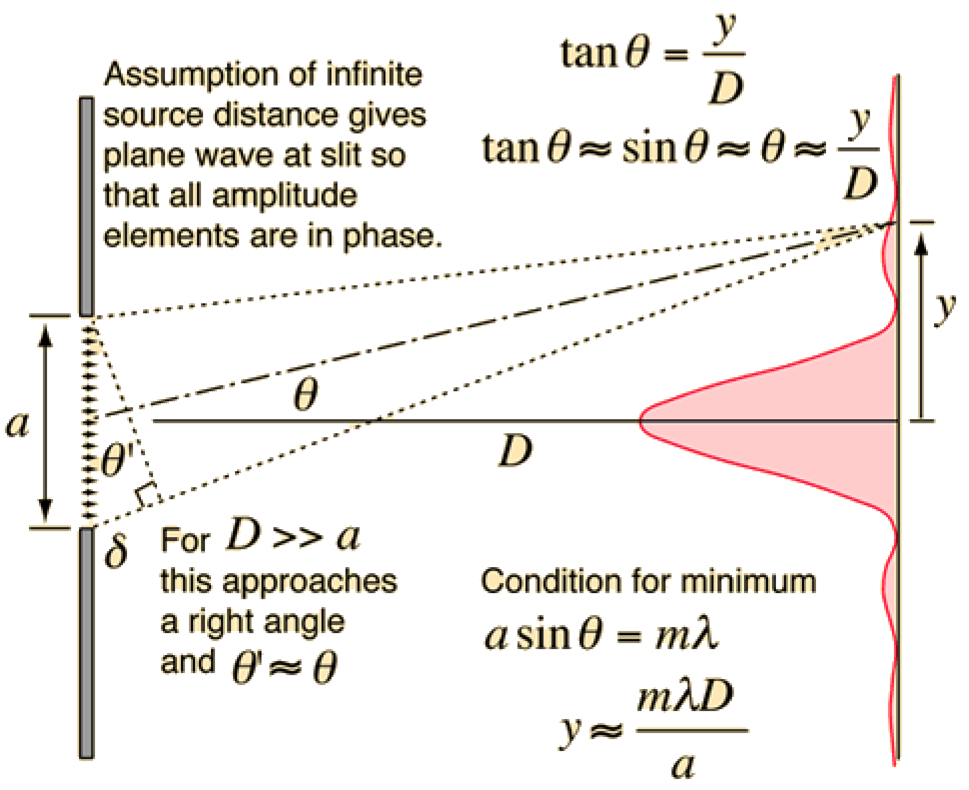
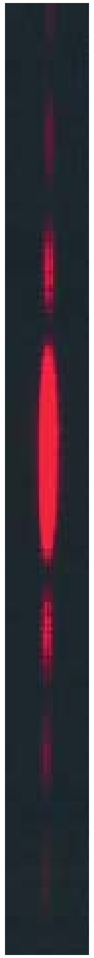
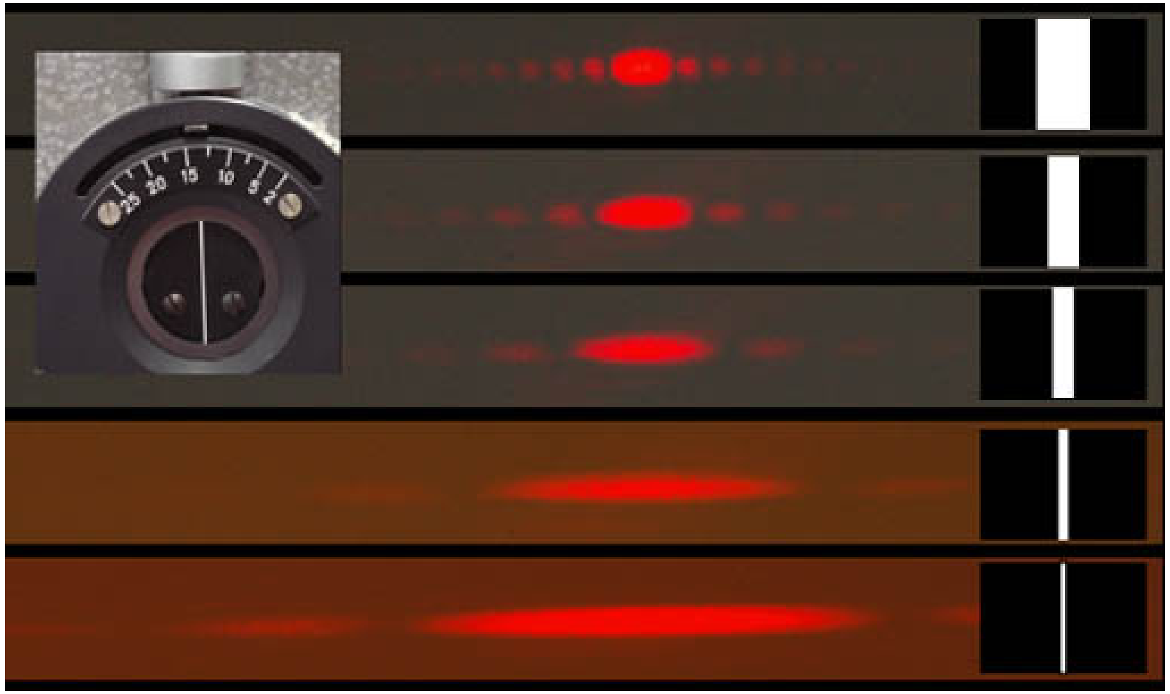
Diffraction and resolution, aberrations

- Last class
  - Microscope components
  - Diffraction
- This class
  - Diffraction and imaging
  - Aberrations

