Microscopy

- It magnifies the objective
- see the virtual image of the object..
- Diffraction and absorption is necessary for image formation.

$$\text{Magnification} = \frac{\text{Virtual size by the objective}}{\text{Real size of the object}}$$

For example, $\frac{0.5\,cm}{2\,\mu m\ E.coli} = 2500$ times Magnification

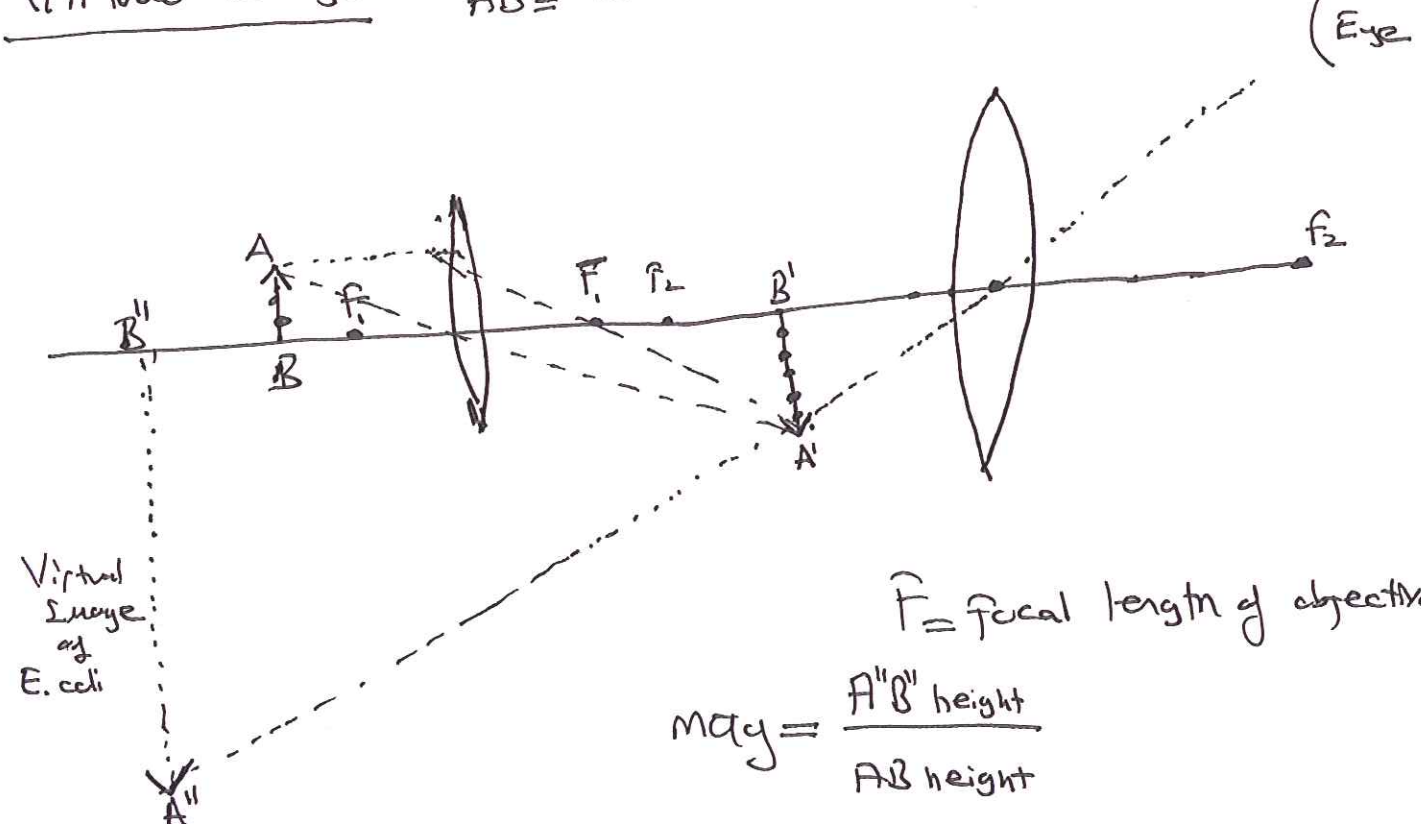
$60X \times 20\times 2$ times $\cong 2400$ times mag.

