STED – extra options
(RESOLFT, RESCue, MINFIELD, DyMIN)

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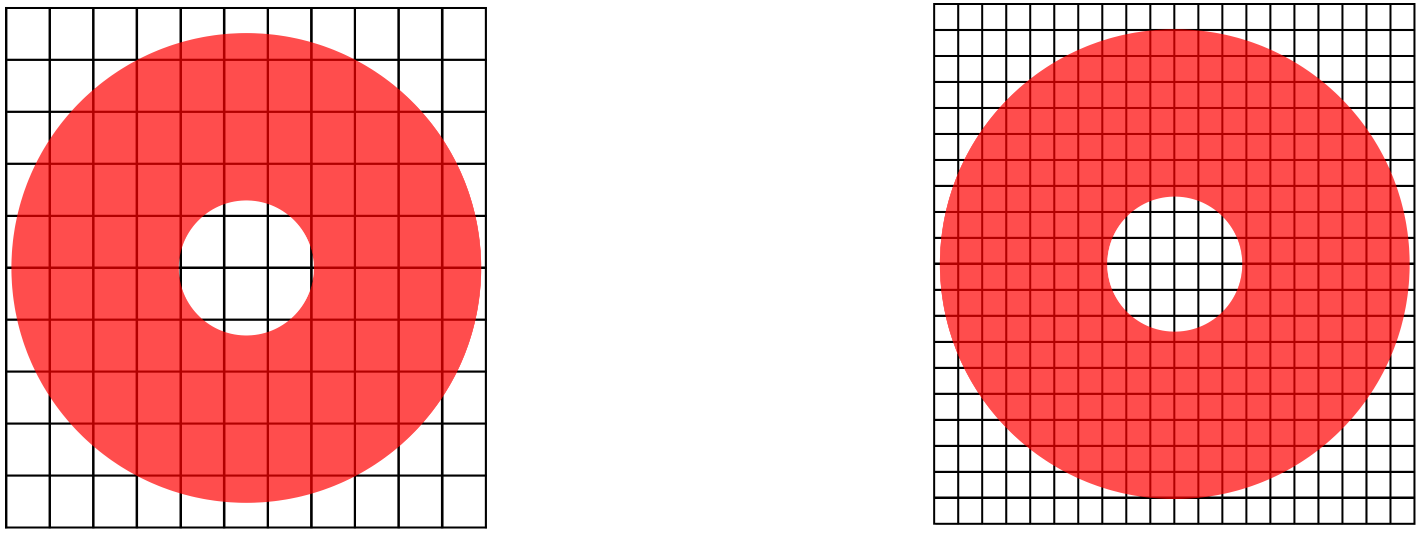
Imaging principles of life

What are the two biggest problems of STED?

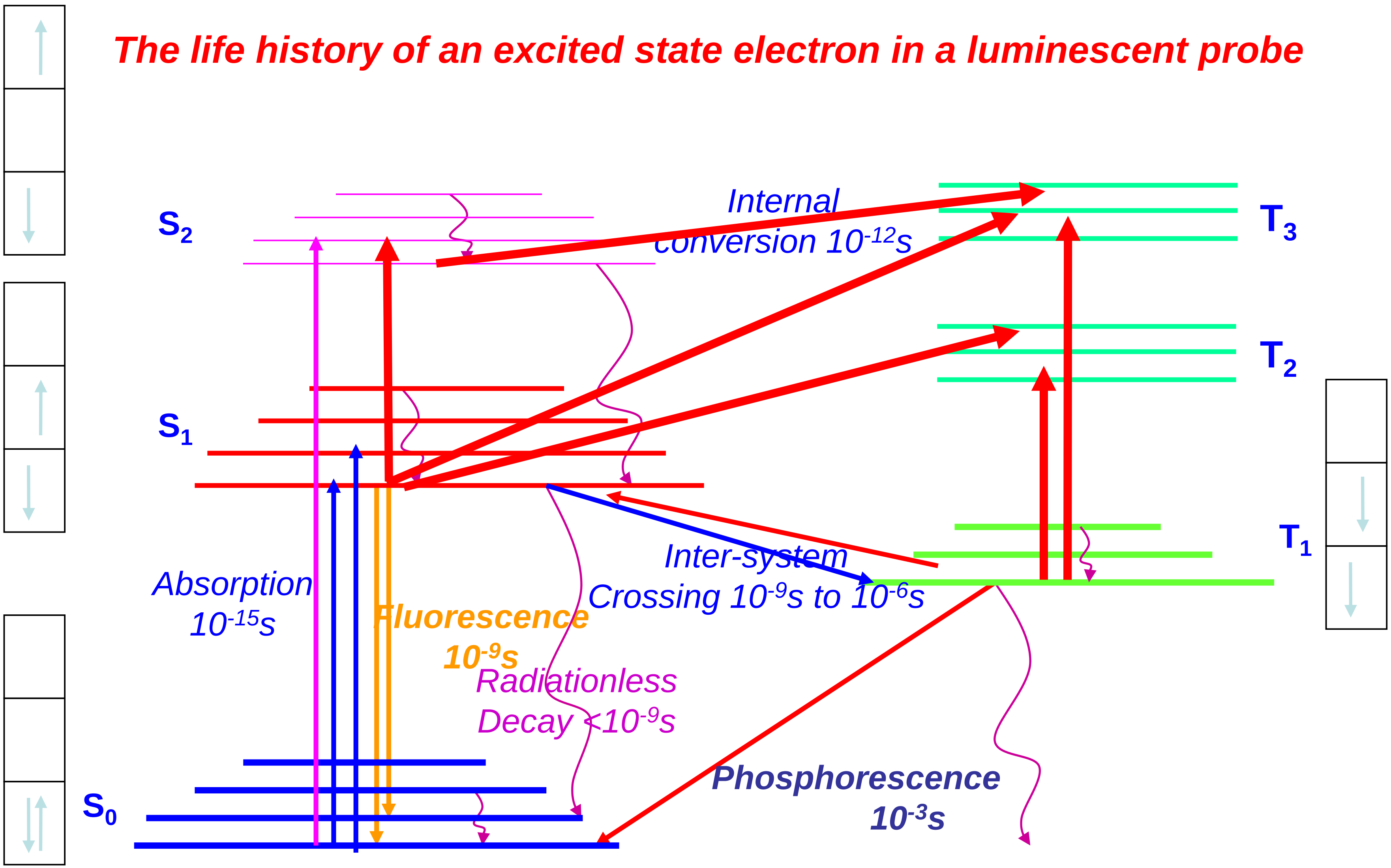
1. Photo-bleaching
2. Live-cell compatibility

Why?

