

Nanoscopy with Focused Light

Stefan W. Hell



**Max Planck Institute for Biophysical Chemistry
Department of NanoBiophotonics
Göttingen**

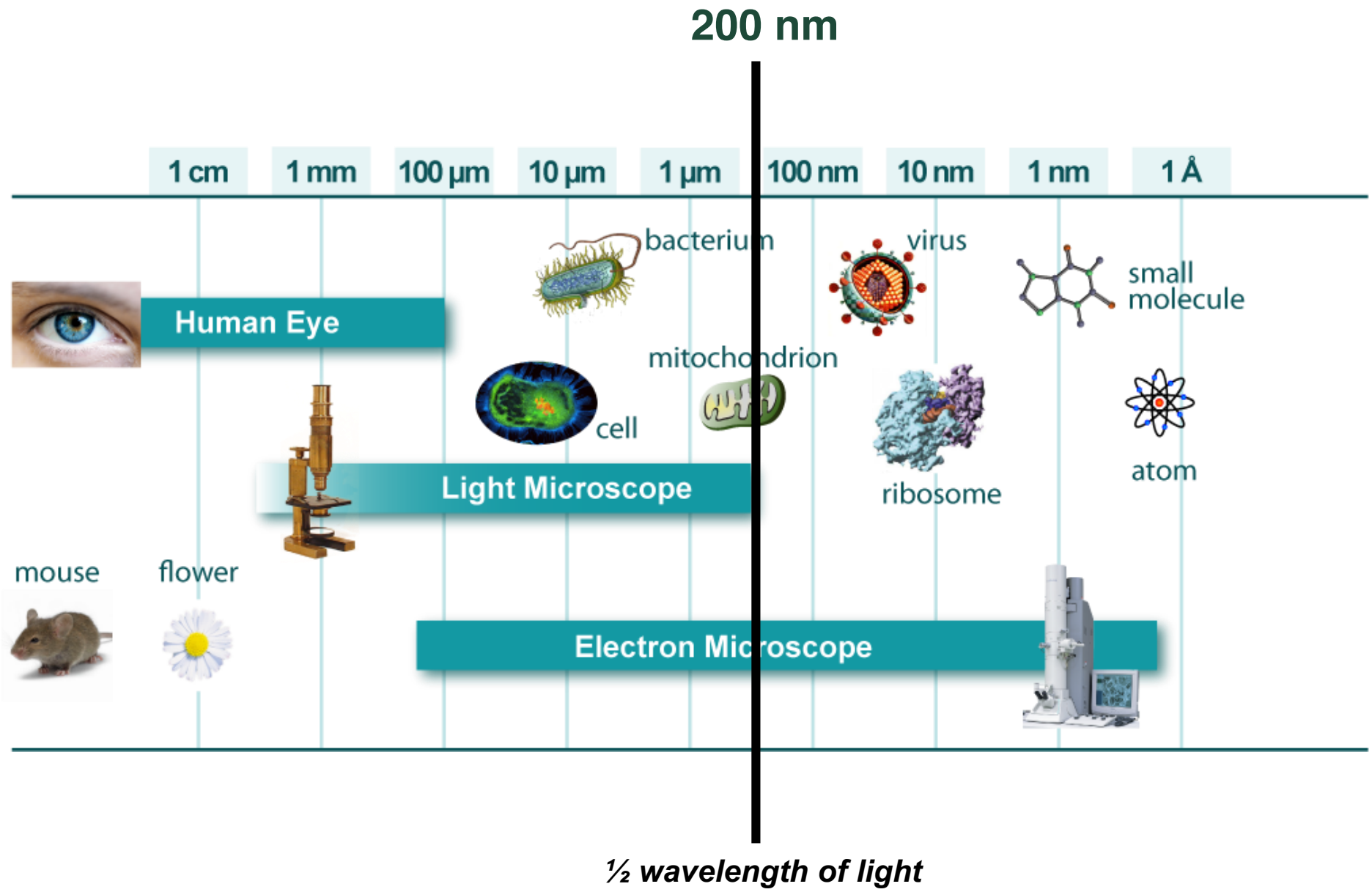
&

**German Cancer Research Center (DKFZ)
Optical Nanoscopy Division
Heidelberg**

dkfz.

Nobel Lecture in Chemistry, Stockholms universitet

8. December 2014



Light microscopy: most popular microscopy technique in life sciences

Electron microscopy, etc.



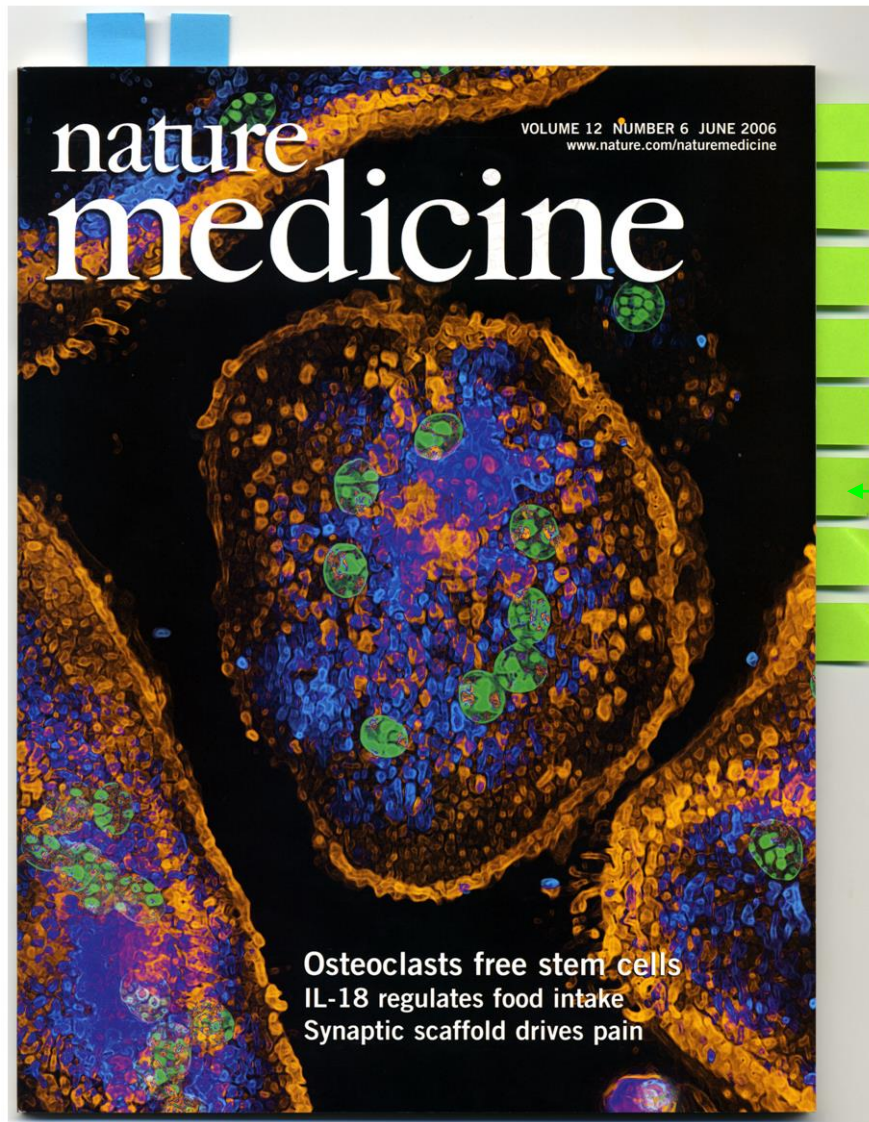
20 %



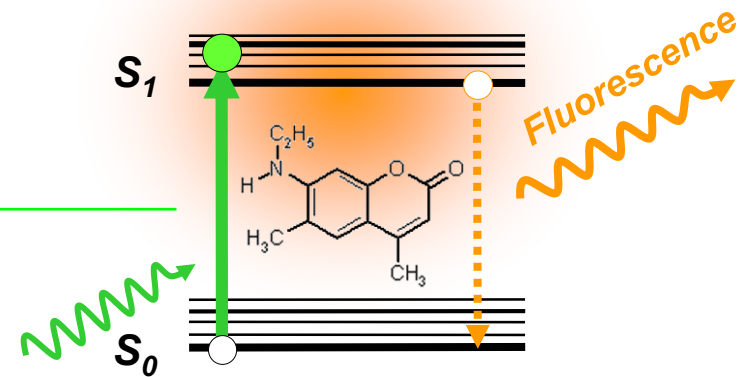
80 %

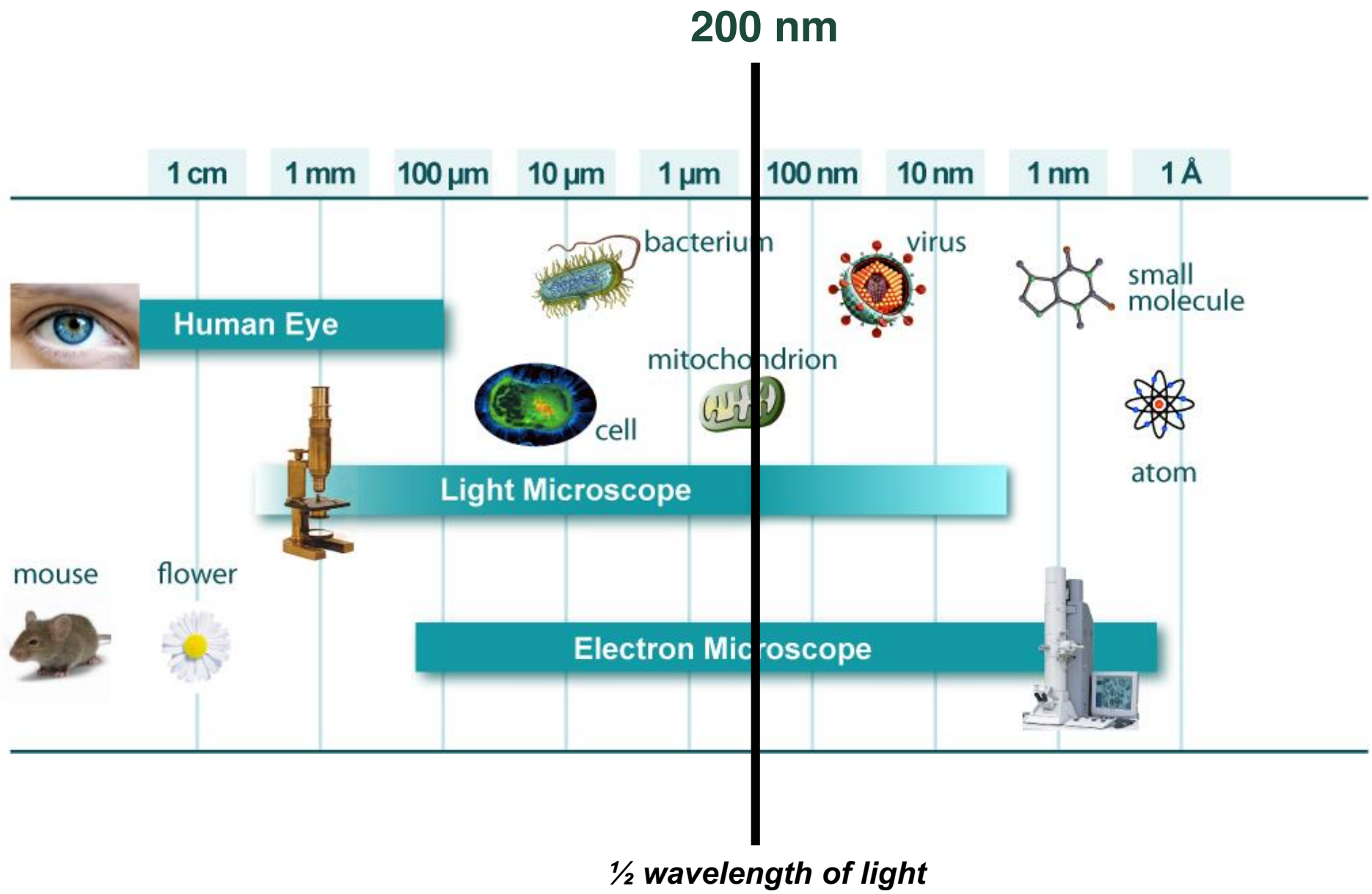
Light microscopy

Fluorescent labels indicate
biomolecule of interest

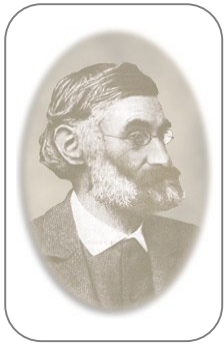


Excitation





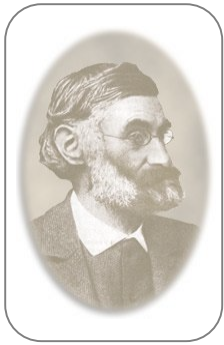
... because of the diffraction barrier:



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

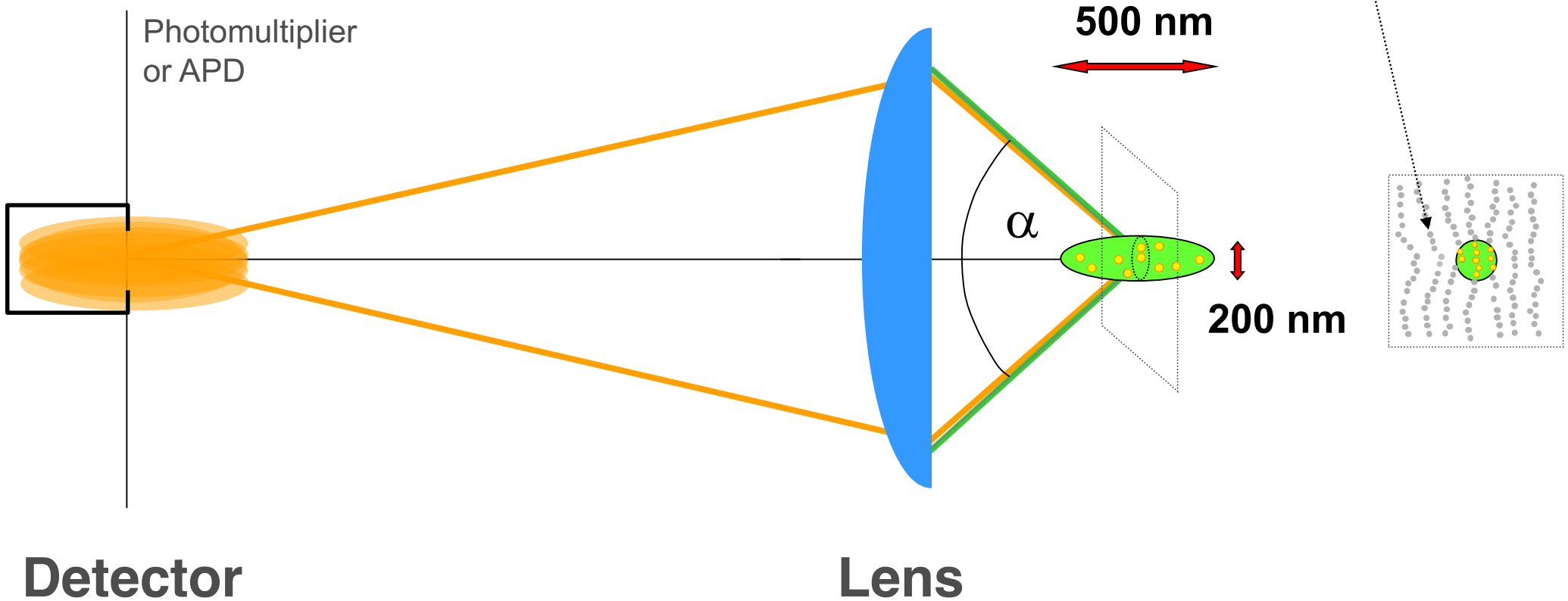


Verdet (1869)
Abbe (1873)
Helmholtz (1874)
Rayleigh (1874)

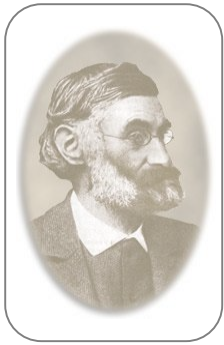


... because of the diffraction barrier:

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

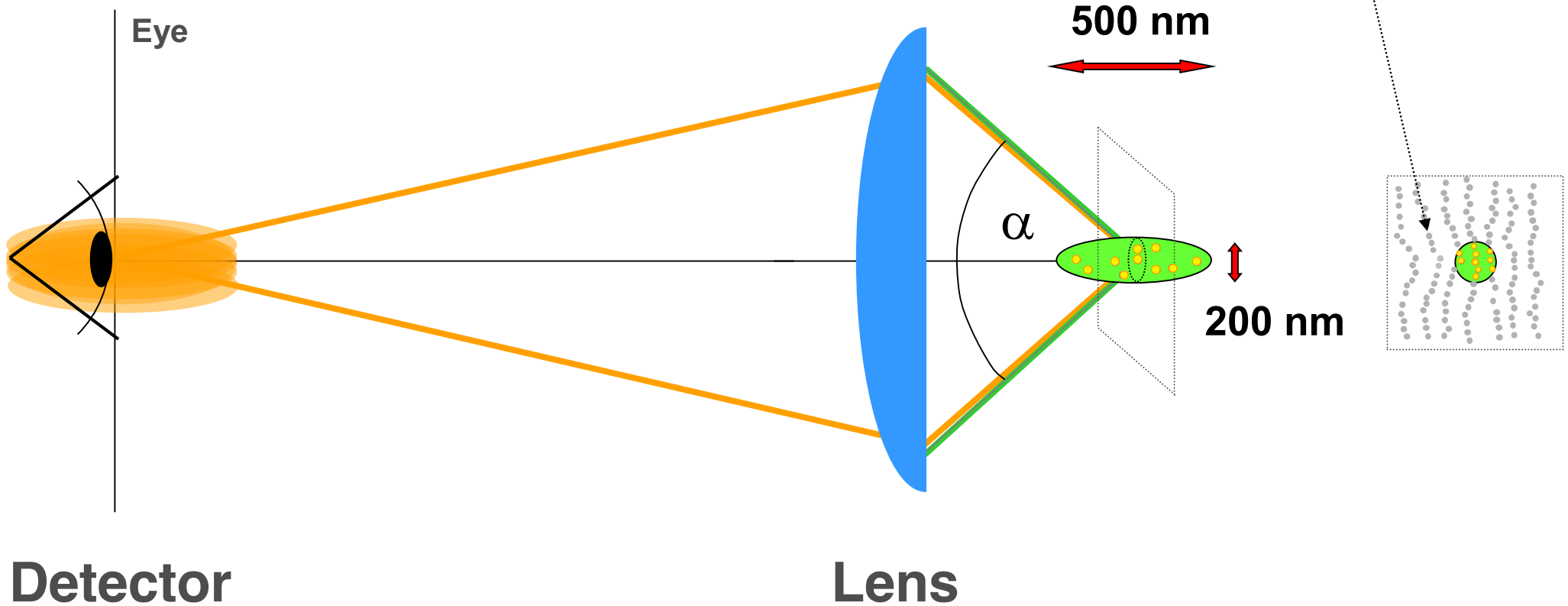


Verdet (1869)
Abbe (1873)
Helmholtz (1874)
Rayleigh (1874)

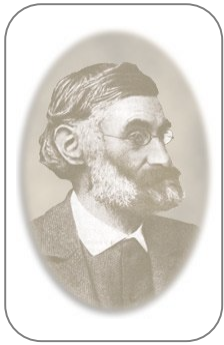


... because of the diffraction barrier:

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

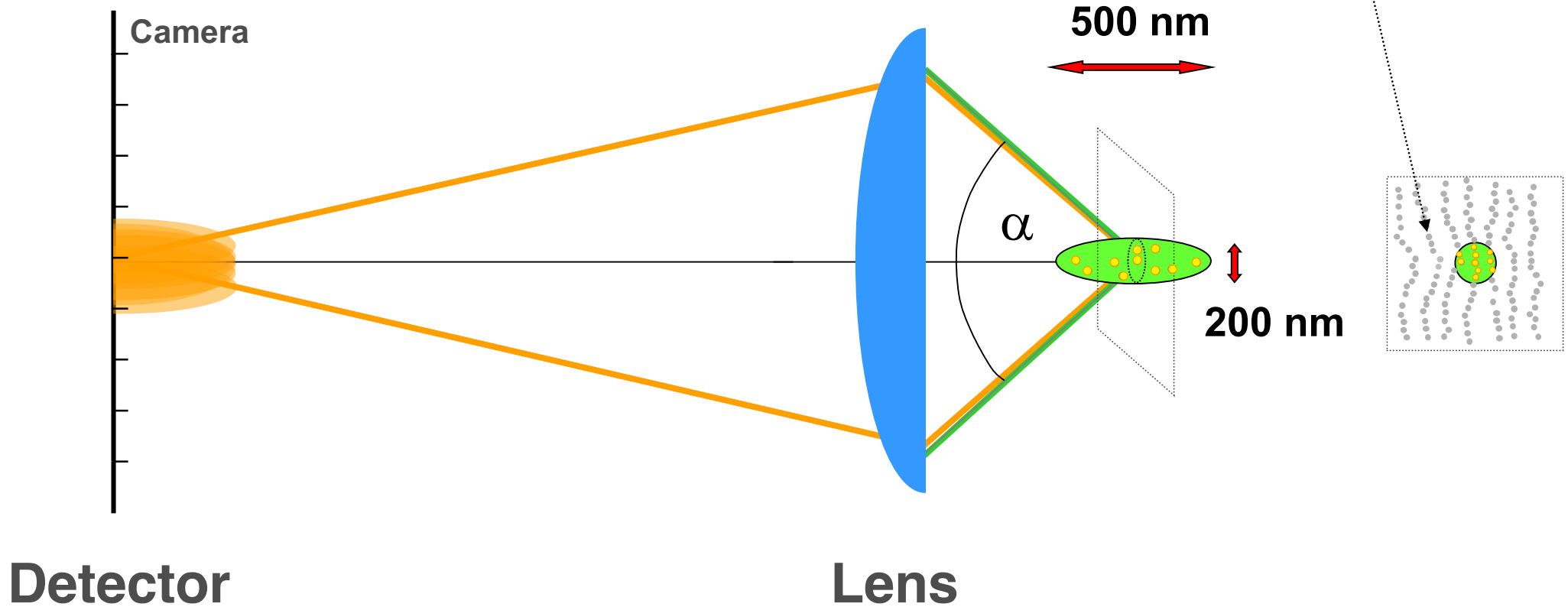


Verdet (1869)
Abbe (1873)
Helmholtz (1874)
Rayleigh (1874)

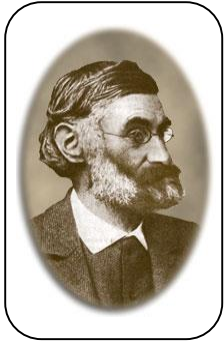


... because of the diffraction barrier:

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



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



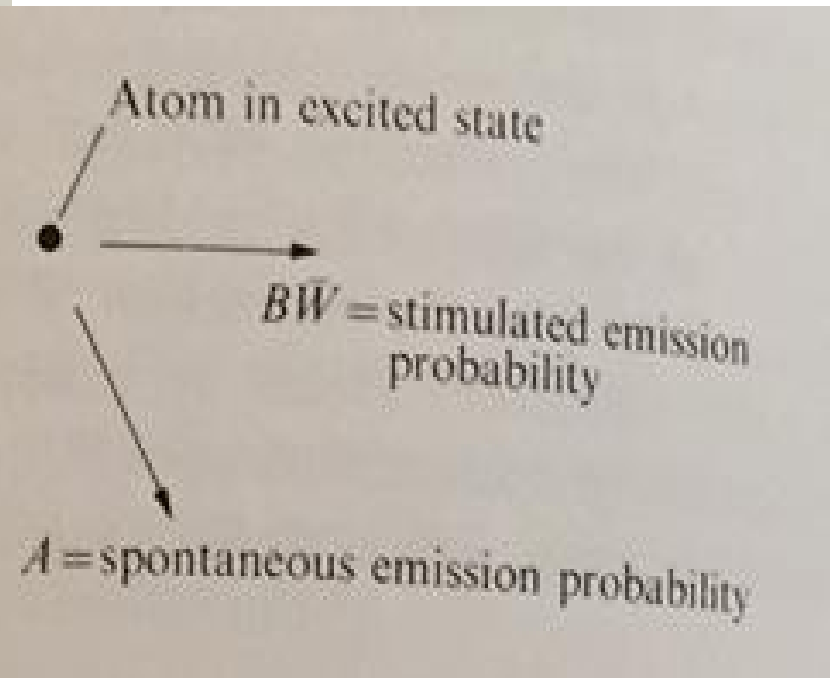
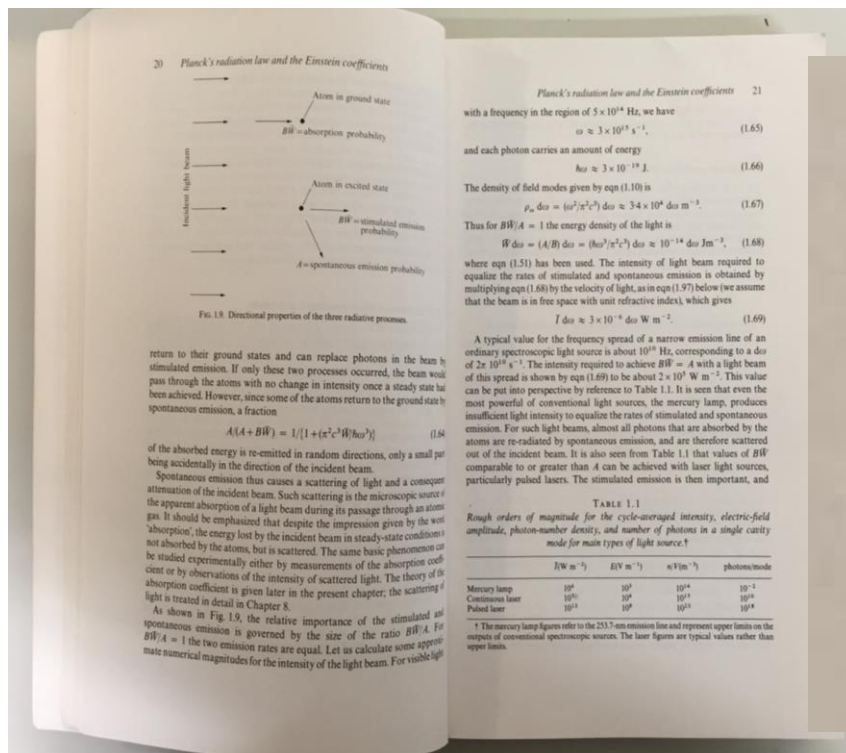
**Jena,
Germany**

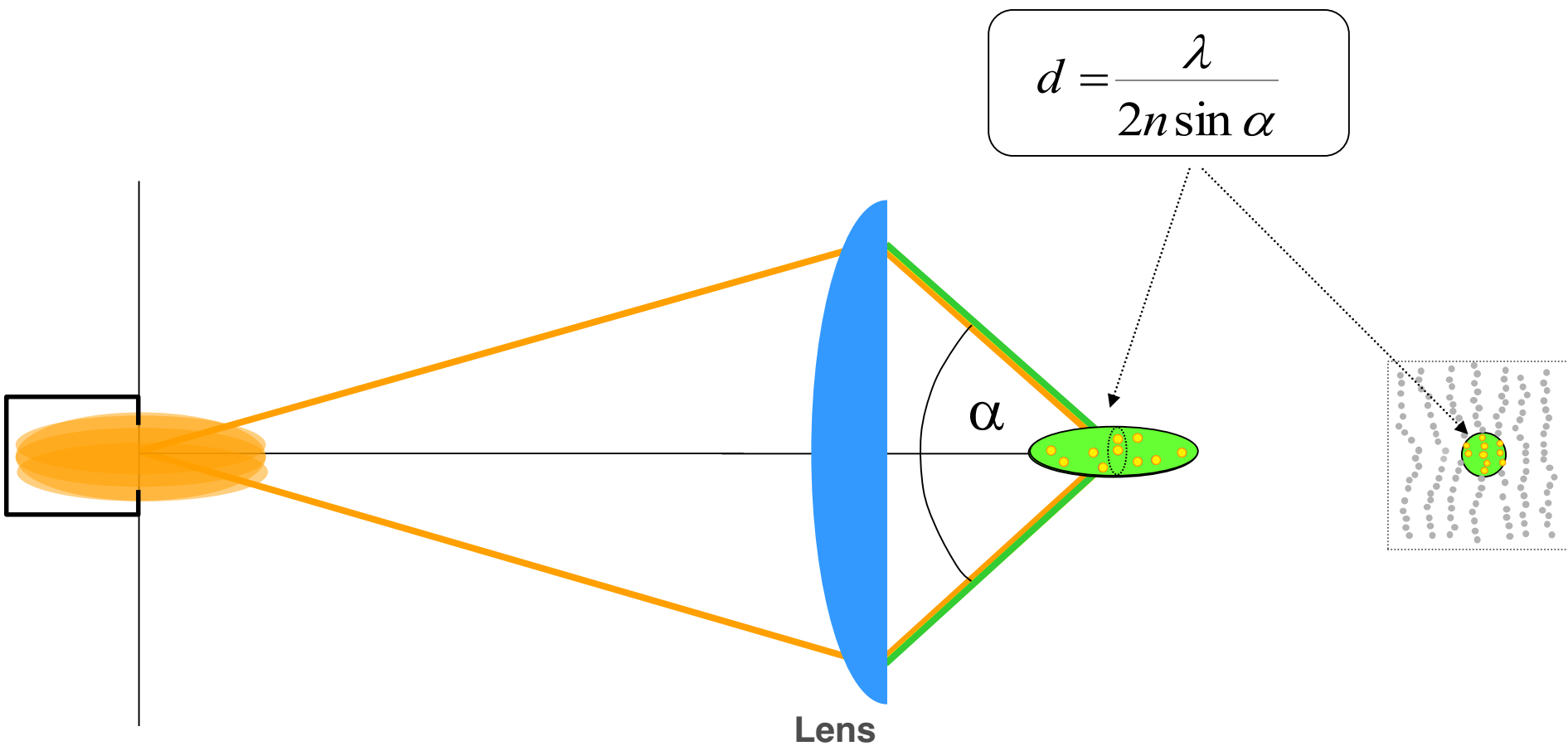
What I believed around 1990:

“... 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.”

What I believed around 1990:

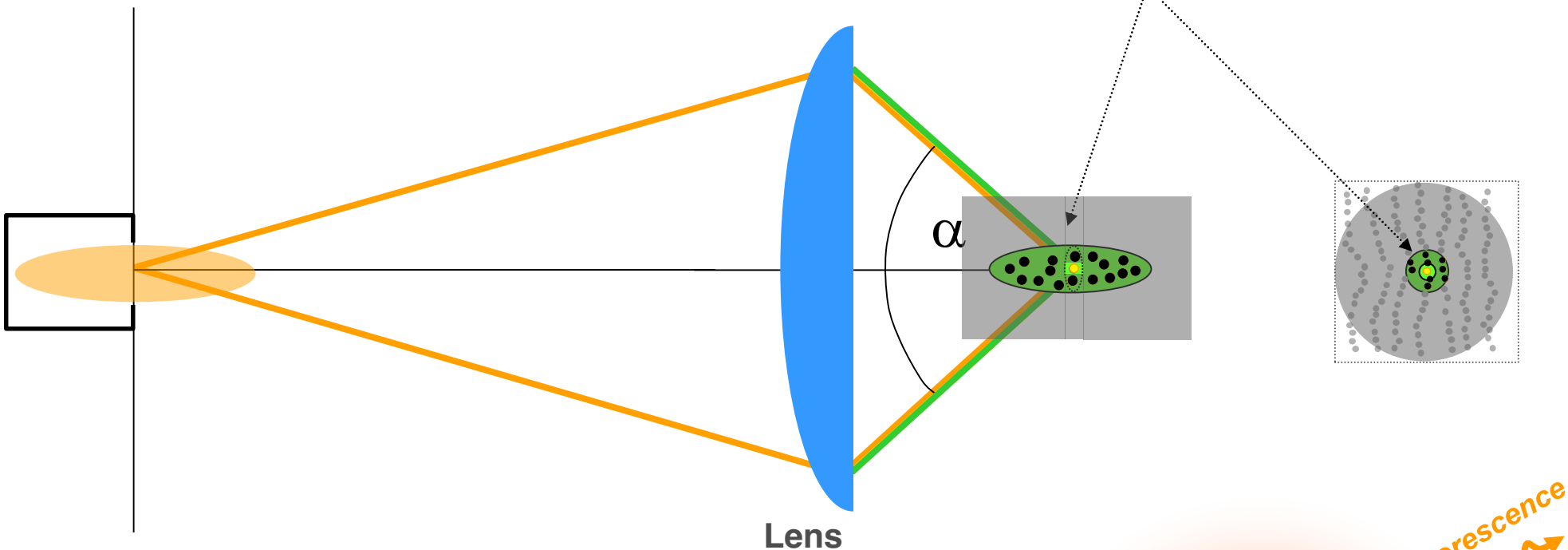
“... the resolution limiting effect of diffraction can be overcome (...) by fully exploiting the properties of the fluorophores. Combined with modern quantum optical techniques the scanning (confocal) microscope has the potential of dramatically improving the resolution in far-field light microscopy.”





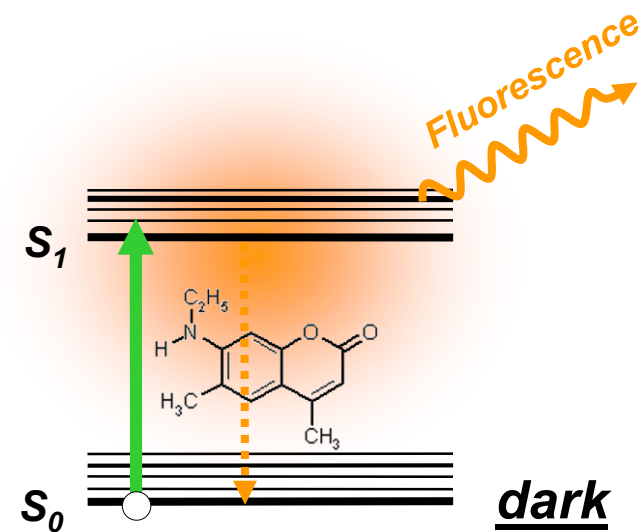
Keep molecules in a dark state !

