# Microscopy

Note Title 4/15/2007

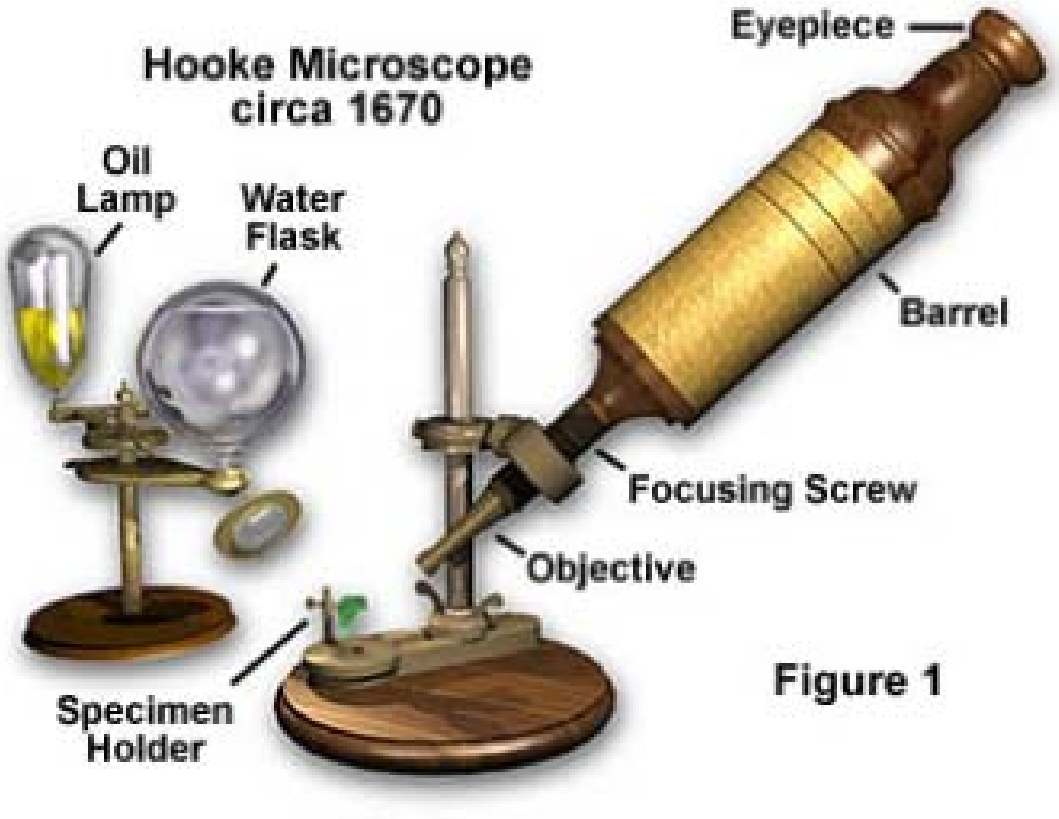



Figure 1

Robert Hooke

# Modern Widefield Microscope

# Numerical Aperature



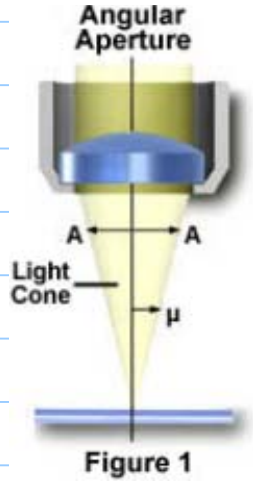



Figure 1

Numerical Aperature(NA) = $n \sin\mu$

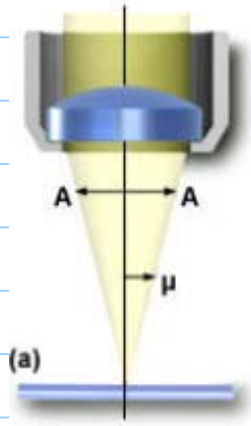
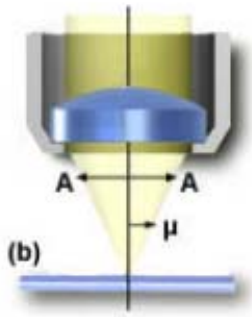



Figure 2

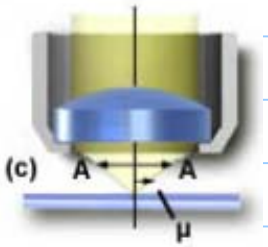



NA = (n)sin(μ)

