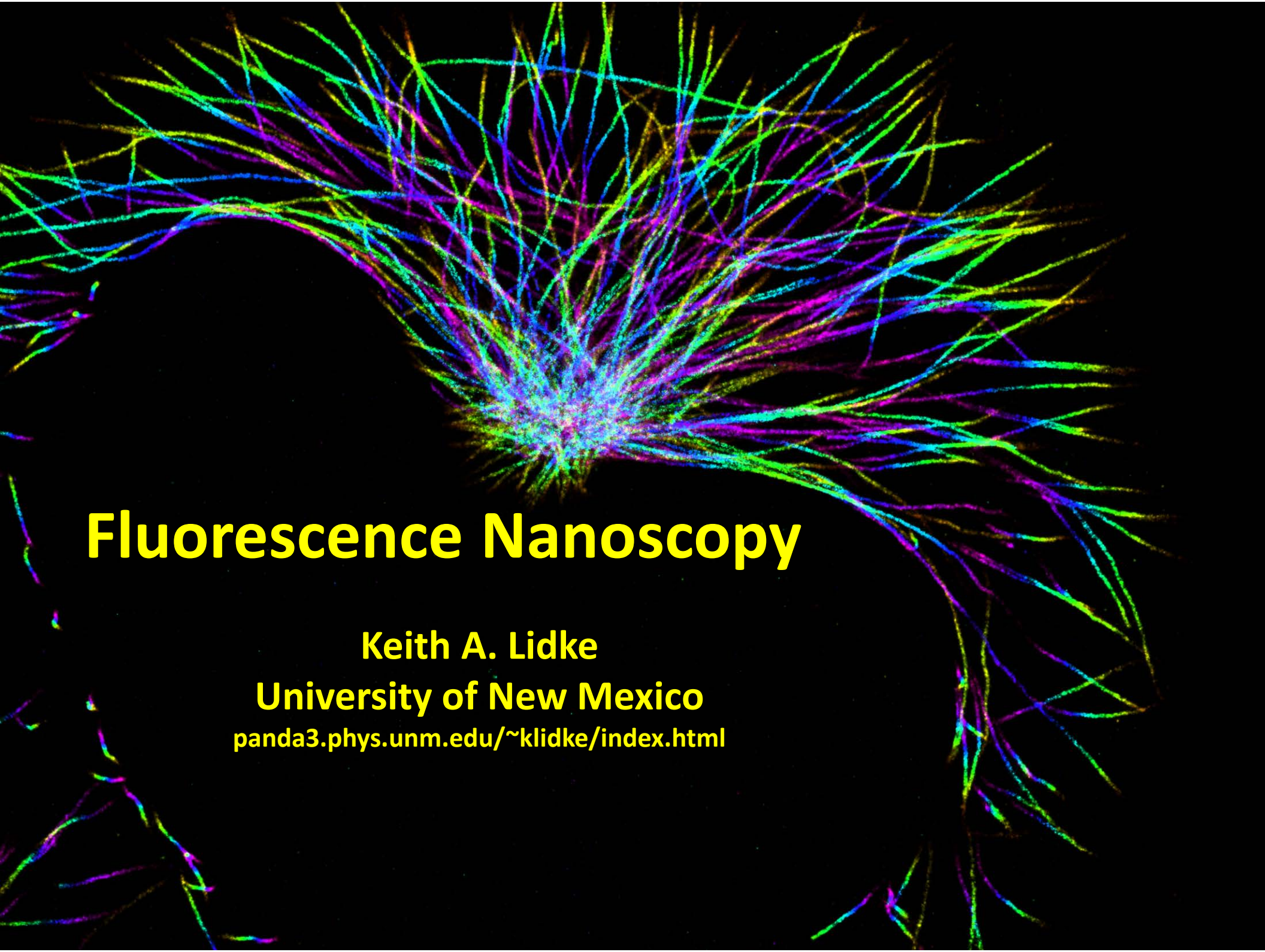


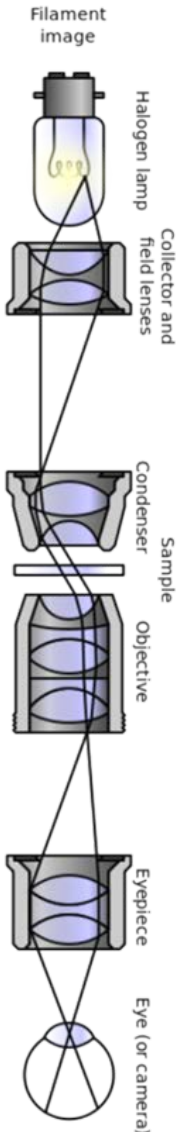
Fluorescence Nanoscopy

Keith A. Lidke

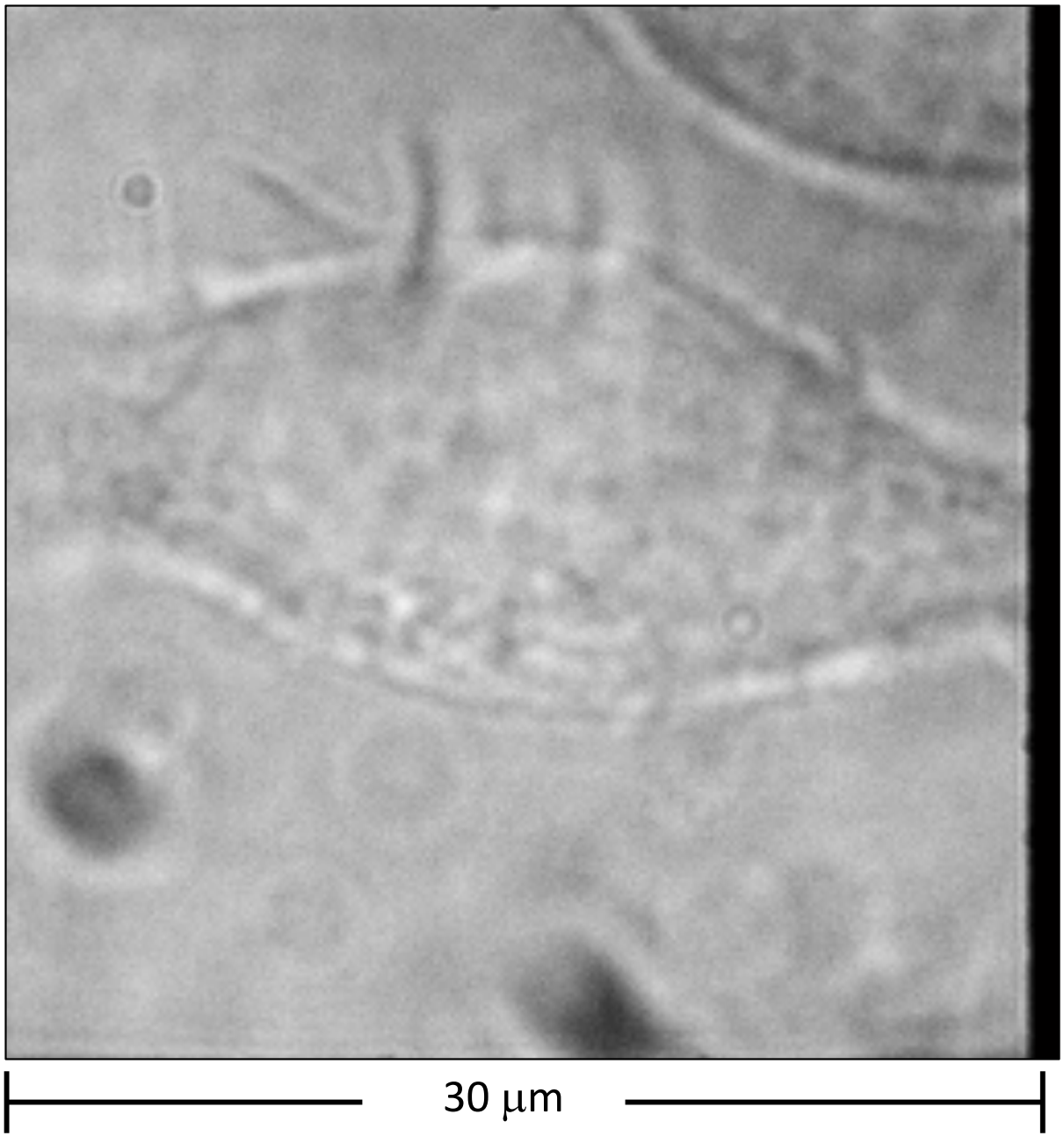
University of New Mexico

panda3.phys.unm.edu/~klidke/index.html

Optical Microscopy

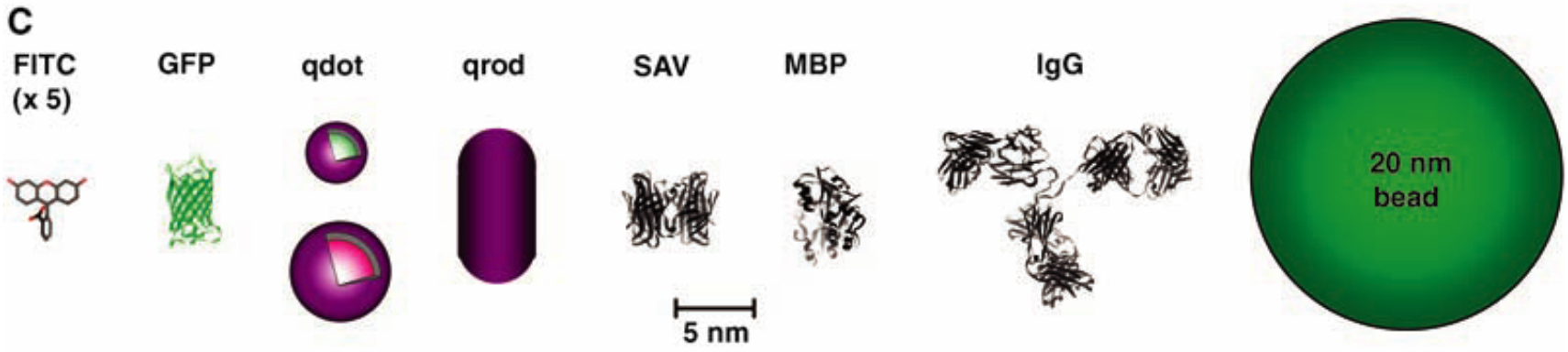


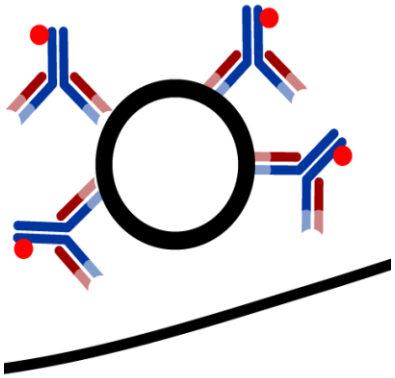
http://en.wikipedia.org/wiki/K%C3%B6hler_illumination

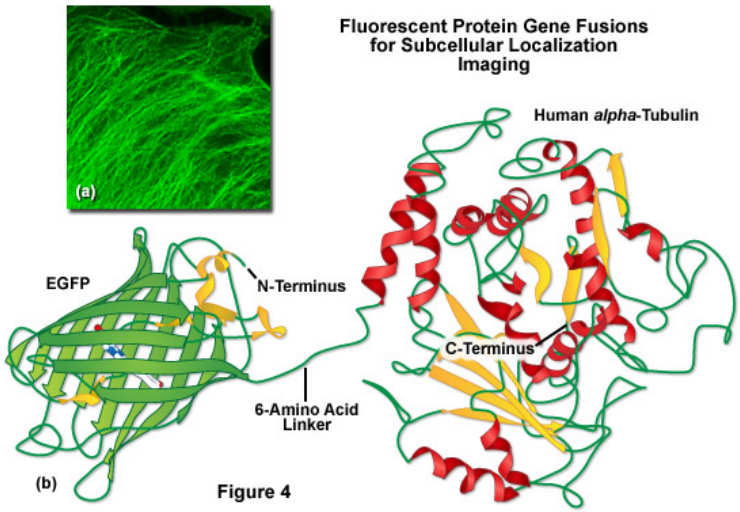


Fluorescent Probes

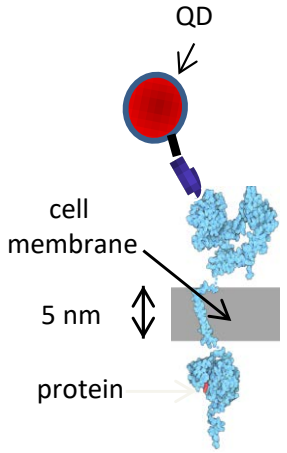
Michalet et. al. Science. Vol 307 p. 538



