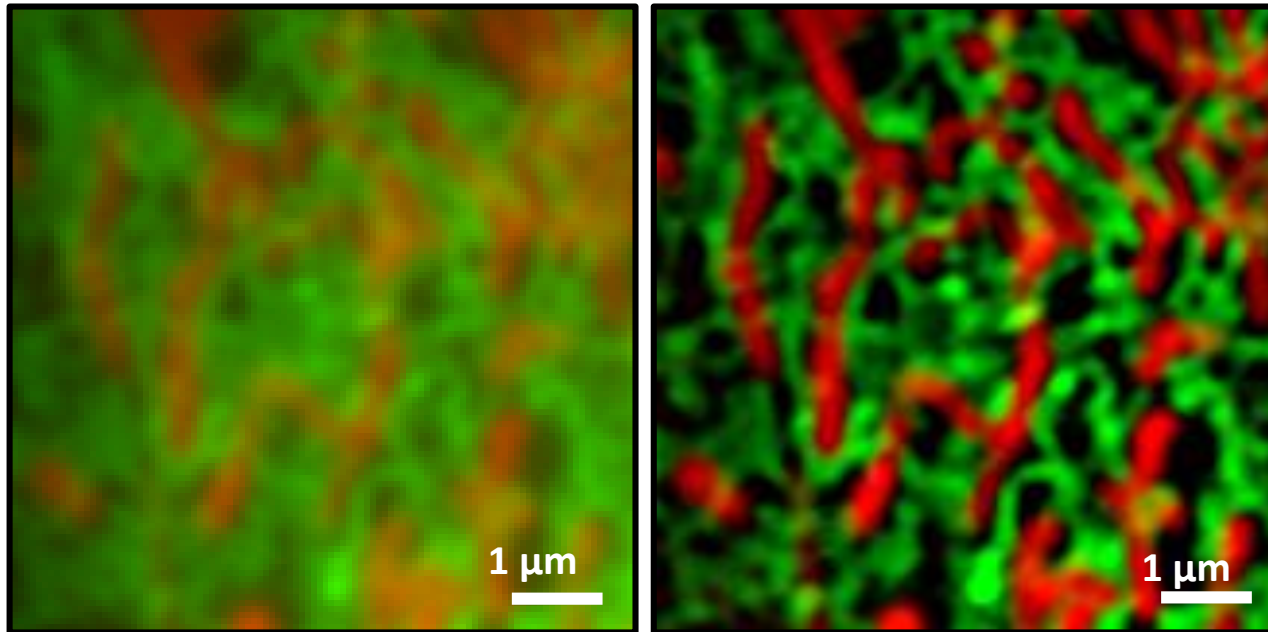


Super-resolution fluorescence imaging

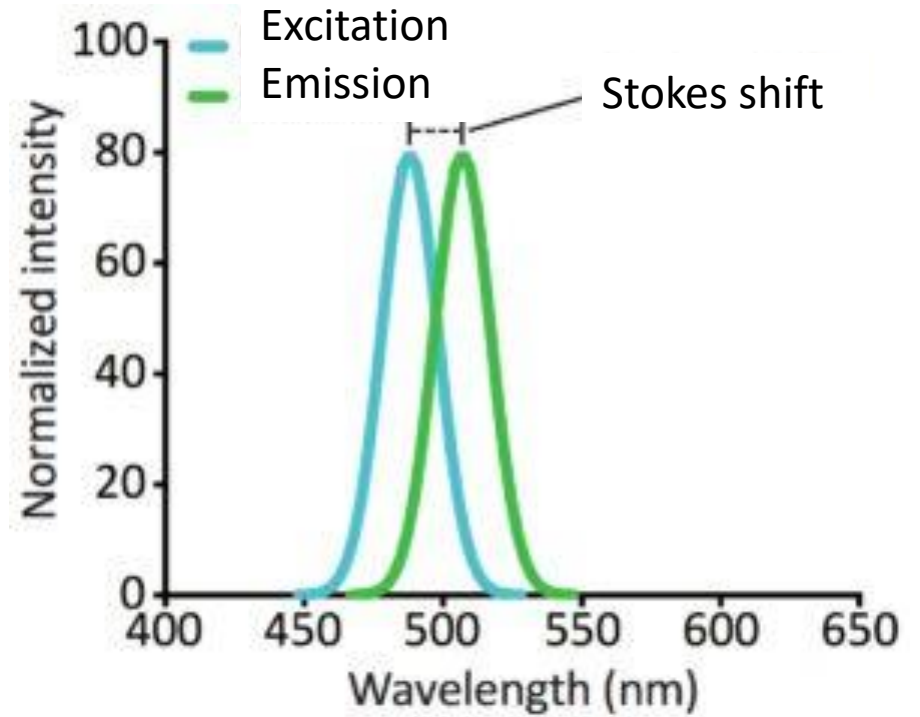
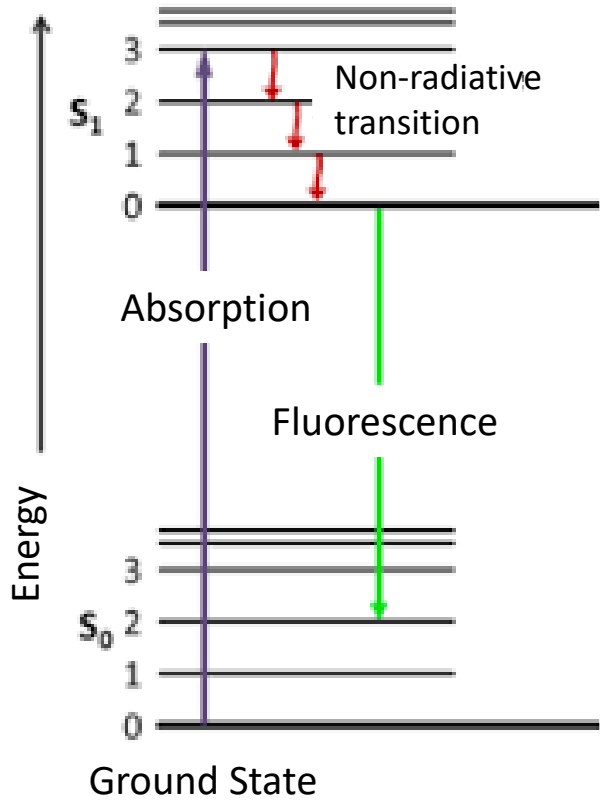


Maia Brunstein

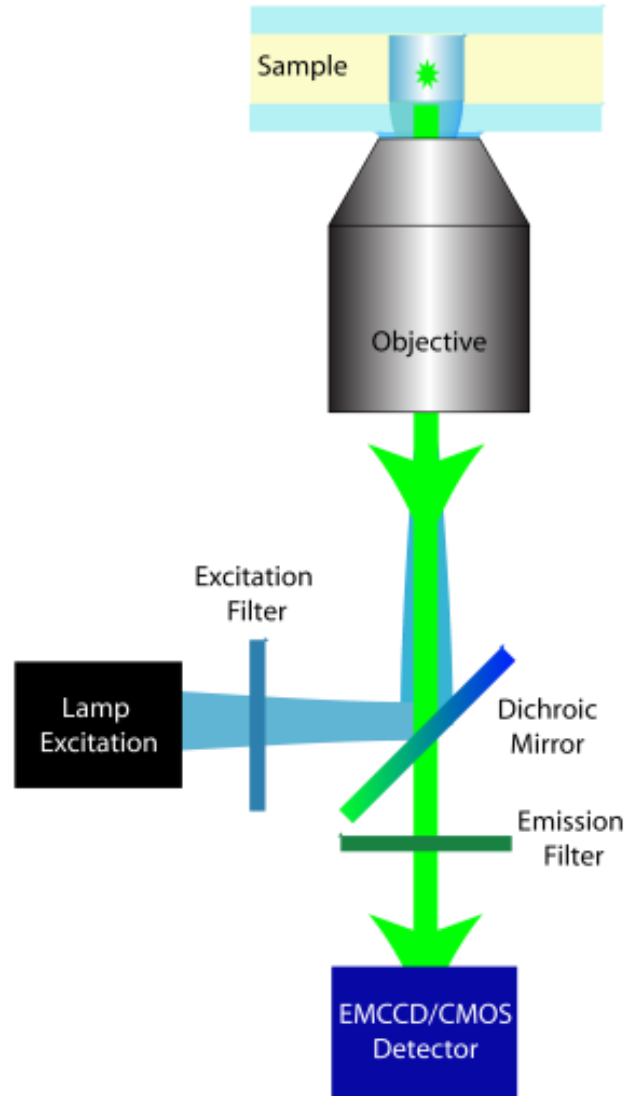
maia.brunstein@pasteur.fr



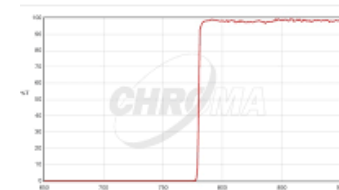
Fluorescence



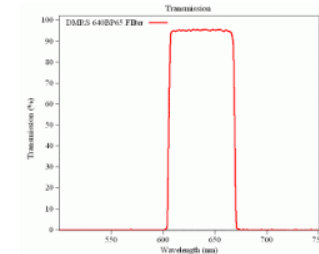
Epifluorescence microscopy



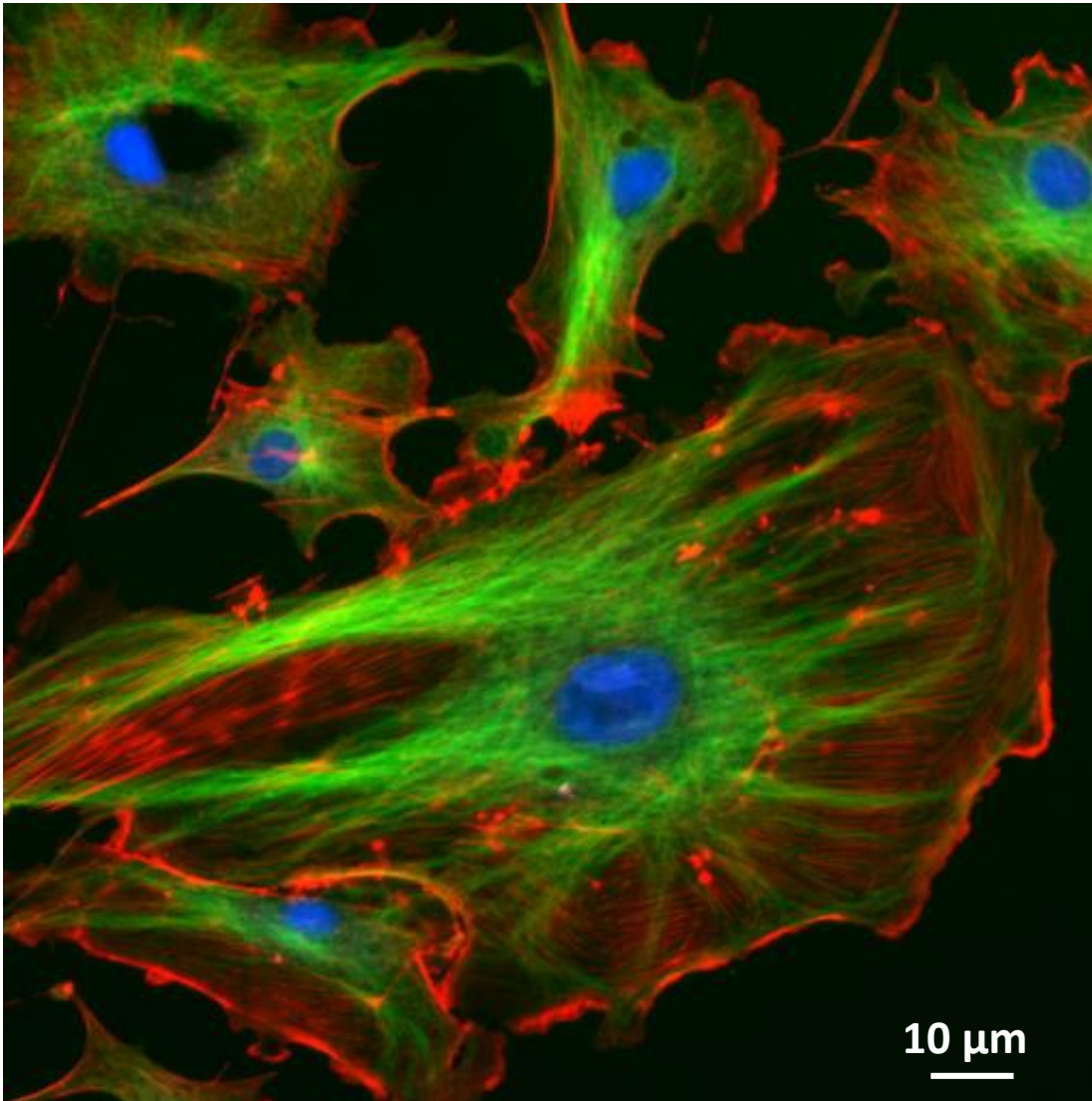
Longpass filter



Bandpass filter

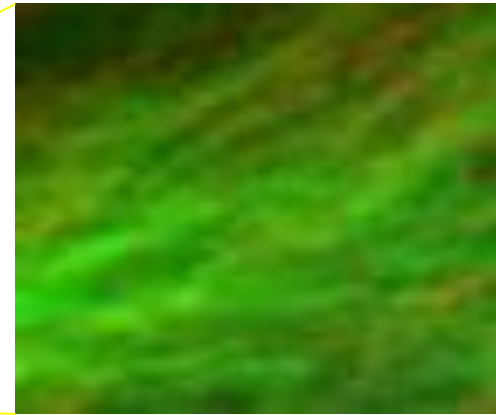
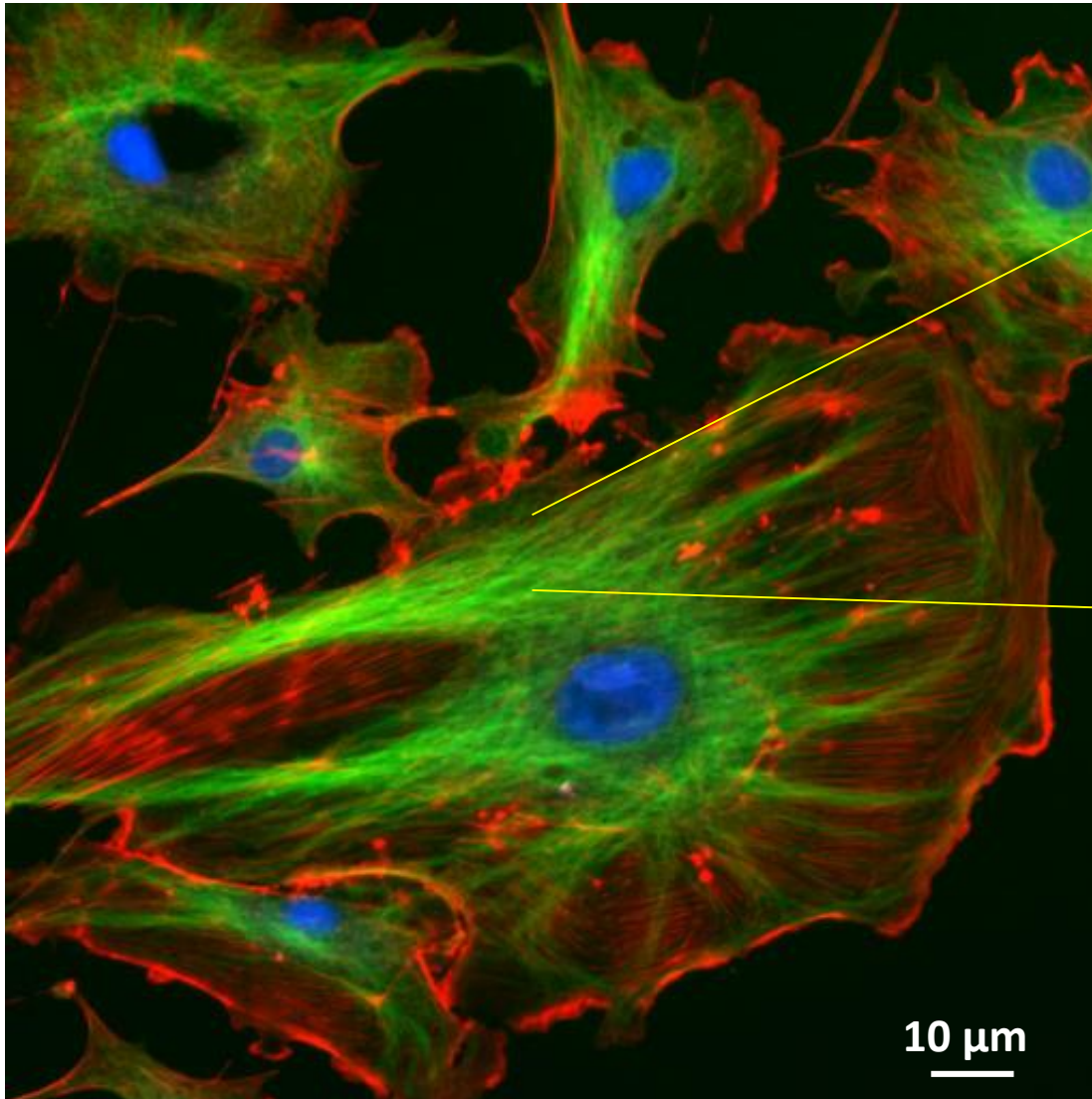


Epifluorescence microscopy



- Specific labelling
- High speed
- High field of view
- Less invasive

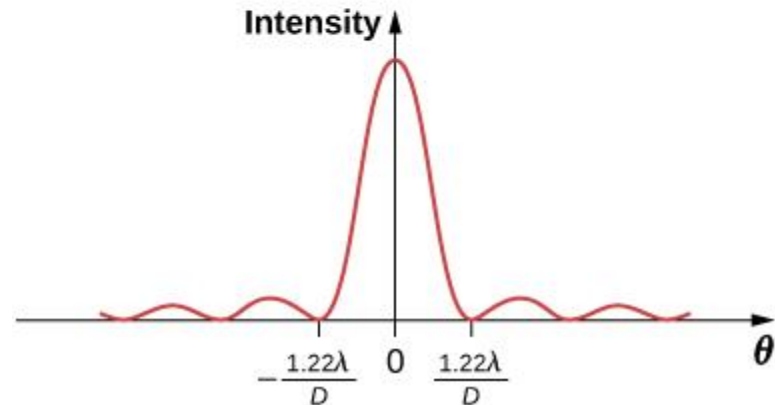
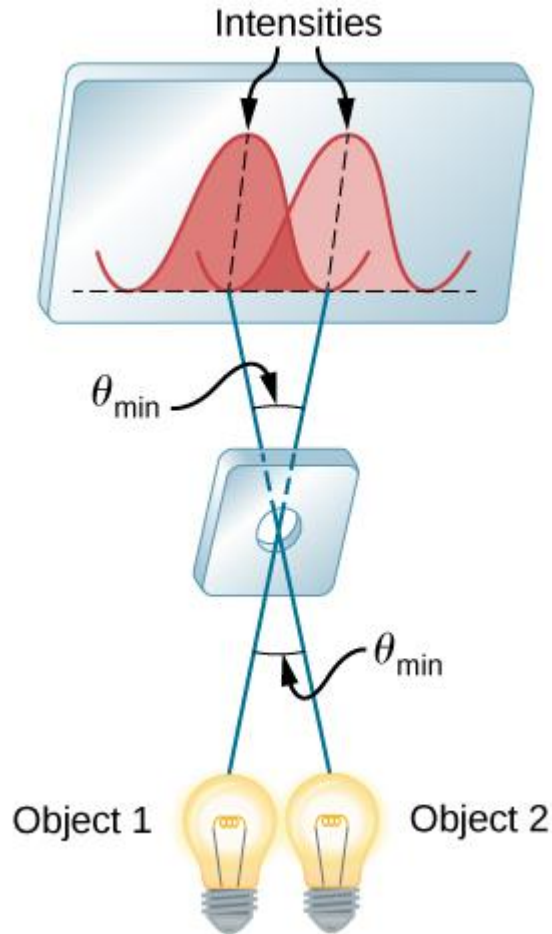
Epifluorescence microscopy



Spatial resolution limited by diffraction

The spatial resolution in an image is limited by diffraction

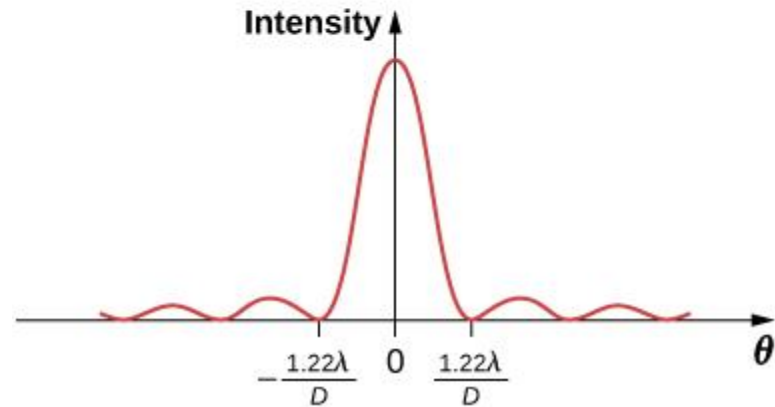
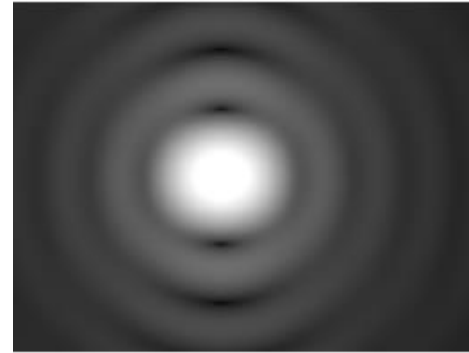
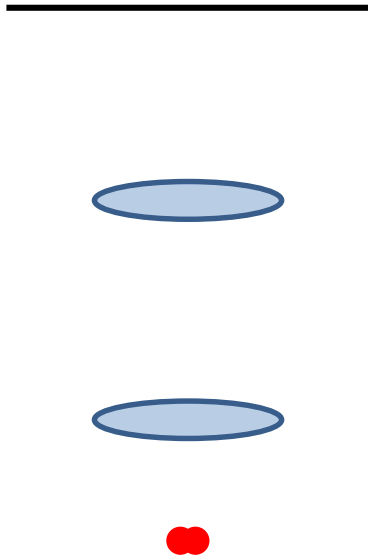
Diffraction through a circular aperture



1st minimum of Bessel function

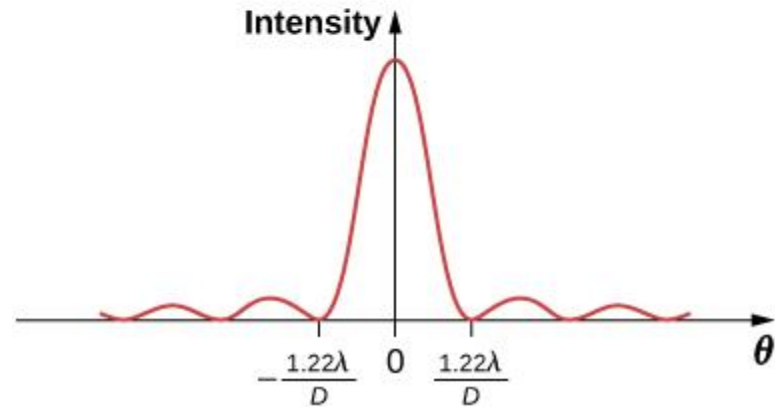
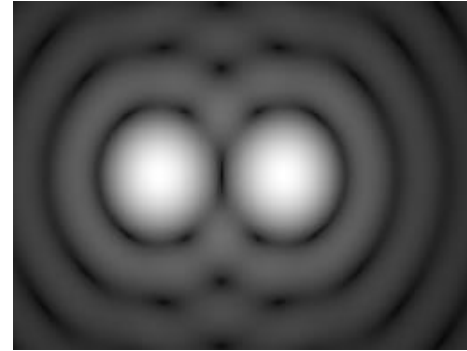
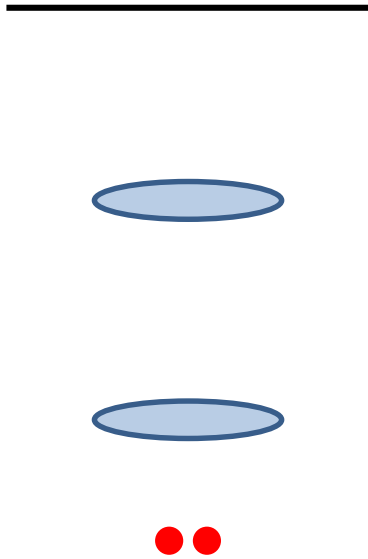
The spatial resolution in an image is limited by diffraction

Diffraction through a circular aperture



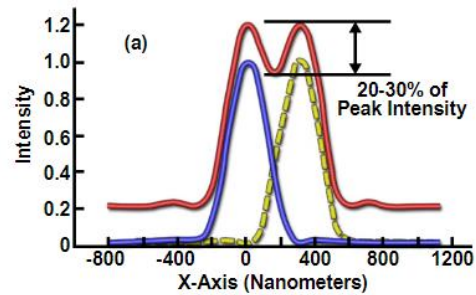
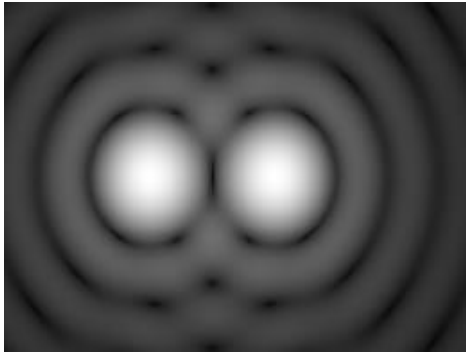
The spatial resolution in an image is limited by diffraction

