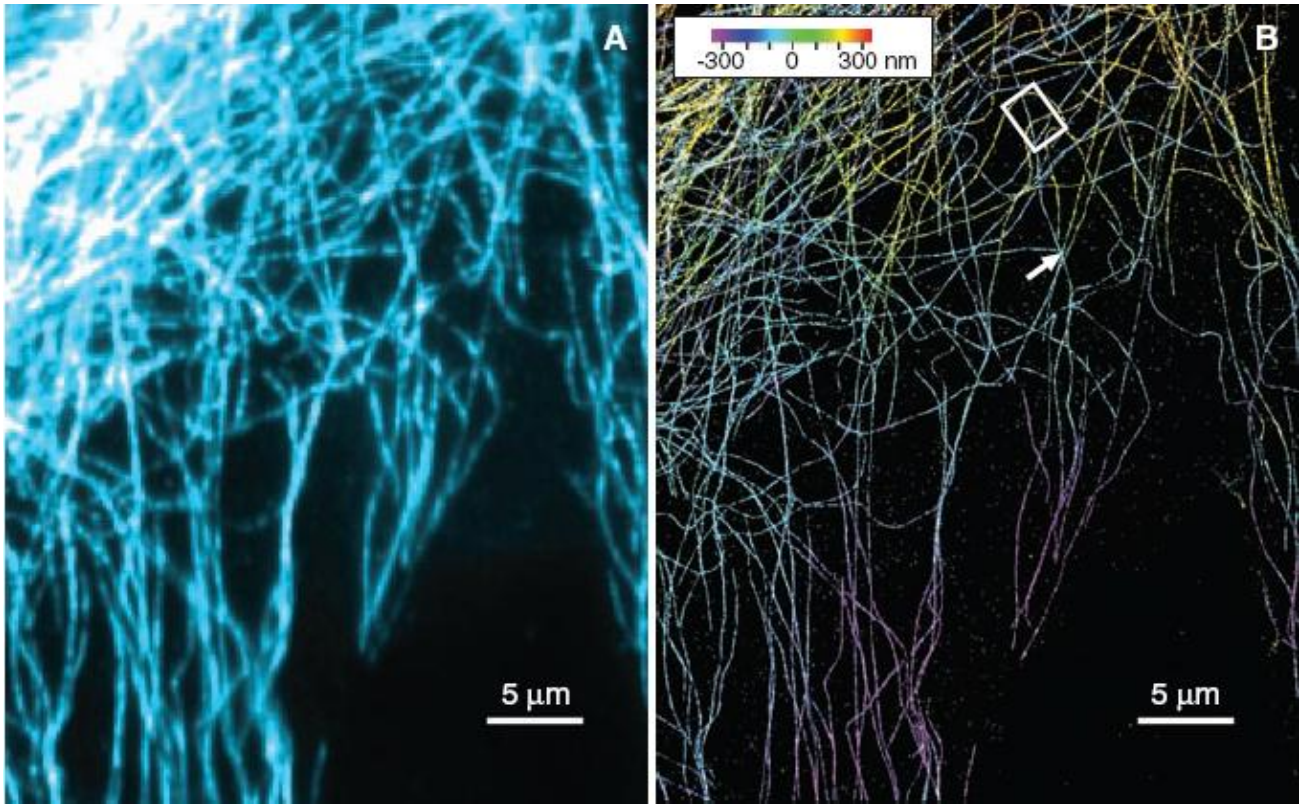
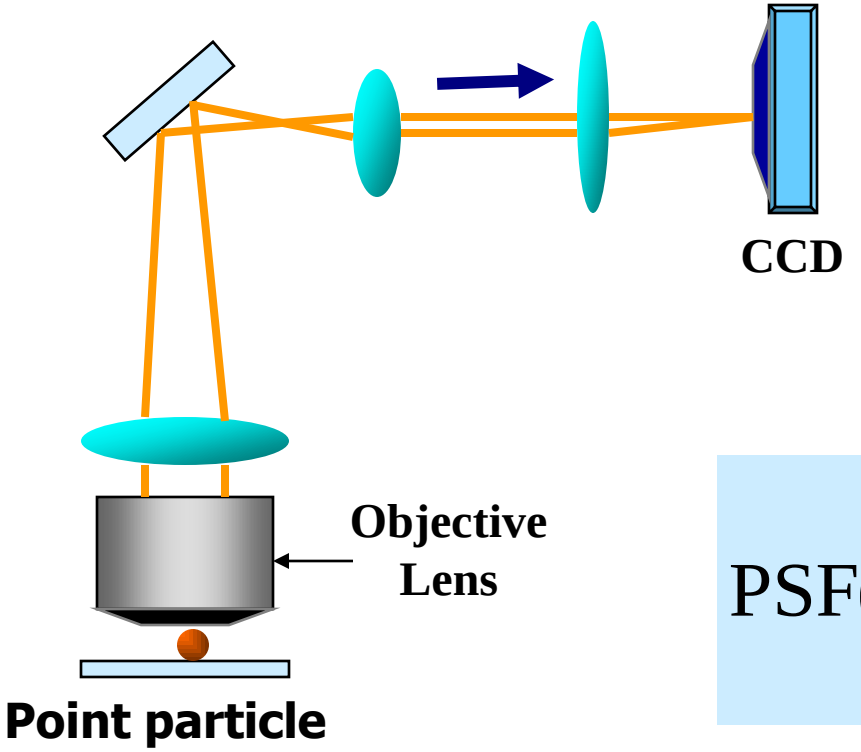
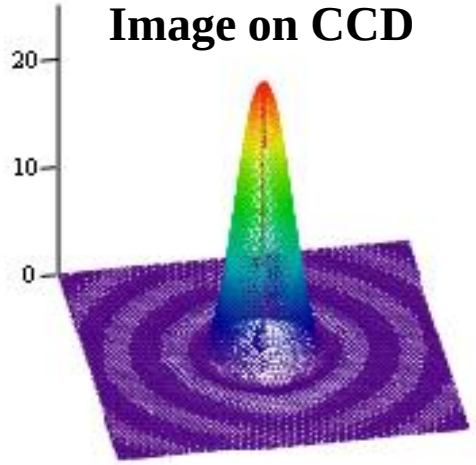
# Super High Resolution Imaging Beyond Diffraction Limit



