluorescence is detected. Otherwise, fluorescence proportional to the excitation intensity is emitted. By placing the center of the excitation doughnut at four positions (cf. blue, purple, red, yellow dots) and detecting the respective fluorescence (see diagram), the molecule position can be calculated. (B) Imaging example of the vimentin network in Vero cells labelled with Alexa 647. Whereas in the confocal image the filaments look blurred, a clear separation of structures is apparent in the MINFLUX image. Measurement data are courtesy of Abberior Instruments, Germany.

give distinct advantages for the MINFLUX instrument to be developed and used for bacterial studies. The spatial information can be used to speed up MINFLUX and reduce possible artifacts caused by the complex cellular environment: Emitters ready for localization (Fig. 1.3b) can be found more rapidly because a larger area is simultaneously probed due to the larger detection area. During the localization or tracking phase, more sophisticated

background compensation algorithms can be implemented, because the area surrounding the molecule being imaged can be observed simultaneously. Such algorithms will be developed in this project, and will give us a decisive edge in less controlled, cellular environments, such as the bacteria-host cell interactions to be studied in this project The approach is not feasible with readily available sCMOS or EMCCD cameras as they lack the necessary timing information. The timing information is needed both for MINFLUX itself (it requires to correlate detection with the position of the moving donut with precision better than 100ns) and for the time gating, which is used to reject both (auto-)fluorescence not coming from short-lifetime NIR fluorophores, as well as from scattering. This requires timing resolutions of <300ps, far beyond what is possible to get with sCMOS and EMCCD cameras.

Laser technology development to combine MINFLUX with stimulated Raman scattering (SRS) and two photon excitation (TPE) microscopy: Together with the nm-localization precision patterns of specific proteins in bacteria and host cells, it is valuable to overlay these patterns onto morphological images. To do this, without adding additional fluorophore markers that would sterically or spectrally interfere with the fluorophore reporters used for MINFLUX, we will demonstrate that two modalities can be run in parallel with the MINFLUX readout: Stimulated Raman scattering (SRS) microscopy and two photon excitation transient state (TPE-TRAST) imaging of auto fluorescent co-enzymes (NAD(P)H and flavins).

SRS is currently under strong development, and morphological as well as chemical imaging of cells is now progressing towards resolutions beyond the diffraction limit (Fig. 1.3c).[5] For the purpose of combining MINFLUX with SRS microscopy, a laser system providing three pulsed, narrow linewidth emission lines, two of them precisely tunable in their emission wavelengths, will be combined with the MINFLUX prototype to be developed in the project. Such laser systems represent the key technology, which has enabled the strong development of SRS microscopy in the last few years.[5] APE (partner 5) is in the absolute forefront of the development of such lasers and has been involved in the development of light sources for Coherent Raman Imaging for 15 years by now. Coherent Raman Imaging requires two ultrafast laser pulses of different wavelength, which are overlapping in space and time at the sample site. The energy difference of these two pulses needs to be tuned to the vibrational band of the sample under investigation. APE has developed synchronously pumped picosecond optical parametric oscillators (OPOs) which overcome many obstacles connected to coherent Raman light sources at the beginning, by providing ease of use, robustness, narrow linewidth for selective excitation, no jitter between the two pulses, multi-MHz operation for fast image acquisition and shot noise limited operation necessary for stimulated Raman imaging. With its current product picoEmerald, APE is the leading light source provider for this application. Over 100 peer-reviewed articles were published using these light sources, including several Nature publications.[11,12] By further optimization of the pulse

[11] Chau YY et al "Visceral and subcutaneous fat have different origins and evidence supports a mesothelial source" *Nat. Cell Biol.*16, 367-375, 2014

characteristics and spectral tuning, and by time-gating and spectral separation of the SRS signal from the fluorescence signal, we will demonstrate that this laser system can be simultaneously used for SRS and for operations required for MINFLUX, in particular for two photon activation. This parallel use of excitation will reduce light exposure (and sample photo-bleaching/photo-toxicity) and speed up image acquisition. SRS microscopy allows us to demonstrate that nanometer-scale MINFLUX protein localization patterns can be correlated with the morphology of the cell. For more complex problems, specific isotope labels can be introduced into the cells that allow generation of chemical images with SRS. This type of correlative imaging, putting the protein localization patterns generated by MINFLUX into a morphological and local chemical/environmental context, will significantly enhance the value of the bacteria-host cell interaction studies in the project.

Moreover, the ability to tune the pulse lengths below ps, will also allow TPE of auto

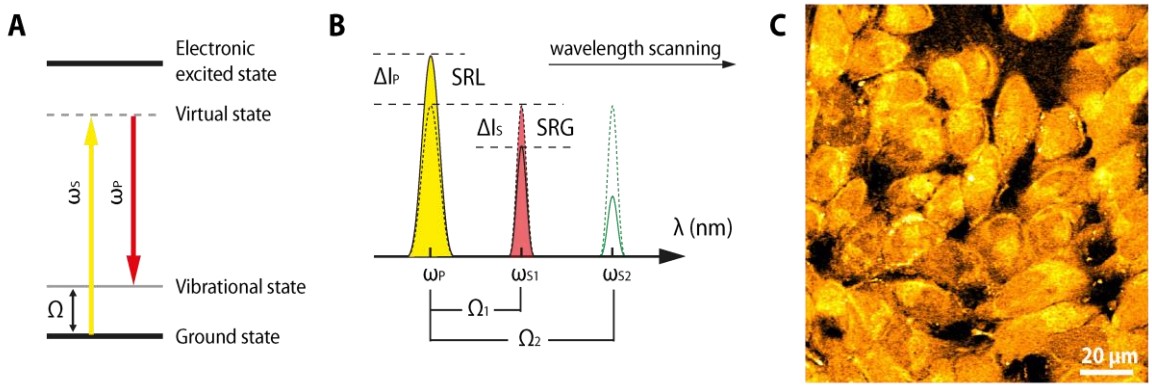

Figure 1.3c: Basic concept of stimulated Raman scattering (SRS). (A) A molecule is driven into a virtual excited state by the absorption of a photon from a pump laser with the frequency ω_p. A second, frequency shifted laser (Stokes-laser ω_S, with $\Omega = \omega_p - \omega_S$) is used to deplete this virtual state by stimulated emission. (B) Depending on the measurement scheme either the loss of the pump laser intensity (stimulated Raman loss, SRL) or the increase of the Stokes-laser intensity (stimulated Raman gain, SRG) is detected. By scanning the wavelength of the Stokes-laser, different vibrational levels can be probed. (C) SRS-imaging example of the fingerprint region at 1445cm^{-1} in living HeLa cells. The image was acquired using a modified Leica TCS SP8 microscope equipped with an APE SRS-detection set. A picoEmerald (APE) with 2ps pulses was used to pump the sample at 897nm with 50mW and deplete the excited state at 1032nm with 200mW. Data are courtesy of G. Hehl and A. Volkmer, Third Institute of Physics, University of Stuttgart.

fluorescent coenzymes (NAD(P)H, flavins). By TPE-TRAST, long-lived, dark, highly environment sensitive states of fluorescent molecules are monitored via the response in the fluorescence to spatio-temporal modulation of the excitation source.[8] These long-lived dark states of the fluorescent molecules, including triplet and photo-ionized states, are highly

[12] Hu F et al "Supermultiplexed optical imaging and barcoding with engineered polyynes" *Nat. Methods* 15, 194-200, 2018

environment sensitive, not the least to parameters strongly influenced by cellular metabolism, such as local oxygen concentrations and redox status in the cells (Fig. 1.3d). TPE-TRAST imaging thus offers the possibility to monitor cellular metabolic states, in a widely applicable manner, and can reflect cellular environmental changes that are difficult, if possible at all, to detect by regular fluorescence parameters. Implementing TPE-TRAST will open the possibility to image also metabolic and micro-environmental conditions in cells in parallel with MINFLUX.

Positioning of the project, from ideas to applications, and from lab to market

Several different positions in this respect can be found in the project, depending on what aspect of the project that is considered.

From the biomedical/bacteriological side, the activities are centred on fundamental research, with the aim to understand the underlying molecular mechanisms of pneumococcal disease, as outlined above. However, findings regarding these mechanisms can also be expected to be quite directly funnelled into efforts to develop new vaccines, antibiotics and diagnostics. The transition of results from the bacteriological side of the project into such efforts will be promoted by the fact that the clinical partner of the project (KI) already has close contacts with pharmaceutical companies and other companies directly involved in e.g. vaccine development.

Also from the biophysics/bioimaging side, much focus is on fundamental research to demonstrate the principles and use of MINFLUX as a next generation super-resolution microscopy technique to reveal nano-scale protein patterns and their role in bacterial virulence, develop and refine acquisition and analyses of nano-scale protein patterns, in a multiplexed manner, extended into the NIR, and with SRS and TPE TRAST imaging providing overlaid morphological, chemical and metabolic images. Here, the plans/ideas will be brought into first demonstrations/applications, which will be reported scientifically, and where the principles for acquisition and analyses can then be implemented by other researchers more broadly, to understand fundamental intra- and inter-cellular processes, as well as how they may be altered upon disease.

Last but not least, **from a technological point of view**, there are three SMEs as partners in the project, which all of them have concrete plans for the development of their products and for how they eventually will be taken to the market. The positioning of the project activities from their points of view are shortly described below:

Partner 3 (AI): Abberior Instruments is an innovation leader in optical nanoscopy and hold exclusive rights to the MINFLUX concept developed by Nobel Laureate Stefan W. Hell at the MPI for biophysical Chemistry. After successfully claiming a significant market share for STED microscopes, AI now intends to make MINFLUX available to the biomedical imaging community as a user-friendly imaging tool offering ten times better resolution than any other optical super-resolution technique available. This project allows AI to partner up with both component developers on one side, and researchers on the other side, to ensure timely development of this next generation imaging system and the demonstration of its potential for biomedical research and development in a ground-breaking lead application. For the

developed microscope system, we expect TRL9 and production transfer no later than one year after the end of the project.

Partner 6 (APE): Requests from users, such as fast tuning for quick spectrum acquisition and the flexibility in pulse length to do both, coherent Raman imaging as well as multiphoton fluorescence imaging, cannot be met with the current product. Within this project APE wants to solve these issues. To achieve this, new physical concepts for optical parametric oscillators and faster electronics are needed. This work starts at a technology readiness level TRL3, with first experimental results indicating the viability of this goal. Within the project we want to achieve at least TRL7 for the fast tuning of the device and TRL6 for the integration of pulse length flexibility from about 300fs to 2ps.

Partner 5 (PII): Pi Imaging Technology's Gen I SPAD array is a 23-pixel detector optimized for emission spectra around 520nm (green). It enables techniques like image scanning microscopy (ISM) to improve the spatial resolution and increasing the number of collected photons. The SPAD array detection electronics output time-tags with 10ns timing resolution. This array has been used by third parties both in research and industry and has TRL7 to TRL8. Within this project, we plan to push the TRL of the Gen I SPAD array by integrating it into a

MINFLUX microscope. We will further improve the timing resolution to 20ps to allow finer time filtering. In parallel to the further development of Gen I SPAD array detection electronics, we will develop a SPAD array with enhanced red and NIR sensitivity. Red and NIR sensitivity is important due to the lower photo-toxicity, lower background, and deeper penetration depth for optical microscopy in this wavelength range, compared to in the visible. Two arrays with the improved NIR sensitivity are planned. These arrays have a TRL of 4, and Pi Imaging Technology will improve the TRL of these arrays to 8 within the project.

Measures for public/societal engagement and use of stakeholders' knowledge

Pneumococcal infections pose major threats to society and there is an urgent need for better preventive and therapeutic options. In the project, the detailed knowledge of pneumococcal surface proteins and their role for pneumococcal virulence and invasiveness will promote both the diagnostic, preventive, and therapeutic abilities, to the benefit of societal health and wellbeing and of large interest to healthcare providers. If we can identify key bacterial proteins and their interaction partners on host cells that affect the ability to develop disease, we may find novel paths for interventions and how to combat the disease. To guide the activities in the project, we will keep a continuous dialogue with clinical bacteriology laboratories, regarding how this knowledge can come into use in their daily activities and about which aspects are likely to be of largest clinical significance. One of the partners, Birgitta Henriques-Normark, is physician and is highly connected to a clinical bacteriology laboratory in one of the biggest hospitals in Sweden (Karolinska University Hospital), as well as to the Public Health Agency of Sweden that is responsible for the national surveillance of contagious diseases in Sweden. We will also seek feedback from these laboratories on how the microscopy system to be developed in the project should be designed to allow a swift transfer and regular use in clinical bacteriology.

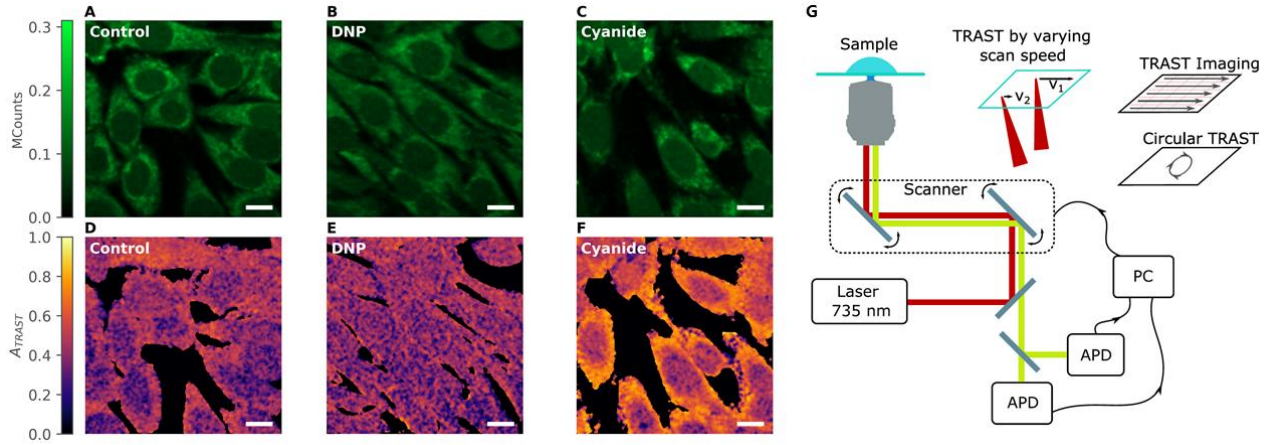

Figure 1.3d: TPE NAD(P)H autofluorescence images (A-C) and TPE-TRAST images (D-F) taken from mouse myoblast cells, subject to a mitochondrial un-coupler (dinitrophenol, DNP) (B and E), and a blocker (cyanide) (C and F). TPE-TRAST images show the photo-oxidative blinking of NAD(P)H, reflecting differences in oxidative environment, not possible to monitor via regular fluorescence parameters. (G) TPE-TRAST is performed following the NAD(P)H autofluorescence variation upon systematically varying the duration of the spotwise TPE excitation, by using different scanning speed on the sample (from Tornmalm et al.[8])

The major concept of this project is to develop a microscope system, capable to resolve nano-scale localization patterns of pneumococcal surface proteins on the bacteria, and with overlaid morphological and chemical/environmental images, thereby providing unique means to understand critical intra- and inter-cellular mechanisms of invasive pneumococcal disease (IPD). However, we expect that this concept will not only be applicable on pneumococcal disease. The same kind of information we aim to retrieve from pneumococci is likely to be critical also for the understanding of how and why other bacteria become virulent and invasive. Even more generally, highly resolved spatial distribution patterns of proteins on cells can likely provide fingerprints and suggest treatment strategies of a range of diseases. As demonstrated in our previous EU project (the FP7 project FLUODIAMON), and in research following after that project,13 super-resolution STED imaging of cancer cells or even of platelets co-incubated with cancer cells, can reveal specific protein distribution patterns, as a possible basis for cellular cancer diagnostics and treatment monitoring. In this project, by combining the 10-fold higher resolution of MINFLUX, with additional multiplexing capabilities and overlaid morphological and chemical images of the cells, we will further demonstrate the validity of this strategy and that it can be further extended. As the project progresses, we will communicate the results to researchers active in bacteriology, virology, cancer research, or with other disease categories where our strategy may find use (see section 2.2b on communication activities for more details). As we predict that the microscope system to be developed in the project will be useful for a large end-user group of biomedical researchers,, we will arrange several workshops in the second half of the project

[13] Bergstrand J et al ”Super-resolution microscopy can identify specific protein distribution patterns in platelets incubated with cancer cells” *Nanoscale* 11(20), 10023-10033, 2019

(D7.4), to inform about the capabilities and potential of the microscopy technique. In addition, we will implement a group of potential end-users for feed-back on the development (D7.3). Thereby, we will receive input from biomedical researchers, on what they judge as the critical major features and performance, so that the company partners of this project can steer the development of the microscope system, as well as their lasers and detectors, towards those critical aspects. To further benefit from the knowledge of this important group of stakeholders, we will in the project also establish one of the developed microscope systems as a test facility open to biomedical researchers (D6.1), thereby providing valuable feedback for further improvements of the microscope system by hands-on experiments by intended end-users. For the three companies in this consortium, the development of the microscope system, the SPAD arrays and the lasers in this project naturally goes hand-in-hand with the regular development and promotion of products to the market, including also feedback from present and potential customers and tuned to their needs and demands (see section 2 on impact).

Other national and international research and innovation activities linked with the project

Previous EU projects: The project will build on long-term experience from two previous EU projects. In these projects Jerker Widengren (KTH), coordinator of this proposal, and Stefan W. Hell (MPIBPC, Göttingen) cooperated, and concepts and procedures for super-resolution imaging and their application for biomedical diagnostics and disease monitoring were successfully developed. In the first of these projects (SPOTLITE, SW Hell coordinator, https://cordis.europa.eu/project/id/5049, 2005 – 2008), fundamental concepts for switching the fluorescence of fluorophore marker molecules on and off at low excitation light levels were investigated, and demonstrated in fluorescence imaging to provide useful mechanisms to break the diffraction resolution limit. Similar photo switching mechanisms will be investigated for NIR dyes in this project, as a basis for their use in MINFLUX imaging (D5.1). In the second project (FLUODIAMON, J Widengren coordinator, https://cordis.europa.eu/project/id/201837, 2008 – 2012), advanced fluorescence microscope techniques were developed for early diagnosis of breast and prostate cancer based on individual cell analyses. In particular, super-resolution STED imaging was successfully pioneered for diagnostic use. Specifically, STED imaging was demonstrated to identify spatial distribution patterns of specific proteins, not resolvable by other microscopic techniques, and was then used as a diagnostic strategy to identify cancer cells from suspect breast and prostate cancer lesions. Two years after the end of the FLUODIAMON project, which has been stated as a highly successful project by the Commission [14,15], Stefan W Hell was awarded the Nobel Prize for the invention of STED and for his seminal contributions to fluorescence-based super-resolution microscopy in general.[9]

Following the FLUODIAMON project, and in the context of several interdisciplinary projects on a national Swedish level, the Widengren research group at KTH has successfully applied

[14] https://horizon-magazine.eu/article/shedding-light-nanoworld.html,

[15] https://ec.europa.eu/programmes/horizon2020/en/news/fluorescence-adds-new-dimension-diagnosing-cancer

STED imaging and analyses of spatial distribution patterns of specific proteins in platelets to understand their role in early cancer development (collaboration with Gert Auer, Karolinska Inst, Stockholm, funded by the Swedish Cancer Foundation and the Stockholm County council).[13] Furthermore, the Widengren group has also a long-term collaboration with the group of Birgitta Henriques-Normark, KI (partner 2) in which STED imaging and analyses of spatial distribution patterns of proteins on bacteria and host cells are a central part.[3,4] This research, funded by Stockholm County council, The Swedish Research Council, and the Swedish Foundation for Strategic research, forms an important starting point and directly links further into the activities in this proposal. In the joint research of the Henriques-Normark and Widengren groups this far, spatial distribution patterns of several pneumococcal surface proteins have been resolved to a resolution of 30nm. Thereby, mechanisms have been elucidated for how pneumococci can evade attacks from the host immune system and how they can bind to host cells with possible invasive pneumococcal disease as a consequence. These mechanisms would not have been possible to resolve without the use of STED imaging, and shows the potential of these analyses. With the 10-fold higher resolution of the protein localization patterns we aim for in this project, correlated with maps of the morphology and chemical environment at the sites of the proteins, we expect to be able to take a very decisive next step towards a more complete understanding of the underlying mechanisms for pneumococcal virulence and invasiveness.

Partner 2 (LLG) has been collaborating in several projects with Stefan W. Hell's department at the Max Planck Institute for Biophysical Chemistry (MPIBPC) as well as with the company Abberior Instruments. Within the framework of the Collaborative Research Centre CRC 755 "Nanoscale Photonic Imaging" funded by the German Research Association, the LLG together with the MPIBPC has advanced STED microscopy for imaging within tissue. In two research alliances within the German Cluster of Excellence "Nanoscale Microscopy and Molecular Physiology of the Brain", the LLG and the MPIBPC collaborated on the development of new microscopy techniques and their quantitative application. Jointly with AI, the LLG has succeeded in developing a system for the correction of sample-induced aberrations in a project funded by the German Federal Ministry of Economics and Energy (see below). Currently, LLG and Abberior, a partner company of AI, are developing a computer-aided molecular design approach as well as a validation platform for live cell compatible fluorescent probes.

Partner 3 (AI) has been active in several interdisciplinary projects on a German national level. In particular, the BMBF Verbundprojekt "Dreidimensionale Lebendzell-Nanoskopie" (LiveCell3DNanoscopy), financed by the German ministry of education and research 2016 – 2019, AI together with MPIBPC, led to the development of a first MINFLUX microscope targeting early adopters and scientists who want to conduct research on the method as such.

AI currently applies for an extension of the project for an additional 36 months. The objective of this project extension will be to speed up image acquisition in MINFLUX systems by a factor of 10 to 50. To this end the synthesis of new, cell-compatible fluorophores, the re-design of the acquisition techniques and largescale application studies are envisaged. While targeted at different aspects of MINFLUX microscopy, NanoVIB and

LiveCell3DNanoscopy will complement one another and together result in an imaging system that is applicable to a wide range of cells and tissues and fluorescent dyes. Moreover, together with partner 4 (LLG), partner 3 (AI) also carried out a project financed by the Central innovation program for small and medium sized businesses of the German ministry of economic affairs and energy, run 2015 – 2017 and called "STED-Mikroskop mit aktiver Aberrationskorrektur & automatischer Justage' (perfectSTED)". This project resulted in: 1) the adaptive optics option for deep tissue imaging now part of Abberior Instruments Expert and Facility Line microscopes[16] and 2) in the automatic alignment option for Expert and Facility Line microscopes[17], which was an integral feature to make our superior STED technology available to technically less-savvy biomedical researchers using STED microscopes in a facility-like setting. The main engineers and scientists participating in this project are still employed by the LLG and Abberior Instruments GmbH and the adaptive optics alignment automation technology developed during the project was the basis for integral parts of our current 3D MINFLUX microscopes and will be instrumental for the expansion to cellular environments and especially to the correlative SRS-MINFLUX approach.

(b) Methodology

Overall methodology of the project

As outlined in the implementation part, section 3.1, this project consists of six WPs and one WP for coordination. A new super-resolution microscope system will be developed in WP1-4, then established and used for a lead pilot application and offered as an open facility (WP5-6). In the lead application (WP6), with the unique resolution and imaging capabilities of the developed microscope system, inter- and intracellular processes underlying pneumococcal virulence and invasiveness will be revealed. Together with the open facility to be established (WP6), this will trigger the interest for the developed technologies and procedures in cell biology and biomedicine, and pave the way for the dissemination, exploitation and use of the developed microscopy system. Most of the activities in the project are heading towards deliverables in the form of demonstrators, but with somewhat different purposes (Fig. 1.3e). Below, we give a brief outline of the overall methodology, the interrelationships of the overall activities in the WPs, their purposes and chronology:

This project has a very strong representation of SMEs, with three companies all having a leading role in their respective fields. Their market leading products in super-resolution microscopy (AI), tunable lasers (APE), and photon detectors (PII) will form an important starting point in the project. In WP3 and WP4, the photon detectors and the tunable lasers will be further developed to meet the demands of the new microscope system to be developed. However, the developed detectors and lasers will on their own merits be attractive for other applications in photonics and biomedicine, and thus the developed detector systems and tunable lasers in WP3 and WP4 will also serve as templates for stand-alone first market

[16] https://www.abberior-instruments.com/products/expert-line/adaptive-optics/

[17] https://www.abberior-instruments.com/products/expert-line/autoalignment/

replications. In WP1, two super-resolution MINFLUX microscope systems will be constructed, and modified to accommodate the new detector system, while optical integration, including the new tunable lasers, will be done in WP2. With feedback from the pilot applications in WP5 and WP6 for the design of image acquisition and analysis algorithms, the developed microscope platform, with the tunable laser offered separately, will also be ready as a commercially available product within at most a year upon the end of the project. Apart from the central role of LLG, the main responsible partner for the optical integration of the lasers and detectors into the microscope platform, the activities in WP1-4 by the SMEs will not only lead to demonstrators providing the prerequisites for the cellular studies in the project (WP5 and 6), but will also pave the way to products which will be commercially available no later than 12 months after the end of the project.

In WP5 and WP6, with their main activities building upon the microscope prototypes developed in WP1-4, a main goal is to demonstrate the capability of this system to resolve intra- and intercellular processes underlying pneumococcal disease. First, the prerequisites in the form of sample preparation, excitation schemes and labeling strategies will be established (WP5), after which the actual bacteriological studies will take place (WP6). The two

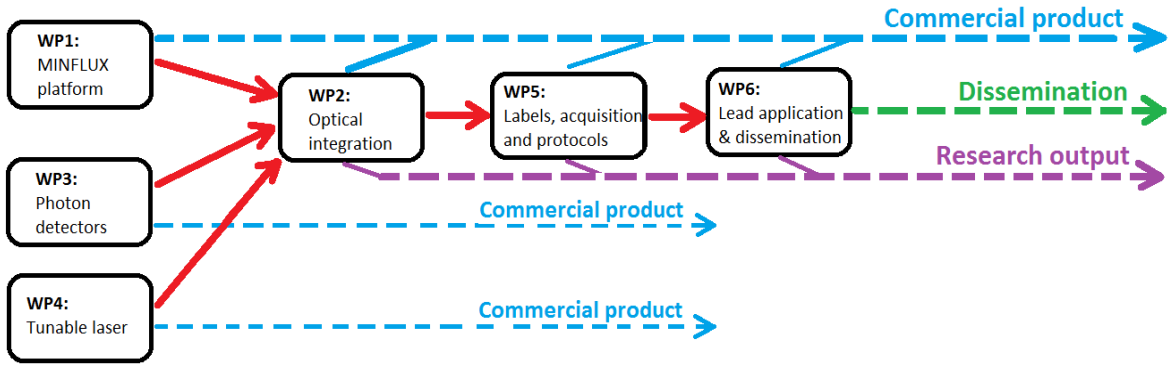

Figure 1.3e: Chart illustrating how the flow of activities in the project (illustrated by red lines) is heading towards outputs in the form of dissemination, research, and commercial products. See also section 3 and the pert chart contained therein for more details.

academic partners (KTH and KI), with very strong records in bioimaging and clinical bacteriology, will have the major roles in these WPs, and research of highest international standard is expected here. However, we expect such research as an outcome already from the optical integration (WP2) stage, shifting in subject from photonics, bioimaging over to clinical bacteriology and cell biology, within the course of the project, and towards WP6.

By generating research of highest international standard, we will also fulfill another important purpose of the activities in WP5 and WP6: to provide a lead example of the capabilities of the developed microscope system to resolve intra- and intercellular processes of large biomedical relevance. Such lead example will spur interest in the technique and its use and promote the demand of the instruments to become commercially available one year after the end of the project. As a complementary strategy for dissemination, a microscope system will also be made available as a facility, open to a broad group of researchers from academia as well as from companies, to further spur the interest in the microscope system,

lasers and detector systems developed in the project and to promote their exploitation.

Gender aspects

From the photonics and microscopy technology side of the project, we have not been able to identify any particular role played by sex and gender with regard to the technology to be developed in this project. However, although we don't see any need to adapt the technology development at this point, we will still keep a constant attention to sex and gender aspects as the development progresses in the project. The technique development in our project will take benefit from feedback given by lead users in the project, as well as from potential end-users, who will test the instrument in the open facility to be established in the project. In the feedback from these groups of users (which we will strive to make diverse), we will pay attention to any gender related aspects, such as if the instrument would not be optimally designed for any of the genders from an ergonomic point of view, if there would be any need to otherwise gender-tailor the instruments in any aspect, and if so, if such measures should better be included as early on as possible, or could be inexpensively adapted at a post-development stage.

From the biomedical/bacteriological side, one may note for some infectious diseases, e.g. in the ongoing Covid-19 pandemic, that men and women are struck somewhat differently by the infection. Pneumococcal infections, to be studied here, affect all age groups and both sexes. However, the youngest children and the elderly population are the most prone to get a severe infection. Other risk groups include immunocompromised individuals and HIV infected patients and those that have been splenectomized. Also, a prior influenza infection sensitizes for a pneumococcal infection. In some studies, it has been shown that among children the proportion of boys that get an invasive pneumococcal infection has been somewhat higher than for girls. In this project, the studies will be performed on a (sub)cellular level, where sex effects are either not relevant or difficult to analyze. Nonetheless, for the studies on cells from mice and human biopsies, we will include the sex of the mice and donors as a parameter to correlate our data against whenever possible in order to identify any existing significant differences in this aspect.

From a project management point of view, a female researcher is leading the tasks of partner KI, one of the two CEOs of APE is female and the department of Optical Nanoscopy of partner LLG, which is responsible for WP2, has a share of over 60% female scientists. In the recruitment of new personnel to the project, the selection will in the first place be based on professional qualifications, but an equal representation of women and men in project will also be a high priority. Overall, in the efforts to adequately include relevant gender aspects into this project, we will take benefit from the significant priority and competence build-up regarding these issues that has taken place at the organization of the coordinator (KTH). As an example, in all recruitment advertisements from KTH, it is explicitly stated that gender equality, diversity and zero tolerance against discrimination and harassment are a natural part of KTH's core values and considered an issue of quality. It goes without saying that the same will apply to this consortium.

1.4 Ambition

This proposal is ambitious in many ways. Below, we describe the state-of-the-art from the bacteriology/biomedical side and from different aspects of the technology development side, and the advances that we foresee in this project:

From the biology/bacteriology side, we have previously used STED super-resolution microscopy for our pathogenesis studies, and then demonstrated the ability to resolve and analyse spatial distribution patterns of specific bacterial surface proteins, and have also shown their relevance for pneumococcal virulence and invasiveness.[3] With these studies, we are indeed in the research front line in the application of super-resolution microscopy in bacteriology research. In this proposal we aim at taking the research to the next level, well above the state-of-the-art.

In this project, we will extend the capabilities of MINFLUX, a next generation super-resolution microscopy technique with ten-fold higher resolution than super-resolution STED imaging, and establish this technique for bacterial studies. Just as STED imaging, with its ten-fold higher resolution than state-of-the-art confocal microscopy, made it possible to reveal the role of specific proteins and their spatial distribution patterns on bacteria, we now foresee to be able to take another major leap in our understanding of bacteria and their virulence, by the additional ten-fold resolution increase of the MINFLUX technique. This will bring us very close to the actual interaction distance of proteins, which is needed when we aim at really understanding the role played by different proteins, their different localization patterns and their interaction sites with the host. Hence, the development of this new technique, which enables a resolution down to a few nm, will revolutionize our view of pathogen-host interactions and will give the possibility to exploit new territories previously not possible to study due to technological limitations. From a bacteriological point of view, the proposal is very ambitious, yet realistic, and will give results well above the state- of-the-art. The aim is not only a significantly increased understanding of disease mechanisms, but also that this understanding will open up new strategies for prevention (vaccines) and treatment (antibiotics). On a yet broader biomedical perspective, nano-scale spatial distribution patterns of proteins are likely to reflect and underpin a whole range of cellular processes. The ambition of the project is therefore also to establish a general approach of acquiring and analyzing such patterns on cells, which will be applicable far outside of the bacteriology field.

On the technology development side, the recent, very exciting progress in super-resolution microscopy is in the forefront of this application. The emerging state-of-the-art is represented by the MINFLUX concept, recently invented by the lab of Stefan W Hell, and then transferred to Abberior Instruments (partner 3) for launching on the market. With the ten-fold higher resolution offered by this concept compared to current state-of-the-art super-resolution microscopes available today, and with additional development potential to be addressed not the least in this project, MINFLUX indeed will be a cornerstone in the next generation of

super-resolution imaging systems. In the project, we will address several aspects, which will further enhance and extend the capacity of MINFLUX:

Extension into the NIR: Up to now (state-of-the-art), super-resolution microscopy in the NIR spectral range has been essentially excluded. A major reason for this is that fluorophores in the NIR have too low brightness and photostability for such applications. A second reason is the lower quantum yields of detectors in this spectral range. In contrast to other super-resolution microscopy techniques however, MINFLUX does not rely on the fluorophore's molecular brightness, and a limited photostability can be tolerated. Rather, a minimized background level is critical for the performance of MINFLUX and can even be better achieved in the NIR than in the visible. We thus see an opportunity to, for the first time, bring (next-generation) super-resolution microscopy into the NIR. This will bring several distinct advantages: i) strongly reduced scattering of both the excitation and fluorescence light by the surrounding tissue, ii) much lower absorption of the signal and significantly reduced sample disturbing autofluorescence, iii) lower phototoxicity, and iv) deeper penetration depths. This provides a good basis for cellular imaging with improved signal-to-background ratio and opens up an additional spectral window, allowing for the simultaneous imaging over multiple, spectrally separated, color channels. In the project, we will identify NIR fluorophores, as well as excitation and photo switching conditions appropriate for MINFLUX. We will also develop photon detector arrays with increased sensitivity in the NIR. The advances foreseen are fully realistic and will represent a major extension of the application range of super-resolution microscopy.

Increased imaging speed and lowered background: In the project, we will implement new array detector technology into MINFLUX to further overcome current application barriers. The additional spatial information of detector arrays compared to single detectors will be used to speed up MINFLUX and reduce possible artifacts caused by a complex cellular environment. Emitters ready for localization can be found more rapidly because a larger area is simultaneously probed due to the larger detection area. During the localization or tracking phase, more sophisticated background compensation algorithms can be implemented, because the area surrounding the molecule being imaged can be observed simultaneously. This will give a decisive edge in less controlled, cellular environments. The approach is not feasible with readily available state-of-the-art sCMOS or EMCCD cameras as they lack the necessary

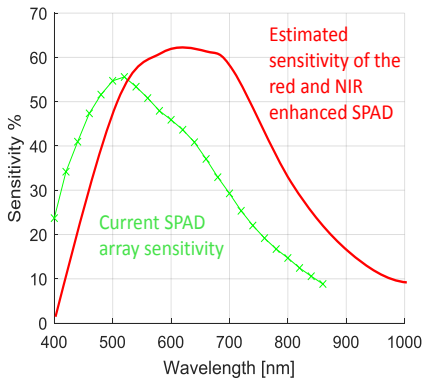

Figure 1.3f: Current (green) and estimated (red) detection quantum yields of the SPAD arrays to be developed in the NanoVIB project.

timing information, both for MINFLUX itself (requiring correlation of fluorescence photon detection with the position of the moving excitation beam with a precision better than 100ns), as well as for time gating, used to reject both (auto-)fluorescence not coming from short-lifetime NIR fluorophores and scattering.

Correlative imaging: Although MINFLUX offers multiplexed mapping of particular protein localizations and co-localizations in cells, the biological significance of this information will be significantly increased if this mapping can be put into a morphological, environmental, or microchemical context. Preferably, this should be made possible without adding additional fluorophore markers that would sterically or spectrally interfere with the fluorophore reporters used for MINFLUX. Therefore, we will demonstrate the implementation of label-free stimulated Raman scattering (SRS) imaging into the MINFLUX prototype, in order to generate overlaid vibrational images of specific lipid- or cytoskeleton-structures in the cells, or of specific metabolites. In addition, label-free two photon excitation TRAST imaging of auto fluorescent co-enzymes will be implemented for provision of metabolic state images of cells overlaid on the MINFLUX images. In this project, we will take benefit of the state-of-the art in SRS[5] and TPE TRAST imaging[8], and rather than progressing these techniques by themselves, we aim for a substantial progress beyond the state-of-the-art, by demonstrating correlative imaging with these imaging techniques together with MINFLUX.

These major lines of development of the MINFLUX technique are in the forefront of this project and form the basis for the subsequent studies on bacteria. However, there will also be significant advances beyond state-of-the-art for both the detector array and laser technologies in their own sense:

Detector arrays with enhanced red and NIR sensitivity: While the majority of scanning confocal microscopes still use point detectors, representing the state-of-the-art, there is a growing trend, not the least for novel microscopy techniques such as image scanning microscopy (ISM) and MINFLUX, to take benefit from array detectors. Currently used array detectors in microscopy are fiber-coupled PMT arrays, with SPAD arrays as an emerging new technology. However, none of these implementations has an optimized red and NIR sensitivity. A main ambition of PII in this project is to design a SPAD array with enhanced red and NIR sensitivity (Fig. 1.3f), which will represent a useful and significant advance beyond state-of-the-art, not only for MINFLUX, but also for other imaging applications.

Tunable lasers for SRS and TPE: State-of-the-art in stimu
by two main approaches: First, by fast and sensitive acc
vibrational bonds, made possible by narrowband picosecond light sources. Second, by setups for simultaneous multispectral stimulated Raman imaging, which however lack in sensitivity. As a third option, spectral acquisition or addressing of multiple vibrational lines is also the stronghold of classical Raman microscopy, but here the acquisition times are extremely long and the 3D resolution is far inferior. To turn the first approach into a useful multispectral readout, fast switching of the wavelength from the light source is necessary. Currently the switching takes about 60 to 120 seconds. In this project, our aim is to reduce this time by more than a factor of 10, aiming for tuning times of a few seconds and thus speeding up multispectral imaging by an order of magnitude.

Regarding the pulse duration, the light sources for coherent Raman imaging, multiphoton imaging and fluorophore activation require different time regimes, from the picosecond down to femtosecond time range, to work efficiently. Currently, to achieve this, either two laser systems are required, or complex opto-mechanical setups, which only could be handled by optics and laser experts. The cost of an additional laser for an extra modality is in the range of 100 to 250k€ depending on the requirement. The aim in this project is to combine both modalities in one laser. This will drastically simplify the setup and reduce system cost, thereby opening up this application to the biomedical community. Both points are ambitious tasks and well beyond the statet-of-the-art, pushing the accessibility and usability of stimulated Raman imaging by a significant improvement of the light source.

Innovation potential

This project harbours a significant innovation potential, in particular on the technology development side, with new products of the three SMEs addressing market needs expected to grow strongly in the next coming years. However, also on the bacteriological side, the outcome of the studies planned in this project is likely to spark the development of new treatments (antibiotics) and vaccine strategies preventing pneumococcal disease.

On the bacteriological side, we expect to be able to identify novel mechanisms for pneumococcal interactions with host cells and how pneumococci cause disease, and the importance of protein localization for their function. We expect the knowledge gained to elucidate new pathways that could be used for intervention, thereby affecting disease development. Furthermore, we envision that the results from this project will form a basis for vaccine development based on for example bacterial exosomes and bacterial protein interactions with the host.

On the technology development side, all three companies in our consortium can identify obvious innovation potential, with new products that are likely to be generated as a result of this project:

MINFUX imaging system as standard microscopy tool for biomedical research (AI): AI's current focus is STED microscopy and its microscopes currently offer the best resolution performance on the market and feature numerous innovations developed by AI, many of them directed towards live-cell imaging. With the invention of MINFLUX which pushes resolution another order of magnitude, it was a logical step for AI to secure exclusive rights to this technology and the company succeeded to acquire such a license. A first generation MINFLUX system operating in the visible range and targeting early adopting users focused on method development and with a high level of technical skills has been developed and will be delivered to 3-4 customers within the year. However, AI aims at wide-spread adoption of the method in the biomedical imaging community, a potential this ground-breaking technology certainly has. Using new detector technology and by demonstrating correlative label-free imaging, the prototype developed as part of this project can be used by biomedical researchers as a robust imaging tool and the lead application will demonstrate this in a convincing way. This will be an essential contribution helping AI to reach acceptance of the method by the imaging community. At the same time the partnership with PII and APE will

allow AI to add innovative features based on array detection and label-free contrasts to its existing line-up of STED microscopes, strengthening its profile as innovation leader and allowing the company to expand its share in the high-end microscopy market. In fact, AI has filed patents in anticipation of array detectors with sufficient sensitivity becoming readily available and conducted patent searches to ensure FTO for these plans. The FTO analysis for adding Stimulated Raman imaging to AI's microscope platform is under way and the details of our preliminary findings are outlined in section 2. In the case of MINFLUX, AI holds all necessary licenses for existing patents and established FTO before the launch of the first-generation platform.

SPAD arrays with red/NIR enhanced sensitivity (PII): Confocal microscopes are the most used high-end microscopes in the world with around 2000 confocal microscopes sold per year. It is predicted that at least 50% of confocal microscopes will feature a detector array in 3 years. This development is primarily driven by the innovation of image scanning microscopy. Secondly, specialized techniques, such as FLIM, FRET and MINFLUX will further drive the introduction of detector arrays. In these applications, detector arrays enable imaging of the single-molecule surrounding and increase imaging speed by parallelization. SPAD arrays developed by Pi Imaging Technology are specially designed to meet the needs of the mentioned applications. A SPAD array with red/NIR enhanced sensitivity, as planned to be developed during this project, will further strengthen Pi's competitive advantage. Pi Imaging Technology has (exclusive) rights for 5 patent applications.