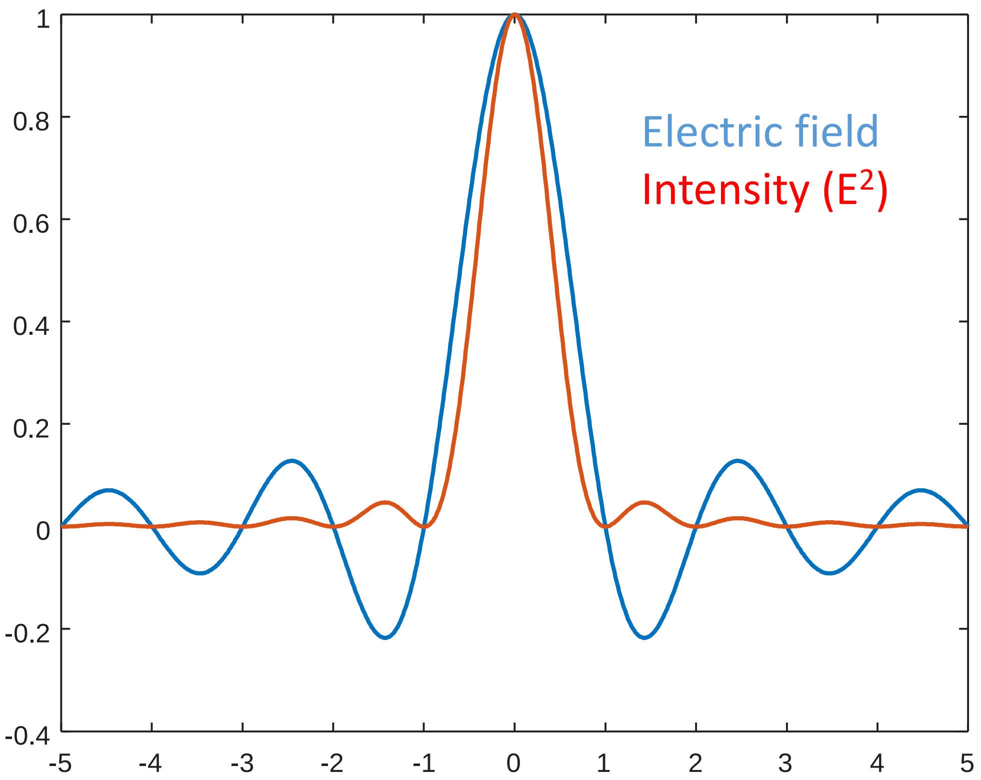
Single slit diffraction







Quick side note



Solution to the diffraction pattern is a sinc function
Intensity is given by $sinc^2$.

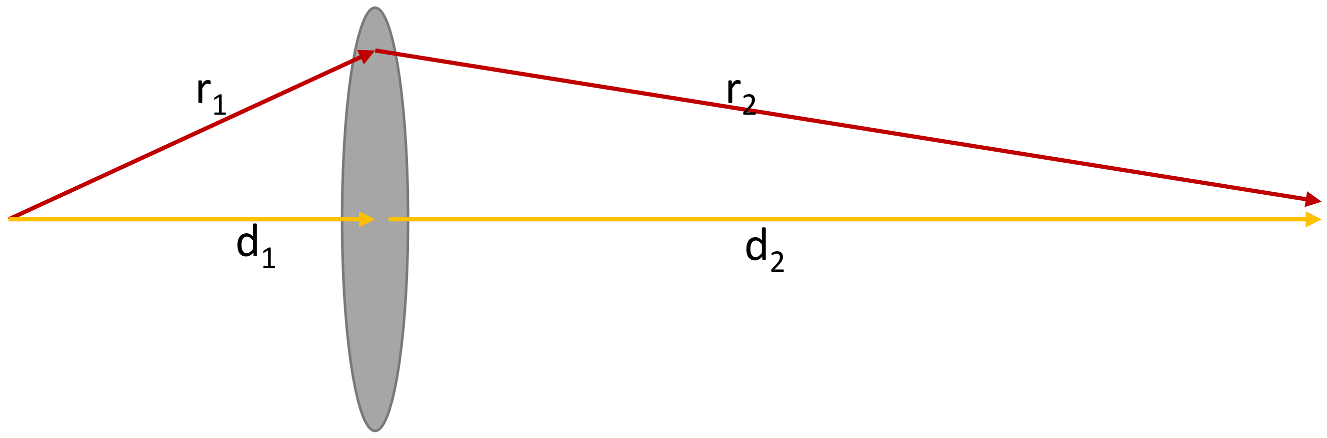
We see INTENSITY of light, measured in watts (energy/sec).

The intensity is the square of the electric field of our plane wave

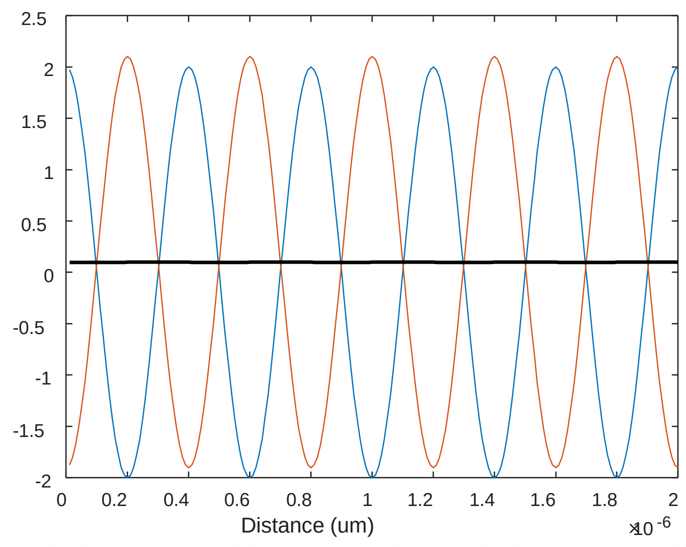
Electric field can be negative, intensity can not.

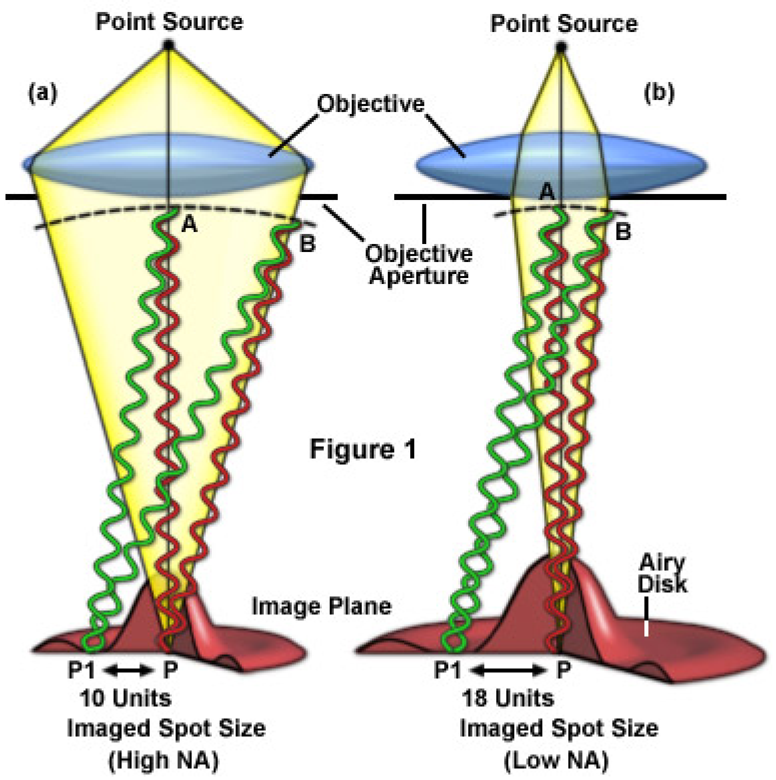
Diffraction of light

Airy disc arises from different paths of light
Destructive interference creates bands of low intensity