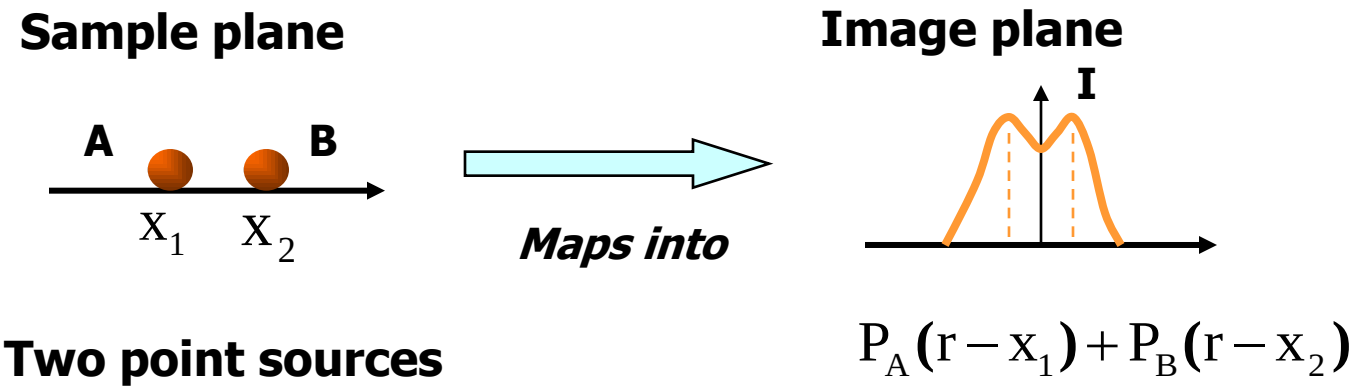
# Point-Spread Function(PSF)




$$\mathrm{PSF}(r) = \left[ \frac{2J_1\left(2\pi r \mathbf{NA}/\lambda\right)}{2\pi r \mathbf{NA}/\lambda} \right]^2$$

# Image Formation in Incoherent Imaging



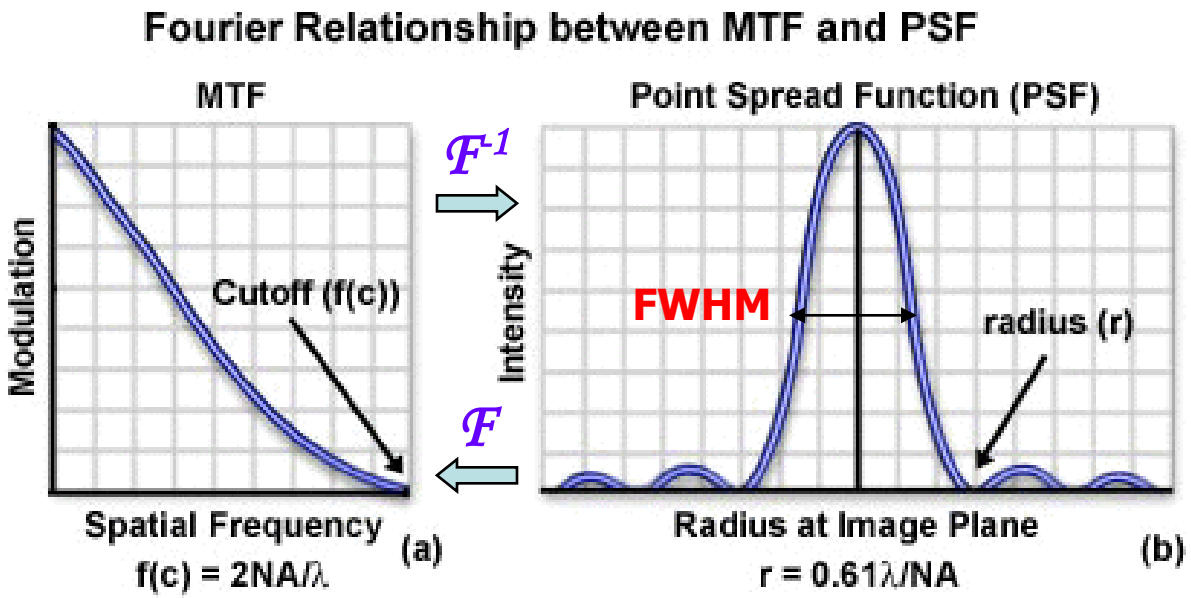

- **Regard any object as a collection of point sources**
- **Image intensity is given as convolution**

