Nanoscopy with Focused Light

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Department of NanoBiophotonics
Göttingen

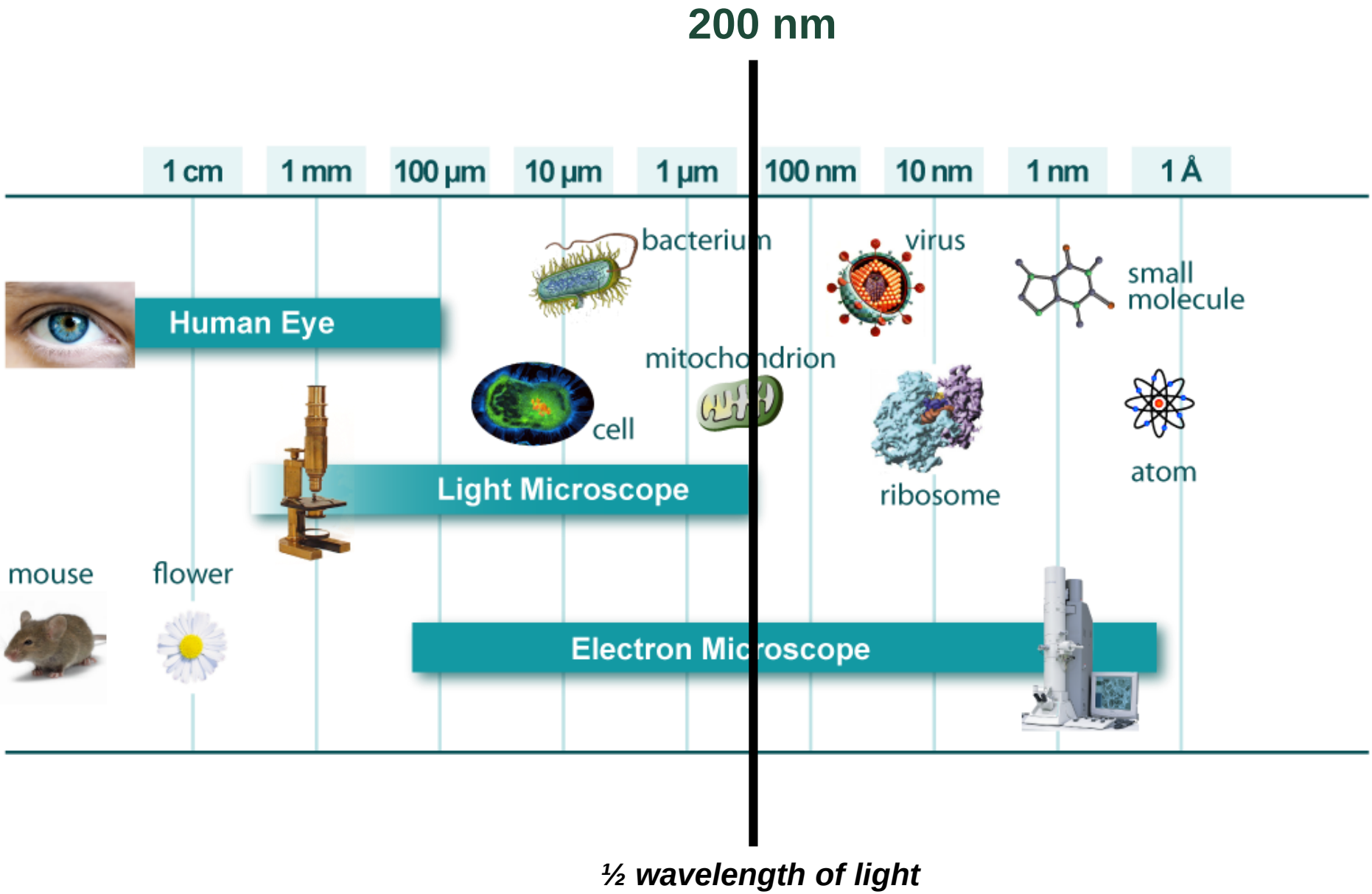
&

German Cancer Research Center (DKFZ)
Optical Nanoscopy Division
Heidelberg

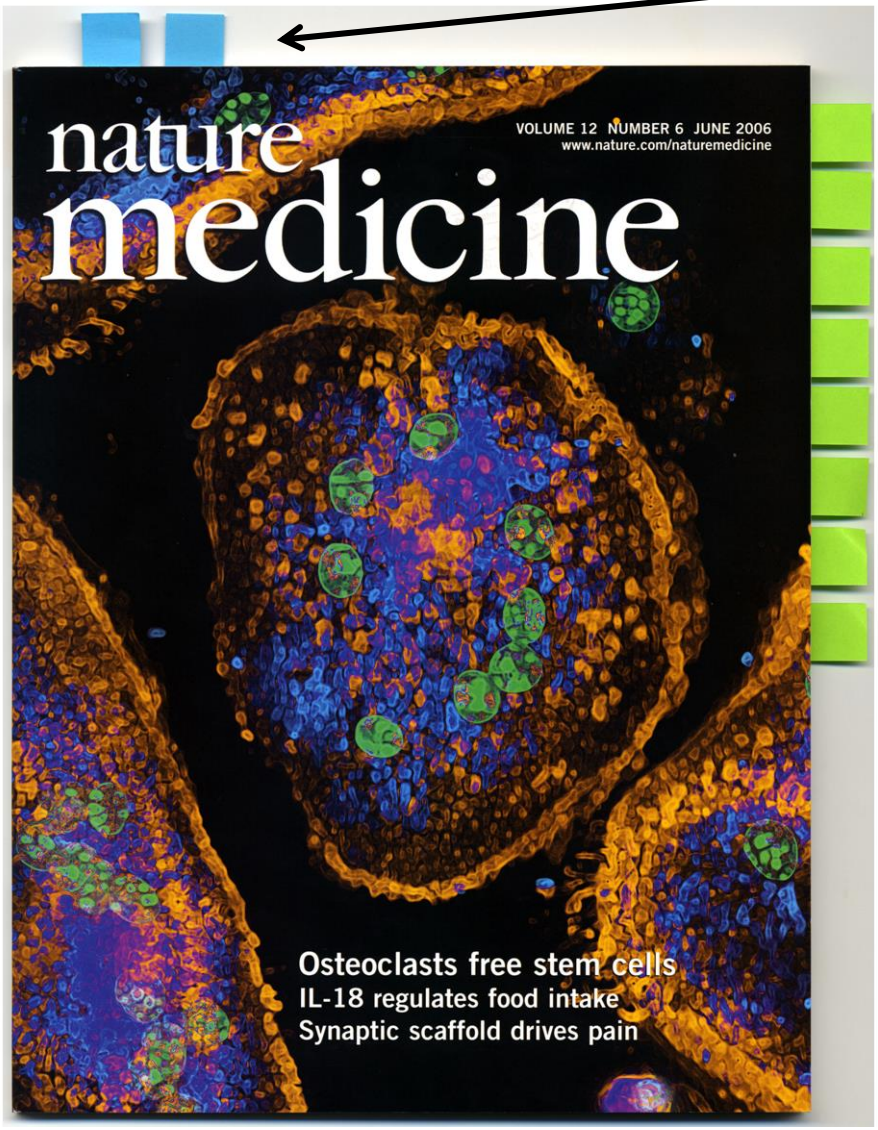
dkfz.

Nobel Lecture in Chemistry, Stockholms universitet

8. December 2014


200 nm
1 cm
1 mm
100 µm
10 µm
1 µm
100 nm
10 nm
1 nm
1 Å
bacterium
virus
small molecule
Human Eye
mitochondrion
cell
Light Microscope
ribosome
atom
mouse
flower
Electron Microscope
½ wavelength of light

Light microscopy: most popular microscopy technique in life sciences



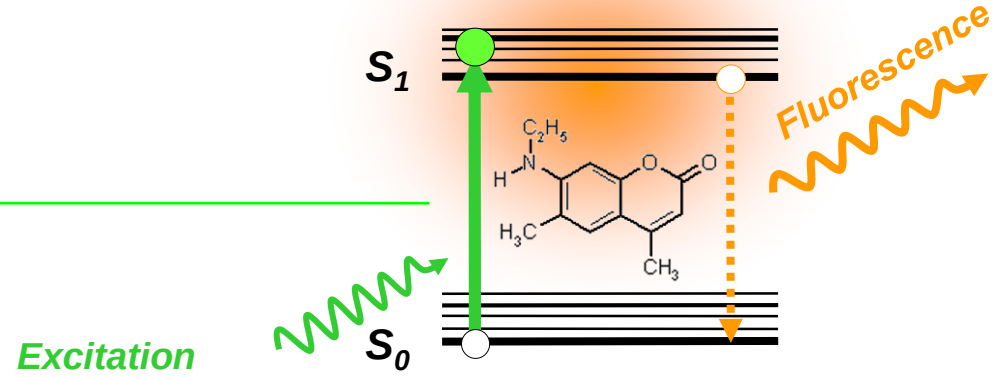
Electron microscopy, etc.

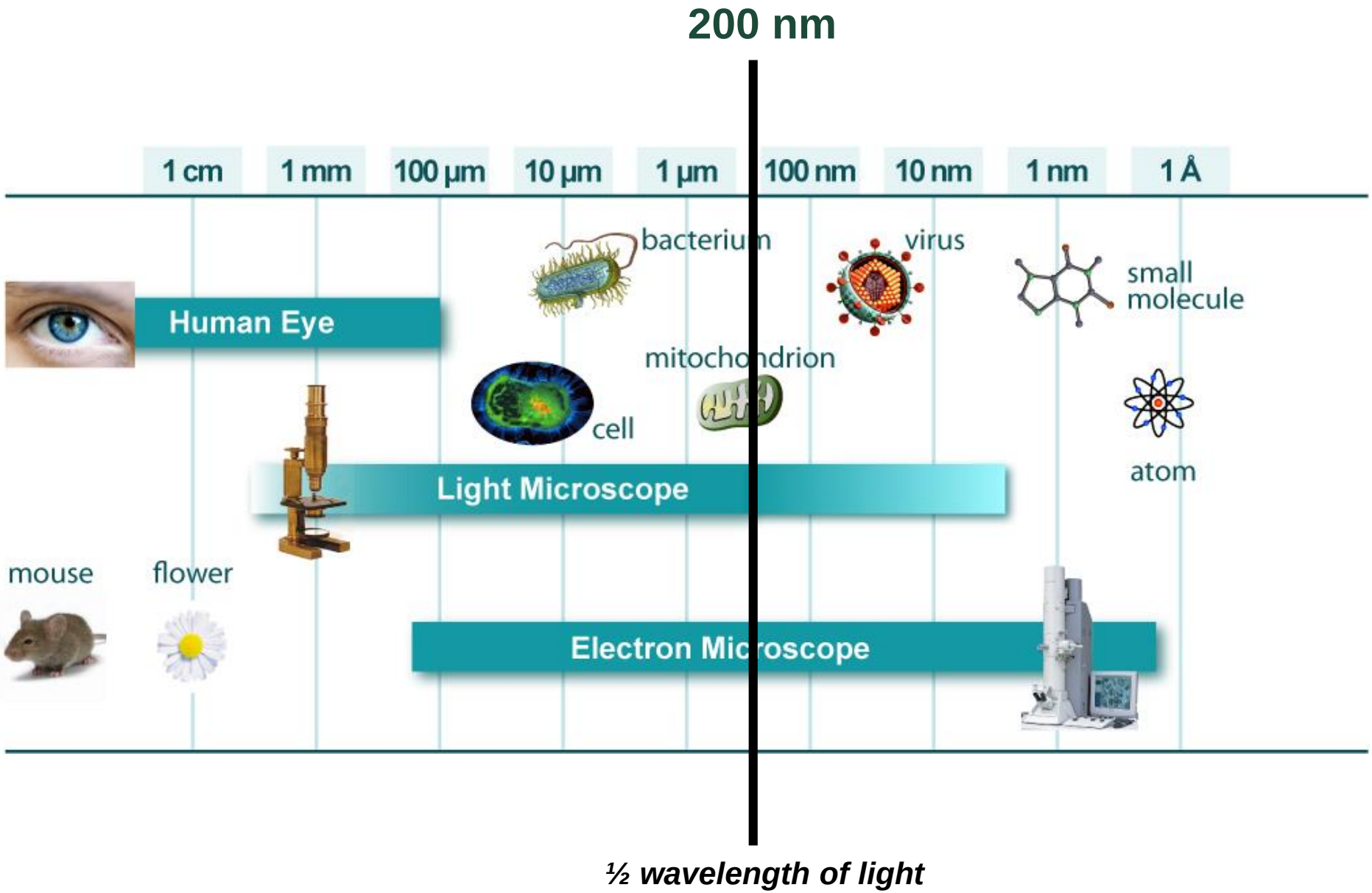
20 %

80 %

Light microscopy

Fluorescent labels indicate biomolecule of interest

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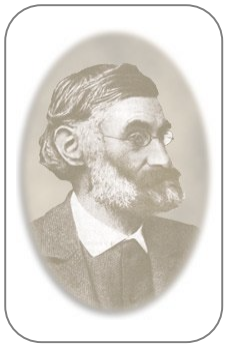
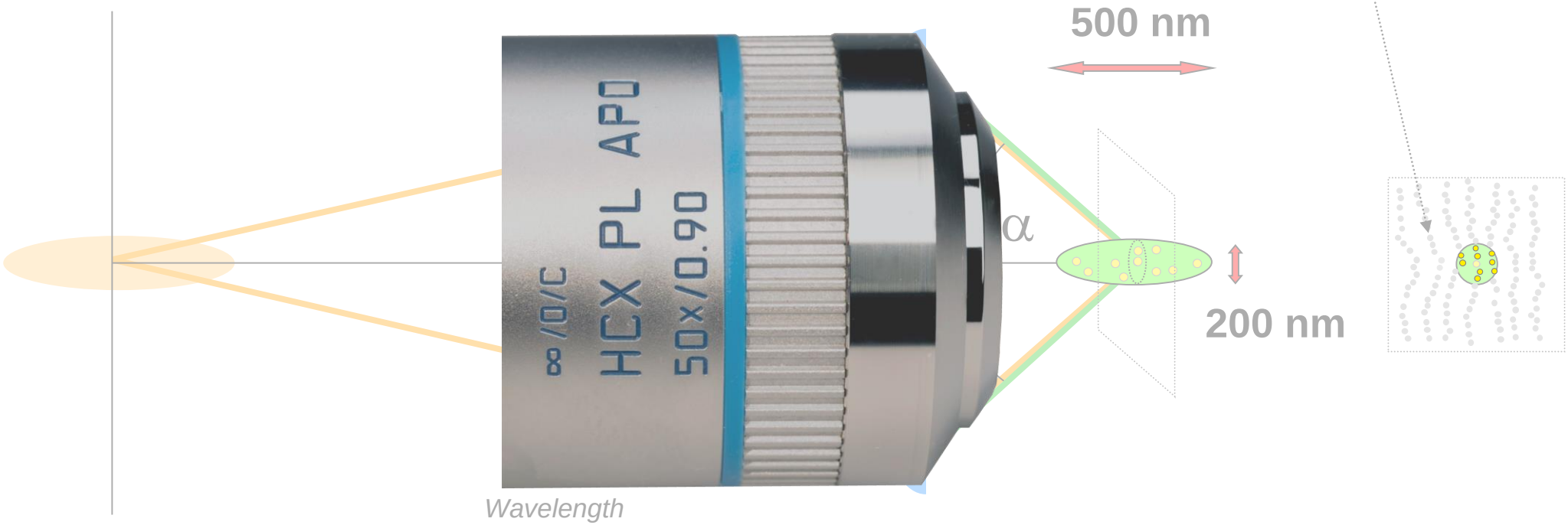
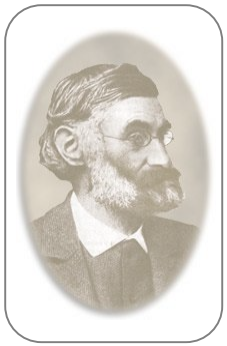


… because of the diffraction barrier:

$$d = \frac{\lambda}{2n\sin\alpha}$$



Verdet (1869)
Abbe (1873)
Helmholtz (1874)
Rayleigh (1874)



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Photomultiplier
or APD

500 nm

α

200 nm

Detector

Lens

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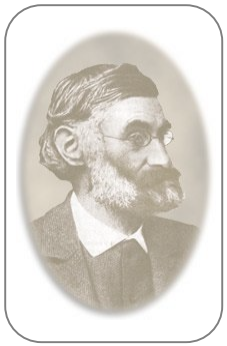
Abbe (1873)
Helmholtz (1874)
Rayleigh (1874)



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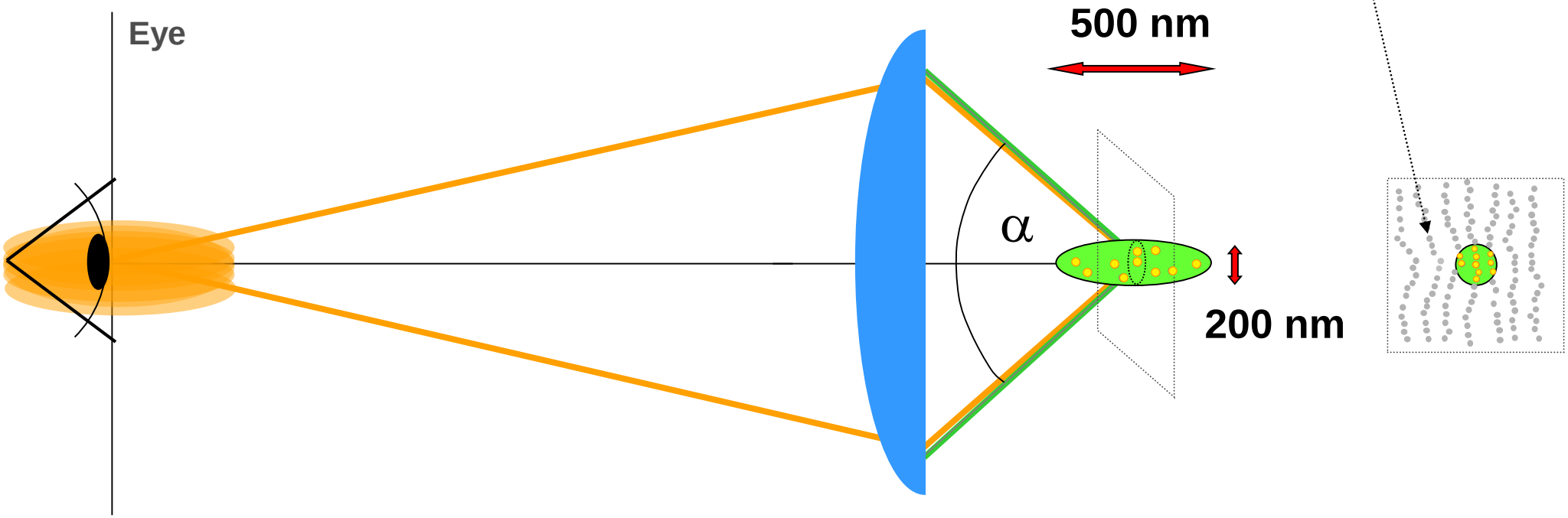

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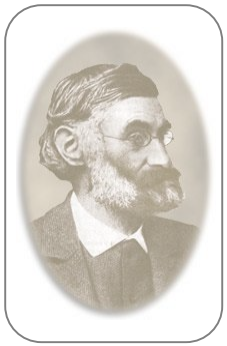

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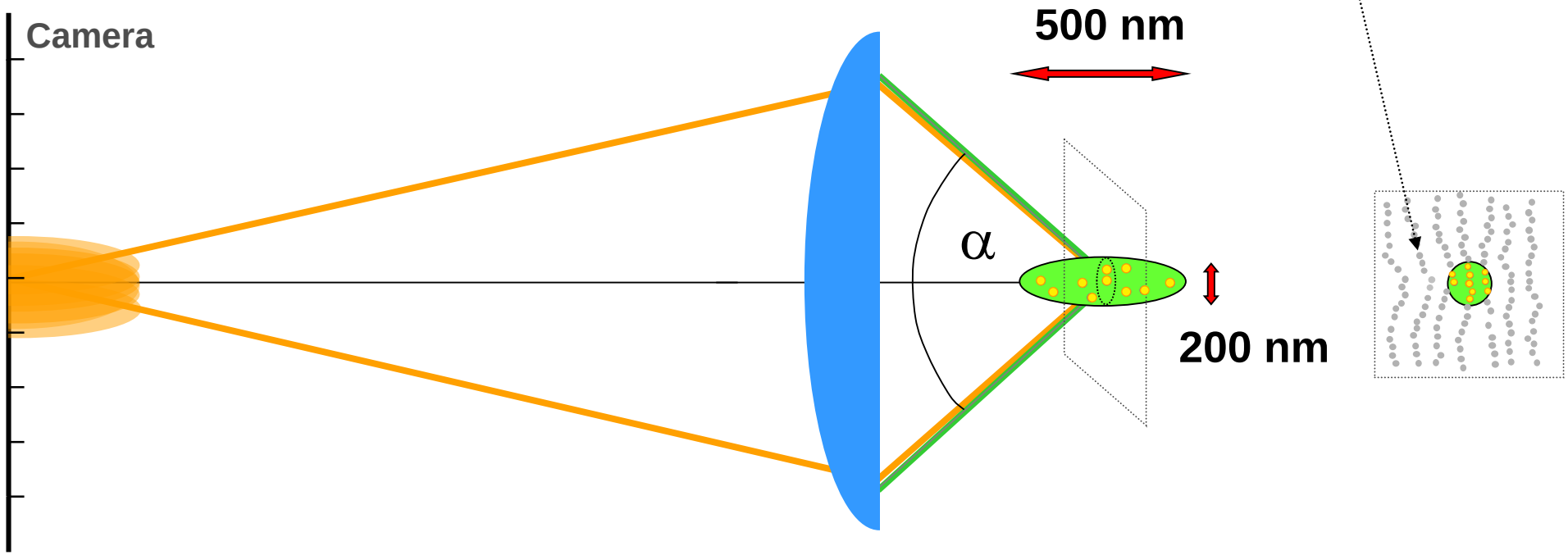

Detector **Lens**

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Jena,
Germany

What I believed around 1990:

"… the resolution limiting effect of diffraction can be overcome (…) by fully **exploiting the properties of the fluorophores**. Combined with modern **quantum optical techniques** the scanning (confocal) microscope has the potential of dramatically improving the resolution in far-field light microscopy."

SWH, Opt. Commun. 106 (1994)
accepted November 1993

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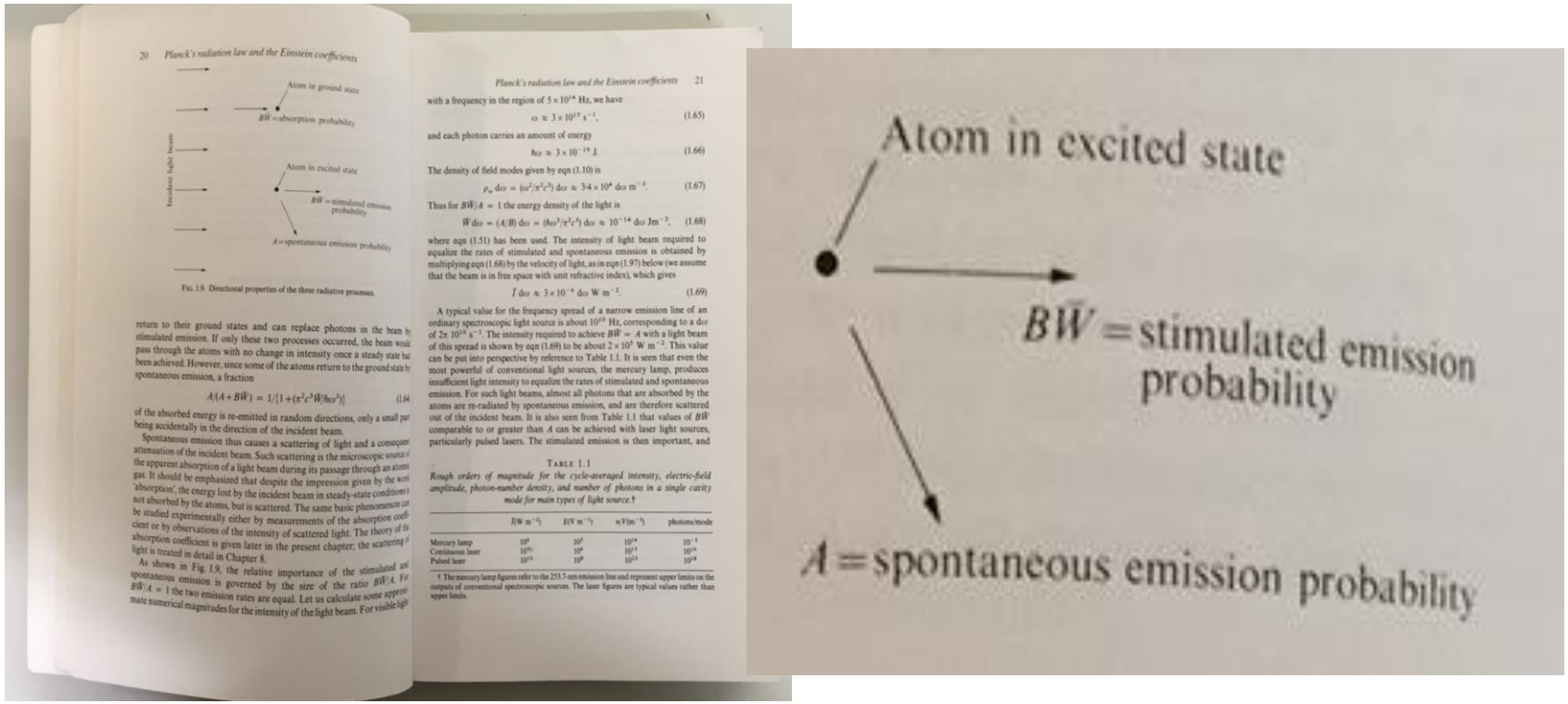

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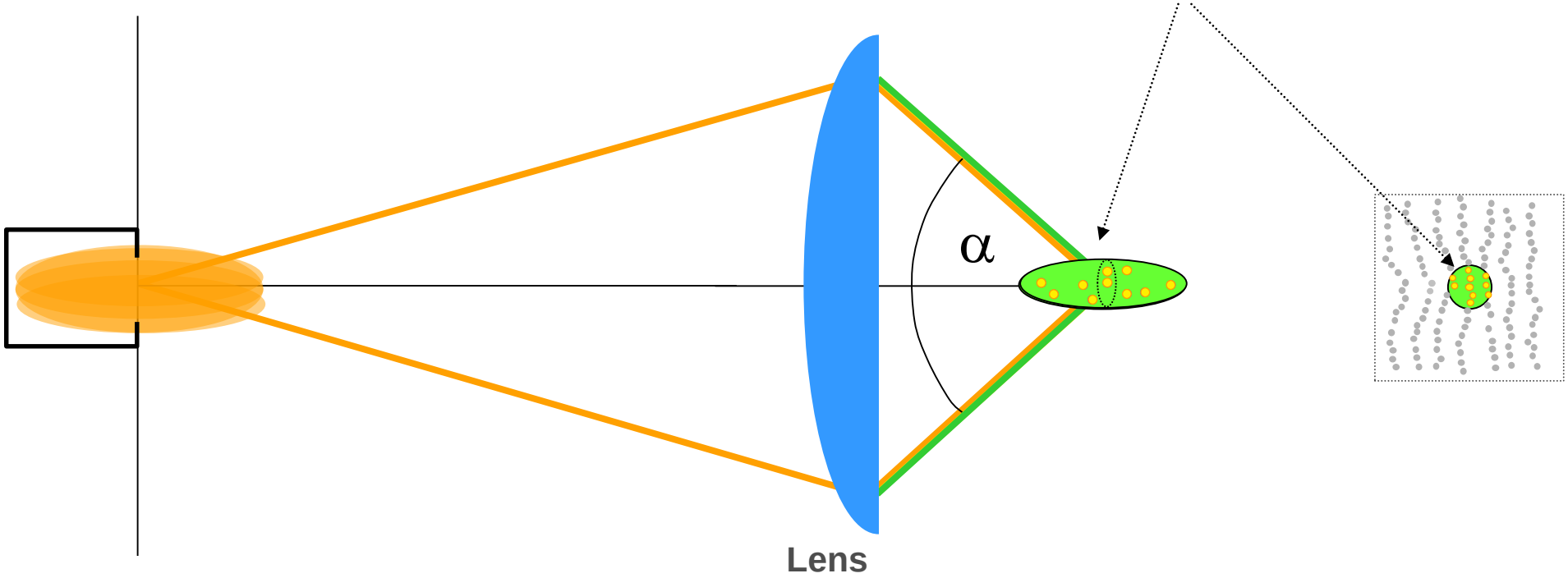

Keep molecules in a dark state !

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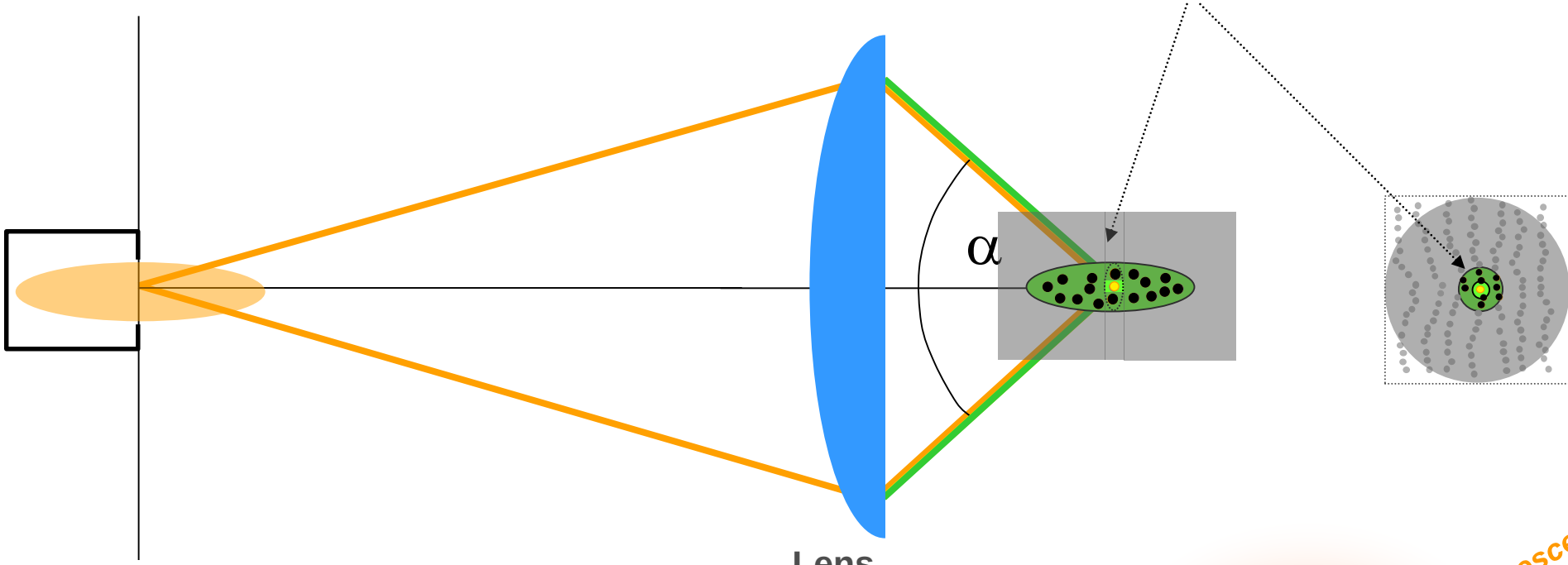

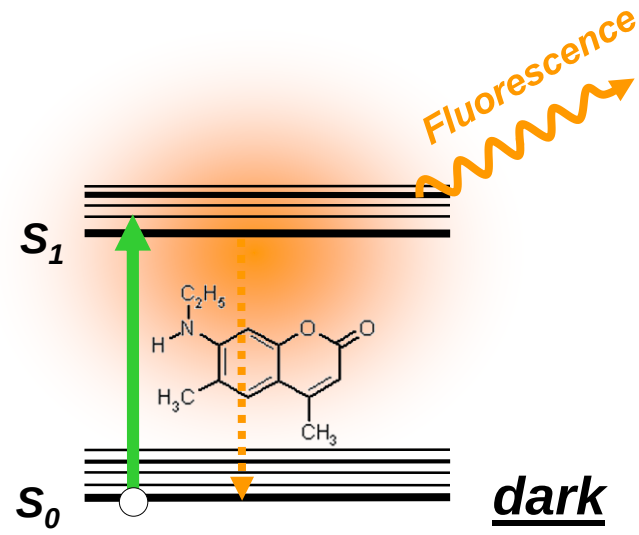

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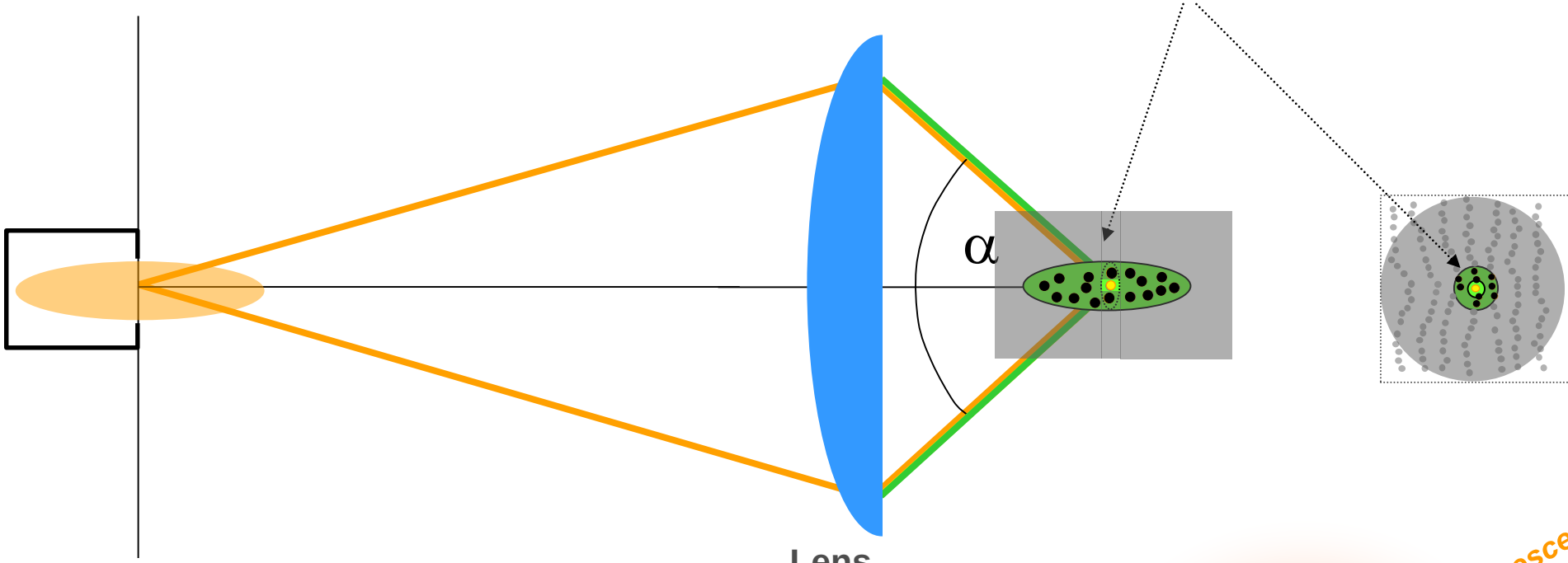
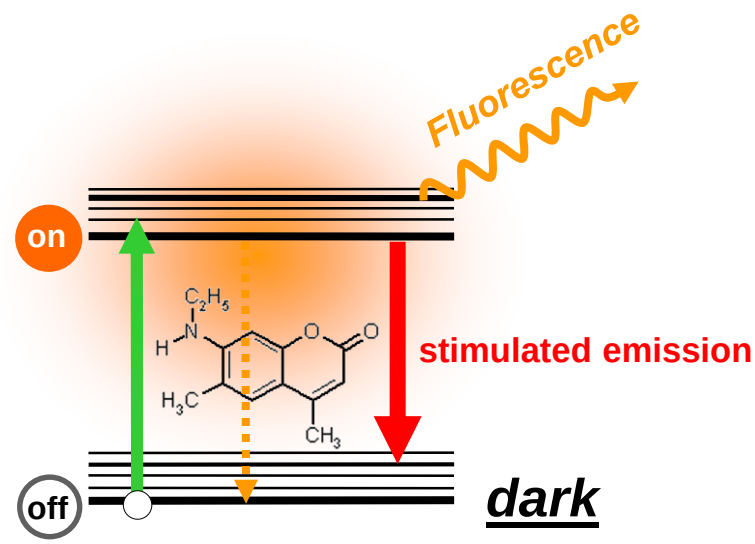
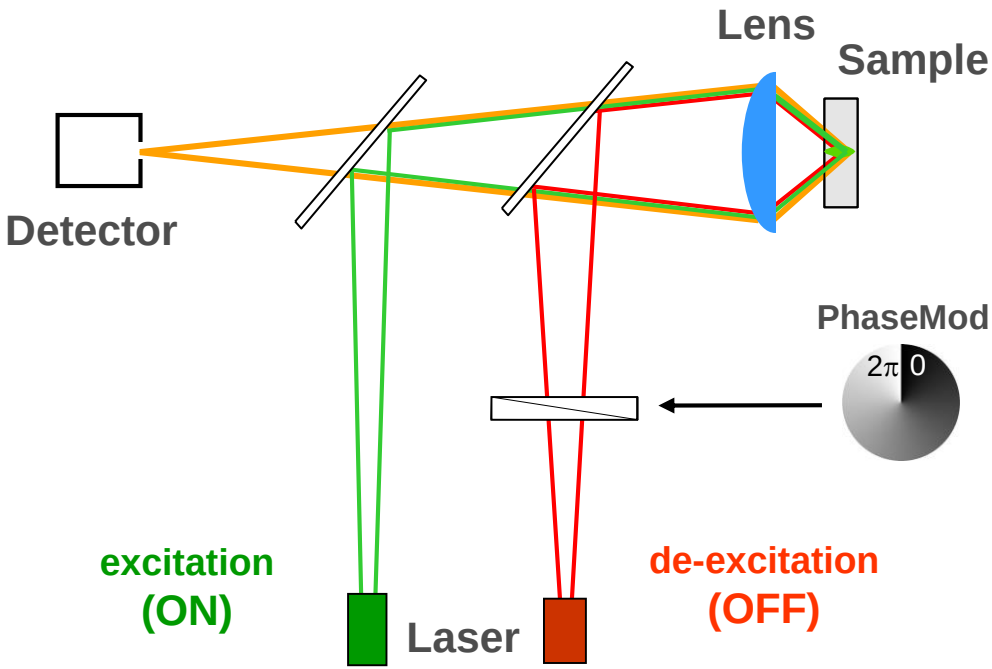


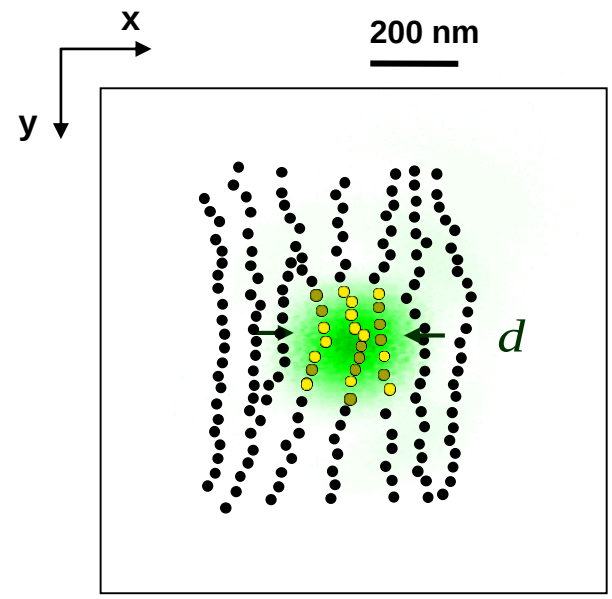


Keep molecules in a dark state !

