A Trojan Horse for Live-Cell Super-Resolution Microscopy

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Journal Club

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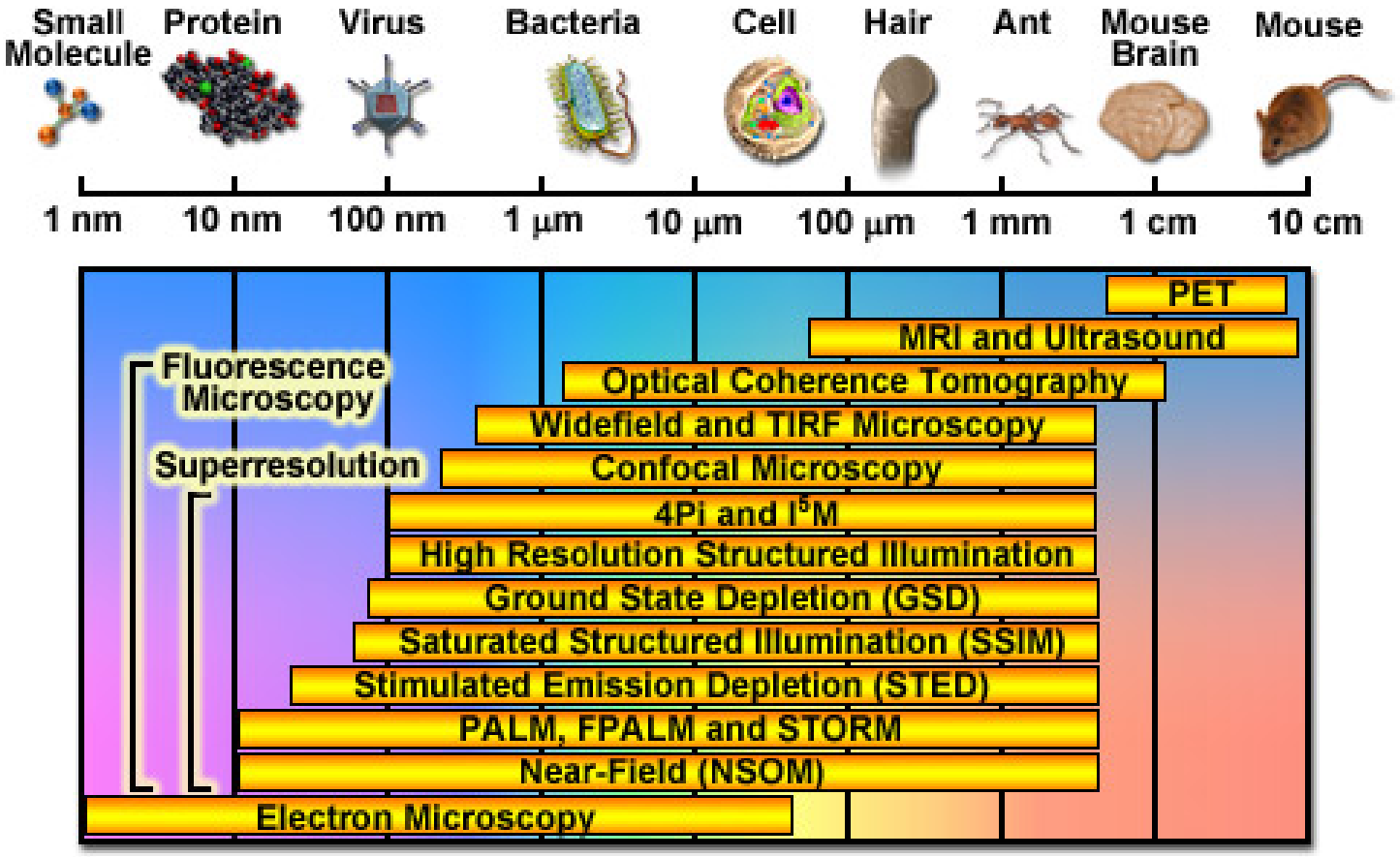
Outline

- Introduction to Super-Resolution
  - STED
  - SIM
  - SMLM: STORM
- A trojan horse for live-cell super-resolution microscopy
- Photoregulated fluoxinal fluorophores for live-cell super-resolution microscopy with no apparent photobleaching

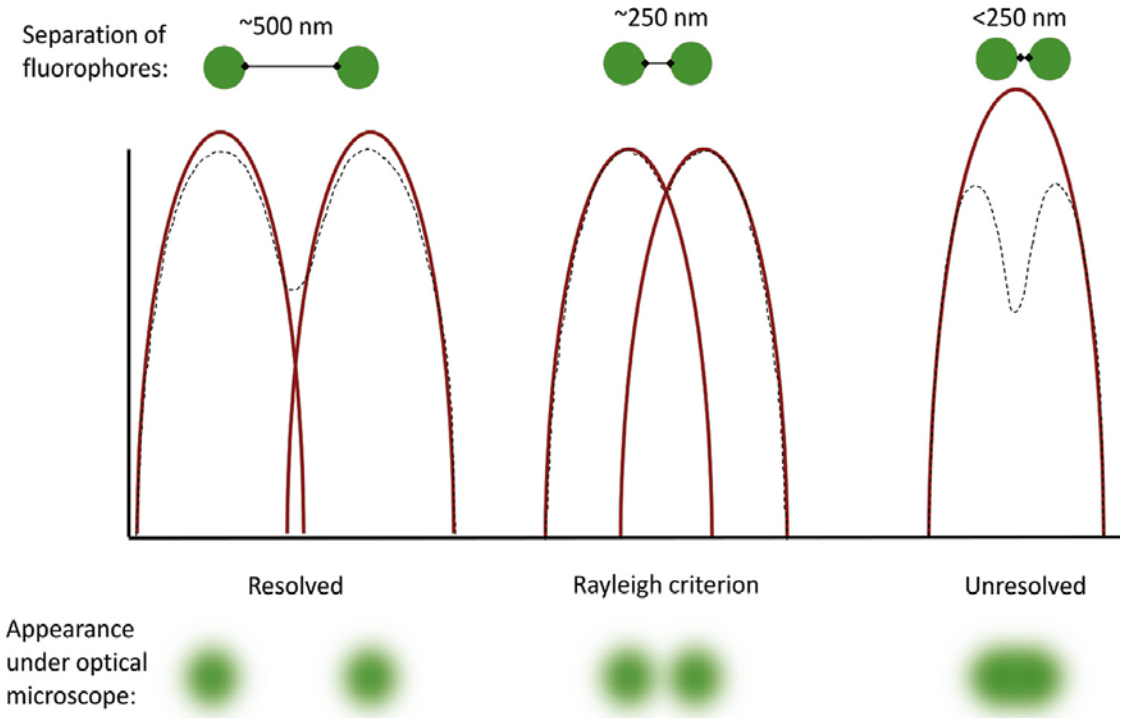
Spatial Resolution of Biological Imaging Techniques