$$I(r)=\int O(r')PSF(r-r')dr'=O(r)\otimes PSF(r)$$

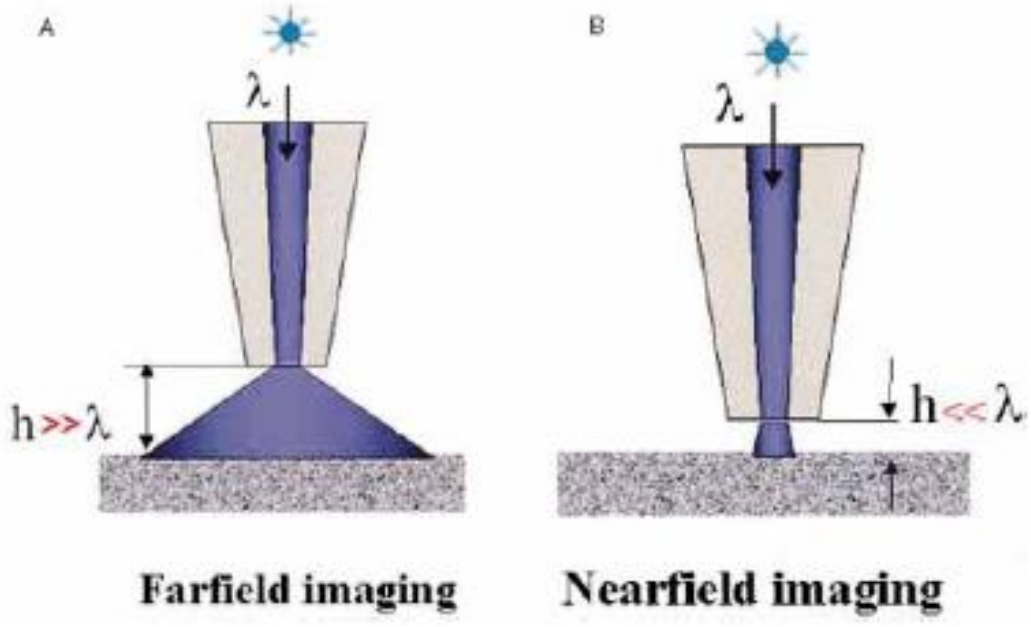
# Lateral Resolution

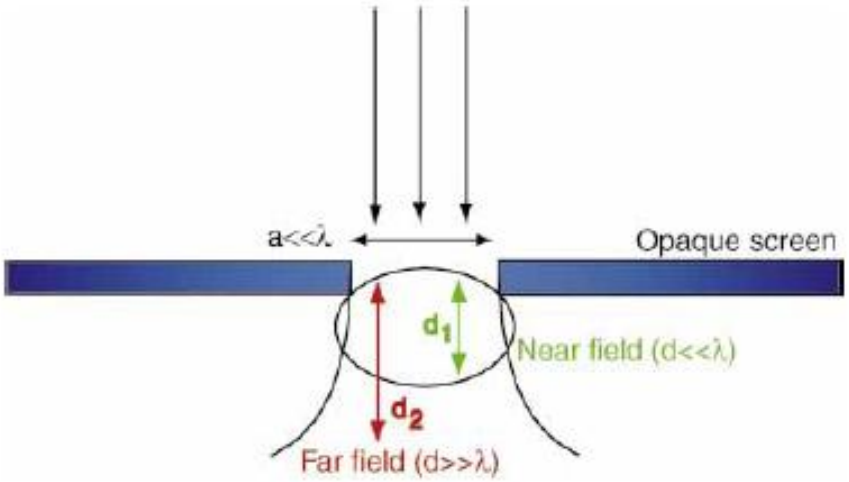
- Resolution: minimum distance between two point objects such that they are seen as distinct
- Quantified by Rayleigh criterion
  - Two objects are resolved when the center of one Airy disk falls on the first min. of the other (i.e. separation > FWHM )
- Frequency domain view

  Modulation Transfer Function(MTF )=|*Fourier Transform* (PSF)|



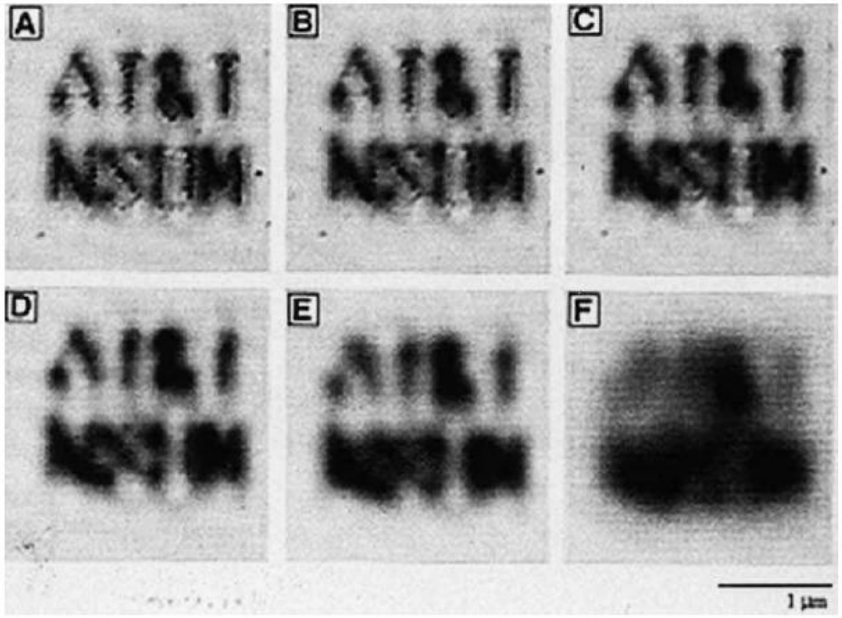
# Near Field Optical Microscopy (NSOM)

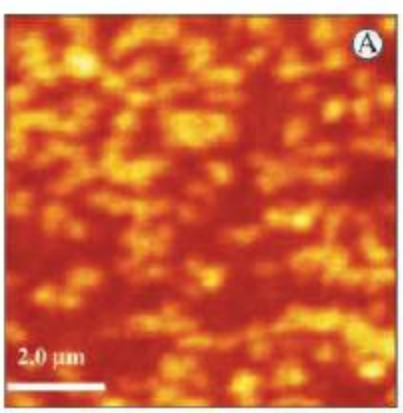


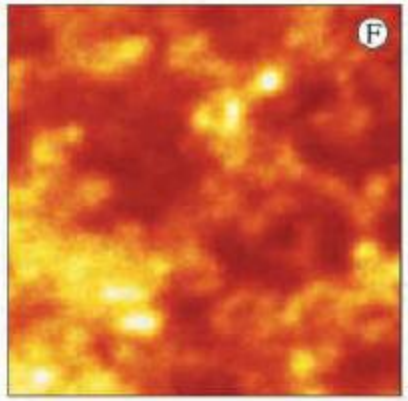




Betzig, Science, 1992
Edidin, Traffic, 2001

# Experimental Results of NSOM




Quiescent Activated

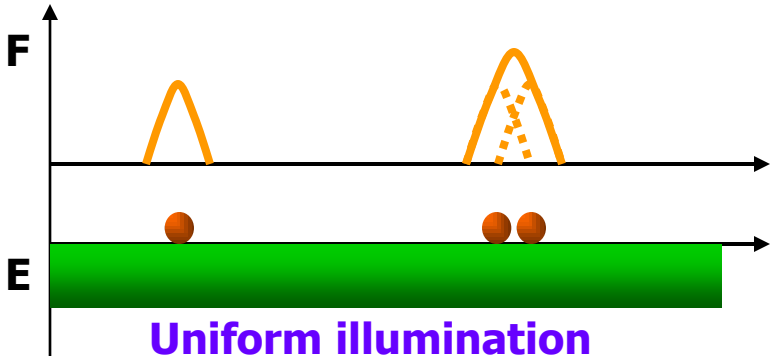
Erb2 receptors on SKBR3 cells

(A, contact, B-F NSOM at 5, 10, 25, 50, 100 nm steps)