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

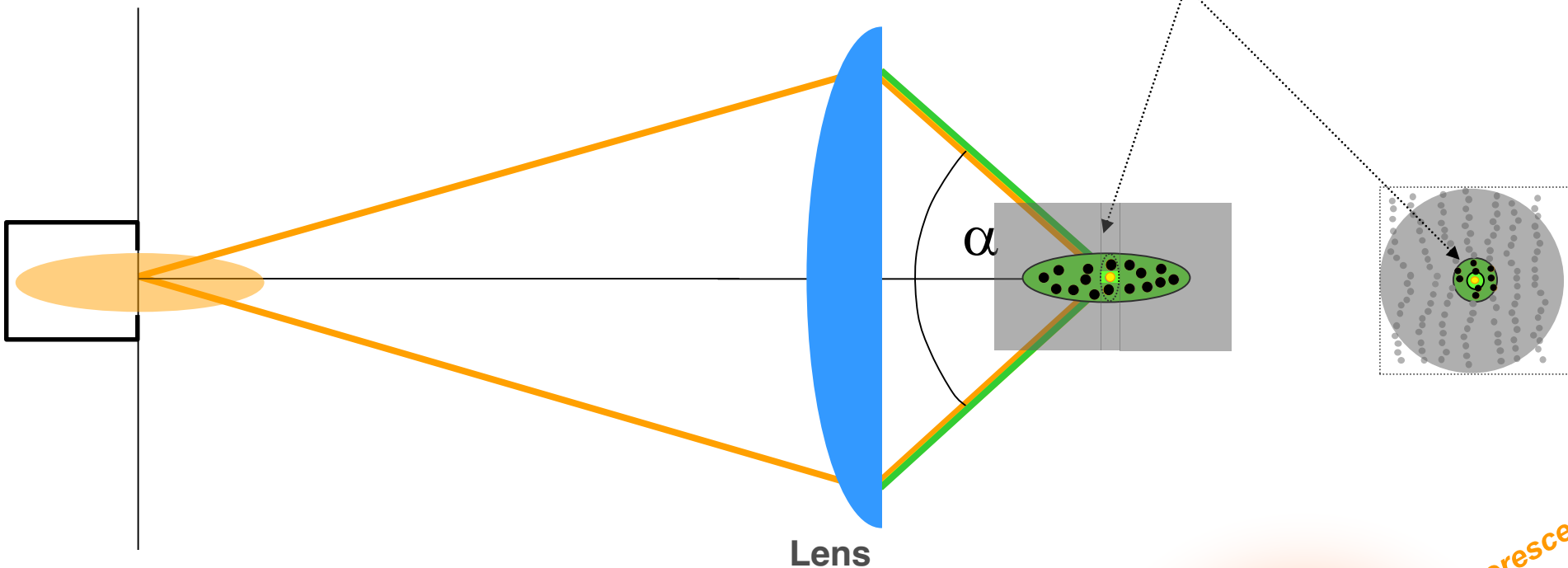


Lens

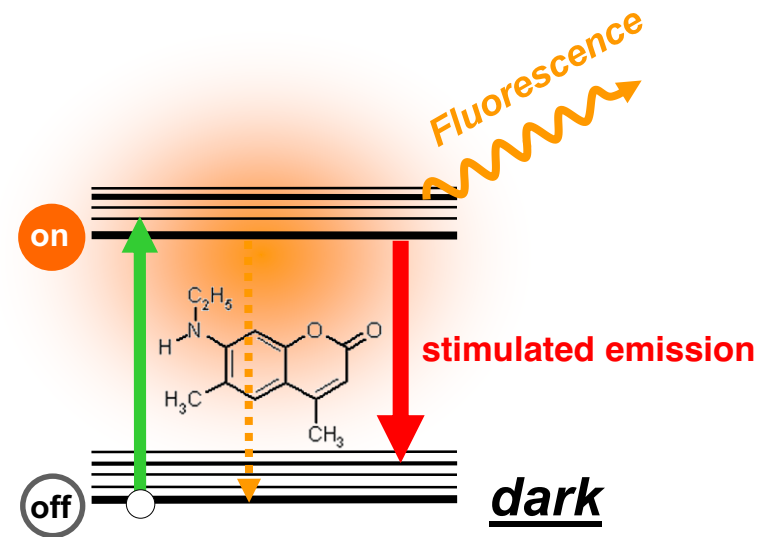
Keep molecules in a dark state !



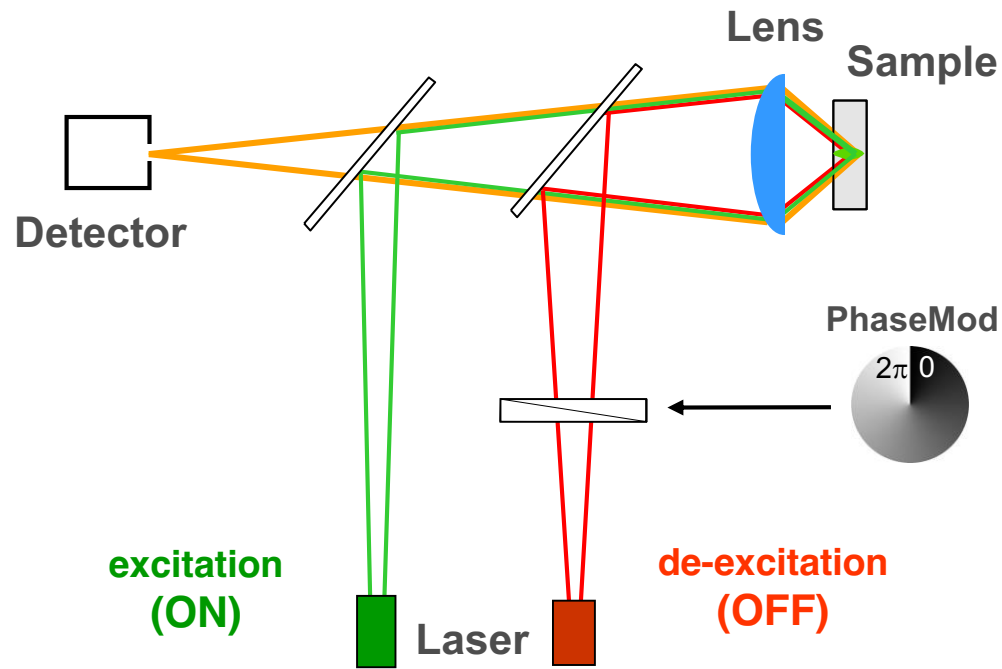
$$d = \frac{\lambda}{2n \sin \alpha}$$



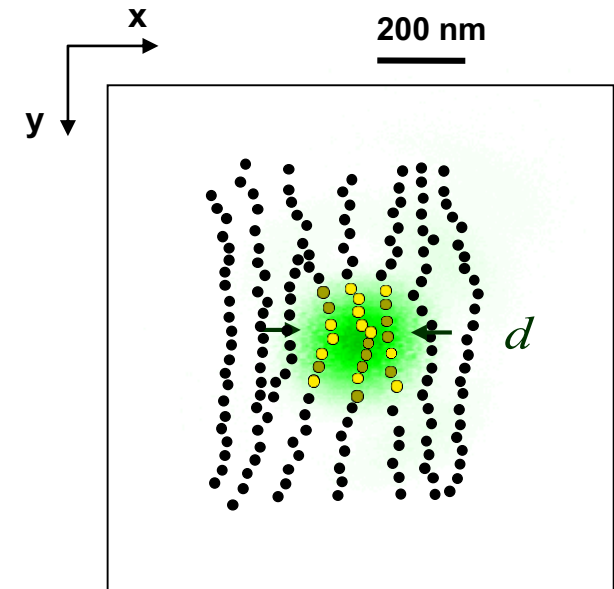
Keep molecules in a dark state !



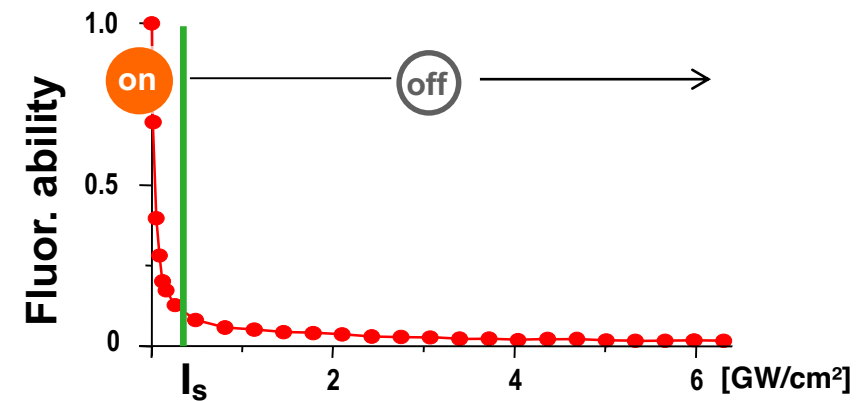
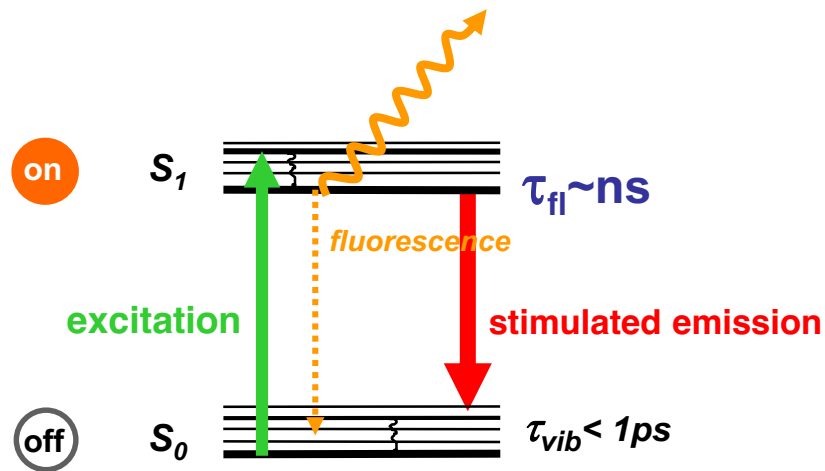
STED microscope:



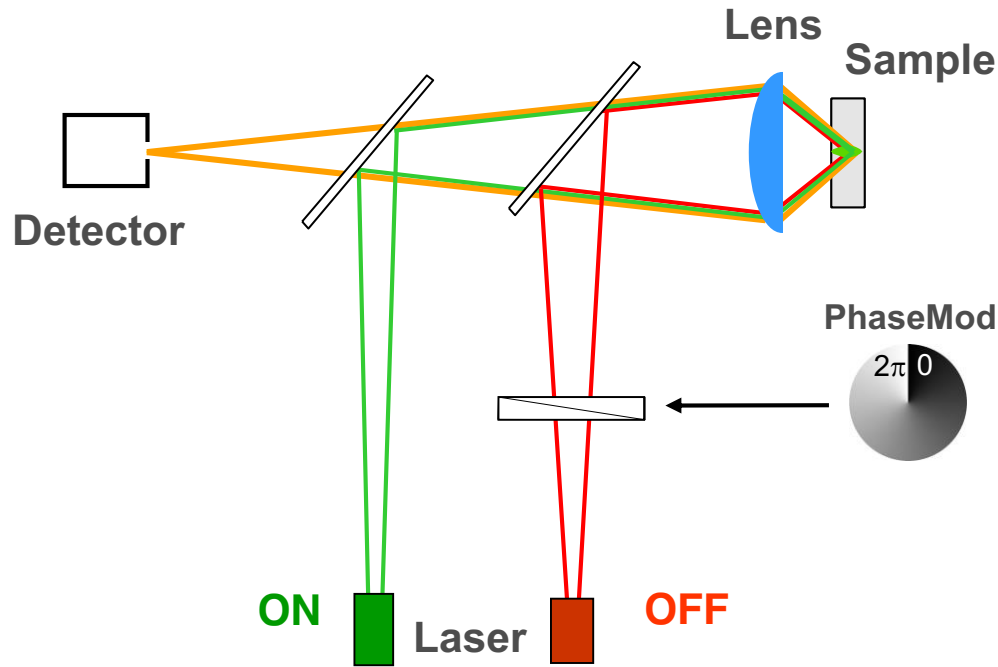
Hell & Wichmann, Opt. Lett. (1994)



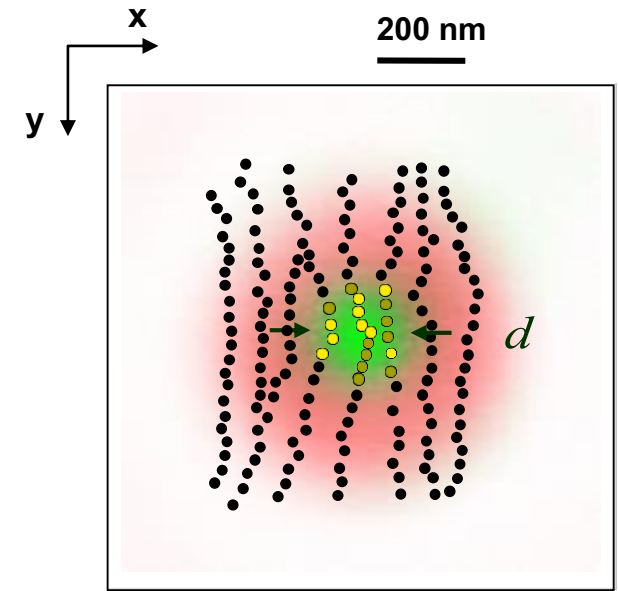
$$d \approx \frac{\lambda}{2n \sin \alpha}$$



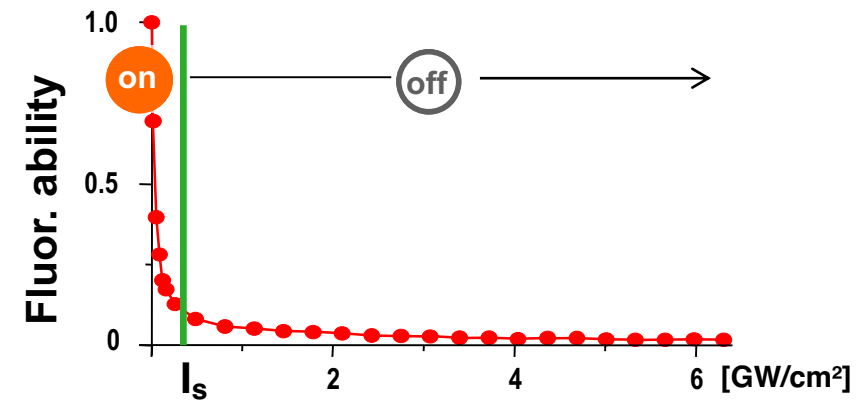
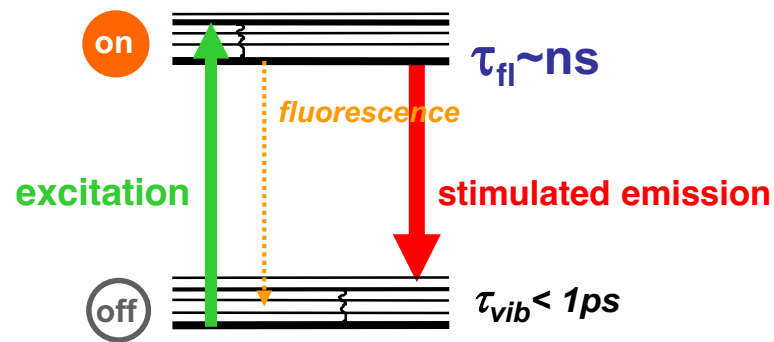
STED microscope:



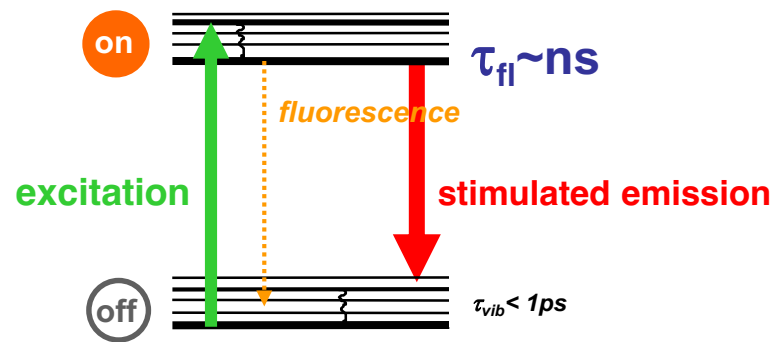
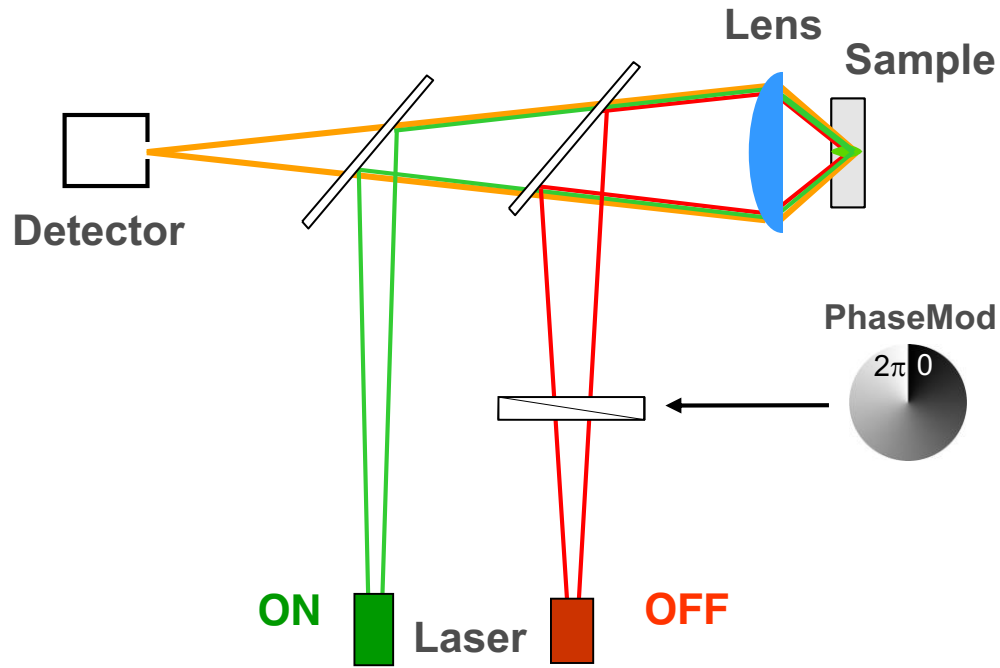
Hell & Wichmann, Opt. Lett. (1994)



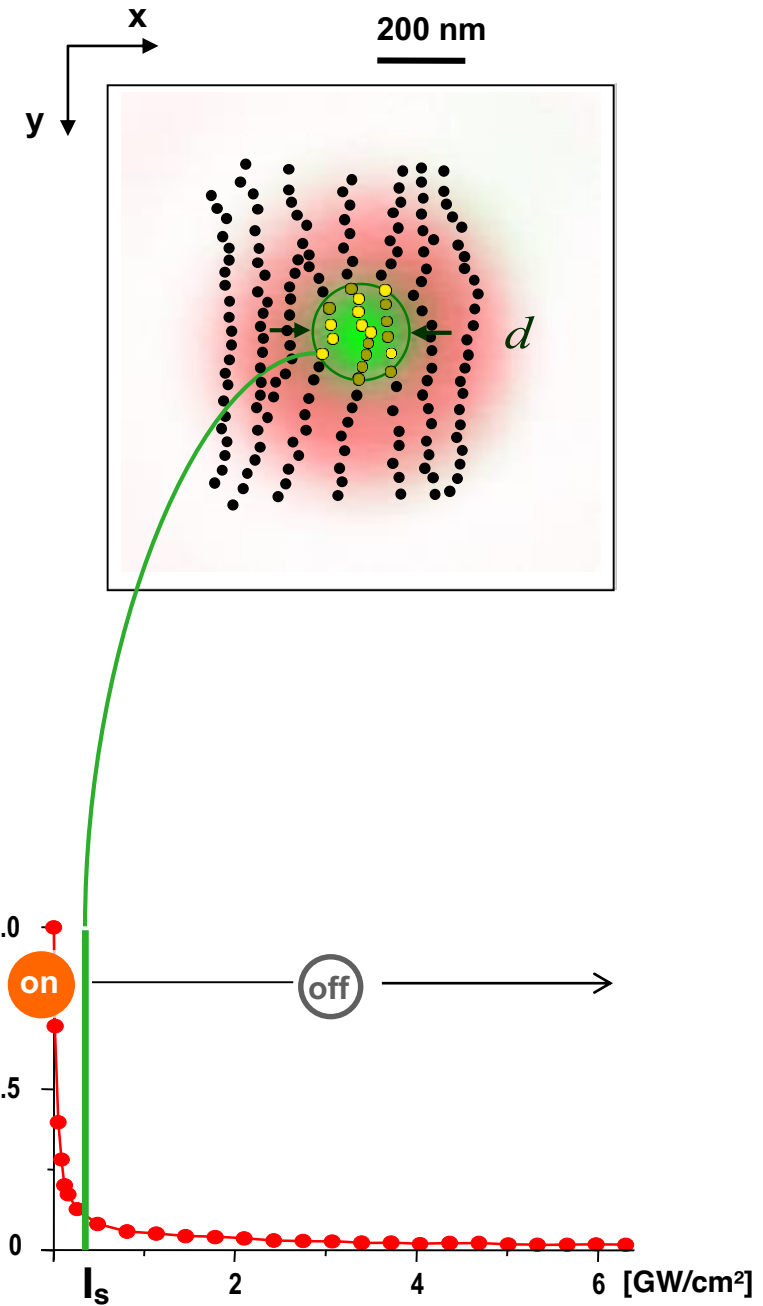
$$d \approx \frac{\lambda}{2n \sin \alpha}$$



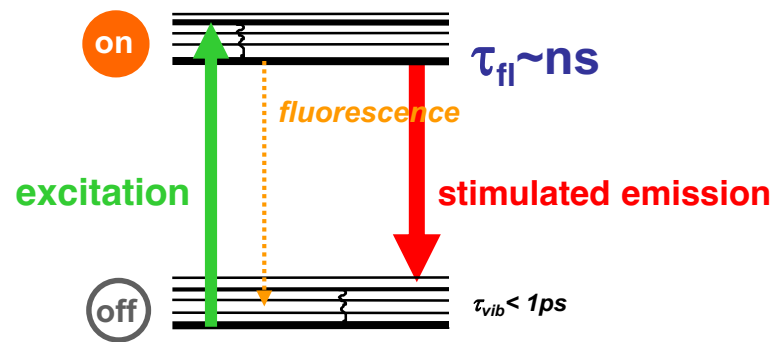
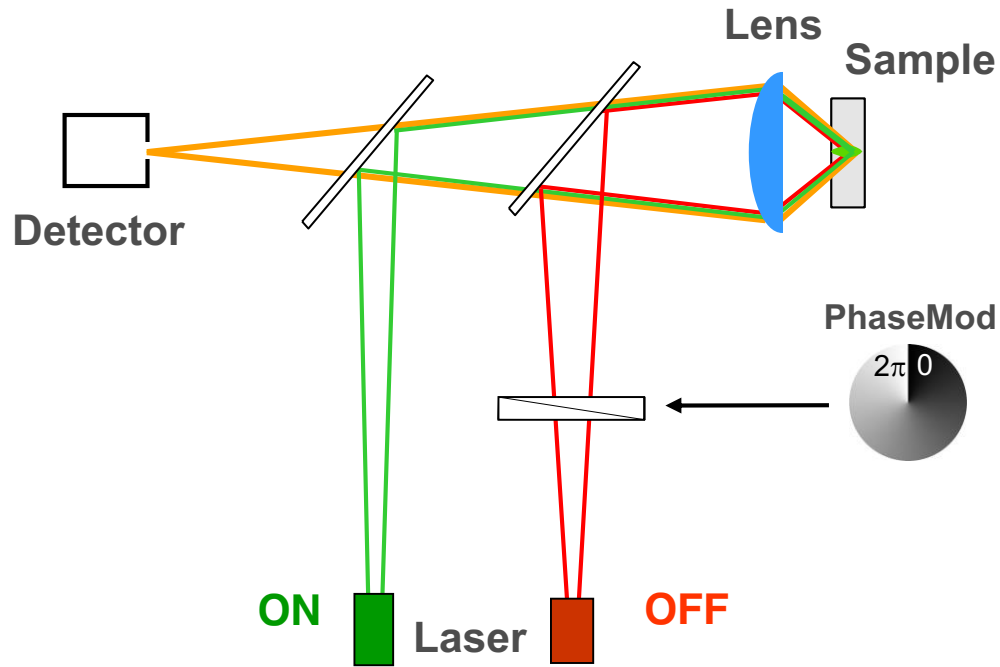
STED microscope:



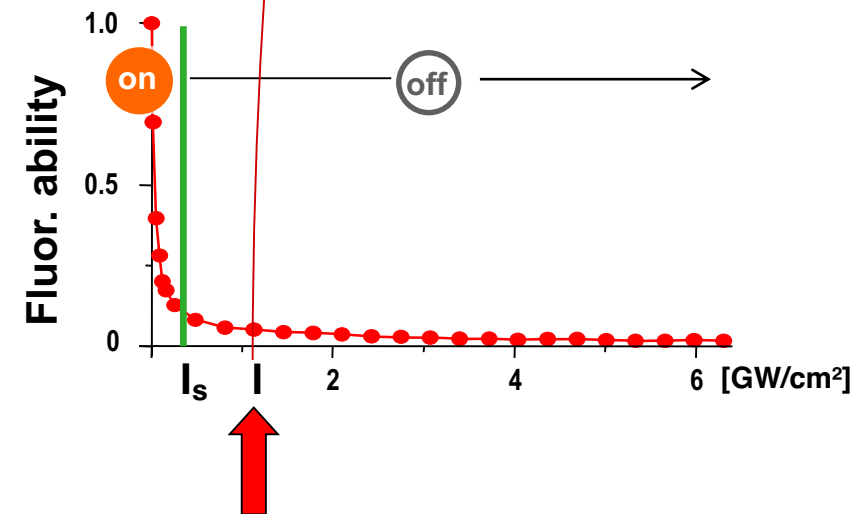
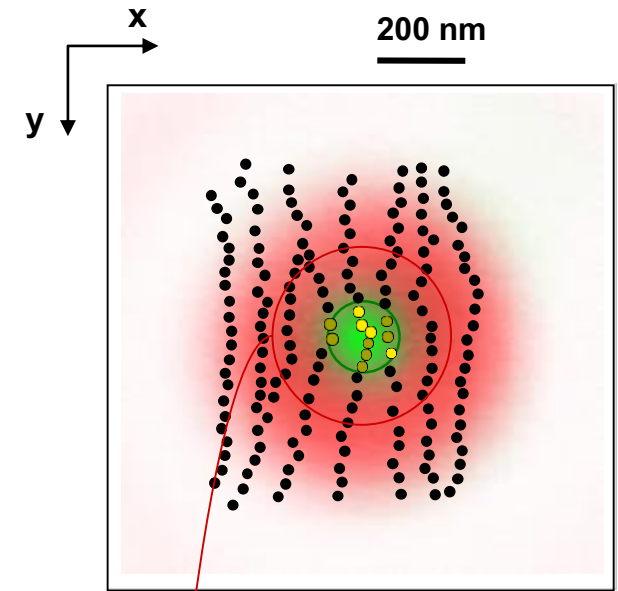
Hell & Wichmann, Opt. Lett. (1994)



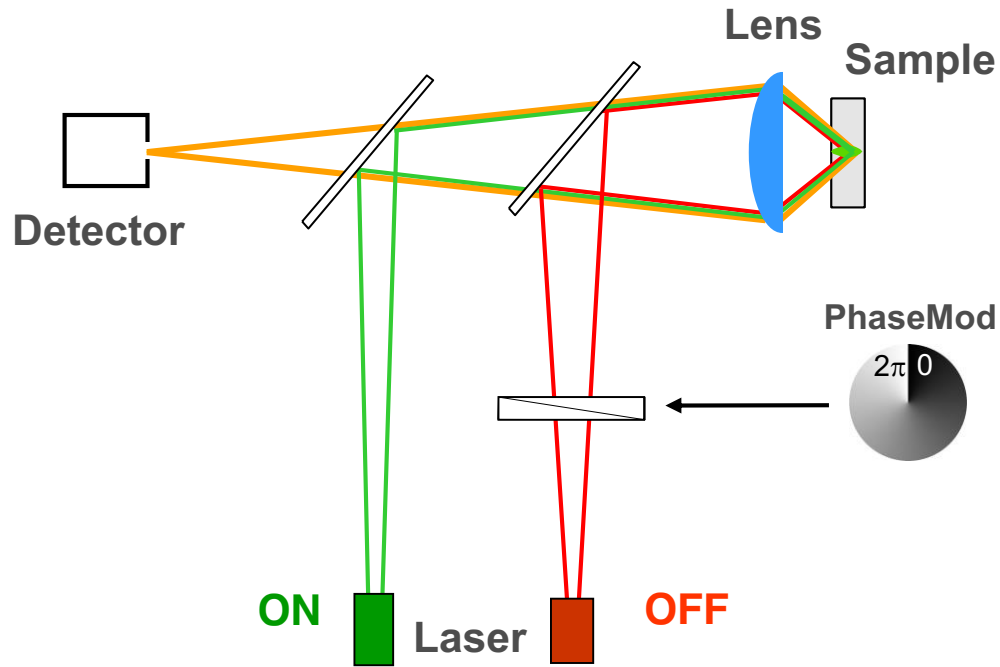
STED microscope:



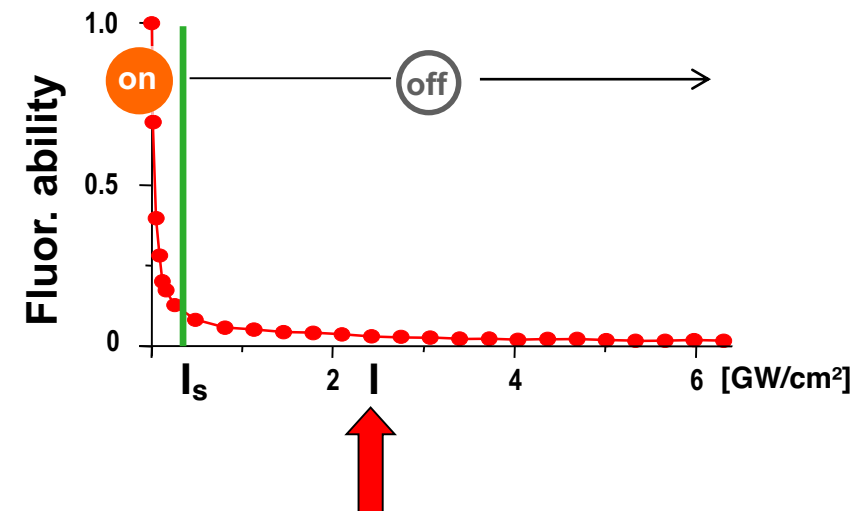
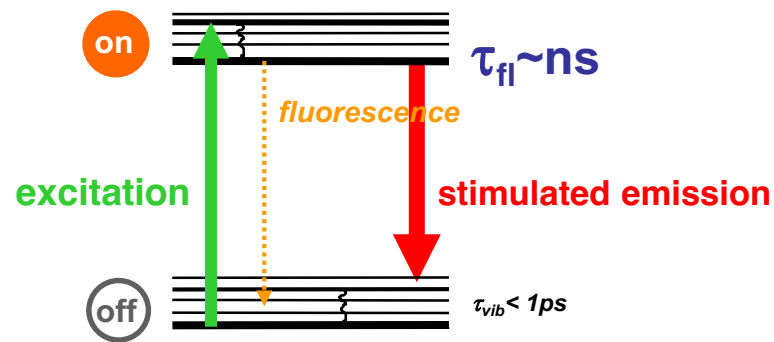
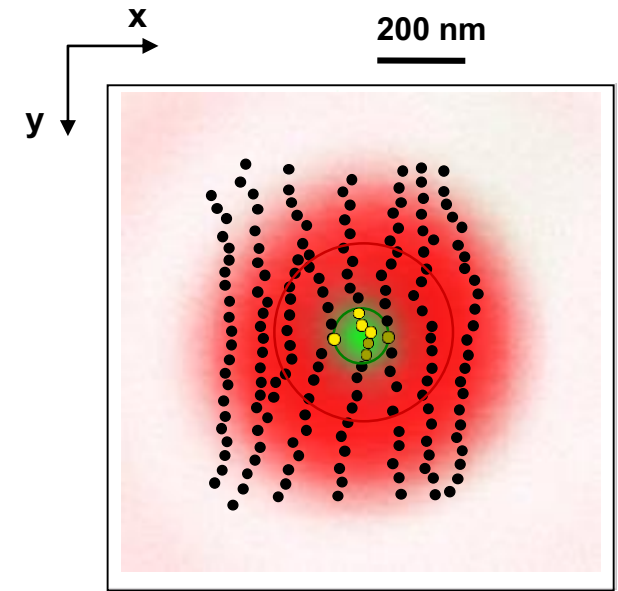
Hell & Wichmann, Opt. Lett. (1994)



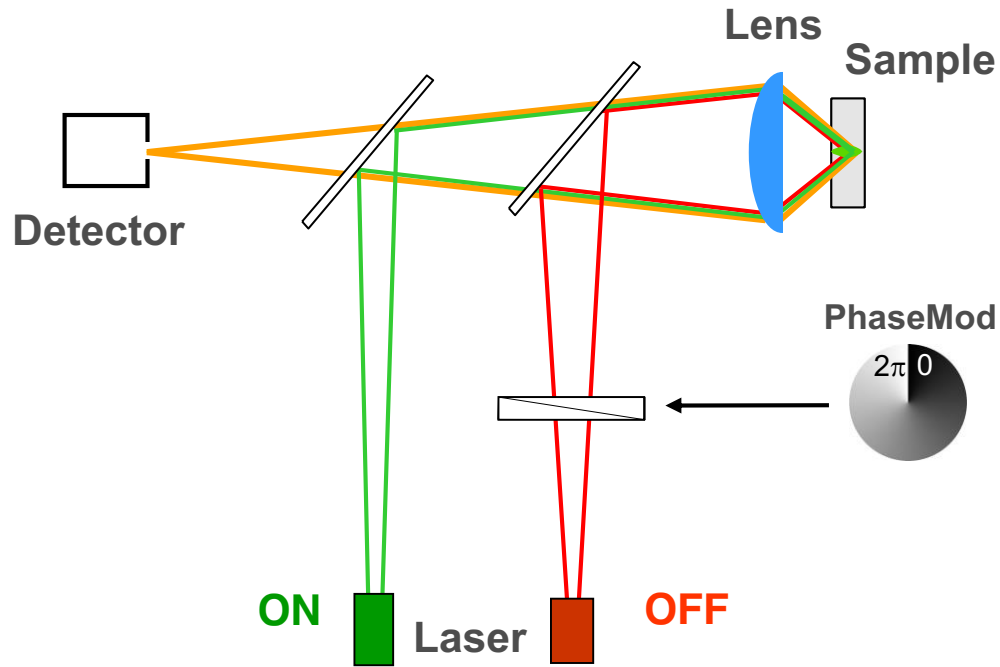
STED microscope:



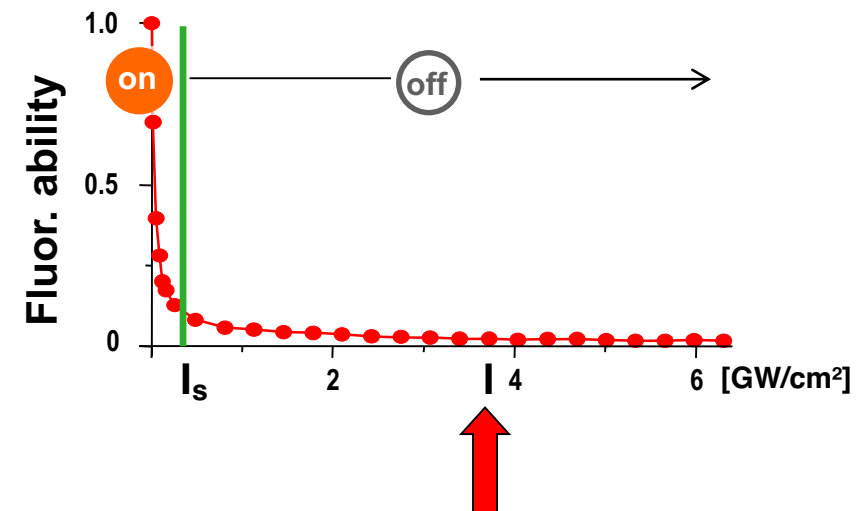
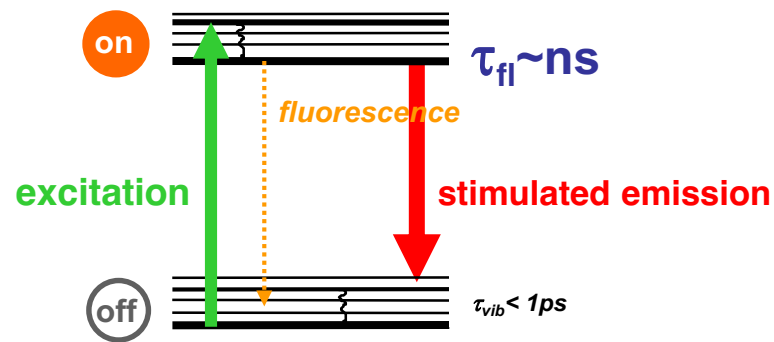
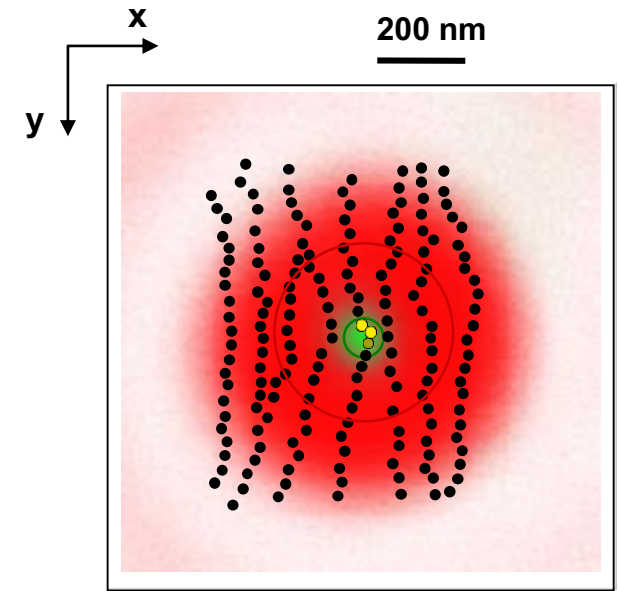
Hell & Wichmann, Opt. Lett. (1994)