Diffraction through a circular aperture

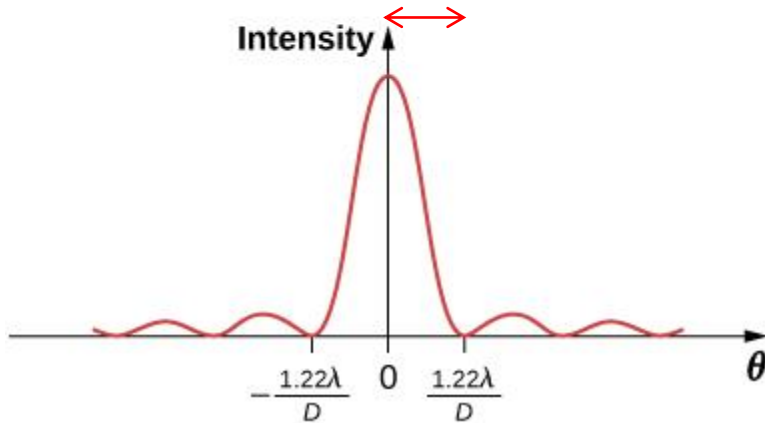


The spatial resolution in an image is limited by diffraction

Diffraction through a circular aperture

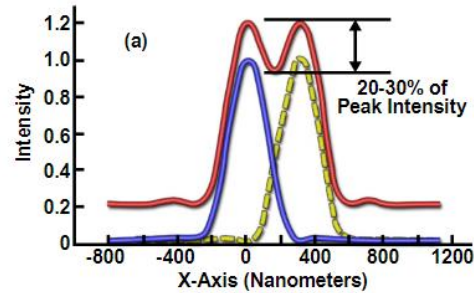
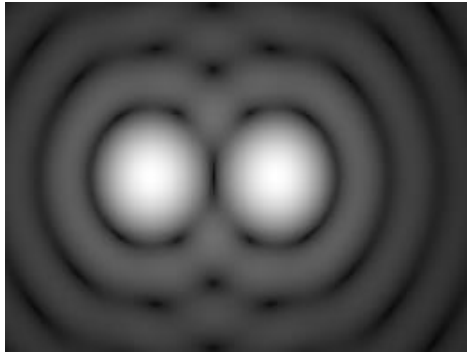


The Rayleigh criterion



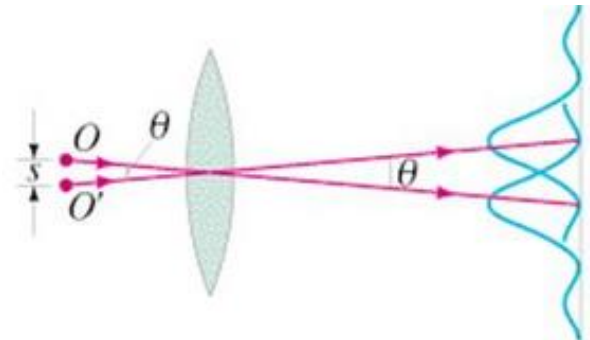
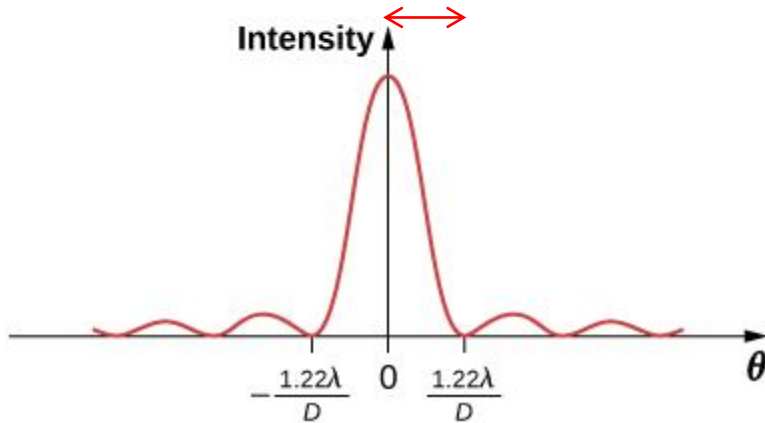
The spatial resolution in an image is limited by diffraction

Diffraction through a circular aperture



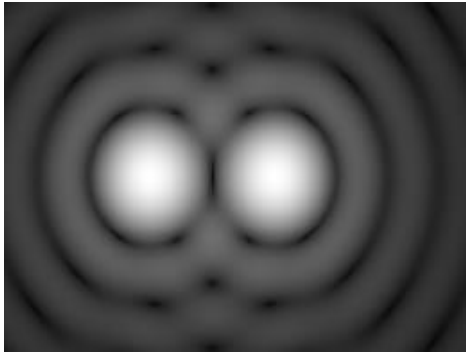
The Rayleigh criterion

$$s = \frac{0.61\lambda}{n \sin(\alpha)} = \frac{0.61\lambda}{NA}$$



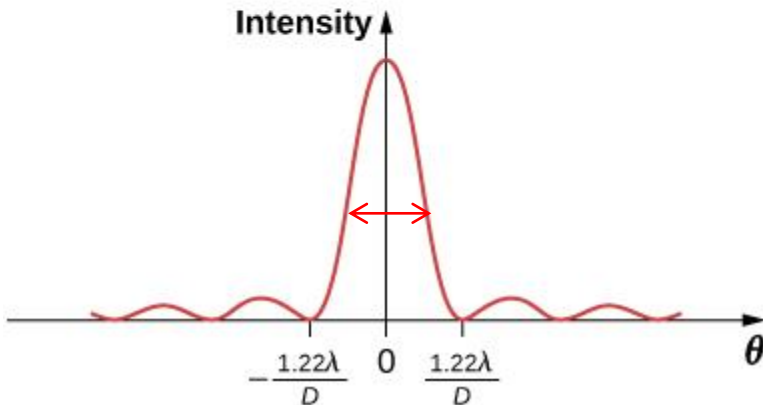
The spatial resolution in an image is limited by diffraction

Diffraction through a circular aperture

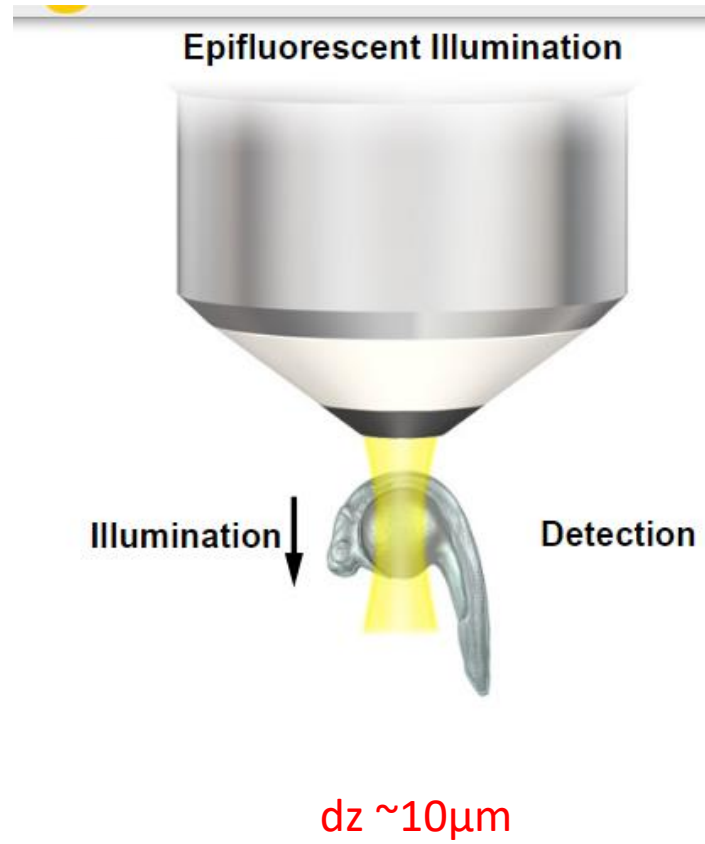


The Abbe criterion

$$dx = \frac{0.5\lambda}{n\sin(\alpha)} = \frac{\lambda}{2NA} \sim 250 \text{ nm}$$

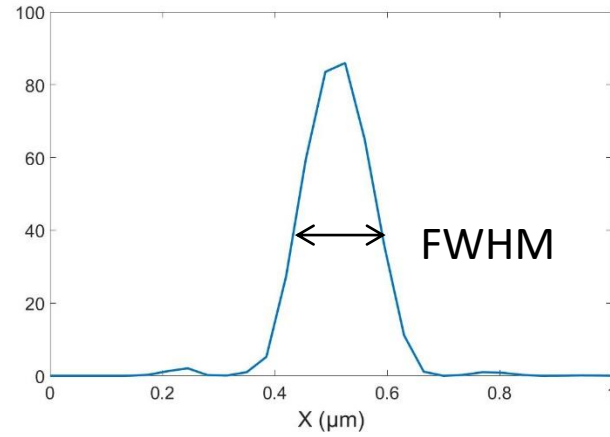
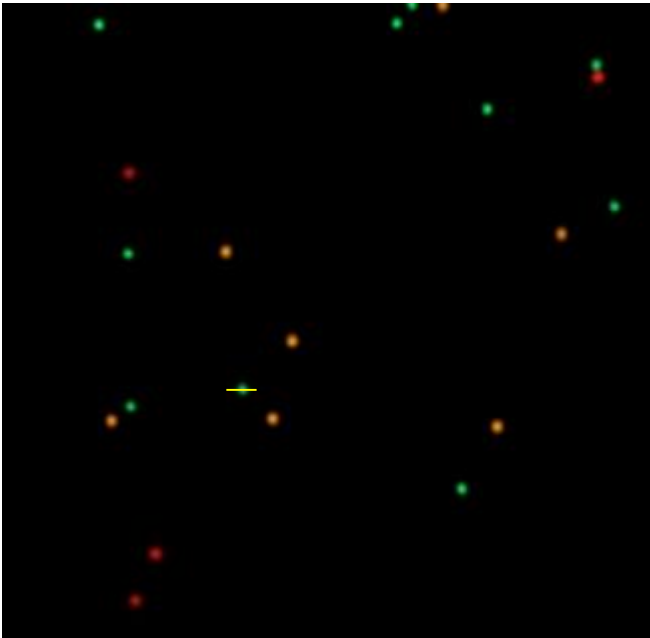


The axial resolution



Measuring the spatial resolution of an optical microscope

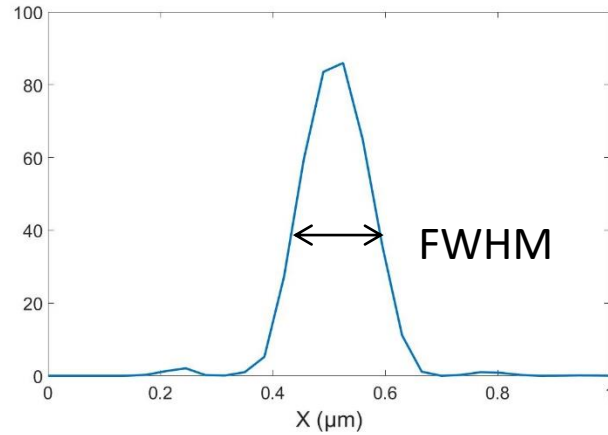
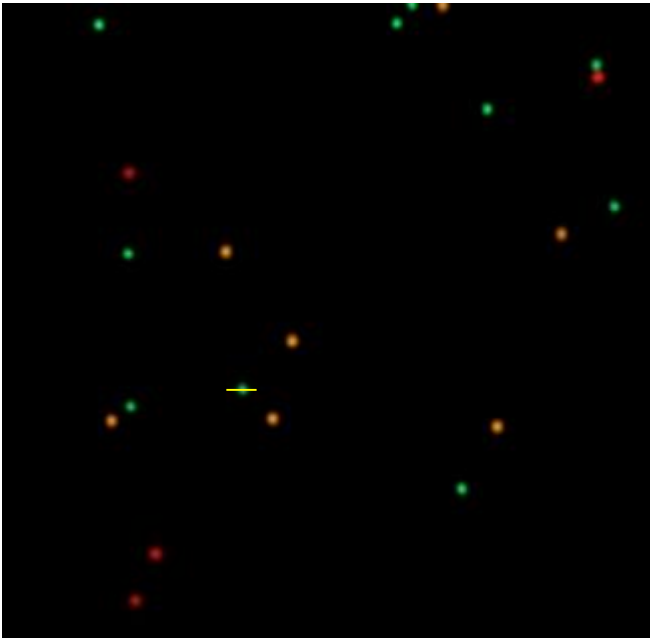
Fluorescent beads



$$FWHM = \frac{0.51\lambda}{NA} \sim \frac{\lambda}{2NA}$$

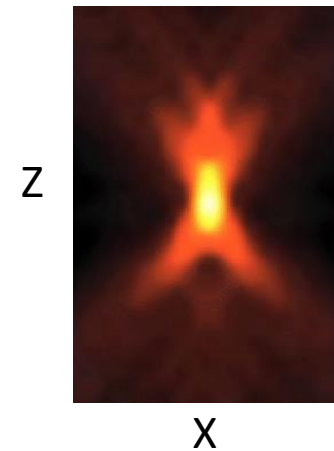
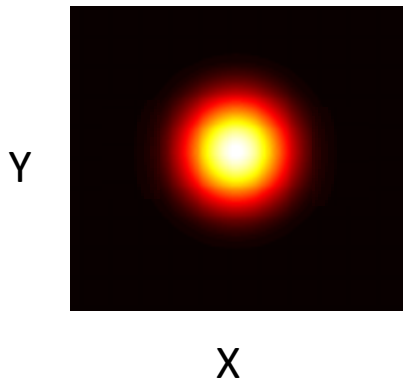
Measuring the spatial resolution of an optical microscope

Fluorescent beads



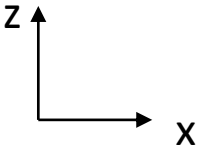
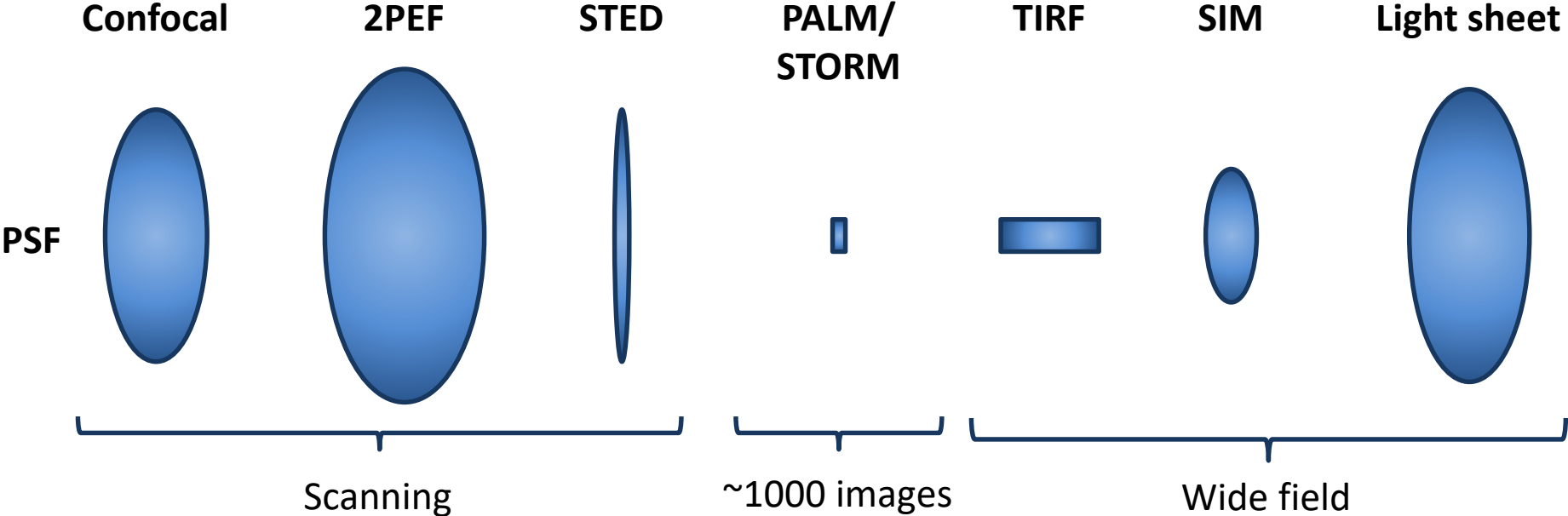
$$FWHM = \frac{0.51\lambda}{NA} \sim \frac{\lambda}{2NA}$$

Point Spread Function (PSF): image of a point source



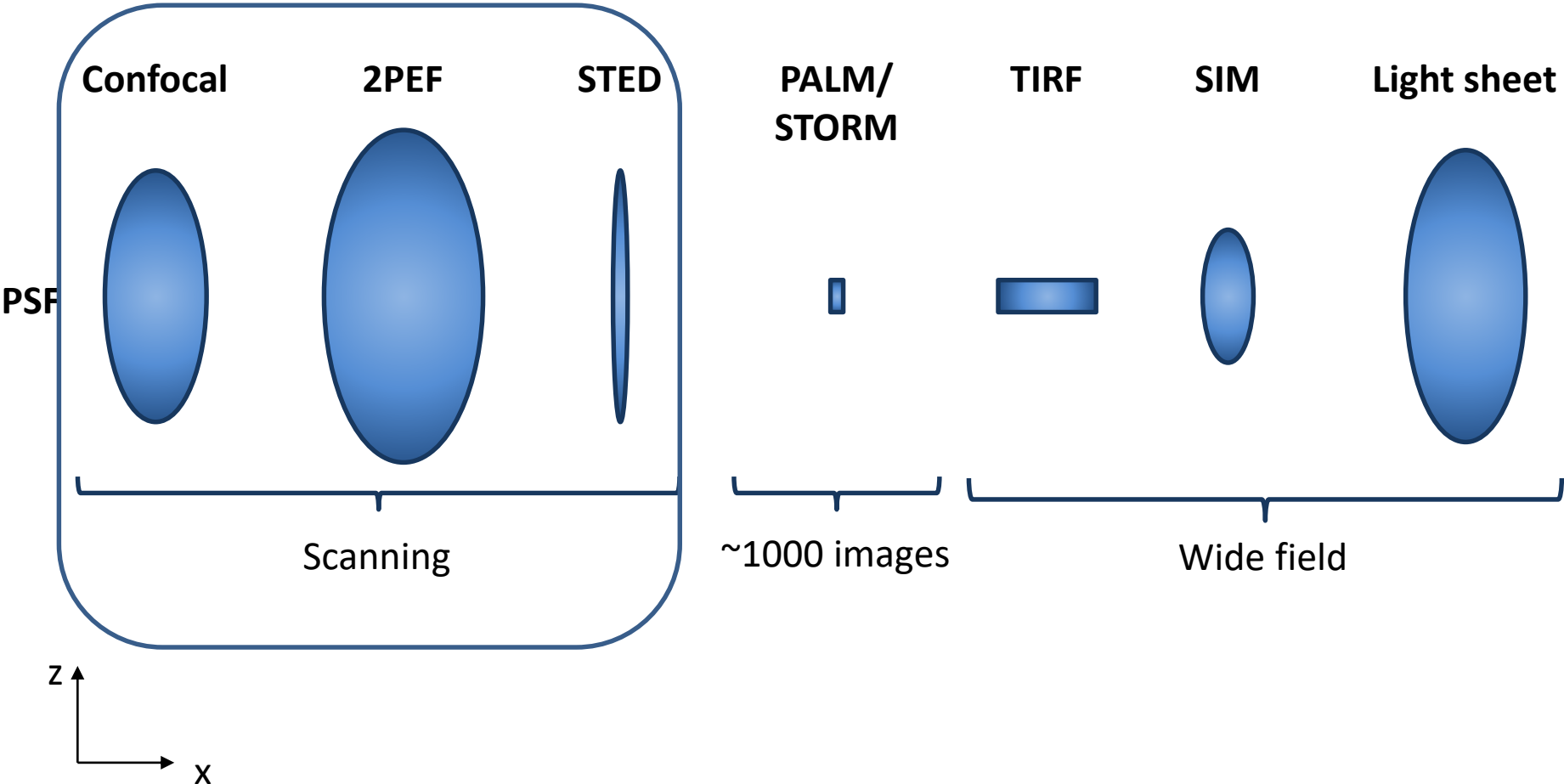
Increasing the spatial resolution of a fluorescence microscope

PSF=Point Spread Function (image of a point source)



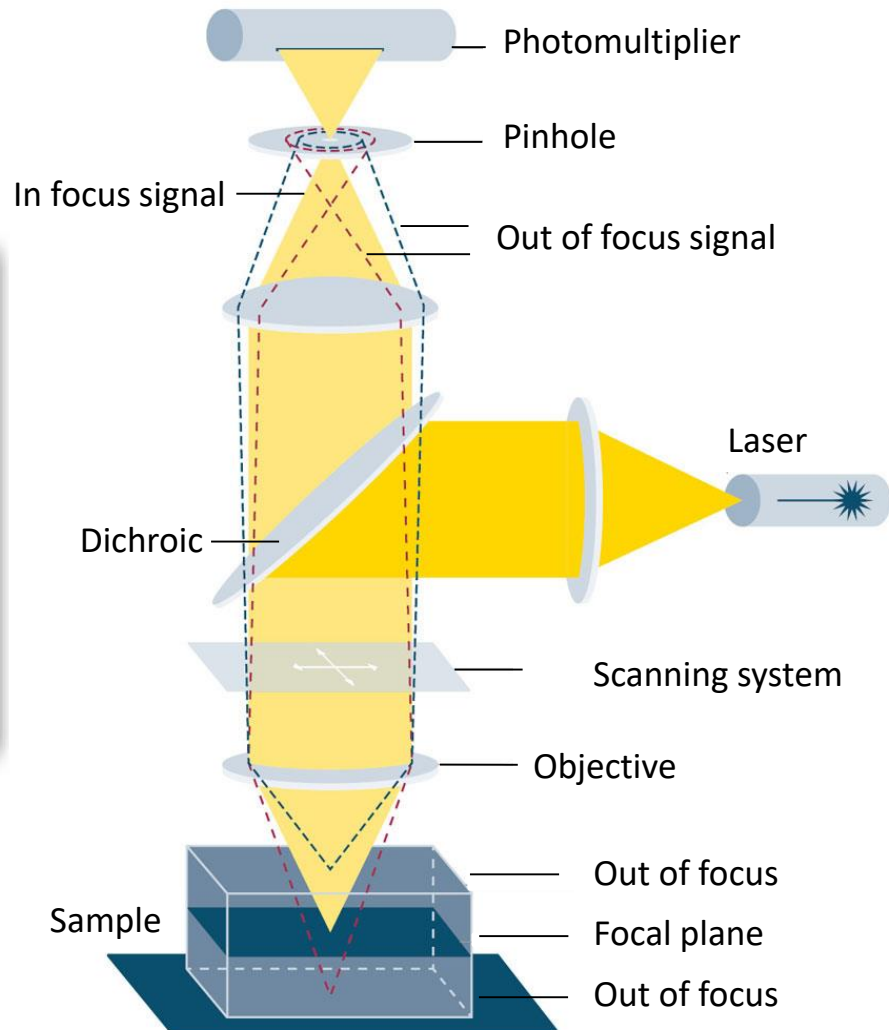
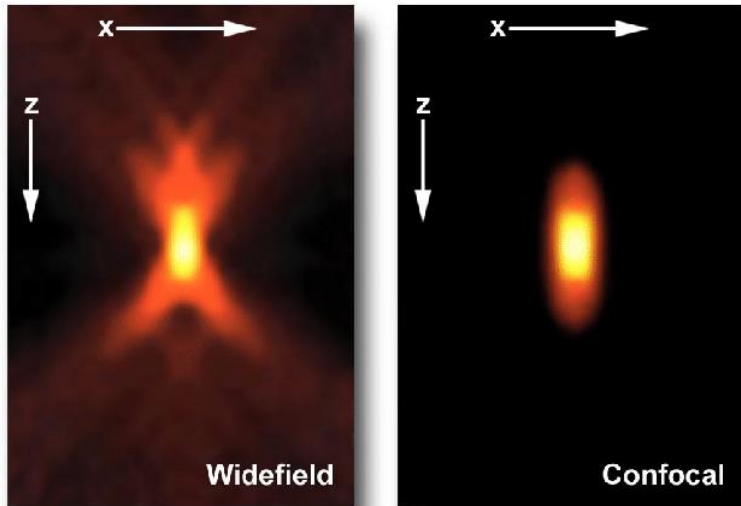
Increasing the spatial resolution of a fluorescence microscope

PSF=Point Spread Function (image of a point source)