Coherent light sources with fast tuning and flexible fs-ps operation (APE): APE is currently market leader for coherent Raman light sources with its product picoEmerald, However it is a narrowband picosecond only system optimized for coherent Raman imaging (CARS and SRS). Further, the tuning time between 60 to 120 seconds is too slow for imaging multiple vibrational lines within an acceptable time. Even though it is possible to image second harmonic generation (SHG, for instance in collagen), or multi-photon excited (MPE) fluorescence with picosecond pulses, this process is much less effective than using femtosecond pulses. In this project, the aim is to develop a new, ease-of-use, fully integrated light source, which is fast tunable, and offers flexible fs-ps operation. To achieve this goal, novel tuning concepts and nonlinear interactions in optical parametric oscillators will be investigated and implemented as described in section 3. The new light source will make the accessibility of stimulated Raman microscopy and the combination with other techniques, such as photo-activation and multiphoton excitation microscopy much easier than before. This combination will give new insight for instance into bacterial research as aimed for as a lead application and being a main objective of the NanoVIB project.

Regarding state-of-the-art, one competitor, MKS/Newport/Spectra-Physics, offers a stimulated Raman solution for its femtosecond laser Insight X3, which is primarily designed to be used for MPE and photo-activation. The concept they follow, called "spectral focussing", is realized in the product SF-TRU. In theory, this setup is capable of similar tasks as we propose. However, the underlying concept is extremely complex and requires laser physicists to operate it, thus preventing the practical use in a biological or medical environment.

An extensive patent search showed that the ideas APE pursues in this project are free of conflicting patents and APE has the freedom to develop the light source described.

2. Impact

2.1 Expected impacts

There are two major parts of this project: First, the development of a next generation super-resolution imaging system, the required detector and laser components resulting in an end-user ready system by the end of the project. Second, the successful application of this microscope system to a pioneering lead application within a highly relevant field of medical biology. Concurrently the system will be operated in an end-user facility during the final year to promote dissemination and further exploitation. All project activities are centered around MINFLUX nanoscopy, a fluorescence imaging concept for structural analysis of biological specimen, which closes the resolution gap between conventional super-resolution microscopy techniques like STED and single molecule localization based approaches (PALM/STORM) on one hand, and electron microscopy and complex spectroscopic techniques like Förster Resonance Energy Transfer (FRET) on the other hand.

Consequently, this project can be expected to generate significant impact in two major areas: (1) the consolidation and extension of Europe's leading position in the development and manufacturing of innovative biomedical imaging devices and (2) the acceleration of fundamental biomedical research.

While the first MINFLUX instruments, operating in the visible wavelength range, will soon be delivered by partner 3 (AI) as a commercial product to laboratories focused on method development, this system is not fit for routine application without support by researchers with a deep understanding of the method, and it is limited to a small subset of available fluorophores and applicable only to certain samples. As it was the case with STED, PALM and STORM nanoscopy a decade ago, the MINFLUX technology has been proven to work but is not yet ready for widespread acceptance. This early phase presents unique opportunities: The sudden availability of a new class of fluorescence images with resolutions an order of magnitude better than existing techniques will potentially lead to important discoveries in biology and to significant business opportunities for highly innovative SMEs, if the most important prerequisite steps towards broad acceptance of a biomedical imaging technique can be swiftly completed: (1) Ensure and demonstrate compatibility of the method with a wide range of fluorescent dyes and labeling techniques, and its fitness to routinely image live cell samples. (2) Interface it with complementary imaging techniques that yield important, additional information and (3) demonstrate its capability to deliver fundamentally new results, in a groundbreaking lead application of significant biomedical and clinical relevance.

By doing just that, this project will ensure that a major part of the expected benefits, both on the industrial side and within the research community, will be reaped within the EU. To meet these expectations, NanoVIB has an ideal constellation of partners: NanoVIB will bring together three highly innovative SMEs with business models already targeting early adopters in the biomedical imaging research community (AI, PII and APE), an institution focusing on

the interface between academic research and industry (LLG) and two academic institutions with an impressive track record of applying novel imaging techniques successfully to real-world biological problems (KTH and the KI).

In WPs 5-6, by establishing the use of this super-resolution imaging system for bacterial studies (WP5), and then showing that it can resolve features underlying bacterial virulence and invasiveness not within reach by other microscopic techniques (WP6), we will demonstrate by a striking example the capacity of this microscopic technique for cellular research. Enabled by the technical development in WPs 1-4, we will thus gain new significant understanding of the inter- and intracellular processes underlying pneumococcal disease. Pneumococci are the cause of worldwide morbidity and many thousands of deaths in Europe alone, and further understanding of these processes can likely pave the way for new antibiotics and vaccines. Thus, fulfilling this objective will directly benefit clinicians and patients and eventually have a huge positive influence on quality of life for all of society. In turn, this reflects on the commercial side, where there is an enormous potential in the development of new vaccines. Huge resources are spent by the pharmaceutical and biotech industry, where not only development of viral vaccines, but also of bacterial vaccines, e.g. against pneumococci, is high on the agenda.

Importantly, resolving cellular proteins on the nanoscale is not only necessary to understand pneumococcal disease, as aimed for in our lead application, but also for cell biology in general and for a broad range of diseases. Bringing this application to a successful end will thus also broadly demonstrate the importance of our new imaging technique, contribute to the commercial success of correlative MINFLUX microscopy, and thus strengthen Europe's position as the prime hub for leading manufacturers of high-end microscopes. Moreover, this project will result in affordable SPAD array detectors with high quantum efficiencies becoming available for the whole visible to NIR range. The development of detection electronics will result in an interface standard with the control logic of the microscope, allowing it to control other parameters based on spatial and temporal information of each detected photon in real time. This is not only a prerequisite for array detection in MINFLUX but will allow array detection to be used in many other advanced imaging techniques and allow research labs to envision and develop innovative new microscopy approaches. The project will also result in the development and commercial availability of a single, robust, and compact laser, compatible with both label-free stimulated Raman based imaging techniques and two photon excitation and activation. For both research labs and system integrators, this project will make all these techniques more accessible, which will inevitably result in their accelerated development and acceptance. This technological development will generate IP which will be secured by the project partners, who will then spearhead their adoption into fluorescence microscopes in general.

Our project, with three market-leading EU companies aligning their development efforts to establish a multi-functional MINFLUX microscope system with ground-breaking performance, and guided by a lead application paving the way for a broad applicability of the microscope system in cellular research, will inevitably strengthen Europe´s industrial position in the biophotonics related market for microscopes and research and development tools.

Below, we describe in more detail how our project will contribute to the expected impacts and how the activities in the project make them link to and support each other:

Significant understanding of inter- and intracellular processes. Here, the deliverables in WP5 and 6 are themselves defined to meet this impact. As a strategy accompanying the activities in WP5 and 6, project partners KTH and KI will act as lead users of the instruments developed in WP1-WP4. By convincingly demonstrating that distribution patterns of proteins can be mapped on bacteria with a ten-fold higher spatial resolution than with any other state-of-the-art fluorescence-based super-resolution imaging technique, and where overlaid SRS and TPE-TRAST images can place the proteins distribution patterns in a morphological, chemical or microenvironmental context (Deliverable D5.5), we expect to trigger a demand from other research groups in biophotonics and biomedicine to use this imaging technology for fundamental cellular research. By showing in WP6 (D6.2) that this information also makes it possible to significantly further our understanding of the disease mechanisms of pneumococci, we will also raise the interest in the bacteriology research community and pharmaceutical industry for using the concepts and instrumentation developed in this project. This will eventually lead to a better understanding of fundamental bacterial disease mechanisms, and subsequent development of new diagnostics, antibiotics and vaccines.

We also envision that the bacterial and host-pathogen interaction data generated as part of our specific lead application will be of commercial interest. New paths and knowledge influencing pneumococcal pathogenesis and disease development can be explored for interventions including novel treatment and prevention. Partner 2, KI, already has promising data for bacterial exosomes as vaccine candidates, something that will be further investigated in this project (Task 6.3). KI has regular contacts with possible industrial partners that will facilitate commercial exploitation of such new inventions, including anti-virulence drugs, antibiotics, and vaccines.

Finally, how specific proteins distribute themselves on or inside cells is not only a key to understand bacterial infections. For instance, the different mechanical, proliferative or adhesive properties of cancer cells can be reflected in the spatial distribution patterns of e.g. membrane, cytoskeletal and cell cycle regulating proteins, which are difficult to resolve by state-of-the-art fluorescence microscopy methods (see e.g. Blom and Widengren[1] for a review). The super-resolution microscope system to be developed in the project is thus likely to find applications not only in fundamental cell biology, or for bacterial diagnostics, vaccine and antibiotics development, but will progress understanding of intra- and intercellular processes, underlying a much broader group of cellular states and diseases.

Therefore, while the project specifically targets a significant gain in understanding of inter- and intracellular processes in the context of pneumococcal disease, we also set as an aim of the project to promote the understanding of such processes and the awareness of the enabling technology more broadly.

As complementary strategies to the lead application, two important dissemination activities are also included in the project: First, one of the microscope systems to be developed will be available in an open facility during the last year of the project. Thereby, biomedical

researchers from academia as well as from biotech and pharma companies can use the unique possibilities of the device to address a broad range of intra- and intercellular processes (D6.1).

Second, a group of potential end-users will be engaged early in the project (D7.4). Their feedback will help us take the needs and concerns of a broad range of potential end users into account when developing the final system and procedures.

By demonstrating a lead application of high biomedical relevance in bacteriology (D6.2), with the open microscope facility (D6.1) and by forming the end-user group (D7.4), we expect to minimize the threshold of acceptance, and shorten the time until the microscopy technique and the developed procedures have found a broader use in academia and industry.

Strengthen Europe´s industrial position in the biophotonics-related market for microscopes and research and development tools. Europe's industrial leadership in high end optical microscopy methods is already remarkable (Confocal, Structured Illumination, Spinning Disk, Single molecule localization based (PALM/STORM), Total internal reflection (TIRF), STED, MINFLUX, Two Photon Excitation (TPE) Microscopy, etc.). Two (Zeiss & Leica) out of the four major optical nanoscopy players (the others are Nikon & Olympus) are European. The total market is estimated to about 1000-1500 units per year, with a total volume of more than 1 billion EURO. These "big four" generate most of their revenue in this segment from traditional high-end microscopes, like confocal laser scanning and camera-based wide field setups.

However, the emergence of "super-resolution techniques" over the last 20 years has transformed the market. As of 2018, an estimated 10% of the units sold feature technology to overcome the traditional resolution limit in optical microscopy. This percentage has been growing steadily over the past years and is expected to grow in accelerated fashion in the future.

The "big four" have adapted to this development by licensing techniques like STED, PALM and STORM, by integrating them with their established system framework, and by marketing them through their traditional channels. This has created an opening for highly innovative SMEs. Among these SMEs there are both system integrators and component manufacturers. The system integrators are focusing on bringing the full potential of these techniques to the end user as fast as possible and on designing systems that can be continuously upgraded as these novel techniques evolve. Component manufacturers often start out targeting the research community. However, they are also ideal OME partners for system integrators with quick innovation cycles as they can "keep up" with this process. Such partnerships are an opportunity to scale up their business.

To take advantage of this opening, Abberior Instruments was established in 2012 as a spin-off from the Max-Planck department of Prof. Stefan W. Hell, one of the Nobel laureates in Chemistry in 2014 and the inventor of STED and MINFLUX microscopy. Stefan W. Hell is strongly supporting this project and has agreed to be on its advisory board. By focusing on quick adoption of novel concepts into commercial products, by closely cooperating both with researchers in instrument and application development, and with its customers in the research

community, the company has managed to conquer a significant market share in optical nanoscopy. At the same time, Europe's research community remains very active in the development of innovative imaging techniques, which has resulted in other, comparable success stories. Such development efforts critically depend on light sources and photonic detectors, along with acquisition and control logic being made available to the scientists. This is something APE has been doing for many years and PII has begun recently in the context of several collaboration projects.

This successful niche of highly innovative SMEs has already led to the creation of many new jobs in Europe, and there is potential for further expansion:

AI alone has not only created more than 50 high-tech jobs within the EU in the past years. By continuously improving its STED microscopes, often based on feedback from lead applications in academia, AI has accelerated their adoption in routine biomedical research. This has increased AI´s market share in comparison with other approaches manufactured outside the EU. While guaranteeing high-tech jobs in the EU, this has also made STED microscopes more affordable and more usable for researchers within and outside the EU. The wide-spread adoption of MINFLUX as an imaging tool, as a result of this project, is expected to create at least 20-30 additional jobs at AI.

Out of the 65 people working at **APE**, more than 20 highly qualified jobs are directly linked to coherent Raman microscopy light sources, developed throughout the last 15 years. Competition from especially American companies, such as MKS/Spectra Physics and Invenio Imaging offering different technologies, has arisen in the last few years. However, they are still behind APE regarding ease of use and flexibility. This project will help APE to bring its coherent Raman light sources to the next level and widen its application to MINFLUX and multiphoton microscopy, thus strengthening and expanding their market position. With this project APE can secure these jobs and expects to create 10 additional high-tech jobs.

PII currently hosts 3 high-tech employees and one full-time consultant and expects to absorb great talent from EU universities working in the domain of SPAD detector arrays. PII focuses on close collaborations within the EU and believes they can elevate the competitive position of their EU partners.

The experiences during the emergence of STED and PALM/STORM a decade ago now offer a blueprint for the development, dissemination, and exploitation of the next generation super-resolution microscope techniques. The MINFLUX technique offers another order of magnitude improvement in resolution and significant potential to become a wide-spread, ground-breaking imaging technique in the biomedical community. The speed with which this can be achieved, ultimately putting a powerful technique into the hands of biomedical and biopharmaceutical researchers, and where the jobs will be created, will depend on how fast and by whom the challenges – which are similar to those faced by STED and PALM/STORM – will be overcome. Several points are critical to make this happen:

- Systematic research into the spectroscopy of label candidates, at wavelengths compatible with specific applications, like live-cell imaging or high throughput screening applications.

- Identification of the most suitable and cost-effective laser and detector technologies, their targeted development, with the requirements for the imaging technique in mind and integration into the microscope.
- Providing a preliminary implementation that allows routine application by biomedical researchers and later by medical staff.
- Successful high-profile projects using the technology and publication of their results to generate acceptance of the technique in the research community.

Essentially, the aim of this project is to ensure that these points are addressed as fast as possible, within the EU, and with all relevant partners for the further exploitation of all findings already in play when the project ends. This is in perfect agreement with the aims of H2020: Our project will achieve a significant gain in understanding of inter- and intracellular processes through a groundbreaking lead application and by putting the imaging technology into the hands of third parties working on promising projects. At the same time it will strengthen Europe´s industrial position in the biophotonics-related market for microscopes and research and development tools by giving AI, APE and PII the possibility to combine their specific strengths to trigger a directed, well guided and concerted technology development. Thereby, we will create an innovative imaging platform for which all major components are manufactured in the EU. This will strengthen AI's position as innovation leader in modern optical nanoscopy, while displaying APE's innovative laser sources and PII's SPAD arrays with intelligent readout electronics. Joint technology development in this project will help promoting these products for a much wider range of applications.

Making the technology more accessible by improving ease of use and saving costs, we will also strive to open this technology to a much wider customer base. Thereby, the technology can better reach out to medical researchers and biologists, who currently refrain from using it due to its current complexity and price. One example in this direction would be a fully automated microscope with laser integration and with less lasers being used, thus saving costs as well as allowing for a much faster data acquisition.

Barriers/obstacles and framework conditions affecting the achievement of the expected impacts

We have not identified any significant barriers that would stop the project partners from achieving the expected high impacts. As with all plans relying on the successful completion of an ambitious development and research project, the most obvious obstacle derives from the implementation risks that are outlined in section 3. There is a medium to low probability that certain parts of the implementation plan (laser / detector / electronics development, identification of suitable dyes, establishing proper labeling and sample preparation protocols) are delayed while additional mitigating actions are taken. However, none of those individual aspects of the development processes has the potential to halt work in other work packages. We are therefore certain that the technological objective of developing a new MINFLUX platform, with a focus on usability and applicability to biomedical imaging, and demonstrating its fitness as a tool for routine research, will be achieved even in a worst case scenario.

Also, while not part of this project, the synergetic integration of SPAD array technology developed in WP1 and WP3 to STED and other imaging methods, as outlined below, will be a major avenue to exploit technology developed here, independently of the work in WP5 and WP6. Similarly, the new fast-tunable fs-ps light source developed by APE will find application and commercial success beyond MINFLUX imaging.

AI has thoroughly analyzed the IP situation for MINFLUX and concluded that we have FTO for the new system development. However, to be able to commercially offer correlated STED- and SRS-MINFLUX microscopes in Germany, France, Great Britain and the US, AI will have to acquire licenses for at least one patent family owned by HARVARD COLLEGE, US. AI has received requests to integrate Raman based imaging with its microscopes in the past. Therefore, the necessary work related to IP has already been started, both at AI and at the LLG, and we plan to enter negotiations with Harvard as soon as possible. However, an agreement cannot be expected until the project is under way. There is thus some risk that licenses will not be granted, or that licensing conditions are unacceptable, which would delay commercial exploitation of the correlated imaging approach in the afore mentioned markets, in worst case until 2029 (max. 5 years after the end of the project), when the patent runs out. On the other hand, APE has successfully marketed SRS imaging packages to end users of confocal microscopes for many years. This strategy can be applied to STED and MINFLUX owners as well and ensure exploitation of Raman-Imaging related results. There are also no IPR-related restrictions in the use of SRS-MINFLUX in the lead application, as planned in WP5 and WP6 in this project. Also, use of APE's lasers for two-photon activation in MINFLUX would not be limited by the current IP situation.

Finally, even with all system and method development on schedule, the lead application in WP6 could prove more challenging than expected, delaying its conclusion and prominent publication, and thereby also the push this would generate during commercial exploitation of the new developments. We are, however, confident that even if this were the case, this project would still achieve its impacts by placing a powerful new tool into the hands of the bioimaging community, eventually leading to important new insights. Similarly, the new technology developed will increase the competitiveness of the three SMEs involved and thus strengthen the Europe's position in the biophotonics market. And as the partners have agreed to leave the two new systems in place after the project is finished and continue to work on MINFLUX applications, the positive effect expected from a high-impact lead application would not be voided but merely delayed.

2.2 Measures to maximize impact

To ensure and maximize the impacts aimed for in this project, KTH, KI, LLG, together with AI will focus on *direct interaction with potential end-users* of the technology and with the target audience for their findings in bacteriology. The lead application conducted in WP5 and WP6 of this project will result in a significant gain in understanding of intra- and intercellular processes. These findings will be disseminated as outlined below. However, by demonstrating the capacity of the microscope developed in this project to reveal intra- and intercellular processes underlying pneumococcal virulence and invasiveness, we also pave

the way for a broader end-use, which will spur the interest for this technology both in academia, in the clinics and in pharmaceutical and biotech industry.

To maximize this effect, we will also establish a microscope facility (D6.1), open to potential end-users of the microscope technology. This facility will not exclude any category of potential end-users and allows researchers from all fields of application to familiarize themselves with the technique and its possibilities. Likewise, we will, for the same reason, recruit people to the end-user group formed in the beginning of the project (D7.4), from as many different relevant categories as possible (researchers in cell biology, biophysics, bacteriology, cancer researchers, representatives from large pharma companies, smaller spin-off companies,…). In this way, we can promote the demand for the microscope systems to be developed, and open new markets for the microscopes, as well as for the lasers and detector systems themselves. In addition, all three companies (AI, PII, APE) will *accompany and follow up development within NanoVIB with synergetic development projects building on the results. These efforts and product marketing through their established channels will be* financed from their regular revenue. This project will therefore establish PII as a manufacturer of next generation SPAD arrays for biomedical imaging, strengthen APE's position as a leading provider of light sources for innovative imaging applications and strengthen AI's position as the innovation leader in super-resolution microscopy. Extension of MINFLUX to the NIR wavelength range and its combination with complementary imaging techniques will help grow the market share of this technique within the super-resolution segment.

For the SMEs involved, the interaction with potential end-users, as planned in this project, will result in better visibility of both the products developed during this project and their dedication to advancing imaging technology in general. We expect this message to be heard by a broad audience, including opinion leaders in the international bioimaging community. This will bring further energy into the photonics branch in Europe in general, will strengthen the competitiveness of the companies in the project, and will allow for their further growth.

a) Dissemination and exploitation of results

The three SME partners have coordinated their plans for exploitation of the newly developed technology, both within the project and as part of synergetic development and subsequent exploitation efforts, in related fields of their business. The planned activities are outlined in Fig. 2.1a, and there also put in context with the deliverables defined in section 3 below. These plans for additional development beyond the scope of NanoVIB, exploiting its results to related imaging techniques, rely on the strong relations formed within our project and on the documented commitment to achieve important technological goals within the four years the project will be active. The R&D personnel hired for and trained during this project will form the backbone for these additional developments. As the primary focus within the project is to deploy prototypes rapidly to our partners, in order to ensure the success of WP5 and WP6, all three companies will also finance the final development steps to TRL9 from their regular revenue and use their established channels for marketing and sales activities. Documenting the SMEs commitment to translate the projects result into commercial success, all other activities listed here are also financed independently of NanoVIB except for those that are

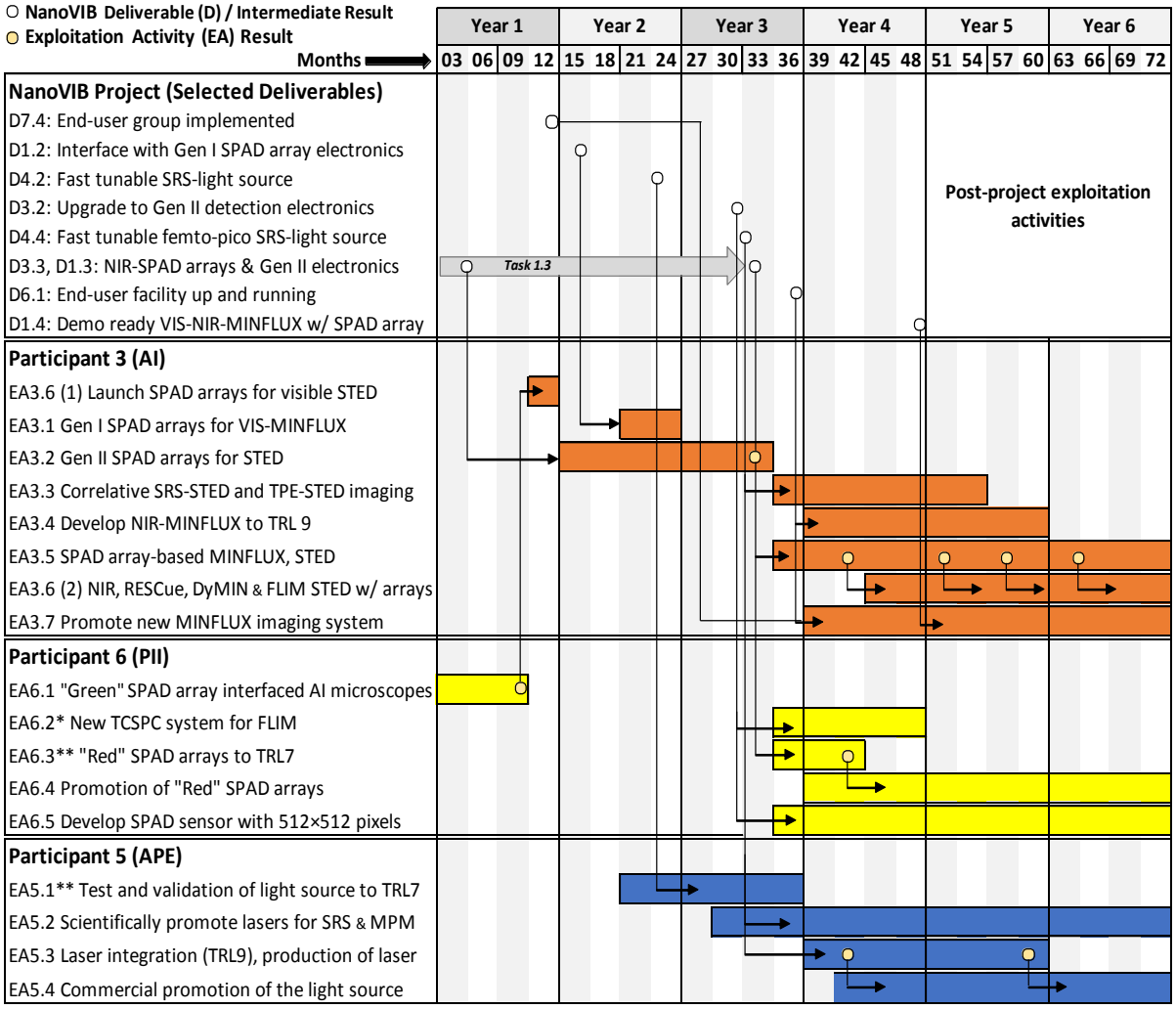

Figure 2.1a: SME exploitation activities (EA) during and after completion of the NanoVIB project.

prerequisite to later project work and thus partly (marked with *) or entirely (marked with **) financed through the project budget.

The paragraphs below highlight the perspective of each of the SMEs involved and give a short description of the individual exploitation activity (EA) planned by each project partner:

Participant 3 (Abberior Instruments GmbH, AI)

The project will result in a MINFLUX imaging prototype demonstrating the applicability of the technology as a standard research tool in biomedical and biopharmaceutical research, with its fitness demonstrated during the lead application in WP5 and WP6.

In a recent joint research project together with the Max-Planck-Institute for Biophysical Chemistry, AI could develop the current, first MINFLUX generation, targeting technically savvy researchers familiar with its technical intricacies and interested in exploring the potential of the method early on. With this achievement and experience as a starting point, and together with the project partners, AI is now well prepared to develop a TRL 5 platform within the project and to advance the prototype to a market-ready device (TRL 9) that can

serve as a standard research tool, no later than a year upon completion of the project.

Facility-like operation of the prototypes created during the project, as envisioned as part of the dissemination efforts, will generate even larger interest and allow us to interest potential customers very early.

The money applied for in this project only covers integration efforts and improvement over the prototypes that can be deployed to our project partners in time, to maximize their productivity in WP5 and WP6. Production transfer, certification and marketing efforts beyond this scope can be covered through AI's regular revenue.

Based on the interest the research community has already shown for the MINFLUX technology we estimate that an instrument, compatible with routine facility operation, will result in 10 - 25 sales within 1-2 years after introduction. This would result in 10-25 M€ additional revenue translating into the creation of an estimated 20-30 jobs at Abberior Instruments in management, marketing, sales, production and service.

In the likely case that the FTO concerns outlined above are mitigated, the know-how resulting from the development of correlative SRS-MINFLUX microscopy in this project would allow Abberior Instruments to immediately develop a similar combination of SRS and STED microscopy in parallel using minimal resources. An SRS channel will then be offered optionally for all Expert Line configurations and will also be available as an upgrade to all current Expert Line customers. This will also make combining STED with two photon excitation (TPE) more attractive, since the same light source allows later integration of coherent Raman imaging without significantly adding to the system price.

Abberior Instruments has received requests to integrate Raman based imaging with its microscopes in the past. Based on the frequency of such requests and its experience with past upgrades and imaging options, like two-photon excitation and fluorescence lifetime imaging, AI expect to sell at least 5 upgrades within 2 years after the initial offering is available and that 10 to 20 percent of future Expert Line microscopes will feature the combined TPE/SRS option. During the first 2 years of introduction this would result in at least 4Mio€ additional revenue and 4 additional jobs being created.

The development of next generation SPAD arrays with high sensitivity in the NIR makes them an ideal candidate for adding image scanning capabilities to STED microscopes. Automatic background subtraction is an obvious application and AI has also filed patents for technology that will bring about significant improvement in 3D STED microscopy based on this approach. The efforts of this project to integrate the next generation of SPAD arrays more deeply with AI´s detection electronics will allow their combination with both fluorescence lifetime imaging and several technologies, offered exclusively by Abberior Instruments (e.g. RESCue STED, DyMIN STED), and that focus on reducing light exposure of the sample in live cell imaging applications. With this integration and NIR sensitive versions of the SPAD available, array detectors could become the standard detector in all of AI's microscopes, thus leveraging the full potential of the optical setup and giving AI a significant edge over its competitors. This will allow AI to further increase its market share in the super-resolution microscopy segment and proportionally create additional jobs not only in

our head-quarter in Göttingen, but also in our development and production facilities in Heidelberg and Basel.

The activities planned by AI during the project and the following two years are listed below. These projects will build on the know-how and work force of the personnel hired and trained as part of NanoVIB and will be conducted in close cooperation with AI's R&D department using AI's established development and exploitation strategy: Upon proof of principle for a new technique or application, it will be integrated into AI's flexible hardware and software platforms. This will essentially guarantee initial commercial exploitation, since it immediately becomes available to all existing customers as an upgrade, and strengthens our product offering as an optional add-on. This reliably generates several initial sales and these success stories support the accompanying marketing and sales efforts, which will be carried out by AI's respective departments using the same approach as for our existing advanced imaging devices. From AI´s side, the individual activities planned are:

EA3.1 Interface modified Gen I SPAD array electronics with visible MINFLUX (M19-M24): Using the preliminary solution developed as part of the project, the modifications to Gen I array electronics will allow AI to also explore the benefits of array detection in the visible range, complementing the work in WP2 and WP5. This will improve MINFLUX as a whole and generate IP within the company securing its future technological leadership beyond the duration of the exclusive license currently held for the technique.

EA3.2 Interface Gen II SPAD array electronics with STED acquisition (M13-M33): As Gen II array electronics becomes available, AI will also re-design its current electronic platform for the Facility-Line and Expert-Line STED-microscopes. This will eventually allow SPAD array detection to play well with its RESCue, DyMIN and FLIM STED products. We will also ensure compatibility of array detection with our flexible rainbow detector unit.

EA3.3 Explore correlative SRS-STED and TPE-STED imaging (M34-M54): AI aims at testing the new APE light source as soon as possible in-house for projects other than NIR-MINFLUX. Provided FTO can be achieved, the expertise gained from NanoVIB will allow us to develop label-free imaging as an add-on for existing STED installations. Independently of FTO concerns, the new light source will also allow us to promote already available options for TPE microscopy and two-photon STED microscopy more aggressively.

EA3.4 Develop NIR-MINFLUX to TRL 9 (M37-M60): After deployment of the two prototype platforms, our optics engineer working on NanoVIB will be tasked to monitor integration progress and address any problems during integration and application. This will allow us to (1) re-deploy solutions to our project partners and (2) start collecting data for the development of the final platform early on. Based on these data and the added experience from facility operation during year 4, AI will then make NIR-MINFLUX part of its overall R&D strategy and we expect TRL9 and production transfer no later than one year after the end of the project.

EA3.5 Develop new SPAD array-based MINFLUX, STED and confocal imaging techniques (M34-M72): Based on NIR- and VIS-sensitive SPAD arrays deeply integrated

into our acquisition electronics, AI will focus on leveraging the added information to improve all its microscopes. Thus, as soon as both VIS- and NIR-sensitive arrays with Gen II electronics and high timing resolution become available, PII and AI will expand their cooperation to finding novel applications of array detection in STED imaging. In addition to the more obvious uses like enabling background reduction and improved 3D deconvolution, AI has already filed patents for potentially game-changing applications in this context.

EA3.6 Launch SPAD arrays as an option for visible STED and confocal imaging (M10 – M12) **and for NIR, RESCue, DyMIN and FLIM STED** (M43-M72): Existing VIS-SPAD arrays can be used with STED with minor modification. Following EA6.1 (PII) will create an upgrade option for all Expert Line customers. Later, building on the results of EA3.5 improved, array-based versions of existing imaging modalities will be launched as Expert and Facility-Line options and offered as upgrades to existing customers as soon as they reach sufficient maturity level. This will give AI an edge over competition in the STED market and help consolidate and grow its market share.

EA3.7 Promote new MINFLUX imaging system (M37-M72): As soon as the open facility (D6.1) is up and running, AI will actively encourage potential customers to evaluate the new NIR-MINFLUX system. AI will guarantee upgradability for MINFLUX systems sold from this point forward and our sales and marketing team will remain in contact with all these potential customers and start advertising through established channels using preliminary results from WP5 and WP6.

Participant 6 (Pi Imaging Technology, PII)

PII's focus is to create new technology that enables microscopy innovations: new imaging and spectroscopy modalities, higher resolution, faster imaging speed, live cell imaging. Confocal microscopes are the most used high-end microscopes in the world. It is predicted that at least 50% of confocal microscopes will feature a SPAD detector array in 3 years. This development is primarily driven by the innovation of image scanning microscopy. Secondly, specialized techniques, such as FLIM, FRET, MINFLUX and STED will further drive the introduction of SPAD detector arrays. In these applications, detector arrays enable imaging of the single molecule surrounding and increase imaging speed by parallelization. SPAD detector arrays developed by Pi Imaging Technology are specially designed to meet the needs of the mentioned applications. With the results from this project, PII will improve its position in the biophotonics related market for research and development tools. By introducing a SPAD array with enhanced red and NIR sensitivity ("red" SPAD), PII will supplement its "green" SPAD arrays and cover the complete visible to NIR spectrum. This enables color/dye specificity without compromising the sensitivity.

To maximize impact of the project results, we plan actions targeted at identified communities:

- Lead users (early adapters in research and industrial community; instrumentation laboratories and innovative companies),

- End users (late adapters in research and industrial community; biologists and large corporations),
- Investors (business angels and venture capital funds).

We will communicate results and offer IP to lead users for early adaptation. Further, we will organize workshops with demo microscopes showcasing the plethora of new microscopy applications enabled by SPAD detector arrays. This will lead to the identification of the most promising applications and targeted marketing to end users. Through participation in a high-profile project like NanoVIB, PII will improve its investment attractiveness. Additional start-up investment will, in turn, fuel faster growth.

PII will, at the end of the project, translate the building block of this project to other applications in spectroscopy, quantum physics, random number generation, material science and laser ranging.

PII's current exploitation plan entails the following activities:

EA6.1 Interface "green" SPAD array with AI's STED microscopes (M1-M9): PII and AI will collaborate to integrate PII's current generation of SPAD arrays into AI's STED microscopes. The focus is going to be placed on hosting multiple SPAD arrays in a compact microscope area. This will improve image quality through background compensation and advanced deconvolution techniques for "green" STED.

EA6.2* Develop new time-correlated single photon counting (TCSPC) system for fluorescence lifetime imaging (FLIM) (M34-M48): PII will develop and market its SPAD array with TCSPC capability. The TCSPC will be implemented on an FPGA hosting an array of time-tagging electronics with 20 ps resolution. This development will be synergic with the development of time-tagging Gen II detection electronics planned for this project. Precise time-tagging enables reduction of auto-fluorescence, gated-STED and FLIM. These features will enrich AI's application possibilities. Time-tagging detection electronics will be also offered as standalone units capable of hosting a plethora of other photon counting detectors.

EA6.3 Test and validation of SPAD arrays with enhanced red and NIR sensitivity in relevant environment (AI) to TRL7** (M34-M42): We will tailor our new SPAD array in a relevant application environment. AI, KTH and LLG will provide valuable feedback on geometry, functionality, and on the electronic interface of the detector. This will lead to TRL7 and pave the way to commercial exploitation of the new SPAD array.

EA6.4 Promotion of SPAD arrays with enhanced red and NIR sensitivity (M37-M72): PII will start marketing the new SPAD array at the end of the third project year. With TRL7 and first application results in MINFLUX microscopy, PII will expand the application field of the new SPADs beyond microscopy. The new applications will include spectroscopy, quantum physics, material science and laser ranging.

EA6.5 Develop SPAD image sensor with 512×512 pixels using the new "red" SPAD as building block (M34-M72): Using the newly developed "red" SPADs as building blocks, PII will develop and exploit large SPAD image sensors with 512×512 pixels. The main benefit of

SPAD image sensors is the elimination of readout noise and the high frame rate. These features pave the way to high-speed photon-counting imaging. A SPAD image sensor will be offered as a replacement for scientific EMCCD cameras.

Participant 5 (Angewandte Physik und Elektronik GmbH, APE)

APE is a SME company and manufacturer with a strong focus on producing light sources for the biophotonic microscopy market, where APE's lasers are used for multi photon excitation (MPE) microscopy and coherent Raman imaging (CARS and SRS). APE is the market leader in coherent Raman light sources, but this application forms only a small fraction of the total MPE market, which is currently dominated by the two American companies Coherent Inc. and MKS/Newport/Spectra-Physics with their tunable fs-lasers. We estimate about 300 MPE laser sales every year, of which about 10% make up for coherent Raman applications and an APE-market share of 50% for the latter. However, MPE lasers are expensive, with prices ranging from 100k€ to 250k€, making it difficult for users to buy two lasers to do both coherent Raman and classical MPE microscopy.

By enhancing the capability of the proposed new light source, to do both efficient MPE and coherent Raman microscopy, APE will considerably strengthen its position in this market. Within this project, APE will develop a version of the light source that is easily integrated by our partners into MINFLUX microscopes, both hardware and software-wise (and, outside the project, into STED microscopes as well). Dissemination to the general market and production transfer is not covered by the money applied for in this grant application. However, to bring this project to a commercial success this step is vital and it will be covered from APE's regular revenue.

APE has the infrastructure necessary to conduct the tasks required for the project and to bring the new prototype to TRL9. TRL9 should be achieved one year after the end of the project at the latest. Investments in infrastructure of 200k€ to 300k€ and in people will then be necessary for scaling up the production, something which APE can cover through its regular revenue. We expect that the new offering will double the sales of coherent Raman light sources and raise the revenue from these products to about 4M€ per year. This will secure 15 highly qualified jobs at APE and generate 10 more within 5 years after product launch. Together with the other industrial partners in this consortium we will thus strengthen the European position in the bio-photonic technology market.

Specific activities to maximize impact are:

EA5.1 Test and validation of light source in relevant environment (LLG and KTH) to TRL7** (M22-M36): In parallel to the two prototypes at LLG and KTH, APE will build a third prototype, to be used in direct interaction with the two partners. The interaction with the users is essential for improving hardware and software, for best performance and ease of use with the SRS- and MINFLUX microscope. This process will bring the prototype to TRL7.

EA5.2 Scientific promotion of light source for SRS and multiphoton microscopy (M28-M72): With the first results from the partners LLG and KTH, APE will start marketing the new light sources on scientific conferences, as a combined light source for SRS and femtosecond multiphoton microscopy. This will be done together with the consortium

partners by presenting microscope and light source data.

EA5.3 Laser qualified with microscope, bringing it to TRL9, production of laser (M37-M60): Together with AI we will qualify and integrate the laser for NIR-MINFLUX and coherent Raman imaging, thus bringing the laser to TRL9. Production transfer will be finalized no later than 12 months after the end of the project. This does include investment into production infrastructure in the order of 200 to 300 k€. Small-scale prototype production for early adopter customers is aimed for M46.

EA5.4 Commercial promotion of the light source (M40-M72): APE will present the new light source on trade shows and through media / social media activities. It will use its international sales and distribution network for promotion. Other microscope companies doing coherent Raman imaging will be contacted. Several of them are already customers of APE. APE is the premier supplier for coherent Raman light sources and well established in the multi-photon microscopy community. The reputation of APE and its scientific and commercial contacts will help to promote the new light source.

Data management

Within the scope of this project, three types of data are in principle generated: 1) software routines for hardware control, 2) software routines for data evaluation and 3) measurement raw data.

Concerning the storage requirements, most of the data is generated by measurements: Typical acquisition times in MINFLUX-based nanoscopy are 5-60 minutes. During this time some 10,000 molecules are localized. The resulting memory requirement for the raw data is currently approx. 1 GB per 60 minutes measurement time. Consequently, a maximum of 2-4 TB of raw data per year will be generated, which is within the range of commercially available hard drives. For SRS recordings the requirements are even one order of magnitude lower. Therefore, short-term and long-term data storage solutions already in place at the partners are sufficient. In addition, all research data associated with a publication will be subject to specific long-term storage in accordance with the rules of good scientific practice and current data guidelines.

To provide access to all data and routines for the entire consortium (shared domain), we will establish a cloud at KTH to which the partners will have access under the consortium agreement. We will make a distinction with regard to access of the general public (public domain) to this data: Software routines for hardware control represent proprietary IP and know-how of the companies of the consortium and are only made publicly available in compiled form in connection with the corresponding products. Software routines for data evaluation and raw measurement data will be freely accessible. However, a general disclosure will only be made after a thorough assessment whether the data and routines have to be embargoed for a certain period of time due to legitimate interests of the scientists (planned publication) or the research institutions (planned patenting). Any lifting of an embargo requires the approval of the project management committee (PMC).

A corresponding **data management plan** (DMP) which meets with H2020 requirements for Open Research Data will be developed and implemented in the scope of Task 7.1 within WP7

during the first six months of the project (D.7.2).

Knowledge management and protection

Results and procedures of potential interest to the project partners and the public will be deposited in a repository as part of our standard publishing procedure in the project. As the SMEs involved are R&D centered and have extensive experience working on emerging technology, policies to ensure proper documentation and archiving of development and research activities are in place. This is of course also the case for the leading research institutions partnering up in this project.

We expect significant IP to emerge from our development efforts, given that the project focuses on a new imaging method and combines array detection and coherent Raman imaging with MINFLUX (array detection and STED as part of EAs) for the first time. During development of the lead application, the project partners will be pursuing optimal solutions to practical problems arising, which routinely results in protectable intellectual property.

Fortunately, several of the researchers involved are inventors of many successfully filed patent families in the past, and therefore the necessary awareness and know-how to secure IPR is guaranteed within the NanoVIB consortium. If needed, the partners from KI, KTH and the LLG are supported by their respective local university patent offices, and AI, APE and PII have IP experts on their staff and procedures in place. As we anticipate a major part of the IP to result from our intensive cooperation, the consortium agreement will contain a non-disclosure agreement (NDA) and a detailed plan how to ensure proper handling of IP in this situation. The partners will also agree on the extent to which they will grant each other automatic licenses for patents filed as a direct result from the joint research conducted as part of the project.

