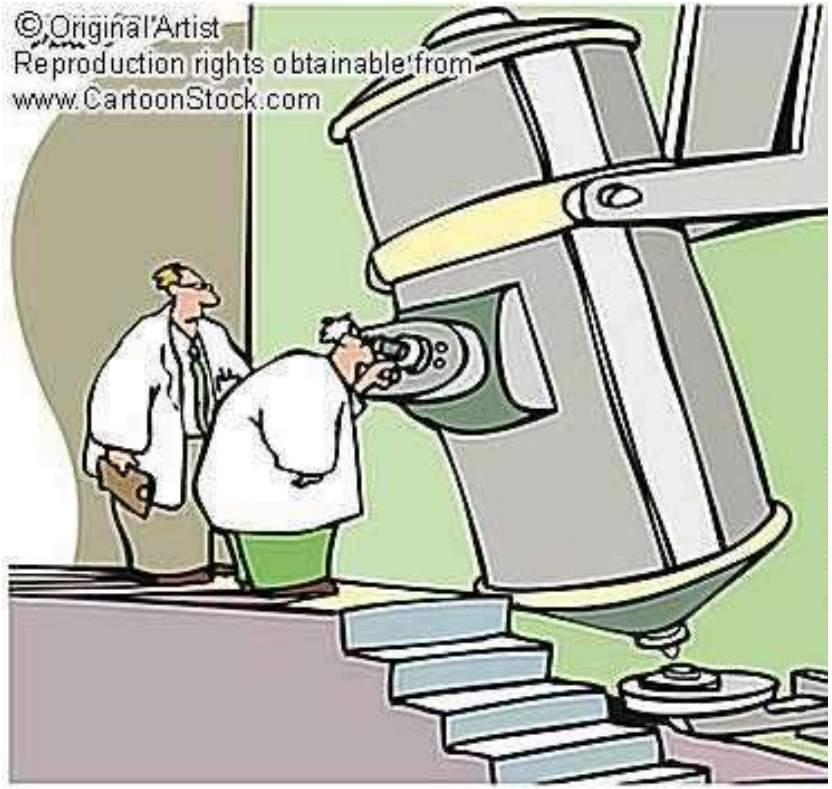
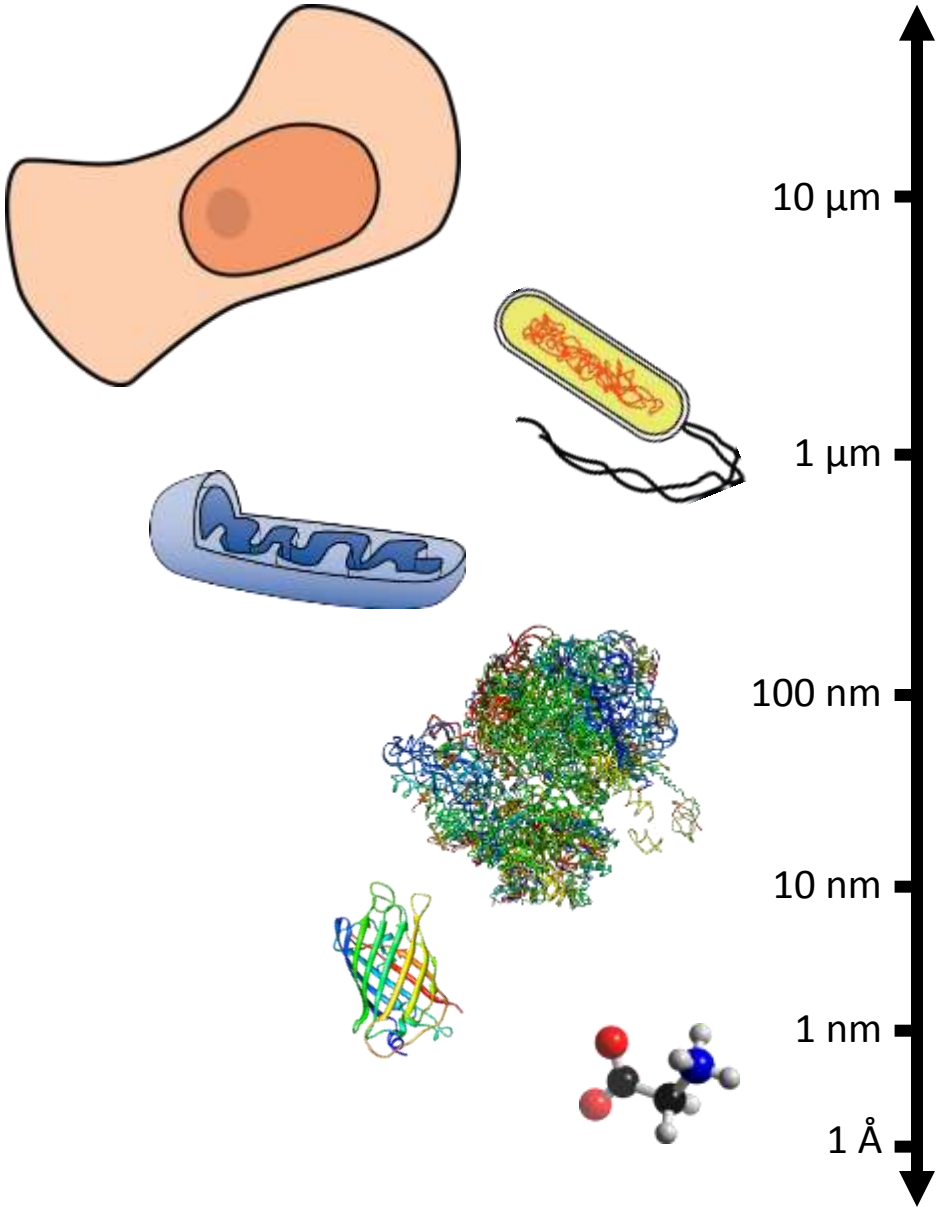
Super-Resolution Microscopy

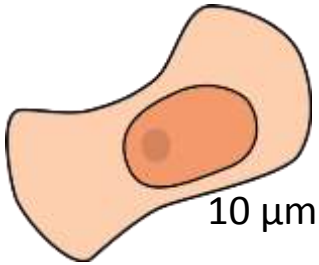
Structured Illumination



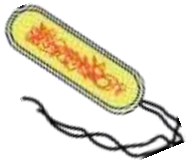
Bo Huang

Looking into microscopic world of life…

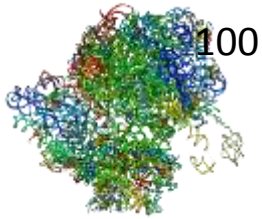




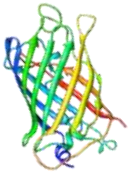
10 µm



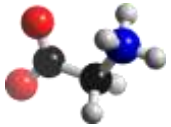
1 µm



100 nm

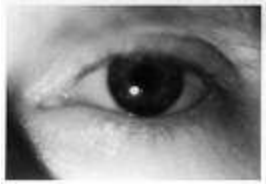


10 nm



1 nm

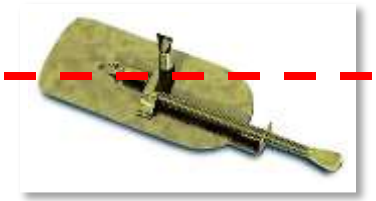
1 Å

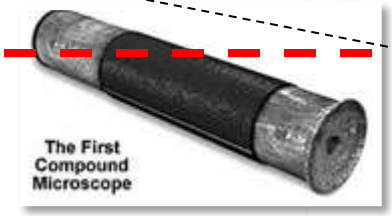


Naked eye: ~ 50-100 µm

1595, Zaccharias and Hans Janssen
First microscope, 9x magnification

Antonie van Leeuwenhoek
(1632-1723), 200x



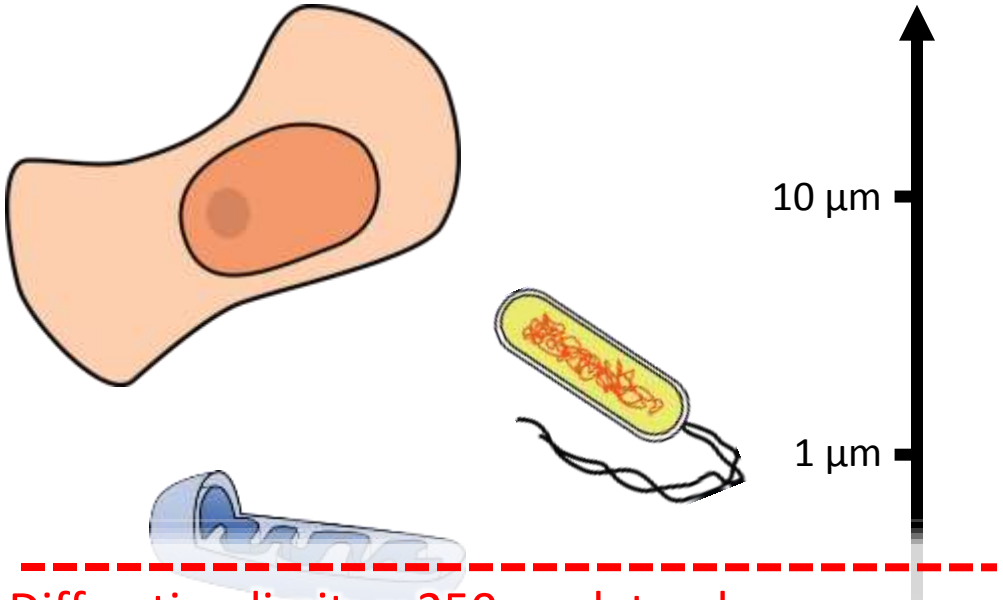


Compound microscope
>1000x

Ernst Abbe (1840-1905)
The "physical" diffraction limit

1600 1700 1800 1900 2000

The diffraction barrier



10 µm

1 µm

Diffraction limit: ~ 250 nm lateral

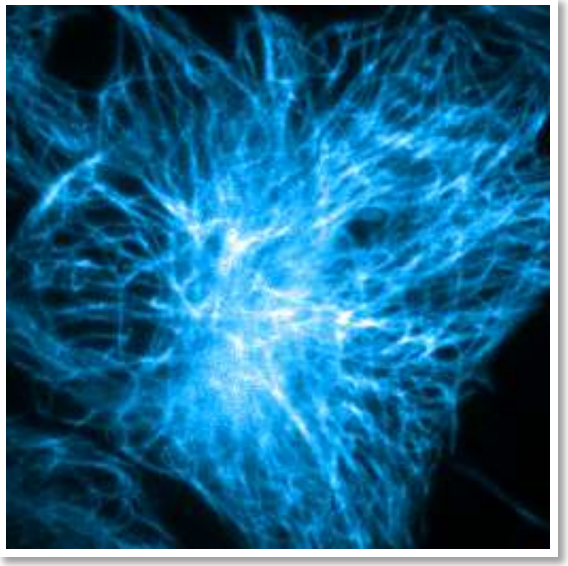
~ 600 nm axial

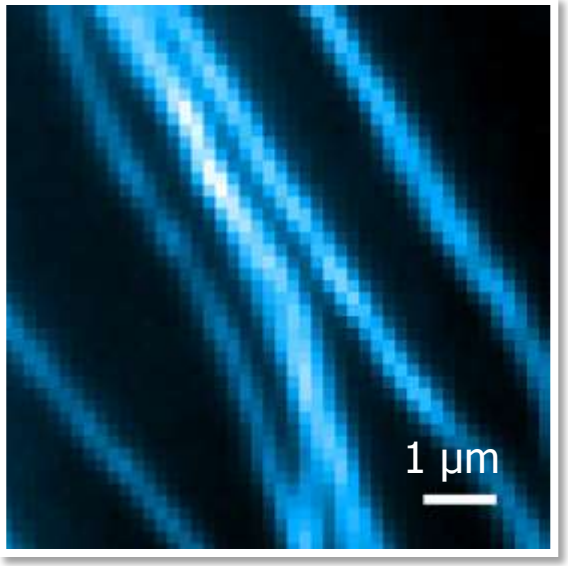
100 nm

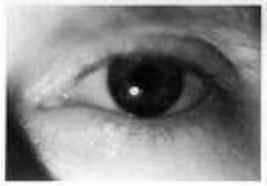
10 nm

1 nm

1 Å

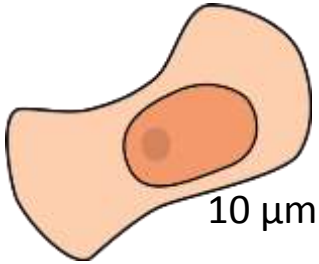






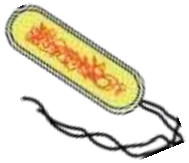
Naked eye: ~ 50-100 µm

$$D \approx \frac{d_{Diffraction}}{S}$$



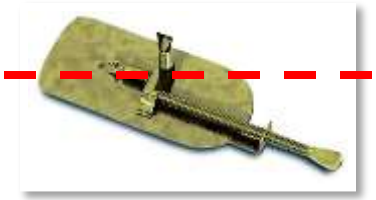
10 µm

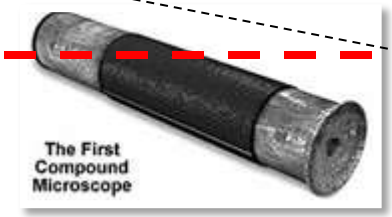
1595, Zaccharias and Hans Janssen
First microscope, 9x magnification



1 µm

Antony Van Leeuwenhoek
(1632-1723), 200x



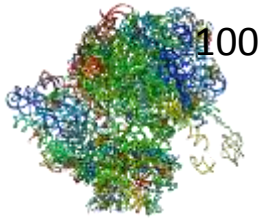


Compound microscope
>1000x

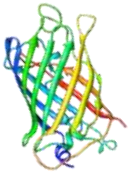
Ernst Abbe (1840-1905)
The "physical" diffraction limit

Super-resolution
Optical Microscopy

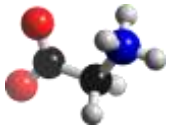
Deconvolution
4-Pi microscopy
...



100 nm



10 nm



1 nm

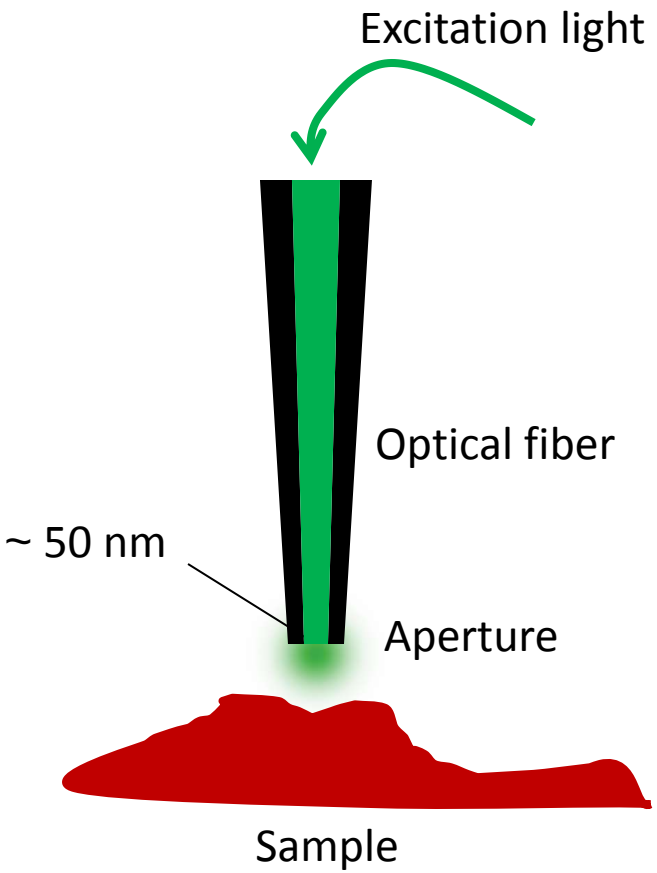
1 Å

1600 1700 1800 1900 2000

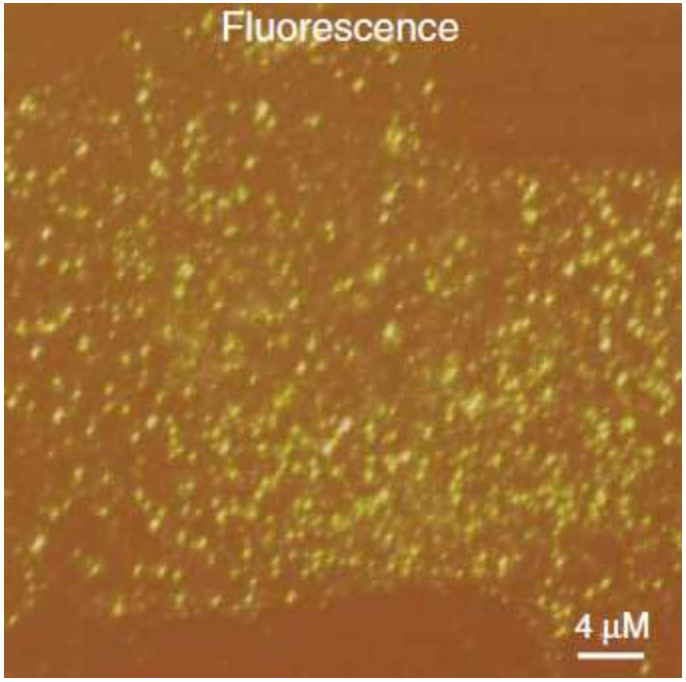
50 years to extend the resolution

- Confocal microscopy (1957)
- Near-field scanning optical microscopy (1972/1984)
- Multiphoton microscopy (1990)
- 4-Pi microscopy / I^5M (1991-1995)
- Structured illumination microscopy (2000)
- Negative refractive index (2006)