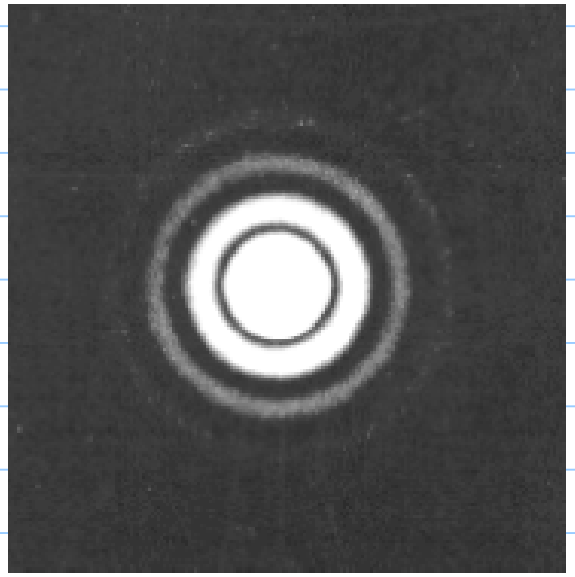
(a) μ = 7° NA = 0.12

(b) μ = 20° NA = 0.34

(c) μ = 60° NA = 0.87



# Diffraction from a pinhole



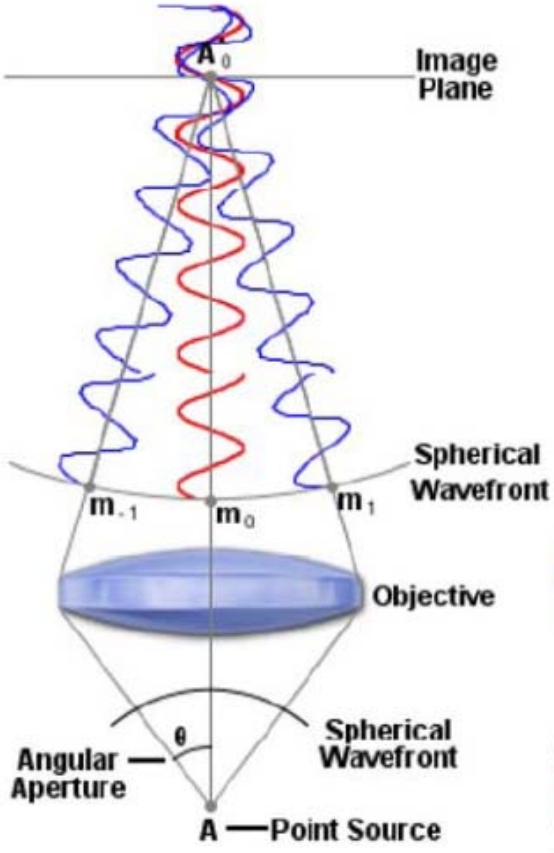
Radius

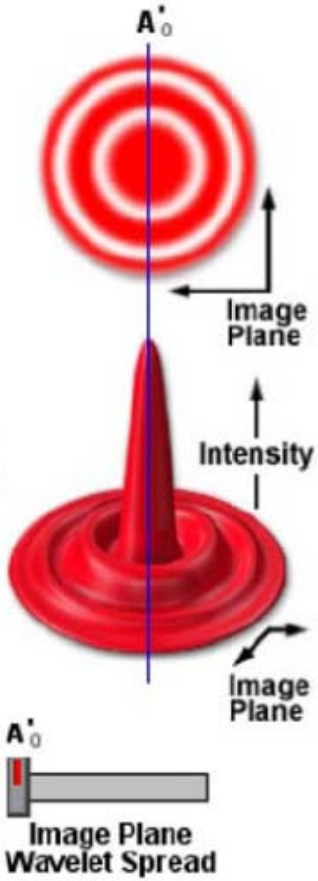
$$r = \frac{.61\,\lambda}{NA}$$

NA: numerical aperature

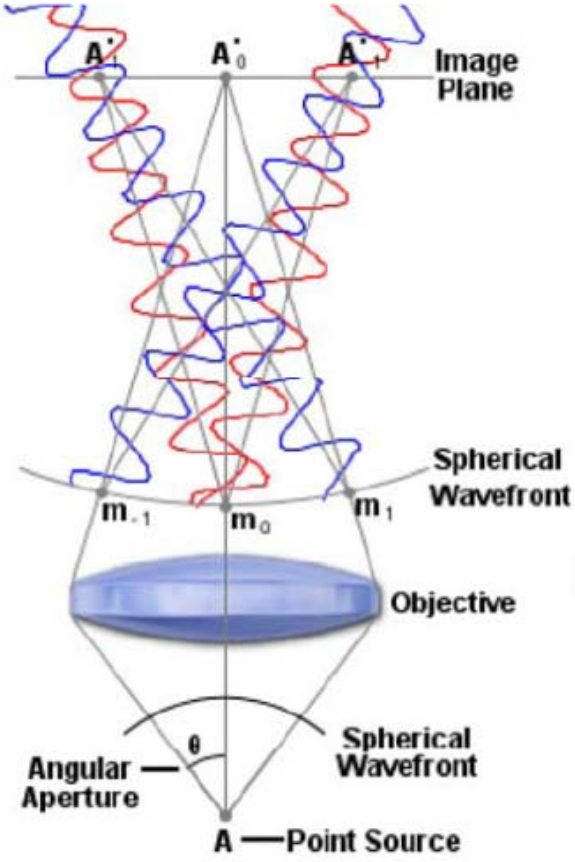
# Airy Disk Formation

On axis




First. Minimum



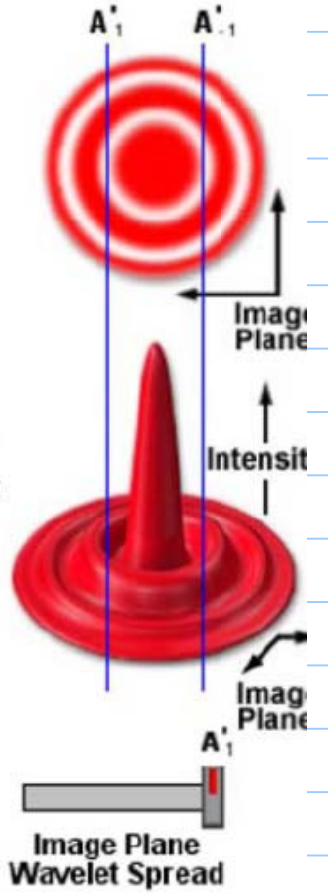
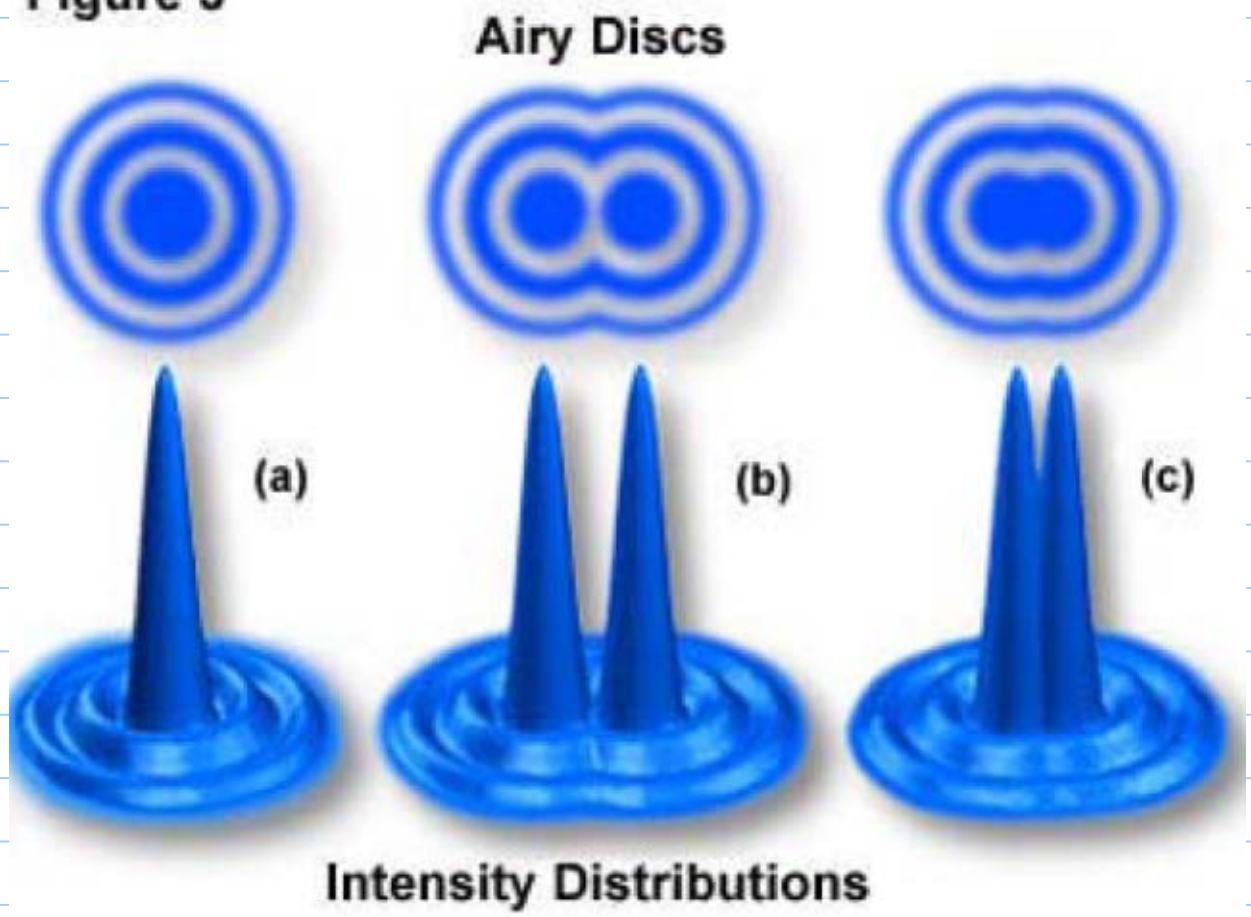
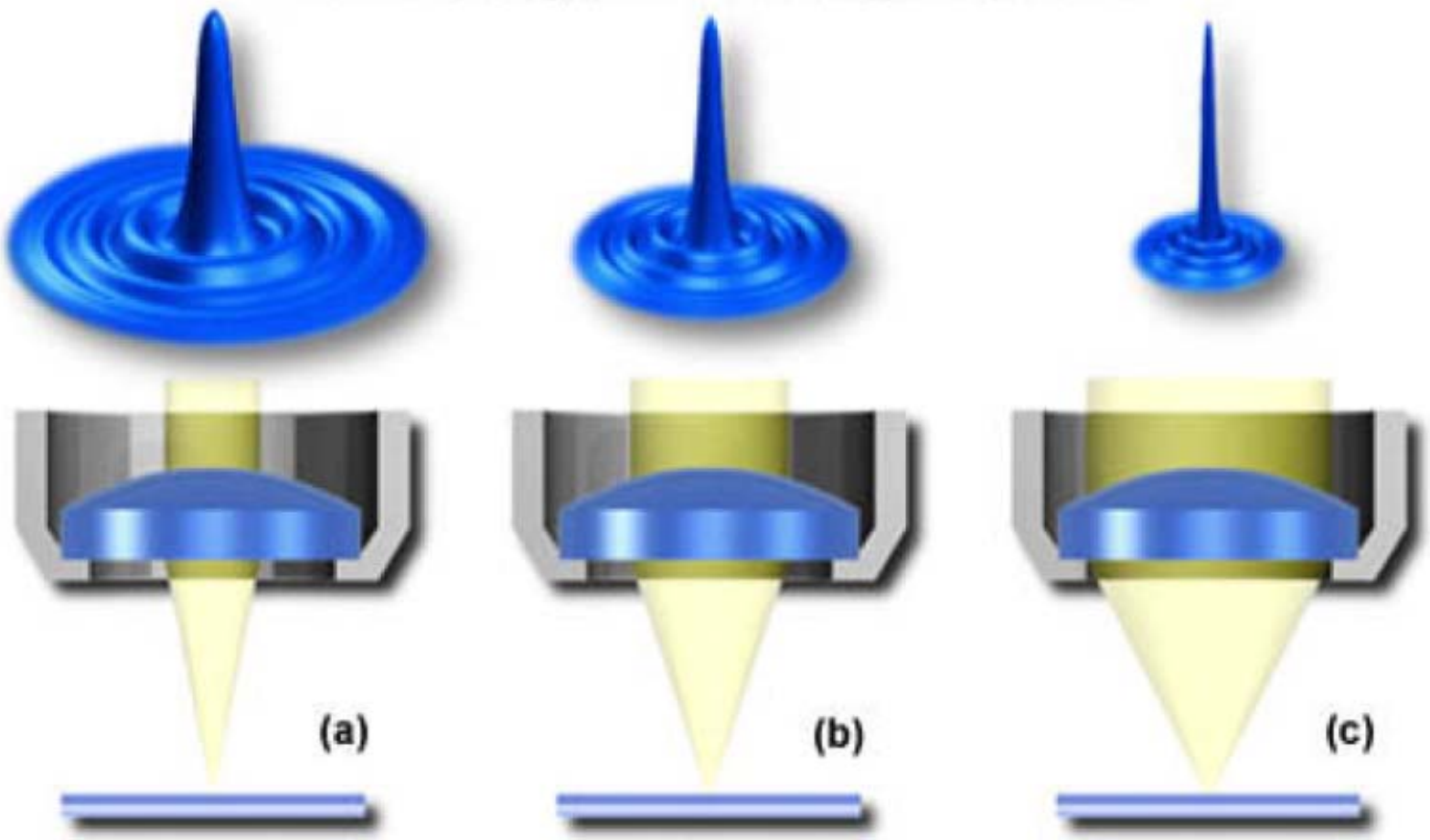



Figure 3



# Numerical Aperture and Airy Disc Size



# Abbe Limit

When the objective NA is large enough to capture the central airy disk

$$\text{Abbe limit} = \frac{\lambda}{2\,NA}$$

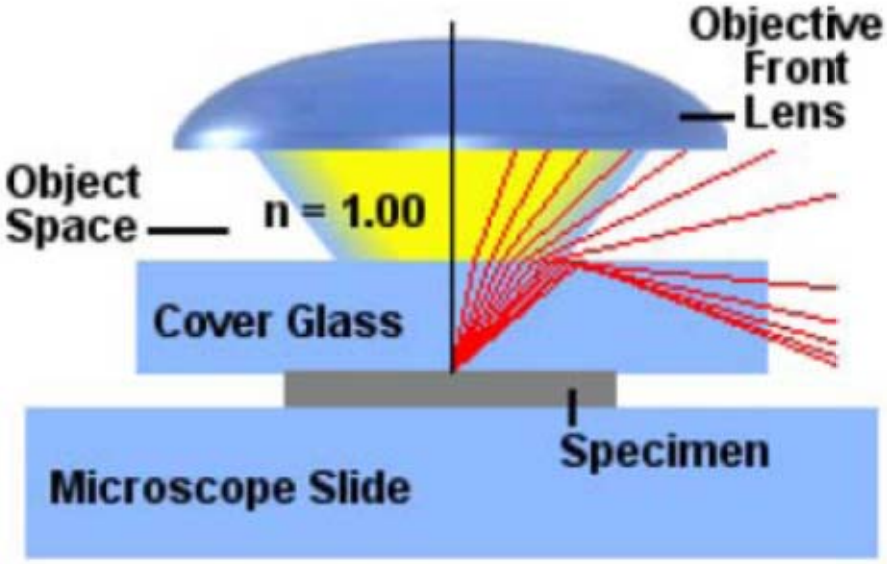
$d$ = lateral resolution

$\lambda$ = wavelength

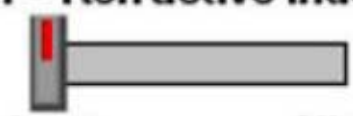
NA = Numerical Aperature of the objective