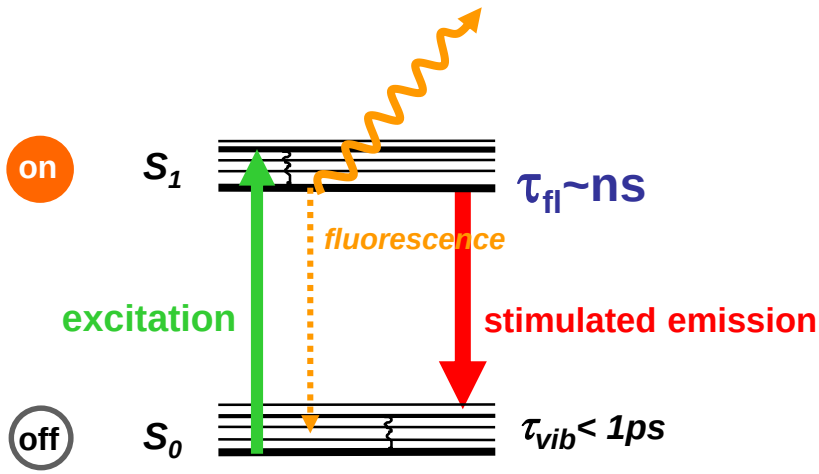
STED microscope:

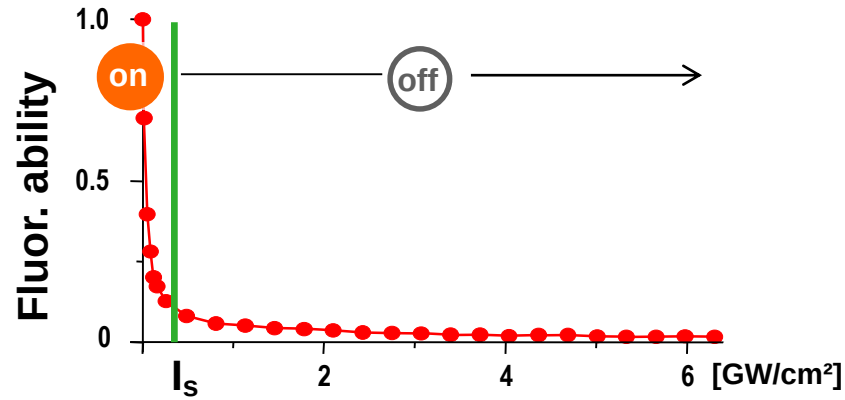
Hell & Wichmann, Opt. Lett. (1994)





$$d \approx \frac{\lambda}{2n \sin \alpha}$$





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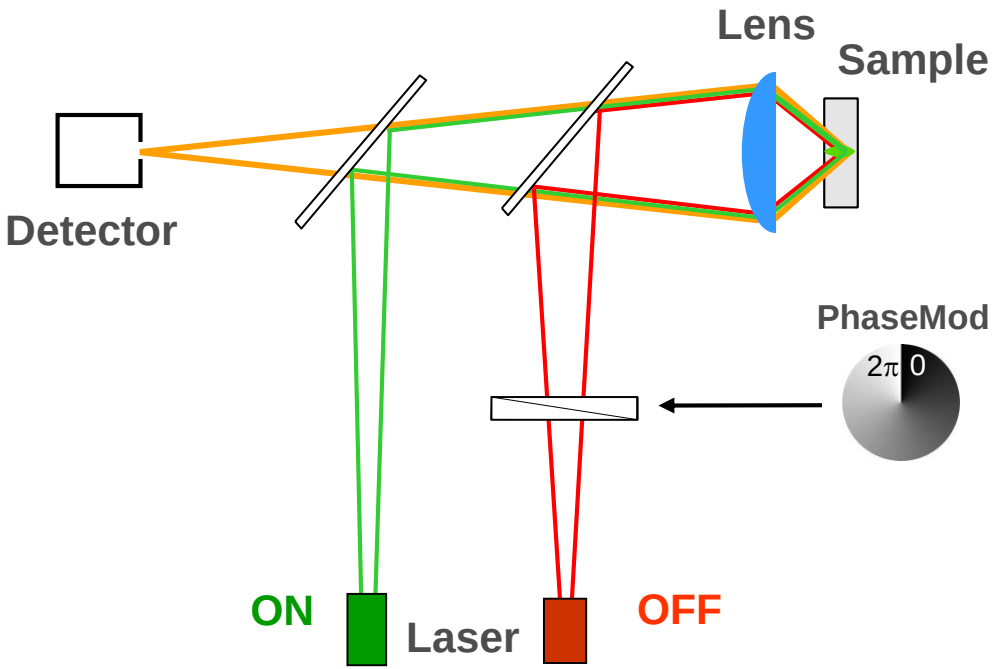

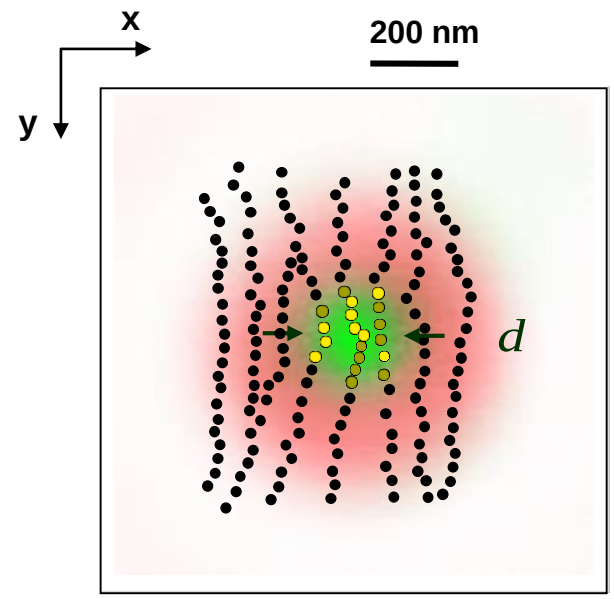

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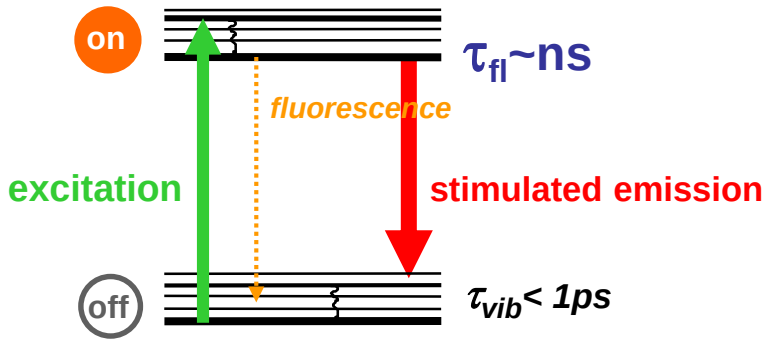

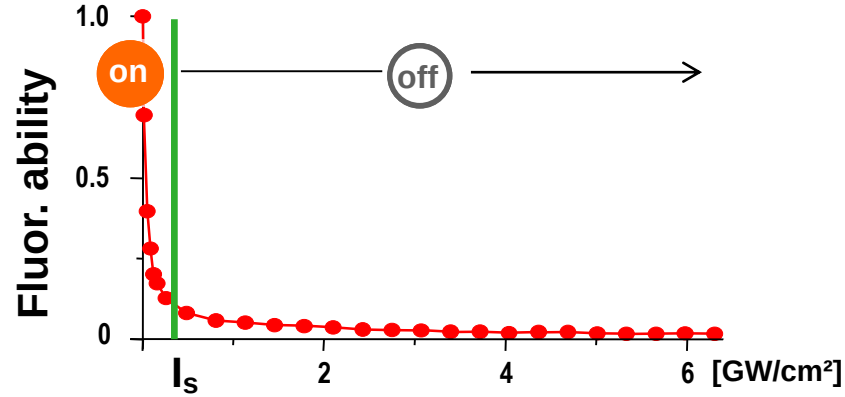

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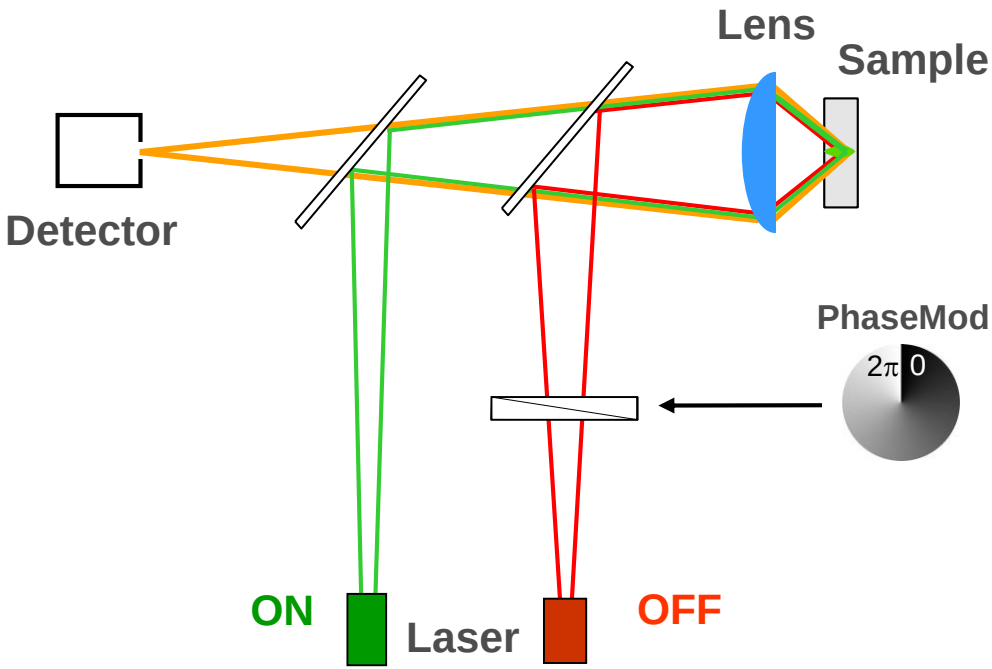

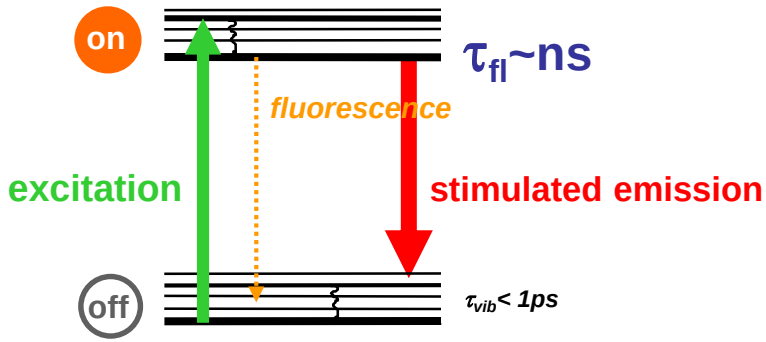

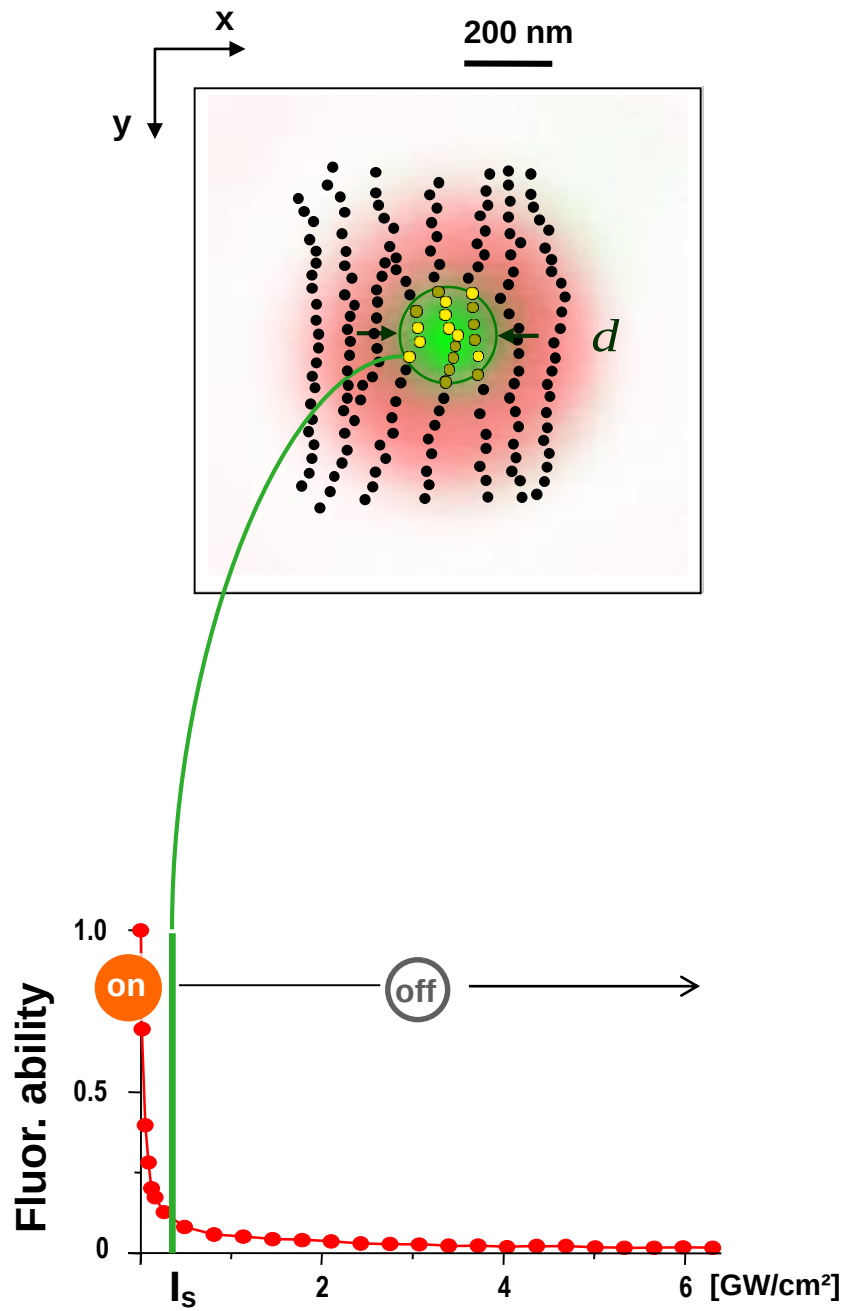

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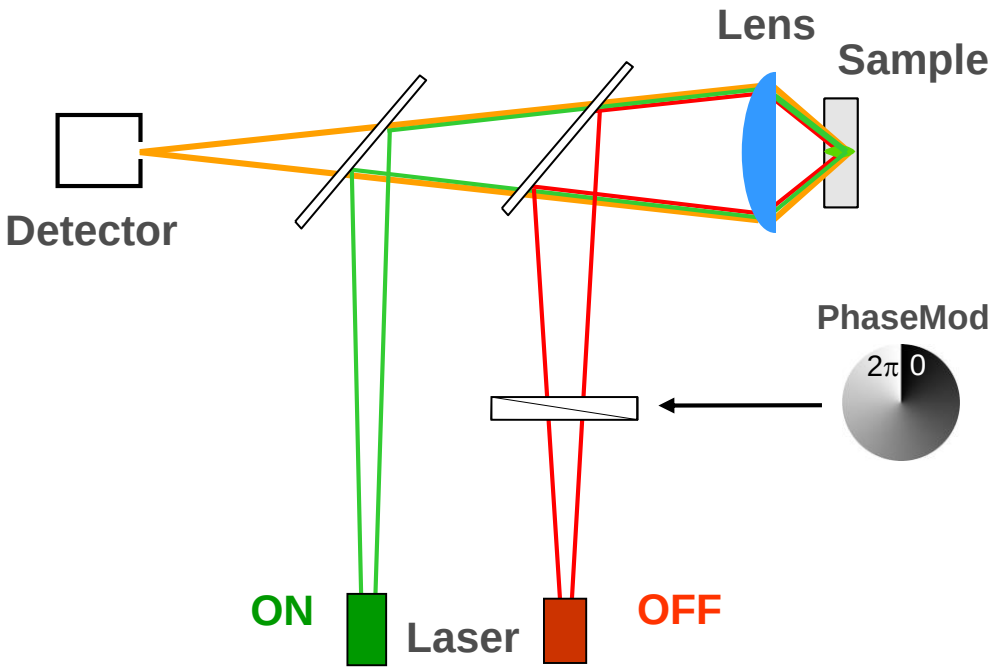

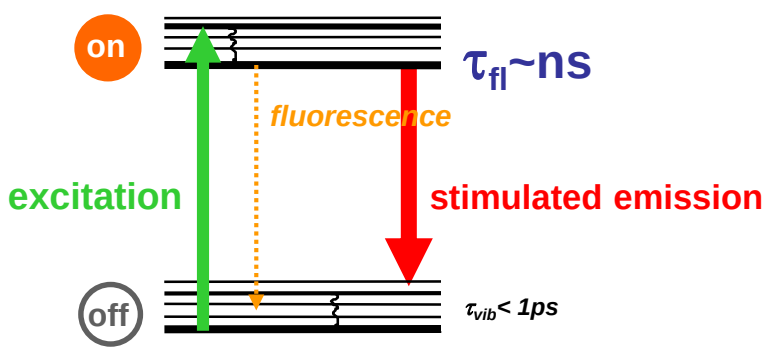

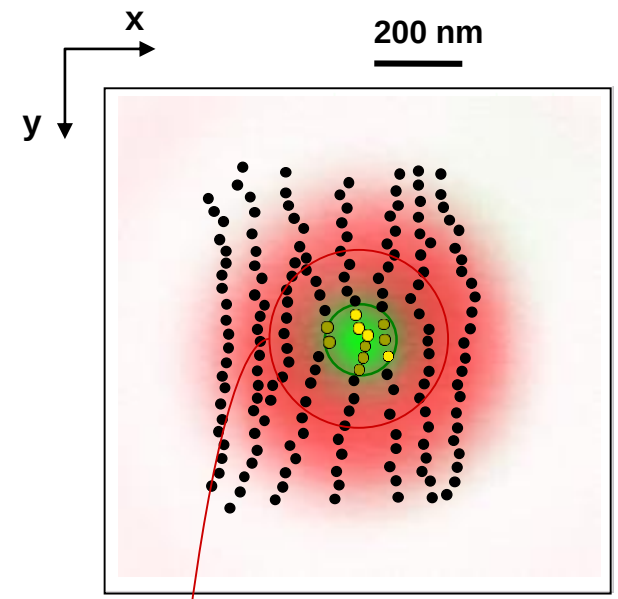

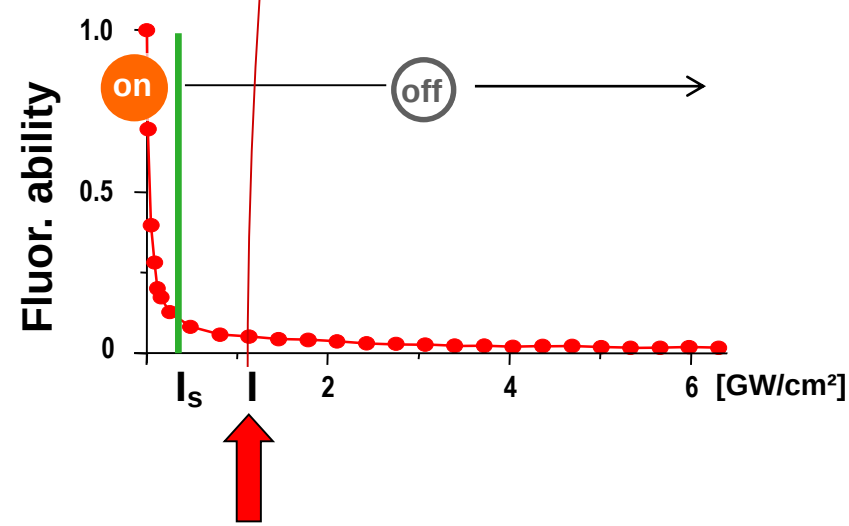

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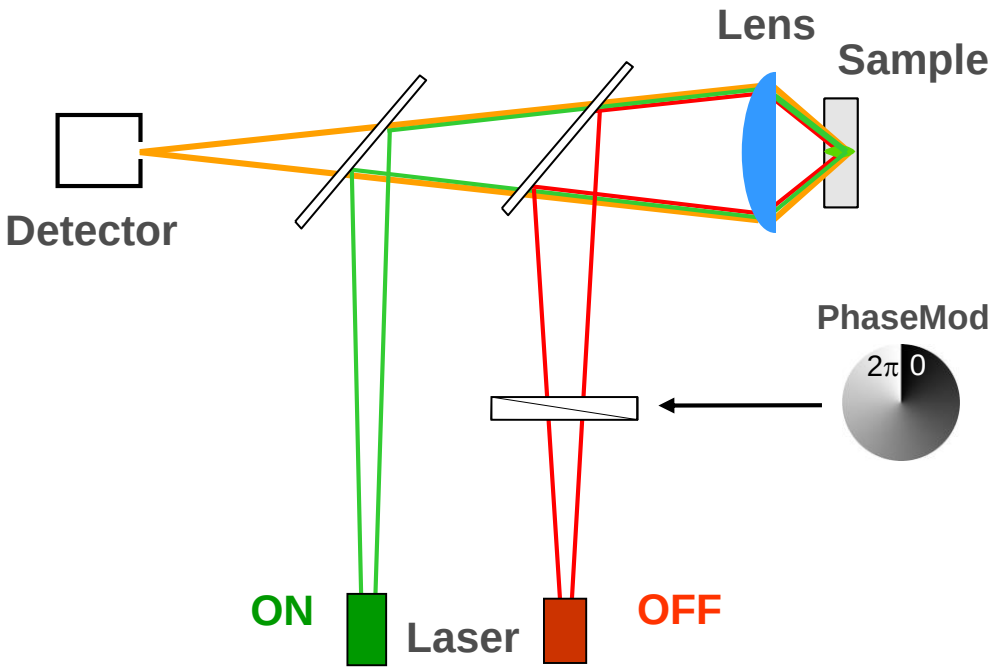

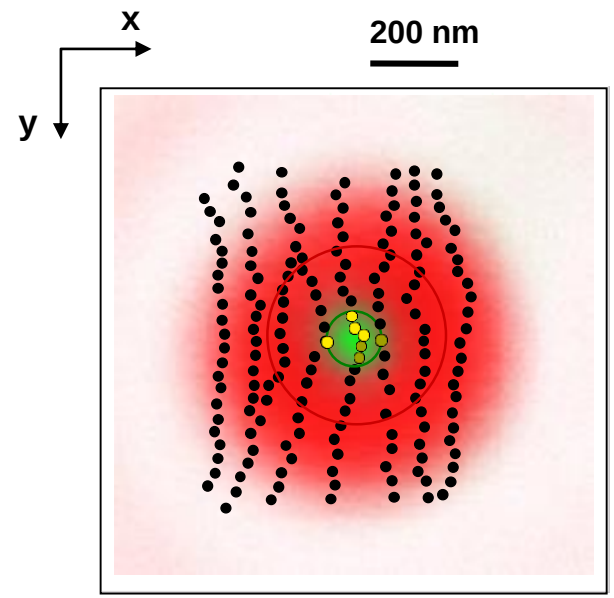

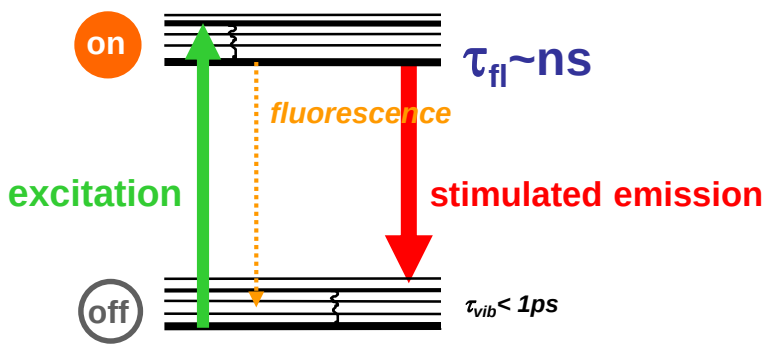

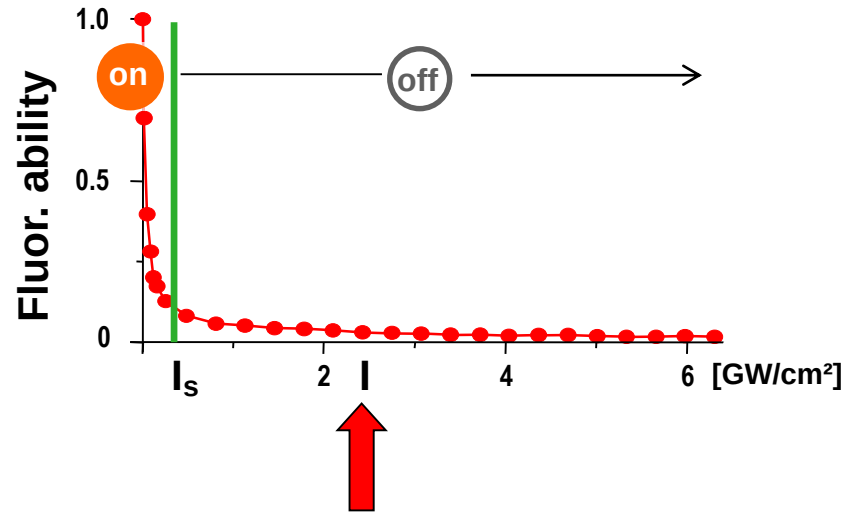

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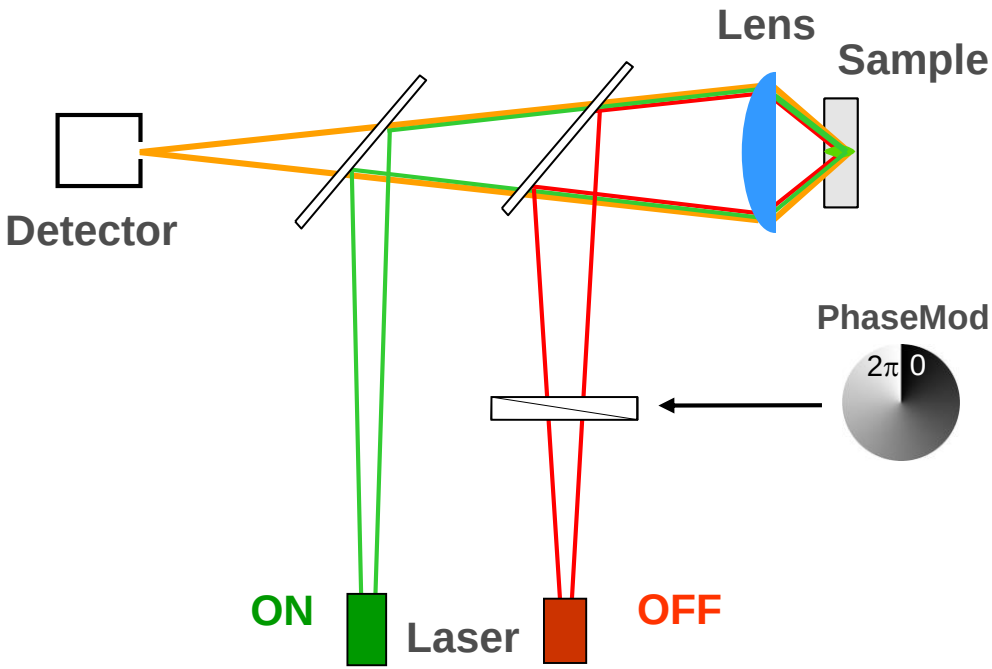