Near-field scanning optical microscopy



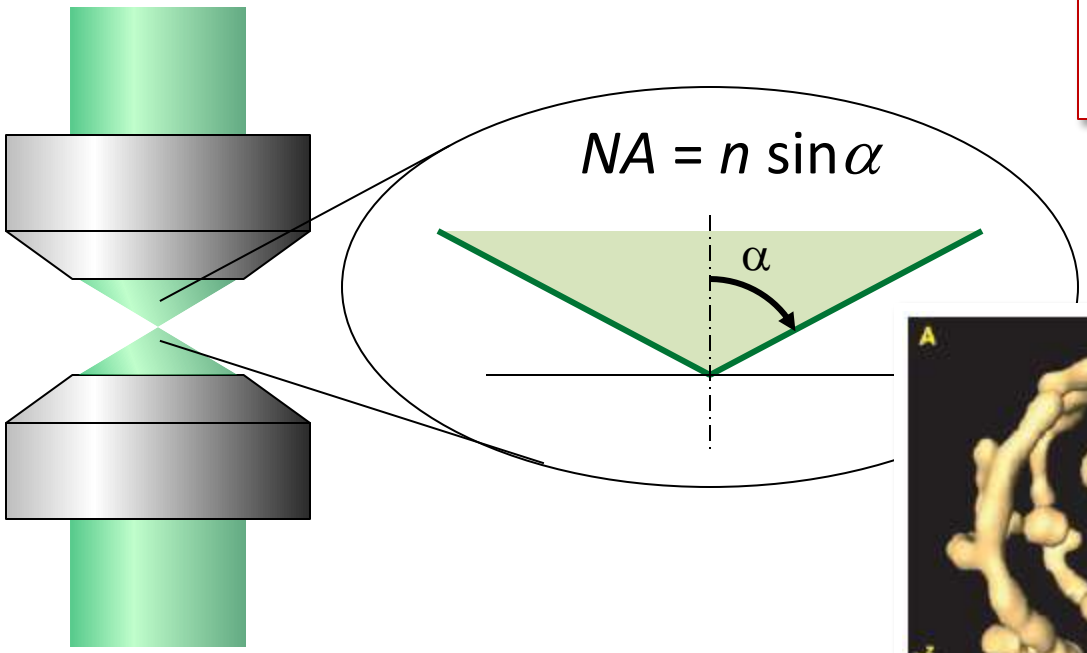
β_2 adrenergic receptor clusters on the plasma membrane

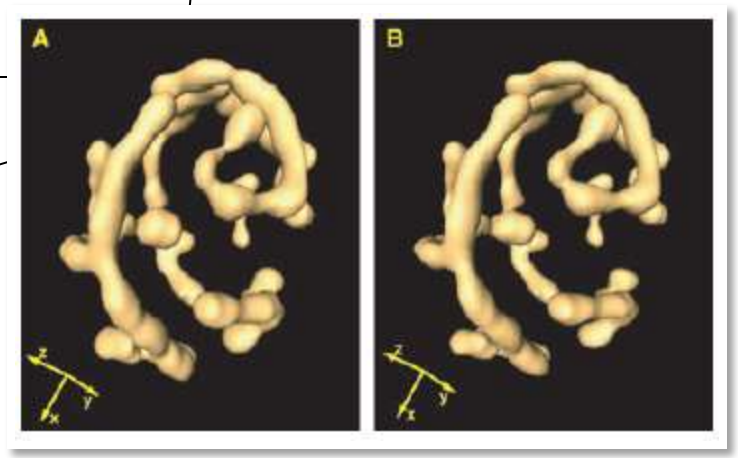


Ianoul et al., 2005

4-Pi / I^{5}M

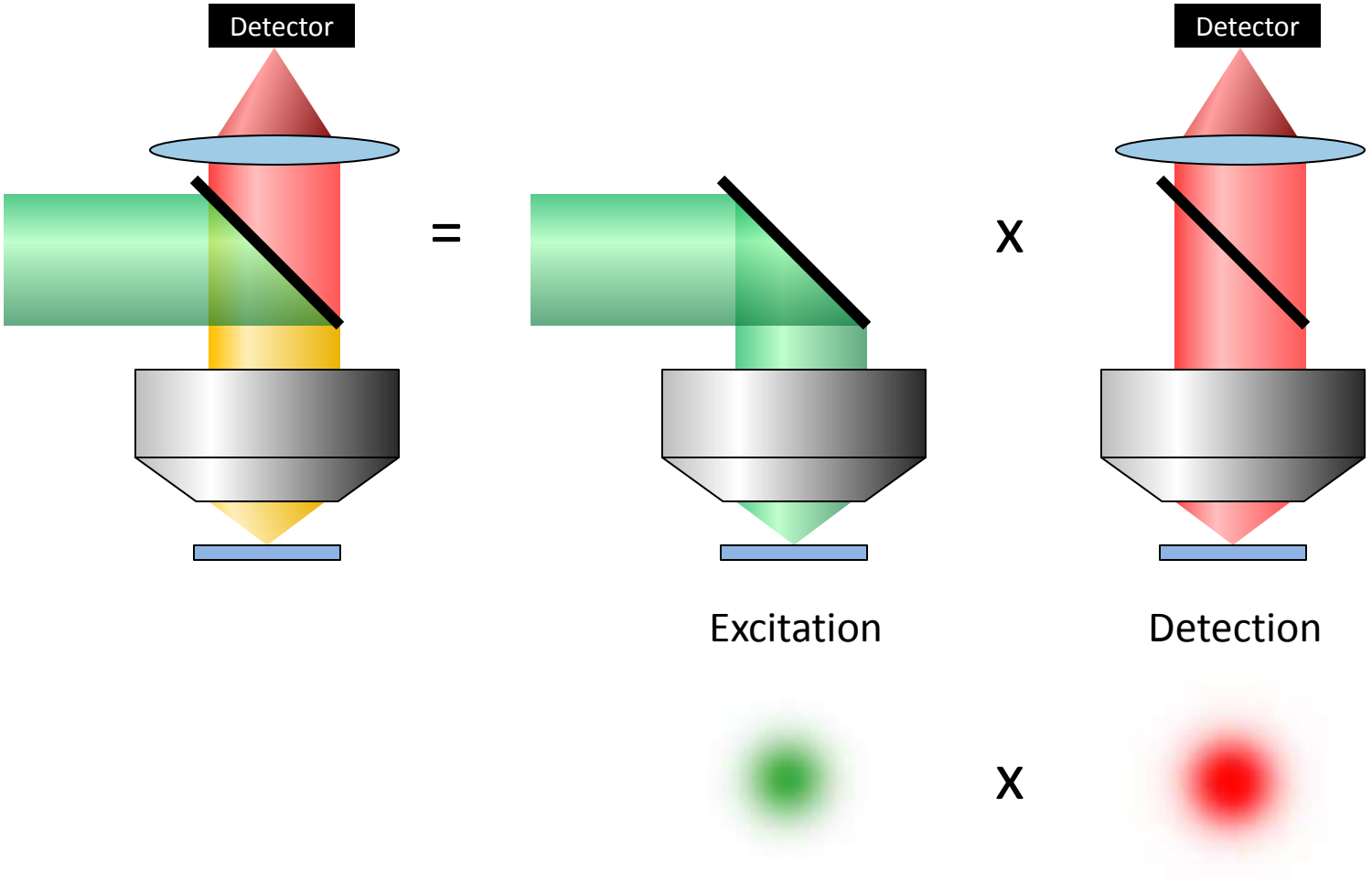
$$d \approx \frac{\lambda}{2\,NA}$$



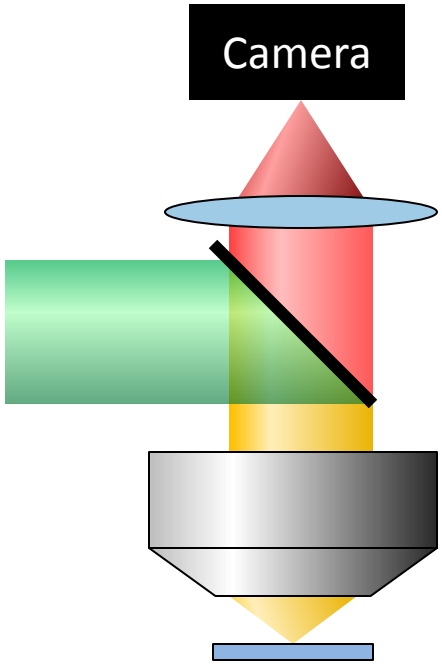


Major advantage:
Similar z resolution as x-y resolution

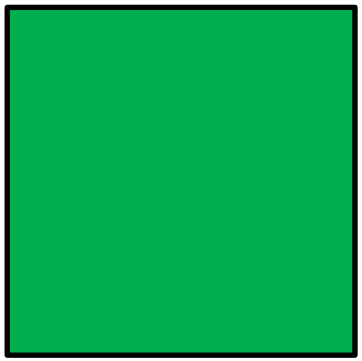
Patterned illumination



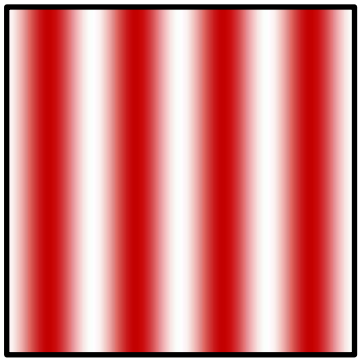
Structured Illumination Microscopy (SIM)



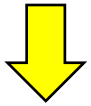
Wide field illumination



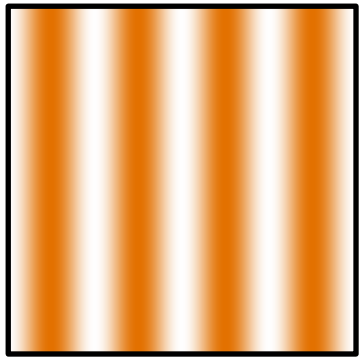
Diffraction-limited detection



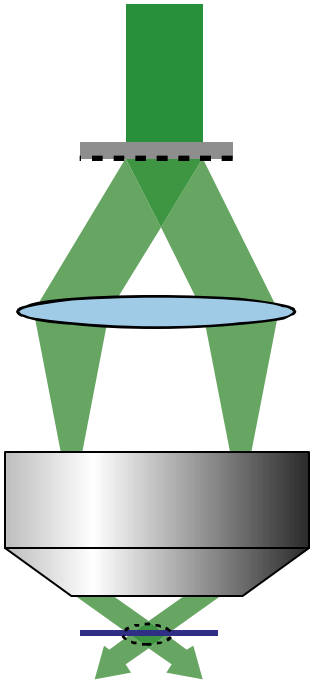
×



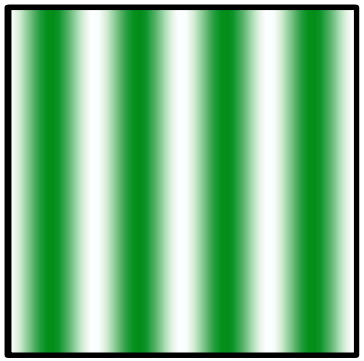
Diffraction-limited image



Structured Illumination Microscopy (SIM)

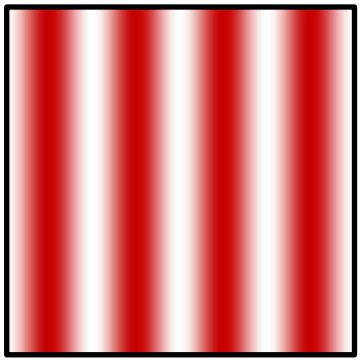


Structured illumination

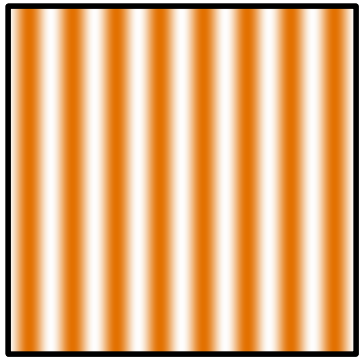


×

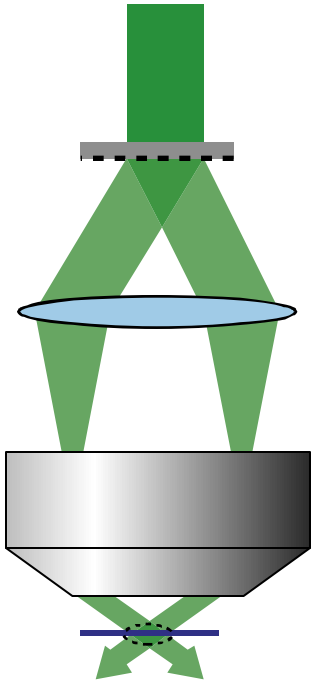
Diffraction-limited detection



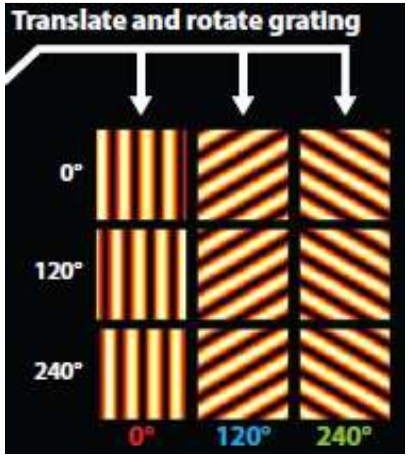
Diffraction-limited image



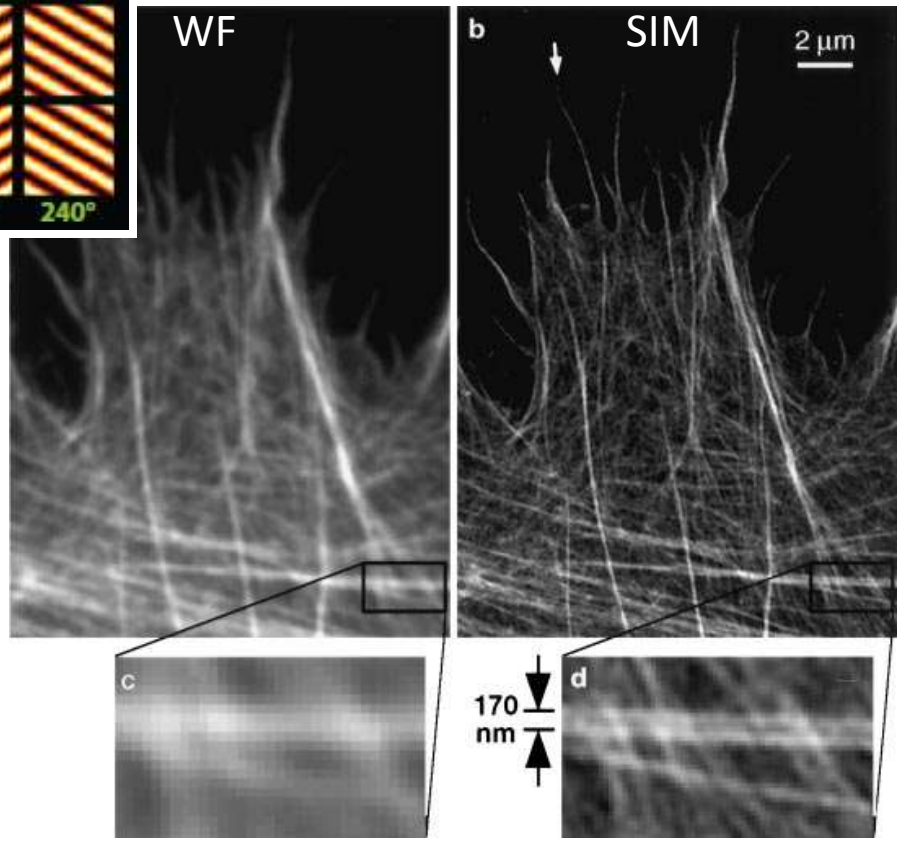
Structured Illumination Microscopy (SIM)

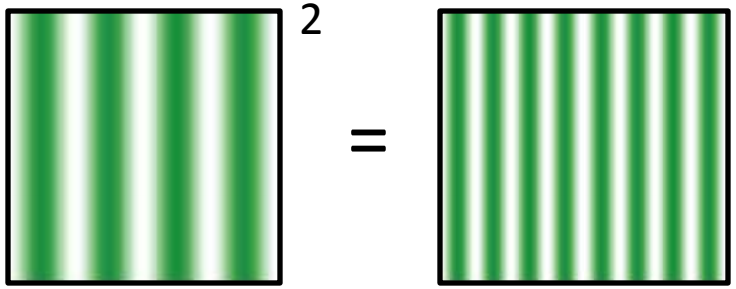


9 images



Reconstruction

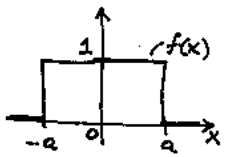




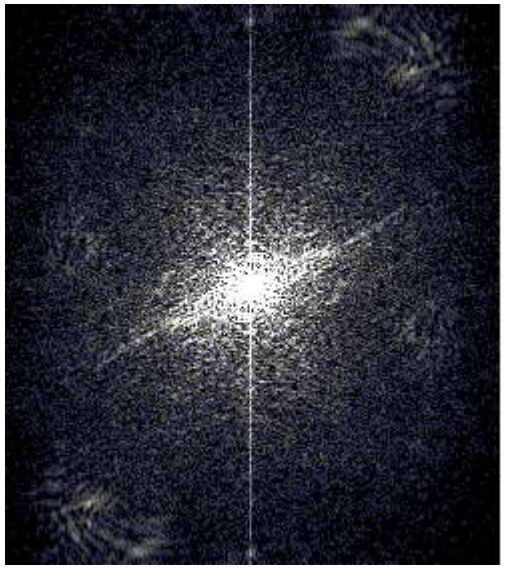
Gustafsson, J Microscopy 2000

Being (slightly) more rigorous about SIM

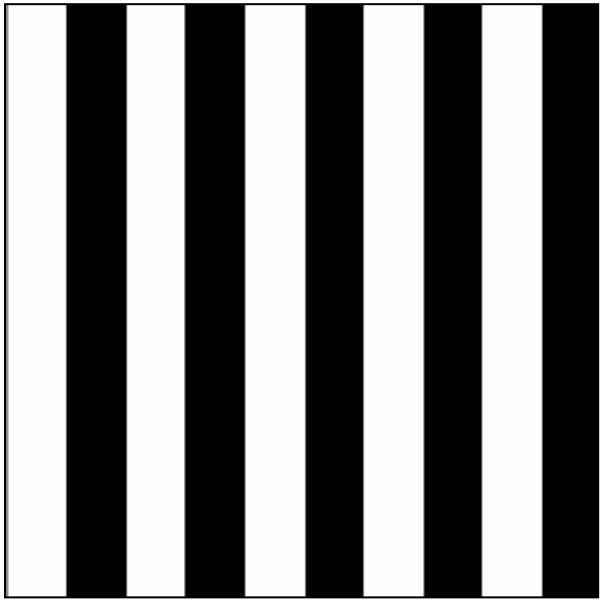
Fourier transform: Examples

- $f(x) = \delta(x) \;\rightarrow\; \tilde{f}(k) = 1$
- $f(x) = 1 \;\rightarrow\; \tilde{f}(k) = 2\pi\,\delta(k)$
- $f(x) = e^{-a|x|} \;\rightarrow\; \tilde{f}(k) = \frac{2a}{k^2 + a^2}$
- $f(x) = \frac{1}{x^2 + a^2} \;\rightarrow\; \tilde{f}(k) = \frac{\pi}{a} e^{-a|k|}$
- $f(x) = e^{-a\frac{x^2}{2}} \;\rightarrow\; \tilde{f}(k) = \sqrt{\frac{2\pi}{a}}\, e^{-\frac{1}{a}\cdot\frac{k^2}{2}}$
- 

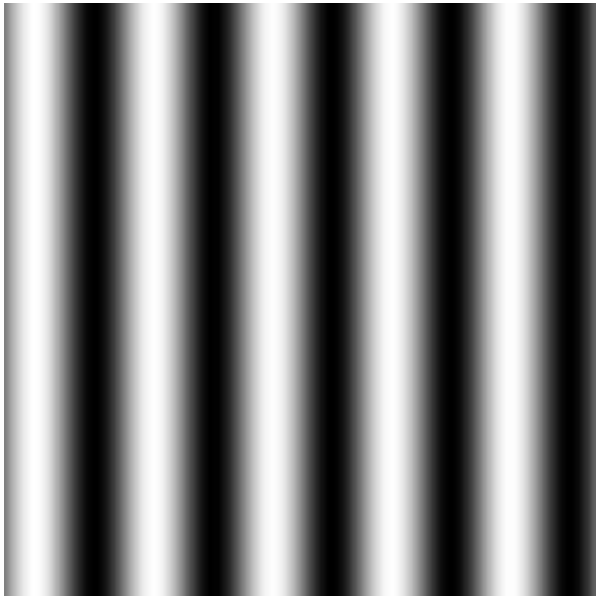
$\;\rightarrow\; \tilde{f}(k) = 2\,\frac{\sin(ak)}{k}$

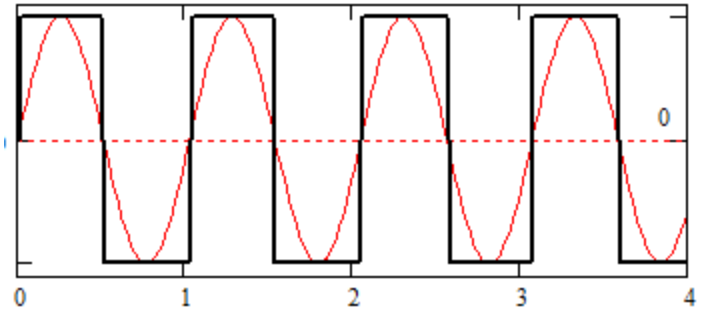


Fourier transform and spatial frequencies

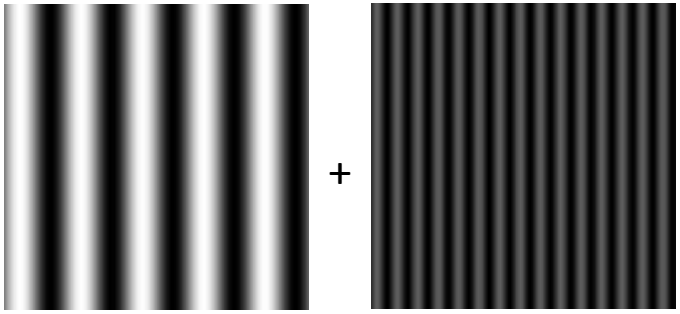


?