Ernst Abbe
(1840-1905)

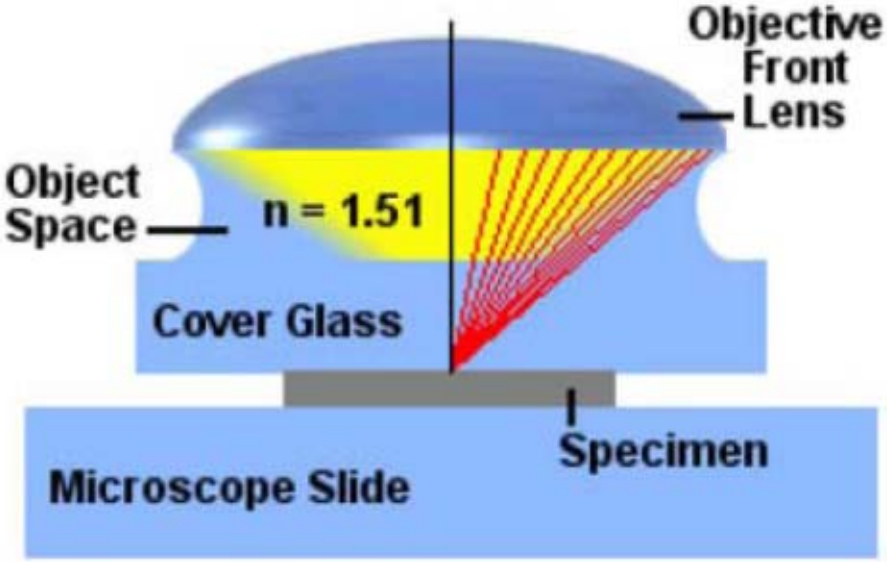
# Oil Immersion




n = Refractive Index




Numerical Aperture (NA) = n sin(θ)
NA = 1.00 sin (65°)
0.90 = 1.00 sin (65°)
θ = Angular Aperture = 65°




n = Refractive Index


Numerical Aperture (NA) = n sin(θ)
NA = 1.51 sin (65°)
1.38 = 1.51 sin (65°)
θ = Angular Aperture = 65°