1. Extreme highly-localized light doze (100s mW per um^2)
2. Need for “high-sampling” (half of targeted resolution)



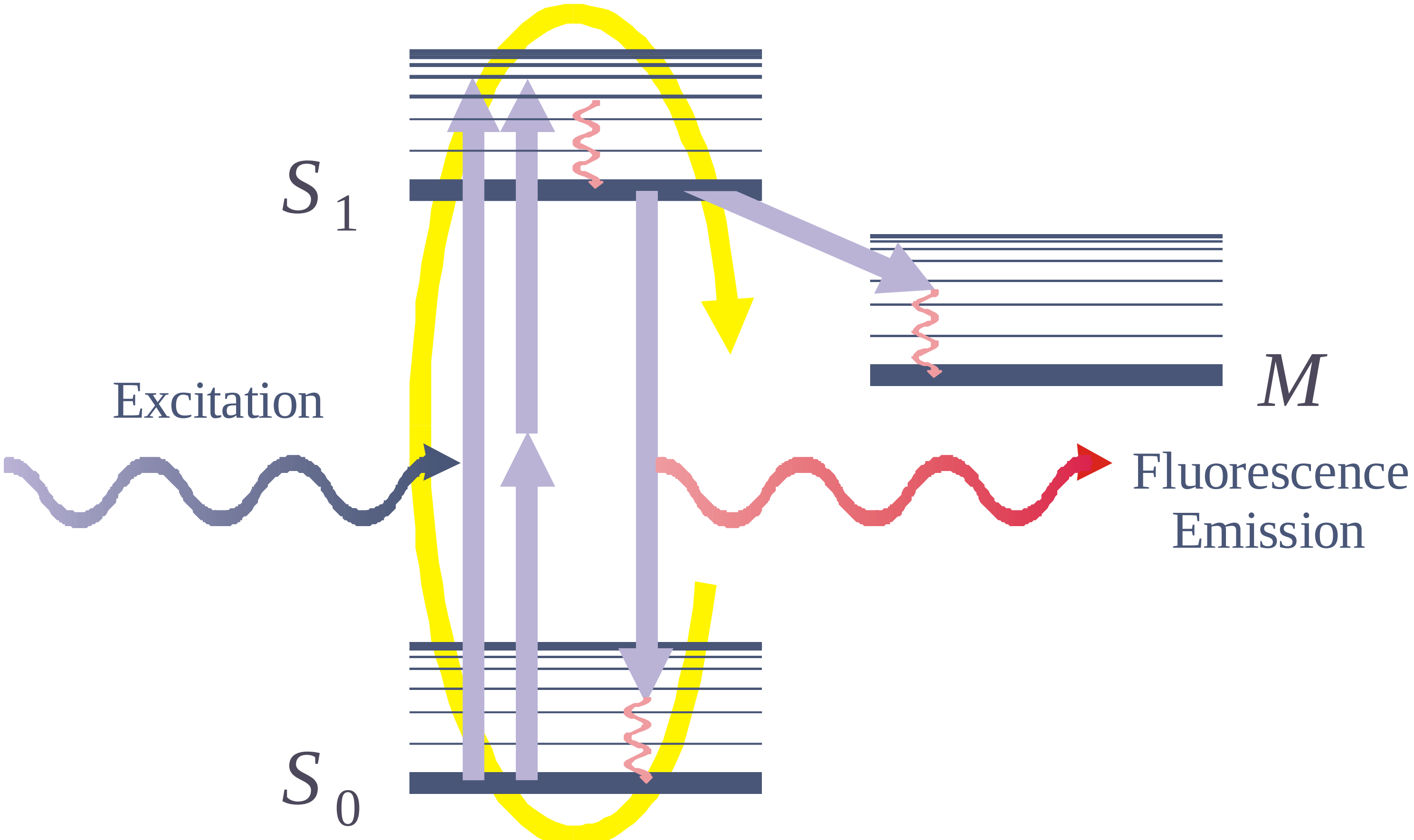
What causes photo-bleaching?



Solutions?

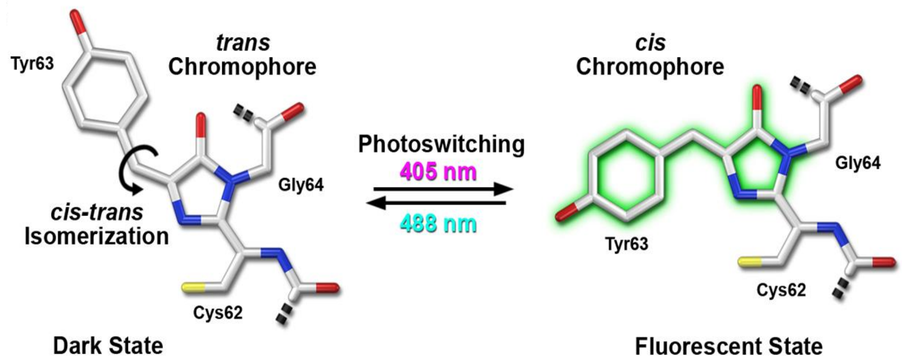
1. Minimize the light-dose
2. Prevent photobleaching
3. Use different mechanism of REversible Saturable OpticaL Fluorescence Transitions (RESOLFT)