http://zeiss-campus.magnet.fsu.edu

Optical Resolution and the Rayleigh Criterion

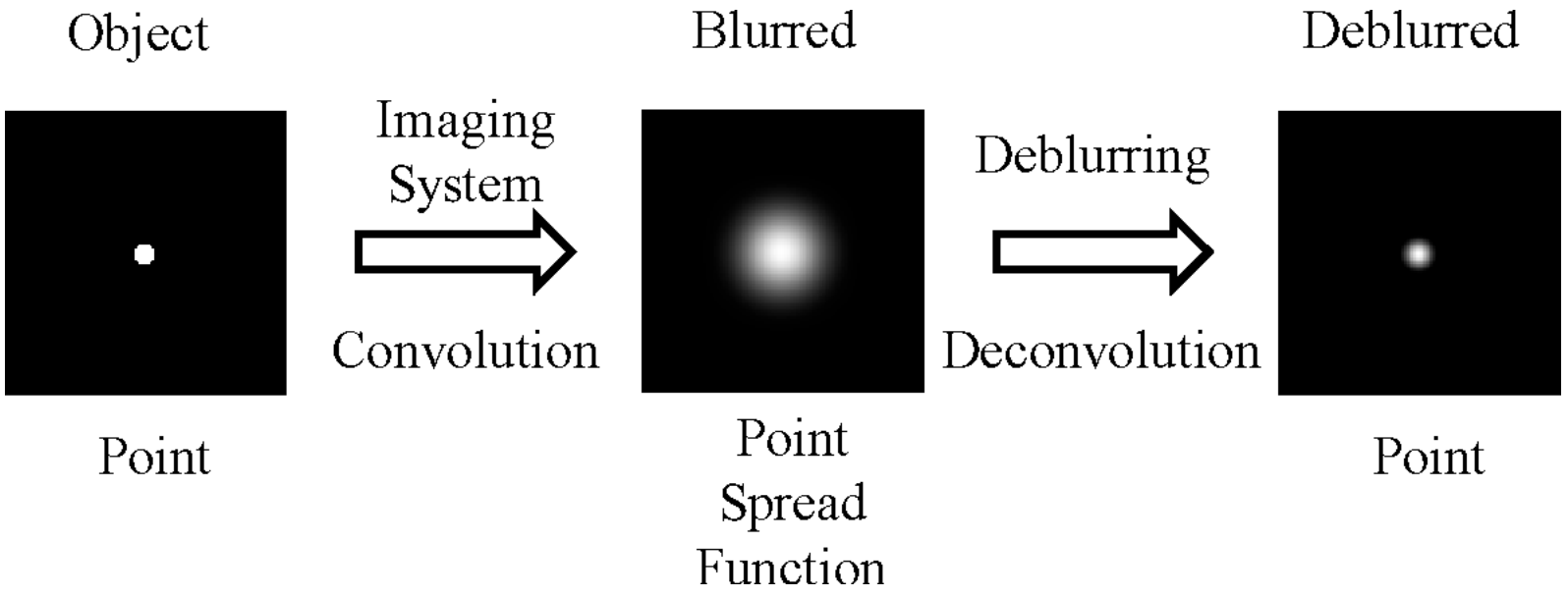
- The optical resolution of microscopes is limited by the diffraction of light, which commonly sets a limit of $\sim\lambda/2$



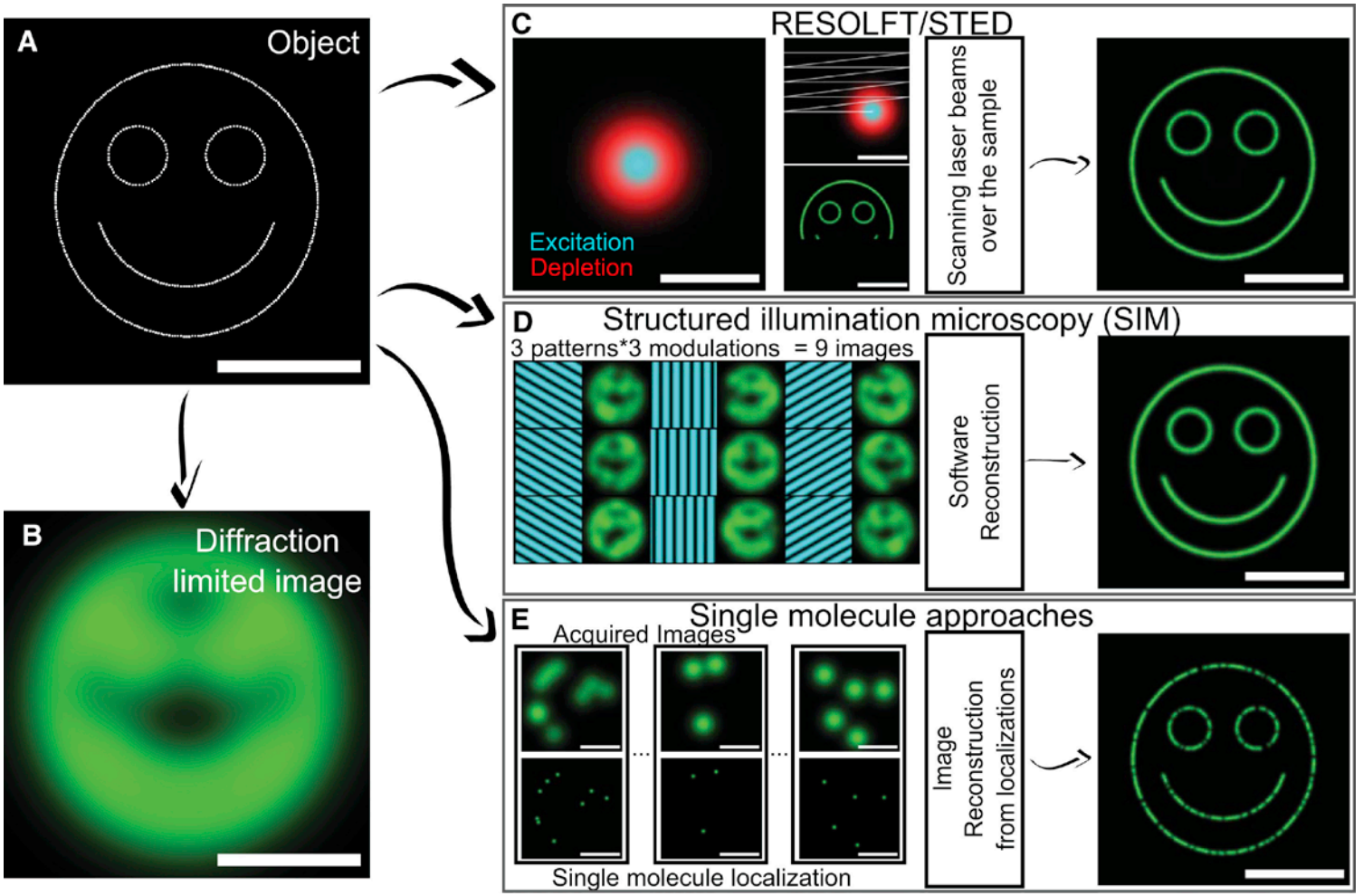
Thorley et al. **2014**

Deconvolution

- Deconvolution is a mathematical operation
- The aim is to get rid of out-of-focus light

