



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











pissimaging



#### 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





# ICT-36-2020 iv.Next generation biophotonicsmethods and devices as research tools to understandthe cellular origin of diseasesActions 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 <u>Impact</u>: 5 out of 5 <u>Implementation</u>: 5 out of 5

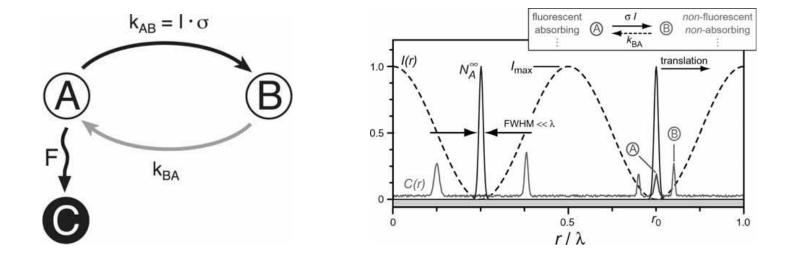


#### Molecular resolution with focused visible light

2005-2007

5 partners

Coordinator: Stefan W Hell

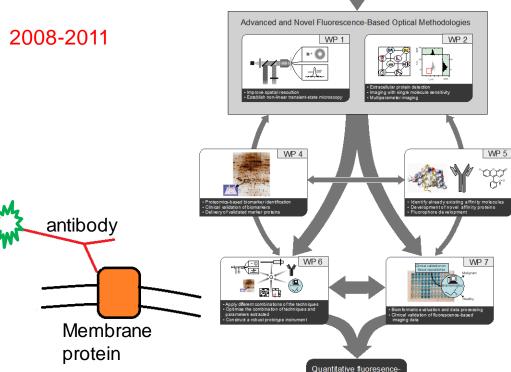


EU-FP7

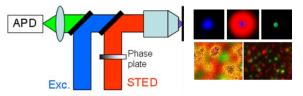


Ultra-high resolution and ultra-sensitive fluorescence methods for objective sub-cellular diagnosis of early disease and disease progression in breast and prostate cancer.

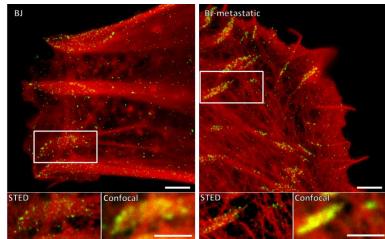
12 partners



based tumor diagnostics

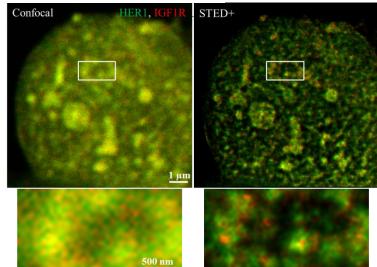


### Cultured fibroblasts

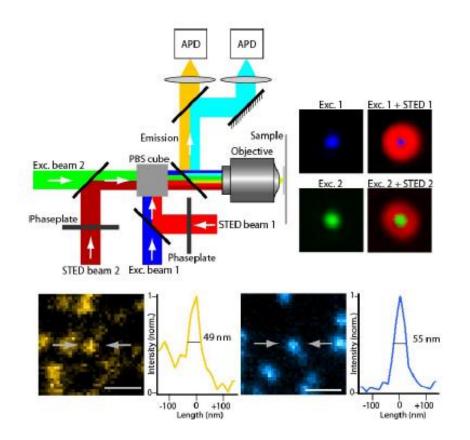


D Rönnlund et al, Cytometry A, 83(9), 855-865, 2013

### FNA sampled cells

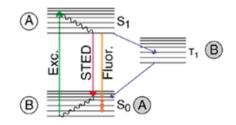


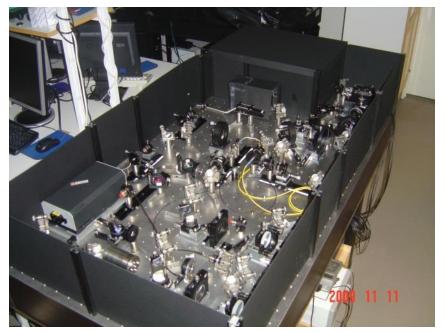
### STED imaging in the Fluodiamon project