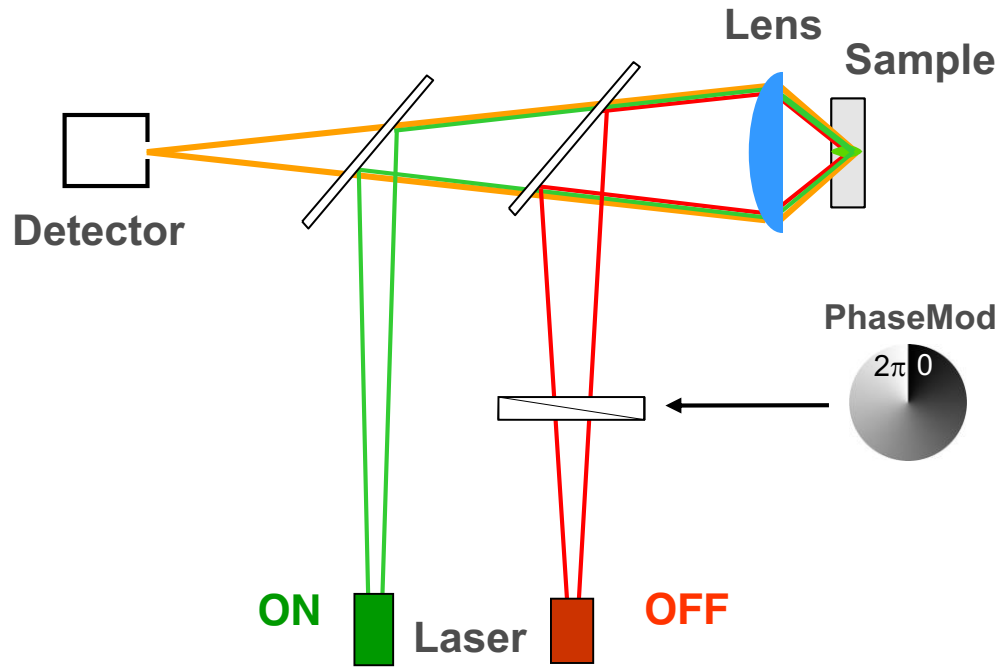
STED microscope:



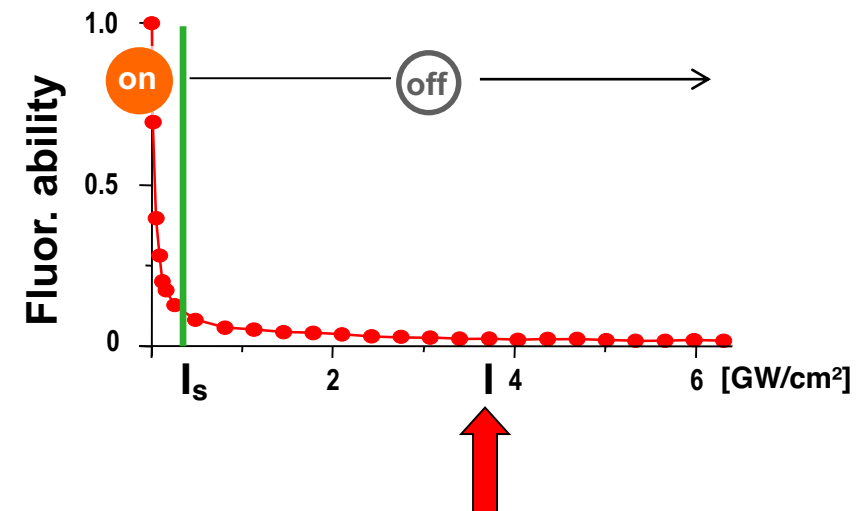
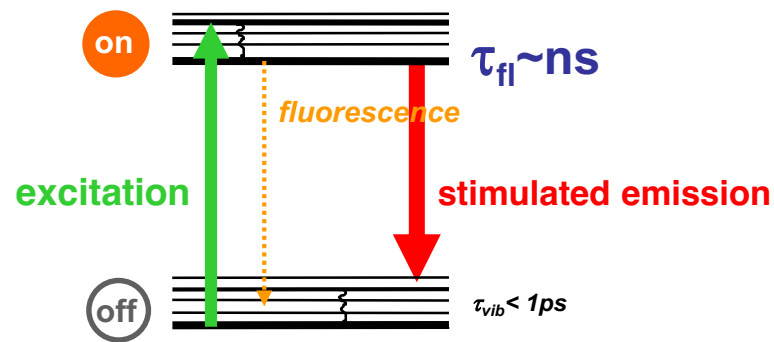
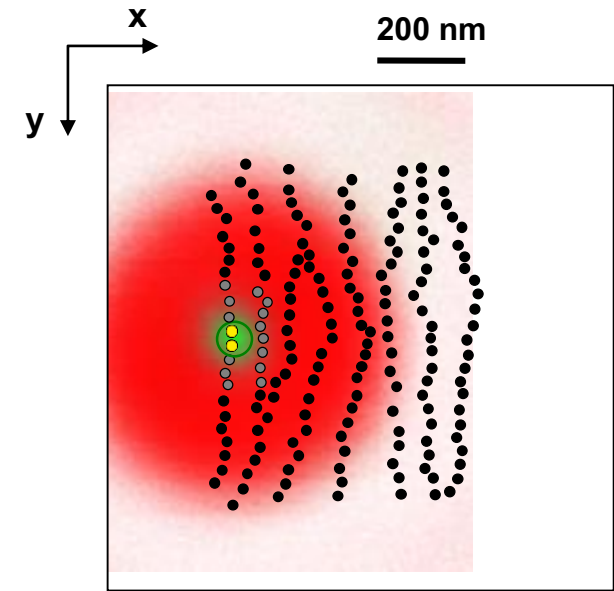
Hell & Wichmann, Opt. Lett. (1994)



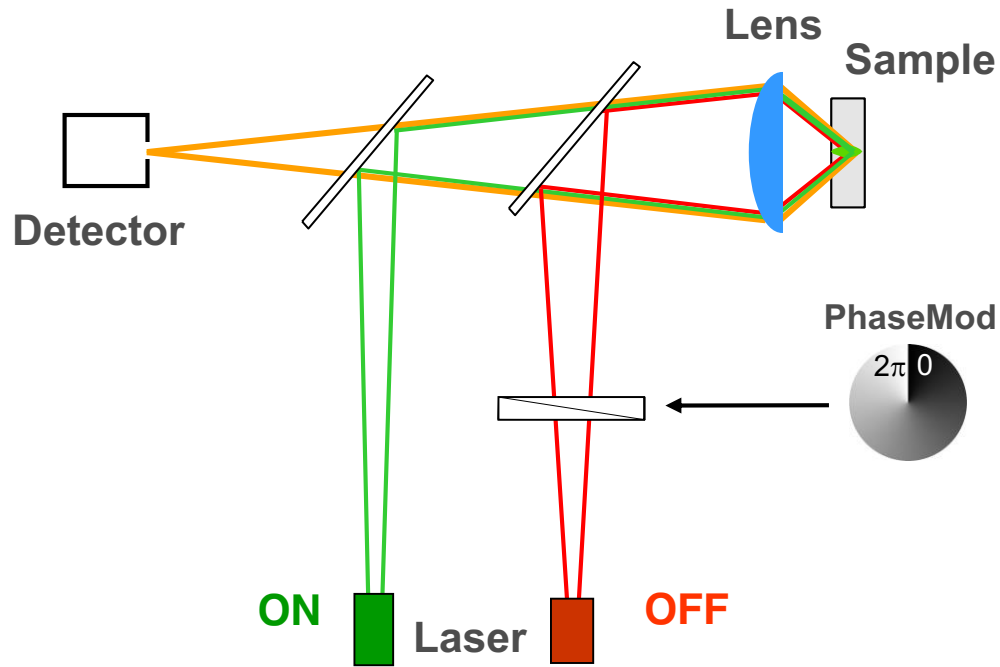
STED microscope:



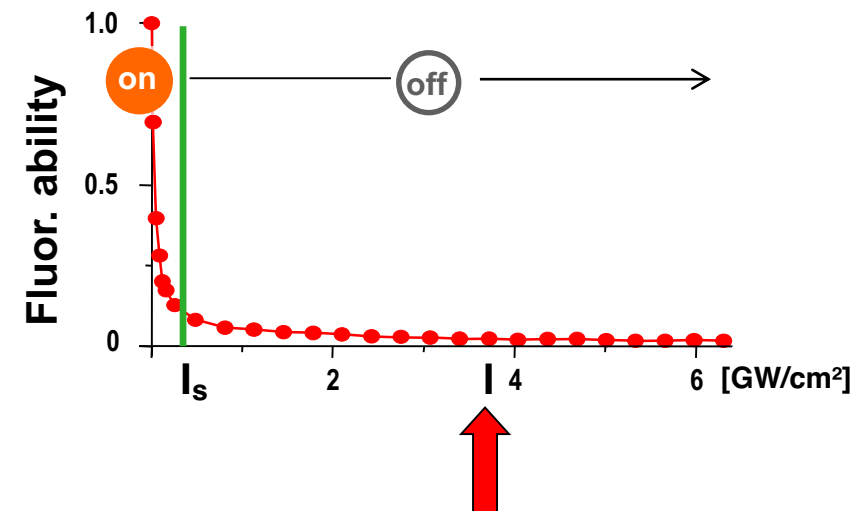
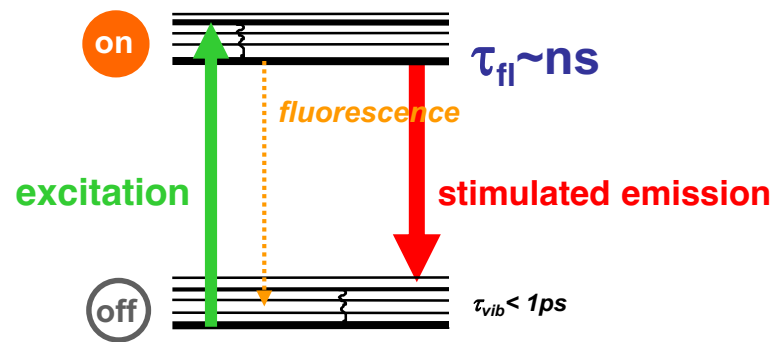
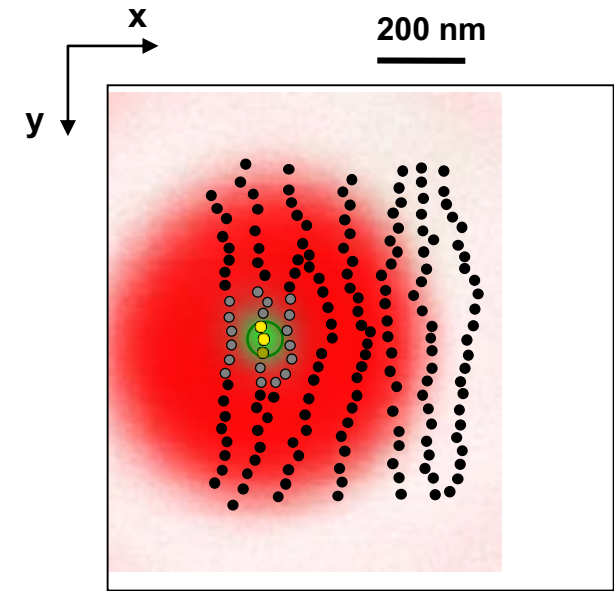
Hell & Wichmann, Opt. Lett. (1994)



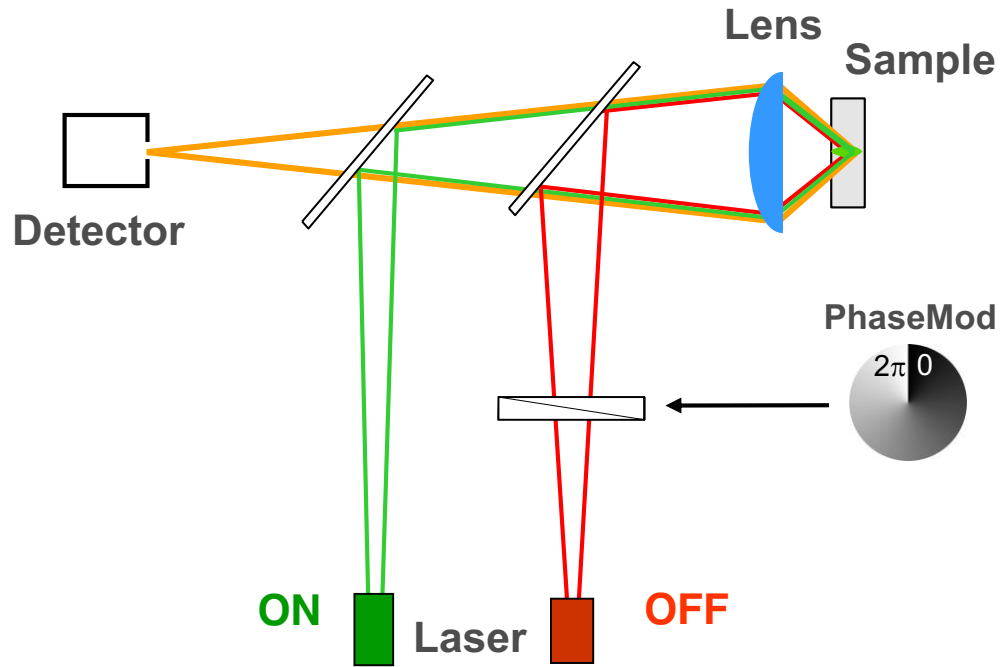
STED microscope:



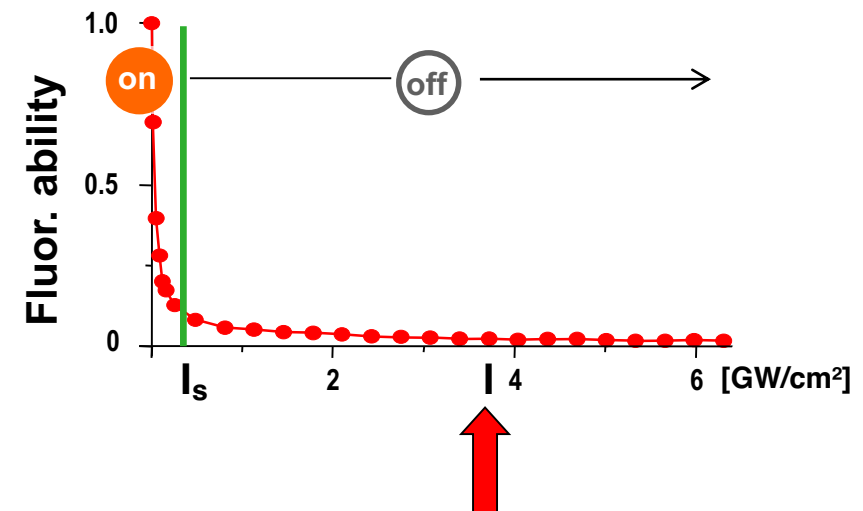
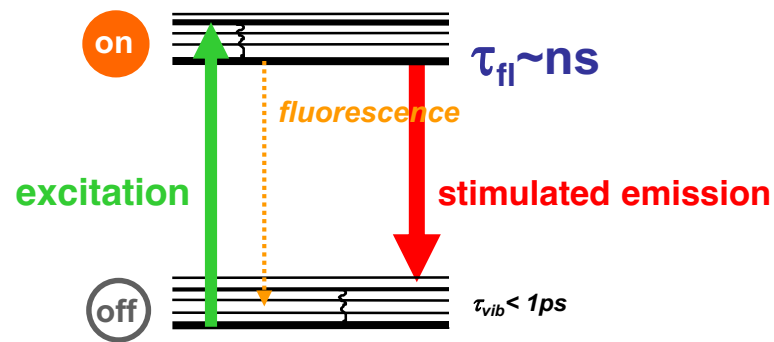
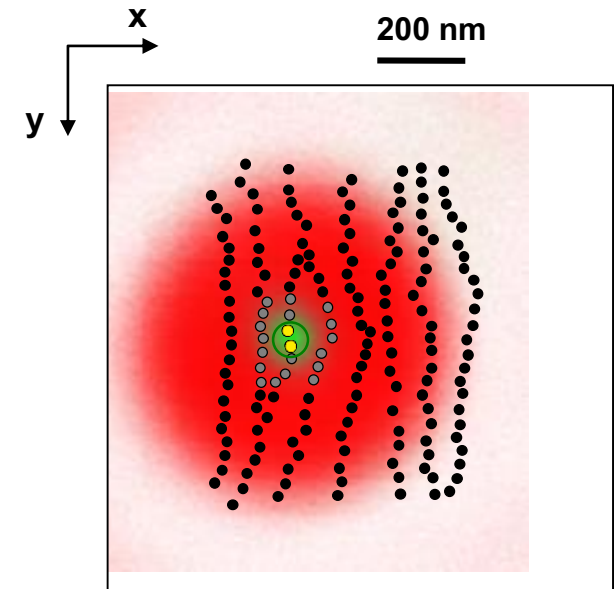
Hell & Wichmann, Opt. Lett. (1994)



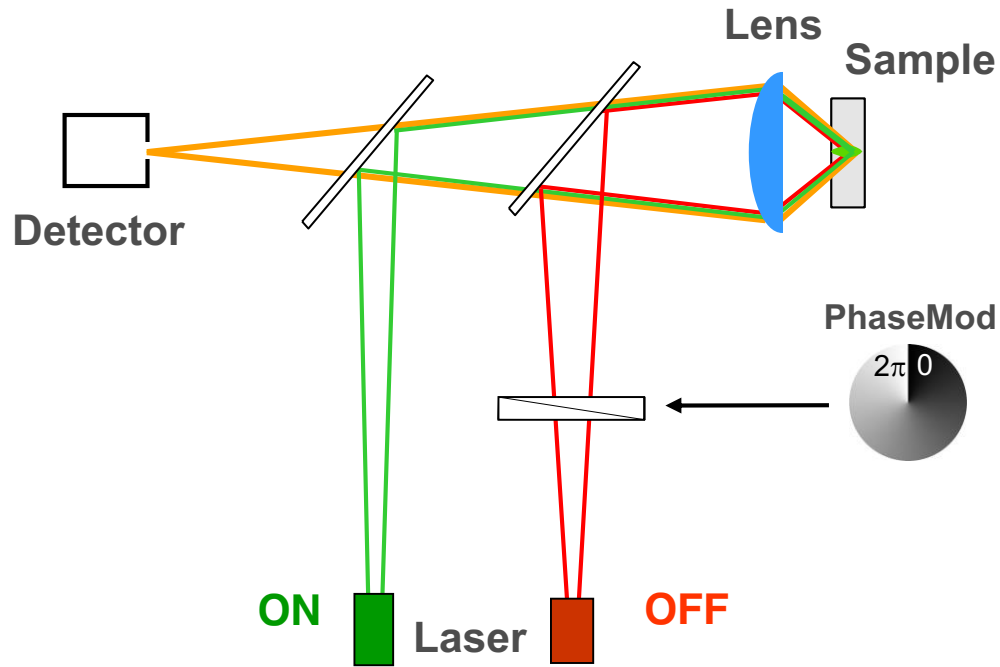
STED microscope:



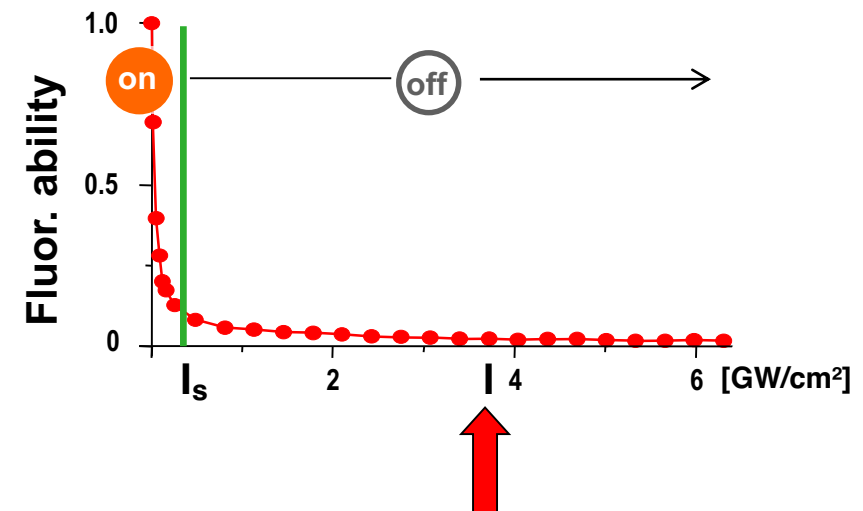
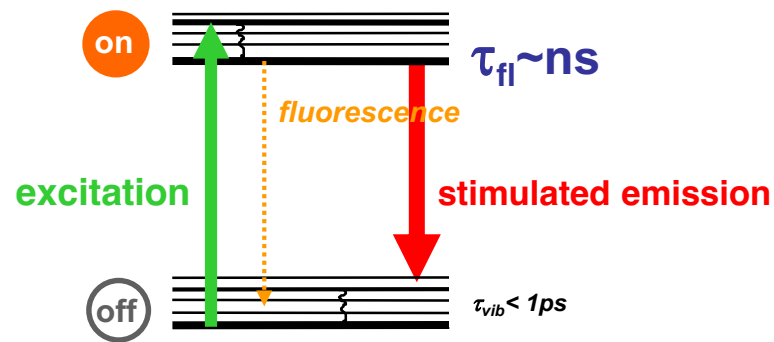
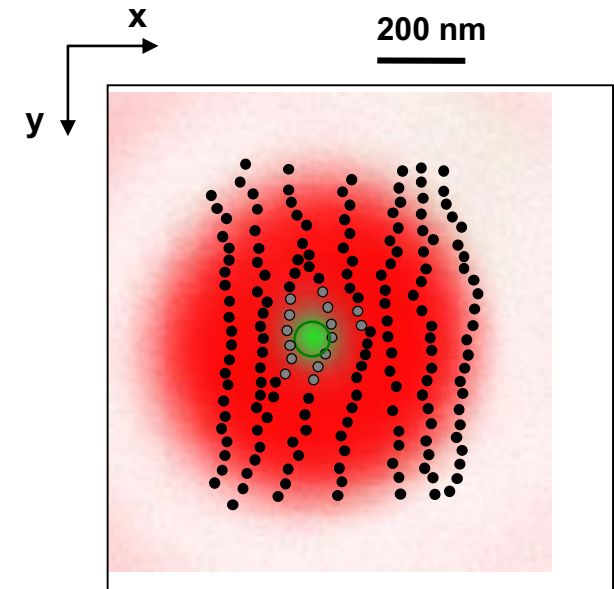
Hell & Wichmann, Opt. Lett. (1994)



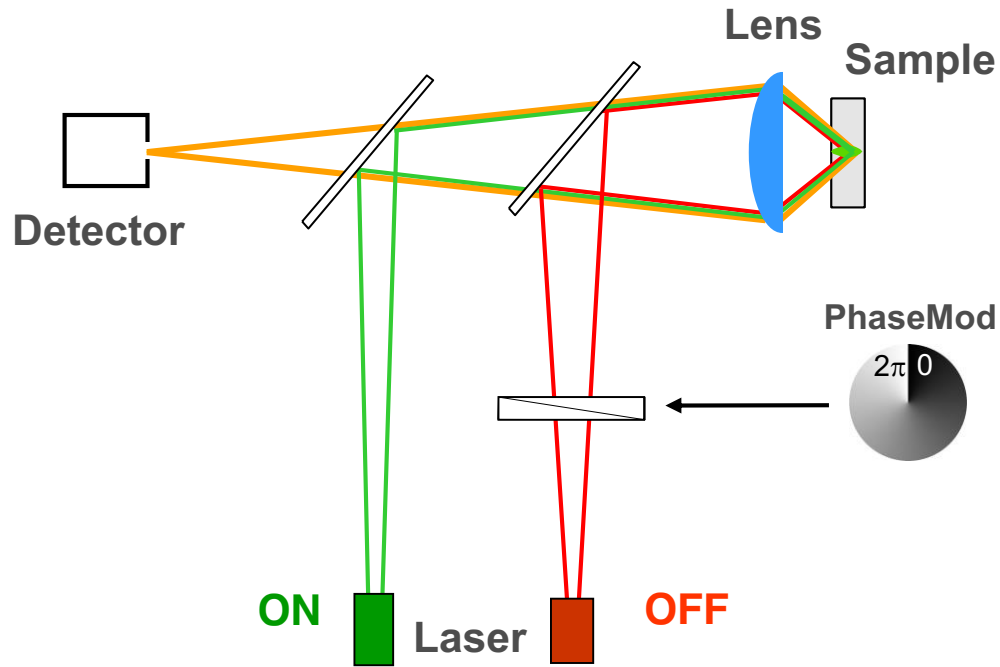
STED microscope:



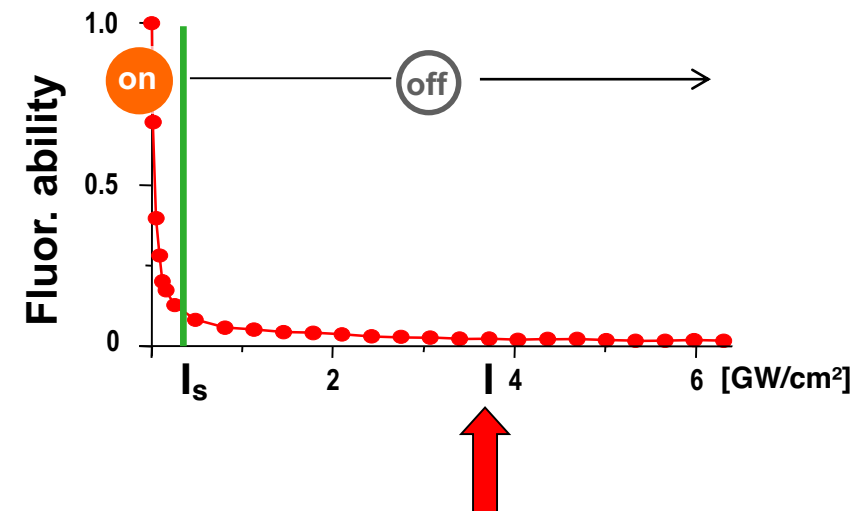
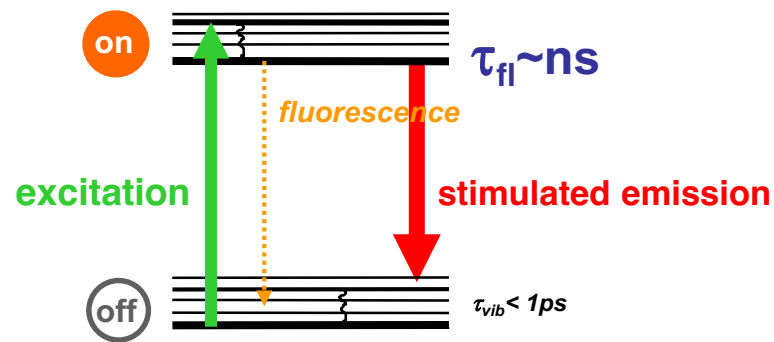
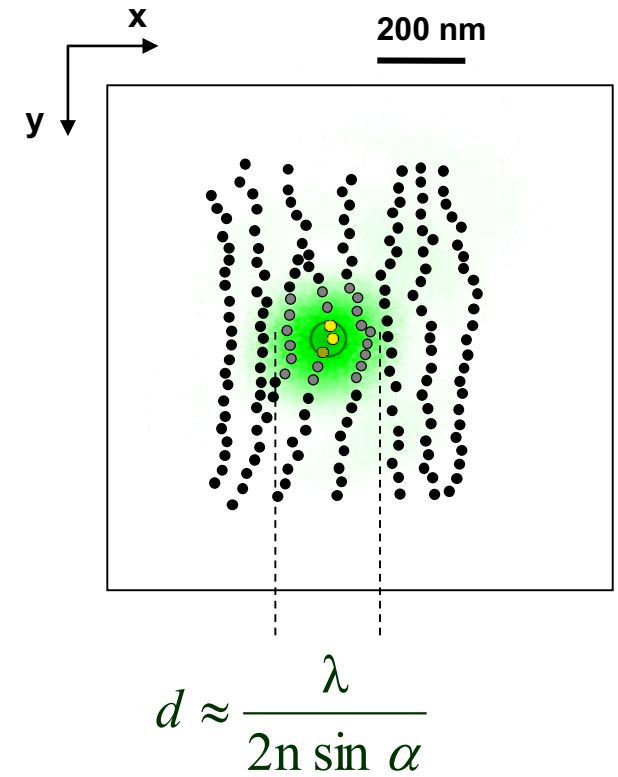
Hell & Wichmann, Opt. Lett. (1994)



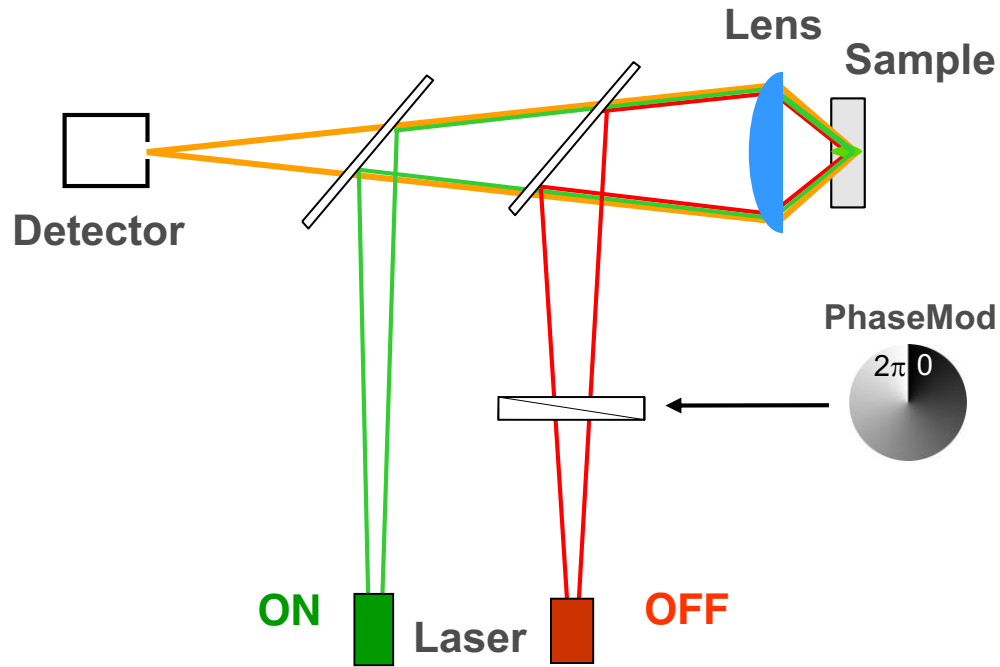
STED microscope:



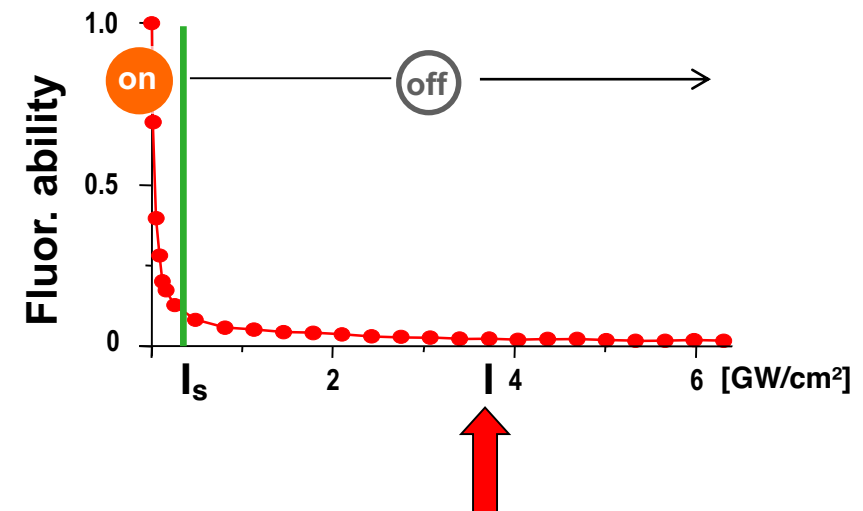
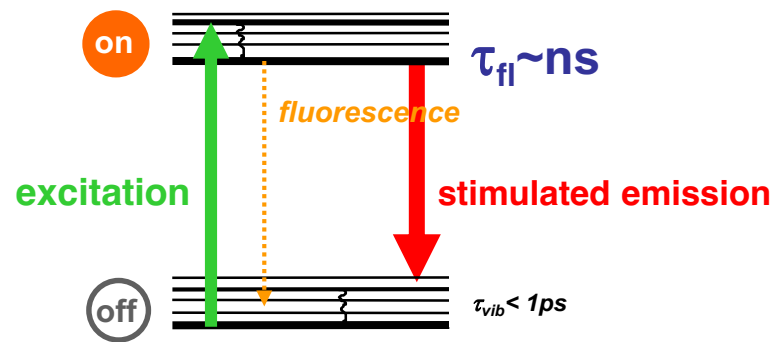
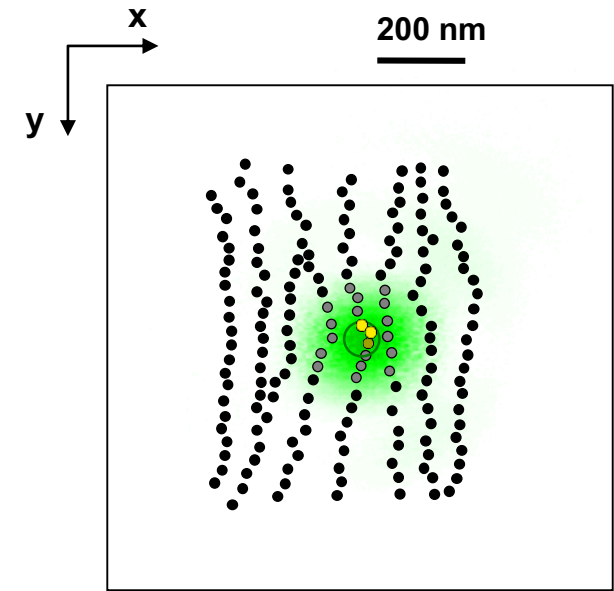
Hell & Wichmann, Opt. Lett. (1994)



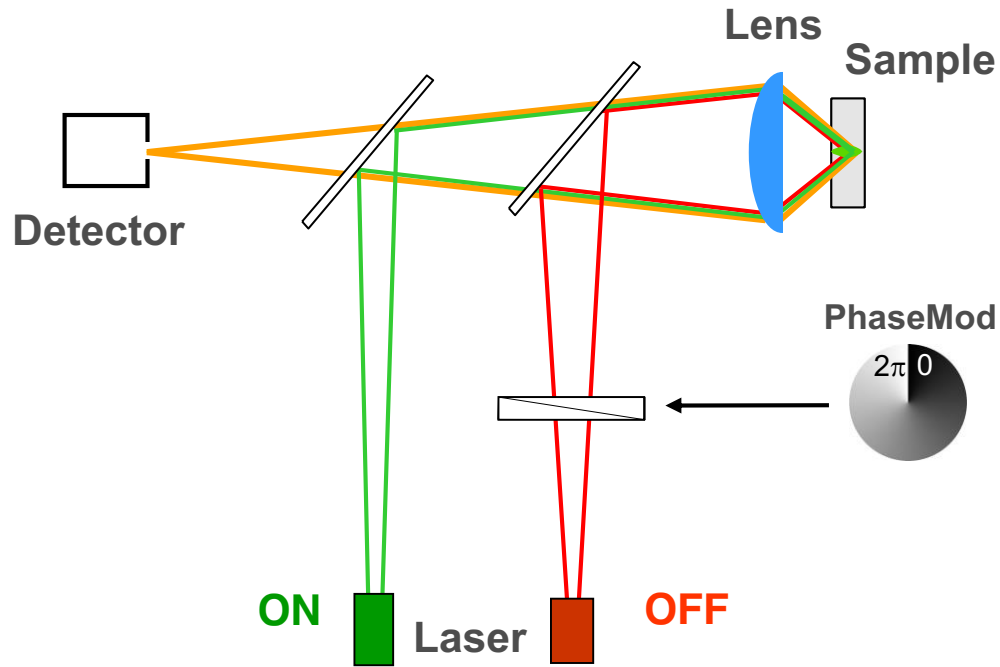
STED microscope:



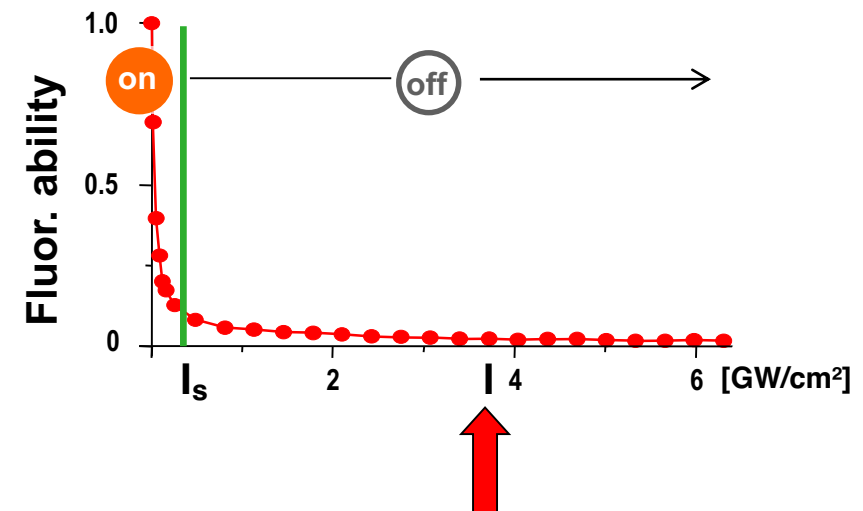
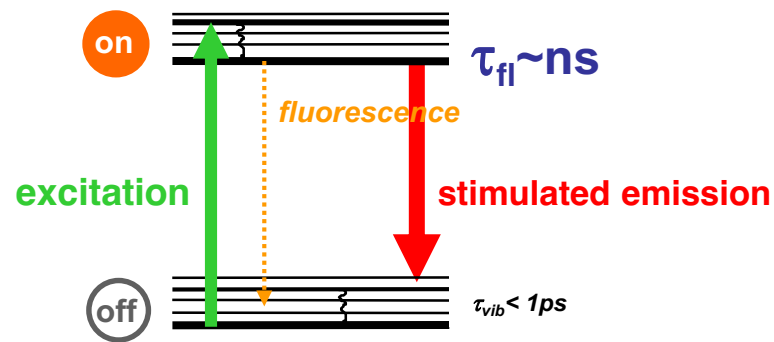
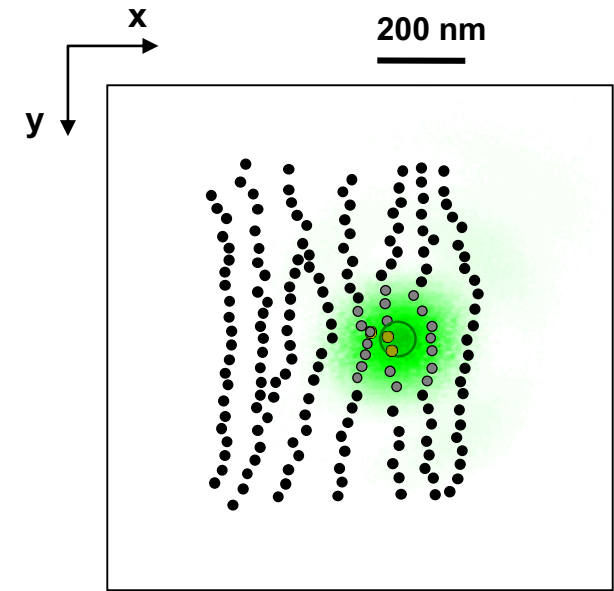
Hell & Wichmann, Opt. Lett. (1994)



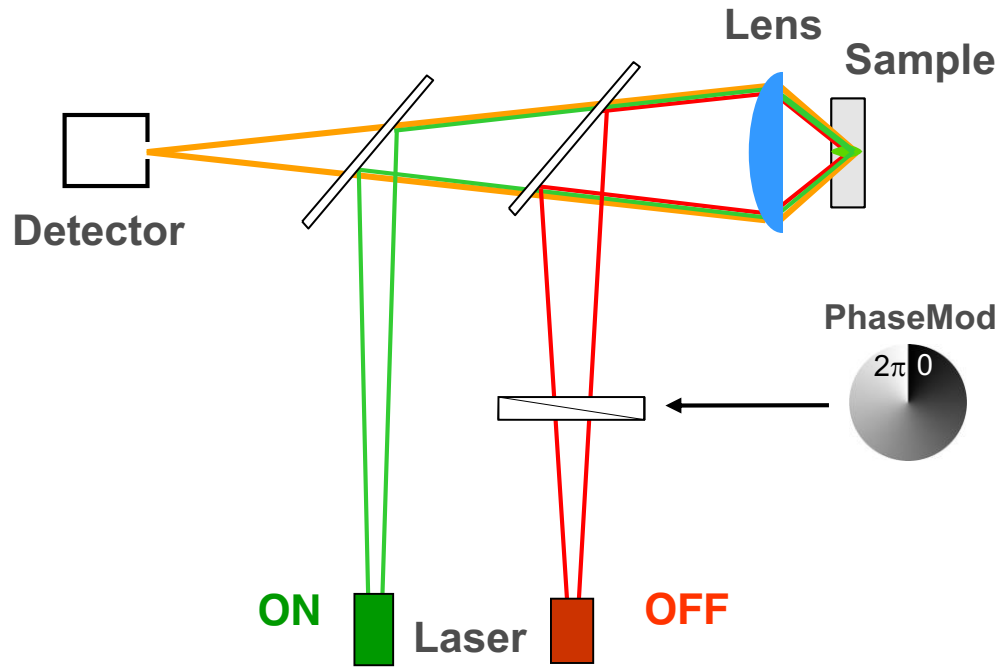
STED microscope:



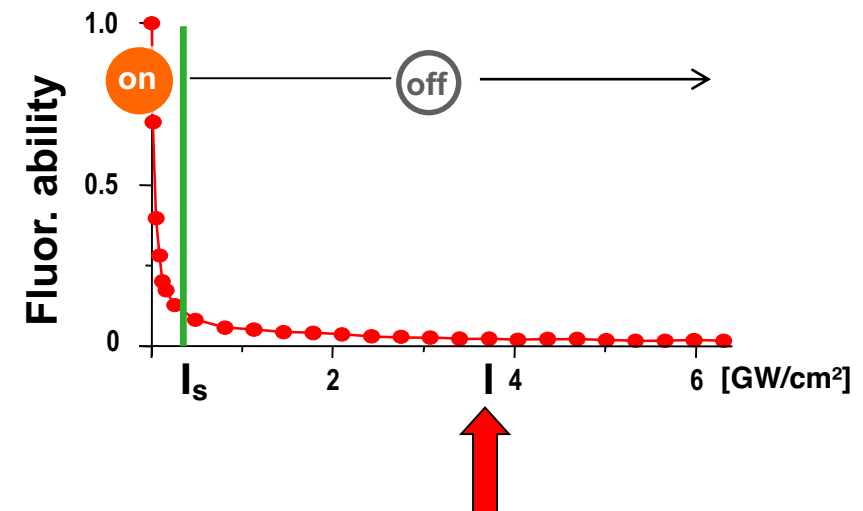
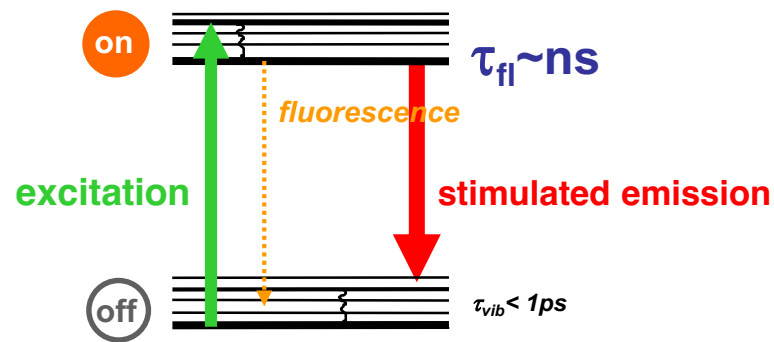
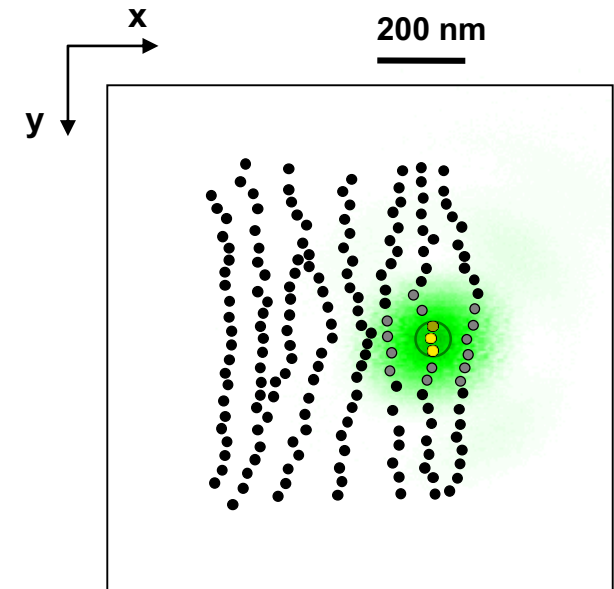
Hell & Wichmann, Opt. Lett. (1994)



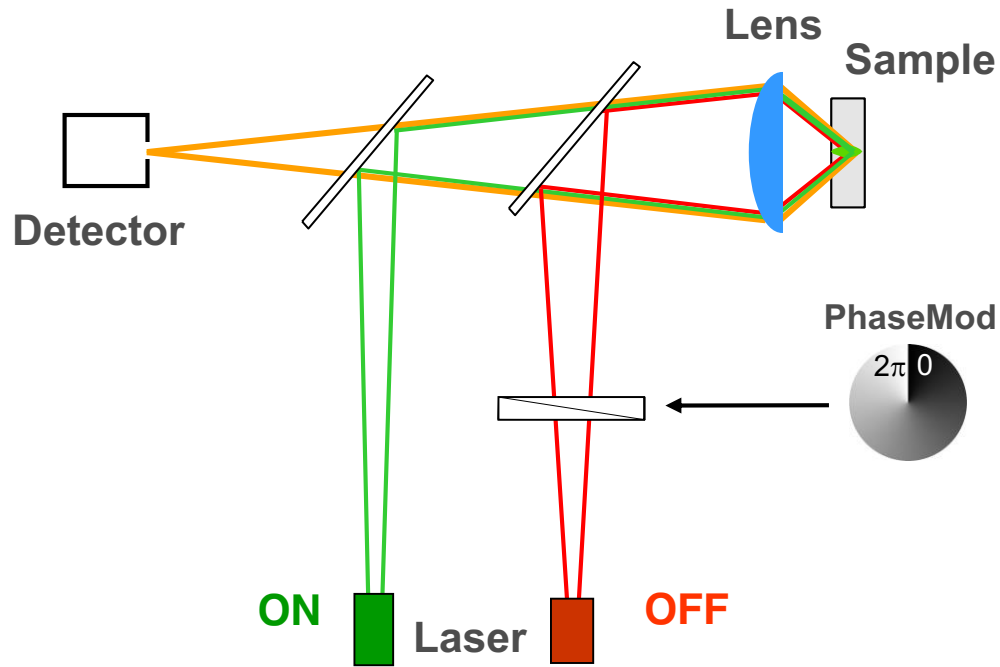
STED microscope:



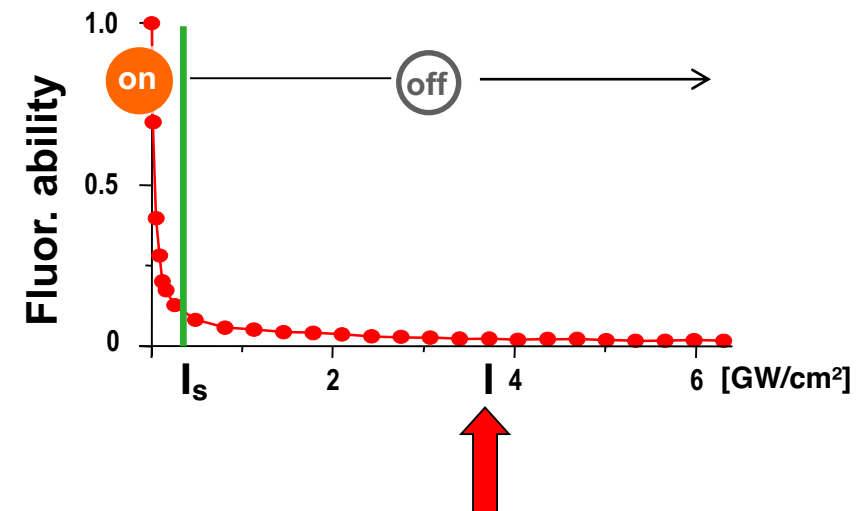
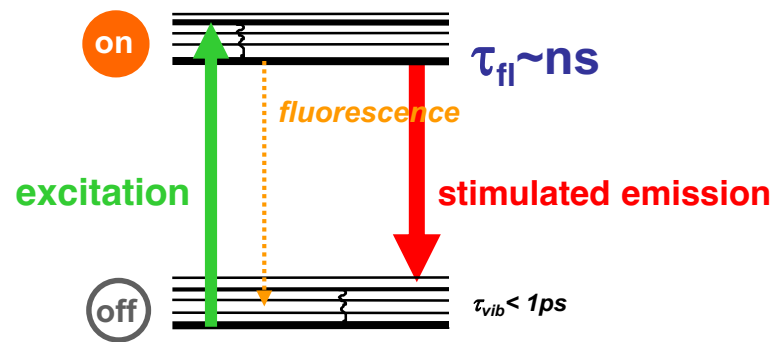
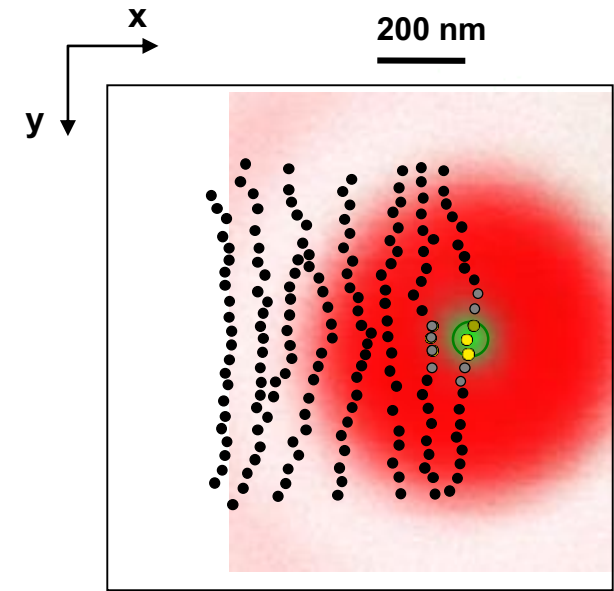
Hell & Wichmann, Opt. Lett. (1994)



STED microscope:

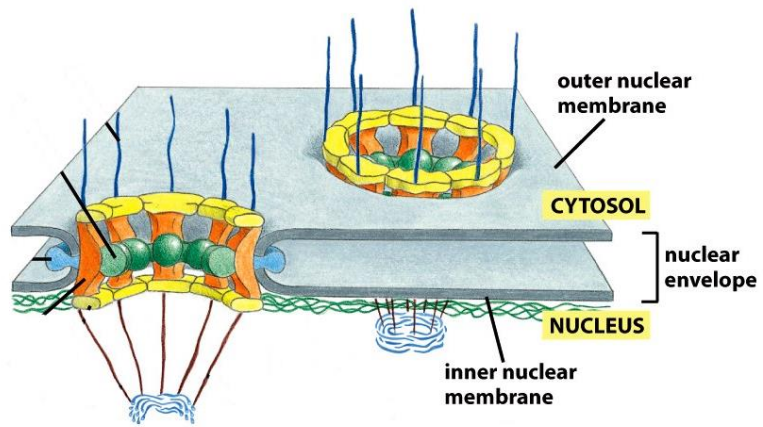


Hell & Wichmann, Opt. Lett. (1994)

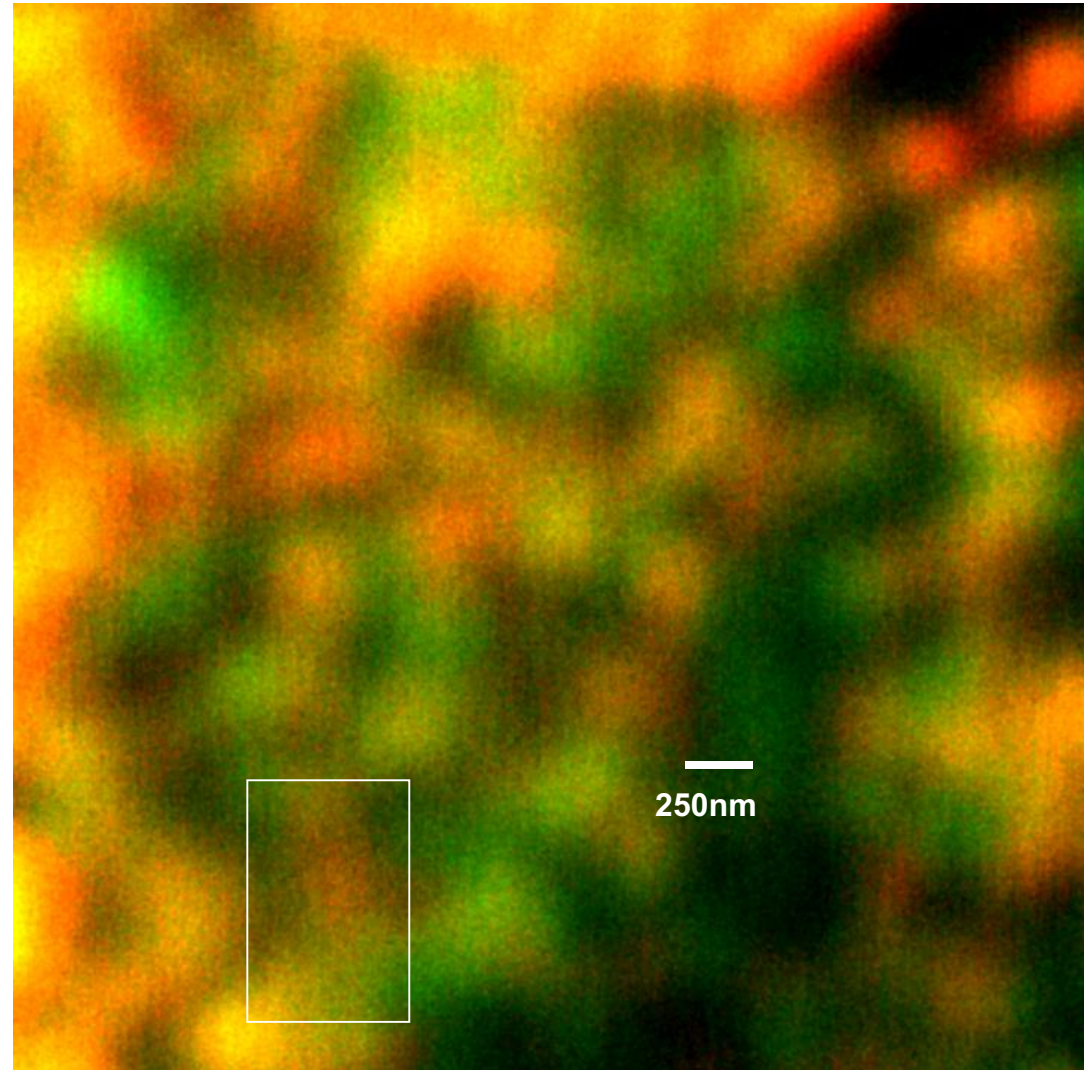


Protein assemblies in cell

Standard (Confocal)

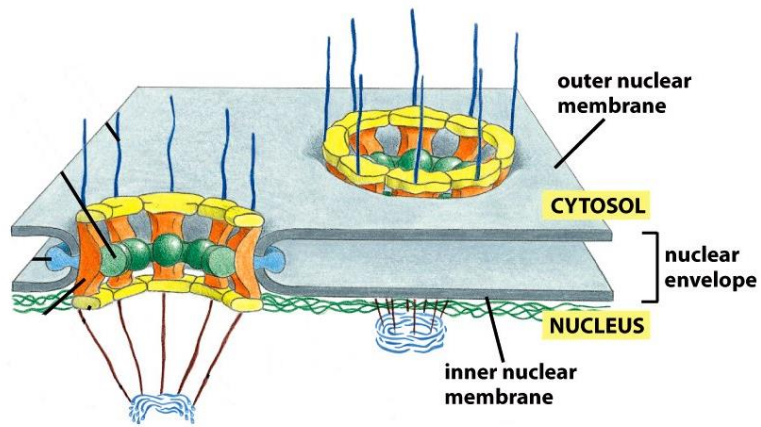


Nuclear pore complex

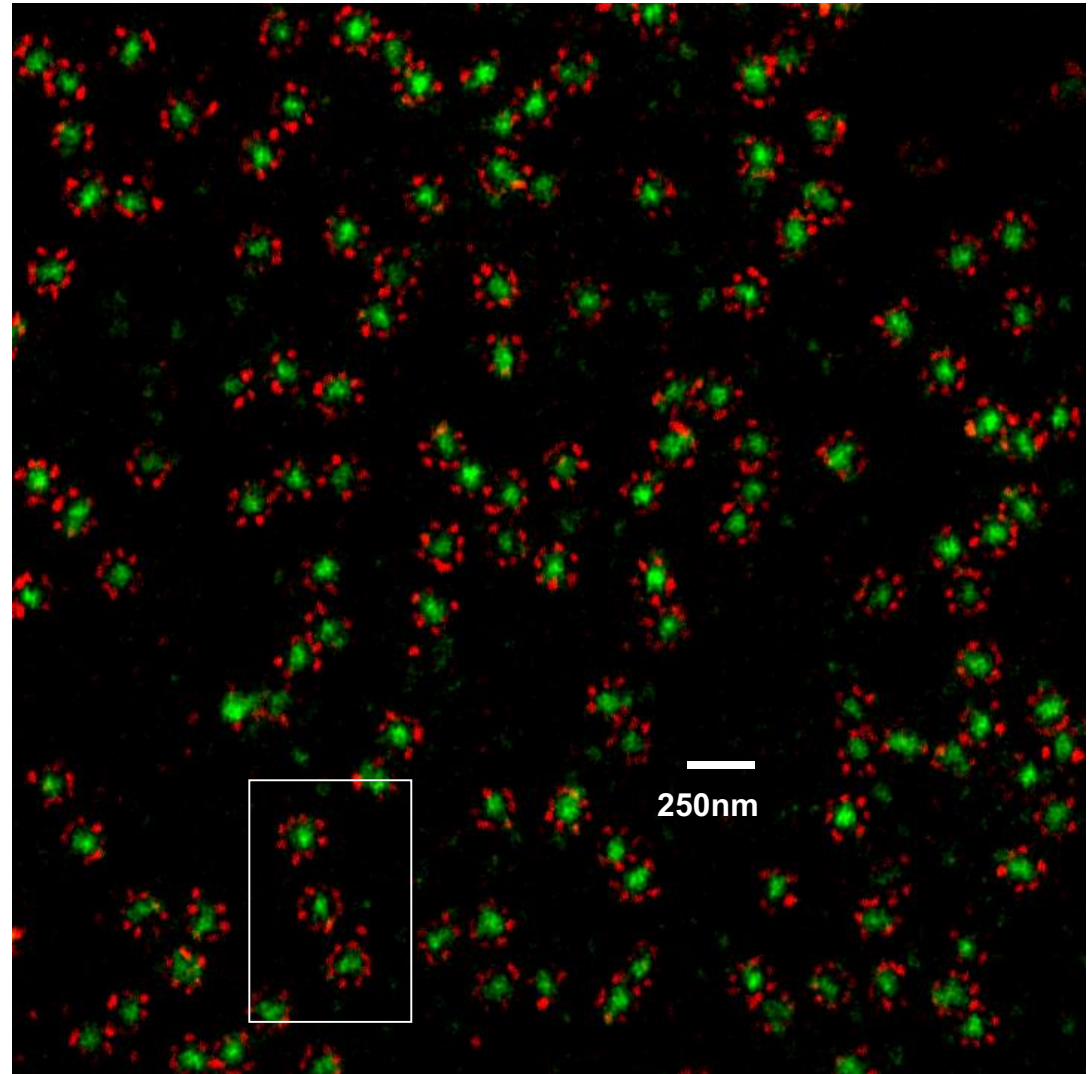


Protein assemblies in cell

STED

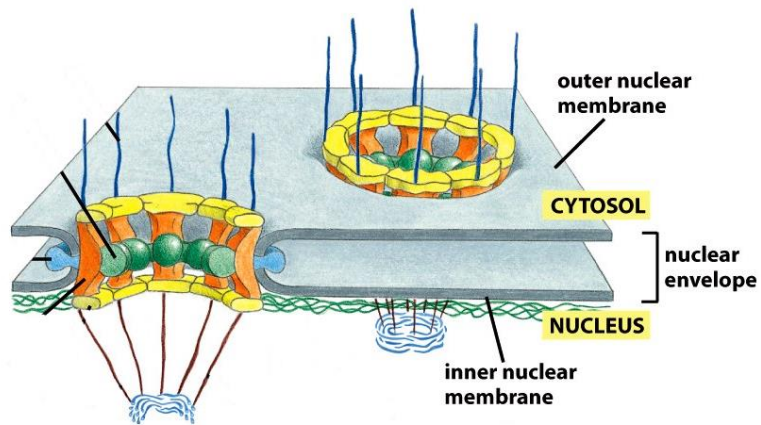


Nuclear pore complex

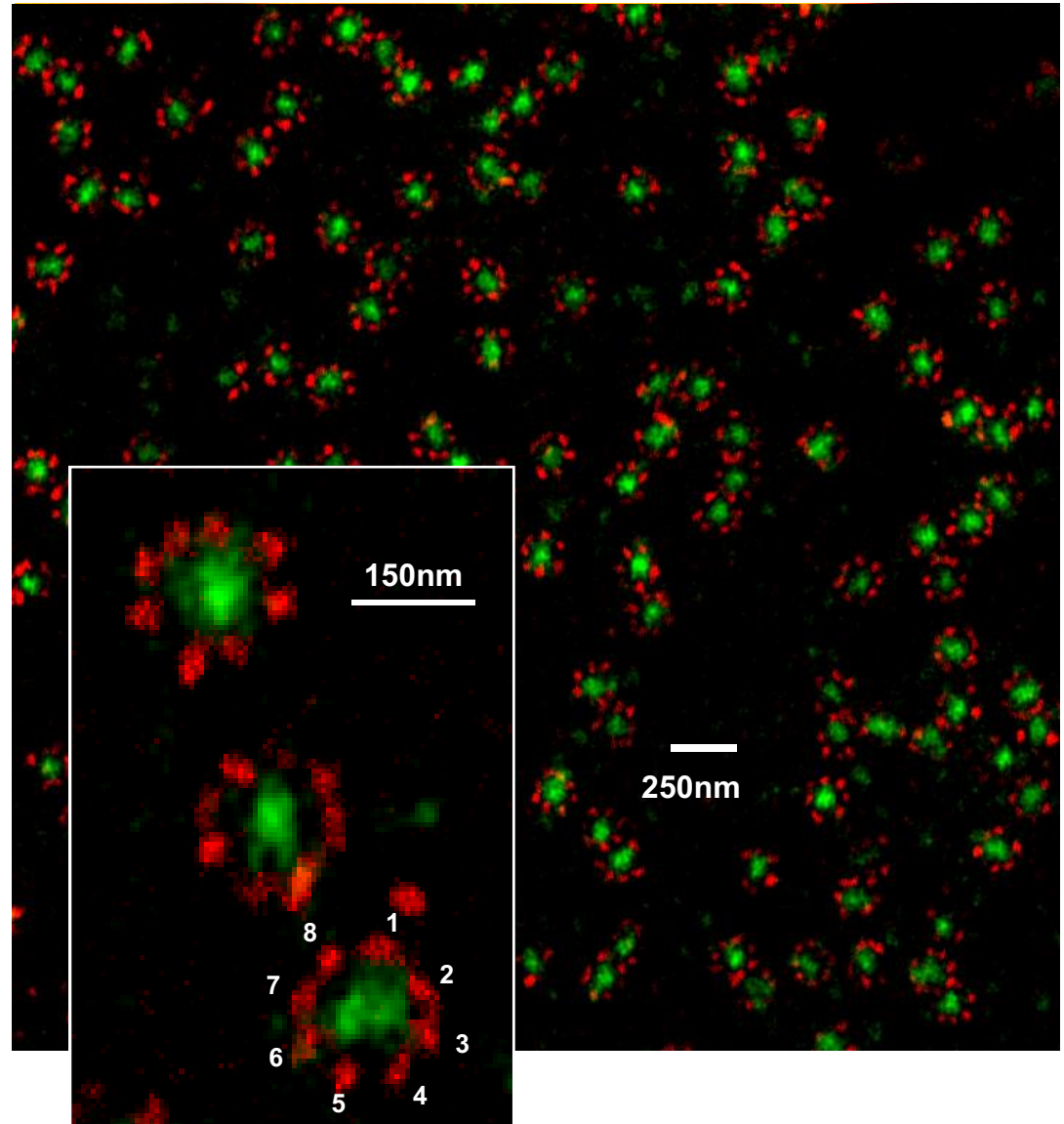


Protein assemblies in cell

STED

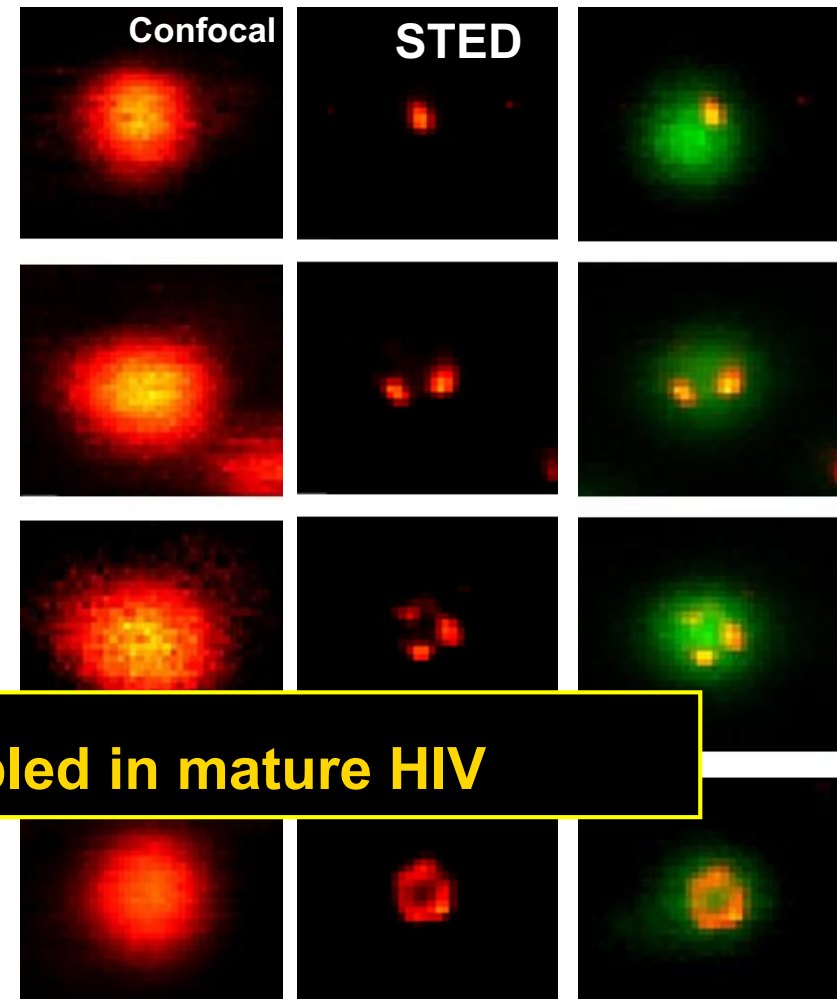
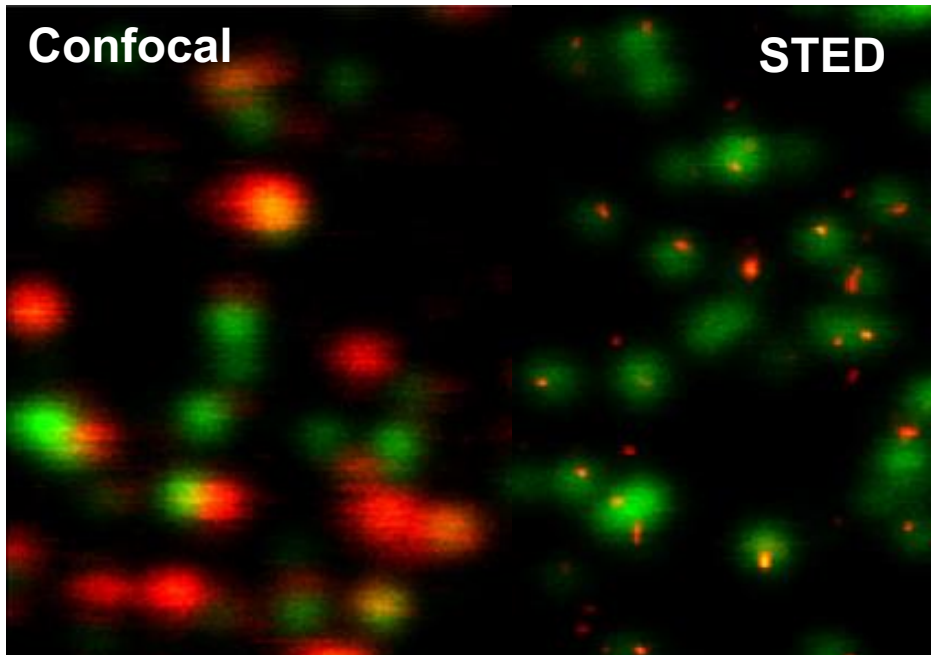
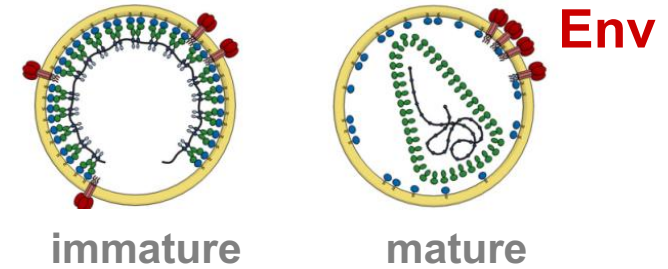


