



PHOTONICS PUBLIC PRIVATE PARTNERSHIP

NANO-scale VIsualization to understand Bacterial Virulence and Invasiveness – Based on fluorescence NANOScopy and VIBrational microscopy





2021-01-14

Kick-off meeting

- 14:00-14:30 Welcome, introduction of all persons at the meeting, including project officer and advisory board
- 14:30-14:45 Project overview
- 14:45-16:00 Participant presentations and their role in the project (KTH, KI, AI, LLG, APE, PII) + Q&A
- 16:00-16:50 Brief outline of plans for the next 6 months (WP-wise, with short presentations of the WP:s) + Q&A
- 16:50-17:00 Other questions/issues, date for the next meeting, concluding remarks



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ICT-36-2020 iv. Next generation biophotonics methods and devices as research tools to understand the cellular origin of diseases

Actions 3-6 M€

Objective is to develop photonics-based in-vivo/in-vitro imaging systems.

Requirements:

- Actions should include medical/clinical doctors or research laboratories with relevant experience.

Expected Impact:

- ❖ Gain significant understanding of inter- and/or intra-cellular processes
- ❖ strengthen Europe's industrial position in the biophotonics-related market for microscopes.

Evaluation: Excellence: 5 out of 5
Impact: 5 out of 5
Implementation: 5 out of 5

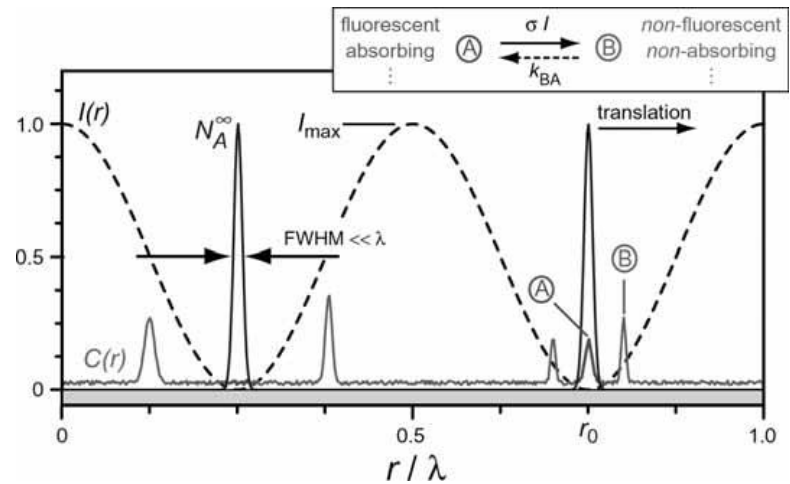
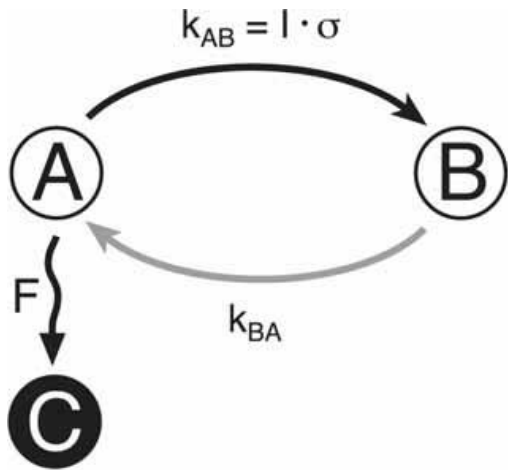


Molecular resolution with focused visible light

2005-2007

5 partners

Coordinator: Stefan W Hell



EU-FP7

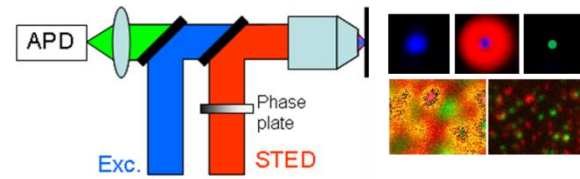


FLUODIAMON

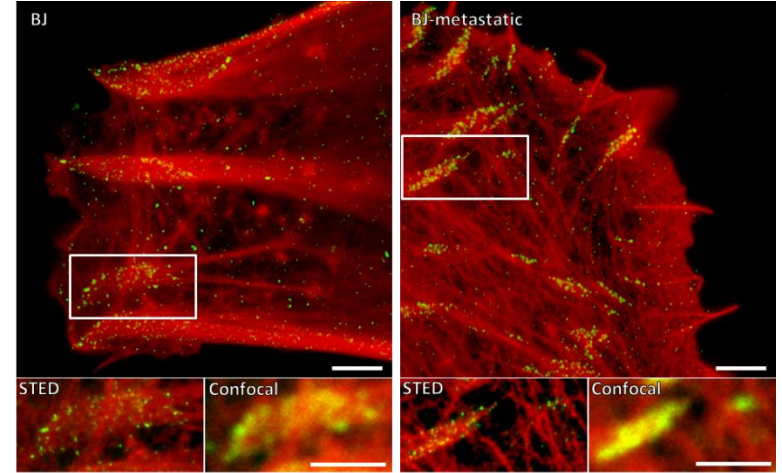
Ultra-high resolution and ultra-sensitive fluorescence methods for objective sub-cellular diagnosis of early disease and disease progression in breast and prostate cancer.