With 400 nm wavelength

best lens $\mu = 68°$

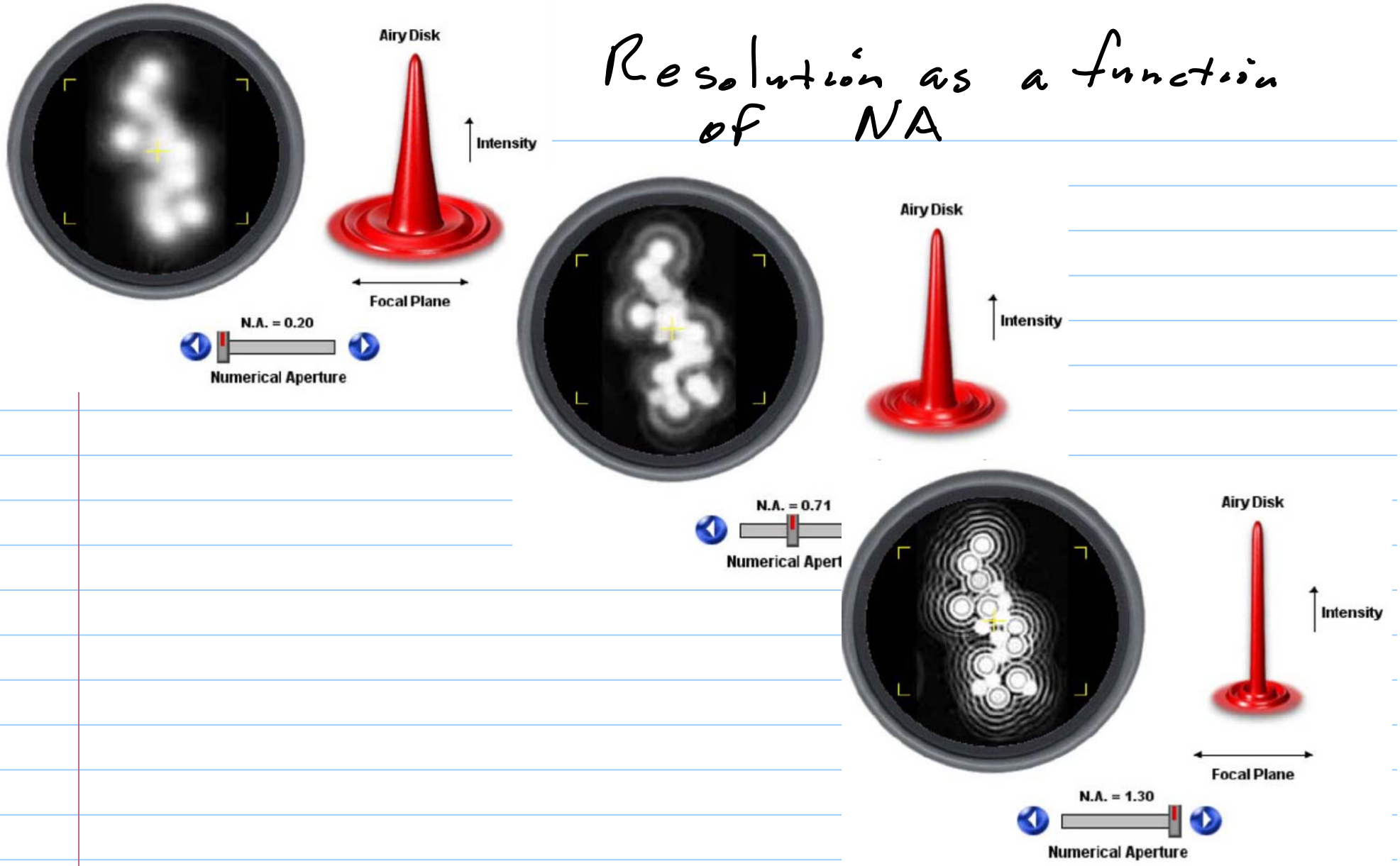
$n = 1.5$ immersion

$$\Delta x = \frac{400\ nm}{\sin(68°)\ 1.5 \times 2} = 144\ nm$$

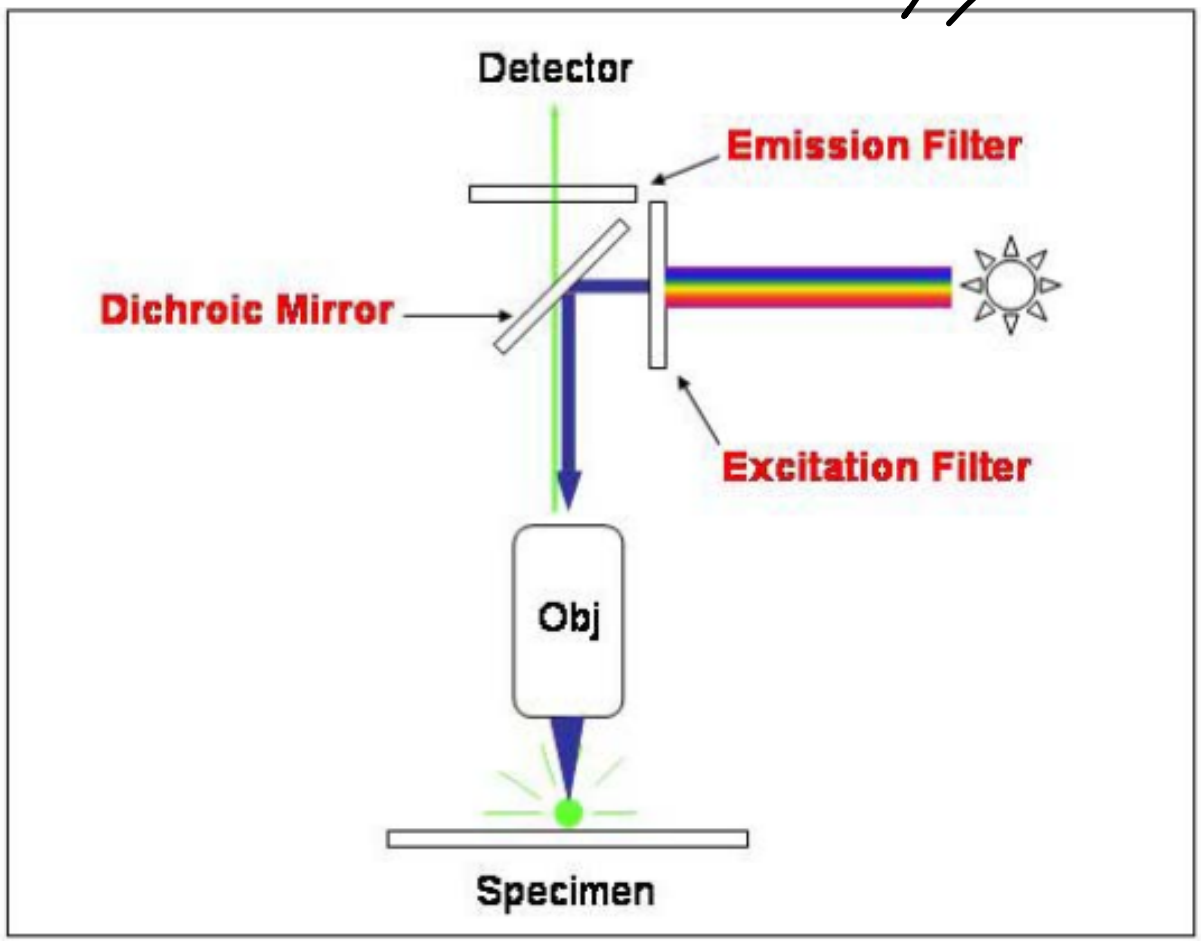
$\Delta z > 500\ nm$

The above are practical limits

# Resolution as a function of NA



# Fluoresence Microscopy



## Photobleaching Rates in Multiply Stained Specimens

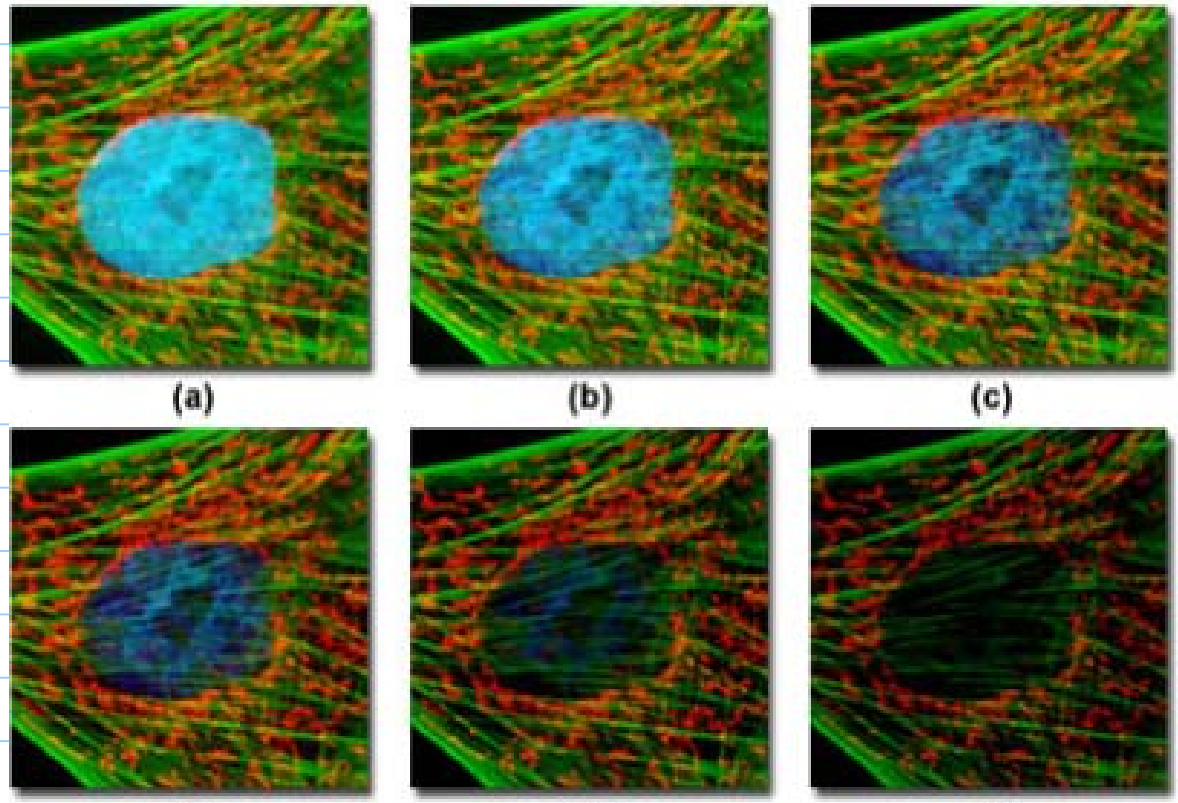
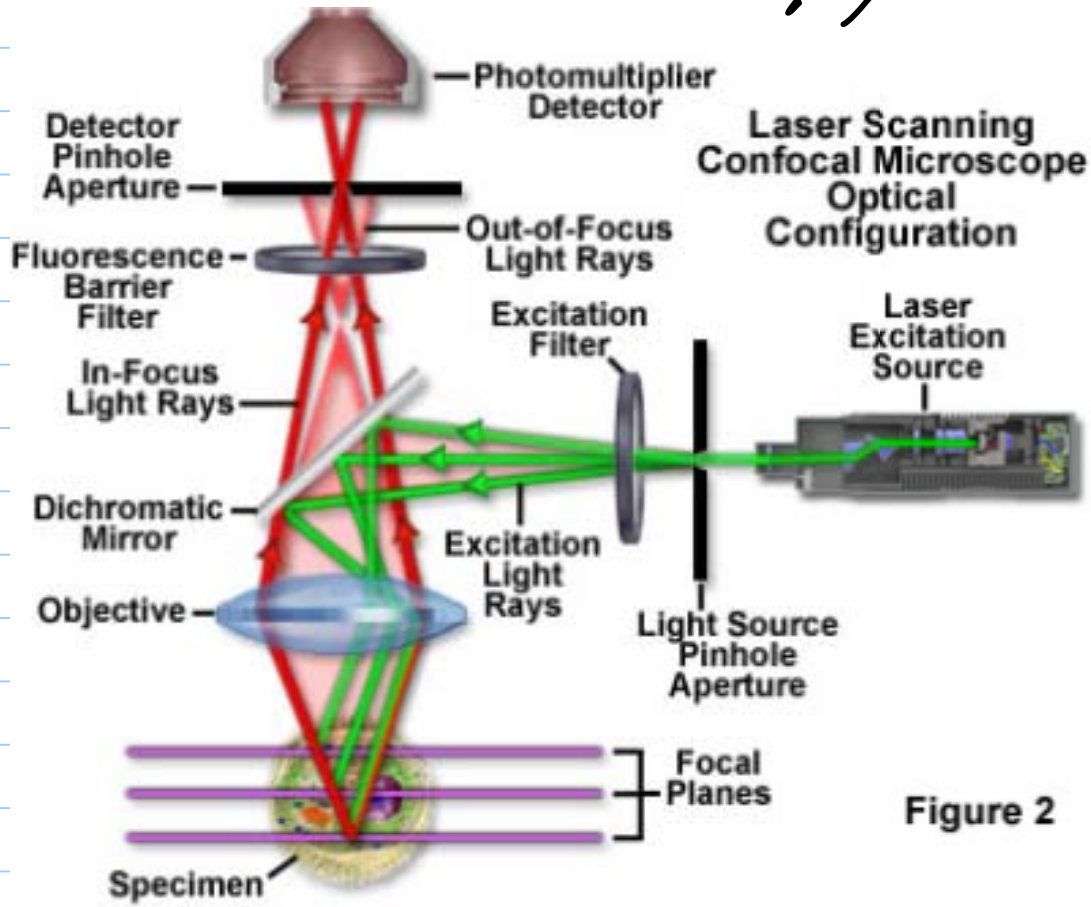