- 60X ↑ by objective
- 20× ↑ by eyepiece or binocular
- 2 times ↖ by microscope

$$Mag = Mag_{objective} \times Mag_{Binocular/eyepiece}$$

Virtual Image

AB = E. coli

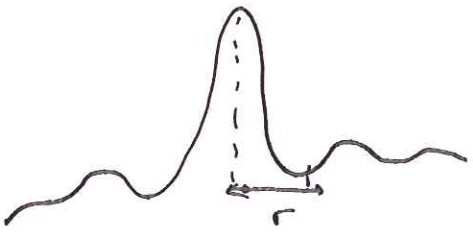


F = focal length of objectives

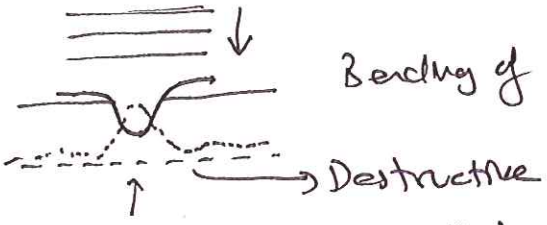
$$Mag = \frac{A''B'' \text{ height}}{AB \text{ height}}$$

Resolution of the Microscope

$$r_{airy} = \frac{1.22\,\lambda}{2\,NA_{objective}}$$

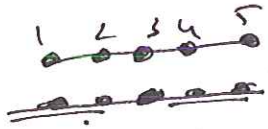


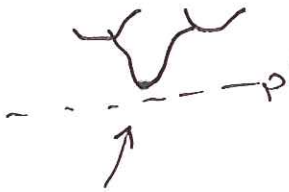
$$d = \frac{\lambda}{2NA} = \frac{\lambda}{2n\sin\theta}$$



Huygen's principle

- Light can be represented by many point like sources.

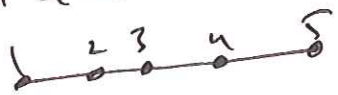




At point A = all waves contribute the same direction and amplitude

All 5 waves contribute.

at point B only 1, 2, 3 and wave from 4 and 5 contribute.

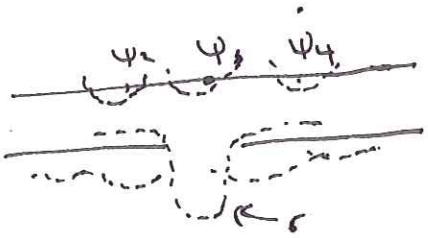


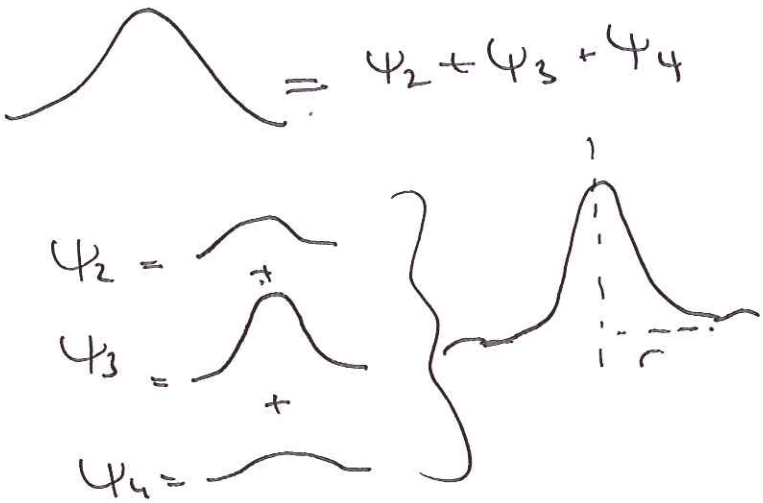
at point A = $\psi(x) = \psi_1 + \psi_2 + \psi_3 + \psi_4 + \psi_5$

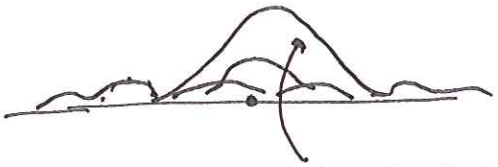
for ex,

at point B = $\psi(x) = \psi_2 + \psi_3 + \psi_4$

$$r = \frac{0.61 \times \lambda}{NA}$$







All point source waves contribute to the formation of the central peak.

Numerical Aperture:

- Range of angles ~~collected~~ of light collected by the objective (lens)