12 partners

2008-2011

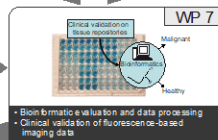
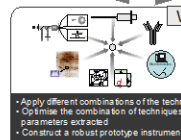
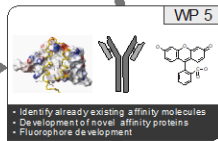
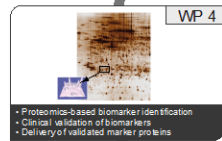
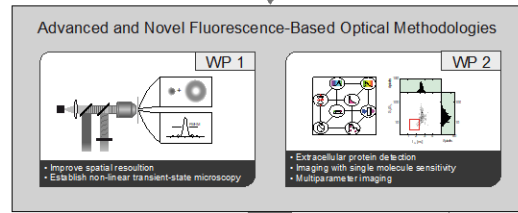
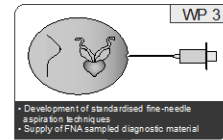
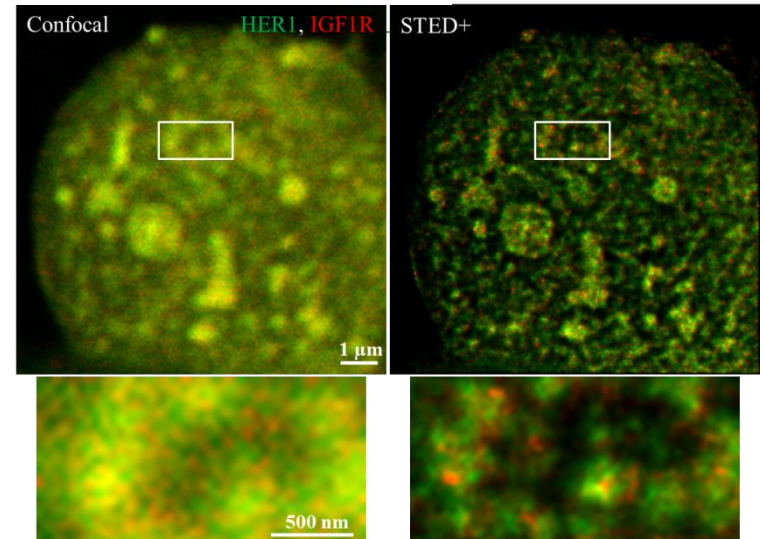