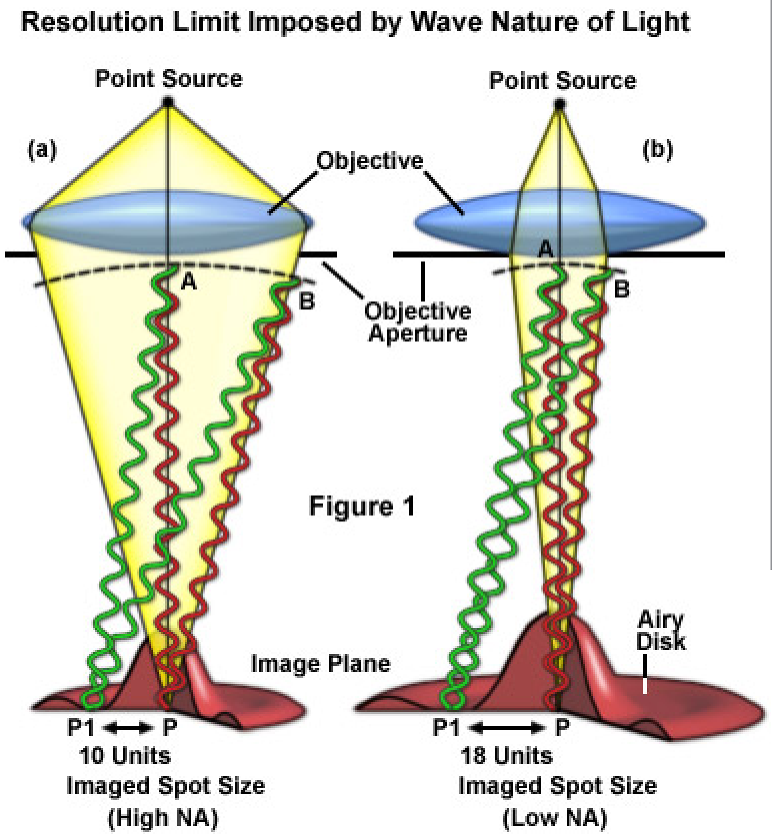


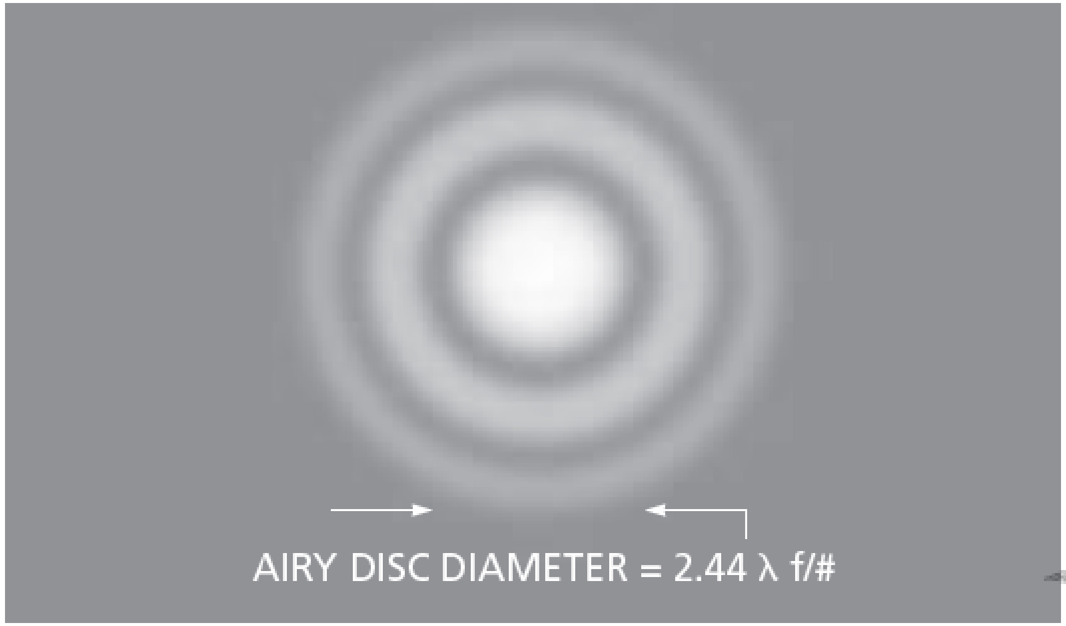
Minimum when length difference d – r = $\lambda/2$
Completely out of phase at that point