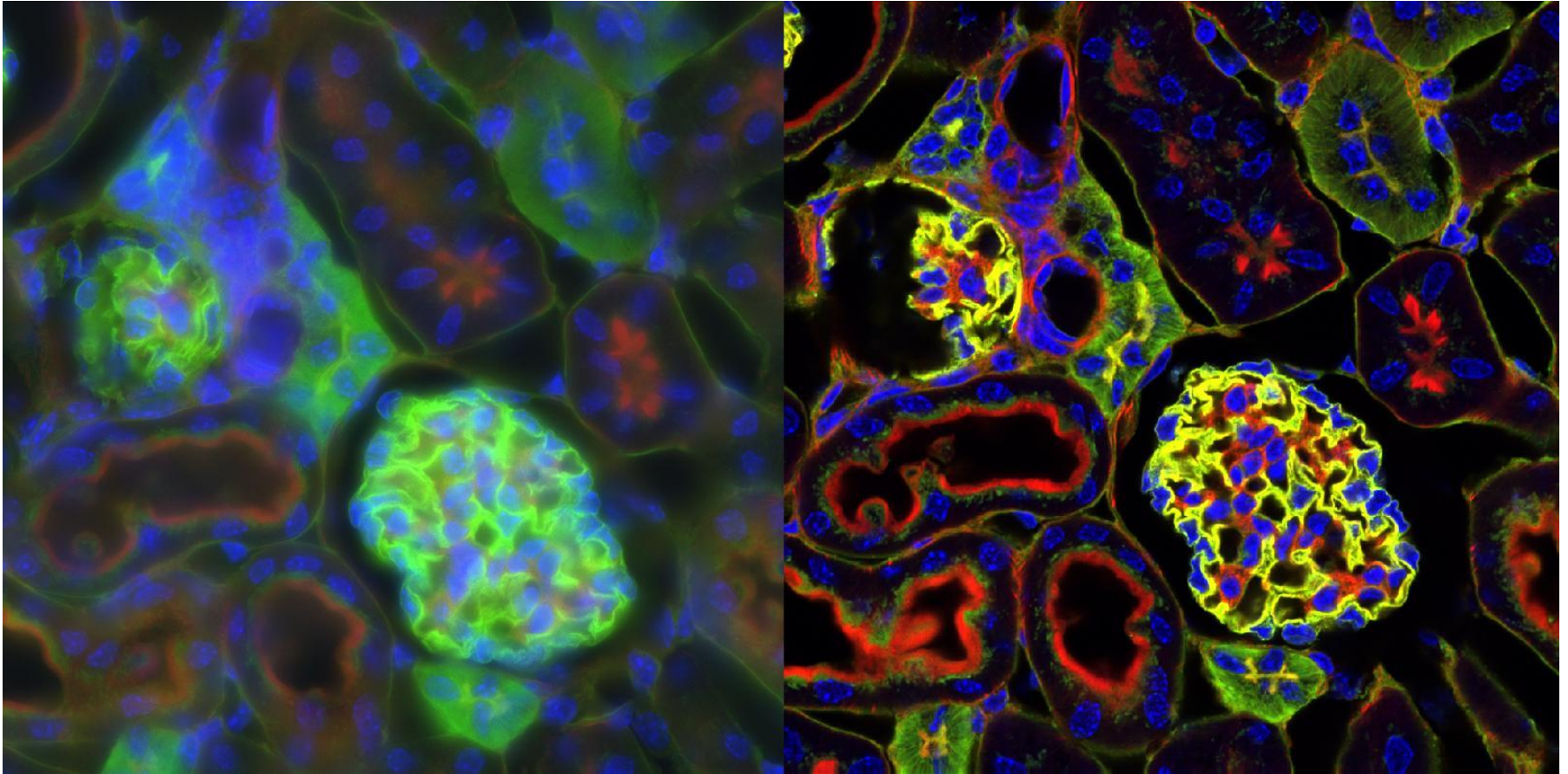


Confocal Microscope

PSF



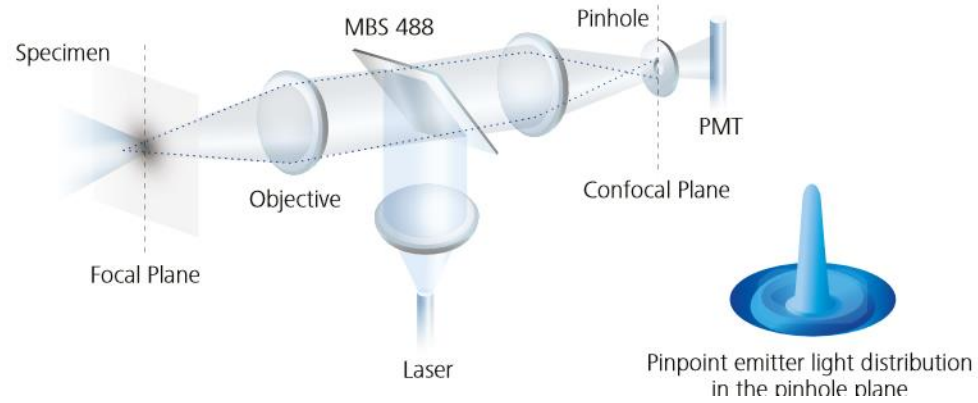
Confocal Microscope



wide field vs confocal images of kidney (Leica)

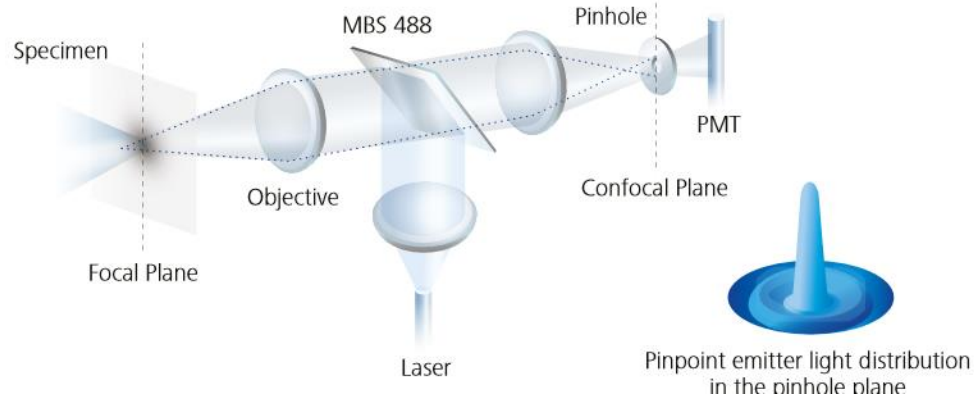
Airyscan

Confocal conventionnel

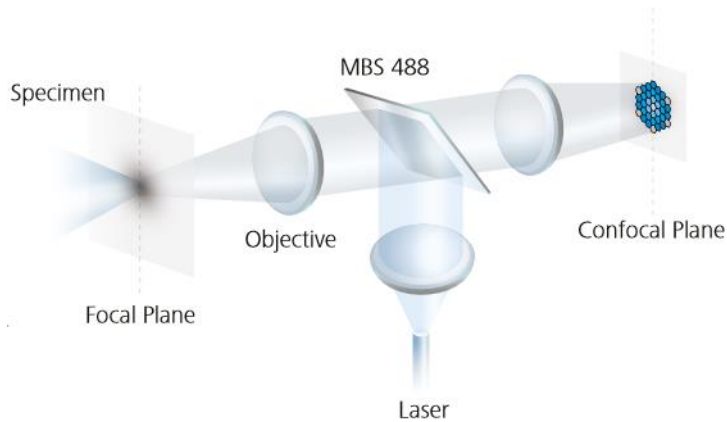


Airyscan

Confocal conventionnel



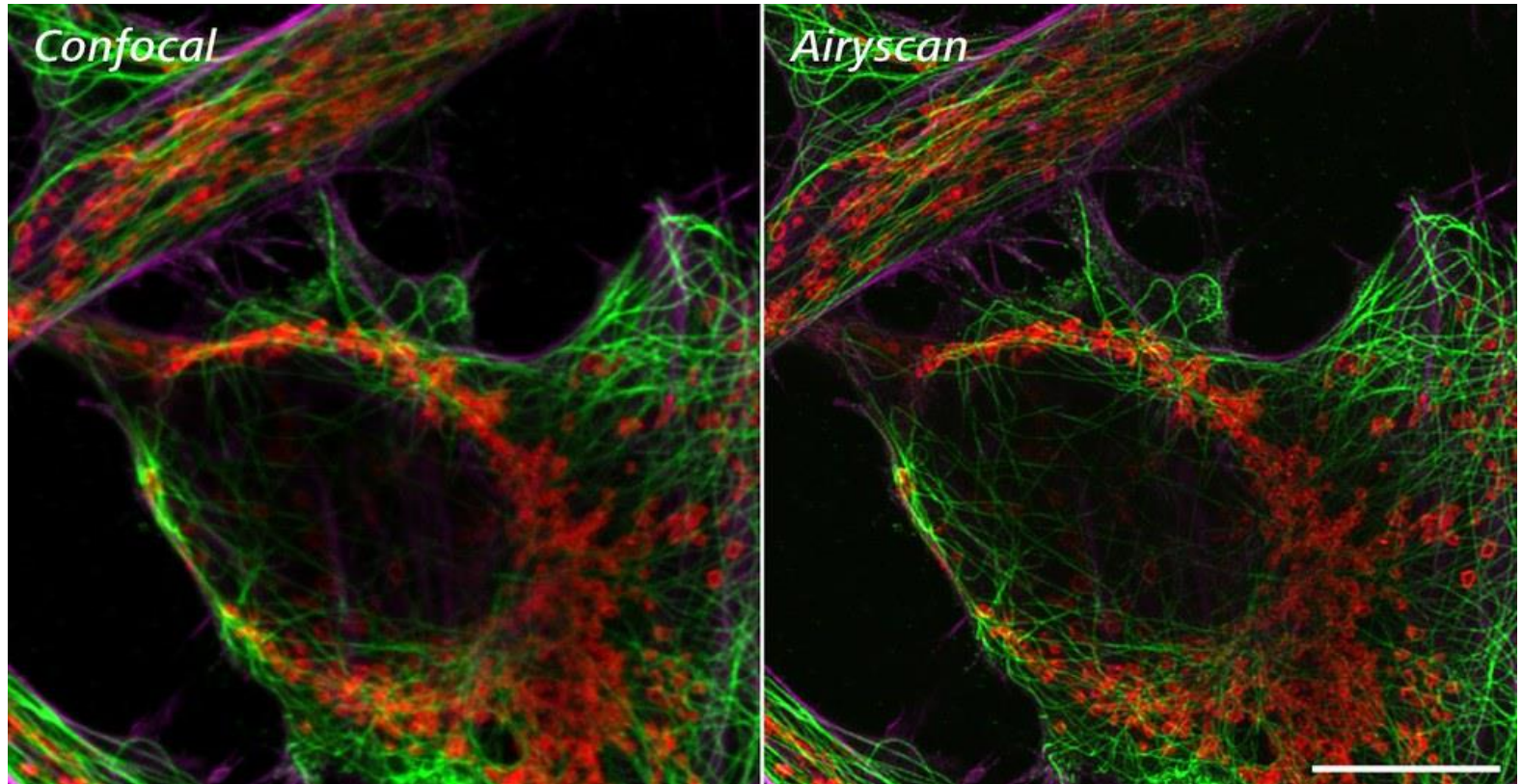
Airyscan



- **More sensibility**
- **Higher resolution**

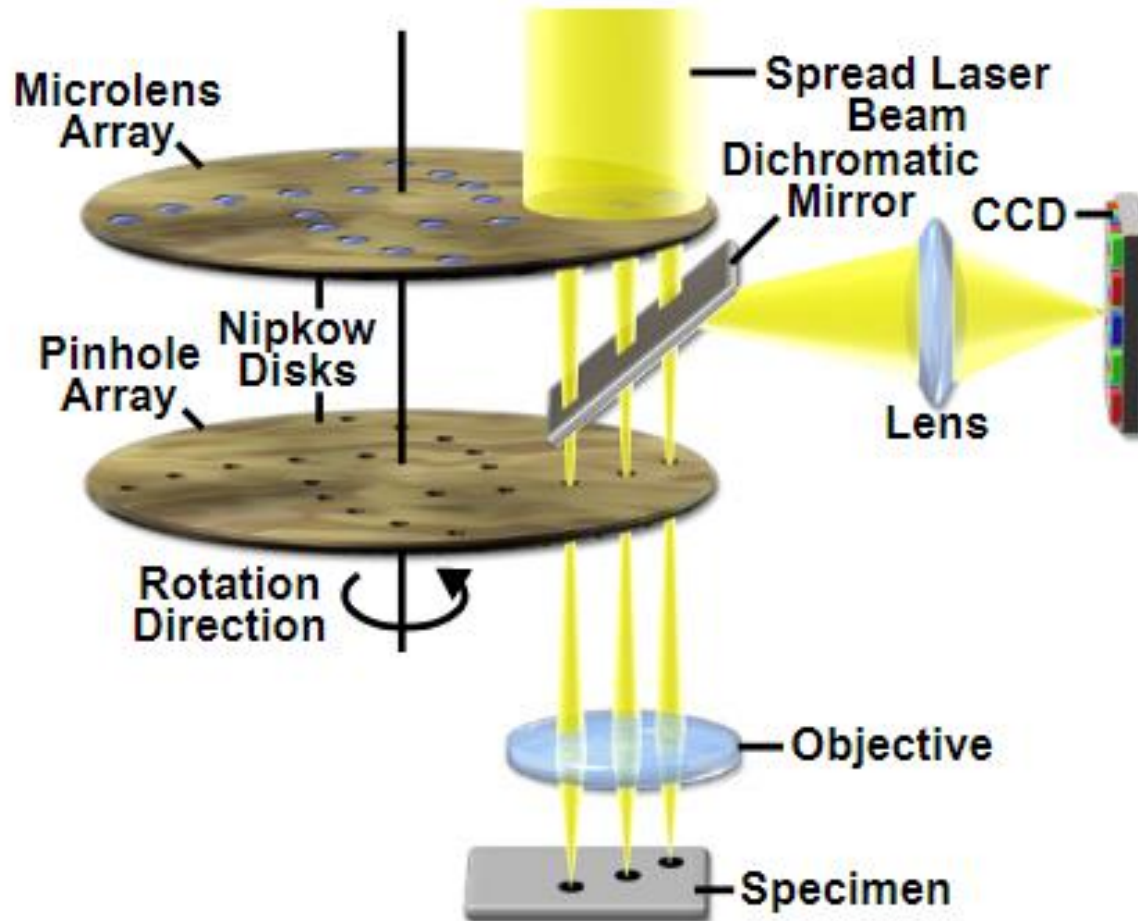
Analogy:
1 confocal with 32 pinholes close
to 0.2 Airy units

AiryScan



HeLa cells, red: mitochondria membrane, green: microtubuli, magenta: actin fibres. Scale bar 10 μm .

Confocal microscope *spinning disk*

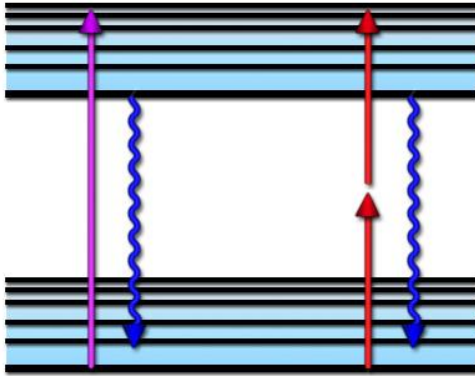


(Olympus)

Two photon microscopy

1 photon
excitation

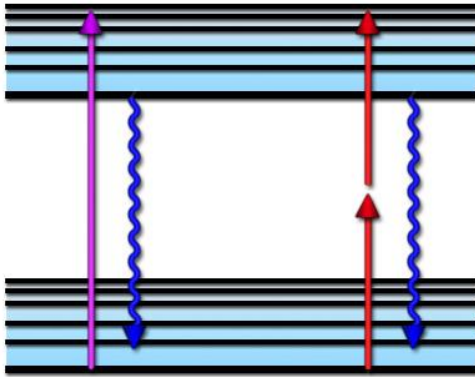
2 photon
excitation



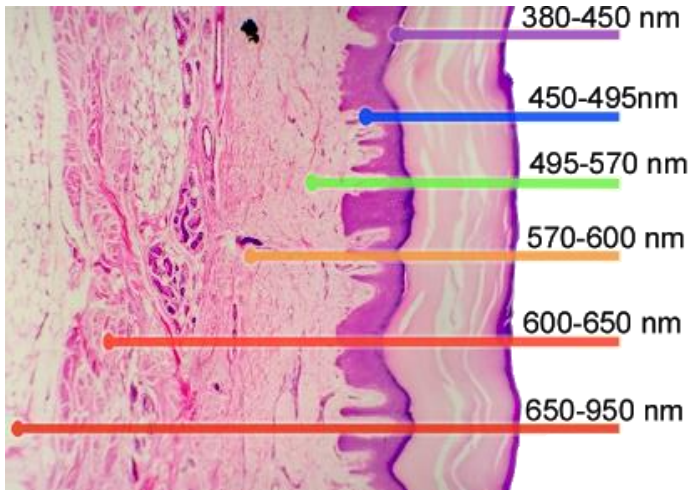
Two photon microscopy

1 photon
excitation

2 photon
excitation



Penetration depth



Two photon absorption coefficient

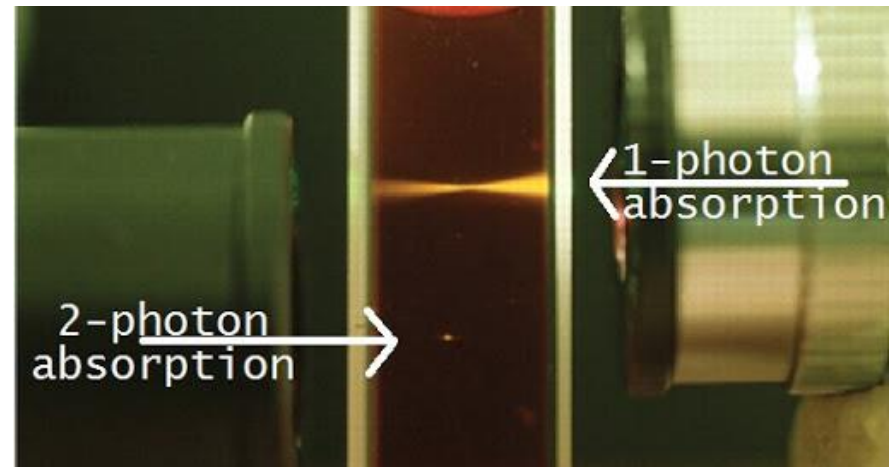
$$\eta = \frac{P_0^2 \delta}{\tau_p f_p^2} \left(\frac{NA^2}{2\hbar c \lambda} \right)^2$$

P_0 : mean power

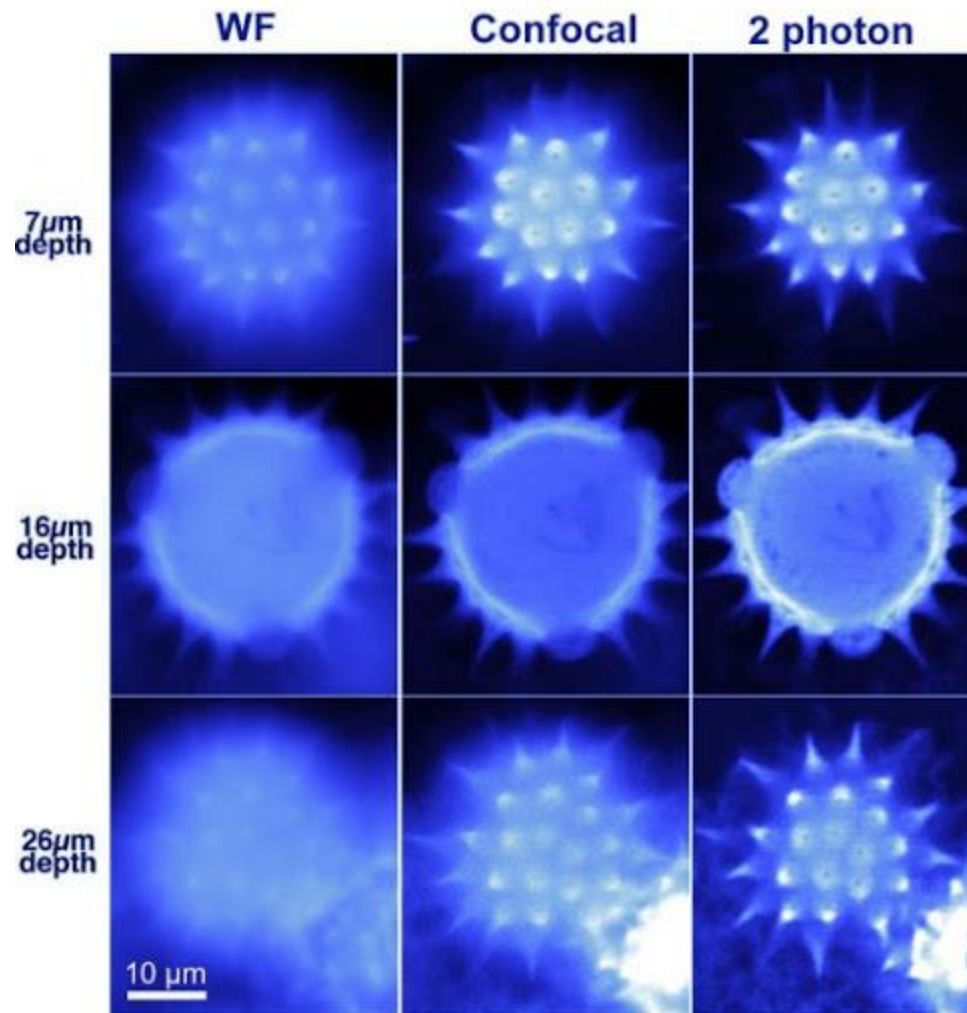
NA : numerical aperture

f_p : repetition rate

τ_p : pulse length



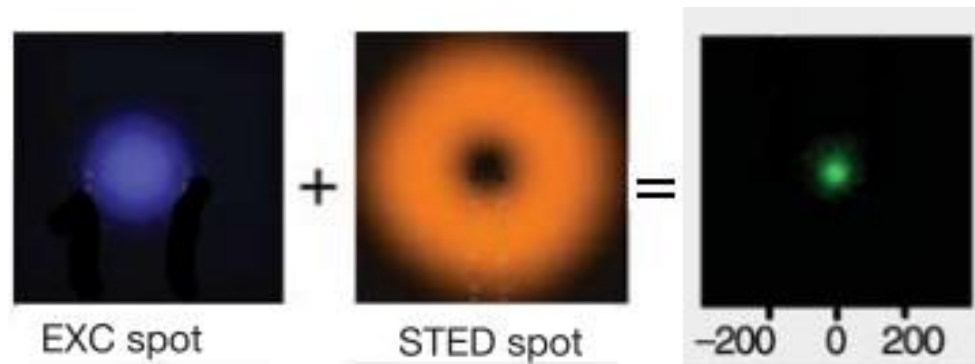
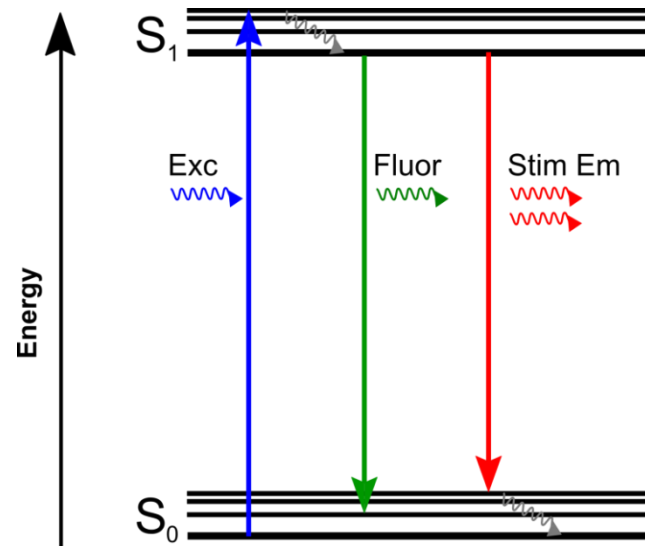
Two photon microscopy