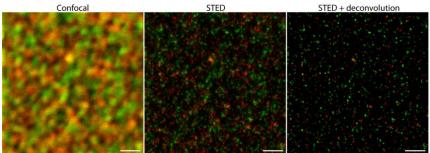


Collab: SW Hell et al, MPIBPC, Göttingen

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







# STED imaging of platelets

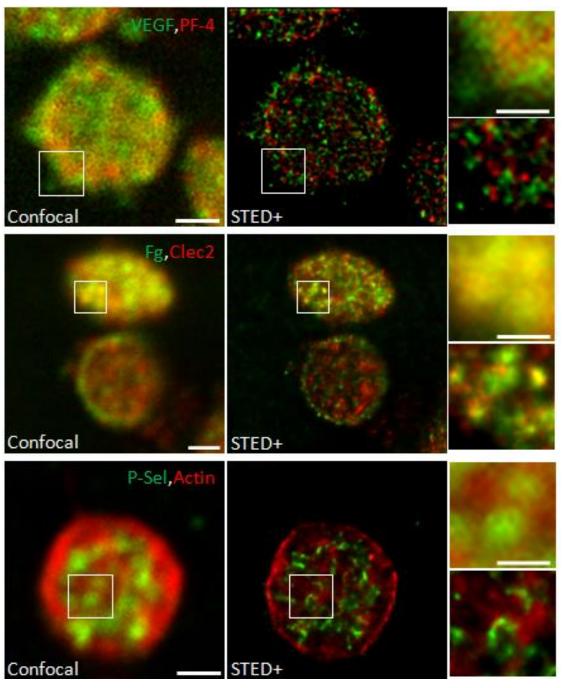
Collaboration: Gert Auer, Karolinska Inst

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

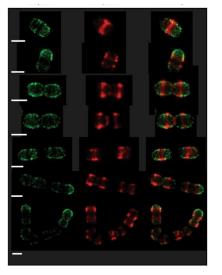
Bergstrand J et al, Nanoscale, 2019

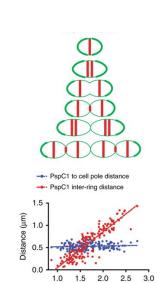


Platelet images from patient with ovarian cancer

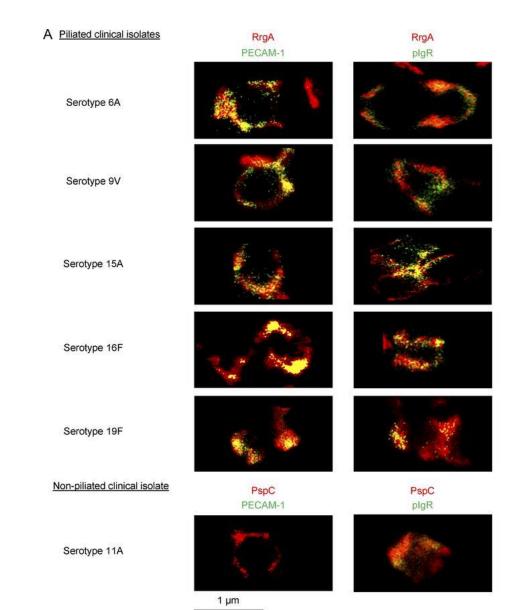
### STED imaging in bacteriology

Collaboration: B. Henriques-Normark, Karolinska Inst.





Cell length (µm)



Pathak et al, Nat. Comm. 9:3398, 2018

Iovino et al, J. Exp. Med. 214(6), 1619-1630, 2017

### SRM studies of pneumococci

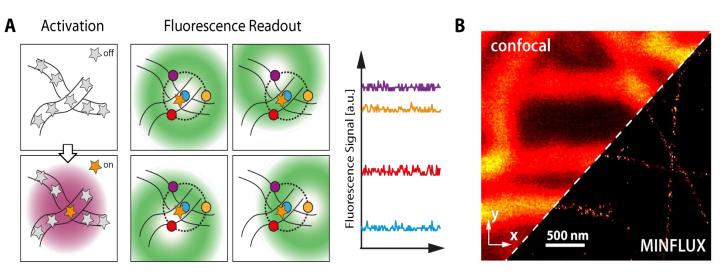
Streptococcus pneumoniae: a major cause of morbidity and mortality world-wide

 Localization patterns of specific bacterial surface proteins and their interactions with host cells

### Next generation super-resolution light microsocpy:

### MINFLUX

- establishing molecular coordinates with minimal emission fluxes

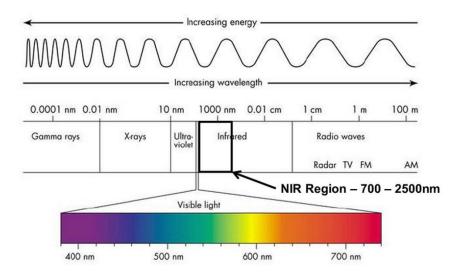


### Compared to any other SRM technique:

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

Balzarotti et al, Science, 2017

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

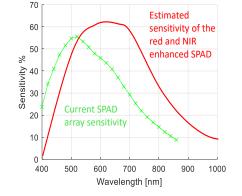


- Strongly reduced scattering
- Lower signal absorption and autofluorescence
- Lower phototoxicity
- Deeper penetration depths
- Provision of an additional spectral window