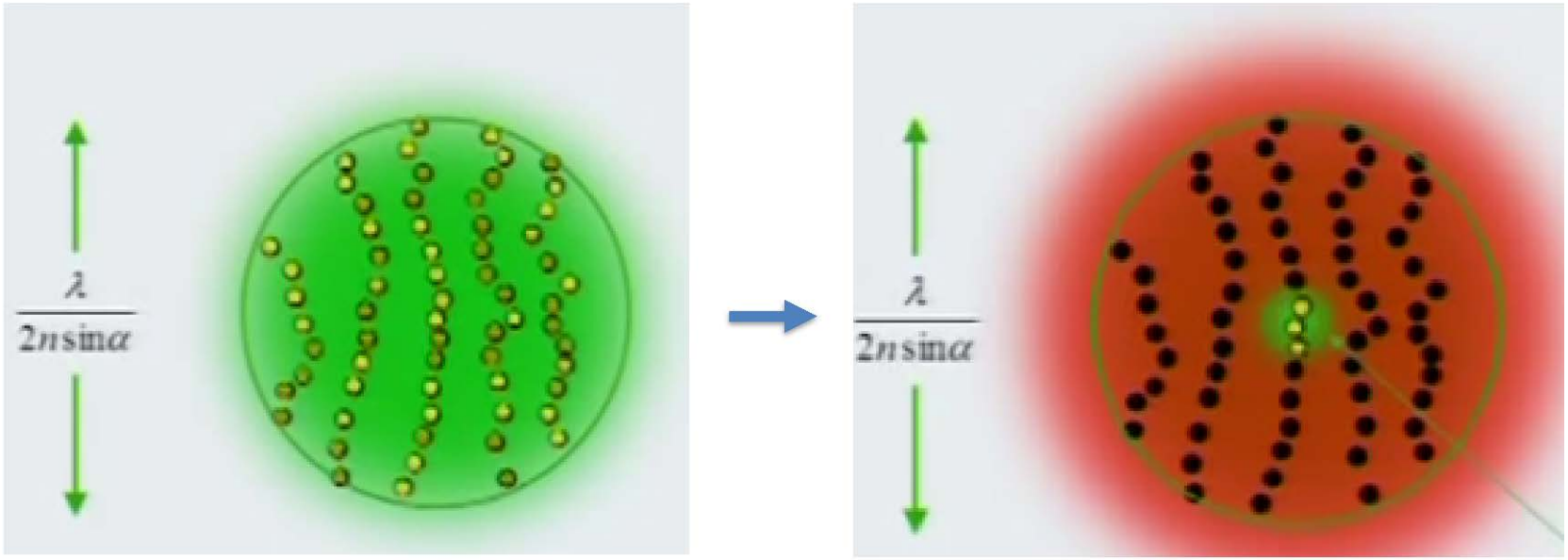


SR Techniques



Godin et al., *Biophysical Review* **2014**

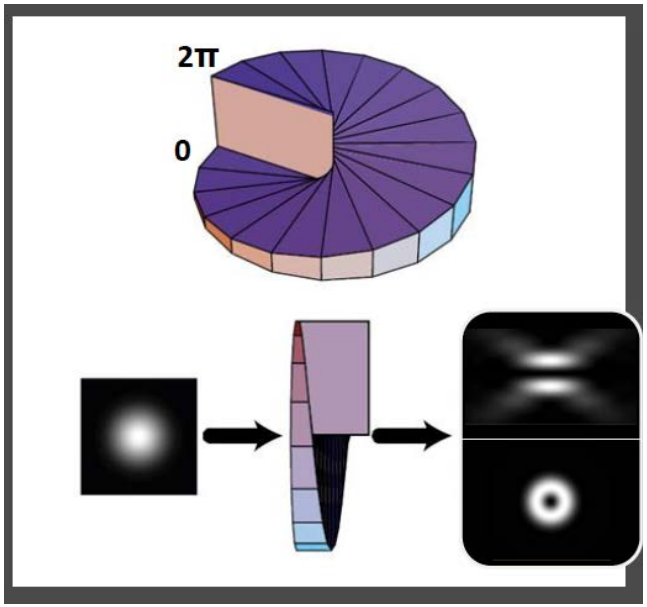
Aim: get a smaller detection spot

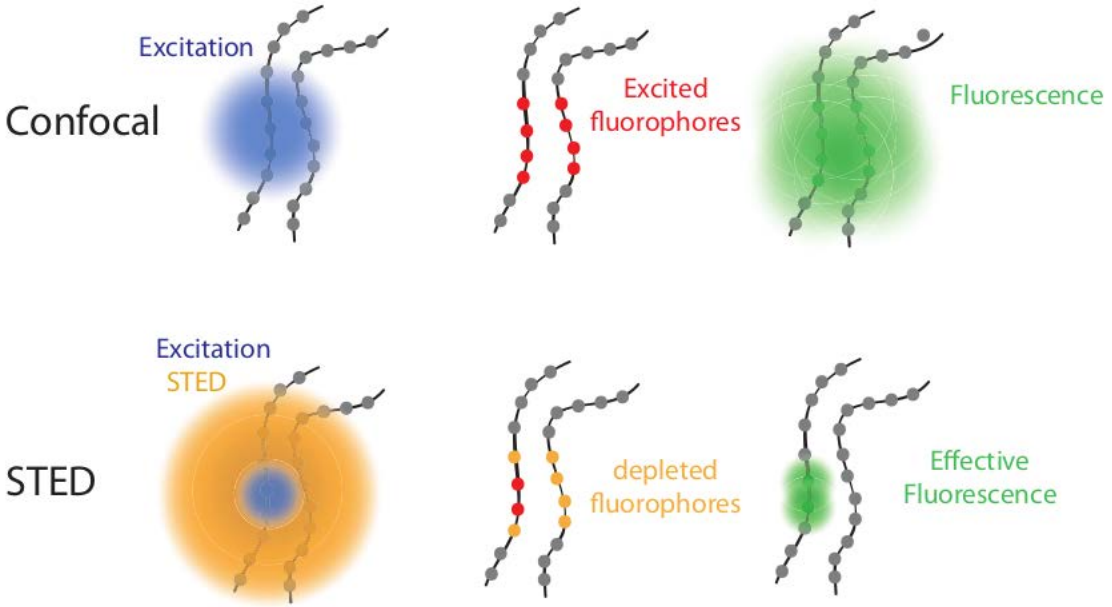


iBiology 2013

STED

- Stimulated emission depletion (STED) uses a **doughnut-shaped depletion beam** to reduce the effective fluorescence volume below the diffraction limit