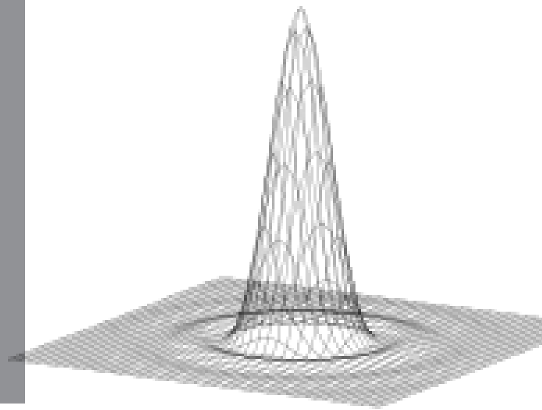




Width of aperture determines size of airy disc

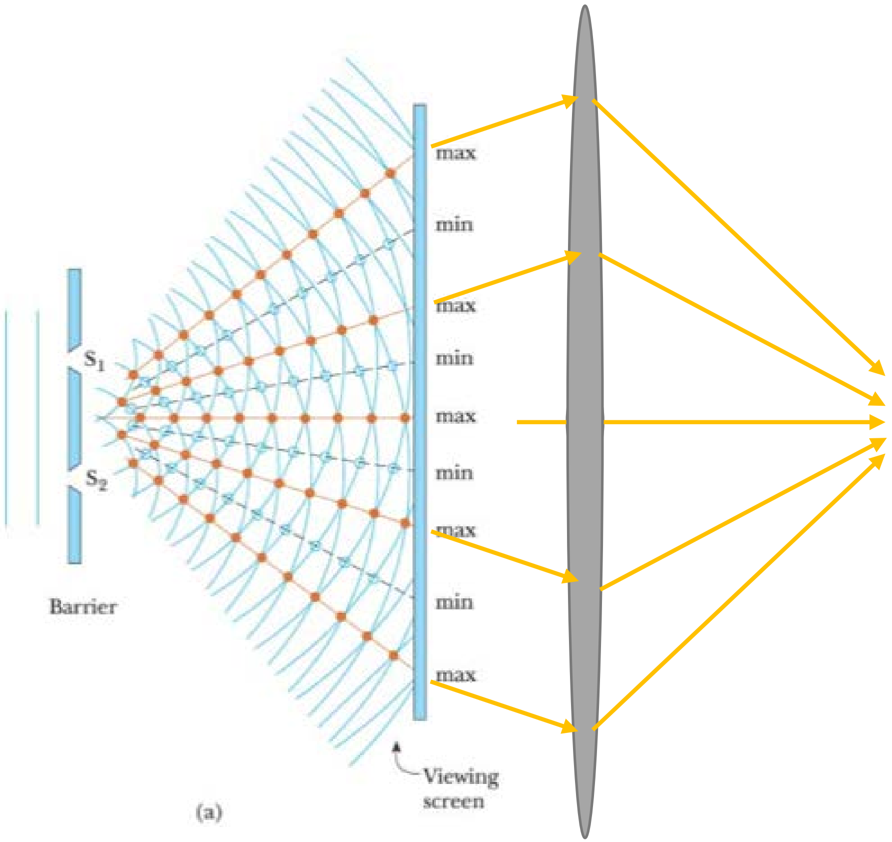




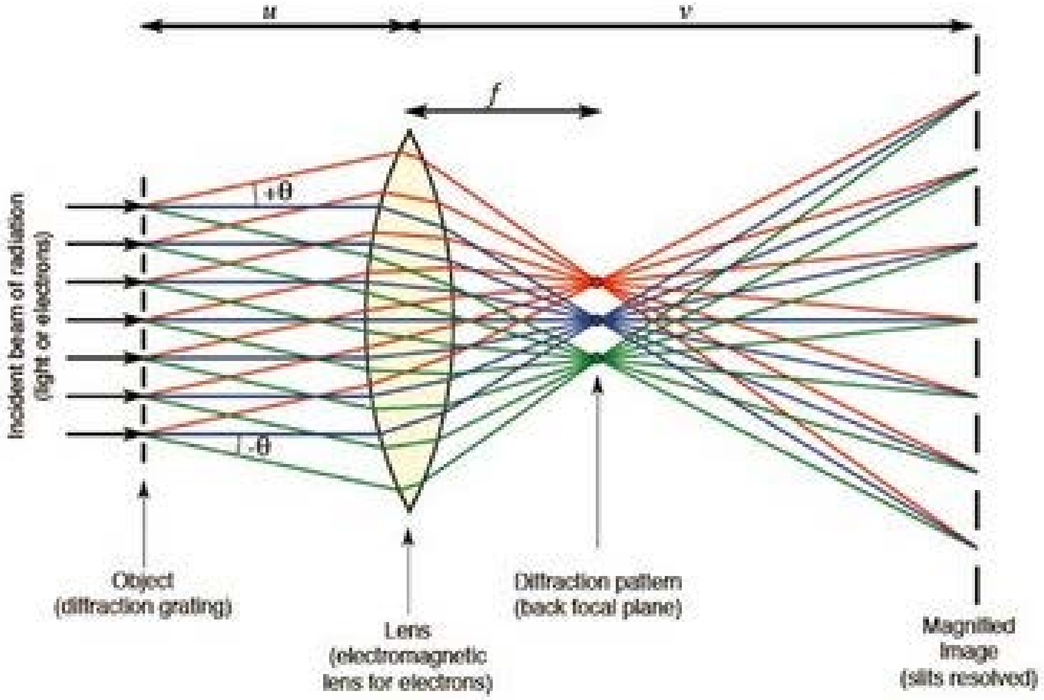


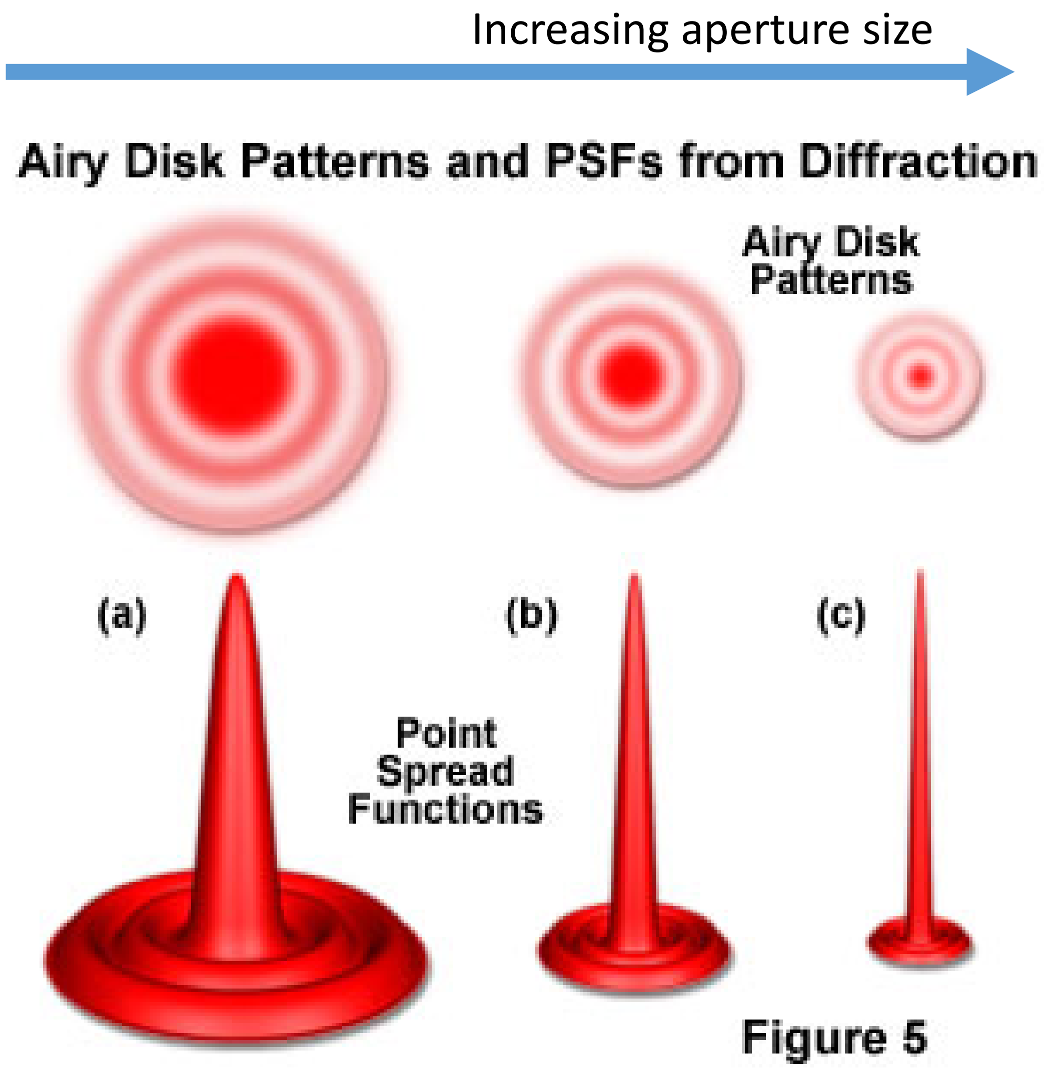
$$I(\theta) = I_0 \left(\frac{2 J_1(ka \sin\theta)}{ka \sin\theta} \right)^2 = I_0 \left(\frac{2 J_1(x)}{x} \right)^2$$

Diffraction is necessary to reform image



Increased aperture allows collection of higher order modes, can create a sharper image (higher resolution)




Increasing aperture size