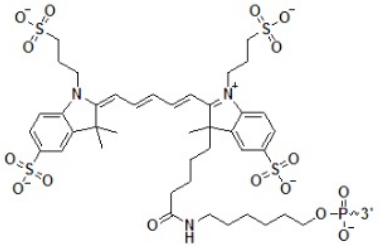


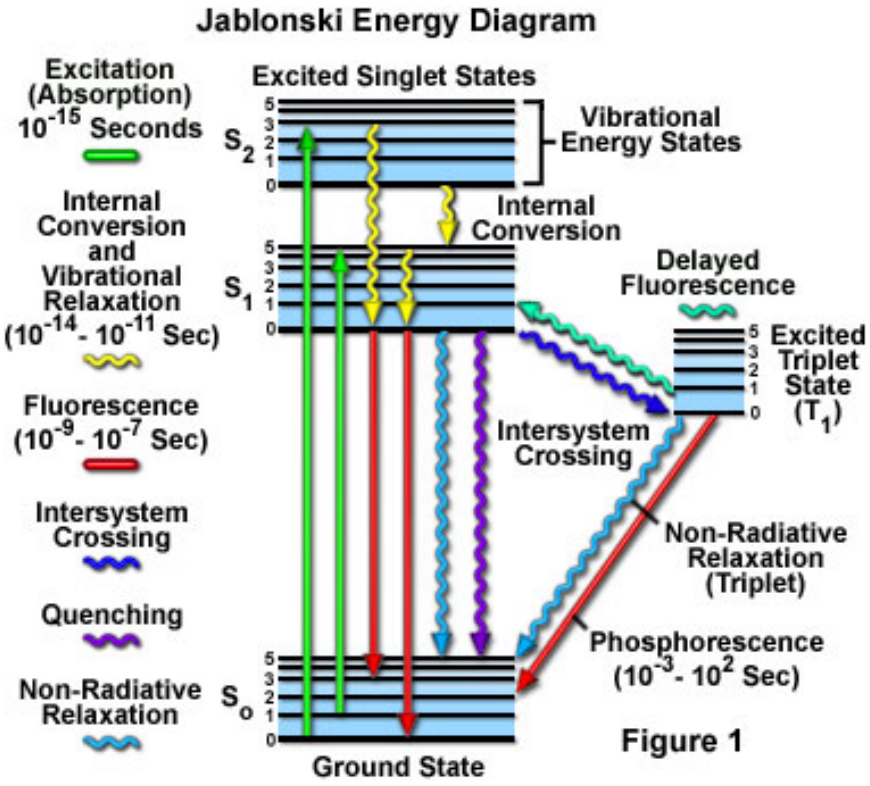


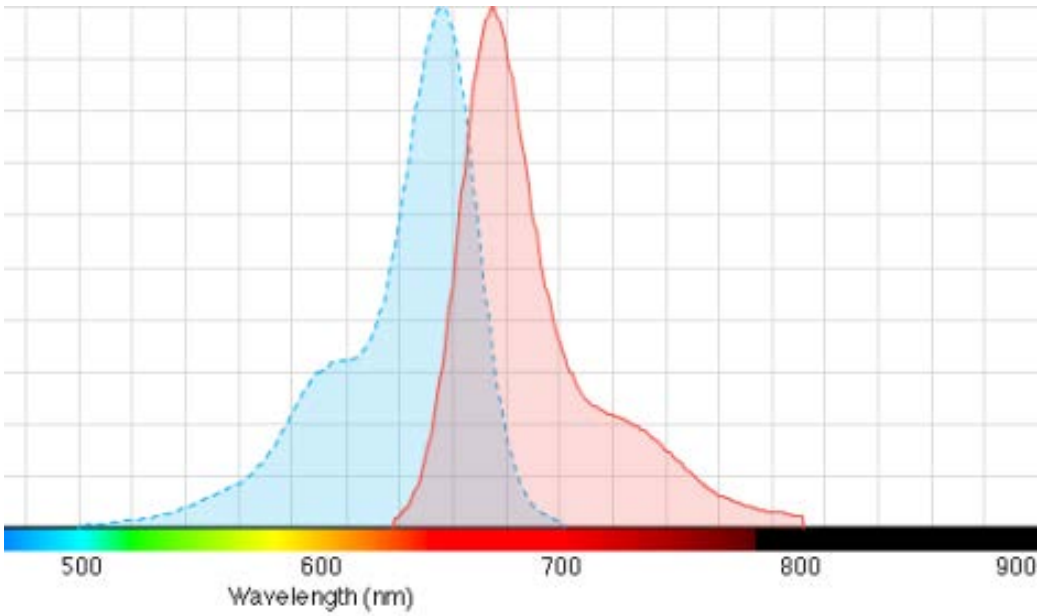
zeiss-campus.magnet.fsu.edu/articles/probes/fpintroduction.html