An explicit aim of this project is to publish our scientific results in peer-reviewed, high impact journals, and to maximize outreach to the scientific community. All scientific publications emanating from the project will be published following the “gold” model, i.e. in online open access journals, such that the publications are immediately freely disseminated by the publisher upon acceptance. If appropriate, the partners will also consider publishing preprints e.g. on arXiv to accelerate dissemination of the scientific results. Open access publication is already an established routine at the academic partners (KTH, KI, LLG), and will be used as a standard also in this project. Prior to submission of manuscripts for publication, due considerations will be made to secure any IPR. For all publications planned in the project, prior notice of one month before submission to all partners in the project is required. During this time, any partner with concerns must respond. Planned publications and their handling with respect to IPR matters will be a standing point on the agenda at the meetings with the project management committee and will be regulated in detail in the consortium agreement.

b) Communication activities

Communication will be set high on the agenda to promote the project actions and its results. In line with this, we have raised the communication activities into a dedicated task (Task 7.2) of WP 7, on the same level as the coordination of the project as a whole, and at the regular

meetings of the project management committee, communication activities will be a standing point on the agenda.

Given the highly multidisciplinary character of the project, its potentially huge implications on societal health and well-being, and its significant exploitation potential, there are many relevant target audiences for our communication activities.

On the exploitation side, the project primarily aims to promote the as to be developed super-resolution microscopy, photon detector and tunable laser technologies, but the outcome of the project may also pave the way for exploitation on the biomedical as well as the biopharmaceutical side, for the development of new antibiotics and vaccines. This further emphasizes the need to reach out to a broad target group for the communication activities, and where the communication also has to be tailored to the different groups. Below, we have listed the main targets groups for our communication activities, the objectives of the communication to each of these groups, and in what form it will take place. All major communication activities are defined as explicit deliverables in the work package 7 (Project management and communication), as referred to in the listing below:

Communication with the general public

In the communication with the public, we want to bring forward three major aspects:

1. This project is a transnational cooperation in a European consortium, funded by the EU, with a scope that is impossible to take on at a national level, but requiring the larger critical mass found on a European level.
2. This project will bring about scientific results in the fields of bioimaging, cell-biology and bacteriology as well as the development of microscopy and photonic products which are world leading.
3. The relevance of the outcome of this project for society, its industrial competitiveness, and how it may contribute to better health and well-being for the society at large. The latter aspect will also be concretized from an individual citizen perspective, emphasizing that cellular originated diseases, such as pneumococcal disease, are a major cause of illness and death, and what a better understanding of the underlying mechanisms can mean for the individual in terms of lowered risk of severe illness, access to new antibiotics and prevention by new vaccines.

Already at the beginning of the project, we will establish a project web page (D7.3). On this web page, a summary of the aims and planned activities will be given, and updates will be done on a regular basis reporting on ongoing studies and achievements. This web page will have two levels, one addressing the public, and one intended for specialists. Second, we will arrange several open lectures to the public (D7.6). These lectures will both be given at the beginning of the project, then mainly communicating the aims, plans and how the outcome is of relevance to the public, and then during the last year of the project, communicating the achievements of our cooperation. These lectures will be publicly announced, given in Stockholm within the regular open lecture series arranged by the academic partner organizations (KTH and KI), as well as in Göttingen, arranged by LLG in collaboration with

the Georg-August University, the University Medical Center as well as the Cluster of Excellence in Göttingen. We will also use the communication office at KTH and the LLG for press releases, to announce the start of the project, and whenever called for, e.g. when exciting new results have been generated in the project.

Communication with potential end-users

By addressing potential end-users of the microscope system to be developed in the project, we want to inform them about what this new technology can add, beyond what is possible with current state-of-the-art microscopy techniques in their different fields of activity. As we see it, this group of end-users is quite broad, and includes not only researchers in bacteriology, cell biology, or cellular biomedicine in general. Also included in this group are users outside of academia, in biotechnology and pharmaceutical companies, in healthcare and at hospitals.

Reaching out to this group serves several purposes; First, we expect that the scientific results and established procedures from the lead application on clinical bacteriology in WP6 can be transferred to several different fields of biomedical and biopharmaceutical research, and help furthering the understanding of the cellular origin of diseases. Second, with a broad group of potential end-users realizing the potential of the developed microscopic technique for their activities, a large surge in the commercial demand can be expected.

To reach out to this group, we will arrange several end-user workshops (D7.5). In conjunction to two of the consortium meetings, a one-day workshop will be arranged, taking place in Stockholm in year two of the project, and in Berlin during year three. In addition, two hands-on workshops for end-users will be arranged in Göttingen during the last year of the project, which in addition to lectures and tutorials also contain practical training at the MINFLUX facility. These workshops will be announced broadly over Europe, and among potential end-users in several different fields and communities. The facility (D6.1), to be established and to be in operation during the last year of the project (and beyond), will thus support both the dissemination and communication outcomes, and will as well promote the exploitation of the developed microscope system, lasers and detectors. For the companies of our consortium an important communication channel to potential end-users is also the participation in fairs and conferences. Here, results from the project will be used and advertised in the form of e.g. demonstrations and application notes. To identify additional end-users, and to adapt the scope and activities of our project efforts for best possible impact, we will establish and then take advice from an end-user group (EUG) (D7.4), representing a broad scope of potential stake-holders.

Outreach to scientists:

With the academic partners of our consortium, and with the three companies having long experience in working very closely with academia, an important communication channel about the outcomes of this project is via scientific publications, lectures, conferences and meetings. We will publish our scientific results in open access, high-impact journals, for maximal spread of our results in the photonics, bioimaging, bacteriology, cell-biology and biopharmaceutical communities and beyond, allowing also others to implement the results

and progress science further. PhD students and postdocs in the project will be given the opportunity to present their results at least at one yearly scientific conference or meeting, in addition to the workshops arranged as part of our agenda (see above).

Communication Requirements

This project is funded by one of the calls under the Photonics Public Private Partnership (PPP). All communication activities related to the project will acknowledge the context of the Photonics PPP, for example by stating that the project is an initiative of the Photonics Public Private Partnership.

Specifically, for workshops, press releases, presentations etc, the EU emblem and Photonics21 logo will be displayed prominently together with the text "Photonics Public Private Partnership". The link www.photonics21.org will also be included. When communicating on Twitter or other social media about project activities, #Photonics will be included together with @Photonics21 and @PhotonicsEU.

The consortium will issue a professional press release at project launch, additional press releases whenever the project has reached a significant milestone or exceptional scientific, economic or societal impact is expected, and a final press release at project end.

A professional communication kit about the project (as a minimum a narrative text, photographs, slides possibly complemented by a video and any other suitable communication material, accompanied with copyright licences for the European Commission and for Photonics21) will be prepared and delivered *at the end of project month 3 at the latest*. The communication kit will target audiences beyond the project's own community, including the broader public and potential end-users, and focus on expected outcomes and related socio-economic benefits for the EU. The communication kit will be updated *at mid-term and at project end* to reflect project progress achieved.

The communication activities will involve public relations experts to ensure the pertinence of messages to target audiences and high outreach at both national and EU level through the use of the relevant national and EU-wide media channels (e.g. newspapers and broadcast).

Project communications such as project press releases, workshops announcements, websites, brochures will respect the principle of fair visibility for all partners. If the logo of any individual beneficiary is included, then the logos of all beneficiaries will be included.

3. Implementation

3.1 Work plan — Work packages, deliverables

The NanoVIB brings together a highly inter-disciplinary consortium, to work closely together in a work plan comprising seven WPs. A next-generation super-resolution microscope system will be developed in WP1-WP4, then established and used for a lead pilot application and offered as an open facility in WP5-WP6. The time frame of all WPs is shown in Fig. 3.1a and the inter-dependence of the scientific tasks is visualized in Fig. 3.1b. At the project's core is the development and realization of a prototype of a super-resolution microscopy platform (WP1 & WP2), to which prototypes of new lasers and photon detectors developed within the project (WP3 & WP4) will be added. During the course of the project, the super-resolution platform is continuously further developed (see Tasks 2.1 – 2.5) and upgraded with new and/or improved components (see Tasks 1.2, 1.3, 3.1 – 3.4, 4.1, 4.2), rendering it faster as well as with new correlative and NIR imaging capabilities. The identification of suitable acquisition strategies as well as sample preparation protocols (see Tasks 2.2, 5.1 – 5.3) gives valuable feedback for the platform development and allows proof-of-concept measurements on different biological samples (see Tasks 5.4 – 5.5). These experiments in turn provide the basis for the biological studies in WP6. In this WP, the performance of the developed microscope system will be demonstrated in a lead application, to reveal detailed molecular mechanisms underlying virulence and invasiveness of pathogenic bacteria with pneumococci as model organism (Tasks 6.1 – 6.4). Furthermore, a user facility will be established to make the system available for testing by researchers outside the project (see Tasks 1.4 & 6.5). WP6 thus provides two complementary means for dissemination and for promoting exploitation of the developed microscope system, its lasers and detectors, but also provides feedback to optimize the platform design and user interface, thereby supporting commercialization also in this way (Task 1.4).

The project is coordinated and steered according to a dedicated management plan (WP 7), which will ensure timely achievements of tasks in the project, and that the WPs are run efficiently and reach the expected results (Tasks 7.1, 7.3-7.4). Furthermore, by dedicated communication and dissemination activities (Task 7.2), it will be ensured that the project results are translated into successful exploitation and leads to scientific outcomes of highest international standard. Each WP has measurable deliverables (Table 3.1c) and intermediate milestones (Table 3.2c) that will be assessed to ensure project progress, and there are robust measures at hand to mitigate critical risks (Table 3.2b).

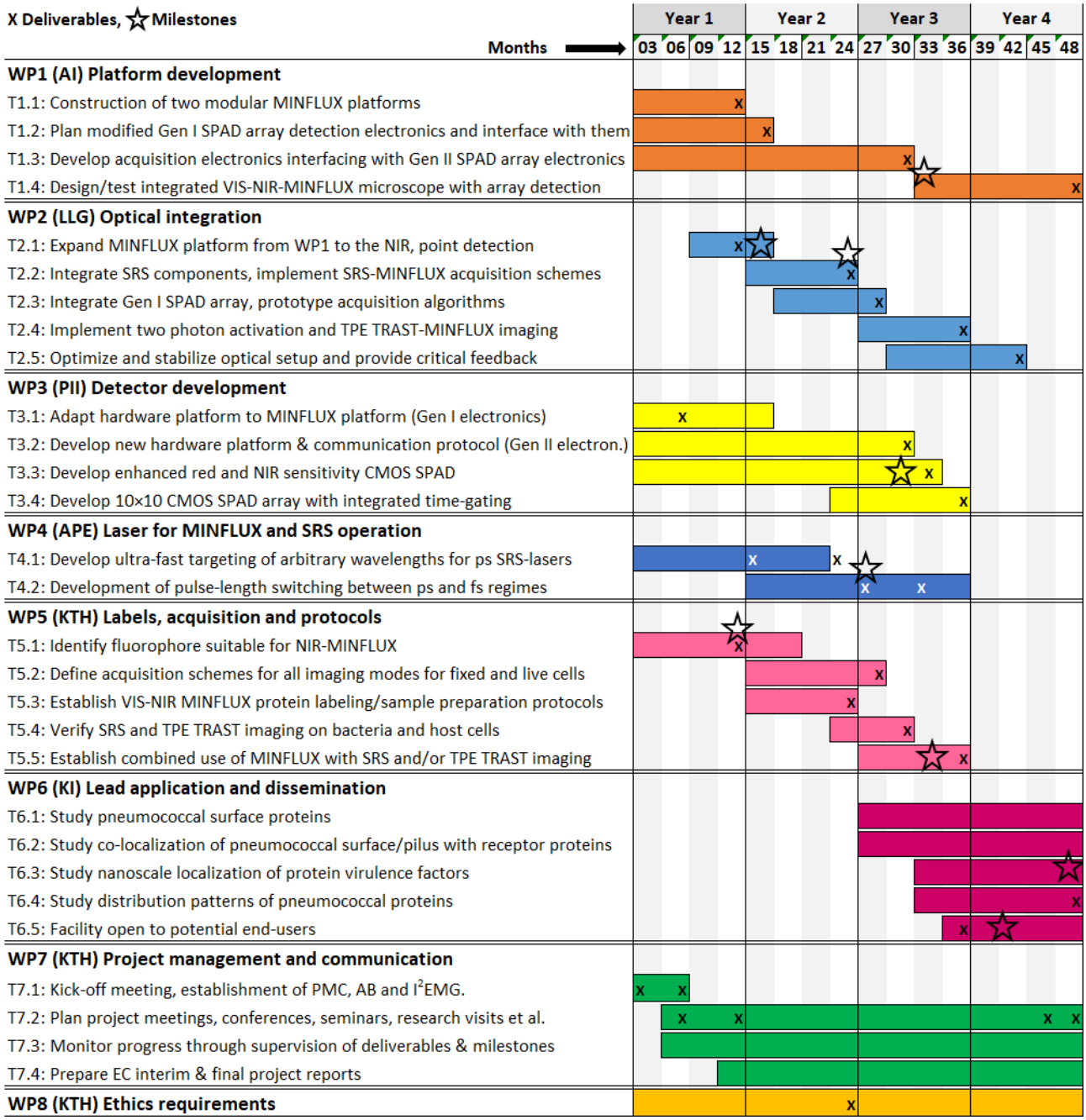

Figure 3.1a Gantt-chart of NanoVIB showing the time-line of the WPs and their different tasks, and the time point of the related deliverables (crosses) and milestones (stars).

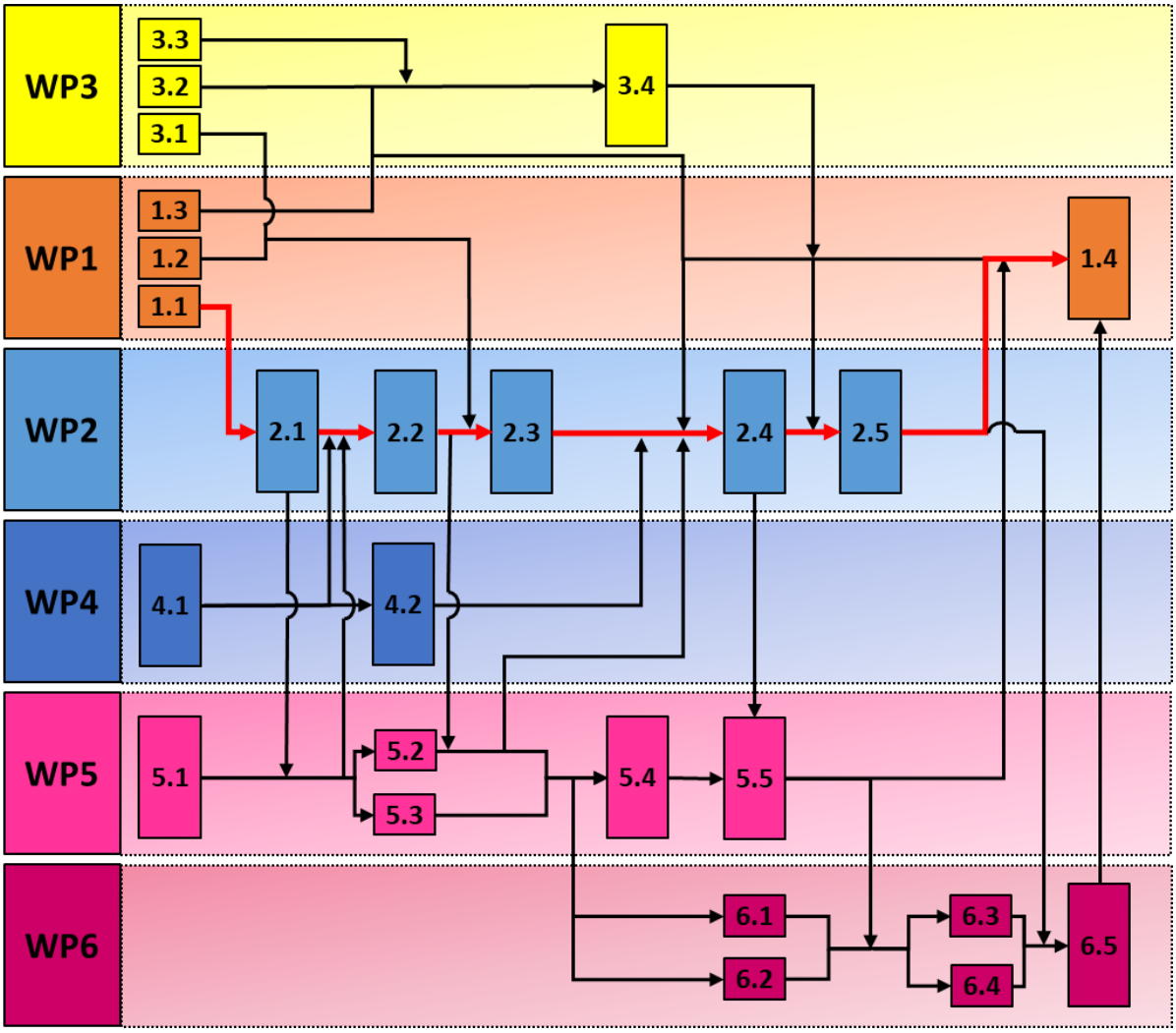

Figure 3.1b: Pert chart, illustrating the overall workflow of NanoVIB, how the different WPs with their main tasks link together, and with the main path in prototyping the next-generation super-resolution microscope system indicated with red arrows.

WP1: Platform Development

The initial aim of work package **WP1** is to provide LLG with two MINFLUX platforms that are fully functional for imaging in the visible wavelength range but feature an open optical design and modular, extensible application software. These platforms are to be equipped with the additional light sources, optics, detectors and control algorithms in order to extend them both to MINFLUX in the NIR wavelength range, to demonstrate correlative TPE TRAST and SRS imaging, as well as to evaluate two photon activation in MINFLUX microscopy (**Task 1.1**). As the project moves forward, AI will then focus on integrating novel SPAD arrays into the platform, developed by PII, that provide additional positional and timing information

about each detected photon, and which have the potential to increase the quality of MINFLUX images greatly (**Task 1.2, 1.3**). Towards the end of the project, the platform will undergo a third transition, to incorporate all insights gained during implementation, testing and successful application of the new technology to challenging biological problems in WP6, into a second generation MINFLUX microscope (**Task 1.4**). The timing of the different tasks is visualized in a Gantt chart (Fig. 3.1a).

The first task (**Task 1.1**) is expected to be completed within one year after starting the joint project, with the first platform being delivered after M6. With the first proof of principle of MINFLUX published in 2017 it is still a novel approach and AI's current implementation, launched as an end-user product in Feb 2019, accommodates this by being flexible both in its optical design and the details of data acquisition and analysis. This makes it an ideal starting point to add optical ports and software interfaces and allows smooth integration of the additional components in WP2**.**

To this end, a dedicated member of our optical engineering team will focus on assembling the platforms and AI will cooperate closely with LLG from the start. This will ensure an efficient deployment of MINFLUX platforms which allows NIR MINFLUX imaging at the KTH and KI site and ensures that a working prototype is available for further integration work at the LLG site.

When both platforms are on place (one at KTH, one at LLG), the optics engineer responsible for platform assembly will have a part of her/his work hours reserved to contribute to WPs 2, 5 and 6 and will collect feedback about optical stability and alignment protocols.

From the start, AI will also begin working on the two-step integration of array detectors into its acquisition logic. For an initial proof of concept, PII will be modifying its Gen I detection electronics to provide a configurable number of electronic pulse outputs, corresponding to photon detection events in different areas of the array. This will allow AI to integrate these novel detectors into the existing acquisition electronics (**Task 1.2),** with minimal changes to the FPGA implementation and electronics as the detectors can essentially be handled in the same way as single photon detectors currently used in MINFLUX microscopy. Nevertheless, by providing the additional spatial information to the acquisition sequencer and the data analysis algorithms, we expect a measurable improvement of the image quality. Through AIs flexible software interface, the partners within the consortium will be able to prototype and test these approaches and provide valuable feedback for the second implementation step.

In a second step, AI will enable the partners within the consortium to leverage all spatial and temporal information provided by PII's Gen II detection electronics **(Task 1.3**). A jointly defined communication protocol will allow the transfer of the arrival time and detector element for each detected photon to the MINFLUX electronics in real time during the acquisition process. However, AI's current platform is not equipped to receive and analyze this 'photon stream'. Therefore, AI will redesign and modularize the detection and acquisition control electronics and implement a new FPGA based platform capable of receiving the data from PII's Gen II detection electronics.

This requires close cooperation with PII who will be developing this Gen II detection

electronics for their SPAD arrays simultaneously. To ensure that partners KTH, KI and LLG have enough time in WP5 and WP6 to fully explore and exploit all possibilities offered by this improvement, both AI and PII will start their respective tasks as soon as NanoVIB is kicked off. Both companies are already engaged in a successful cooperation aimed at leveraging SPAD arrays for STED microscopy. Thus, with all the key personnel already acquainted, AI and PII will be able to design and finalize the specification of a communication protocol for detector data within 3 months after the starting date of the task. During the remaining part of the task PII and AI will continue to cooperate closely providing the respective partner with test hardware and consulting each other.

On AI's side, an electronics engineer will be hired as soon as possible and exclusively tasked with this project. As AI's current STED and MINFLUX platforms all employ similar techniques, the engineer will be provided with all necessary resources from the start and the project will be overseen by the lead developers of the STED and MINFLUX FPGA platforms. After the design phase, a test implementation will be realized on a commercially available FPGA evaluation board and verified with simulated detector input based on the specifications of the communication protocol.

This test implementation will be the basis to task a C/C++ developer with providing an interface that allows integration in the instrument control software, while the electronics engineer proceeds to the design, layout and production of required components in order to provide a module that is also compatible with traditional single photon detectors and the Gen I SPAD array detection electronics. In this way, testing can commence independently of the WP3 timeline. This module will replace the previous generation electronics in both deployed MINFLUX platforms by M32 making them ready for the integration of Gen II SPAD arrays.

At this time, the platforms will already have been in operation since M7, application development and regular imaging operation will have been under way since M9 and M25 respectively. With the AI optics engineer and the AI project coordinator closely tied into WP2, WP5 and WP6, sufficient feedback on improving both details of the optical design and the user interface will have been collected to start making design changes aimed at getting the technology ready to be demonstrated to and tested by researchers outside the project (**Task 1.4**). Those changes which can be incorporated into the existing platforms will be implemented directly and others will be used to design the final product.

Operation of the prototypes at both sites, within a lead application and as an open facility, will attract early adopters that both give additional feedback and form a pool of potential customers for the new MINFLUX generation. By the end of our project, AI will be in position to demonstrate NIR-MINFLUX in collaboration with KTH/KI and LLG.

WP2: Optical integration

The aim of work package **WP2** is, on the one hand, the demonstration of a multimodal super-resolution platform for correlative MINFLUX, SRS and TRAST imaging (**Tasks 2.2**, **2.4**) and on the other hand, the further development of the MINFLUX method as such (**Tasks 2.1**, **2.3**). The focus lies on the optical and mechanical integration of the components provided by the partners AI, PII and APE into the platform and on the development of acquisition

protocols, which are the basis for specialized strategies for bacteria and host cell studies within WP5 and WP6. To ensure know-how transfer between Göttingen and Stockholm, several research visits of scientists from KTH are planned. Optimization of the platform in **Task 2.5** will generate valuable feedback for the design of the partners' prototypes. The timing of the different tasks is visualized in a Gantt chart (Fig. 3.1a).

Already in the construction of the modular base MINFLUX platforms by AI (in Task 1.1), the requirements of the later device integration steps must be considered. This is especially true with regard to the wavelength-dependent optical properties of the components, since the final microscope platform will cover a wide wavelength range from UV to NIR. Therefore, the LLG will be involved in the platform design from the start of the project and supports AI in Task 1.1. Further platform development will be performed at LLG, with each step being accompanied by proof-of-concept measurements on test samples. During the course of the project, the platform is repeatedly upgraded with (improved) components provided by APE, PII and AI. After successful verification of each development step, a second identical platform at KTH will be upgraded to the new standard in order to allow for the parallel establishment of best practices and new labeling protocols (WP5).

In detail, as a first step (**Task 2.1**), the MINFLUX method will be extended to the NIR by upgrading two modular MINFLUX platforms with suitable excitation lasers and point detection modules. Platform I will be provided by AI at the beginning of the work package; platform II will follow at the end of year 1. A PhD student from KTH will support the LLG in Göttingen and familiarize himself/herself with the microscope and its usage. Upon successful implementation, platform I will be delivered to KTH and installed there by the end of year 1. The complex and sophisticated MINFLUX acquisition scheme, whose central part is an elaborate fluorophore localization procedure in which the fluorescent signal of a single fluorophore is iteratively analyzed for different positions of a doughnut-shaped excitation focus, is sensitive towards various parameters, like focus quality, background fluorescence and fluorophore photophysics. Therefore, taking into account the results of Task 5.1 and the therein identified suitable NIR fluorophore, the acquisition protocol will be adapted and optimized using the Python interface which allows the acquisition parameters, as well as the FPGA-controlled acquisition sequences to be accessed and modified.

Platform II at LLG will be used for initial research on combining MINFLUX with SRS (**Task 2.2**). This requires the implementation of an SRS-light source, which will be developed and then provided by APE within WP4, taking benefit from APE's expertise in SRS imaging. For correlative imaging, the mutual influence of both imaging modalities needs to be carefully analyzed in order to prevent artifacts, e.g. due to pre-bleaching of the fluorophores by the SRS acquisition. Therefore, proof-of-principle measurements for correlated SRS-MINFLUX imaging will be combined with the development of suitable acquisition strategies, maximizing the useful information content while minimizing the light dose and thus sample damage.

MINFLUX itself can be conceptually improved by using an array detector instead of a point detector (**Task 2.3**). LLG will therefore integrate a SPAD array into the setup in close

collaboration with PII, who modified the Gen I detection electronics in Task 3.1, and AI, who integrated the array into their acquisition electronics in Task 1.2. Taking the spatial information into account, new data analysis algorithms for background suppression and faster molecule localization will be developed using the Python interface of AI's application software.

With the upgrade of the SRS-light source with the femto-pico conversion add-on (from Task 4.2), the SRS-laser can be switched between picosecond and femtosecond pulse length operation. Using the latter allows to exploit the intrinsic optical sectioning capability of two photon processes in **Task 2.4**. Therefore, MINFLUX will be combined with two photon activation of the fluorophores, which spatially restricts fluorophore on-switching to the focal plane and thus potentially reduces the resolution-limiting background signal. As well, label-free two photon excitation TRAST metabolic imaging will be implemented for correlative TRAST-MINFLUX imaging, as performed within Tasks 5.5 and 6.2. Again, viable acquisition schemes are developed which take into account possible adverse mutual influences of the correlative imaging modalities.

The optimization of the platform in **Task 2.5** includes mechanical stabilization and workflow improvements. Further, the newly designed CMOS SPAD arrays will be evaluated and the photon stream-data made available by the Gen II detection electronics and AI's new FPGA platform will be used for extended data analysis. Our feedback on handling and performance of the components provided by the partners will give valuable insight for a commercial prototype design by AI in Task 1.4.

The WP is headed by Prof. Alexander Egner who is supported by Dr. Claudia Geisler, an experienced research assistant. The work in connection with optical and mechanical implementation and acquisition strategies will be performed by a PhD student in close cooperation with a postdoc and the visiting scientists from KTH.

WP3: Detector Development

The aim of WP3 is, on the one hand, to enable scalable integration of SPAD arrays into MINFLUX microscopes, and, on the other hand, to develop complementary metal–oxide–semiconductor (CMOS) SPAD arrays with enhanced red and NIR sensitivity. The main specifications to be achieved are as follows:

- Time-tagging electronics with 20ps timing resolution
- A peak quantum efficiency above 50% at 640nm
- Timing jitter of the SPAD with less than 250ps FWMH at 640nm
- On-chip time-gating with 1ns gate windows

PII will first modify existing detection electronics to integrate Gen I SPAD arrays in MINFLUX microscopes. Further, to utilize all 23 channels of the current SPAD array, PII will develop Gen II detection electronics and in parallel design new SPAD arrays with enhanced red and NIR sensitivity. Lastly, PII will develop larger SPAD arrays with chip-level time-gating. The timing of the different tasks is visualized in a Gantt chart (Fig. 3.1a).

In **Task 3.1** PII will modify its existing detection electronics (field programmable gate array

and printed circuit board, i.e. FPGA and PCB) to allow seamless integration of its green-detection-optimized 23-pixel SPAD arrays into AI's MINFLUX microscopes. The Gen I detection electronics will forward software-selectable SPAD array channels with intrinsic jitter through a reconfigurable FPGA. SPAD channels are going to be forwarded to 4 input channels available in the MINFLUX system.

Further in **Task 3.2**, PII will develop the Gen II detection electronics together with AI. Because the MINFLUX technique requires feedback loops (scan positioning depends on detector output), the firmware functionalities of photon counting/time-tagging and microscope control will be merged through a dedicated communication protocol. This will enable utilization of all 23 pixels of the SPAD array with full timing resolution. PII and AI will explore scalable electronics platforms with both parallel and serial protocols for communication. PII will further develop time-tagging functionalities with 20ps timing resolution and explore non-uniformities in terms of differential and integral non-linearity, as well as power versus area trade-offs.

To supplement its current SPAD array optimized for detection around the green spectra, PII will develop SPAD arrays with enhanced red and NIR sensitivity, targeting a peak quantum efficiency (QE) above 50% at 640nm and specially enhanced QE spectra between 700nm and 850nm (**Task 3.3**). The array will be optimized for off-chip time-gating, with a timing jitter of less than 250ps at 640nm. The new SPAD array will be made platform compatible with Gen II detection electronics. PII will analyze fill factor (ratio between detection area and pixel area) versus noise trade-offs.

As an outcome of these activities, PII will develop larger (10×10 pixels) SPAD arrays (small image sensors with 1Mframes per second) and develop a chip architecture to dynamically pre-select relevant photon events by chip-level time-gating in **Task 3.4**. Both chips will be supplemented with microlenses to utilize the intrinsic sensitivity of the SPAD array. PII will analyze and optimize additive and imprint microlens fabrication procedures. The larger SPAD array will be developed by integrating our partners' feedback on the deliverable of **Task 3.1**. Chips will be fully electro-optically characterized.

PII will hire a chip and FPGA designer to supplement the PII's experienced team. The designer will work in a close collaborative setup throughout **Task 3.1** and **Task 3.2**, visiting our partners AI, LLG and KTH for the development of Gen I and Gen II detection electronics. She/he will further continue designing novel SPAD arrays tailored for the MINFLUX application. Special emphasis is going to be placed on early data processing on the FPGA, utilizing the parallelized computation power of the FPGAs.

WP4: Laser for MINFLUX and SRS operation

The aim of **WP4** is to develop a new, easy to use one-box light source, which provides picosecond pulses for SRS imaging and femtosecond pulses for two-photo-activation in MINFLUX imaging and thus makes the usage of two separate complex and expensive laser systems obsolete. The light source should be completely computer controlled allowing for integration into microscope setups. Further, the tuning time of the current SRS light source picoEmerald should be improved by more than a factor of 10, from typically 60 to 120

seconds to less than 5 seconds in order to enable fast multispectral SRS imaging. The main parameters to be achieved are as follows:

- Automatic temporal and spectral overlap of pump and stokes beam for SRS
- Noise of the pump beam around 160dBc at 20MHz; modulation of the stokes beam at 20 MHz
- Wavelength tuning at least 700 to 990nm / 400 – 4600cm^{-1} wavelength difference for SRS, tuning time <5s
- Bandwidth of ps-mode ~10cm^{-1}, pulse length about 2ps, pulse length of fs-mode ~300fs.
- Several hundred mW output power in each beam in the femto- and the picosecond mode

To decouple the two major development steps, we have divided the work into two tasks. **Task 4.1** will concentrate on the fast tuning and **Task 4.2** on pulse length switching between femto- and picosecond operation. The timing of the two tasks is visualized in a Gantt chart (Fig. 3.1a).

The light source is based on an optical parametric oscillator (OPO) pumped by a ps-fiber laser. To achieve the goals described, a different tuning concept needs to be developed and the OPO needs to be completely redesigned. Further, APE plans to implement a modern and flexible electronic and software platform. A laser and optics physicist will be assigned to the work package throughout the project to develop optical schemes, perform optical tests, define requirements on electronics, mechanical design and software requirements and validate these results.

First, APE will focus on developing ultra-fast targeting of arbitrary wavelengths for picosecond lasers in **Task 4.1**. The current OPO tuning concept in APE's SRS light source is based on temperature tuning of nonlinear crystals. Even though the heating elements are optimized for speed, the limitations are tuning times of one to two minutes. To overcome this, different nonlinear interactions will be evaluated to tune the OPO by mechanical shift or rotation of nonlinear crystals as well as fast cavity length adjustments. Next to the specifications mentioned above, the following parameters need to be verified: nonlinear crystal degradation, conversion efficiency, beam quality and beam pointing over tuning. Mechanical actuators need to be validated regarding the required tuning speed and accuracy. A first assessment of the femto-pico conversion based on spectral broadening in the cavity will be performed. After this evaluation phase mechanical design of the OPO cavity and the overall light source will start. A mechanical design engineer at APE will be assigned to this task for about 6 months. To minimize the risk, the current picoEmerald platform has to be used as a starting point for the mechanical design. The electronic and software architecture used in the picoEmerald is not flexible to adapt to new tuning schemes and new features. Therefore, the electronic hardware will be switched to a modular architecture based on a mixture of CAN and I2C bus communication. The platform architecture described does already exist at APE, which does allow for efficient implementation and requires only 3 months work from an electronics engineer. For software development, a similar modular approach will be followed. APE will use a LabVIEW based modular software architecture, established at APE. Moreover, a software architect will be assigned at APE at the beginning

to allow for basic, scripting based tuning tests and later for the full software integration of the laser (7 months).

LLG plans to start Task 2.2 already in M13. APE will only be able to deliver a first prototype of a fast tunable SRS-light source in M22 (D4.2). To bridge the time and enable LLG to start working, APE will provide a loan SRS laser (picoEmerald) to LLG with D4.1 in M13.

APE will work together with LLG to demonstrate that the prototype can be integrated into the microscope setup and support them to demonstrate correlative SRS imaging with its expertise. To support LLG efficiently, APE needs to build a second prototype to verify and reproduce problems and fix possible issues. This prototype will also be the basis for the following task.

Task 4.2 will concentrate on switching the pulse length between about 2ps and 300fs to enable narrowband SRS imaging as well as MINFLUX with the same light source.

The work will start with the evaluation of spectral broadening of the pulses inside the optical parametric oscillator. Proof-of-concept work is already done in **Task 4.1** during the evaluation of new oscillator and tuning concepts. APE will study the concept in detail and implement it into software tuning and stabilization routines. Since the spectrally broadened pulses from the OPO will still have picosecond pulse lengths, it will be needed to develop pulse compressor designs based on diffractive gratings. The challenge here is to cover the whole tuning range of the picoEmerald with a high compressor efficiency of at least 50% and maintain beam quality and beam pointing. The coupling between spectral broadening and optimized compressor settings will be evaluated. APE will deliver a prototype femto-pico conversion upgrade of SRS-light source (D4.3) as an add-on for the prototype light source to LLG in M25. This is milestone M4 of the NanoVIB project.

A second compressor will be built to do software development and more close integration of the compressor and the light source. The feedback from LLG is taken to further improve software and optics. LLG will receive regular software updates for the light source and the compressor as well as hardware upgrades if necessary. For this work package APE will allocate 3 months of software engineering, 2 months of mechanical design and one month of electronic engineering resources in addition to the leading physicist. A matured prototype fast tuneable femto-pico SRS light source will be delivered to KTH in M31 (D4.4). APE will also produce a third prototype light source and compressor to be able to support KTH and LLG and finalize software and mechanical design within 6 months until end of M36. APE will allocate 4 months of software engineering and 1 month of mechanical design in addition to the laser physicist to this task.

WP5: Labels, acquisition and protocols

WP5 has two major aims: First, to identify fluorophore labels in the NIR suitable for MINFLUX imaging (**Tasks 5.1, 5.2**), second, to establish and optimize acquisition and sample protocols for the bacterial studies in WP6, allowing operation of MINFLUX (in the visible as well as in the NIR), SRS and TPE TRAST imaging by their own or in combination (**Tasks 5.3 – 5.5**). The timing of the different tasks is visualized in a Gantt chart (Fig. 3.1a).

Although MINFLUX does not require exceptional photo-stability of the NIR fluorophores, they need to display stable, controlled photo-switching properties to qualify for MINFLUX imaging, which adds other requirements on the fluorophores and on the sample preparation. In **Task 5.1**, KTH will therefore investigate a range of NIR fluorophores with respect to their photophysical properties in order to identify dyes with switching properties which lend them suitable for MINFLUX imaging, and KTH will study under which sample and excitation conditions such switching can be induced. In the first place, KTH will look for suitable NIR dyes within the same category of dyes as currently used for MINFLUX in the visible wavelength range. In the red visible range, the cyanine dye Alexa 647 (excitation at 640nm, photo-switching at 405nm) has been found quite suitable for MINFLUX imaging, and KTH will thus explore if the suitability can be extrapolated to other cyanine dyes in the NIR, and if so, verify their usefulness for cellular measurements, and under what sample and excitation conditions they work optimally.

Apart from taking MINFLUX into the NIR (with the benefits of an extended spectral range, opening for further multiplexing, lowered photo-toxicity, autofluorescence as well as scattering background), a major methodological progress aimed for in this project is to combine MINFLUX with SRS and TPE TRAST for correlative imaging. In **Task 5.2**, KTH will explore the prerequisites for combined two photon activation for MINFLUX (of NIR as well as visible dyes) and label-free TPE TRAST imaging based on NADH/Flavin autofluorescence, specifically, the parallel use of the excitation source, to minimize photo-bleaching/toxicity and to speed up image acquisition. Also, KTH will investigate the possible combined use of the laser developed in WP4 for simultaneous SRS and NIR dye excitation. For SRS the emission wavelength of the laser beams can be set over a broader range. As an option, the wavelength of one of them can then be set to match the excitation maximum of the NIR dye. Similarly, also here the benefit would be a lowered photo-bleaching/toxicity and a faster image acquisition. The outcome of the studies in **Task 5.1** and **Task 5.2** will provide useful feedback to the activities in WP4 and WP2.

Following the photophysical investigations above, the second major task of **WP5** is to establish and optimize the use of the MINFLUX instrument developed in WP2 for bacterial and host cell studies (to be carried out in WP6). The bacteria to be used here are mainly suitable mutants of pneumococci, and the host cells mainly cultured lung epithelial cells and immune cells. With the acquisition procedures determined in WP2 and in **Task 5.1/5.2** as a starting point, and using the MINFLUX instrument provided to KTH, KI will together with KTH first establish labeling protocols and sample handling/preparation of bacteria and host cells for VIS- and NIR-MINFLUX imaging (**Task 5.3**). For the most suitable VIS and NIR dyes, as identified in **Task 5.1**, we will then establish labeling procedures to specific relevant bacterial and host cell proteins and optimize the sample preparation to maximize the fluorescence and switching performance of the dyes in the samples. As studies will be performed on both fixed and live cells, different labeling strategies will be used, based on dye-labeled antibodies, as well as on modified dyes binding to genetically tagged proteins, following recently developed procedures for cellular studies by MINFLUX.[10] Likewise, the sample preparation and optimization of dye switching performance will be adapted

depending on whether the imaging is to be performed in live cells or not, and will be largely based on standard switching buffers, as used in single-molecule localization super-resolution.[18] Next, with support from visiting scientists from LLG, we will verify SRS and TPE TRAST imaging on bacteria and host cells (**Task 5.4**). SRS imaging, as established in WP2 and using a laser established in WP4, will be verified on bacteria and host cells, by analyzing major lipid vibrational bands in these cells (e.g. the 3015cm^{-1} band associated with C=C-H stretching modes in unsaturated fatty acids, or the CH_2 stretching mode of lipids at 2845cm^{-1}). Moreover, the cells will be grown in deuterated glucose, and SRS imaging of isotopically shifted bands will be verified, as an approach to be further used for isotopic labeling and the exosome studies in WP6. For TPE TRAST imaging, KTH will take its recently developed procedures[8] as a starting point, to establish and optimize this imaging on bacteria and host cells. To start with, KTH will use the same instrumentation as in Tornmalm et al.[8] for these studies, and then switch over to the fs-pulsed laser to be developed in WP4. Finally, within the constraints given by the dye photophysics, acquisition procedures and sample preparation, KTH will demonstrate the combined use of MINFLUX with SRS and/or TPE TRAST imaging (**Task 5.5**). This demonstration will be based on several preceding steps in the project, in particular on the integration and procedures established in WP2 and the preceding steps of this WP, and will establish best practices and the protocols for the bacterial studies in WP6. The gained knowledge will be transferred to AI in order to be considered in the commercial prototype design.

The WP is headed by Prof. Jerker Widengren who is supported by Dr. Joachim Piguet, an experienced researcher. The work in connection with tasks 5.1 – 5.5 will be performed by a PhD student in close cooperation with a postdoc. They will be supported for tasks 5.4 and 5.5 by visiting scientists from LLG and for task 5.3 by a postdoc from KI.