Cultured fibroblasts

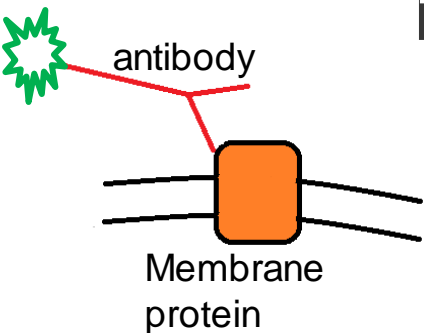


D Rönnlund *et al*, *Cytometry A*, 83(9), 855-865, 2013

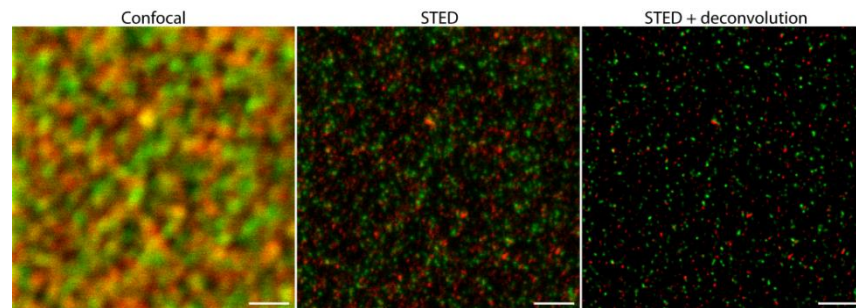
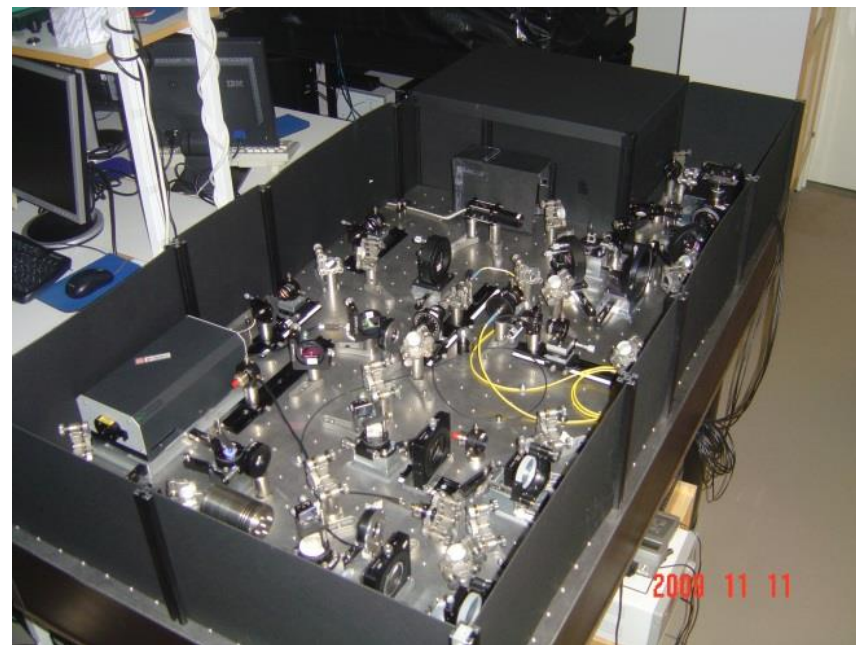
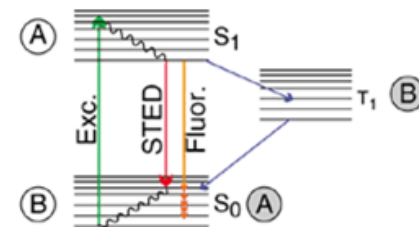
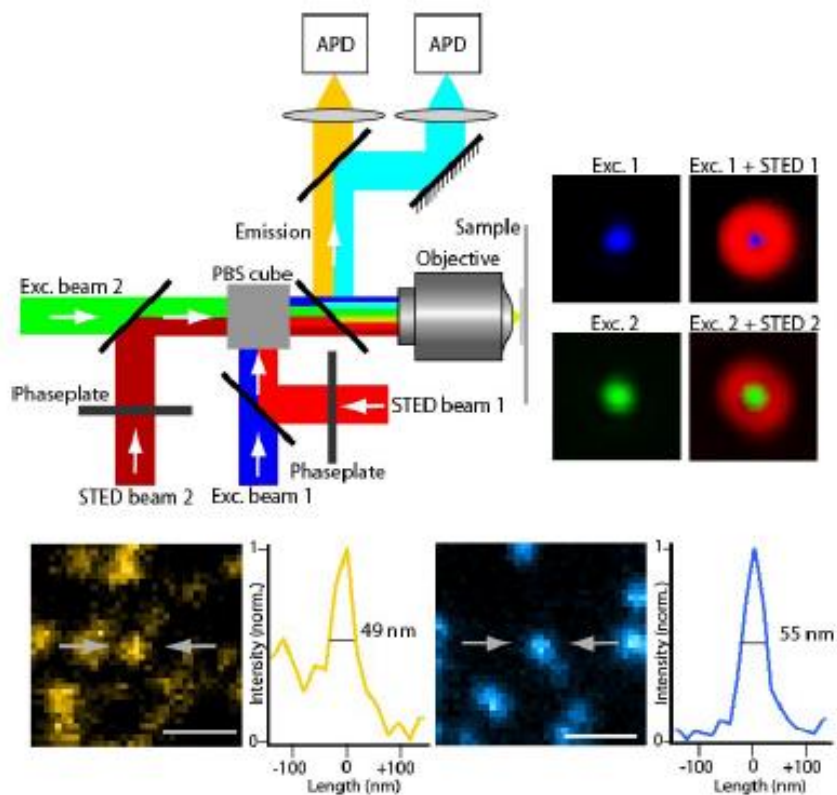
FNA sampled cells