Fluorophore Energy Levels

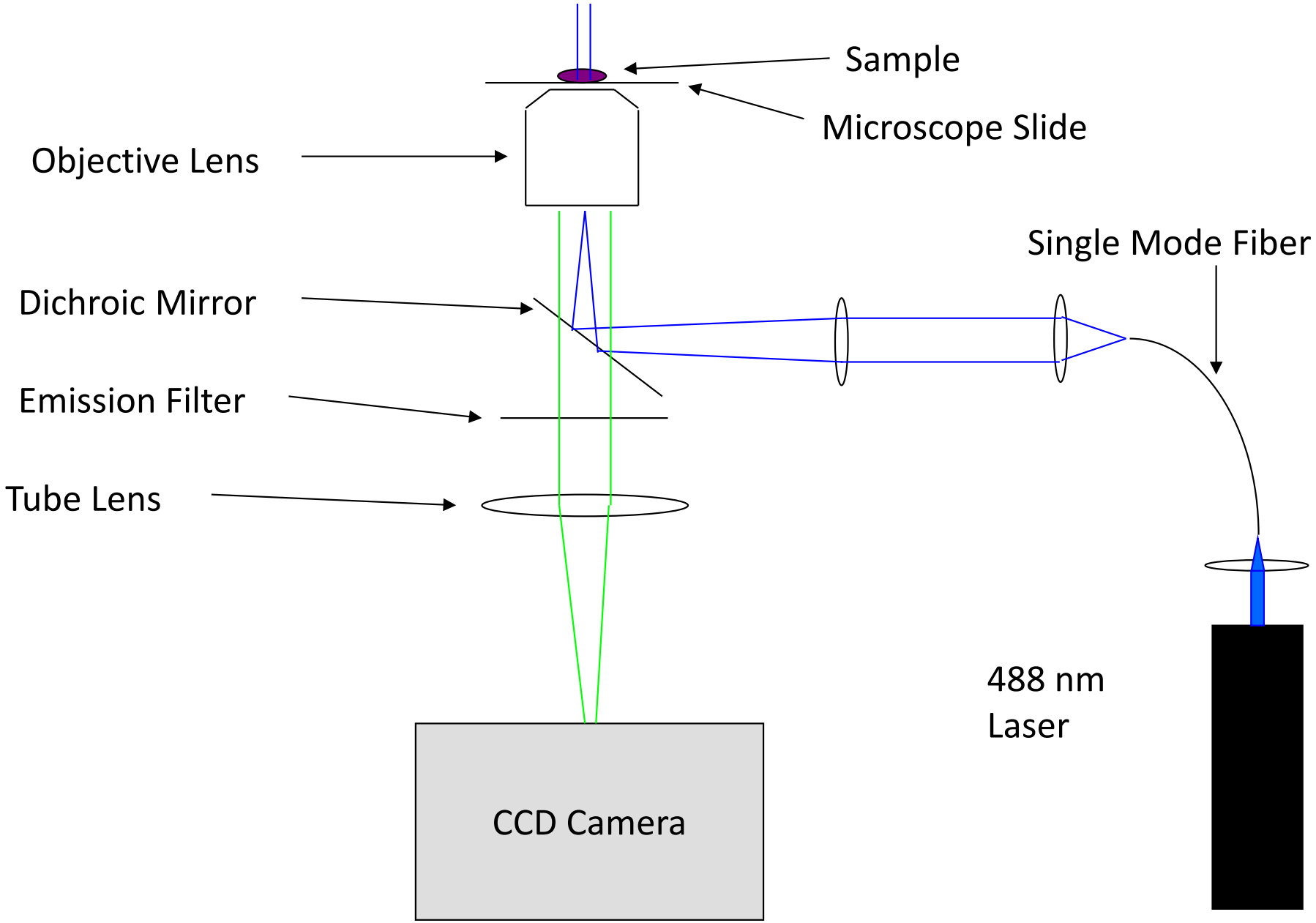




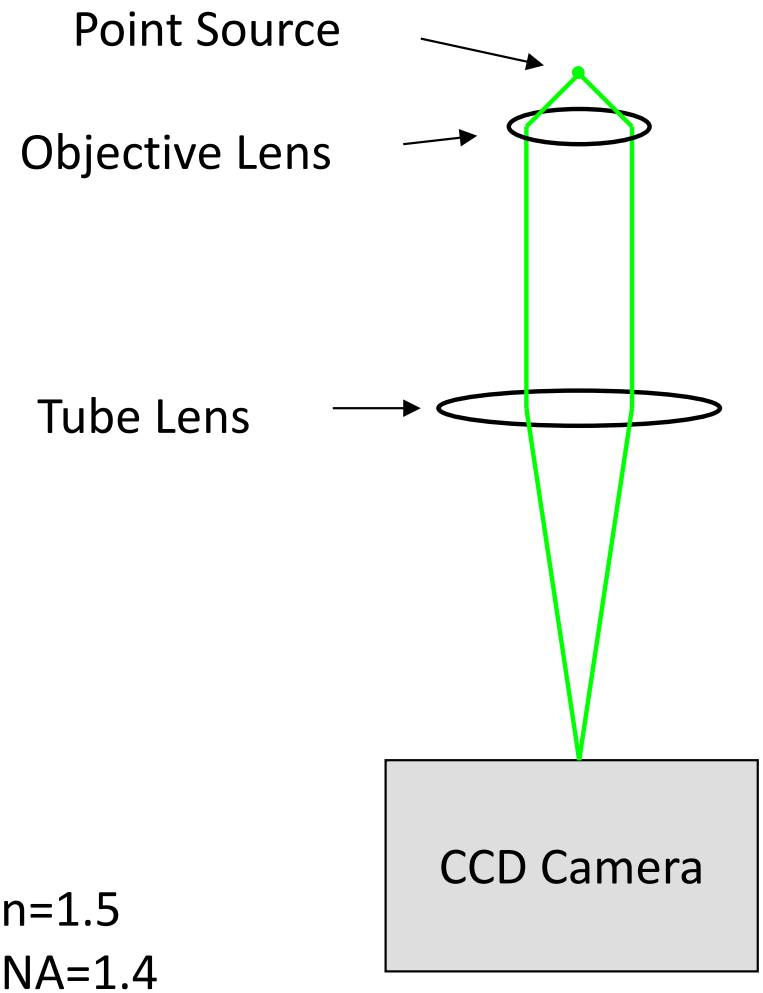


http://micro.magnet.fsu.edu/primer/java/jablonski/lightandcolor/

Epi-Fluorescence Setup

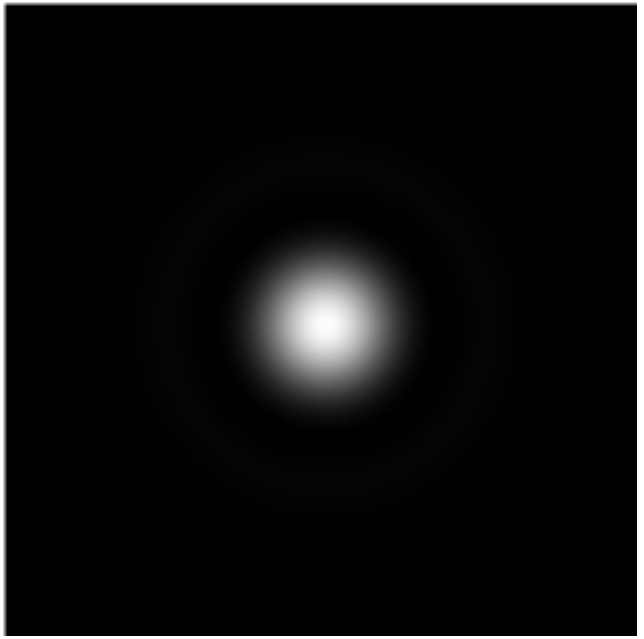


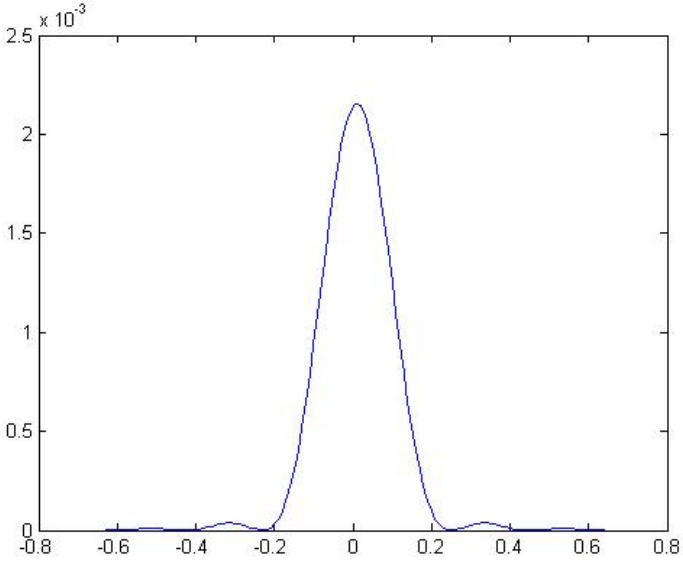
Imaging in a light microscope



n=1.5
NA=1.4
lambda=.55

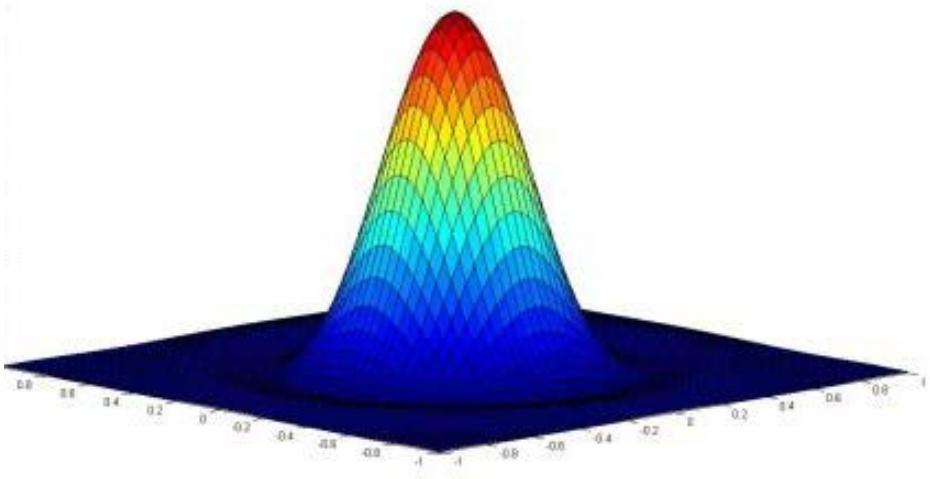
Point Spread Function (PSF)





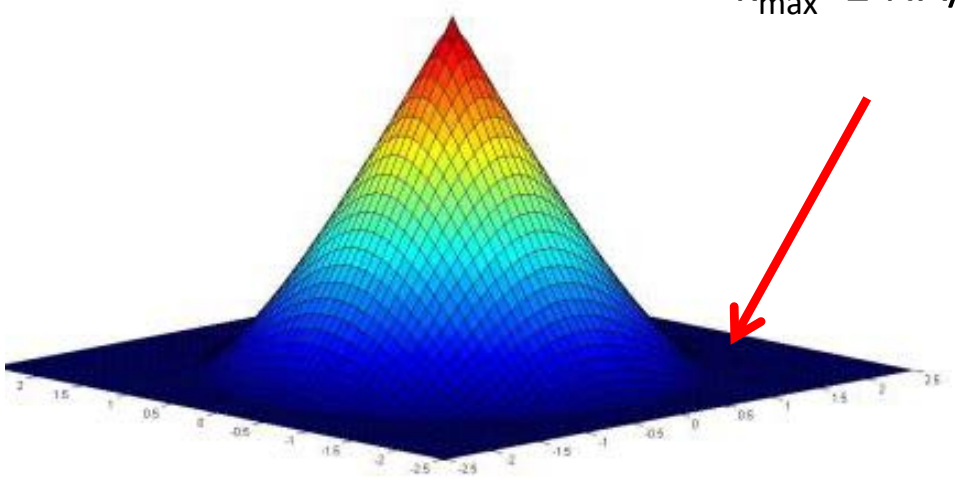
Point Spread Function and Optical Transfer Function

PSF



OTF

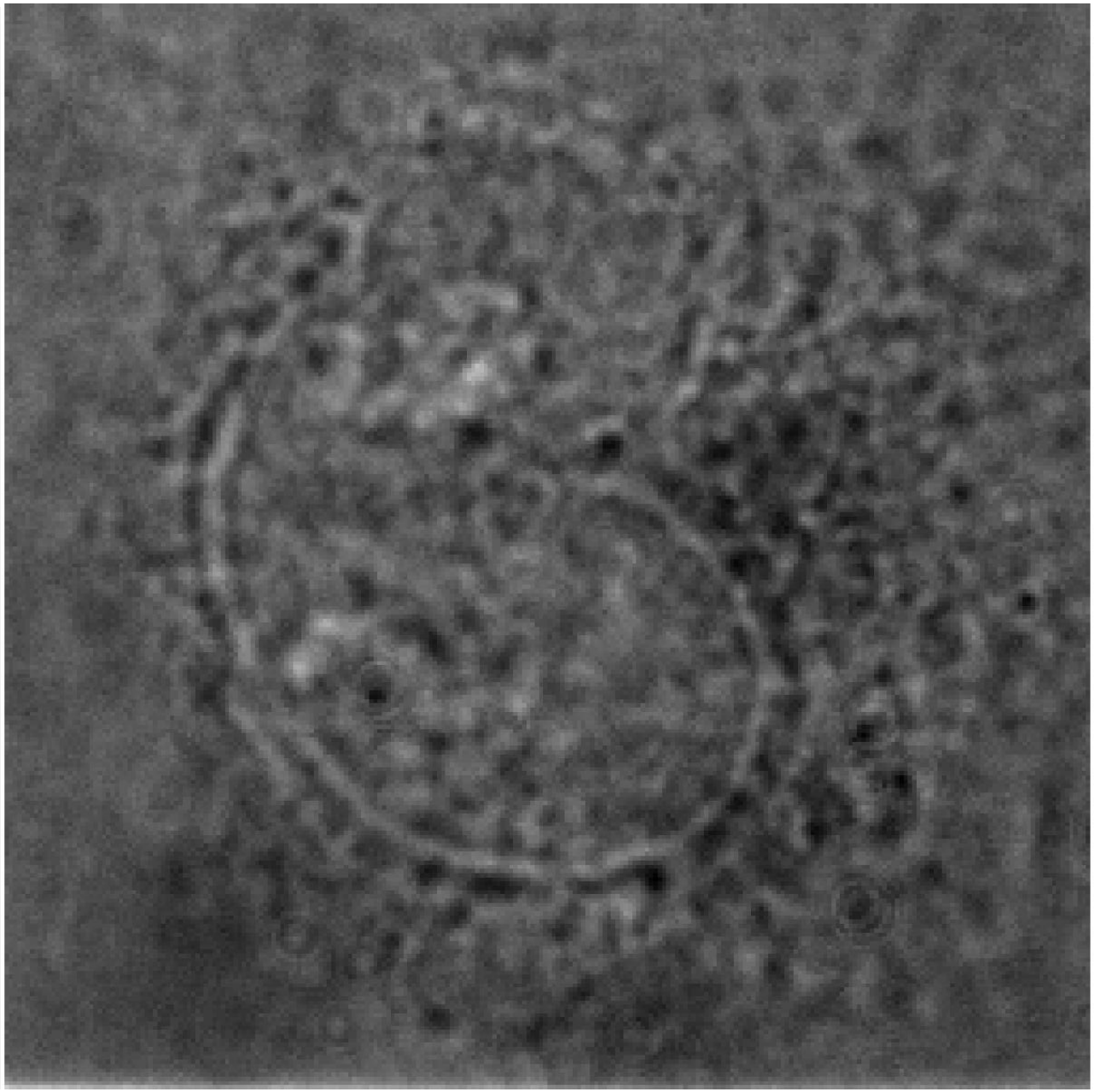
$k_{max}=2\ NA/\lambda$



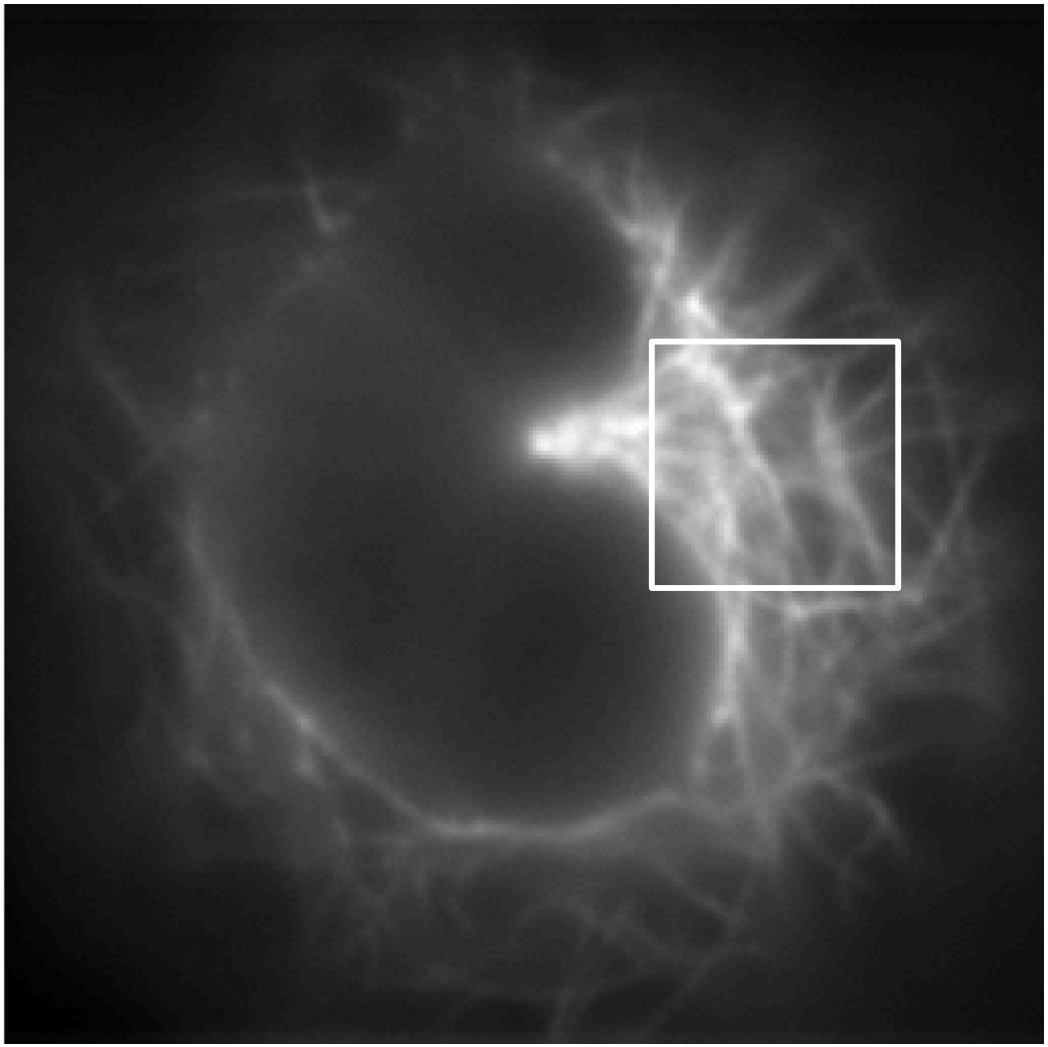
OTF is the Fourier Transform of the PSF

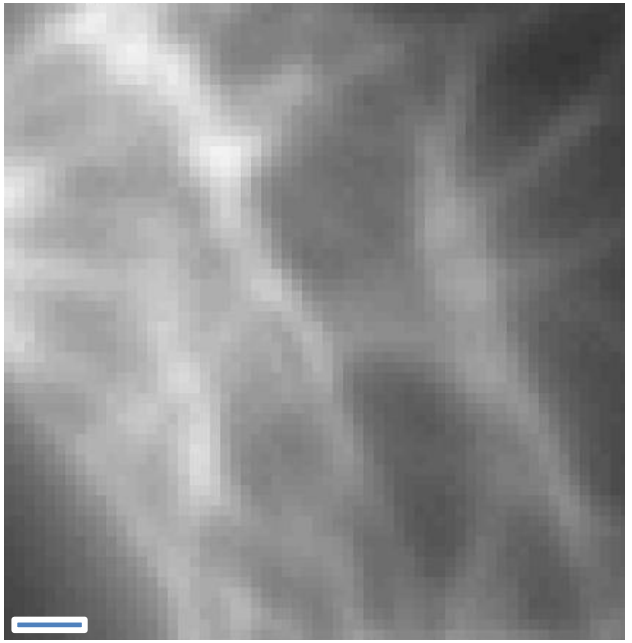

$d = \frac{\lambda}{2n\sin\alpha}$
ERNST ABBE
1840-1905
FRIEDRICH SCHILLER
UNIVERSITÄT JENA