WP6: Lead application and dissemination

WP6 will effectuate two important final outcomes of the NanoVIB project: First, using platform I and as a lead application of the VIS-NIR-MINFLUX system developed in this project (WP1-4), utilizing the unique resolution and imaging capabilities of this microscope system, KI and KTH will reveal inter- and intracellular processes underlying pneumococcal virulence and invasiveness. Second, based on platform II, LLG will establish an open end-user facility to a broad group of potential end-users, to trigger the interest for the developed technologies and procedures in cell biology and biomedicine, and pave the way for the dissemination, exploitation and use of the developed microscopy system.

Lead application (**Tasks 6.1 – 6.4**): Pneumococcal virulence and invasiveness strongly depend on the properties of certain proteins on their surface, how they distribute themselves on the surface, and how their localization patterns are related to e.g. the cell cycle and the local cellular and outer cellular environment. In recent work by partners KTH and KI it was demonstrated that specific spatial distribution patterns of pneumococcal surface proteins can

[18] van de Linde S et al "Direct stochastic optical reconstruction microscopy with standard fluorescent probes" *Nat. Protocols* 6, 991 – 1009, 2011

be resolved by super-resolution STED microscopy, revealing important information about underlying mechanisms for disease and invasiveness of the bacteria.[3,4] In this WP, with the ten-fold higher resolution offered by the MINFLUX microscope system as compared to other super-resolution microscopes, KI will demonstrate that an additional major leap in the understanding of these mechanisms can be achieved. With this resolution increase, coming even closer to the actual spatial scale of the protein interactions, with correlative morphological and environmental images by SRS and TPE TRAST, and with protocols and procedures for bacteria-host cell studies established in WP5, KI will address several central aspects of pneumococcal proteins and their role in disease:

Bacterial virulence and host defense evasion (**Task 6.1**): KI will study surface-associated pneumococcal proteins and focus on two proteins with central, but yet incompletely understood roles in virulence and host defense evasion. The studies will be done on pneumococci in different states of cell division, and on different strains with deleted or modified forms of these proteins. First, KI will study the surface protein PspC, known to bind factor H, in turn preventing immunological attacks by complement binding. KI will study how different variants of the surface protein PspC distribute on the bacterial surfaces, to what extent they bind factor H, cover particularly vulnerable division zones of the bacteria, and if and how this can be affected by additives having potential antibiotic or bacteriostatic effects. LytA, a pneumococcal protein that mediates autolysis of the cell wall, will be studied in a similar way: how it is brought to the surface from the cytosol and distributes on and between bacteria. KI aims to better understand how it is activated upon penicillin treatment, as well as the function of this protein, which still remains controversial. These studies will be performed on fixed cells, following similar labeling protocols as for previous STED studies,[3] but now with the ten-fold higher resolution of MINFLUX, and with the protein distribution patterns correlated to the cellular morphology by SRS imaging.

Invasiveness (**Task 6.2**): Pneumococci, the main cause of bacterial meningitis globally, pass the blood-brain-barrier (BBB) with help of pilus proteins binding to receptor proteins on epithelial cells of the BBB. In a recent work by KTH and KI,[4] based on protein co-localization studies using STED, KI could identify two receptor proteins on the epithelial cells to which the pneumococcal adhesion proteins RrgA (a pilus protein) and PspC bind. In this WP, KI will use MINFLUX, with its ten-fold higher resolution, to take these studies to a next level. KI will study the co-localization of pneumococcal surface and pilus proteins with receptor proteins on epithelial cells of the BBB, in brain biopsies from patients who have died from pneumococcal meningitis and from mice models. Samples will be prepared following established protocols, with possible modifications for MINFLUX imaging based on the outcome in WP5. With MINFLUX extended into the NIR, these studies can be performed with significantly lower background, scattering, deeper penetration, and with less cross-talk. With overlaid SRS images, the protein (co)localization patterns can also be correlated to the morphology of the bacteria and the cells of the BBB. In samples from mice, KI will study effects of adding anti-bodies competing for binding to the BBB epithelial cell receptors as a potential approach to prevent pneumococcal meningitis.

Bacterial exosomes, their possible role in disease and as a basis for vaccines (**Task 6.3**): Recent studies by KI have shown that pneumococci can form extracellular vesicles (bacterial exosomes), that they can be internalized into host cells and elicit cytolysis and inflammatory reactions,[7] but can also generate immunization with serotype-independent protection in mice (unpublished). To better understand the role of pneumococcal exosomes in disease development, how they are formed, and their potential as candidates for new vaccines, KI will use the MINFLUX microscopy system to study the nanoscale localization of protein virulence factors, including the cytotoxin pneumolysin, PspC, LytA, RrgA etc, within the exosomes. KI will investigate if there are differences between different pneumococcal strains and how the vesicles bind and internalize with host cells (epithelial and immune cells). By overlaying the protein localizations onto SRS images showing morphological cytoskeleton and lipid membrane maps of bacteria, host cells and vesicles, KI will further elucidate the role of the proteins in vesicle formation and in subsequent host cell interactions. Inflammatory reactions on host cells will be studied by TPE TRAST imaging, adding whole bacteria, pneumococcal vesicles, or specific bacterial antigens (e.g. pneumolysin). These images, reflecting local redox status and oxygenation in the cells, will then be correlated to nano-scale localization patterns of protein virulence factors.

Pneumococcal-viral coinfection (**Task 6.4**): By using the next generation super-resolution microscopy of this proposal, KI will study distribution patterns of pneumococcal surface proteins, coupled to sustained bacterial growth in lungs of pneumococcal-influenza virus co-infected mice with a focus on the pneumococcal protease HtrA. HtrA is central for bacterial growth during inflammatory conditions and protects and removes misfolded (oxidized) proteins. KI will study how the distribution patterns and localization of HtrA correlate with the microenvironment of the host, presence of antioxidants, and with the ability of the bacteria to evade immunological attack by imaging the degree of complement deposition on their surface. In this way, we expect to unravel important metabolic aspects in bacterial-viral co-infections, which seem to be a major driving force in such infections, and thereby also find better strategies to curb these infections.

End-user facility (**Task 6.5**): During the last year of the project, one of the next-generation super-resolution MINFLUX microscope systems established in the project will be set up by LLG as an end-user facility, open to a broad group of researchers from academia as well as from companies. Thereby, potential end-users will get the possibility to evaluate the capabilities of the MINFLUX microscope system for their applications, via guided, hands-on pilot experiments. Together with the lead application (**Tasks 6.1 – 6.4**), this will additionally spur the interest for the microscope system, lasers and detector systems developed in the project, and pave the way for the further dissemination, exploitation and use of the developed microscopy system.

Finally, the experience from both the lead application above and from the pilot end-user experiments in the open facility will be brought back to the SMEs in this project (AI, PII, APE), providing them with important feedback for further refinement on their technologies.

The WP is headed by Prof. Birgitta Henriques-Normark who is supported by Dr. Anuj

Pathak, an experienced researcher. The work in connection with **Tasks 6.1** – **6.4** will be performed by two postdocs from KI and one postdoc from KTH. Work in connection with the end-user facility will be performed by one postdoc from KI and one postdoc from LLG.

WP7: Project management and communication

Activities within this WP are outlined in detail in section 3.2.

WP8: Ethics requirements

Ethical aspects and the three deliverables within this WP are covered in section 5.1.

3.2 Management structure, milestones and procedures

NanoVIB is an ambitious multidisciplinary project and will need to be managed intelligently to overcome technical hurdles, mitigate risks, and achieve its scientific targets. Best-practice management approaches will be used, aligned with the Grant Agreement and the pre-signed Consortium Agreement. The project will be led by the **Project Coordinator (PC)** and managed by the **Project Management Committee (PMC)**. Each Work Package will be led by a **Work Package Coordinator (WPC)**. The PC will be assisted in the administrative and management tasks, as outlined below, by a **Project Manager (PM)**.

The **Project Coordinator (PC), Prof. Jerker Widengren**, will be responsible for administrative and financial co-ordination as well as for monitoring the overall progress of the project. He will steer the scientific and innovative direction of the project, ensuring cross-fertilization and synergies, and manage overall project activities and liaising with the EC. He will chair the meetings of the PMC, composed of one executive representative of each of the participants, take on all tasks and responsibilities of the coordinator as identified in the Commission grant agreement and work closely with the WPCs to ensure excellence in research and technology development, timely progress and reporting of deliverables and milestones (see tables 3.1c and 3.2a). The Coordinator will assemble all reports required by the EC and execute action points raised by the PMC. Project reporting periods are M19 (PR1), M37 (PR2) and M49 (PR3).

Prof. Widengren has extensive previous experience of project management from several national, bilateral, as well as multi-national research projects, funded by EU or by other sources. He was the coordinator of the highly successful EU FP7 project FLUODIAMON, which pioneered the use of super-resolution STED imaging for diagnostic applications, and from which this proposal in several ways is starting off. He is presently heading a successful research unit of more than 30 persons and has organized and chaired many larger international conferences and meetings, in Sweden and elsewhere. Both in research, education and innovation, Prof. Widengren has taken on several tasks as a coordinator to promote cross-disciplinary activities, in particular between KTH and KI, Stockholm. Being both a physicist and a physician, Prof. Widengren combines in-depth knowledge in ultrasensitive and ultrahigh resolution fluorescence spectroscopy/imaging, physics and clinical medicine in a unique way, which in particular for this multi-disciplinary project is a strong asset.

For financial and legal aspects Prof. Widengren will have major support from the administrative functions at KTH. KTH is one of the leading research organizations in Sweden taking part in the EU framework programmes and has so far been involved in more than 200 and managed more than 15 H2020 projects.

The **Project Management Committee (PMC)** will have one permanent representative from each participating organization. Each organization will also be asked to nominate a proxy. The PMC will meet at least every 3 months, alternating between video conferences and face-

to-face meetings, at milestone and key decision points, or more often if needed. The PMC will support the coordinator in monitoring project progress and ensuring that deliverables are completed in line with the commitments of each partner. The PMC has responsibility for the success of the project, and all the technical, legal, ethical and regulatory aspects. The PMC will also take decisions concerning patenting, dissemination and exploitation of the results. Key items on the agenda will furthermore be the identification of potential problems and the possible need for revisions of the project plan. The PMC will log and assess risks and give recommendations for solutions in case critical risks would occur (see table 3.2b). The PMC will seek consensus when making decisions. Through the PMC, the Coordinator will facilitate the resolution of disagreements through fair and transparent decision-making processes. Each organization represented will have 1 vote with the coordinator having a casting vote in case of a tie. The aim is however to have consensus in all major decisions.

The **Work Package Coordinators (WPCs)** will be responsible for coordinating the work of all partners in the respective work package, overseeing that deliverables and milestones are reached on time, reporting progress within the work package in project meetings and in the contractual reports to the Commission and having regular contacts with the PC concerning progress within their work package. The WPCs are Schönle (WP 1), Egner (WP 2), Antolovic (WP 3), Rimke (WP 4), Widengren (WP 5, 7, 8) and Henriques-Normark (WP 6).

An **Advisory Board (AB)** consisting of a scientific representative (**Nobel Laureate, Stefan W. Hell**), a representative of the optical industry (**former CEO of Leica Microsystems, Martin Haase**) and the biopharmaceutical industry (**Science Relations Director AstraZeneca, Anna Sandström**) will closely follow the progress of the project. The AB members will provide valuable feedback and advice from their different expertise, experiences and perspectives. The AB members will take part at yearly meetings with the PMC, where topics such as project progress, management, and supervision of innovations as well as measures on exploitation and dissemination will be discussed.

An **Innovation, IPR and Exploitation Management Group (I^{2}EMG)** led by the PC will promote an effective management of the intended innovations, the protection of intellectual property and the sustainable exploitation of results. Core task of **innovation management** is the regular and systematic screening of the current scientific and market development in the targeted fields with the objective to open up new opportunities and competitive advantages for the consortium. Potential challenges will also be addressed in this context. Regarding **IPR**, recommendations will be compiled as to how the acquired intellectual property can be optimally protected with respect to the project's overall objective, while simultaneously considering the individual interests of all partners. Furthermore it is reviewed whether 3rd party components have to be licensed in. While **commercial exploitation** (Fig. 2.1a) will be mainly driven by the industrial partners at their own expense, the I^{2}EMG will monitor that the actions in the project to promote exploitation remain effective and optimize the final outcome. **Scientific exploitation** will be achieved by the academic partners through publications and conference contributions. I^{2}EMG will be responsible for the coordination of these activities.

Before the start of the project a **consortium agreement** will be set up, containing legally enforceable provisions relating to dispute resolution and notice periods. It will specify especially **IPR** aspects of the project, but also aspects concerning project coordination, governance structure, responsibilities and financial provisions. The Research Support Office of KTH, together with its innovation office (KTH Innovation) have extensive experience of handling IPR matters and formulation of consortium agreements for EU projects, and will provide active expertise assistance in the preparation of the consortium agreement. Such an agreement will be in place before the start of the project. The overall responsibility of the IPR management within the project will fall onto the PC and the PMC.

Consortium wide NanoVIB meetings, will be held twice annually to ensure cohesiveness between different WPs, and will alternate between video and face-to-face meetings. The location of the annual face-to-face meetings will alternate between the facilities of the consortium partners, The main part of the meetings will cover scientific project work and will be open to everybody working actively in the project, also the PhD students. Before each of the following meetings the WPCs will compile reports on progress and submit these to the PC, who will circulate them to all PMC members.

The **PMC will meet directly after each project meeting**. Standard items on the agenda will be;

1) Minutes from last meeting
2) Assessment of project work in relation to deliverables and milestones
3) If necessary, decisions on corrective measures based on project progress or on findings of the I^2EMG
4) Planned activities during the next period
5) Communication activities
6) Scientific publications and IPR matters, including identification of potentially patentable results
7) Financial matters
8) Ethical and gender issues
9) Other relevant issues.

A **project web page** will be set up directly at the start of the project. It will contain one part open to the public (D7.3), providing general information about scope, objectives and results, and one part with password access only for project participants (D7.1). The closed page will be the main route for internal communication and will contain all project key documents, such as the grant agreement with all annexes, especially the description of work. There will be information and templates for reporting and cost statements, and guidelines for administrative matters. Continuously updated information about progress made in the respective work packages will be available, as well as all information in relation to different meetings, such as agendas and minutes. A well-functioning web page will be the

responsibility of the coordinator.

As already explained in section 2.2, a **data management plan (DMP)** which meets with H2020 requirements for Open Research Data will also be developed and implemented (D7.2) during the first six months of the project. This enables access to all data and routines for the entire consortium (shared domain). Due to IPR considerations we will have to make a distinction with regard to access of the general public (public domain) to this data: Software routines for hardware control represent proprietary IP and know-how of the companies of the consortium and are only made publicly available in compiled form in connection with the corresponding products. Software routines for data evaluation and raw measurement data will be freely accessible. However, a general disclosure will only be made after a thorough assessment whether the data and routines have to be embargoed for a certain period of time due to legitimate interests of the scientists (planned publication) or the research institutions (planned patenting). Any lifting of an embargo requires the approval of the project management committee (PMC).

The **consortium** is set up as a **highly multidisciplinary co-operation and a public-private partnership**. To facilitate the necessary communication over the disciplines, and better mutual understanding of the possibilities, needs and prerequisites within the other fields and sectors involved, means have been allocated in the budget for **frequent exchange and visits of staff between the different partners**. In addition, the opportunity will be taken in connection to the project meetings to arrange internal as well as open training sessions and seminars (D.7.6), as well as site visits to the different facilities of the project partners.

Implementation risks and mitigating actions

While every ambitious research project has the inherent risk of not producing the expected outcome, we feel that the risks associated with the technology development part are minimal except for the specific aspects outlined below which do not threaten the core technological objective of the project: Extending MINFLUX to the NIR wavelength range and to the image live cells thus making the technique a potential standard imaging tool in biomedical research. This goal will require complex research into the spectroscopy of dye candidates and into novel strategies for data acquisition and analysis and will indeed pose challenges. However, these challenges are not greater than those that have been overcome by the project partners AI and LLG together with the MPI for Biophysical Chemistry during the development of the current STED-microscopes, and mitigation strategies successful then will help us achieve the expected results.

Developing suitable protocols for sample preparation and imaging of the targeted bacteria may prove more challenging than we expect, giving our lead application not enough time to produce excellent, convincing results within the project. However, concerning fluorophores, sample preparation, as well as excitation and photo-activation procedures, there are numerous established protocols used in current imaging applications, bearing major similarities to the ones planned in this project. The probability that no one of them would be viable under the conditions used in this project is small. Moreover, the two MINFLUX prototypes that will be constructed in WPs 1-4 will remain at KTH and LLG after the project is finished, as

permanent loans. In worst case, if there would not be time to produce the expected results in the lead application, this will ensure that the study proposed herein, projects started by third parties during the demonstration phase, and all possible, promising follow-up studies, can continue without interruption even after the four-year period of the project. Again in the worst case, we anticipate that this would merely result in a delay of our exploitation plans would result while the main objectives of our proposal would still be accomplished.

The objective of combining MINFLUX in the NIR with coherent Raman based imaging methods may limit the number of usable dyes if both methods are to be applied simultaneously or iteratively due to interaction of certain dye molecules with wavelengths used for Raman imaging. In this case we would focus on selecting the best possible MINFLUX dyes, even if it will initially limit us to Raman imaging only after the MINFLUX analysis is complete. Even in this combination, the complementary structural and chemical information will be very appreciable and the search for suitable dye candidates for correlative imaging without restrictions can be postponed. Similarly, two-photon activation (TPA) for MINFLUX with the novel laser developed as part of this project could turn out to put too much strain on some of our dye candidates. Weighing this against the potential benefits (reduced background, limitation of activation to a single plane in 3D samples, live cell compatibility) is part of WP5. Even if TPA will turn out to be unusable for all dyes considered, addition of the fs pulse feature to the coherent Raman laser would still add significant value, enabling two-photon excitation (TPE) microscopy and other nonlinear imaging techniques to be performed on the system.

Some specific risks have been identified by APE and PII in their development of the red sensitive SPAD arrays and the fast tuning fs-ps laser. Both companies have identified several possible mitigation strategies and the overall risk of significant delay is low. The project can move forward even if deployment of the light source is delayed: As outlined above, TPA is not vital to the overall success of our project, and even if fast tuning in SRS imaging were available later than anticipated, proof of principle could still be achieved with the loaned state-of-the art light source. If production of “red” SPAD arrays is delayed, we will use the “green” optimized version to verify our strategies for exploiting spatial information and have the option to either use single APDs until the new array becomes available, or temporarily compromise on quantum efficiency using the “green” version.

To achieve better MINFLUX images by exploiting the large amount of additional information about each detected photon (provided by the next generation SPAD arrays) requires the development of novel approaches to data acquisition, both on the electronics and the software side. To avoid a possible delay of WP2, WP3 and WP5, PII and AI have agreed on the following strategies to mitigate the implementation risks a re-development of this scale poses: (1) We will provide a preliminary solution, only delivering partial spatial information through a minor modification of the existing technology, so implementation and testing of the analysis tools needed can commence as soon as possible (Task 3.1 and 1.2). (2) The new electronics will rely on programmable FPGA hardware that allows fast development cycles without the need of physically changing hardware in the prototypes and (3) we will use ready-to-use demonstration/OEM boards wherever possible to avoid undue delay. As the

focus during the project is on providing usable prototypes as fast as possible, achieving commercial grade stability of operation and subsequent production transfer may prove more challenging than we currently anticipate. In case of such a delay AI will start exploitation using the preliminary solution, as described above, and will guarantee an upgrade as soon as the development of the new electronic components is finished. It is AI's experience that this is acceptable for a significant number of early customers who appreciate the head start with the new technology. Also, because MINFLUX is exclusively licensed to AI and currently unrivalled in resolution, we only anticipate a delayed generation of revenue as the worst-case scenario.

Finally, there is a low risk that we over-estimate the significance of the nanoscale protein distribution patterns of pneumococcal surface proteins. Our previous data using STED microscopy support that localization and distribution patterns of pneumococcal surface proteins are closely associated with their function. However, even conclusively showing that spatial organization of several pneumococcal surface proteins down to the molecular level is not correlated to the virulence of pneumococci would constitute a major scientific contribution and lay the ground for further investigation into alternative proteins or mechanisms beyond the timeline of this project. The goal of showcasing the new MINFLUX system would still be achieved. In addition, due to our early engagement with the end user group and direct contact to the biomedical research community, we expect to identify worthwhile projects to be started during open facility operation. This would help us identify other early applications that will help establishing the method.

Communication and dissemination activities

Communication will be set high on the agenda to promote the project actions and its results. Given the highly multidisciplinary character of the project, its potentially huge implications on societal health and well-being, and its significant exploitation potential, there are many relevant target audiences for the communication activities in this project. The plans for the communication activities and the main target groups are detailed in section 2.2b. As part of the communication activities, and to promote dissemination and exploitation, an **end-user group (EUG)** will be formed at the beginning of the project (D7.4). The members of the EUG will represent a broad scope of potential stake-holders of this project, and a major purpose of this group is to identify end-users, and to provide advice on how to adapt the scope and activities of our communication efforts to reach these, and for best possible impact.

Dissemination is also a major priority in the project. As a major strategy for dissemination, a microscope system in the project (platform II) will be made available as a facility, open to a broad group of researchers from academia as well as from companies. This will spur the interest in the microscope system, lasers and detector systems developed in the project and promote their exploitation. This strategy will be complemented by a second strategy: By generating research of highest international standard in the project, providing a lead example of the capabilities of the developed microscope system to resolve intra- and intercellular processes of large biomedical relevance, we will further promote interest in the technique and its use, and thereby increase the demand for the instruments to become commercially available by the end of the project.

3.3 Consortium as a whole

The call addressed in this application is quite multidisciplinary in its character, and it is thus a challenge to cover the full disciplinary span of expertise needed for a consortium in this call. Also, the expected impacts the project will contribute to are ambitious and multi-faceted, including fundamental insights in biology and medicine (significant gain in understanding of inter- and intra-cellular processes) as well as impact on the industrial exploitation side in biophotonics (strengthen Europe's industrial position in the biophotonics-related market for microscopes and research and development tools). However, our consortium has a profile of expertise well adapted to the objectives of the project, and the necessary interdisciplinary width, spanning from solid state physics, detector technology, optics, laser technology, microscopy, data acquisition and processing, fluorescence and Raman spectroscopy, over fluorophore chemistry and photo physics, molecular and cell biology, all the way to clinical bacteriology. Moreover, the consortium includes three SMEs active in biophotonics (AI, APE, PII) with long successful track records of close interaction with academia, a research institute placed in the border zone between private industry and academia (LLG), and two academic partners from large renowned universities (KTH, KI), such that we have the competence, experience and prerequisites to meet the expected impacts.

In the forefront of this application is the recent, very exciting progress in super-resolution microscopy, where the MINFLUX imaging technique has raised the level even further. With the ten-fold higher resolution offered by this technique compared to state-of-the-art super-resolution microscopes available today, and with additional development potential to be addressed e.g. in this project, MINFLUX indeed will be a cornerstone in the next generation of super-resolution microscopes. **Abberior Instruments GmbH (AI, partner 3)** is a spin-off company from the research group of Stefan W. Hell (Nobel laureate 2014), where also the MINFLUX technique was invented. AI is the unquestionable market leader in super-resolution microscopy. With numerous strong recruitments of postdocs and senior researchers from the research group of S. W. Hell, AI has by no comparison stronger competence in super-resolution microscopy than any other microscopy company worldwide. With the MINFLUX technique in the forefront of this project, AI adds considerable strength to this consortium, and the contributions from AI will be instrumental, in particular to the detailed objectives I, V and VII as set out for this project. AI will be main responsible partner for WP1.

Yet, while a ten-fold increase in spatial resolution compared to current super-resolution microscopy techniques already gives a substantial potential for the MINFLUX technique to enable prominent gain in the understanding of inter- and intra-cellular processes, we aim in this project to raise this potential even further:

First, we will expand the spectral range of MINFLUX into the NIR. This will make it possible to perform imaging with lower background, increased penetration, and with extended multiplexing, with less cross-talk and higher specificity. To make this expansion into the NIR possible, **Pi Imaging SA (PII, partner 5)** will develop photon detector arrays with enhanced sensitivity in the NIR. PII is world-leading in the development of single-

photon sensitive detector arrays, and the expertise of PII will be critical to establish such detector arrays into MINFLUX. Together with AI, PII will implement their detector arrays into the MINFLUX microscope platform, which will result in lower background, faster image acquisition and access to the NIR spectral range for imaging and increased multiplexing. PII's contribution to this activity is an absolute requirement, and so is its contribution to the detailed objectives II, III, V and VII. PII will be the main responsible partner for WP3.

Second, although MINFLUX offers multiplexed mapping of particular protein localizations and co-localizations in cells, the biological significance of this information will be considerably increased if this mapping can be put into a morphological, environmental, or microchemical context. To make this possible, we will implement stimulated Raman scattering (SRS) imaging into the MINFLUX prototype platform, to provide vibrational imaging of specific lipid- or cytoskeleton-structures in the cells, or of their metabolites. **Angewandte Physik und Elektronik GmbH (APE, partner 6)** is a world-leading provider of pulsed, narrow line-width lasers for SRS, and in their use for SRS microscope imaging. In the project, APE will provide laser prototypes, precisely tunable not only spectrally but also with respect to the pulse widths. These laser prototypes will be implemented into the MINFLUX platform for multifunctional use, for SRS imaging, dye photo-activation (as required for MINFLUX operation) as well as for two photon excitation TRAST imaging for provision of metabolic state images of cells overlaid with the MINFLUX images. The laser prototypes developed by APE for this project will be necessary for the addition of the SRS and TRAST imaging capabilities into the MINFLUX platform, which will require a close interplay between in particular AI and LLG in the project. The contributions from APE are required for several of the detailed objectives (IV, V, VI and VII), and APE will be the main responsible partner of WP4.

The central task of optical integration of lasers and NIR detector arrays rendering a multi-functional MINFLUX instrument lies on **Laser-Laboratorium Göttingen e. V. (LLG, partner 4)**. As a research institute with strong resources and world-leading competence in super-resolution microscopy and laser technology, LLG has the capacity to take on this task, which would have been difficult to do by any of the SMEs, which by necessity have to focus their activities towards development of their core technologies. Also, the technical and engineering effort required for this task lies a bit outside of what an academic research group at a university would be able to handle within their research activities. The contributions of LLG in many ways link the overall instrument development in the project together and prepare for the lead application and dissemination parts to follow and are crucial for the project. LLGs contributions are key for the project objectives I, II, V and VII. LLG is also main responsible for WP2 and has a key role for the dissemination by establishing and operating the open MINFLUX facility in the project.

Following the establishment of a multi-functional MINFLUX microscope platform extended into the NIR, the next step is to implement it for cellular studies, and more specifically for studying bacteria-host cell interactions and revealing mechanisms underlying pneumococcal disease. To render this possible, considerable effort must first be paid on optimizing the sample preparation, explore and identify fluorophores in the NIR that are compatible with

MINFLUX and identify the proper excitation, photo-activation and acquisition conditions. The partner group from the **Royal Institute of Technology (KTH, partner 1)** has a very strong record in fluorescence-based single molecule spectroscopy, not the least in the photophysical aspects. Moreover, KTH also has long experience in super-resolution imaging and pioneered its use for subcellular diagnostics, first in the cancer field (e.g. as the coordinator of the EU FP7 project FLUODIAMON), then in bacteriology (together with the KI partner in this project). Based on this experience, KTH will be ideally suited to establish the multifunctional MINFLUX instrument for bacteria-host cell studies, and then together with the KI partner, take the full benefits out of this instrument in the studies of the inter- and intra-cellular processes underlying pneumococcal disease. KTH's contributions will be absolutely necessary for the objectives V, VI and VIII. KTH is the main responsible partner for WP5, and with Jerker Widengren having a background both in engineering physics and clinical medicine, and with a successful track record as coordinator of a larger EU project on a similar topic (FLUODIAMON) KTH will have the proper experience and competence to take on also the task as coordinator of this project (WP7).

The partner group from **Karolinska Institutet (KI, partner 2)** has a very strong research record in clinical bacteriology, in particular on pneumococci, where the group is internationally leading. Since the research of the group goes all the way from studies on the molecular mechanism underlying pneumococcal disease, to studies on mice, patient samples and even epidemiology, the studies in this project can be efficiently guided towards retrieving the clinically most relevant information. KI also already has all the contacts, resources and competence necessary to provide the samples needed for these studies. Thereby, and in close interaction with KTH, and with the unique information that can be acquired by the MINFLUX system, we will indeed be well settled to achieve a significant gain in the understanding of inter- and intra-cellular processes underlying pneumococcal disease. In the project KI is main responsible for WP6, and for the fulfillment of the objectives VI and VIII, the participation of KI is absolutely required.

As indicated above, the consortium covers a broad interdisciplinary expertise. All the consortium members adequately complement each other and are all required to take the chain of activities all the way, from the start to the end. Since the project builds on the development and subsequent use of a prototype microscope system, harnessed with new laser and photon detector technology, a significant part of the project budget had to be allocated to equipment costs at the SMEs (AI, PII, APE), where the prototypes of microscope system, lasers and detector arrays will be developed and made available to the project, and at LLG, where the optical integration of the microscope will take place. The equipment costs (as specified in section 3.4) and the person months of each partner are adequate and proportionate to their contributions in the project. In the project, some competence overlaps do exist among the partners. For instance, partners AI, LLG and KTH all have a strong record in super-resolution imaging, however, from different points of view (AI, LLG: optics, instrument development, KTH: implementation, sub-cellular diagnostics). Also, APE and LLG both have a strong competence in laser technology, but again of a complementary character (APE: specialist in pulsed, tunable, narrow-band lasers, LLG: implementation of lasers in

microscopy/spectroscopy). Overall, this competence overlap will be necessary to bridge the different activities in the project.

The two academic partners (KTH and KI), with very strong records in bioimaging and clinical bacteriology, will have the major roles in the last two WPs, and research of highest international standard is expected here. However, we expect such research as an outcome already from the optical integration (WP2) stage, shifting in subject from photonics, bioimaging over to clinical bacteriology and cell biology, within the course of the project, and towards WP6.

By generating research of highest international standard in WP5 and WP6, we will provide a lead example of the capabilities of the prototyped microscope system to resolve intra- and intercellular processes of large biomedical relevance. Such lead example will spur interest in the technique and its use and promote the demand of the instruments to become commercially available after the end of the project. As a complementary strategy to promote the interest in the microscope system, lasers and detector systems developed in the project and to promote their exploitation, one of the microscope prototypes will also be made available as a facility, open to a broad group of researchers, from academia as well as from companies. This facility will be located at LLG, which has the resources and competence to maintain its operation, and as a partly industry-financed entity, also has the channels to act as a link between end-users and the SMEs.

Industrial/commercial involvement in the project

The strong representation of SMEs in this consortium, with each of the three SMEs having ample experience from close collaborations with academia, and of how to take benefit of such collaborations to promote their products, will further facilitate the industrial exploitation outcome of this project:

APE: The aim of APE is to develop a prototype of an easy to use and robust light source, which is capable to do SRS, two photon activation as well as TPE. To ensure the commercial success of such a product in the end, a system integrator and lead users of the overall system are absolutely necessary for practical validation. Only within this framework APE is able to test and optimize the light source for the given application. Even though such product will not be exclusive for the microscope setup being prototyped in this project, or the lead application aimed for in this project, a common marketing and sales based on the project will strengthen the market position of APE, as well as of all the three commercial partners.

PII: PII has a strong commercial interest to develop SPAD arrays with enhanced red and NIR sensitivity. There is an industrial need for it, driven by eye safety regulations in the automotive market and by the need to reduce photo-toxicity and expand the spectral detection range in life-sciences. The consortium will give PII the opportunity to develop the new SPAD technology in an application driven environment. This will bring the technology closer to lead users (instrumentation laboratories and innovative companies), the key community for the exploitation of SPAD arrays.

AI: Rapid commercialization of novel and groundbreaking nanoscopy methods, typically

customized, is at the core of Abberior Instruments' business model. The short development cycles necessary to claim these new markets can only be achieved by very close and trusting collaboration with both, component manufacturers and researchers developing, applying and validating those new methods. This consortium brings together the ideal mix of partners to establish MINFLUX as an essential addition to the toolbox of biomedical and biopharmaceutical research.

3.4 Resources to be committed

We have ensured sufficient resources to deliver the work plan, based on experience of the partners in executing complex projects. Total budget is € 5.635.530 with 480 person months (PM), which is distributed across the WPs according to Table 3.4a. The PMs correspond to 64.7% of the total budget. The travel budget covers the necessary meetings between individual partners, the whole consortium as well as conferences. Within this context, longer research visits of KTH project staff at LLG, and vice versa, are included for joint efforts and to ensure substantial know-how transfer. The expenses for other direct costs exceed 15% of the personnel costs for the partners mainly working on prototype and method development (AI, LLG, PII and APE). This is a consequence of the extensive instrument development for building the prototypes of the next-generation fluorescence super-resolution platform (main objective I), the SPAD arrays with enhanced NIR sensitivity (main objective III) and the pulsed – narrow linewidth – multi-line laser (main objective IV). These costs cover mainly the purchase of components needed to build those prototypes (equipment in table 3.4b) and have been carefully planned. It is important to note that the overall costs for all prototypes (€ 1.2 million) are significantly lower than the current market price of a single high-end microscope (€ 1.5 million). Personnel costs are standardised to 2020 rates for each individual partner and verified by the partner organisations.

Table 3.4b: 'Other direct cost' items

Participant 3/AI	**Cost (€)**	**Justification**
Travel	18.500	2 research visits at PII (1-2 persons), 2 research visits at KTH (1-2 persons), 4 annual project meetings (2 persons)
Equipment	688.000	Components for 2 MINFLUX Platform prototypes to remain at KTH and LLG. As costs for construction of a prototype they will be declared as (full) direct costs: Microscope stand (2x € 80.000), lasers (2x € 40.000), optomechanics (2x € 62.000), electronics (2x € 30.000), optics (2x € 36.500), detectors (2x € 12.000), scanners (2x € 36.000), custom mirror coatings (€ 60.000). Components for Gen2 electronics board prototypes (€ 35.000)
Other goods and services	5.000	Audit
Total	711.500	

Participant 4/LLG	Cost (€)	Justification
Travel	45.000	4 research visits at KTH (1-2 persons for 3 weeks), 4 international conferences (1 person), 4 annual project meetings (3-4 persons)
Equipment	172.600	Components for integration of IR, SRS and SPAD into the 2 MINFLUX Platform prototypes which remain at KTH and LLG. As costs for construction of a prototype they will be declared as (full) direct costs: NIR laser (2x € 20.000), NIR detector (2x € 5.000), NIR spatial light detector (2x € 15.000), optics (€ 38.300), mechanics (€ 30.300), filters and dichroics (24.000)
Other goods and services	69.000	Consumables: Chemicals (1.000 p.a.), dyes (1.500 p.a.), cell culture supplies (€ 2.500 p.a.), optical supplies (€ 3.500 p.a.), mechanical supplies (€ 2.500 p.a.) and electronic supplies (€ 1.000 p.a.) Three open access publications (€ 2.000 each), organisation of two workshops (invitations, flyers, consumables, catering, € 10,000) Audit (€ 5.000)
Total	286.600	

Participant 5/APE	Cost (€)	Justification
Travel	14.000	2 research visits at LLG (1-2 persons), 1 research visit at KTH (1-2 persons), 4 annual project meetings (2 persons), installation of picoEmerald at KTH and LLG
Equipment	190.000	Components for 2 prototype light sources to remain at KTH and LLG, 1 reference for APE. As costs for construction of a prototype they will be declared as (full) direct costs: Pump Laser for light source (3x € 50.000), optics (€ 20.000), mechanics (€ 12.500), electronics (€ 7.500)
Other goods and services	5.000	Audit
Total	209.000	

Participant 6/PII	Cost	Justification

	(€)	
Travel	12.500	2 research visits at AI/LLG (1-2 persons), 4 annual project meetings (2 persons)
Equipment	182.000	Fabrication of SPAD array prototypes. As costs for construction of a prototype they will be declared as (full) direct costs: Chip fabrication of 5x5 mm2 (€ 137.000), FPGA electronics (€ 5.000), Chip bonding (€ 3.000), microlenses (€ 35.000), chip packages (€ 2000)
Other goods and services	5.000	Audit
Total	199.500	

4. Members of the consortium

4.1. Participants

Participant 1 - Royal Institute of Technology (KTH), Sweden

Royal Institute of Technology (KTH), founded in 1827, is one of Europe's leading technical and engineering universities and the largest technical research and learning institution in Sweden, with 13,000 full-time students, over 1,700 PhD students and approximately 3,600 full-time employees (about 1/3 are women and 2/3 men, in all the three categories). KTH is addressing world leading, high-impact research and education in natural sciences and all branches of engineering, and is part of extensive international research collaborations and participate in a large number of educational exchange or joint programs with universities and colleges in Europe and worldwide. Almost two-thirds of the total turnover of KTH (500 million Euro) relates to research. Life Science Technology (LST) is a strong area of research at KTH, and is appointed as one of its six focus areas, converging engineering, natural and mathematical sciences with life sciences, and with numerous examples of world-leading research. KTH maintains strong interaction with industry, and provides competent innovation support to its researchers via the KTH Innovation office, with a long and successful track record in taking research and ideas from KTH into exploitation outside of academia.

The Experimental Biomolecular Physics research group, headed by Prof. Jerker Widengren, consists of 10 persons (1 professor, 3 researchers, 2 postdocs, 4 PhD students), and contributes to the strong status of LST research at KTH. Most of the members have an engineering physics background, but also educational backgrounds in chemistry, molecular biology and medicine, together forming a solid multi-disciplinary research competence of the group. The Widengren research group belongs to the pioneers in Fluorescence Correlation Spectroscopy (FCS), and is still in the very forefront in the development, application and use of FCS and single-molecule fluorescence methods for studies of biomolecules, their dynamics and interactions. Over the years, Widengren and his research group have studied extensively the photophysical prerequisites for fluorescence-based single-molecule analyses, and the influence of excitation conditions and sample conditions on fluorophore brightness, blinking properties and photo-stability. These parameters are critical for ultrasensitive fluorescence spectroscopy/imaging. Moreover, they are also fundamental for all forms of fluorescence super-resolution imaging. In two EU projects (described above) performed in collaboration with the group of Stefan W. Hell, Göttingen, the Widengren group early on started to explore photophysical switching properties of fluorophores for super-resolution imaging (EU SPOTLITE project, coordinated by S. W. Hell), and then pioneered the use of super-resolution STED imaging for diagnostic applications, demonstrating cellular diagnostics of breast and prostate cancer, based on spatial distribution patterns of specific

proteins in the cells (EU FLUODIAMON project, coordinated by J. Widengren). In recent years, the Widengren group has applied super-resolution STED imaging for studying the role of platelets in early cancer development, and together with the Henriques-Normark group at KI, to elucidate the role of pneumococcal surface proteins for the virulence and invasiveness of these bacteria. Finally, of relevance for this project, the Widengren group has also invented the TRAST imaging technique, which emanated from the strong photophysical track record of the group, and uses long-lived dark transient states of fluorophores as very sensitive reporters of several physiologically relevant environmental parameters (as described above).

Infrastructure:

The Widengren lab at KTH is well equipped, with several FCS, multi-parameter single-molecule spectroscopy, STED and TRAST instruments, and multiple laser sources, including tunable supercontinuum lasers and a laser for TPE, making it possible to get started with core activities in WP2 and WP5 from day one. There are also facilities for cell culturing and a biochemistry lab next to the fluorescence lab for use in the project, and the Applied Physics department of KTH offers workshop and research engineering resources and an overall very strong environment within the opto-bio-nano fields of physics.

Main tasks in this project:

The particular expertise and experience of partner KTH is in several aspects very well aligned with the project needs. First, partner KTH will work on the evaluation/identification of NIR fluorophores (Task 5.1), optimization of excitation, photo-activation and illumination schemes (Task 5.2) and their integration into the MINFLUX platforms (Task 2.4). In these tasks, the profound experience of partner KTH in ultrasensitive and ultrahigh resolution fluorescence methods, and its leading expertise in fluorophore photophysics will come to its full right. Second, partner KTH will also take a major role in the establishment of VIS-NIR MINFLUX, SRS, TPE TRAST and combinations thereof, for bacterial and host cell studies (Tasks 5.3-5.5), and in the subsequent studies in WP6, to demonstrate how the developed imaging platform can diagnose bacteria, as well as to identify and characterize their intricate virulence and invasion mechanisms. These tasks will strongly benefit from the experience and expertise of KTH in both super-resolution imaging and TPE TRAST imaging, as well as in the fact that KTH pioneered the use of super-resolution imaging for cellular diagnostic and bacterial studies, specifically focusing on analyses of protein localization patterns in the cells. Finally, partner KTH will take on the role as coordinator of this project (WP7). Scientifically, Prof. Widengren has both the necessary expertise and interdisciplinary competence to take on this task, and has also a successful record as coordinator of the EU FP7 project FLUODIAMON (see below). For financial and legal aspects, Prof. Widengren will have major support from administrative functions at KTH. As one of the leading research organizations in Sweden, KTH has a long and successful track record as coordinator of EU projects. This will guarantee that all administrative support needed for this project will be provided.