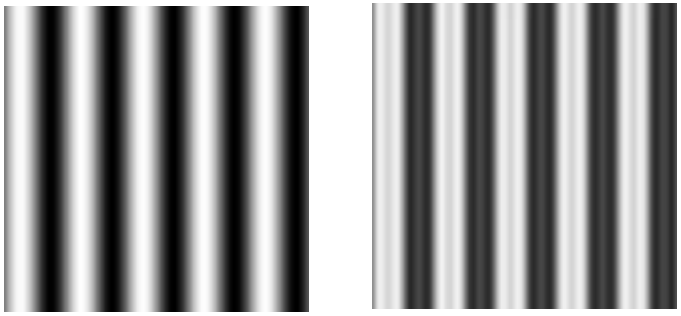


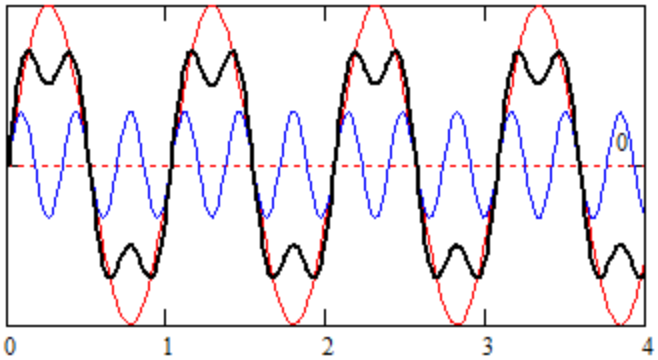
Fourier transform and spatial frequencies



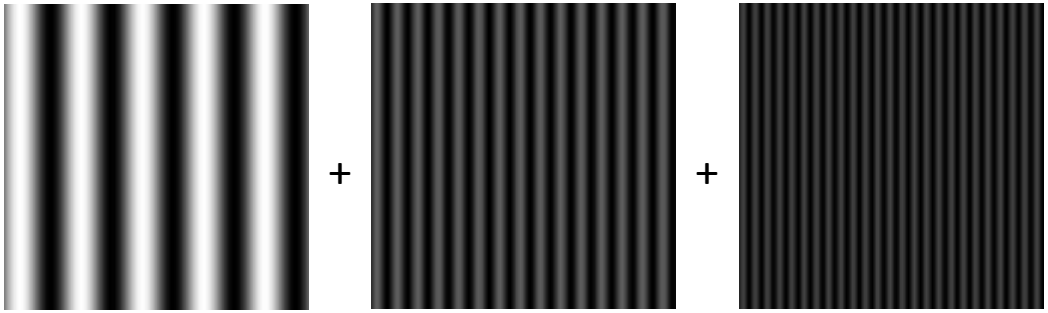
+

Summed image

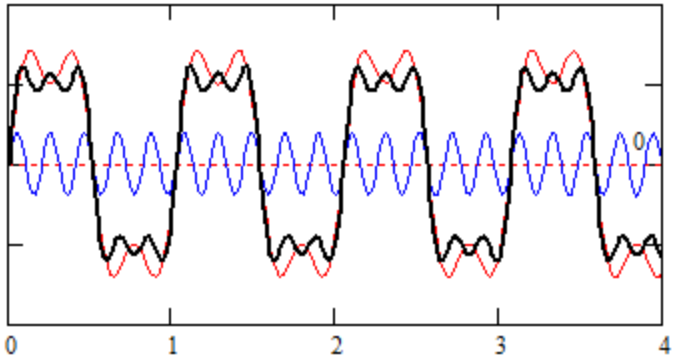




Fourier transform and spatial frequencies

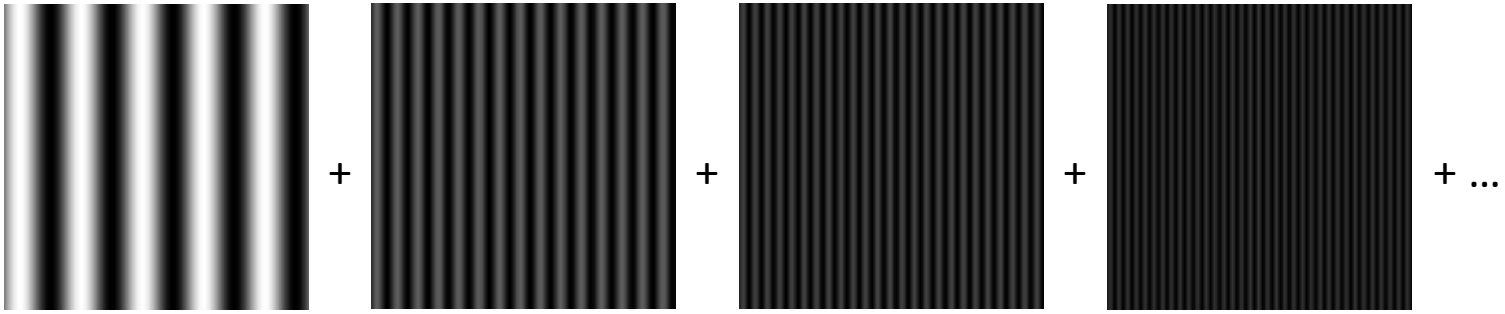


Summed image



Fourier transform and spatial frequencies

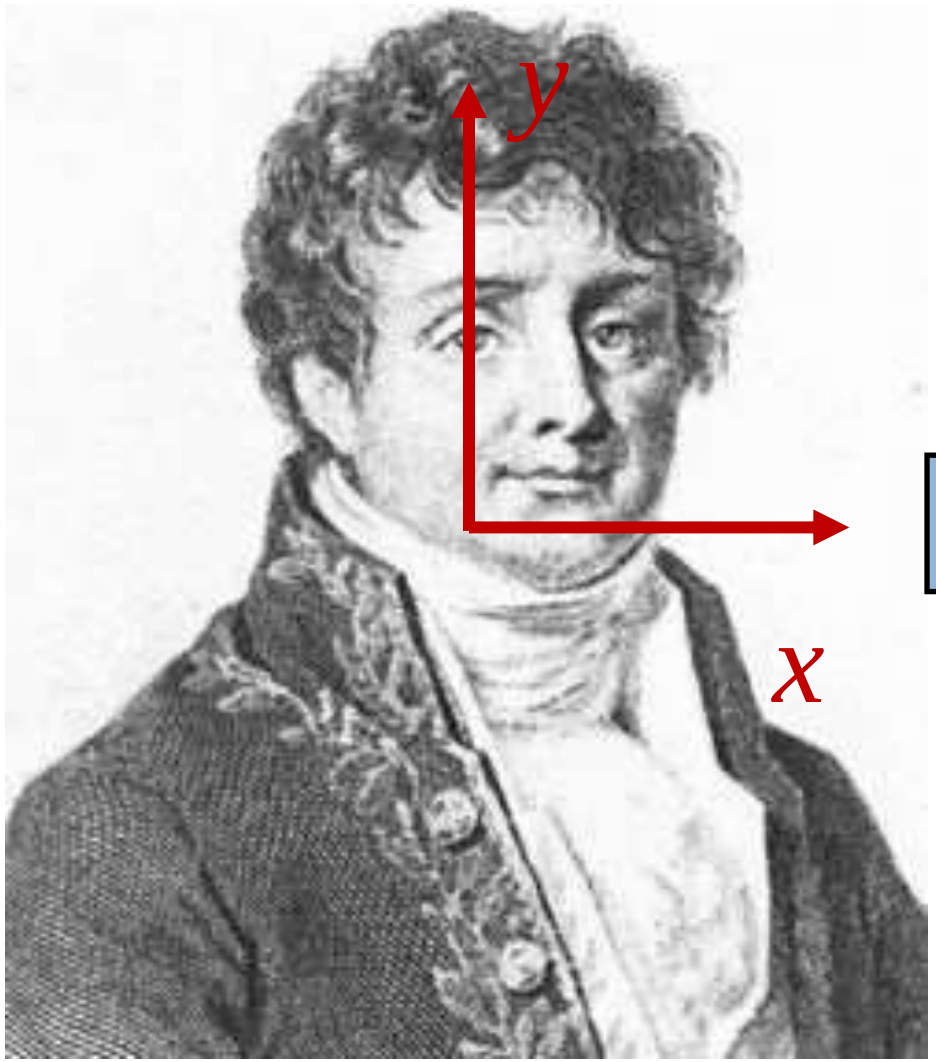
Discrete spatial frequencies

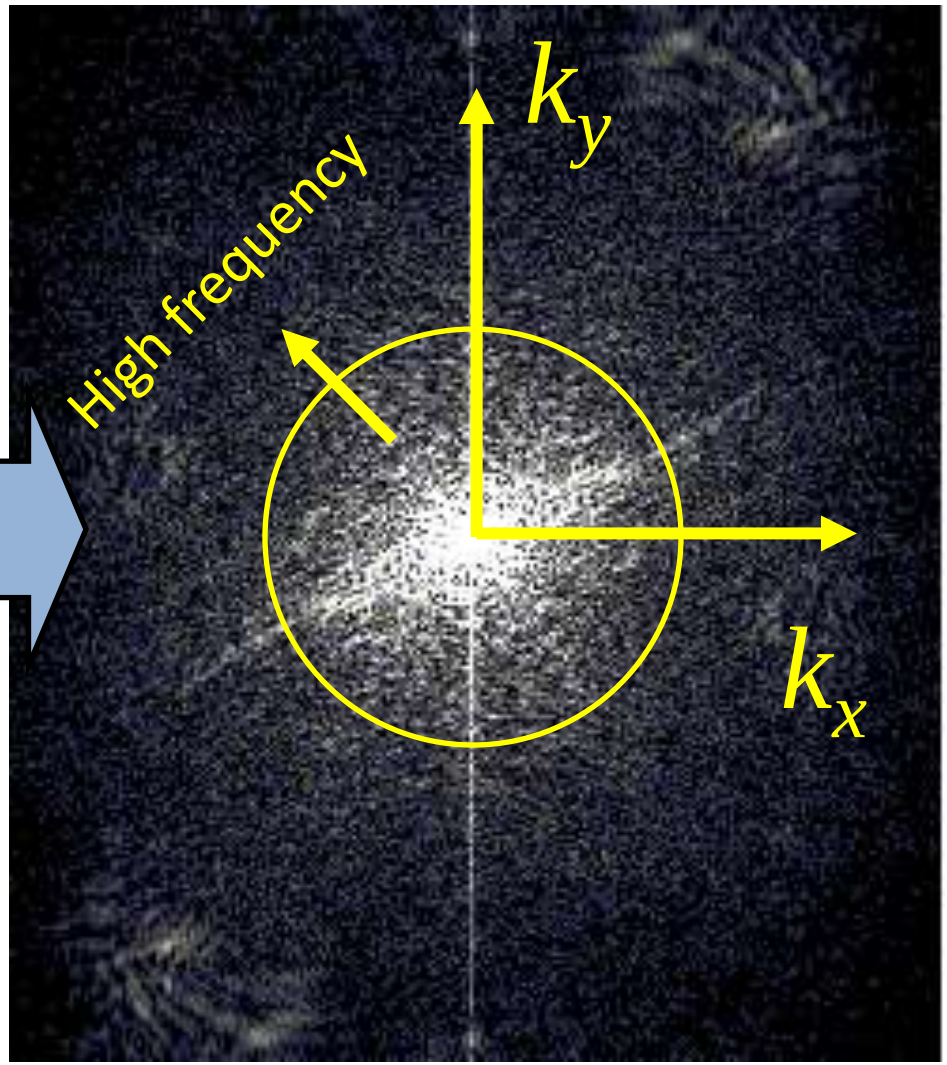


Summed image

$$G(x) = \sum F(k) \sin(k\,x)$$

Fourier transform and spatial frequencies

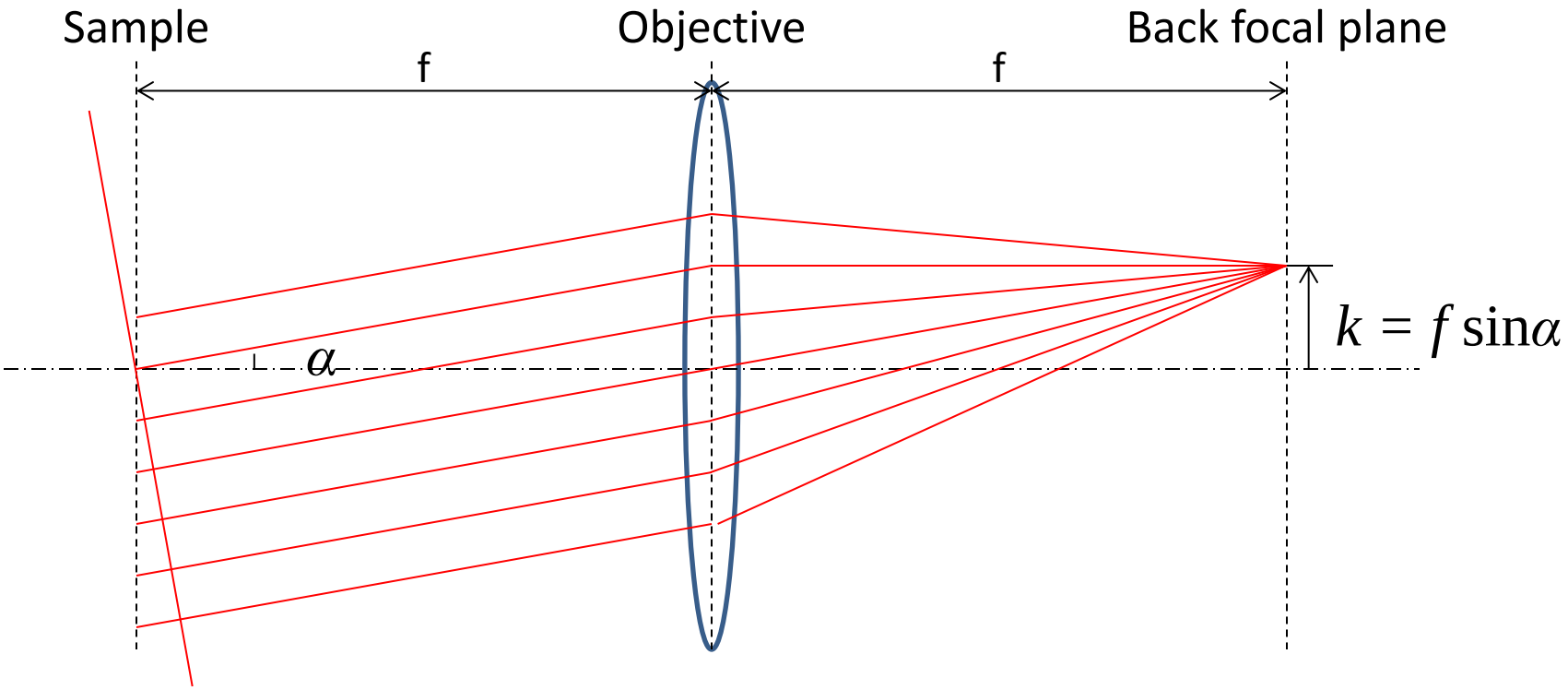




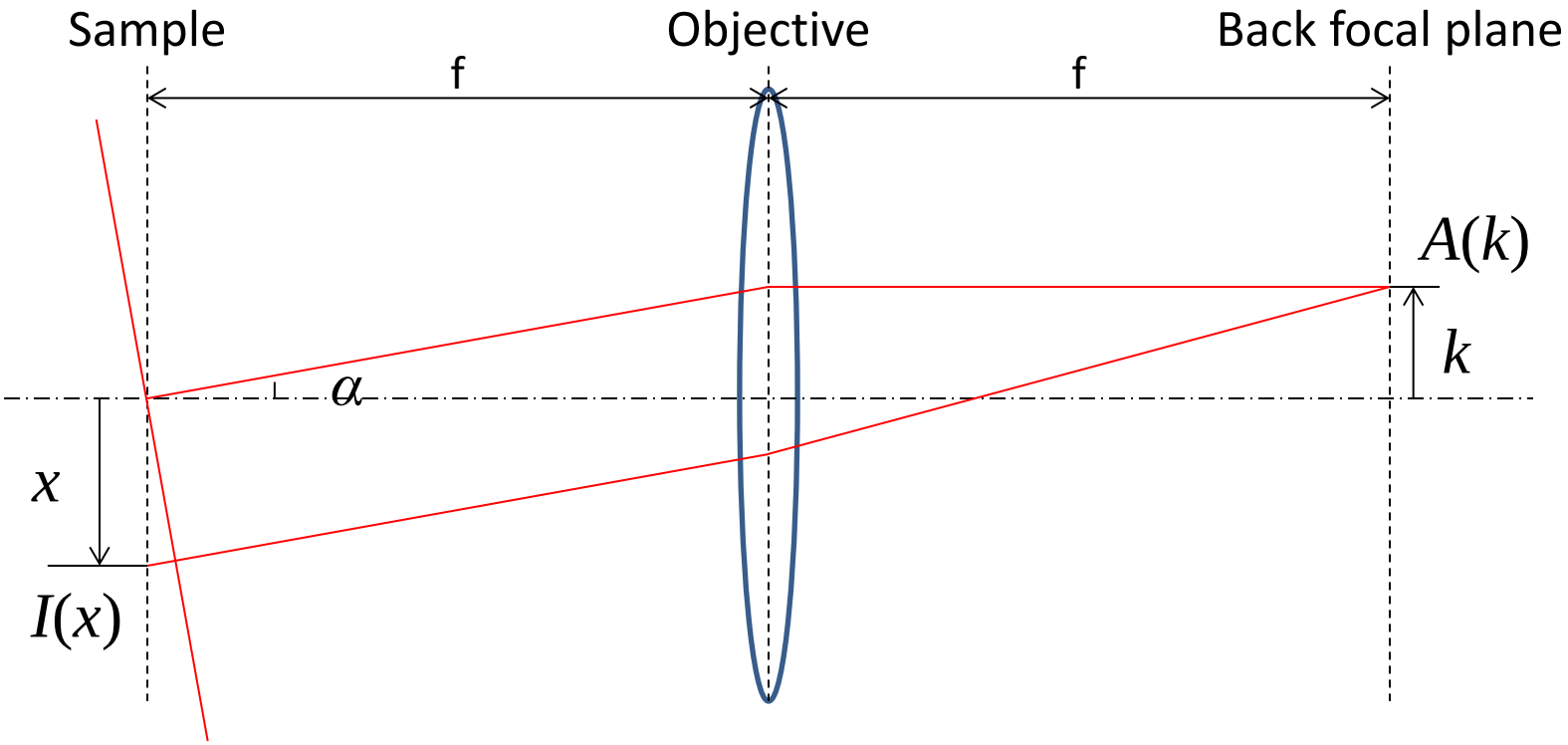
Original Image (real space)