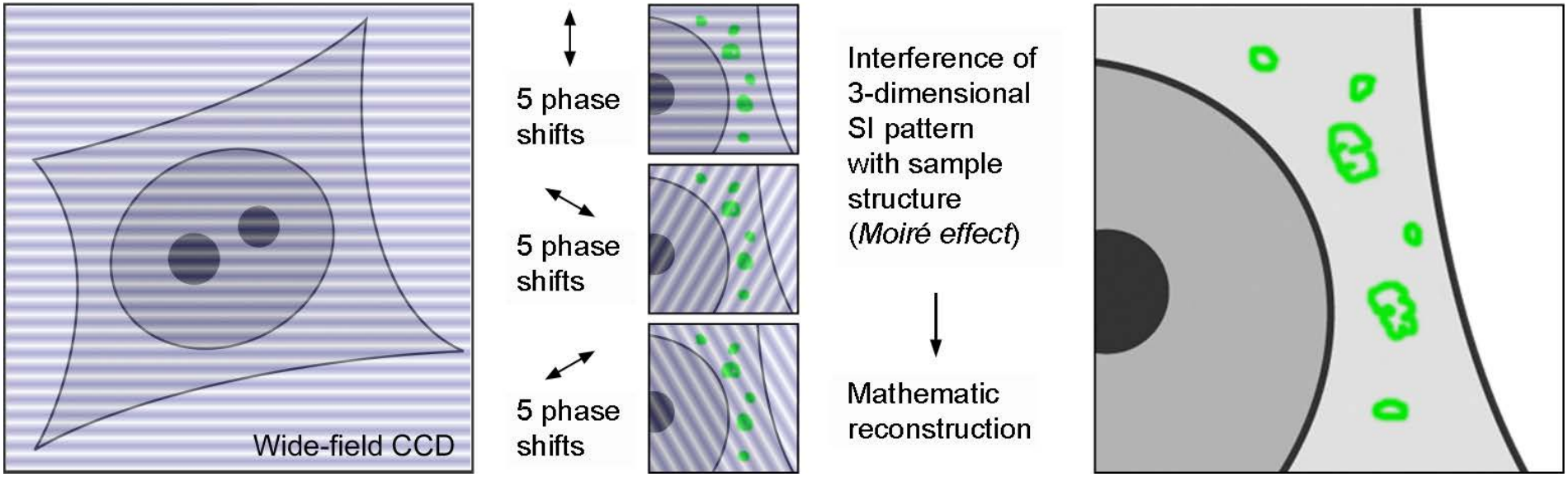




https://svi.nl/STEDMicroscopy

Structured Illumination Microscopy (SIM)

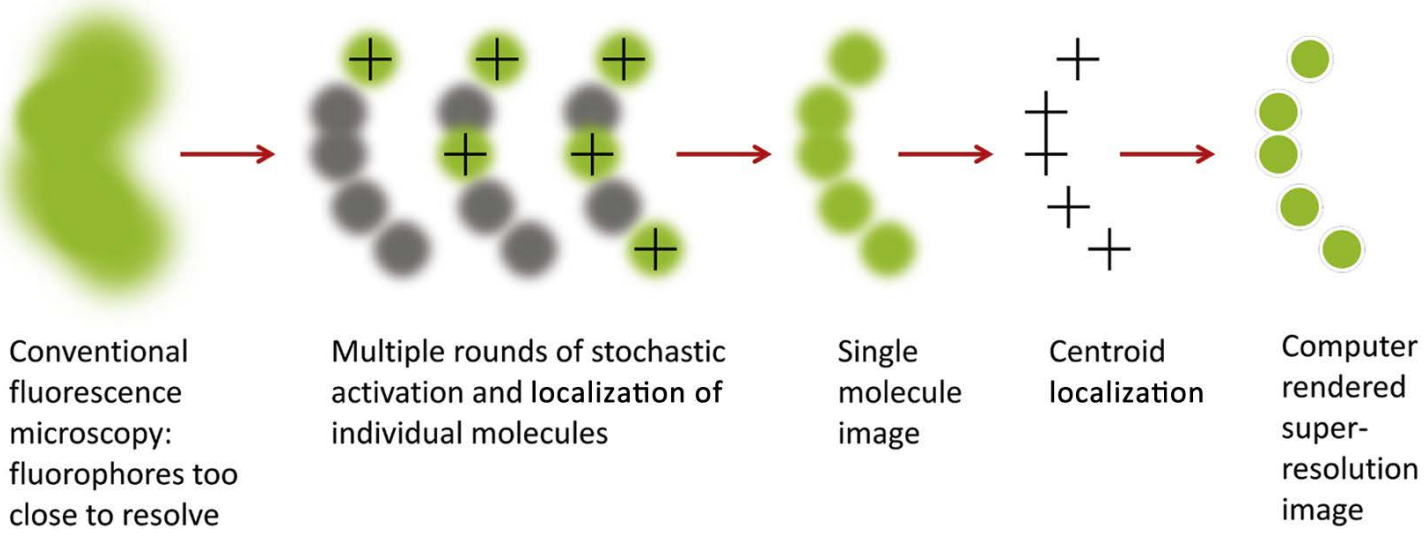
- SIM is based on standard wide-field microscopy but it uses **nonuniform illuminations** with known spatial patterns (e.g., originally a sinusoidal grid, stripes)



www.micron.ox.ac.uk

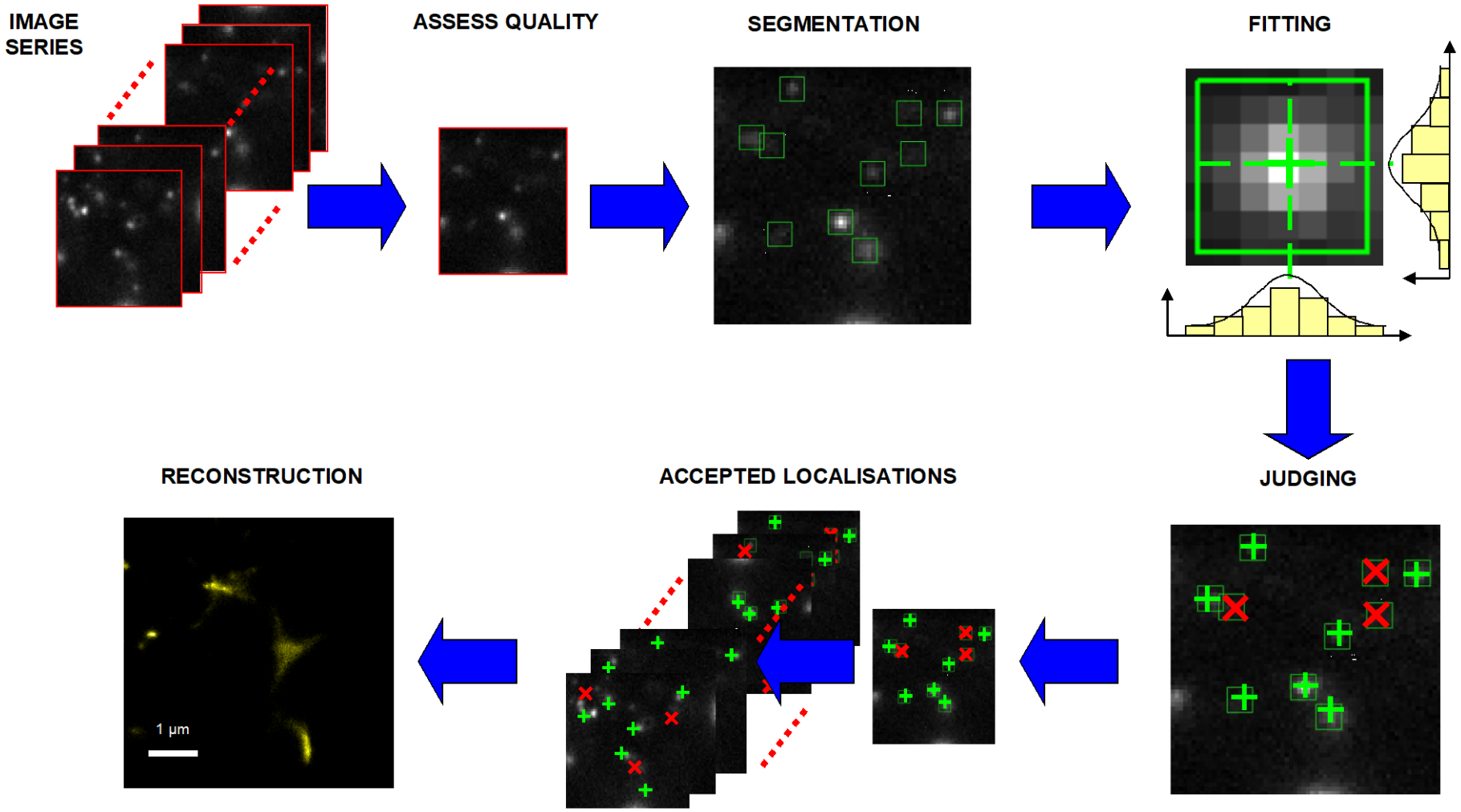
Single-Molecule Localization Microscopy (SMLM)