Quantitative fluorescence-based tumor diagnostics



STED imaging in the Fluodiamon project

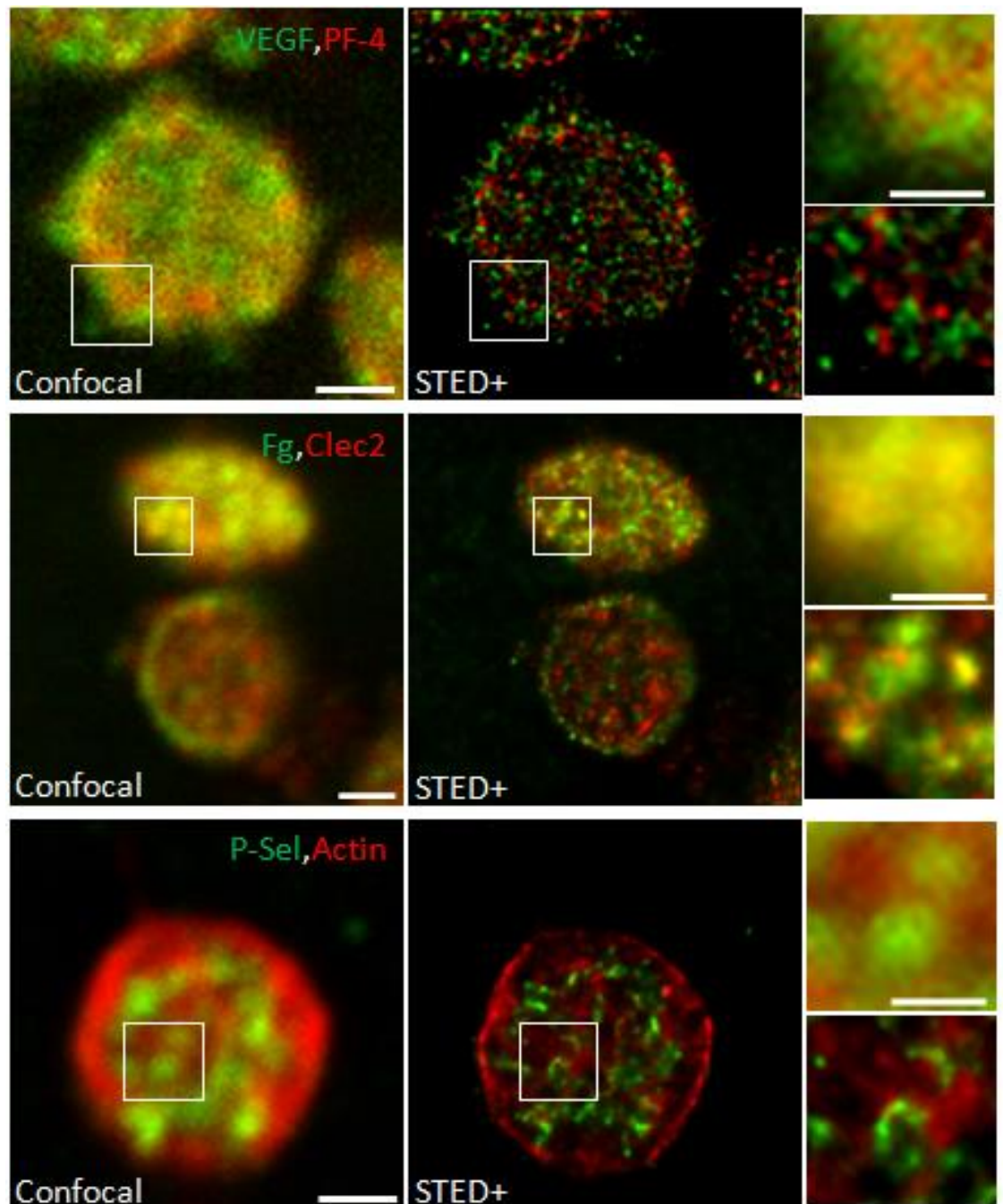


Collab: SW Hell et al, MPIBPC, Göttingen

Wildanger et al, *Optics Express* 16, 9614-9621, 2008

STED imaging of platelets

Collaboration:
Gert Auer, Karolinska Inst



Rönnlund *et al*, *Adv Healthcare Mater.*
1(6), 707-713, 2012

Rönnlund *et al*, *ACS Nano*, 5,
4358-4365, 2014

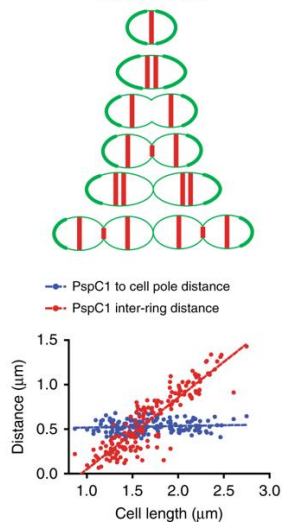
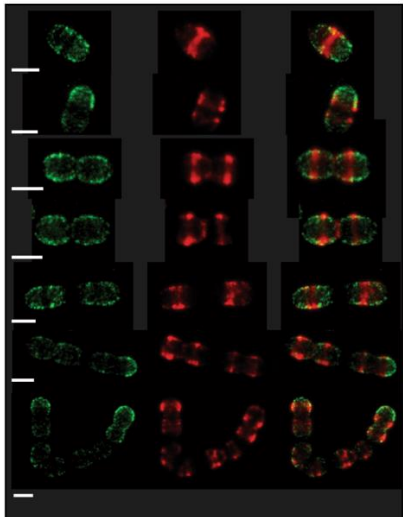
Blom H and Widengren J
Chemical Reviews, 2017

Bergstrand J *et al*, *Nanoscale*, 2019

Platelet images from patient with ovarian cancer

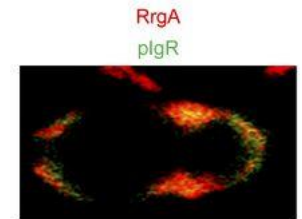
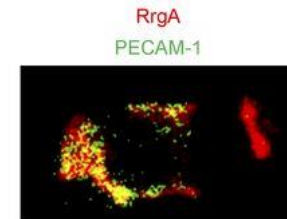
STED imaging in bacteriology

Collaboration:
B. Henriques-Normark, Karolinska Inst.