Airy Disk Patterns and PSFs from Diffraction
Airy Disk Patterns
(a)
(b)
(c)
Point Spread Functions
Figure 5

Increasing resolution

$$d = \frac{\lambda}{2n\sin\theta}$$

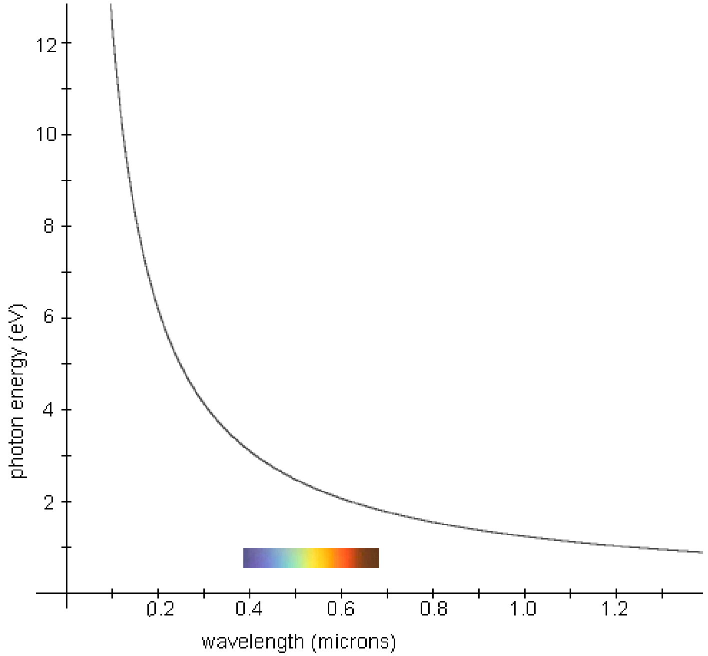
NA = nsin(θ)

$$d = \frac{\lambda}{2 * NA}$$

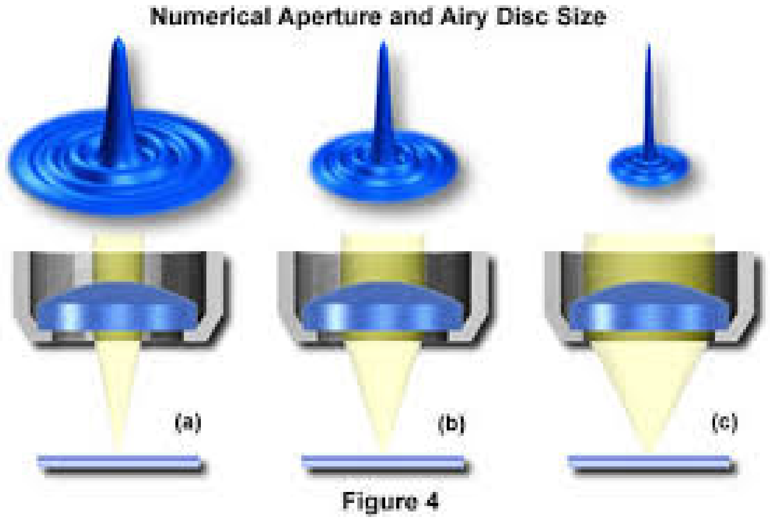
Bigger aperture

Bigger optics are more expensive
Take up more room
Every downstream optic must also have >= NA

Smaller wavelength



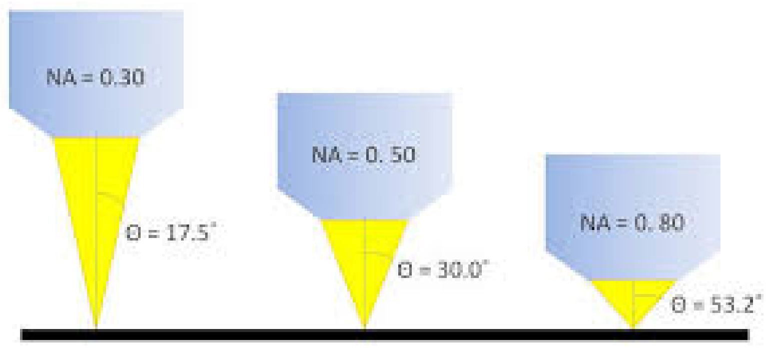
Higher energy
Damage to sample
harder to produce enough intensity