- Reconstructing an image from single molecule localizations retrieved from a large number of movie frames (typically thousands)



Thorley et al. **2014**

Stochastic Optical Reconstruction Microscopy (STORM)



Summary of Super-Resolution techniques

Modality	Resolution	Illumination	Probes	Acquisition Time	Post-Acquisition Processing	Data Size	Other Concerns
Standard fluorescence microscopy	250 nm	Epi/TIRF/confocal	Conventional fluorescent probes				
SIM	100 nm	Wide-field (epi/TIRF)	Conventional fluorescent probes	Short (seconds)	Yes (Fourier transforms)	Small (9-15 frames)	Out of focus signal and sample thickness
STED	30-70 nm	Laser scanning confocal	Not all conventional probes- see text	Short (seconds)	No	Small (1 image)	Photobleaching
PALM/fPALM	10-55 nm	Wide-field (epi/TIRF)	Photo-activatable fluorescent proteins (e.g. PA-GFP)	Long (minutes)	Yes (centroid identification)	Large (many frames)	Over/under labelling artefacts
STORM/ dSTORM	10-55 nm	Wide-field (epi/TIRF)	Photoswitchable dyes (e.g Cy3/5) Blinking fluorophores (e.g. Alexaflour-647)	Long (minutes)	Yes (centroid identification)	Large (many frames)	Over/under labelling artefacts

Thorley et al. **2014**

NEWS & VIEWS Open Access

A Trojan Horse for live-cell super-resolution microscopy

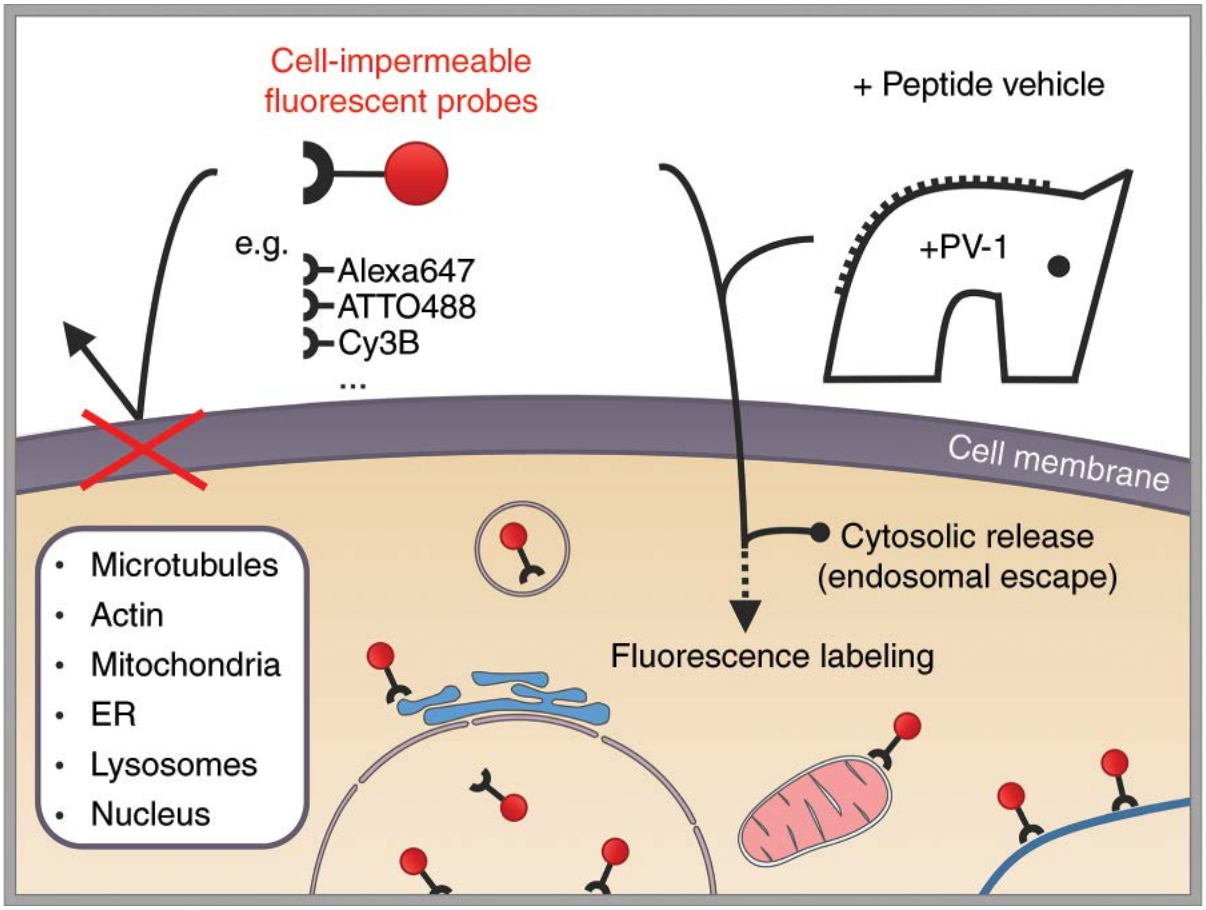
Gerti Beliu[1] and Markus Sauer[1]

ARTICLE Open Access

Simple and efficient delivery of cell-impermeable organic fluorescent probes into live cells for live-cell superresolution imaging

Meng Zhang[1,2], Meihua Li[1,2], Wenting Zhang[1,2], Yubing Han[1,2] and Yu-Hui Zhang[1,2]