Figure 4

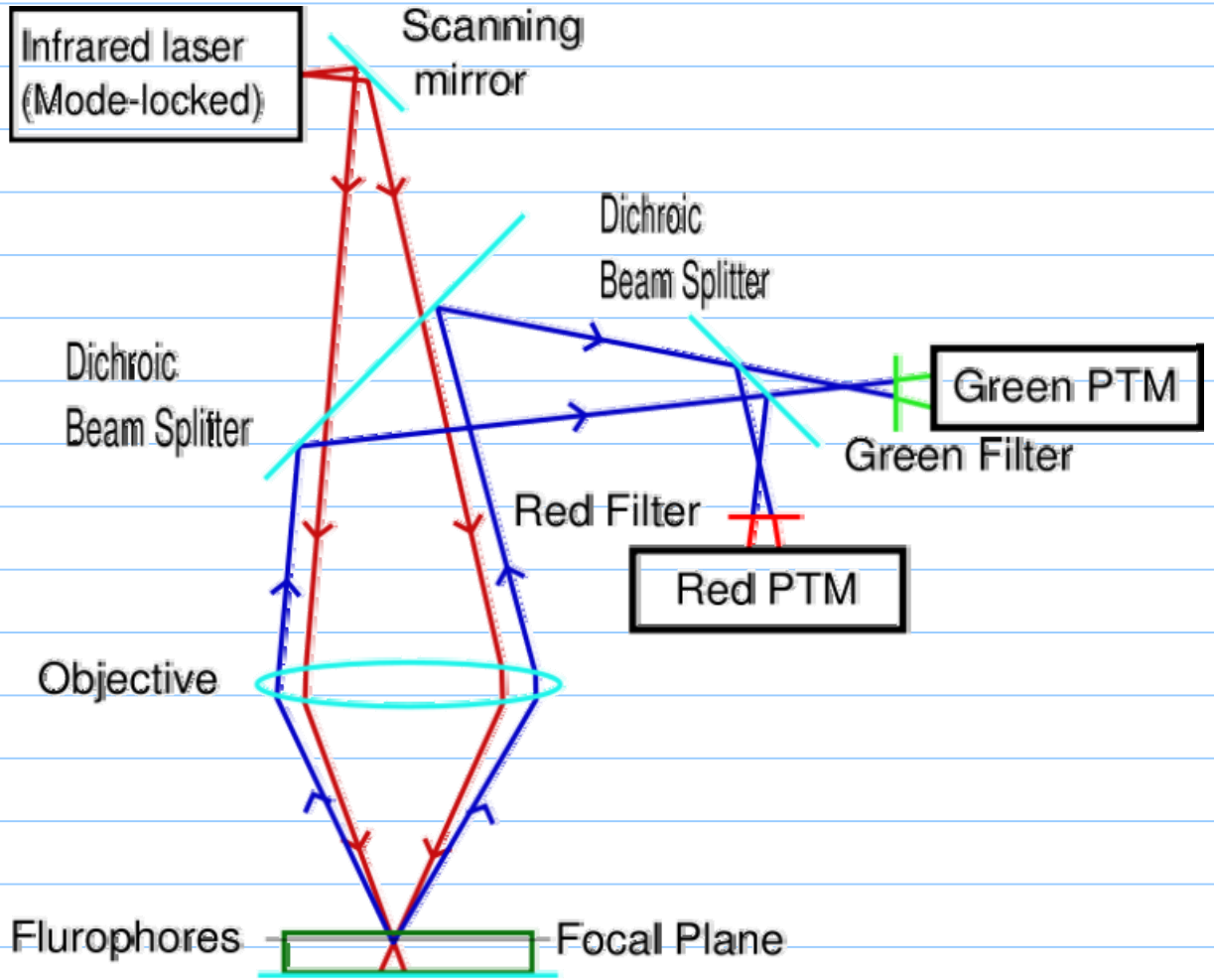
# Confocal Microscopy




Laser Scanning Confocal Microscope Optical Configuration

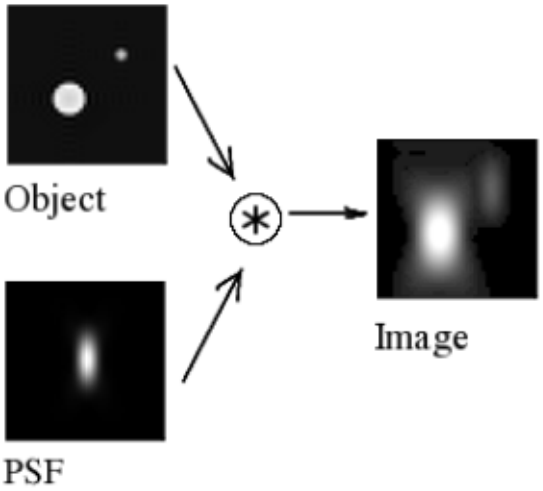
Figure 2

# Two Photon Fluorescence Microscopy



Can we do better than the Abbe limit?