Numerical aperture

NA = nsin(θ)

n = 1 (Air)
= 1.33 (Water)
= 1.48 (Oil)



Olympus objective:
60x magnification, NA 1.45

NA = n sin θ
$\theta = \sin^{-1}$ (NA/n)
$= \sin^{-1}$ (1.45/1.48)
= 79 °

Pct of light collected

Pct = -1/2 (cos (θ) – 1)
= -.5 * (cos(79) – 1)
= 40 %

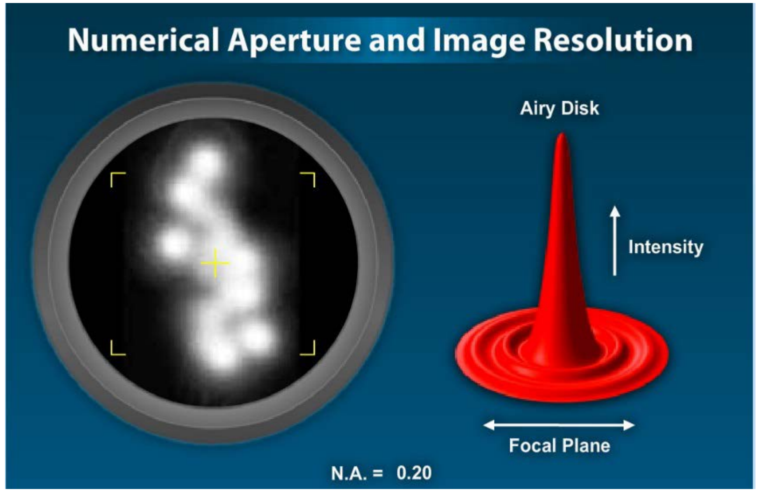
Olympus objective:
60x magnification, NA 1.35

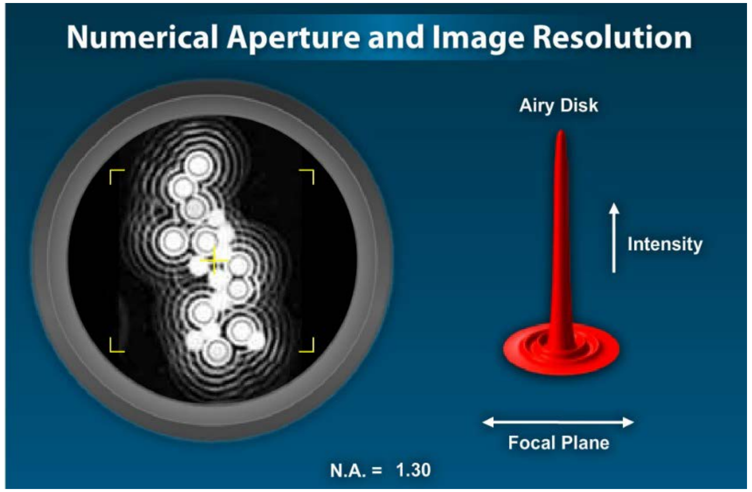
NA = n sin θ
$\theta = \sin^{-1}$ (NA/n)
$= \sin^{-1}$ (1.35/1.48)
= 65 °

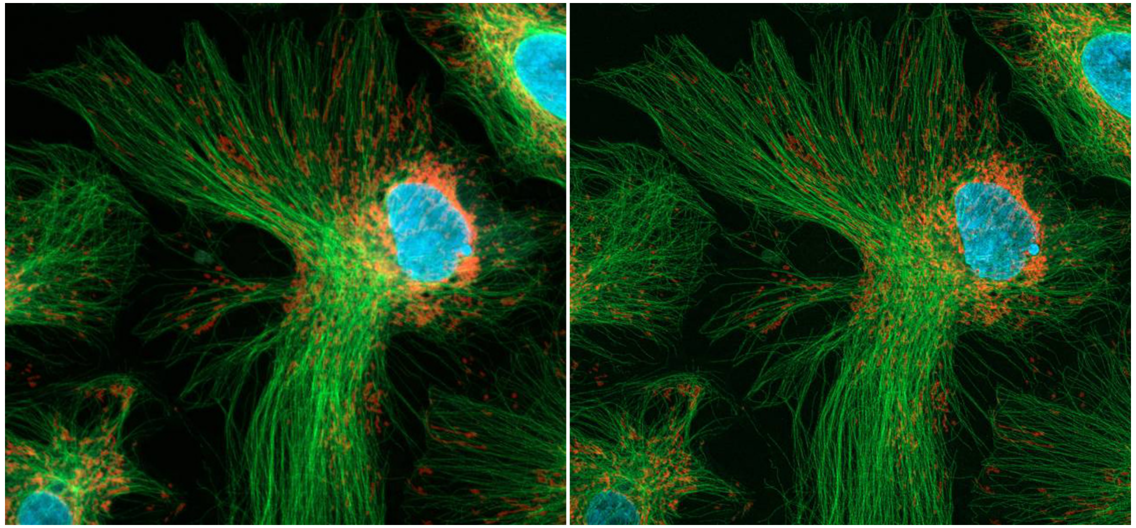
Pct of light collected

Pct = -1/2 (cos (θ) – 1)
= -.5 * (cos(65) – 1)
= 28 %

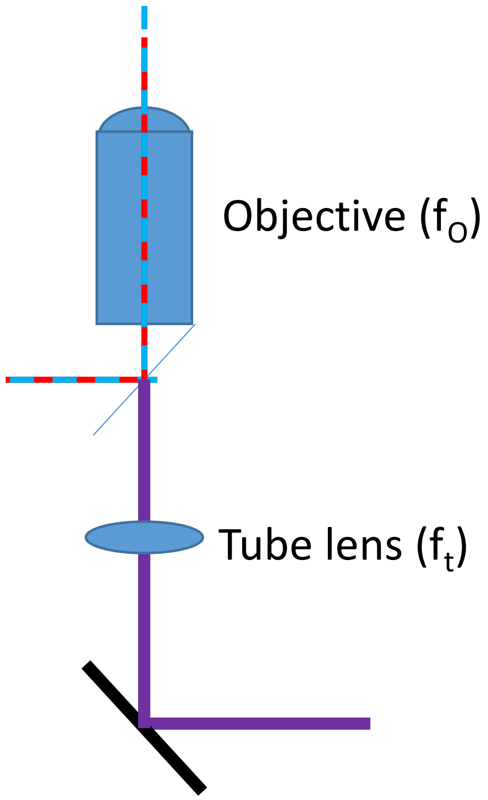
NA and resolution







Another word on NA



Olympus objectives expect a tube lens of 18 cm.