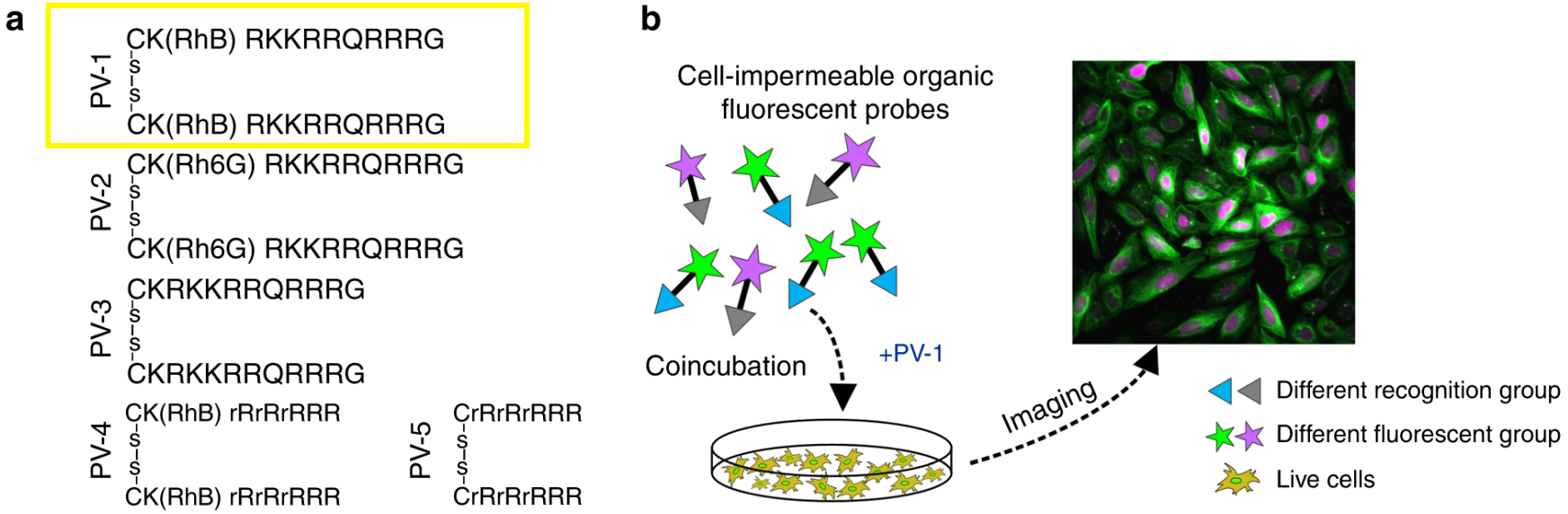
How to efficiently label inside the cell?



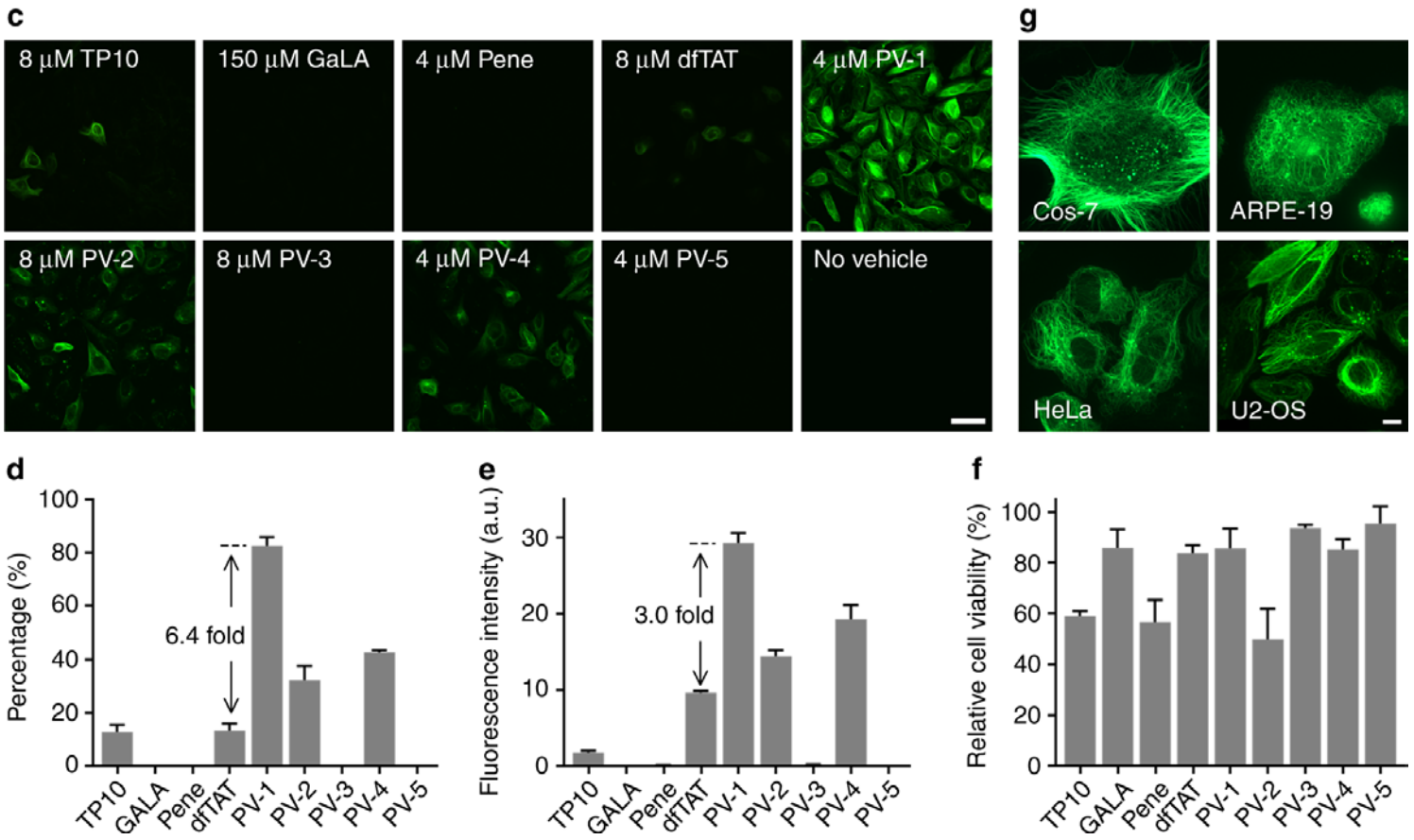
Beliu and Sauer, **2020**

CPPs

- Cell-penetrating peptides
- PV-1 (peptide vehicle)