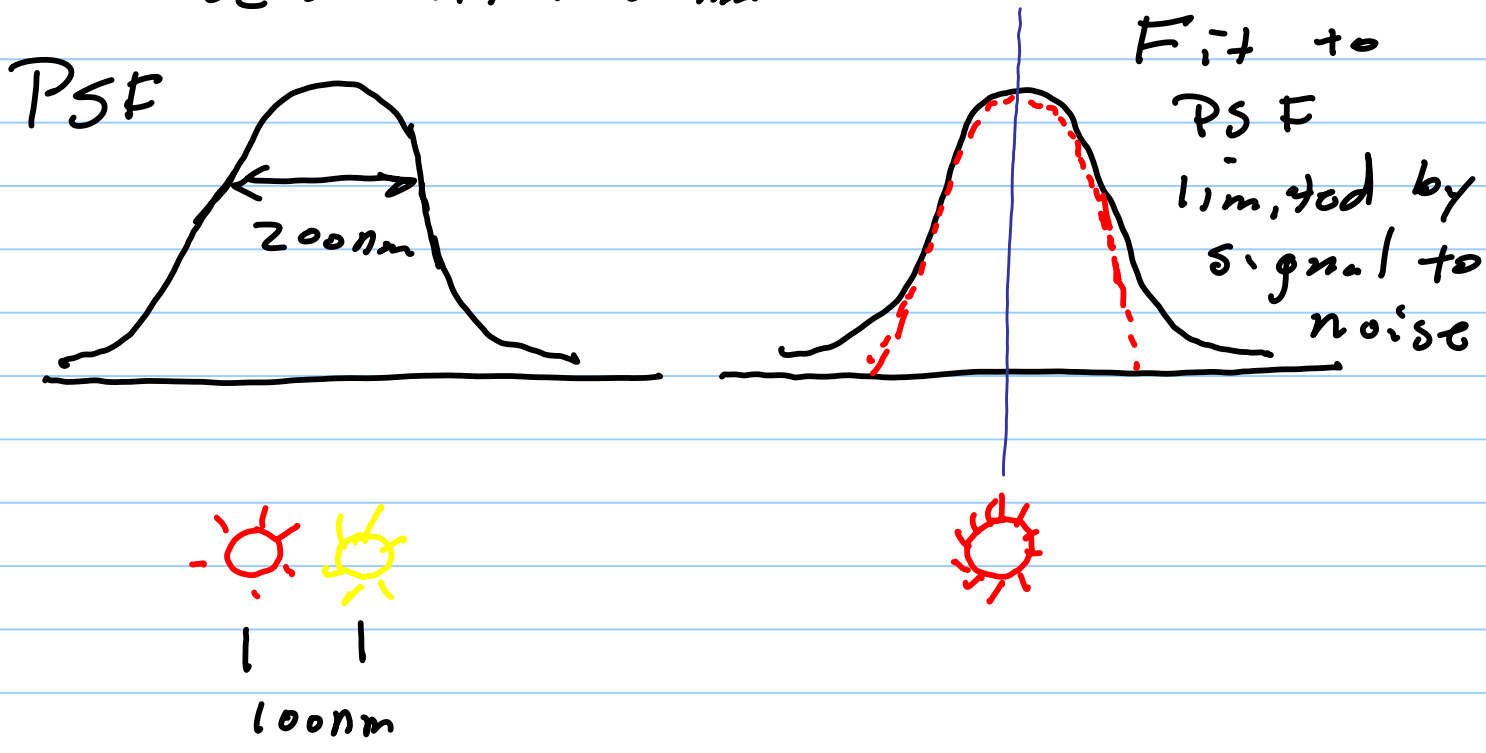
Concept: Point Spread function



Note Title 11/14/2006

# Imaging at nanometer resolution

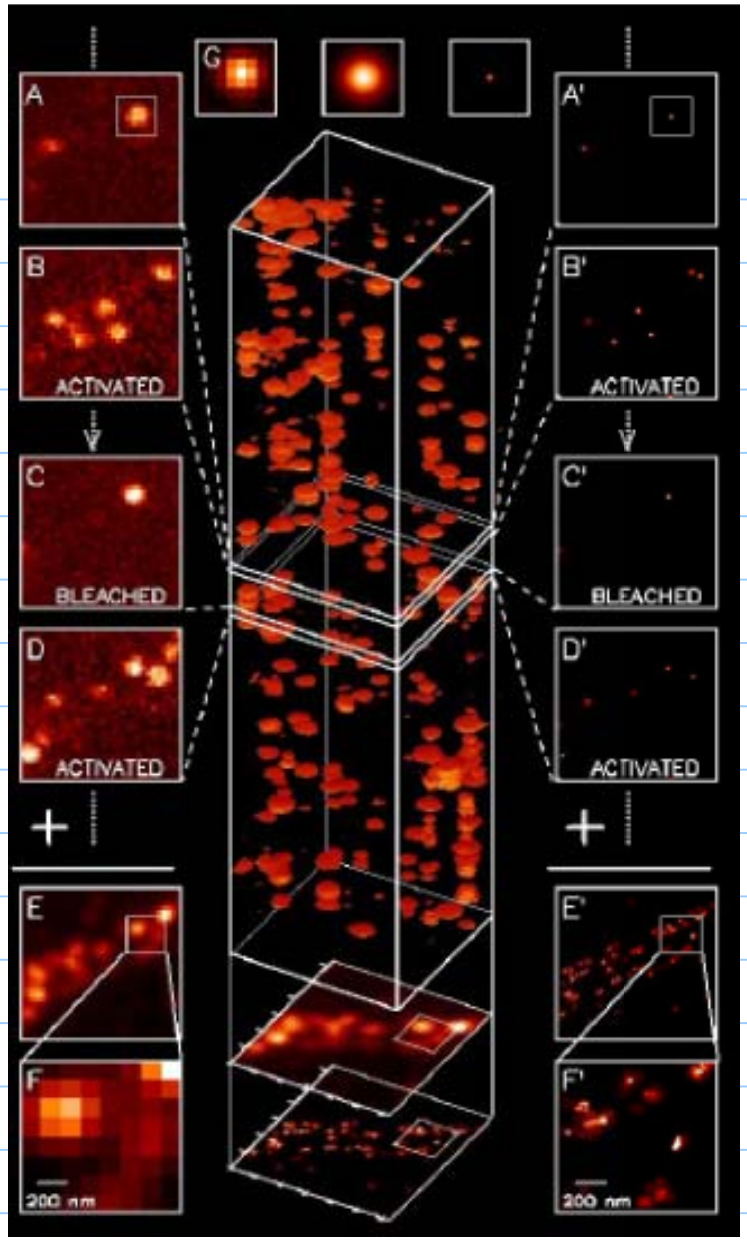
Abbe Limit $\lambda = 500nm$



# PALM Concept

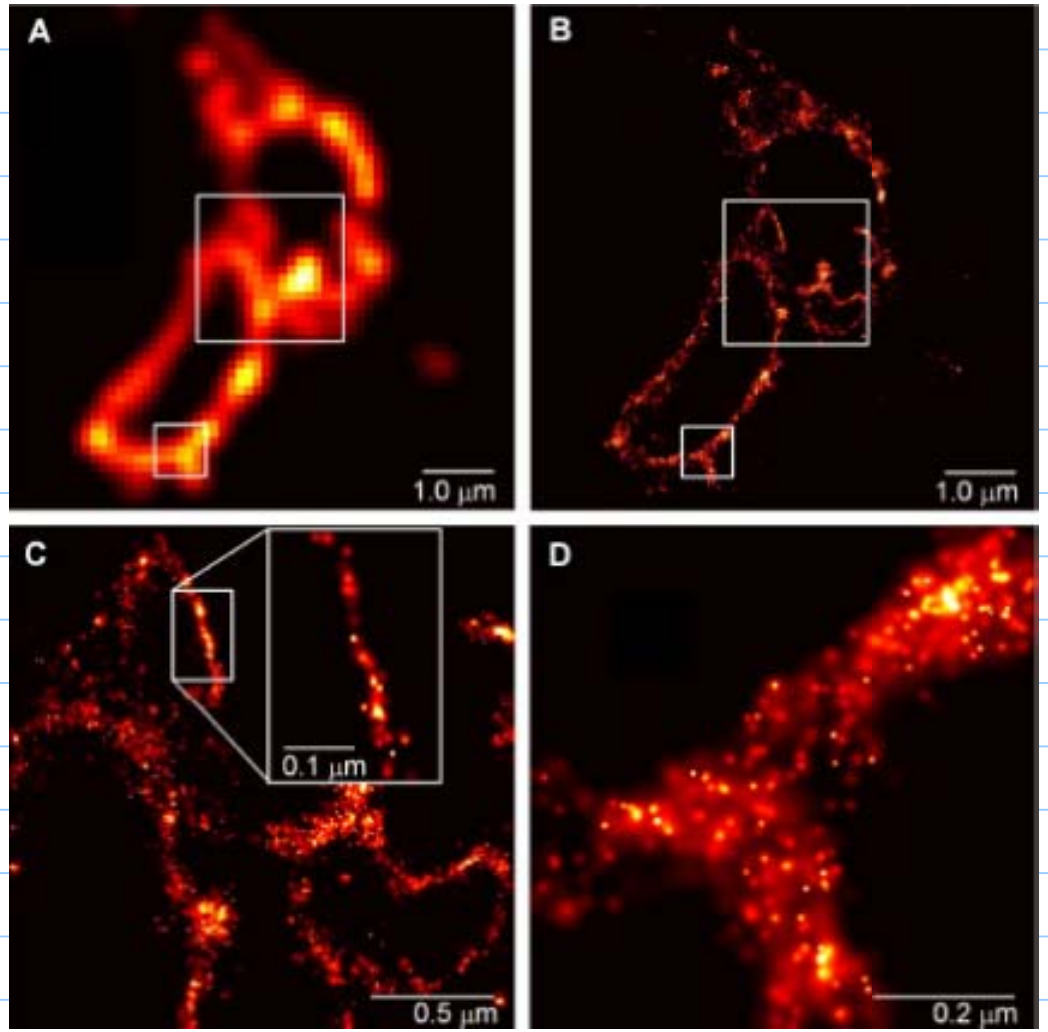
- Use photoactivated fluorescent protein molecules
- Protein attaches randomly to sample
- Use sparse labeling
- Determine location from fit, taking data until bleaching
- Reactivate

# Photo Activated Localization Microscopy



TIRF

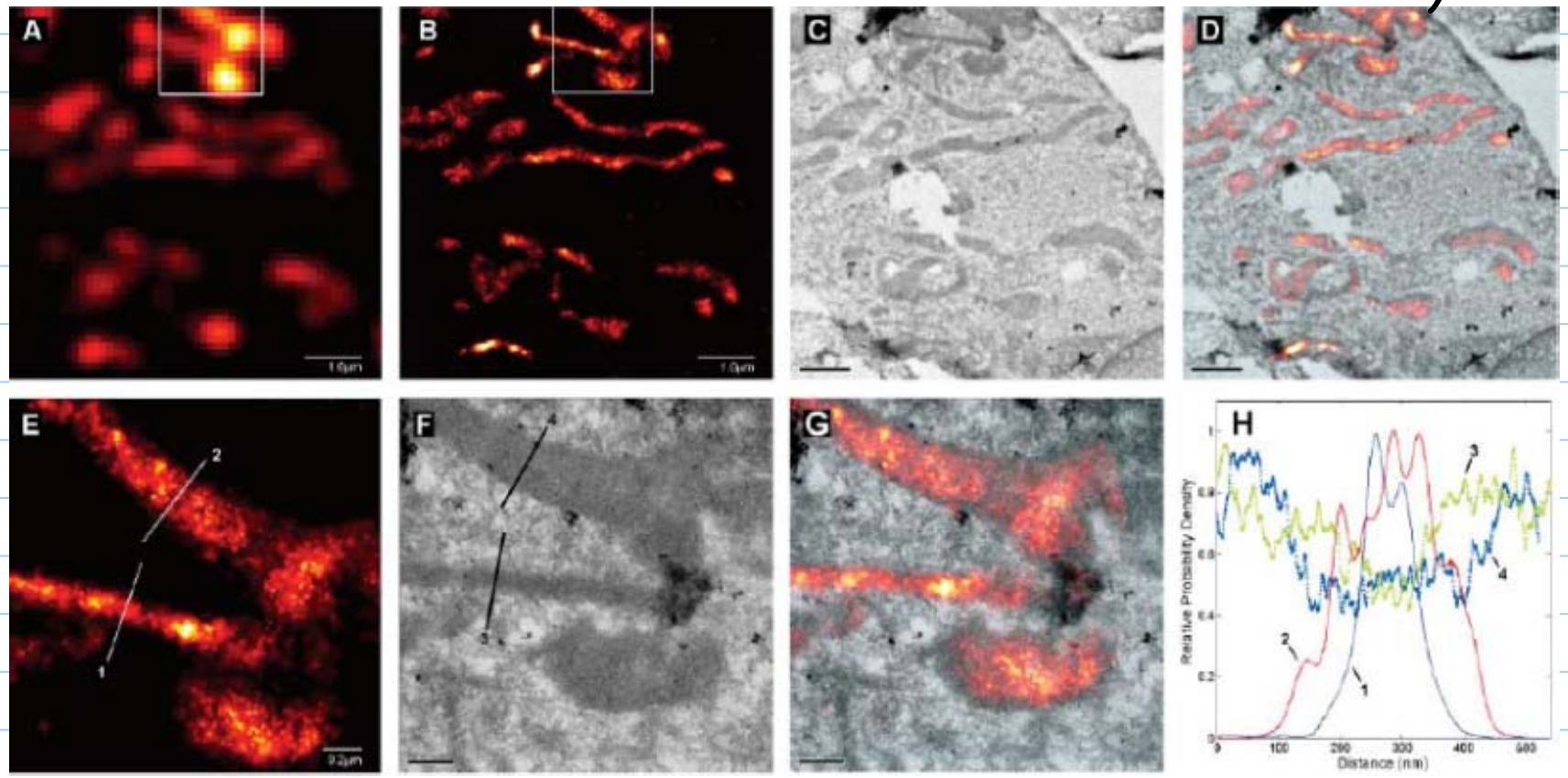
PALM




Membranes

Smaller region B

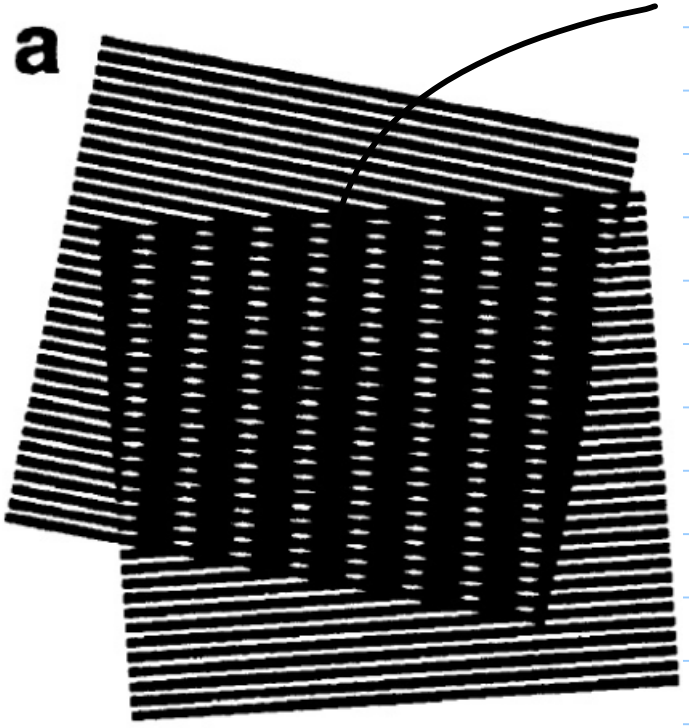
TIRF PALM TEM Overlay




PALM Higher M TEM Overlay Mol Comparison

Structured illumination "getting a factor of two"