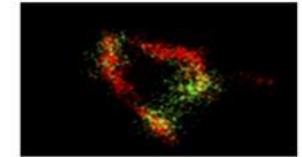
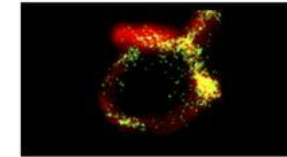


A Piliated clinical isolates

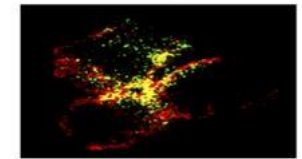
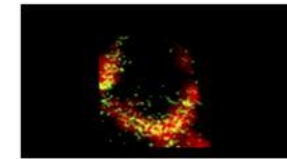
Serotype 6A



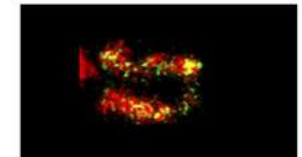
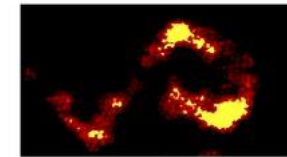
Serotype 9V



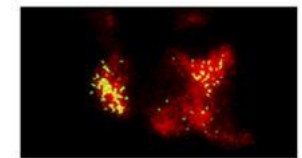
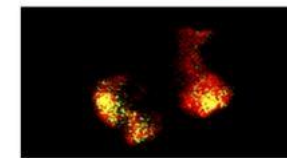
Serotype 15A



Serotype 16F

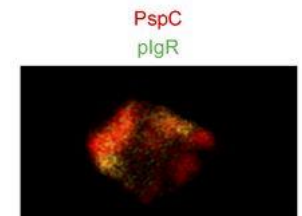
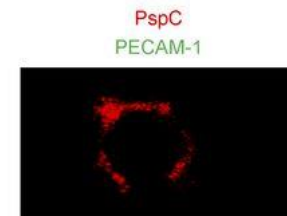


Serotype 19F



Non-piliated clinical isolate

Serotype 11A



1 μ m

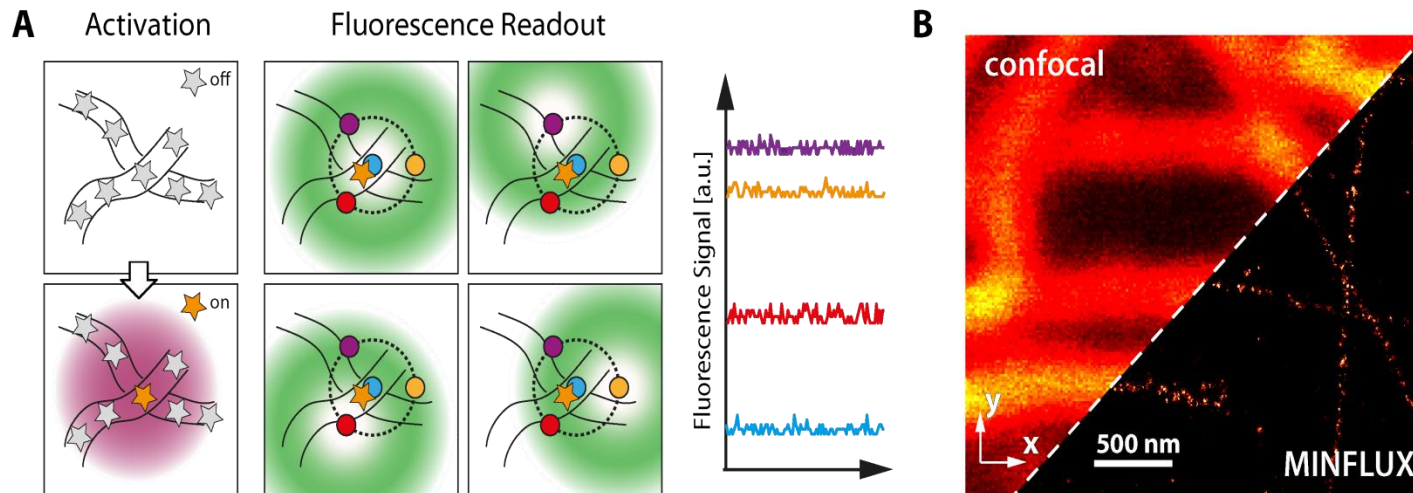
SRM studies of pneumococci

- *Streptococcus pneumoniae*: a major cause of morbidity and mortality world-wide
- Localization patterns of specific bacterial surface proteins and their interactions with host cells

Next generation super-resolution light microscopy:

MINFLUX

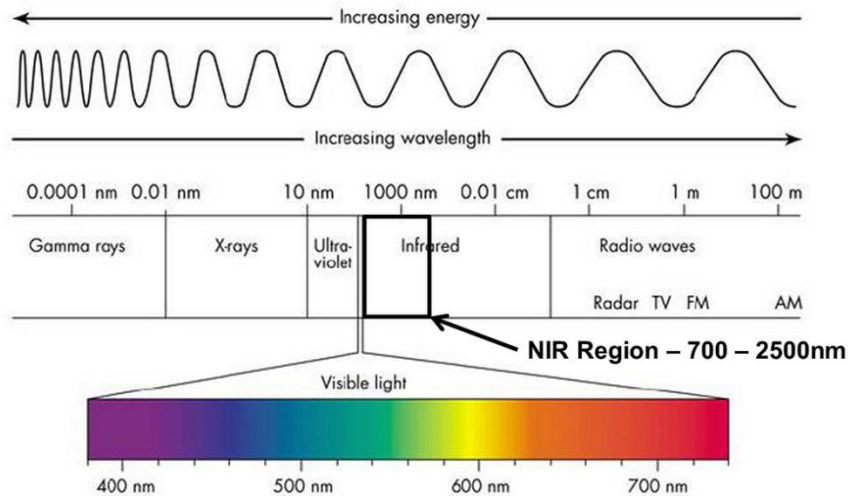
- establishing molecular coordinates with minimal emission fluxes