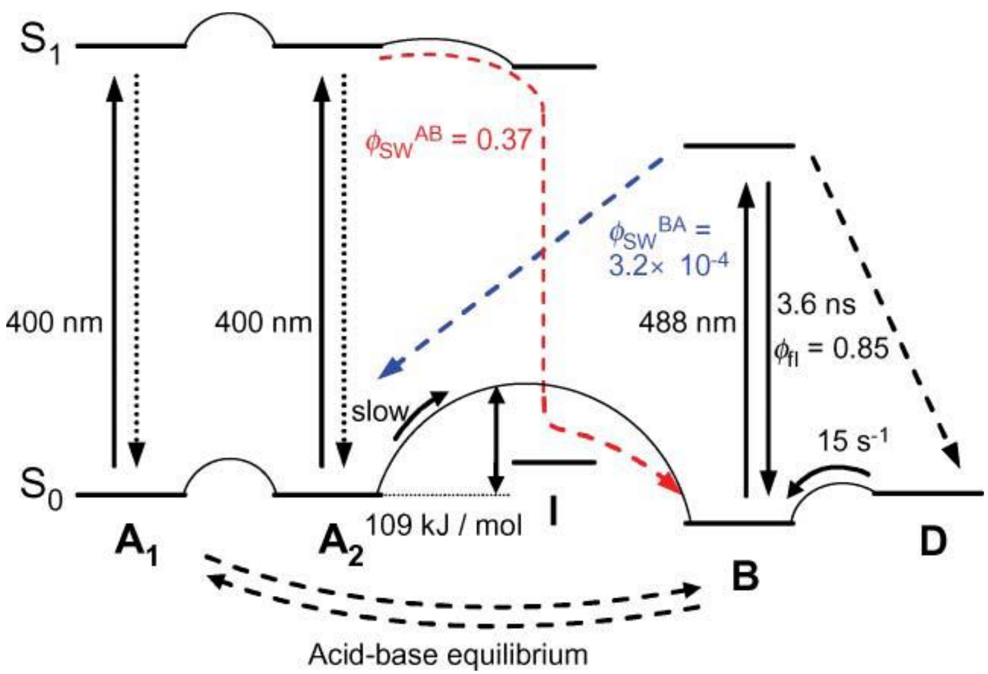
Ground state depletion into metastable state (switchable chromophores)



Reversibly photoswitchable dyes

(usually fluorescent proteins, some reports on organic dyes)