Wide Field Immunofluorescence



Wide Field Immunofluorescence



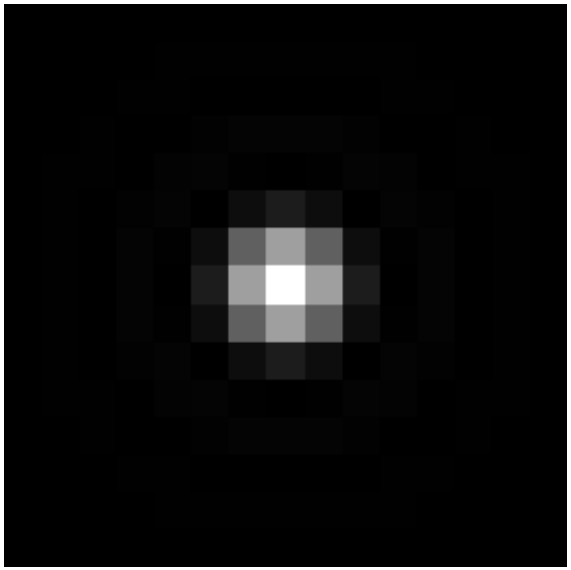


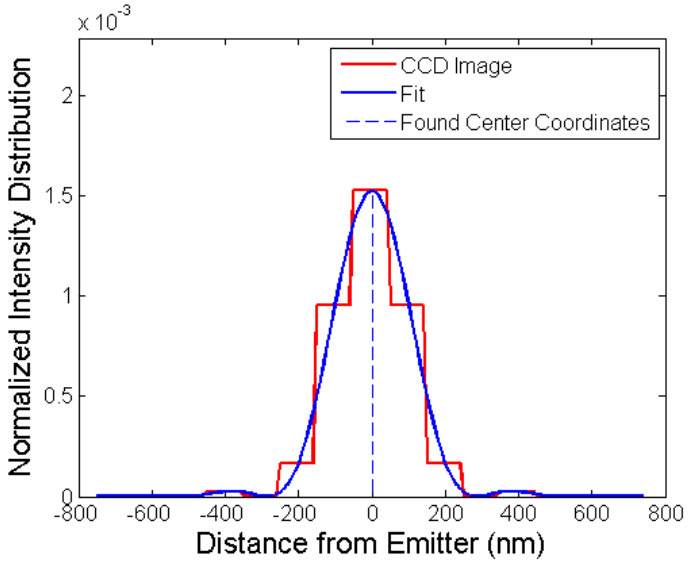
1 μm

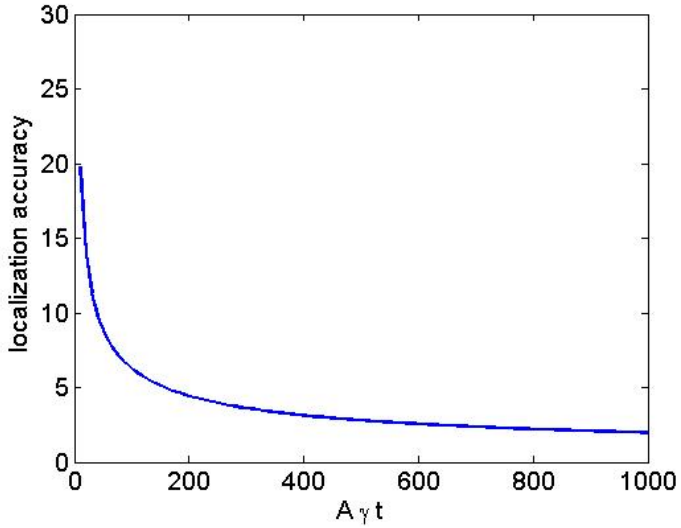
Single-Molecule Localization-Based Super-Resolution

f(PALM), (d)STORM, Pointillism, GSDIM,
PAINT, Blink Microscopy, …

Single Molecule Localization





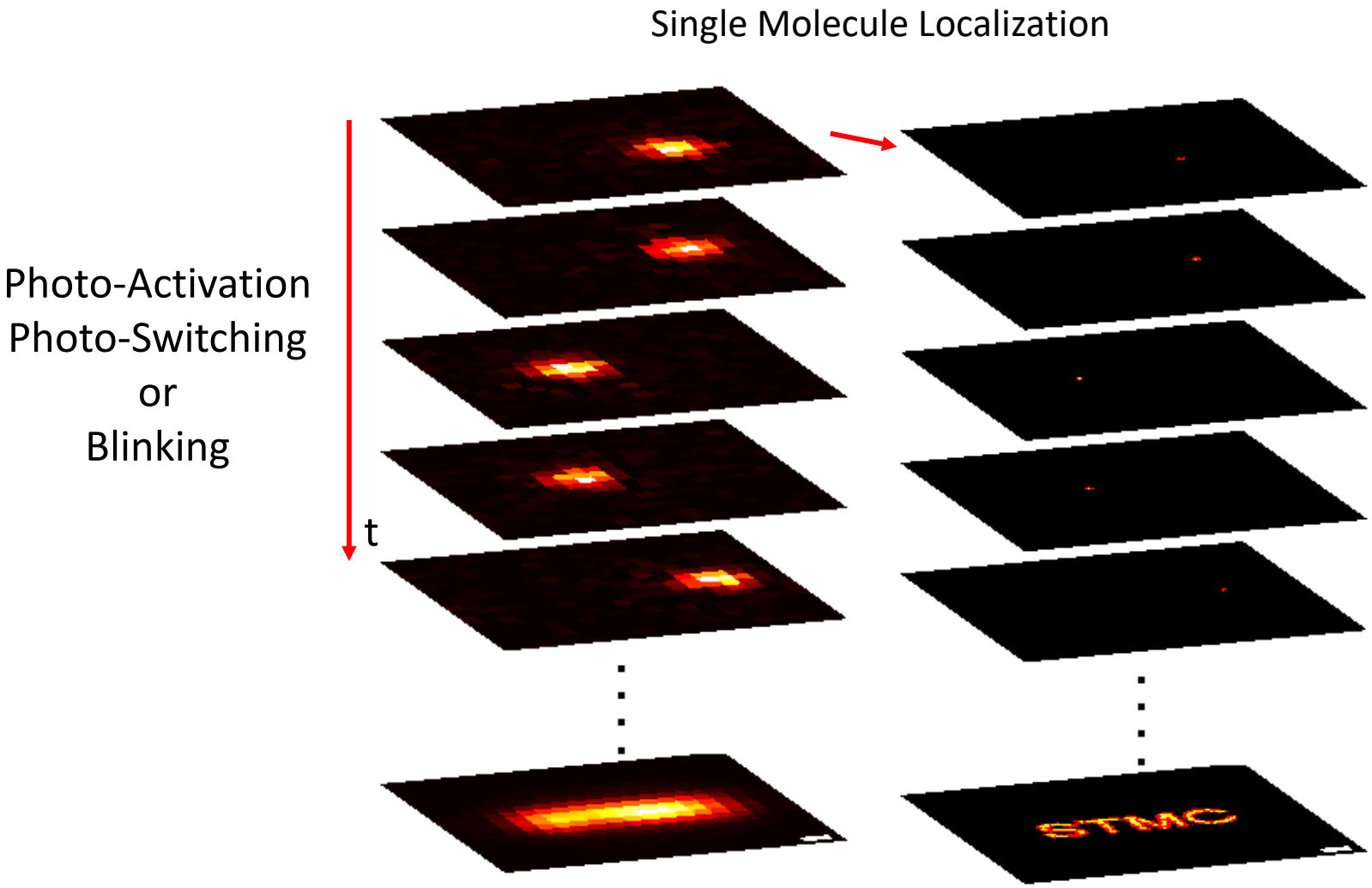


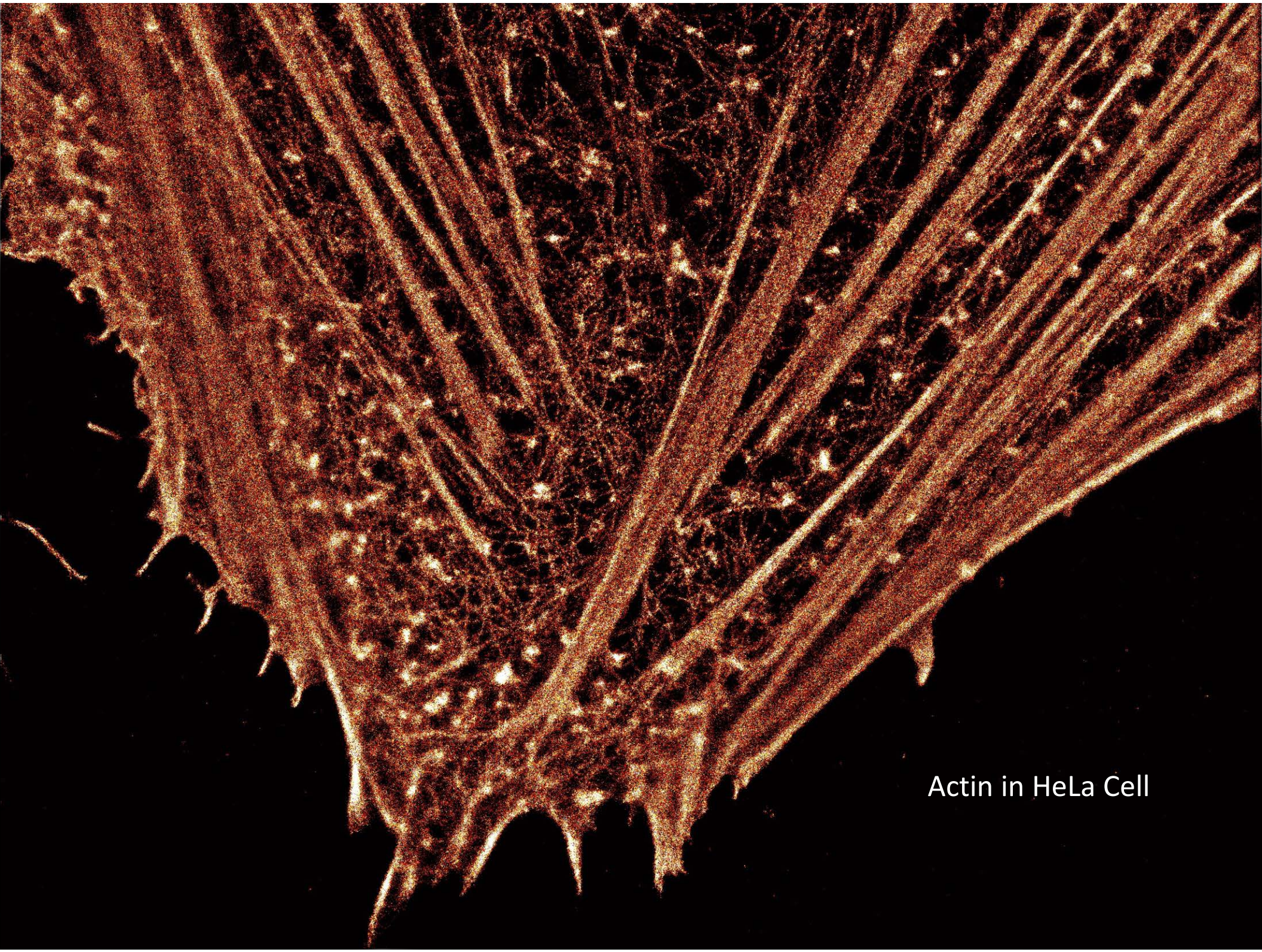
$$\frac{\lambda_{em}}{2\pi n_a \sqrt{\gamma A t}}$$

λ_{em}=emission wavelength
γ=collection efficiency
A= emission rate

Ober, R.J., *et al*, Biophysical Journal, 2004. **86**(2): p. 1185-1200.