Fourier transform (frequency space)

Fourier optics and microscope resolution



Fourier optics and microscope resolution



Phase delay from the mid-point

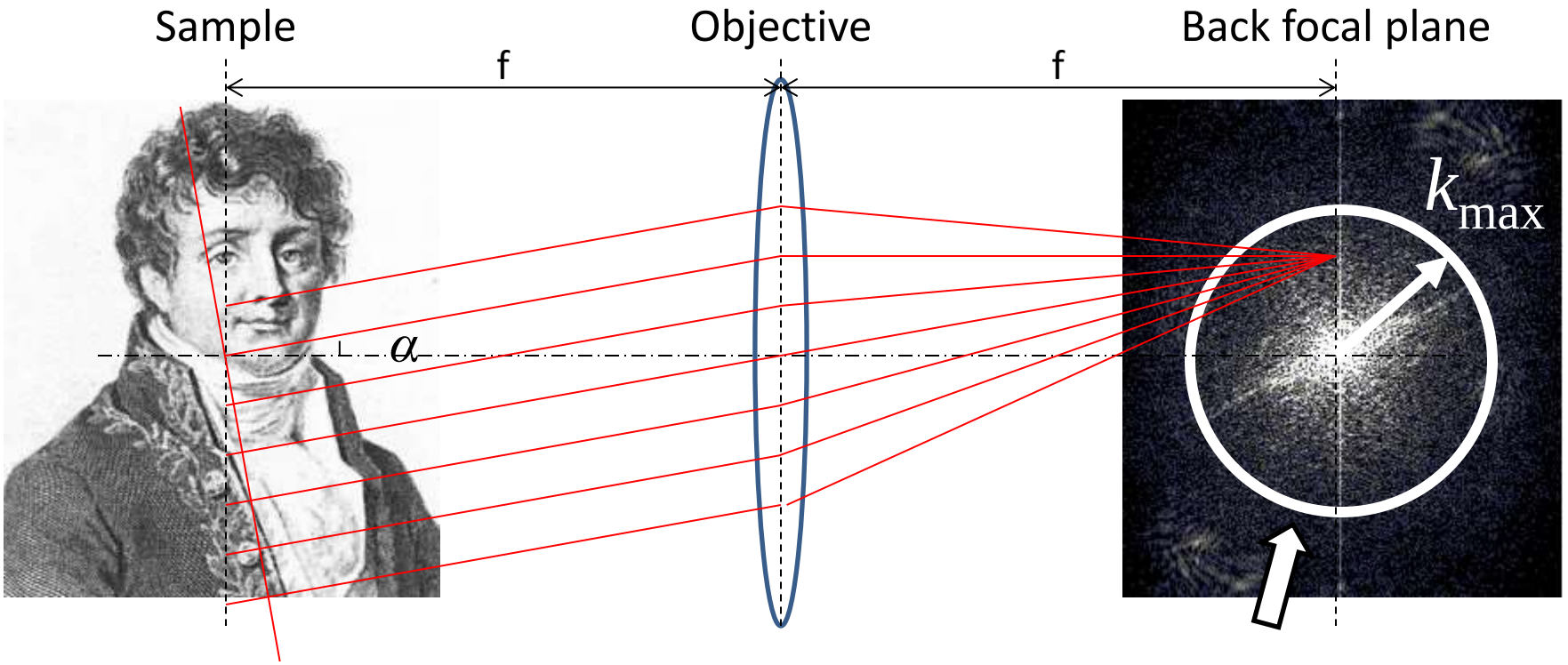
$$\Delta\varphi = x \sin\alpha\ (2\pi/\lambda) = x\ (k / f\ )\ (2\pi/\lambda)$$ assuming refractive index = 1

Light intensity at the sample plane

Fourier Transform!

$$I(x) = \Sigma A(k) \sin(\Delta\varphi) = \Sigma\ A(k) \sin\ (x\ k\ 2\pi/\lambda f)$$

Fourier optics and microscope resolution



Size of the back focal plane

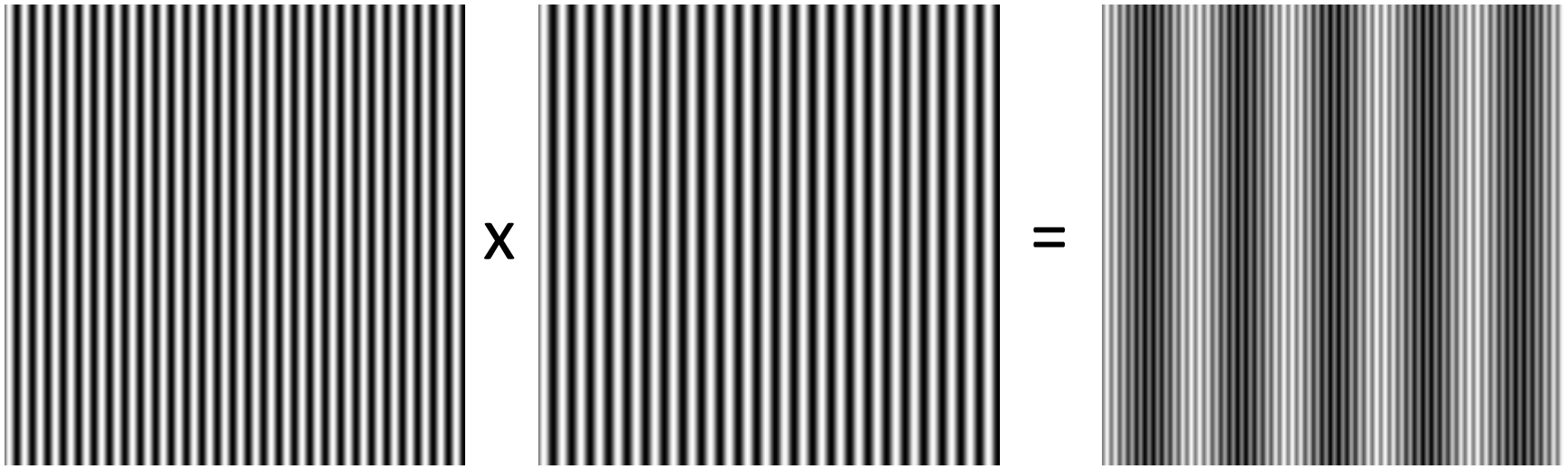
Spatial frequency = $k \cdot 2\pi/\lambda f$

$k_{max} = f \sin\alpha_{max} = f \cdot NA$

Resolution = $\lambda / 2NA$

Extending the measurable freq. range

$$\text{Excitation}(x) \times \text{Sample}(x) = \text{Observed Signal}(x)$$

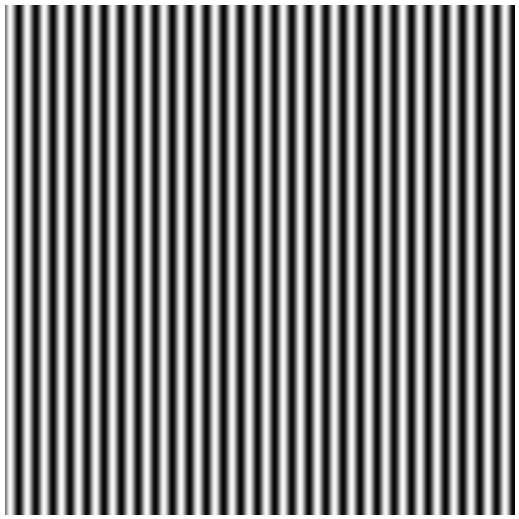


Freq = 30 Freq = 25 Freq = 55 & 5

$$\sin A \cdot \sin B = (\cos(A - B) - \cos(A + B)) / 2$$

Extending the measurable freq. range

Excitation(x) × Sample(x) = Observed Signal(x)