Dronpa and its variants

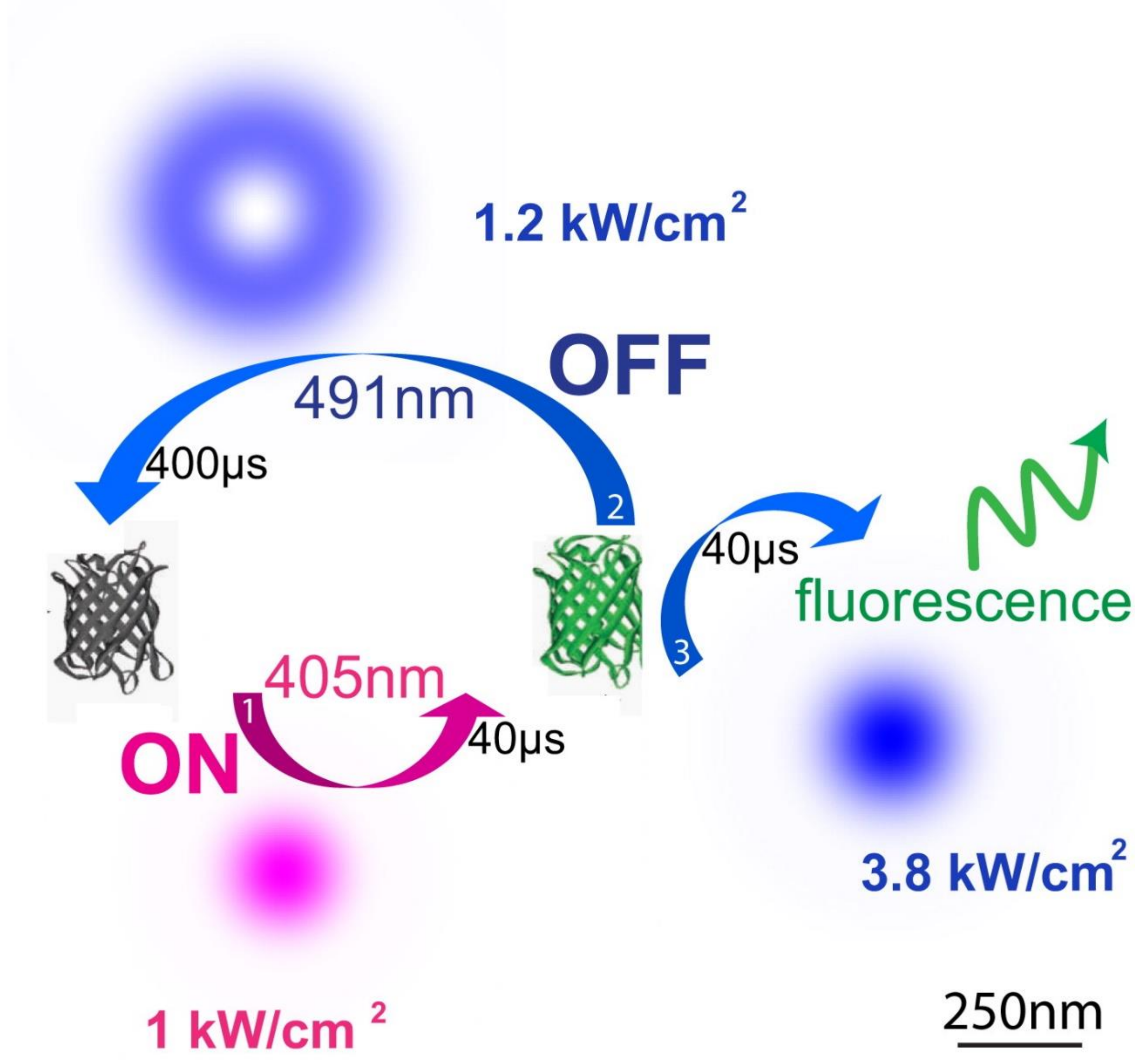
rsEGFP

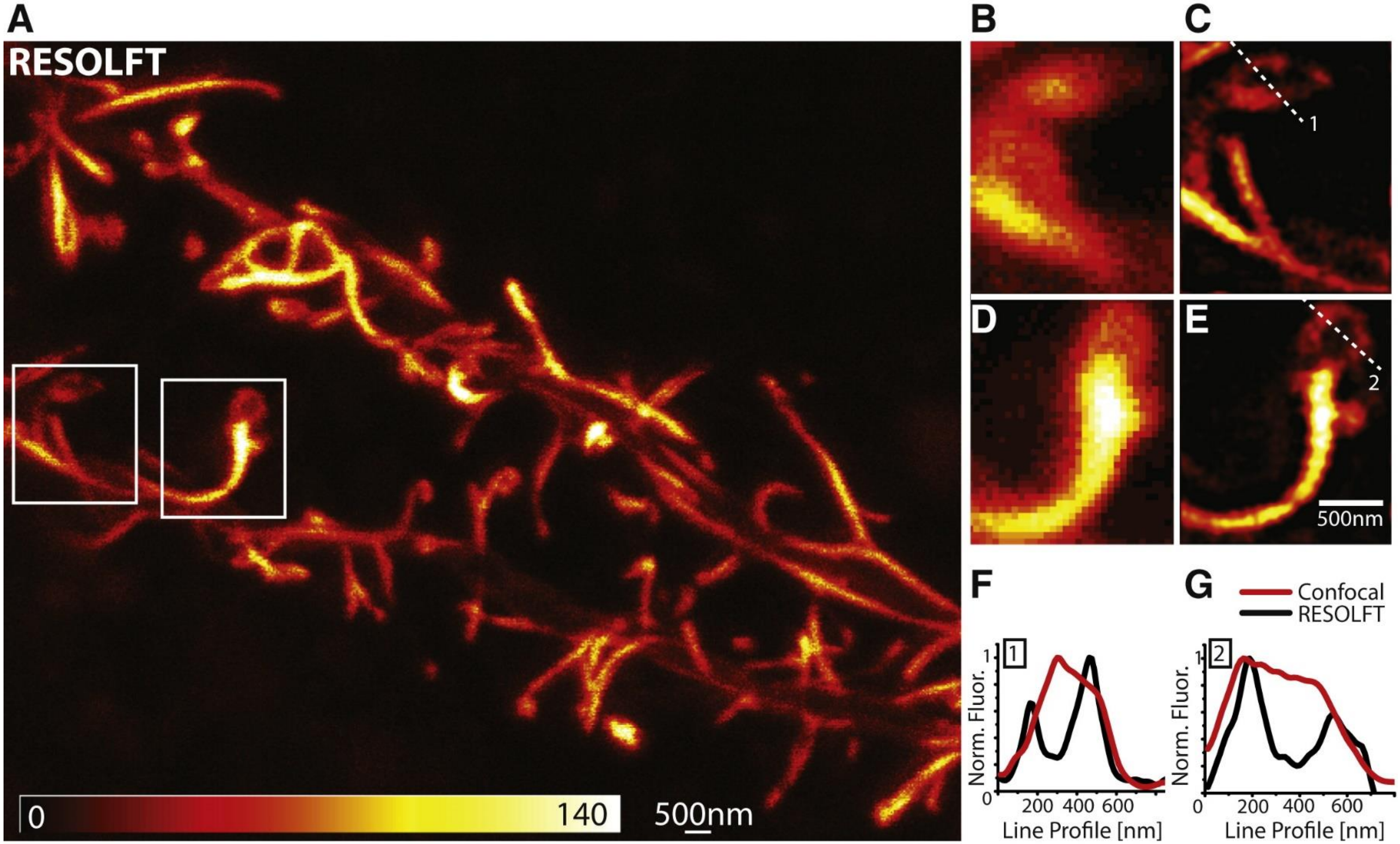
Dreiklang

Nanoscopy of Living Brain Slices with Low Light Levels

Ilaria Testa, Nicolai T. Urban, Stefan Jakobs, Christian Eggeling, Katrin I. Willig, Stefan W. Hell

Neuron, Volume 75, Issue 6, Pages 992-1000 (September 2012) DOI: 10.1016/j.neuron.2012.07.028





Dronpa-M159T, 5–50 µm deep beneath the surface of the living brain slices
4.2 × 3 µm^2 fast scans at **7 s / frame, 70 nm resolution**

0.5–3 µW cw

Nanoscopy with more than 100,000 'doughnuts'
Chmyrov et al., Nature Methods 10, 737–740 (2013)

What if 70 nm is not enough?
How to decrease the photobleaching in standard STED?

Triplet-Relaxation microscopy (T-REX)

Resonant Scanning with Large Field of View Reduces Photobleaching and Enhances Fluorescence Yield in STED Microscopy

Yong Wu, Xundong Wu, Rong Lu, Jin Zhang, Ligia Toro & Enrico Stefani

Scientific Reports 5, Article number: 14766

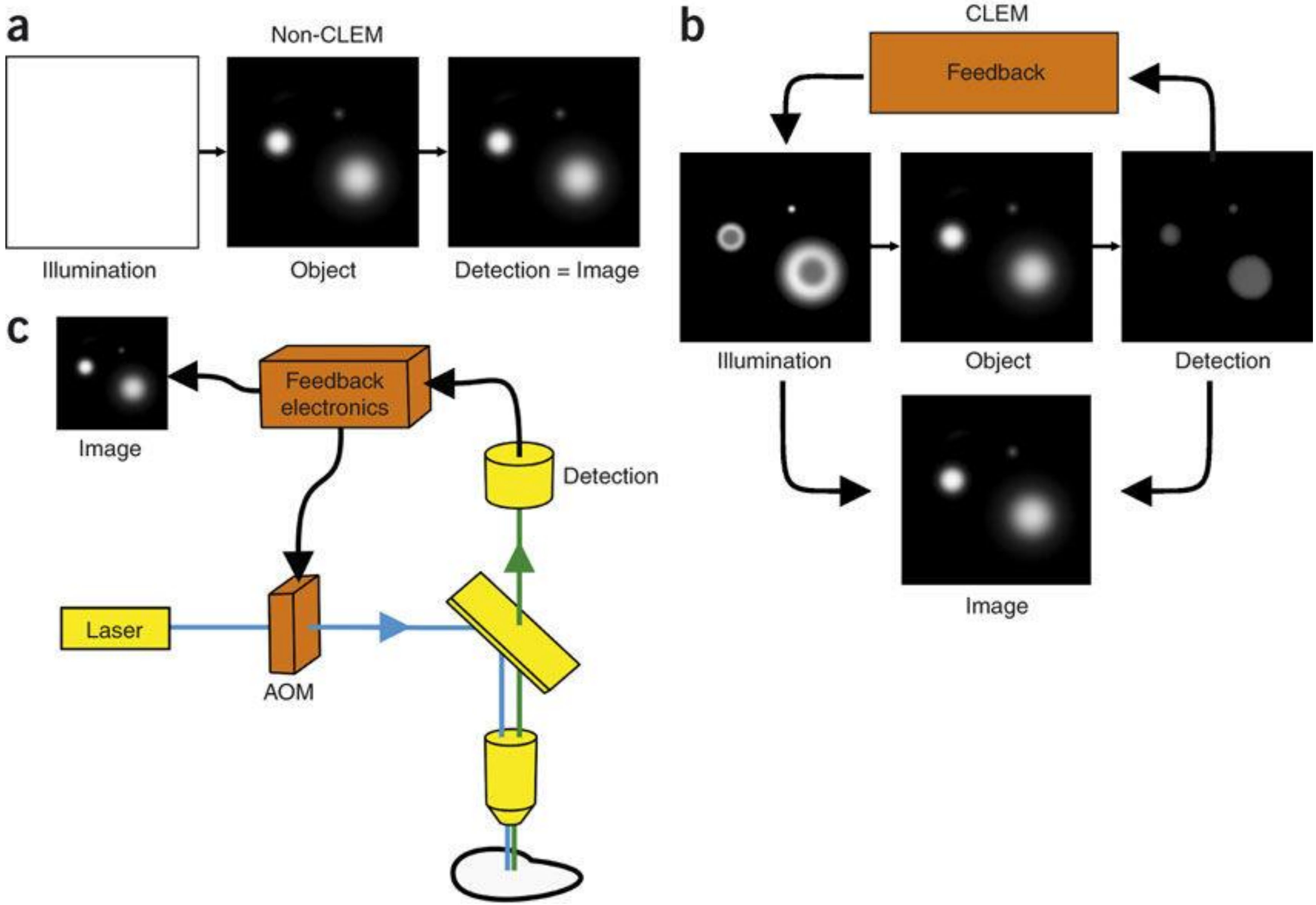
What if the photobleaching reduction is not sufficient?