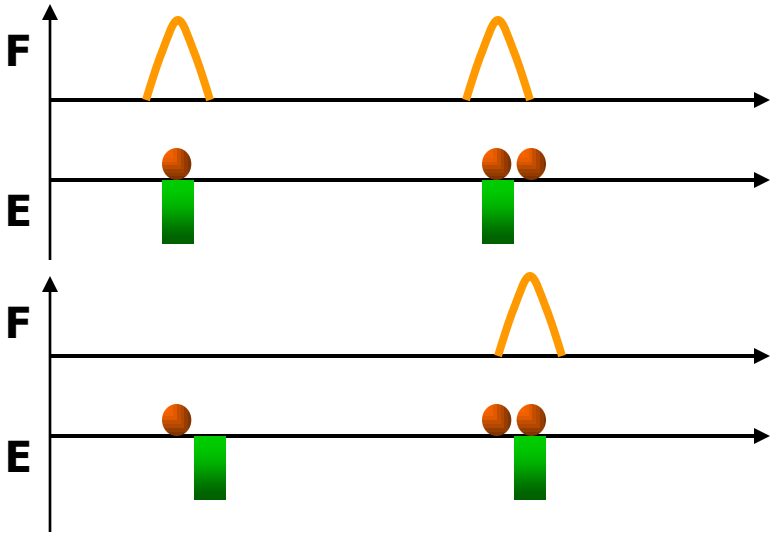
Betzig, Science, 1992

Nagy, JCS1999

# Resolution Improvement Based on Structured Illumination




**Uniform illumination**

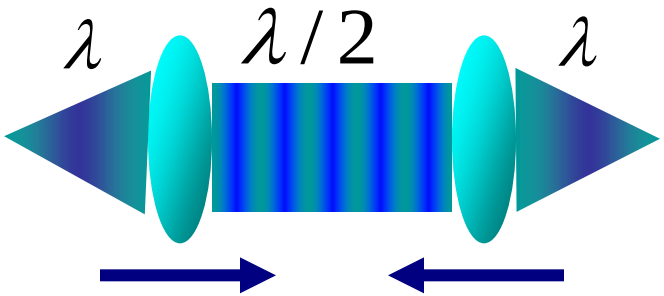



**Translated high frequency illumination**

- Diffraction in the imaging path function effectively as a low pass filter obscures the presence of closely spaced objects under uniform illumination
- Even with high frequency illumination, point objects are still broadened by diffraction
- The presence of closely spaced objects can be discerned if the high frequency excitation field can be translated
- Resolution improvement is possible by phase shifting structured illumination containing high spatial frequency

(F: Fluorescent image, E: Excitation illumination region)

# Standing Wave Microscopy
# (SWM, Bailey, Nature, 1993, Krishnamurthi, SPIE, 1996)




$$k = \lambda / 2\pi$$

$$E_1(x,t) = E_0 \cos(kx - \omega t) \qquad E_2(x,t) = E_0 \cos(kx + \omega t)$$

- Interference of two-counter propagating plane waves generates standing-wave

$$E(x,t) = E_1(x,t) + E_2(x,t) = 2E_0 \cos(kx)\cos(\omega t)$$

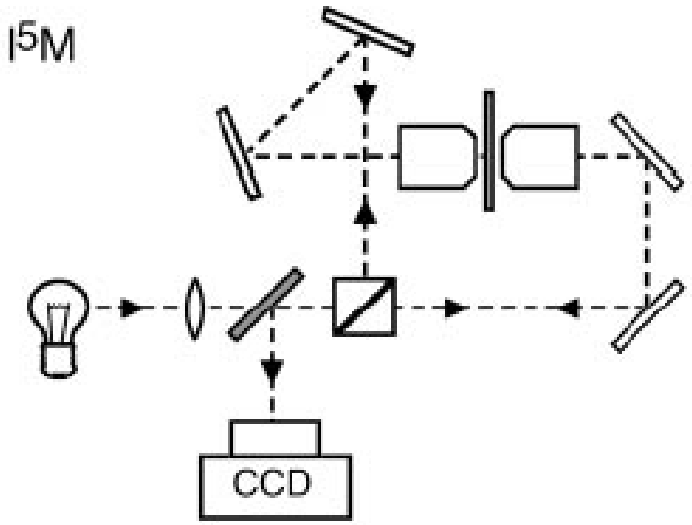
- Intensity distribution

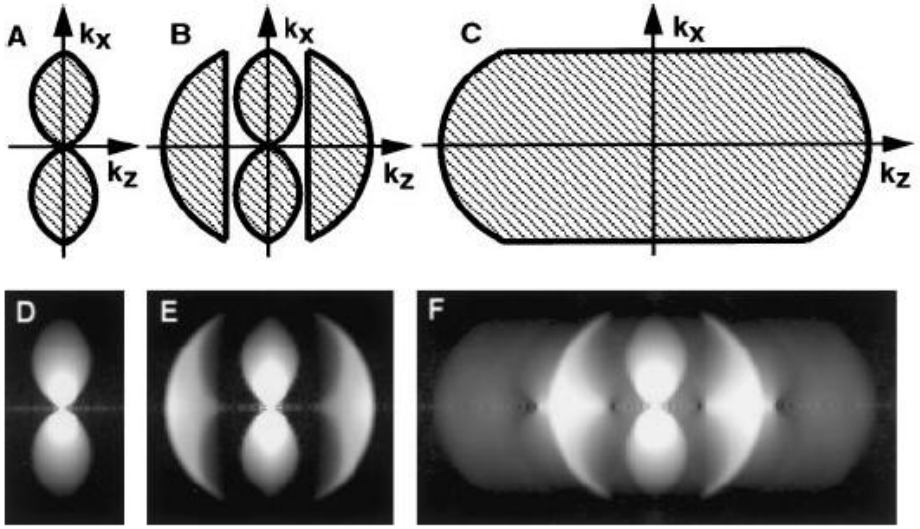
$$I(x) = \left\langle E^2 \right\rangle = I_0 \cos^2(kx) = \frac{1}{2} I_0 \left[1 + \cos(Kx)\right]$$