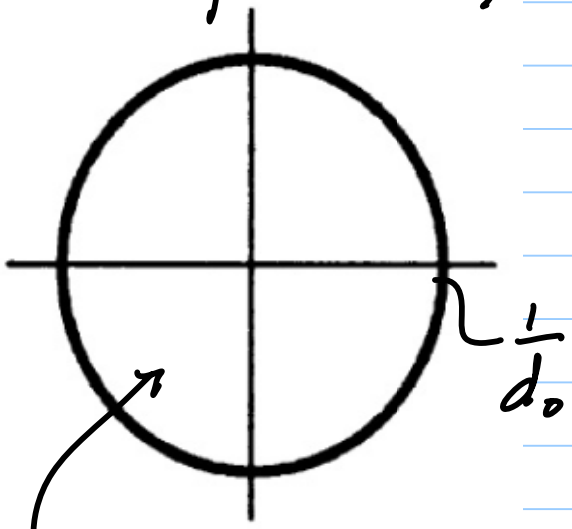
Moiré effect




fringes contain high resolution information

Conventional microscope limited by diffraction

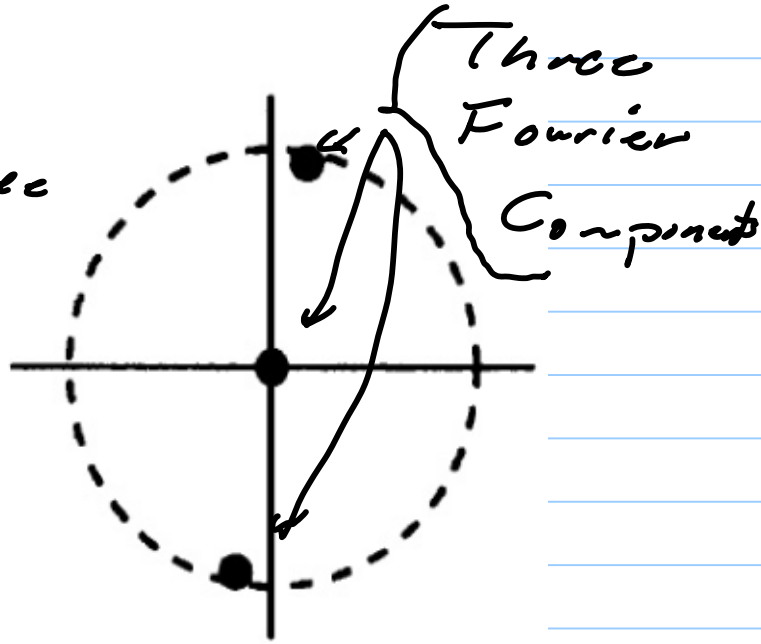
Reciprical Space




Observable region

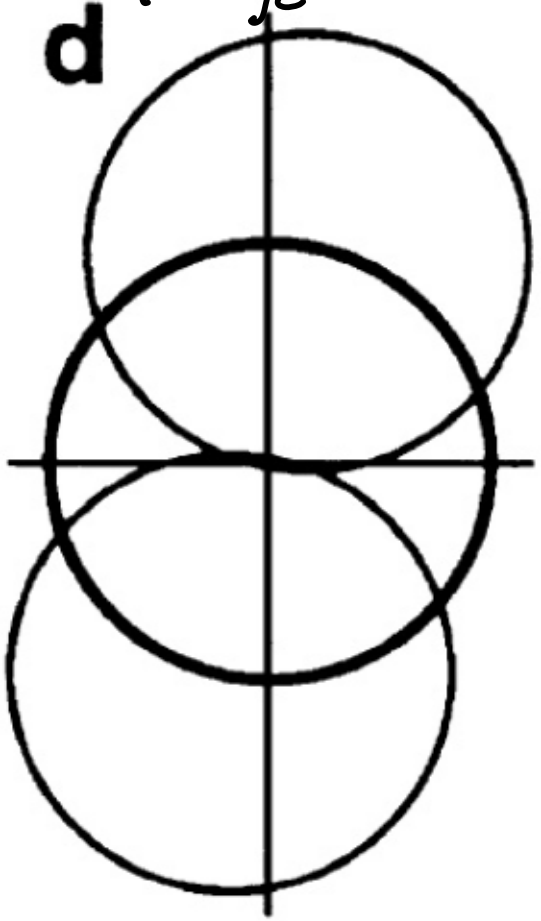
Sinusoidal Illumination

Three Fourier Components

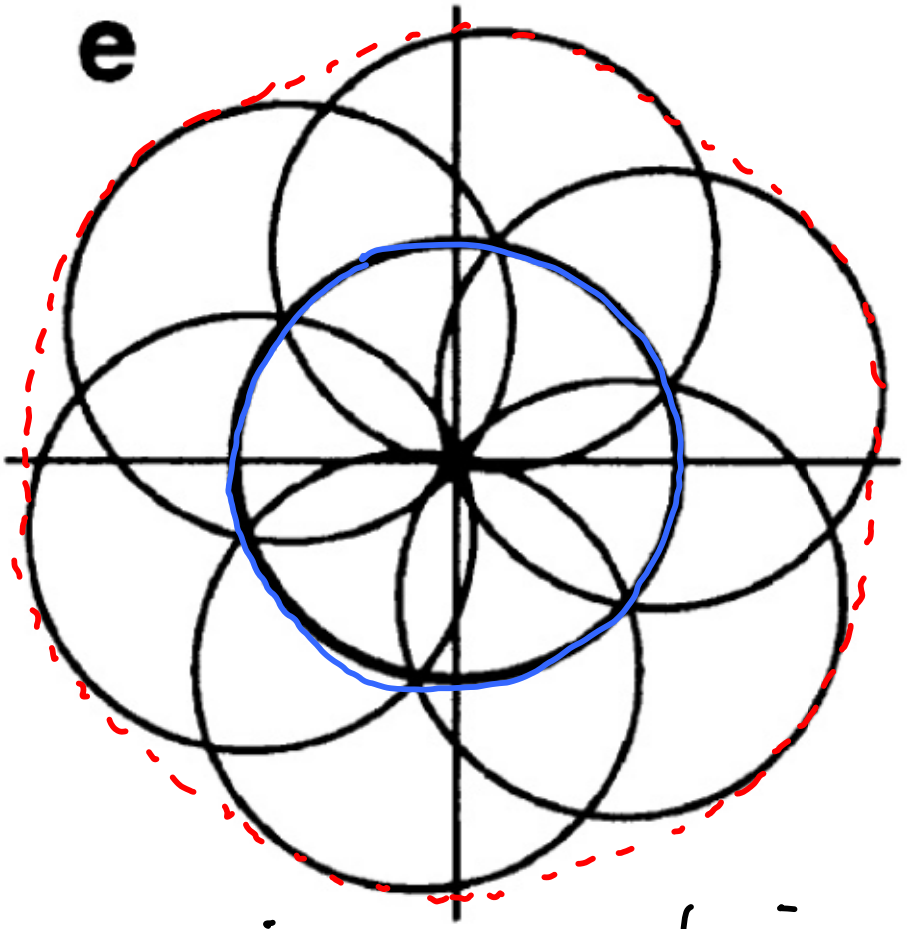


Struct. Illumination

Image




Sum of 3 Images

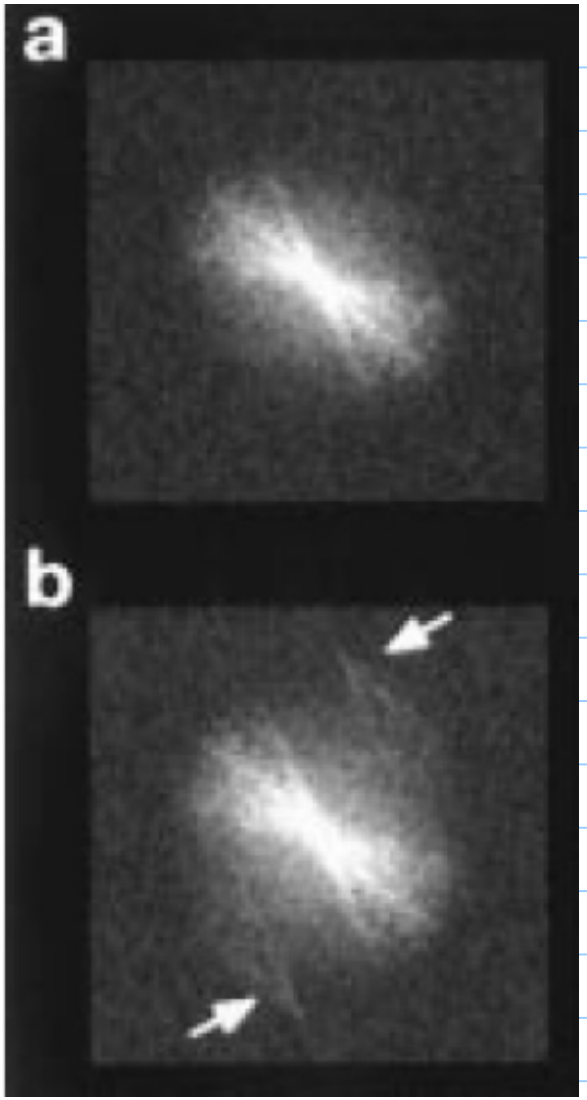