How to decrease the light-dose?

Inspiration from “Controlled light-exposure microscopy (CLEM)”

Controlled light-exposure microscopy reduces photobleaching and phototoxicity in fluorescence live-cell imaging

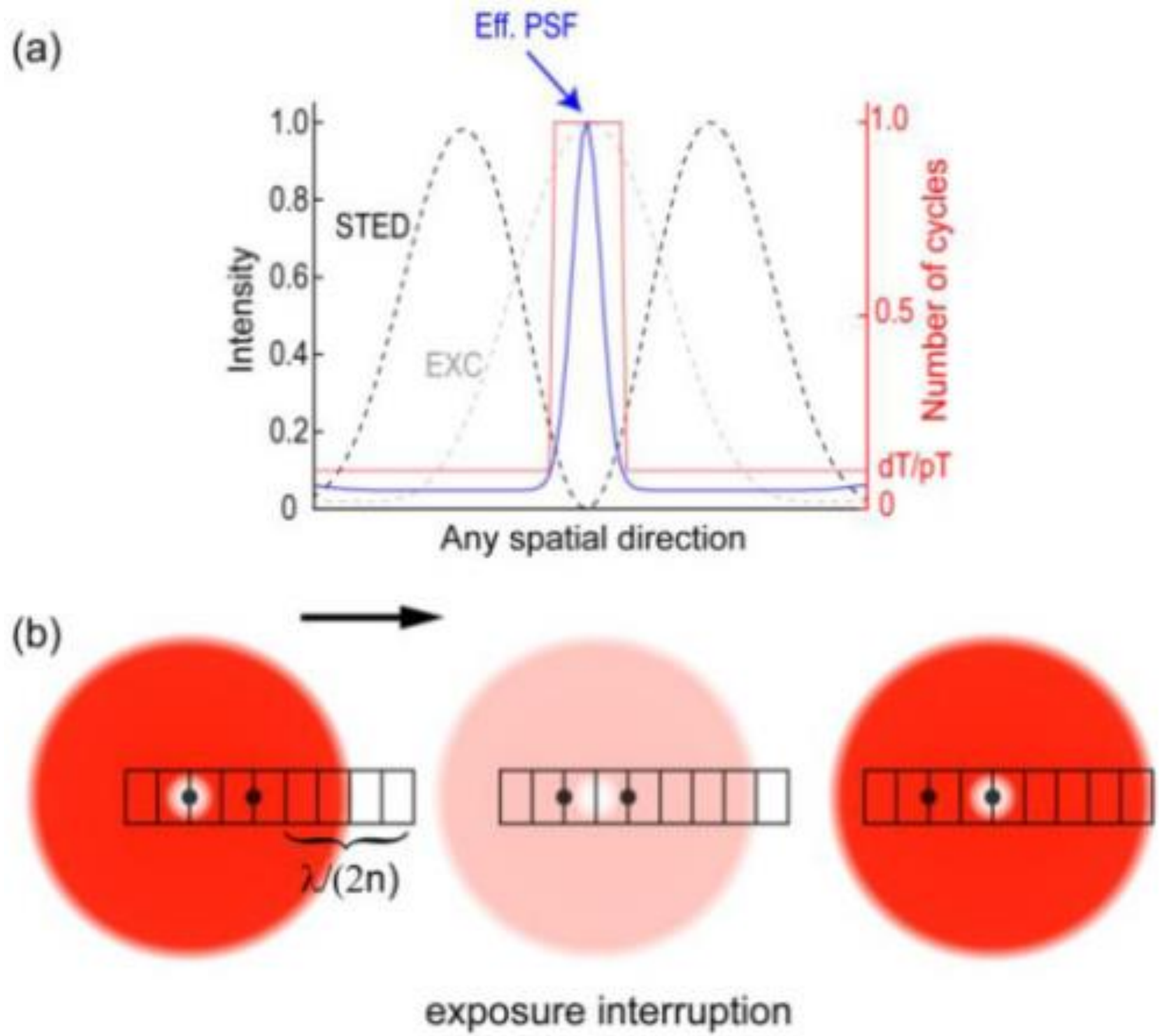
R A Hoebe et al., Nature Biotechnology 25, 249–253 (2007)



REduction of State transition Cycles (RESCue STED)

Far-field optical nanoscopy with reduced number of state transition cycles

Thorsten Staudt et al., Optics Express Vol. 19, Issue 6, pp. 5644-5657 (2011)