Profile of Prof. Jerker Widengren:

Prof. Jerker Widengren (male) will be responsible for the coordination and planning of all project contributions of KTH, and will also take on the task as the coordinator of the project as a whole. Starting as a PhD student in 1990 at Karolinska Institutet, Stockholm (supervisor Rudolf Rigler), Jerker Widengren has a 30 year successful track record in fluorescence-based ultrasensitive and super-resolution based spectroscopy/microscopy. Jerker Widengren belongs to the pioneers of fluorescence-based single-molecule detection (SMD) and fluorescence correlation spectroscopy (FCS), and contributed already as a PhD student to the breakthrough of this technique for biomolecular and cellular studies, in academia as well as in biotechnology and pharma industry. As a postdoc (with C. A. M. Seidel at the Max Planck Institute for Biophysical Chemistry in Göttingen) Jerker Widengren with colleagues established so-called single-molecule multi-parameter detection (smMFD), demonstrated its diagnostic potential by detection and identification of single DNA molecules and its use for fundamental protein conformational studies. Jerker Widengren has also extensively studied the photophysical aspects of single-molecule and FCS measurements, with several highly cited papers describing the mechanisms and remedies for such measurements. Since 2003, Jerker Widengren leads a successful research group at KTH with a focus on development of fluorescence-based ultrasensitive and super-resolution spectroscopy/microscopy techniques, and their application for biomolecular and cellular studies. Jerker Widengren and his group were one of the first outside of the S. W. Hell lab in Göttingen to start with fluorescence-based super-resolution STED imaging, and pioneered its use for sub-cellular characterization and diagnostics. With Birgitta Henriques-Normark, KI, Jerker Widengren has thereafter successfully applied STED imaging to resolve and characterize the role of pneumococcal surface proteins in the virulence and invasiveness of these bacteria. Jerker Widengren is also the inventor of the TRAST imaging technique, and Jerker Widengren and his group have in several articles demonstrated how this technique can transform the limiting photophysical aspects of single-molecule spectroscopy into highly informative imaging parameters.

Jerker Widengren has more than **100 publications** and **h-index of 31**, and his work has resulted in several patents and patent applications on fluorescence microscopy/spectroscopy techniques. He has supervised 12 PhDs and 16 postdocs. Currently there are 2 postdocs and 4 PhD students under his direct supervision. He has given more than 40 invited lectures and talks (Sweden and abroad) during the last 10 years and was chairman and organizer of several international workshops and meetings on single-molecule spectroscopy, FCS and related techniques.

As an engineering physicist (M.Sc.) as well as clinical physician (M.D.) by training, Jerker Widengren combines in-depth knowledge of ultrasensitive and super-resolution spectroscopy/microscopy and clinical medicine in a unique way. This will be particularly useful given his coordinator role in this project, and very well fits with the highly multi-disciplinary scope of the project.

Education:

1. Jerker Widengren, born 1965, received his **M.Sc. in Engineering Physics** from KTH in 1989 and his **Ph.D. in Medical Biophysics** from Karolinska Institutet, Stockholm in 1996.
2. After his graduation from medical school (**M.D./Physician**, Karolinska Institutet) in 1998, he was a postdoctoral researcher at the **Max Planck Institute for Biophysical Chemistry** in Göttingen as well as Docent in medical Biophysics at Karolinska Institutet.
3. Since 2003 he heads the **Experimental Biomolecular Physics** research group at KTH.

Scientific Career (Detail):

- 1985 – 1989: M.Sc. in Engineering Physics, KTH
- 1990 – 1996: Ph.D., Medical Biophysics, Karolinska Institutet, Stockholm
- 1998: M.D./Physician, Karolinska Institutet (KI)
- 1998, 2001: Internship as Physician (AT) Karolinska Univ. Hospital
- 1999 – 2001: Postdoctoral researcher, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany
- 2001: Docent in medical Biophysics, Karolinska Institutet
- since May 2003: Professor in Experimental Biomolecular Physics, KTH
- 2006: Invited guest professor at EPFL, Lausanne
- 2008 – 2012: Head of the research studies at the department of Applied Physics, KTH
- 2008 – 2012: Coordinator of the EU FP7 project FLUODIAMON with 12 European partners
- 2009: Invited guest professor at Joseph Fourier University, Grenoble
- 2013 – 2015: Director of the Life Science Technology platform of KTH
- 2015-2017: Deputy Head (pro-prefekt) of the department of Applied Physics, KTH
- since 2018: Head of the Quantum and Biophotonics unit/Applied Physics, KTH, with >30 scientists

Memberships:

- Elected member of the Swedish Academy of Engineering Sciences (IVA), since 2017

Top 5 relevant achievements:

Publications:

- Tornmalm, J.; Sandberg, E.: Rabasovic, M. & Widengren, J. "Local redox conditions in cells imaged via non-fluorescent transient states of NAD(P)H" *Scientific Reports* 9, 15070 (2019)
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- Pathak, A.; Bergstrand, J.; Sender, V.; Spelmink, L.; Aschtgen, M.-S.; Widengren, J. & Henriques-Normark, B. "Factor H binding proteins protect division septa on encapsulated Streptococcus pneumoniae against complement C3b deposition and amplification" *Nature Comm.* 9, 3398 (2018)
- Blom, H. & Widengren, J. "Stimulated Emission Depletion Microscopy" *Chemical Reviews* 117(11), 7377-7427 (2017)
- Iovino, F.; Engelen-Lee, J. Y.; Brouwer, M.; van de Beek, D.; van der Ende, A.; Valls Seron, M.; Mellroth, P.; Muschiol, S.; Bergstrand, J.; Widengren, J. & Henriques-Normark, B. "pIgR and PECAM-1 bind to pneumococcal adhesins RrgA and PspC mediating bacterial brain invasion" *The Journal of Experimental Medicine* 214(6), 1619-1630 (2017)

Up to 5 relevant projects or activities:

The NanoVIB project builds further several previous EU and national Swedish projects, in which the Widengren group at KTH has taken part, and have clear synergies (but no overlap) with one recently started national project:

- EU FP6 project SPOTLITE, 2004-2006 (Stefan W. Hell coord): Development of nanoscopy, based on blinking of long-lived dark transient states in fluorophores.
- EU FP7 project FLUODIAMON, 2007-2012 (Jerker Widengren coordinator): Development of super-resolution STED imaging, TRAST imaging and other advanced fluorescence-based imaging techniques for early subcellular diagnosis of breast and prostate cancer.
- Swedish Foundation for Strategic Research (SSF) project Immunomodulation of host-microbe interactions in infections caused by commensal pathogens - MOHICAN, 2014-2018 (B. Henriques-Normark coord): A multidisciplinary project targeting the molecular mechanisms through which the major global pathogen *Streptococcus pneumoniae* interacts with the host immune defense system. Localization and roles played by different pneumococcal surface proteins and their interactions with the immune defense system such as the complement system resolved by super-resolution STED microscopy were studied.
- Swedish Cancer Research Foundation (Cancerfonden) project, 2018-2020 (PI: Jerker Widengren): Super-resolution STED imaging analyses to elucidate the role of platelets in early cancer development, and the possible use of highly resolved protein distribution patterns on the platelets as a diagnostic marker.
- Swedish Foundation for Strategic Research (SSF) project Bacterial exosomes and their nano-mimics as vaccine - BENVAC, 2019-2023 (B. Henriques-Normark coord): With the main goal to generate new nano-vaccines based on bacterial exosomes, they will be studied in vitro and in vivo. For this, we will apply fluorescence-based ultrasensitive, super-resolution imaging and spectroscopy techniques to reveal underlying molecular mechanisms and host-bacterial exosome interactions.

Participant 2 - Karolinska Institutet (KI), Sweden

Karolinska Institutet (KI) is one of the world's leading medical universities that was founded by King Karl XIII in 1810. Since 1901, the Nobel Assembly at Karolinska Institutet selects the Nobel laureates in Physiology or Medicine. The vision of Karolinska Institutet is to significantly contribute to the improvement of human health. Karolinska Institutet is Sweden's single largest center of medical academic research and offers the country's widest range of medical courses and programs. About 40-50% of medical research in Sweden is placed at Karolinska Institutet, and the research spans the entire medical field, from basic experimental research to patient-oriented and nursing research. The research is conducted in 22 departments, most of which are situated or adjacent to Stockholm's teaching hospitals. This creates ample opportunities for translational research in which new experimental results are rapidly implemented for patient benefit, and where clinical observations provide a basis for new research ideas. Also, the close proximity of the Karolinska University Hospital and other teaching hospitals in the Stockholm area plays an important role during education. Karolinska Institutet offers the widest range of medical education under one roof in Sweden. Several of the programs include clinical training or other training within the healthcare system. Approximately 6,000 full-time students are taking educational and single subject courses at Bachelor and Master levels at Karolinska Institutet. Also, Karolinska Institutet carries out 12% of Swedish doctoral/third cycle education at universities or university colleges and it has collaboration agreements in research and education with a large number of universities all over the world, with companies in the biomed and biotech sectors and also with individual countries. In 2019, Karolinska Institutet had 5,088 full time employees. In addition to this a large number of people without formal employment, especially visiting scientists, fellows and unpaid docents were active at KI. Karolinska Institutet's turnover in 2019 was SEK 7,120 million.

Birgitta Henriques-Normark is professor at Karolinska Institutet (KI), senior consultant/head physician in clinical microbiology at Karolinska University hospital and associated to the Public Health Agency of Sweden (FOHM). Since about 25 years she has studied pneumococcal infections with a broad and translational approach going from clinical studies and epidemiological investigations also on the molecular level, to more basic understanding of mechanisms important for disease development. **The research group of Birgitta Henriques-Normark** is located at the Karolinska University hospital close to Karolinska Institutet and consists of about 35 researchers with a mix of different competences from clinicians to basic researchers including PhD students, Postdocs and Assistant professors, a Senior professor, and Senior researchers. Competences in the group include molecular epidemiology, infectious disease epidemiology, clinical microbiology, bacterial molecular biology, genomics, sequencing and data analyses, proteomics, RNA technologies, visualization, animal models, antibiotic resistance development, and vaccination studies in mice. Birgitta Henriques-Normark has supervised 18 PhD students as main and 10 as co-supervisor, and more than 50 postdocs. Collaborators add competences in biophysics, chemistry, structure biology, and visualization. Hence, all competences needed for the project

are present within the group or by collaborators. At KI and at the Karolinska University hospital and at FOHM we have access to all core facilities needed, as stated below, and access to all clinical pneumococcal isolates including serotype data and patient samples, as well as huge collections of other bacterial strains. The research environment includes researchers at all level from Master students, and PhD students to assistant professors, associate professors and professors and clinicians, molecular biologists and microbiologists. We are responsible for a seminar series in infections and we have group meetings every week where the latest research findings are presented and discussed.

Infrastructure:

The research environment is outstanding with access to all equipment and infrastructure needed such as molecular biology tools, FACS, microscopy facilities, different omics approaches such as genomics, transcriptomics, proteomics, metabolomics etc., both in our laboratories, but also in the core facilities at the Karolinska hospital, at KI, and at Science for Life Laboratory. Also, there are excellent animal facilities with large collections of mutant mice. Strains, patient samples and diagnostic competence are all found in the clinical microbiology laboratory and at FOHM.

Main tasks in this project:

Birgitta Henriques-Normark and her group will be responsible for the microbiology part of the project and applications of the visualization tools that will be developed in the project. Birgitta Henriques-Normark will lead WP6 where the aims will be to demonstrate how the developed imaging platform with super-resolution can be used to distinguish pathogenic bacteria from commensal bacteria, and to identify and characterize their intricate virulence and invasion mechanisms. Her group has an extensive expertise in microbiological and molecular tools for pathogenesis studies both in vitro and in vivo mice. The group of Birgitta Henriques-Normark together with the partners in the project will investigate protein localization patterns on the bacterial surface and on membrane vesicles, not previously possible due to resolution issues. Moreover, they aim at studying the biological relevance of identified protein localization patterns and their role for virulence and disease outcome. This will be investigated using microbiological technologies that are already in place in the laboratory of Birgitta Henriques-Normark. The data generated are expected to form a basis for development of novel approaches for prevention and treatment of bacterial diseases.

Profile of Prof. Birgitta Henriques-Normark:

Birgitta Henriques-Normark (female) is professor and senior consultant/head physician (M.D.) in Clinical microbiology at the Karolinska Institutet (KI) and Karolinska University Hospital. She is also a visiting professor at Nanyang Technical University (NTU) in Singapore. Her research mainly targets respiratory tract and invasive infections, with a focus on pneumococcal infections. Her studies go from clinical and epidemiological research to studies on host-pathogen interactions, and more basic understanding on microbial and host immune factors that determine disease outcome. Birgitta Henriques-Normark has published over **200 publications** in the field, **h-index of 49**, and has been the supervisor of over 25 PhD

students (18 as main supervisor) that have defended their thesis. She has evaluated research on numerous occasions and for many different organizations such as for the European Research Council, ERC, and the European commission, the Helmholtz association in Germany, Max Planck in Germany, and she has been a member of the steering board for Medicine and Health at the Swedish Research Council for 6 years. She has been the vice dean for recruitment of higher positions (professors, associated professor etc.) and the chair of the recruitment committee at KI for almost 6 years and is now Academic vice president for research at KI. She has participated in several EU networks/projects: 3 in the 5th framework (EURIS, DEAR, STREP-EURO), 7 in the 6th fp (Europathogenomics, GRACE, OmVac, INCA, EIMID, IMO-train, PREVIS), 3 in the 7th fp (Pneumopath, EIMID-IAPP, Lapaso), 2 JPIAMR projects, ERA-Infect project. She was the coordinator of the EU program PREVIS (Molecular mechanisms of resistance, virulence and epidemicity in Streptococcus pneumoniae) within the 6th framework. She has several international and national collaborators both in industry and academia. She also participated in networks governed by DG research and ECDC for the following pathogens: Diphtheria, H. influenzae, and S. pneumoniae. Birgitta Henriques-Normark is a member of the European Academy of Microbiology (EAM), and of the American Academy of Microbiology (AAM). She is also a member of the Royal Swedish Academy of Sciences and of the Nobel assembly at Karolinska Institutet, awarding the Nobel prize in Physiology or Medicine, as well as a member of EMBO (European Molecular Biology Organization).

Education:

1. Birgitta Henriques-Normark, born in 1958, became **Medical Doctor (M.D.)** at the Karolinska Institutet (KI), Stockholm, Sweden in 1983. She was licensed as physician (Leg Läk) in 1987, and in 1994 she got here **specialization in Clinical Bacteriology**.
2. In year 2000, she got her **Doctoral degree, Ph.D., in Infectious disease control**, and in 2004 she became Associate professor/Docent, at KI. In year 2000 she was appointed as Senior consultant/Head physician at Smittskyddsinstitutet (SMI, today Public Health Agency of Sweden), and in 2008 she became **Professor in Medical Microbial Pathogenesis**, Dep Microbiology, Tumor- and Cellbiology, MTC, at KI.
3. In 2011 she became **Professor in Clinical microbiology at KI**, as well as Senior consultant/Head physician at the Karolinska University Hospital. Since 2016 she is also Guest professor at Nanyang Technological University, NTU, in Singapore.

Scientific Career (Detail):

- 1983: Medical Doctor (M.D.) at the Karolinska Institutet (KI), Stockholm, Sweden
- 1983 – 1985: Physician Södertälje/Huddinge hospital Ear-Nose- and Throat Department
- 1985 – 1987: Internship physician Huddinge hospital/Södertälje hospital
- 1988 – 1993: Physician at the National Bacteriological Laboratory
- 1993 – 1994: Physician at the Department of Infectious Diseases at Huddinge hospital

- 1993 – 2014: Physician at the Swedish Institute for Infectious Disease Control (Smittskydds-institutet, SMI)
- since 2000: Head physician, SMI, since 2014 Public Health Agency of Sweden (FOHM)
- 2001 – 2007: Member of the steering group for SMI
- 2001 – 2003: Head Department of Molecular Epidemiology and Biotechnology SMI (~50 employees)
- 2001 – 2010: Head Section Respiratory tract/Invasive infections at SMI (ca 25 employees)
- 2004 – 2007: Head Department of Bacteriology at SMI (~100 employees)
- 2010 – 2012: Chair evaluation committee for Infection and Global Health at the Swedish Research Council
- since 2010: Member of the reference group for Clinical Microbiology appointed by FKM (association for clinical microbiology in Sweden)
- since 2011: Chair of the Centre for Infectious Disease Control, CID, at KI
- 2012 – 2016, since 2018: Member of the steering group of Dep MTC at KI
- 2012 – 2017: Chair of the Research and education committee in Clinical microbiology Karolinska University hospital
- 2012 – 2019: Evaluator at the European Research Council, ERC
- 2013 – 2018: Member of the steering board of Medicine and Health, the Swedish Research Council (VR)
- 2014 – 2019: Vice dean for recruitment at KI responsible for recruitments of higher positions at KI (professors, lecturers, centrally financed positions such as Assistant professors and researchers at KI)
- 2014 – 2019: Chairman of the recruitment committee at KI
- since 2014: Editor of FEMS Microbiology Reviews (IF 13,231)
- 2017 – 2020: Chair of the Evaluation committee of the Helmholtz Centre for Infection Research, Braunschweig, Germany, Member of the Strategic Advisory Board for Helmholtz Health
- 2017 – 2021: Member of the board of Umeå Centre for Microbial Research (UCMR) and Molecular Infection Medicine Sweden (MIMS) at Umeå University
- 2019 – 2022: Academic Vice President for Research at KI, and Chairman of the Committee for Research at KI

Memberships:

- Elected Member of the European Academy of Microbiology (EAM), since 2013
- Elected Member of the American Academy of Microbiology (AAM), since 2015
- Appointed Wallenberg Clinical Scholars (one out of two in 2017) in national competition, since 2017
- Elected member of the Royal Swedish Academy of Sciences (KVA), since 2018
- Elected member of the Nobel Assembly at KI, awarding the Nobel Prize in Physiology or Medicine, since 2019
- Elected member of EMBO (European Molecular Biology organization), since 2019

Top 5 relevant achievements:

Publications:

- Subramanian, K.; Neill, D.; Malak, H. A.; Spelmink, L.; Khandaker, S.; Dalla Libera Marchiori, G.; Dearing, E.; Kirby, A.; Yang, M.; Achour, A.; Nilvebrant, J.; Nygren, P. A.; Plant, L.; Kadioglu, A. & Henriques-Normark, B. "Pneumolysin binds to the Mannose-Receptor C type 1 (MRC-1) leading to anti-inflammatory responses and enhanced pneumococcal survival" *Nature Microbiology* 4(1), 62-70 (2019)
- Pathak, A.; Bergstrand, J.; Sender, V.; Spelmink, L.; Aschtgen, M. S.; Muschiol, S.; Widengren, J. & Henriques-Normark, B. "Factor H binding proteins protect division septa on encapsulated Streptococcus pneumoniae against complement C3b deposition and amplification" *Nature Commun.* 9(1), 3398 (2018)
- Iovino, F.; Engelen-Lee, J. Y.; Brouwer, M.; van de Beek, D.; van der Ende, A.; Valls Seron, M.; Mellroth, P.; Muschiol, S.; Bergstrand, J.; Widengren, J. & Henriques-Normark, B. "pIgR and PECAM-1 bind to pneumococcal adhesins RrgA and PspC mediating bacterial brain invasion" *The Journal of Experimental Medicine* 214(6), 1619-1630 (2017)
- Iovino, F.; Hammarlöf, D. L.; Garriss, G.; Browall, S.; Nannapaneni, P. & Henriques-Normark, B. "Pneumococcal meningitis is promoted by single cocci expressing pilus adhesin RrgA" *J Clin Invest.* 126(8), 2821-2826 (2016)
- Hentrich, K.; Löfling, J.; Pathak, A.; Nizet, V.; Varki, A. & Henriques-Normark, B. "Streptococcus pneumoniae senses a human-like sialic acid profile via the response regulator CiaR" *Cell Host&Microbe* 20(3), 307-317 (2016)

Up to 5 relevant projects or activities:

- Swedish Foundation for Strategic Research (SSF) project Immunomodulation of host-microbe interactions in infections caused by commensal pathogens - MOHICAN, 2014-2018 (B. Henriques-Normark main applicant and coordinator): A multidisciplinary project targeting the molecular mechanisms through which the major global pathogen *Streptococcus pneumoniae* interacts with the host immune defense system. Localization and roles played by different pneumococcal surface proteins and their interactions with the immune defense system such as the complement system resolved by super-resolution STED microscopy were studied.
- Swedish Foundation for Strategic Research (SSF) project Bacterial exosomes and their nano-mimics as vaccine - BENVAC, 2019-2023 (B. Henriques-Normark main applicant and coordinator): With the main goal to generate new nano-vaccines based on bacterial exosomes, they will be studied in vitro and in vivo. For this, we will apply fluorescence-based ultrasensitive, super-resolution imaging and spectroscopy techniques to reveal underlying molecular mechanisms and host-bacterial exosome interactions.

Participant 3 – Abberior Instruments GmbH (AI), Germany

Abberior Instruments GmbH (AI) was founded in April 2012 as a spin-off from the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany by Nobel prize laureate Prof. Stefan W. Hell and several senior scientists from his department. Around this core, AI has built the world's strongest development team in the area of fluorescence nanoscopy, with a proven track record of rapidly commercializing both, in house developments and innovations emerging from academic research. AI has thereby significantly advanced the performance of nanoscopy in biomedical research and its applicability to biomedical research.

Already in its first full fiscal year 2013, Abberior Instruments grossed 1.3 Mio€ yielding a positive result. Based on its technological leadership in fluorescence nanoscopy, the company has been profitable and exponentially growing ever since. Currently, Abberior Instruments has 55 employees. With its headquarters in Göttingen, Germany and business locations in Heidelberg, Germany and Basel, Switzerland, AI is in direct contact with many of Europe's leading researchers in both development and application of biomedical imaging techniques. AI is present on all important international markets.

To further strengthen this position and aggressively drive world-wide expansion, AI has founded its US subsidiary Abberior Instruments America (AIA) and plans to open a business location in Asia within the year. Based on the current order volume, AI expects to greatly increase its revenue in 2020 despite the effects caused by the outbreak of SARS-CoV-2.

Since its inception, AI has acquired exclusive licenses for groundbreaking super-resolution imaging techniques, and simultaneously built a strong portfolio of its own intellectual property. After acquiring exclusive rights to the MINFLUX technique developed in Stefan Hell's department at the MPI, the company developed a market-ready microscope within three years after the first published proof of concept. In STED microscopy – a mature super-resolution technique – AI has achieved times-to-market as short as half a year.

Being able to always offer the latest technology in STED, RESOLFT and MINFLUX imaging has helped AI to build a strong customer base especially among early adopters, method developers and, more generally speaking, researchers that drive scientific discovery by leveraging their instrument's full potential.

This has ensured that AI's new developments have always been used on real-life scientific problems within weeks after their implementation and has helped the company to build an exceptional understanding of how to design instruments that facilitate method development during the early adoption phase and to create an atmosphere of openness necessary for such collaborations.

The company employs highly skilled optical engineers, software, FPGA and electronics developers and application scientists, which have been involved in the development of three STED and one MINFLUX based super-resolution products, and has proven to possess one of the most innovative development teams in the industry. At the same time AI maintains close ties with the research community and is continuously involved in research activities through

projects funded by the German Ministry of Education and Research. In addition, Abberior Instruments will hire one additional optics developer and one electronics- and FPGA-developer as part of NanoVIB project to address specific tasks related to the integration of detector arrays and an SRS imaging path into the microscope and to develop a market-ready microscope in the later stages of the project.

Infrastructure:

AI's development infrastructure is geared towards rapid development of novel microscopy techniques. The company staff includes 6 former postdoctoral researchers that worked with Stefan Hell during the development of STED and MINFLUX microscopy. Our R&D team consist of 10 optics, electronics and software engineers and routinely prototypes novel microscopy hardware based on standard and custom produced optical components, microprocessors, and FPGAs, with both hardware and firmware development conducted in-house. Integrating custom light sources, detectors and beam paths into our Expert Line microscopes is an important part of AI's business model. To this end AI runs 3 fully equipped optical laboratories and routinely uses optical simulation software, CAD for mechanical design and software for electronics design and simulation. Acquisition software and the firmware for our hardware control electronics is developed using C/C++, Python, LabVIEW, VHDL and JAVA and AIs software department employs expert users of all these tools. Abberior Instruments also considers IP management as an important part of its business and currently employs two specialists. In the past two years (2018 and 2019) AI has filed 13 original patents.

Main tasks in this project:

AI's main task in this project is to extend its current implementation (optics, electronics and software) of MINFLUX imaging to allow (1) integration of additional light sources for NIR-MINFLUX, two photon activation and label-free imaging (2) integration of position-sensitive detectors (SPAD arrays) and (3) implementation and test of advanced data acquisition and analysis schemes within the instrument control software. As the design and production of custom microscope configurations is one of AI's unique selling points to advanced microscope users and because AI's competitiveness is largely based on the company's ability to quickly adopt new technology into their existing line of products, all infrastructure and know-how required for these tasks is available and AI has demonstrated this ability repeatedly: Among others, the RESOLFT, Easy3D, RESCue STED, DyMIN STED and MINFLUX imaging techniques were all either developed completely in-house or licensed from research institutions long before they could be considered mature and developed to TRL9 and offered as a commercial product within 1-3 years. Similar FPGA technology as is necessary for the integration of SPAD arrays is already at the core of AI's current products and AI's R&D staff has extensive experience with all the tools and techniques needed for the task at hand. Providing optimal starting points for custom integration of additional hardware and optics into AI's microscopes is part of its business model and will not pose significant challenges.

Profile of Dr. Andreas Schönle:

Dr. Andreas Schönle (male) will be responsible for coordination and planning of all project contributions by Abberior Instruments. He will join the lead developers of AI's current MINFLUX product in conceiving the open MINFLUX platform, he will recruit a qualified FPGA developer and lead the development necessary to integrate SPAD arrays into the MINFLUX electronics and oversee all necessary software development at AI. He will also plan and oversee all modifications that might become necessary due to the critical feedback from other project partners.

After heading the software department and contributing to many of AI's important technology and product developments until fall 2019, Andreas Schönle is now the head of AI's newly established "Intelligence and Innovation" section. There he focusses on highly innovative long-term development projects and technology scouting.

During his academic career, Andreas Schönle has worked in the field of optical microscopy beyond the diffraction barrier with far-field optics since 1996. He has worked on theoretical modelling of novel super-resolution techniques based on photo-switching and nonlinear spectroscopy, experimental development and theoretical analysis of STED microscopy and related concepts. During his time at the Max Planck Institute for Biophysical Chemistry under Stefan Hell he also developed imaging software that was used to operate and analyze the data of many of the 4Pi, STED and localization based super-resolution microscopes, making him part of a large number of successful research projects. He co-authored more than 40 scientific publications in the field of fluorescence nanoscopy and book chapters about the fundamentals of super-resolution. His work has also resulted in numerous inventions and several important patents in the field.

Education and professional career:

1. Andreas Schönle, born 1973 in Munich, studied **Physics, Mathematics and Economics** at Osnabrück University, Germany, Kent State University, USA and Heidelberg University, Germany. From Heidelberg University he received his "Diplom" in Physics in 1998 and his Doctorate in Physics in 2003.
2. He was subsequently postdoctoral researcher and later senior scientist in the **Department of NanoBiophotonics** at the **Max Planck Institute for Biophysical Chemistry**, Göttingen.
3. In 2012 he became **co-founder of Abberior Instruments GmbH,** a spinoff from the Department of NanoBiophotonics and became head of software development. Since October 2019 he is head of the "Intelligence and Innovation" department at AI.

Top 5 relevant achievements:

Products:

Abberior Instruments VIS-MINFLUX microscope:

Within 3 years after the initial proof of concept, Abberior Instruments has developed its current MINFLUX offering and received orders for this novel type of microscope from

several leading research institutions, including the EMBL in Heidelberg. The platform has an open design to allow optimization due to feedback from applicants in this early phase and with some modifications will offer an ideal base platform for the NanoVIB project.

(https://www.abberior-instruments.com/products/minflux/)

Imspector Software:

The software was first created in Stefan Hell's group (now the Department of NanoBiophotonics) at the Max Planck Institute for Biophysical Chemistry as a flexible base for incorporation and synchronized operation of lasers, detectors, optomechanical components and acquisition and control electronics in order to quickly design and build microscopes based on novel super-resolution concepts and allow their test and operation by non-engineers. An early version has been licensed to LaVision Biotec to operate their two-photon microscopes and numerous research labs to operate their home-built STED microscopes by the Max Planck Society. At AI, Imspector was systematically streamlined, further modularized and improved and is now at the core of the Abberior Instruments Expert Line and Facility Line microscopes, and a major facilitator for both AI's rapid innovation cycles and the ability of AI's customers to find innovative applications of AI's technology beyond the current state of the art.

Publications:

- Heine, J.; Wurm, C. A.; Keller-Findeisen, J.; Schönle, A.; Harke, B.; Reuss, M.; Winter, F. R. & Donnert, G. "Three dimensional live-cell STED microscopy at increased depth using a water immersion objective" *Review of Scientific Instruments* 89(5) (2018)
- Heine, J.; Reuss, M.; Harke, B.; D'Este, E.; Sahl, S. & Hell, S. W. "Adaptive-illumination STED nanoscopy" *PNAS* 114(37), 9797-9802 (2017)
- Schloetel, J.-G.; Heine, J.; Cowman, A. F. & Pasternak, M. "Guided STED nanoscopy enables super-resolution imaging of blood stage malaria parasites" *Nature Scientific Reports* 9(4674) (2019)

Up to 5 relevant projects or activities:

- Optical nanoscopy for three-dimensional imaging of live cells ('LiveCell3DNanoskop', German ministry of Education and Research, 2017-2020): Cooperation with Abberior GmbH, Göttingen and Max Planck Institute for Biophysical Chemistry, Göttingen. Project ongoing.
- STED microscope with active aberration correction & automatic adjustment: Integration of relevant optics into existing microscope and software-based integration of expert knowledge into image acquisition software. Cooperation with the LLG. Product launched in 2019.
- Automated STED-nanoscopy for high-throughput analysis in cell biology ('ScreeningSTED', German ministry for Education and Research): Cooperation with Bayer AG, Leverkusen.

- Development of Easy3D STED: AI has developed an adaptive optics solution to create and overlay two independent intensity patterns using a single beam path. The resulting stability is critical for routine 3D STED nanoscopy. The resulting Easy3D module is a core component of most Expert Line and Facility Line microscopes and one of AI's important USPs.
- Development of intelligent light dose management in confocal and STED microscopy: Cooperation with the Max Planck Institute for Biophysical Chemistry. Illumination intensities are dynamically adjusted during acquisition based on real-time analysis of detector count-rates to avoid unnecessary illumination of the sample with low information density. Corresponding products: RESCue STED, DyMIN STED.

Participant 4 - Laser-Laboratorium Göttingen e.V. (LLG), Germany

Laser-Laboratorium Göttingen (LLG) was founded in 1987 as a non-profit making special-purpose enterprise, being institutionally supported by the State of Lower Saxony. Promotion of optical technologies is successfully realized by applied pure research and knowledge transfer between research institutions and industry in the form of collaboration projects, research assignments, consultancy and training. Research and development results are usually marketed or distributed by companies under license.

The LLG divisions "**Short Pulses/Nanostructures**", "**Optics/Short Wavelengths**", "**Photonic Sensor Technology**" and "**Optical Nanoscopy**" have gained worldwide acceptance in various fields of photonics. Research activities range from development of non-contacting laser measurement technology, manufacturing of new products, laser based product processing and development of new laser systems to applications in medical technology and the life sciences.

The LLG receives important institutional sponsorship from the Federal State of Lower Saxony, substantial third party funding for contract research from the industry as well as from projects of the Federal Government and the DFG (German Research Association). The LLG employed 52 employees in 2019 and is well networked with small and medium-sized enterprises as well as conglomerates. In the education of Bachelor, Master as well as PhD students, the LLG collaborates closely with the Georg August University Göttingen and the University of Applied Sciences Hildesheim/Holzminden/Göttingen. Furthermore, it regularly offers insights into modern optical research to trainees.

The **Department of Optical Nanoscopy**, to be directly involved in the NanoVIB project, focuses on basic research and applications in the field of super-resolution fluorescence microscopy. For this purpose, switchable optical transitions are used to bypass restrictions imposed by the laws of diffraction, which are inherent to all optical far-field techniques. One of the main activities of the department is the development of new optics, tools and microscopes that can be routinely used by life scientists without the need for optical expertise. The department cooperates closely with the Department of NanoBiophotonics at the Max Planck Institute for Biophysical Chemistry led by Stefan W. Hell as well as the Department of Photonic Sensor Technology of the LLG, which among other things focuses on Raman scattering-based analytics.

Infrastructure:

The department has a 120 meter square lab space fully equipped for optical nanoscopy research purposes as well as an additional laboratory equipped for routine biological research. Among other things, the laboratories contain: two STED nanoscopes with single color imaging capability, three STED nanoscopes with multicolor imaging capability, two PALM/STORM nanoscopes with multicolor capability, an isoSTED microscope which can also be used as an interferometric PALM/STORM nanoscope, a scanning electron

microscope and an atomic force microscope. The Department has access to the core light microscopy facility of the Max Planck Institute for Biophysical Chemistry and the DFG Clusters of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC) as well as the cell culture of the department of NanoBiophotonics at the Max Planck Institute for Biophysical Chemistry.

Main tasks in this project:

LLG's main task in this project is the optical integration of several components, developed by the partners, in order to realize a NIR-MINFLUX system capable of correlative SRS-MINFLUX as well as correlative TRAST-MINFLUX imaging. The optical planning is completed by the implementation of acquisition strategies and the prototyping of e.g. molecule finding algorithms. The Department of Optical Nanoscopy, led by Alexander Egner, will therefore be responsible for the step-wise development of the super-resolution system as specified in WP2 and will provide partner KTH with an update to the platform after each step. Alexander Egner and his group have extensive experience and expertise in the design and implementation of microscopy systems for various super-resolution techniques. The development of new tools as well as the integration of novel components and approaches is part of the department's main activities, alongside with the analysis and implementation of acquisition strategies for super-resolution imaging. Equipped with several optic laboratories as well as a laboratory dedicated to biological research and sample preparation, the department has all the knowledge and infrastructure necessary to start with the optical integration as well as to assist the partners from the very beginning.

Profile of Prof. Alexander Egner:

Prof. Alexander Egner (male) will be primarily responsible for carrying out the LLG based research and innovation activities in NanoVIB. He will lead WP2 "optical integration" and will work directly on the project tasks.

Prof. Egner is the Director of the LLG and also heads the Department of Optical Nanoscopy. His research is focused on: working on optical microscopy beyond the diffraction barrier with far-field optics since 1996; experimental development and theoretical analysis of 4Pi microscopy; development of STED microscopy and related concepts using photo-switching of fluorophores for far-field optical resolution on the nanoscale; and development of PALM/STORM microscopy and related concepts using photo-switching of fluorophores for far-field optical resolution on the nanoscale.

He has **53 publications** and **h-index of 37**, and his work has resulted in more than 8 patent applications in the field. He has supervised 17 PhDs. There are currently 4 postdocs, 3 PhD students, and 1 Master student in Egner's team, focusing on basic research and the resulting practical implementations for use in the field of super-resolution fluorescence microscopy. Egner's group has attracted over €8.000.000 in the past 5 years alone. He has a number of significant national and international collaborations, including: Tel Aviv University, "The mitochondrion-plasma membrane junction at super-resolution microscopy"; Ljubljana University, "super-resolution fluorescence microscopy (nanoscopy) for live imaging of

subcellular organelles by fluorescence means in living cells"; Abberior Instruments GmbH, "automated STED-nanoscope"; Göttingen University, DFG Clusters of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells".

Education:

1. Alexander Egner, born 1970 in Mannheim, studied **Physics** at **Heidelberg University**, where he received his **Doctorate in Physics** in 2002.
2. He was subsequently postdoctoral researcher and later senior scientist in the **Department of NanoBiophotonics** at the **Max Planck Institute for Biophysical Chemistry**.
3. In 2010 he became **Director of the Laser-Laboratorium Göttingen** where he also heads the **Department of Optical Nanoscopy**.

Scientific Career (Detail):

- 1991 – 1997: Studied in Physics, University of Heidelberg
- 1997: Diploma in Physics, University of Heidelberg, Prof. Dr. S. W. Hell
- 1998: Visiting Scientist, Department of Applied Physics, University of Osaka, Prof. Dr. S. Kawate
- 1998 – 2002: Ph.D. (Dr. rer. nat.), Physics, University of Heidelberg, Prof. Dr. S. W. Hell
- 2002 – 2003: Postdoctoral Researcher, Max Planck Institute for Biophysical Chemistry, Göttingen, Prof. Dr. S. W. Hell
- 2003 – 2010: Senior scientist, Max Planck Institute for Biophysical Chemistry, Göttingen, Prof. Dr. S. W. Hell
- 2005 – 2010: Head of Central Light Microscopy Facility, Max Planck Institute for Biophysical Chemistry, Göttingen
- since 2010: Managing Director and Head of Department of Optical Nanoscopy, Laser-Laboratorium Göttingen e.V.
- 2014: Habilitation, Physics, University of Göttingen
- 2017: Adj. Prof., Physics, University of Göttingen

Memberships:

- Executive board member, "Microscopy at the Nanometer Range" section of the "Cluster of Excellence: Nanoscale Microscopy and Molecular Physiology of the Brain", 2011 – 2019
- Faculty member, Göttingen Graduate School for Neurosciences, Biophysics and Molecular Biosciences, since 2011
- Steering committee member, DFG Collaborative Research Center 755 "Nanoscale Photonic Imaging", 2015 – 2019
- Spokesperson of the northern regional group, German Industrial Research Association Konrad Zuse, since 2017
- Member, DFG Clusters of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells", since 2019

Top 5 relevant achievements:

Publications:

- Klar, T. A.; Jakobs, S.; Dyba, M.; Egner, A. & Hell, S. W. "Fluorescence microscopy with diffraction resolution barrier broken by stimulated emission" *Proc. Natl. Acad. Sci. U. S. A.* 97, 8206-8210 (2000)
- Egner, A.; Jakobs, S.; Hell, S. W. "Fast 100-nm resolution three-dimensional microscope reveals structural plasticity of mitochondria in live yeast" *Proc. Natl. Acad. Sci. U. S. A.* 99, 3370-3375 (2002)
- Schmidt, R.; Wurm, C. A.; Jakobs, S.; Engelhardt, J.; Egner, A. & Hell, S. W. "Spherical nanosized focal spot unravels the interior of cells" *Nat. Methods* 5, 539-544 (2008)
- Aquino, D.; Schönle, A.; Geisler, C.; von Middendorf, C.; Wurm, C. A.; Okamura, Y.; Lang, T.; Hell, S. W. & Egner, A. "Two-color nanoscopy of three-dimensional volumes by 4Pi detection of stochastically switched fluorophores" *Nat. Methods* 8, 353-359 (2011)
- Vinçon, B.; Geisler, C.; Egner, A. "Pixel hopping enables fast STED nanoscopy at low light dose" *Opt. Express* 28, 4516-4528 (2020)

Up to 5 relevant projects or activities:

- Nanoscale analysis of the molecular interactions that control insertion and elimination of the complement C5b-9 complex (Volkswagen Foundation): Development of labelling strategies and acquisition protocols for super-resolution imaging based characterization of the induced translocation of mitochondria and mitochondrial proteins to the plasma membrane.
- isoSTED microscopy within tissue (DFG): Expand isoSTED microscopy to imaging under strongly aberrated imaging conditions, such as those introduced by biological tissue.
- STED microscope with active aberration correction & automatic adjustment (Federal Ministry of Economics and Energy): Integration of relevant optics into existing microscope and software-based integration of expert knowledge into image acquisition software. Cooperation with Abberior Instruments. Product launched in 2019.
- Fast monochromatic reflection nanoscopy by absorption modulation (German Research Council): Transfer of the STED principle to photochromic molecules and from the visible to the ultraviolet spectral range.
- Super-resolution microscopy with smart and sample-specific scanning patterns (German Research Council, within the framework of the German excellence initiative): Acceleration of STED and MINFLUX microscopy by using smart algorithms to allow the microscope to autonomously recognize the structure to be examined. In cooperation with Stefan W. Hell.

Participant 5 – Angewandte Physik und Elektronik GmbH (APE), Germany

Angewandte Physik & Elektronik GmbH (APE) was founded in 1992 and employs about 70 people at present. Headquartered in Berlin, APE is a worldwide leading supplier in the field of ultrashort laser pulse diagnostics and tunable wavelength conversion. With development expenditures of 30% of total revenues we constantly improve our products and develop new ones to serve the scientific community as well as the industrial ultrafast market.

The company is a hidden champion in the photonics industry: highly specialized, in the ultrashort pulse niche closely connected with users and customers, but for many rather unknown. However, due to its highly specialized focus on nonlinear optics, APE is a sought-after OEM development partner and supplier for laser manufacturers, microscopy companies and other well-known technology companies.

Today, the APE repertoire encompasses a large range of nonlinear optics equipment, from spectrometers to harmonic generators, from pulse compressors to quantum-dot single photon generation sources. Over the years, APE has built up an impressive array of more than 30 products entirely developed and produced within its headquarters in Berlin. APE's products are used worldwide in the leading universities and research institutes as well as by all large ultrafast laser manufacturers. They are sold and serviced by APE GmbH in Berlin and its partner company APE Inc. in Freemont, California, as well as through a worldwide distribution network.

In the field of microscopy APE has amassed experience over the last 20 years and offers several products.

- APE provided visible OPOs for second generation STED microscopes especially in the laboratory of Stefan W. Hell.
- Multi-photon microscopy is supported by APE with several products and APE has been the first on the market for many of them, such as light sources for two-photon excitation above 1000nm ("Chameleon compact OPO"), light sources for three-photon excitation ("AVUS-SP"), microscope autocorrelators ("Carpe") and precompressors to compensate the chirp of the microscopes ("femtocontrol").
- The company also provides infrared light sources for near field microscopy such as s-SNOM (scattering scanning nearfield optical microscopy) → "Carmina"

APE has been developing and building OPO-based light sources for coherent Raman microscopy (CARS and SRS) since 2005. In close cooperation with the leading scientists in this field, such as Sunney Xie, Andreas Zumbusch, Herve Rigneault, Jürgen Popp, Ji-Xin Cheng, Wei Min and many others we have developed easy to use and robust light sources and detectors for this type of microscopy. As of now, APE is the leading supplier of such light sources. The latest APE products for coherent Raman microscopy are the picoEmerald laser and the SRS detection set, with completely hands free and computer controlled operation to

enable easy setup and operation of coherent Raman microscopy.