  - where K=2k
  - double the spatial frequency of the excitation

# I5M Microscopy

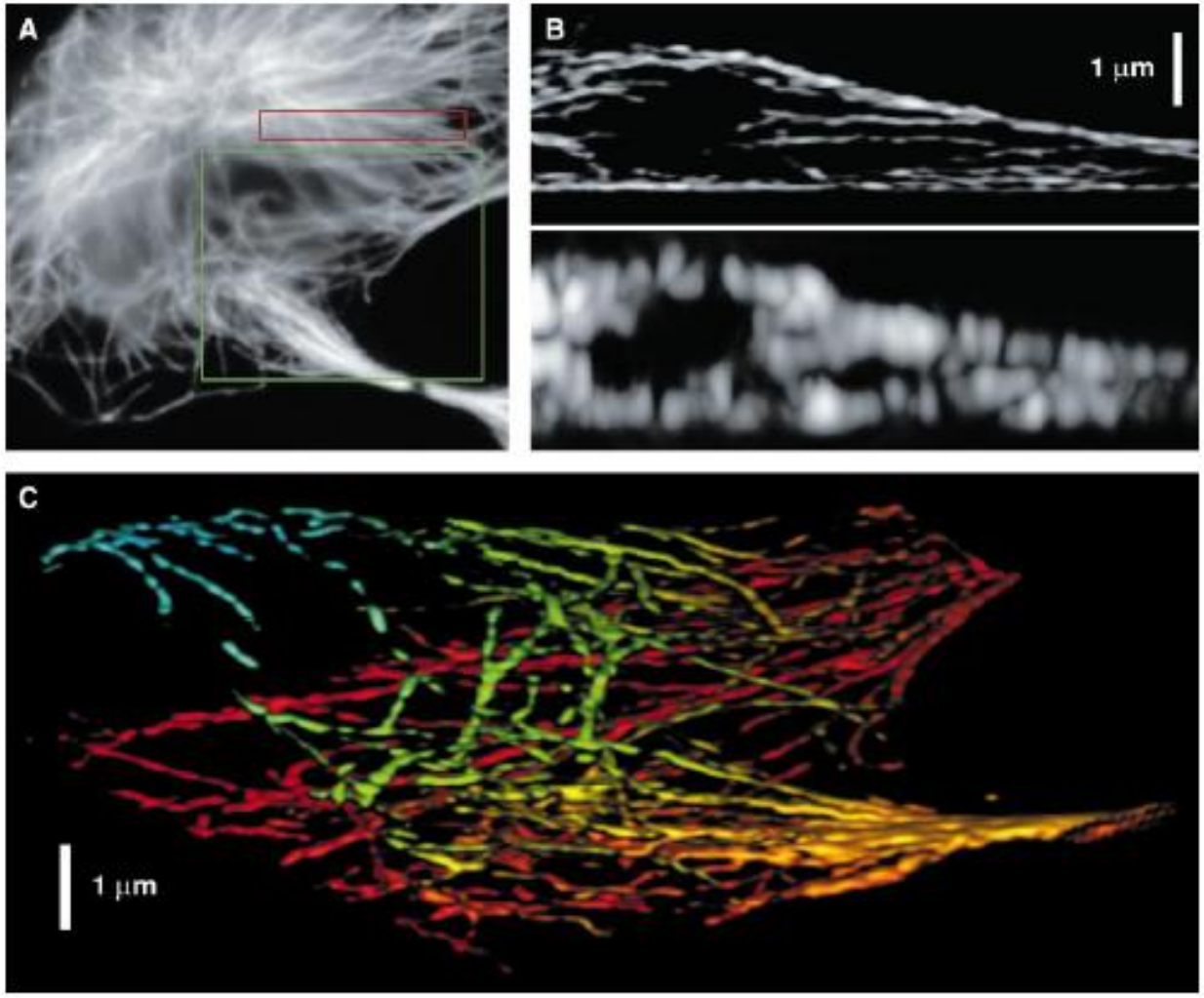
It is essentially similar to standing wave microscopy but interferes the “low coherence” excitation signal and interferes the fluorescence signal at the detector.