Fluorescent pollen grain

STED microscope

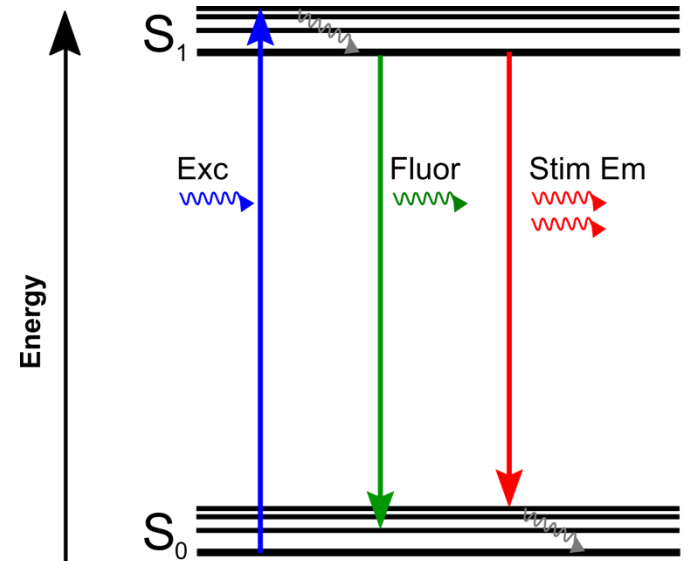
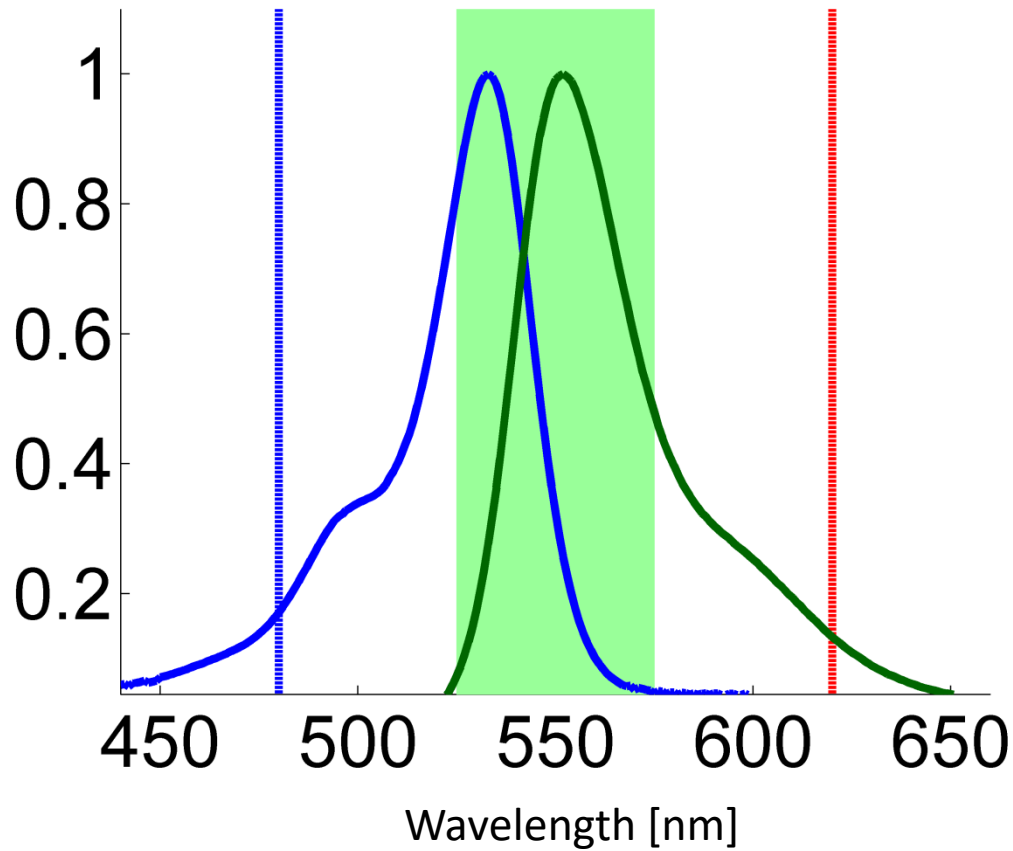
Stimulated emission depletion



STED microscope

Stimulated emission depletion

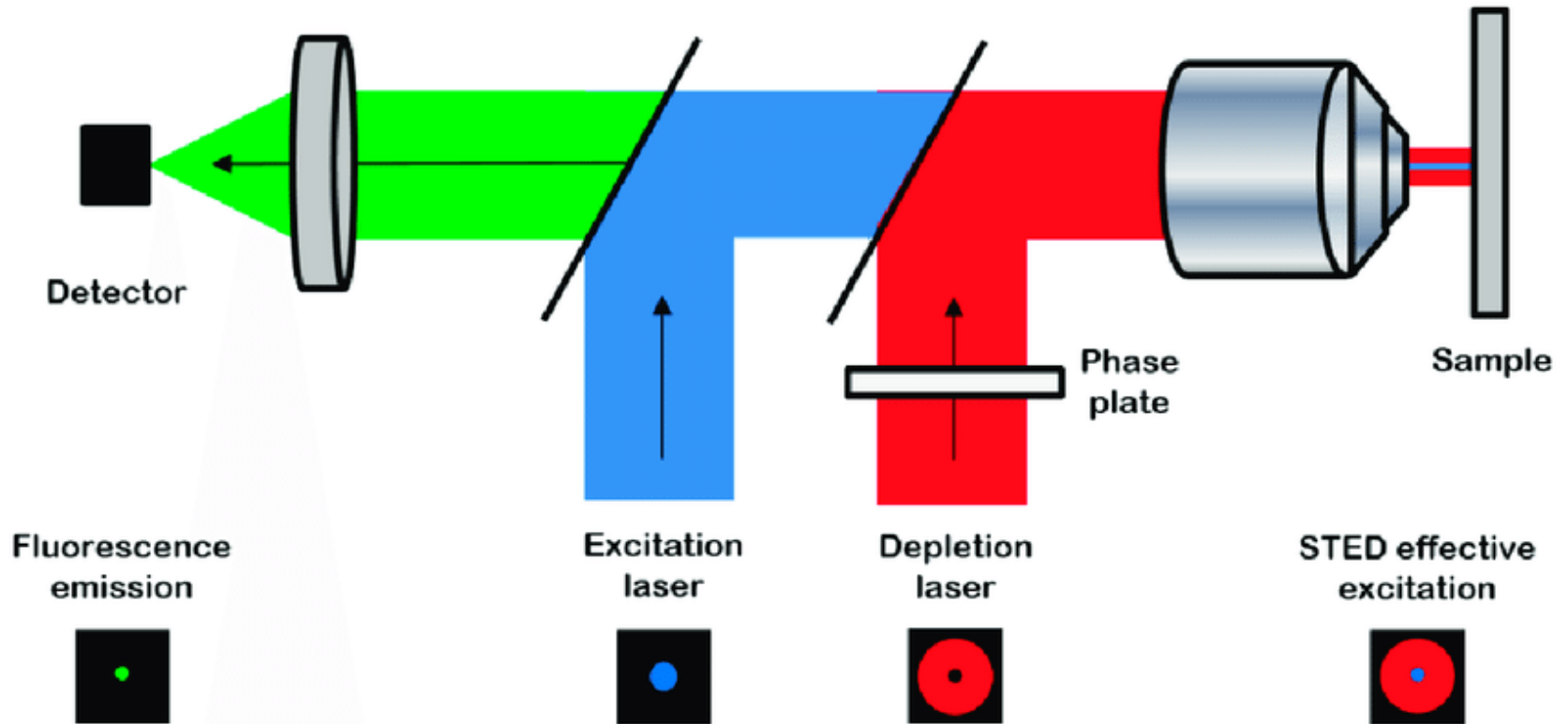
Spectral separation



STED microscope

Stimulated emission depletion

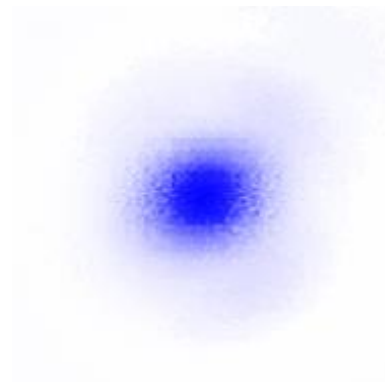
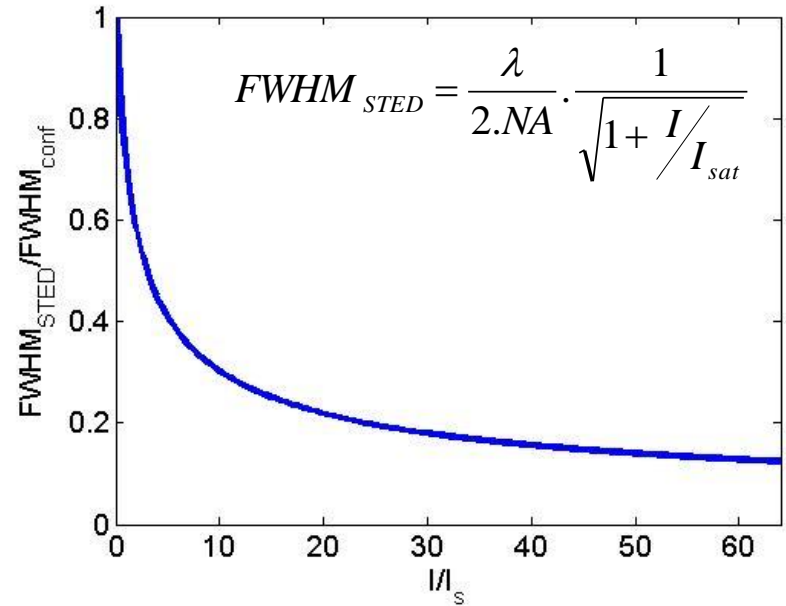
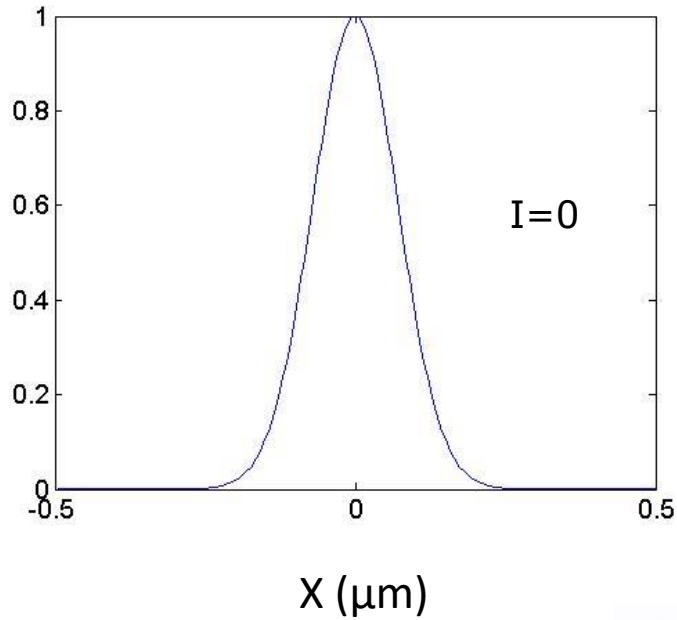
The excitation beam is followed (before the fluorophore emission) by a “donuts shape” beam producing the stimulated emission depletion



STED microscope

Stimulated emission depletion

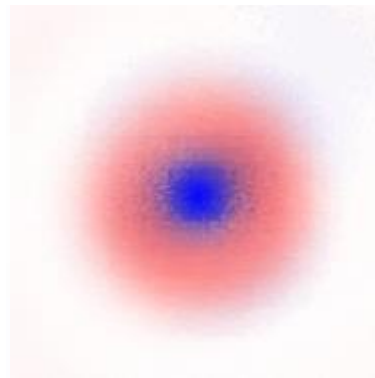
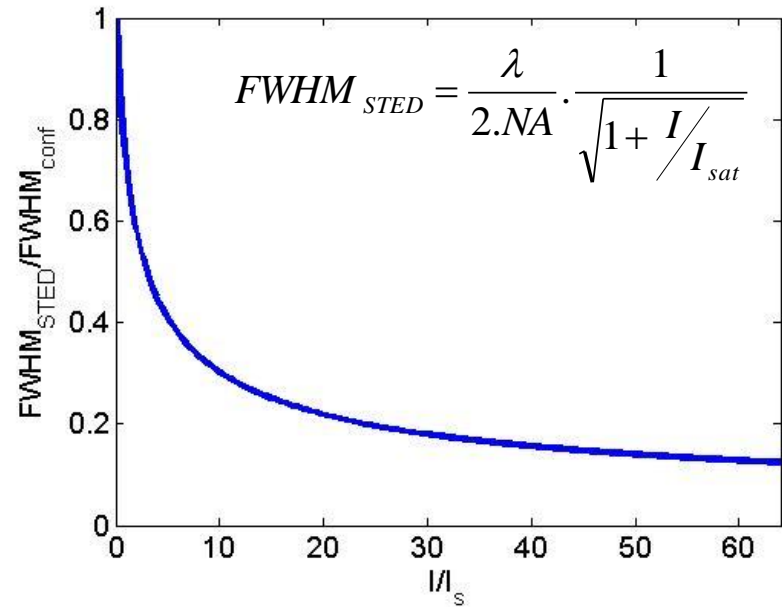
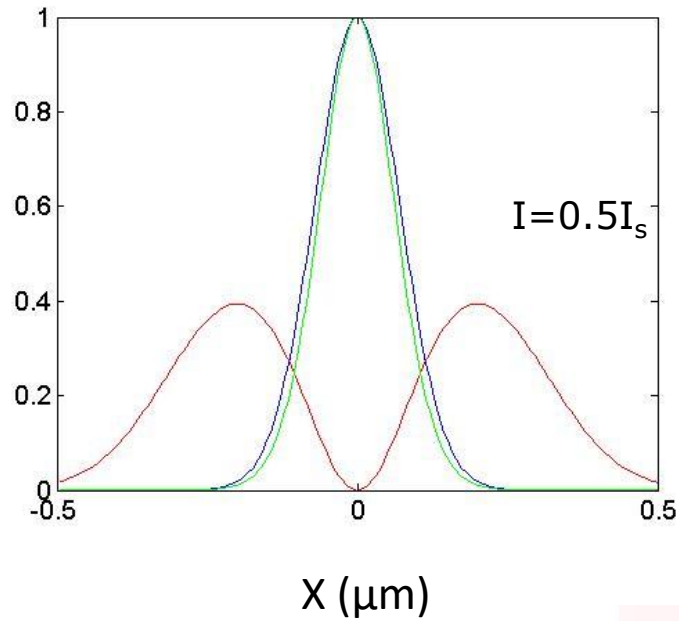
Spatial resolution



STED microscope

Stimulated emission depletion

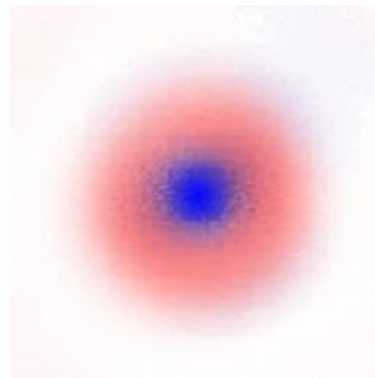
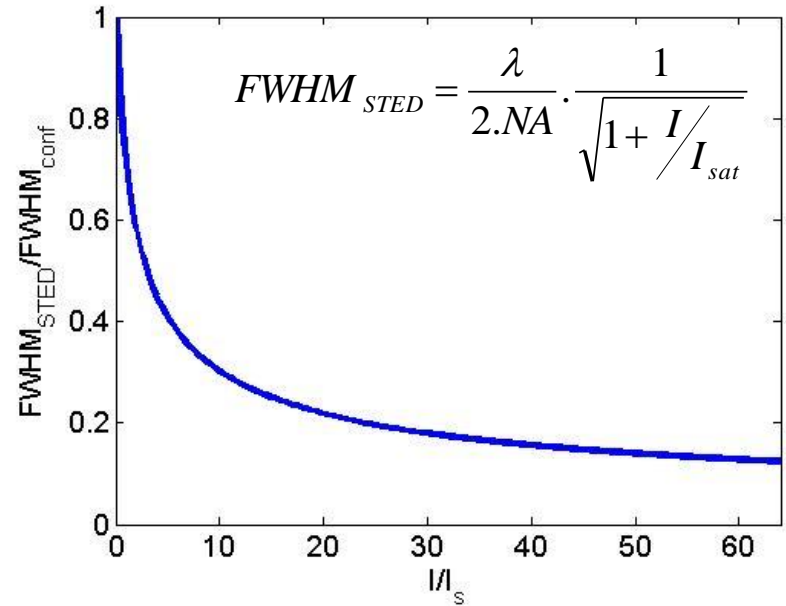
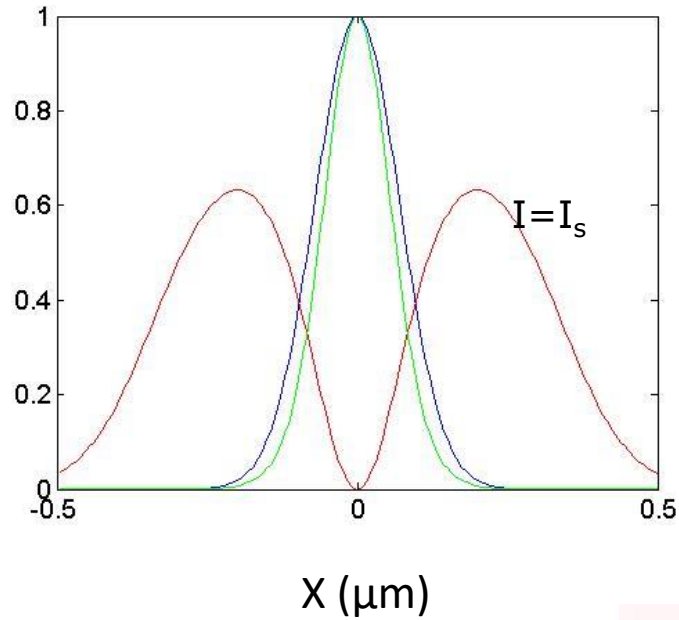
Spatial resolution



STED microscope

Stimulated emission depletion

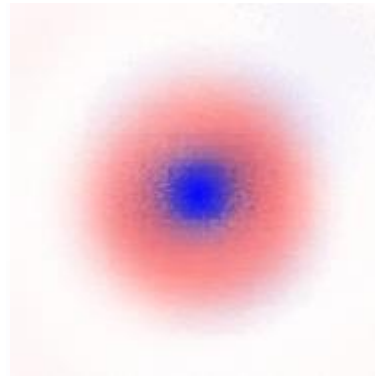
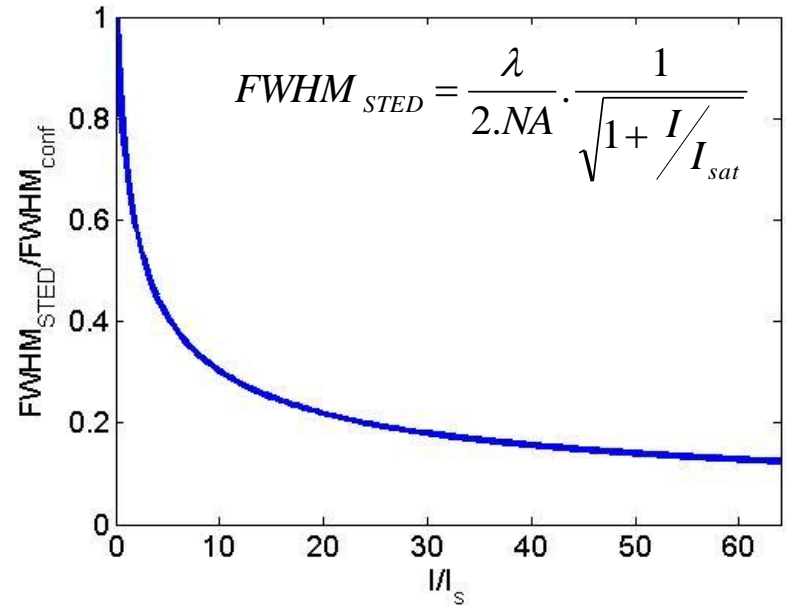
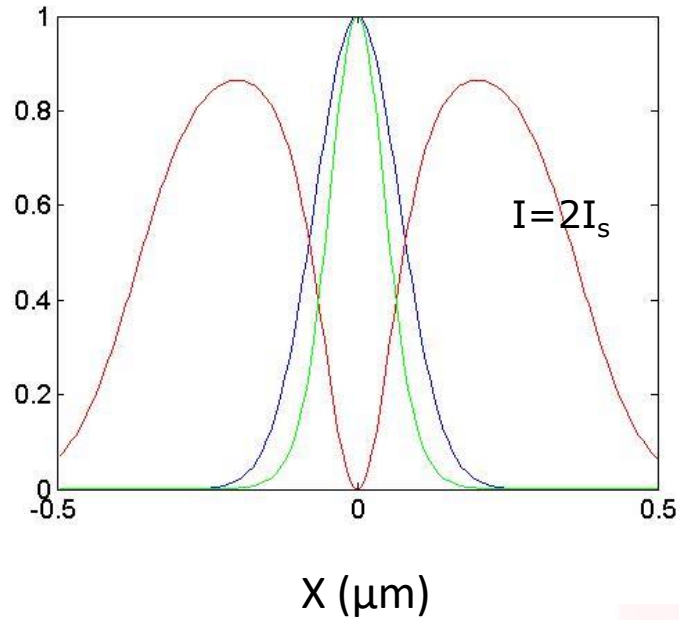
Spatial resolution



STED microscope

Stimulated emission depletion

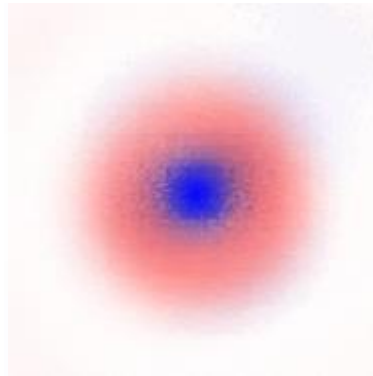
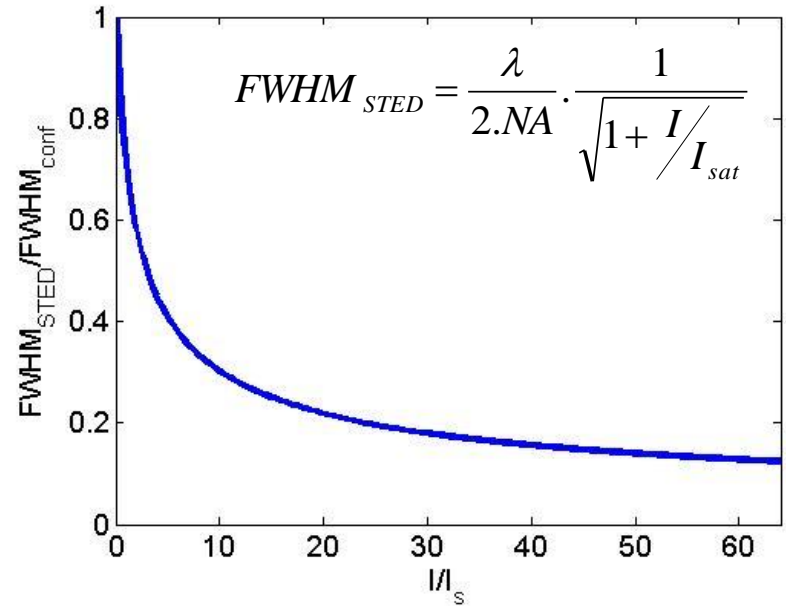
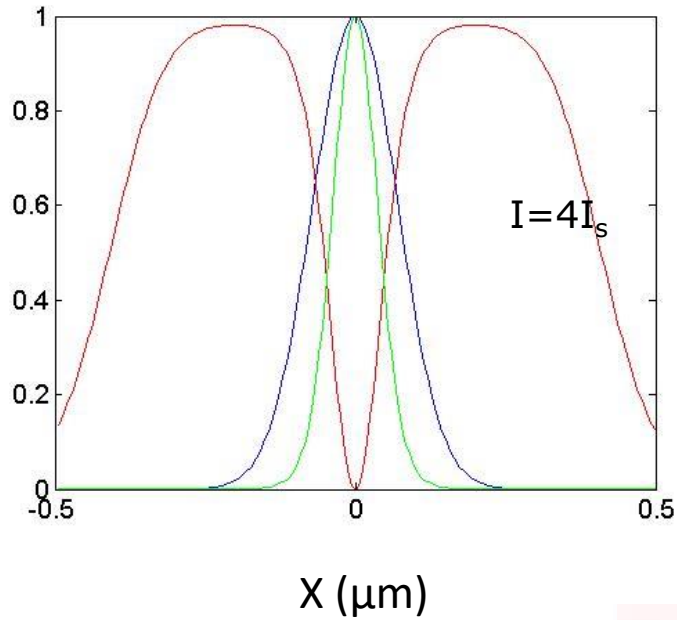
Spatial resolution