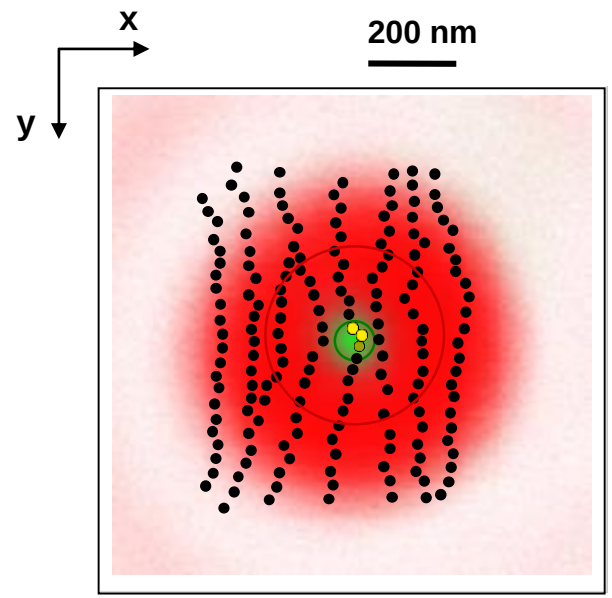

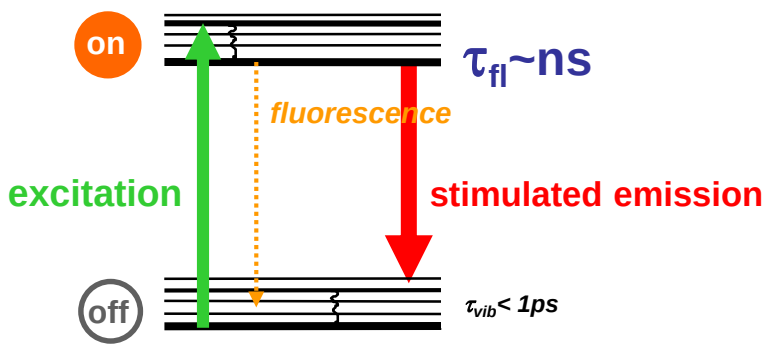

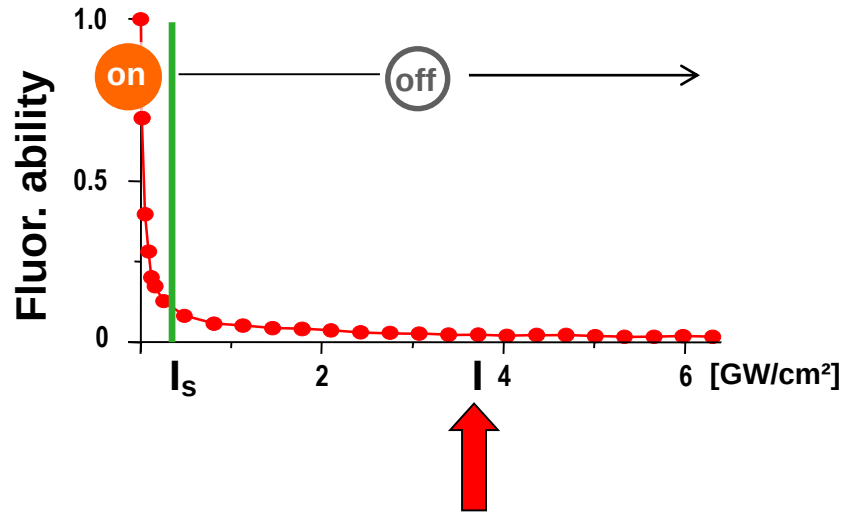

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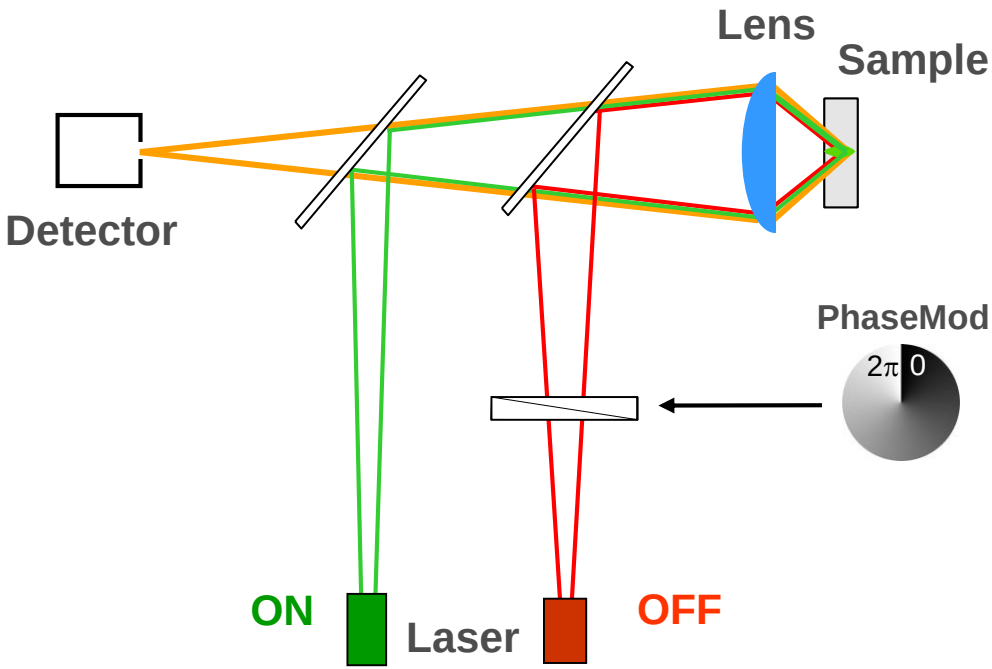

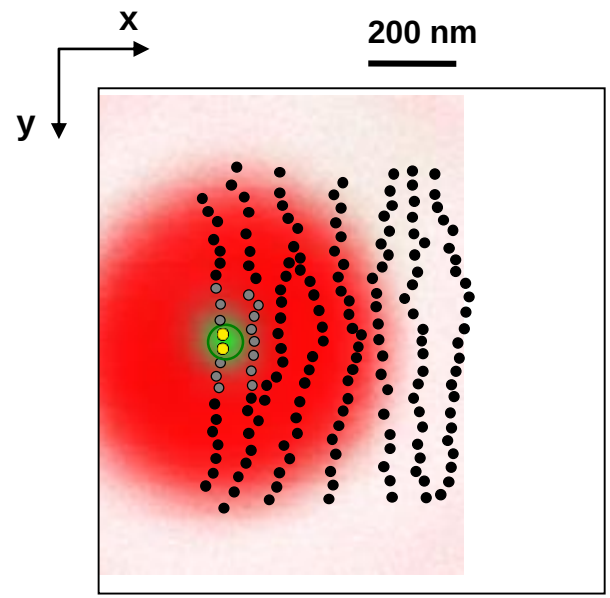

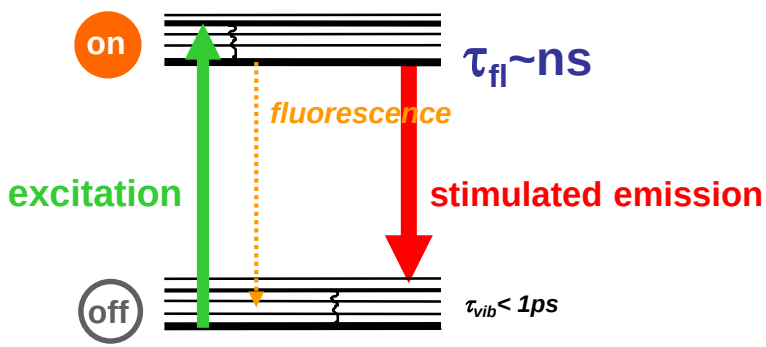

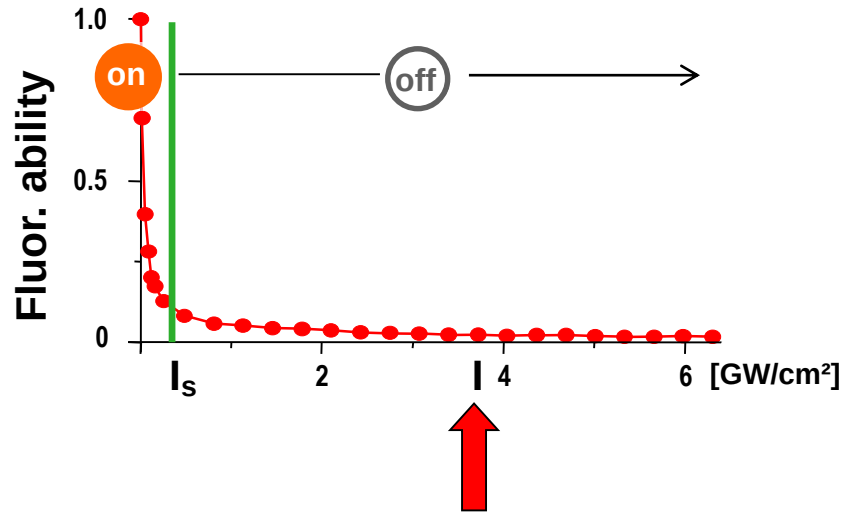

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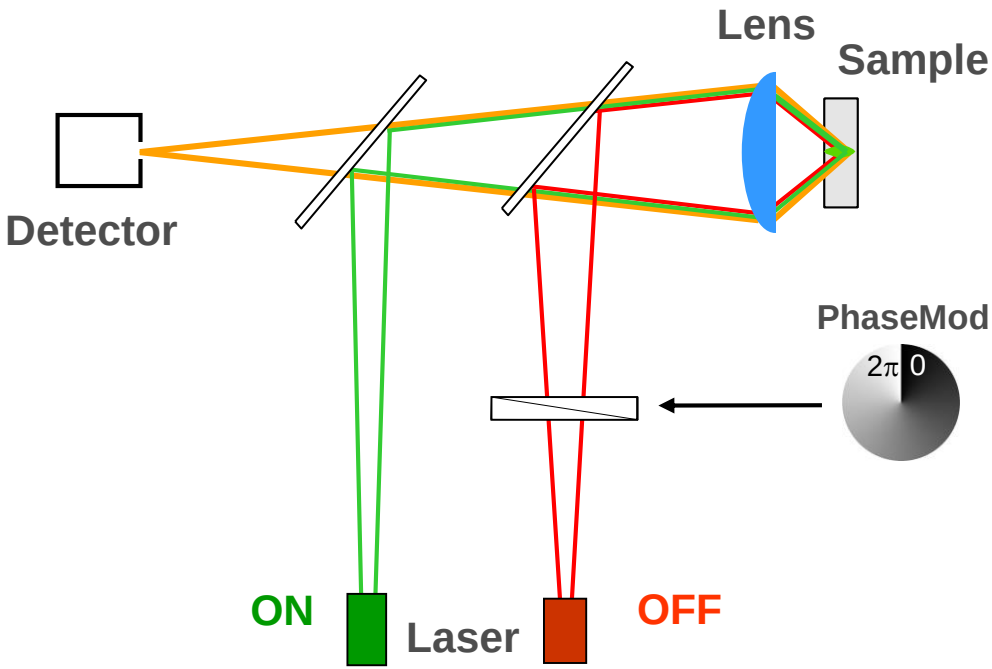

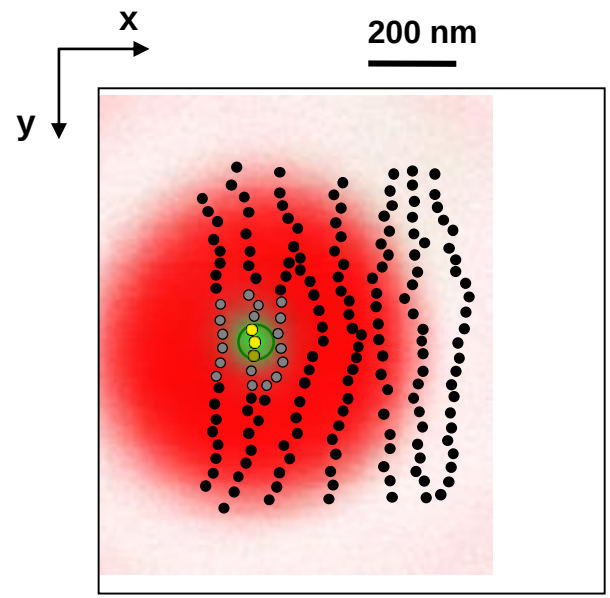

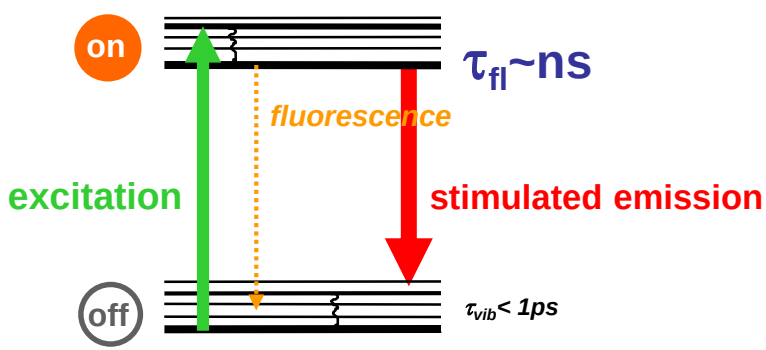

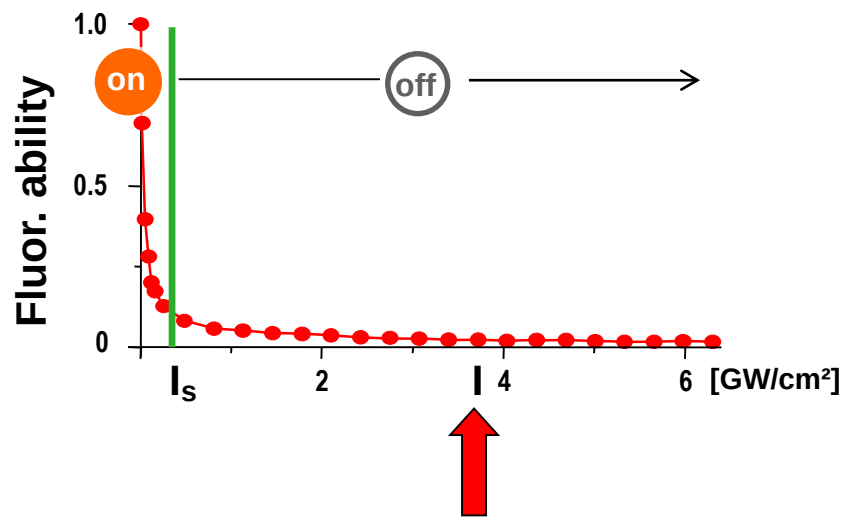

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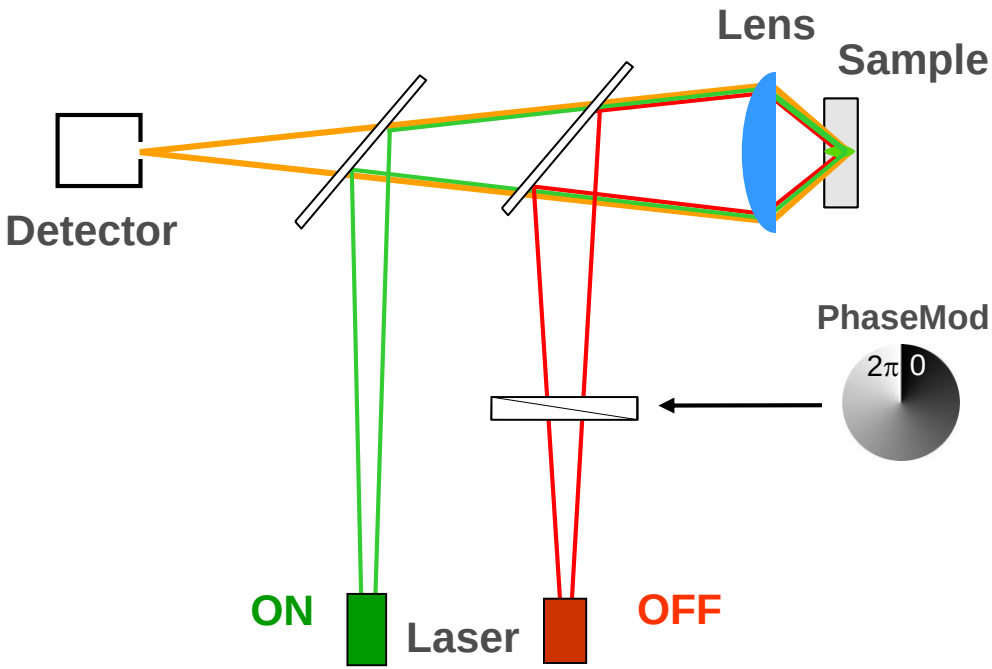
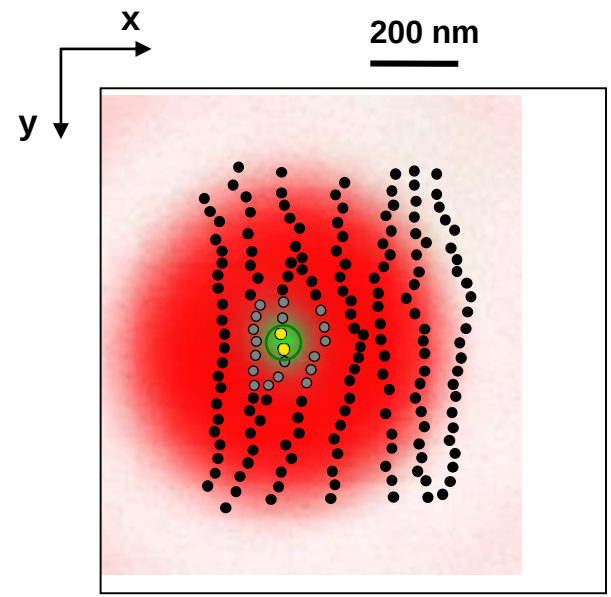
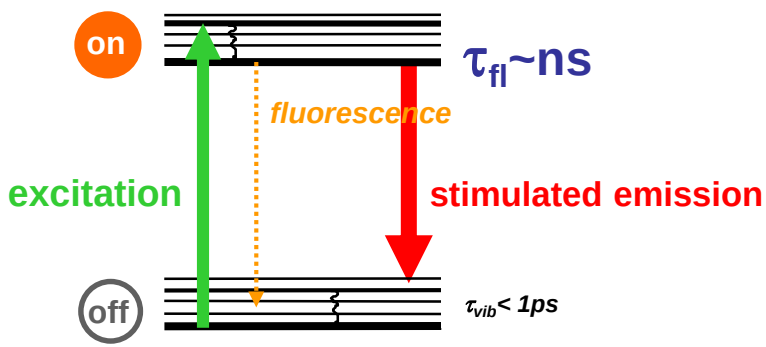
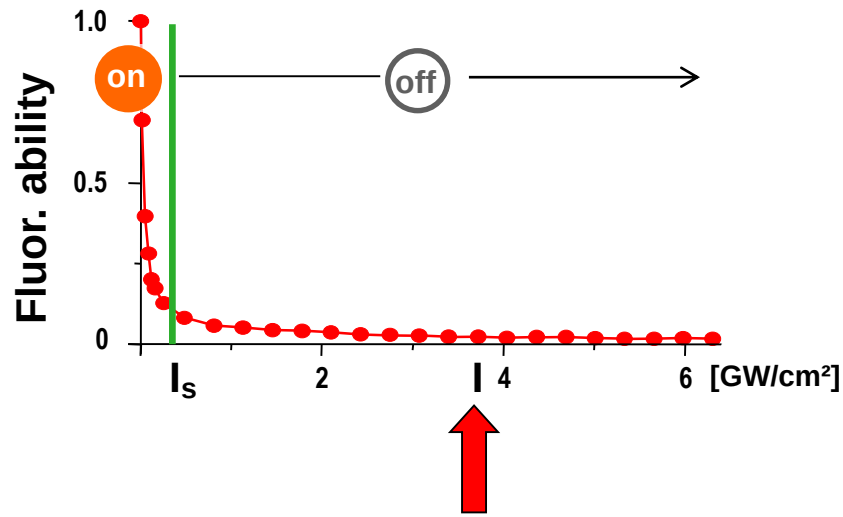


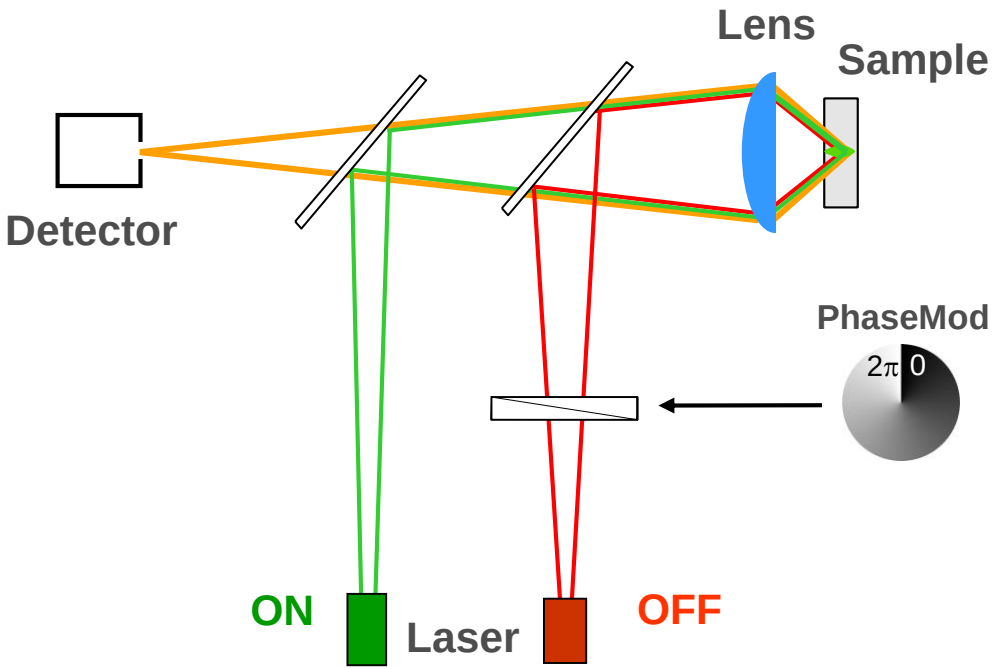


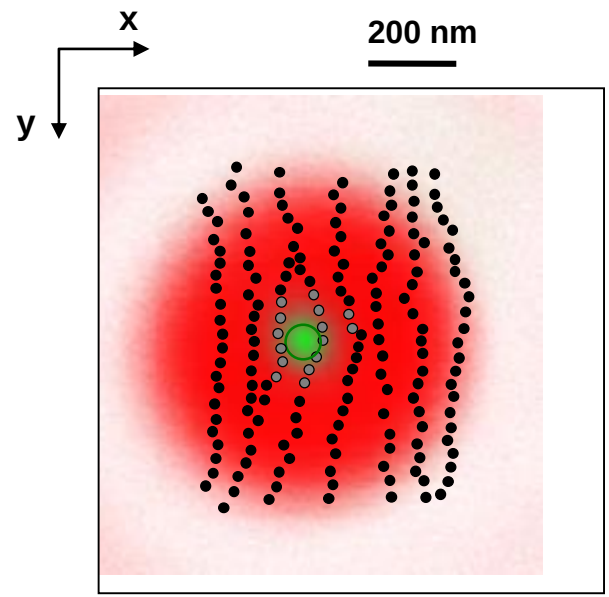


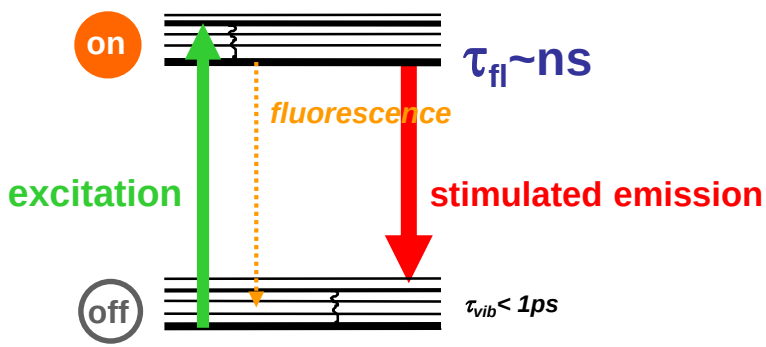


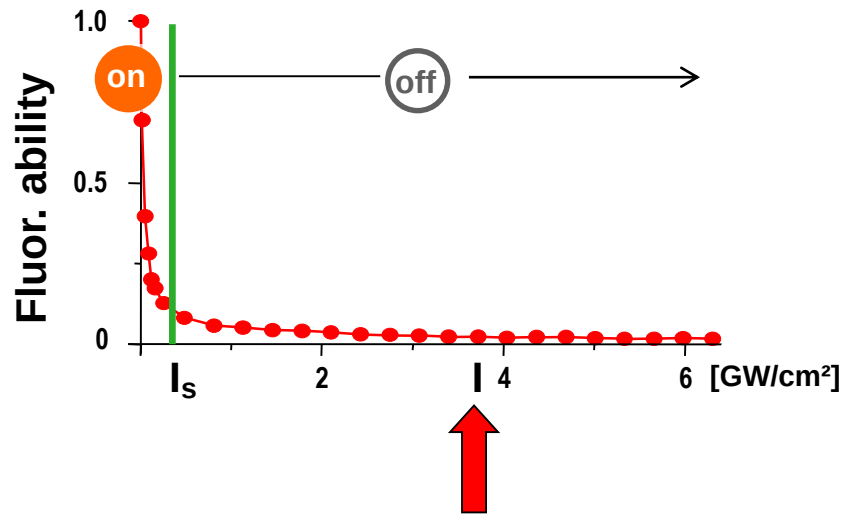
STED microscope:

Hell & Wichmann, Opt. Lett. (1994)



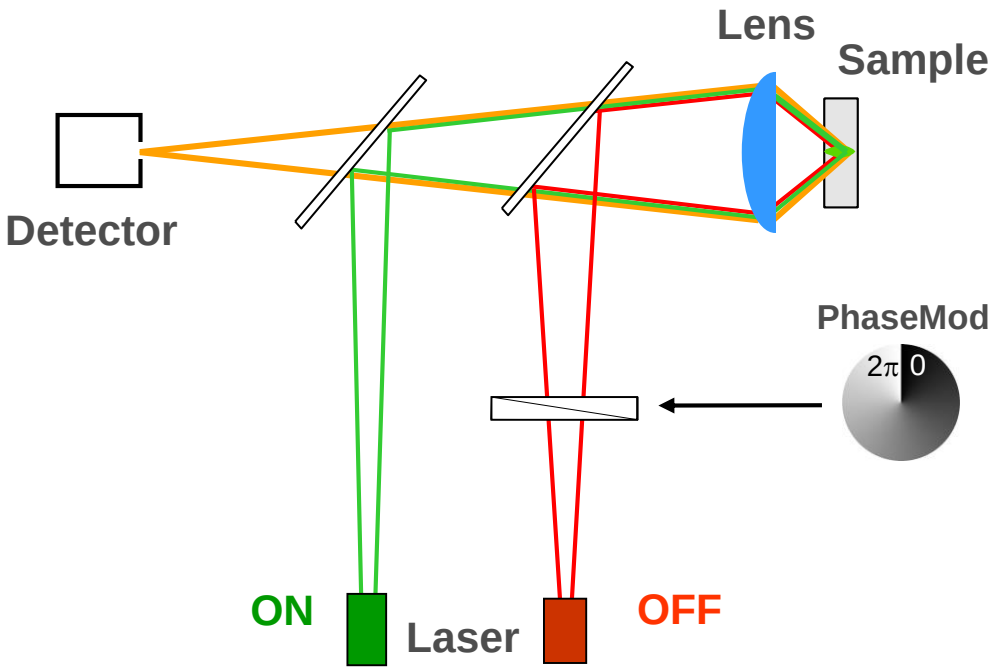


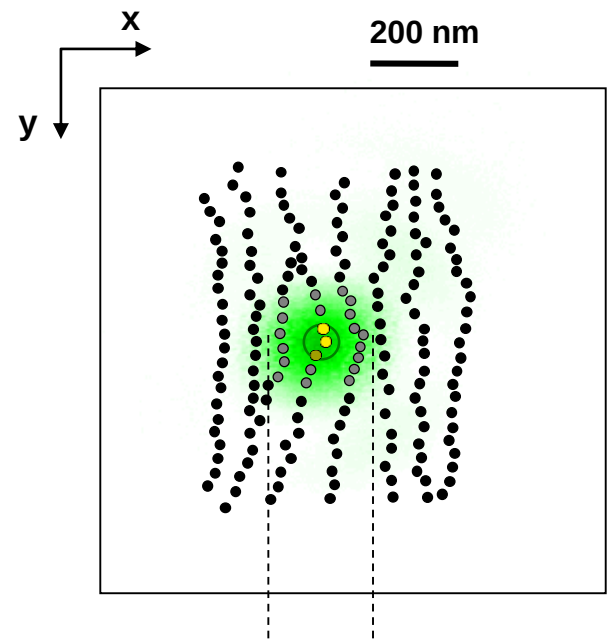




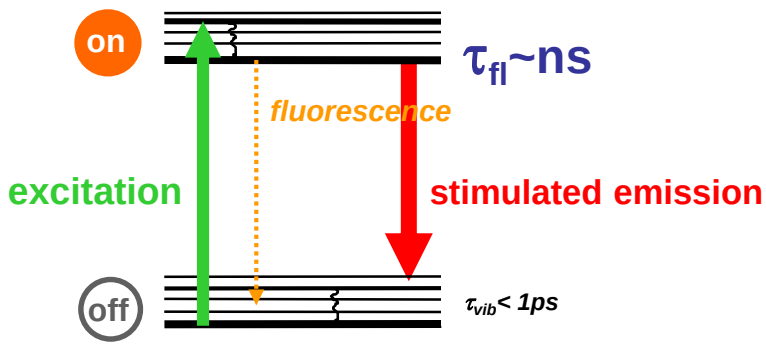
STED microscope:

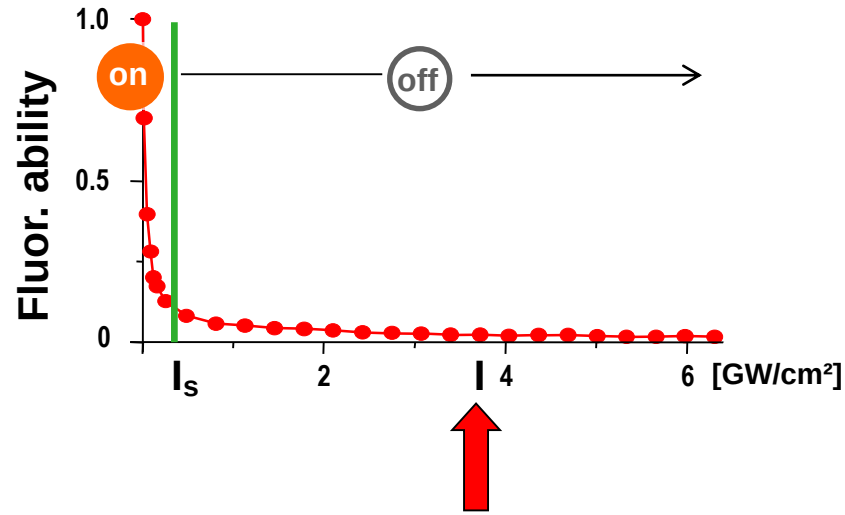
Hell & Wichmann, Opt. Lett. (1994)





$$d \approx \frac{\lambda}{2n \sin \alpha}$$





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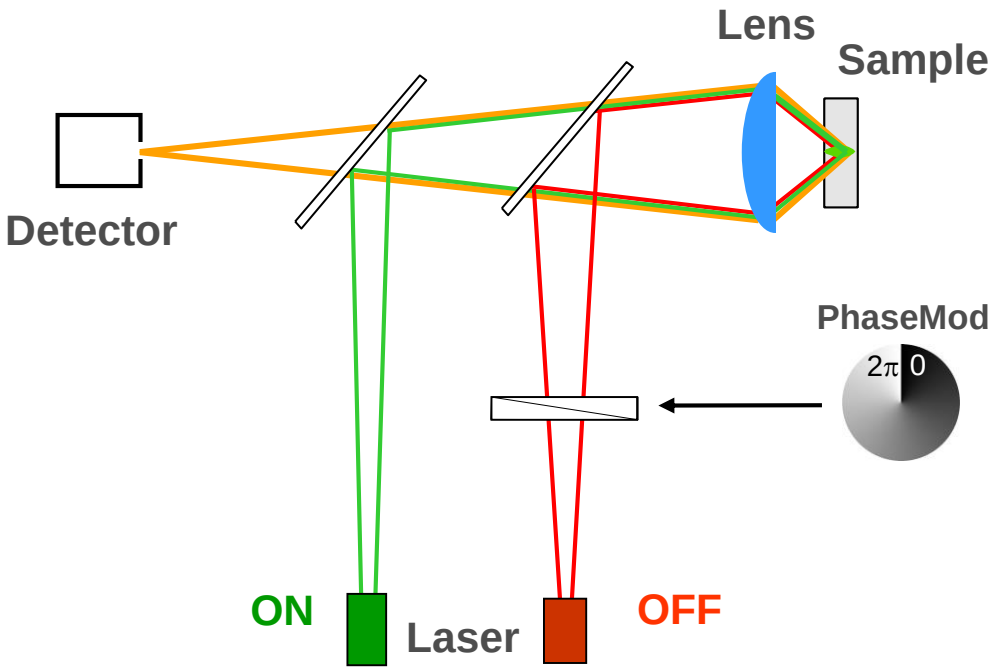

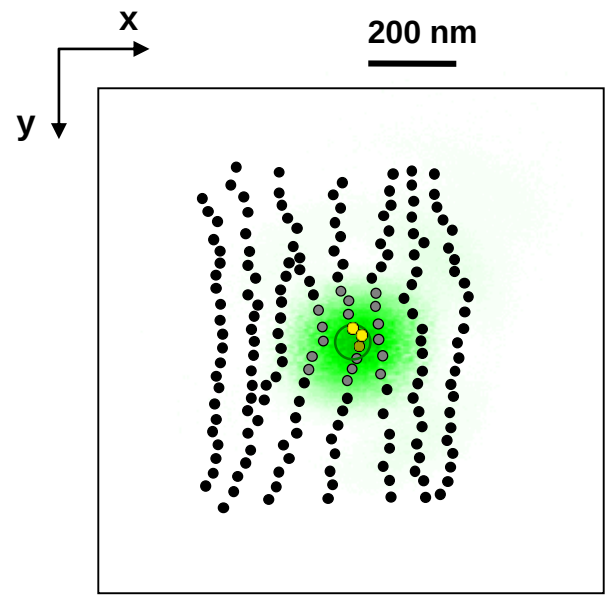

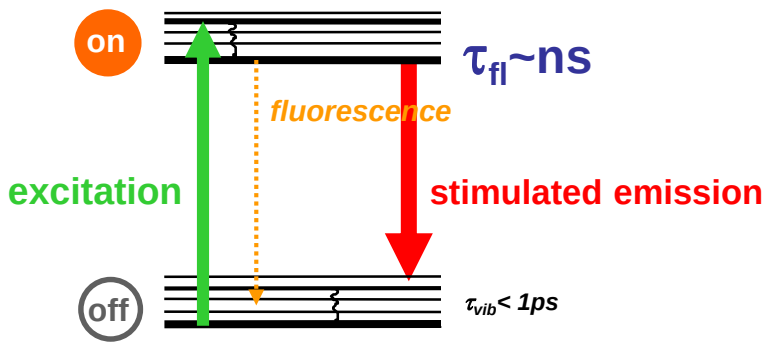

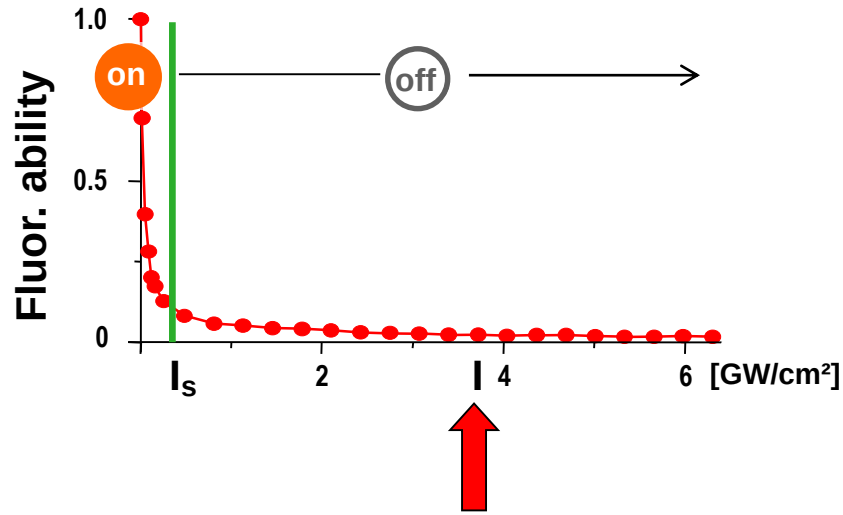

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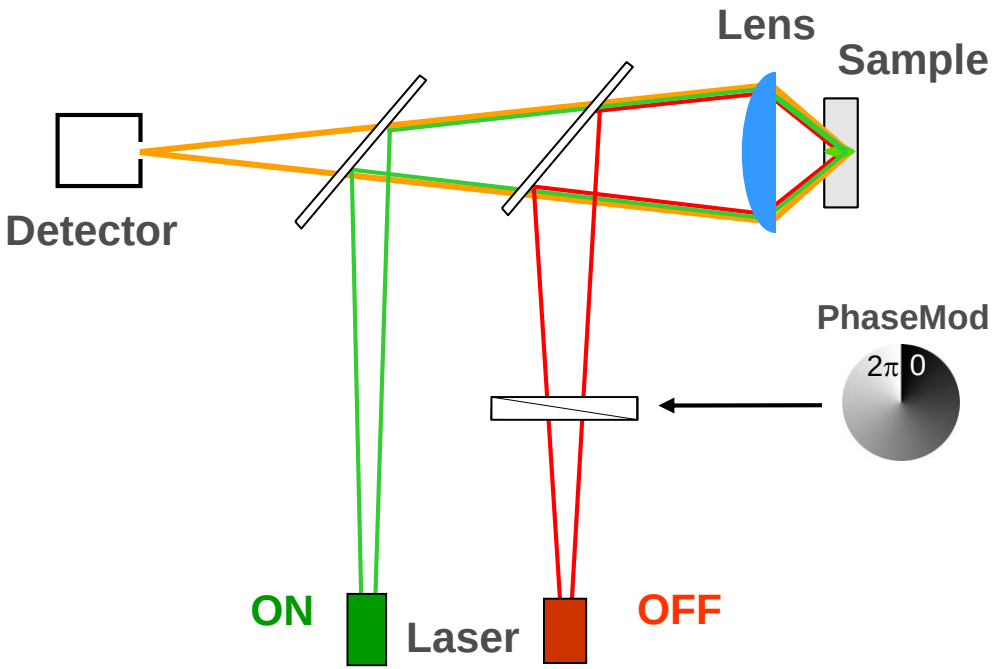