Single Molecule Super-Resolution Concept



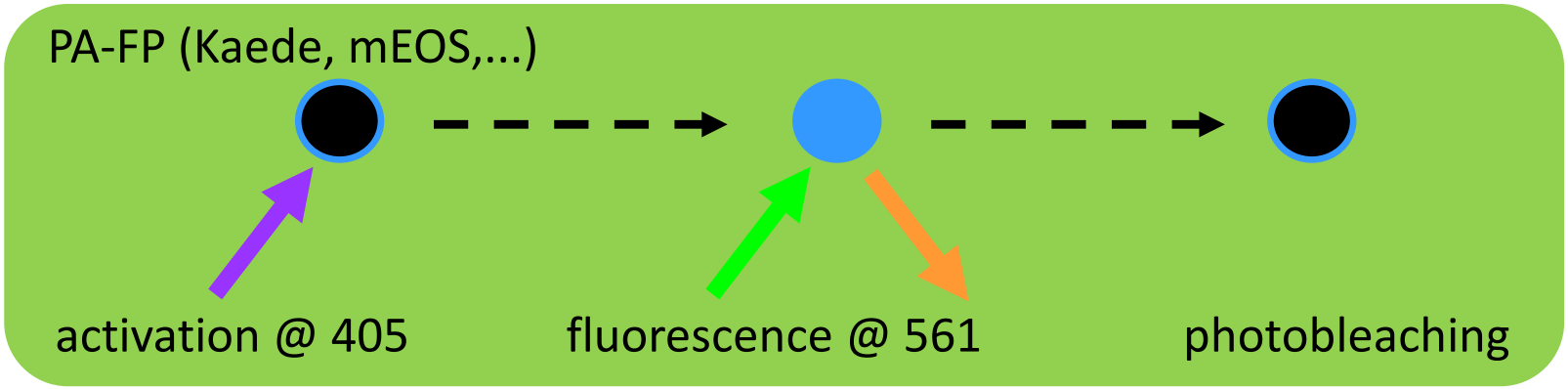

Actin in HeLa Cell

Mechanisms for “on” / “off” switching

(f)PALM = (fluorescence) Photo-Activation Localization Microscopy

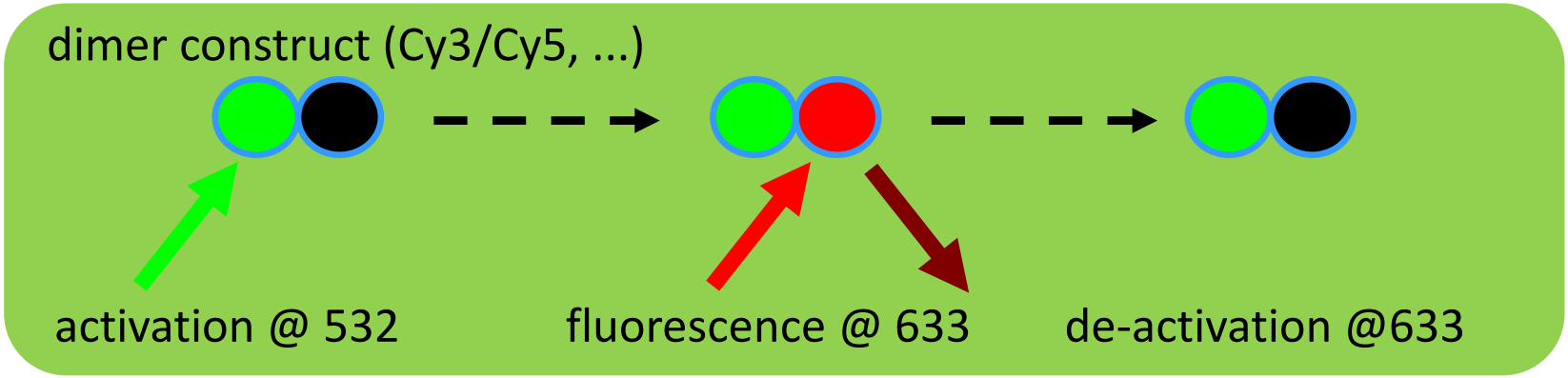
Betzig/ H.Hess, Science 2006

S. Hess, Biophysical Journal 2006



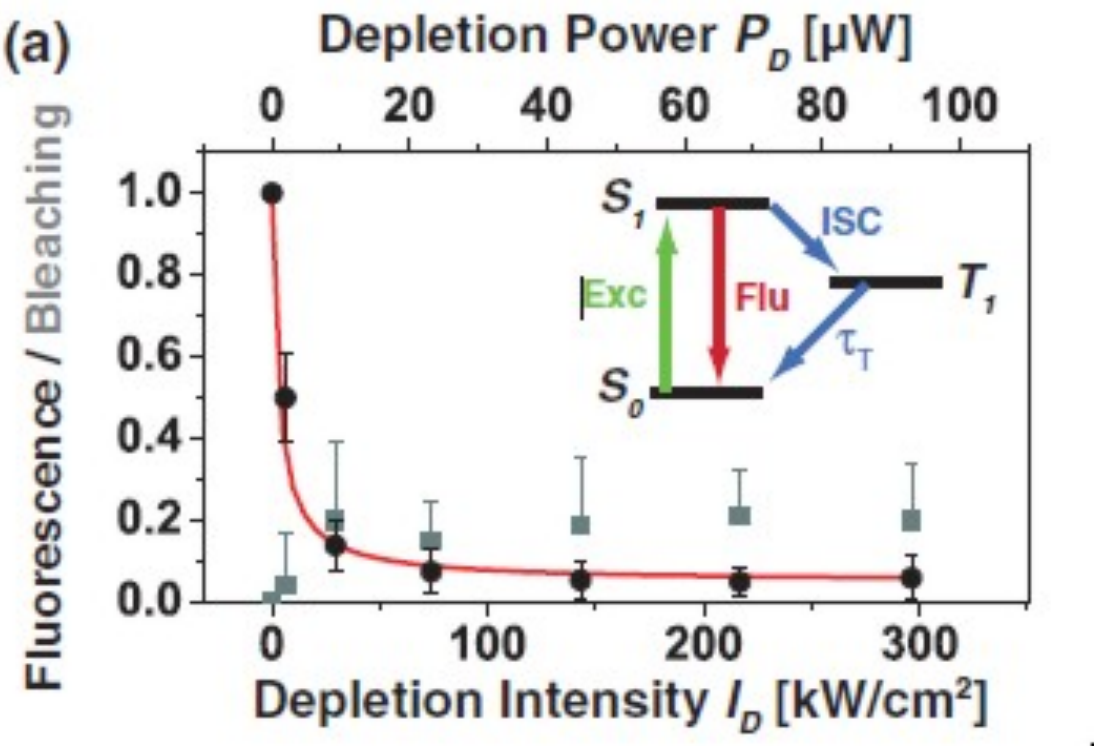
Mechanisms for “on” / “off” switching

STORM = STochastical Optical Reconstruction Microscopy
(Zhuang, Nat. Methods 2006)



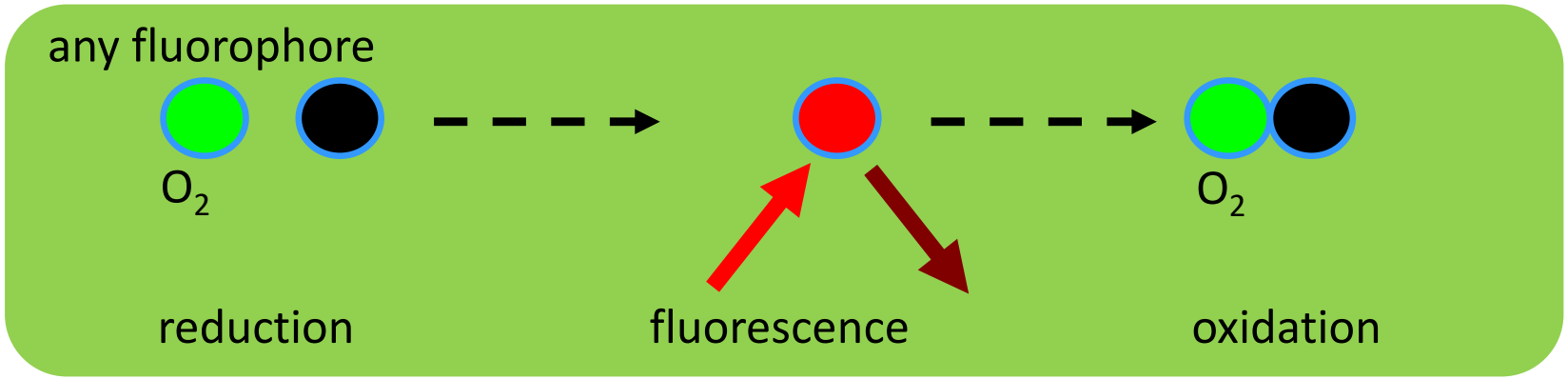
Mechanisms for “on” / “off” switching

GSDIM = Ground State Depletion followed by single molecule IMaging (Hell, PRL 2007)



Mechanisms for “on” / “off” switching

dSTORM = "direct" STORM (Heilemann, Angew. Chem. 2008)



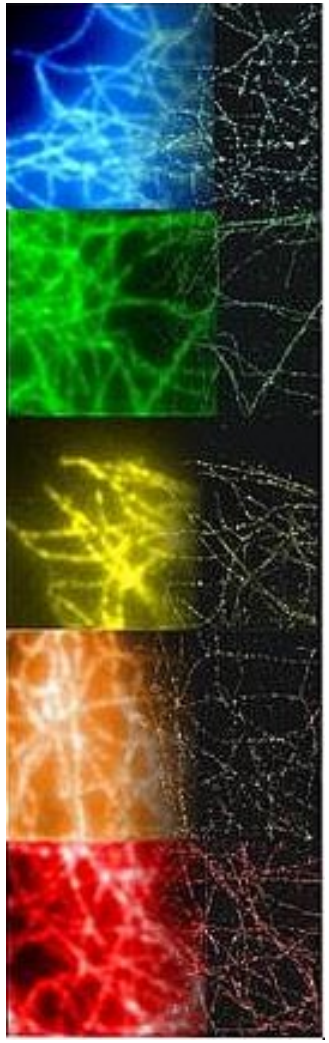
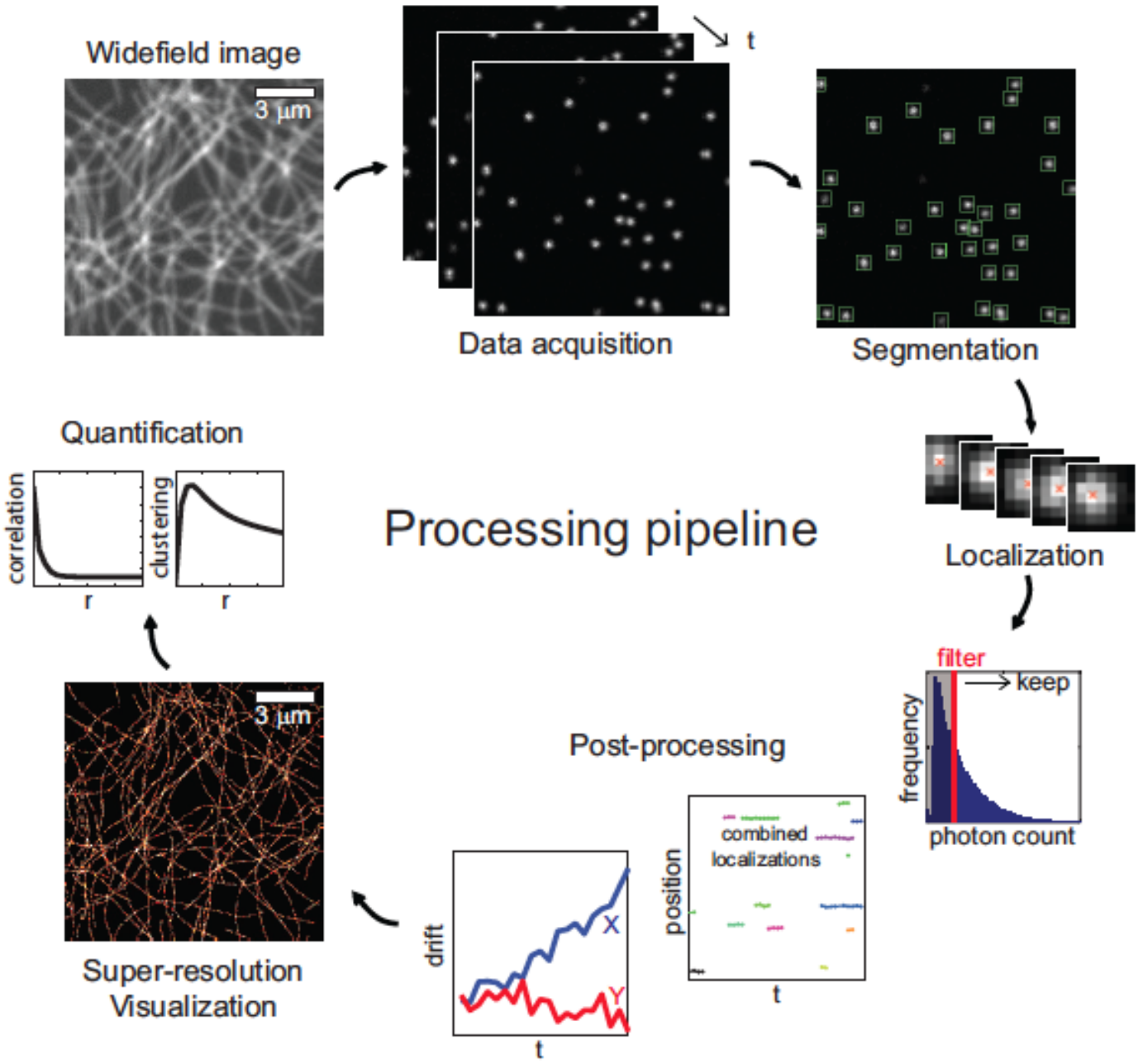
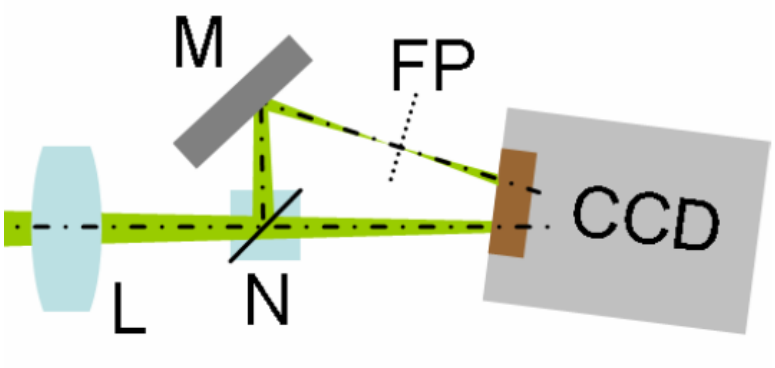