Nuclear pore complex



Viral infection

HIV **Env** envelope protein on single virions

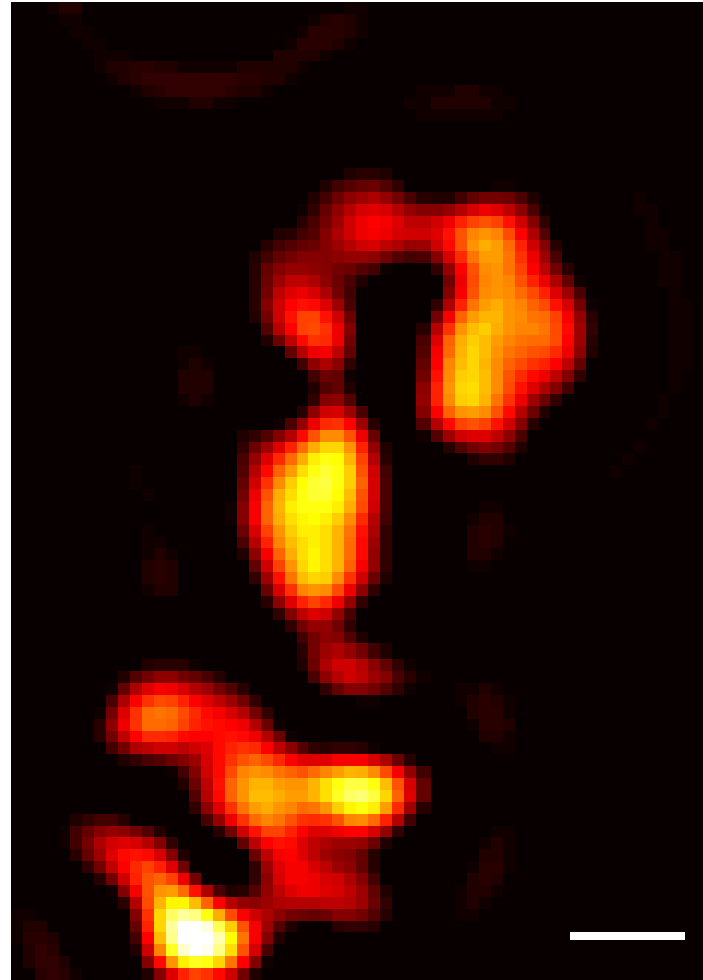


Insight: **Env proteins are assembled in mature HIV**

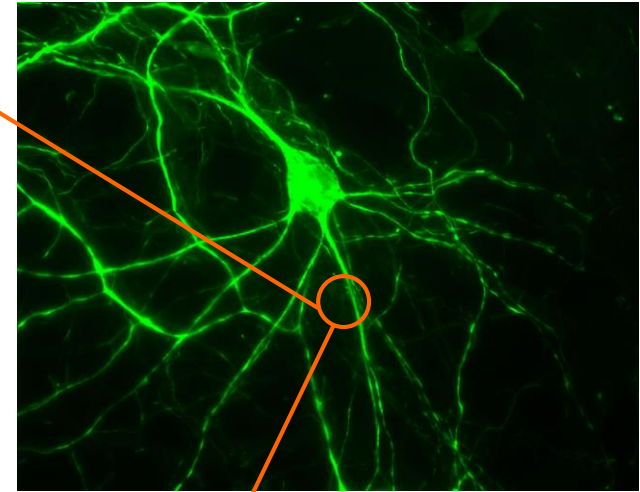
HIV (Vpr.eGFP)
Env (Ab 2G12)

Synaptic vesicles in axon of living hippocampal neuron

Standard (Confocal)
snapshot



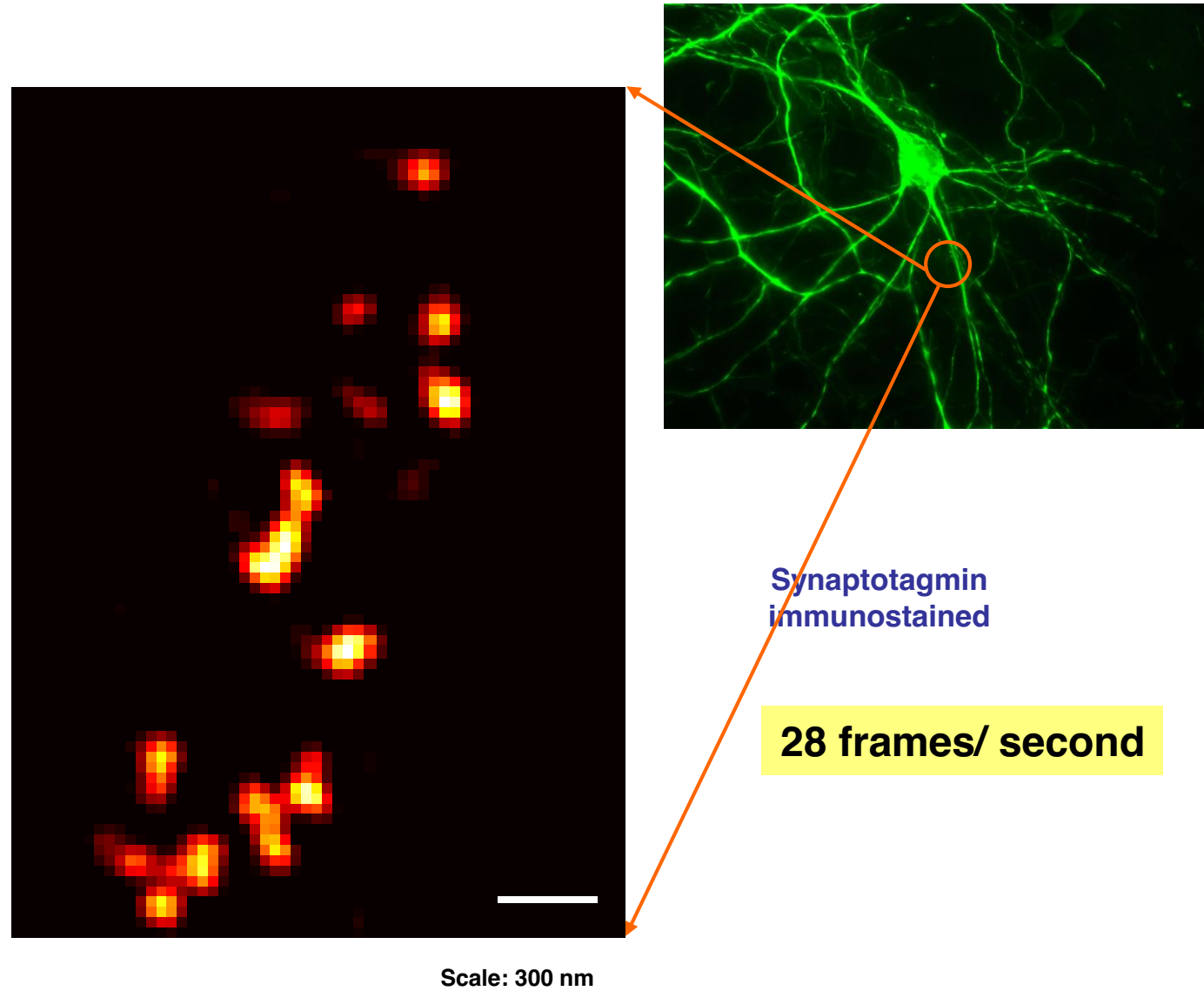
Scale: 300 nm



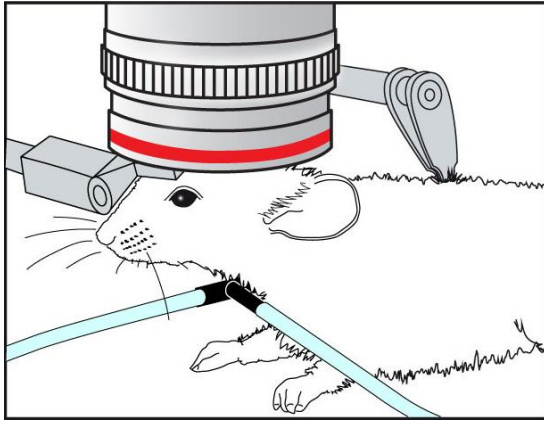
Synaptotagmin
immunostained

Synaptic vesicles in axon of living hippocampal neuron

Video rate STED

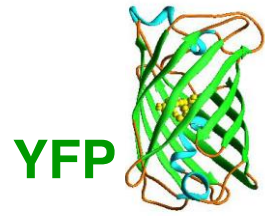


Neurophysiology



in living mouse brain

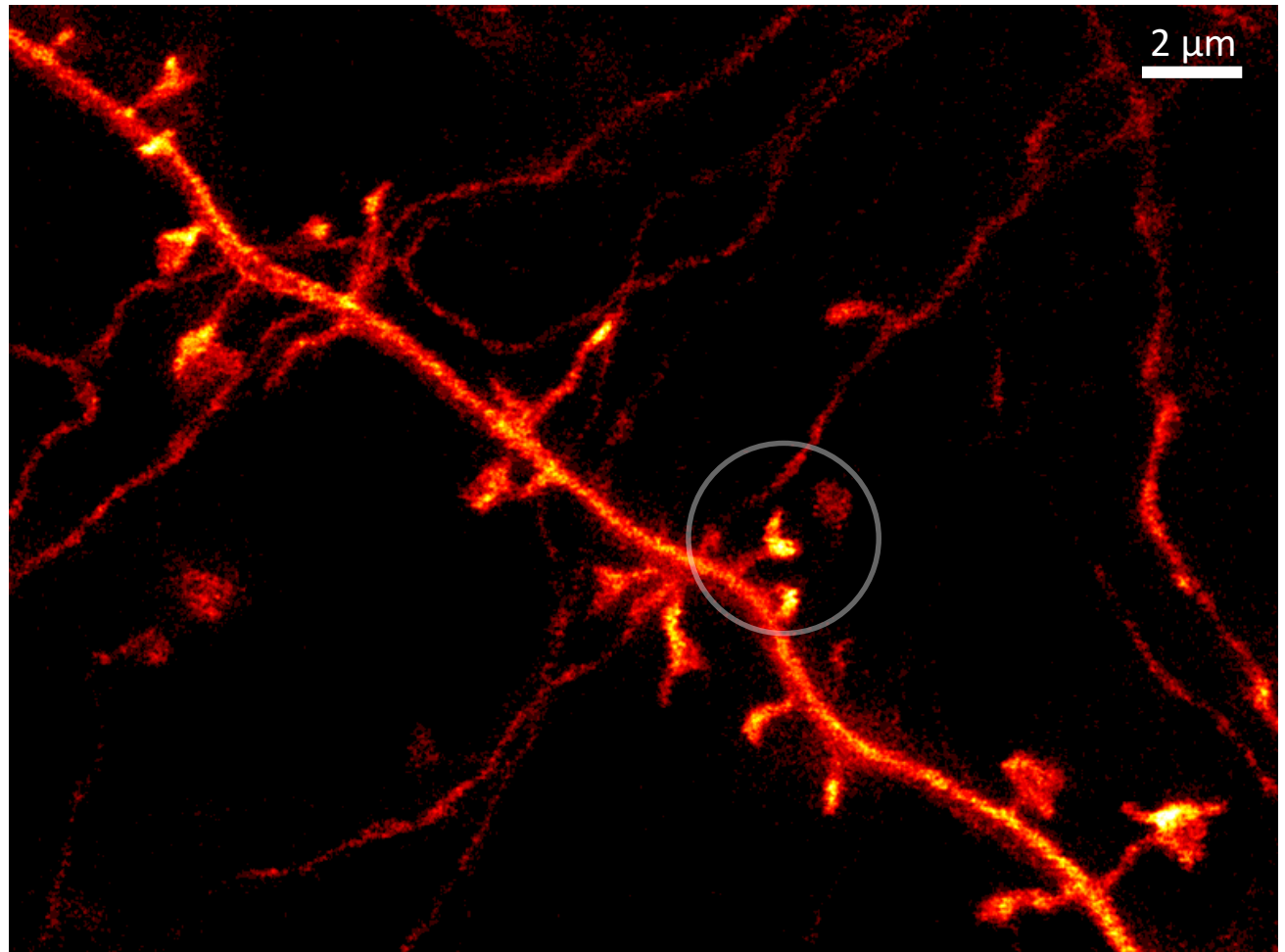
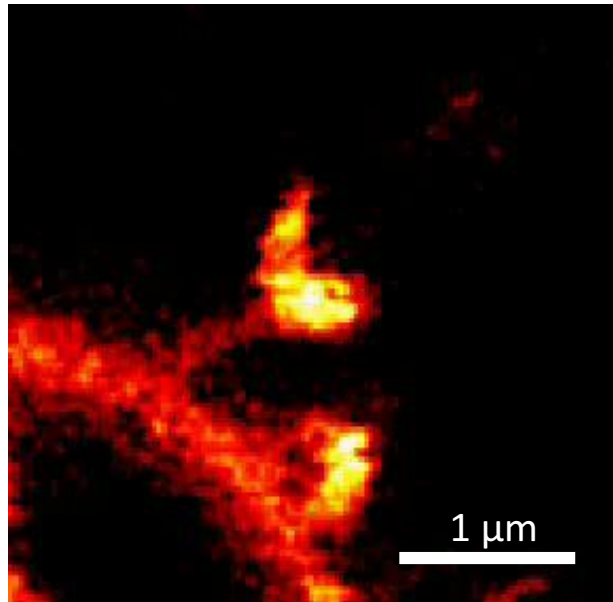
STED



**Cortical neurons expressing
cytoplasmic EYFP**

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

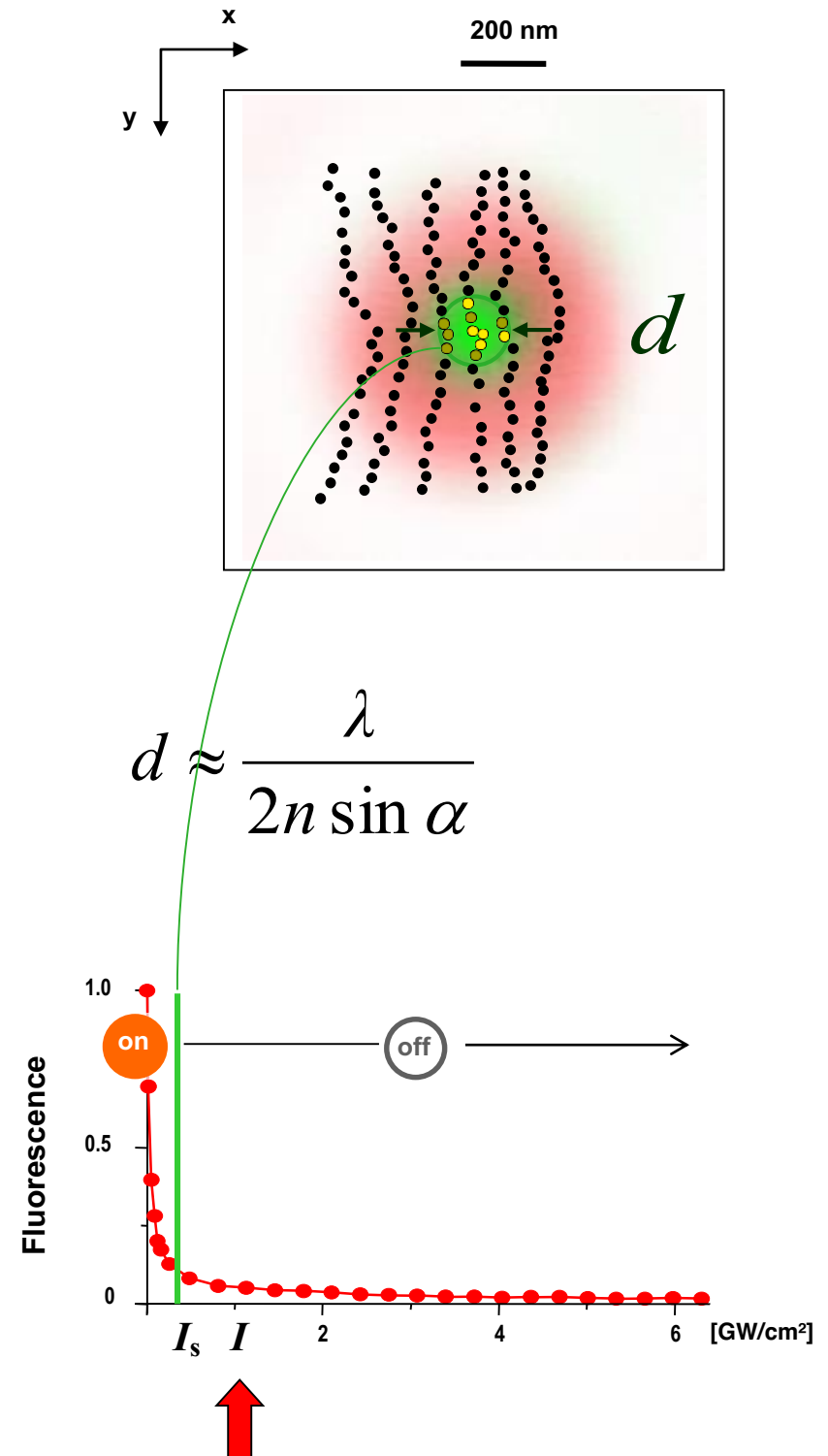
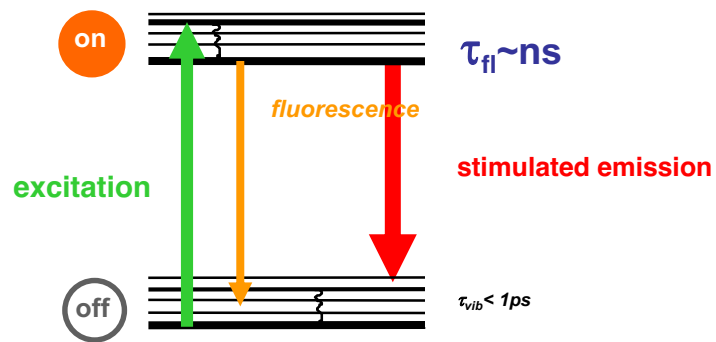
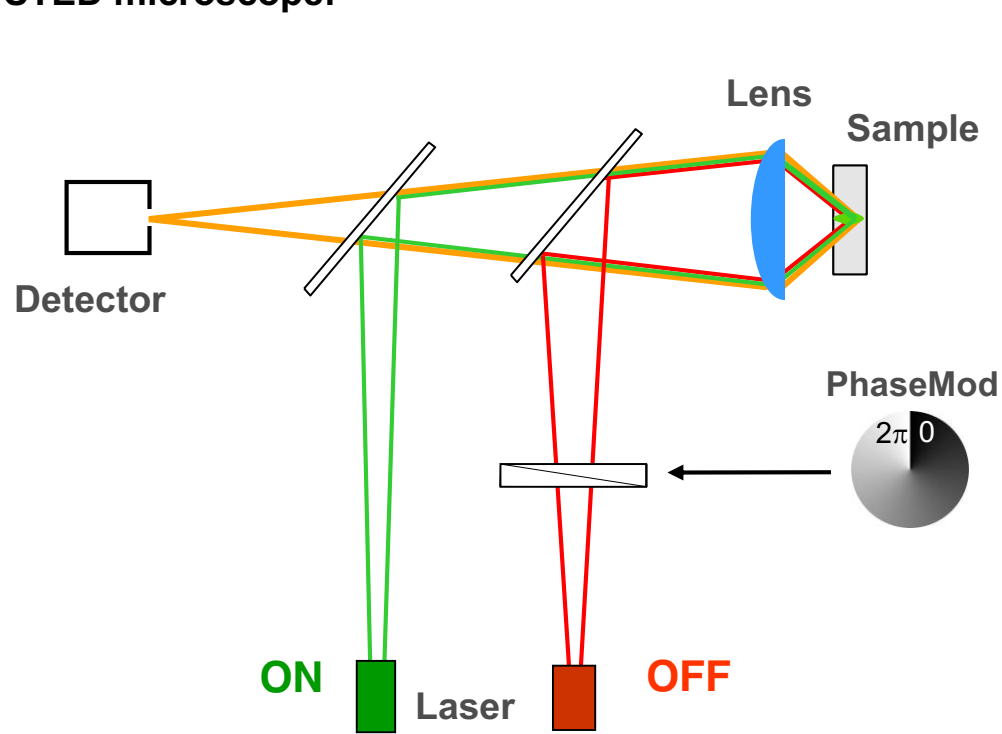
~20 μm deep



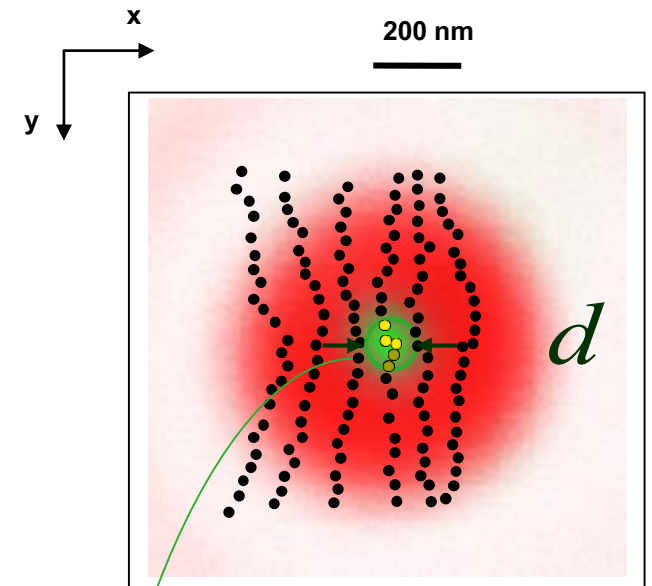
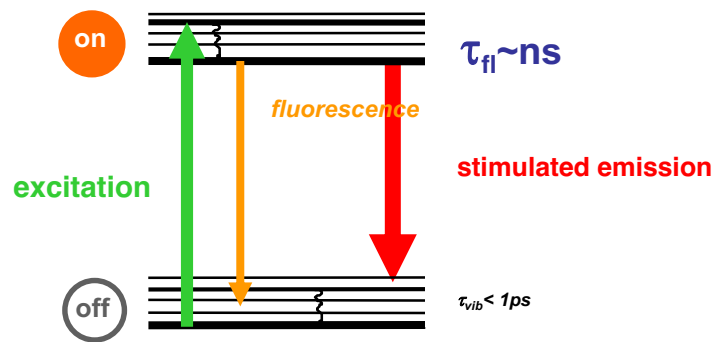
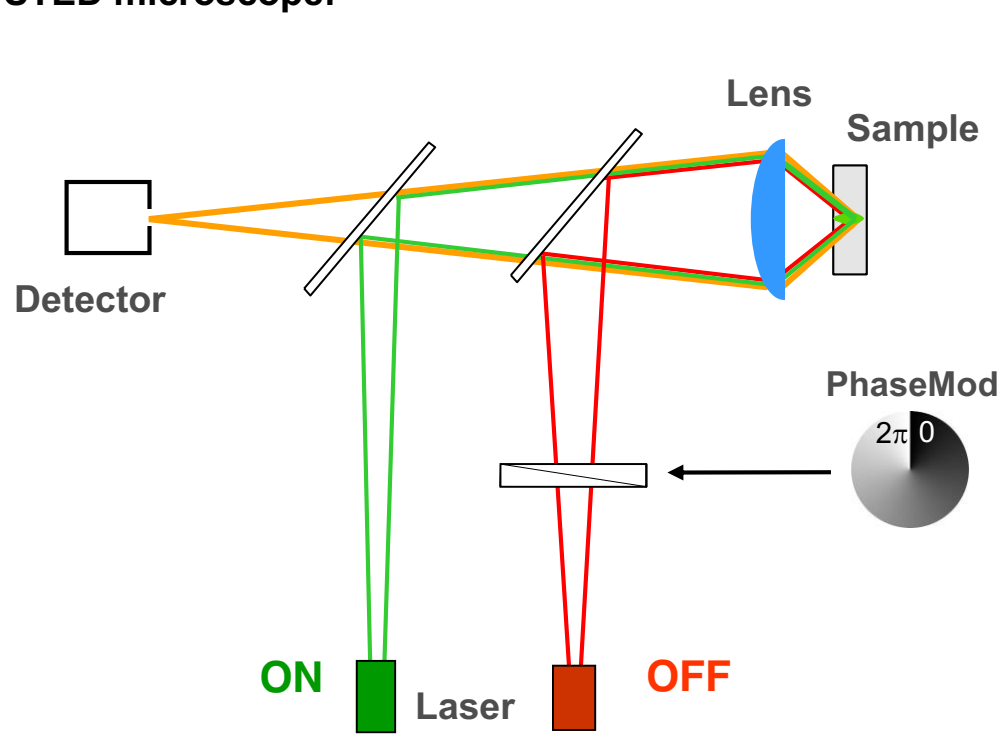
Berning et al, *Science* (2012)

The resolution

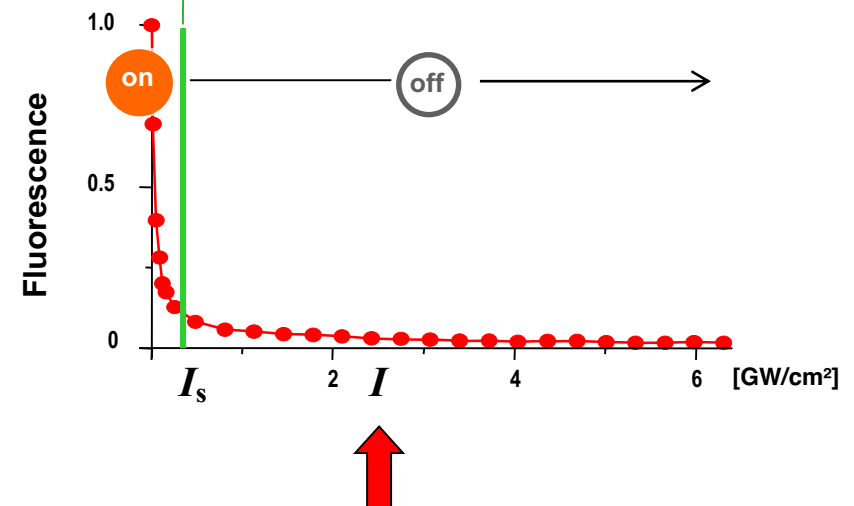
STED microscope:



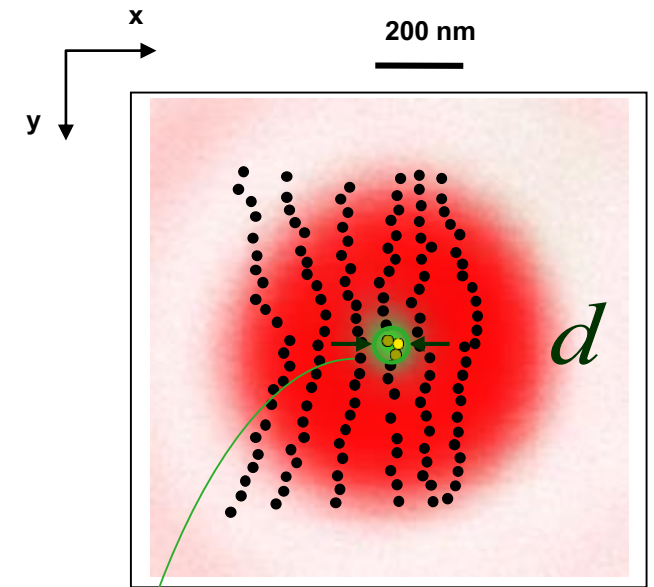
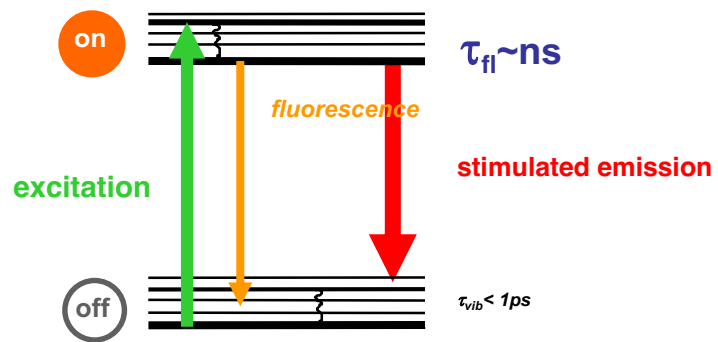
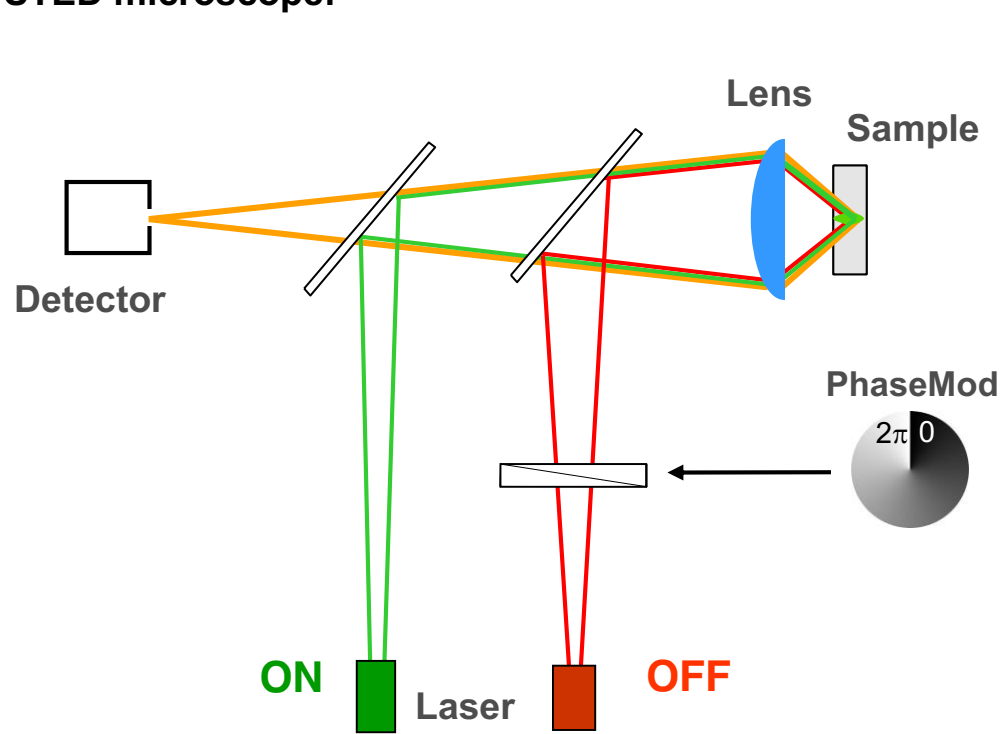
STED microscope:



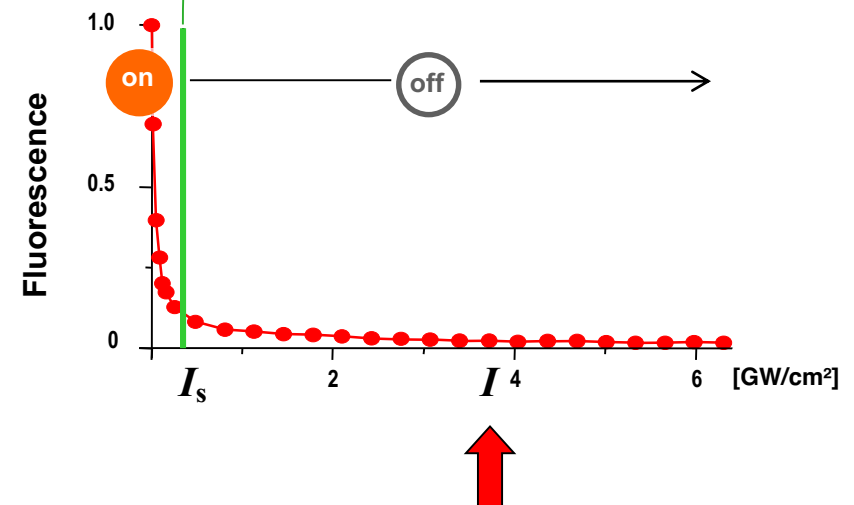
$$d \approx \frac{\lambda}{2n \sin \alpha}$$



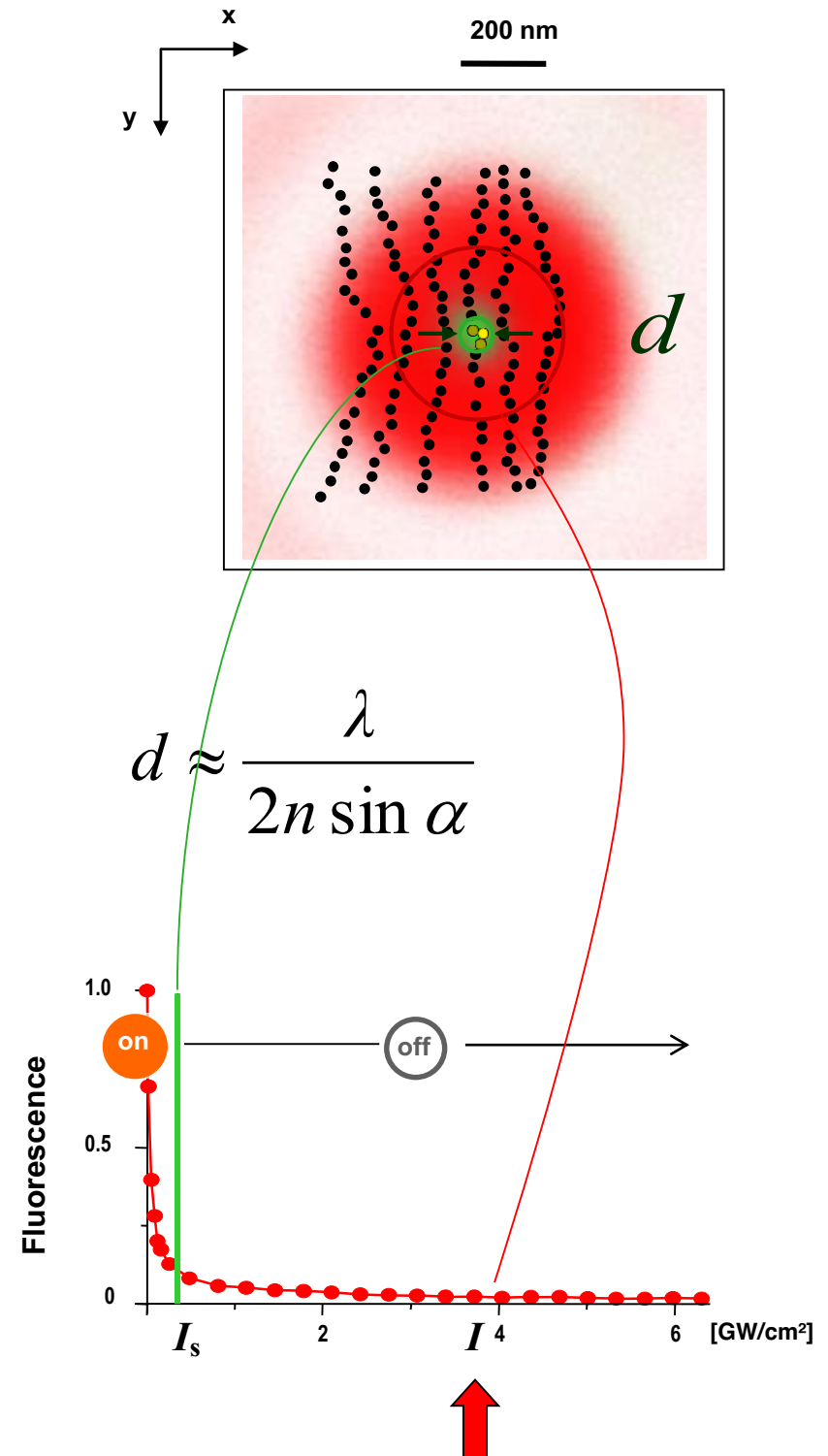
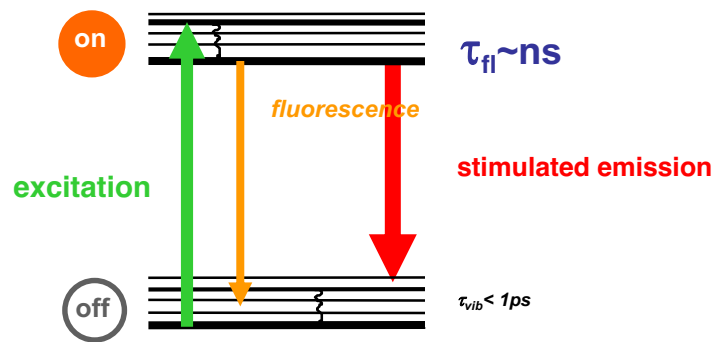
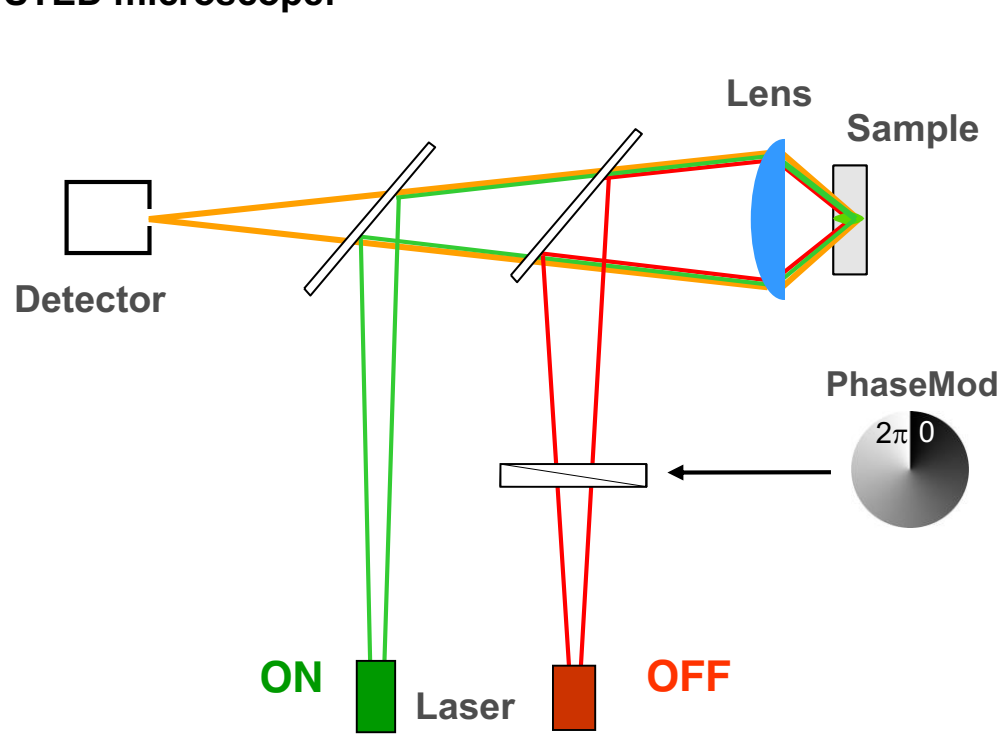
STED microscope:



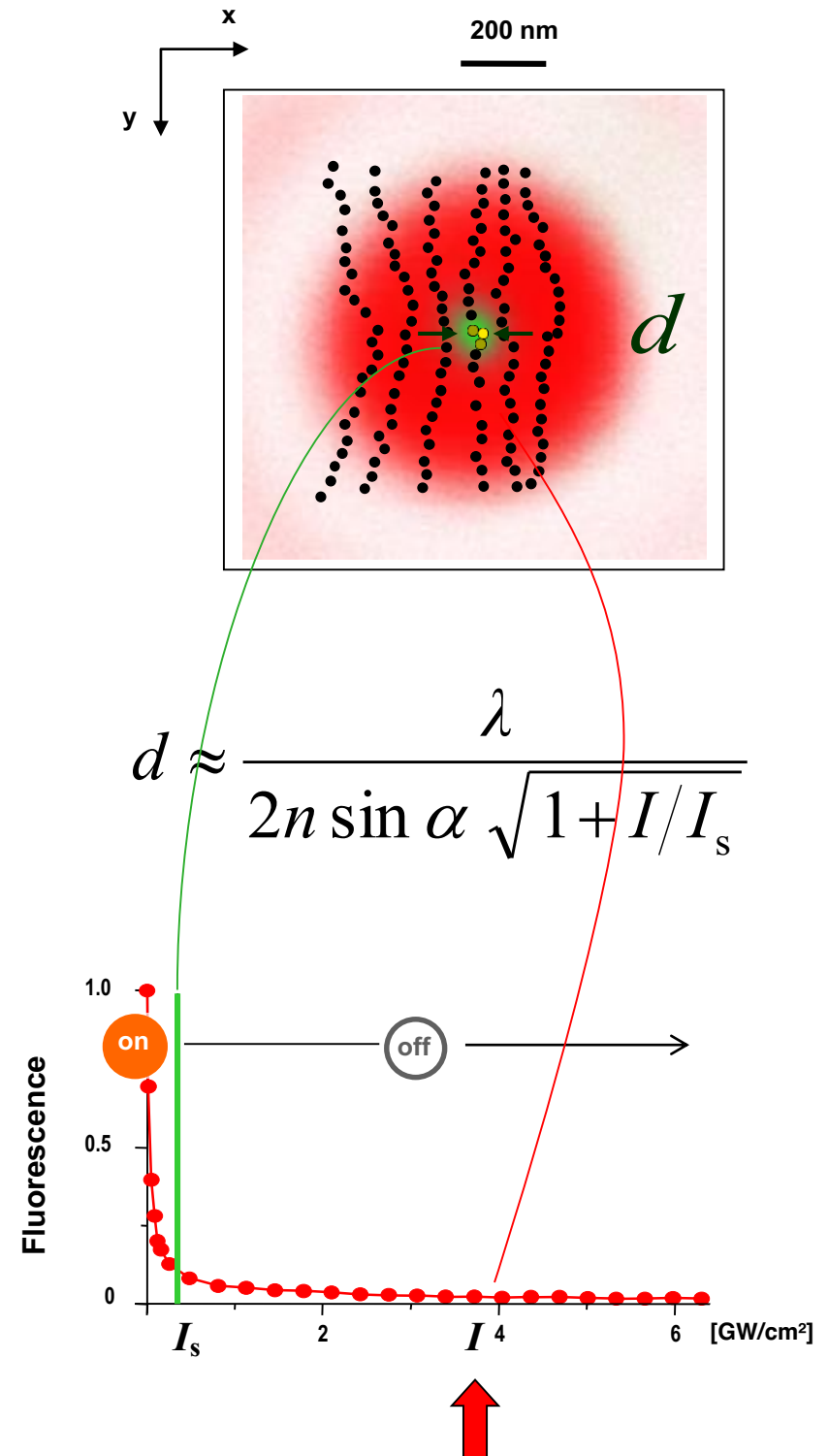
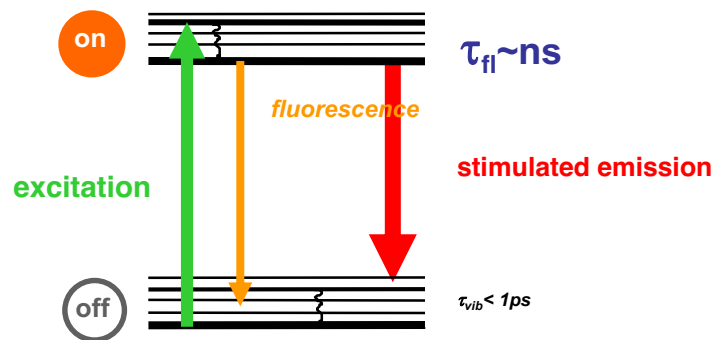
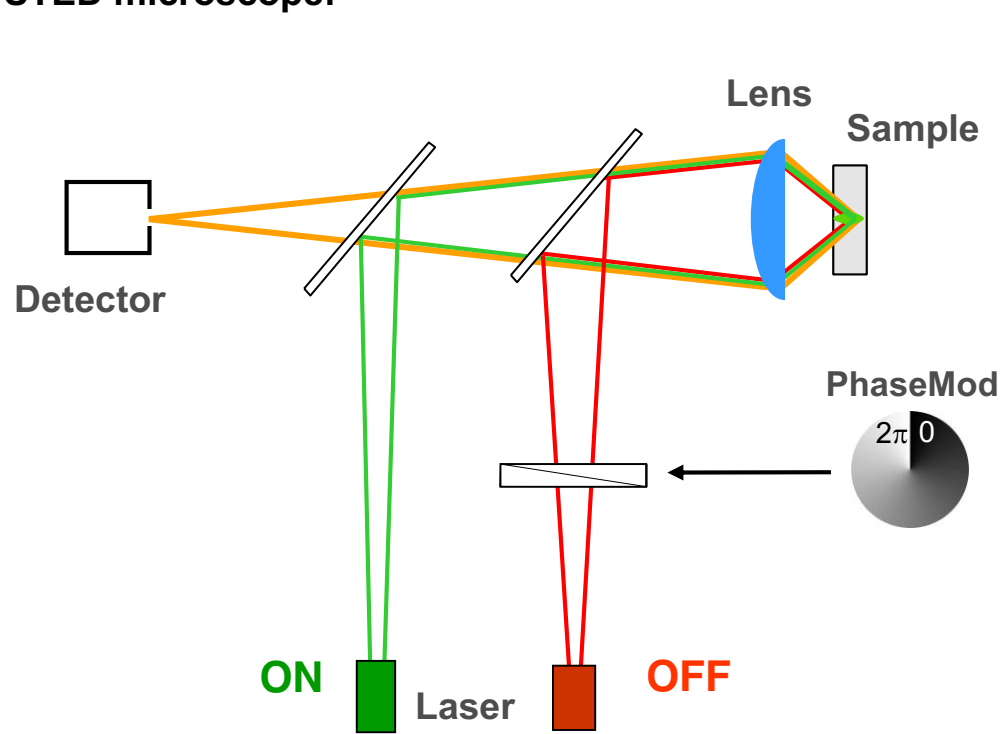
$$d \approx \frac{\lambda}{2n \sin \alpha}$$



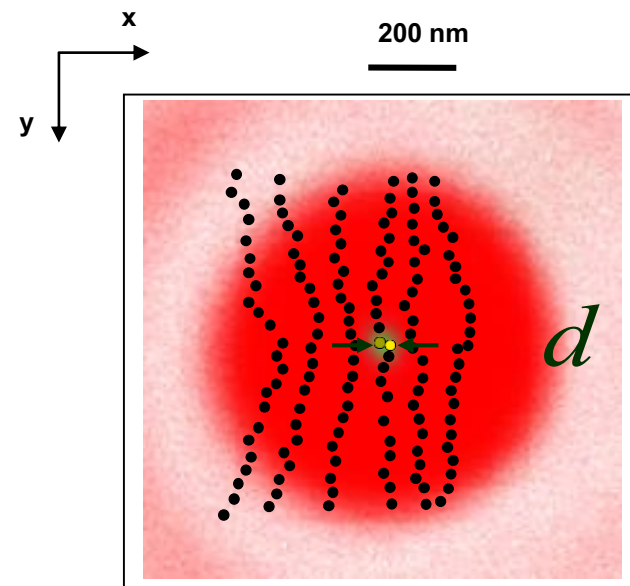
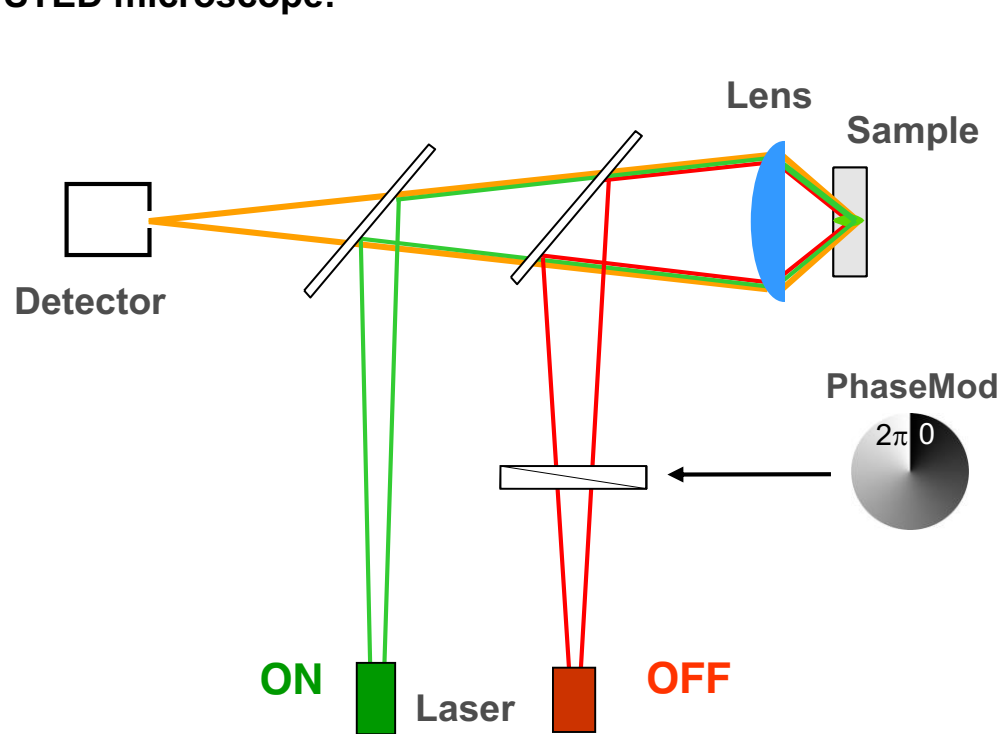
STED microscope:



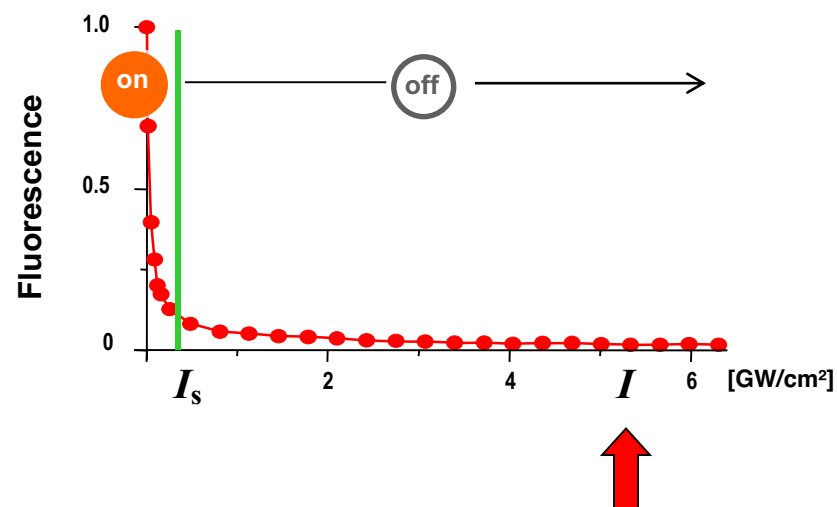
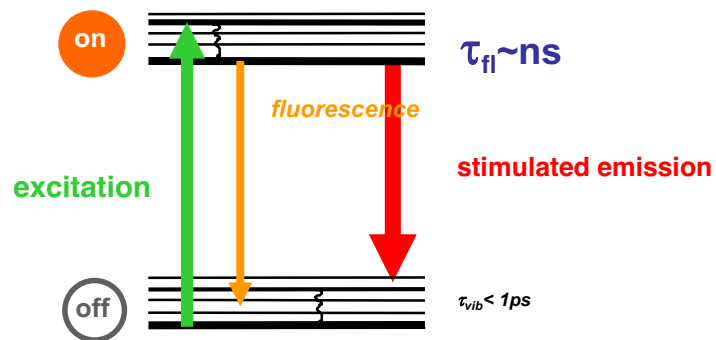
STED microscope:



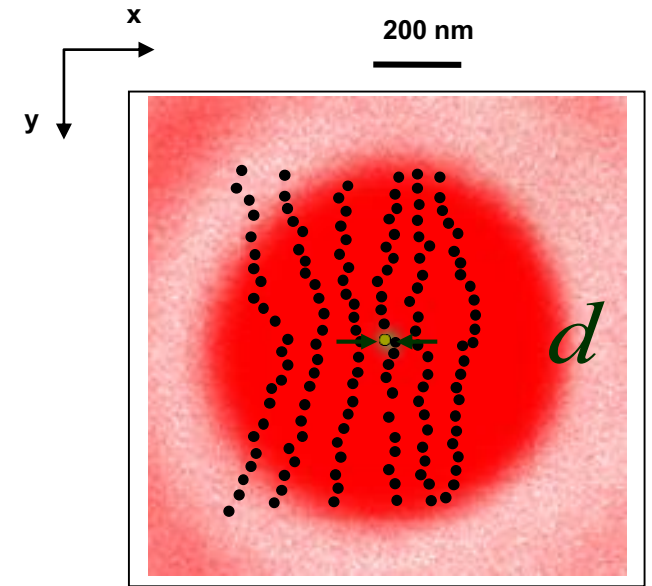
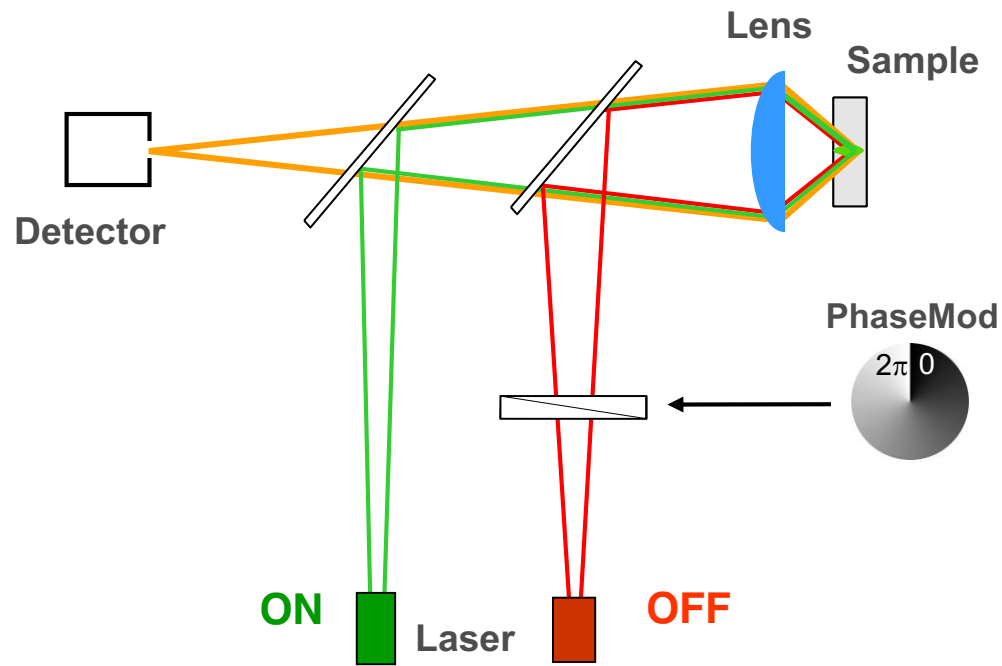
STED microscope:



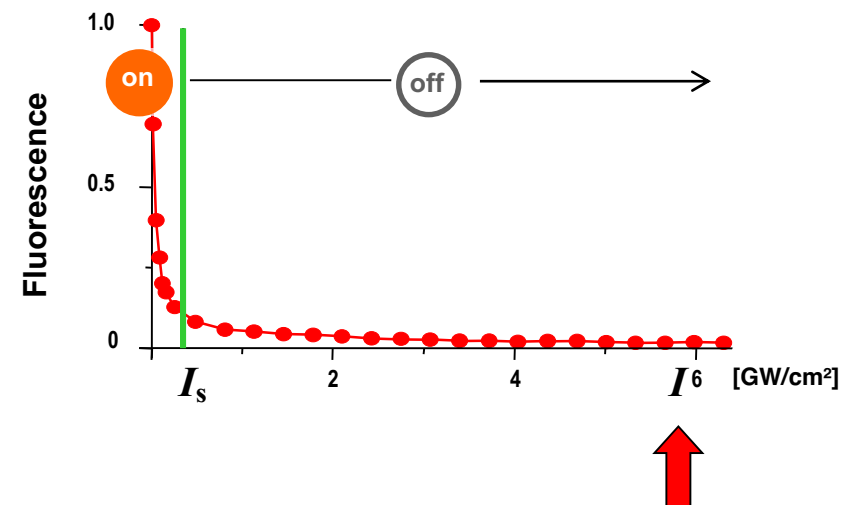
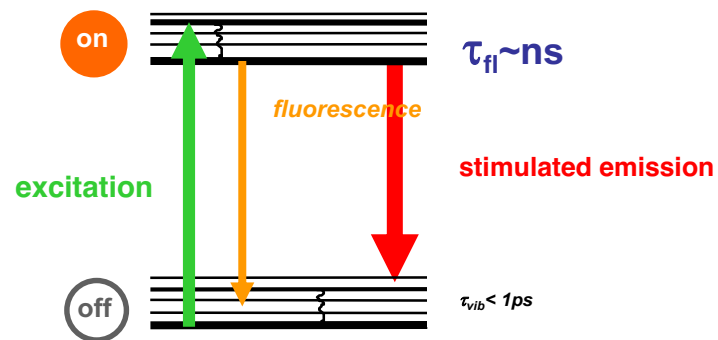
$$d \approx \frac{\lambda}{2n \sin \alpha \sqrt{1 + I/I_s}} \rightarrow 0$$



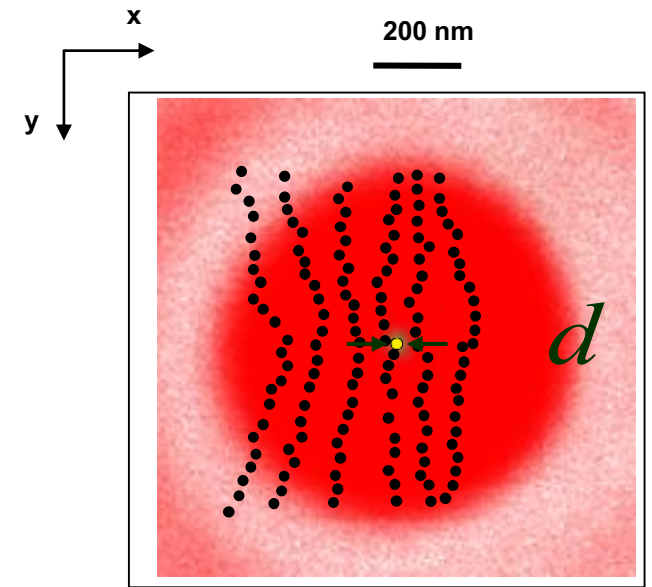
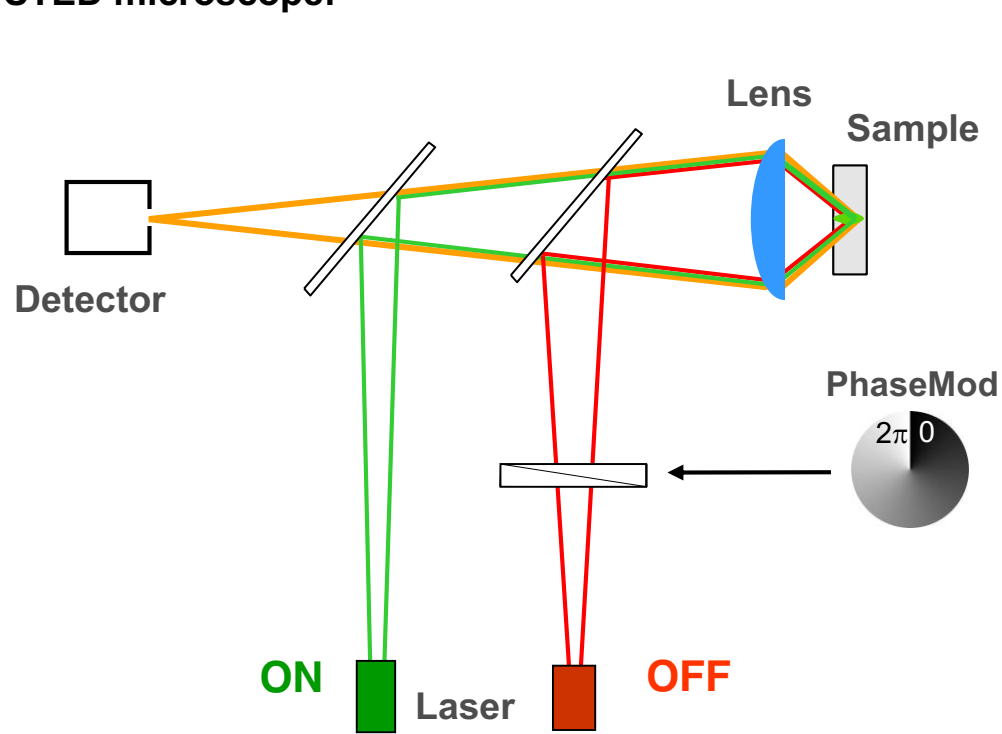
STED microscope:



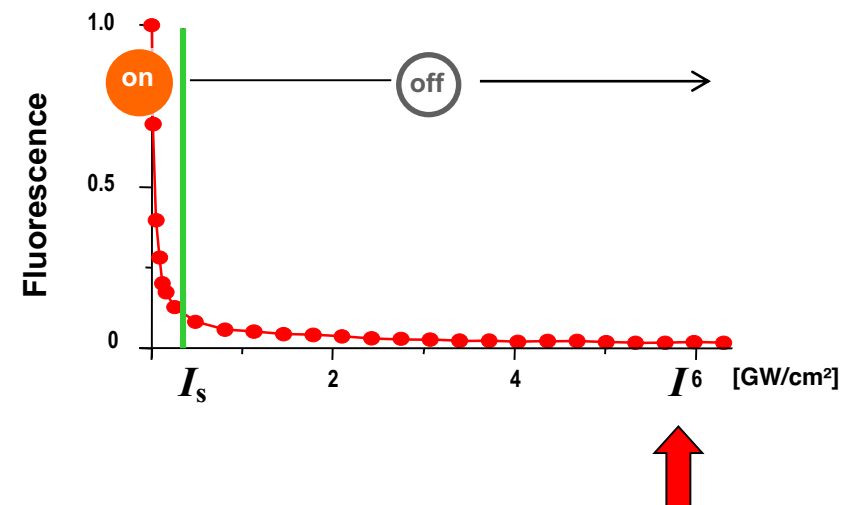
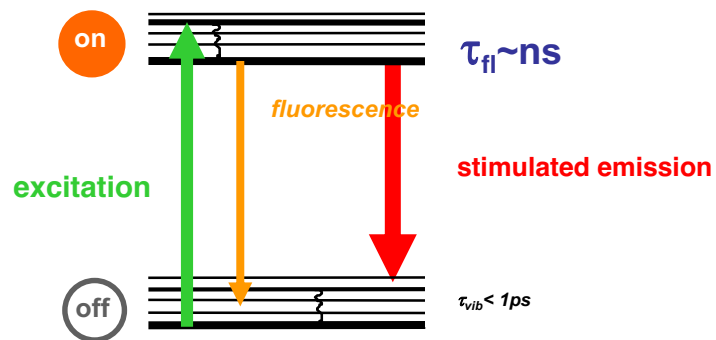
$$d \approx \frac{\lambda}{2n \sin \alpha \sqrt{1 + I/I_s}} \rightarrow 0$$



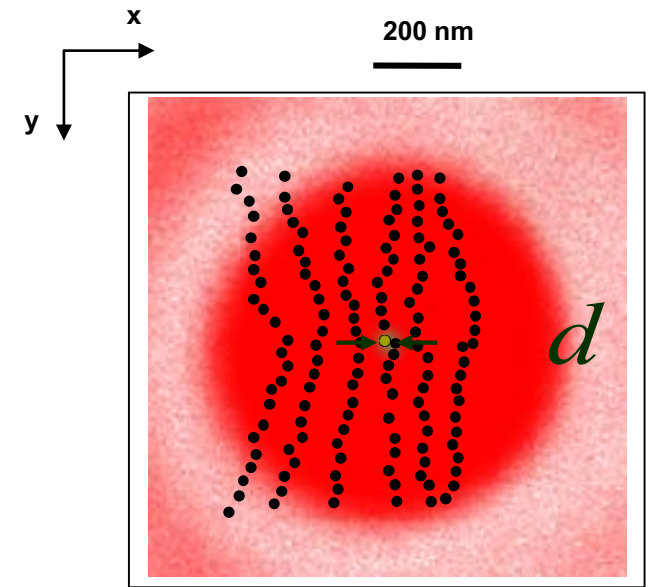
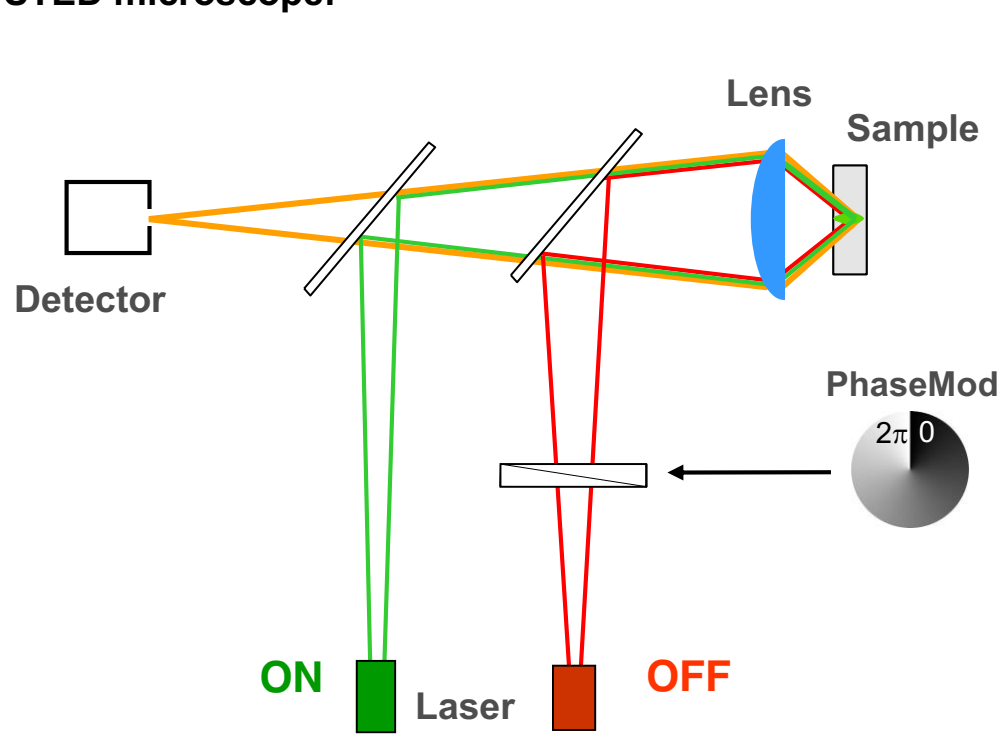
STED microscope:



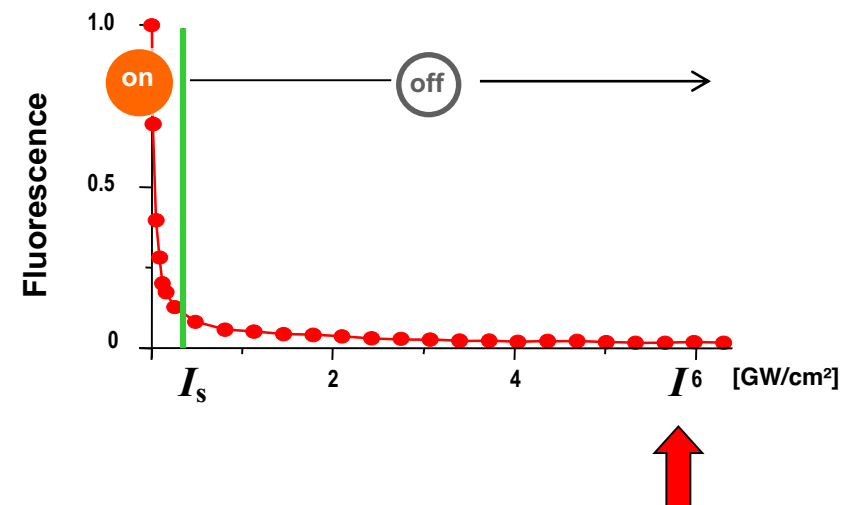
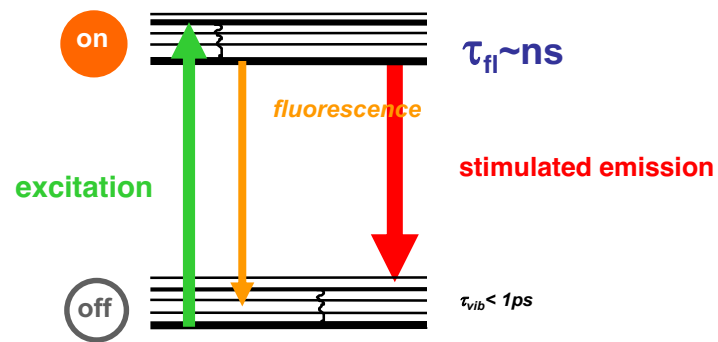
$$d \approx \frac{\lambda}{2n \sin \alpha \sqrt{1 + I/I_s}} \rightarrow 0$$



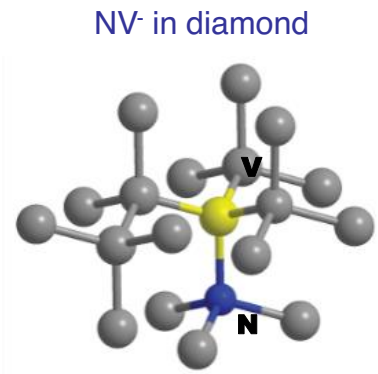
STED microscope:



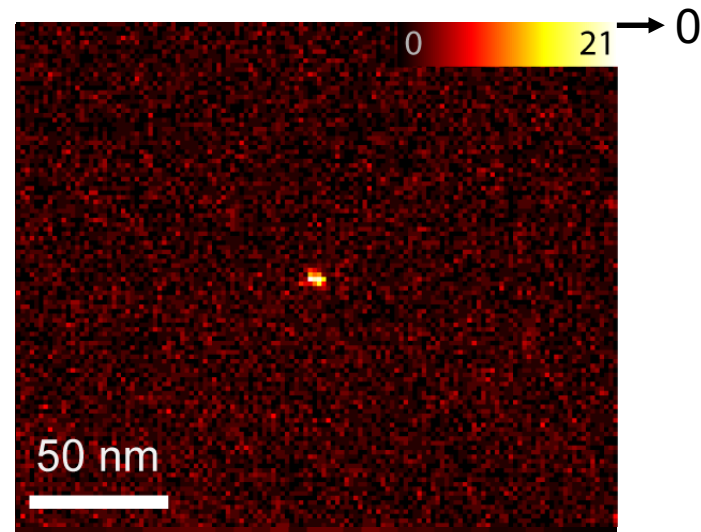
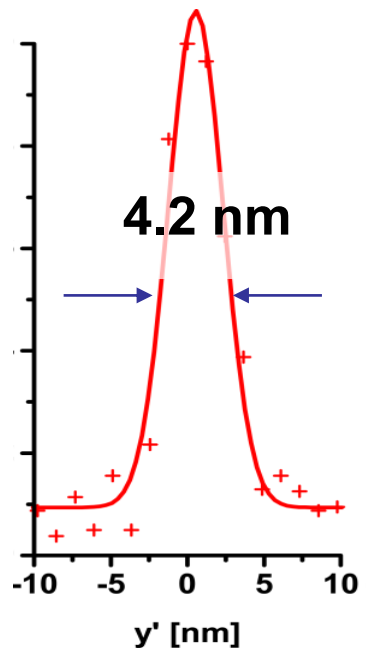
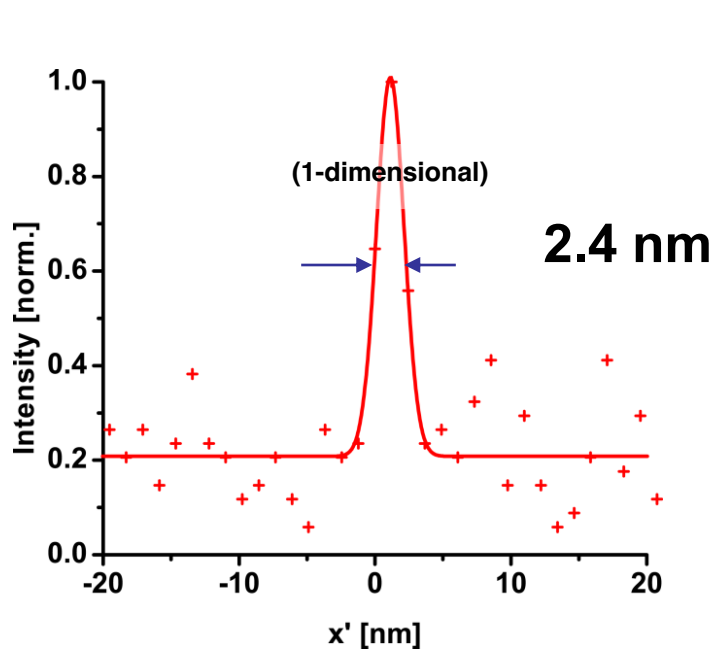
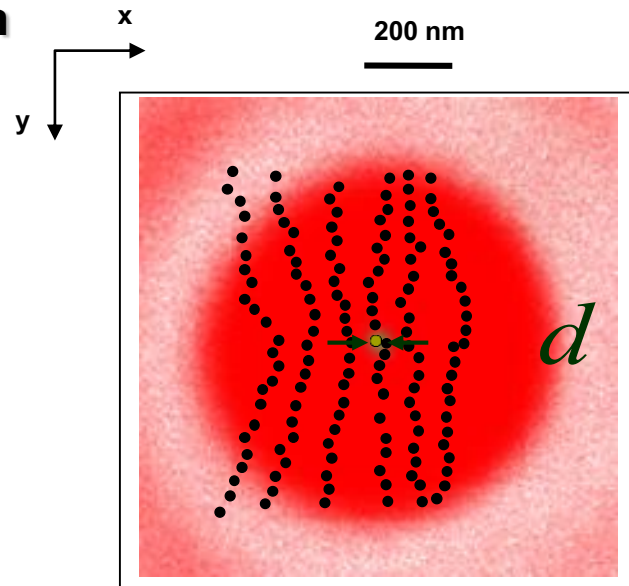
$$d \approx \frac{\lambda}{2n \sin \alpha \sqrt{1 + I/I_s}} \rightarrow 0$$