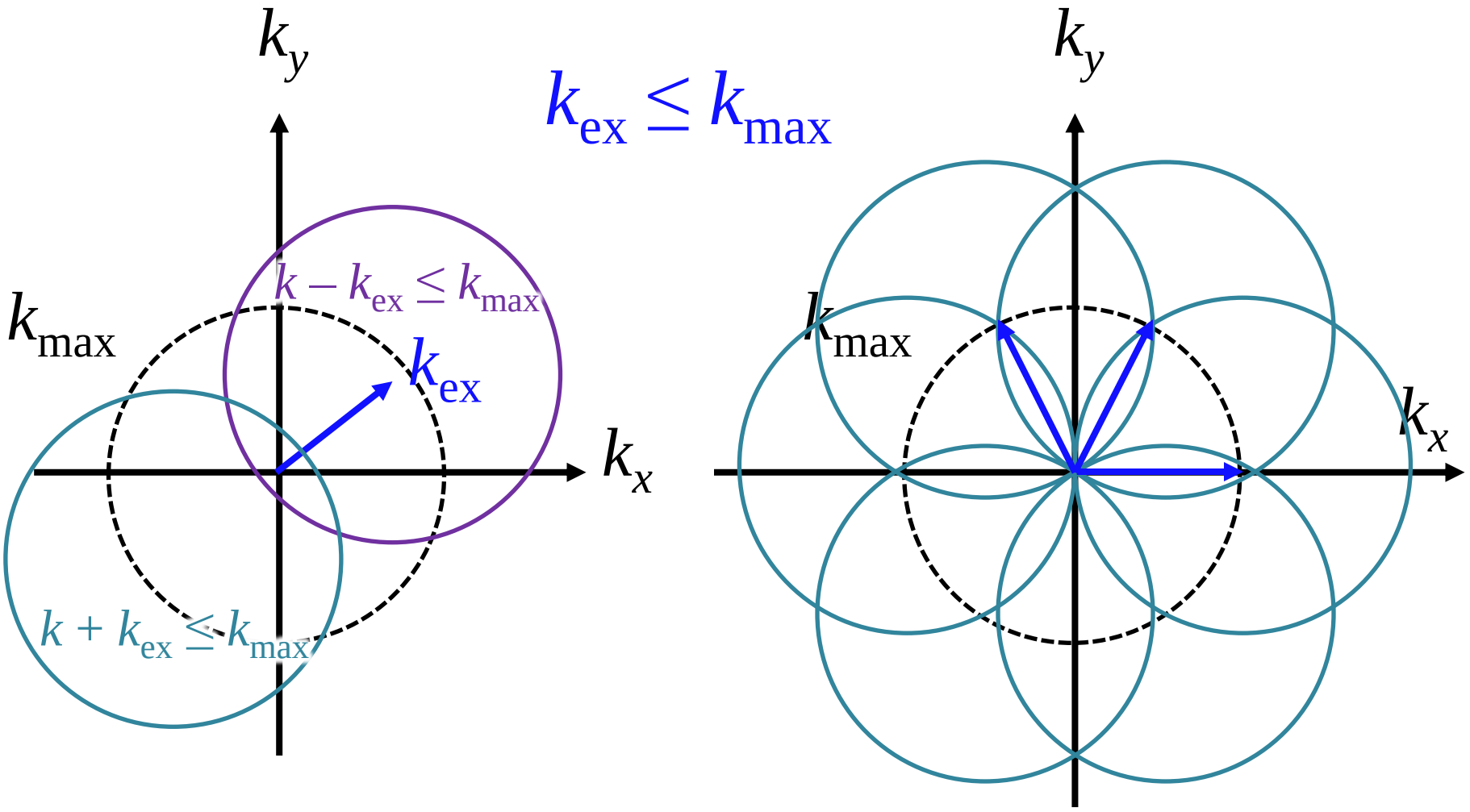


Freq = 30

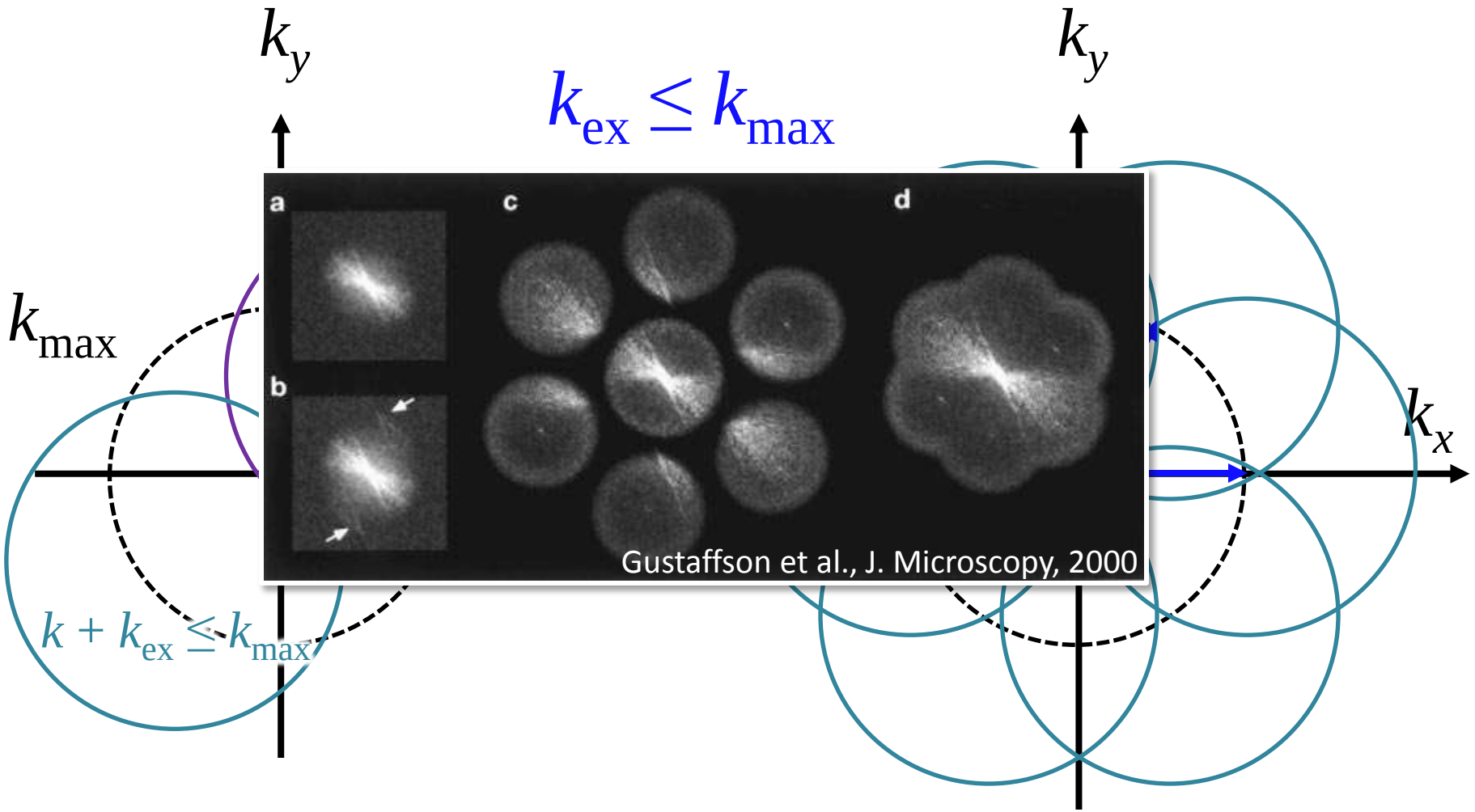
X

$\sin A \cdot \sin$

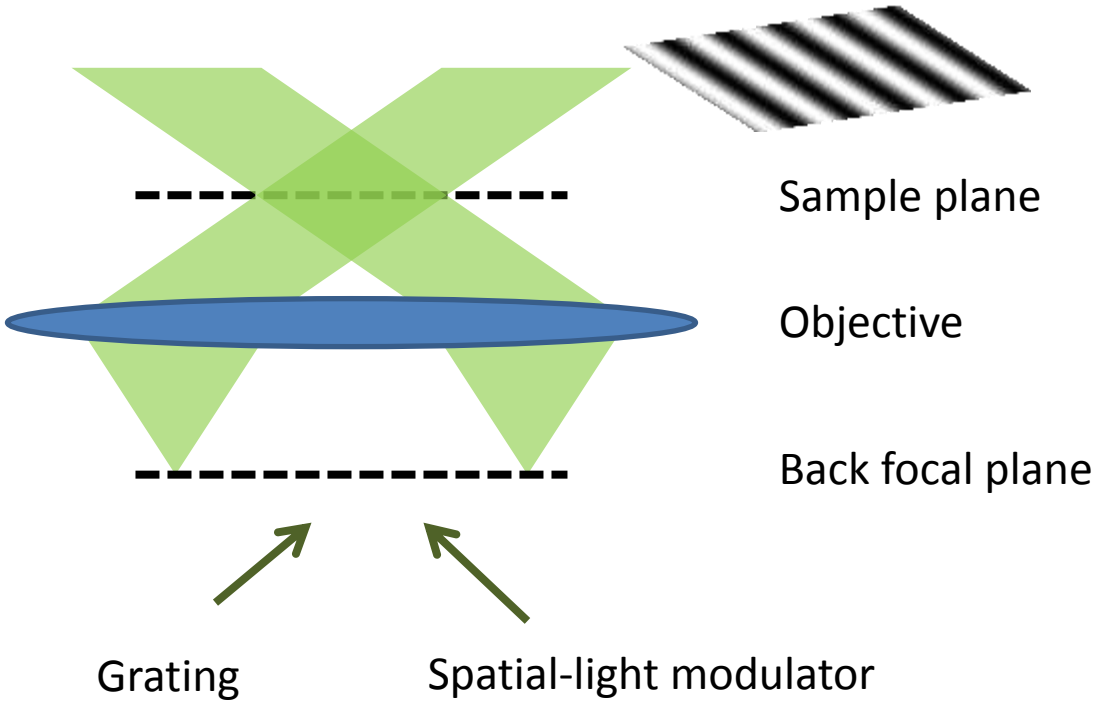
Extending the measurable freq. range

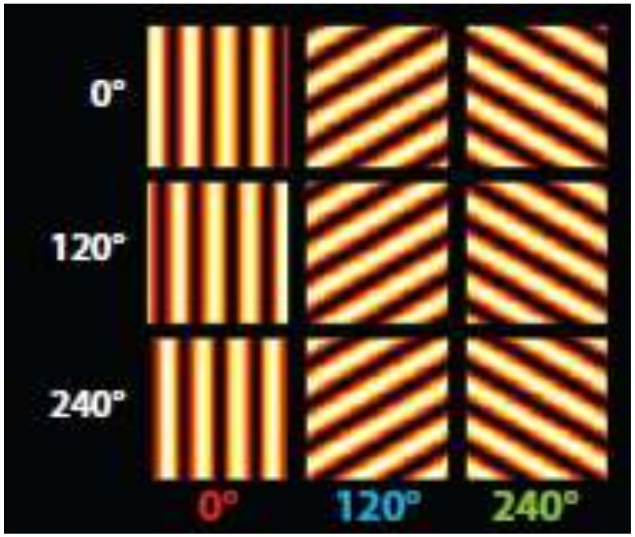


Extending the measurable freq. range

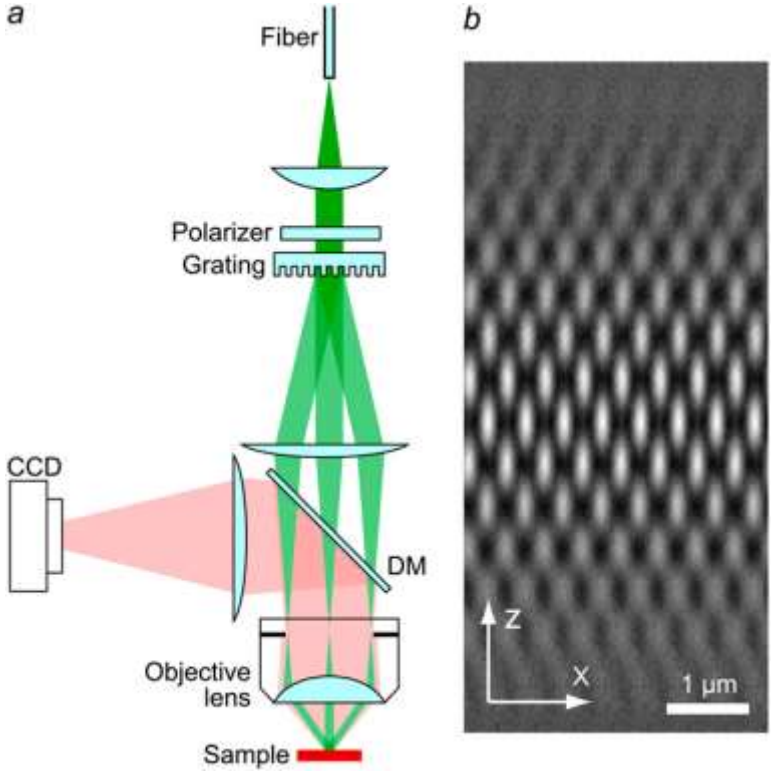


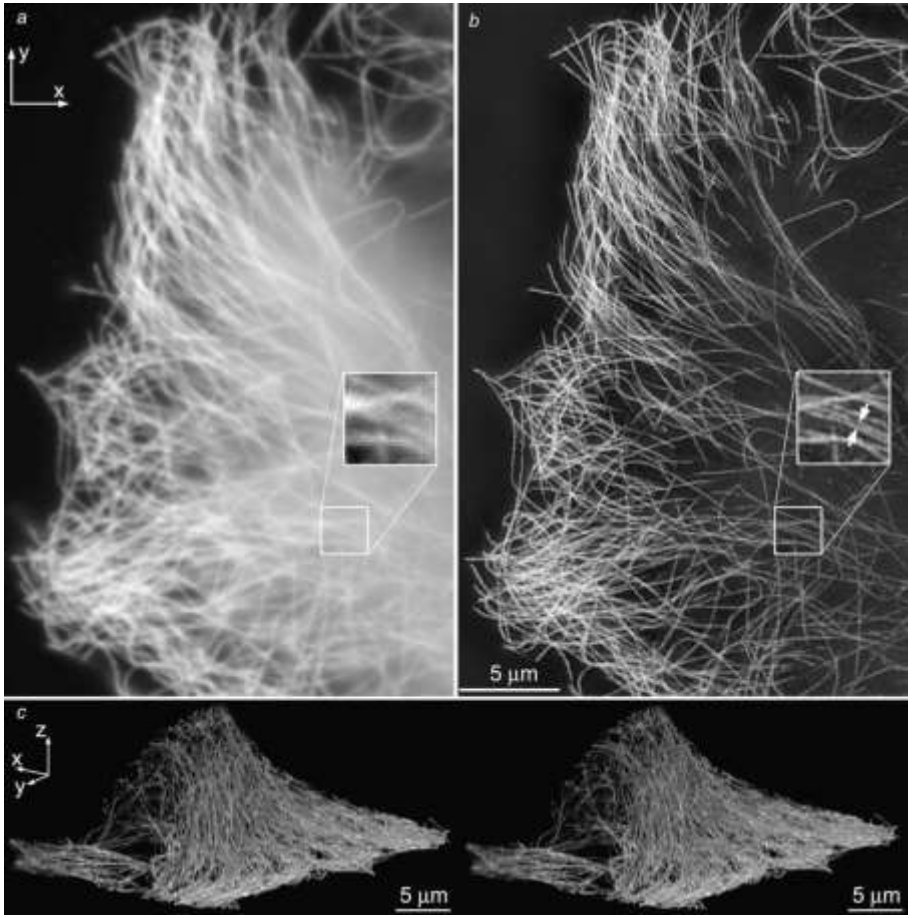
Generating the illumination pattern





3D SIM: better resolution + optical sectioning

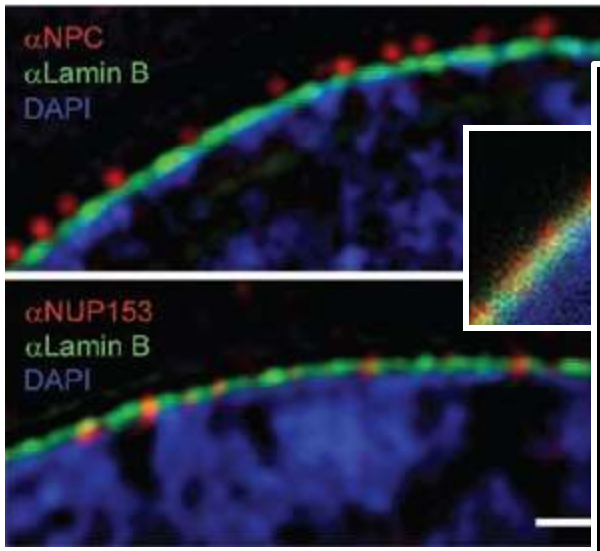


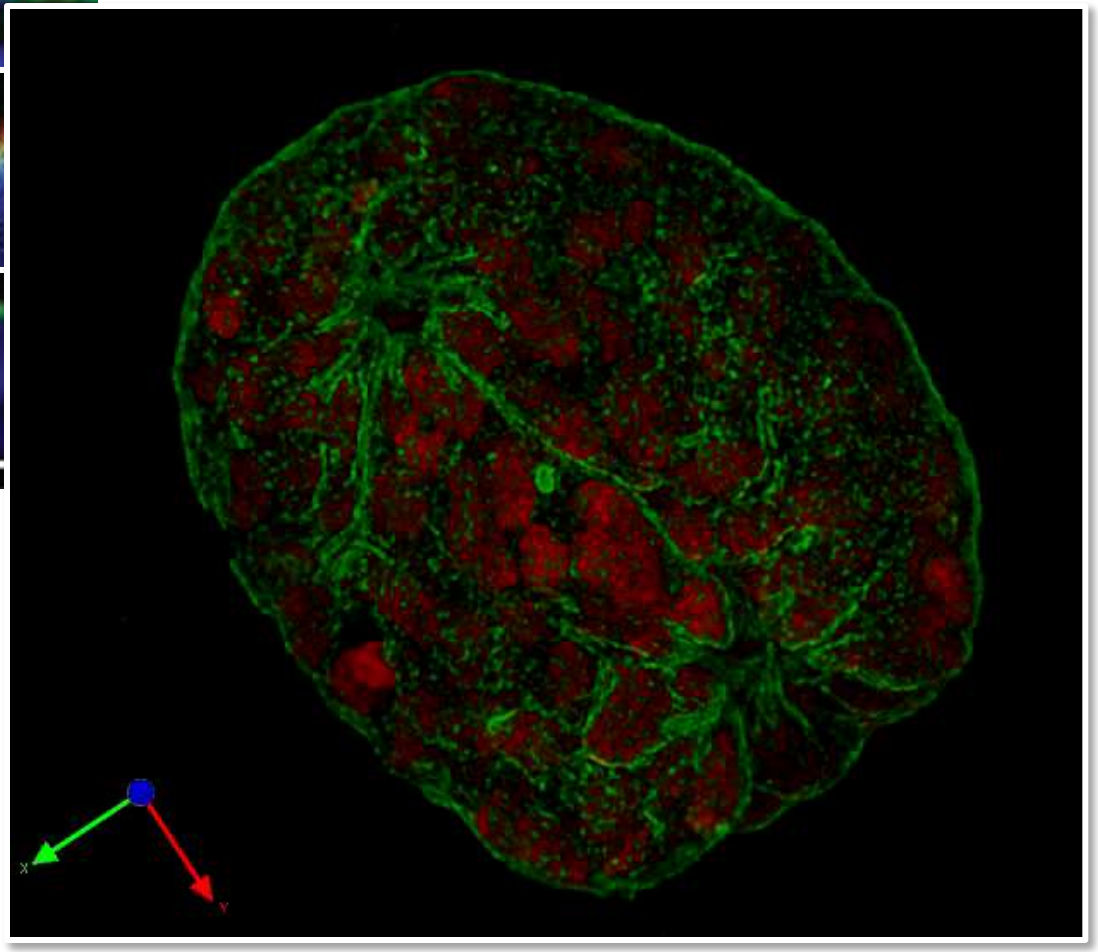


Schermellech et al., Science 2008, Gustafsson et al., Biophys J. 2008

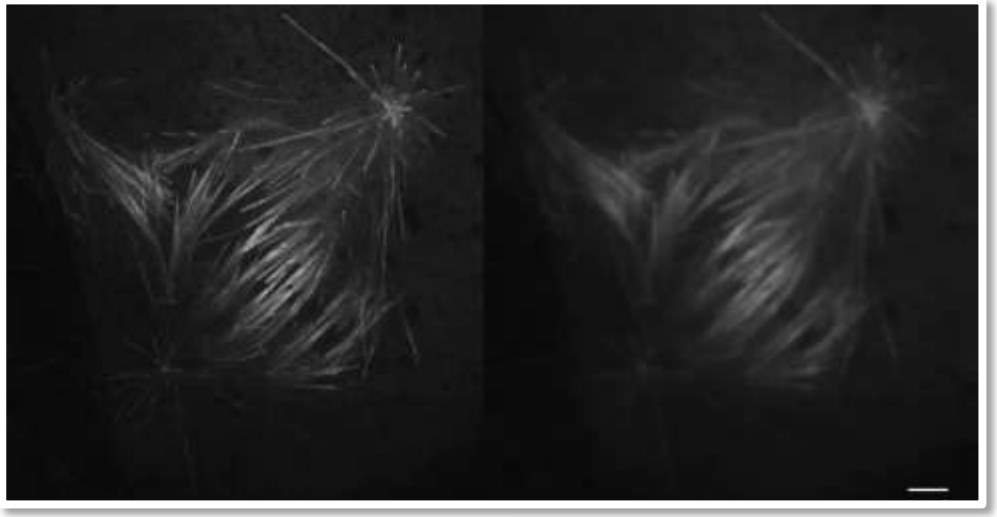
Multicolor SIM

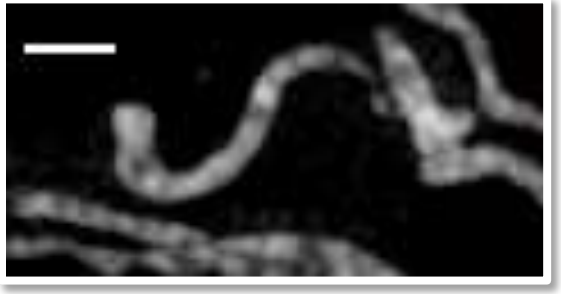
Same as conventional fluorescence microscopy!



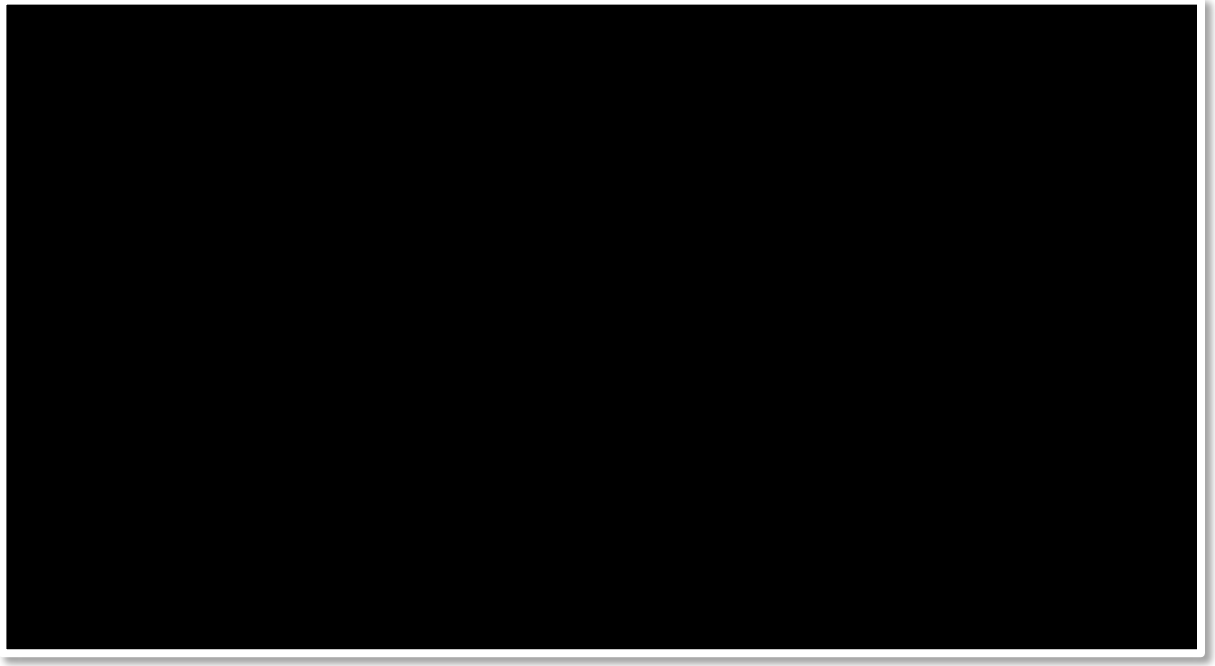


Live imaging with SIM





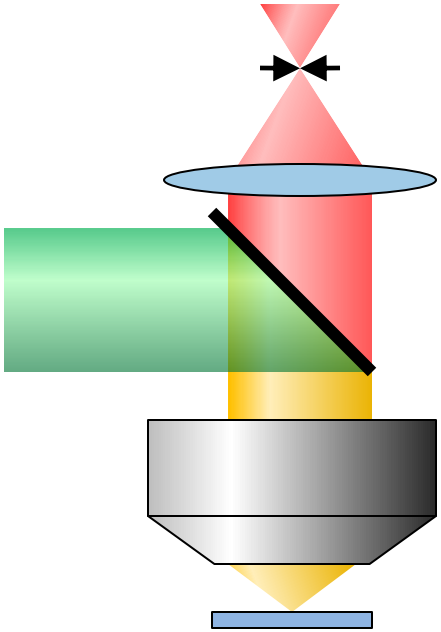
Kner, Chhun et al., Nat Methods,



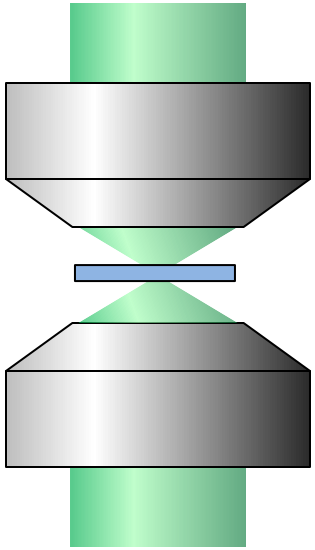
Shao et al., Nat Methods, 2011

The diffraction limit still exists

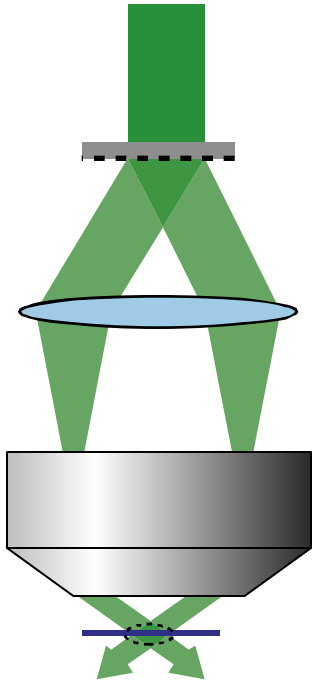
$$d \geq \frac{1}{2} \cdot \frac{\lambda}{2NA}$$