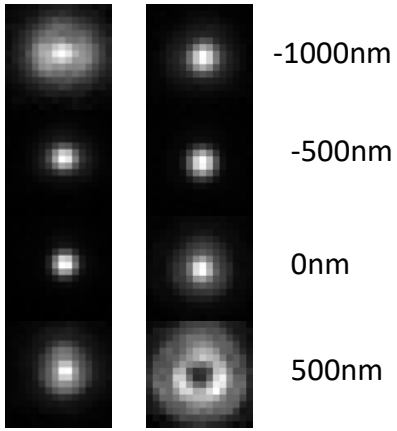
Image Processing Pipeline



3D Localization Techniques

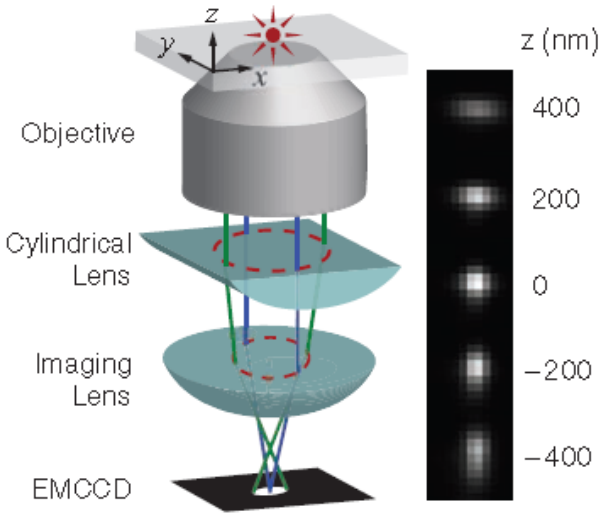
Dual Focal Plane





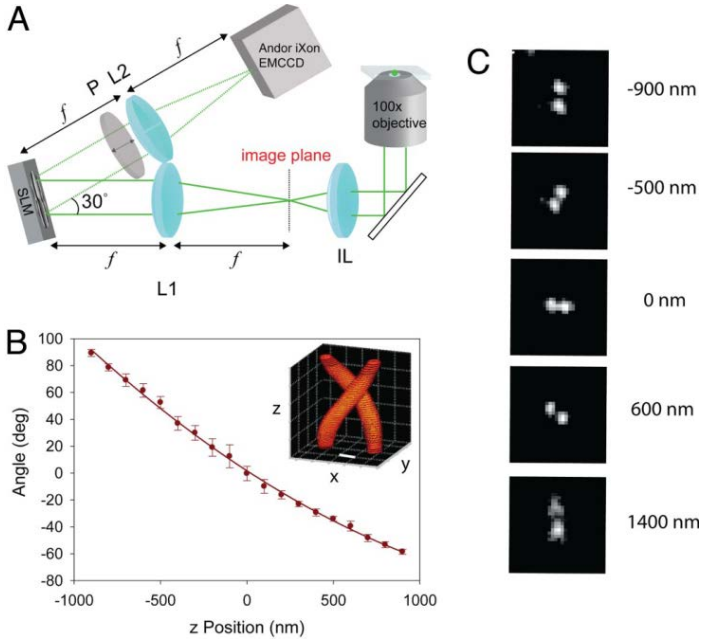
Juette, Nat. Meth.,2008
Ram, Biophys. J., 2008

Astigmatism



Huang, Science, 2008

Double-helix PSF



Pavani , PNAS, 2009

Dealing with reality: Aberrations

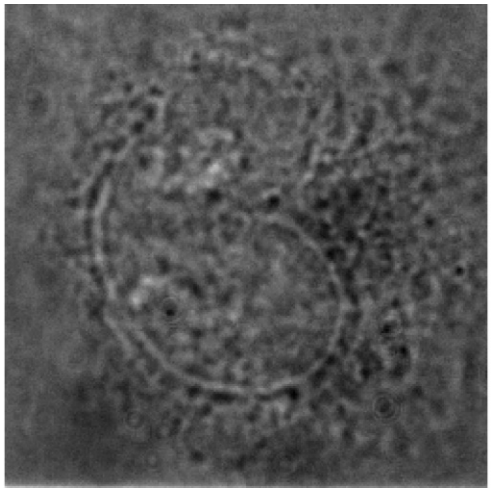
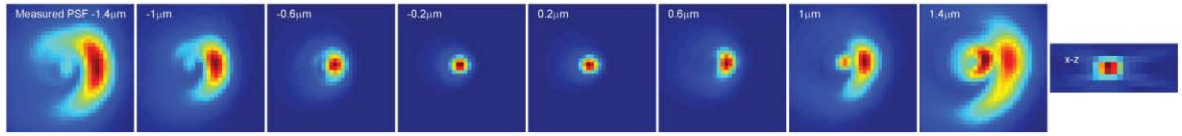


Image of a point source at various defocus

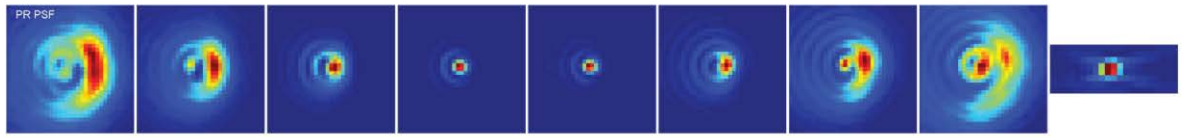
Pupil Function

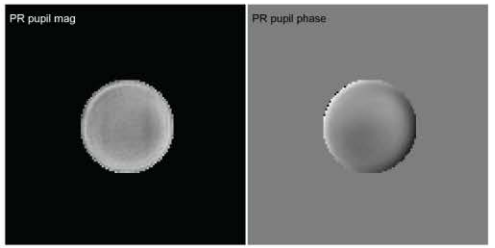
coverslip thickness is 0.21 mm, aberration collar at 0.18, on glass

Data



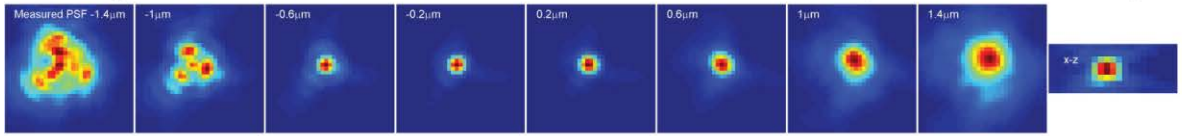
Model



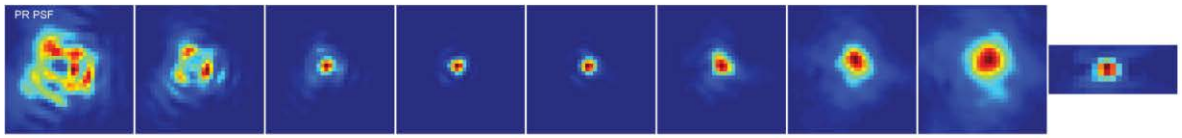


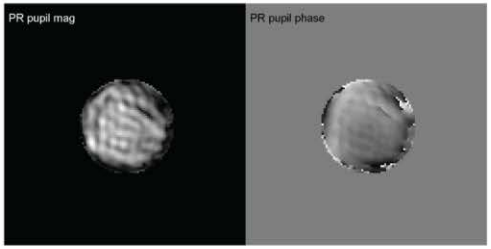
coverslip thickness is 0.21 mm, aberration collar at 0.18, 10 μm above coverslip