Compared to any other SRM technique:

- An order of magnitude higher resolution
- Much lower excitation/depletion irradiances required
- Relies on far fewer detected photons

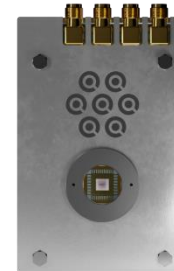
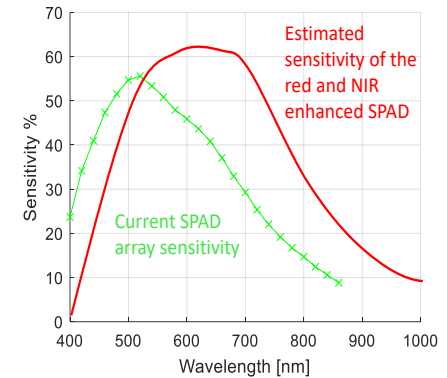
Near Infrared (NIR) – a hitherto unexplored spectral range in SRM



- Strongly reduced scattering
- Lower signal absorption and autofluorescence
- Lower phototoxicity
- Deeper penetration depths
- Provision of an additional spectral window

Single photon avalanche photo diodes (SPADs) in the NIR:

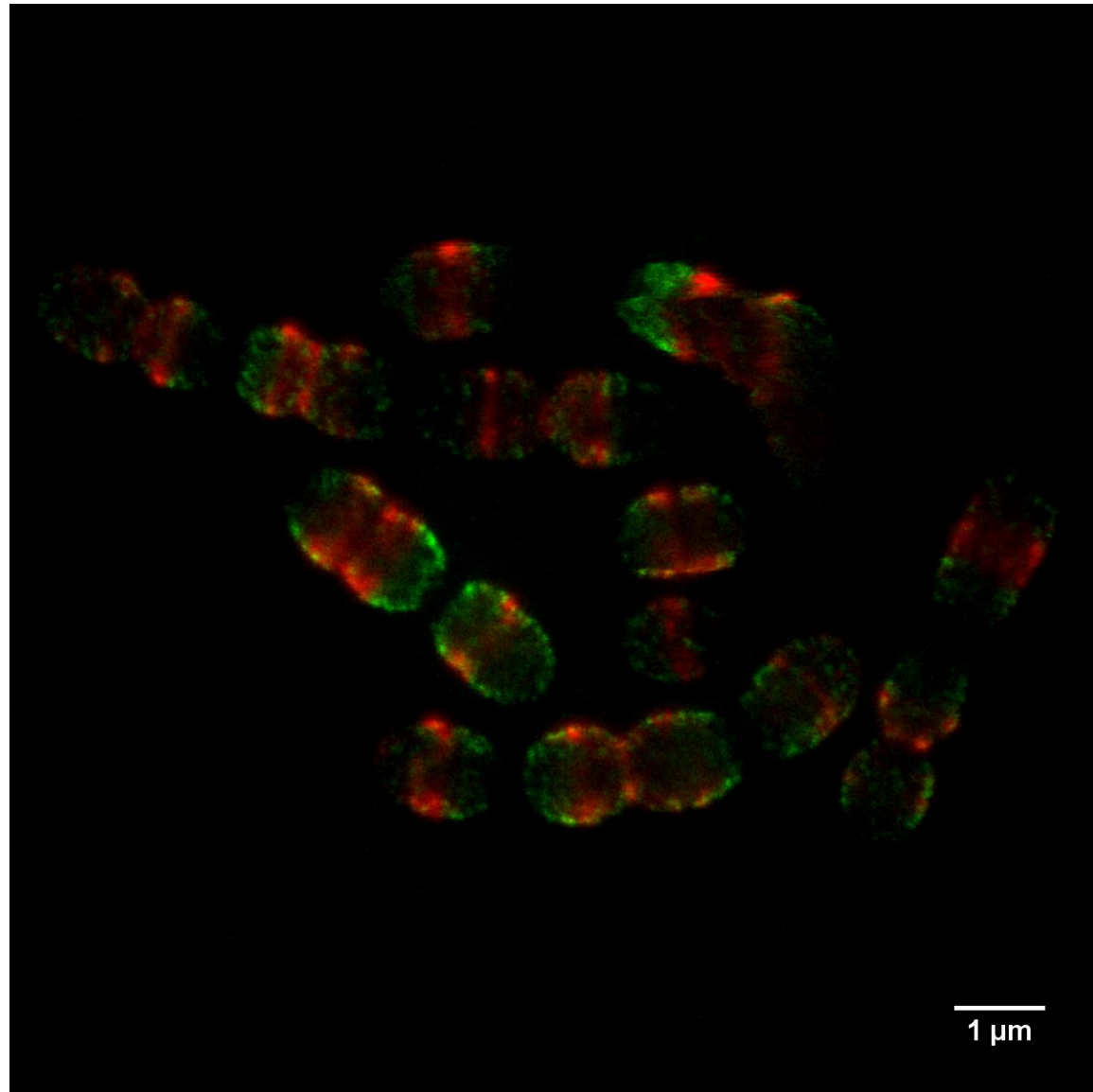
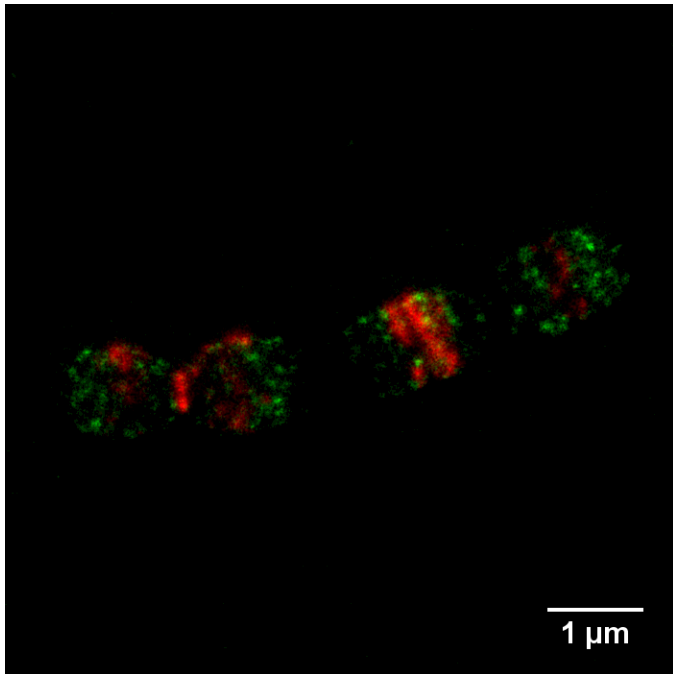
- Better detection quantum yield in the NIR
- Lowered dark count rates
- Time gating by high time resolution
- SPAD arrays -> faster emitter localization by MINIFLUX with reduction of possible artifacts