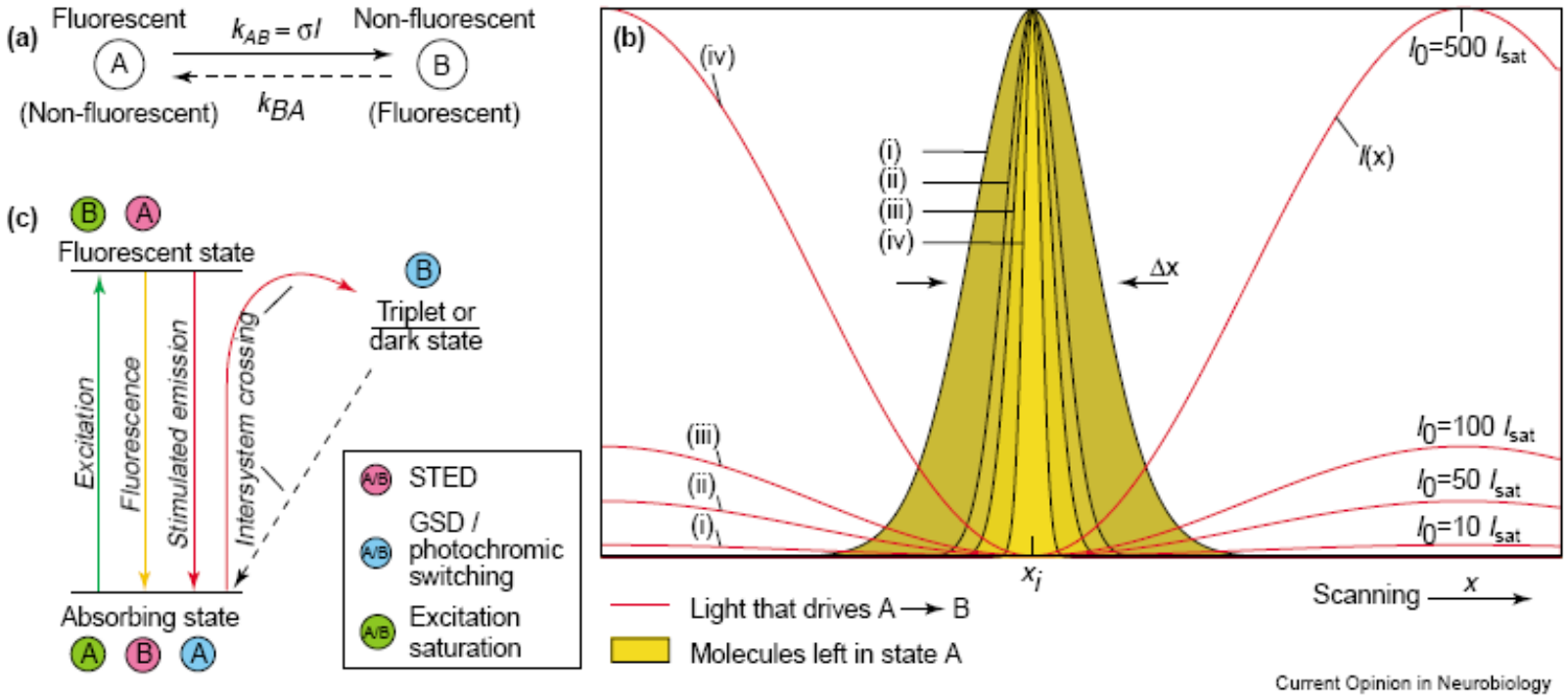

Gutstaffson, JM 1999

# Experimental Results of I5M




Gutstaffson, JM 1999

# Reversible Saturable Optical Transition




$$\frac{dN_A}{dt} = -\frac{dN_B}{dt} = N_B k_{BA} - N_A k_{AB}$$

$$I(\vec{r}) = I_0 f(\vec{r})$$

For standing wave $f(x) = \sin^2(2\pi n x / \lambda)$

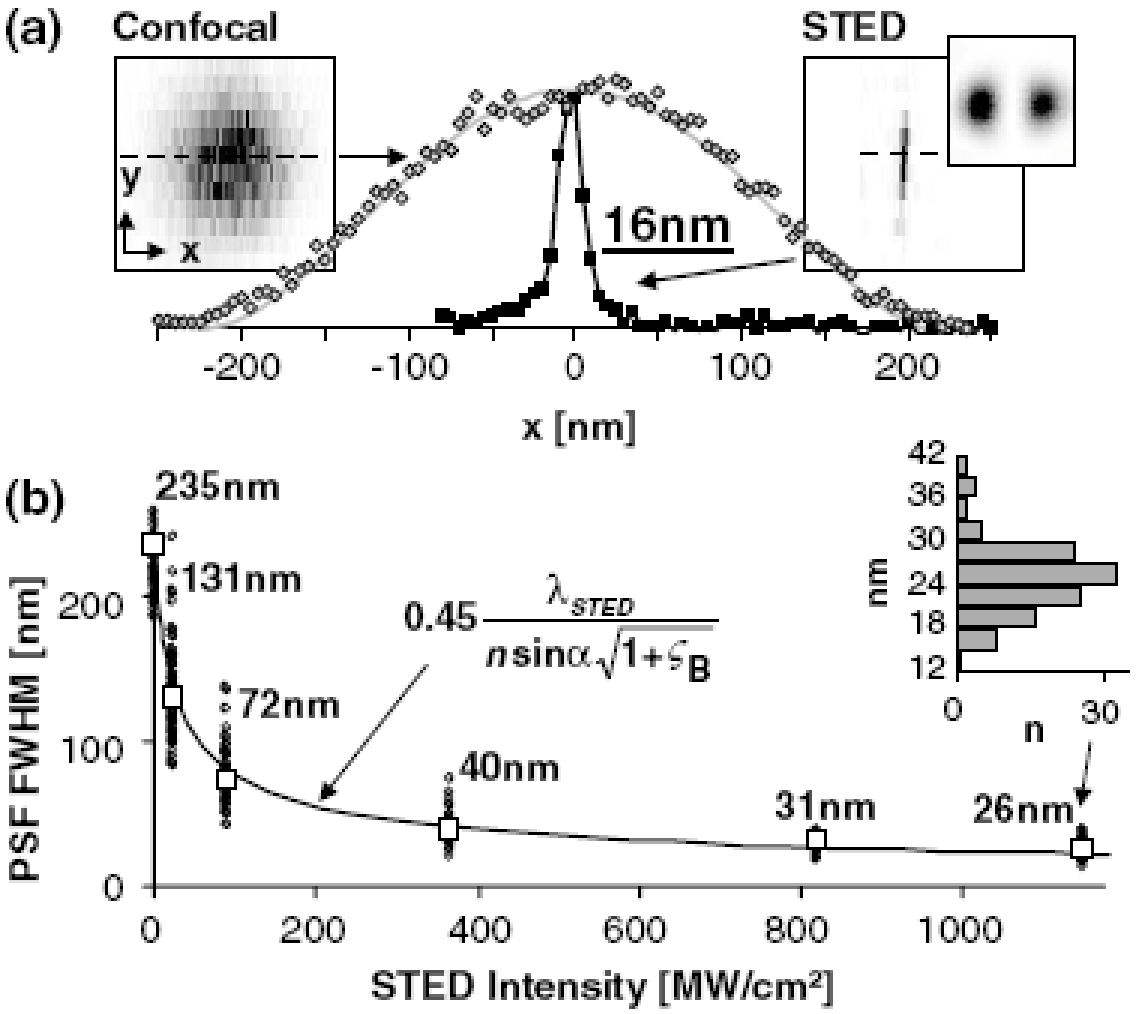
$$k_{AB} = \sigma I \qquad N_A^{\infty} = \frac{k_{BA}}{(\sigma I + k_{BA})}$$

Define: $I_{Sat} = \frac{k_{BA}}{\sigma}$ $\quad I >> I_{sat};\quad N_A^{\infty} = \frac{k_{BA}}{\sigma I}$

$$\Delta x = \frac{\lambda}{\pi n} \arcsin\left(\sqrt{\frac{k_{BA}}{\sigma I_0}}\right) \approx \frac{\lambda}{\pi n \sqrt{I_0 / I_{Sat}}}$$