Data

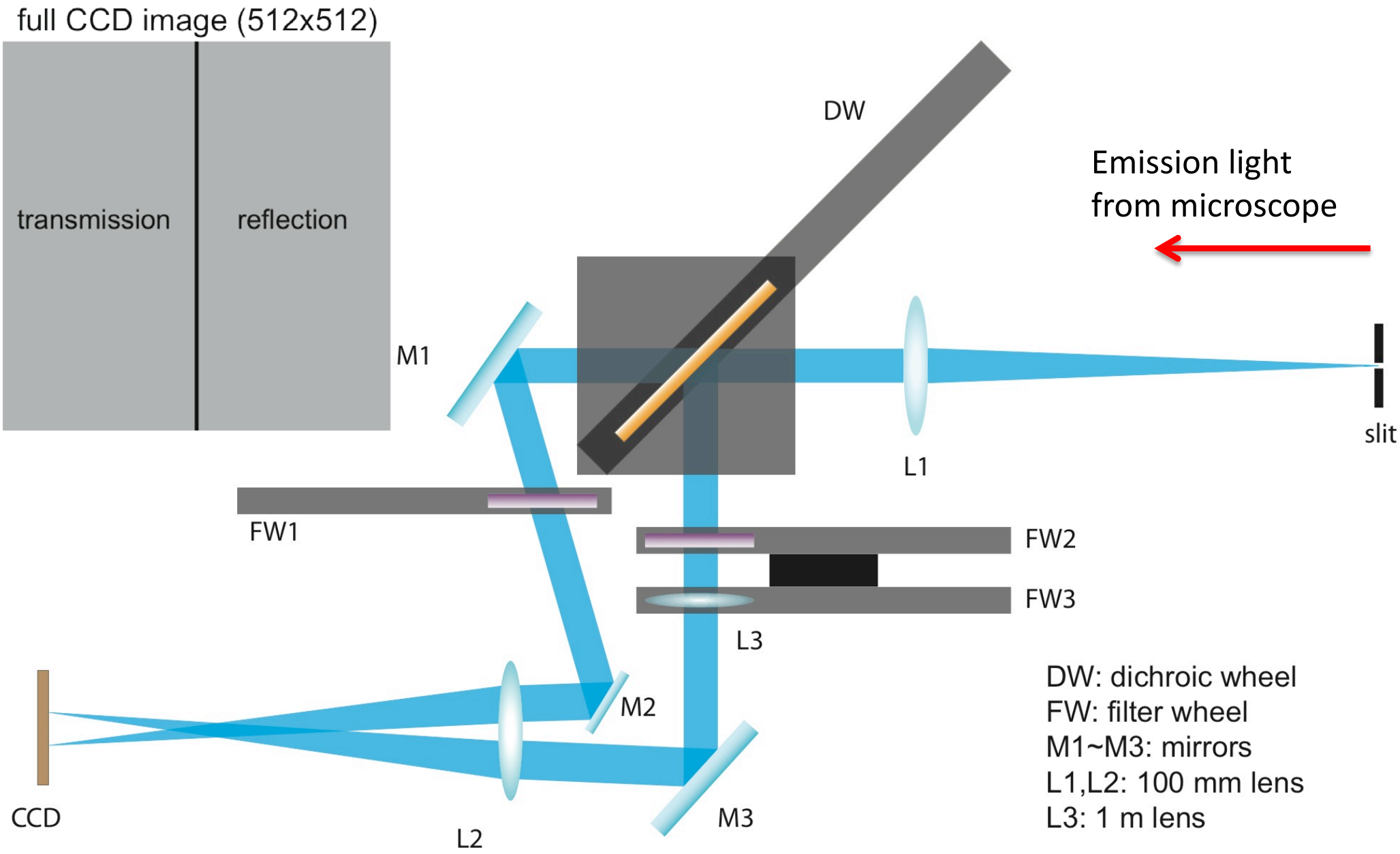


Model

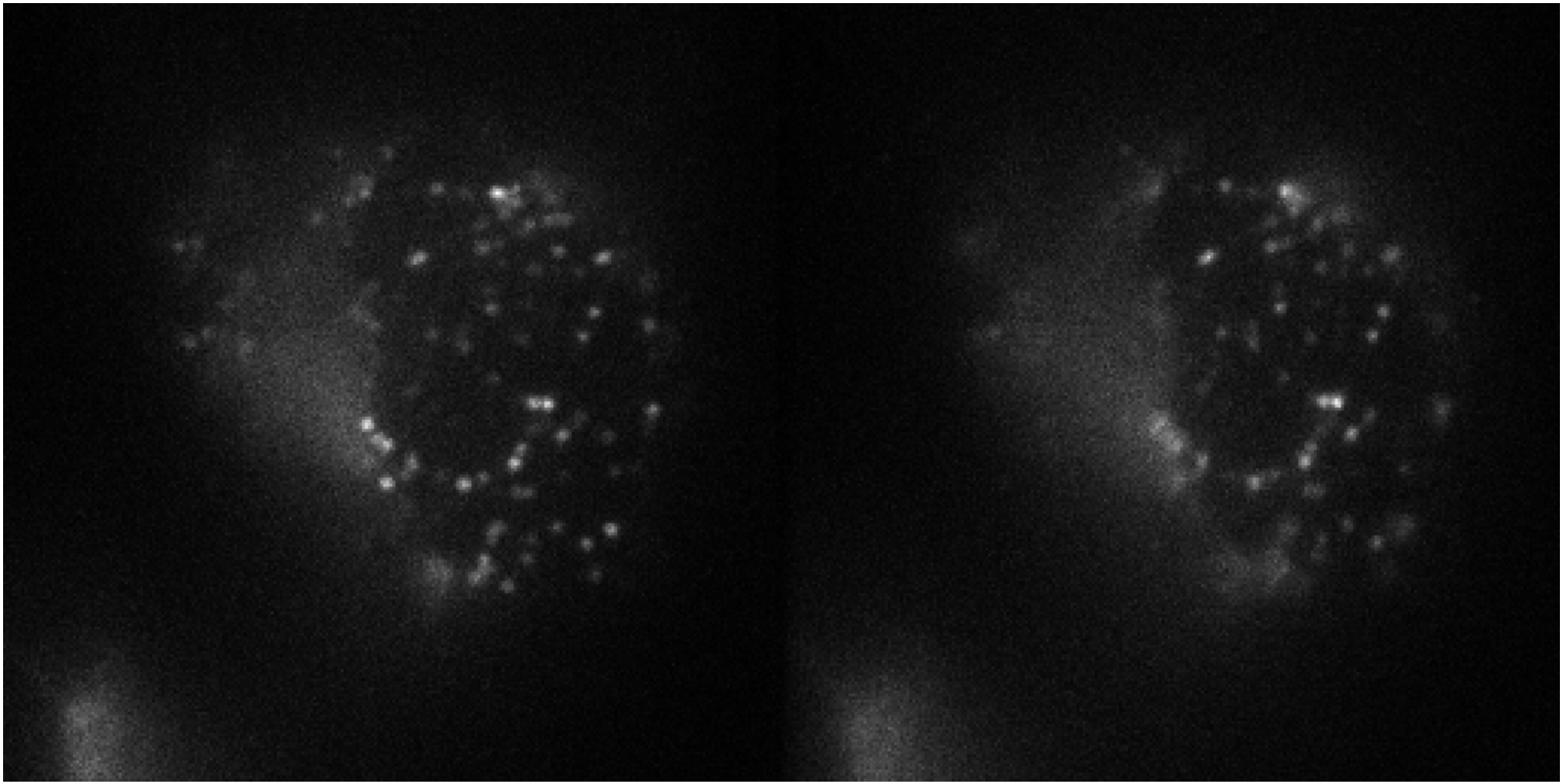




Dual focal plane setup

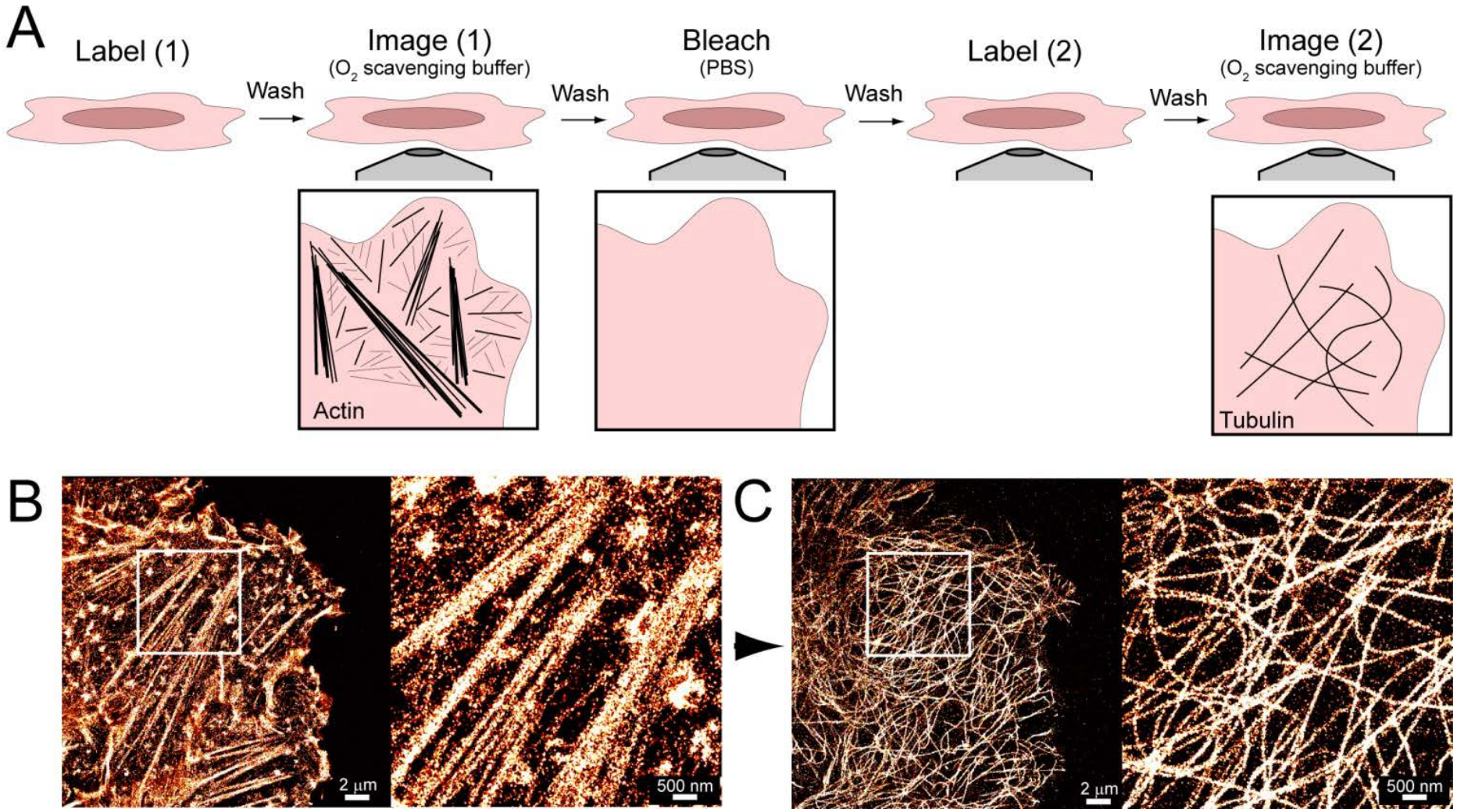


Dual Focal Plane 3D SR Data

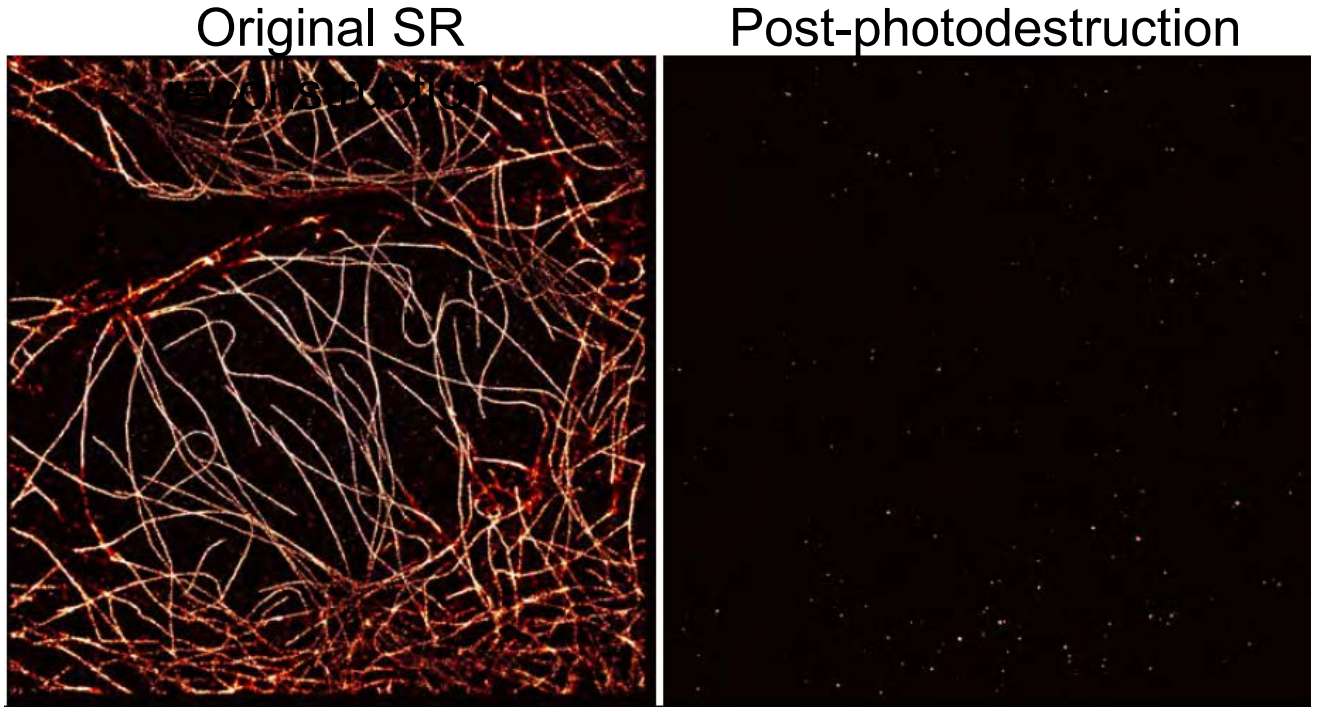


3D Super-resolution using Dual Focal Planes

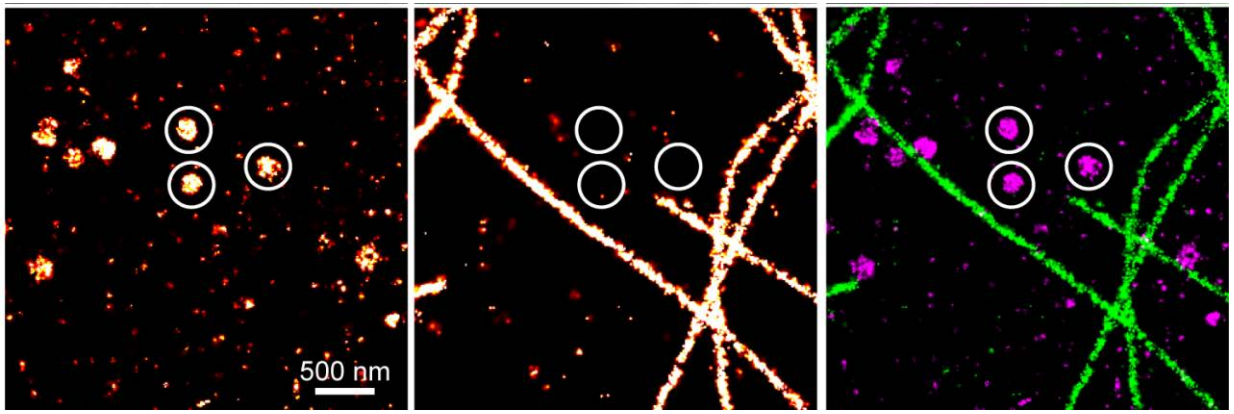
Can we image multiple targets with a single fluorophore?