### 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 MINFLUX with reduction of possible artifacts

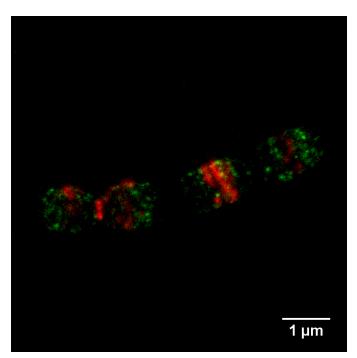


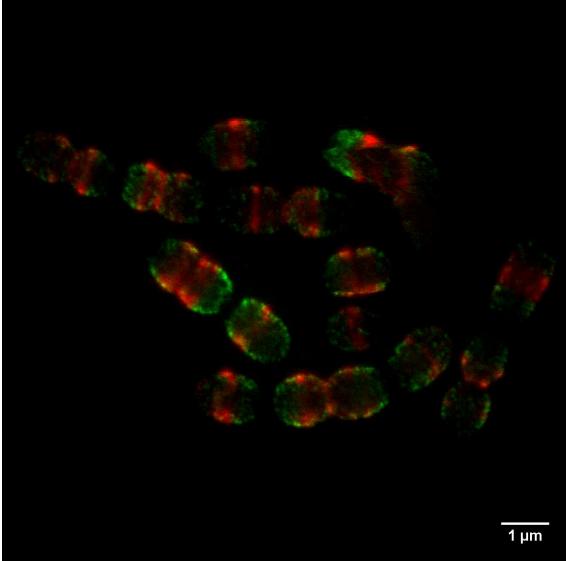


### STED imaging of factor H binding proteins:

PspC2 and PspC1

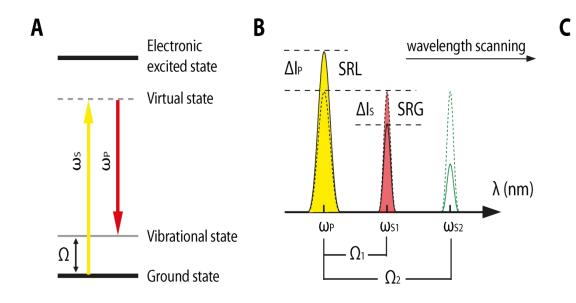
### STED imaging

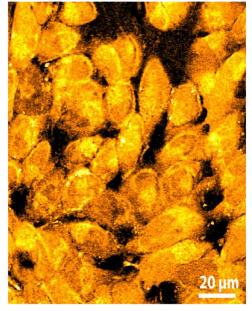




Pathak et al, Nat Comm. 2017

### 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:**

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

X Deliverables, 샀 Milestones		Year 1			Year 2				Year 3				Year 4		
Months	03	06	09	12	15	18	21	24	27	30	33	36	39	42 4	48
WP1 (AI) Platform development															
T1.1: Construction of two modular MINFLUX platforms				Х											
T1.2: Plan modified Gen I SPAD array detection electronics and interface with them					Х										
T1.3: Develop acquisition electronics interfacing with Gen II SPAD array electronics										X	لم				
T1.4: Design/test integrated VIS-NIR-MINFLUX microscope with array detection											$\sim$				Х
WP2 (LLG) Optical integration															
T2.1: Expand MINFLUX platform from WP1 to the NIR, point detection				X	ž			$\overline{\mathbf{v}}$	•						
T2.2: Integrate SRS components, implement SRS-MINFLUX acquisition schemes								X							
T2.3: Integrate Gen I SPAD array, prototype acquisition algorithms									Х						
T2.4: Implement two photon activation and TPE TRAST-MINFLUX imaging												Х			
T2.5: Optimize and stabilize optical setup and provide critical feedback		_												Х	
WP3 (PII) Detector development															
T3.1: Adapt hardware platform to MINFLUX platform (Gen I electronics)		Х													
T3.2: Develop new hardware platform & communication protocol (Gen II electron.)										Х					
T3.3: Develop enhanced red and NIR sensitivity CMOS SPAD										ণ্ন	X				
T3.4: Develop 10×10 CMOS SPAD array with integrated time-gating												Х			
WP4 (APE) Laser for MINFLUX and SRS operation															
T4.1: Develop ultra-fast targeting of arbitrary wavelengths for ps SRS-lasers					х			×4	ح۸						
T4.2: Development of pulse-length switching between ps and fs regimes									x		X				
WP5 (KTH) Labels, acquisition and protocols				2	•										
T5.1: Identify fluorophore suitable for NIR-MINFLUX				Ŷ											
T5.2: Define acquisition schemes for all imaging modes for fixed and live cells									Х						
T5.3: Establish VIS-NIR MINFLUX protein labeling/sample preparation protocols								Х							
T5.4: Verify SRS and TPE TRAST imaging on bacteria and host cells										Х					
T5.5: Establish combined use of MINFLUX with SRS and/or TPE TRAST imaging											<del>び</del>	'x			
WP6 (KI) Lead application and dissemination															
T6.1: Study pneumococcal surface proteins															
T6.2: Study co-localization of pneumococcal surface/pilus with receptor proteins															
T6.3: Study nanoscale localization of protein virulence factors															X
T6.4: Study distribution patterns of pneumococcal proteins															Х
T6.5: Facility open to potential end-users												Х	ど	3	
WP7 (KTH) Project management and communication															
T7.1: Kick-off meeting, establishment of PMC, AB and I <sup>2</sup> EMG.	х	х													
T7.2: Communication activities		X		Х											x x
T7.3: Monitor progress through supervision of deliverables & milestones															
T7.4: Prepare EC interim & final project reports															

#### <u>Consortium meetings:</u> Every 6 months