Infrastructure:

APEs infrastructure encompasses 13 fully equipped laser laboratories including 3 clean rooms for production and development as well as more than 2000m² of office and manufacturing space. With a 20 people strong R&D team, APE covers the expertise for development in optics, mechanical design, electronics and software. Tools such as optical simulation software, CAD for mechanical design and software for electronics design and simulation are used. The software department has the capability of programming FPGA and microcontrollers as well as high level control software in LabVIEW, Java, C and Python. APE has the in-house capability of small scale in-house prototyping of mechanical and electronics parts. Furthermore, all products are designed and manufactured directly at APE in Berlin, ensuring short reaction times and direct means of communication.

Main tasks in this project:

The main task of APE in this project is to develop a laser for MINFLUX and SRS imaging operation. The two main goals are:

1. Increasing the tuning speed by more than an order of magnitude to <5s
2. Femto-picosecond switching for enabling SRS and photoactivation with a single light source

The expertise and product line of APE proves that APE understands the requirements for SRS and multiphoton microscopy and is able to develop hands free and easy to use microscopy light sources. The core technology of APE's light sources are optical parametric oscillators, which will be implemented in this development. With 25 years of experience in developing synchronously pumped OPOs in the femto- and picosecond regime, over 20 different OPO-based products and more than 800 installed systems within this time there is no other company in the world with this degree of expertise. This knowledge does help us to implement new nonlinear interactions and tuning regimes to speed up the tuning time and to implement the femto-picosecond switching. For the required pulse compression, APE has shown its expertise as well with its product "femtocontrol". Therefore, APE's profile is perfectly matching the tasks in this project.

Profile of Dr. Ingo Rimke:

Dr. Ingo Rimke (male) will be primarily responsible from APE for carrying out the proposed research and innovation activities in NanoVIB. He will lead WP4 "Laser for MINFLUX and SRS imaging operation".

Dr. Ingo Rimke is director of the research and development department at APE. He joined APE in 2001 and was since involved in all OPO related product developments at APE, either as the lead physics engineer, project manager or project supervisor.

He further coordinates APE's activities in the field of Coherent Raman microscopy and acts as a scientific advisor.

Education and professional career:

1. Ingo Rimke, born 1970 in Berlin, studied **Physics** at Humboldt University Berlin, the University of Manchester and the Free University Berlin. He received his **Doctorate in Physics** from the Free University in 1999.
2. In 2000 he joined **Bioptic Laser GmbH** to develop a diode pumped, frequency tripled ns-laser for mass spectrometer applications (MALDI-TOF).
3. From 2001 Ingo Rimke was working as **Manager Product Development at APE**.
4. In 2015 Ingo Rimke was appointed as the **Director of Research and Development at APE**.

Top 5 relevant achievements:

Products:

- picoEmerald (one-box, hands free light source for coherent Raman microscopy)
- https://www.ape-berlin.de/en/cars-srs/
- SRS detection set (detector and lock-in amplifier combination for easy SRS-detection) https://www.ape-berlin.de/content/uploads/2020/04/APE-SRS-Detection-Set-Rev-3-2-0.pdf

Publications:

- Rimke, I.; Hehl, G.; Beutler, M.; Volz, P.; Volkmer, A. & Büttner, E. "Tunable dual-wavelength two-picosecond light source for coherent Raman scattering microscopy" *Proc SPIE* 894816 (2014)
- Stiebing, C.; Meyer, T.; Rimke, I.; Matthäus, C.; Schmitt, M.; Lorkowski, S. & Popp, J. "Real- time Raman and SRS imaging of living human macrophages reveals cell- to- cell heterogeneity and dynamics of lipid uptake" *J. Biophoton.* 10, 1217-1226 (2017)
- Audier, X.; Heuke, S.; Volz, P.; Rimke, I. & Rigneault, H. "Noise in stimulated Raman scattering measurement: From basics to practice" *APL Photonics* 5, 011101 (2020)

Up to 5 relevant projects or activities:

- Development of the Levante OPO in 2005, the first jitter free light source for CARS microscopy
- Development of the Levante Emerald OPO in 2007 for CARS microscopy, a completely redesigned ps-OPO with much larger tuning range and improved handling
- First one-box, fully integrated light source for CARS microscopy – picoEmerald, Prism award finalist in 2009
- BMBF funded project MicroQuant (2011-2014) in the call "optical technologies in life sciences" to develop a hands free and computer controlled light source for stimulated Raman microscopy. Partners were among others Leica Microsystems, Univ. Konstanz and Univ. Stuttgart. The results of this project were used to develop

the second generation of picoEmerald lasers, now capable to do SRS, as well as a SRS detection unit.

- EU-Attract project SRS-Histology (2019-2020) together with Institute Fresnel, Marseille to develop building blocks to generate stimulated Raman based histopathology images for cancer research

Participant 6 – Pi Imaging Technology SA (PII), Switzerland

Pi Imaging Technology SA is a spin-off from EPFL, based on the know-how acquired during research work of Dr. Ivan Michel Antolovic, Dr. Claudio Bruschini and Prof. Edoardo Charbon on detectors counting single quanta of light, photons. The co-founders have an accumulated experience of more than 35 years in the field of photon-counting detectors. The company is headquartered in Neuchâtel and has offices in Ecublens.

Our company creates highly efficient photon-counting arrays in standard semiconductor technology, which enables unlimited number of pixels, and miniaturized shapes and architectures. The implemented photon-counting arrays directly transform photons to digital pulses. Our first product is a 23-pixel photon-counting array with excellent sensitivity, dynamic range and speed. In microscopy, this can improve the image quality by a factor of 2, reducing photo-toxicity of living cells. In cyber-security, a high dynamic range enables true random numbers at 1Gbps for data encryption. In machine vision, our arrays image in dark conditions at speeds faster than 1MHz.

Infrastructure:

Pi Imaging Technology is a spin-off from EPFL, with headquarters in Neuchâtel and offices in EPFL Innovation Park, Ecublens. Pi Imaging Technology's equipment consists of tools for electro-optical characterization and testing of photodetection systems. Pi Imaging Technology has access to fast electronic signal analyzers, fast picosecond-pulse lasers, temperature chambers and integrating spheres, and to the advanced nano-fabrication facilities at Center of MicroNanoTechnology, EPFL. This facility comprises photolithography, electron beam lithography, physical and chemical etching and material deposition machines. Moreover, Pi Imaging Technology has extensive experience in custom chip design with the emphasis on single-photon detectors and software packages Cadence (chip design), Xilinx ISE/Vivado (firmware design) and Altium Designer (electronics design).

Furthermore, Pi Imaging Technology has access to all infrastructure of EPFL Innovation Park, where more than 200 technological young companies with an impact on our economy and society create a unique ecosystem.

Main tasks in this project:

Pi Imaging Technology's main task is to design a new application specific SPAD array with special emphasis on increasing the sensitivity in the red spectrum. Pi Imaging will also adapt its current SPAD array detector to enable time filtering of autofluorescence and imaging of the fluorophore surrounding, both important features in MINFLUX searching algorithms. These tasks will strongly benefit from the experience and expertise of Pi Imaging in designing SPAD devices and semiconductor chips specifically for microscopy applications. Dr. Antolovic has both the necessary expertise and interdisciplinary competence to take on this task, gained through industrial and research projects.

Profile of Dr. Ivan Michel Antolovic and Dr. Harald Homulle:

Dr. Ivan Michel Antolovic (male)

Ivan Michel Antolovic obtained his Ph.D. in 2018 at TU Delft. His Ph.D. thesis on SPAD arrays for super resolution microscopy was awarded the 2018 Else Kooi Award for young researchers in the field of applied semiconductor research conducted in the Netherlands. After being a scientist at EPFL for two years, he co-founded Pi Imaging Technology with Prof. Edoardo Charbon, Dr. Claudio Bruschini and Dr. Ron Hoebe and is currently leading the company. Dr. Antolovic authored and co-authored over 15 articles in technical journals and conference proceedings and 5 patent applications.

Education and professional career:

1. Ivan Michel Antolovic, born 1988, studied **Electrical Engineering and Computing** at University of Zagreb and TU Graz. He received his **Ph.D.** in SPAD arrays for super-resolution microscopy from TU Delft in 2018.
2. He was subsequently postdoctoral researcher and scientist at **EPFL**.
3. In 2018, he became **co-founder of Pi Imaging Technology,** a spinoff from EPFL and is currently CEO.

Dr. Harald Homulle (male)

Harald Homulle obtained his Ph.D. (*cum laude*) in 2019 at TU Delft. During his Ph.D., he worked on both quantum computing electronic interfaces and single-photon avalanche diode systems. At the same, he was employed as consultant at Fastree3D for the implementation of SPAD arrays for automotive light detection and ranging systems. After the Ph.D., he joined Pi Imaging Technology as a Senior Design Engineer. He authored and co-authored over 25 technical journal articles and conference papers.

Education and professional career:

1. Harald Homulle, born 1990 in Den Haag, studied **Microelectronics** at TU Delft and EPFL. He received his **Ph.D.** in quantum electronic from TU Delft in 2019.
2. During his Ph.D., he worked as **consultant at Fastree3D**, a SME focused on SPAD arrays for automotive light detection and ranging systems.
3. He joined Pi Imaging Technology in 2020 as **Senior Design Engineer**.

Top 5 relevant achievements:

Products:

- 23-pixel SPAD array optimized for green detection, designed in a standard CMOS process (scalable), matched with excellent sensor performance and low system complexity. The SPAD active area is 107µm^2. The SPAD pixels feature a QE of 45%, a median dark count rate of 140cps, and a FWHM timing jitter of less than 130ps. Afterpulsing and crosstalk are below 0.1%. This SPAD array represents a compact solution for advanced scanning techniques such as fluorescence lifetime imaging

(FLIM), fluorescence correlation spectroscopy (FCS), image scanning microscopy (ISM) and stimulated emission depletion (STED).

Services:

- Custom design of SPAD arrays and systems with SPAD arrays. Pi Imaging offers top-down (starting from specifications) services of SPAD array design and manufacturing. Design services are based on building blocks verified in previous Pi Imaging products. On the system level, Pi Imaging offers advanced photon time-tagging and counting modalities implemented on FPGA.

Publications:

- Antolovic, I. M.; Burri, S.; Bruschini, C.; Hoebe, R. A. & Charbon, E. "SPAD imagers for super resolution localization microscopy enable analysis of fast fluorophore blinking" *Sci. Rep.* 7, 44108 (2017)
- Bruschini, C.; Homulle, H.; Antolovic, I. M.; Burri, S. & Charbon, E. "Single-photon avalanche diode imagers in biophotonics: review and outlook" *Light Sci. Appl.* 8, 1–28 (2019)
- Ulku, A.; Ardelean, A.; Antolovic, I. M.; Weiss, S.; Charbon, E.; Bruschini, C. & Michalet, X. "Wide-field time-gated SPAD imager for phasor-based FLIM applications" *Methods Appl. Fluoresc.* 8, 024002 (2020)

Up to 5 relevant projects or activities:

Pi Imaging Technology currently participates in multiple industrial projects aimed at utilizing SPAD arrays in microscopy, spectroscopy and random number generation. Within his scientific activity, Dr. Antolovic participated in scientific projects related to the activities in this proposal:

- NWO project "Ultra-fast GSDIM super resolution microscopy using a SPAD-array camera"
- NWO project "L3SPAD: A Single-Photon, Time-Resolved Image Sensor for Low-Light-Level Vision"
- SNSF project "Three-Dimensionally Integrated, Ultra-Fast Cameras for Time-Resolved Multi-Wavelength Fluorescence Imaging"

4.2. Third parties involved in the project

No third parties involved.

5. Ethics and Security

5.1 Ethics

How the proposal meets the national legal and ethical requirements: All animal experiments and studies on human tissue samples will take place in Stockholm, within WP6 of the project. They will be performed in accordance with all Swedish national regulations from the concerned authorities:

- Arbetsmiljöverket (the Swedish Work Environment Authority), regarding laboratory security, where handling of bacteria is regulated. In the project, all work using bacterial pathogens including mutants (BSL2 pathogens) will be taking place in laboratories classified at biosecurity level 2 (BSL2), all available to us, and approved by this authority.
- Jordbruksverket (the Swedish Board of Agriculture) and the local ethical committee for animal experiments (Stockholms Norra Djurförsöksetiska nämnd), regarding handling of animals in the research with ethical approval from the local ethical committee (Stockholms Norra Djurförsöksetiska nämnd).
- Etikprövningsmyndigheten (the Swedish Ethical Review Committee) and the local ethical committee for human experiments (KI Forskningsetik-kommitté Nord), regarding handling of human tissue material.

Research objectives, methodologies and potential impact of the research: In this project, all research involving animals (wild-type and genetically modified mice), human tissues and cells, as well as pathogenic bacteria will be performed within WP6. In WP5 we will develop sample preparation procedures for the use of the de developed microscopy techniques, and for the research in WP6, but this work will not be based on animal or human tissue samples. WP1-WP4 will entirely focus on microscope system development and will also not involve samples or activities raising any ethical issues. In WP6, two tasks concern the use of animals or human tissue:

In task 6.2, we will study possible co-localization of pneumococcal surface and pilus proteins with receptor proteins on epithelial cells of the blood-brain-barrier (BBB), in brain biopsies from patients who died from pneumococcal meningitis and in brain biopsies from mice models. The brain biopsies will either be obtained via collaboration with the Department of Neurology, Academic Medical Center, University of Amsterdam (Prof Diederik van de Beek), or locally from the clinics at Karolinska University Hospital, Stockholm, with ethical approval from the ethical committee when needed. The biopsies will come from deceased persons, who are fully anonymized; with no other information connected to the samples than that they are from individuals who died from pneumococcal meningitis, and there will be permission for autopsies by family and relatives of the deceased persons. The research will not involve any human participant with interventions, and no personal data collection and/or processing, or further processing of previously collected

personal data. Tissue samples will only be used in accordance with obtained approvals, or if necessary, after additional application for approval to the appropriate ethical committee. Human cells that will be included are bought from commercial sources such as A549 epithelial cells, or THP-1 macrophages from American Type Culture Collection (ATCC). No human embryos will be used.

In samples from mice, we will study effects of adding antibodies competing for binding to the BBB epithelial cell receptors, as a potential approach to prevent pneumococcal meningitis. Both wild type mice and genetically modified mice are planned to be used. Mice samples will only be used in accordance with obtained approvals, or if necessary, after additional application for approval to the appropriate ethical committee.

In task 6.4, we will use the developed microscopy of this proposal to study distribution patterns of pneumococcal surface proteins, coupled to sustained bacterial growth, in lungs of pneumococcal-influenza virus co-infected mice. The goal is to unravel important metabolic aspects in bacterial-viral co-infections, which seem to be a major driving force in such infections, to find better strategies to curb these infections. As for task 6.2, both wild type mice and genetically modified mice are planned to be used, and the mice and the samples from them will be handled in accordance with obtained ethical approvals, or if necessary, after additional application for approval to the appropriate ethical committee.

For both tasks 6.2 and 6.4, we see no detrimental impact as a consequence of the research planned in these work packages. The mice will be kept in a dedicated center for animals at Karolinska Insitutet, with well-established routines and extensive experience in these activities. Likewise, research on live pathogenic bacteria will be performed in BSL2 labs, with researchers and personnel well-trained and educated about any potential hazard in handling bacteria. No research will be performed in non-EU countries.

For the ethical approvals of the planned studies, we refer to the following approved ethical applications for the suggested mice experiments:

11367-2019: Study of pathogen and host responses in infections caused by Streptococcus pneumoniae, S. aureus and K. pneumoniae (approval dated 2015-12-30), pdf-file: N235-15_co-infection.pdf.

English summary: First page is the decision page from the local ethical committee for animal experiments (Stockholms Norra Djurförsöksetiska nämnd). “Concerning Your application(s) for ethical approval of animal experiments, Stockholms Norra Djurförsöksetiska nämnd have at this meeting on the 17th of December 2015 approved Your application(s) regarding ethical approval of animal experiments number N235/15”. On the second page, the head page of the application approved, Birgitta Henriques-Normark is named as the PI (försöksledaren), and the title of the application (as stated above) is given in box 1 at the bottom of the page

N235-15: Study of pathogen and host response in co-infections caused by influenza virus and Streptococcus pneumoniae (approval dated 2019-09-12), pdf-file: 11367-

2019_general_permit.pdf

English summary: The first page is the decision page from the local ethical committee for animal experiments (Stockholms Norra Djurförsöksetiska nämnd). “Stockholms Djurförsöksetiska nämnd approves your application and your planned experiment from an ethical point of view, and you can hereby perform your experiment, following the conditions below: 1. PI is Birgitta Henriques-Normark, 2. Director is Elisabeth Andersson, 3. The experiments are performed on sites specified in supplementary part A, 4. The experiments are performed according to supplementary part A”. The title of the ethical application approved (as stated above) is given in section 1.5 in the application, p.5(45).

Ethical approvals needed for the studies using brain biopsied postmortem will be applied for and will not start until ethical approvals have been granted.

As requested by the European Commission all the ethics related documents will be kept on file. The project will also submit three deliverables on M24 as part of WP8:

D8.1: Copies of relevant documents for using, producing or collecting human cells or tissues (e.g., ethics approval, import licence, accreditation/designation/authorisation/licensing) must be kept on file.

D8.2: Copies of relevant authorisations for animal experiments (covering also the work with genetically modified animals, if applicable) must be kept on file. If applicable, copies of training certificates/personal licenses of the staff involved in animal experiments must be kept on file (to be specified in the grant agreement).

D8.3: General information on the procedures to ensure animal welfare and adherence to the Three Rs principle must be submitted as a deliverable. The number of animals that will be used, in the context of the 3-Rs principle, must be specified.

5.2 Security

This project will involve:

- activities or results raising security issues: NO
- 'EU-classified information' as background or results: NO

ESTIMATED BUDGET FOR THE ACTION

Associated with document Ref. Ares(2020)7177005 - 29/11/2020

	Estimated eligible[1] costs (per budget category)										EU contribution			Additional information		
	A. Direct personnel costs				B. Direct costs of subcontracting	*[C. Direct costs of fin. support]*	D. Other direct costs		E. Indirect costs[2]	Total costs	Reimbursement rate %	Maximum EU contribution[3]	Maximum grant amount[4]	Information for indirect costs	Information for auditors	Other information:
	A.1 Employees (or equivalent) A.2 Natural persons under direct contract A.3 Seconded persons *[A.6 Personnel for providing access to research infrastructure]*		A.4 SME owners without salary A.5 Beneficiaries that are natural persons without salary				D.1 Travel D.2 Equipment D.3 Other goods and services *[D.4 Costs of large research infrastructure]*	D.5 Costs of internally invoiced goods and services						Estimated costs of in-kind contributions not used on premises	Declaration of costs under Point D.4	Estimated costs of beneficiaries/ linked third parties not receiving funding/ international partners
Form of costs[6]	Actual	Unit[7]	Unit[8]		Actual	Actual	Actual	Unit[9]	Flat-rate[10] 25%							
	a	Total b	No hours	Total c	d	*[e]*	f	Total g	h = 0,25 x (a +b+c+f+g +*[i1]*[13]+*[i2]*[13]-n)	j = a+b+c+d +*[e]*+f+g+h +*[i1]*+*[i2]*	k	l	m	n	Yes/No	
1. KTH	790 400.00	0.00	0.00	0.00	0.00	0.00	118 000.00	0.00	227 100.00	1 135 500.00	100.00	1 135 500.00	1 135 500.00	0.00	No	n/a
2. KI	454 080.00	0.00	0.00	0.00	0.00	0.00	68 000.00	0.00	130 520.00	652 600.00	100.00	652 600.00	652 600.00	0.00	No	n/a
3. AI	530 799.00	0.00	0.00	0.00	0.00	0.00	711 500.00	0.00	310 574.75	1 552 873.75	100.00	1 552 873.75	1 552 873.00	0.00	No	n/a
4. LLG	451 125.00	0.00	0.00	0.00	0.00	0.00	286 600.00	0.00	184 431.25	922 156.25	100.00	922 156.25	922 156.00	0.00	No	n/a
5. APE	353 420.00	0.00	0.00	0.00	0.00	0.00	209 000.00	0.00	140 605.00	703 025.00	100.00	703 025.00	703 025.00	0.00	No	n/a
6. PII	336 000.00	0.00	0.00	0.00	0.00	0.00	199 500.00	0.00	133 875.00	669 375.00	100.00	669 375.00	669 375.00	0.00	No	n/a
Total consortium	2 915 824.00	0.00		0.00	0.00	0.00	1 592 600.00	0.00	1 127 106.00	5 635 530.00		5 635 530.00	5 635 529.00			0.00

[1] See Article 6 for the eligibility conditions.
[2] Indirect costs already covered by an operating grant (received under any EU or Euratom funding programme; see Article 6.5.(b)) are ineligible under the GA. Therefore, a beneficiary/linked third party that receives an operating grant during the action's duration cannot declare indirect costs for the year(s)/reporting period(s) covered by the operating grant, unless it can demonstrate that the operating grant does not cover any costs of the action (see Article 6.2.E).
[3] This is the theoretical amount of EU contribution that the system calculates automatically (by multiplying all the budgeted costs by the reimbursement rate). This theoretical amount is capped by the 'maximum grant amount' (that the Commission decided to grant for the action) (see Article 5.1).
[4] The 'maximum grant amount' is the maximum grant amount decided by the Commission. It normally corresponds to the requested grant, but may be lower.
[5] Depending on its type, this specific cost category will or will not cover indirect costs. Specific unit costs that include indirect costs are: costs for energy efficiency measures in buildings, access costs for providing trans-national access to research infrastructure and costs for clinical studies.
[6] See Article 5 for the forms of costs.
[7] Unit : hours worked on the action; costs per unit (hourly rate) : calculated according to the beneficiary's usual accounting practice.
[8] See Annex 2a 'Additional information on the estimated budget' for the details (costs per hour (hourly rate)).
[9] Unit and costs per unit : calculated according to the beneficiary's usual accounting practices.
[10] Flat rate : 25% of eligible direct costs, from which are excluded: direct costs of subcontracting, costs of in-kind contributions not used on premises, direct costs of financial support, and unit costs declared under budget category F if they include indirect costs (see Article 6.2.E).
[11] See Annex 2a 'Additional information on the estimated budget' for the details (units, costs per unit).
[12] See Annex 2a 'Additional information on the estimated budget' for the details (units, costs per unit, estimated number of units, etc).
[13] Only specific unit costs that do not include indirect costs.
[14] See Article 9 for beneficiaries not receiving funding.
[15] Only for linked third parties that receive funding.

ANNEX 2a

ADDITIONAL INFORMATION ON THE ESTIMATED BUDGET

- **Instructions and footnotes in blue will not appear in the text generated by the IT system (since they are internal instructions only).**
- **For options *[*in square brackets*]*: the applicable option will be chosen by the IT system. Options not chosen will automatically not appear.**
- **For fields in [grey in square brackets] (even if they are part of an option as specified in the previous item): IT system will enter the appropriate data.**

⚠ **Transitory period: Until SyGMa fully supports Annex 2a, you must prepare it manually (using this template by choosing and deleting the options/entering the appropriate data).**
For the 'unit cost tables': either fill them out manually or use currently existing tables from Annex 1 or the proposal.
The document can then be uploaded in SyGMa and attached to the grant agreement.

Unit cost for SME owners/natural beneficiaries without salary

1. Costs for a *[*SME owner*][*beneficiary that is a natural person*]* not receiving a salary

Units: hours worked on the action

Amount per unit ('hourly rate'): calculated according to the following formula:

{the monthly living allowance for researchers in MSCA-IF actions / 143 hours}
multiplied by
{country-specific correction coefficient of the country where the beneficiary is established}

The monthly living allowance and the country-specific correction coefficients are set out in the Work Programme (section 3 MSCA) in force at the time of the call:

- for calls *before* Work Programme 2018-2020:
  - for the monthly living allowance: **EUR 4 650**
  - for the country-specific correction coefficients: see Work Programme 2014-2015 and Work Programme 2016-2017 (available on the Participant Portal Reference Documents page)
- for calls *under* Work Programme 2018-2020:
  - for the monthly living allowance: **EUR 4 880**
  - for the country-specific correction coefficients: see Work Programme 2018-2020 (available on the Participant Portal Reference Documents page)

[additional OPTION for beneficiaries/linked third parties that have opted to use the unit cost (in the proposal/with an amendment): For the following beneficiaries/linked third parties, the amounts per unit (hourly rate) are fixed as follows:

- beneficiary/linked third party [short name]: EUR [insert amount]
- beneficiary/linked third party [short name]: EUR [insert amount]

[same for other beneficiaries/linked third parties, if necessary] *]*

Estimated number of units: see Annex 2

Energy efficiency measures unit cost

2. Costs for energy efficiency measures in buildings

Unit: m^2 of eligible 'conditioned' (i.e. built or refurbished) floor area

Amount per unit*: see (for each beneficiary/linked third party and BEST table) the 'unit cost table' attached

* Amount calculated as follows:
{EUR 0.1 x estimated total kWh saved per m^2 per year x 10}

Estimated number of units: see (for each beneficiary/linked third party and BEST table) the 'unit cost table' attached

Unit cost table (energy efficiency measures unit cost)[1]

Short name beneficiary/linked third party	BEST No	Amount per unit	Estimated No of units	Total unit cost (cost per unit x estimated no of units)

[1] Data from the 'building energy specification table (BEST)' that is part of the proposal and Annex 1.

Research infrastructure unit cost

3. Access costs for providing trans-national access to research infrastructure

Units[2]: see (for each access provider and installation) the 'unit cost table' attached

Amount per unit*: see (for each access provider and installation) the 'unit cost table' attached

* Amount calculated as follows:

$$\frac{\text{average annual total access cost to the installation (over past two years[3])}}{\text{average annual total quantity of access to the installation (over past two years[4])}}$$

Estimated number of units: see (for each access provider and installation) the 'unit cost table' attached

Unit cost table (access to research infrastructure unit cost)[5]

Short name access provider	Short name infrastructure	Installation		Unit of access	Amount per unit	Estimated No of units	Total unit cost (cost per unit x estimated no of units)
		No	Short name				

Clinical studies unit cost

4. Costs for clinical studies

Units: patients/subjects that participate in the clinical study

Amount per unit*: see (for each sequence (if any), clinical study and beneficiary/linked third party) the 'unit cost table' attached

* Amount calculated, for the cost components of each task, as follows:

For **personnel costs**:

For personnel costs of doctors: 'average hourly cost for doctors', i.e.:

{certified or auditable total personnel costs for doctors for year N-1

{1720 * number of full-time-equivalent for doctors for year N-1}

multiplied by

estimated number of hours to be worked by doctors for the task (per participant)**}**

For personnel costs of other medical personnel: 'average hourly cost for other medical personnel', i.e.:

{certified or auditable total personnel costs for other medical personnel for year N-1

{1720 * number of full-time-equivalent for other medical personnel for year N-1}

---

2 Unit of access (e.g. beam hours, weeks of access, sample analysis) fixed by the access provider in proposal.

3 In exceptional and duly justified cases, the Commission/Agency may agree to a different reference period.

4 In exceptional and duly justified cases, the Commission/Agency may agree to a different reference period.

5 Data from the 'table on estimated costs/quantity of access to be provided' that is part of the proposal and Annex 1.

multiplied by
estimated number of hours to be worked by other medical personnel for the task (per participant)**}**

For personnel costs of technical personnel: **'**average hourly cost for technical personnel', i.e.:

{certified or auditable total personnel costs for technical personnel for year N-1

{1720 * number of full-time-equivalent for technical personnel for year N-1}
multiplied by
estimated number of hours to be worked by technical personnel for the task (per participant)**}**

'total personnel costs' means actual salaries + actual social security contributions + actual taxes and other costs included in the remuneration, provided they arise from national law or the employment contract/equivalent appointing act

For **consumables**:

For each cost item: 'average price of the consumable', i.e.:

{{certified or auditable total costs of purchase of the consumable in year N-1

total number of items purchased in year N-1}
multiplied by
estimated number of items to be used for the task (per participant)**}**

'total costs of purchase of the consumable' means total value of the supply contracts (including related duties, taxes and charges such as non-deductible VAT) concluded by the beneficiary for the consumable delivered in year N-1, provided the contracts were awarded according to the principle of best value- for-money and without any conflict of interests

For **medical equipment**:

For each cost item: 'average cost of depreciation and directly related services per unit of use', i.e.:

{{ certified or auditable total depreciation costs in year N-1 + certified or auditable total costs of purchase of services in year N-1 for the category of equipment concerned}

total capacity in year N-1
multiplied by
estimated number of units of use of the equipment for the task (per participant)**}**

'total depreciation costs' means total depreciation allowances as recorded in the beneficiary's accounts of year N-1 for the category of equipment concerned, provided the equipment was purchased according to the principle of best value for money and without any conflict of interests + total costs of renting or leasing contracts (including related duties, taxes and charges such as non-deductible VAT) in year N-1 for the category of equipment concerned, provided they do not exceed the depreciation costs of similar equipment and do not include finance fees

For **services**:

For each cost item: 'average cost of the service per study participant', i.e.:

{certified or auditable total costs of purchase of the service in year N-1

total number of patients or subjects included in the clinical studies for which the service was delivered in year N-1**}**

'total costs of purchase of the service' means total value of the contracts concluded by the beneficiary (including related duties, taxes and charges such as non-deductible VAT) for the specific service delivered in year N-1 for the conduct of clinical studies, provided the contracts were awarded according to the principle of best value for money and without any conflict of interests

For **indirect costs**:

{{{cost component 'personnel costs' + cost component 'consumables' + cost component 'medical equipment'}

minus

{costs of in-kind contributions provided by third parties which are not used on the beneficiary's premises + costs of providing financial support to third parties (if any)}**}**

multiplied by

25%**}**

The estimation of the resources to be used must be done on the basis of the study protocol and must be the same for all beneficiaries/linked third parties/third parties involved.

The year N-1 to be used is the last closed financial year at the time of submission of the grant application.

Estimated number of units: see (for each clinical study and beneficiary/linked third party) the 'unit cost table' attached

Unit cost table: clinical studies unit cost[6]

Task, Direct cost categories	**Resource per patient**	**Costs year N-1 Beneficiary 1 [short name]**	**Costs year N-1 Linked third party 1a [short name]**	**Costs year N-1 Beneficiary 2 [short name]**	**Costs year N-1 Linked third party 2a [short name]**	**Costs year N-1 Third party giving in-kind contributions 1 [short name]**
Sequence No. 1						
Task No. 1 Blood sample						
(a) **Personnel costs:** - Doctors	n/a					
- Other Medical Personnel	Phlebotomy (nurse), 10 minutes	8,33 EUR	11,59 EUR	10,30 EUR	11,00 EUR	9,49 EUR
- Technical Personnel	Sample Processing (lab technician), 15 minutes	9,51 EUR	15,68 EUR	14,60 EUR	15,23 EUR	10,78 EUR
(b) **Costs of consumables**:	Syringe	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
	Cannula	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
	Blood container	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
(c) **Costs of medical equipment**:	Use of -80° deep freezer, 60 days	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
	Use of centrifuge, 15 minutes	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
(d) **Costs of services**	Cleaning of XXX	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
(e) **Indirect costs** (25% flat-rate)		XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
Task No. 2						
…						
Amount per unit (unit cost sequence 1):		**XX EUR**	**XX EUR**	**XX EUR**	**XX EUR**	**XX EUR**
Sequence No. 2						
Task No. 1						

[6] Same table as in proposal and Annex 1.

XXX						
(a) **Personnel costs:** - Doctors	XXX	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
- Other Medical Personnel	XXX	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
- Technical Personnel	XXX	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
(b) **Costs of consumables**:	XXX	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
	XXX	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
	XXX	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
(c) **Costs of medical equipment**:	XXX	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
	XXX	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
(d) **Costs of services**	XXX	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
(e) **Indirect costs** (25% flat-rate)		XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
Task No. 2						
…						
Amount per unit (unit cost sequence 2):		**XX EUR**	**XX EUR**	**XX EUR**	**XX EUR**	**XX EUR**
…						
Amount per unit (unit cost entire study):		**XX EUR**	**XX EUR**	**XX EUR**	**XX EUR**	**XX EUR**

1

Associated with document Ref. Ares(2020)7177005 - 29/11/2020

ANNEX 3

ACCESSION FORM FOR BENEFICIARIES

KAROLINSKA INSTITUTET (KI), established in Nobels Vag 5, STOCKHOLM 17177, Sweden, VAT number: SE202100297301, ('the beneficiary'), represented for the purpose of signing this Accession Form by the undersigned,

hereby agrees

to become beneficiary No ('2')

in Grant Agreement No 101017180 ('the Agreement')

between KUNGLIGA TEKNISKA HOEGSKOLAN **and** the European Union ('the EU'), represented by the European Commission ('the Commission'),

for the action entitled 'NANO-scale VIsualization to understand Bacterial virulence and invasiveness - based on fluorescence NANOscopy and VIBrational microscopy (NanoVIB)'.

and mandates

the coordinator to submit and sign in its name and on its behalf any **amendments** to the Agreement, in accordance with Article 55.

By signing this Accession Form, the beneficiary accepts the grant and agrees to implement it in accordance with the Agreement, with all the obligations and conditions it sets out.

SIGNATURE

For the beneficiary

Bjorn KULL with ECAS id nkulbjor signed in the Participant Portal on 08/12/2020 at 16:24:21 (transaction id SigId-44780-LDgZCUXdbXhS67MMRNax7GBLjsIp8qg1ATdsV4lTUxOMXVkt4vVWjQpeYsPn8aQzmEVSOKL4cT3WpK7NGzrpqg-rS0vSrmBGYCMvs3SGKskZa-wBa7elQtChOYJO7L47zX4pNCuaqzw53lvMVTfMJitOXaNzU8xpczxH84YRAtiwrpEmA3mVBptvkghpfApNwCKJG). Timestamp by third party at 2020.12.08 16:24:26 CET

ANNEX 3

ACCESSION FORM FOR BENEFICIARIES

ABBERIOR INSTRUMENTS GMBH (AI), established in HANS ADOLF KREBS WEG 1, GOTTINGEN 37077, Germany, VAT number: DE283588727, ('the beneficiary'), represented for the purpose of signing this Accession Form by the undersigned,

hereby agrees

to become beneficiary No ('3')

in Grant Agreement No 101017180 ('the Agreement')

between KUNGLIGA TEKNISKA HOEGSKOLAN **and** the European Union ('the EU'), represented by the European Commission ('the Commission'),

for the action entitled 'NANO-scale VIsualization to understand Bacterial virulence and invasiveness - based on fluorescence NANOscopy and VIBrational microscopy (NanoVIB)'.

and mandates

the coordinator to submit and sign in its name and on its behalf any **amendments** to the Agreement, in accordance with Article 55.

By signing this Accession Form, the beneficiary accepts the grant and agrees to implement it in accordance with the Agreement, with all the obligations and conditions it sets out.

SIGNATURE

For the beneficiary

Andreas SCHOENLE with ECAS id nsoenlan signed in the Participant Portal on 06/01/2021 at 19:57:12 (transaction id SigId-262231-K1lGL3VGd4OEzJ1MYDwBEehmldvMPuaORcskjojUm55DGdDmzcZrWKqJXUR32Z1zUTli6pLt10bfK4CeNHzIhzR-rS0vSrmBGYCMvs3SGKskZa-chWczPC4d4zkIqnFl2qoJRRoVqvFJuqWG1mp43BmQdAHEq3H7xQyMxZF1yahgsPaLkFCUUEAXUAsxdwljkAP5zW). Timestamp by third party at 2021.01.06 19:57:17 CET

ANNEX 3

ACCESSION FORM FOR BENEFICIARIES

LASER-LABORATORIUM GOTTINGEN EV (LLG), established in HANS-ADOLF-KREBS WEG 1, GOTTINGEN 37077, Germany, VAT number: DE115312817, ('the beneficiary'), represented for the purpose of signing this Accession Form by the undersigned,

hereby agrees

to become beneficiary No ('4')

in Grant Agreement No 101017180 ('the Agreement')

between KUNGLIGA TEKNISKA HOEGSKOLAN **and** the European Union ('the EU'), represented by the European Commission ('the Commission'),

for the action entitled 'NANO-scale VIsualization to understand Bacterial virulence and invasiveness - based on fluorescence NANOscopy and VIBrational microscopy (NanoVIB)'.

and mandates

the coordinator to submit and sign in its name and on its behalf any **amendments** to the Agreement, in accordance with Article 55.

By signing this Accession Form, the beneficiary accepts the grant and agrees to implement it in accordance with the Agreement, with all the obligations and conditions it sets out.

SIGNATURE

For the beneficiary

Roland Becker with ECAS id n0041dpw signed in the Participant Portal on 08/12/2020 at 14:14:21 (transaction id SigId-40946-imJ4hP PzMQynkEEbLQbCf1YsmxmnDjNOL6HQ8F4fxNGZI42P4nfqmS2Uq7 Z0Mj8JCwtvIDTFAdaJDwOZlCaCS0-rS0vSrmBGYCMvs3SGKskZa-twyo aHPRA6cyzHhzzxzk4hrUbpQ05LeMNv2N0ueQ3E5LVS7MdY56tPxX1 bJ7zUEDfr9M9hyNjyGJyzKuIceD3dc0). Timestamp by third party at 2020.12.08 15:14:26 CET

ANNEX 3

ACCESSION FORM FOR BENEFICIARIES

APE ANGEWANDTE PHYSIK UND ELEKTRONIK GMBH (APE), established in PLAUENER STRASSE 163 165, BERLIN 13053, Germany, VAT number: DE155557053, ('the beneficiary'), represented for the purpose of signing this Accession Form by the undersigned,

hereby agrees

to become beneficiary No ('5')

in Grant Agreement No 101017180 ('the Agreement')

between KUNGLIGA TEKNISKA HOEGSKOLAN **and** the European Union ('the EU'), represented by the European Commission ('the Commission'),

for the action entitled 'NANO-scale VIsualization to understand Bacterial virulence and invasiveness - based on fluorescence NANOscopy and VIBrational microscopy (NanoVIB)'.

and mandates

the coordinator to submit and sign in its name and on its behalf any **amendments** to the Agreement, in accordance with Article 55.

By signing this Accession Form, the beneficiary accepts the grant and agrees to implement it in accordance with the Agreement, with all the obligations and conditions it sets out.

SIGNATURE

For the beneficiary

Ingo Rimke with ECAS id n00354e7 signed in the Participant Portal on 08/12/2020 at 13:45:28 (transaction id SigId-40203-8rnOqa6TTd zlgqzS5phiJZGfgzOwh2t4vk8ByRxuPdwYqT070KPxg8FQHdZgv9G8 5yB7FGpF1UXfMZ8OzzaIflg-rS0vSrmBGYCMvs3SGKskZa-tkyoMtlrq nvSzxHsnpXdPH880GzLaqAfPPqhzNDHw44Ym2lzgMhm7NJVdNZX jJi7staPvdSI42pIVgWKPEnUZVS). Timestamp by third party at 2020.12.08 15:45:38 CET

ANNEX 3

ACCESSION FORM FOR BENEFICIARIES

PI IMAGING TECHNOLOGY SA (PII), established in RUE DE LA PIERRE A MAZEL 39, NEUCHATEL 2000, Switzerland, VAT number: CHE496873037TVA, ('the beneficiary'), represented for the purpose of signing this Accession Form by the undersigned,

hereby agrees

to become beneficiary No ('6')

in Grant Agreement No 101017180 ('the Agreement')

between KUNGLIGA TEKNISKA HOEGSKOLAN **and** the European Union ('the EU'), represented by the European Commission ('the Commission'),

for the action entitled 'NANO-scale VIsualization to understand Bacterial virulence and invasiveness - based on fluorescence NANOscopy and VIBrational microscopy (NanoVIB)'.

and mandates

the coordinator to submit and sign in its name and on its behalf any **amendments** to the Agreement, in accordance with Article 55.

By signing this Accession Form, the beneficiary accepts the grant and agrees to implement it in accordance with the Agreement, with all the obligations and conditions it sets out.