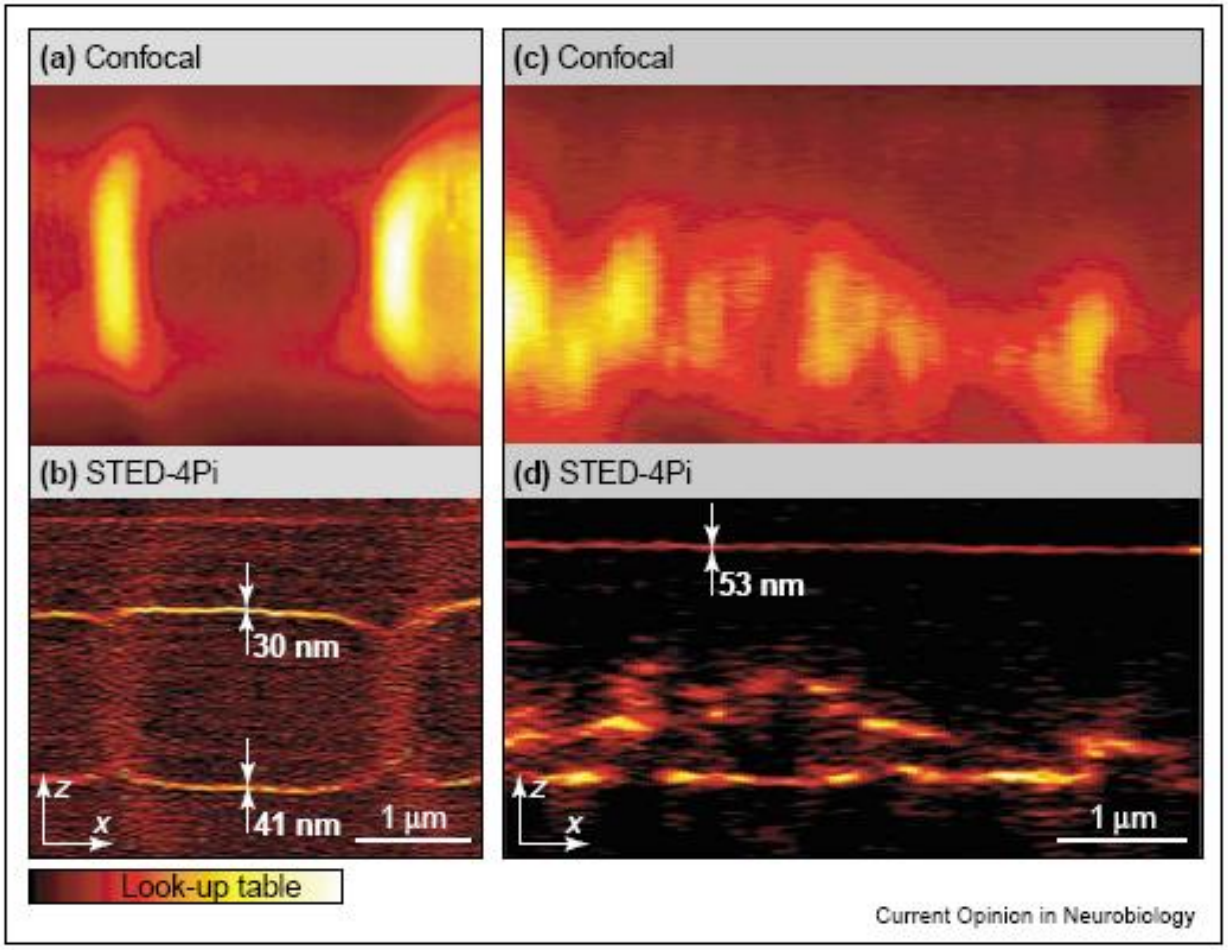
Hell, NBT 2003

# Implementation of Stimulated Emission Depletion Microscopy (STED)




Westphal, PRL 2004

# STED Images



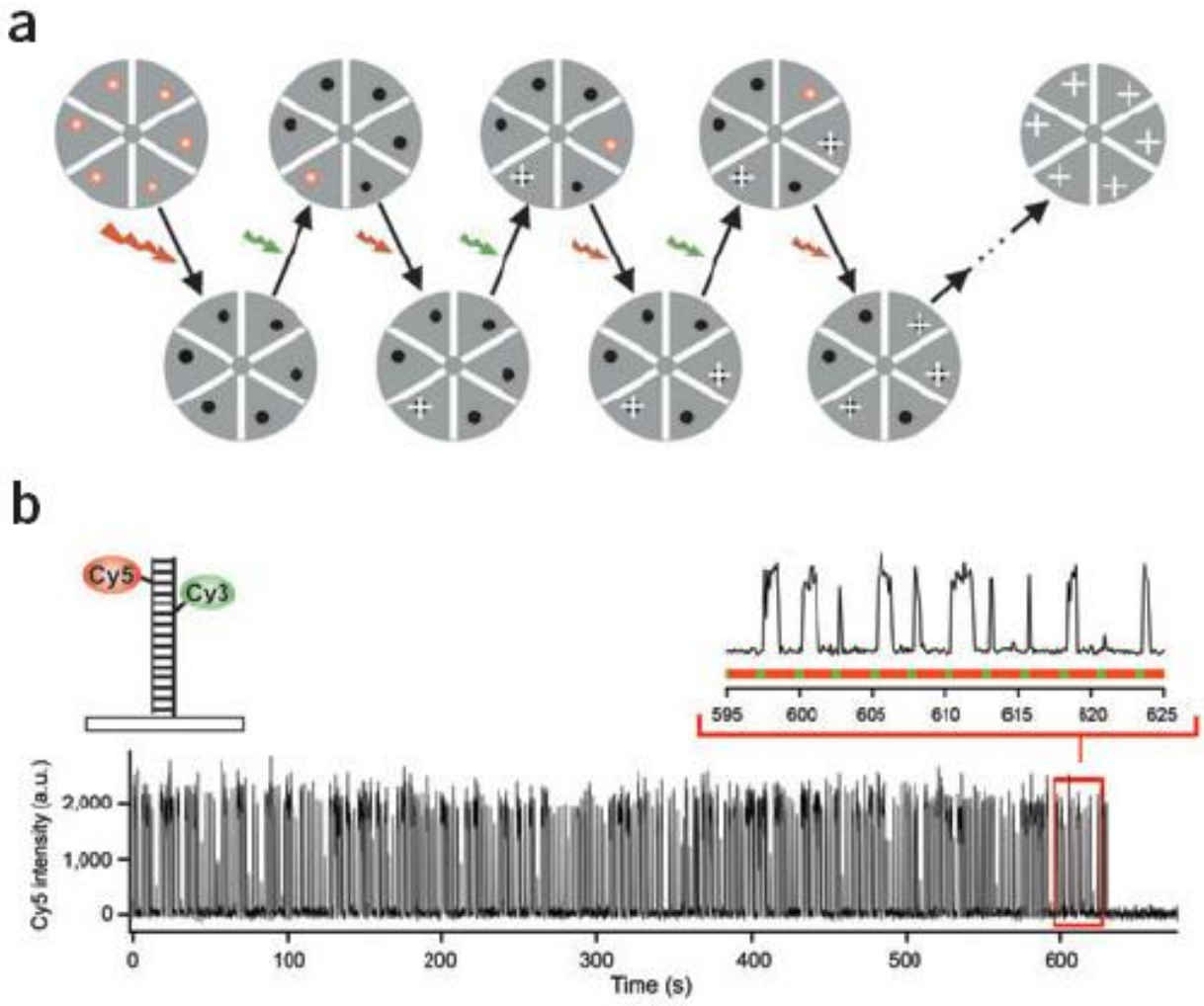

Bacteria

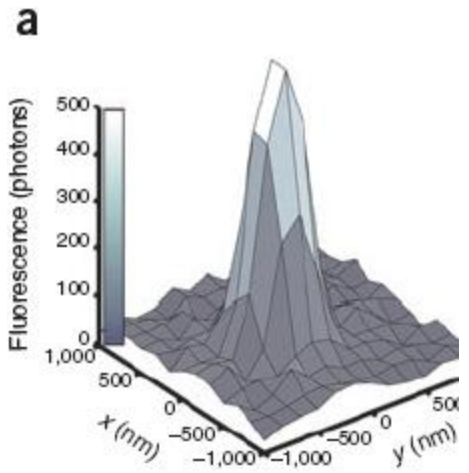
Microtubule in Kidney Cells