$NA = n(\sin\theta)$

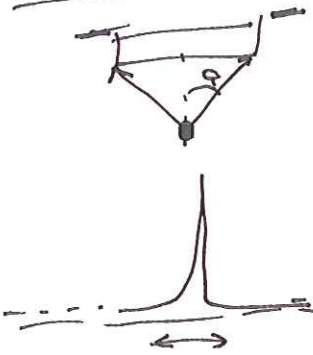
$NA = 0.87$ if $\theta = 60°$

$NA = 0.12$ if $\theta = 7°$

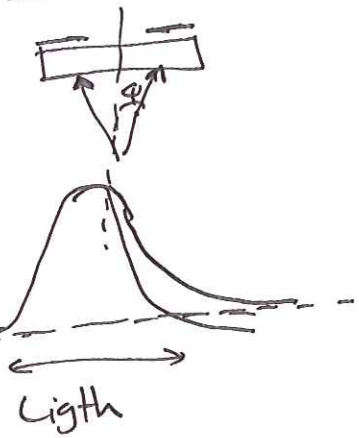
n = index of refraction

- Air = 1.00
- Water = 1.33
- Oil = 1.52

Less bending



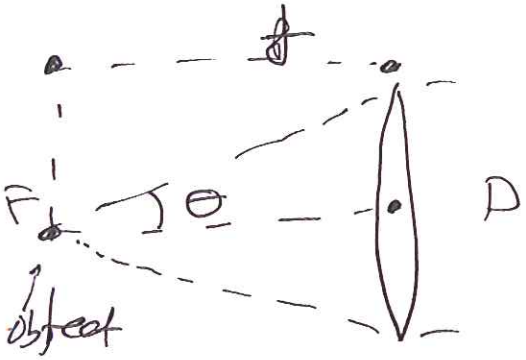
Large bending



Light pass a large slit and therefore less bending and diffraction occurs.

If NA is small, that means it a a smaller ~~diff~~ slit and large diffraction occurs.

- it has no dimension



$NA = n \times \sin\theta$

$N = \frac{f}{D}$ } photography they use f-number

$$\underbrace{\text{Mag(obj)} \times 0.61 \times \frac{\lambda}{NA}}_{} = 2.3 \times \text{pixel size}$$

$$\frac{10\,\mu m}{2} \cong 5\,\mu m \text{ pixel size required}$$

⇒ Sampling interval (pixel size) required adequately reconstruct an analogue signal must be at least ture the maximum frequency measured.

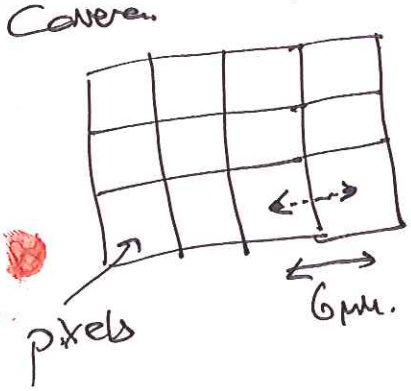
$$\text{pixel frequency} \geq \frac{\text{sampling frequency}}{2}$$

Numeral

Camera Pixel Size

Andor – 6μm

Camera



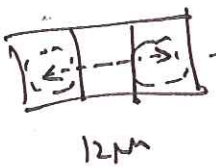
$$0.61 \times \frac{480}{1.4} \times 60x \cong 12\mu m.$$

$$r = 209nm.$$

$$12 = 2.3 \times \text{pixel size}$$

$$\text{Pixel Size} = 5.2\,\mu m$$

If you use Andor, ya may have the the resolution g 209 nm.



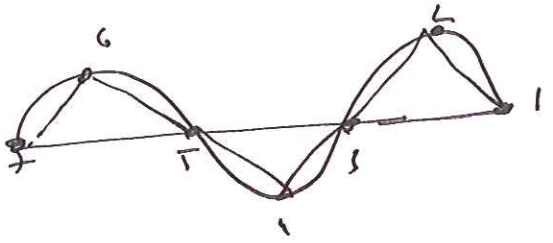
→ you can resolve two objects if their separation as small as 209 nm.

Nyquist Theorm.

To reconstruction analog signal

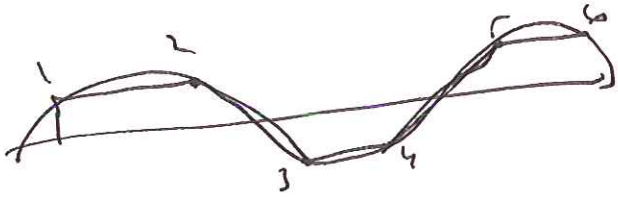
for example; $r_{xy} = \frac{0.61 \times \lambda}{NA} = \frac{0.6 \times 500nm}{1.2} = 250\,nm$ theoretical resolution

pixel size $= \frac{250nm}{2} = 125nm$



$f = 10$ kHz,

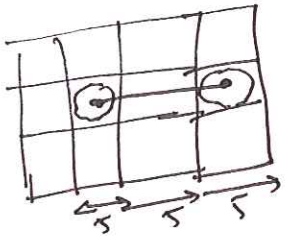
frequency of reading $\geq 2 \times f$ sample.



In accuracy:

Size of the pixel should be two thes smaller ther size of Magnyfet object.

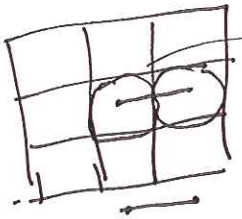
10μm object Magnyfet, smatt, largest pixel size x 5μm



10μm.

1

what if you have a ~~smaller~~ large size pixel?



you can not separate the object with a large pixel size camera.

10μm

Recording frequency

frequency of reading $\geq$ 2x sample size

Pixel are used to identify objects.