STED microscope

Stimulated emission depletion

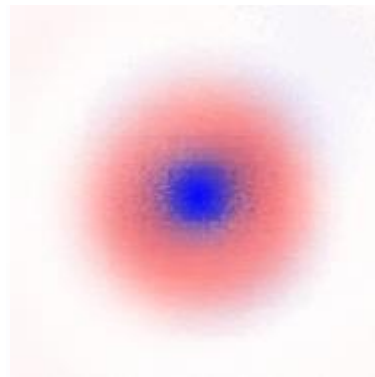
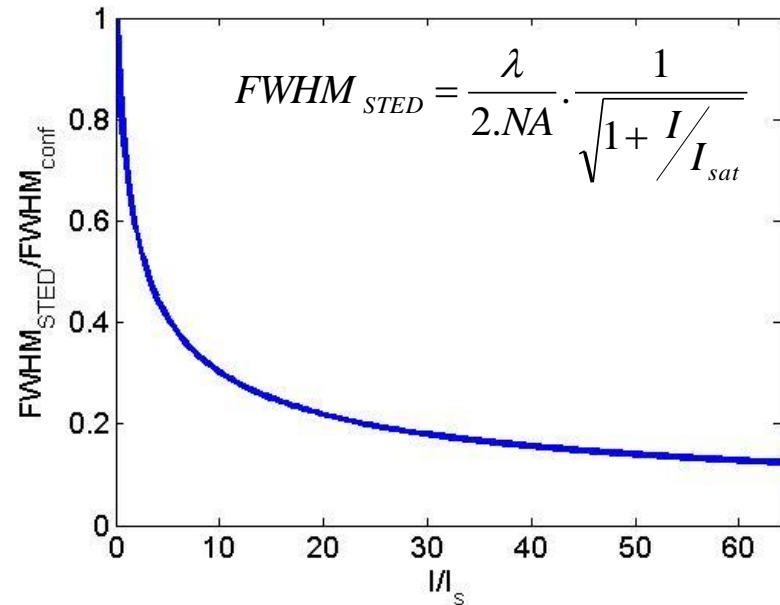
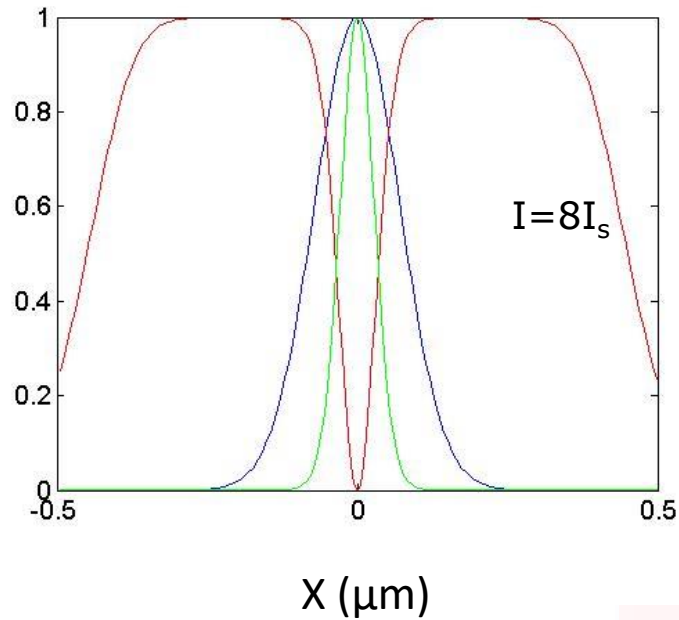
Spatial resolution



STED microscope

Stimulated emission depletion

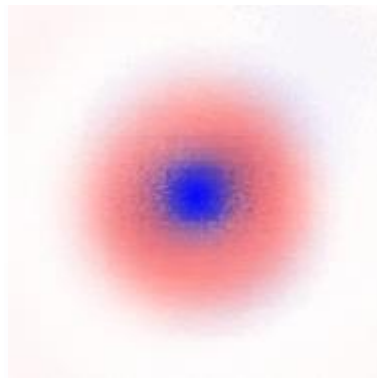
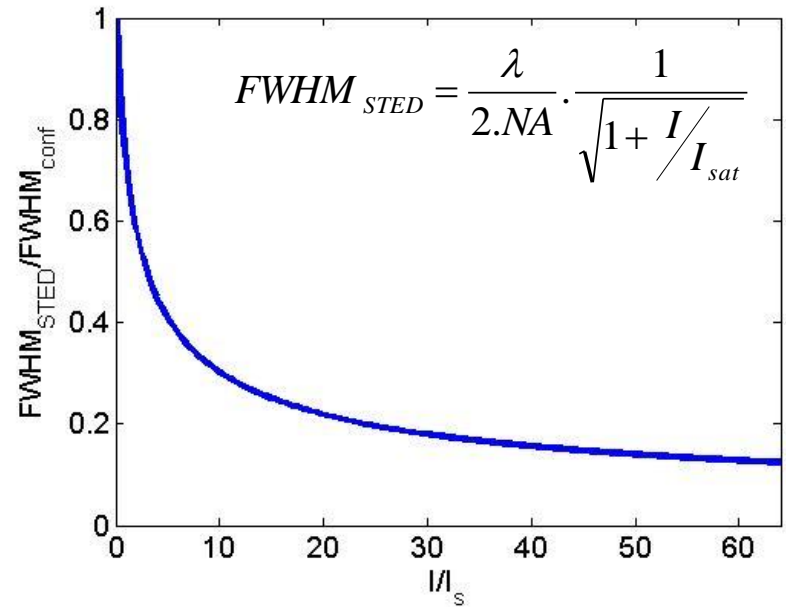
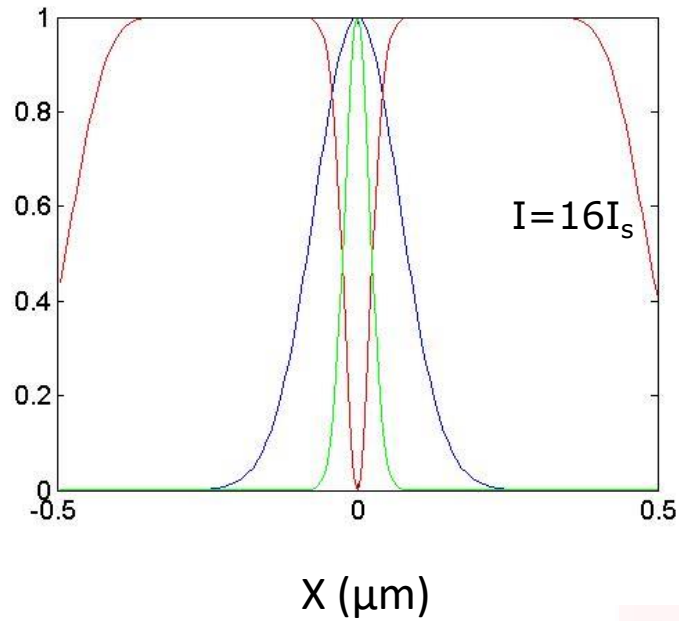
Spatial resolution



STED microscope

Stimulated emission depletion

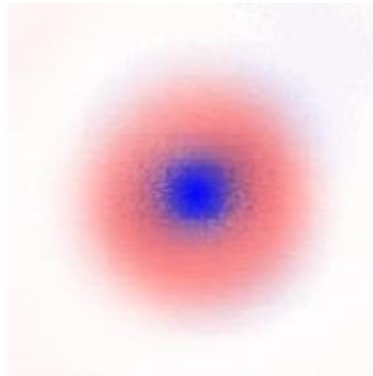
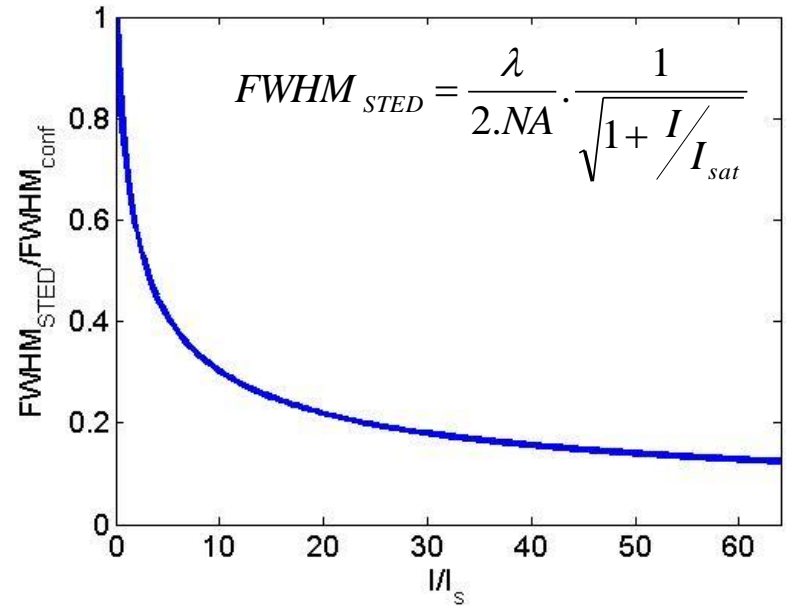
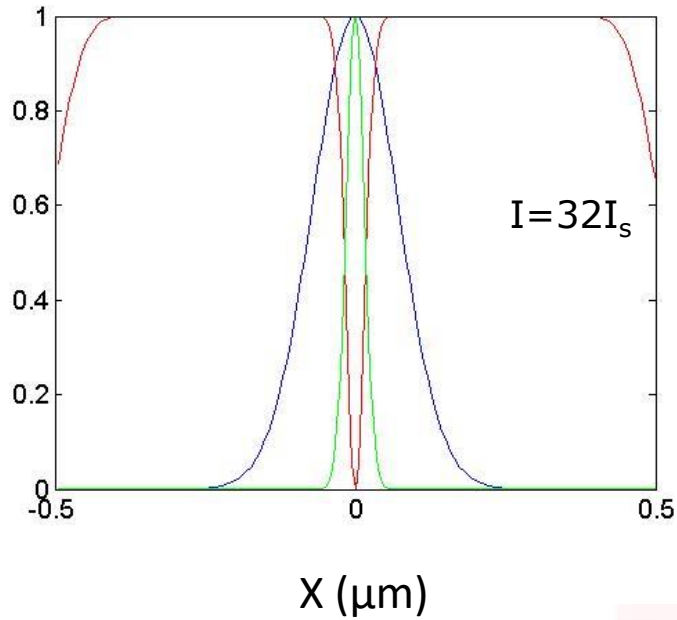
Spatial resolution



STED microscope

Stimulated emission depletion

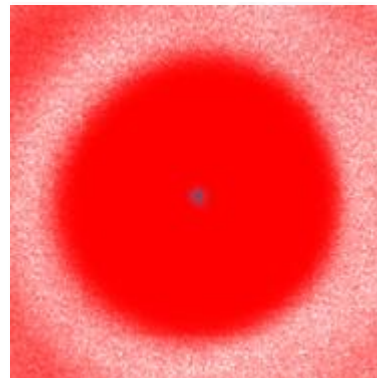
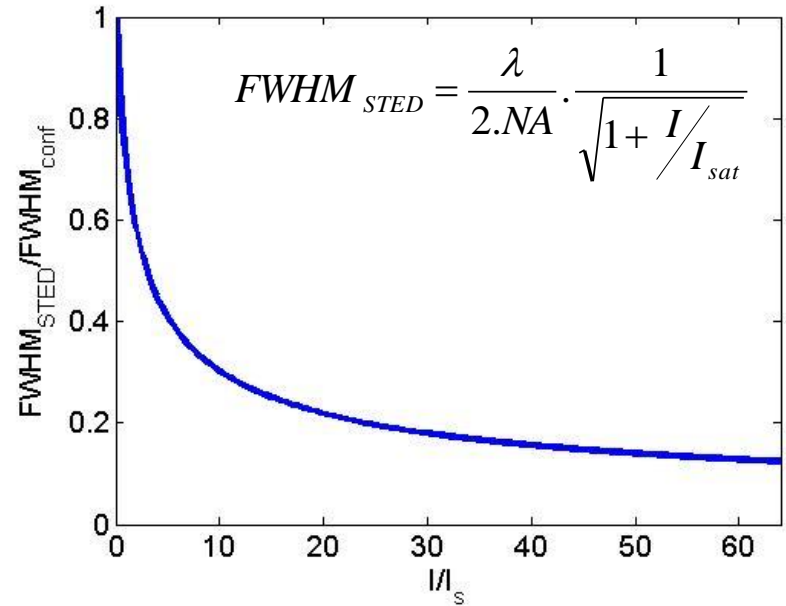
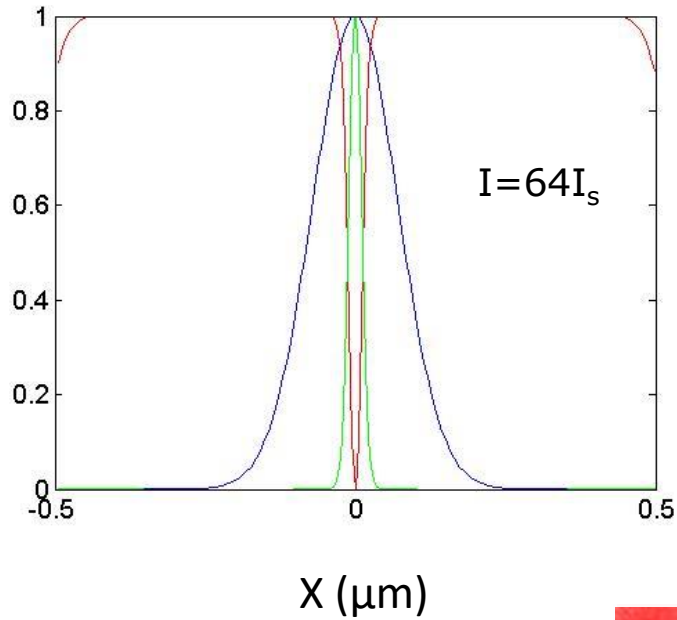
Spatial resolution



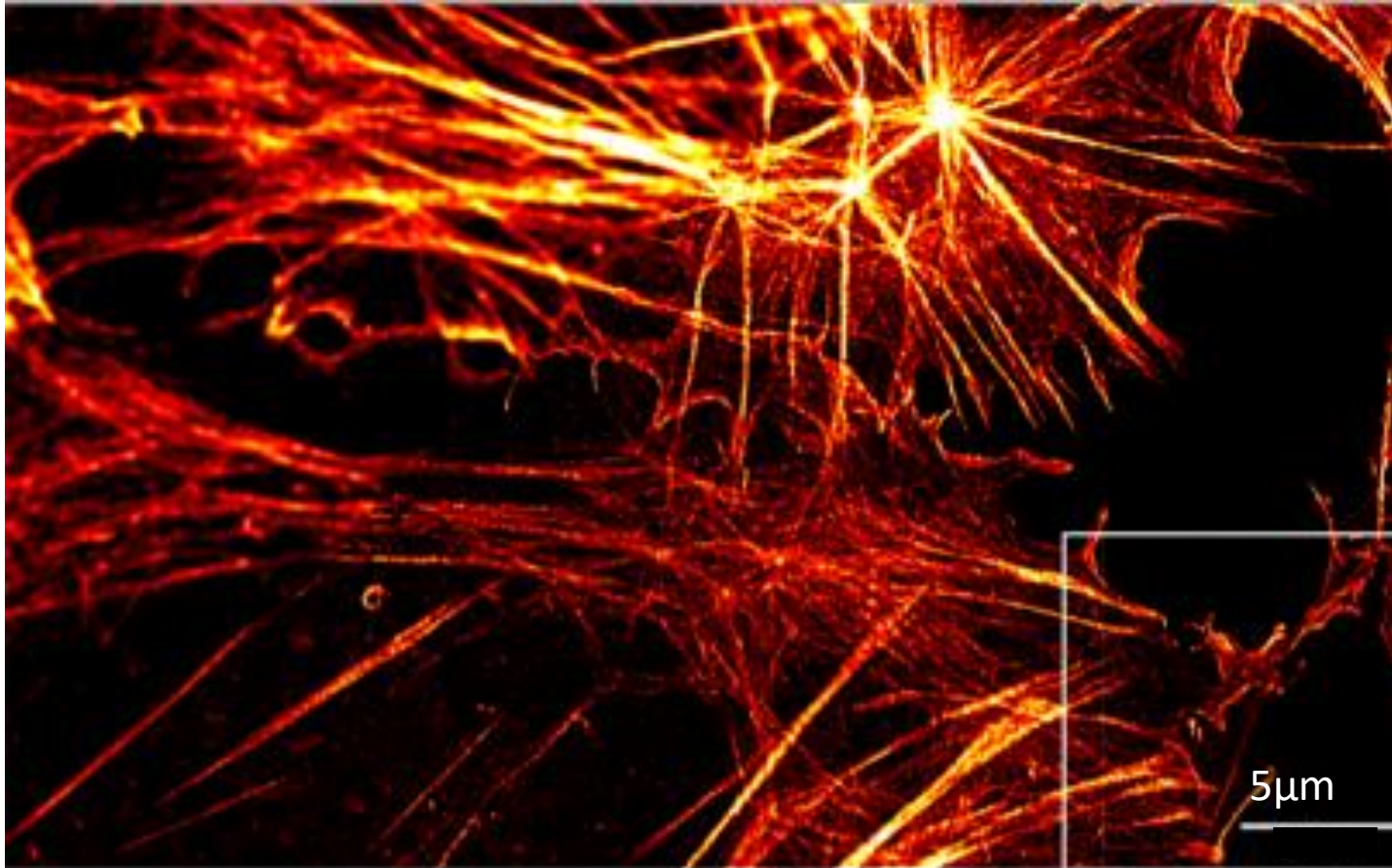
STED microscope

Stimulated emission depletion

Spatial resolution



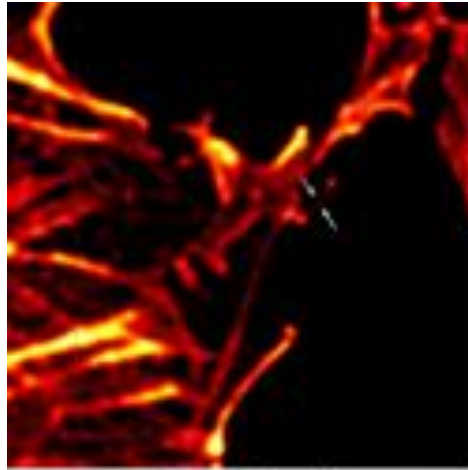
STED microscope
Stimulated emission depletion



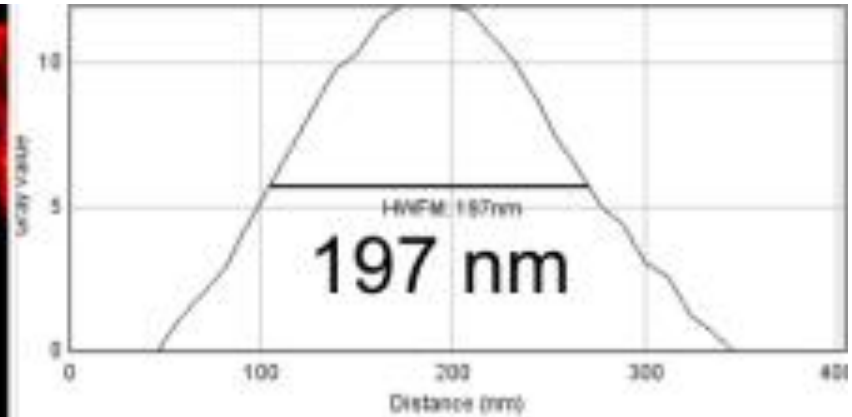
Actin filaments

STED microscope

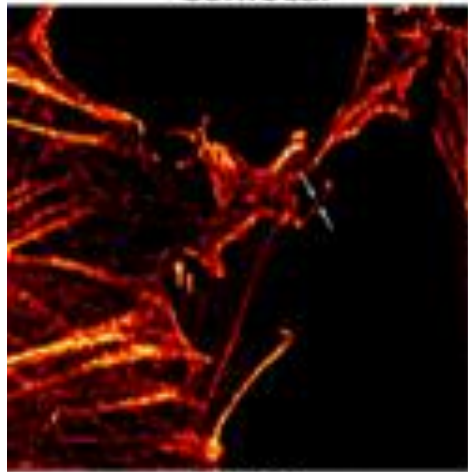
Stimulated emission depletion



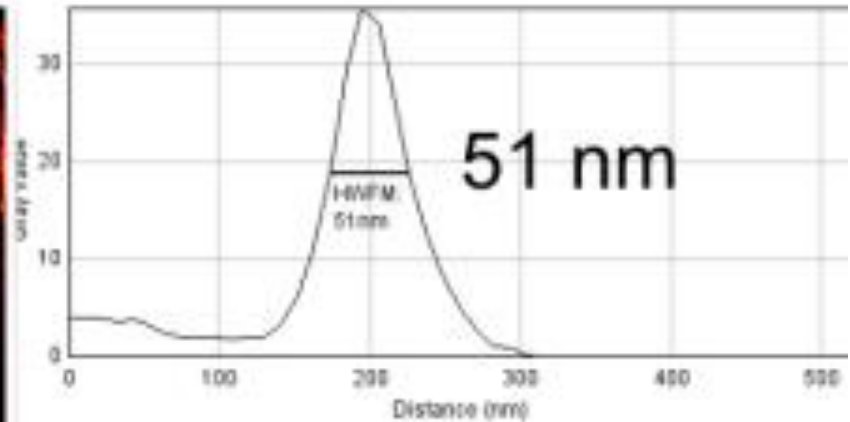
Confocal



Confocal



STED

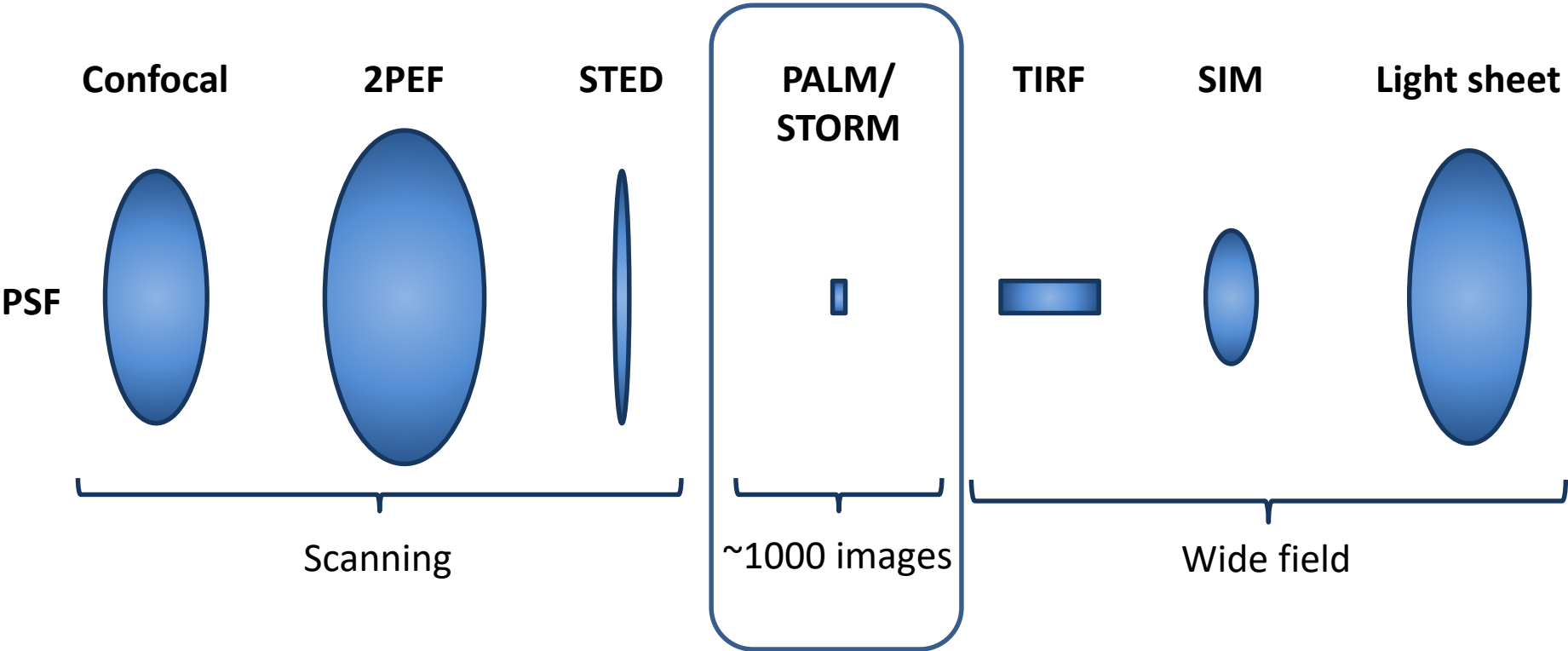


STED

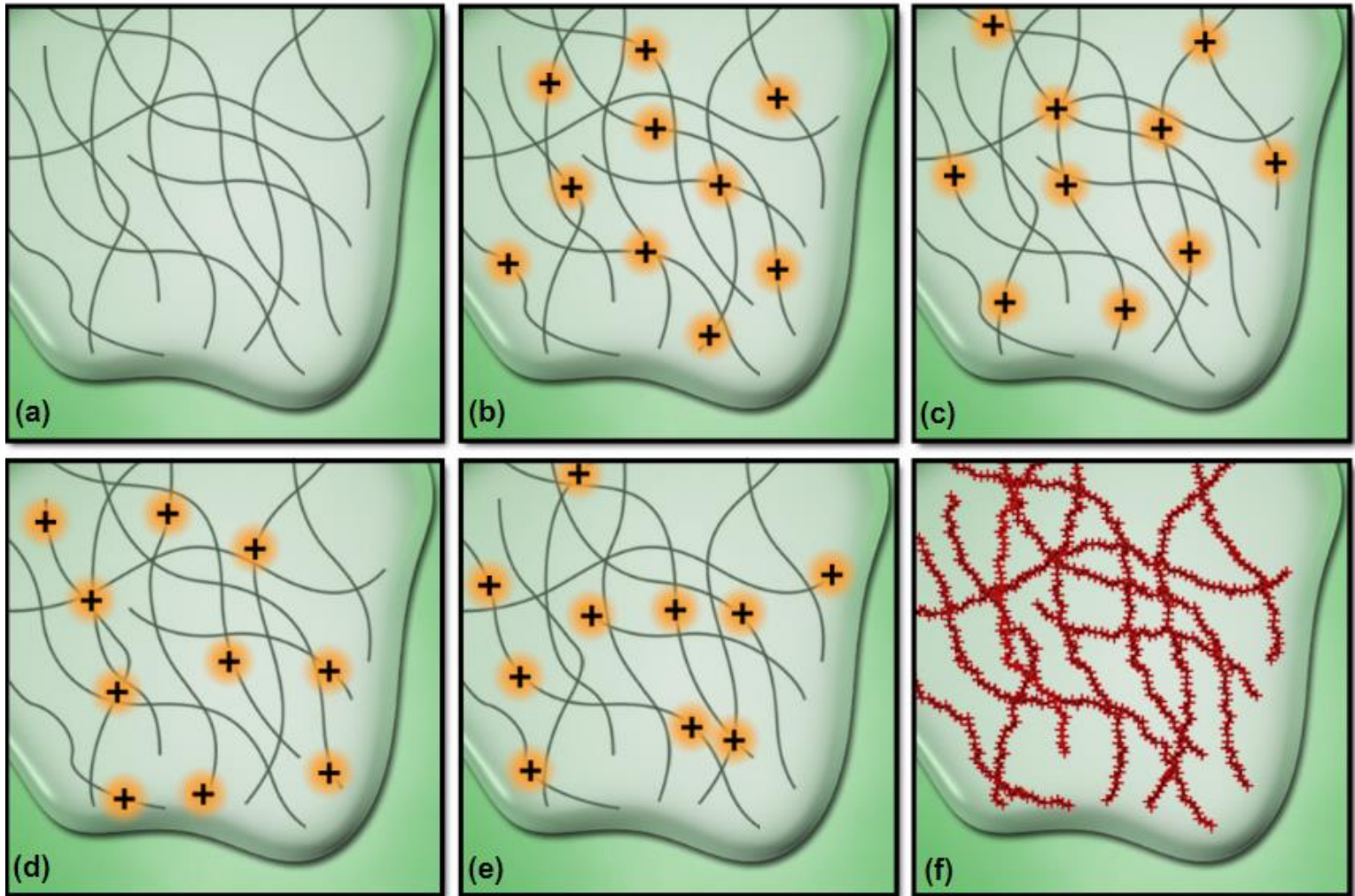
Actin filaments

Increasing the spatial resolution of a fluorescence microscope

PSF=Point Spread Function (image of a point source)