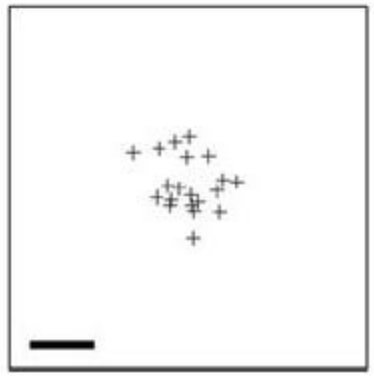
Hell, NBT 2003

# STORM & PALM

## High Resolution based on Single Chromophore “Signature”

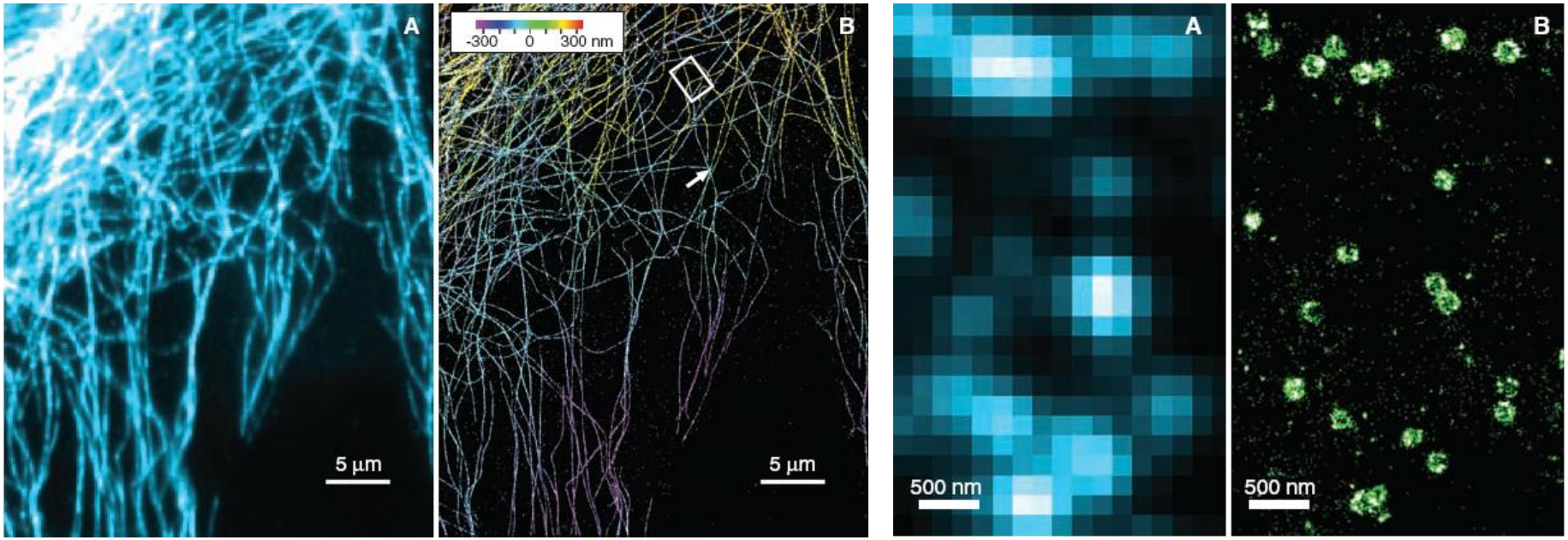


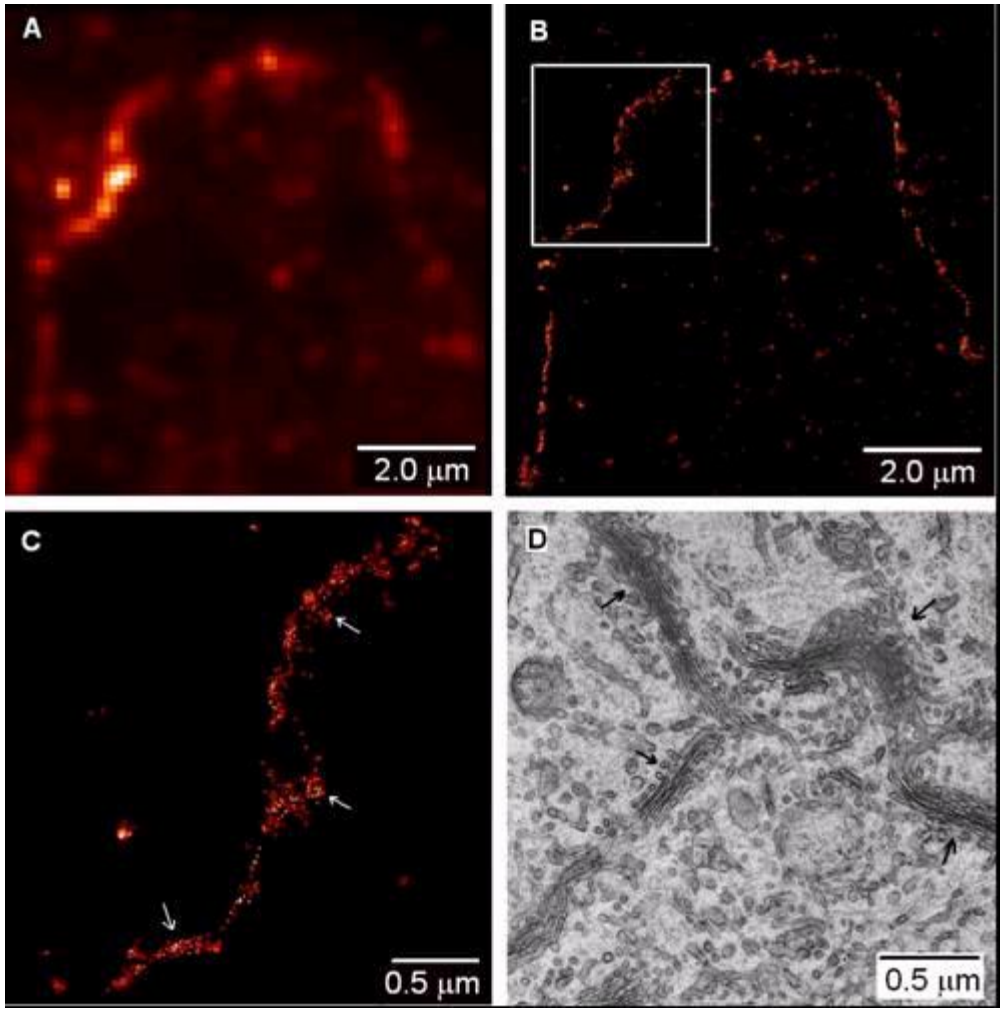

Rust, Nat Meth 2006

# STORM




Huang, Science 2008

# PALM




Betzig et al, Science, 2006