Consider 6.5 μm pixel size
60x mag -> 108 nm

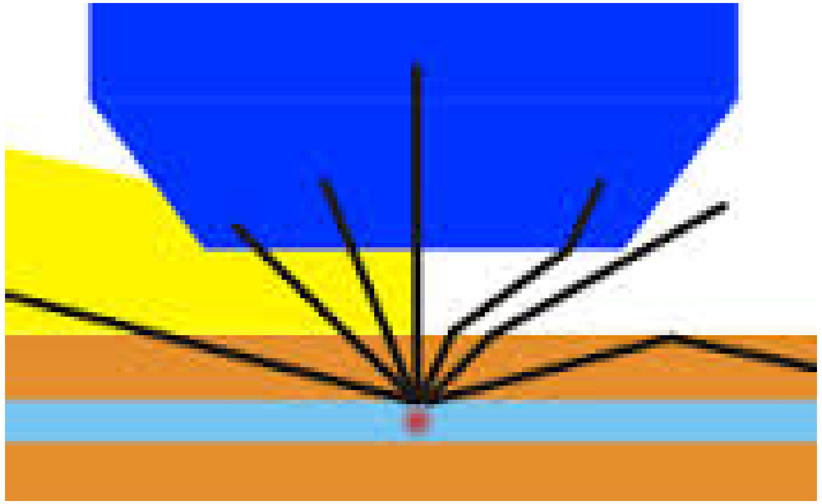
Diffraction Limit:

d = λ/2 * NA
= 700 nm / (2*1.45)
= 241 nm (AT BEST)

Nyquist frequency says sample at 2x minimum resolvable feature

100x doesn’t buy any additional resolution.
Adds noise as each pixel produces noise

Why oil



Oil has the same index as glass
Allows a larger cone

$NA_{max} = Na_{immersion} < NA_{glass}$

Oil objectives cost more money
You have to deal with oil

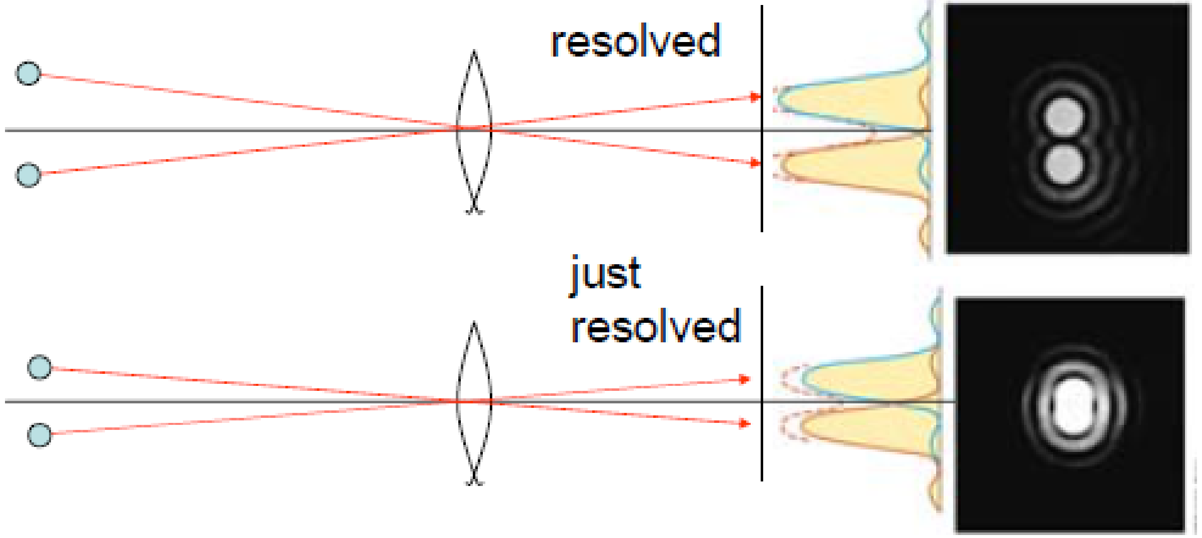
Only way to do TIRF microscopy

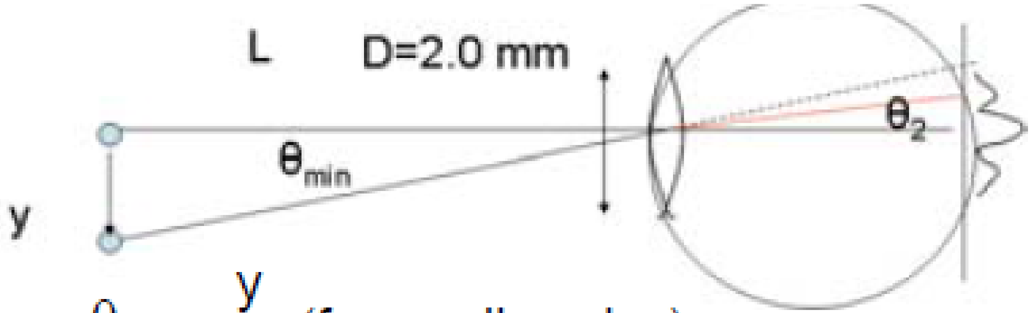
$\theta_c = \sin^{-1}(n_1/n_2)$
$= \sin^{-1}(1.33/1.49)$
$= 64^{\circ}$

Because they are index matched, the amount of reflection at each interface is also reduced

Diffraction limits the resolution

Resolution of two images by a lens





$\theta_{min} = \frac{y}{L}$ (for small angles)