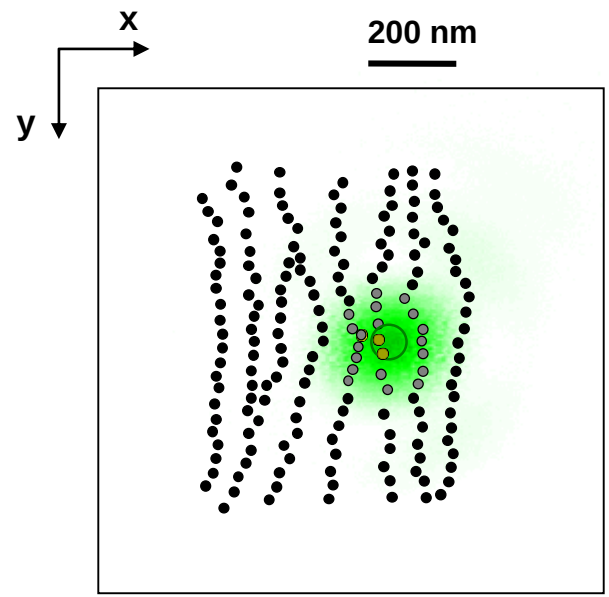

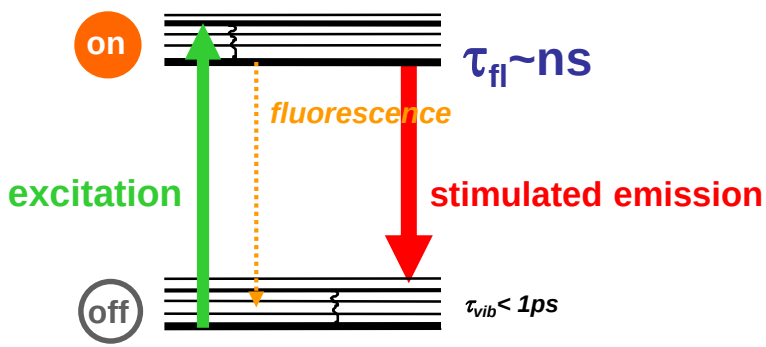

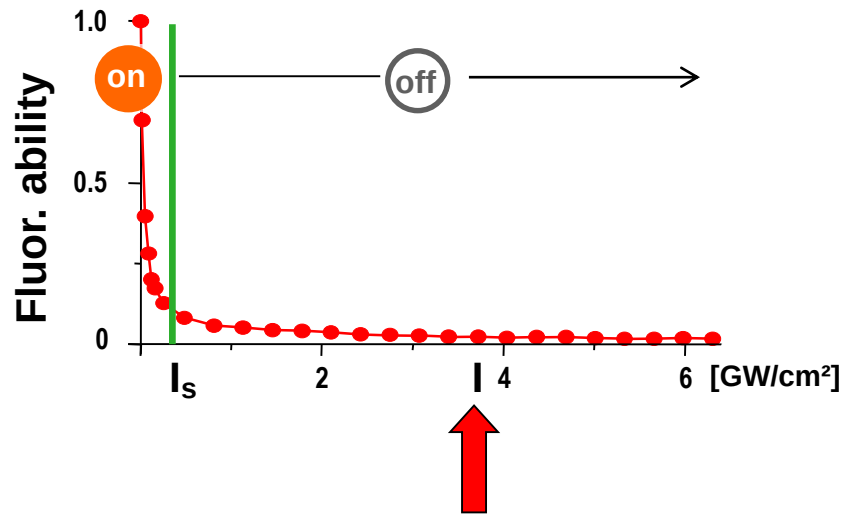

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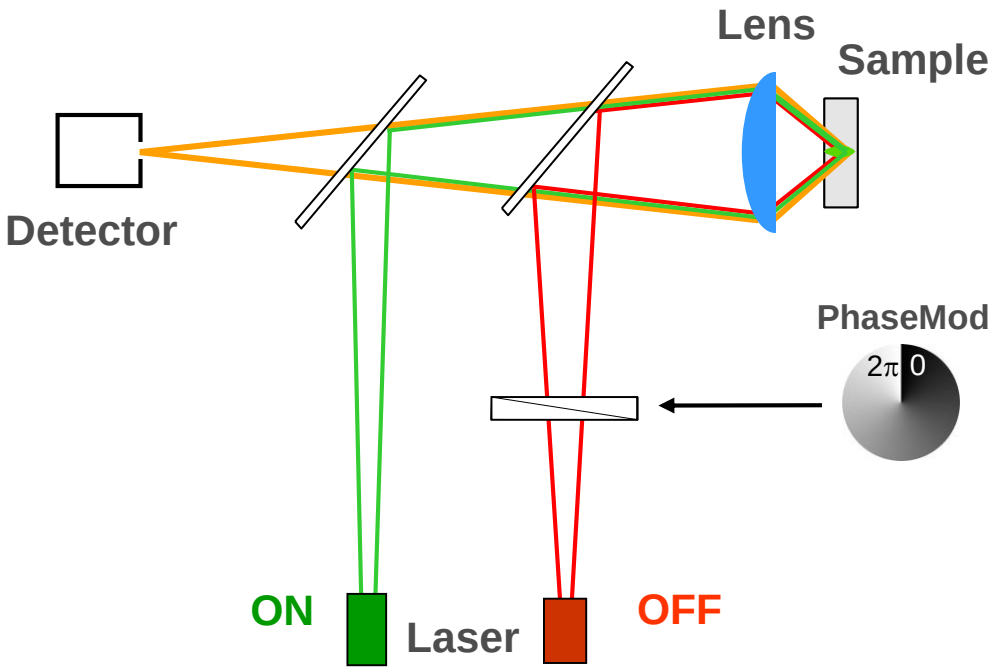

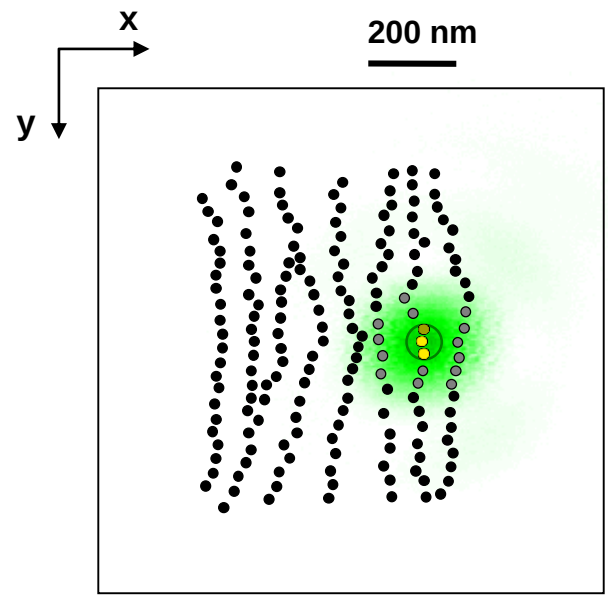

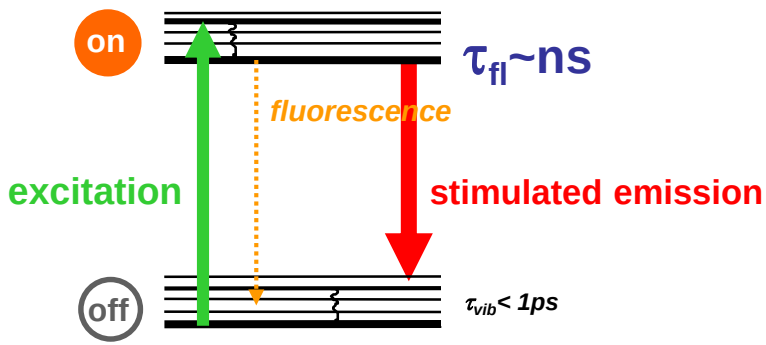

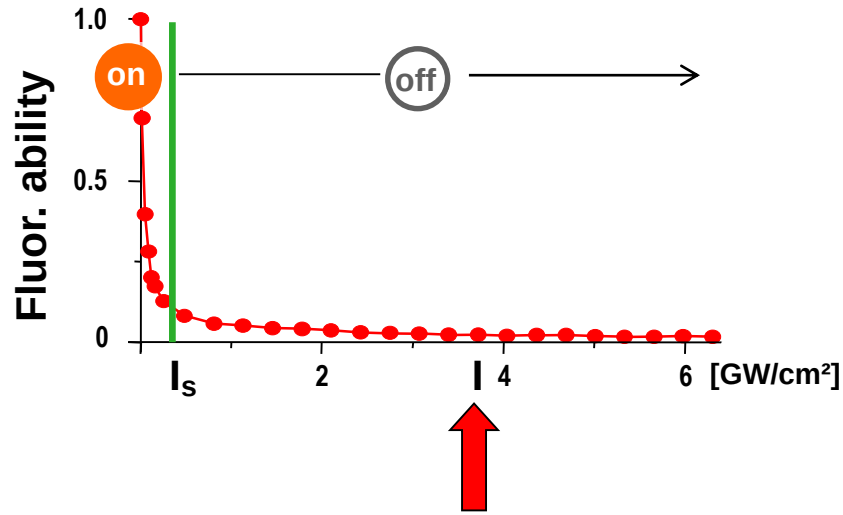

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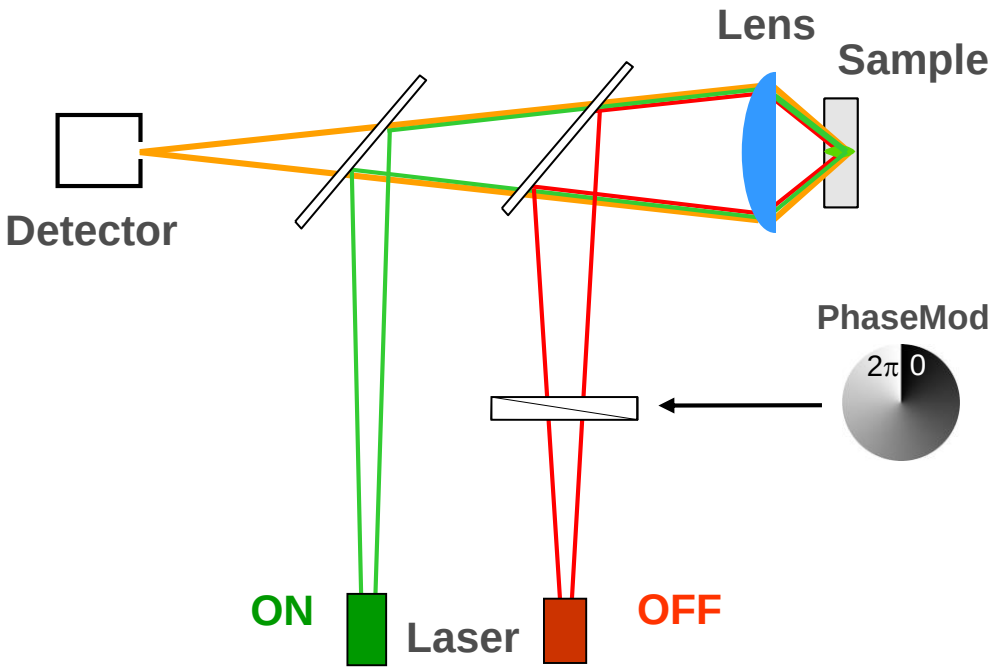
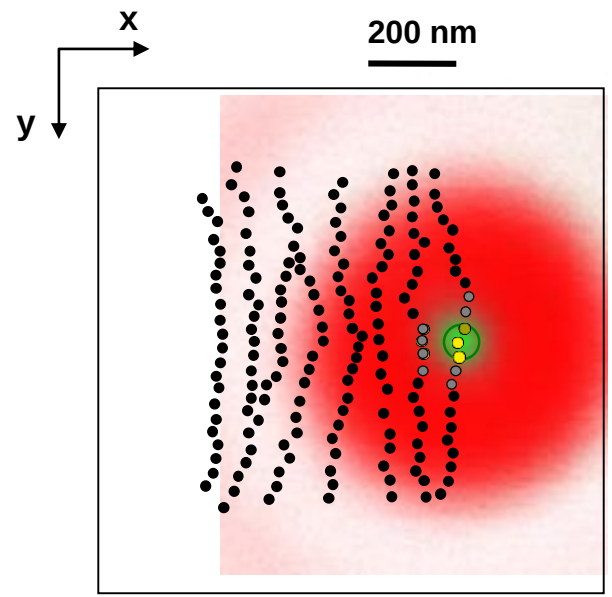
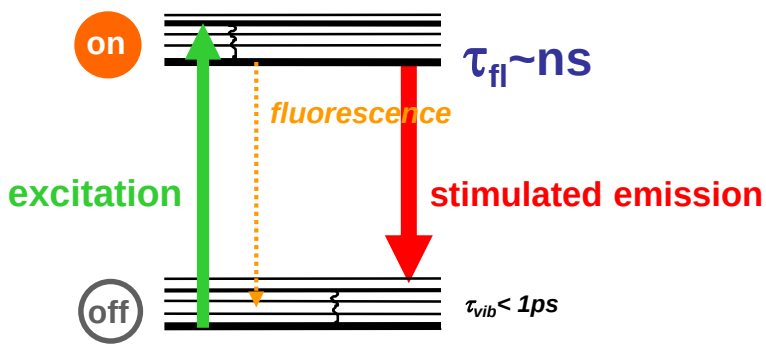
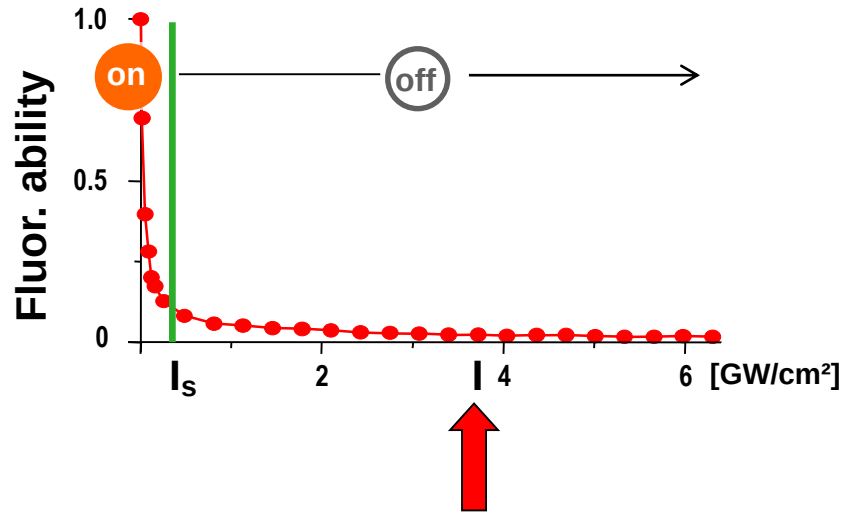
Hell & Wichmann, Opt. Lett. (1994)

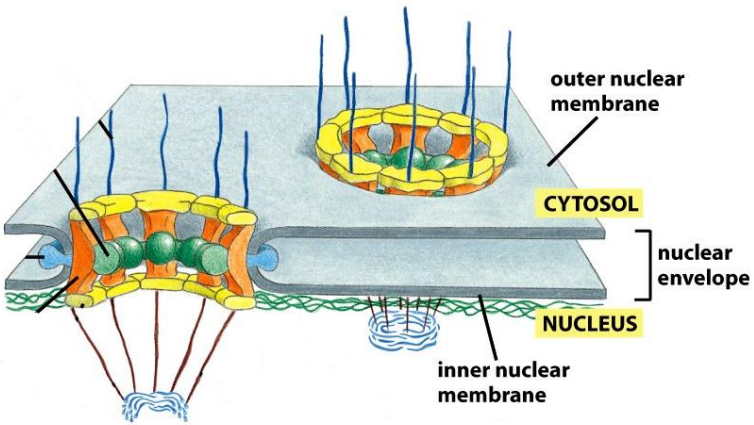






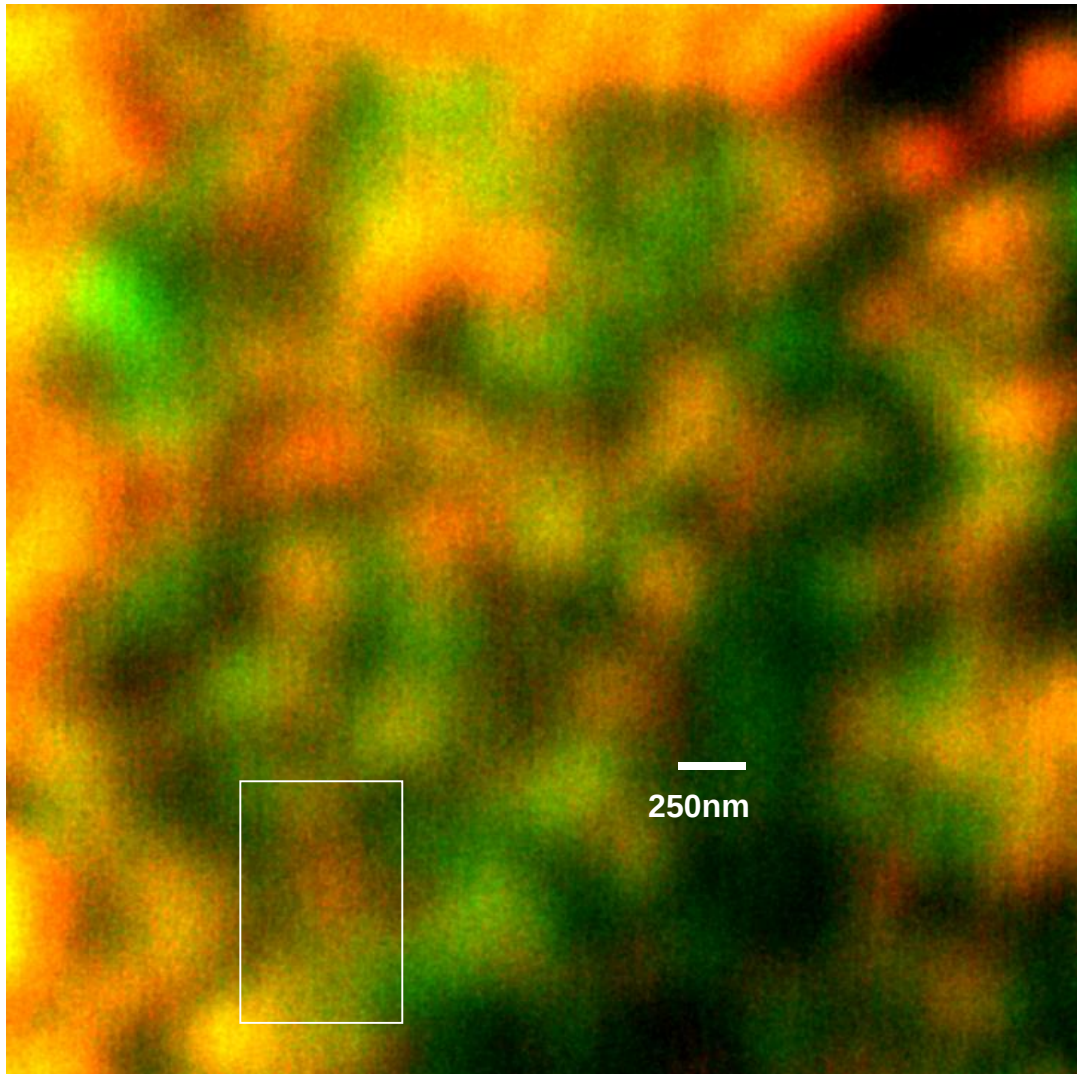


Protein assemblies in cell



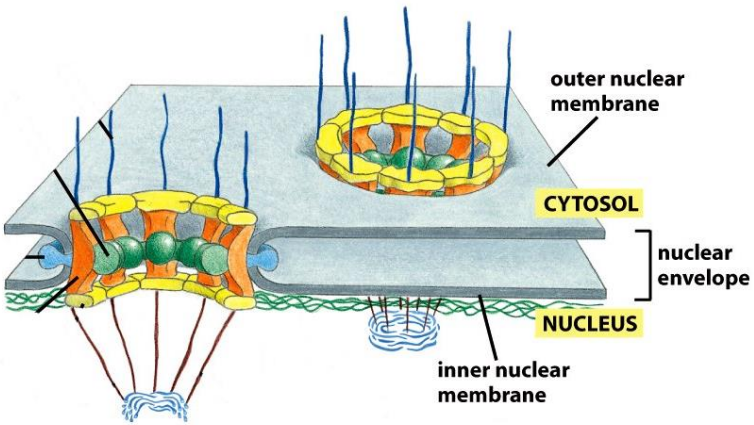
Nuclear pore complex

Standard (Confocal)



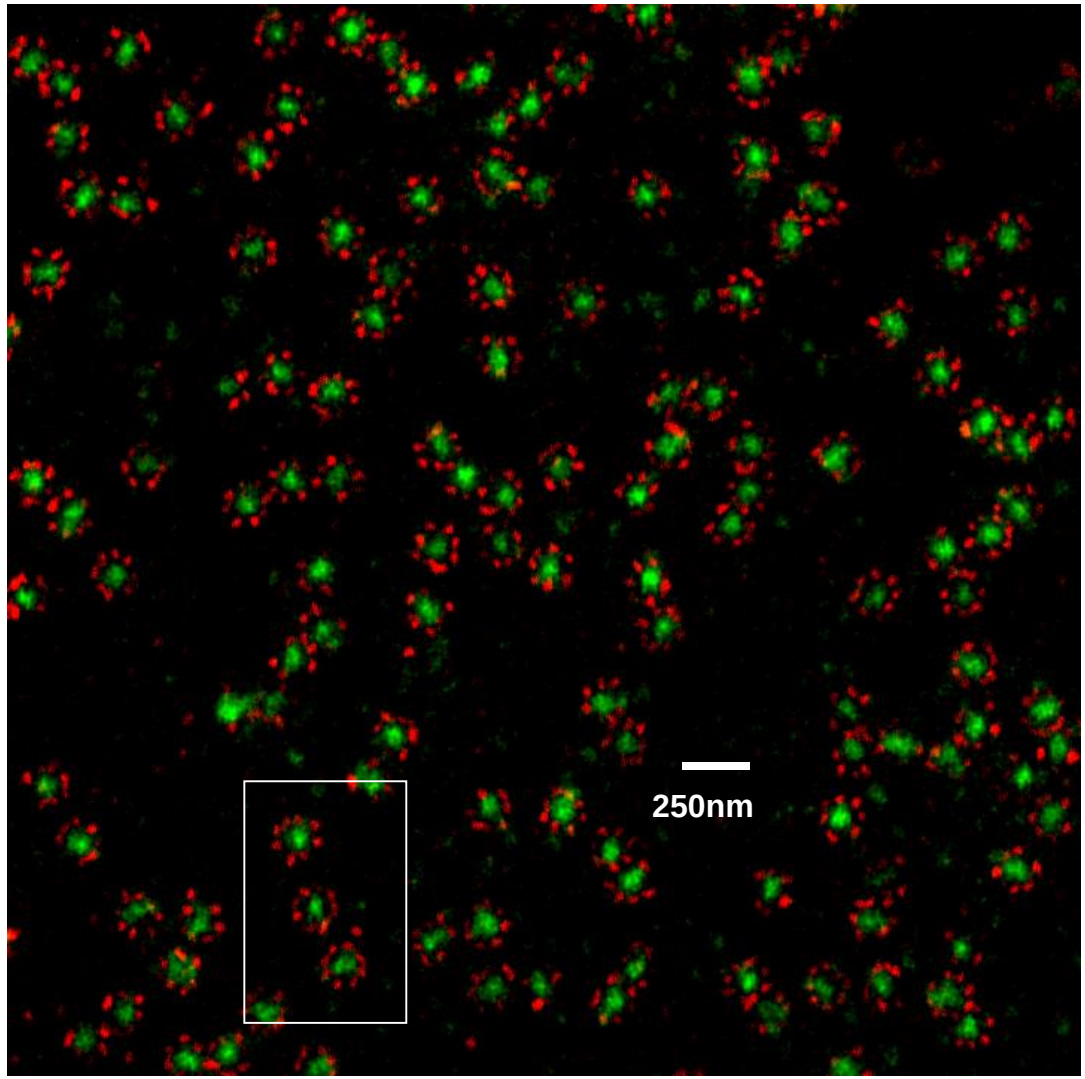
Göttfert, Wurm *et al* Biophys J (2013)

Protein assemblies in cell



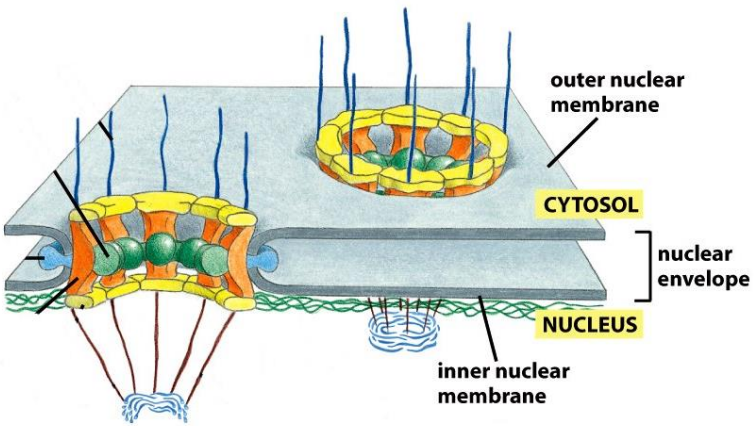
Nuclear pore complex

STED



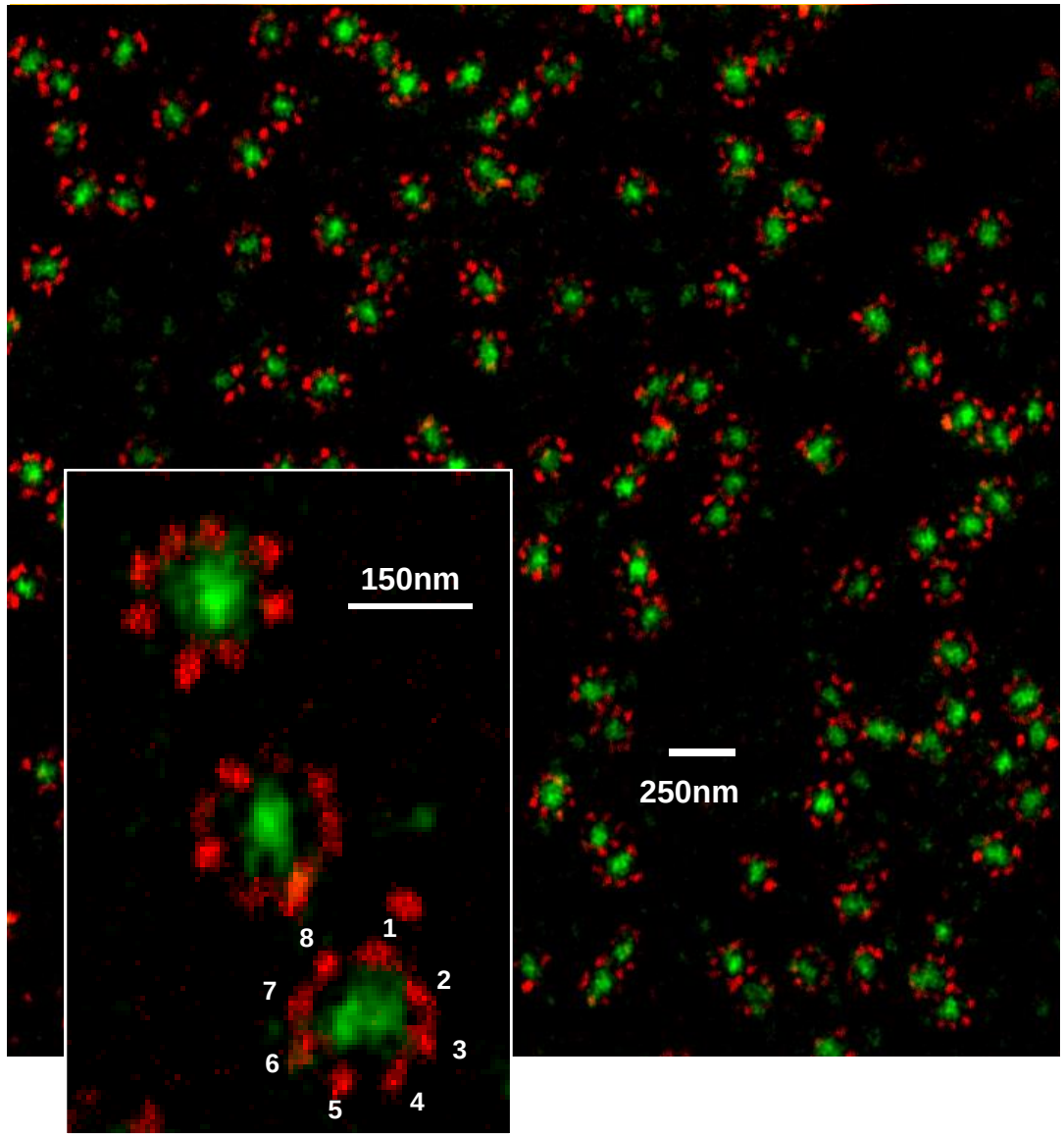
Göttfert, Wurm *et al* Biophys J (2013)

Protein assemblies in cell



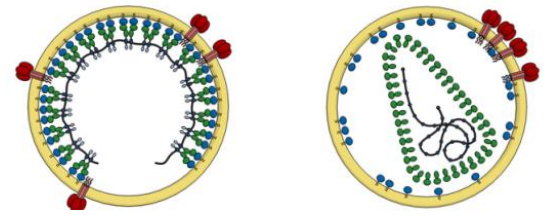
Nuclear pore complex

STED

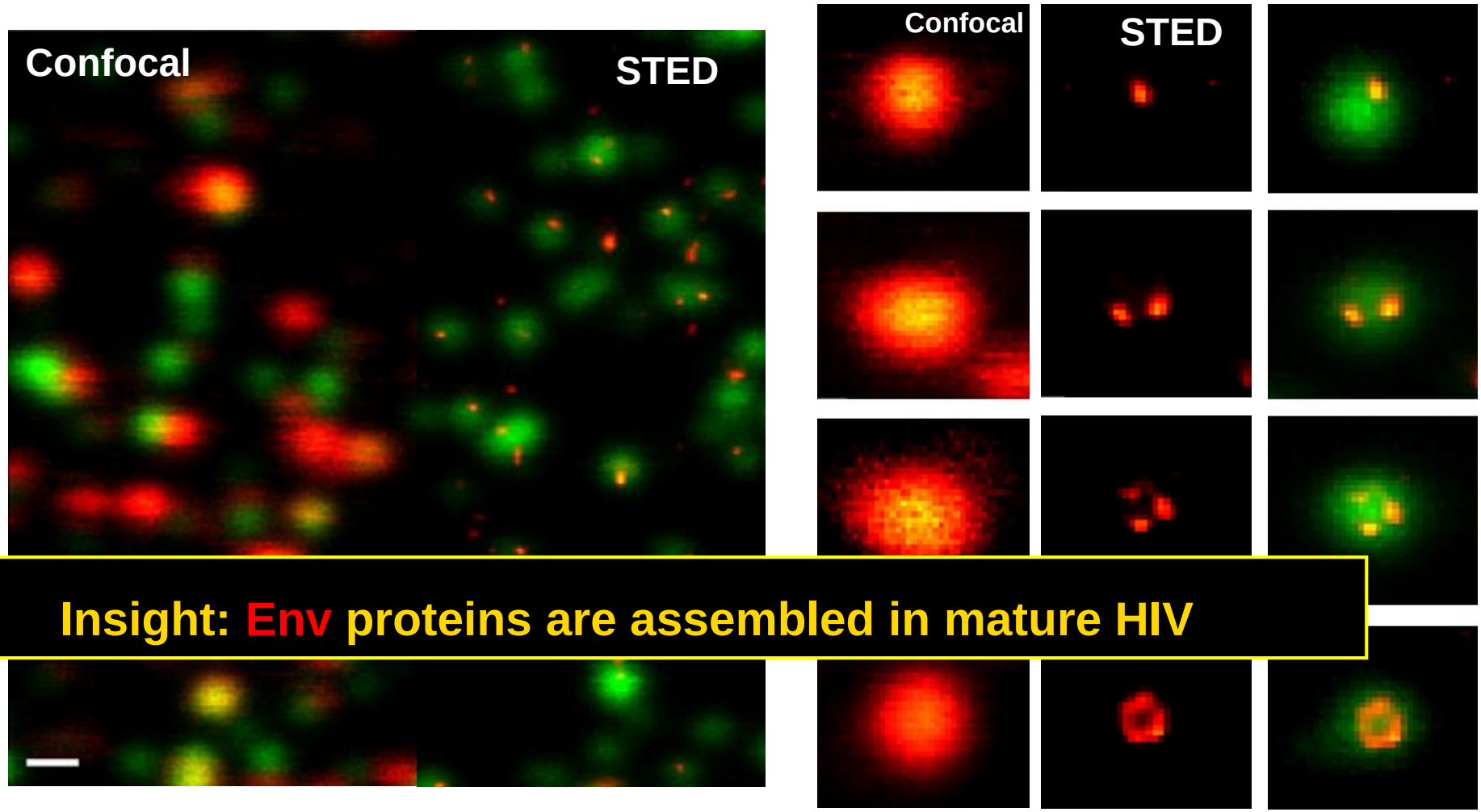


Göttfert, Wurm *et al* Biophys J (2013)