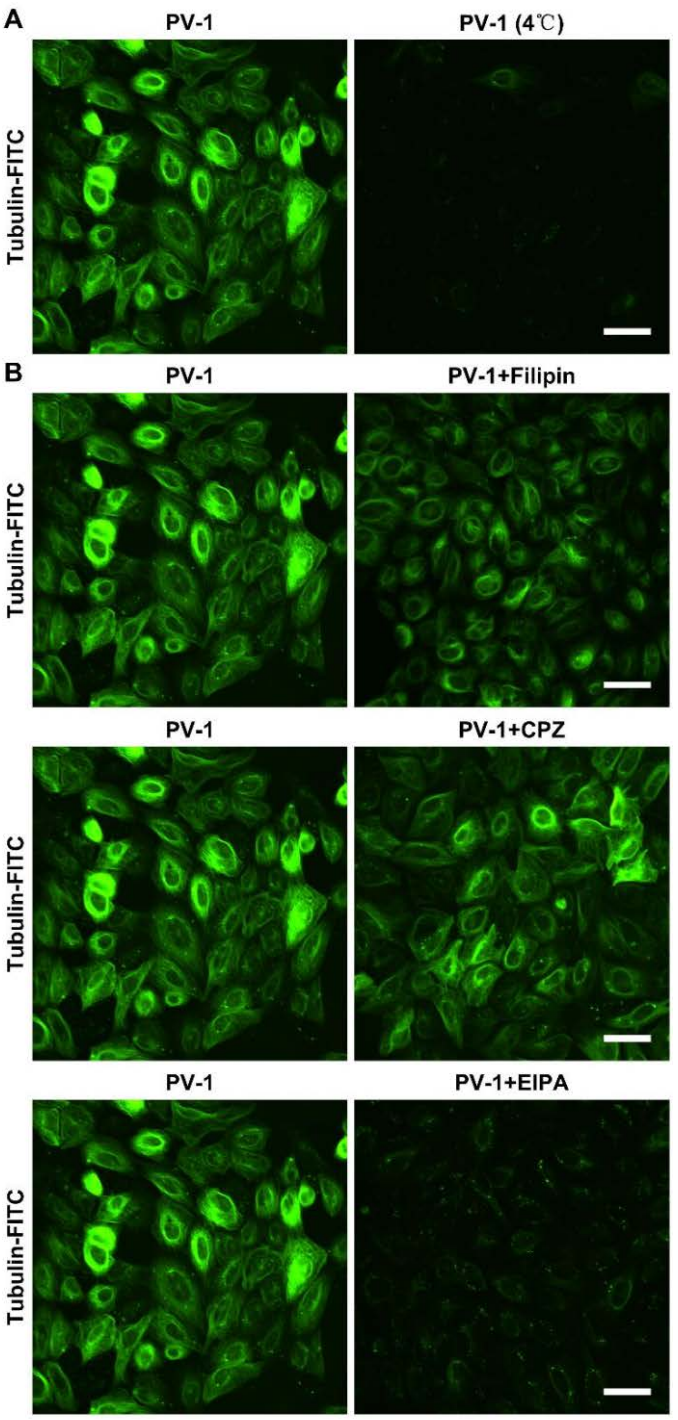


Labelling efficiency of different PV

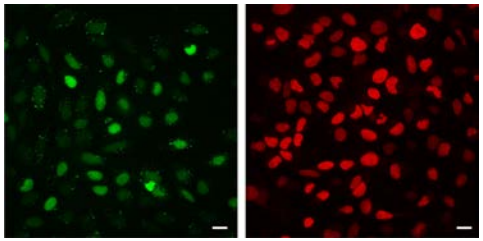


How does the "Trojan-Horse" work?

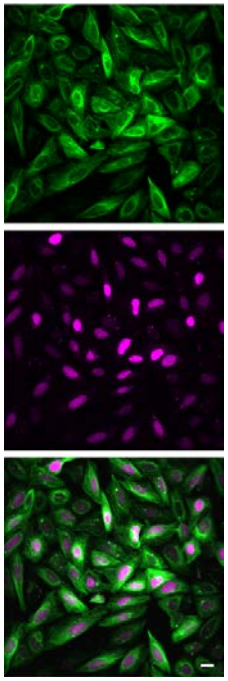
- Via endocytic pathways
- Effect of temperature
- 3 inhibitors tested
- EIPA: inhibitor of **macropinocytosis**



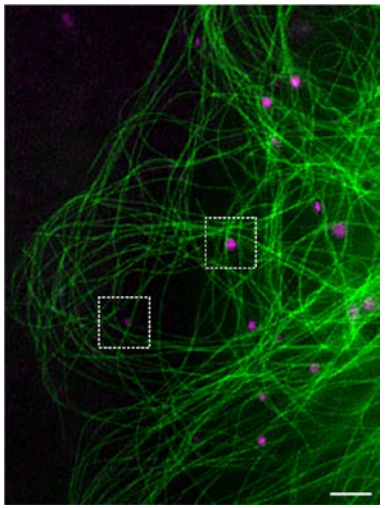
PV-1 efficiently delivers SNAP-CLIP probes



Simultaneous delivery of 3 fluorophores



Dynamics of the cell with live-cell SIM Imaging



nature COMMUNICATIONS

ARTICLE

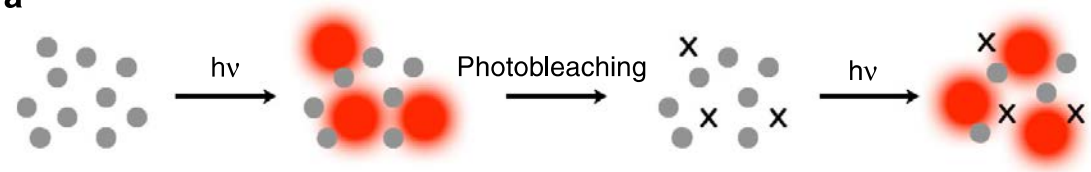
https://doi.org/10.1038/s41467-019-09217-7 OPEN

Photoregulated fluxional fluorophores for live-cell super-resolution microscopy with no apparent photobleaching

Elias A. Halabi[1], Dorothea Pinotsi[2] & Pablo Rivera-Fuentes[1]