Crosstalk – NaBH4 and Photo-bleaching

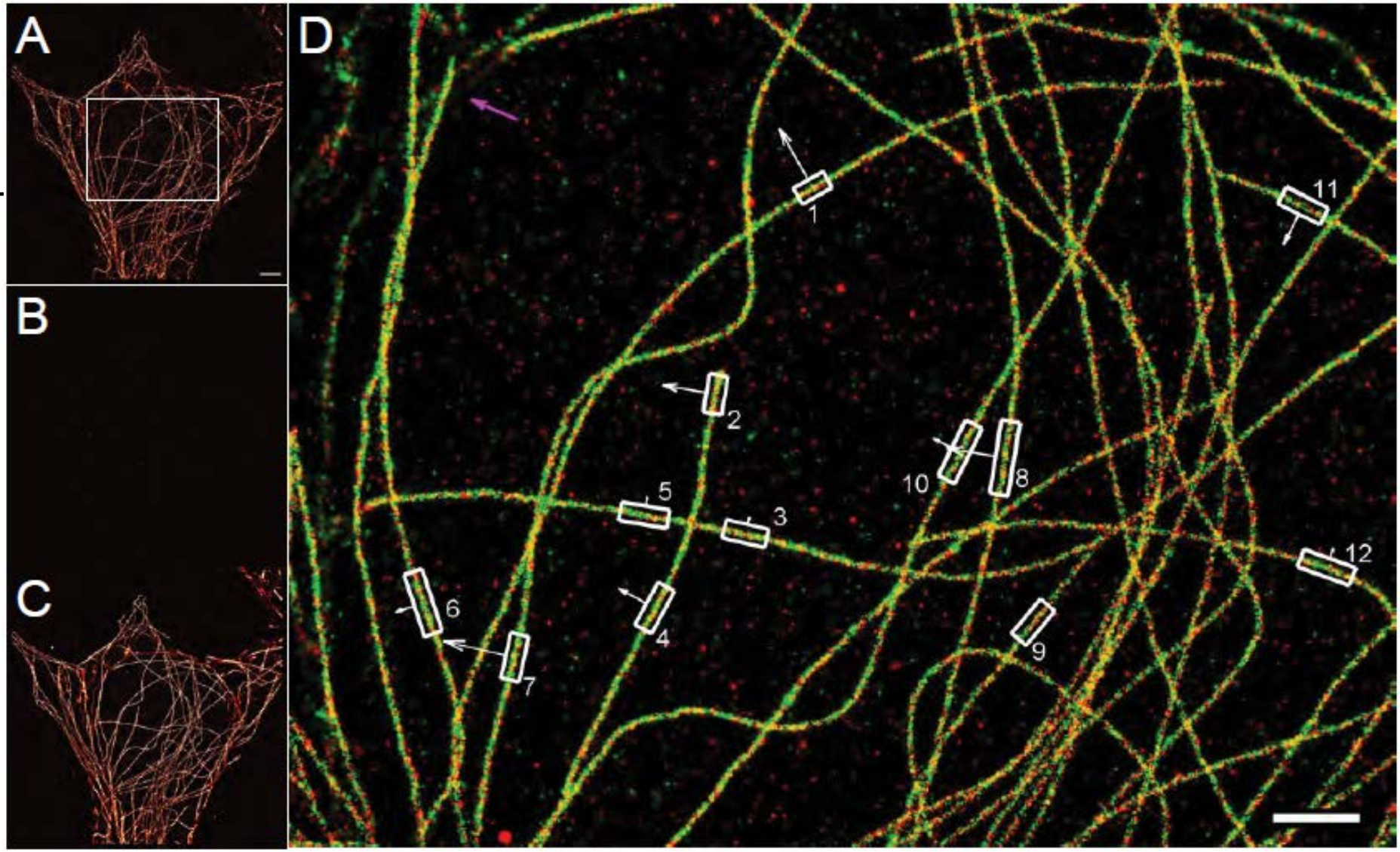


No significant cross-talk

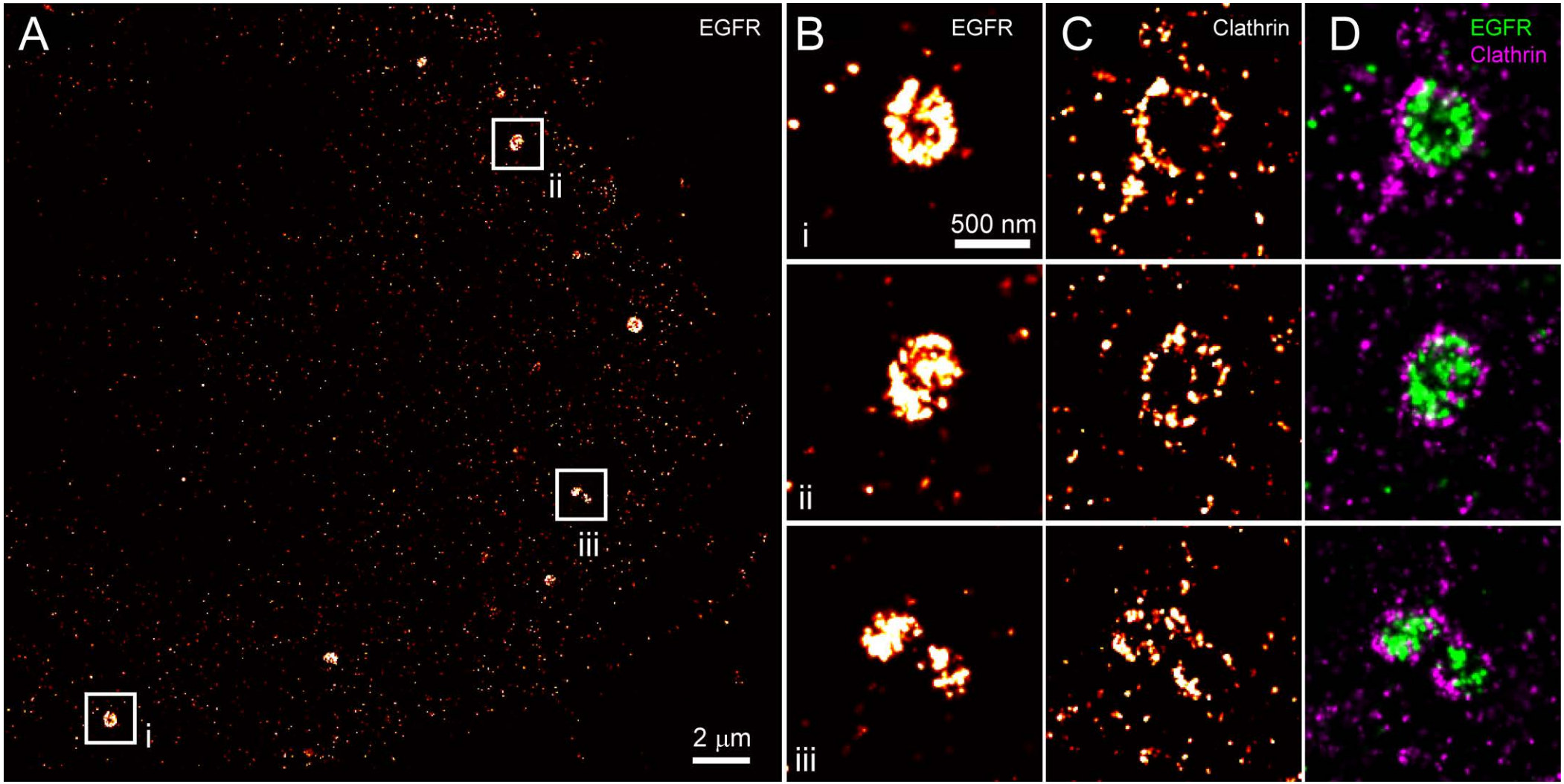


Brightfield registration and alignment

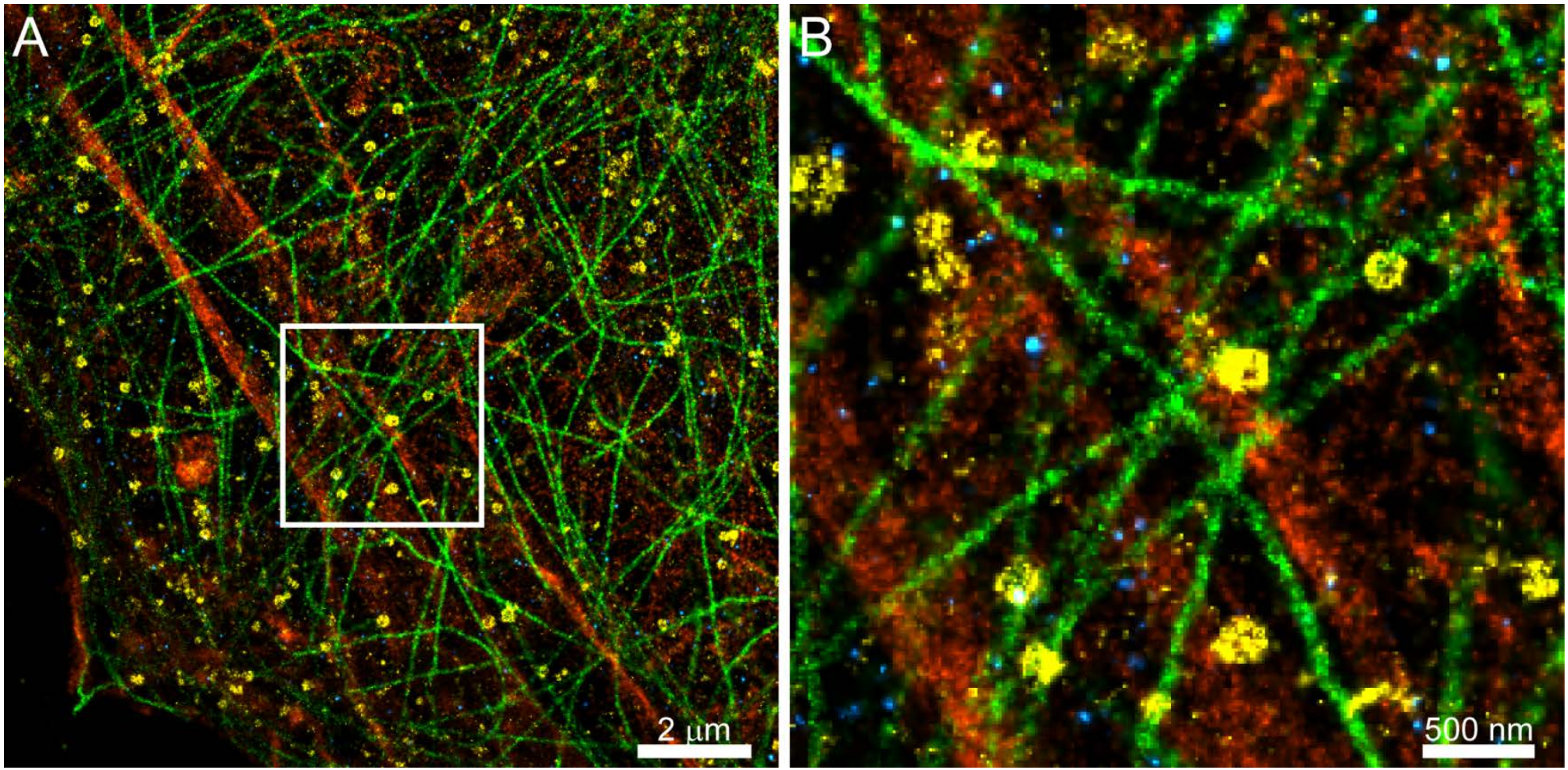
Sequential imaging of β-tubulin and α-tubulin, overlay using BF alignment



Localization of EGFR within clathrin-coated vesicles



Four color super-resolution imaging with a single fluorophore, and negligible crosstalk



Valley CC, Liu S, Lidke DS, Lidke KA (2015) Sequential Superresolution Imaging of Multiple Targets Using a Single Fluorophore. PLoS ONE