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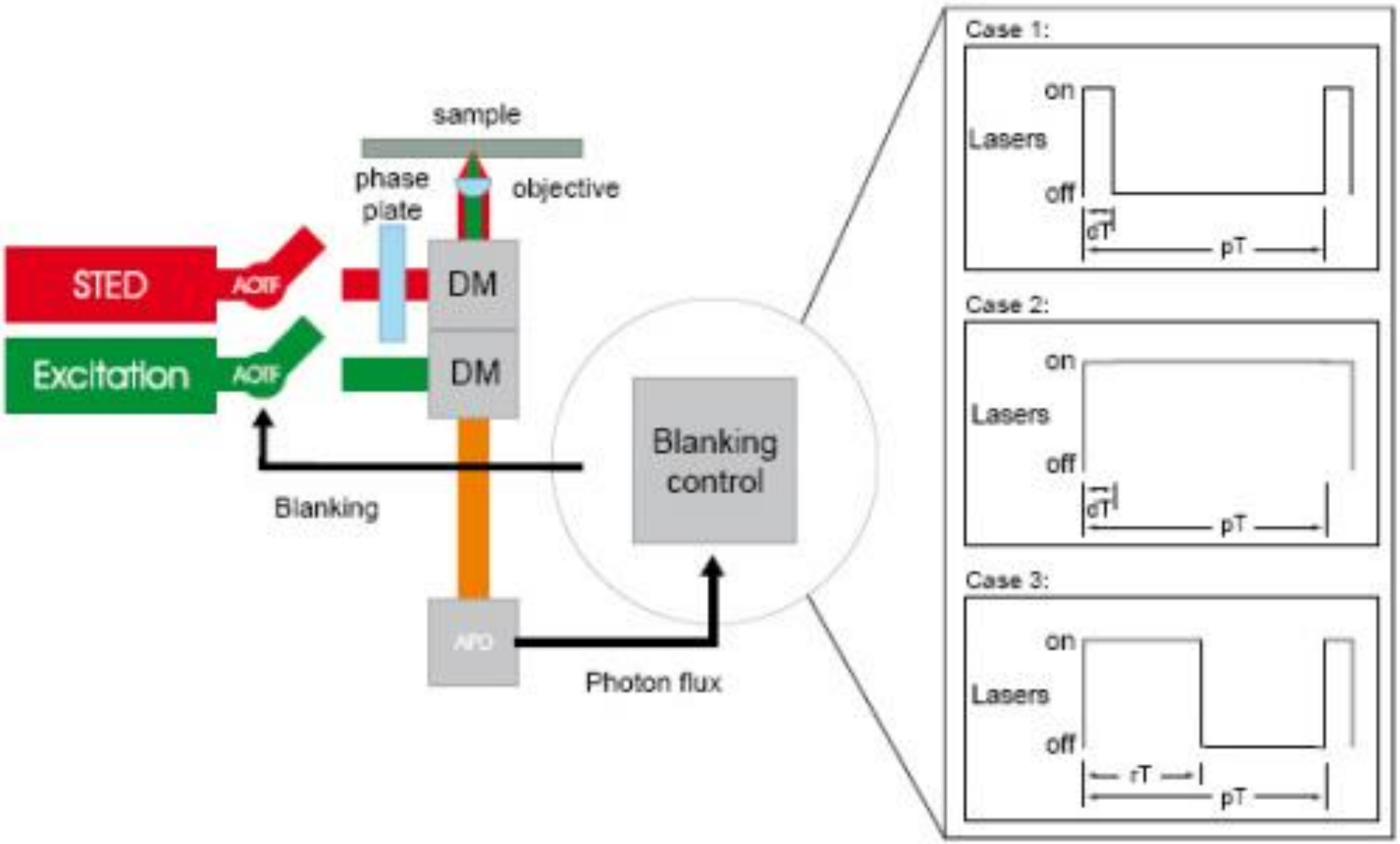