Classical SM

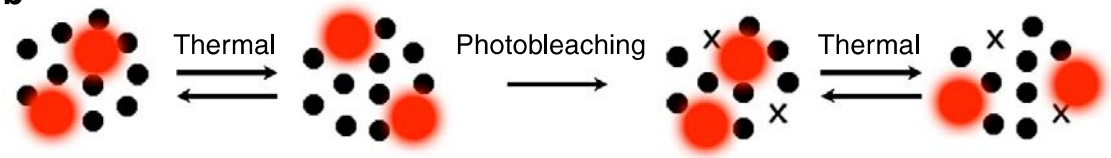
a



☒ High phototoxicity
☒ Fast photobleaching
✓ Medium insensitive

Spontaneous Blinking Dyes

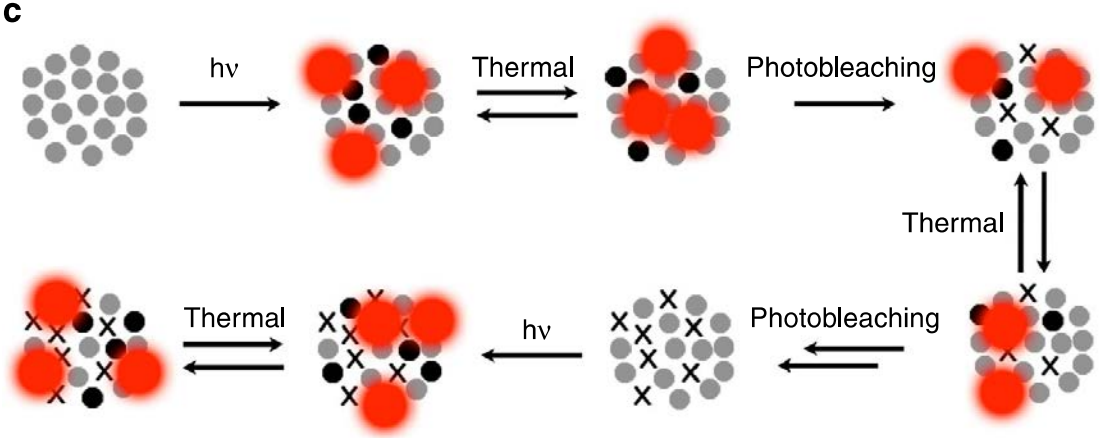
b



✓ Low phototoxicity
✓ Slow photobleaching
☒ Medium sensitive

Photoactivation + Fluoxionality

c

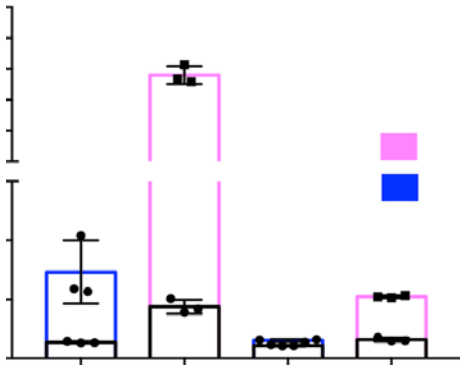


✓ Low phototoxicity
✓ Slow photobleaching
✓ Medium insensitive

● Molecule before photoactivation ● Dark molecule ● Fluorescent molecule X Photobleached molecule

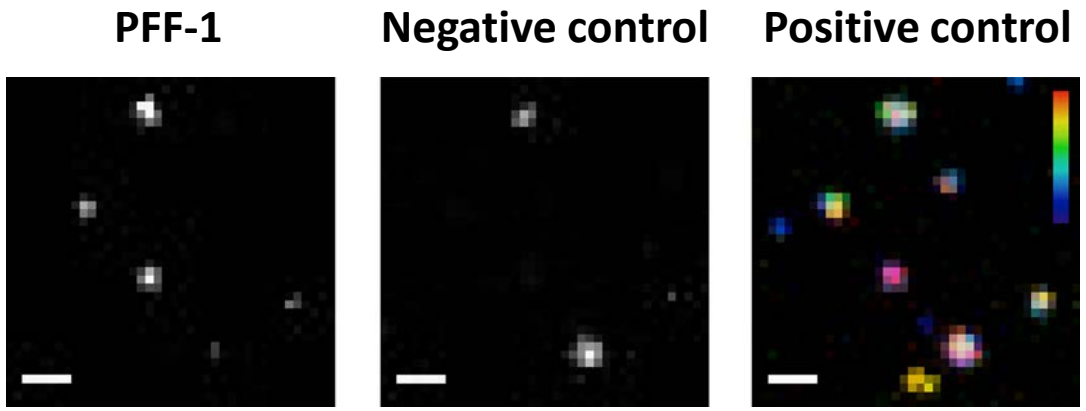
Mechanism: E-Z equilibrium

Photoactivation

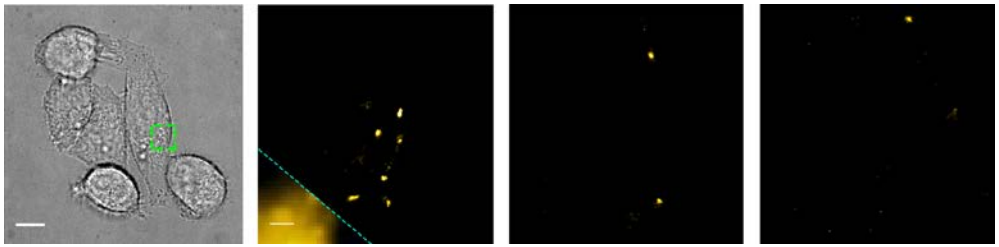


DFT

Blinking “test” with TIRF



Lysosome tracking

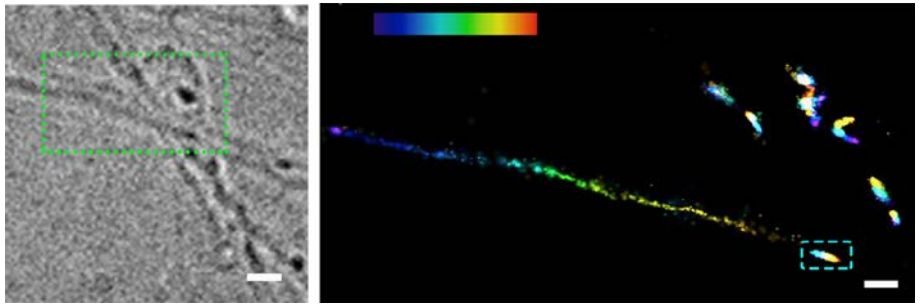


Single pulse-excitation vs pulse every 10 min

Optimisation of pulse and labelling

3D SMLM with astigmatism and adaptive optics

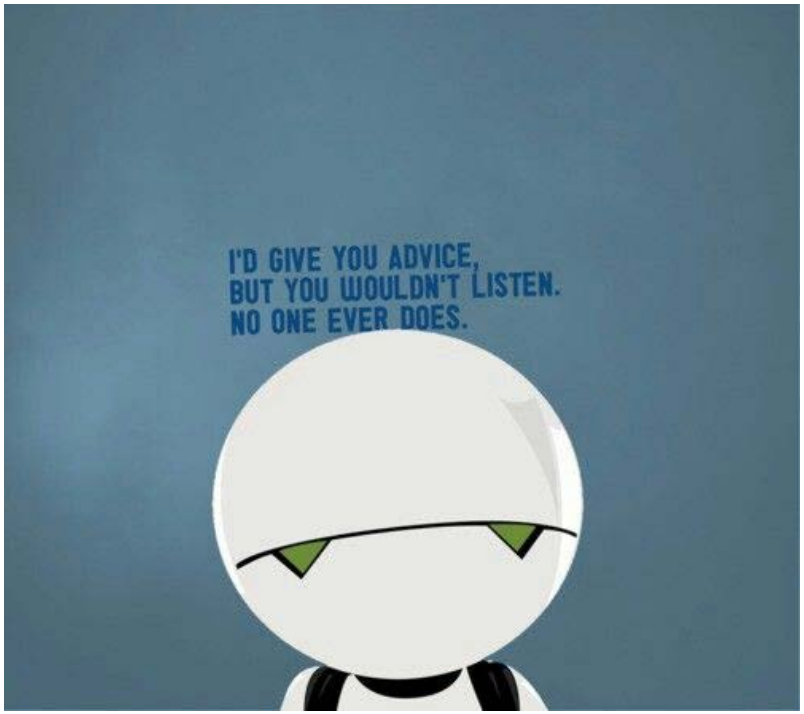
Single-vesicle tracking in live neurons



- Subsecond time resolution
- Resolve 2 vesicles within a limited diffraction spot (250 x 250 nm)
- Diffusion coefficients could be very small and very large (slow and fast)
- Slow vesicles accumulate in hotspots
- Fast vesicles move along axons

Conclusions

- Super-resolution requires **a lot of light (photons):**
  - In all super-resolution methods, breaking the diffraction limit on a given field of view comes at the expense of the acquisition time
- Limitations for live-cell SR:
  - **Cytotoxicity**
  - **Photobleaching**
  - **Lack of fluorophores**
  - Large time-scales for observation
- PV-1 effectively delivers organic fluorophores (up to 3)
- PFF-1 enables high resolution in space and time (up to 30 min)

Thanks for your attention

Questions?