SIGNATURE

For the beneficiary

Michel Antolovic with ECAS id n0038axy signed in the Participant Portal on 09/12/2020 at 14:02:33 (transaction id SigId-60632-1Pj4Jd8Xl9YHWcVs5i34NiOYhEpI8fhgtZexhwBzUehYY7ukkKE9czWxaK864MNUVooKtrwchtZoBQIzK3iLzcy-rS0vSrmBGYCMvs3SGKskZa-OtxRXtbL87I4wOKob8vyuQKnbtnZwr5JiffdsGStZtTOQFZzOA4zOtK3ozOP6kGiAp6Fu5ufWiCWvBv8EBRnUMm). Timestamp by third party at 2020.12.09 14:02:38 CET

ⓘ print format A4 landscape

MODEL ANNEX 4 FOR H2020 GENERAL MGA — MULTI

Associated with document Ref. Ares(2020)7177005 - 29/11/2020

FINANCIAL STATEMENT FOR *[BENEFICIARY [name]/ LINKED THIRD PARTY [name]]* FOR REPORTING PERIOD [reporting period]

	Eligible[1] costs (per budget category)													Receipts	EU contribution			Additional information
	A. Direct personnel costs				B. Direct costs of subcontracting	*[C. Direct costs of fin. support]*	D. Other direct costs			E. Indirect costs[2]	*[F. Costs of …]*		Total costs	Receipts	Reimbursement rate %	Maximum EU contribution[3]	Requested EU contribution	Information for indirect costs :
	A.1 Employees (or equivalent) A.2 Natural persons under direct contract A.3 Seconded persons *[A.6 Personnel for providing access to research infrastructure]*		A.4 SME owners without salary A.5 Beneficiaries that are natural persons without salary			*[C.1 Financial support]* *[C.2 Prizes]*	D.1 Travel D.2 Equipment D.3 Other goods and services	*[D.4 Costs of large research infrastructure]*	D.5 Costs of internally invoiced goods and services		*[F.1 Costs of …]*	*[F.2 Costs of …]*		Receipts of the action, to be reported in the last reporting period, according to Article 5.3.3				Costs of in-kind contributions not used on premises
Form of costs[4]	Actual	Unit	Unit		Actual	Actual	Actual	Actual	Unit	Flat-rate[5] 25%	Unit		*[Unit][Lump sum]*					
	a	Total b	No hours	Total c	d	*[e]*	f	*[g]*	Total h	i=0,25 x (a+b+ c+f+*[g]* + h+ *[j 1]*[6] +*[j2]*[6] -p)	No units	Total *[j1]*	Total *[j2]*	k = a+b+c+d+*[e]* +*f* + *[g]* +h+ i + *[j1]* +*[j2]*	l	m	n	o	p
[short name beneficiary/linked third party]																		

The beneficiary/linked third party hereby confirms that:
The information provided is complete, reliable and true.
The costs declared are eligible (see Article 6).
The costs can be substantiated by adequate records and supporting documentation that will be produced upon request or in the context of checks, reviews, audits and investigations (see Articles 17, 18 and 22).
For the last reporting period: that all the receipts have been declared (see Article 5.3.3).

ⓘ Please declare all eligible costs, even if they exceed the amounts indicated in the estimated budget (see Annex 2). Only amounts that were declared in your individual financial statements can be taken into account lateron, in order to replace other costs that are found to be ineligible.

[1] See Article 6 for the eligibility conditions

[2] The indirect costs claimed must be free of any amounts covered by an operating grant (received under any EU or Euratom funding programme; see Article 6.2.E). If you have received an operating grant during this reporting period, you cannot claim indirect costs unless you can demonstrate that the operating grant does not cover any costs of the action.

[3] This is the *theoretical* amount of EU contribution that the system calculates automatically (by multiplying the reimbursement rate by the total costs declared). The amount you request (in the column 'requested EU contribution') may be less,

[4] See Article 5 for the forms of costs

[5] Flat rate : 25% of eligible direct costs, from which are excluded: direct costs of subcontracting, costs of in-kind contributions not used on premises, direct costs of financial support, and unit costs declared under budget category F if they include indirect costs (see Article 6.2.E)

[6] Only specific unit costs that do not include indirect costs

ANNEX 5

MODEL FOR THE CERTIFICATE ON THE FINANCIAL STATEMENTS

- **For options [*in italics in square brackets*]: choose the applicable option. Options not chosen should be deleted.**
- **For fields in [grey in square brackets]: enter the appropriate data**

TABLE OF CONTENTS

Terms of Reference for an Independent Report of Factual Findings on costs declared under a Grant Agreement financed under the Horizon 2020 Research and Innovation Framework Programme

This document sets out the '**Terms of Reference (ToR)**' under which

[OPTION 1: [insert name of the beneficiary] ('the Beneficiary')] [OPTION 2: [insert name of the linked third party] ('the Linked Third Party'), third party linked to the Beneficiary [insert name of the beneficiary] ('the Beneficiary')]

agrees to engage

[**insert legal name of the auditor**] ('the Auditor')

to produce an independent report of factual findings ('the Report') concerning the Financial Statement(s)[1] drawn up by the *[Beneficiary] [Linked Third Party]* for the Horizon 2020 grant agreement [insert number of the grant agreement, title of the action, acronym and duration from/to] ('the Agreement'), and

to issue a Certificate on the Financial Statements' ('CFS') referred to in Article 20.4 of the Agreement based on the compulsory reporting template stipulated by the Commission.

The Agreement has been concluded under the Horizon 2020 Research and Innovation Framework Programme (H2020) between the Beneficiary and *[OPTION 1: the European Union, represented by the European Commission ('the Commission')][OPTION 2: the European Atomic Energy Community (Euratom,) represented by the European Commission ('the Commission')][OPTION 3: the [Research Executive Agency (REA)] [European Research Council Executive Agency (ERCEA)] [Innovation and Networks Executive Agency (INEA)] [Executive Agency for Small and Medium-sized Enterprises (EASME)] ('the Agency'), under the powers delegated by the European Commission ('the Commission').]*

The *[Commission] [Agency]* is mentioned as a signatory of the Agreement with the Beneficiary only. The *[European Union][Euratom][Agency]* is not a party to this engagement.

1.1 Subject of the engagement

The coordinator must submit to the *[Commission][Agency]* the final report within 60 days following the end of the last reporting period which should include, amongst other documents, a CFS for each beneficiary and for each linked third party that requests a total contribution of EUR 325 000 or more, as reimbursement of actual costs and unit costs calculated on the basis of its usual cost accounting practices (see Article 20.4 of the Agreement). The CFS must cover all reporting periods of the beneficiary or linked third party indicated above.

The Beneficiary must submit to the coordinator the CFS for itself and for its linked third party(ies), if the CFS must be included in the final report according to Article 20.4 of the Agreement.

The CFS is composed of two separate documents:

- The Terms of Reference ('the ToR') to be signed by the *[Beneficiary] [Linked Third Party]* and the Auditor;

[1] By which costs under the Agreement are declared (see template 'Model Financial Statements' in Annex 4 to the Grant Agreement).

- The Auditor's Independent Report of Factual Findings ('the Report') to be issued on the Auditor's letterhead, dated, stamped and signed by the Auditor (or the competent public officer) which includes the agreed-upon procedures ('the Procedures') to be performed by the Auditor, and the standard factual findings ('the Findings') to be confirmed by the Auditor.

If the CFS must be included in the final report according to Article 20.4 of the Agreement, the request for payment of the balance relating to the Agreement cannot be made without the CFS. However, the payment for reimbursement of costs covered by the CFS does not preclude the Commission *[Agency,]* the European Anti-Fraud Office and the European Court of Auditors from carrying out checks, reviews, audits and investigations in accordance with Article 22 of the Agreement.

1.2 Responsibilities

The *[Beneficiary] [Linked Third Party]:*

- must draw up the Financial Statement(s) for the action financed by the Agreement in compliance with the obligations under the Agreement. The Financial Statement(s) must be drawn up according to the *[Beneficiary's] [Linked Third Party's]* accounting and book-keeping system and the underlying accounts and records;
- must send the Financial Statement(s) to the Auditor;
- is responsible and liable for the accuracy of the Financial Statement(s);
- is responsible for the completeness and accuracy of the information provided to enable the Auditor to carry out the Procedures. It must provide the Auditor with a written representation letter supporting these statements. The written representation letter must state the period covered by the statements and must be dated;
- accepts that the Auditor cannot carry out the Procedures unless it is given full access to the *[Beneficiary's] [Linked Third Party's]* staff and accounting as well as any other relevant records and documentation.

The Auditor:

- *[Option 1 by default:* is qualified to carry out statutory audits of accounting documents in accordance with Directive 2006/43/EC of the European Parliament and of the Council of 17 May 2006 on statutory audits of annual accounts and consolidated accounts, amending Council Directives 78/660/EEC and 83/349/EEC and repealing Council Directive 84/253/EEC or similar national regulations*]*.
- *[Option 2 if the Beneficiary or Linked Third Party has an independent Public Officer:* is a competent and independent Public Officer for which the relevant national authorities have established the legal capacity to audit the Beneficiary*]*.
- *[Option 3 if the Beneficiary or Linked Third Party is an international organisation:* is an [internal] [external] auditor in accordance with the internal financial regulations and procedures of the international organisation*]*.

The Auditor:

- must be independent from the Beneficiary *[and the Linked Third Party]*, in particular, it must not have been involved in preparing the *[Beneficiary's] [Linked Third Party's]* Financial Statement(s);
- must plan work so that the Procedures may be carried out and the Findings may be assessed;
- must adhere to the Procedures laid down and the compulsory report format;
- must carry out the engagement in accordance with this ToR;
- must document matters which are important to support the Report;
- must base its Report on the evidence gathered;
- must submit the Report to the *[Beneficiary] [Linked Third Party]*.

The Commission sets out the Procedures to be carried out by the Auditor. The Auditor is not responsible for their suitability or pertinence. As this engagement is not an assurance engagement, the Auditor does not provide an audit opinion or a statement of assurance.

1.3 Applicable Standards

The Auditor must comply with these Terms of Reference and with[2]:

- the International Standard on Related Services ('ISRS') 4400 *Engagements to perform Agreed-upon Procedures regarding Financial Information* as issued by the International Auditing and Assurance Standards Board (IAASB);
- the *Code of Ethics for Professional Accountants* issued by the International Ethics Standards Board for Accountants (IESBA). Although ISRS 4400 states that independence is not a requirement for engagements to carry out agreed-upon procedures, the *[Commission][Agency]* requires that the Auditor also complies with the Code's independence requirements.

The Auditor's Report must state that there is no conflict of interests in establishing this Report between the Auditor and the Beneficiary *[and the Linked Third Party]*, and must specify - if the service is invoiced - the total fee paid to the Auditor for providing the Report.

1.4 Reporting

The Report must be written in the language of the Agreement (see Article 20.7).

Under Article 22 of the Agreement, the Commission*[, the Agency],* the European Anti-Fraud Office and the Court of Auditors have the right to audit any work that is carried out under the action and for which costs are declared from [*the European Union*] [*Euratom*] budget. This includes work related to this engagement. The Auditor must provide access to all working papers (e.g. recalculation of hourly rates, verification of the time declared for the action) related to this assignment if the Commission *[, the Agency]*, the European Anti-Fraud Office or the European Court of Auditors requests them.

1.5 Timing

The Report must be provided by *[*dd Month yyyy*]*.

1.6 Other terms

[*The [Beneficiary] [Linked Third Party] and the Auditor can use this section to agree other specific terms, such as the Auditor's fees, liability, applicable law, etc. Those specific terms must not contradict the terms specified above.*]

[legal name of the Auditor]	[legal name of the *[Beneficiary][Linked Third Party]*]
[name & function of authorised representative]	[name & function of authorised representative]
[dd Month yyyy]	[dd Month yyyy]
Signature of the Auditor	Signature of the *[Beneficiary][Linked Third Party]*

[2] Supreme Audit Institutions applying INTOSAI-standards may carry out the Procedures according to the corresponding International Standards of Supreme Audit Institutions and code of ethics issued by INTOSAI instead of the International Standard on Related Services ('ISRS') 4400 and the Code of Ethics for Professional Accountants issued by the IAASB and the IESBA.

Independent Report of Factual Findings on costs declared under Horizon 2020 Research and Innovation Framework Programme

(To be printed on the Auditor's letterhead)

To
[name of contact person(s)], [Position]
[[*Beneficiary's*] [*Linked Third Party's*] name]
[Address]
[dd Month yyyy]

Dear [Name of contact person(s)],

As agreed under the terms of reference dated [dd Month yyyy]

with *[OPTION 1: [insert name of the beneficiary] ('the Beneficiary')] [OPTION 2: [insert name of the linked third party] ('the Linked Third Party'), third party linked to the Beneficiary [insert name of the beneficiary] ('the Beneficiary')],*

we

[name of the auditor] ('the Auditor'),

established at

[full address/city/state/province/country],

represented by

[name and function of an authorised representative],

have carried out the procedures agreed with you regarding the costs declared in the Financial Statement(s)[3] of the *[Beneficiary] [Linked Third Party]* concerning the grant agreement [insert grant agreement reference: number, title of the action and acronym] ('the Agreement'),

with a total cost declared of
[total amount] EUR,

and a total of actual costs and unit costs calculated in accordance with the [*Beneficiary's*] [*Linked Third Party's*] usual cost accounting practices' declared of

[sum of total actual costs and total direct personnel costs declared as unit costs calculated in accordance with the *[Beneficiary's] [Linked Third Party's]* usual cost accounting practices] EUR

and **hereby provide our Independent Report of Factual Findings ('the Report')** using the compulsory report format agreed with you.

The Report

Our engagement was carried out in accordance with the terms of reference ('the ToR') appended to this Report. The Report includes the agreed-upon procedures ('the Procedures') carried out and the standard factual findings ('the Findings') examined.

[3] By which the Beneficiary declares costs under the Agreement (see template 'Model Financial Statement' in Annex 4 to the Agreement).

The Procedures were carried out solely to assist the *[Commission] [Agency]* in evaluating whether the *[Beneficiary's] [Linked Third Party's]* costs in the accompanying Financial Statement(s) were declared in accordance with the Agreement. The *[Commission] [Agency]* draws its own conclusions from the Report and any additional information it may require.

The scope of the Procedures was defined by the Commission. Therefore, the Auditor is not responsible for their suitability or pertinence. Since the Procedures carried out constitute neither an audit nor a review made in accordance with International Standards on Auditing or International Standards on Review Engagements, the Auditor does not give a statement of assurance on the Financial Statements.

Had the Auditor carried out additional procedures or an audit of the *[Beneficiary's] [Linked Third Party's]* Financial Statements in accordance with International Standards on Auditing or International Standards on Review Engagements, other matters might have come to its attention and would have been included in the Report.

Not applicable Findings

We examined the Financial Statement(s) stated above and considered the following Findings not applicable:

Explanation (to be removed from the Report):

*If a Finding was not applicable, it must be marked as '**N.A.**' ('Not applicable') in the corresponding row on the right-hand column of the table and means that the Finding did not have to be corroborated by the Auditor and the related Procedure(s) did not have to be carried out.*

The reasons of the non-application of a certain Finding must be obvious i.e.

i) if no cost was declared under a certain category then the related Finding(s) and Procedure(s) are not applicable;

ii) if the condition set to apply certain Procedure(s) are not met the related Finding(s) and those Procedure(s) are not applicable. For instance, for 'beneficiaries with accounts established in a currency other than euro' the Procedure and Finding related to 'beneficiaries with accounts established in euro' are not applicable. Similarly, if no additional remuneration is paid, the related Finding(s) and Procedure(s) for additional remuneration are not applicable.

List here all Findings considered not applicable for the present engagement and explain the reasons of the non-applicability.

….

Exceptions

Apart from the exceptions listed below, the *[Beneficiary] [Linked Third Party]* provided the Auditor all the documentation and accounting information needed by the Auditor to carry out the requested Procedures and evaluate the Findings.

Explanation (to be removed from the Report):

- *If the Auditor was not able to successfully complete a procedure requested, it must be marked as '**E**' ('Exception') in the corresponding row on the right-hand column of the table. The reason such as the inability to reconcile key information or the unavailability of data that prevents the Auditor from carrying out the Procedure must be indicated below.*
- *If the Auditor cannot corroborate a standard finding after having carried out the corresponding procedure, it must also be marked as '**E**' ('Exception') and, where possible, the reasons why the Finding was not fulfilled and its possible impact must be explained here below.*

List here any exceptions and add any information on the cause and possible consequences of each exception, if known. If the exception is quantifiable, include the corresponding amount.

….

Example (to be removed from the Report):

1. *The Beneficiary was unable to substantiate the Finding number 1 on … because ….*
2. *Finding number 30 was not fulfilled because the methodology used by the Beneficiary to calculate unit costs was different from the one approved by the Commission. The differences were as follows: …*
3. *After carrying out the agreed procedures to confirm the Finding number 31, the Auditor found a difference of _____ EUR. The difference can be explained by …*

Further Remarks

In addition to reporting on the results of the specific procedures carried out, the Auditor would like to make the following general remarks:

Example (to be removed from the Report):

1. *Regarding Finding number 8 the conditions for additional remuneration were considered as fulfilled because …*
2. *In order to be able to confirm the Finding number 15 we carried out the following additional procedures: ….*

Use of this Report

This Report may be used only for the purpose described in the above objective. It was prepared solely for the confidential use of the *[Beneficiary] [Linked Third Party]* and the [*Commission*] [*Agency*], and only to be submitted to the *[Commission] [Agency]* in connection with the requirements set out in Article 20.4 of the Agreement. The Report may not be used by the *[Beneficiary] [Linked Third Party]* or by the *[Commission] [Agency]* for any other purpose, nor may it be distributed to any other parties. The *[Commission] [Agency]* may only disclose the Report to authorised parties, in particular to the European Anti-Fraud Office (OLAF) and the European Court of Auditors.

This Report relates only to the Financial Statement(s) submitted to the *[Commission] [Agency]* by the *[Beneficiary] [Linked Third Party]* for the Agreement. Therefore, it does not extend to any other of the *[Beneficiary's] [Linked Third Party's]* Financial Statement(s).

There was no conflict of interest[4] between the Auditor and the Beneficiary *[and Linked Third Party]* in establishing this Report. The total fee paid to the Auditor for providing the Report was EUR _____ (including EUR_____ of deductible VAT).

We look forward to discussing our Report with you and would be pleased to provide any further information or assistance.

[legal name of the Auditor]
[name and function of an authorised representative]
[dd Month yyyy]
Signature of the Auditor

[4] A conflict of interest arises when the Auditor's objectivity to establish the certificate is compromised in fact or in appearance when the Auditor for instance:
- was involved in the preparation of the Financial Statements;
- stands to benefit directly should the certificate be accepted;
- has a close relationship with any person representing the beneficiary;
- is a director, trustee or partner of the beneficiary; or
- is in any other situation that compromises his or her independence or ability to establish the certificate impartially.

Agreed-upon procedures to be performed and standard factual findings to be confirmed by the Auditor

The European Commission reserves the right to i) provide the auditor with additional guidance regarding the procedures to be followed or the facts to be ascertained and the way in which to present them (this may include sample coverage and findings) or to ii) change the procedures, by notifying the Beneficiary in writing. The procedures carried out by the auditor to confirm the standard factual finding are listed in the table below.

If this certificate relates to a Linked Third Party, any reference here below to 'the Beneficiary' is to be considered as a reference to 'the Linked Third Party'.

The 'result' column has three different options: 'C', 'E' and 'N.A.':

- 'C' stands for 'confirmed' and means that the auditor can confirm the 'standard factual finding' and, therefore, there is no exception to be reported.
- 'E' stands for 'exception' and means that the Auditor carried out the procedures but cannot confirm the 'standard factual finding', or that the Auditor was not able to carry out a specific procedure (e.g. because it was impossible to reconcile key information or data were unavailable),
- 'N.A.' stands for 'not applicable' and means that the Finding did not have to be examined by the Auditor and the related Procedure(s) did not have to be carried out. The reasons of the non-application of a certain Finding must be obvious i.e. i) if no cost was declared under a certain category then the related Finding(s) and Procedure(s) are not applicable; ii) if the condition set to apply certain Procedure(s) are not met then the related Finding(s) and Procedure(s) are not applicable. For instance, for 'beneficiaries with accounts established in a currency other than the euro' the Procedure related to 'beneficiaries with accounts established in euro' is not applicable. Similarly, if no additional remuneration is paid, the related Finding(s) and Procedure(s) for additional remuneration are not applicable.

Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
A	**ACTUAL PERSONNEL COSTS AND UNIT COSTS CALCULATED BY THE BENEFICIARY IN ACCORDANCE WITH ITS USUAL COST ACCOUNTING PRACTICE**		
	The Auditor draws a sample of persons whose costs were declared in the Financial Statement(s) to carry out the procedures indicated in the consecutive points of this section A. (*The sample should be selected randomly so that it is representative. Full coverage is required if there are fewer than 10 people (including employees, natural persons working under a direct contract and personnel seconded by a third party), otherwise the sample should have a minimum of 10 people, or 10% of the total, whichever number is the highest)* The Auditor sampled _____ people out of the total of _____ people.		

Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
A.1	**PERSONNEL COSTS** For the persons included in the sample and working under an employment contract or equivalent act (general procedures for individual actual personnel costs and personnel costs declared as unit costs) To confirm standard factual findings 1-5 listed in the next column, the Auditor reviewed following information/documents provided by the Beneficiary: o a list of the persons included in the sample indicating the period(s) during which they worked for the action, their position (classification or category) and type of contract; o the payslips of the employees included in the sample; o reconciliation of the personnel costs declared in the Financial Statement(s) with the accounting system (project accounting and general ledger) and payroll system; o information concerning the employment status and employment conditions of personnel included in the sample, in particular their employment contracts or equivalent; o the Beneficiary's usual policy regarding payroll matters (e.g. salary policy, overtime policy, variable pay); o applicable national law on taxes, labour and social security and o any other document that supports the personnel costs declared. The Auditor also verified the eligibility of all components of the retribution (see Article 6 GA) and recalculated the personnel costs for employees included in the sample.	1) The employees were i) directly hired by the Beneficiary in accordance with its national legislation, ii) under the Beneficiary's sole technical supervision and responsibility and iii) remunerated in accordance with the Beneficiary's usual practices.	
		2) Personnel costs were recorded in the Beneficiary's accounts/payroll system.	
		3) Costs were adequately supported and reconciled with the accounts and payroll records.	
		4) Personnel costs did not contain any ineligible elements.	
		5) There were no discrepancies between the personnel costs charged to the action and the costs recalculated by the Auditor.	
	Further procedures if 'additional remuneration' is paid To confirm standard factual findings 6-9 listed in the next column, the Auditor: o reviewed relevant documents provided by the Beneficiary (legal form, legal/statutory	6) The Beneficiary paying "additional remuneration" was a non-profit legal entity.	

Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
	obligations, the Beneficiary's usual policy on additional remuneration, criteria used for its calculation, the Beneficiary's usual remuneration practice for projects funded under national funding schemes…); o recalculated the amount of additional remuneration eligible for the action based on the supporting documents received (full-time or part-time work, exclusive or non-exclusive dedication to the action, usual remuneration paid for projects funded by national schemes) to arrive at the applicable FTE/year and pro-rata rate (see data collected in the course of carrying out the procedures under A.2 'Productive hours' and A.4 'Time recording system'). *'ADDITIONAL REMUNERATION' MEANS ANY PART OF THE REMUNERATION WHICH EXCEEDS WHAT THE PERSON WOULD BE PAID FOR TIME WORKED IN PROJECTS FUNDED BY NATIONAL SCHEMES.* *IF ANY PART OF THE REMUNERATION PAID TO THE EMPLOYEE QUALIFIES AS "ADDITIONAL REMUNERATION" AND IS ELIGIBLE UNDER THE PROVISIONS OF ARTICLE 6.2.A.1, THIS CAN BE CHARGED AS ELIGIBLE COST TO THE ACTION UP TO THE FOLLOWING AMOUNT:* *(A) IF THE PERSON WORKS FULL TIME AND EXCLUSIVELY ON THE ACTION DURING THE FULL YEAR: UP TO EUR 8 000/YEAR;* *(B) IF THE PERSON WORKS EXCLUSIVELY ON THE ACTION BUT NOT FULL-TIME OR NOT FOR THE FULL YEAR: UP TO THE CORRESPONDING PRO-RATA AMOUNT OF EUR 8 000, OR* *(C) IF THE PERSON DOES NOT WORK EXCLUSIVELY ON THE ACTION: UP TO A PRO-RATA AMOUNT CALCULATED IN ACCORDANCE TO ARTICLE 6.2.A.1.*	7) The amount of additional remuneration paid corresponded to the Beneficiary's usual remuneration practices and was consistently paid whenever the same kind of work or expertise was required.	
		8) The criteria used to calculate the additional remuneration were objective and generally applied by the Beneficiary regardless of the source of funding used.	
		9) The amount of additional remuneration included in the personnel costs charged to the action was capped at EUR 8,000 per FTE/year (up to the equivalent pro-rata amount if the person did not work on the action full-time during the year or did not work exclusively on the action).	
	Additional procedures in case "unit costs calculated by the Beneficiary in accordance with its usual cost accounting practices" is applied: Apart from carrying out the procedures indicated above to confirm standard factual findings 1-5 and, if applicable, also 6-9, the Auditor carried out following procedures to confirm standard	10) The personnel costs included in the Financial Statement were calculated in accordance with the Beneficiary's usual cost accounting practice. This methodology was consistently	

Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
	factual findings 10-13 listed in the next column: o obtained a description of the Beneficiary's usual cost accounting practice to calculate unit costs;. o reviewed whether the Beneficiary's usual cost accounting practice was applied for the Financial Statements subject of the present CFS; o verified the employees included in the sample were charged under the correct category (in accordance with the criteria used by the Beneficiary to establish personnel categories) by reviewing the contract/HR-record or analytical accounting records; o verified that there is no difference between the total amount of personnel costs used in calculating the cost per unit and the total amount of personnel costs recorded in the statutory accounts; o verified whether actual personnel costs were adjusted on the basis of budgeted or estimated elements and, if so, verified whether those elements used are actually relevant for the calculation, objective and supported by documents.	used in all H2020 actions.	
		11) The employees were charged under the correct category.	
		12) Total personnel costs used in calculating the unit costs were consistent with the expenses recorded in the statutory accounts.	
		13) Any estimated or budgeted element used by the Beneficiary in its unit-cost calculation were relevant for calculating personnel costs and corresponded to objective and verifiable information.	
	<u>For natural persons included in the sample and working with the Beneficiary under a direct contract other than an employment contract, such as consultants (no subcontractors).</u> To confirm standard factual findings 14-17 listed in the next column the Auditor reviewed following information/documents provided by the Beneficiary: o the contracts, especially the cost, contract duration, work description, place of work, ownership of the results and reporting obligations to the Beneficiary; o the employment conditions of staff in the same category to compare costs and; o any other document that supports the costs declared and its registration (e.g. invoices, accounting records, etc.).	14) The natural persons worked under conditions similar to those of an employee, in particular regarding the way the work is organised, the tasks that are performed and the premises where they are performed.	
		15) The results of work carried out belong to the Beneficiary, or, if not, the Beneficiary has obtained all necessary rights to fulfil its obligations as if those	

Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
		results were generated by itself.	
		16) Their costs were not significantly different from those for staff who performed similar tasks under an employment contract with the Beneficiary.	
		17) The costs were supported by audit evidence and registered in the accounts.	
	<u>For personnel seconded by a third party and included in the sample (not subcontractors)</u> To confirm standard factual findings 18-21 listed in the next column, the Auditor reviewed following information/documents provided by the Beneficiary: o their secondment contract(s) notably regarding costs, duration, work description, place of work and ownership of the results; o if there is reimbursement by the Beneficiary to the third party for the resource made available (in-kind contribution against payment): any documentation that supports the costs declared (e.g. contract, invoice, bank payment, and proof of registration in its accounting/payroll, etc.) and reconciliation of the Financial Statement(s) with the accounting system (project accounting and general ledger) as well as any proof that the amount invoiced by the third party did not include any profit; o if there is no reimbursement by the Beneficiary to the third party for the resource made available (in-kind contribution free of charge): a proof of the actual cost borne by the Third Party for the resource made available free of charge to the Beneficiary such as a statement of costs incurred by the Third Party and proof of the registration in the Third Party's accounting/payroll;	18) Seconded personnel reported to the Beneficiary and worked on the Beneficiary's premises (unless otherwise agreed with the Beneficiary).	
		19) The results of work carried out belong to the Beneficiary, or, if not, the Beneficiary has obtained all necessary rights to fulfil its obligations as if those results were generated by itself..	
		If personnel is seconded against payment: 20) The costs declared were supported with documentation and recorded in the	

Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
	○ any other document that supports the costs declared (e.g. invoices, etc.).	Beneficiary's accounts. The third party did not include any profit.	
		If personnel is seconded free of charge: 21) The costs declared did not exceed the third party's cost as recorded in the accounts of the third party and were supported with documentation.	
A.2	**PRODUCTIVE HOURS** To confirm standard factual findings 22-27 listed in the next column, the Auditor reviewed relevant documents, especially national legislation, labour agreements and contracts and time records of the persons included in the sample, to verify that: ○ the annual productive hours applied were calculated in accordance with one of the methods described below, ○ the full-time equivalent (FTEs) ratios for employees not working full-time were correctly calculated. If the Beneficiary applied method B, the auditor verified that the correctness in which the total number of hours worked was calculated and that the contracts specified the annual workable hours. If the Beneficiary applied method C, the auditor verified that the 'annual productive hours' applied when calculating the hourly rate were equivalent to at least 90 % of the 'standard annual workable hours'. The Auditor can only do this if the calculation of the standard annual workable	22) The Beneficiary applied method [*choose one option and delete the others*] [**A**: 1720 hours] [**B**: the 'total number of hours worked'] [**C**: 'standard annual productive hours' used correspond to usual accounting practices]	
		23) Productive hours were calculated annually.	
		24) For employees not working full-time the full-time equivalent (FTE) ratio was correctly applied.	

Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
	hours can be supported by records, such as national legislation, labour agreements, and contracts. *BENEFICIARY'S PRODUCTIVE HOURS' FOR PERSONS WORKING FULL TIME SHALL BE ONE OF THE FOLLOWING METHODS:* ***A**. 1720 ANNUAL PRODUCTIVE HOURS (PRO-RATA FOR PERSONS NOT WORKING FULL-TIME)* ***B**. THE TOTAL NUMBER OF HOURS WORKED BY THE PERSON FOR THE BENEFICIARY IN THE YEAR (THIS METHOD IS ALSO REFERRED TO AS 'TOTAL NUMBER OF HOURS WORKED' IN THE NEXT COLUMN). THE CALCULATION OF THE TOTAL NUMBER OF HOURS WORKED WAS DONE AS FOLLOWS: ANNUAL WORKABLE HOURS OF THE PERSON ACCORDING TO THE EMPLOYMENT CONTRACT, APPLICABLE LABOUR AGREEMENT OR NATIONAL LAW PLUS OVERTIME WORKED MINUS ABSENCES (SUCH AS SICK LEAVE OR SPECIAL LEAVE).* ***C**. THE STANDARD NUMBER OF ANNUAL HOURS GENERALLY APPLIED BY THE BENEFICIARY FOR ITS PERSONNEL IN ACCORDANCE WITH ITS USUAL COST ACCOUNTING PRACTICES (THIS METHOD IS ALSO REFERRED TO AS 'STANDARD ANNUAL PRODUCTIVE HOURS' IN THE NEXT COLUMN). THIS NUMBER MUST BE AT LEAST 90% OF THE STANDARD ANNUAL WORKABLE HOURS.* *'ANNUAL WORKABLE HOURS' MEANS THE PERIOD DURING WHICH THE PERSONNEL MUST BE WORKING, AT THE EMPLOYER'S DISPOSAL AND CARRYING OUT HIS/HER ACTIVITY OR DUTIES UNDER THE EMPLOYMENT CONTRACT, APPLICABLE COLLECTIVE LABOUR AGREEMENT OR NATIONAL WORKING TIME LEGISLATION.*	*If the Beneficiary applied method B.* 25) The calculation of the number of 'annual workable hours', overtime and absences was verifiable based on the documents provided by the Beneficiary. 25.1) The Beneficiary calculates the hourly rates per full financial year following procedure A.3 (method B is not allowed for beneficiaries calculating hourly rates per month). *If the Beneficiary applied method C.* 26) The calculation of the number of 'standard annual workable hours' was verifiable based on the documents provided by the Beneficiary.	

Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
		27) The 'annual productive hours' used for calculating the hourly rate were consistent with the usual cost accounting practices of the Beneficiary and were equivalent to at least 90 % of the 'annual workable hours'.	
A.3	**HOURLY PERSONNEL RATES** I) For unit costs calculated in accordance to the Beneficiary's usual cost accounting practice (unit costs): If the Beneficiary has a "Certificate on Methodology to calculate unit costs " (CoMUC) approved by the Commission, the Beneficiary provides the Auditor with a description of the approved methodology and the Commission's letter of acceptance. The Auditor verified that the Beneficiary has indeed used the methodology approved. If so, no further verification is necessary. If the Beneficiary does not have a "Certificate on Methodology" (CoMUC) approved by the Commission, or if the methodology approved was not applied, then the Auditor: o reviewed the documentation provided by the Beneficiary, including manuals and internal guidelines that explain how to calculate hourly rates; o recalculated the unit costs (hourly rates) of staff included in the sample following the results of the procedures carried out in A.1 and A.2. II) For individual hourly rates: The Auditor: o reviewed the documentation provided by the Beneficiary, including manuals and internal guidelines that explain how to calculate hourly rates;	28) The Beneficiary applied [*choose one option and delete the other*]: [Option I: "Unit costs (hourly rates) were calculated in accordance with the Beneficiary's usual cost accounting practices"] [Option II: Individual hourly rates were applied] *For option I concerning unit costs and if the Beneficiary applies the methodology approved by the Commission (CoMUC):* 29) The Beneficiary used the Commission-approved methodology to calculate hourly rates. It corresponded to the organisation's usual cost accounting practices and was applied consistently for all	

Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
	○ recalculated the hourly rates of staff included in the sample (recalculation of all hourly rates if the Beneficiary uses annual rates, recalculation of three months selected randomly for every year and person if the Beneficiary uses monthly rates) following the results of the procedures carried out in A.1 and A.2; ○ (only in case of monthly rates) confirmed that the time spent on parental leave is not deducted, and that, if parts of the basic remuneration are generated over a period longer than a month, the Beneficiary has included only the share which is generated in the month. *"UNIT COSTS CALCULATED BY THE BENEFICIARY IN ACCORDANCE WITH ITS USUAL COST ACCOUNTING PRACTICES":* *IT IS CALCULATED BY DIVIDING THE TOTAL AMOUNT OF PERSONNEL COSTS OF THE CATEGORY TO WHICH THE EMPLOYEE BELONGS VERIFIED IN LINE WITH PROCEDURE A.1 BY THE NUMBER OF FTE AND THE ANNUAL TOTAL PRODUCTIVE HOURS OF THE SAME CATEGORY CALCULATED BY THE BENEFICIARY IN ACCORDANCE WITH PROCEDURE A.2.* *HOURLY RATE FOR INDIVIDUAL ACTUAL PERSONAL COSTS:* *IT IS CALCULATED FOLLOWING ONE OF THE TWO OPTIONS BELOW:* *A) [OPTION BY DEFAULT] BY DIVIDING THE ACTUAL ANNUAL AMOUNT OF PERSONNEL COSTS OF AN EMPLOYEE VERIFIED IN LINE WITH PROCEDURE A.1 BY THE NUMBER OF ANNUAL PRODUCTIVE HOURS VERIFIED IN LINE WITH PROCEDURE A.2 (FULL FINANCIAL YEAR HOURLY RATE);* *B) BY DIVIDING THE ACTUAL MONTHLY AMOUNT OF PERSONNEL COSTS OF AN EMPLOYEE VERIFIED IN LINE WITH PROCEDURE A.1 BY 1/12 OF THE NUMBER OF ANNUAL PRODUCTIVE HOURS VERIFIED IN LINE WITH PROCEDURE A.2.(MONTHLY HOURLY RATE).*	activities irrespective of the source of funding.	
		For option I concerning unit costs and if the Beneficiary applies a methodology not approved by the Commission: 30) The unit costs re-calculated by the Auditor were the same as the rates applied by the Beneficiary.	
		For option II concerning individual hourly rates: 31) The individual rates re-calculated by the Auditor were the same as the rates applied by the Beneficiary. 31.1) The Beneficiary used only one option (per full financial year or per month) throughout each financial year examined. 31.2) The hourly rates do not include additional remuneration.	

Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
A.4	**TIME RECORDING SYSTEM** To verify that the time recording system ensures the fulfilment of all minimum requirements and that the hours declared for the action were correct, accurate and properly authorised and supported by documentation, the Auditor made the following checks for the persons included in the sample that declare time as worked for the action on the basis of time records: o description of the time recording system provided by the Beneficiary (registration, authorisation, processing in the HR-system); o its actual implementation; o time records were signed at least monthly by the employees (on paper or electronically) and authorised by the project manager or another manager; o the hours declared were worked within the project period; o there were no hours declared as worked for the action if HR-records showed absence due to holidays or sickness (further cross-checks with travels are carried out in B.1 below) ; o the hours charged to the action matched those in the time recording system. *ONLY THE HOURS WORKED ON THE ACTION CAN BE CHARGED. ALL WORKING TIME TO BE CHARGED SHOULD BE RECORDED THROUGHOUT THE DURATION OF THE PROJECT, ADEQUATELY SUPPORTED BY EVIDENCE OF THEIR REALITY AND RELIABILITY (SEE SPECIFIC PROVISIONS BELOW FOR PERSONS WORKING EXCLUSIVELY FOR THE ACTION WITHOUT TIME RECORDS).*	32) All persons recorded their time dedicated to the action on a **daily/ weekly/ monthly** basis using a **paper/computer-based** system. (*delete the answers that are not applicable)*	
		33) Their time-records were authorised at least monthly by the project manager or other superior.	
		34) Hours declared were worked within the project period and were consistent with the presences/absences recorded in HR-records.	
		35) There were no discrepancies between the number of hours charged to the action and the number of hours recorded.	
	<u>If the persons are working exclusively for the action and without time records</u> For the persons selected that worked exclusively for the action without time records, the Auditor verified evidence available demonstrating that they were in reality exclusively dedicated to the action and that the Beneficiary signed a declaration confirming that they have worked exclusively for the action.	36) The exclusive dedication is supported by a declaration signed by the Beneficiary and by any other evidence gathered.	

Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
B	**COSTS OF SUBCONTRACTING**		
B.1	**The Auditor obtained the detail/breakdown of subcontracting costs and sampled _____ cost items selected randomly** (*full coverage is required if there are fewer than 10 items, otherwise the sample should have a minimum of 10 item, or 10% of the total, whichever number is highest*). To confirm standard factual findings 37-41 listed in the next column, the Auditor reviewed the following for the items included in the sample: o the use of subcontractors was foreseen in Annex 1; o subcontracting costs were declared in the subcontracting category of the Financial Statement; o supporting documents on the selection and award procedure were followed; o the Beneficiary ensured best value for money (key elements to appreciate the respect of this principle are the award of the subcontract to the bid offering best price-quality ratio, under conditions of transparency and equal treatment. In case an existing framework contract was used the Beneficiary ensured it was established on the basis of the principle of best value for money under conditions of transparency and equal treatment). In particular, i. if the Beneficiary acted as a contracting authority within the meaning of Directive 2004/18/EC (or 2014/24/EU) or of Directive 2004/17/EC (or 2014/25/EU), the Auditor verified that the applicable national law on public procurement was followed and that the subcontracting complied with the Terms and Conditions of the Agreement. ii. if the Beneficiary did not fall under the above-mentioned category the Auditor verified that the Beneficiary followed their usual procurement rules and respected the Terms and Conditions of the Agreement..	37) The use of claimed subcontracting costs was foreseen in Annex 1 and costs were declared in the Financial Statements under the subcontracting category.	
		38) There were documents of requests to different providers, different offers and assessment of the offers before selection of the provider in line with internal procedures and procurement rules. Subcontracts were awarded in accordance with the principle of best value for money. *(When different offers were not collected the Auditor explains the reasons provided by the Beneficiary under the caption "Exceptions" of the Report. The Commission will analyse this information to evaluate whether these costs might be accepted as eligible)*	
		39) The subcontracts were not awarded to other Beneficiaries	

Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
	For the items included in the sample the Auditor also verified that: o the subcontracts were not awarded to other Beneficiaries in the consortium; o there were signed agreements between the Beneficiary and the subcontractor; o there was evidence that the services were provided by subcontractor;	of the consortium.	
		40) All subcontracts were supported by signed agreements between the Beneficiary and the subcontractor.	
		41) There was evidence that the services were provided by the subcontractors.	
C	**COSTS OF PROVIDING FINANCIAL SUPPORT TO THIRD PARTIES**		
C.1	**The Auditor obtained the detail/breakdown of the costs of providing financial support to third parties and sampled _____ cost items selected randomly** (*full coverage is required if there are fewer than 10 items, otherwise the sample should have a minimum of 10 item, or 10% of the total, whichever number is highest*). The Auditor verified that the following minimum conditions were met: a) the maximum amount of financial support for each third party did not exceed EUR 60 000, unless explicitly mentioned in Annex 1; b) the financial support to third parties was agreed in Annex 1 of the Agreement and the other provisions on financial support to third parties included in Annex 1 were respected.	42) All minimum conditions were met	

D	OTHER ACTUAL DIRECT COSTS		
D.1	**COSTS OF TRAVEL AND RELATED SUBSISTENCE ALLOWANCES** **The Auditor sampled _____ cost items selected randomly** (*full coverage is required if there are fewer than 10 items, otherwise the sample should have a minimum of 10 item, or 10% of the total, whichever number is the highest*). The Auditor inspected the sample and verified that: o travel and subsistence costs were consistent with the Beneficiary's usual policy for travel. In this context, the Beneficiary provided evidence of its normal policy for travel costs (e.g. use of first class tickets, reimbursement by the Beneficiary on the basis of actual costs, a lump sum or per diem) to enable the Auditor to compare the travel costs charged with this policy; o travel costs are correctly identified and allocated to the action (e.g. trips are directly linked to the action) by reviewing relevant supporting documents such as minutes of meetings, workshops or conferences, their registration in the correct project account, their consistency with time records or with the dates/duration of the workshop/conference; o no ineligible costs or excessive or reckless expenditure was declared (see Article 6.5 MGA).	43) Costs were incurred, approved and reimbursed in line with the Beneficiary's usual policy for travels.	
		44) There was a link between the trip and the action.	
		45) The supporting documents were consistent with each other regarding subject of the trip, dates, duration and reconciled with time records and accounting.	
		46) No ineligible costs or excessive or reckless expenditure was declared.	
D.2	**DEPRECIATION COSTS FOR EQUIPMENT, INFRASTRUCTURE OR OTHER ASSETS** **The Auditor sampled _____ cost items selected randomly** (*full coverage is required if there are fewer than 10 items, otherwise the sample should have a minimum of 10 item, or 10% of the total, whichever number is the highest*). For "equipment, infrastructure or other assets" [from now on called "asset(s)"] selected in the sample the Auditor verified that: o the assets were acquired in conformity with the Beneficiary's internal guidelines and procedures;	47) Procurement rules, principles and guides were followed.	
		48) There was a link between the grant agreement and the asset charged to the action.	
		49) The asset charged to the action was traceable to the accounting records and the underlying documents.	