SMLM microscope (PALM/STORM) “Single Molecule Localization Microscopy”

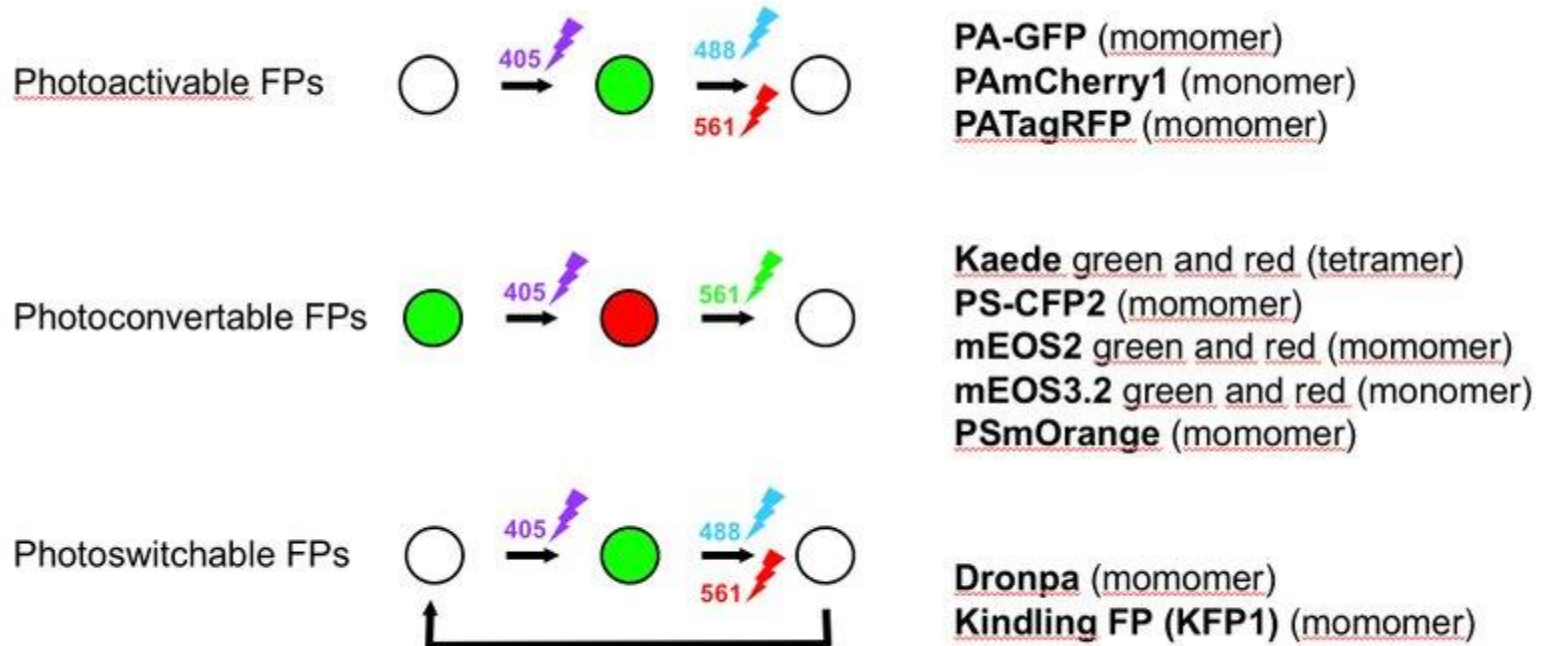


<https://www.microscopyu.com>

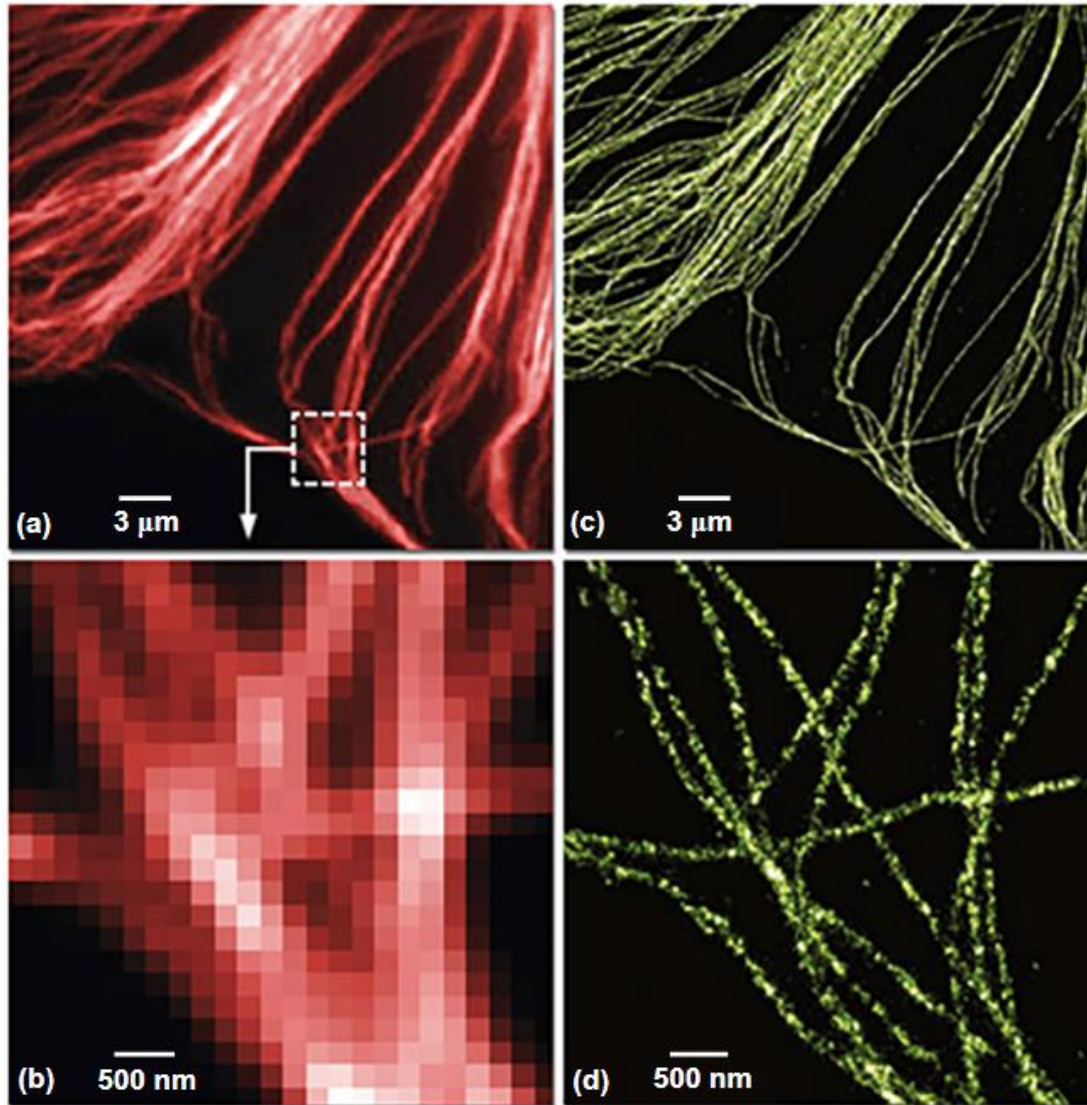
Resolution $\sigma \approx s/\sqrt{N}$, s : gaussian width, N number of photons

SMLM microscope (PALM/STORM)

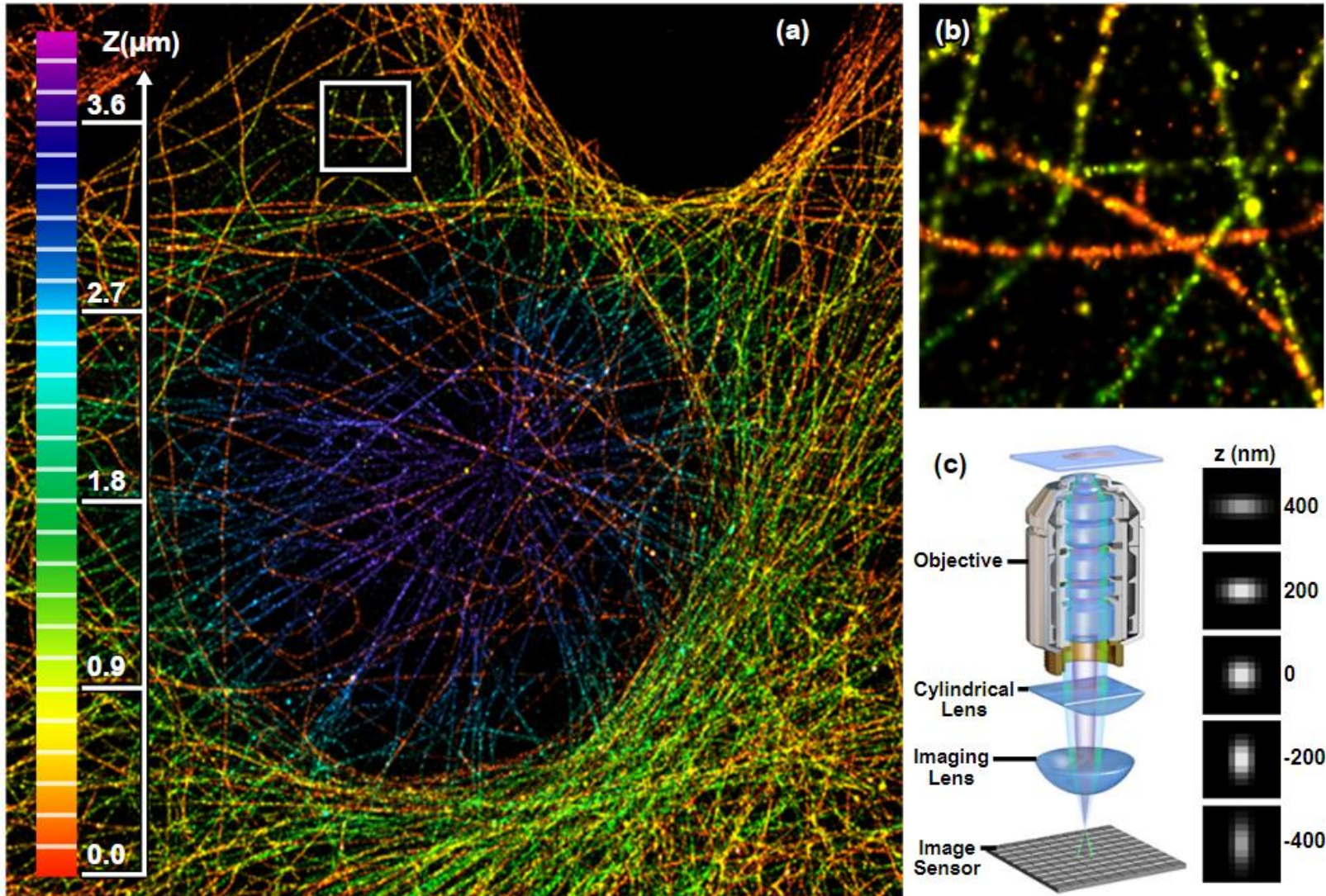
“Single Molecule Localization Microscopy”



SMLM microscope (PALM/STORM)
“Single Molecule Localization Microscopy”



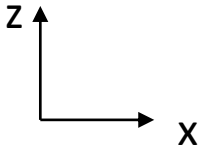
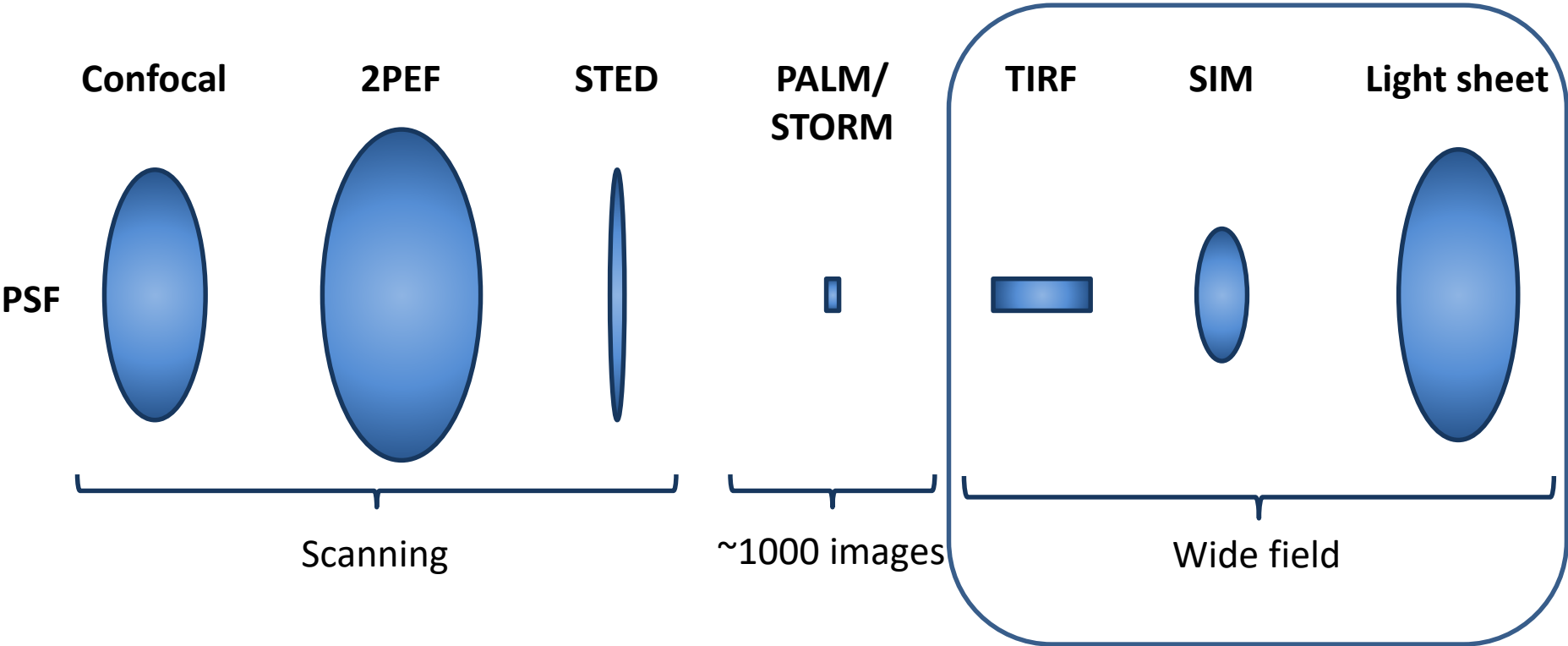
SMLM microscope (PALM/STORM) “Single Molecule Localization Microscopy”



Microtubules, *Alexa Fluor 647*, <https://www.microscopyu.com>

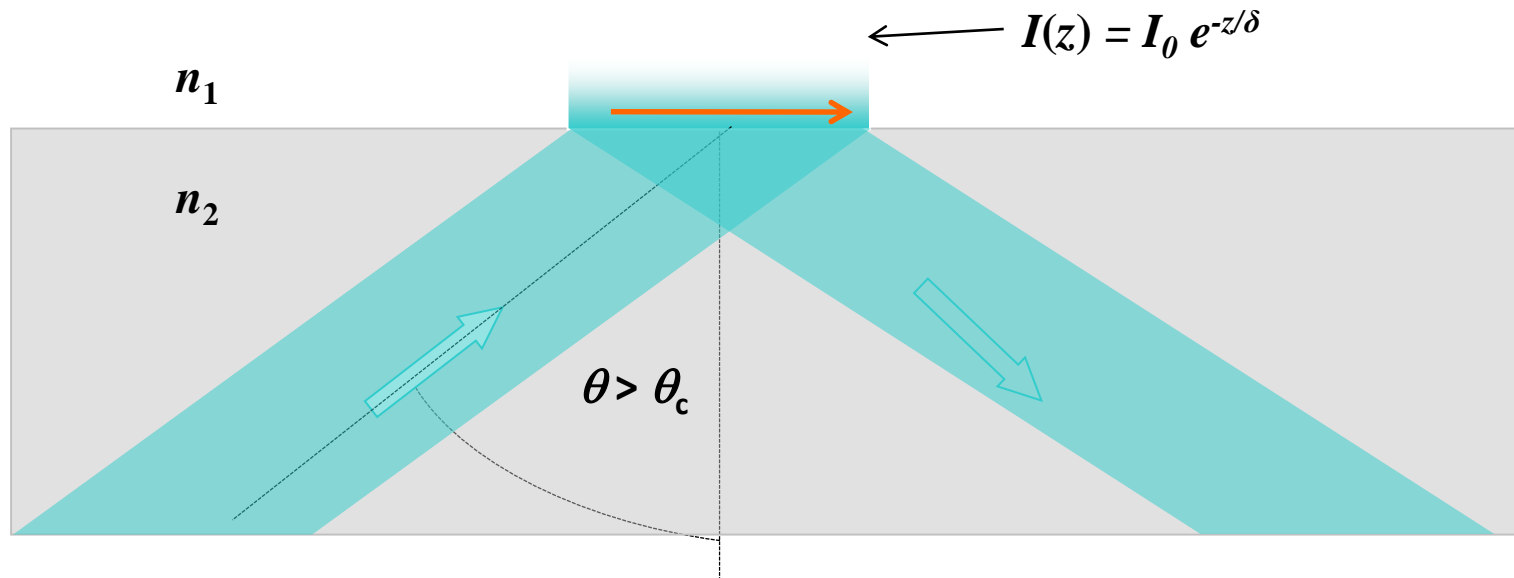
Increasing the spatial resolution of a fluorescence microscope

PSF=Point Spread Function (image of a point source)



Total internal reflection (TIRF)

Evanescent wave



Penetration depth

$$\delta = \lambda_{ex} / [4\pi(n_2^2 \sin^2(\theta) - n_1^2)^{1/2}]$$

Ex : interface glass / water: $n_1 = 1.33$; $n_2 = 1.514$ (BK7) $\lambda_{ex} = 488$ nm



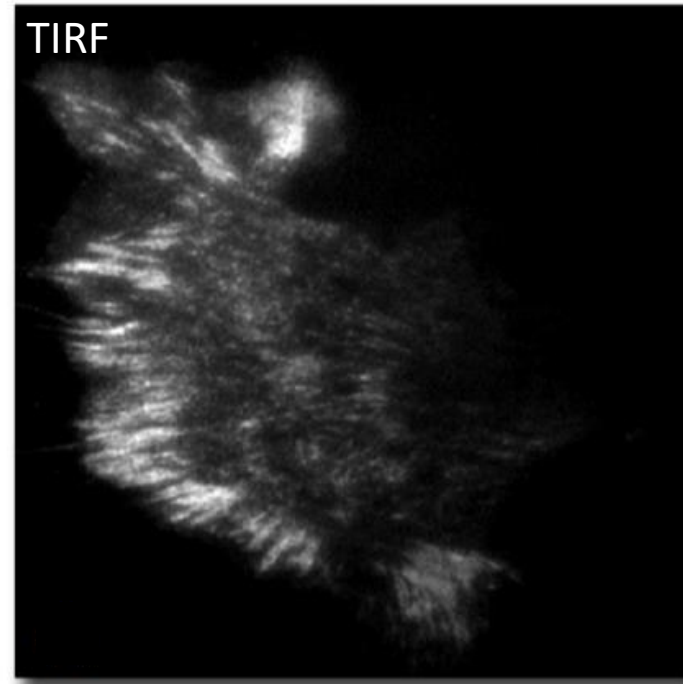
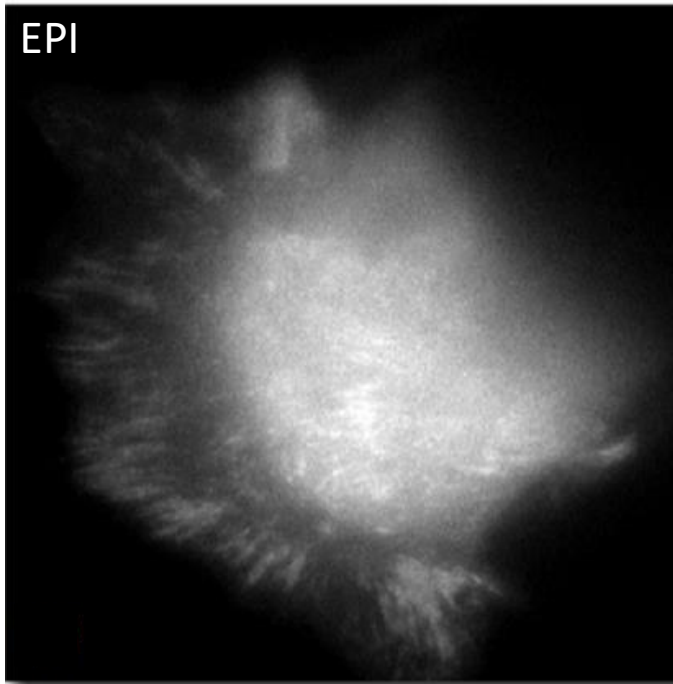
$$\theta_c \sim 61^\circ$$

$$\delta (\theta = 67^\circ) \sim 93 \text{ nm}$$

Total internal reflection microscopy (TIRF)

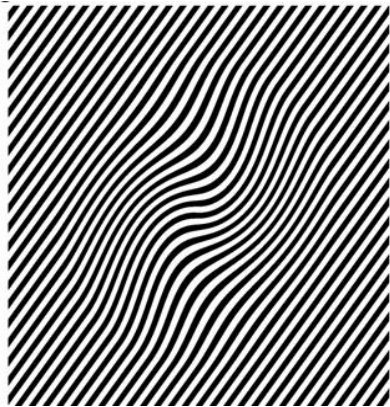
Increase of the signal to noise ratio

Cell focal adhesions



SIM microscope (*“Structured Illumination Microscope”*)

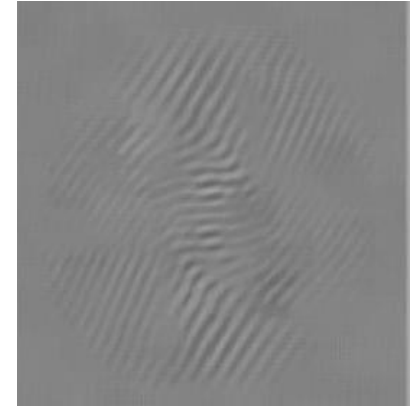
Objet



Illumination

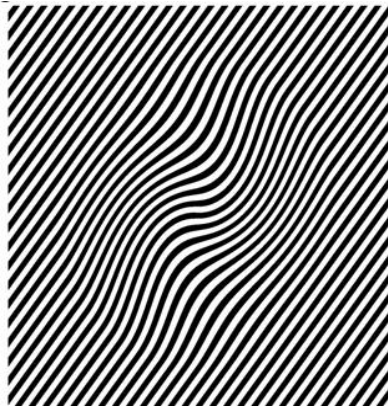


Image

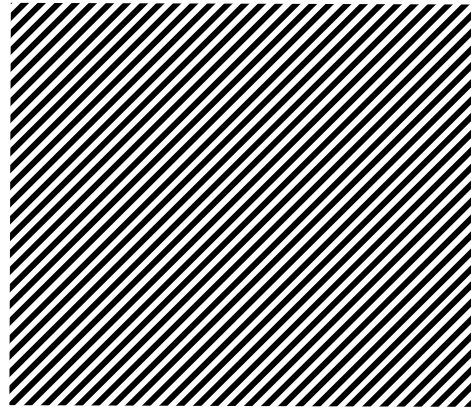


SIM microscope (“*Structured Illumination Microscope*”)