Confocal



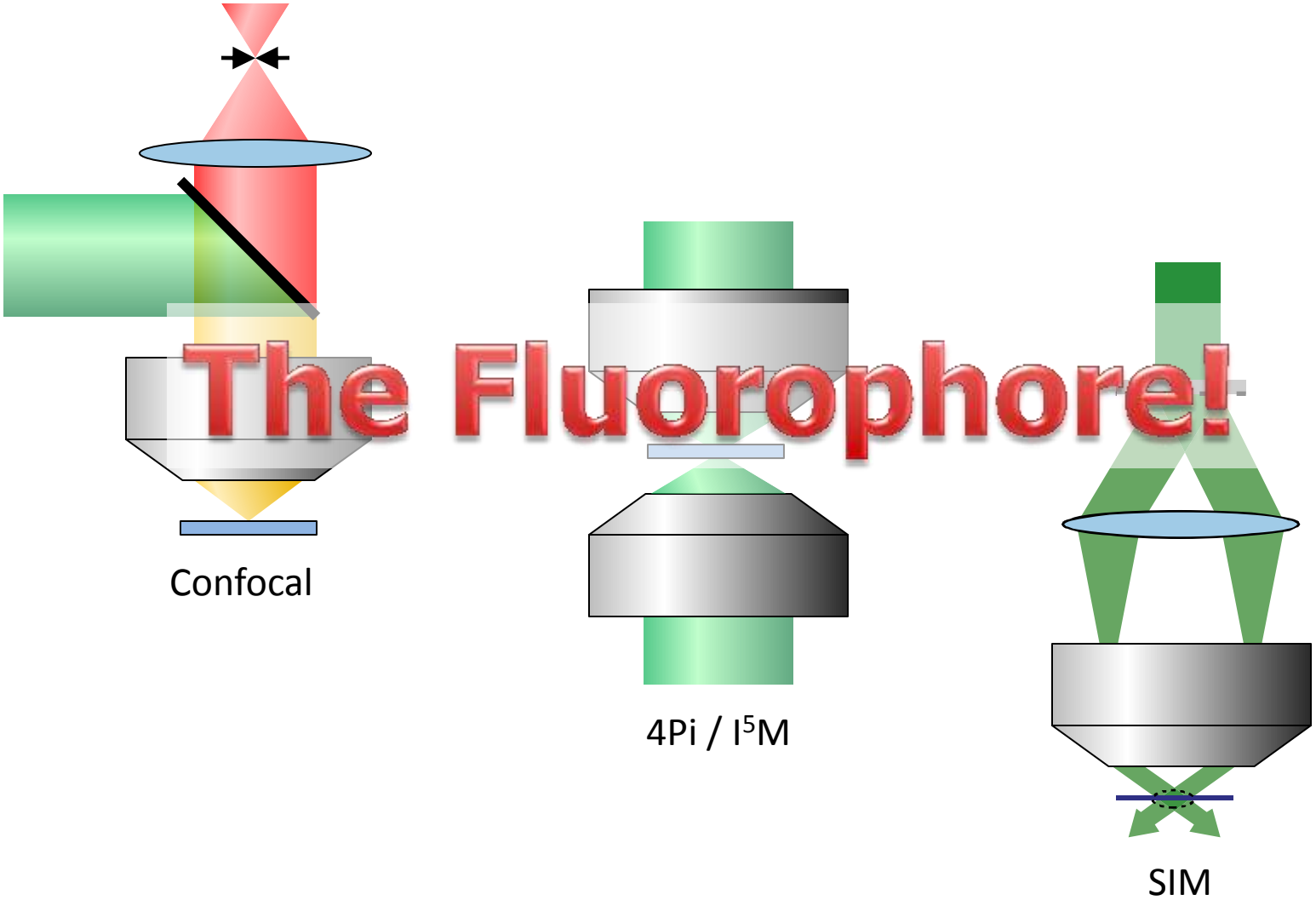
4Pi / I^{5}M



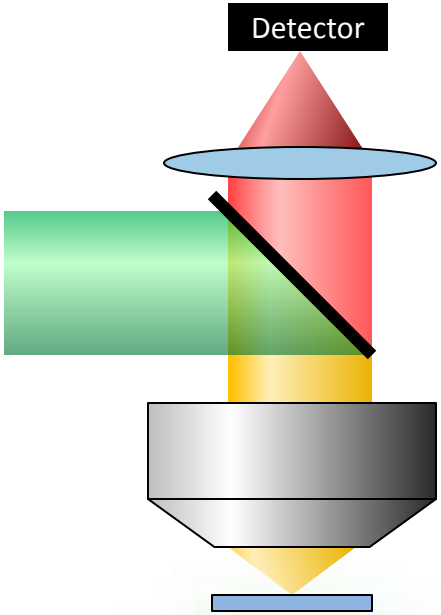
SIM

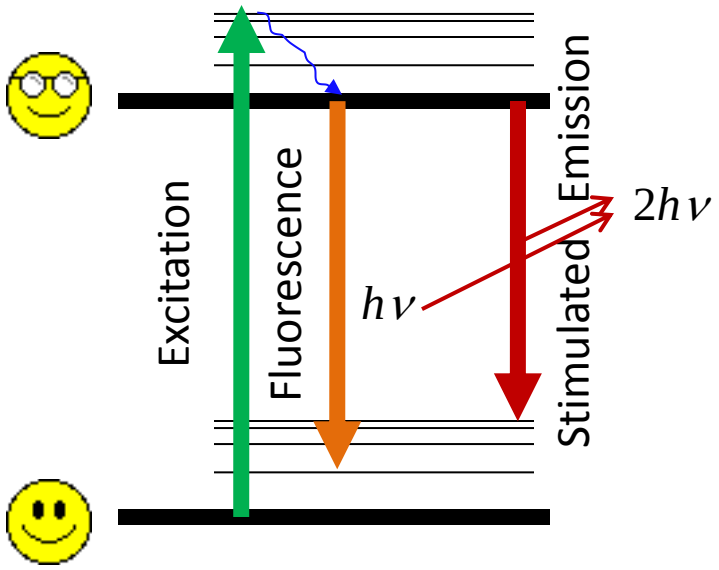
Breaking the diffraction barrier

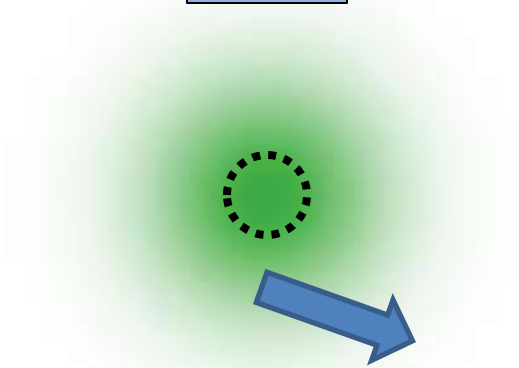
Breaking the diffraction barrier



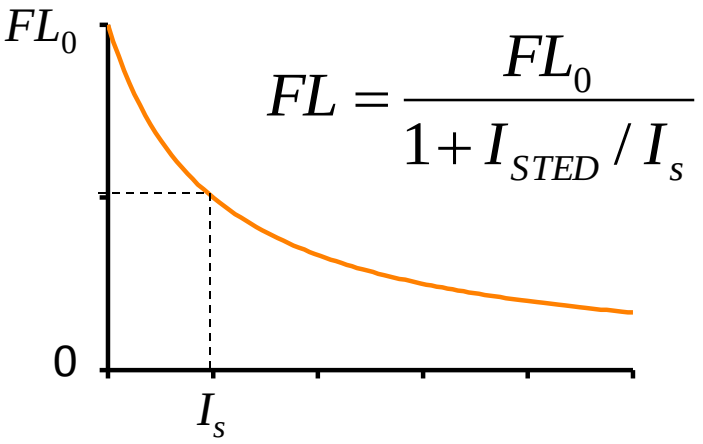
Stimulated Emission Depletion (STED)