	○ they were correctly allocated to the action (with supporting documents such as delivery note invoice or any other proof demonstrating the link to the action) ○ they were entered in the accounting system; ○ the extent to which the assets were used for the action (as a percentage) was supported by reliable documentation (e.g. usage overview table); The Auditor recalculated the depreciation costs and verified that they were in line with the applicable rules in the Beneficiary's country and with the Beneficiary's usual accounting policy (e.g. depreciation calculated on the acquisition value). The Auditor verified that no ineligible costs such as deductible VAT, exchange rate losses, excessive or reckless expenditure were declared (see Article 6.5 GA).	50) The depreciation method used to charge the asset to the action was in line with the applicable rules of the Beneficiary's country and the Beneficiary's usual accounting policy. 51) The amount charged corresponded to the actual usage for the action. 52) No ineligible costs or excessive or reckless expenditure were declared.	
D.3	**COSTS OF OTHER GOODS AND SERVICES** **The Auditor sampled _____ cost items selected randomly** (*full coverage is required if there are fewer than 10 items, otherwise the sample should have a minimum of 10 item, or 10% of the total, whichever number is highest*). For the purchase of goods, works or services included in the sample the Auditor verified that: ○ the contracts did not cover tasks described in Annex 1; ○ they were correctly identified, allocated to the proper action, entered in the accounting system (traceable to underlying documents such as purchase orders, invoices and accounting); ○ the goods were not placed in the inventory of durable equipment; ○ the costs charged to the action were accounted in line with the Beneficiary's usual accounting practices; ○ no ineligible costs or excessive or reckless expenditure were declared (see Article 6 GA). In addition, the Auditor verified that these goods and services were acquired in conformity with	53) Contracts for works or services did not cover tasks described in Annex 1. 54) Costs were allocated to the correct action and the goods were not placed in the inventory of durable equipment. 55) The costs were charged in line with the Beneficiary's accounting policy and were adequately supported. 56) No ineligible costs or excessive or reckless expenditure were declared. For internal invoices/charges only the cost element was charged, without any mark-ups.	

	the Beneficiary's internal guidelines and procedures, in particular: o if Beneficiary acted as a contracting authority within the meaning of Directive 2004/18/EC (or 2014/24/EU) or of Directive 2004/17/EC (or 2014/25/EU), the Auditor verified that the applicable national law on public procurement was followed and that the procurement contract complied with the Terms and Conditions of the Agreement. o if the Beneficiary did not fall into the category above, the Auditor verified that the Beneficiary followed their usual procurement rules and respected the Terms and Conditions of the Agreement. For the items included in the sample the Auditor also verified that: o the Beneficiary ensured best value for money (key elements to appreciate the respect of this principle are the award of the contract to the bid offering best price-quality ratio, under conditions of transparency and equal treatment. In case an existing framework contract was used the Auditor also verified that the Beneficiary ensured it was established on the basis of the principle of best value for money under conditions of transparency and equal treatment); *SUCH GOODS AND SERVICES INCLUDE, FOR INSTANCE, CONSUMABLES AND SUPPLIES, DISSEMINATION (INCLUDING OPEN ACCESS), PROTECTION OF RESULTS, SPECIFIC EVALUATION OF THE ACTION IF IT IS REQUIRED BY THE AGREEMENT, CERTIFICATES ON THE FINANCIAL STATEMENTS IF THEY ARE REQUIRED BY THE AGREEMENT AND CERTIFICATES ON THE METHODOLOGY, TRANSLATIONS, REPRODUCTION.*	57) Procurement rules, principles and guides were followed. There were documents of requests to different providers, different offers and assessment of the offers before selection of the provider in line with internal procedures and procurement rules. The purchases were made in accordance with the principle of best value for money. *(When different offers were not collected the Auditor explains the reasons provided by the Beneficiary under the caption "Exceptions" of the Report. The Commission will analyse this information to evaluate whether these costs might be accepted as eligible)*	
D.4	**AGGREGATED CAPITALISED AND OPERATING COSTS OF RESEARCH INFRASTRUCTURE** The Auditor ensured the existence of a positive ex-ante assessment (issued by the EC Services) of the cost accounting methodology of the Beneficiary allowing it to apply the guidelines on direct costing for large research infrastructures in Horizon 2020.	58) The costs declared as direct costs for Large Research Infrastructures (in the appropriate line of the Financial Statement) comply with the methodology described in the positive ex-ante assessment report.	

	In the cases that a positive ex-ante assessment has been issued *(see the standard factual findings 58-59 on the next column)***,** The Auditor ensured that the beneficiary has applied consistently the methodology that is explained and approved in the positive ex ante assessment; ***In the cases that a positive ex-ante assessment has NOT been issued*** *(see the standard factual findings 60 on the next column),* The Auditor verified that no costs of Large Research Infrastructure have been charged as direct costs in any costs category; ***In the cases that a draft ex-ante assessment report has been issued with recommendation for further changes*** *(see the standard factual findings 60 on the next column),* • The Auditor followed the same procedure as above (when a positive ex-ante assessment has NOT yet been issued) and paid particular attention (testing reinforced) to the cost items for which the draft ex-ante assessment either rejected the inclusion as direct costs for Large Research Infrastructures or issued recommendations.	59) Any difference between the methodology applied and the one positively assessed was extensively described and adjusted accordingly. 60) The direct costs declared were free from any indirect costs items related to the Large Research Infrastructure.	
D.5	**Costs of internally invoiced goods and services** **The Auditor sampled cost items selected randomly** (*full coverage is required if there are fewer than 10 items, otherwise the sample should have a minimum of 10 item, or 10% of the total, whichever number is highest*). To confirm standard factual findings 61-65 listed in the next column, the Auditor: ○ obtained a description of the Beneficiary's usual cost accounting practice to calculate costs of internally invoiced goods and services (unit costs); ○ reviewed whether the Beneficiary's usual cost accounting practice was applied for the Financial Statements subject of the present CFS; ○ ensured that the methodology to calculate unit costs is being used in a consistent manner, based on objective criteria, regardless of the source of funding; ○ verified that any ineligible items or any costs claimed under other budget categories, in particular indirect costs, have not been taken into account when calculating the costs of	61) The costs of internally invoiced goods and services included in the Financial Statement were calculated in accordance with the Beneficiary's usual cost accounting practice. 62) The cost accounting practices used to calculate the costs of internally invoiced goods and services were applied by the Beneficiary in a consistent manner based on objective criteria regardless of the source of funding. 63) The unit cost is calculated using the actual costs for the good or service recorded in the Beneficiary's accounts, excluding any ineligible cost or costs included in other	

	internally invoiced goods and services (see Article 6 GA); ○ verified whether actual costs of internally invoiced goods and services were adjusted on the basis of budgeted or estimated elements and, if so, verified whether those elements used are actually relevant for the calculation, and correspond to objective and verifiable information. ○ verified that any costs of items which are not directly linked to the production of the invoiced goods or service (e.g. supporting services like cleaning, general accountancy, administrative support, etc. not directly used for production of the good or service) have not been taken into account when calculating the costs of internally invoiced goods and services. ○ verified that any costs of items used for calculating the costs internally invoiced goods and services are supported by audit evidence and registered in the accounts.	budget categories. 64) The unit cost excludes any costs of items which are not directly linked to the production of the invoiced goods or service. 65) The costs items used for calculating the actual costs of internally invoiced goods and services were relevant, reasonable and correspond to objective and verifiable information.	
E	**USE OF EXCHANGE RATES**		
E.1	a) For Beneficiaries with accounts established in a currency other than euros **The Auditor sampled _____ cost items selected randomly and verified that the exchange rates used for converting other currencies into euros were in accordance with the following rules established in the Agreement** (*full coverage is required if there are fewer than 10 items, otherwise the sample should have a minimum of 10 item, or 10% of the total, whichever number is highest)*: *COSTS RECORDED IN THE ACCOUNTS IN A CURRENCY OTHER THAN EURO SHALL BE CONVERTED INTO EURO AT THE AVERAGE OF THE DAILY EXCHANGE RATES PUBLISHED IN THE C SERIES OF OFFICIAL JOURNAL OF THE EUROPEAN UNION (https://www.ecb.int/stats/exchange/eurofxref/html/index.en.html), DETERMINED OVER THE CORRESPONDING REPORTING PERIOD.* *IF NO DAILY EURO EXCHANGE RATE IS PUBLISHED IN THE OFFICIAL JOURNAL OF THE EUROPEAN UNION FOR THE CURRENCY IN QUESTION, CONVERSION SHALL BE MADE AT THE AVERAGE OF THE MONTHLY ACCOUNTING RATES ESTABLISHED BY THE COMMISSION AND PUBLISHED ON ITS WEBSITE (http://ec.europa.eu/budget/contracts_grants/info_contracts/inforeuro/inforeuro_en.cfm),*	66) The exchange rates used to convert other currencies into Euros were in accordance with the rules established of the Grant Agreement and there was no difference in the final figures.	

	DETERMINED OVER THE CORRESPONDING REPORTING PERIOD.		
	b) For Beneficiaries with accounts established in euros **The Auditor sampled _____ cost items selected randomly and verified that the exchange rates used for converting other currencies into euros were in accordance with the following rules established in the Agreement** (*full coverage is required if there are fewer than 10 items, otherwise the sample should have a minimum of 10 item, or 10% of the total, whichever number is highest)*: *COSTS INCURRED IN ANOTHER CURRENCY SHALL BE CONVERTED INTO EURO BY APPLYING THE BENEFICIARY'S USUAL ACCOUNTING PRACTICES.*	67) The Beneficiary applied its usual accounting practices.	

[legal name of the audit firm]
[name and function of an authorised representative]
[dd Month yyyy]
<Signature of the Auditor>

ANNEX 6

MODEL FOR THE CERTIFICATE ON THE METHODOLOGY

- **For options [*in italics in square brackets*]: choose the applicable option. Options not chosen should be deleted.**
- **For fields in [grey in square brackets]: enter the appropriate data.**

TABLE OF CONTENTS

Terms of reference for an audit engagement for a methodology certificate in connection with one or more grant agreements financed under the Horizon 2020 Research and Innovation Framework Programme

This document sets out the '**Terms of Reference (ToR)**' under which

[OPTION 1: [insert name of the beneficiary] ('the Beneficiary')] [OPTION 2: [insert name of the linked third party] ('the Linked Third Party'), third party linked to the Beneficiary [insert name of the beneficiary] ('the Beneficiary')]

agrees to engage

[**insert legal name of the auditor**] ('the Auditor')

to produce an independent report of factual findings ('the Report') concerning the *[Beneficiary's] [Linked Third Party's]* usual accounting practices for calculating and claiming direct personnel costs declared as unit costs ('the Methodology') in connection with grant agreements financed under the Horizon 2020 Research and Innovation Framework Programme.

The procedures to be carried out for the assessment of the methodology will be based on the grant agreement(s) detailed below:

[title and number of the grant agreement(s)] ('the Agreement(s)')

The Agreement(s) has(have) been concluded between the Beneficiary and *[OPTION 1: the European Union, represented by the European Commission ('the Commission')][OPTION 2: the European Atomic Energy Community (Euratom,) represented by the European Commission ('the Commission')][OPTION 3: the [Research Executive Agency (REA)] [European Research Council Executive Agency (ERCEA)] [Innovation and Networks Executive Agency (INEA)] [Executive Agency for Small and Medium-sized Enterprises (EASME)] ('the Agency'), under the powers delegated by the European Commission ('the Commission').]*.

The *[Commission] [Agency]* is mentioned as a signatory of the Agreement with the Beneficiary only. The *[European Union] [Euratom] [Agency]* is not a party to this engagement.

1.1 Subject of the engagement

According to Article 18.1.2 of the Agreement, beneficiaries *[and linked third parties]* that declare direct personnel costs as unit costs calculated in accordance with their usual cost accounting practices may submit to the *[Commission] [Agency]*, for approval, a certificate on the methodology ('CoMUC') stating that there are adequate records and documentation to prove that their cost accounting practices used comply with the conditions set out in Point A of Article 6.2.

The subject of this engagement is the CoMUC which is composed of two separate documents:

- the Terms of Reference ('the ToR') to be signed by the *[Beneficiary] [Linked Third Party]* and the Auditor;

- the Auditor's Independent Report of Factual Findings ('the Report') issued on the Auditor's letterhead, dated, stamped and signed by the Auditor which includes; the standard statements ('the Statements') evaluated and signed by the *[Beneficiary] [Linked Third Party]*, the agreed-upon procedures ('the Procedures') performed by the Auditor and the standard factual findings

('the Findings') assessed by the Auditor. The Statements, Procedures and Findings are summarised in the table that forms part of the Report.

The information provided through the Statements, the Procedures and the Findings will enable the Commission to draw conclusions regarding the existence of the *[Beneficiary's] [Linked Third Party's]* usual cost accounting practice and its suitability to ensure that direct personnel costs claimed on that basis comply with the provisions of the Agreement. The Commission draws its own conclusions from the Report and any additional information it may require.

1.2 Responsibilities

The parties to this agreement are the *[Beneficiary] [Linked Third Party]* and the Auditor.

The *[Beneficiary] [Linked Third Party]:*

- is responsible for preparing financial statements for the Agreement(s) ('the Financial Statements') in compliance with those Agreements;
- is responsible for providing the Financial Statement(s) to the Auditor and enabling the Auditor to reconcile them with the *[Beneficiary's] [Linked Third Party's]* accounting and bookkeeping system and the underlying accounts and records. The Financial Statement(s) will be used as a basis for the procedures which the Auditor will carry out under this ToR;
- is responsible for its Methodology and liable for the accuracy of the Financial Statement(s);
- is responsible for endorsing or refuting the Statements indicated under the heading 'Statements to be made by the Beneficiary/ Linked Third Party' in the first column of the table that forms part of the Report;
- must provide the Auditor with a signed and dated representation letter;
- accepts that the ability of the Auditor to carry out the Procedures effectively depends upon the *[Beneficiary] [Linked Third Party]* providing full and free access to the *[Beneficiary's] [Linked Third Party's]* staff and to its accounting and other relevant records.

The Auditor:

- *[Option 1 by default: is qualified to carry out statutory audits of accounting documents in accordance with Directive 2006/43/EC of the European Parliament and of the Council of 17 May 2006 on statutory audits of annual accounts and consolidated accounts, amending Council Directives 78/660/EEC and 83/349/EEC and repealing Council Directive 84/253/EEC or similar national regulations].*
- *[Option 2 if the Beneficiary or Linked Third Party has an independent Public Officer: is a competent and independent Public Officer for which the relevant national authorities have established the legal capacity to audit the Beneficiary].*
- *[Option 3 if the Beneficiary or Linked Third Party is an international organisation: is an [internal] [external] auditor in accordance with the internal financial regulations and procedures of the international organisation].*

The Auditor:

- must be independent from the Beneficiary *[and the Linked Third Party]*, in particular, it must not have been involved in preparing the Beneficiary's *[and Linked Third Party's]* Financial Statement(s);
- must plan work so that the Procedures may be carried out and the Findings may be assessed;
- must adhere to the Procedures laid down and the compulsory report format;
- must carry out the engagement in accordance with these ToR;
- must document matters which are important to support the Report;
- must base its Report on the evidence gathered;
- must submit the Report to the *[Beneficiary] [Linked Third Party]*.

The Commission sets out the Procedures to be carried out and the Findings to be endorsed by the Auditor. The Auditor is not responsible for their suitability or pertinence. As this engagement is not an assurance engagement the Auditor does not provide an audit opinion or a statement of assurance.

1.3 Applicable Standards

The Auditor must comply with these Terms of Reference and with[1]:

- the International Standard on Related Services ('ISRS') 4400 *Engagements to perform Agreed-upon Procedures regarding Financial Information* as issued by the International Auditing and Assurance Standards Board (IAASB);
- the *Code of Ethics for Professional Accountants* issued by the International Ethics Standards Board for Accountants (IESBA). Although ISRS 4400 states that independence is not a requirement for engagements to carry out agreed-upon procedures, the Commission requires that the Auditor also complies with the Code's independence requirements.

The Auditor's Report must state that there was no conflict of interests in establishing this Report between the Auditor and the Beneficiary *[and the Linked Third Party]* that could have a bearing on the Report, and must specify – if the service is invoiced - the total fee paid to the Auditor for providing the Report.

1.4 Reporting

The Report must be written in the language of the Agreement (see Article 20.7 of the Agreement).

Under Article 22 of the Agreement, the Commission, *[the Agency]*, the European Anti-Fraud Office and the Court of Auditors have the right to audit any work that is carried out under the action and for which costs are declared from *[the European Union] [Euratom]* budget. This includes work related to this engagement. The Auditor must provide access to all working papers related to this assignment if the Commission*[, the Agency]*, the European Anti-Fraud Office or the European Court of Auditors requests them.

1.5 Timing

The Report must be provided by [dd Month yyyy].

1.6 Other Terms

[The [Beneficiary] [Linked Third Party] and the Auditor can use this section to agree other specific terms, such as the Auditor's fees, liability, applicable law, etc. Those specific terms must not contradict the terms specified above.]

[legal name of the Auditor]	[legal name of the [Beneficiary] [Linked Third Party]]
[name & title of authorised representative]	[name & title of authorised representative]
[dd Month yyyy]	[dd Month yyyy]
Signature of the Auditor	Signature of the *[Beneficiary] [Linked Third Party]*

[1] Supreme Audit Institutions applying INTOSAI-standards may carry out the Procedures according to the corresponding International Standards of Supreme Audit Institutions and code of ethics issued by INTOSAI instead of the International Standard on Related Services ('ISRS') 4400 and the Code of Ethics for Professional Accountants issued by the IAASB and the IESBA.

Independent report of factual findings on the methodology concerning grant agreements financed under the Horizon 2020 Research and Innovation Framework Programme

(To be printed on letterhead paper of the auditor)

To
[name of contact person(s)], [Position]
[[Beneficiary's] [Linked Third Party's] name]
[Address]
[dd Month yyyy]

Dear [Name of contact person(s)],

As agreed under the terms of reference dated [dd Month yyyy]

with *[OPTION 1: [insert name of the beneficiary] ('the Beneficiary')] [OPTION 2: [insert name of the linked third party] ('the Linked Third Party'), third party linked to the Beneficiary [insert name of the beneficiary] ('the Beneficiary')]*,

we

[name of the auditor] ('the Auditor'),

established at

[full address/city/state/province/country],

represented by

[name and function of an authorised representative],

have carried out the agreed-upon procedures ('the Procedures') and provide hereby our Independent Report of Factual Findings ('the Report'), concerning the *[Beneficiary's] [Linked Third Party's]* usual accounting practices for calculating and declaring direct personnel costs declared as unit costs ('the Methodology').

You requested certain procedures to be carried out in connection with the grant(s)

[title and number of the grant agreement(s)] ('the Agreement(s)').

The Report

Our engagement was carried out in accordance with the terms of reference ('the ToR') appended to this Report. The Report includes: the standard statements ('the Statements') made by the *[Beneficiary] [Linked Third Party]*, the agreed-upon procedures ('the Procedures') carried out and the standard factual findings ('the Findings') confirmed by us.

The engagement involved carrying out the Procedures and assessing the Findings and the documentation requested appended to this Report, the results of which the Commission uses to draw conclusions regarding the acceptability of the Methodology applied by the *[Beneficiary] [Linked Third Party]*.

The Report covers the methodology used from [dd Month yyyy]. In the event that the *[Beneficiary] [Linked Third Party]* changes this methodology, the Report will not be applicable to any Financial Statement[1] submitted thereafter.

The scope of the Procedures and the definition of the standard statements and findings were determined solely by the Commission. Therefore, the Auditor is not responsible for their suitability or pertinence.

Since the Procedures carried out constitute neither an audit nor a review made in accordance with International Standards on Auditing or International Standards on Review Engagements, we do not give a statement of assurance on the costs declared on the basis of the *[Beneficiary's] [Linked Third Party's]* Methodology. Had we carried out additional procedures or had we performed an audit or review in accordance with these standards, other matters might have come to its attention and would have been included in the Report.

Exceptions

Apart from the exceptions listed below, the *[Beneficiary] [Linked Third Party]* agreed with the standard Statements and provided the Auditor all the documentation and accounting information needed by the Auditor to carry out the requested Procedures and corroborate the standard Findings.

List here any exception and add any information on the cause and possible consequences of each exception, if known. If the exception is quantifiable, also indicate the corresponding amount.

…..

Explanation of possible exceptions in the form of examples (to be removed from the Report):

i. the [Beneficiary] [Linked Third Party] did not agree with the standard Statement number … because…;

ii. the Auditor could not carry out the procedure … established because …. (e.g. due to the inability to reconcile key information or the unavailability or inconsistency of data);

iii. the Auditor could not confirm or corroborate the standard Finding number … because ….

Remarks

We would like to add the following remarks relevant for the proper understanding of the Methodology applied by the [Beneficiary] [Linked Third Party] or the results reported:

Example (to be removed from the Report):

Regarding the methodology applied to calculate hourly rates …

Regarding standard Finding 15 it has to be noted that …

The [Beneficiary] [Linked Third Party] explained the deviation from the benchmark statement XXIV concerning time recording for personnel with no exclusive dedication to the action in the following manner: …

Annexes

Please provide the following documents to the auditor and annex them to the report when submitting this CoMUC to the Commission:

[1] Financial Statement in this context refers solely to Annex 4 of the Agreement by which the Beneficiary declares costs under the Agreement.

1. Brief description of the methodology for calculating personnel costs, productive hours and hourly rates;
2. Brief description of the time recording system in place;
3. An example of the time records used by the [*Beneficiary] [Linked Third Party*];
4. Description of any budgeted or estimated elements applied, together with an explanation as to why they are relevant for calculating the personnel costs and how they are based on objective and verifiable information;
5. A summary sheet with the hourly rate for direct personnel declared by the [*Beneficiary*] [*Linked Third Party*] and recalculated by the Auditor for each staff member included in the sample (the names do not need to be reported);
6. A comparative table summarising for each person selected in the sample a) the time claimed by the [*Beneficiary*] [*Linked Third Party*] in the Financial Statement(s) and b) the time according to the time record verified by the Auditor;
7. A copy of the letter of representation provided to the Auditor.

Use of this Report

This Report has been drawn up solely for the purpose given under Point 1.1 Reasons for the engagement.

The Report:

- is confidential and is intended to be submitted to the Commission by the [*Beneficiary*] [*Linked Third Party*] in connection with Article 18.1.2 of the Agreement;
- may not be used by the [*Beneficiary*] [*Linked Third Party*] or by the Commission for any other purpose, nor distributed to any other parties;
- may be disclosed by the Commission only to authorised parties, in particular the European Anti-Fraud Office (OLAF) and the European Court of Auditors.
- relates only to the usual cost accounting practices specified above and does not constitute a report on the Financial Statements of the [*Beneficiary*] [*Linked Third Party*].

No conflict of interest[2] exists between the Auditor and the Beneficiary *[and the Linked Third Party*] that could have a bearing on the Report. The total fee paid to the Auditor for producing the Report was EUR _____ (including EUR _____ of deductible VAT).

We look forward to discussing our Report with you and would be pleased to provide any further information or assistance which may be required.

Yours sincerely

[legal name of the Auditor]
[name and title of the authorised representative]
[dd Month yyyy]
Signature of the Auditor

[2] A conflict of interest arises when the Auditor's objectivity to establish the certificate is compromised in fact or in appearance when the Auditor for instance:
- was involved in the preparation of the Financial Statements;
- stands to benefit directly should the certificate be accepted;
- has a close relationship with any person representing the beneficiary;
- is a director, trustee or partner of the beneficiary; or
- is in any other situation that compromises his or her independence or ability to establish the certificate impartially.

Statements to be made by the Beneficiary/Linked Third Party ('the Statements') and Procedures to be carried out by the Auditor ('the Procedures') and standard factual findings ('the Findings') to be confirmed by the Auditor

The Commission reserves the right to provide the auditor with guidance regarding the Statements to be made, the Procedures to be carried out or the Findings to be ascertained and the way in which to present them. The Commission reserves the right to vary the Statements, Procedures or Findings by written notification to the Beneficiary/Linked Third Party to adapt the procedures to changes in the grant agreement(s) or to any other circumstances.

If this methodology certificate relates to the Linked Third Party's usual accounting practices for calculating and claiming direct personnel costs declared as unit costs any reference here below to 'the Beneficiary' is to be considered as a reference to 'the Linked Third Party'.

Please explain any discrepancies in the body of the Report.	
Statements to be made by Beneficiary	**Procedures to be carried out and Findings to be confirmed by the Auditor**
A. Use of the Methodology I. The cost accounting practice described below has been in use since *[*dd Month yyyy*]*. II. The next planned alteration to the methodology used by the Beneficiary will be from [dd Month yyyy*]*.	**Procedure:** ✓ The Auditor checked these dates against the documentation the Beneficiary has provided. **Factual finding:** 1. The dates provided by the Beneficiary were consistent with the documentation.
B. Description of the Methodology III. The methodology to calculate unit costs is being used in a consistent manner and is reflected in the relevant procedures. *[Please describe the methodology your entity uses to calculate personnel costs, productive hours and hourly rates, present your description to the Auditor and annex it to this certificate]* *[If the statement of section "B. Description of the methodology" cannot be endorsed by the Beneficiary or there is no written methodology to calculate unit costs it should be listed here below and reported as exception by the Auditor in the main Report of Factual Findings:* - *…]*	**Procedure:** ✓ The Auditor reviewed the description, the relevant manuals and/or internal guidance documents describing the methodology. **Factual finding:** 2. The brief description was consistent with the relevant manuals, internal guidance and/or other documentary evidence the Auditor has reviewed. 3. The methodology was generally applied by the Beneficiary as part of its usual costs accounting practices.

Please explain any discrepancies in the body of the Report.	
Statements to be made by Beneficiary	**Procedures to be carried out and Findings to be confirmed by the Auditor**
C. **Personnel costs** General IV. The unit costs (hourly rates) are limited to salaries including during parental leave, social security contributions, taxes and other costs included in the remuneration required under national law and the employment contract or equivalent appointing act; V. Employees are hired directly by the Beneficiary in accordance with national law, and work under its sole supervision and responsibility; VI. The Beneficiary remunerates its employees in accordance with its usual practices. This means that personnel costs are charged in line with the Beneficiary's usual payroll policy (e.g. salary policy, overtime policy, variable pay) and no special conditions exist for employees assigned to tasks relating to the European Union or Euratom, unless explicitly provided for in the grant agreement(s); VII. The Beneficiary allocates its employees to the relevant group/category/cost centre for the purpose of the unit cost calculation in line with the usual cost accounting practice; VIII. Personnel costs are based on the payroll system and accounting system. IX. Any exceptional adjustments of actual personnel costs resulted from relevant budgeted or estimated elements and were based on objective and verifiable information. *[Please describe the 'budgeted or estimated elements' and their relevance to personnel costs, and explain how they were reasonable and based on objective and verifiable information, present your explanation to the Auditor and annex it to this certificate].* X. Personnel costs claimed do not contain any of the following ineligible costs: costs related to return on capital; debt and debt service charges; provisions for future losses or debts; interest owed; doubtful debts; currency exchange losses; bank costs charged by the Beneficiary's bank for transfers from the Commission/Agency; excessive or reckless expenditure; deductible VAT or costs incurred during suspension of the implementation of the action. XI. Personnel costs were not declared under another EU or Euratom grant	**Procedure:** *The Auditor draws a sample of employees to carry out the procedures indicated in this section C and the following sections D to F.* *[The Auditor has drawn a random sample of 10 employees assigned to Horizon 2020 action(s). If fewer than 10 employees are assigned to the Horizon 2020 action(s), the Auditor has selected all employees assigned to the Horizon 2020 action(s) complemented by other employees irrespective of their assignments until he has reached 10 employees.].* For this sample: ✓ the Auditor reviewed all documents relating to personnel costs such as employment contracts, payslips, payroll policy (e.g. salary policy, overtime policy, variable pay policy), accounting and payroll records, applicable national tax , labour and social security law and any other documents corroborating the personnel costs claimed; ✓ in particular, the Auditor reviewed the employment contracts of the employees in the sample to verify that: i. they were employed directly by the Beneficiary in accordance with applicable national legislation; ii. they were working under the sole technical supervision and responsibility of the latter; iii. they were remunerated in accordance with the Beneficiary's usual practices; iv. they were allocated to the correct group/category/cost centre for the purposes of calculating the unit cost in line with the Beneficiary's usual cost accounting practices; ✓ the Auditor verified that any ineligible items or any costs claimed under other costs categories or costs covered by other types of grant or by other grants financed from the European Union budget have not been taken into account when calculating the personnel costs; ✓ the Auditor numerically reconciled the total amount of personnel costs used to calculate the unit cost with the total amount of personnel costs recorded in the statutory accounts and the payroll system.

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Statements to be made by Beneficiary	**Procedures to be carried out and Findings to be confirmed by the Auditor**
(including grants awarded by a Member State and financed by the EU budget and grants awarded by bodies other than the Commission/Agency for the purpose of implementing the EU or Euratom budget in the same period, unless the Beneficiary can demonstrate that the operating grant does not cover any costs of the action). <u>If additional remuneration as referred to in the grant agreement(s) is paid</u> XII. The Beneficiary is a non-profit legal entity; XIII. The additional remuneration is part of the beneficiary's usual remuneration practices and paid consistently whenever the relevant work or expertise is required; XIV. The criteria used to calculate the additional remuneration are objective and generally applied regardless of the source of funding; XV. The additional remuneration included in the personnel costs used to calculate the hourly rates for the grant agreement(s) is capped at EUR 8 000 per full-time equivalent (reduced proportionately if the employee is not assigned exclusively to the action). *[If certain statement(s) of section "C. Personnel costs" cannot be endorsed by the Beneficiary they should be listed here below and reported as exception by the Auditor in the main Report of Factual Findings:* *- …]*	✓ to the extent that actual personnel costs were adjusted on the basis of budgeted or estimated elements, the Auditor carefully examined those elements and checked the information source to confirm that they correspond to objective and verifiable information; ✓ if additional remuneration has been claimed, the Auditor verified that the Beneficiary was a non-profit legal entity, that the amount was capped at EUR 8 000 per full-time equivalent and that it was reduced proportionately for employees not assigned exclusively to the action(s). ✓ the Auditor recalculated the personnel costs for the employees in the sample. **Factual finding:** 4. All the components of the remuneration that have been claimed as personnel costs are supported by underlying documentation. 5. The employees in the sample were employed directly by the Beneficiary in accordance with applicable national law and were working under its sole supervision and responsibility. 6. Their employment contracts were in line with the Beneficiary's usual policy; 7. Personnel costs were duly documented and consisted solely of salaries, social security contributions (pension contributions, health insurance, unemployment fund contributions, etc.), taxes and other statutory costs included in the remuneration (holiday pay, thirteenth month's pay, etc.); 8. The totals used to calculate the personnel unit costs are consistent with those registered in the payroll and accounting records; 9. To the extent that actual personnel costs were adjusted on the basis of budgeted or estimated elements, those elements were relevant for calculating the personnel costs and correspond to objective and verifiable information. The budgeted or estimated elements used are: — (indicate the elements and their values). 10. Personnel costs contained no ineligible elements; 11. Specific conditions for eligibility were fulfilled when additional

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	remuneration was paid: a) the Beneficiary is registered in the grant agreements as a non-profit legal entity; b) it was paid according to objective criteria generally applied regardless of the source of funding used and c) remuneration was capped at EUR 8 000 per full-time equivalent (or up to up to the equivalent pro-rata amount if the person did not work on the action full-time during the year or did not work exclusively on the action).
D. Productive hours XVI. The number of productive hours per full-time employee applied is *[delete as appropriate]*: A. 1720 productive hours per year for a person working full-time (corresponding pro-rata for persons not working full time). B. the total number of hours worked in the year by a person for the Beneficiary C. the standard number of annual hours generally applied by the beneficiary for its personnel in accordance with its usual cost accounting practices. This number must be at least 90% of the standard annual workable hours. <u>If method B is applied</u> XVII. The calculation of the total number of hours worked was done as follows: annual workable hours of the person according to the employment contract, applicable labour agreement or national law plus overtime worked minus absences (such as sick leave and special leave). XVIII. 'Annual workable hours' are hours during which the personnel must be working, at the employer's disposal and carrying out his/her activity or duties under the employment contract, applicable collective labour agreement or national working time legislation. XIX. The contract (applicable collective labour agreement or national working time legislation) do specify the working time enabling to calculate the annual workable hours.	**Procedure (same sample basis as for Section C: Personnel costs):** ✓ The Auditor verified that the number of productive hours applied is in accordance with method A, B or C. ✓ The Auditor checked that the number of productive hours per full-time employee is correct. ✓ If method B is applied the Auditor verified i) the manner in which the total number of hours worked was done and ii) that the contract specified the annual workable hours by inspecting all the relevant documents, national legislation, labour agreements and contracts. ✓ If method C is applied the Auditor reviewed the manner in which the standard number of working hours per year has been calculated by inspecting all the relevant documents, national legislation, labour agreements and contracts and verified that the number of productive hours per year used for these calculations was at least 90 % of the standard number of working hours per year. **Factual finding:** <u>General</u> 12. The Beneficiary applied a number of productive hours consistent with method A, B or C detailed in the left-hand column. 13. The number of productive hours per year per full-time employee was accurate. <u>If method B is applied</u> 14. The number of 'annual workable hours', overtime and absences was

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Statements to be made by Beneficiary	**Procedures to be carried out and Findings to be confirmed by the Auditor**
If method C is applied XX. The standard number of productive hours per year is that of a full-time equivalent. XXI. The number of productive hours per year on which the hourly rate is based i) corresponds to the Beneficiary's usual accounting practices; ii) is at least 90% of the standard number of workable (working) hours per year. XXII. Standard workable (working) hours are hours during which personnel are at the Beneficiary's disposal preforming the duties described in the relevant employment contract, collective labour agreement or national labour legislation. The number of standard annual workable (working) hours that the Beneficiary claims is supported by labour contracts, national legislation and other documentary evidence. *[If certain statement(s) of section "D. Productive hours" cannot be endorsed by the Beneficiary they should be listed here below and reported as exception by the Auditor:* - *…]*	verifiable based on the documents provided by the Beneficiary and the calculation of the total number of hours worked was accurate. 15. The contract specified the working time enabling to calculate the annual workable hours. If method C is applied 16. The calculation of the number of productive hours per year corresponded to the usual costs accounting practice of the Beneficiary. 17. The calculation of the standard number of workable (working) hours per year was corroborated by the documents presented by the Beneficiary. 18. The number of productive hours per year used for the calculation of the hourly rate was at least 90% of the number of workable (working) hours per year.
E. Hourly rates The hourly rates are correct because: XXIII. Hourly rates are correctly calculated since they result from dividing annual personnel costs by the productive hours of a given year and group (e.g. staff category or department or cost centre depending on the methodology applied) and they are in line with the statements made in section C. and D. above. *[If the statement of section 'E. Hourly rates' cannot be endorsed by the Beneficiary they should be listed here below and reported as exception by the Auditor:* - *…]*	**Procedure** ✓ The Auditor has obtained a list of all personnel rates calculated by the Beneficiary in accordance with the methodology used. ✓ The Auditor has obtained a list of all the relevant employees, based on which the personnel rate(s) are calculated. For 10 employees selected at random (same sample basis as Section C: Personnel costs): ✓ The Auditor recalculated the hourly rates. ✓ The Auditor verified that the methodology applied corresponds to the usual accounting practices of the organisation and is applied consistently for all activities of the organisation on the basis of objective criteria irrespective of the source of funding. **Factual finding:**

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	19. No differences arose from the recalculation of the hourly rate for the employees included in the sample.
F. Time recording XXIV. Time recording is in place for all persons with no exclusive dedication to one Horizon 2020 action. At least all hours worked in connection with the grant agreement(s) are registered on a **daily/weekly/monthly** basis *[delete as appropriate]* using a **paper/computer-based system** *[delete as appropriate]*; XXV. For persons exclusively assigned to one Horizon 2020 activity the Beneficiary has either signed a declaration to that effect or has put arrangements in place to record their working time; XXVI. Records of time worked have been signed by the person concerned (on paper or electronically) and approved by the action manager or line manager at least monthly; XXVII. Measures are in place to prevent staff from: i. recording the same hours twice, ii. recording working hours during absence periods (e.g. holidays, sick leave), iii. recording more than the number of productive hours per year used to calculate the hourly rates, and iv. recording hours worked outside the action period. XXVIII. No working time was recorded outside the action period; XXIX. No more hours were claimed than the productive hours used to calculate the hourly personnel rates. *[Please provide a brief description of the time recording system in place together with the measures applied to ensure its reliability to the Auditor and annex it to the*	**Procedure** ✓ The Auditor reviewed the brief description, all relevant manuals and/or internal guidance describing the methodology used to record time. The Auditor reviewed the time records of the random sample of 10 employees referred to under Section C: Personnel costs, and verified in particular: ✓ that time records were available for all persons with not exclusive assignment to the action; ✓ that time records were available for persons working exclusively for a Horizon 2020 action, or, alternatively, that a declaration signed by the Beneficiary was available for them certifying that they were working exclusively for a Horizon 2020 action; ✓ that time records were signed and approved in due time and that all minimum requirements were fulfilled; ✓ that the persons worked for the action in the periods claimed; ✓ that no more hours were claimed than the productive hours used to calculate the hourly personnel rates; ✓ that internal controls were in place to prevent that time is recorded twice, during absences for holidays or sick leave; that more hours are claimed per person per year for Horizon 2020 actions than the number of productive hours per year used to calculate the hourly rates; that working time is recorded outside the action period; ✓ the Auditor cross-checked the information with human-resources records to verify consistency and to ensure that the internal controls have been effective. In addition, the Auditor has verified that no more hours were charged to Horizon 2020 actions per person per year than the number of productive hours per year used to calculate the hourly rates, and verified that

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present certificate[1]]. *[If certain statement(s) of section "F. Time recording" cannot be endorsed by the Beneficiary they should be listed here below and reported as exception by the Auditor:* - *…]*	no time worked outside the action period was charged to the action. **Factual finding:** 20. The brief description, manuals and/or internal guidance on time recording provided by the Beneficiary were consistent with management reports/records and other documents reviewed and were generally applied by the Beneficiary to produce the financial statements. 21. For the random sample time was recorded or, in the case of employees working exclusively for the action, either a signed declaration or time records were available; 22. For the random sample the time records were signed by the employee and the action manager/line manager, at least monthly. 23. Working time claimed for the action occurred in the periods claimed; 24. No more hours were claimed than the number productive hours used to calculate the hourly personnel rates; 25. There is proof that the Beneficiary has checked that working time has not been claimed twice, that it is consistent with absence records and the number of productive hours per year, and that no working time has been claimed outside the action period. 26. Working time claimed is consistent with that on record at the human-resources department.

[1] The description of the time recording system must state among others information on the content of the time records, its coverage (full or action time-recording, for all personnel or only for personnel involved in H2020 actions), its degree of detail (whether there is a reference to the particular tasks accomplished), its form, periodicity of the time registration and authorisation (paper or a computer-based system; on a daily, weekly or monthly basis; signed and countersigned by whom), controls applied to prevent double-charging of time or ensure consistency with HR-records such as absences and travels as well as it information flow up to its use for the preparation of the Financial Statements.

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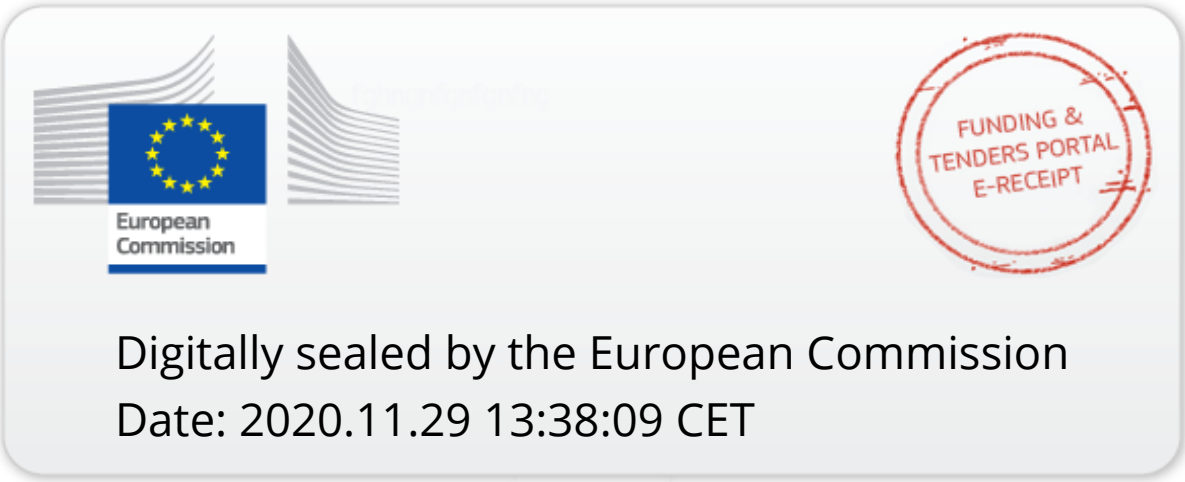
[official name of the *[Beneficiary] [Linked Third Party]**]* ***[name and title of authorised representative]*** ***[dd Month yyyy]*** ***<Signature of the*** *[Beneficiary] [Linked Third Party]>*	***[official name of the Auditor]*** ***[name and title of authorised representative]*** ***[dd Month yyyy]*** ***<Signature*** *of the Auditor>*



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