Viral infection



HIV Envelope protein on single virions



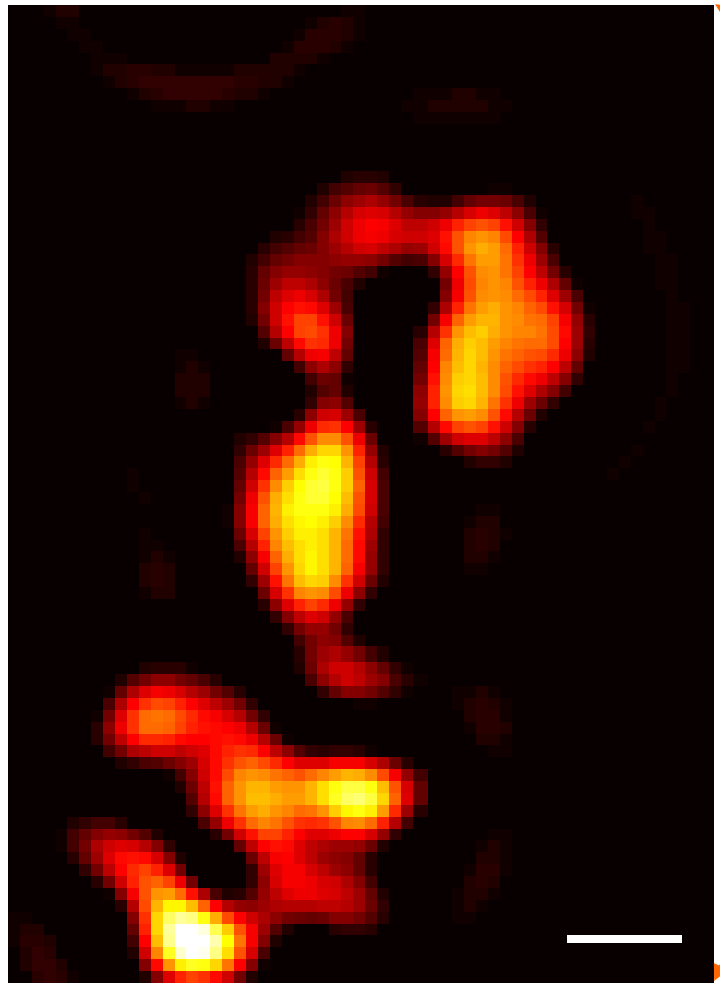
Insight: Env proteins are assembled in mature HIV

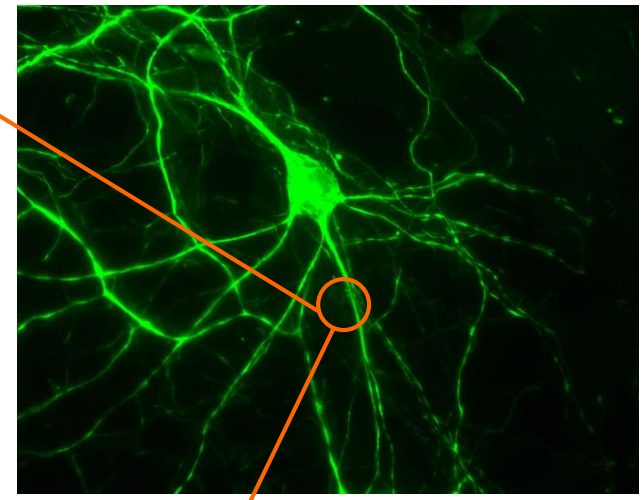
HIV (Vpr.eGFP)
Env (Ab 2G12)

J Chojnacki,..,SWH, HG Kräusslich, *Science* (2012)

Synaptic vesicles in axon of living hippocampal neuron

Standard (Confocal) snapshot





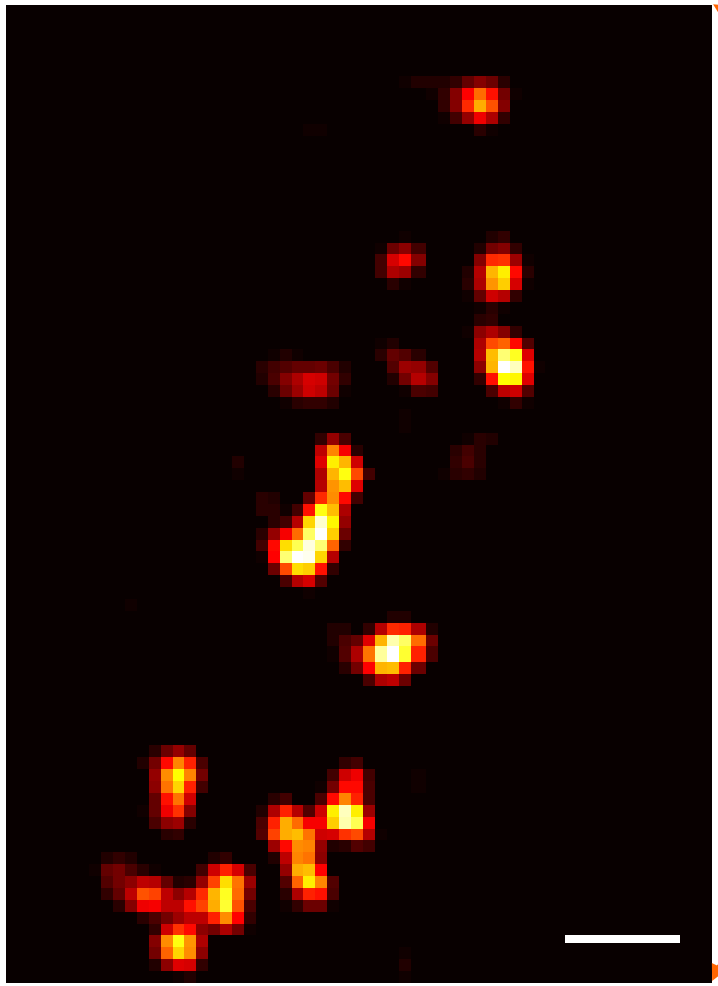
Synaptotagmin immunostained

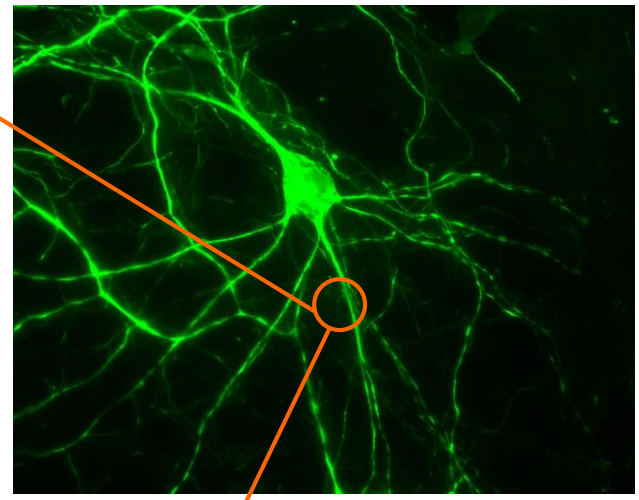
Scale: 300 nm

Westphal, Rizzoli, Lauterbach, Jahn, SWH, Science (2008)

Synaptic vesicles in axon of living hippocampal neuron

Video rate STED





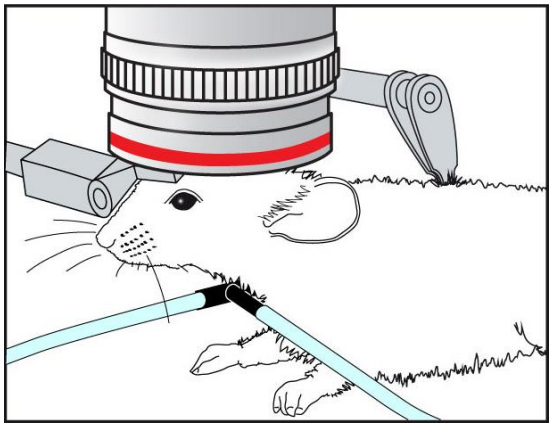
Synaptotagmin immunostained

28 frames/ second

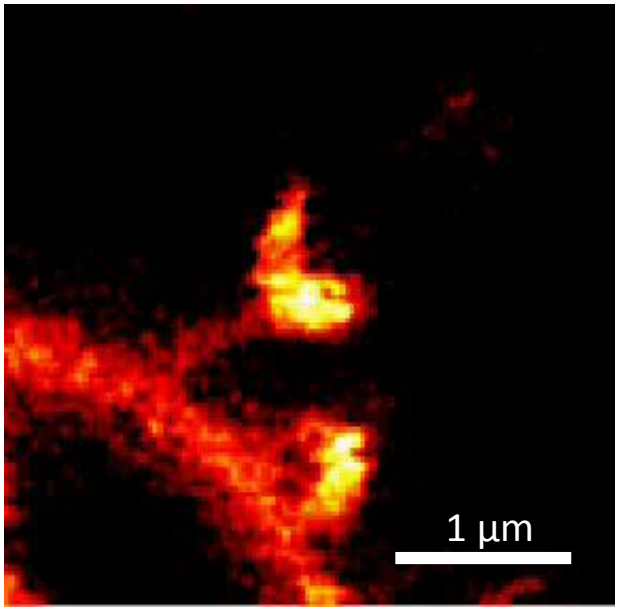
Scale: 300 nm

Westphal, Rizzoli, Lauterbach, Jahn, SWH, Science (2008)

Neurophysiology



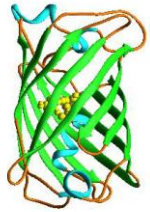
Cortical neurons expressing cytoplasmic EYFP



Berning et al, *Science* (2012)

STED

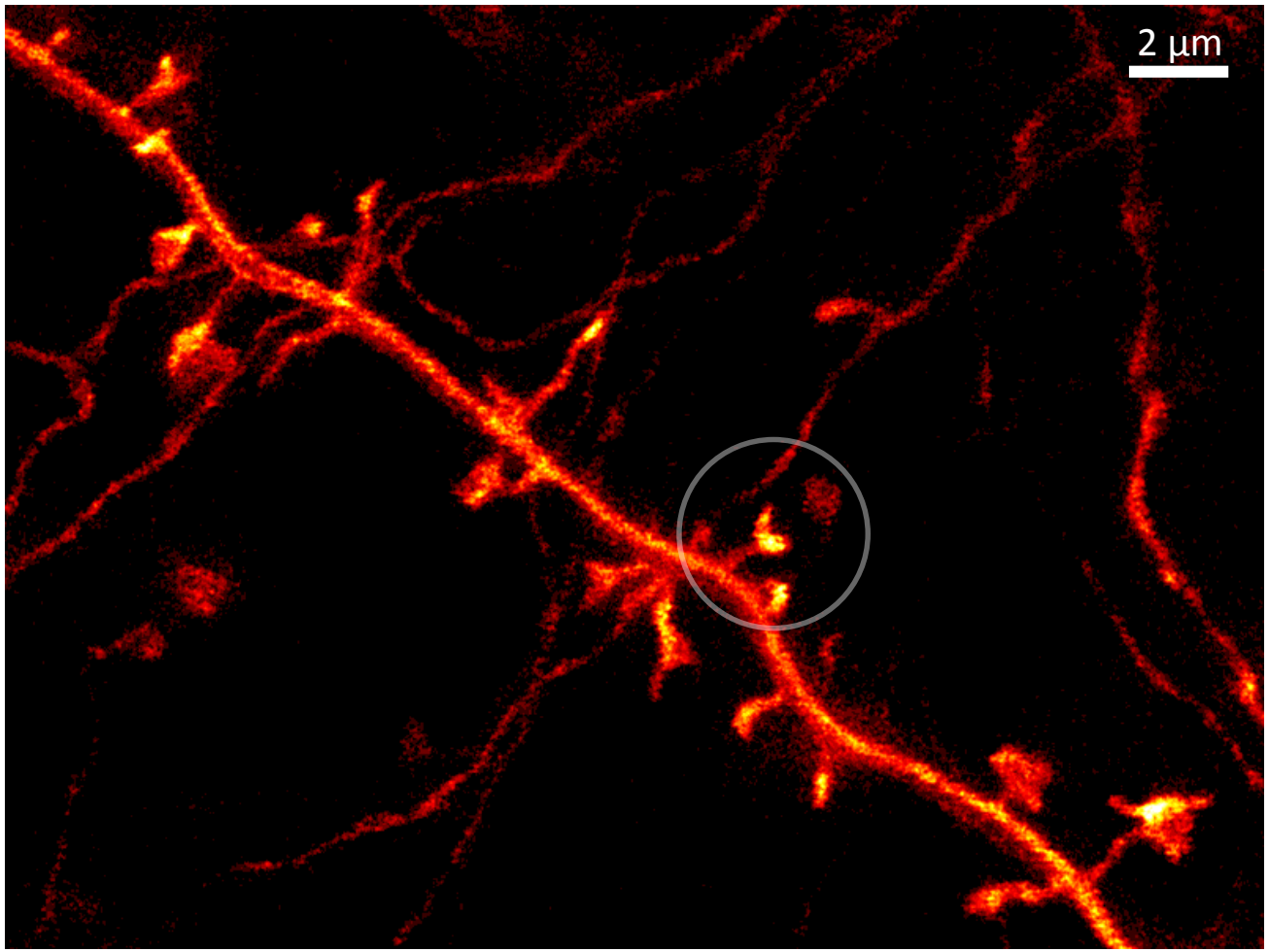
YFP



in living mouse brain

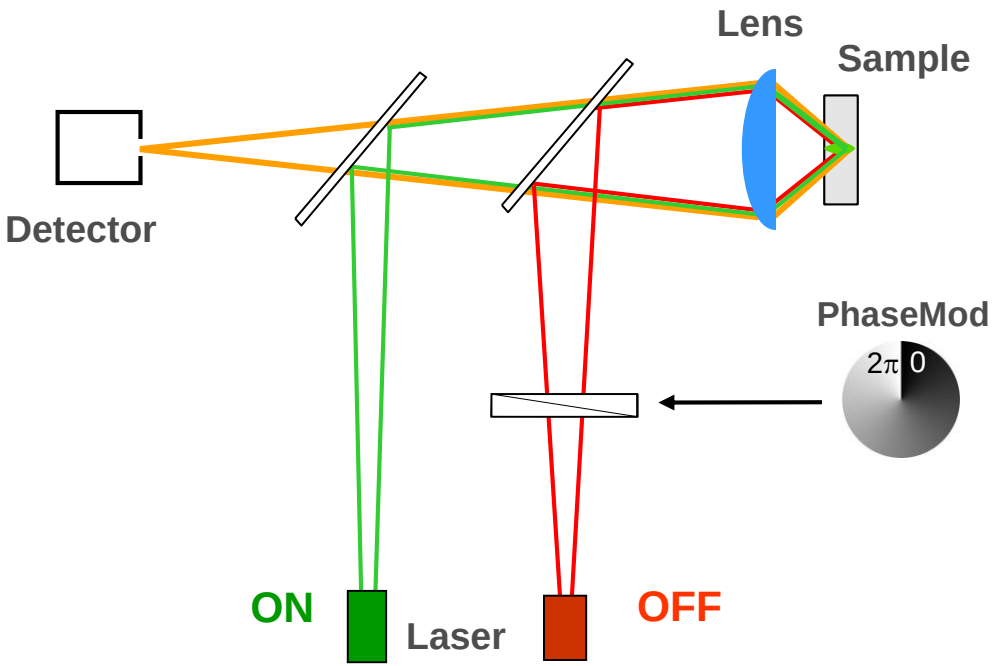
23 x 18 x 3 µm, 10µs / px, 800 x 600 x 5 px, interval 5 min

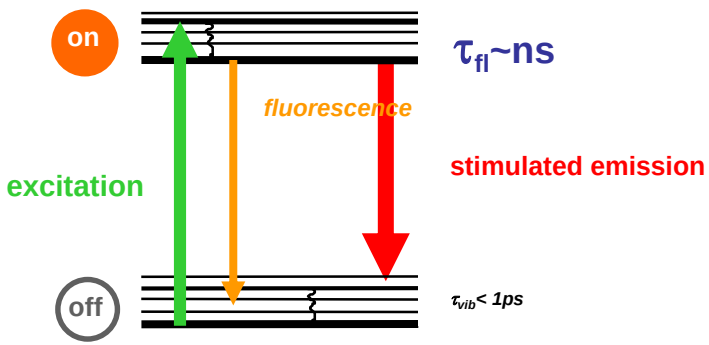
~20 µm deep

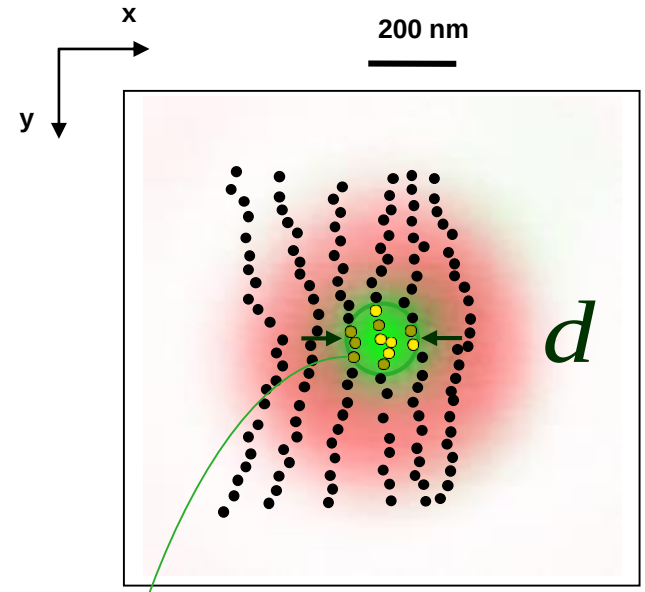


The resolution

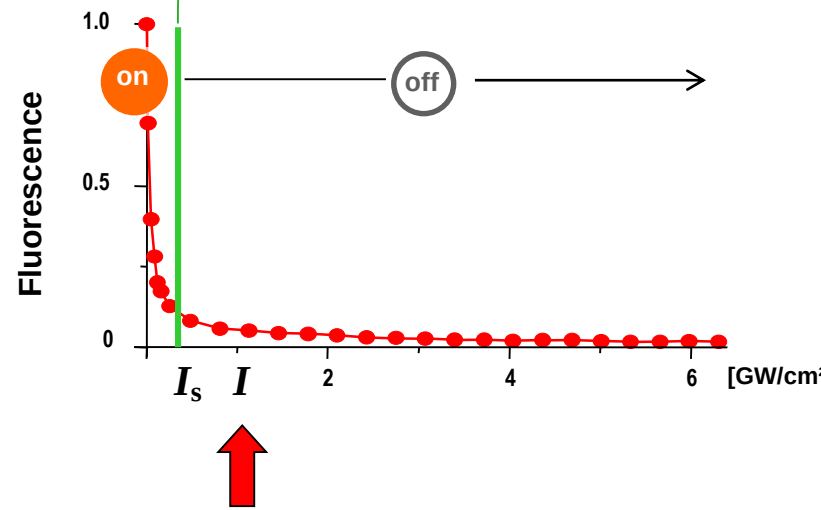
STED microscope:







$$d \approx \frac{\lambda}{2n \sin \alpha}$$



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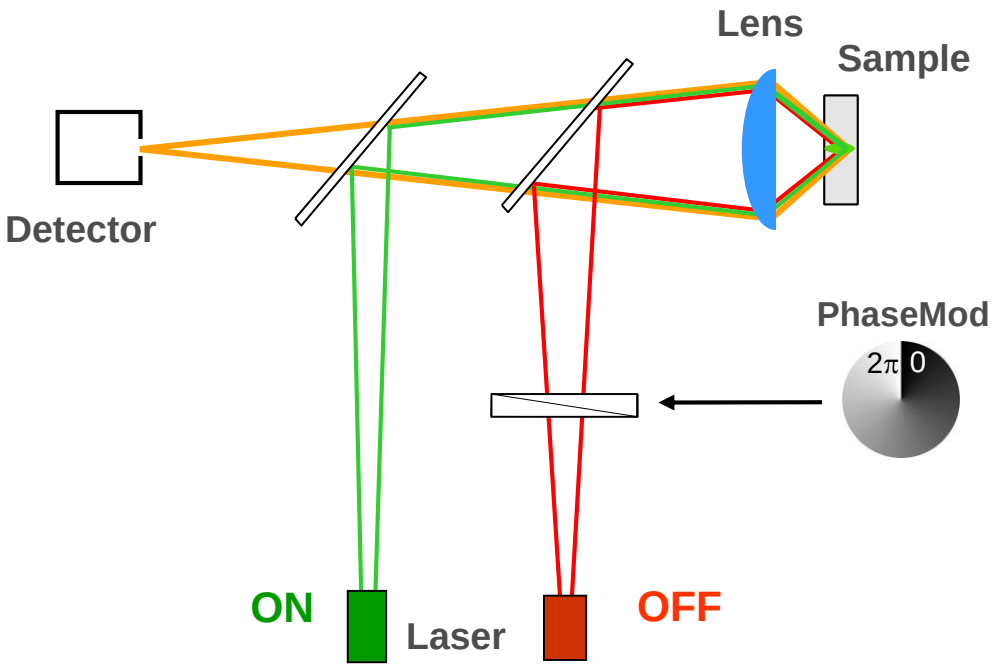

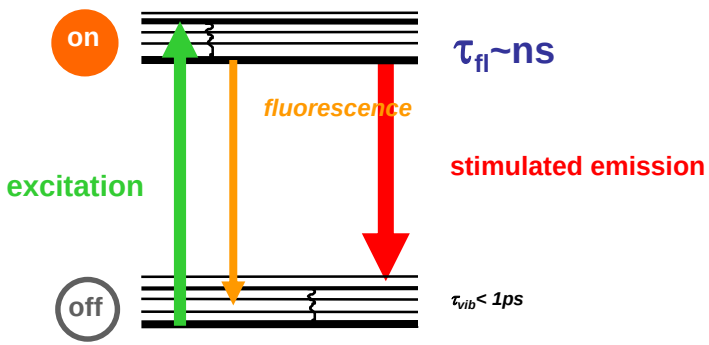

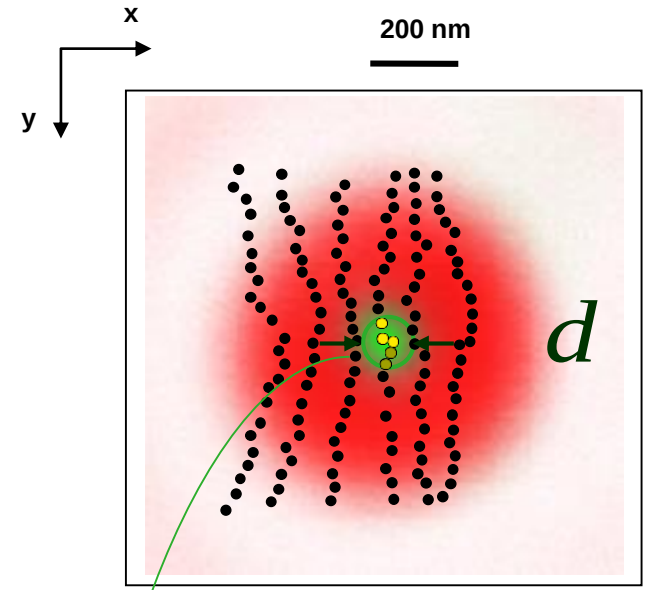

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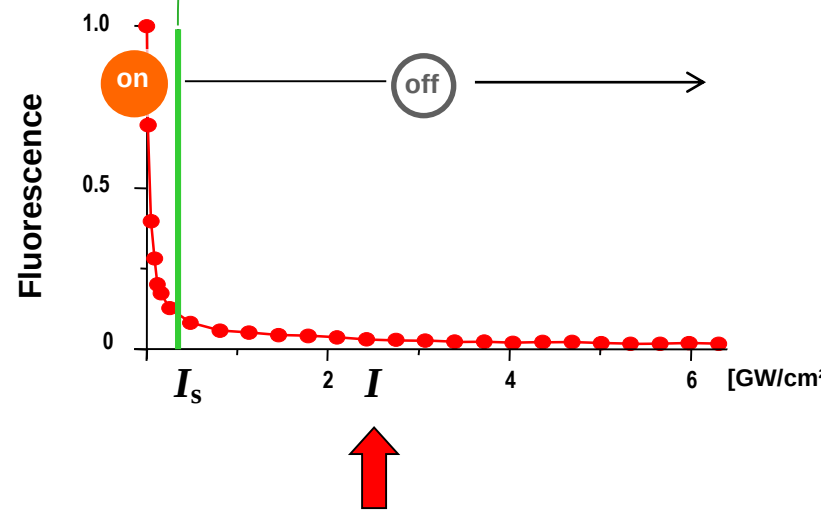

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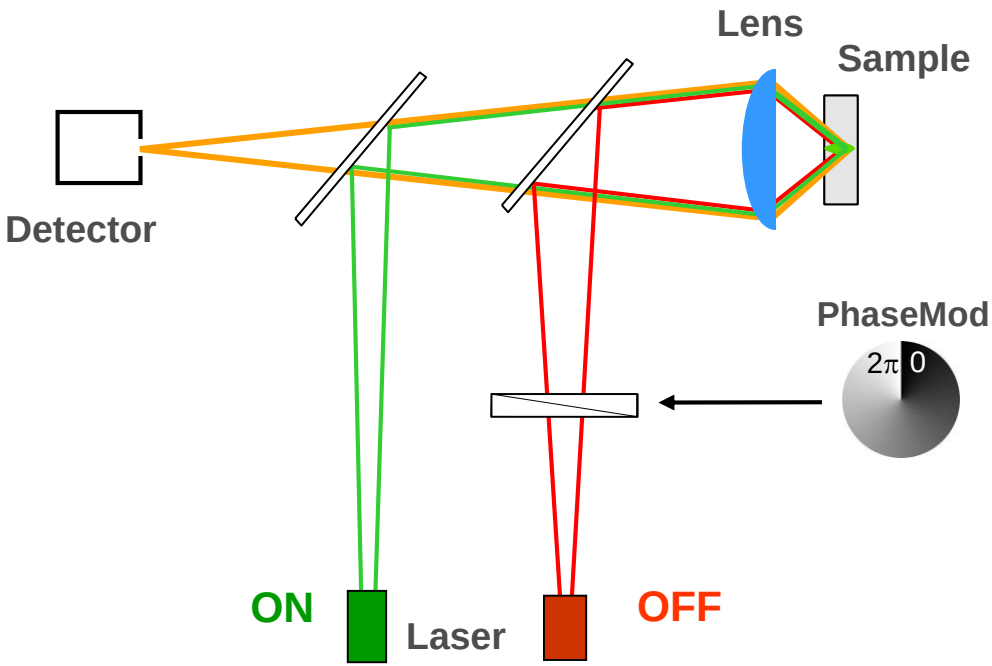
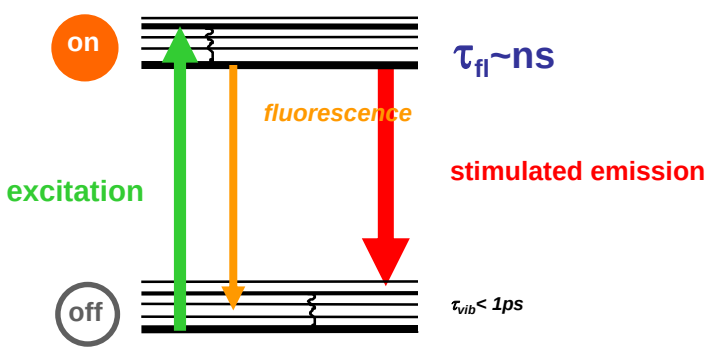
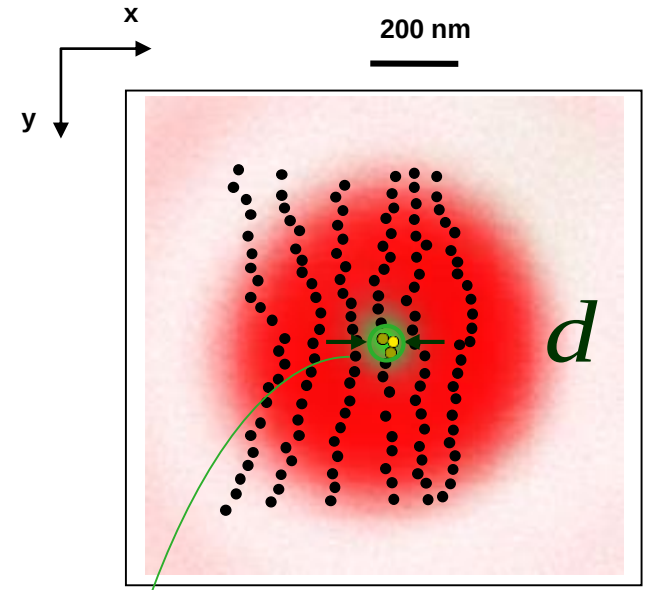
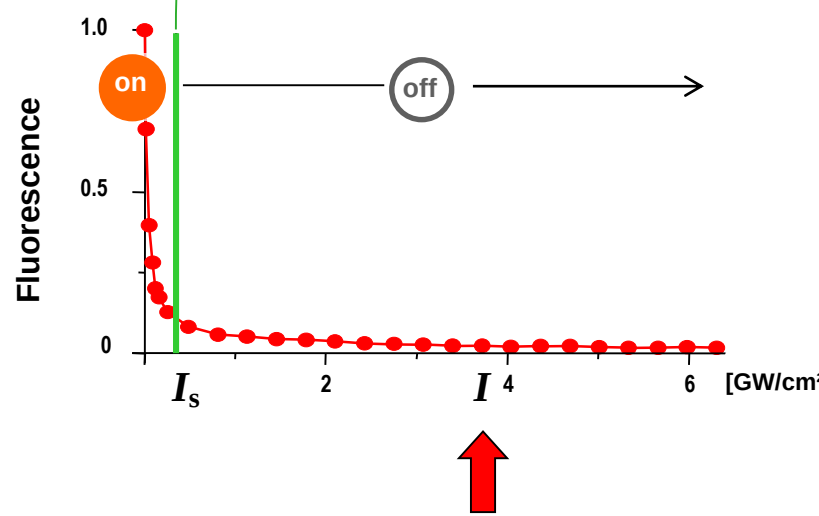


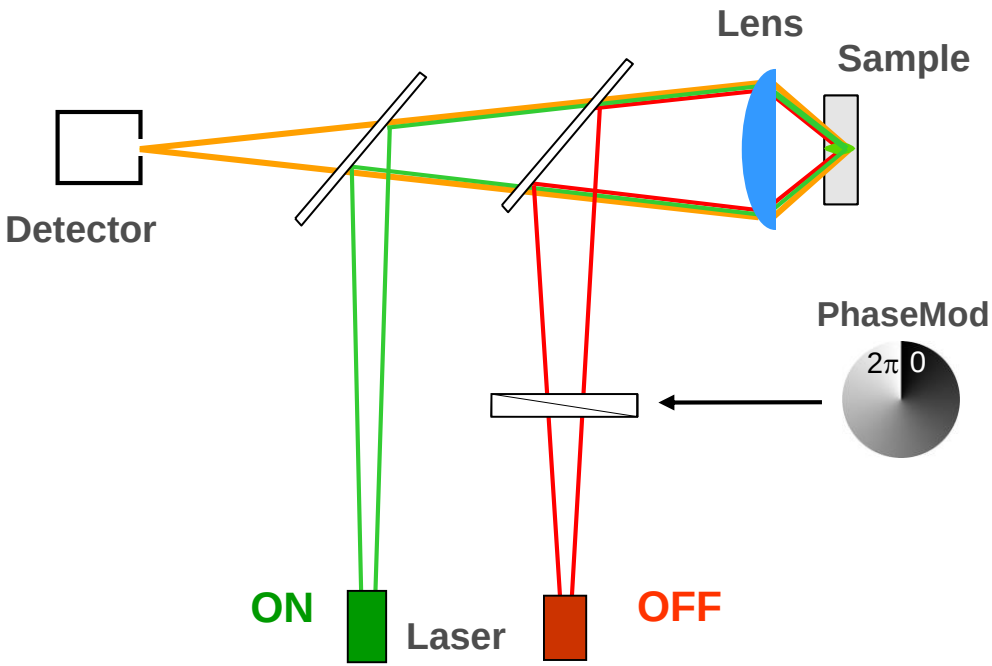


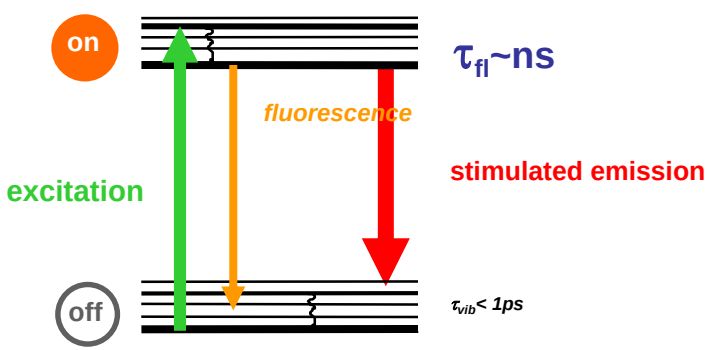


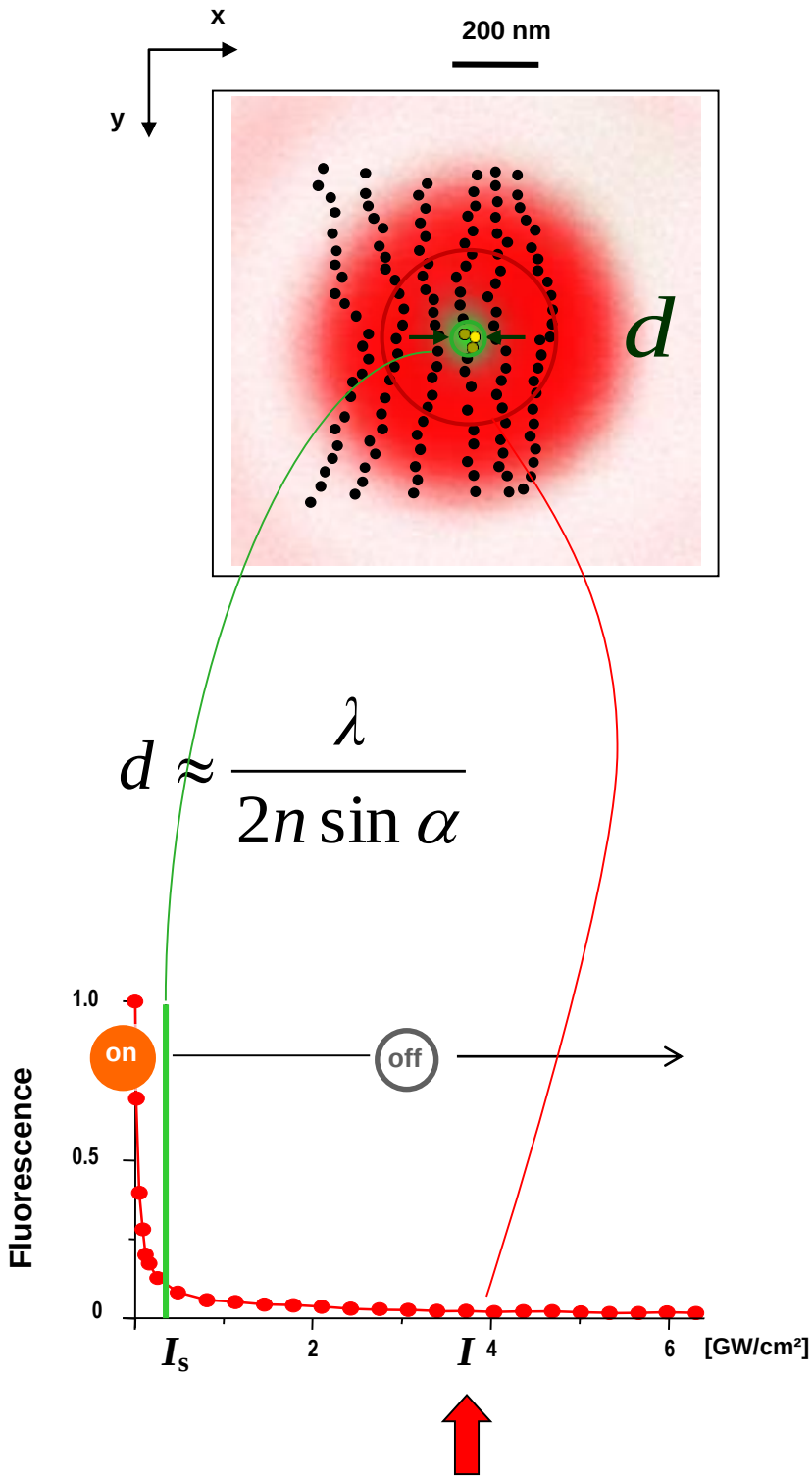
$$d \approx \frac{\lambda}{2n \sin \alpha}$$