Material sciences, magnetic sensing, quantum information



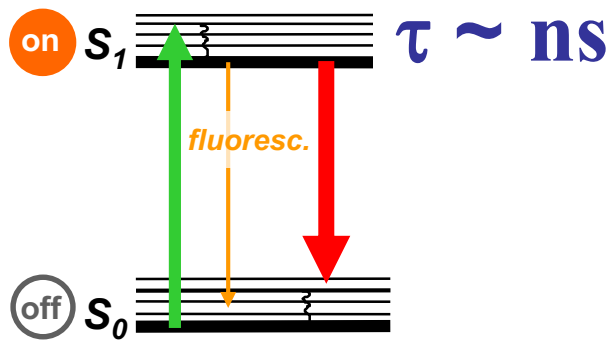
Color centers



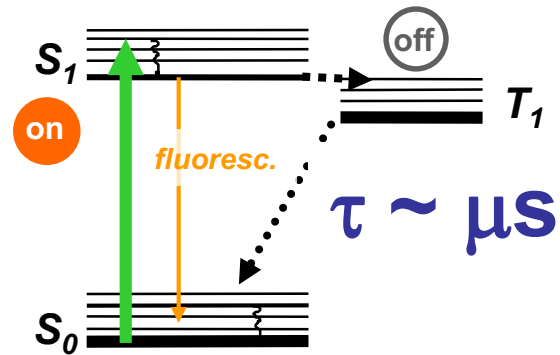
$\lambda = 775 \text{ nm}$

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

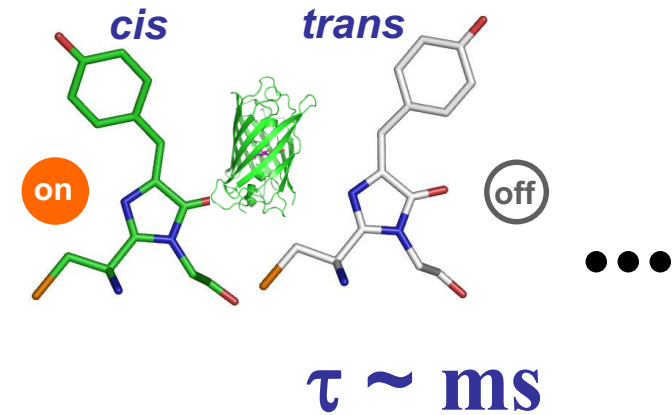
STED



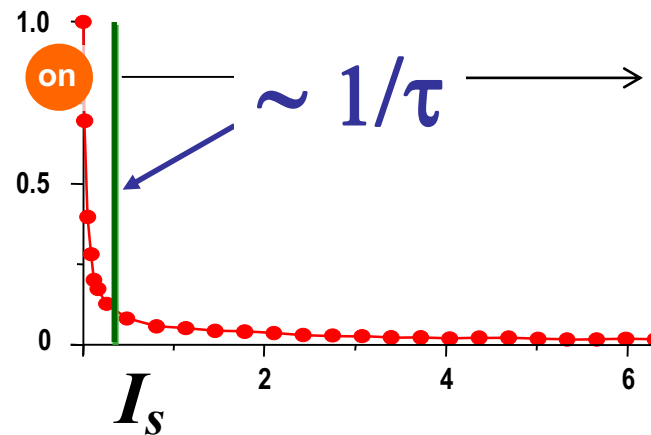
GSD (metastable dark state)



RESOLFT

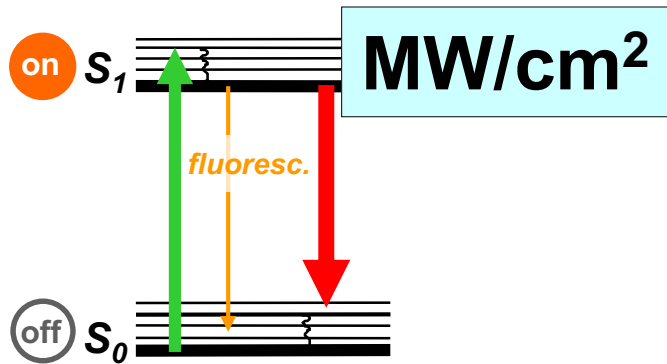


$$d \approx \frac{\lambda}{2n \sin \alpha \sqrt{1 + I/I_s}}$$

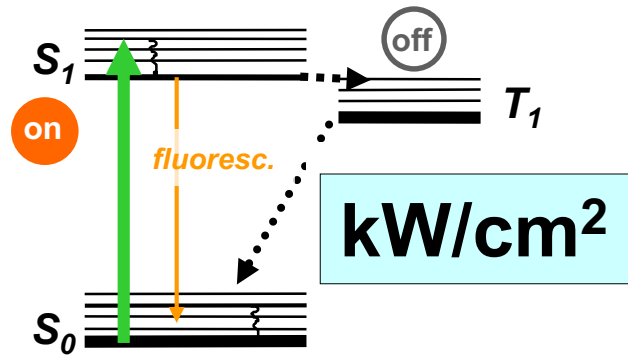


Principle: Discern by **ON / OFF** states in the sample

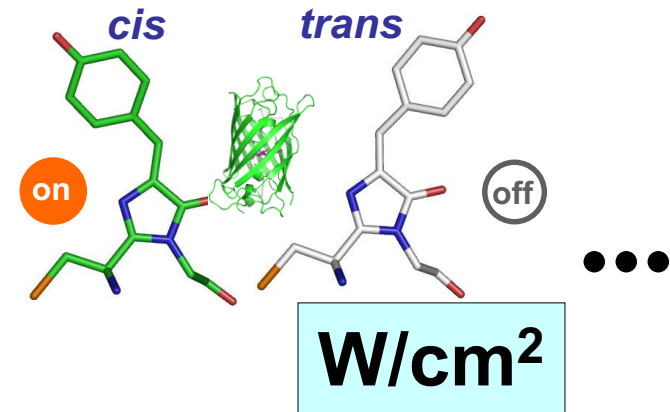
STED



GSD (metastable dark state)

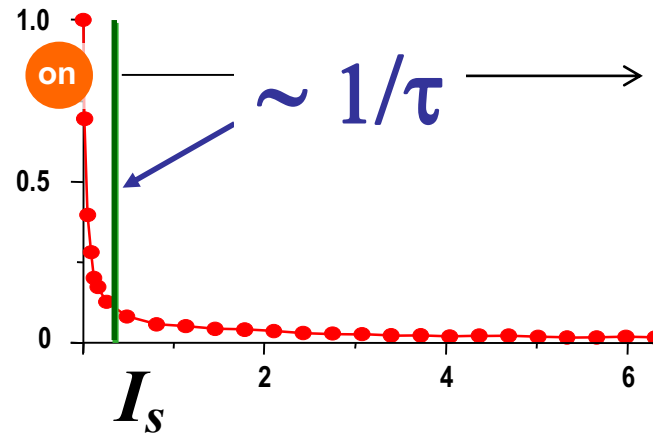


RESOLFT



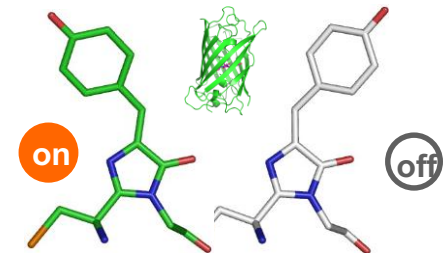
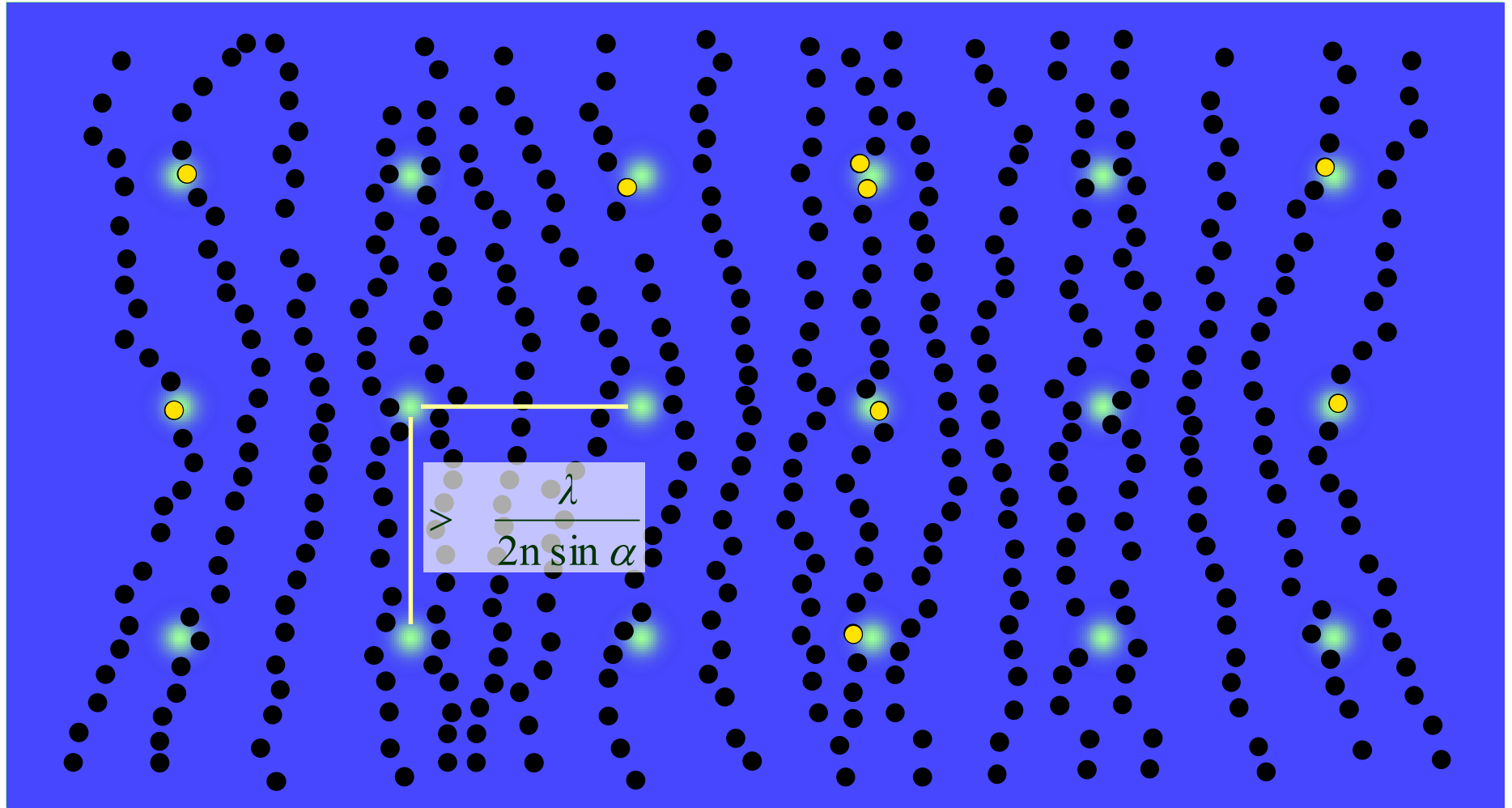
$$d \approx \frac{\lambda}{2n \sin \alpha \sqrt{1 + I/I_s}}$$

↓
↓



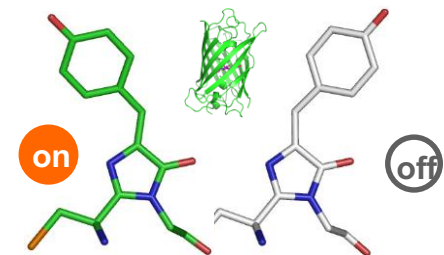
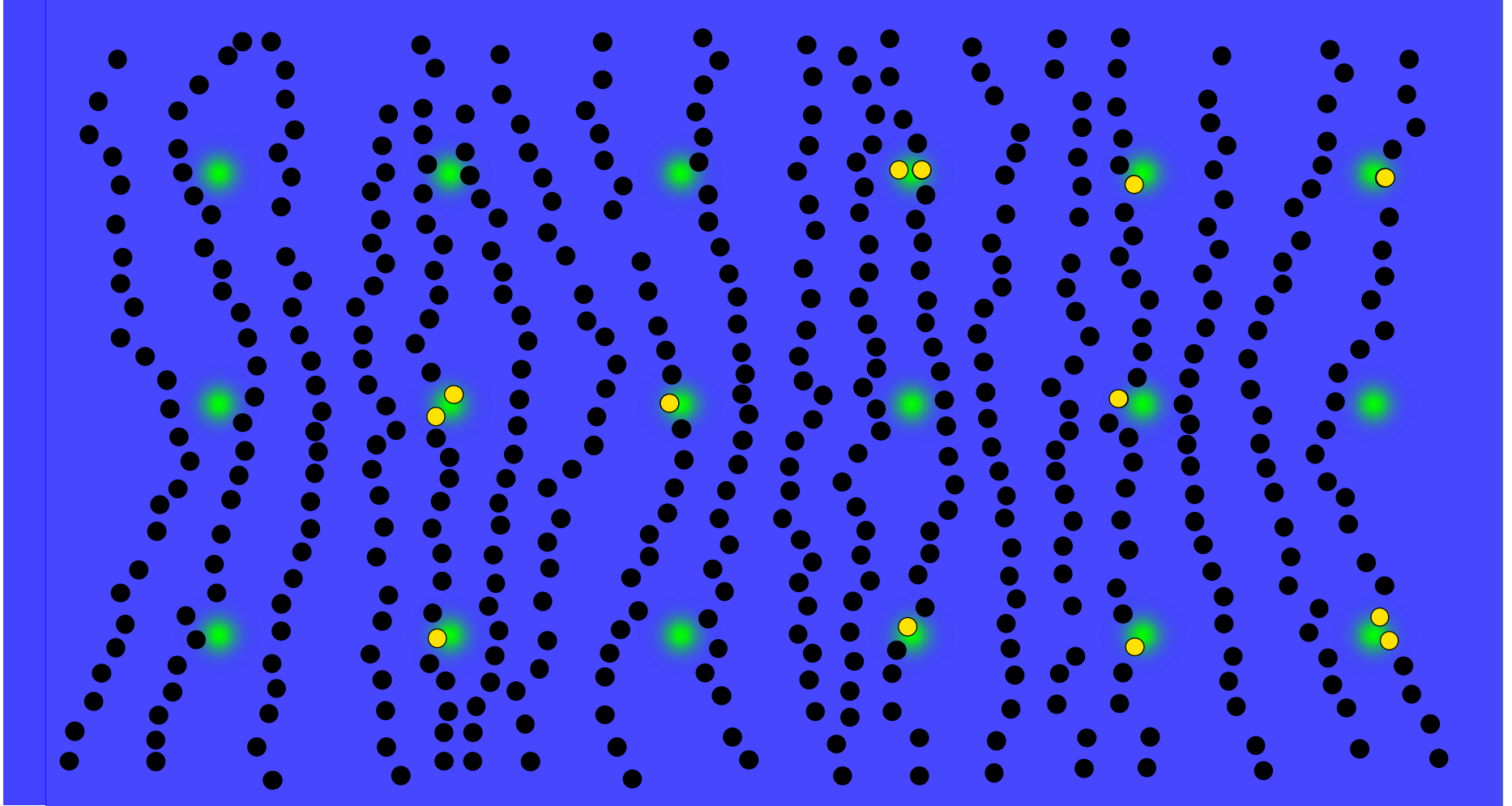
RESOLFT:

many ,doughnuts' (zeros) in parallel



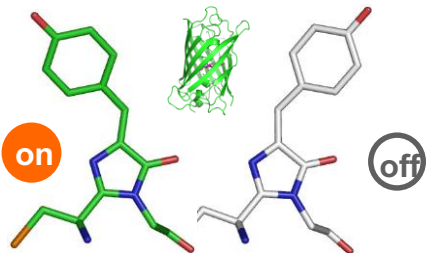
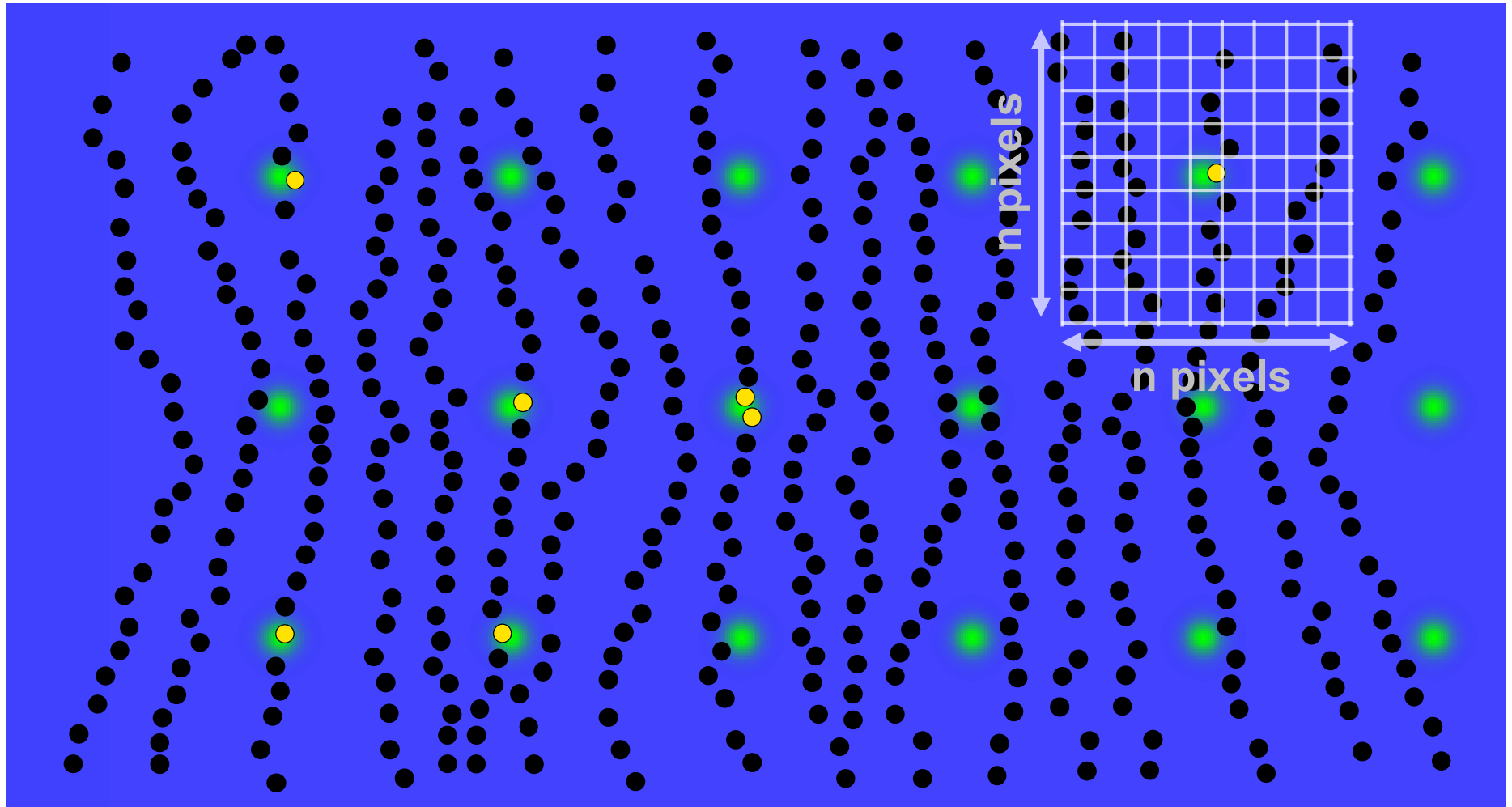
... because of low intensity operation.

RESULT: many ,doughnuts' (zeros) in parallel



... because of low intensity operation.

RESULT: many ,doughnuts' (zeros) in parallel



... because of low intensity operation.

RESOLFT

Keratin filaments in living kidney epithelial cells

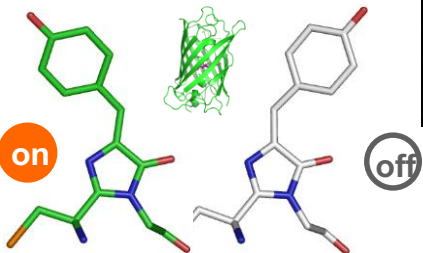
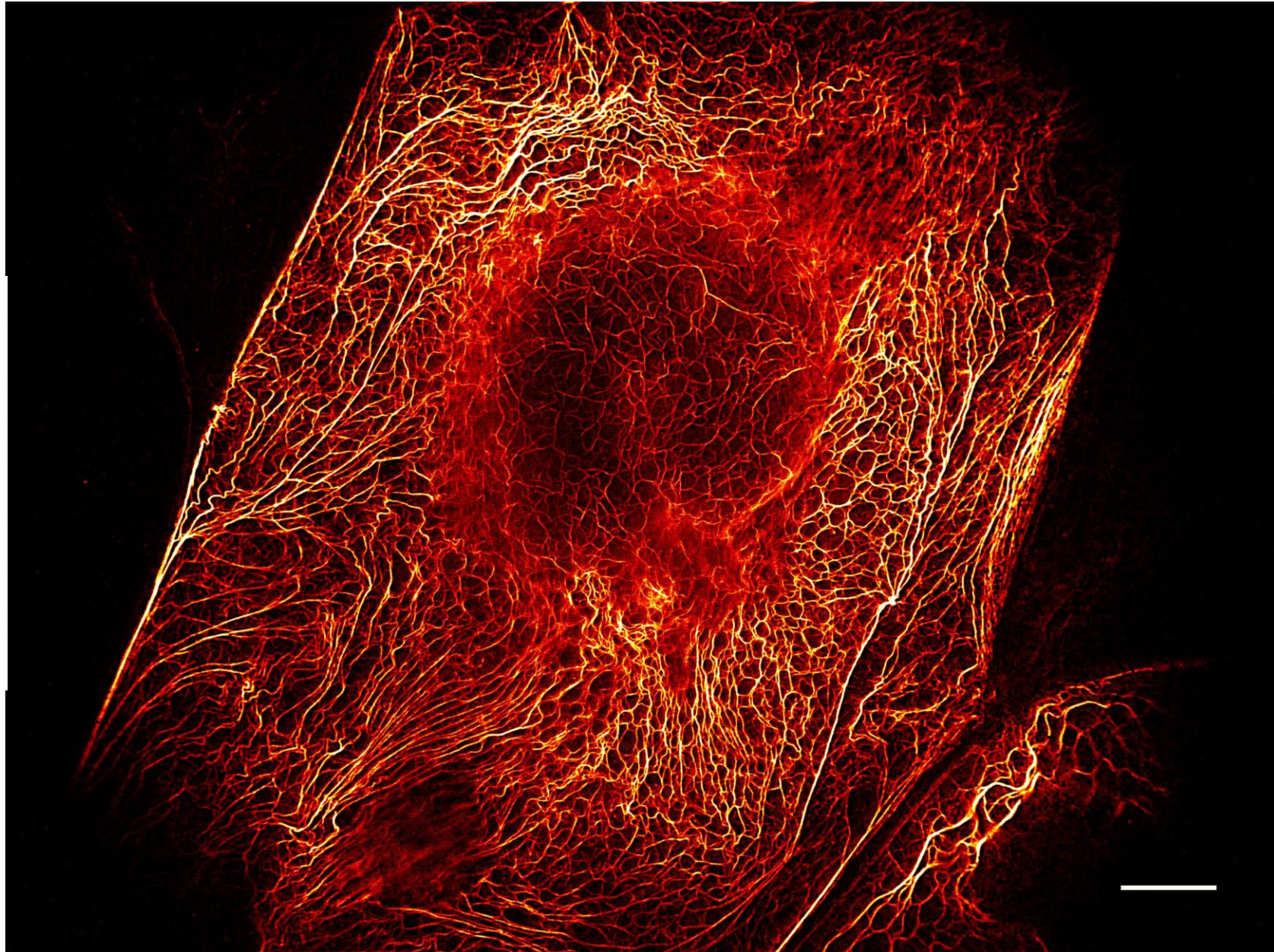
recorded with

>100,000

,doughnuts‘

in

2 seconds



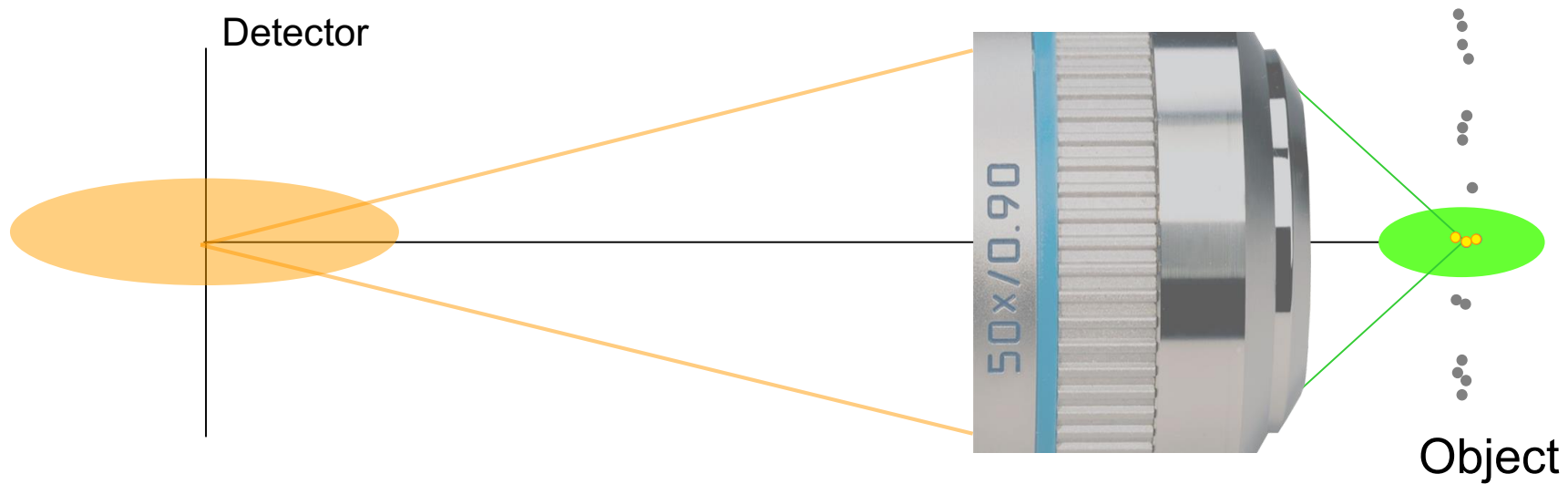
Chmyrov et al, *Nature Meth* (2013)

Scale bar: 10 μ m

What does it take to get the best resolution ?

20th century:

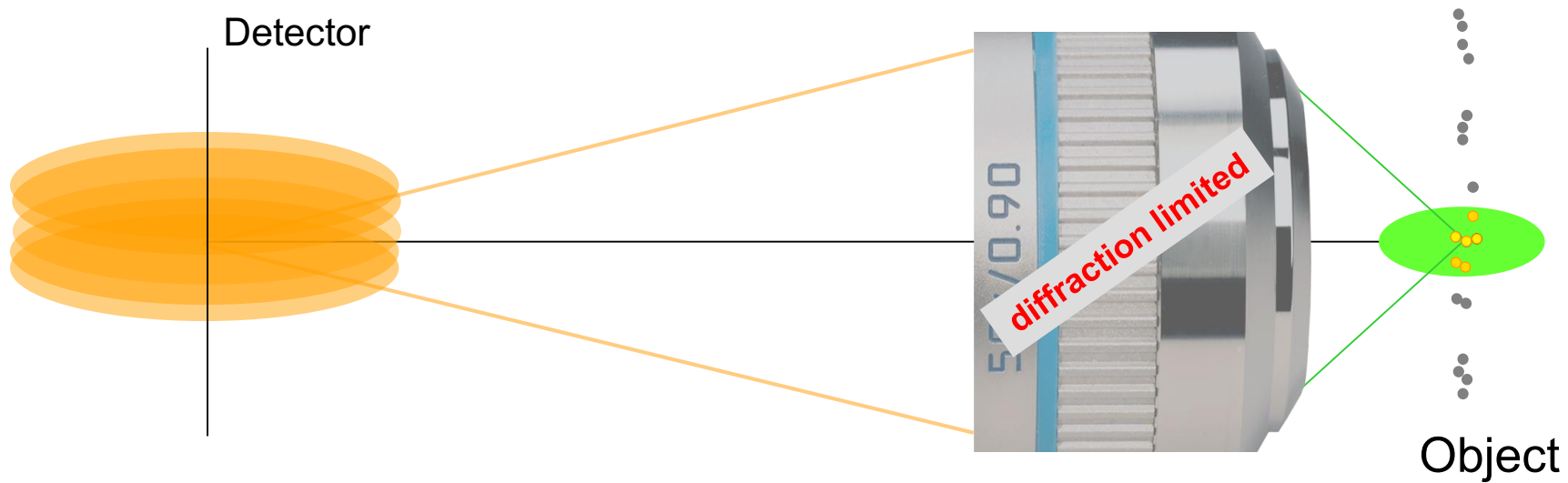
... separate features by ***focusing light***



Good lenses !

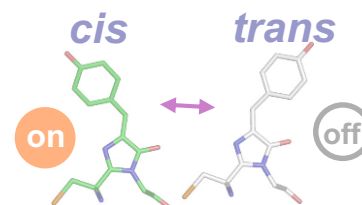
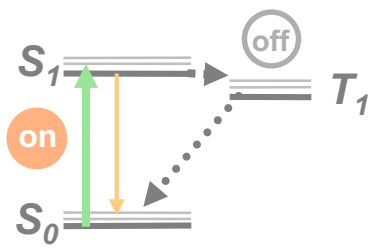
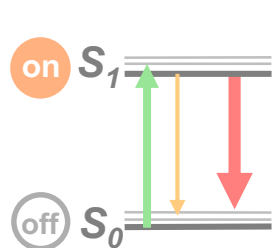
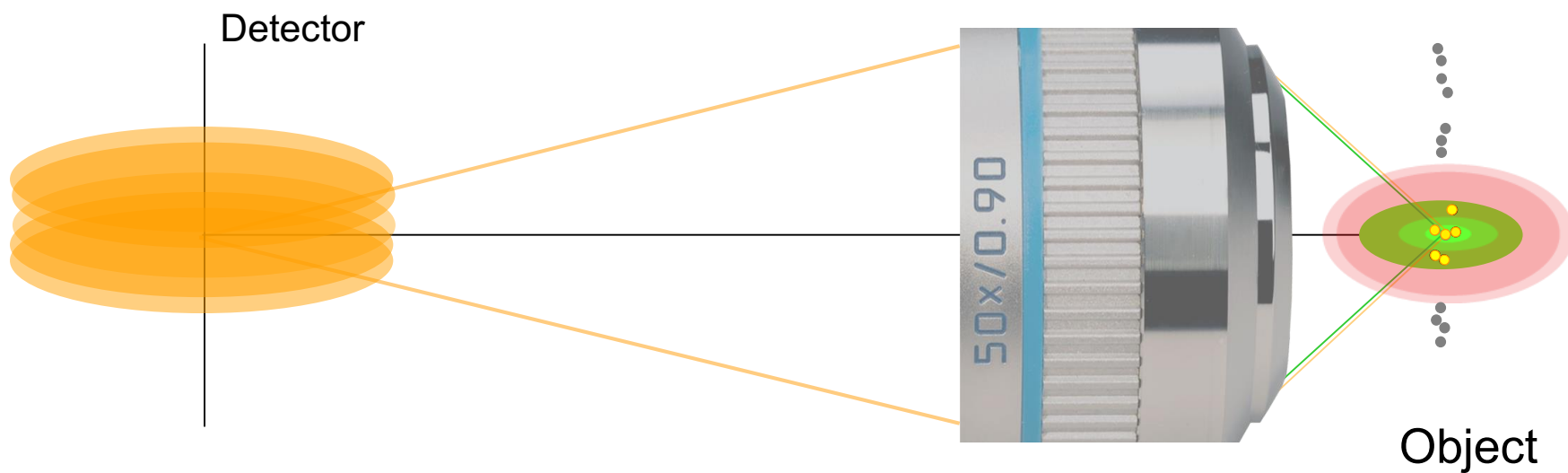
20th century:

... separate features by **focusing light**



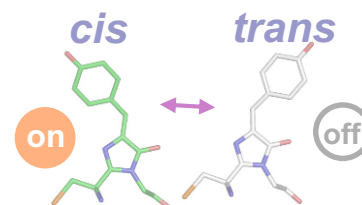
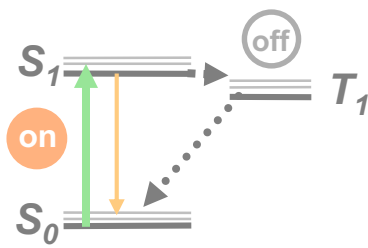
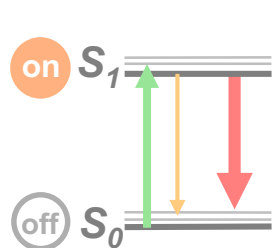
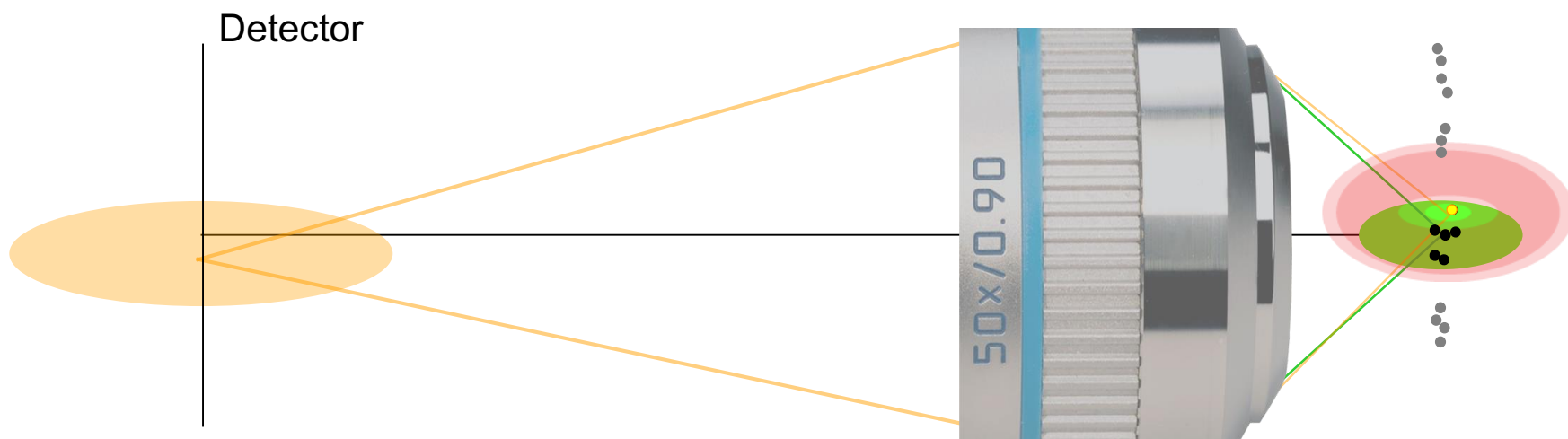
Solution:

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



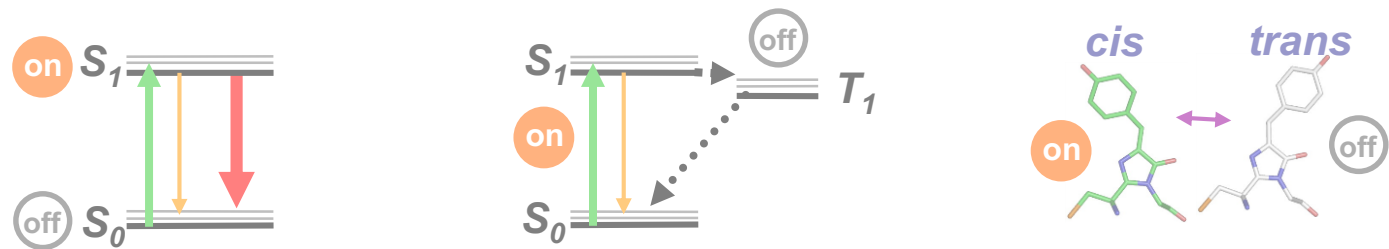
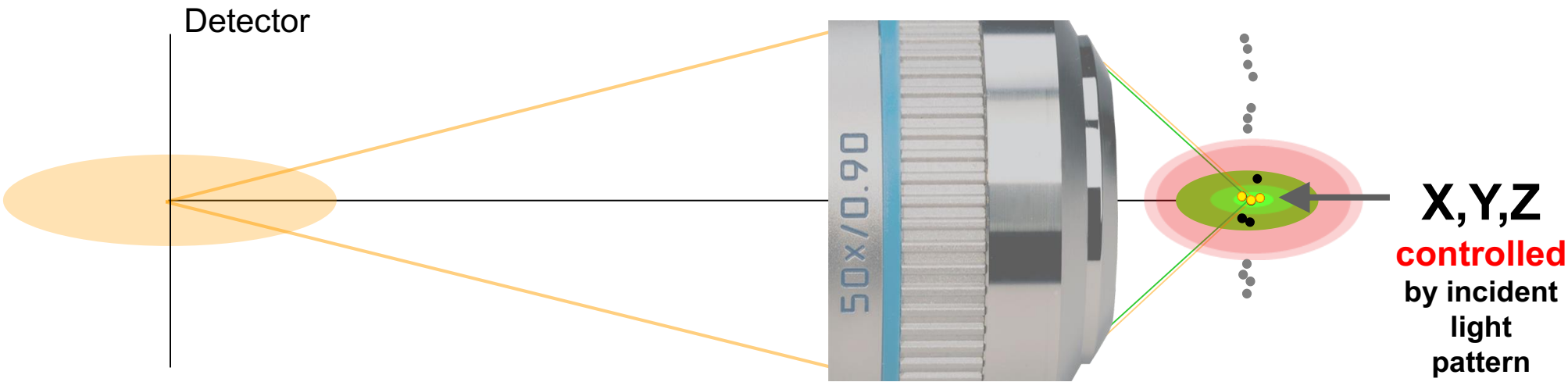
etc.