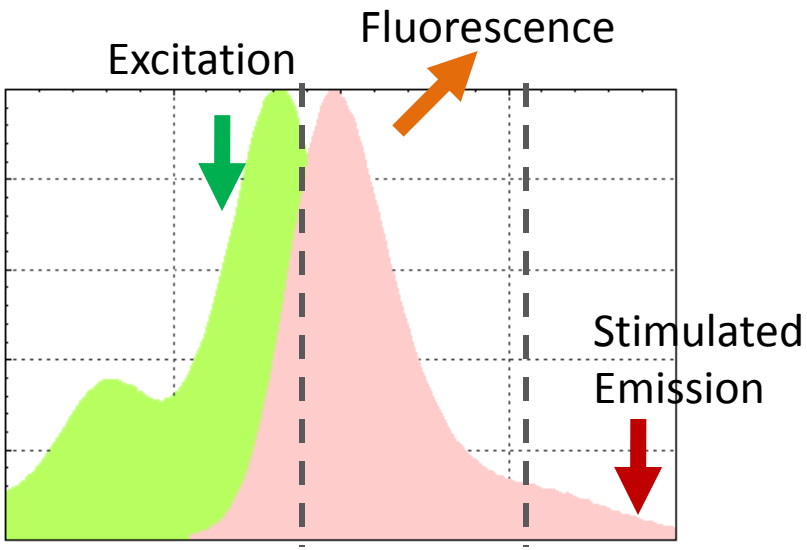


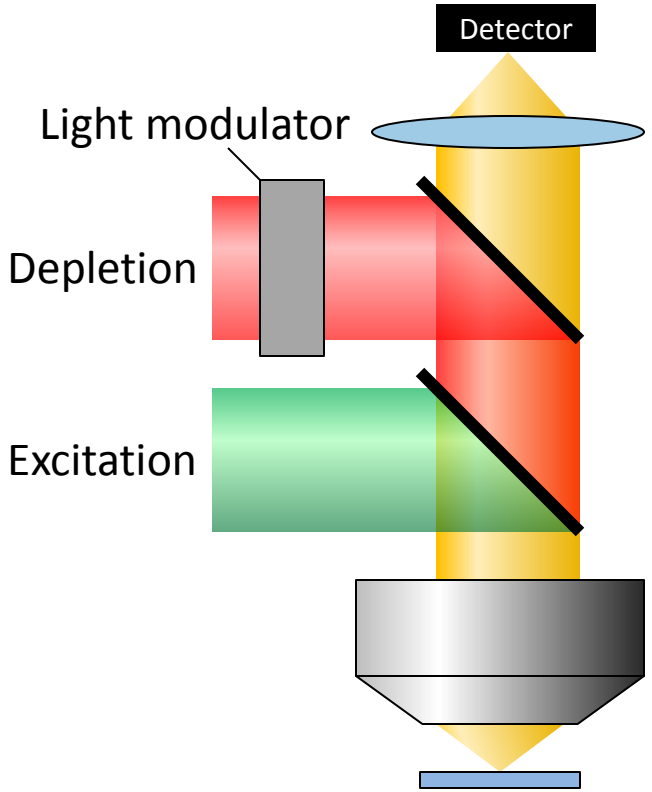
Send to a dark state

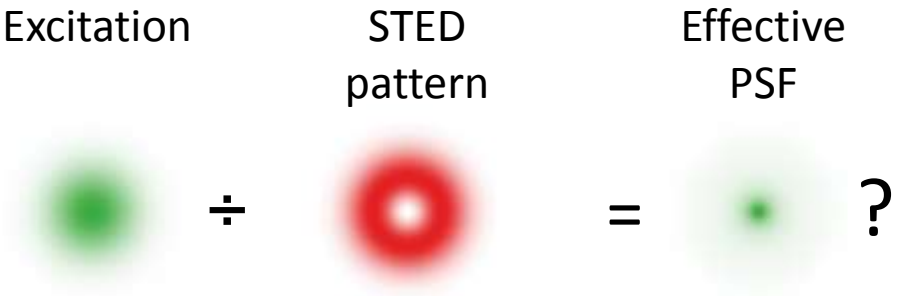


$$FL = \frac{FL_0}{1 + I_{STED} / I_s}$$

STED microscopy

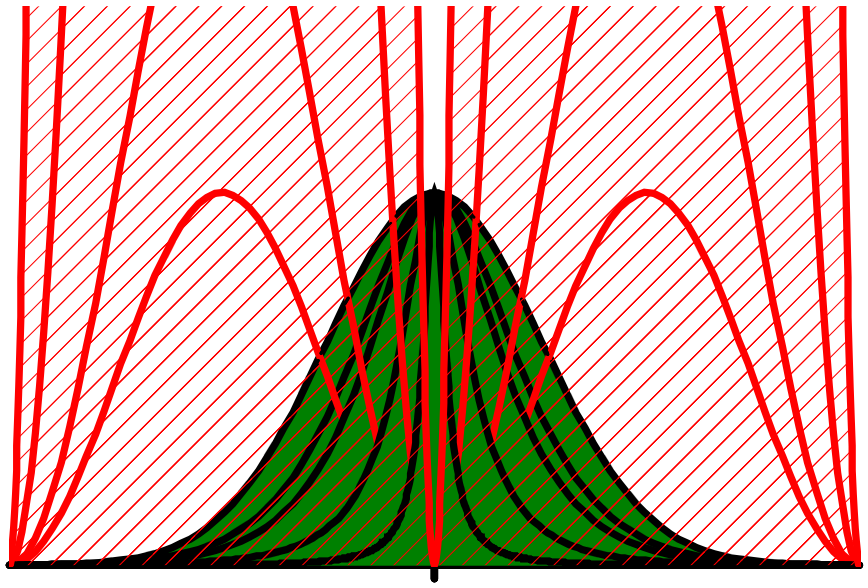






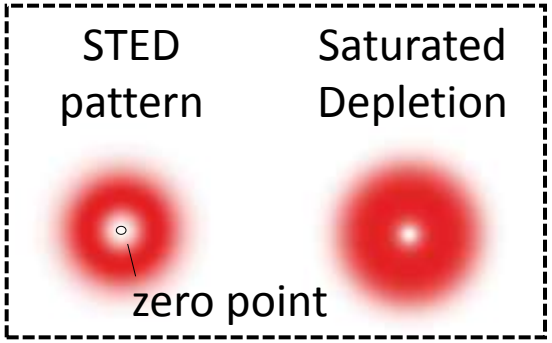
Hell 1994, Hell 2000

Saturated depletion

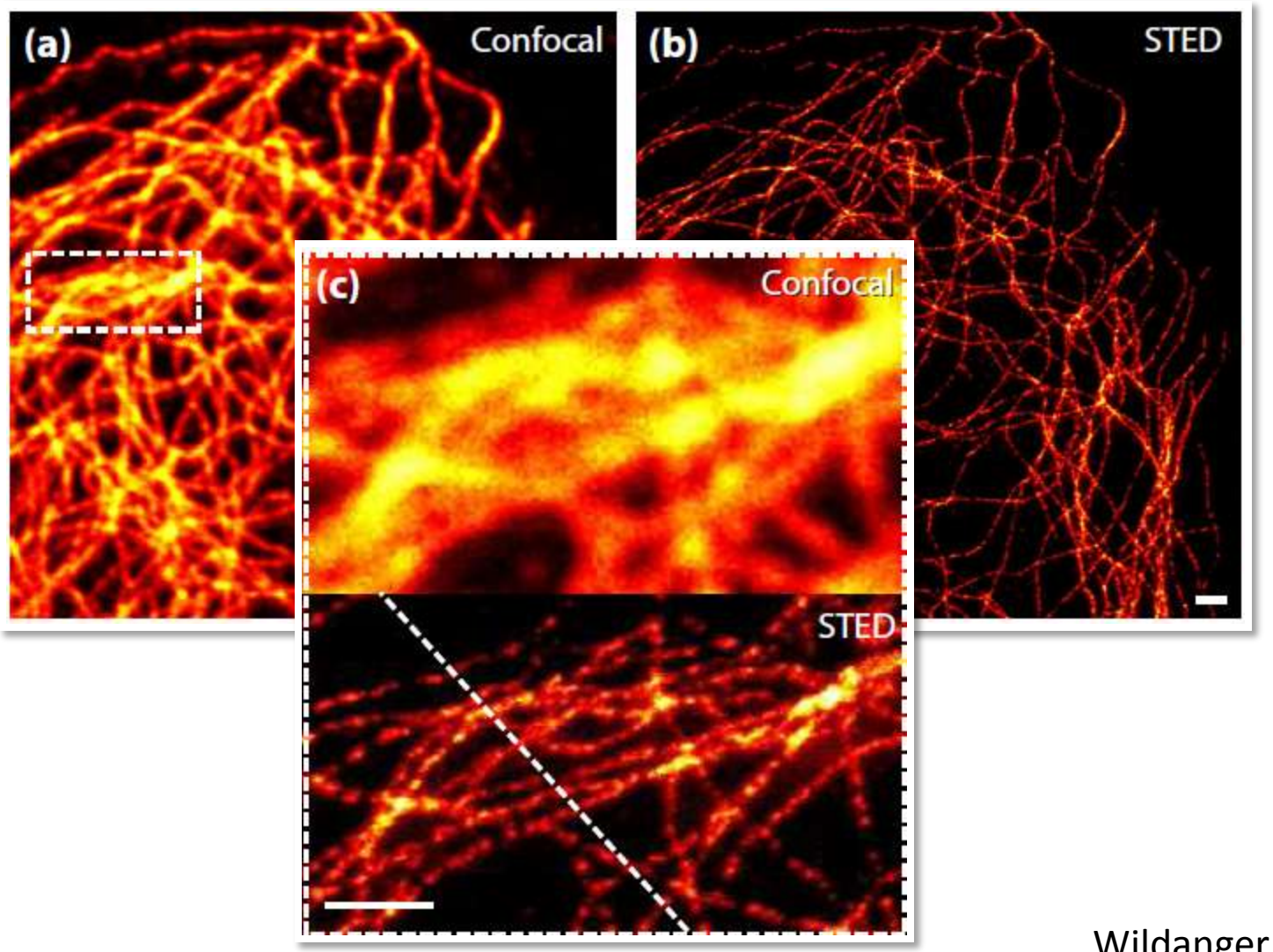


$$I_{\mathrm{STED}} = 200 I_S$$

$$D = \frac{1}{\sqrt{1 + I / I_s}} \cdot \frac{\lambda}{2NA}$$

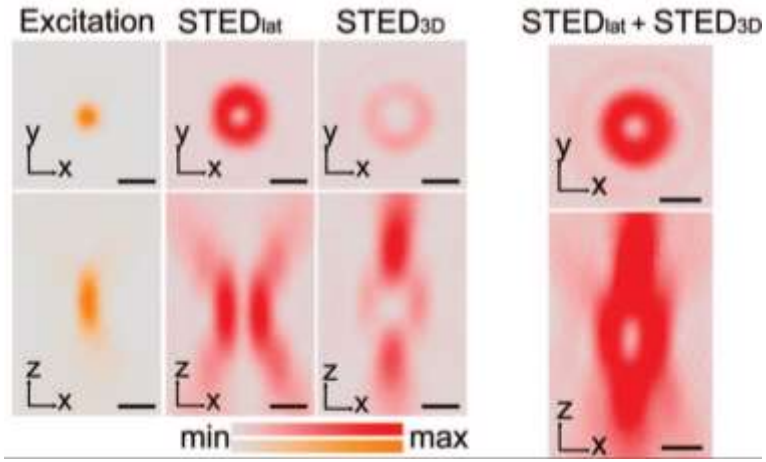


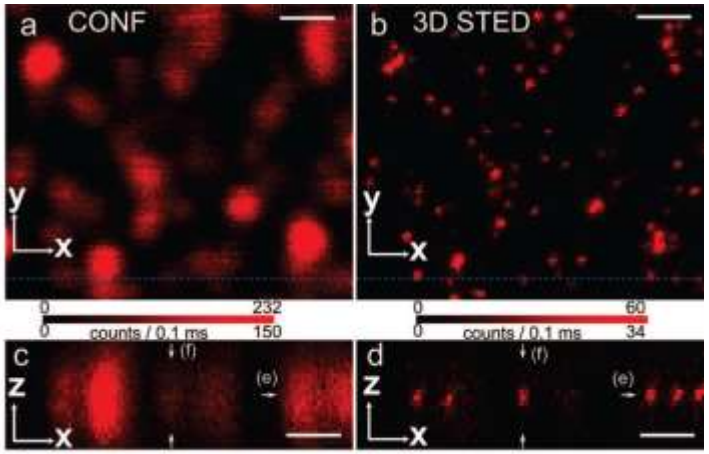
STED images of microtubules

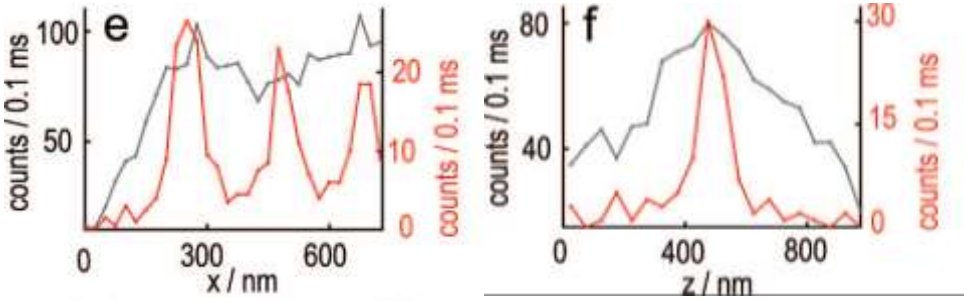


Wildanger et al., 2009

3D STED

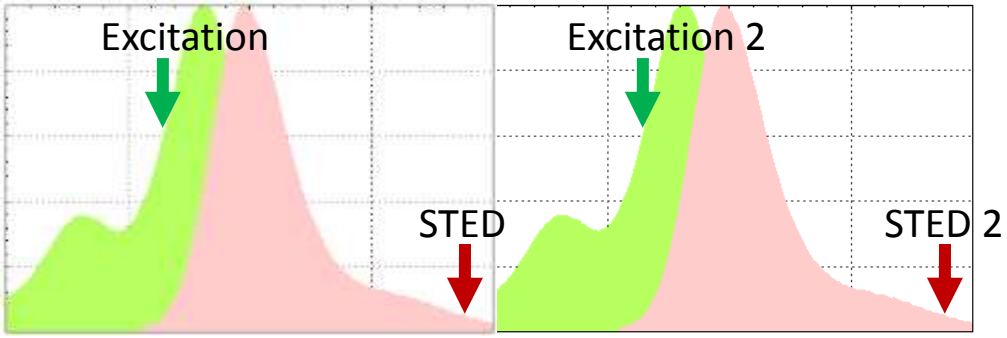




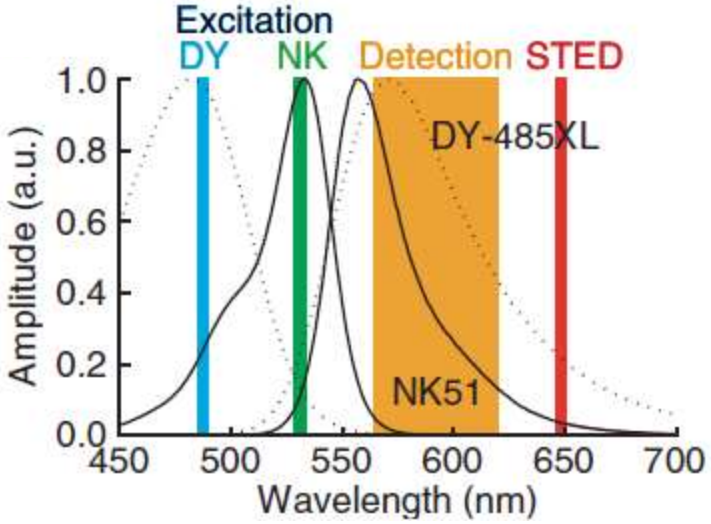


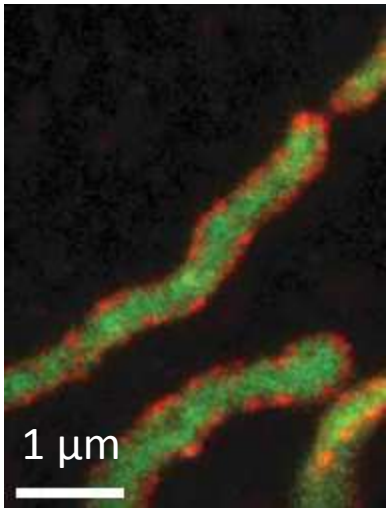
Harke et al., Nano Lett, 2008

Muticolor STED



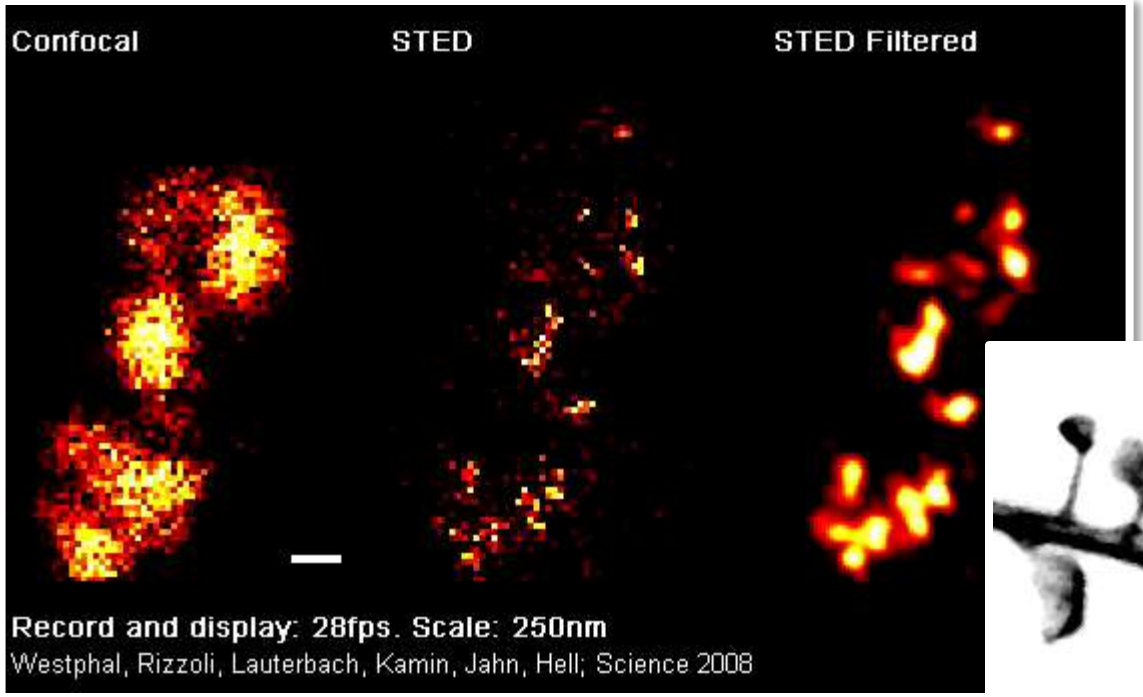
2 color isoSTED resolving
the inner and outer membrane
of mitochondria



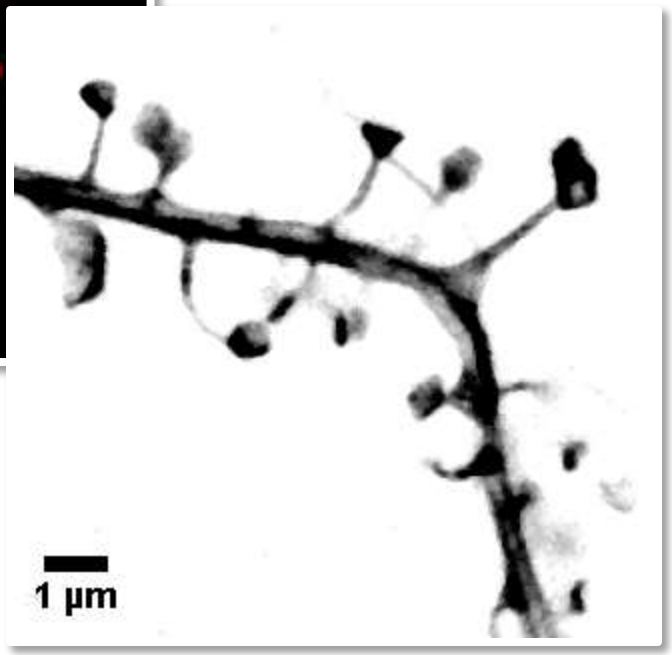


Schmidt et al., Nat Methods 2008

Live STED



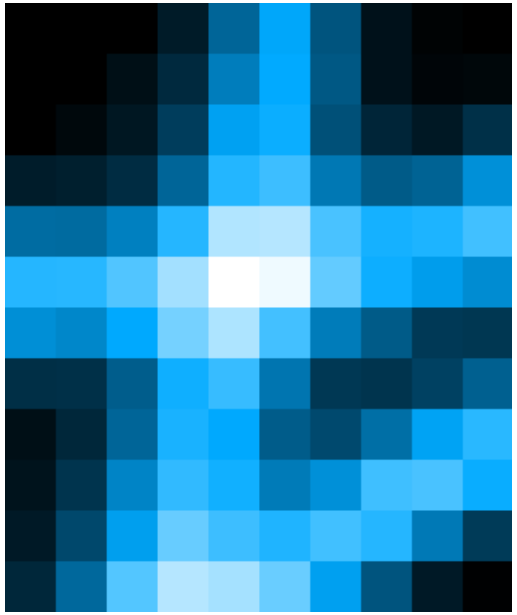
Westaphl et al., Science, 2008



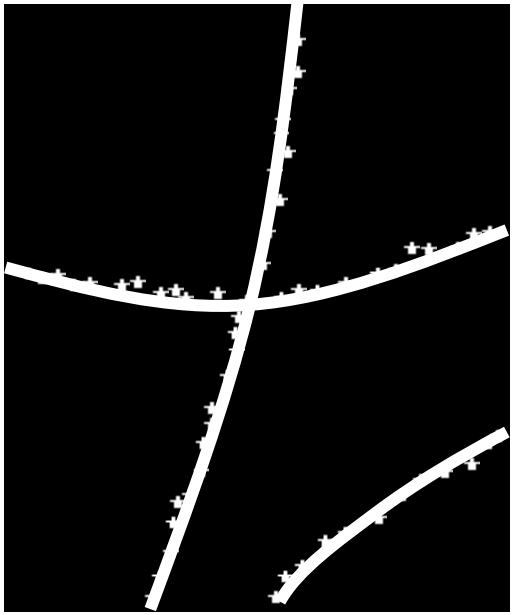
Nagerl et al., PNAS, 2008

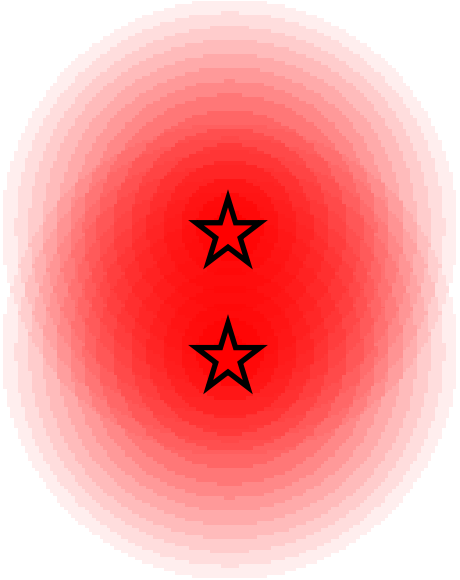
Super-resolution by…

Fluorescence image



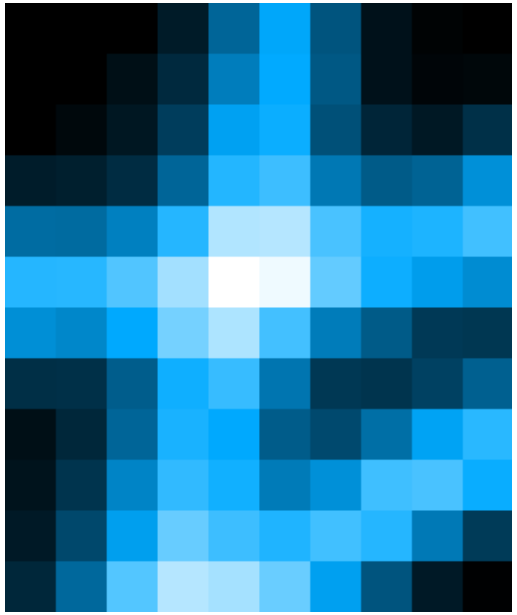
Underlying structure



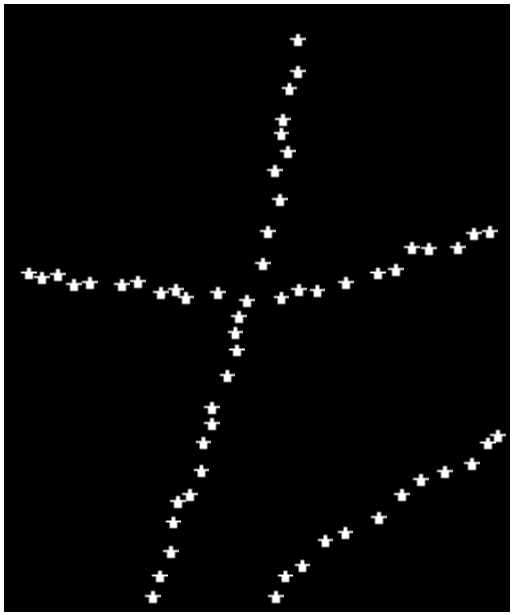


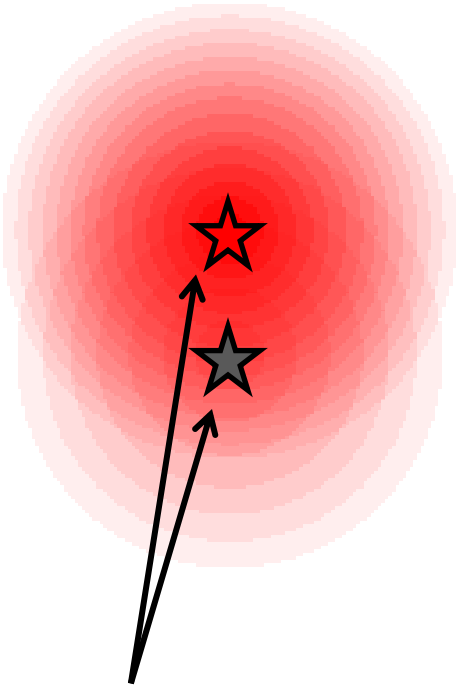
Super-resolution by spatial modulation

Fluorescence image



Underlying structure

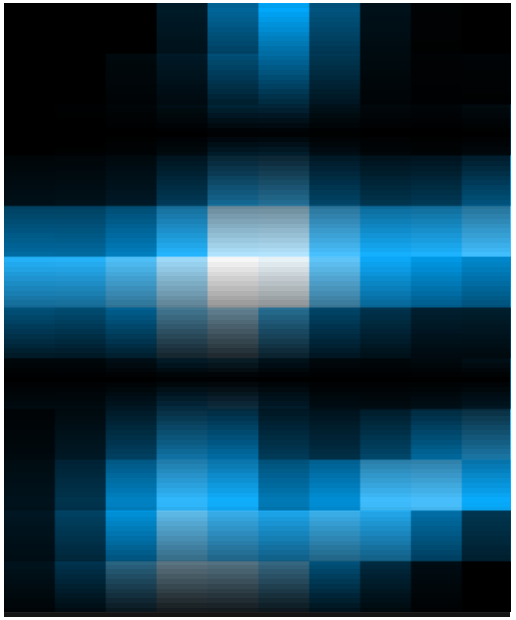




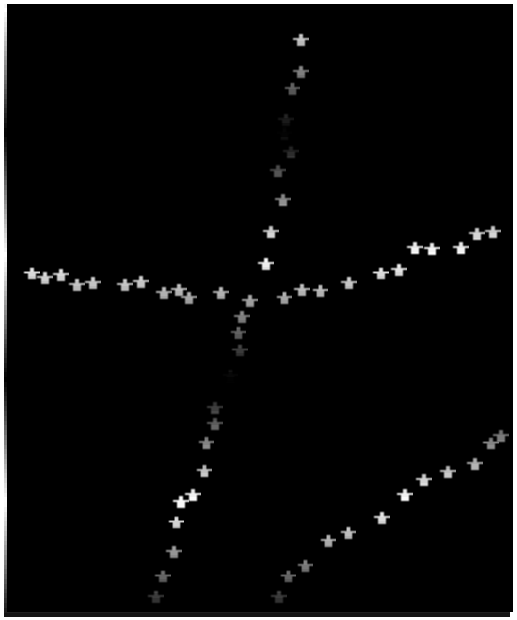
Differential modulation
of the fluorescence response