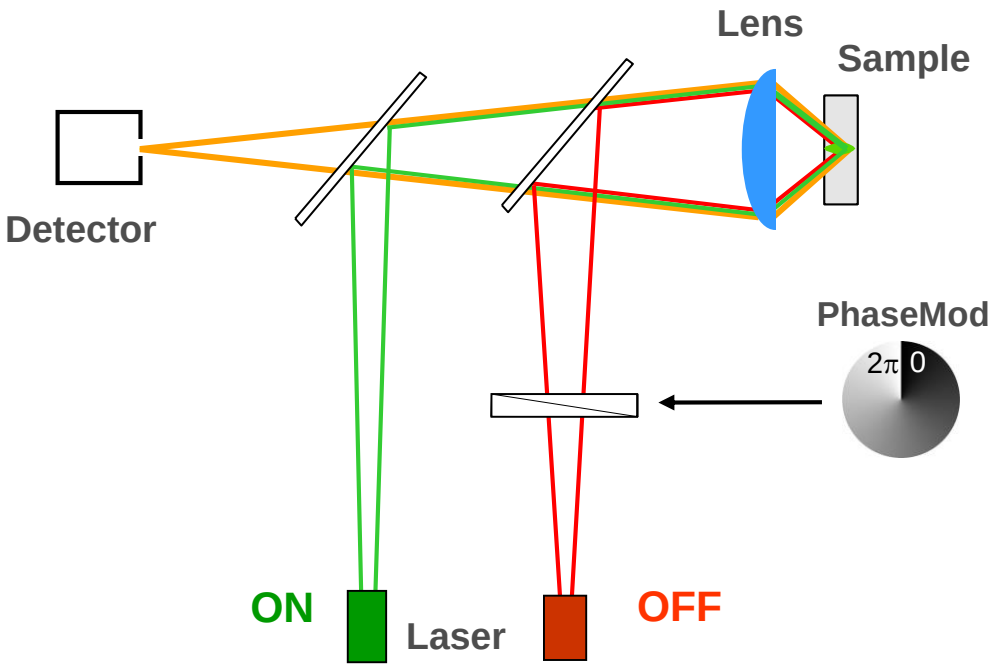
STED microscope:

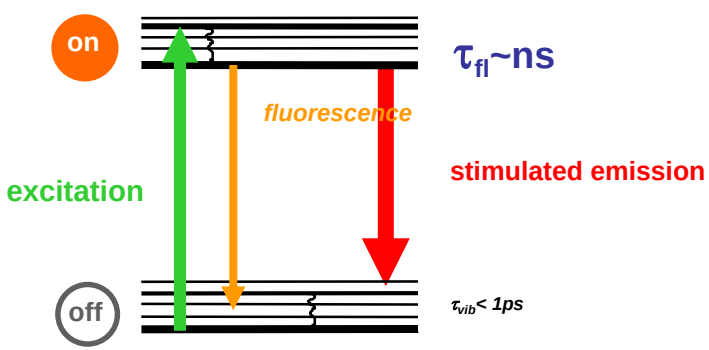


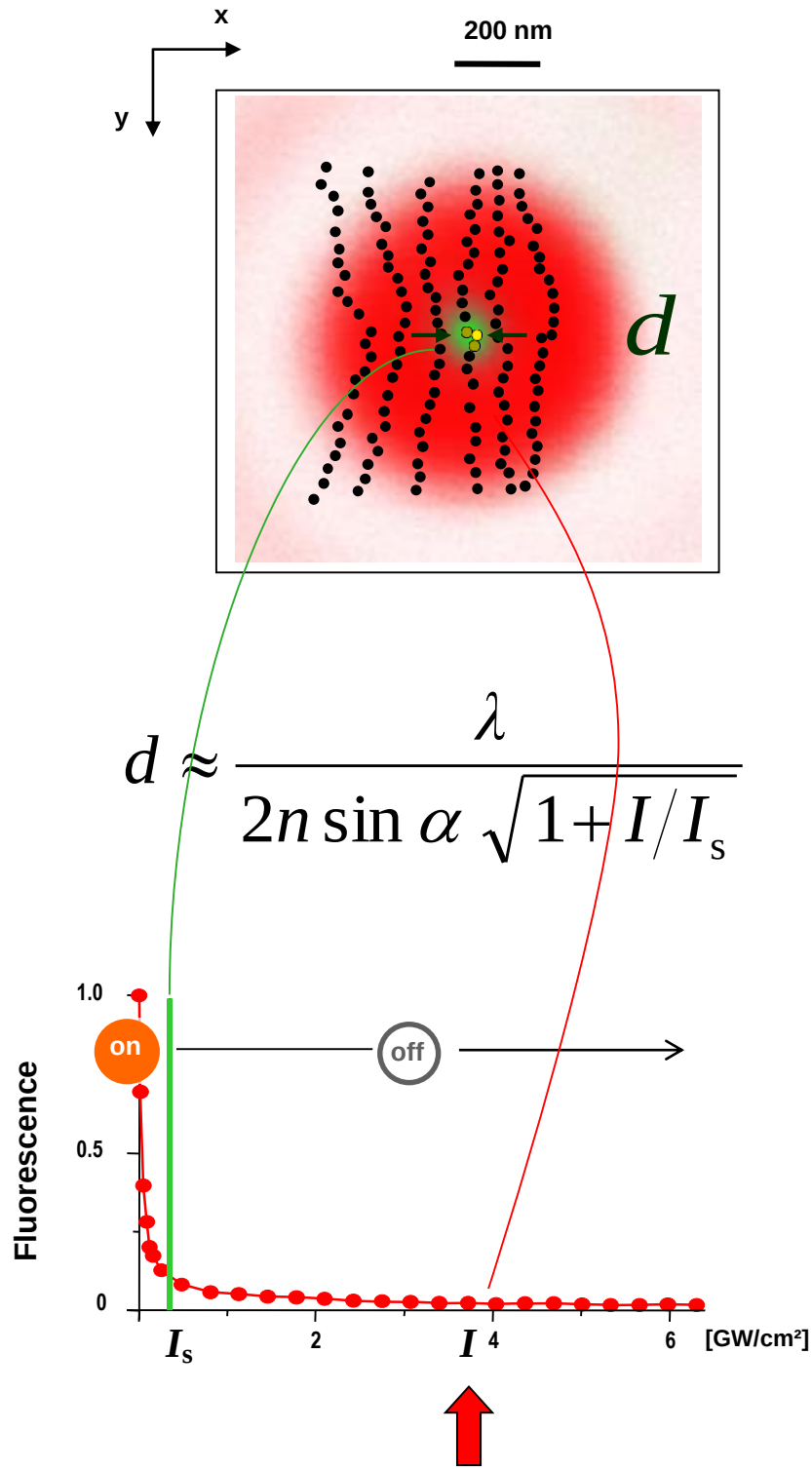




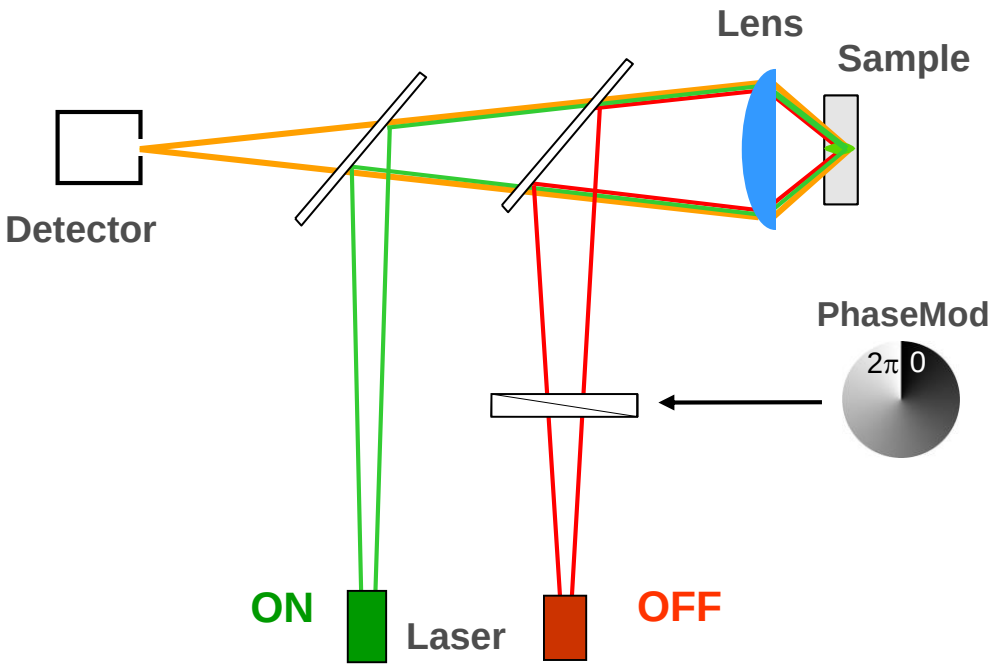
STED microscope:

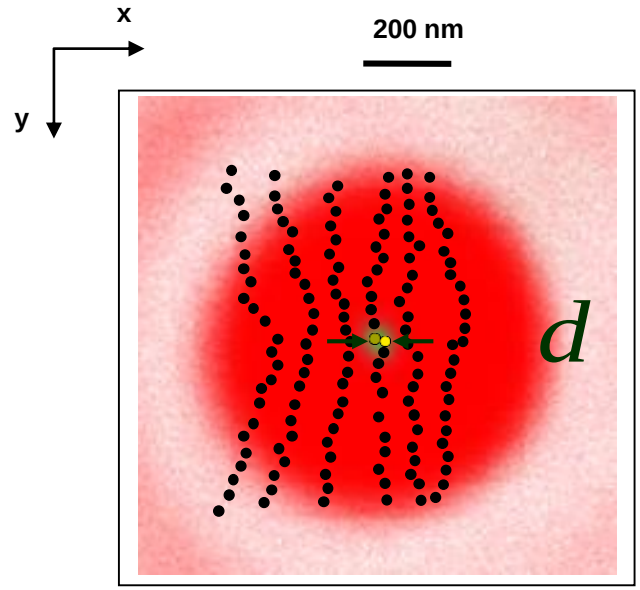




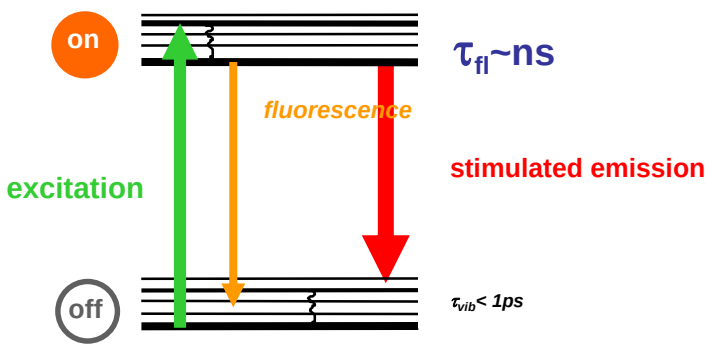


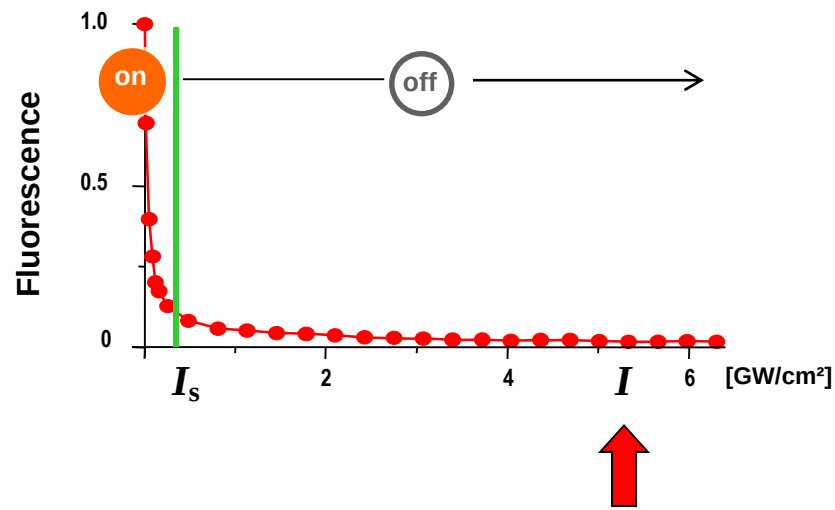
STED microscope:



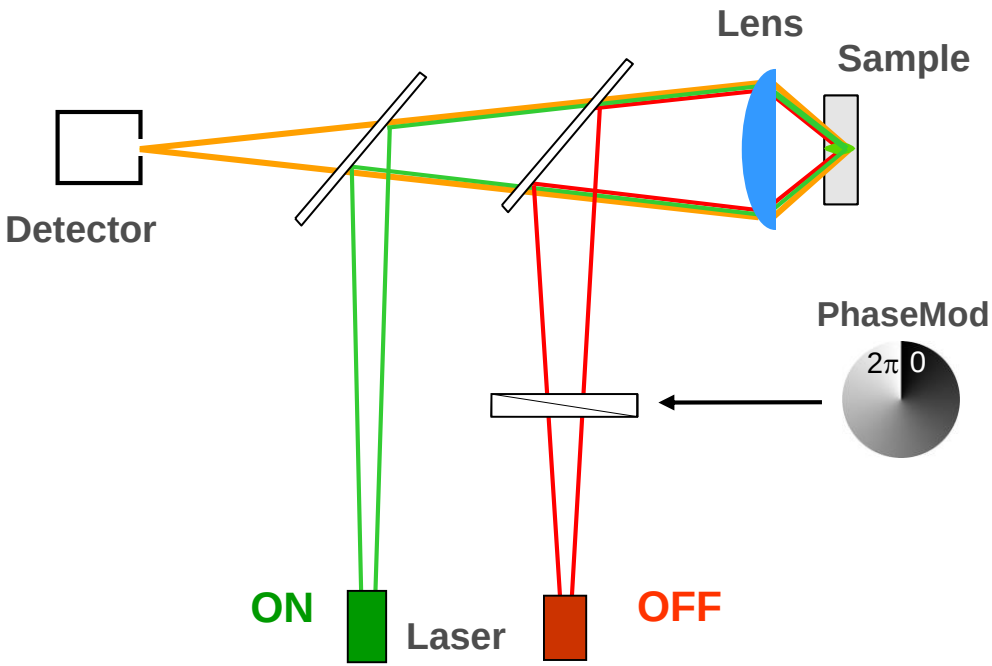


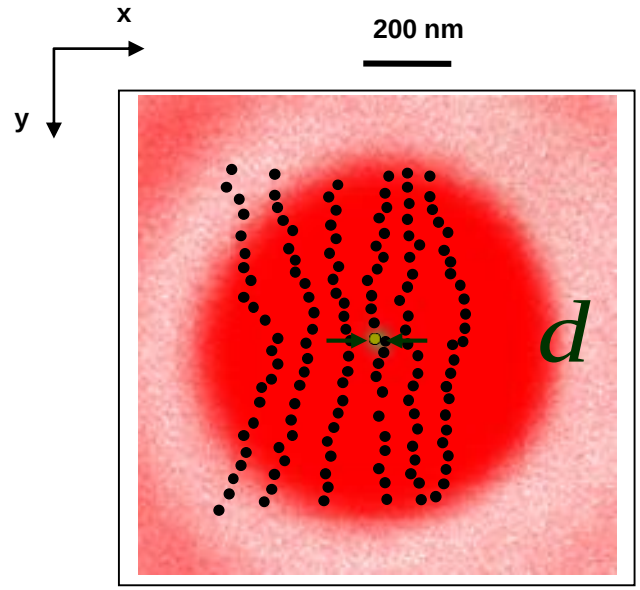
$$d \approx \frac{\lambda}{2n \sin\alpha \sqrt{1 + I/I_s}} \rightarrow 0$$



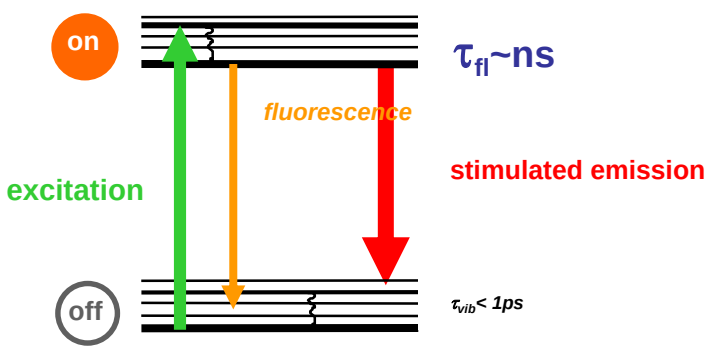


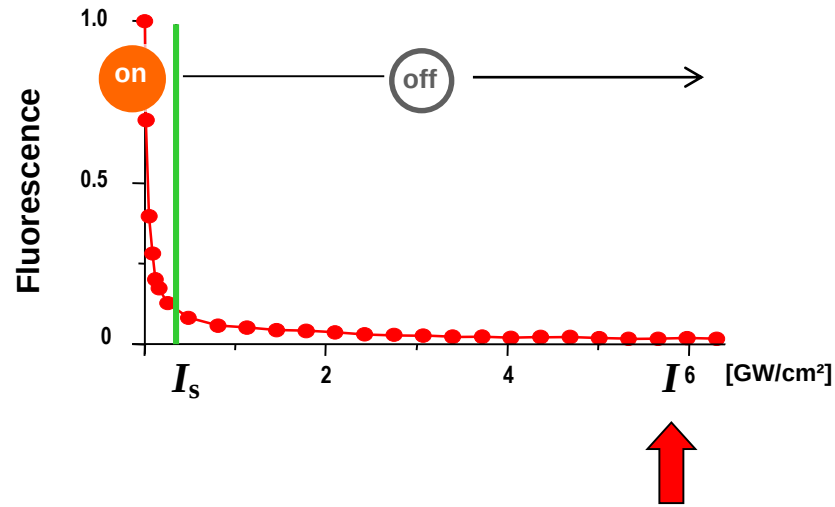
STED microscope:



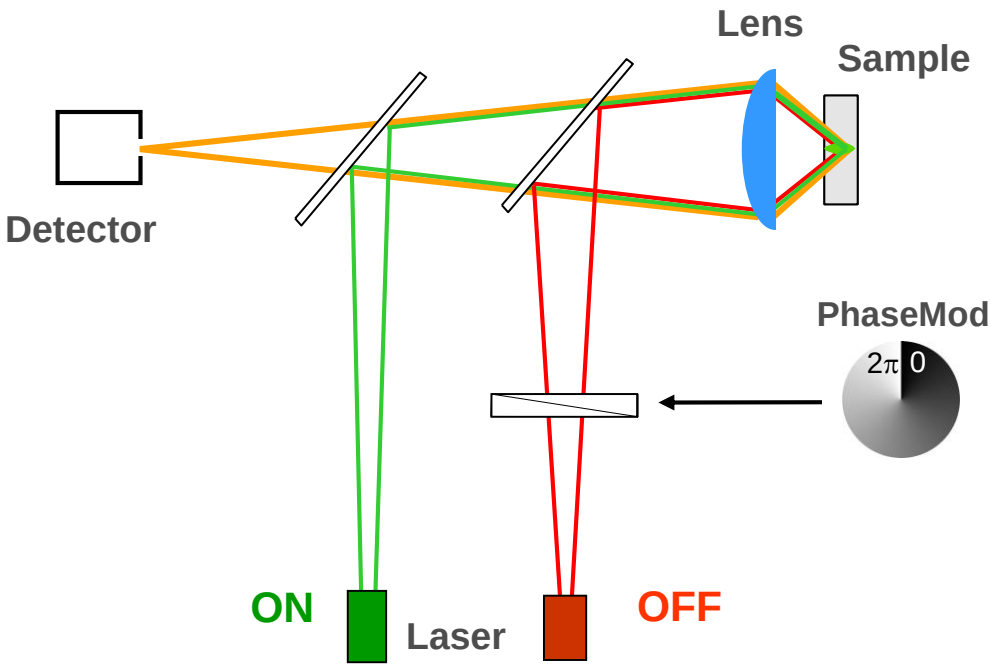


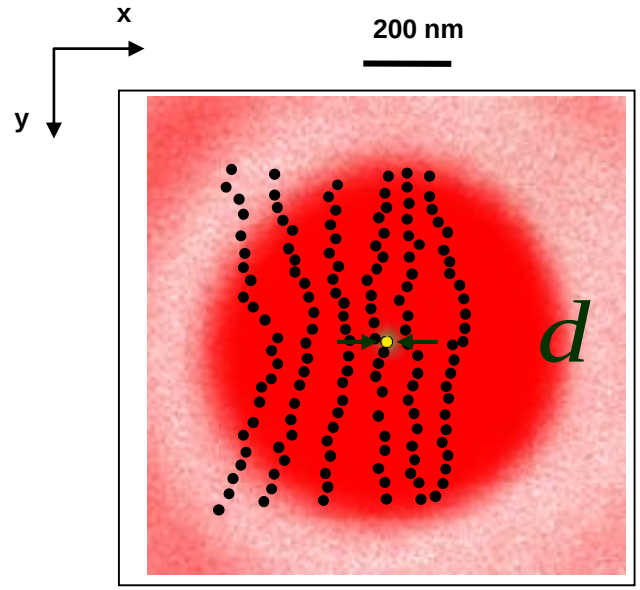
$$d \approx \frac{\lambda}{2n \sin\alpha \sqrt{1 + I/I_s}} \rightarrow 0$$



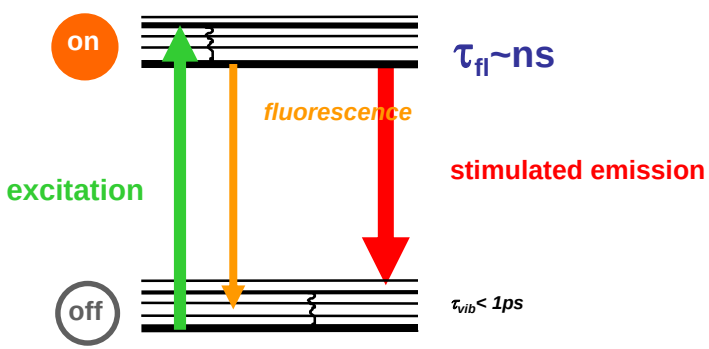


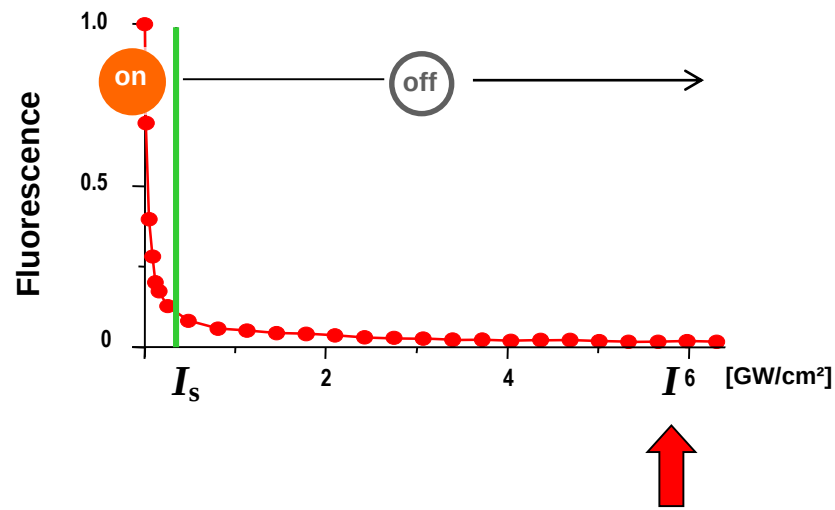
STED microscope:



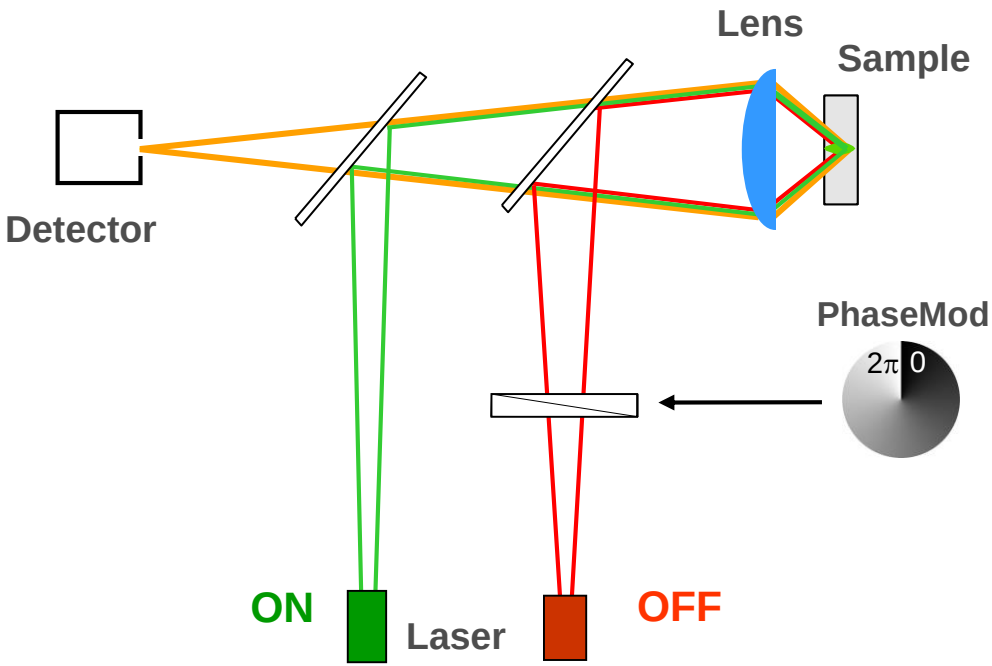


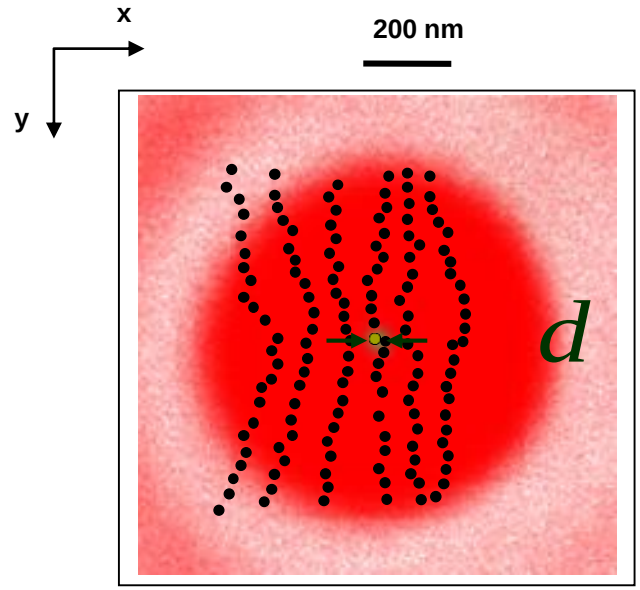
$$d \approx \frac{\lambda}{2n \sin\alpha \sqrt{1 + I/I_s}} \rightarrow 0$$



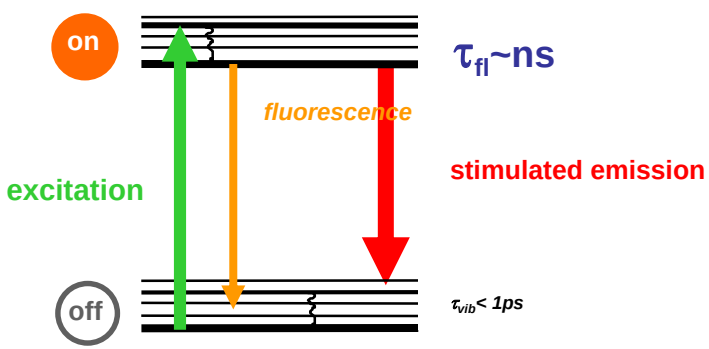


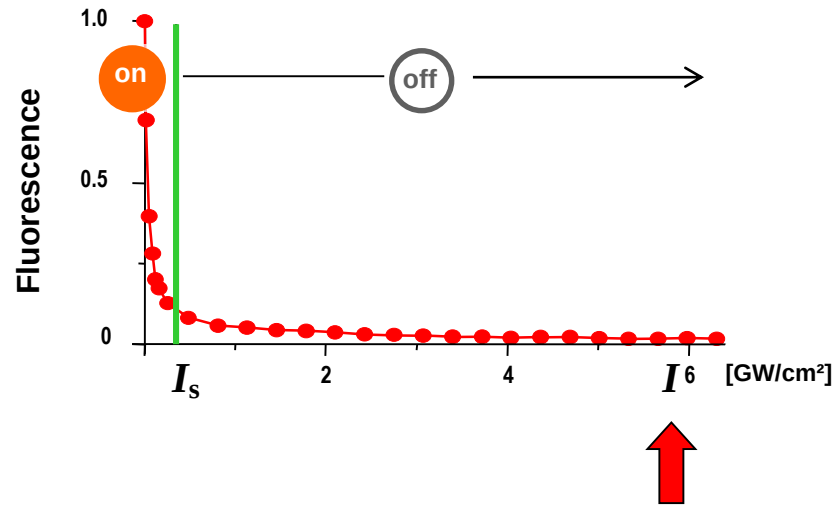
STED microscope:





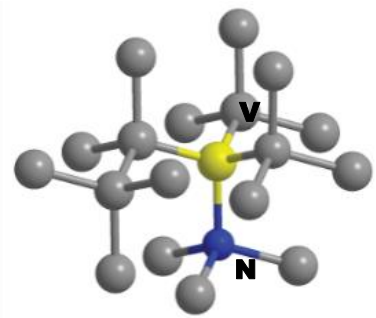
$$d \approx \frac{\lambda}{2n \sin\alpha \sqrt{1 + I/I_s}} \rightarrow 0$$



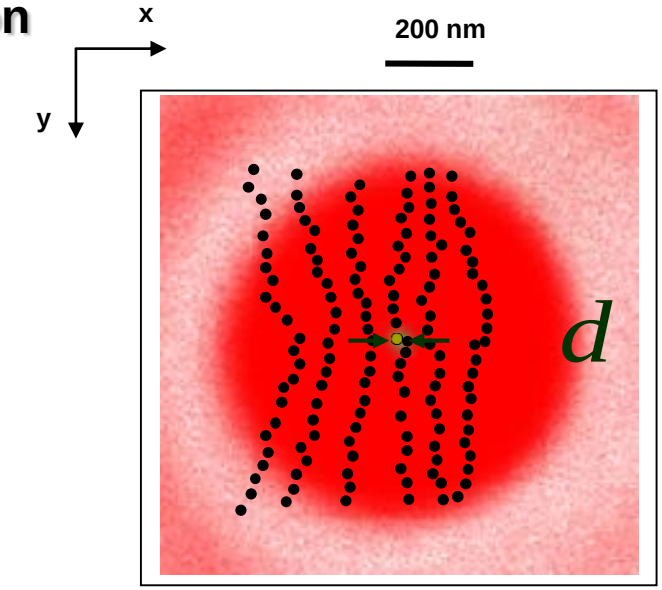


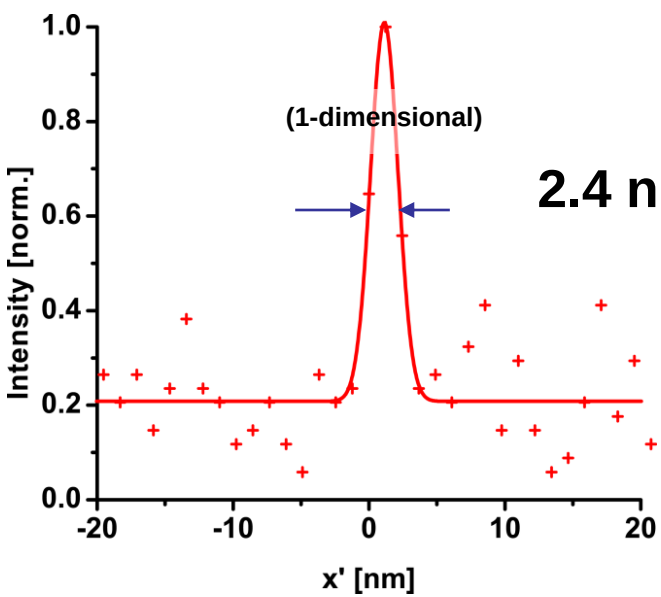
Material sciences, magnetic sensing, quantum information

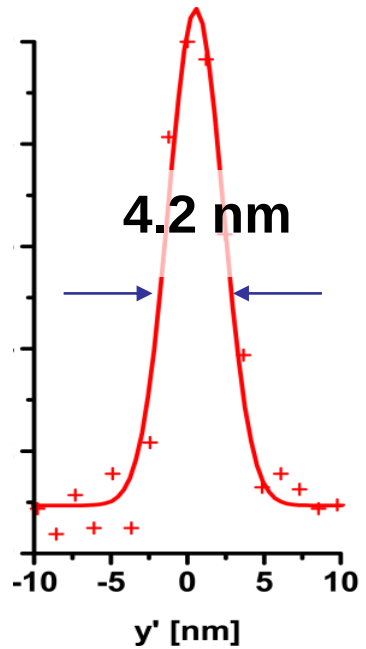
NV$^-$ in diamond

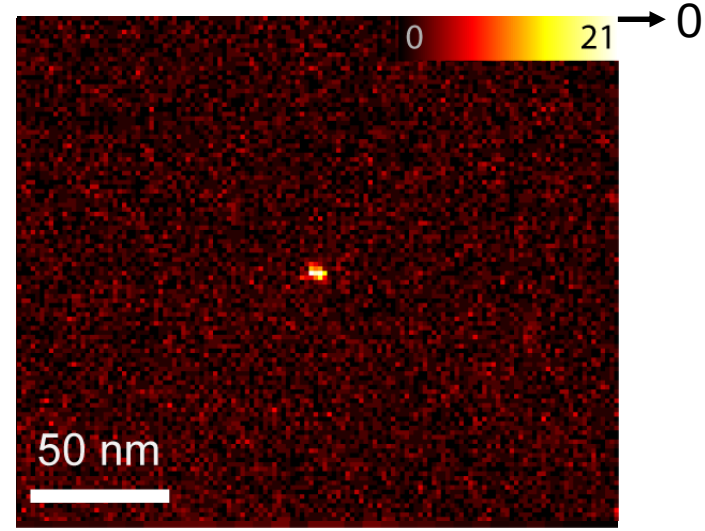


Color centers





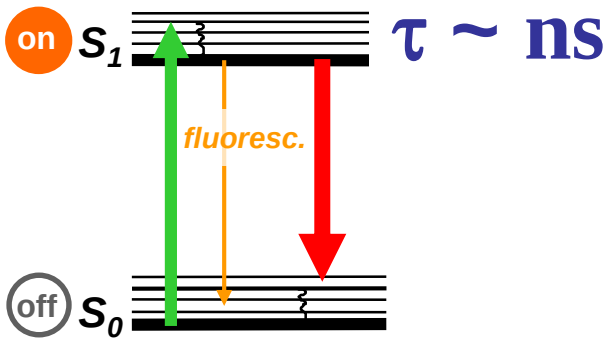




λ = 775 nm

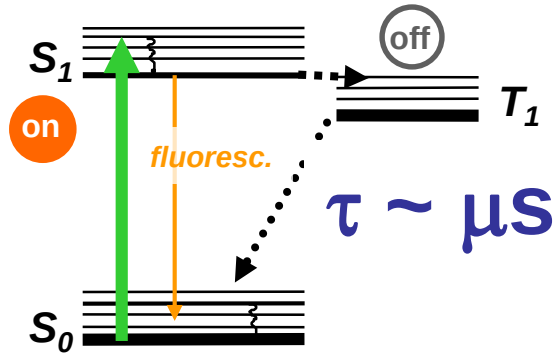
Principle: Discern by **ON / OFF** states in the sample