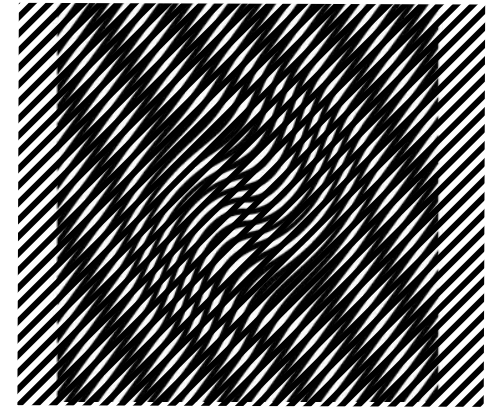
Objet



Illumination



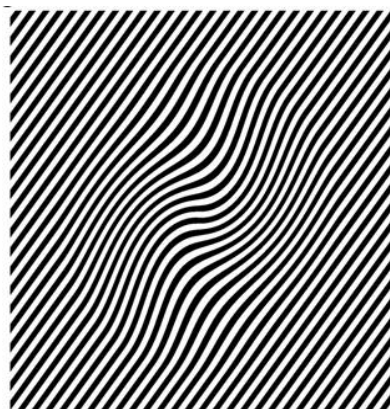
Image



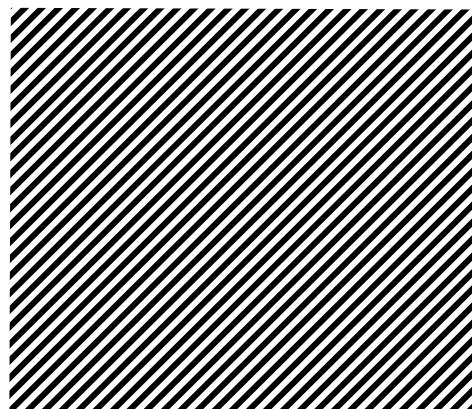
Moiré pattern

SIM microscope (*“Structured Illumination Microscope”*)

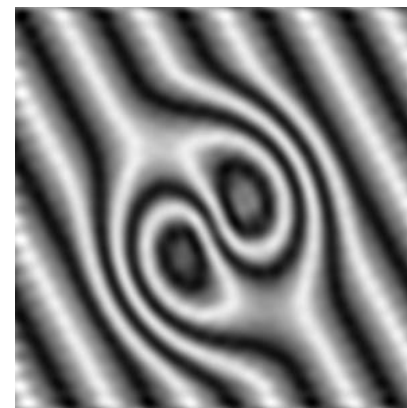
Objet



Illumination



Image

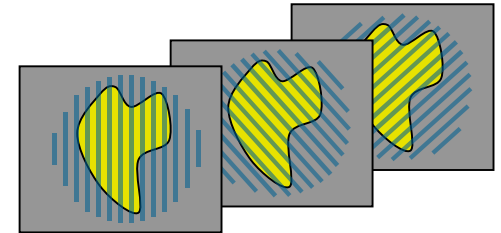
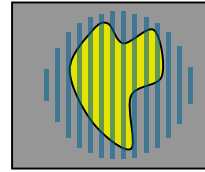
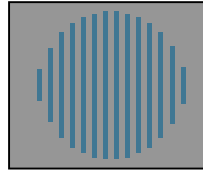


After reconstruction

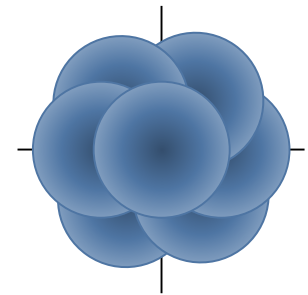
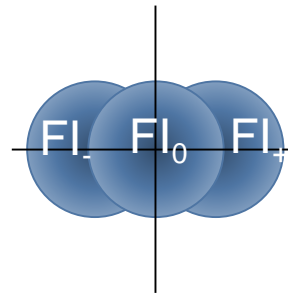
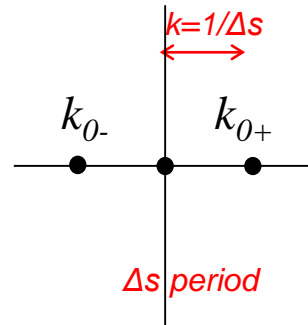
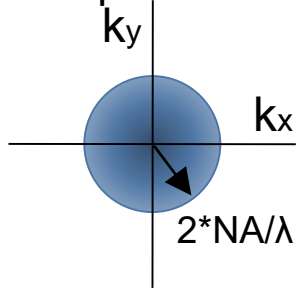
SIM microscope (“*Structured Illumination Microscope*”)

Fourier Space

Real space



Fourier space

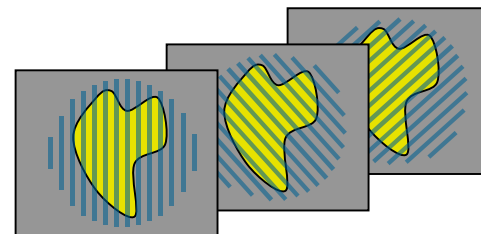
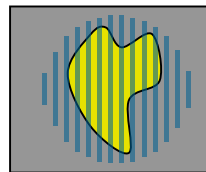
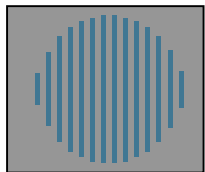


$$\text{SIM FT} = \text{FI}_0 + e^{i\varphi} \text{FI}_+ + e^{-i\varphi} \text{FI}_-$$

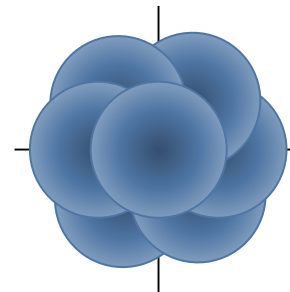
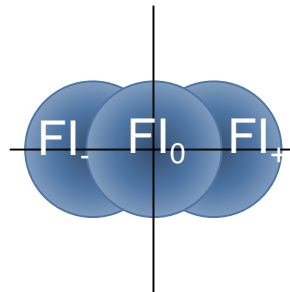
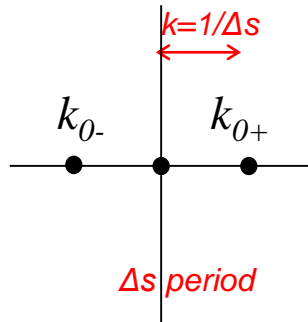
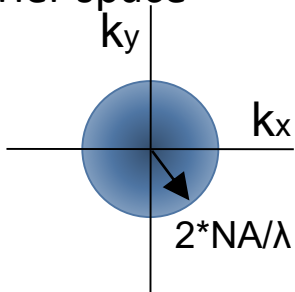
SIM microscope ("Structured Illumination Microscope")

Fourier Space

Real space



Fourier space

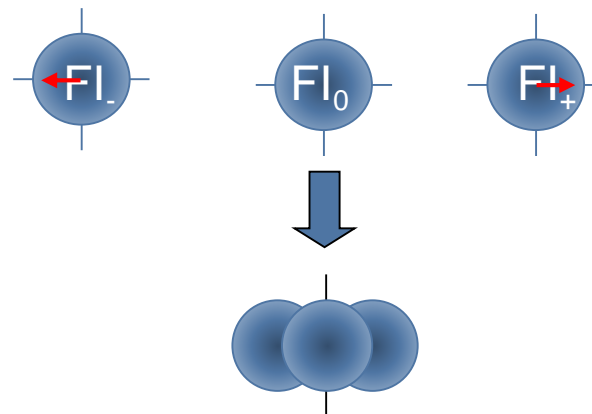


$$\text{SIM FT} = \text{FI}_0 + e^{i\phi} \text{FI}_+ + e^{-i\phi} \text{FI}_-$$

3 pattern phase shifted images

$$\begin{pmatrix} \tilde{\text{I}}_1 \\ \tilde{\text{I}}_2 \\ \tilde{\text{I}}_3 \end{pmatrix} = \begin{pmatrix} 1 & e^{i\phi_1} & e^{-i\phi_1} \\ 1 & e^{i\phi_2} & e^{-i\phi_2} \\ 1 & e^{i\phi_3} & e^{-i\phi_3} \end{pmatrix} \begin{pmatrix} \text{FI}_0 \\ \text{FI}_+ \\ \text{FI}_- \end{pmatrix}$$

$$\tilde{\text{I}} = \text{M FI} \Rightarrow \text{FI} = \text{M}^{-1} \tilde{\text{I}}$$

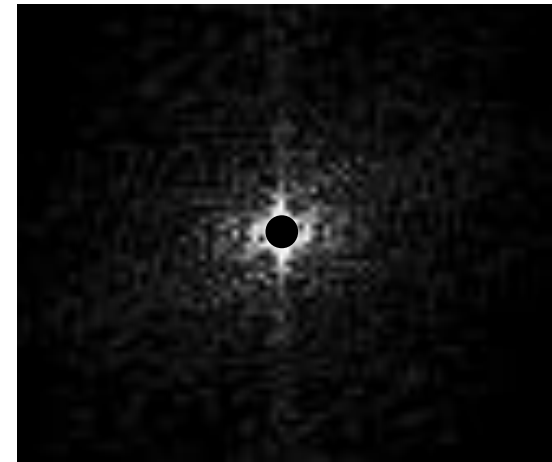
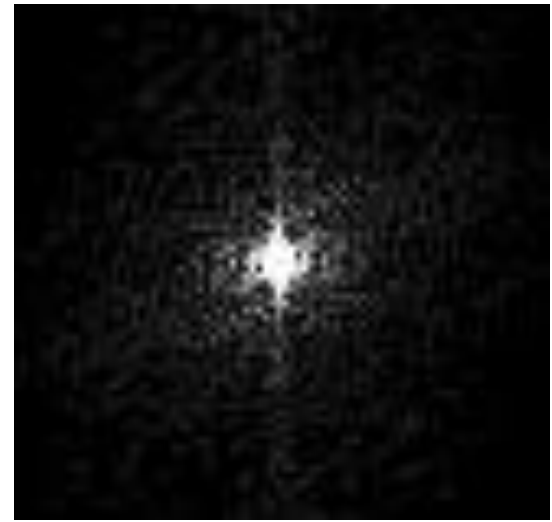


Smaller the period higher the resolution, ~120 nm

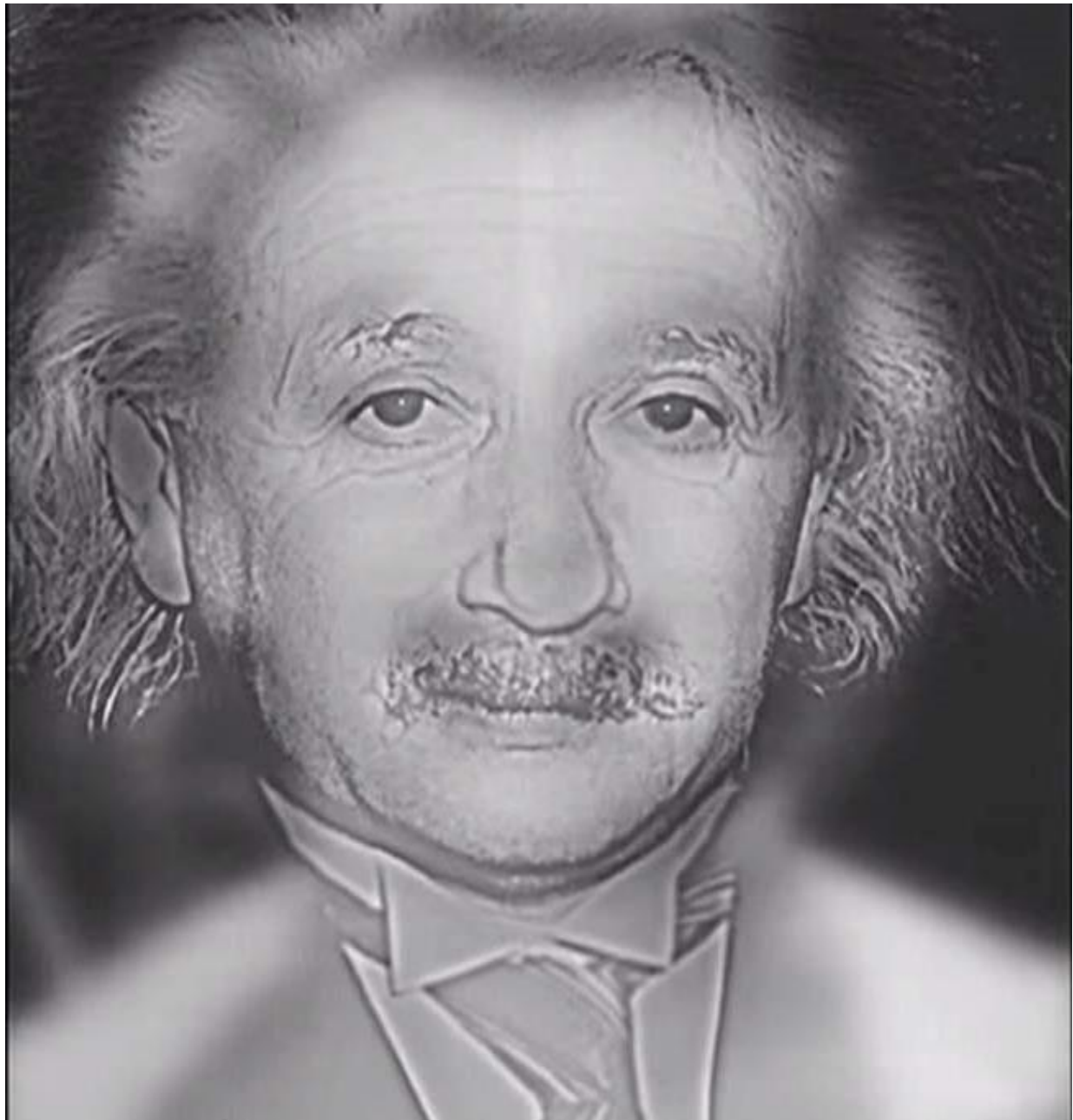
Real space

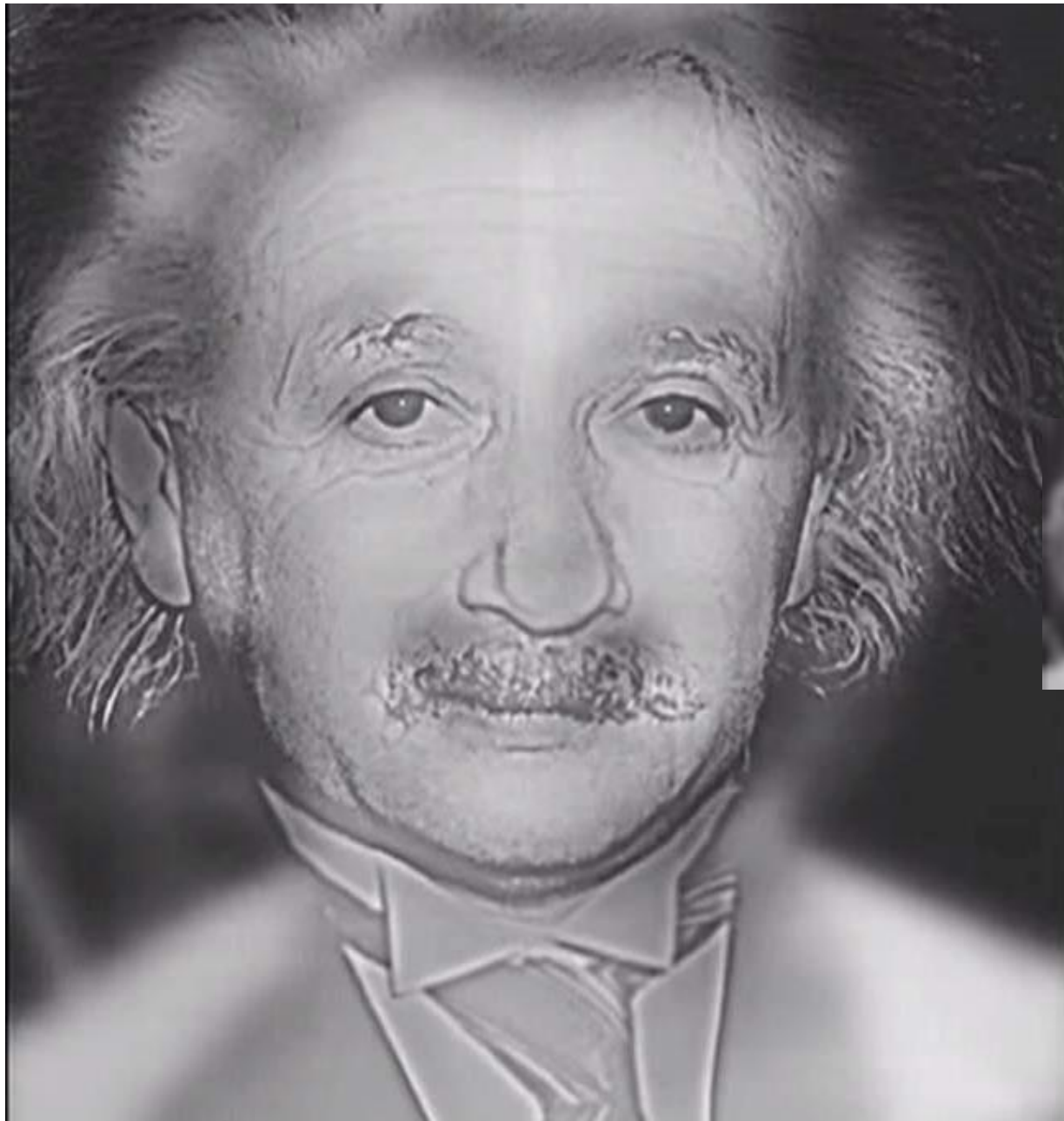


Frequency space



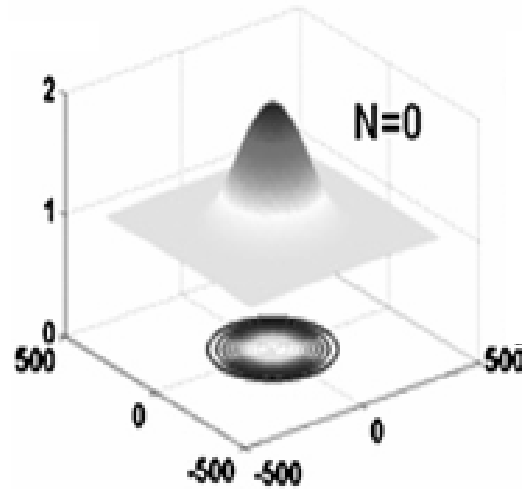
High frequencies  Sample details



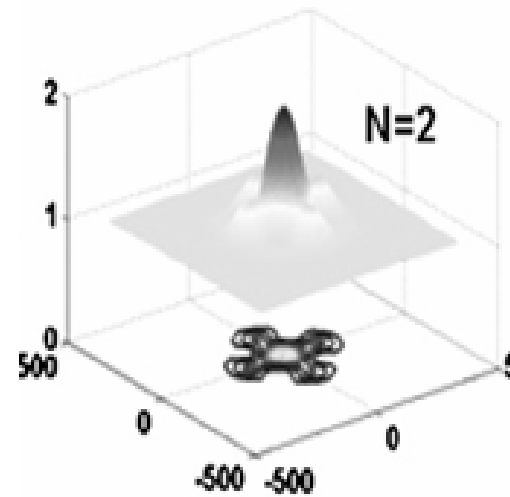
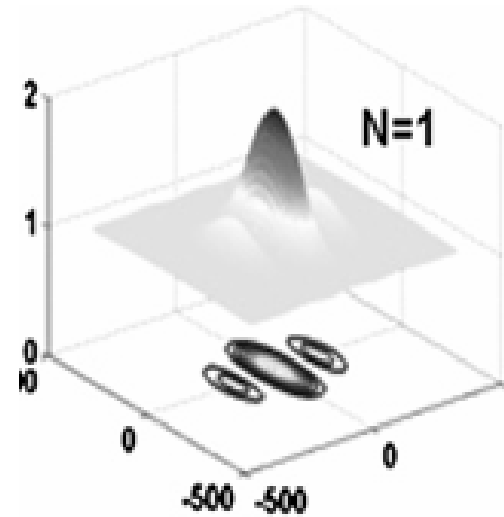