No signal

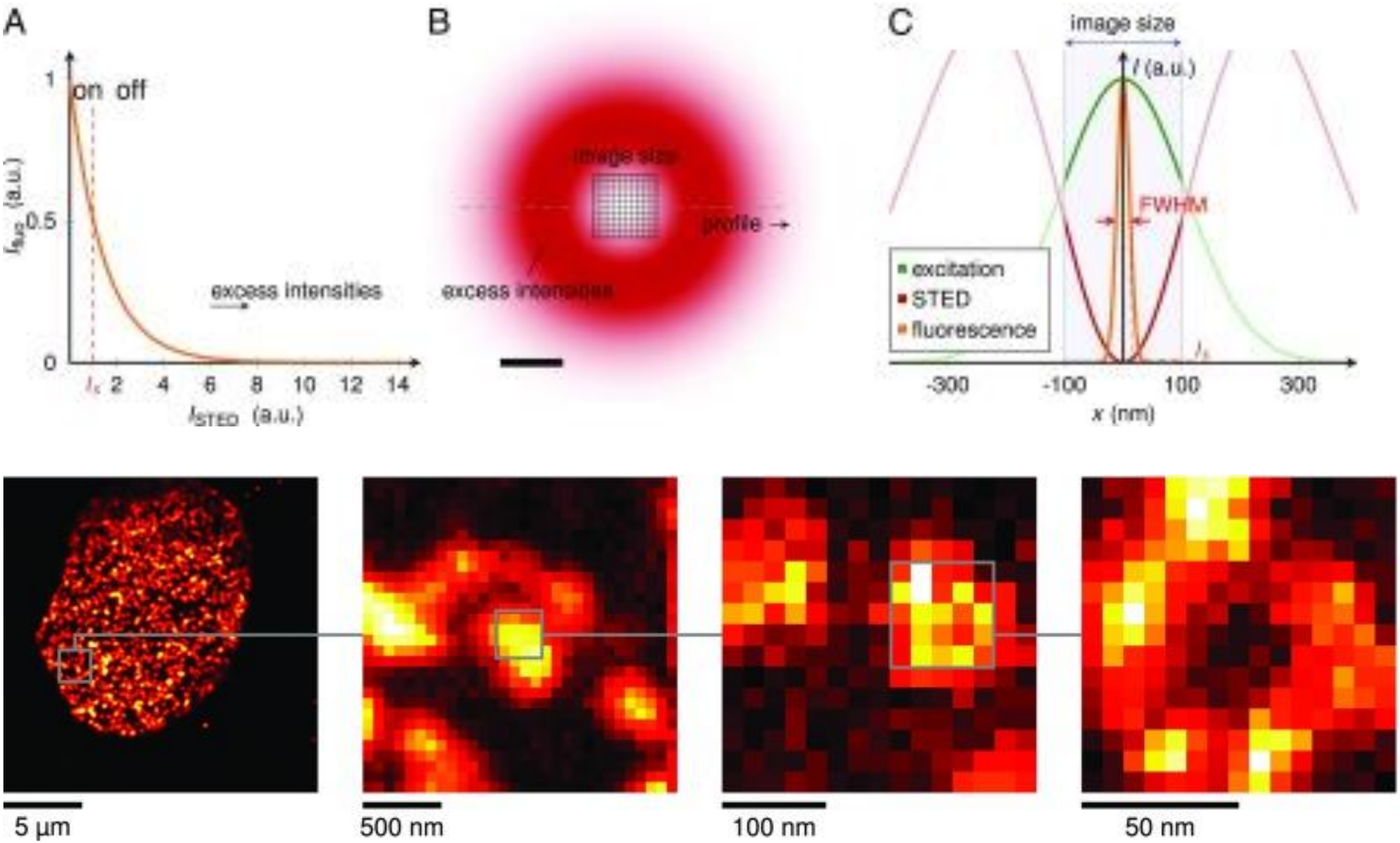
Standard signal

Strong signal

MINFIELD STED

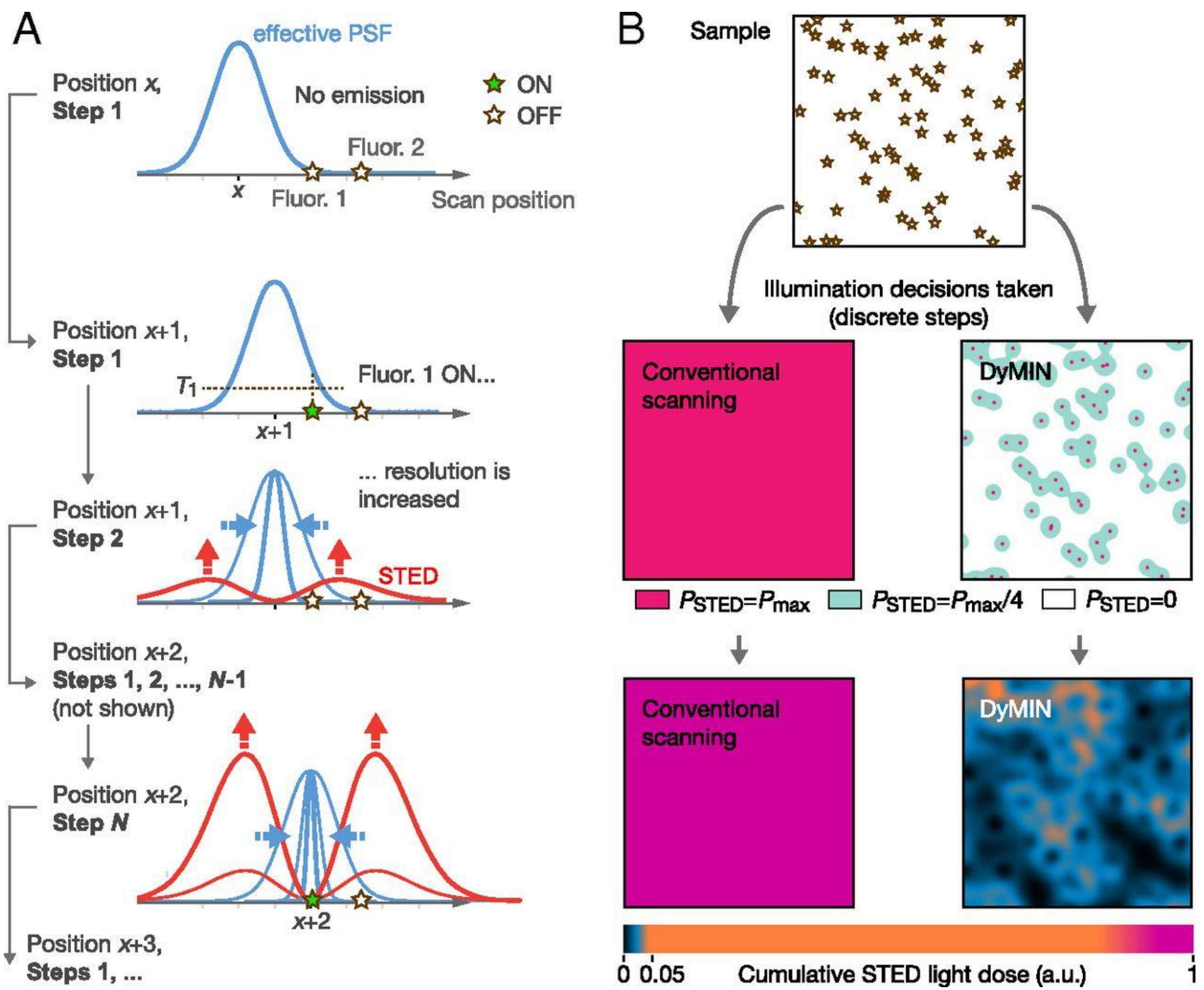
Strong signal increase in STED fluorescence microscopy by imaging regions of subdiffraction extent

Göttfert et al., PNAS 2017 Feb 28; 114(9): 2125–2130.



Dynamic Minimum (DyMIN STED)