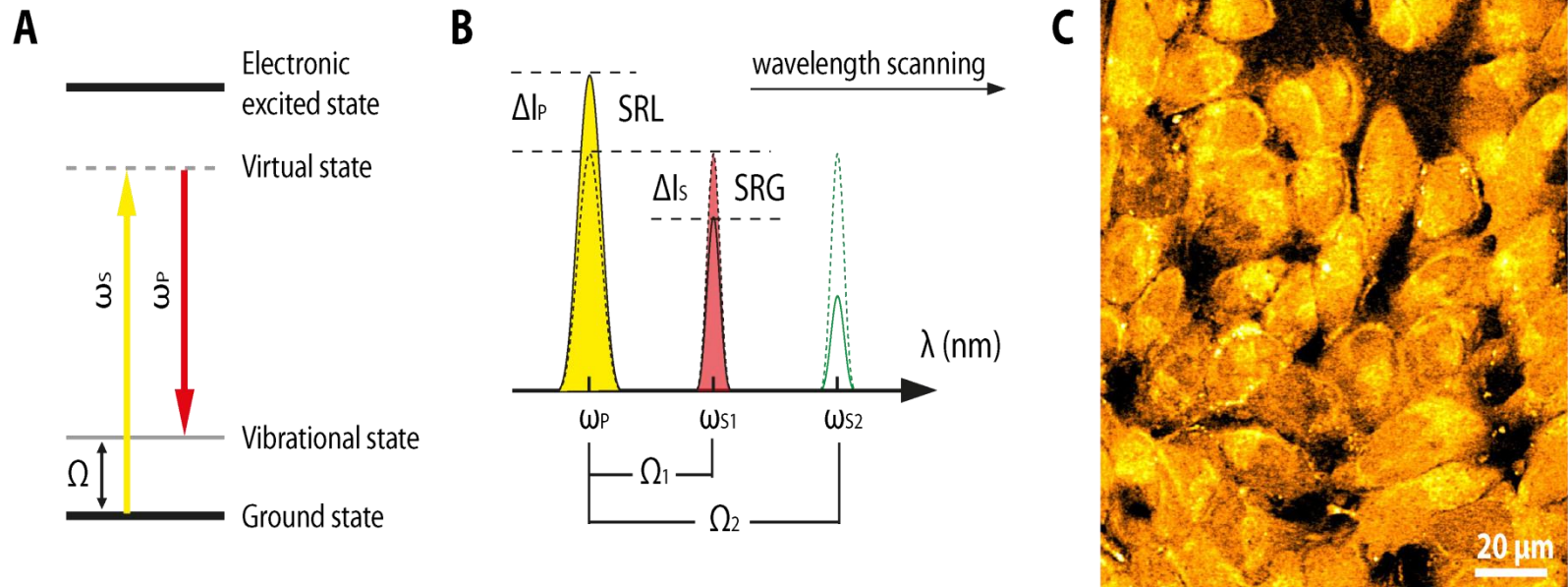
STED imaging of factor H binding proteins:

PspC2 and PspC1

STED imaging



Stimulated Raman scattering (SRS) imaging:



Courtesy of G Hehl and A Volkmer, Univ Stuttgart

Key technology for SRS: Pulsed, tunable, multiple line, narrow linewidth laser systems

Laser technology development

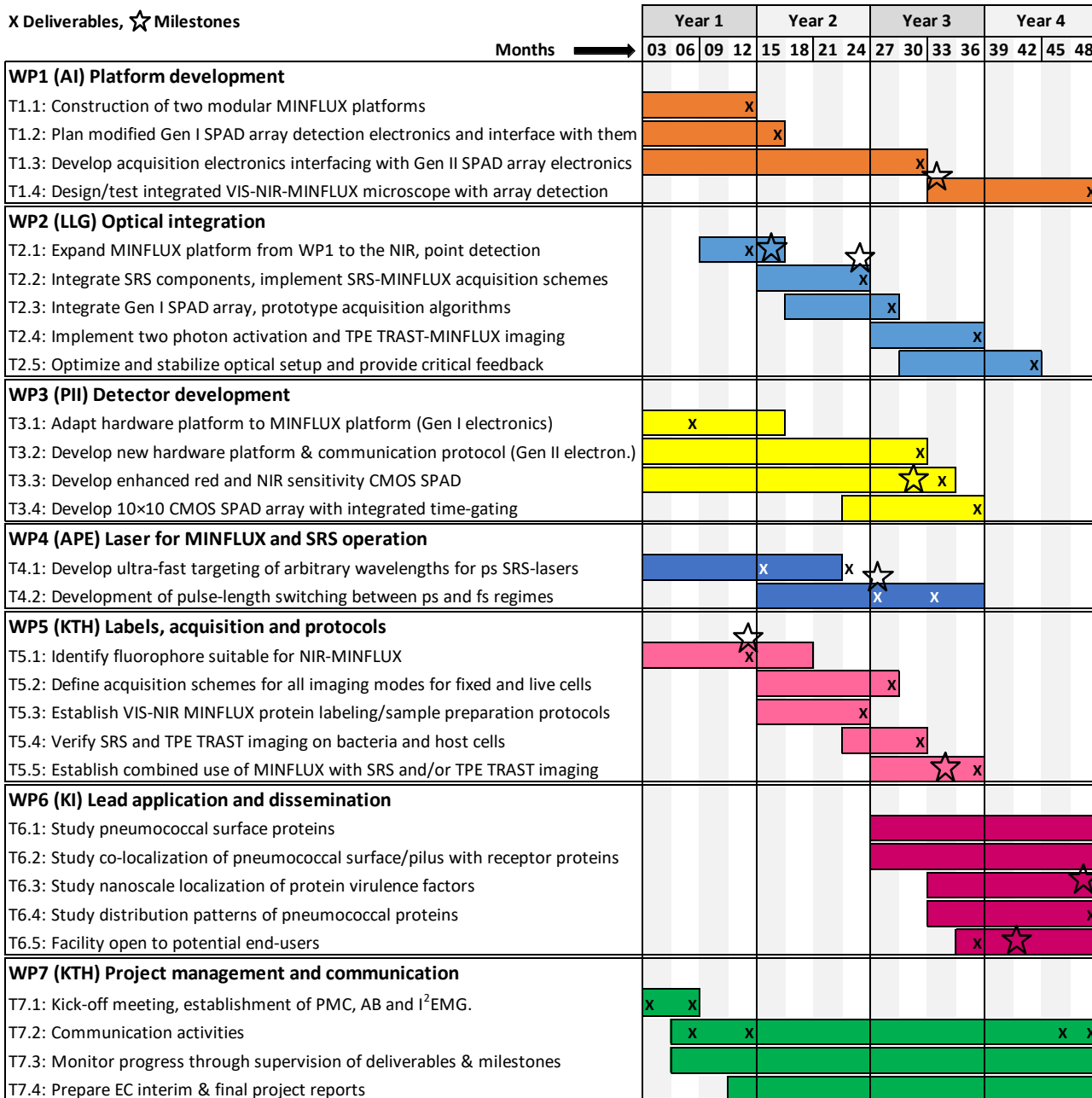


- Combine MINFLUX with SRS and two-photon excitation (TPE) imaging
- Label-free correlative imaging (morphological/chemical/environmental mapping)
- Requirement: laser with several pulsed, narrow linewidth emission lines, tunable in wavelength and pulse width.

Overall objectives:

- Technological: Development of a prototype of a combined SRS/TPE and MINFLUX imaging system.
- Biomedical: Significantly increase our understanding of diseases, on a cellular, as well as sub- and intra-cellular level.
 - Lead application: Pneumococcal virulence and invasiveness
 - Open facility for pilot studies

X Deliverables, ☆ Milestones



Consortium meetings:
Every 6 months