Twice the resolution

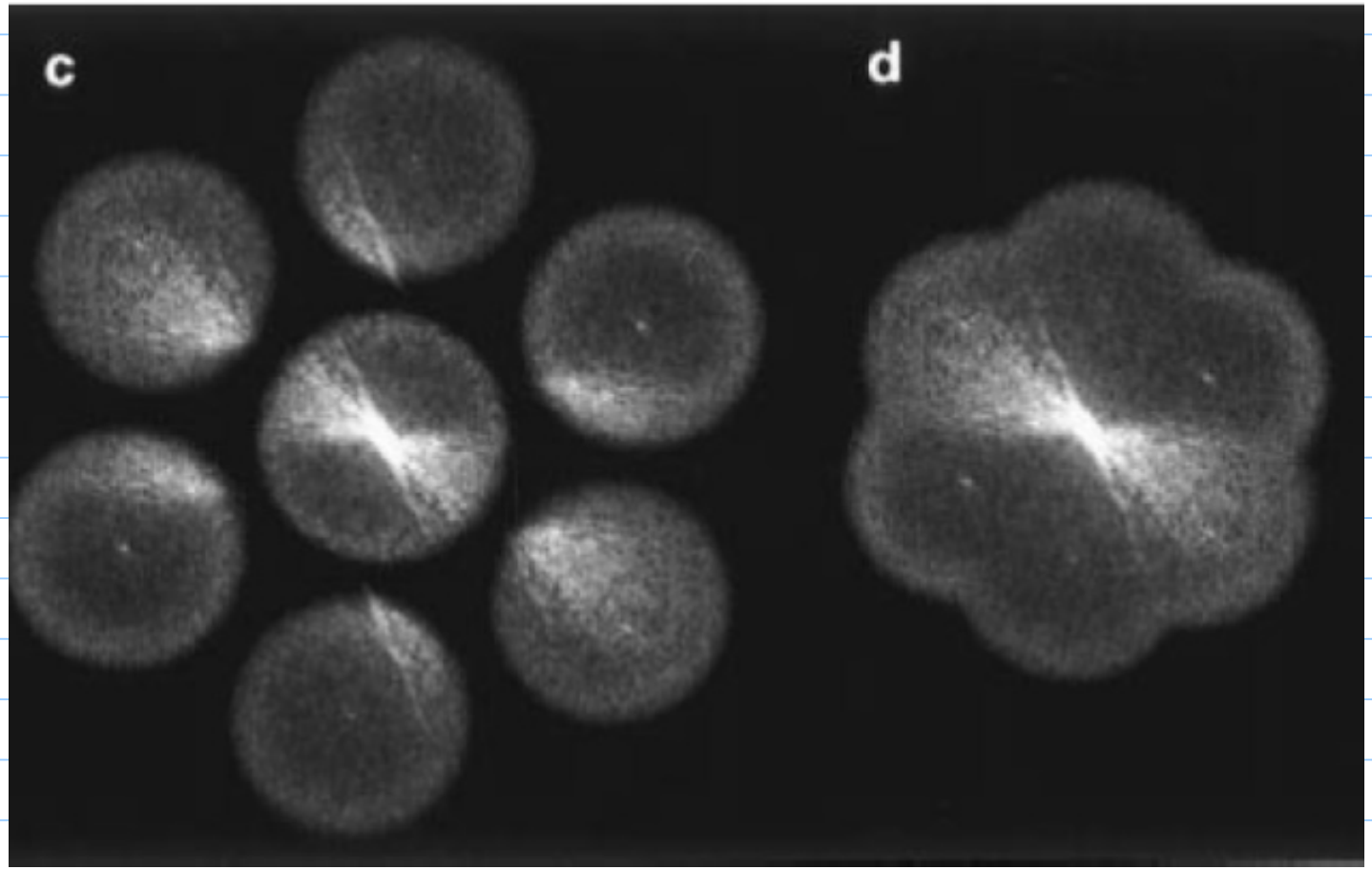
# Fourier Transform Images

Normal Illumination

Sinusoidal Illumination



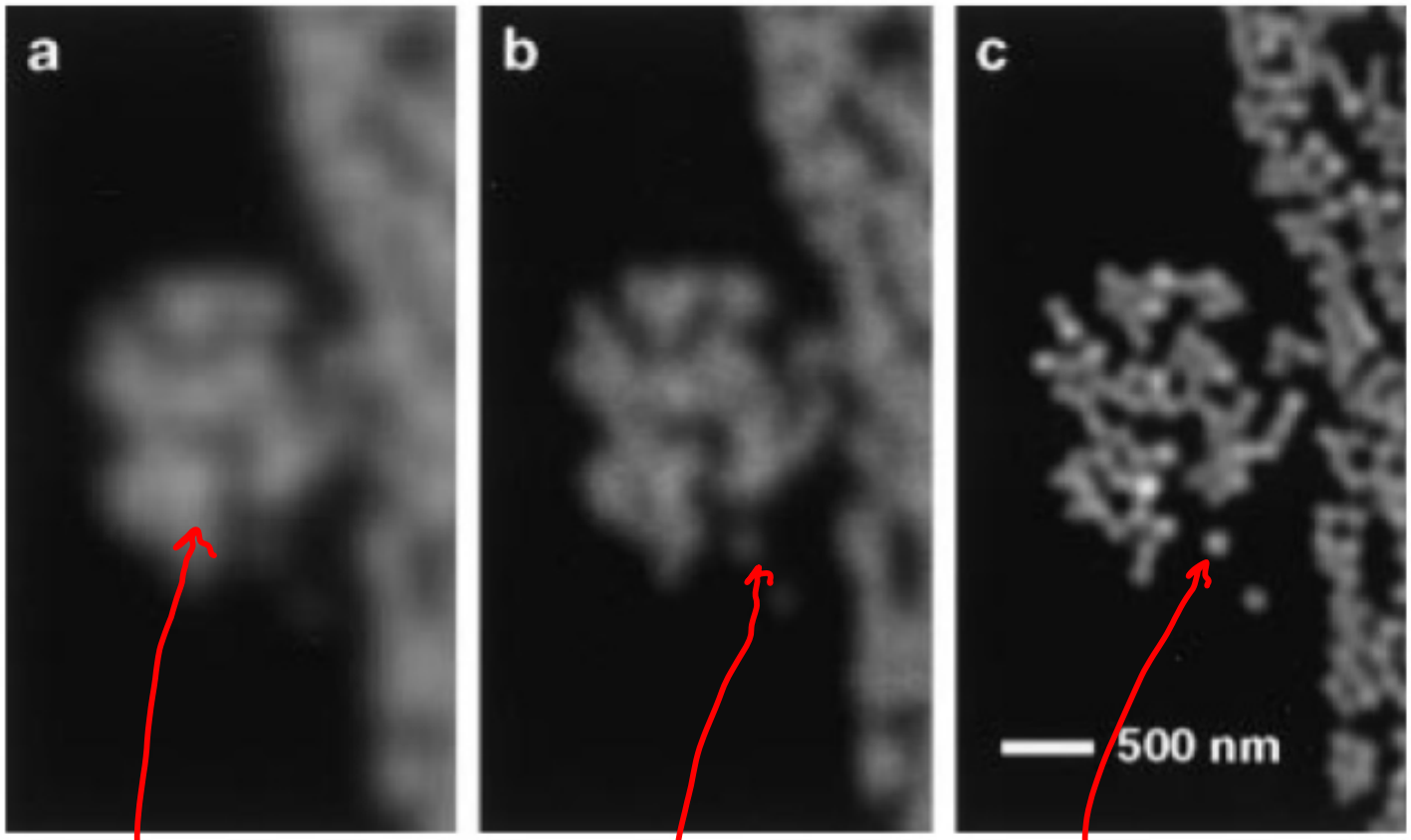
Extracted Fourier Images  Reconstruction




doubled resolution

Three images fluorescent microspheres (121nm)

Conventional | Confocal | Struct. Illumin

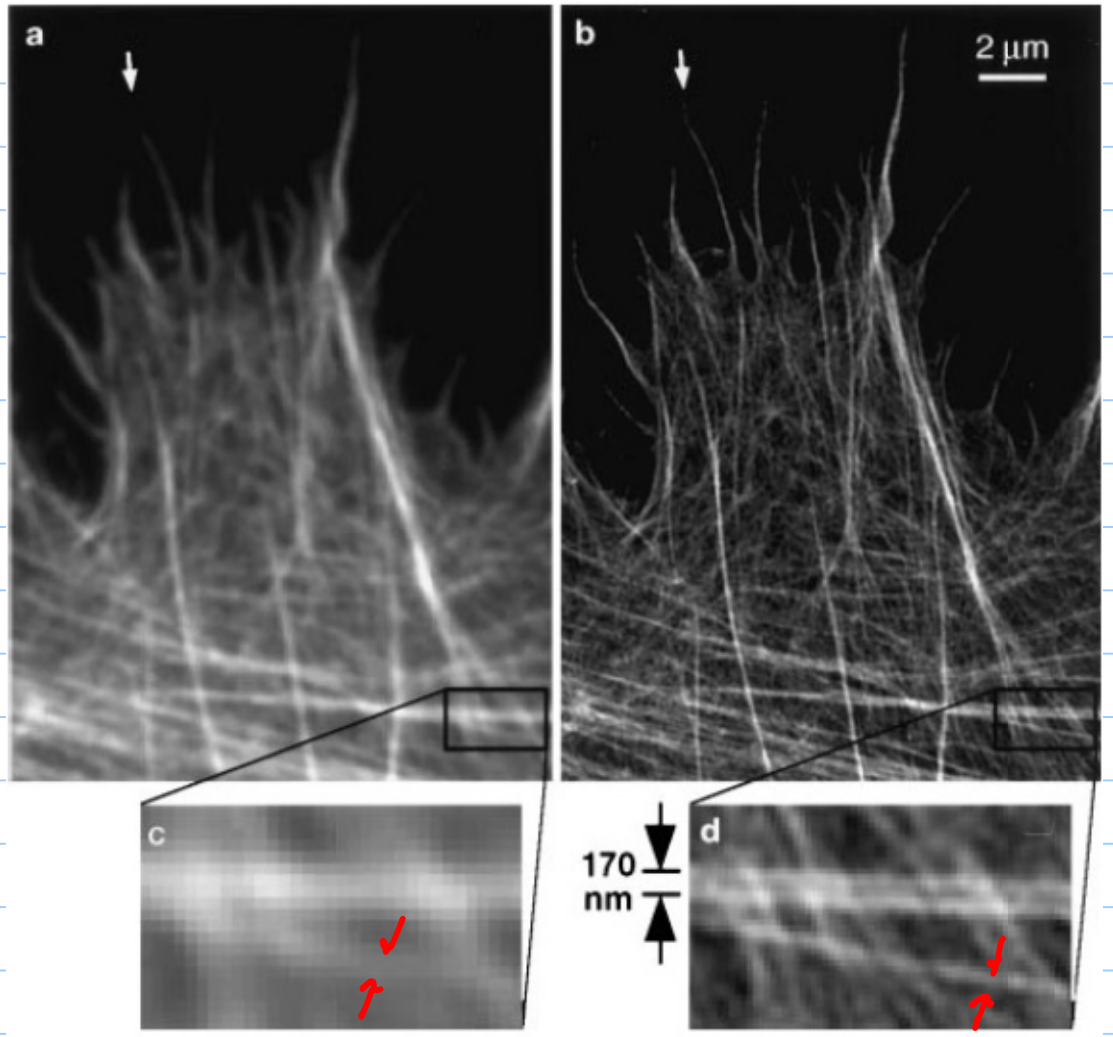



290 nm

210nm

130nm

Conventional  Struct. Illumin




280–300 nm

110 nm