Super-resolution by differential excitation

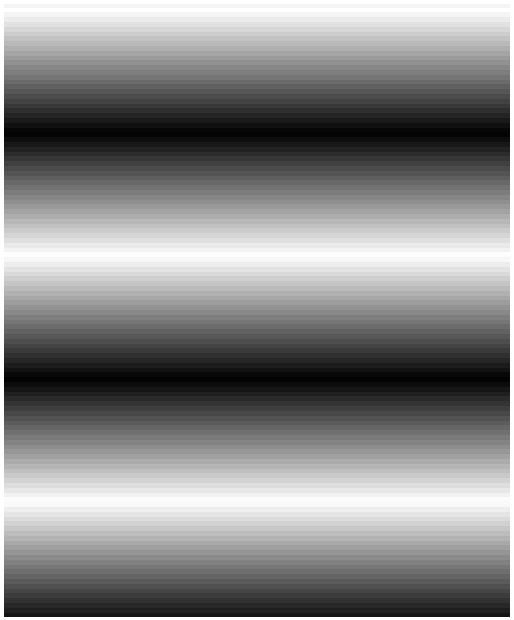
Fluorescence image

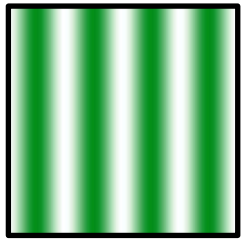


Underlying structure

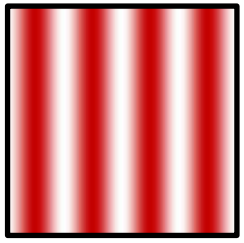


Excitation pattern

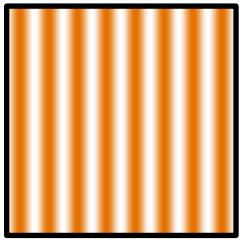




×



=



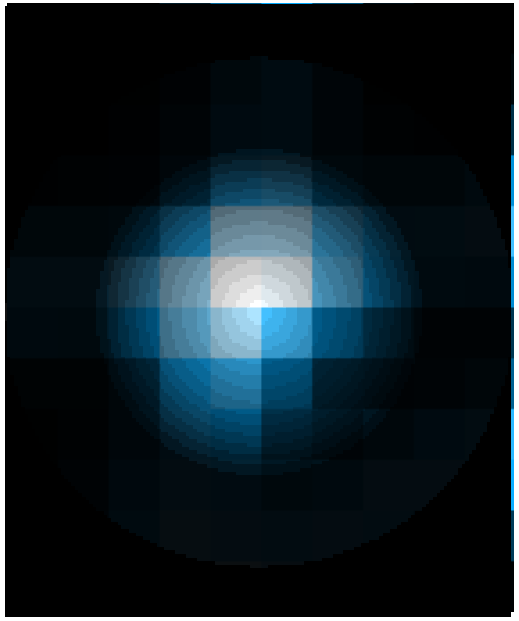
SIM (Gustafsson / Heintzmann)
SSIM (Gustafsson 2005)

Diffraction limited excitation and emission

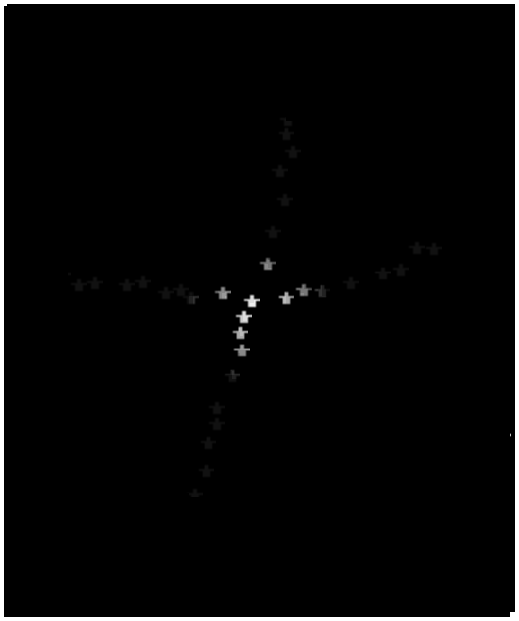
Doubled resolution

Super-resolution by differential depletion

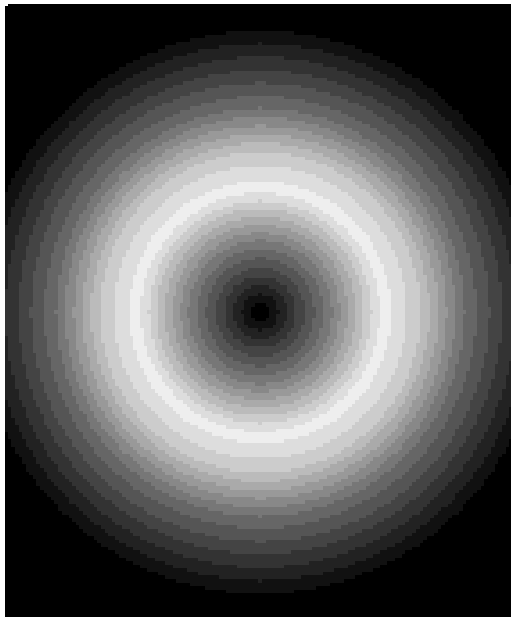
Fluorescence image



Underlying structure



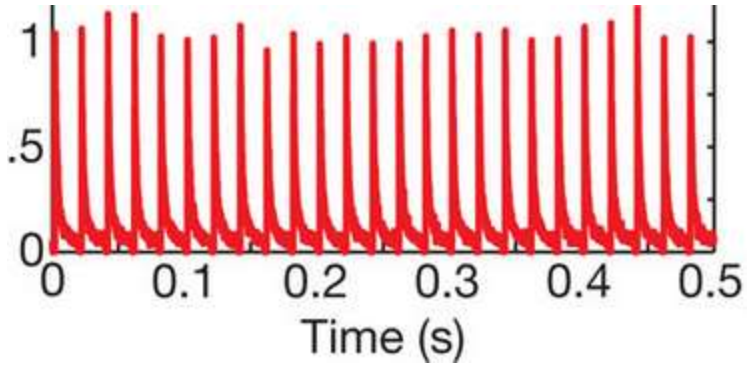
Depletion pattern

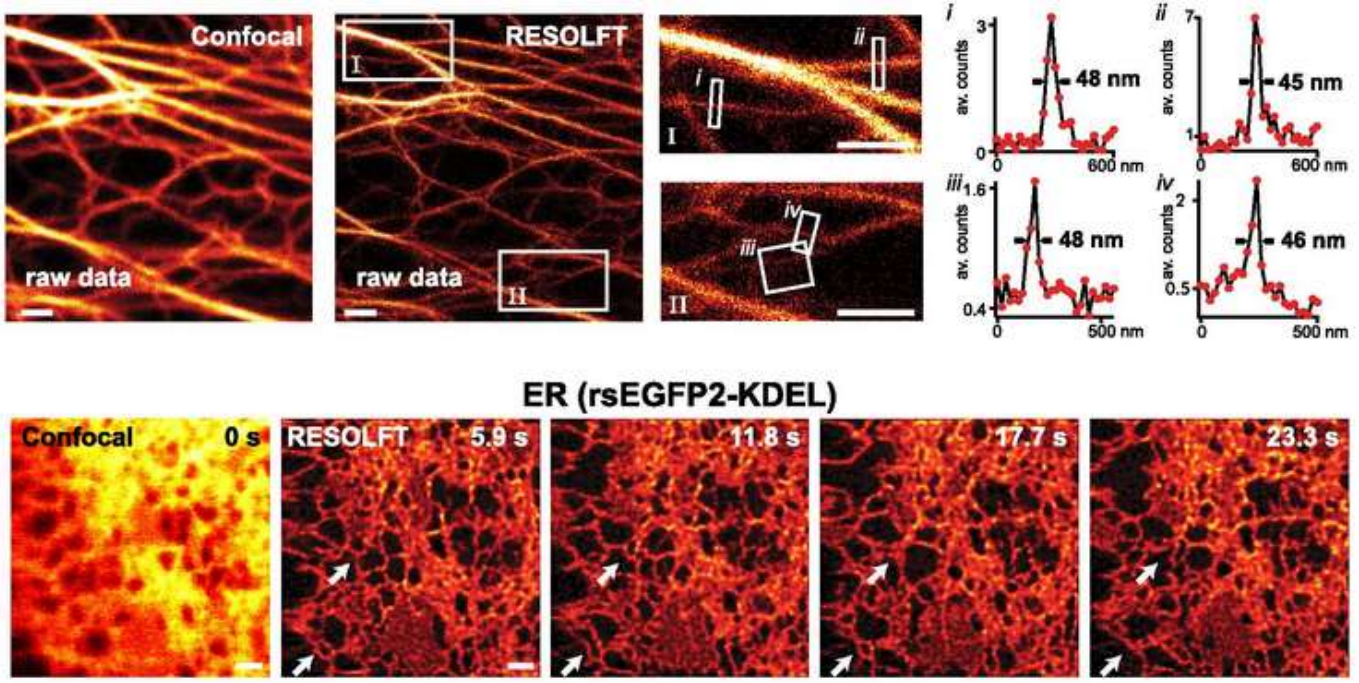


Diffraction limited PSF Saturated depletion Smaller effective PSF

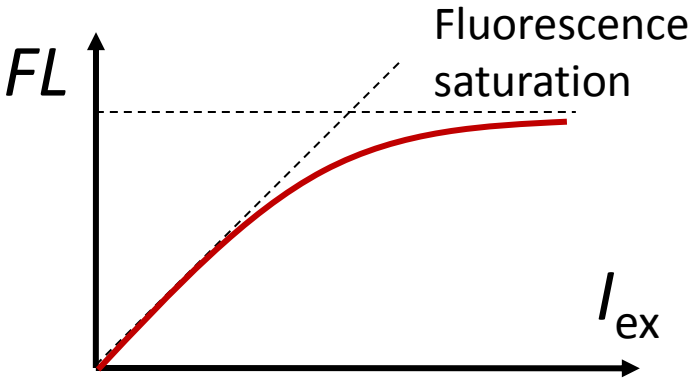
STED (Hell 1994, Hell 1999)
GSD (Hell 1995, Hell 2007)
RESOLFT (Hell 2003, Hell 2011)

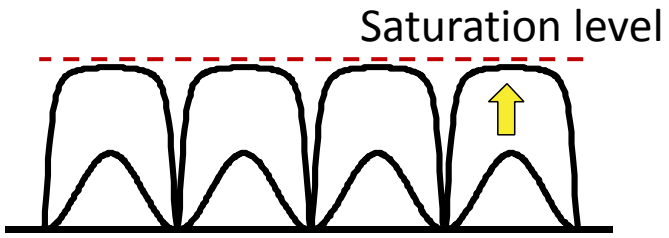
RESOLFT by rsEGFP and rsEGFP2



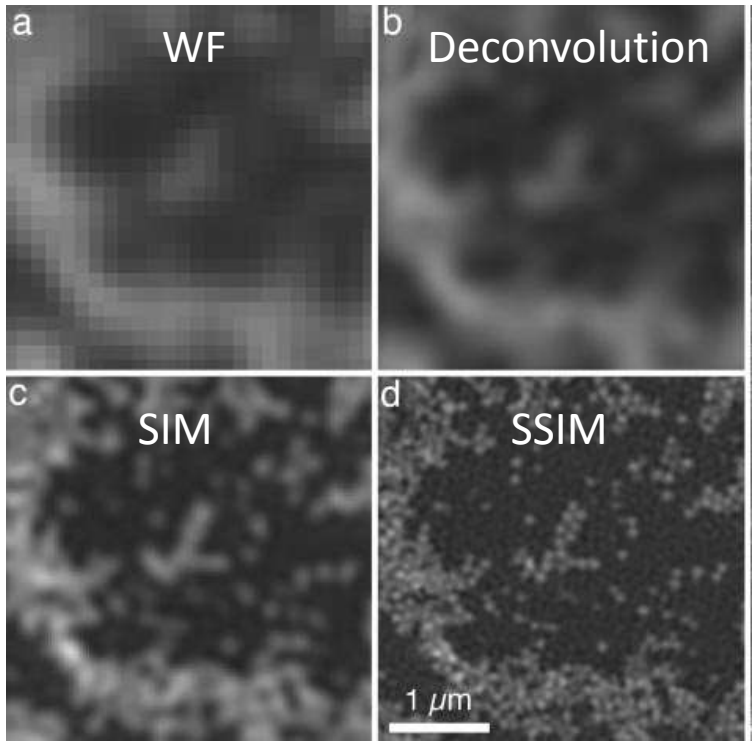


Saturated SIM





Saturated illumination pattern



50 nm resolution

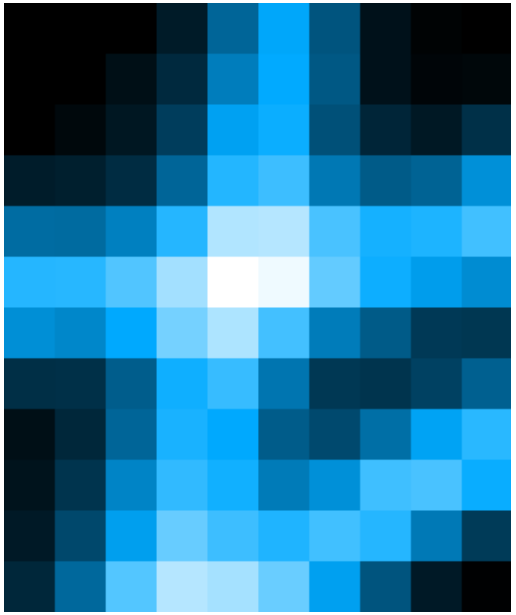
Suffers from fast photobleaching under saturated excitation condition

Gustaffson, PNAS 2005

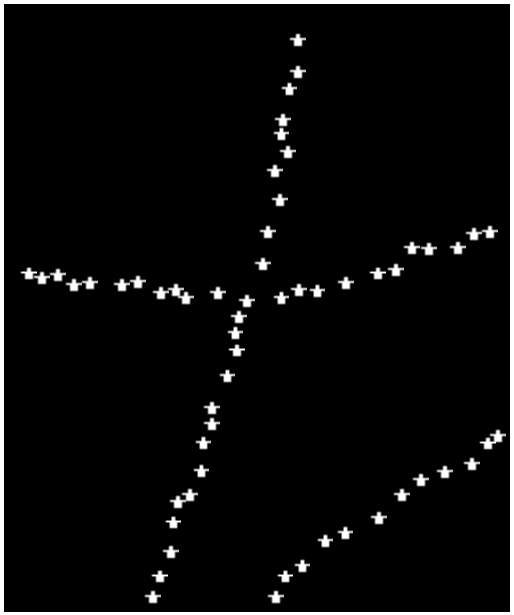
Super-resolution by single-molecule switching

Super-resolution by single-molecule switching

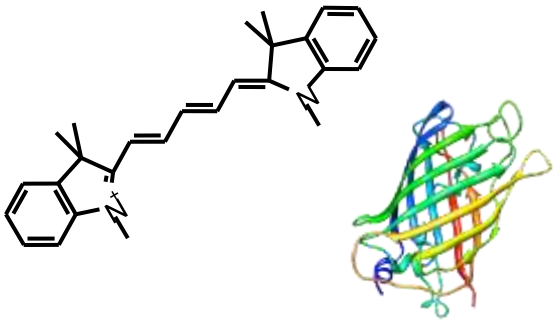
Fluorescence image

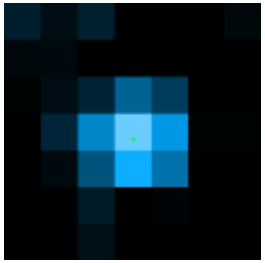


Underlying structure

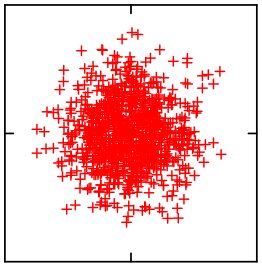


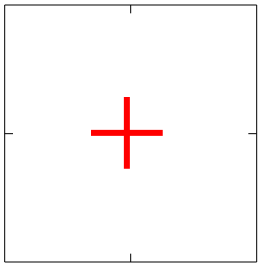
Photoswitchable molecules





=





$$D \approx d / \sqrt{N}$$

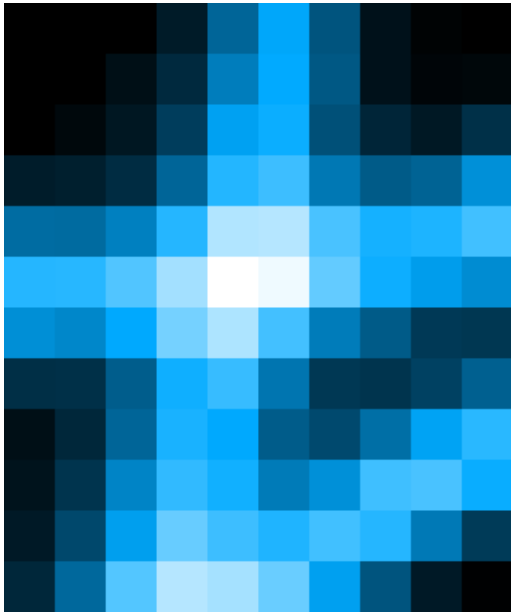
Single molecule image

N photons

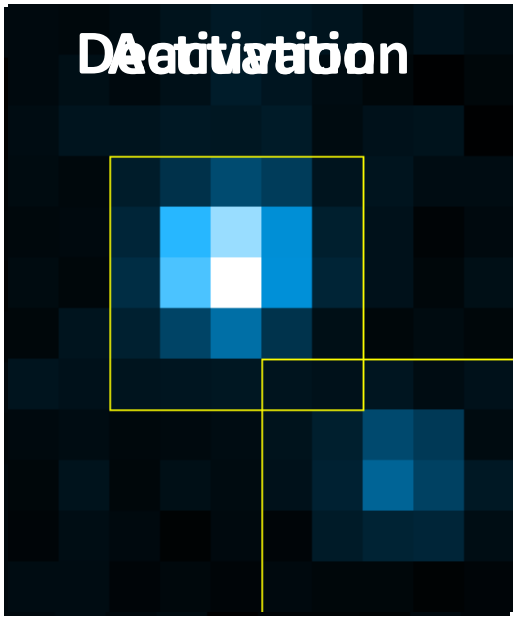
Single-molecule localization

Super-resolution by single-molecule switching

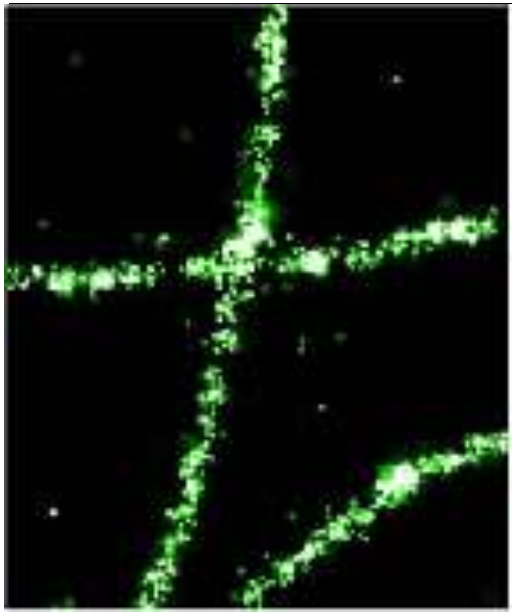
Fluorescence image



Raw images



STORM Image



2x real time

STORM = Stochastic Optical Reconstruction Microscopy (Zhuang 2006)
PALM = Photoactivated Localization Microscopy (Betzig & Hess 2006)
FPALM = Fluorescence Photoactivation Localization Microscopy (Hess 2006)
PALMIRA (Hell 2007), GSDIM (Hell 2008), dSTORM (Sauer 2008), SMACM (Moerner 2008)
PAINT (Hochstrasser 2006), SPRAYPAINT (Moerner 2011), SOFI (Weiss 2009)

Drosophila motoneuron dendrites