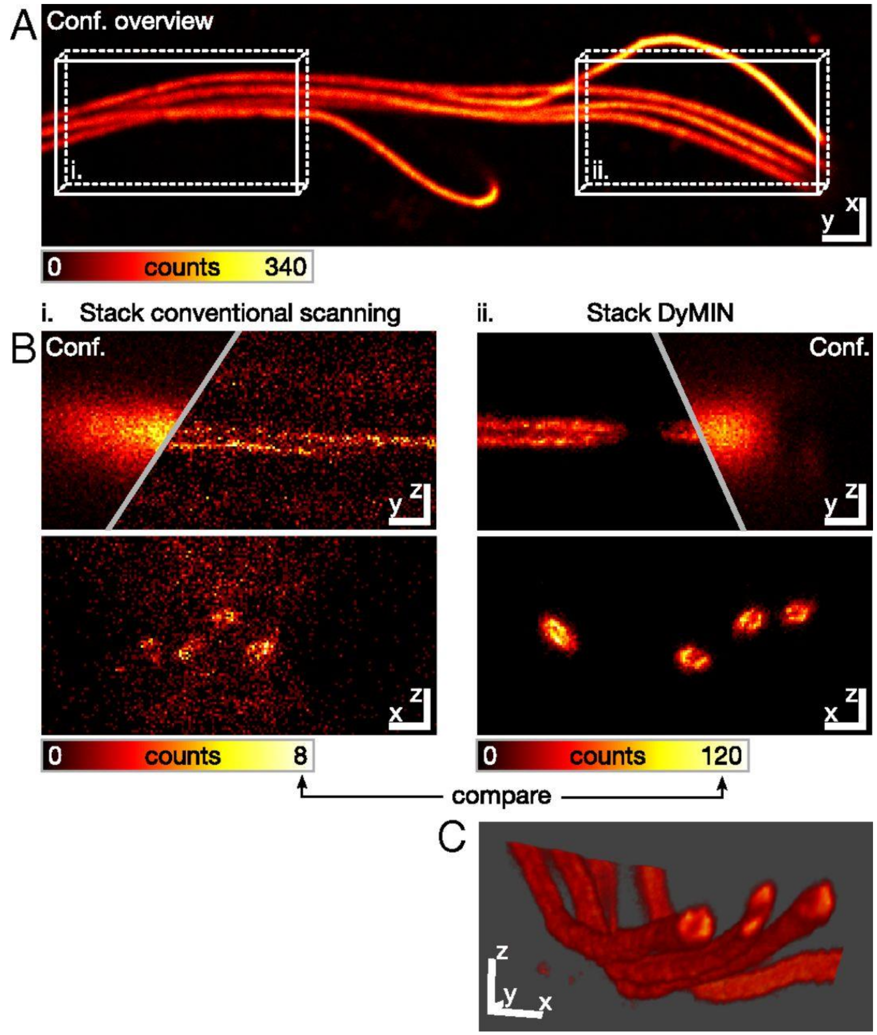
Nanoscopy with DyMIN adaptive illumination.



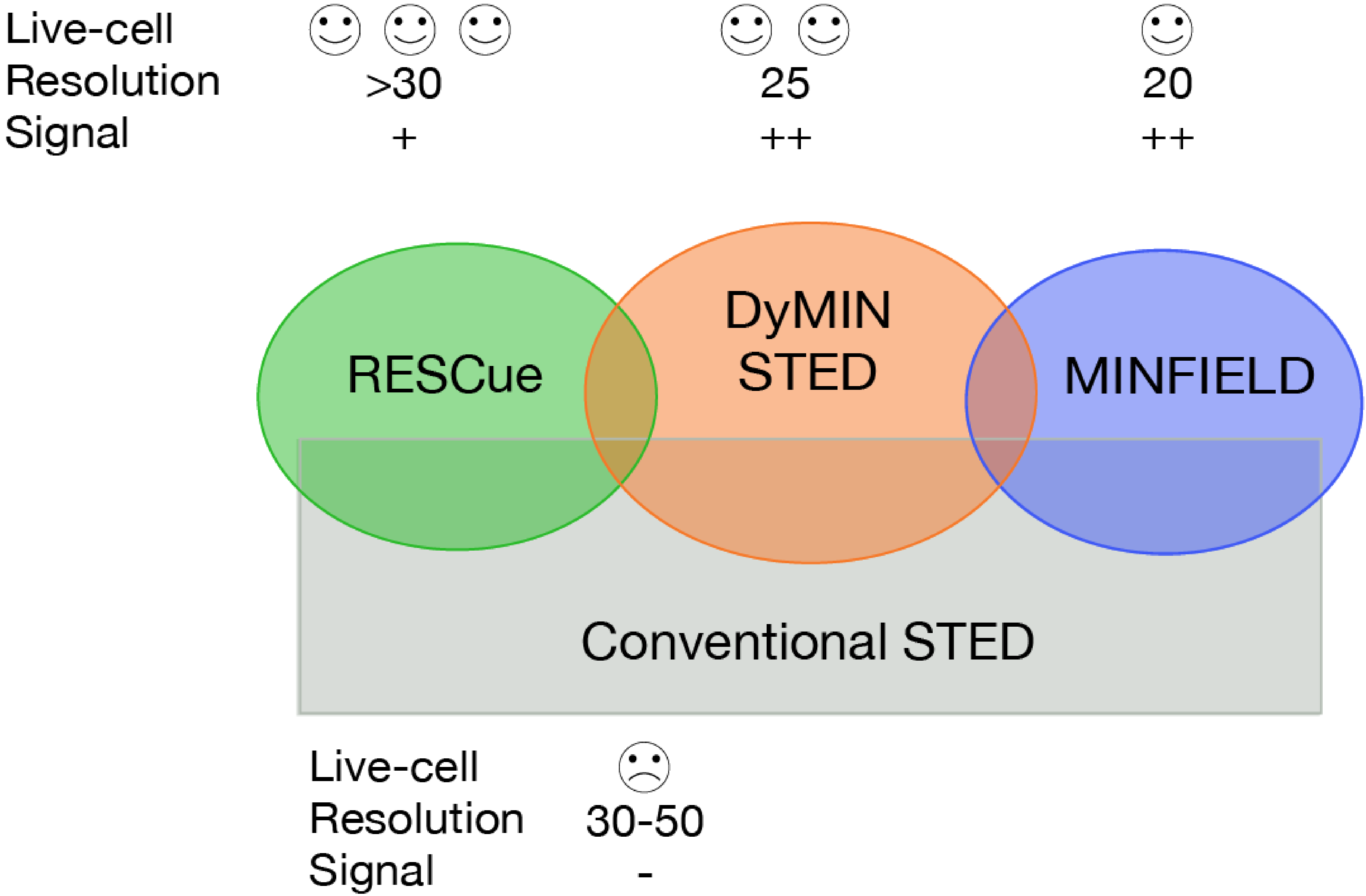
Jörn Heine et al. PNAS 2017;114:9797-9802

PNAS

Pronounced signal increase in DyMIN STED nanoscopy enables 3D visualization of tubulin in the axonemes of mouse spermatozoa.



Jörn Heine et al. PNAS 2017;114:9797-9802



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www.biocev.eu/corefacilit/imaging-methods/