Rayleigh criterion for resolution

$\theta_{min} = 1.22\ \lambda/D$

Resolution by your eye

Depth of field

Depth of field decreases with cone angle (NA)
Can be good (trying to exclude regions, sectioning0
Can be bad (very little is visible)

$$\frac{\lambda\sqrt{n^2 - (NA)^2}}{(NA)^2}$$

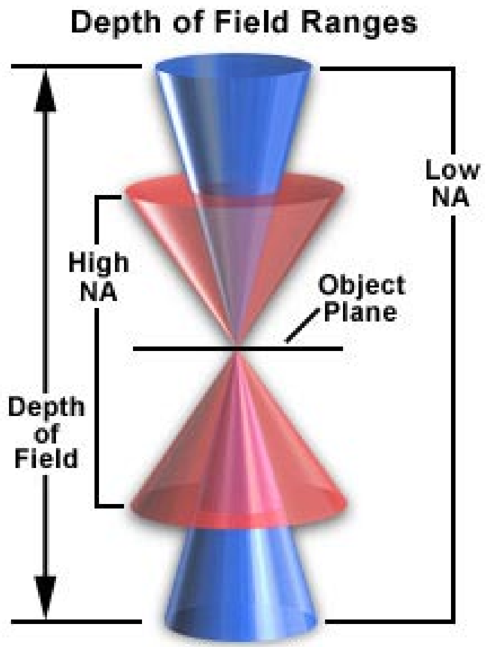


Figure 1

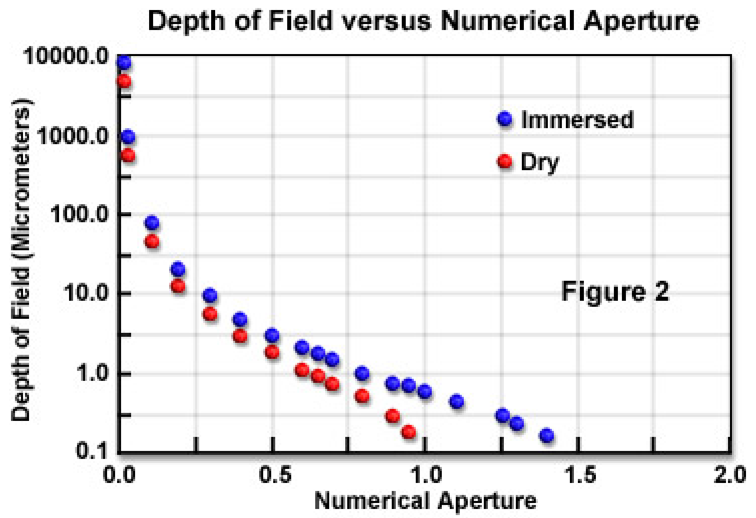
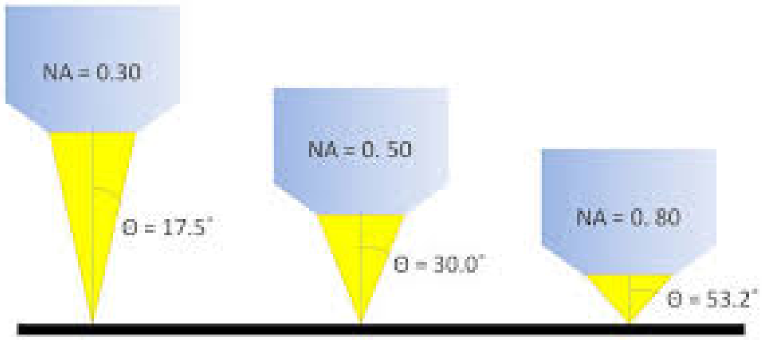


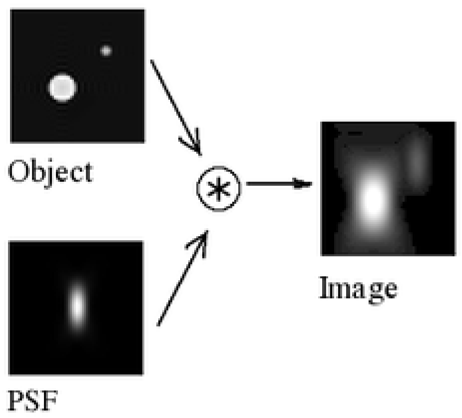
Figure 2

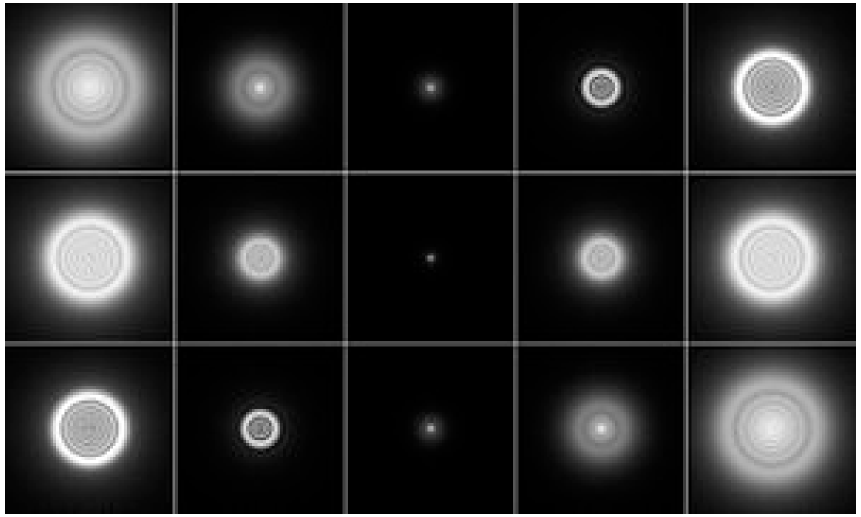


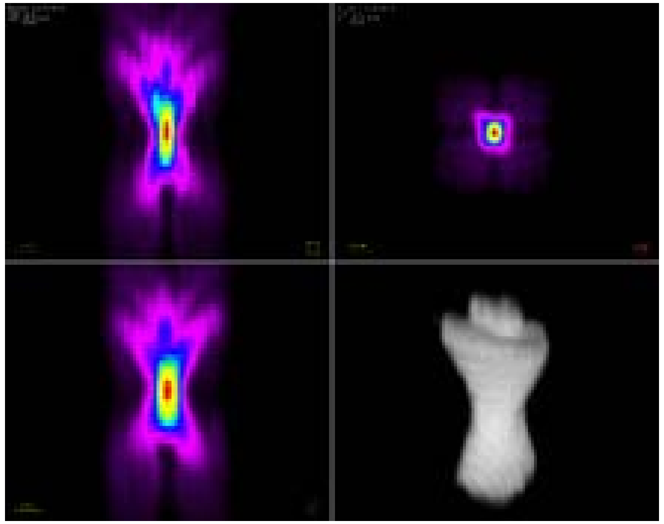
Point spread function

Defines resolution of entire system

Response of a point light emitter







Three dimensional response of all the optics in the microscope.

On to Matlab…