STED



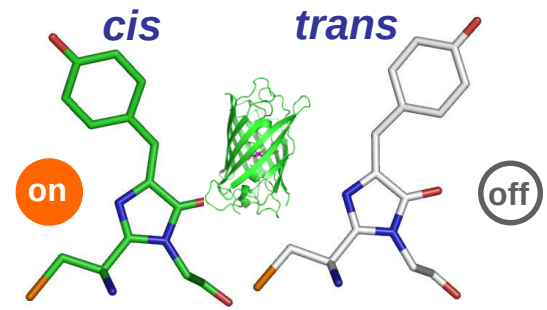
$\tau \sim$ ns

GSD (metastable dark state)



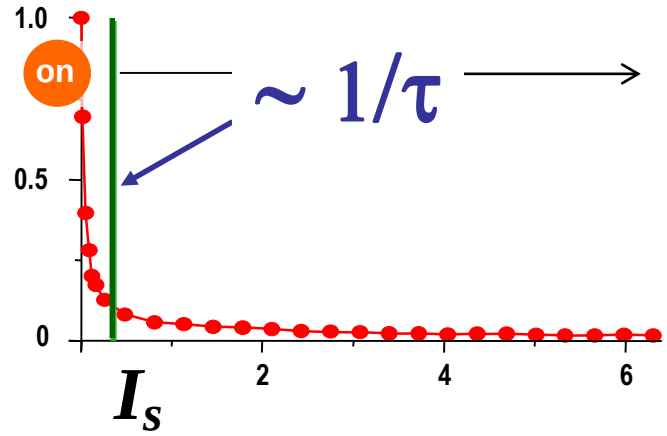
$\tau \sim \mu$s

RESOLFT



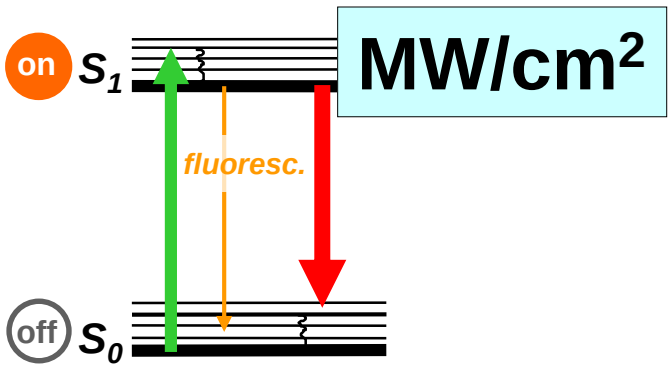
$\tau \sim$ ms

$$d \approx \frac{\lambda}{2n \sin\alpha \sqrt{1 + I/I_s}}$$

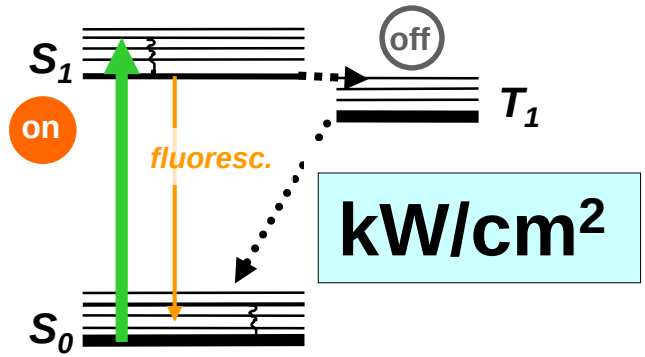


Principle: Discern by **ON / OFF** states in the sample

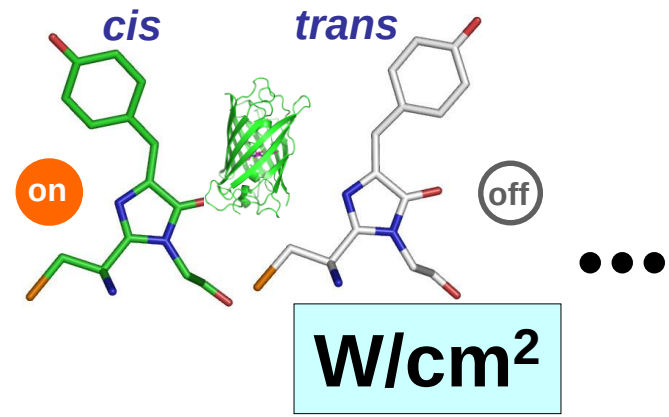
STED



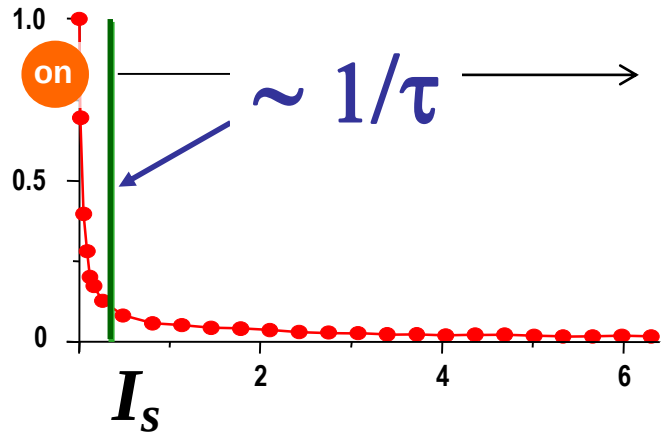
GSD (metastable dark state)



RESOLFT

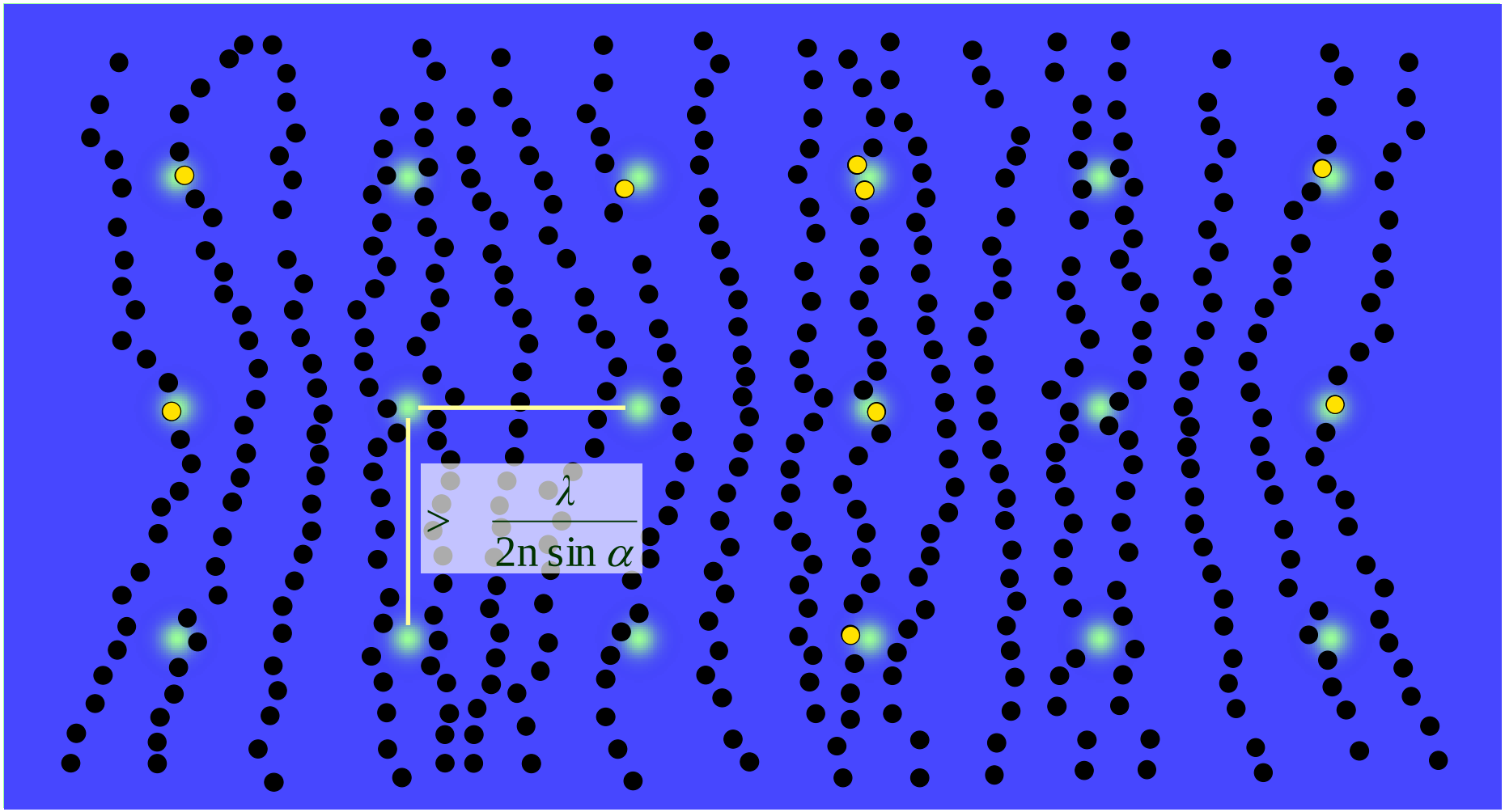


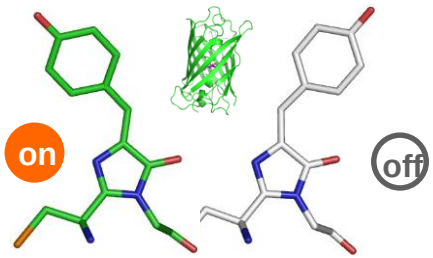
$$d \approx \frac{\lambda}{2n \sin\alpha \sqrt{1 + I/I_s}}$$



RESOLFT:

many ‚doughnuts' (zeros) in parallel

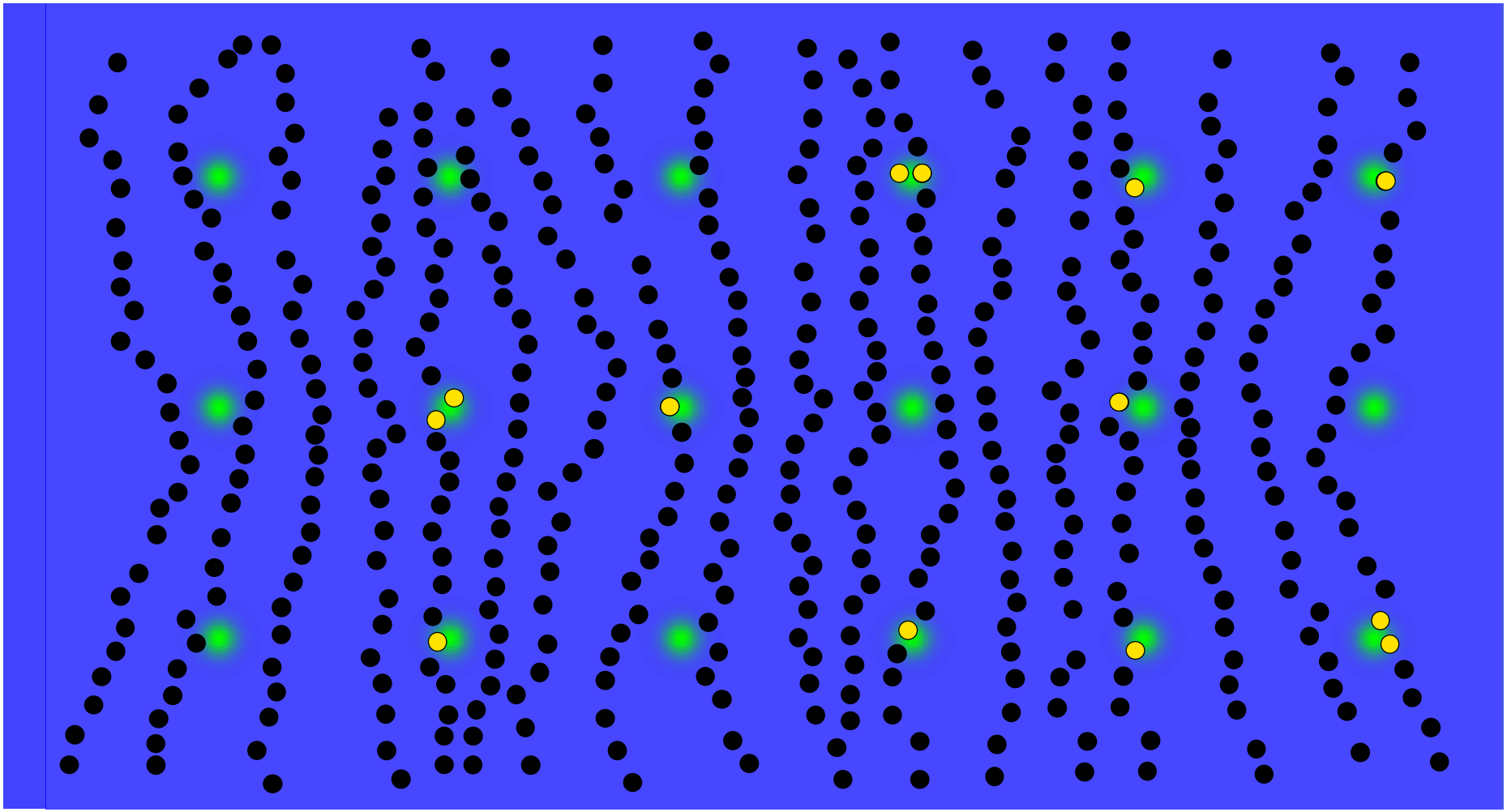


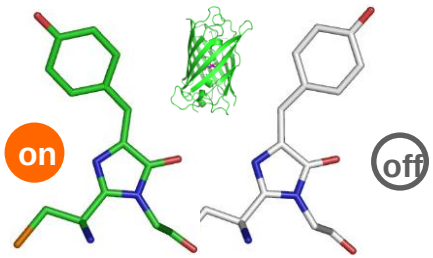


… because of low intensity operation.

RESOLFT:

many ‚doughnuts' (zeros) in parallel





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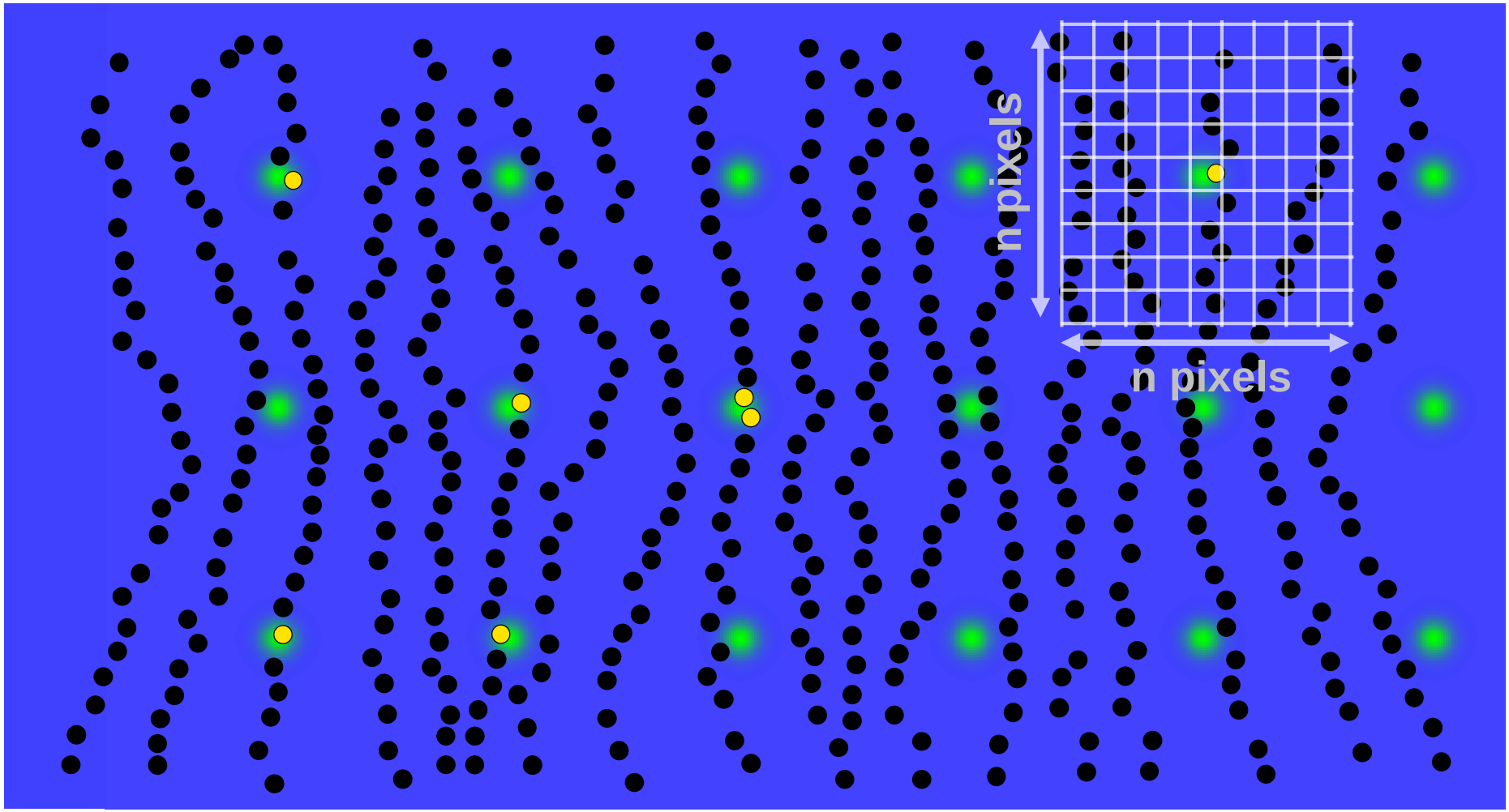
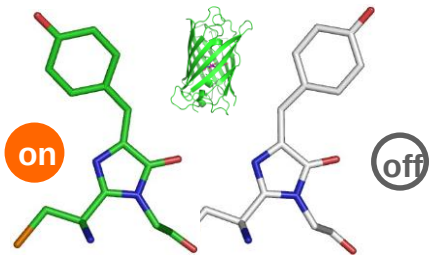




… because of low intensity operation.

RESOLFT

Keratin filaments in living kidney epithelial cells

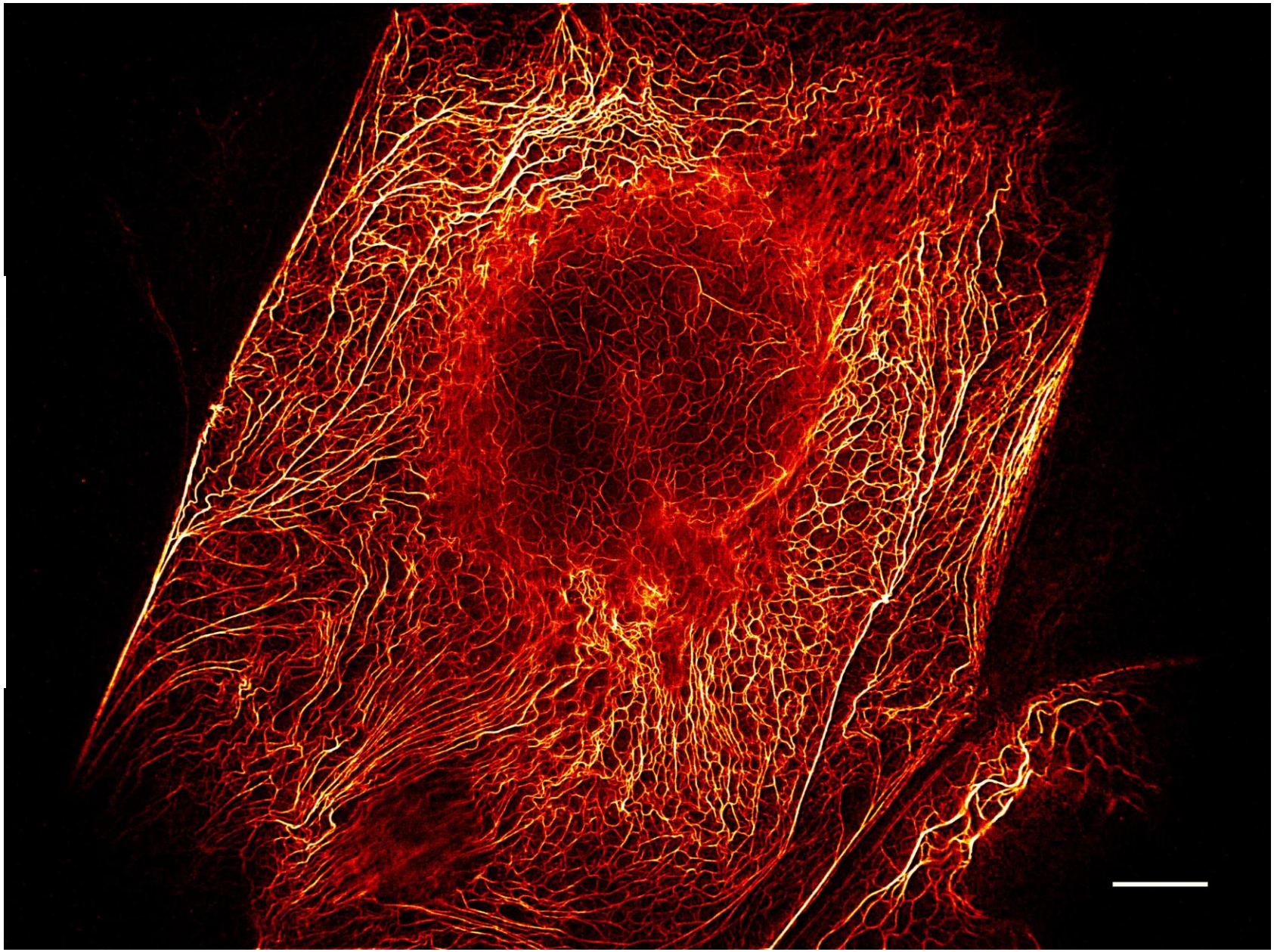
recorded with

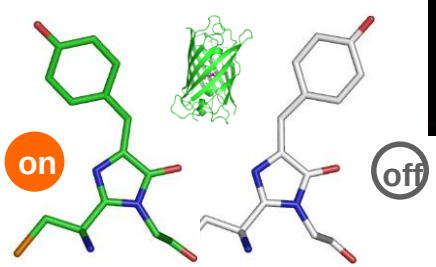
>100,000

‚doughnuts'

in

2 seconds





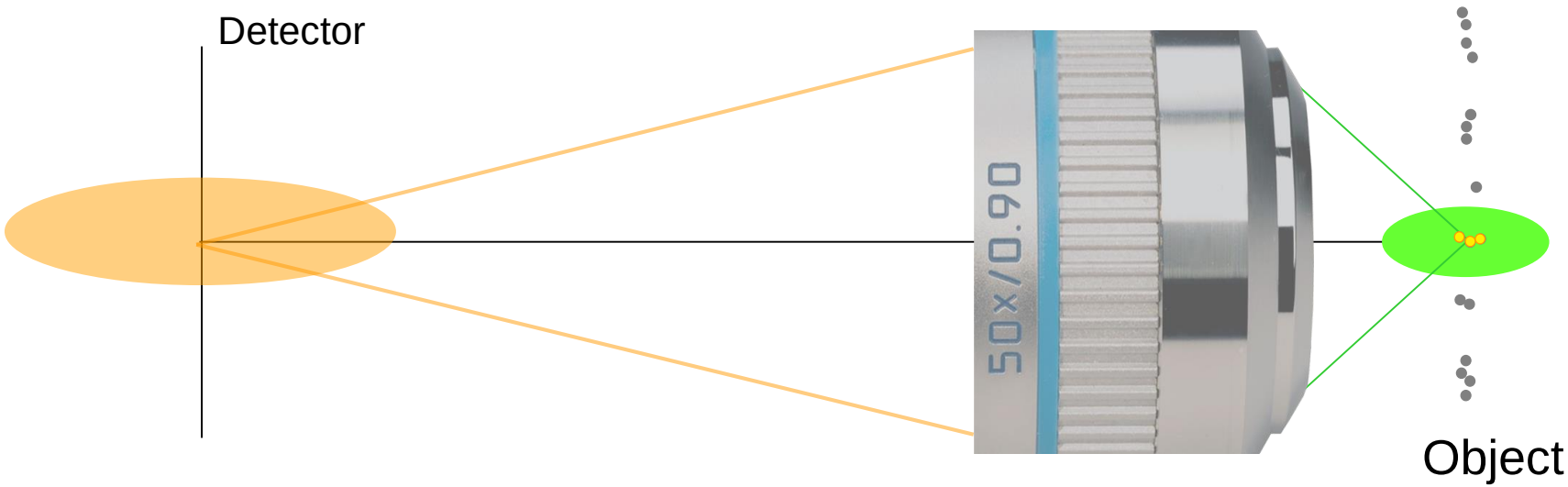
Chmyrov et al, *Nature Meth* (2013)

Scale bar: 10 µm

What does it take to get the best resolution ?

20th century:

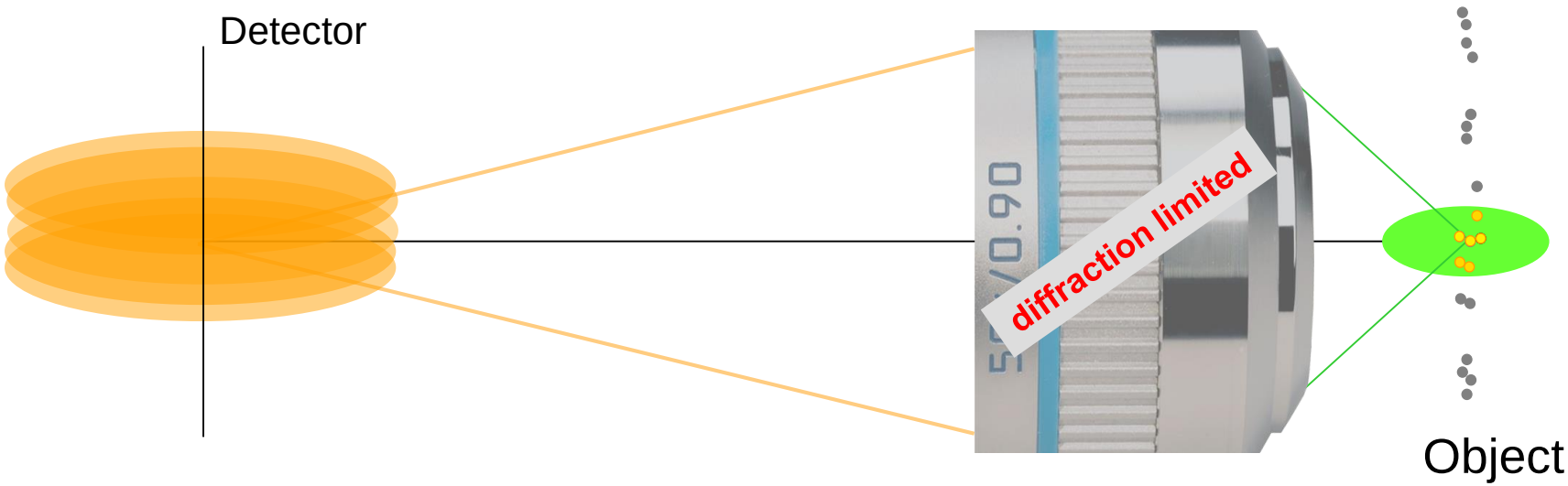
… separate features by ***focusing light***



Good lenses !

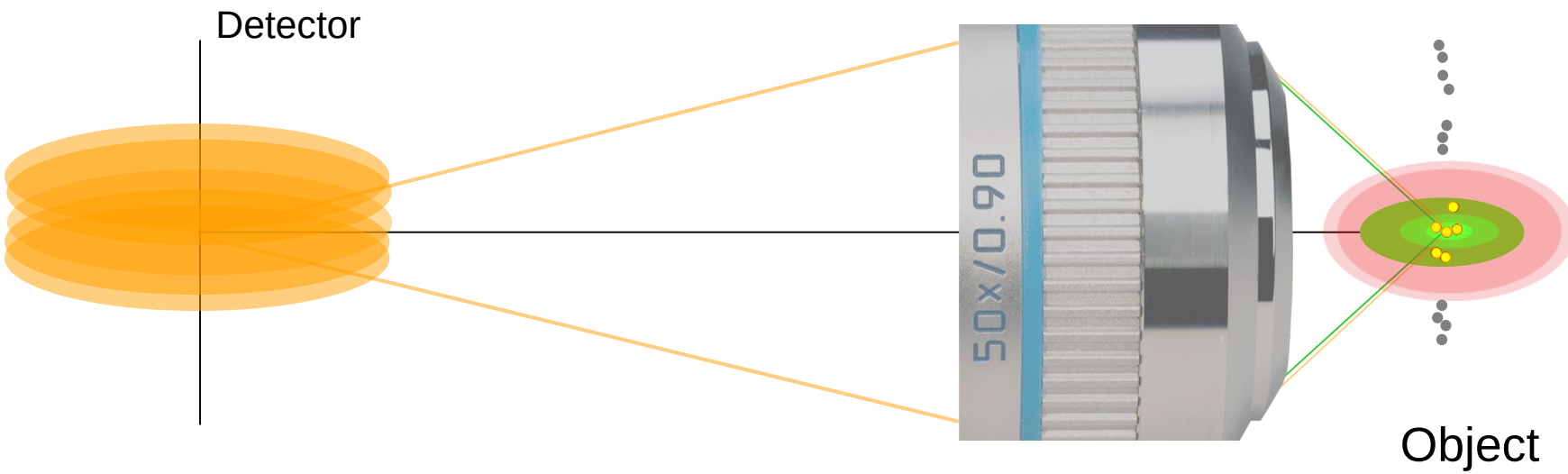
20th century:

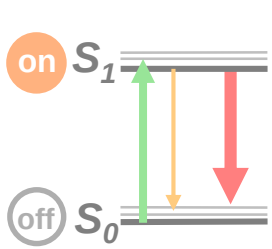
… separate features by ***focusing light***

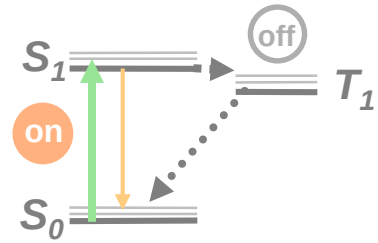


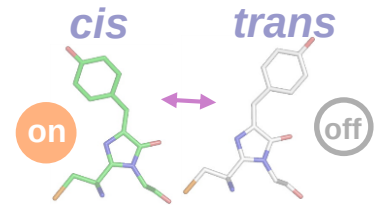
Solution:

… separate by molecular (on/off) ***states***





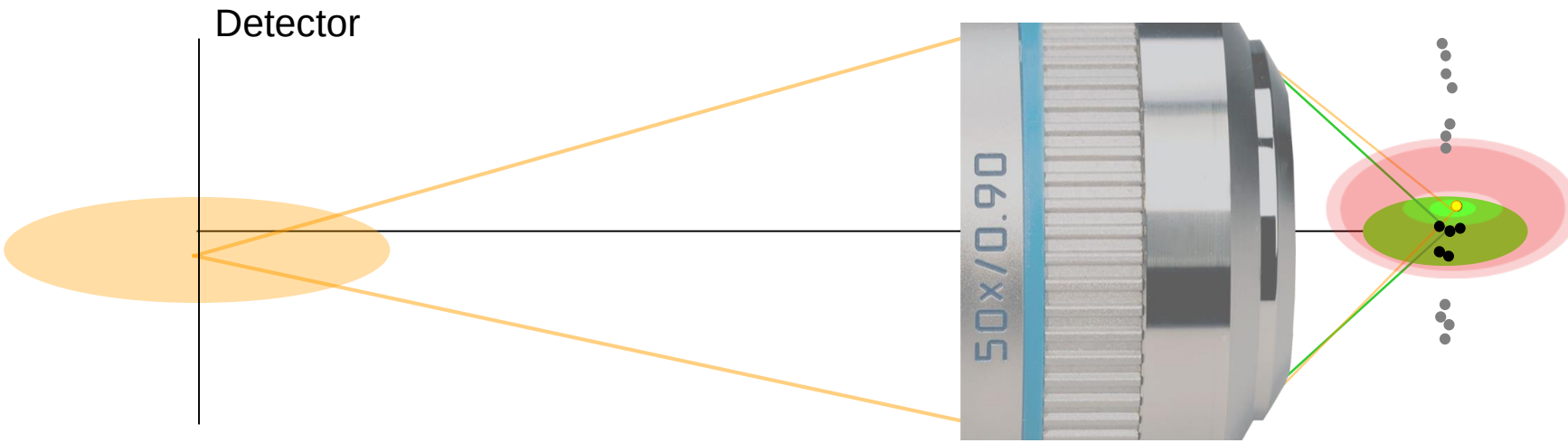


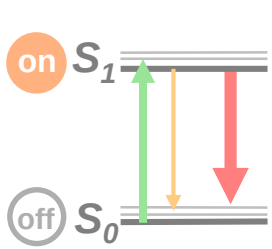


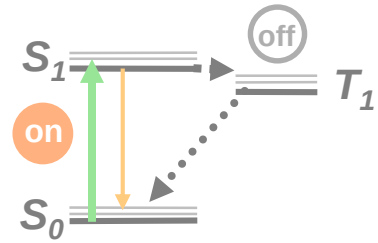
etc.