Point Spread Function: point source image in SIM

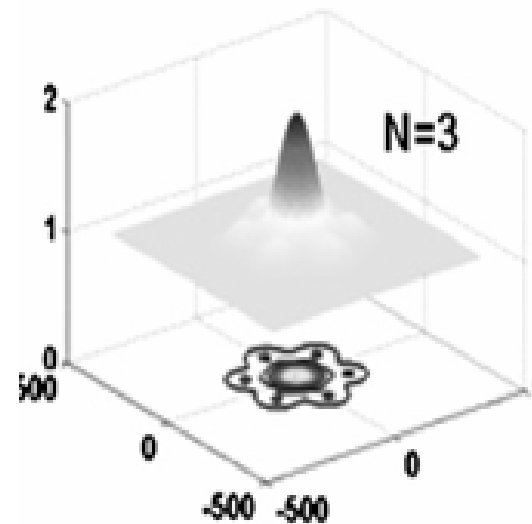
Homogeneous illumination



1 direction pattern



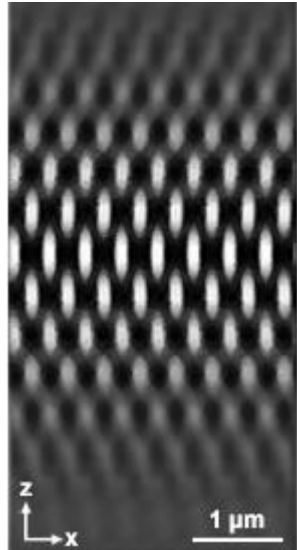
2 directions



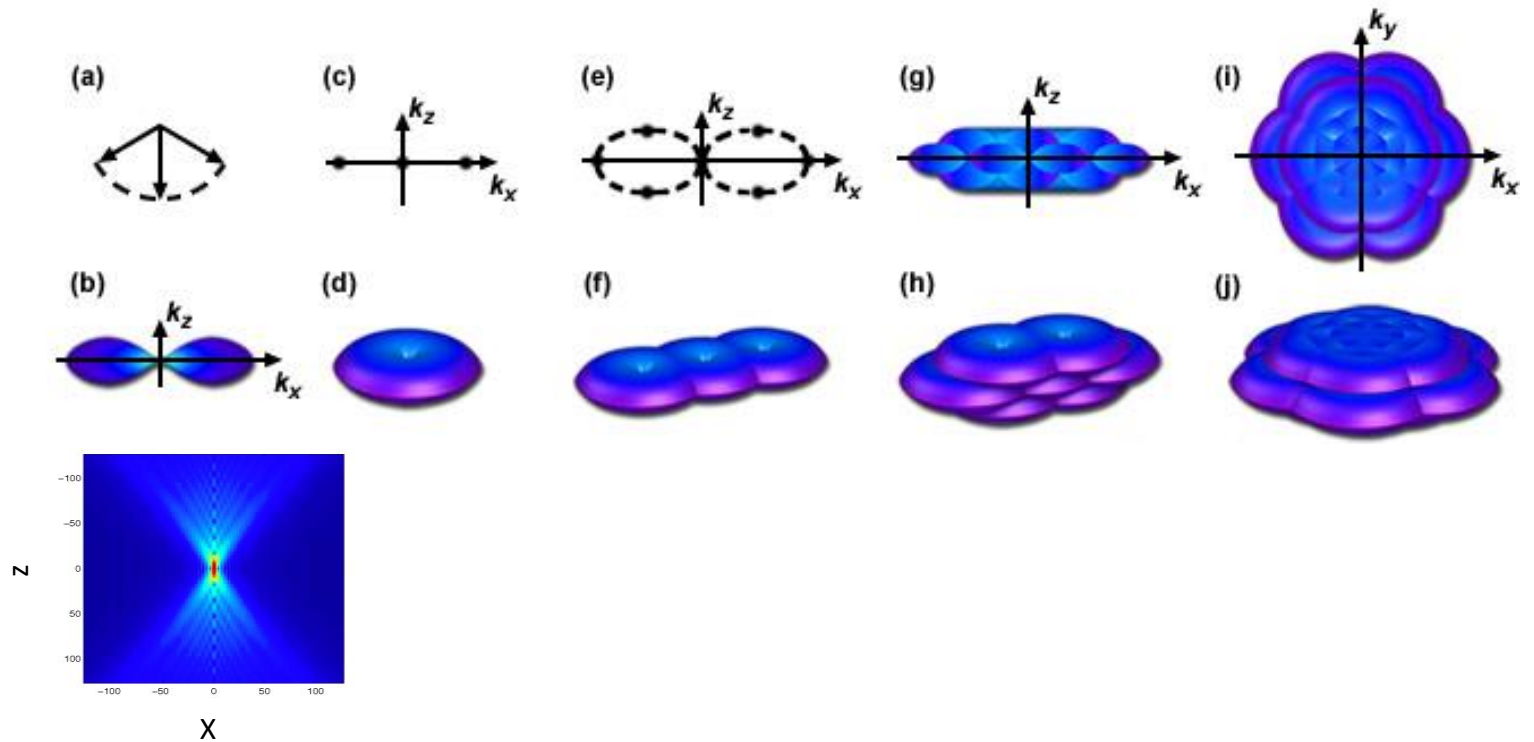
3 directions

Axial resolution in SIM

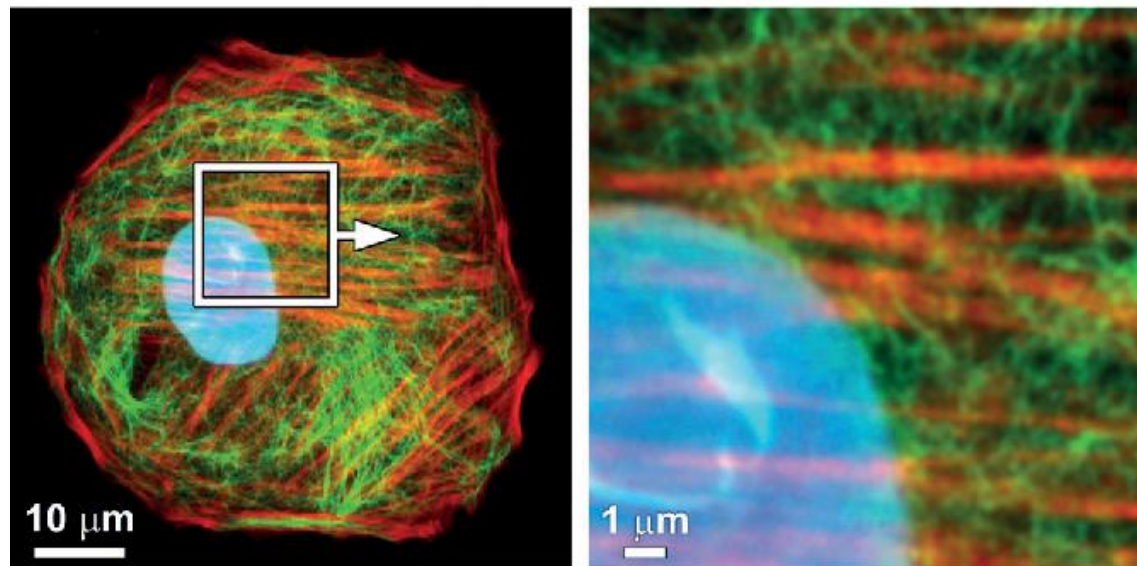
3D-SIM



OTF Support in Three-Dimensional Structured Illumination Microscopy



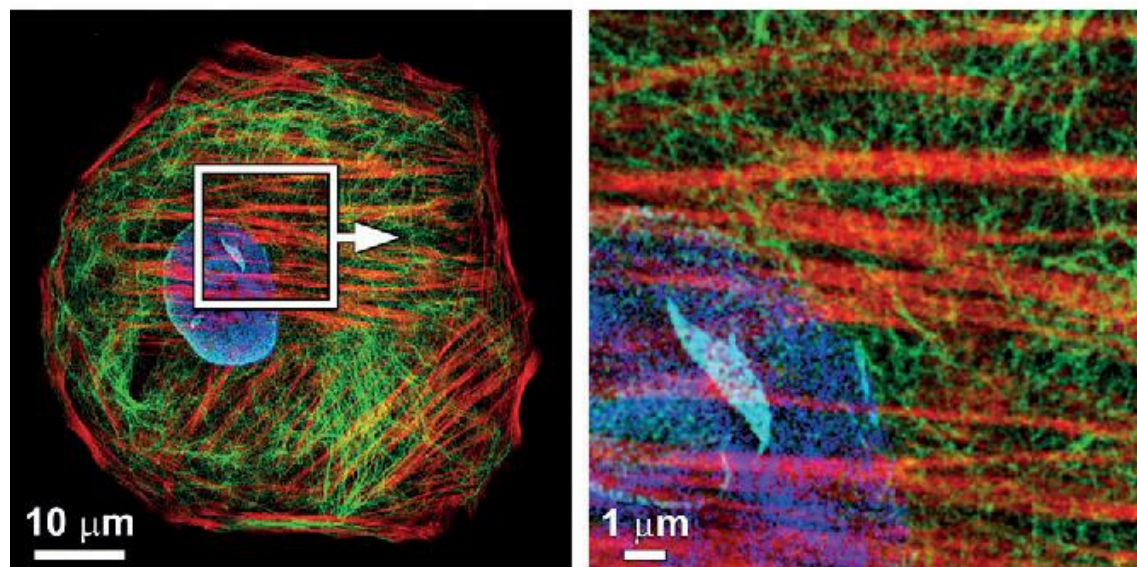
Four-Color SR-SIM of Mink Uterus Epithelium Cells



Widefield

(a)

(b)

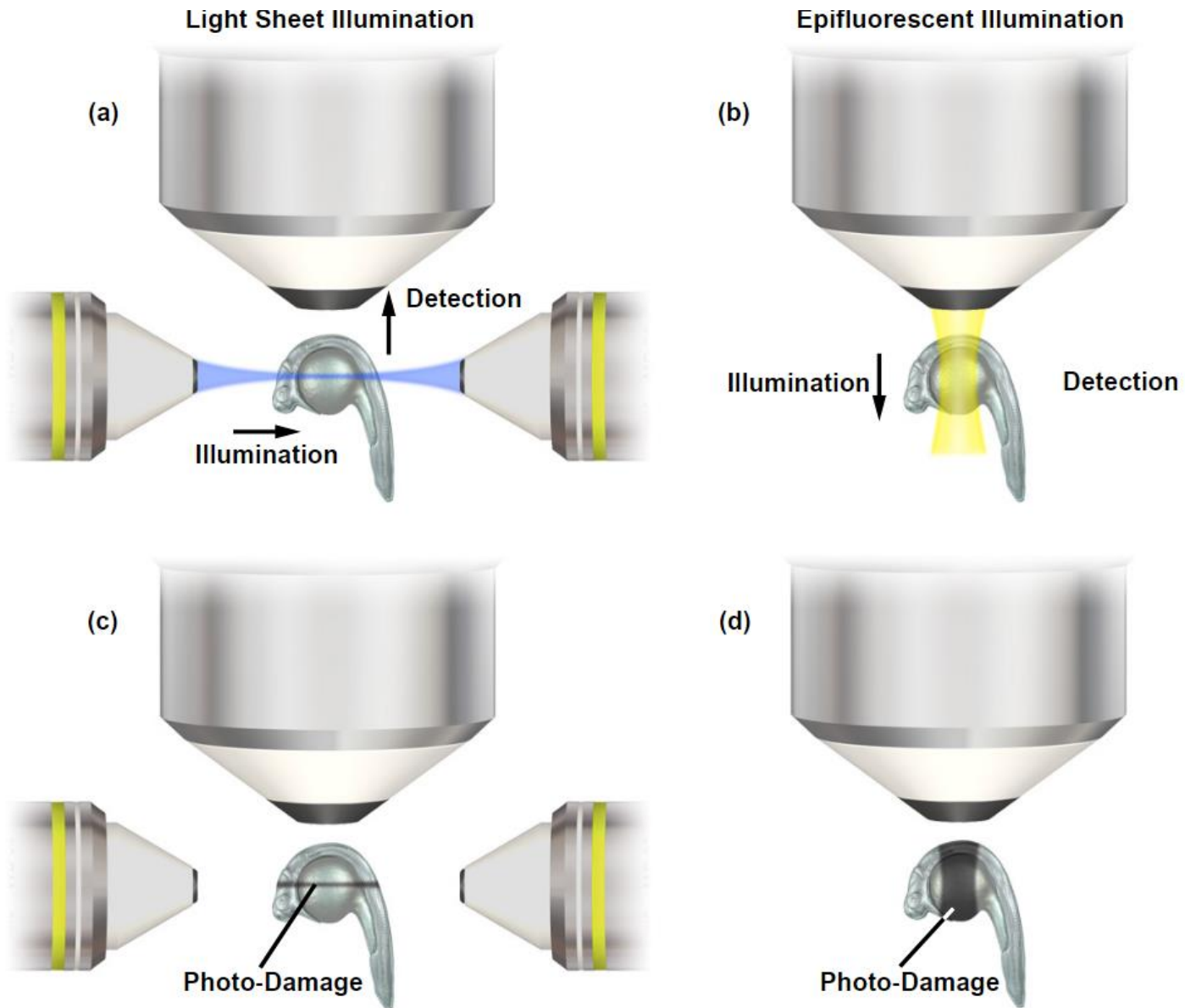


SIM

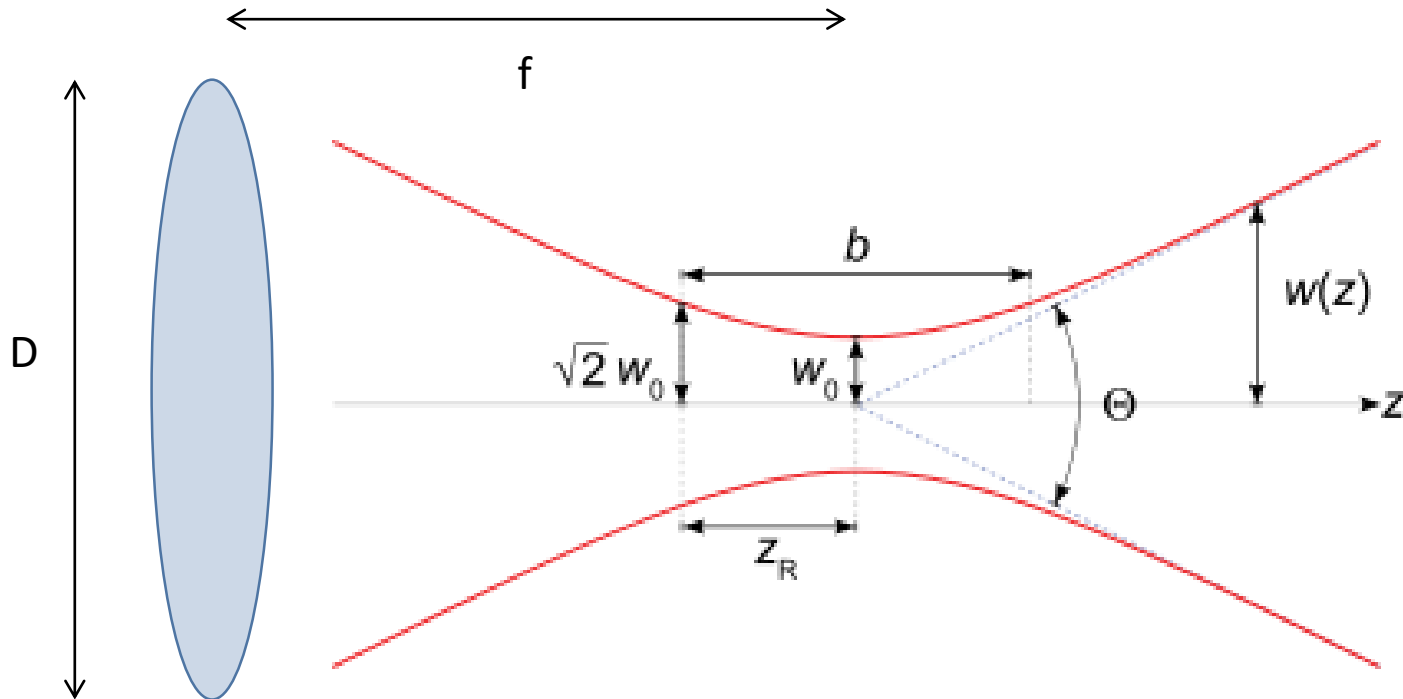
(c)

(d)

Axial resolution: "light sheet"



Axial resolution: "light sheet"

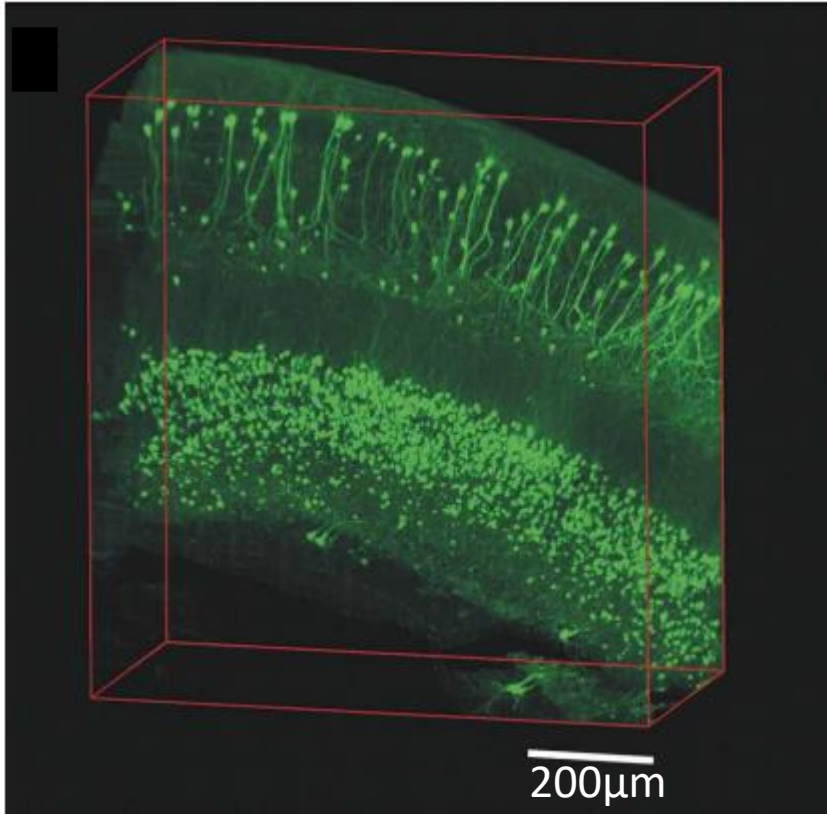


$$\omega_0 = \frac{2\lambda f}{n\pi D}$$

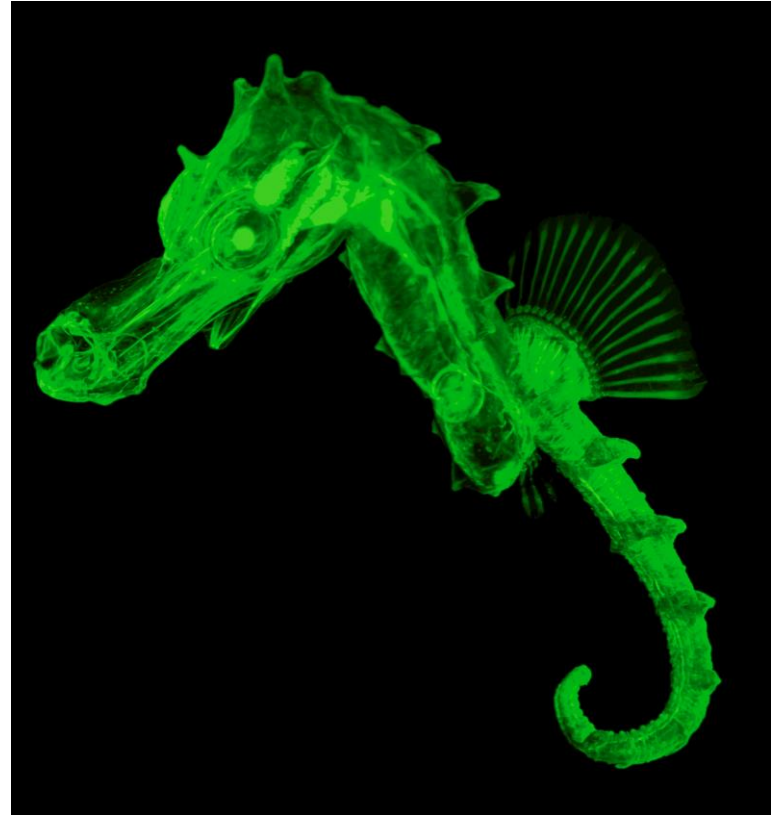
$$b = \frac{2\pi n \omega_0^2}{\lambda} = \frac{n \omega_0}{NA}$$

Si $\omega_0 \sim 0.5 \mu\text{m}$ \rightarrow $b \sim 1 \mu\text{m}$

Axial resolution: “light sheet”

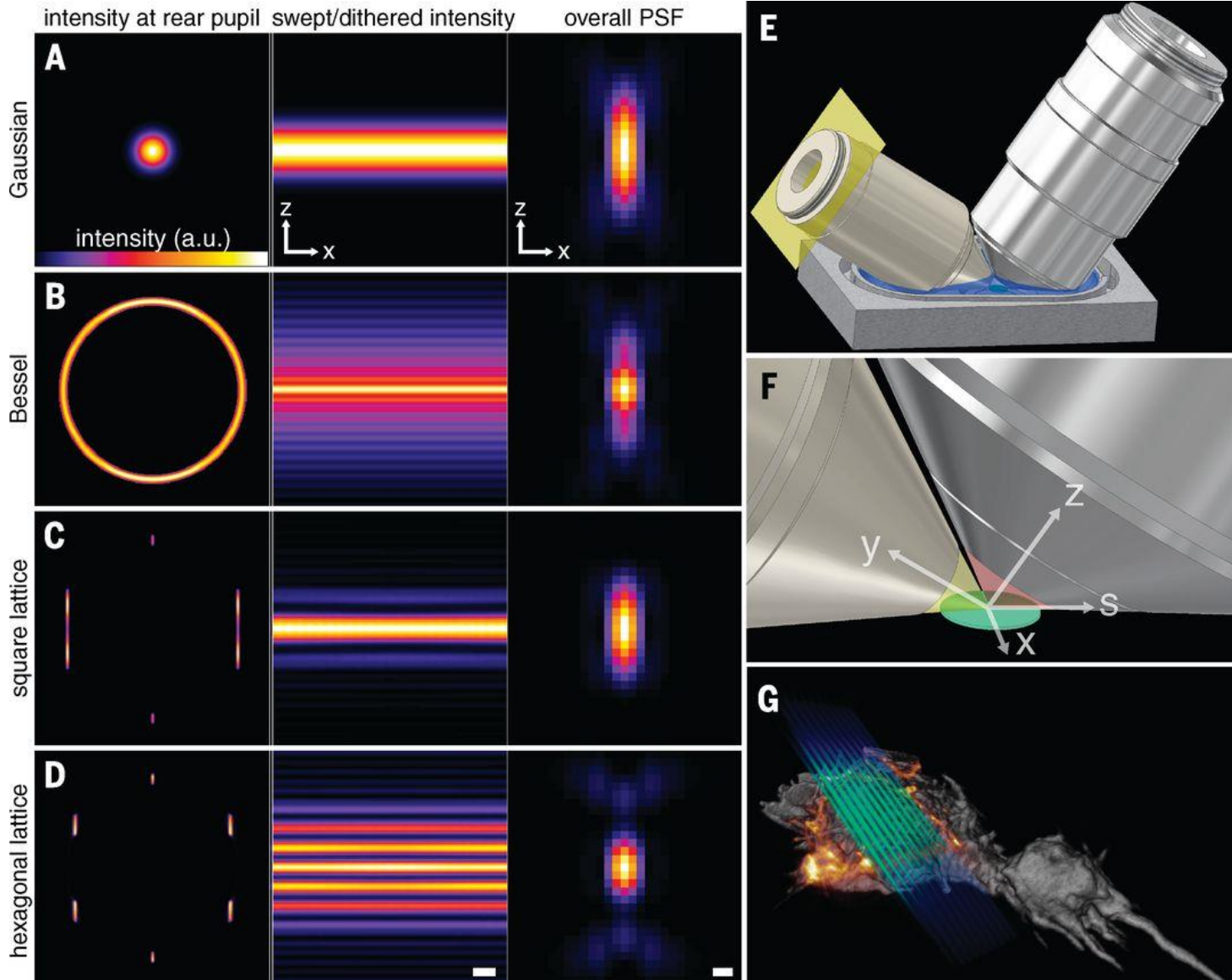


Hans-Ulrich Dodt et al Nature Methods 4, 2007

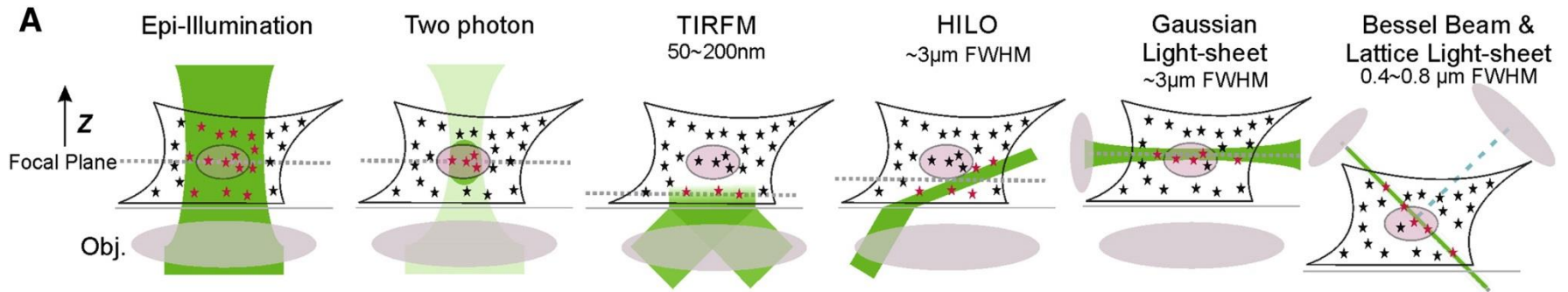


<https://www.miltenyibiotec.com>

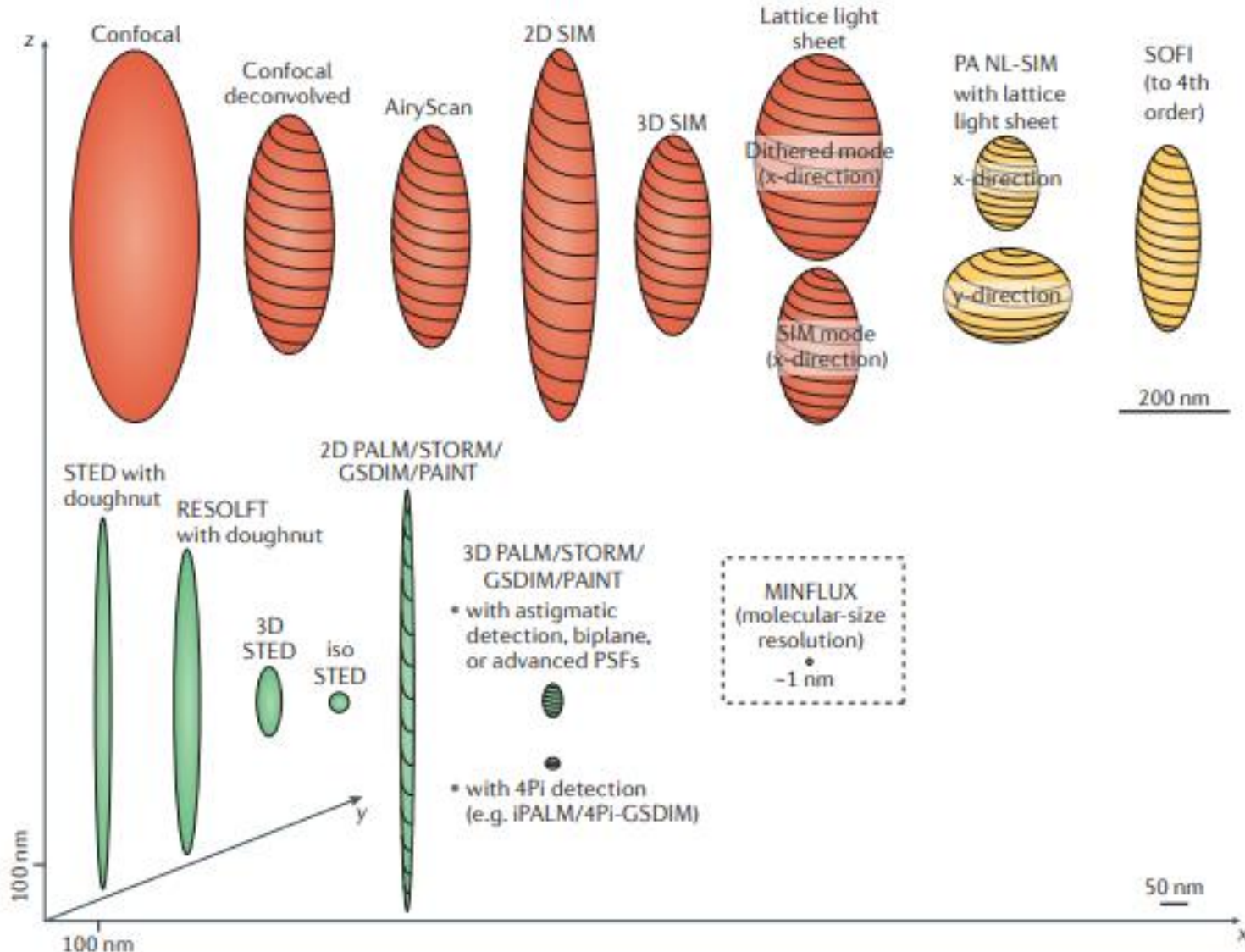
Axial resolution: "light sheet"



Confinamiento axial



Conclusion





L'IdA vise à élucider les principes du fonctionnement du système auditif, de la perception et de la cognition auditives, et de l'intégration multisensorielle, pour comprendre la plasticité du système auditif, pour déchiffrer comment le génome et l'environnement sonore interagissent.

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<https://www.institut-audition.fr/fr>

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