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



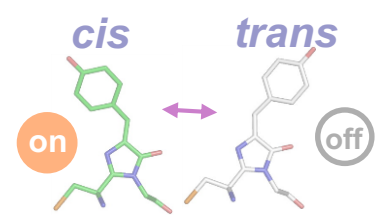
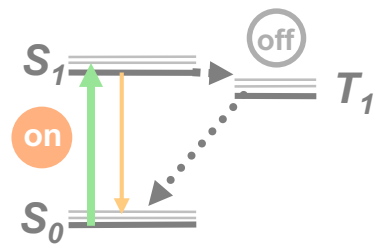
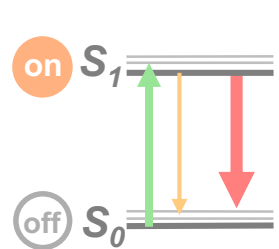
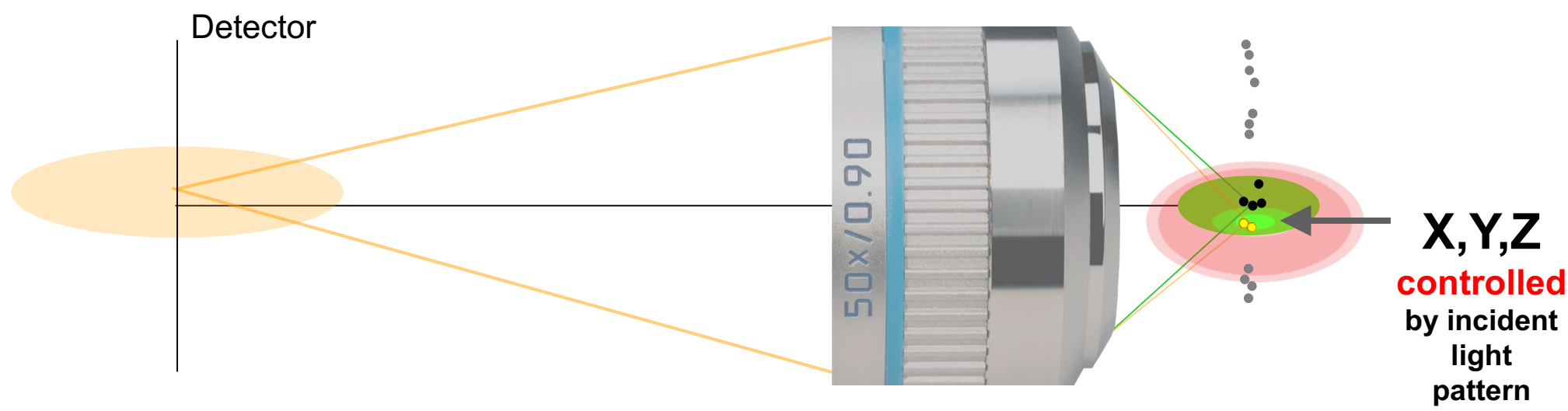
etc.

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



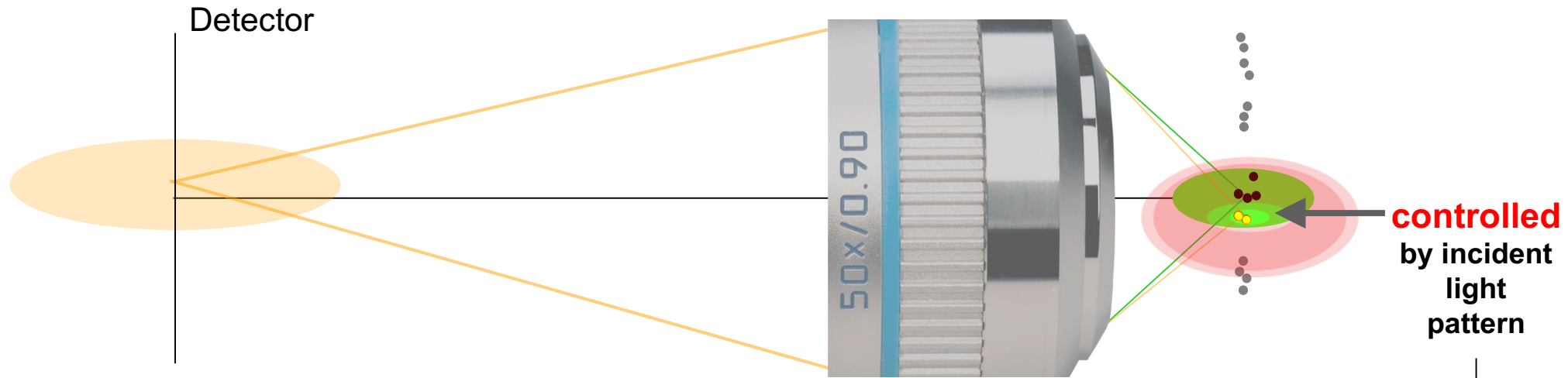
etc.

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



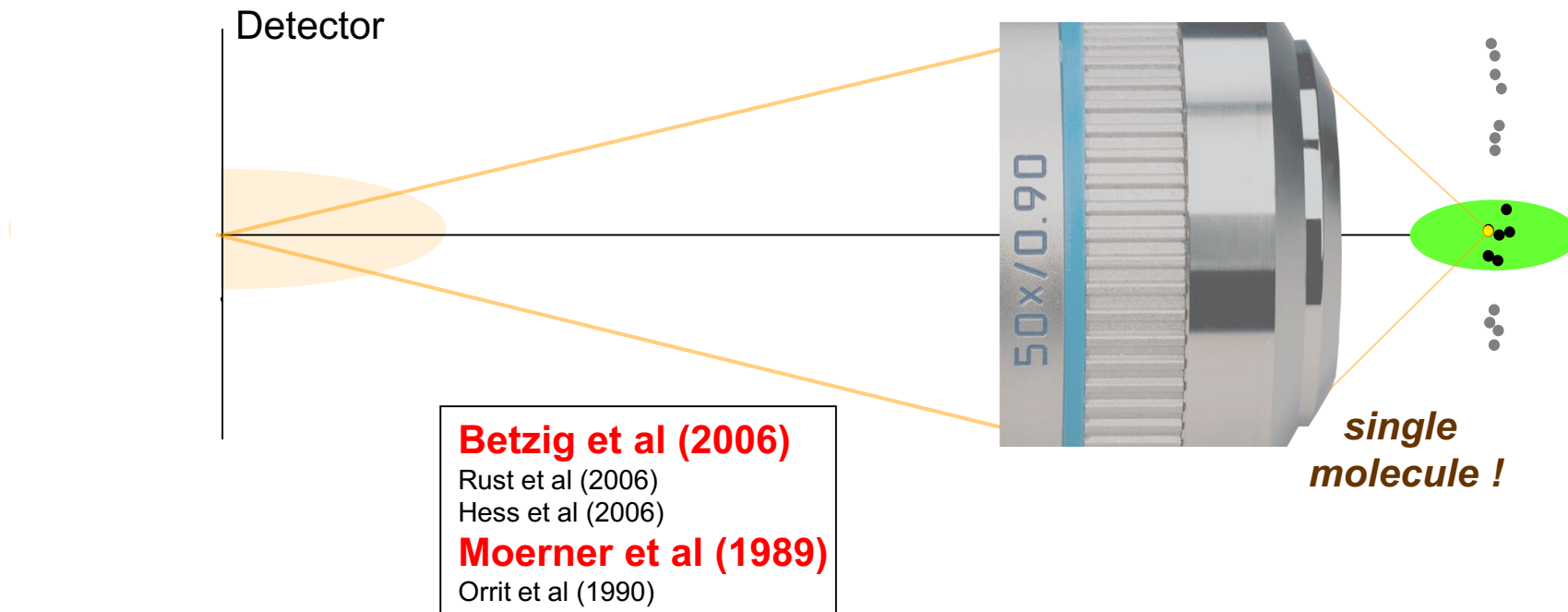
etc.

STED, GSD, SSIM, RESOLFT,...

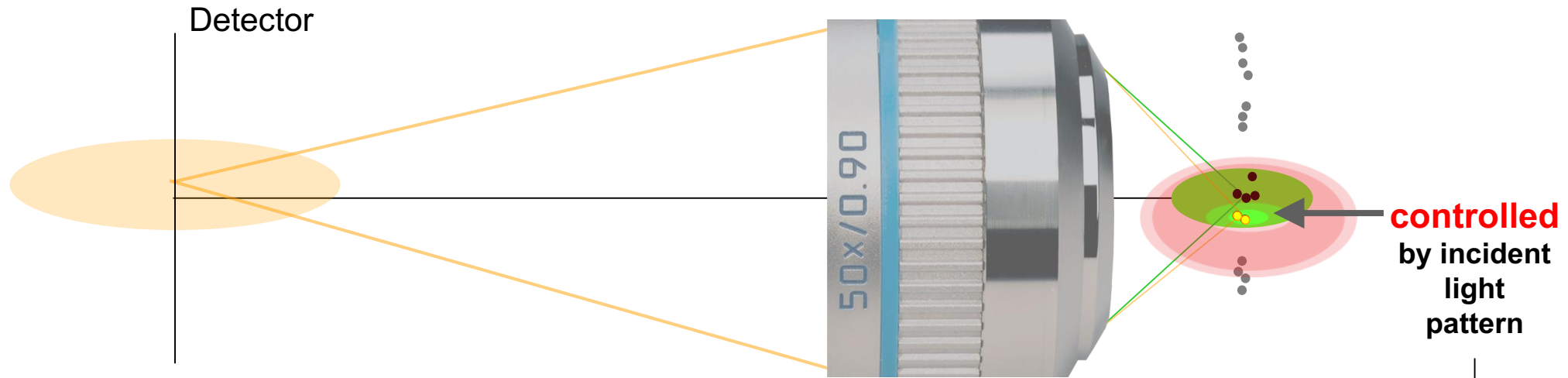


PALM, STORM, PAINT, GSDIM,...

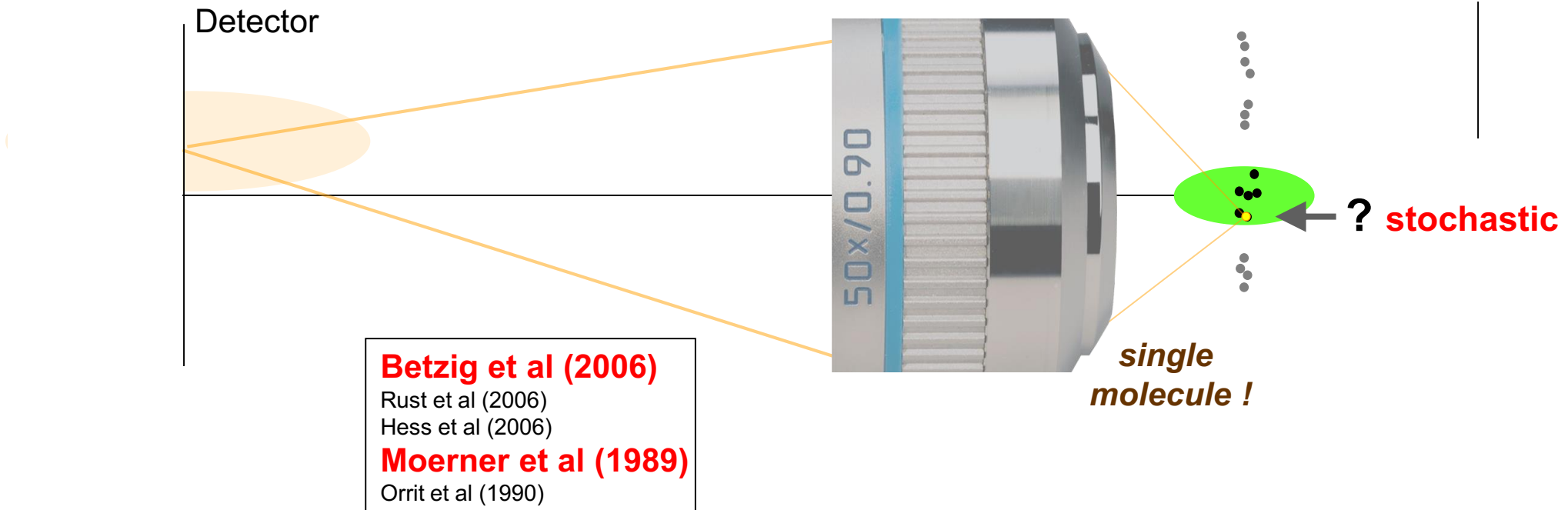
X,Y,Z



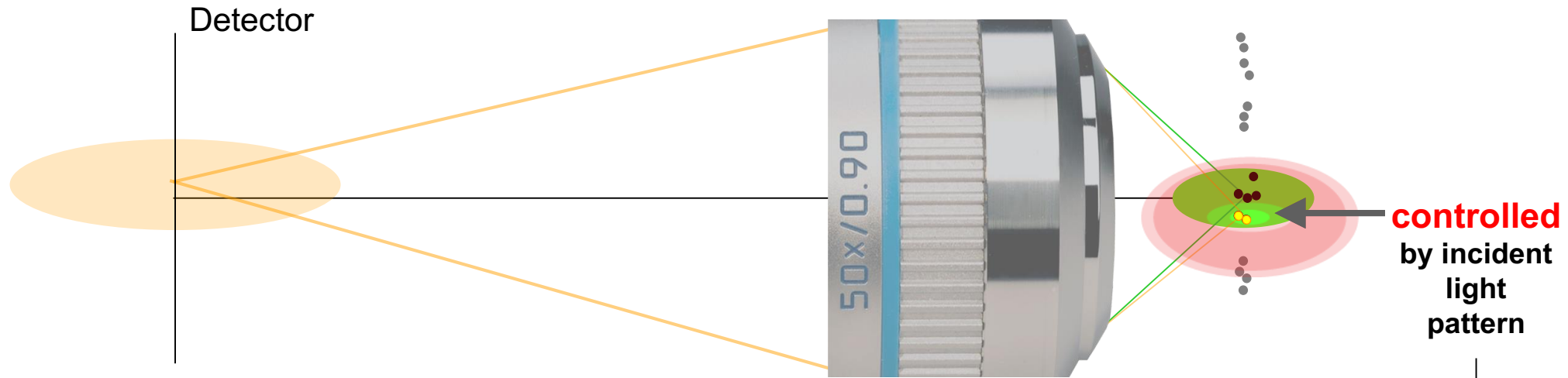
STED, GSD, SSIM, RESOLFT,...



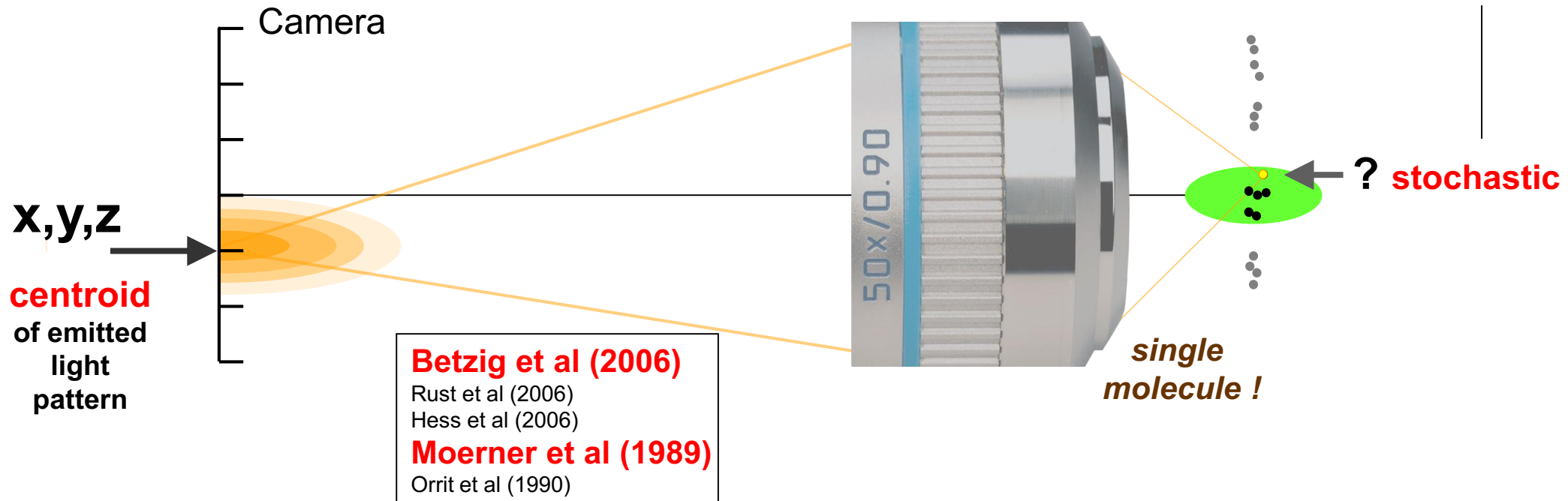
PALM, STORM, PAINT, GSDIM,...



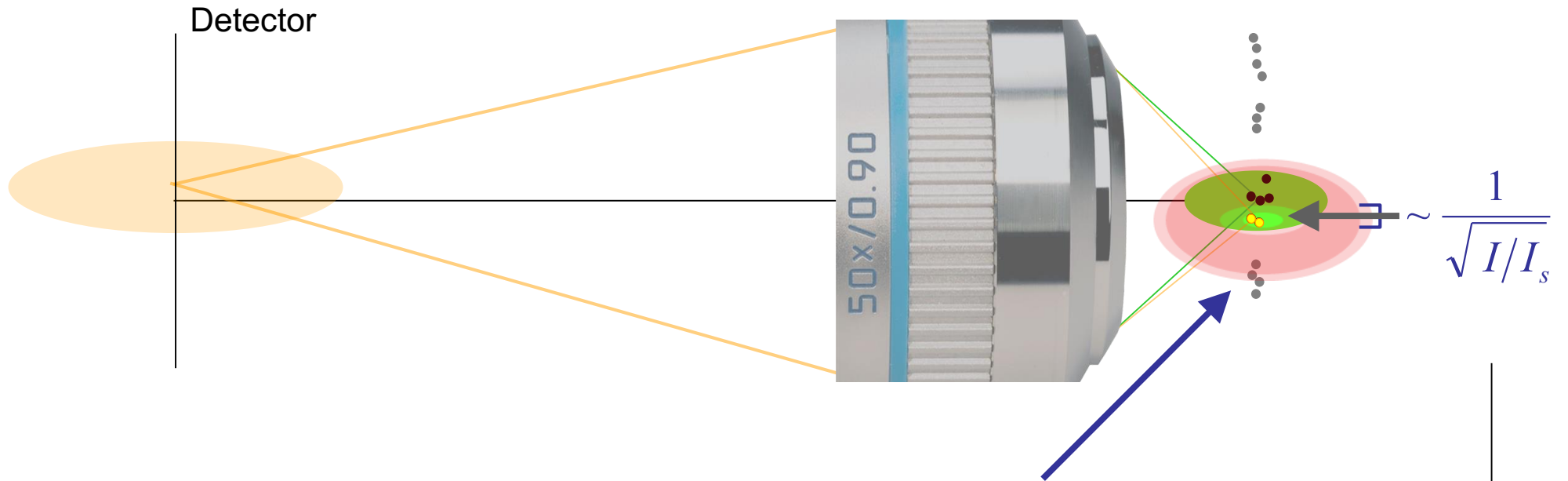
STED, GSD, SSIM, RESOLFT,...



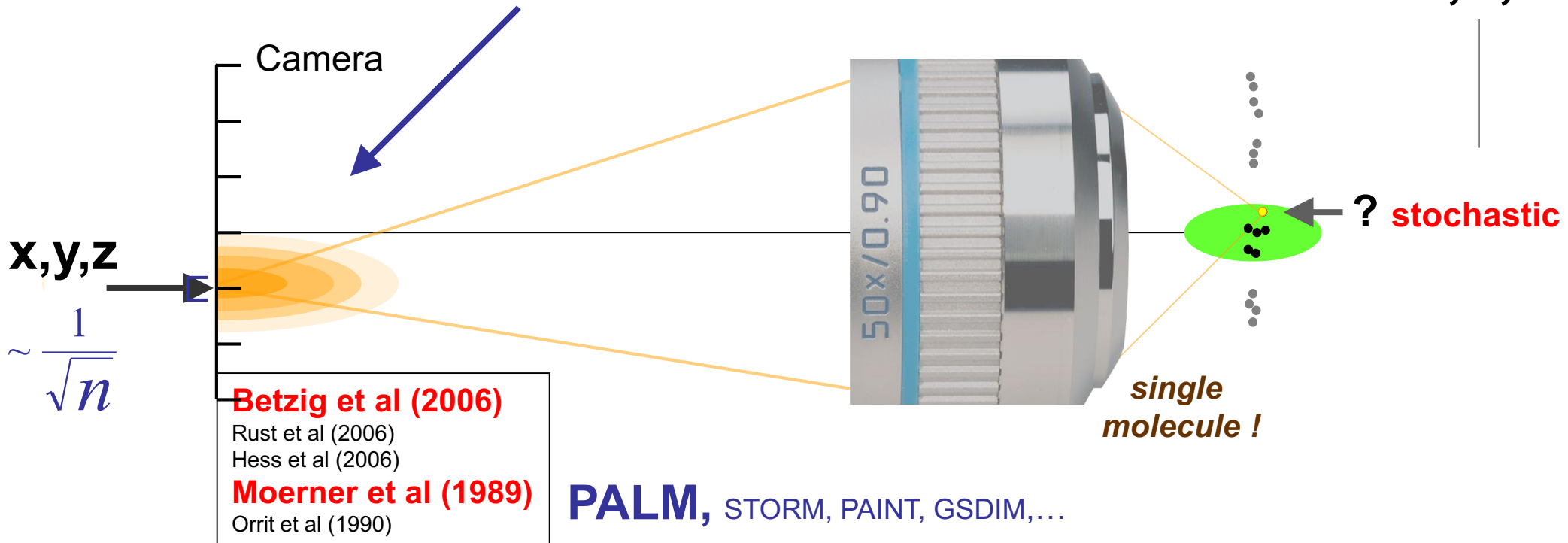
PALM, STORM, PAINT, GSDIM,...



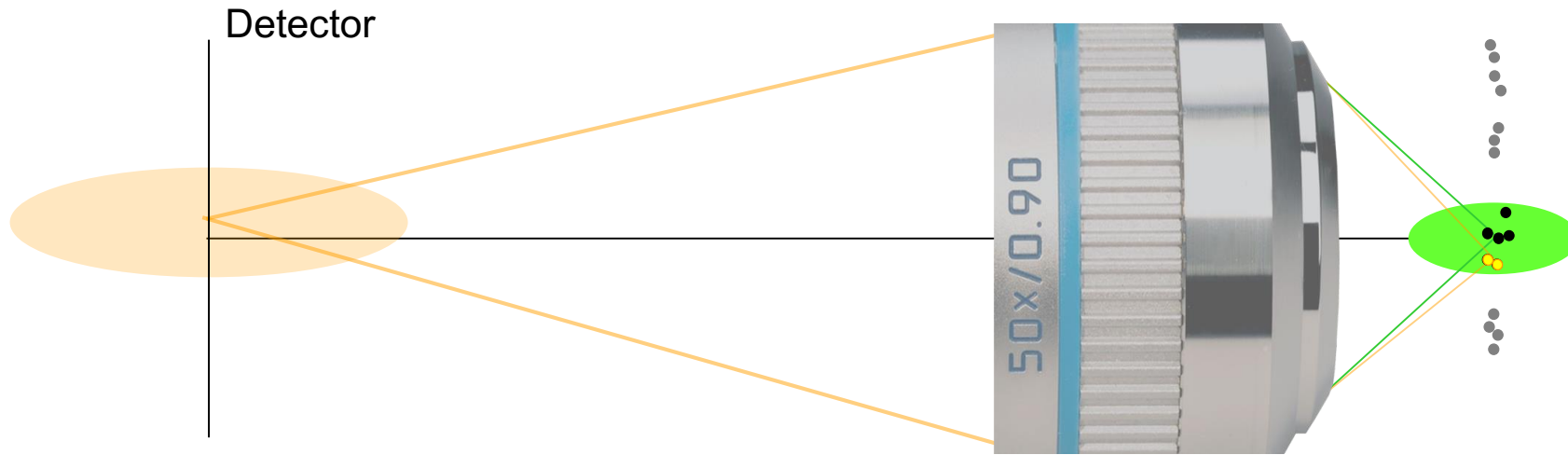
STED, GSD, SSIM, RESOLFT,...



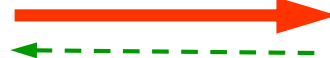
... many photons for X,Y,Z precision



STED, GSD, SSIM, RESOLFT,...

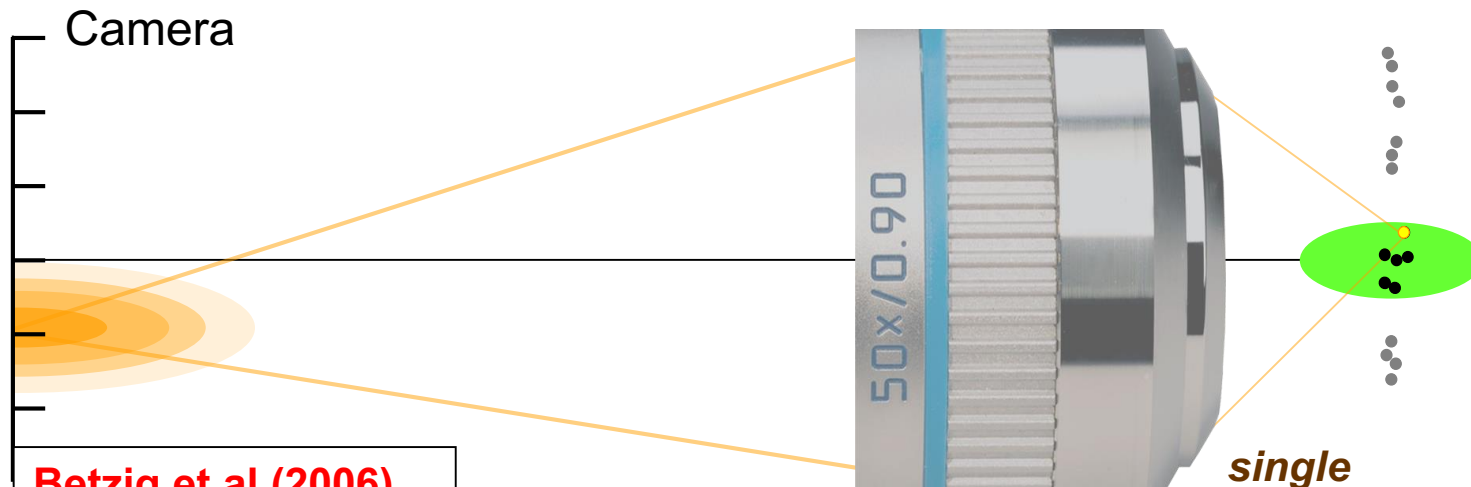


on



off

separates the features



Betzig et al (2006)

Rust et al (2006)

Hess et al (2006)

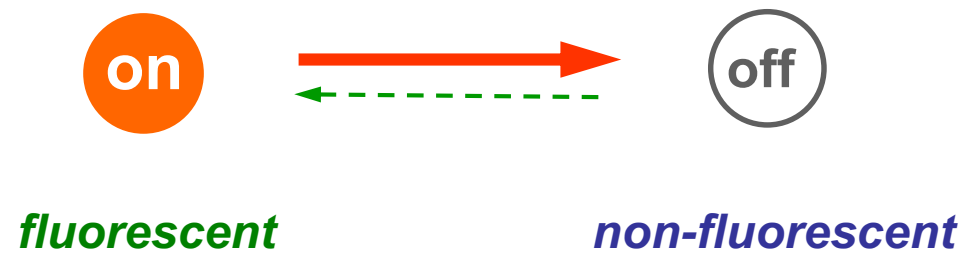
Moerner et al (1989)

Orrit et al (1990)

PALM, STORM, PAINT, GSDIM,...

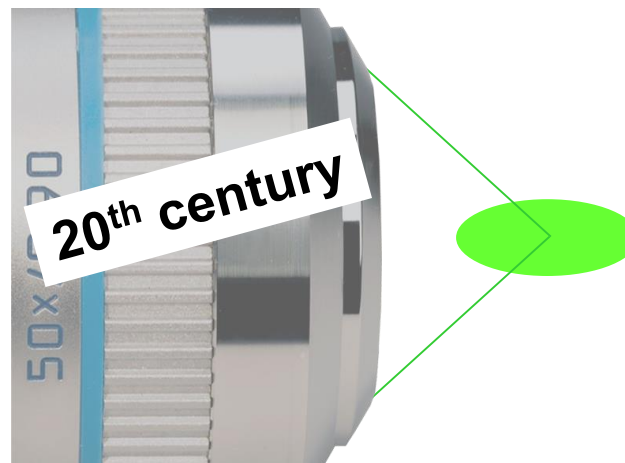
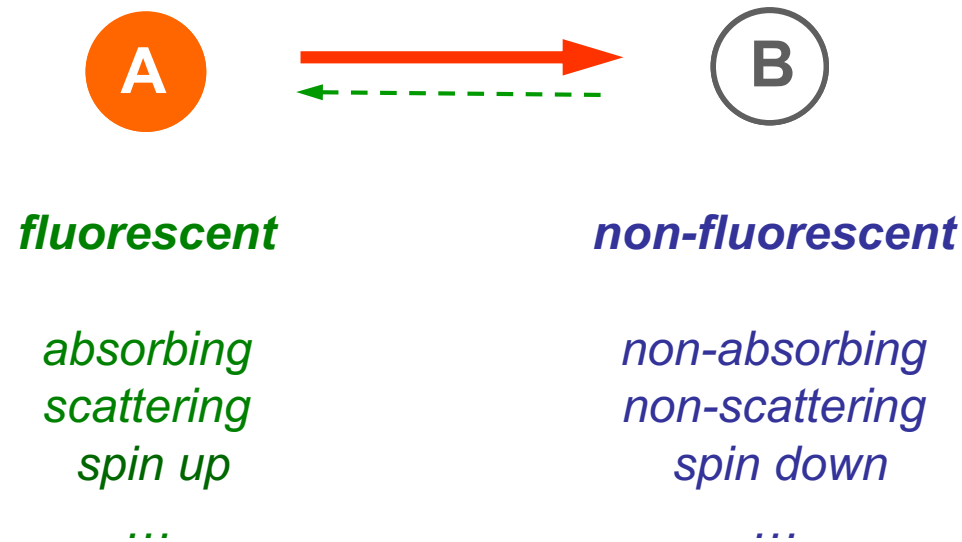
Superresolution

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



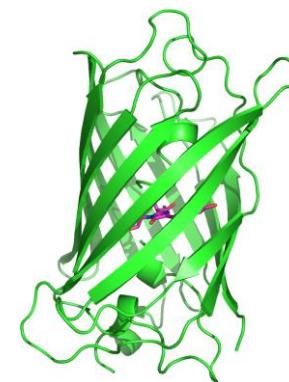
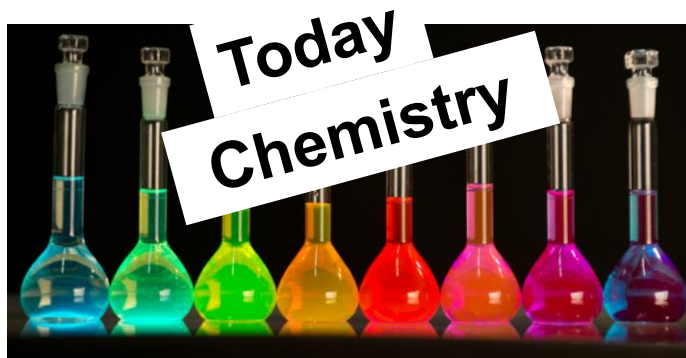
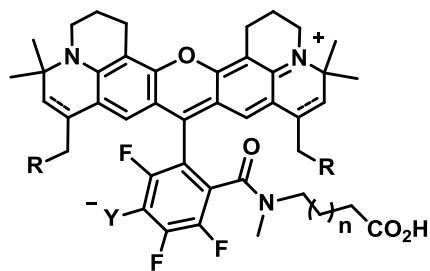
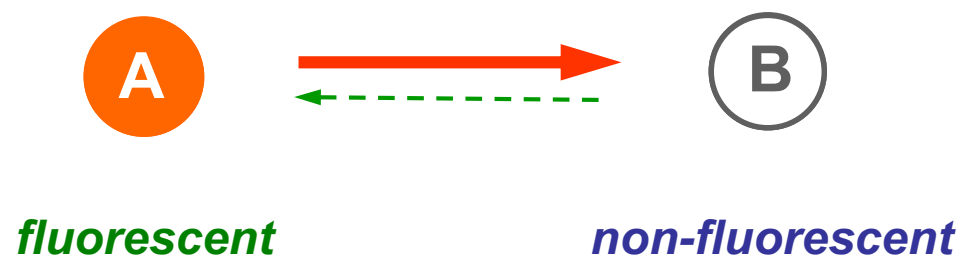
Superresolution

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



Superresolution

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





Former lab members



Thomas Klar



Stefan Jakobs



Katrin Willig



Alexander Egner



Lars
Kastrup



Andreas Schönle



Benjamin Harke

Current lab members



Volker Westphal



Roman Schmidt



Jan Keller



Tim
Grotjohann



Vladimir Belov



Christian
Wurm



Johann Engelhardt

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

... down to molecular scale.