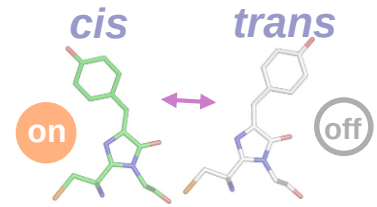
STED, GSD, SSIM, RESOLFT,…

… separate by molecular (on/off) ***states***





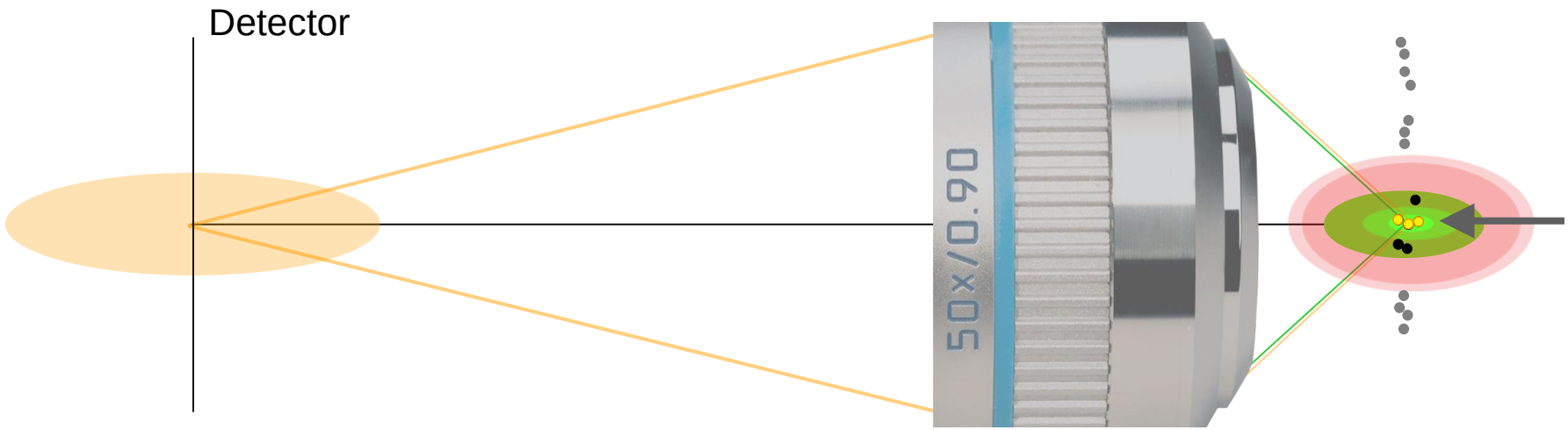


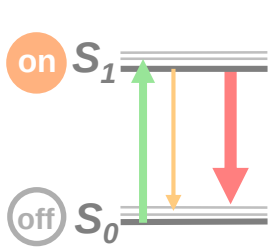


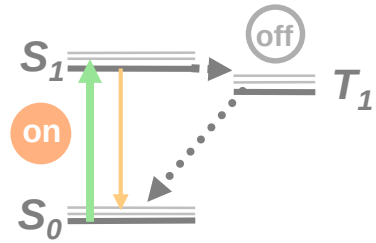
etc.

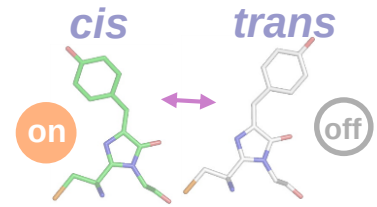
STED, GSD, SSIM, RESOLFT,…

… separate by molecular (on/off) ***states***





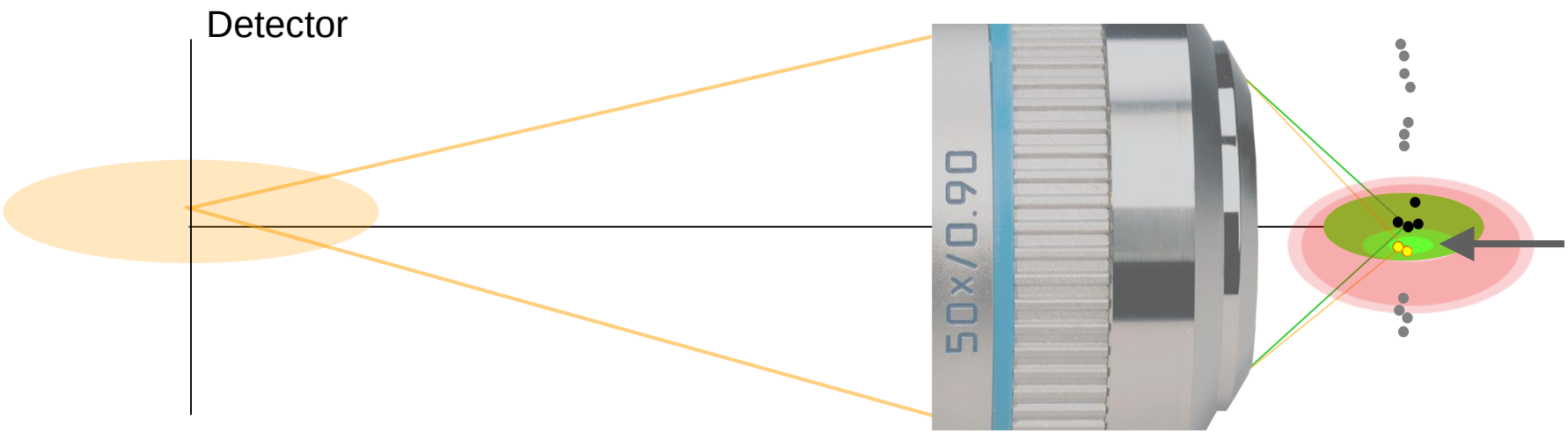




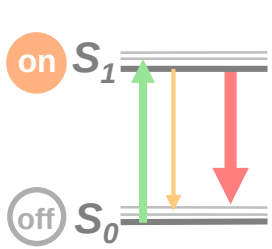
etc.

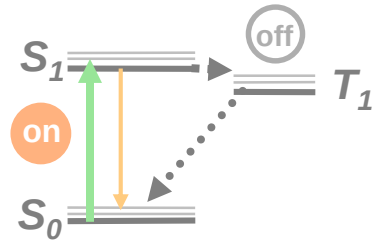
STED, GSD, SSIM, RESOLFT,…

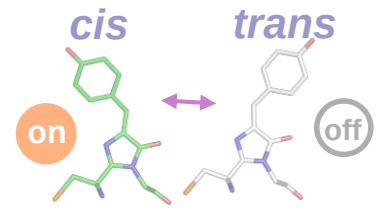
… separate by molecular (on/off) ***states***



X,Y,Z
controlled
by incident light pattern

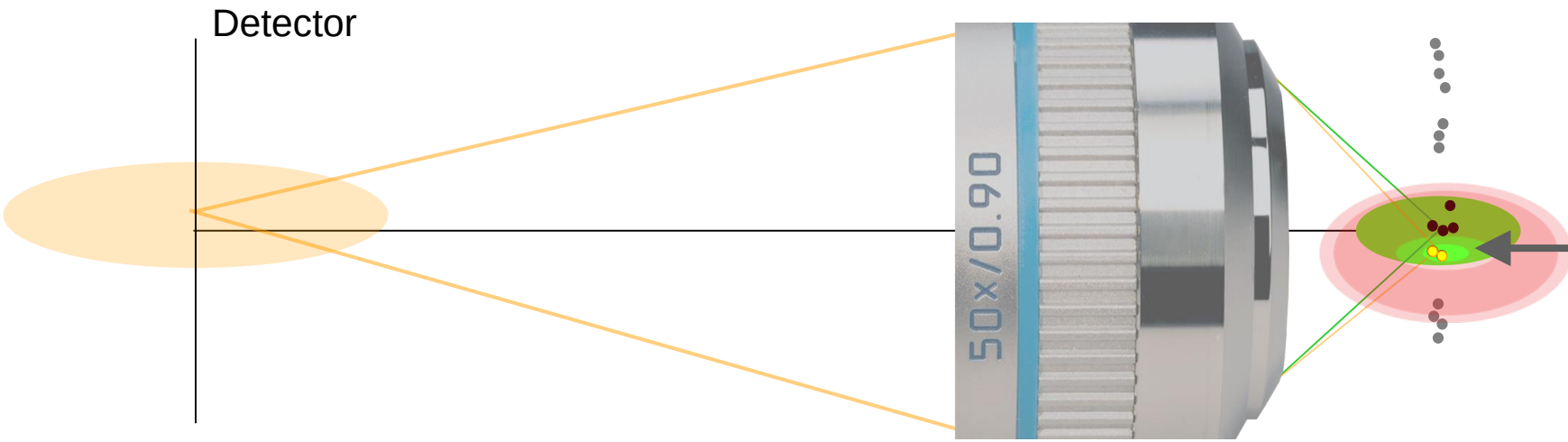






etc.

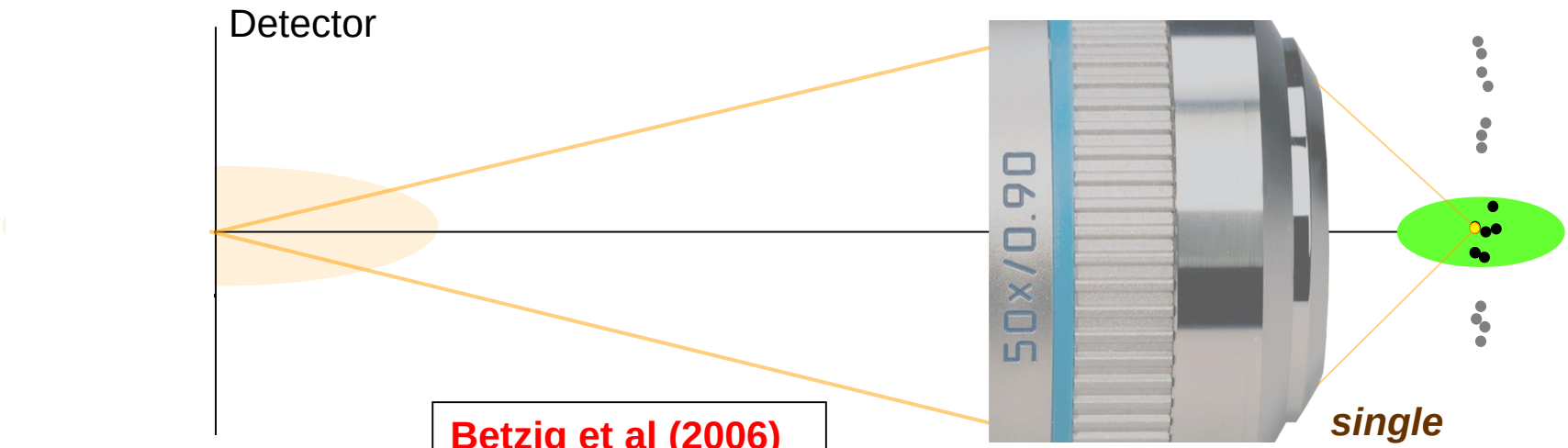
STED, GSD, SSIM, RESOLFT,…



controlled by incident light pattern

PALM, STORM, PAINT, GSDIM,…

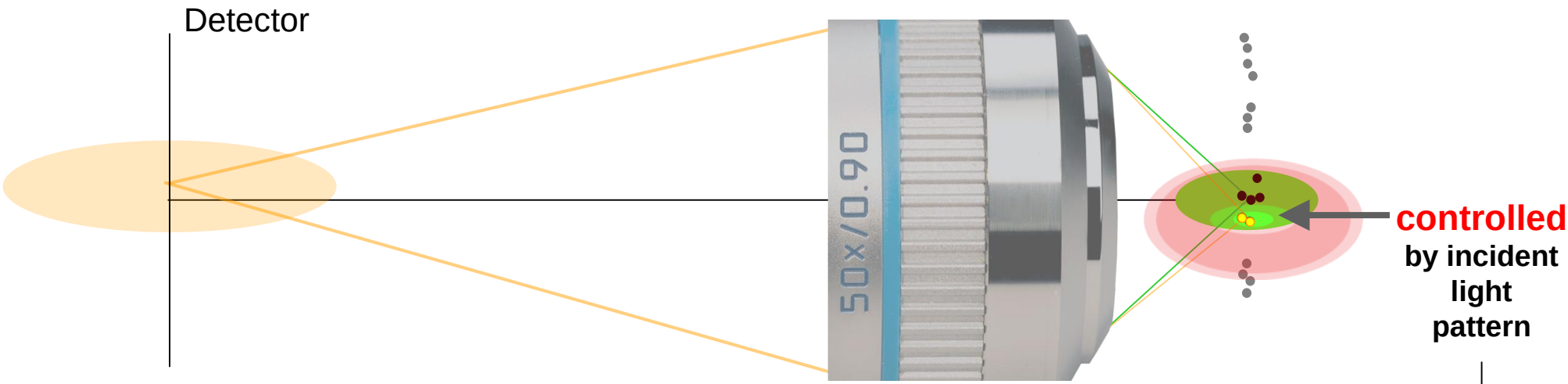
X,Y,Z



single molecule !

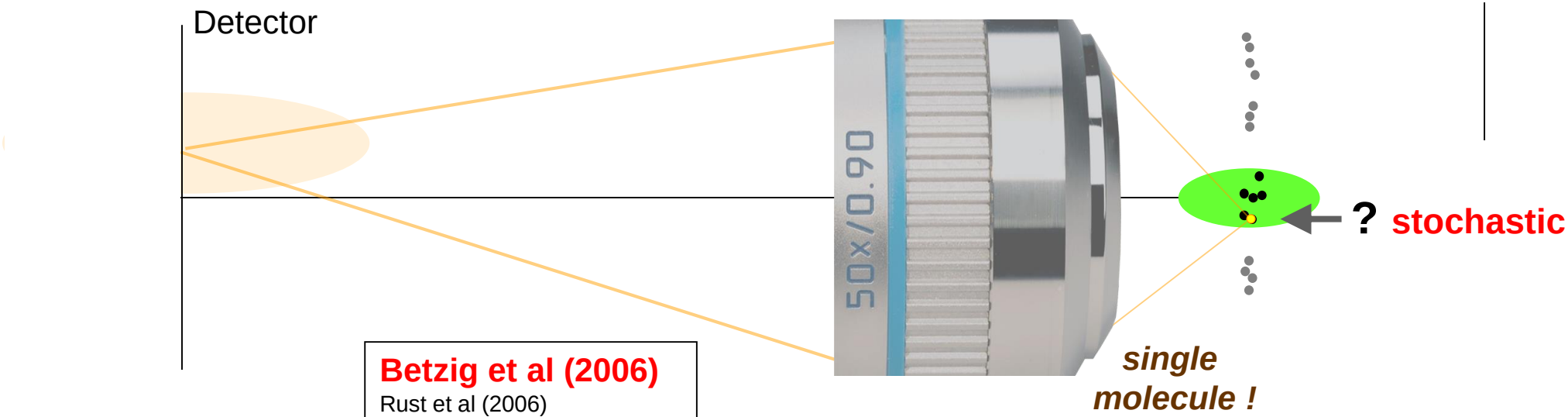
Betzig et al (2006)
Rust et al (2006)
Hess et al (2006)
Moerner et al (1989)
Orrit et al (1990)

STED, GSD, SSIM, RESOLFT,…



PALM, STORM, PAINT, GSDIM,…

X,Y,Z



Betzig et al (2006)
Rust et al (2006)
Hess et al (2006)
Moerner et al (1989)
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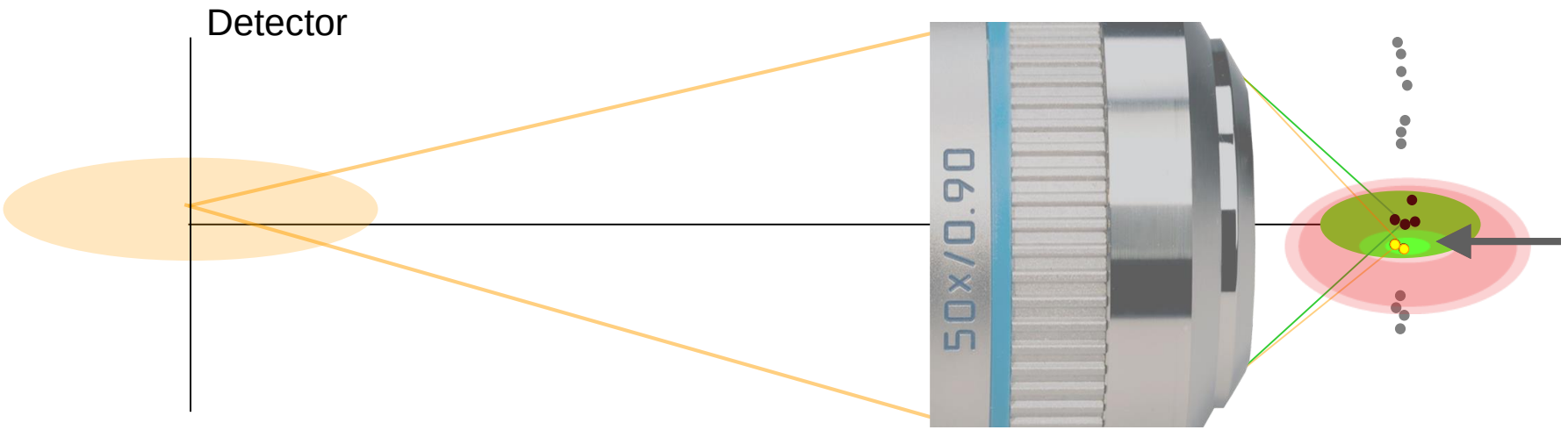
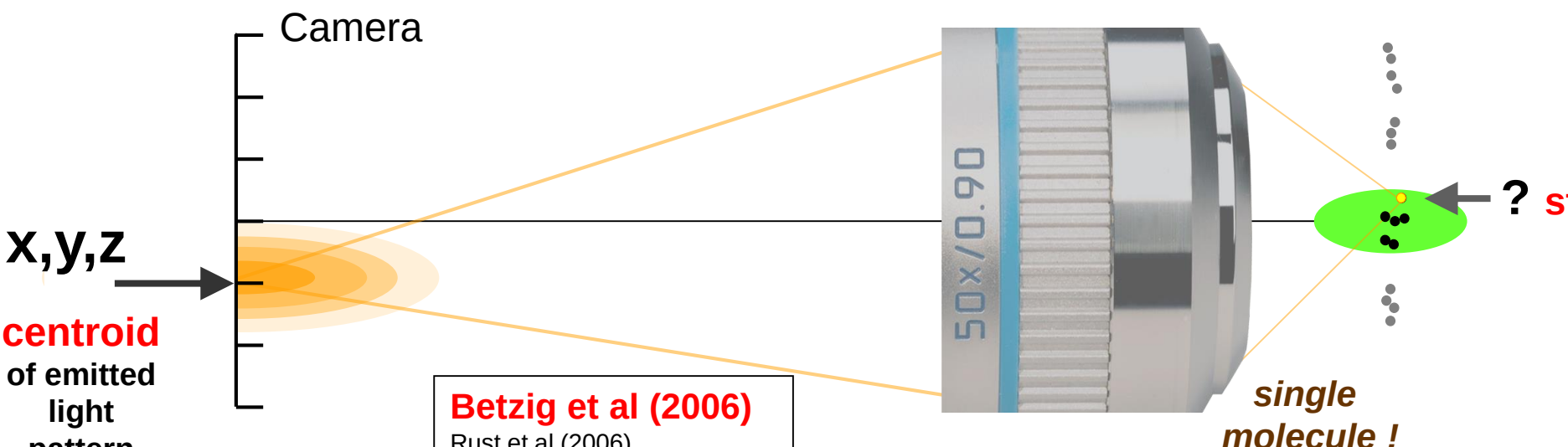
STED, GSD, SSIM, RESOLFT,…



controlled by incident light pattern

X,Y,Z

PALM, STORM, PAINT, GSDIM,…



x,y,z

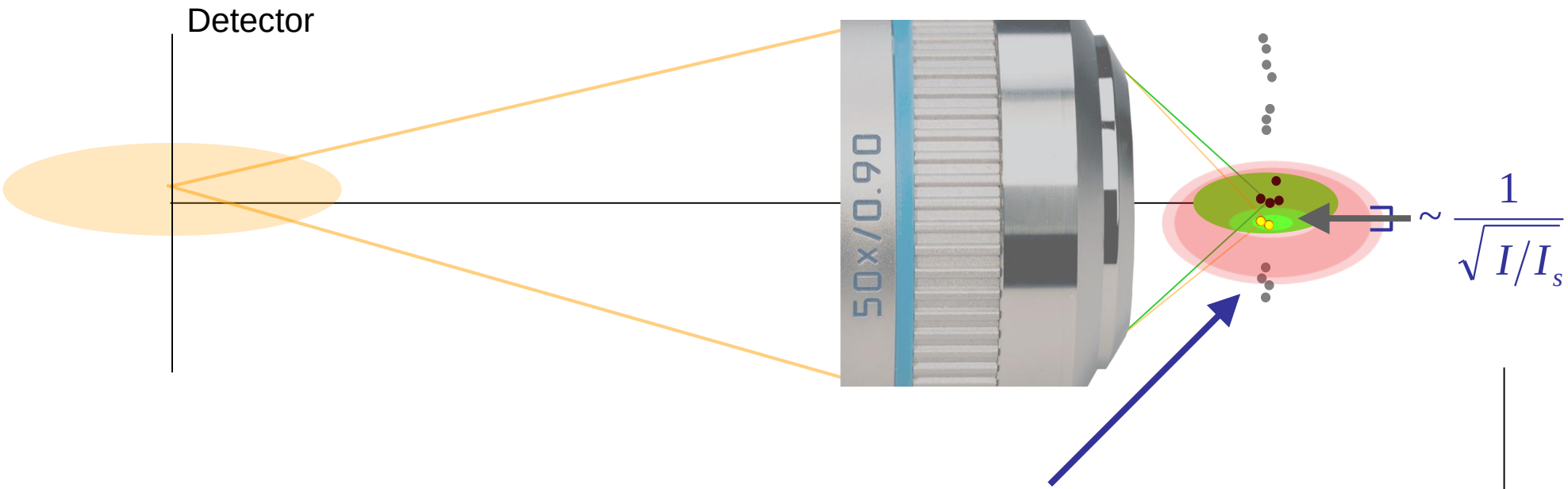
centroid of emitted light pattern

? stochastic

single molecule !

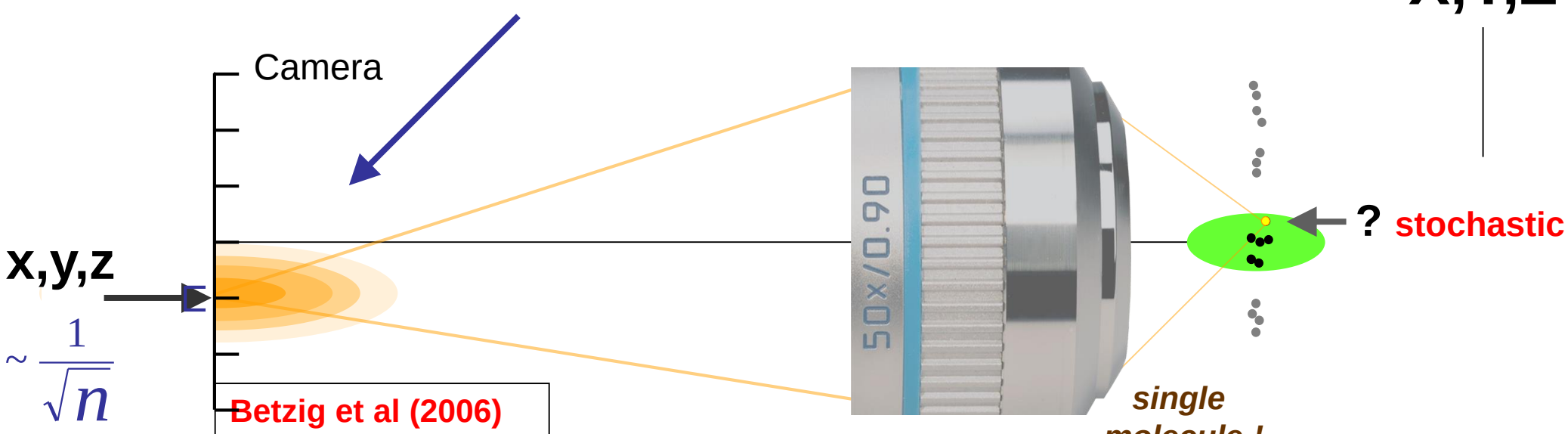
Betzig et al (2006)
Rust et al (2006)
Hess et al (2006)
Moerner et al (1989)
Orrit et al (1990)

STED, GSD, SSIM, RESOLFT,…



… many photons for X,Y,Z precision

X,Y,Z



Betzig et al (2006)
Rust et al (2006)
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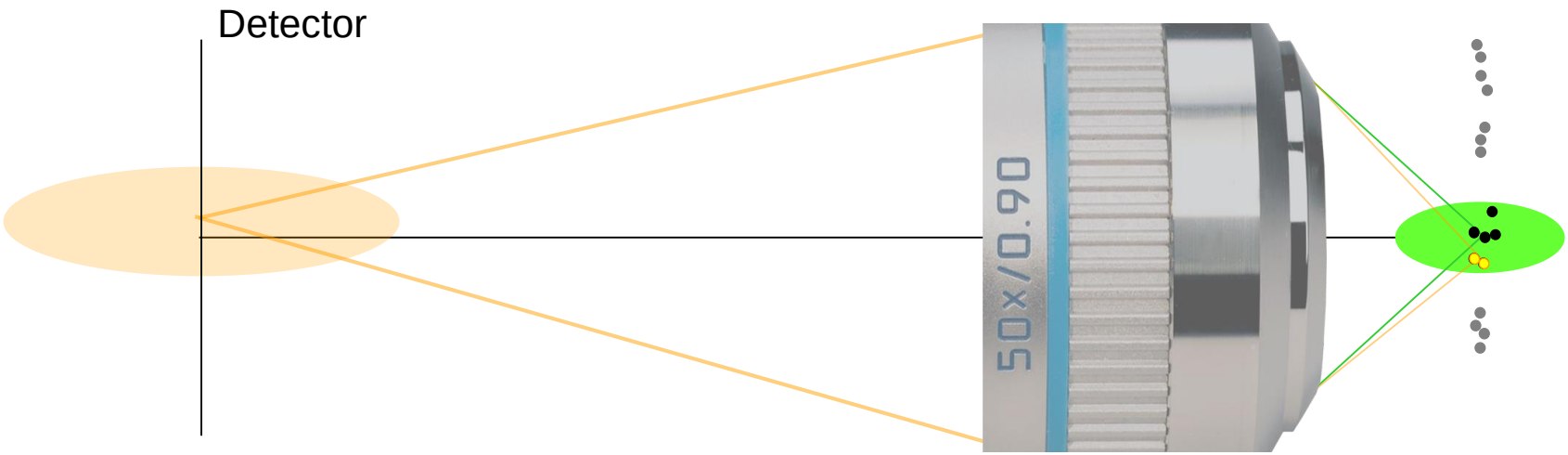
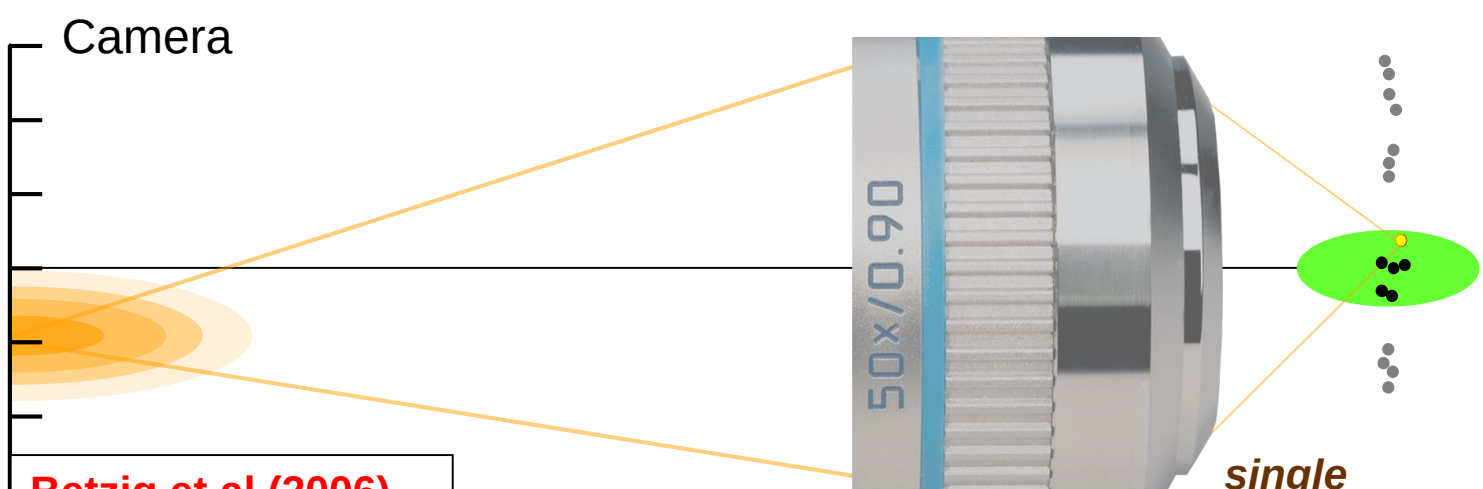
PALM, STORM, PAINT, GSDIM,…

STED, GSD, SSIM, RESOLFT,…



separates the features



single molecule !

Betzig et al (2006)
Rust et al (2006)
Hess et al (2006)
Moerner et al (1989)
Orrit et al (1990)

PALM, STORM, PAINT, GSDIM,…

Superresolution

separates features using (at least) 2 molecular ***states***

fluorescent ***non-fluorescent***

Superresolution

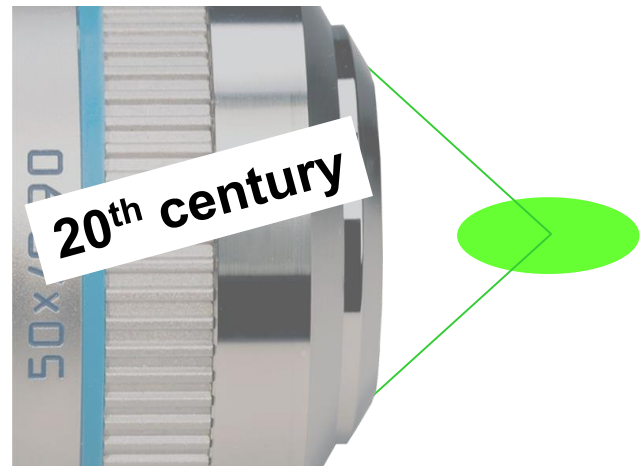
separates features using (at least) 2 molecular ***states***

fluorescent

absorbing
scattering
spin up
…

non-fluorescent

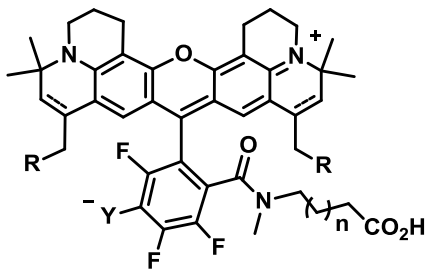
non-absorbing
non-scattering
spin down
…

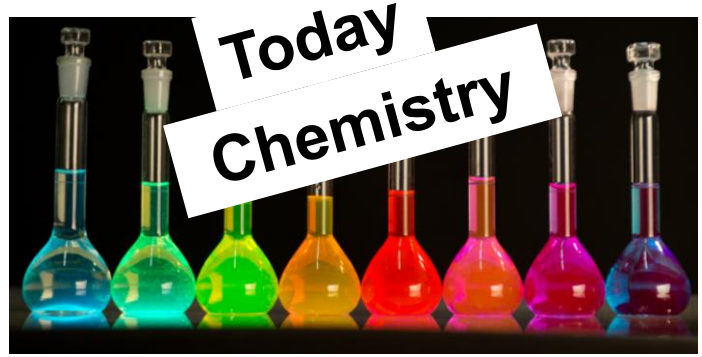


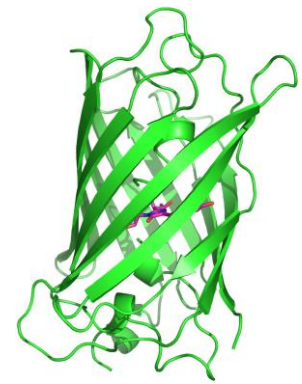
Superresolution

separates features using (at least) 2 molecular ***states***

fluorescent ***non-fluorescent***







Turun yliopisto
University of Turku

1993-1996

since 1997

dkfz.

since 2003

Former lab members

Thomas Klar

Stefan Jakobs

Katrin Willig

Alexander Egner

Andreas Schönle

Lars Kastrup

Christian Eggeling

Benjamin Harke

Current lab members

Volker Westphal

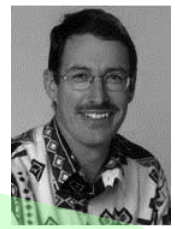
Roman Schmidt

Jan Keller

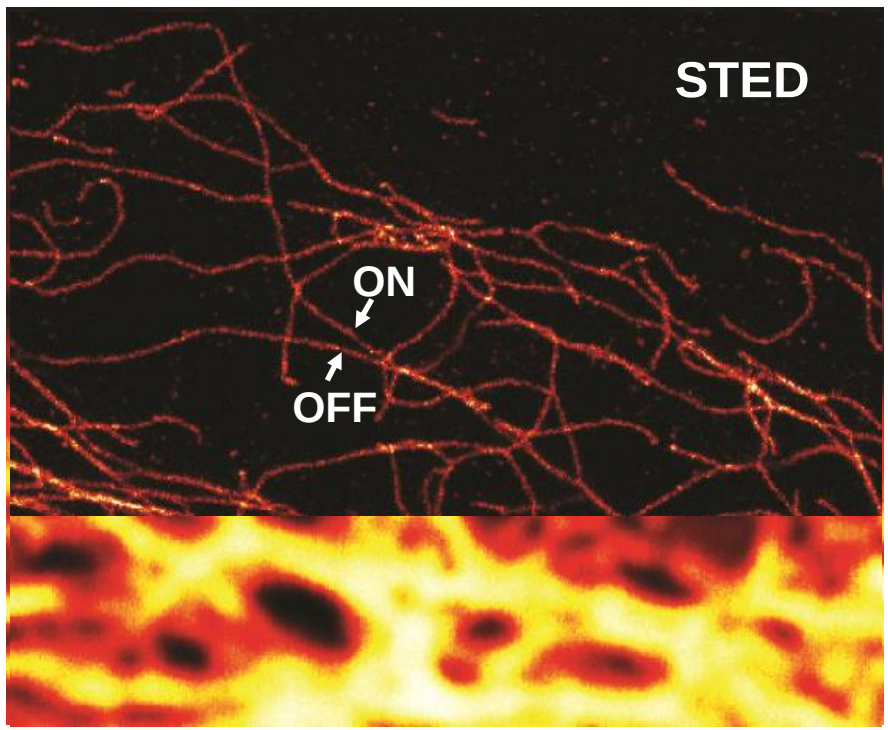
Tim Grotjohann

Vladimir Belov

Christian Wurm


Johann Engelhardt

Collaborators: P. Hänninen, E. Soini, R. Jahn, F. Barrantes, S. Sigrist, H.G. Kräusslich, S. Rizzoli

$$d = \frac{\lambda}{2n \sin\alpha \sqrt{1 + J/J_s}} \longrightarrow 0$$

… down to molecular scale.