

2009 spring

***Microstructural Characterization  
of  
Materials***

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# ***Contents for previous class***

- ***Types of optical microscopy***

(1) Simple OM:

(2) Stereo OM:

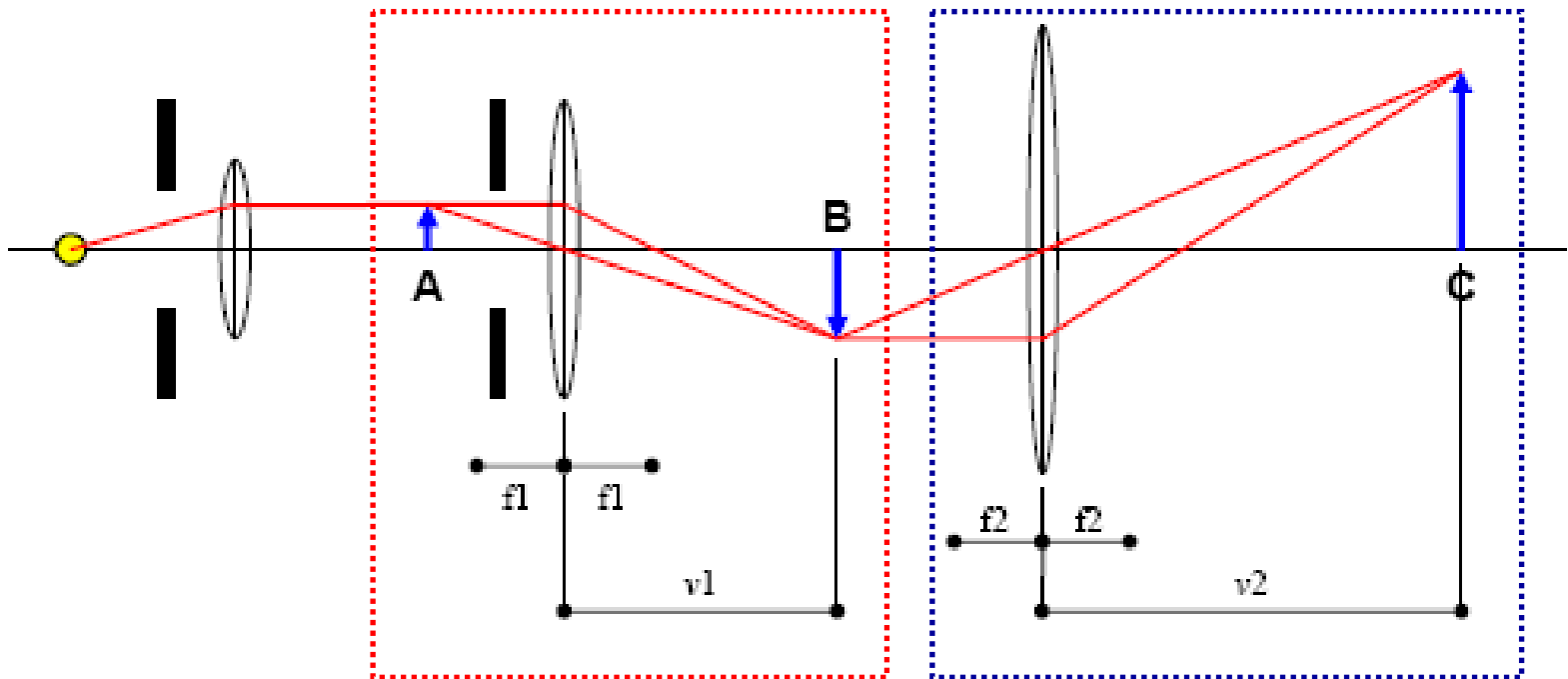
(3) Compound OM:



- ***Microscope Components***

(1) Illumination system: **Lamps, Lenses, Filters, Diaphragm**

(2) Optical System: **Objective Lens, Projector Lens, Eyepiece**



- Let  $v$  be the distance between the magnified image and the lens (also known as the image distance). Let  $f$  be the focal distance

OBJECTIVE LENS MAGNIFICATION

$$M1 = (v1 - f1) / f1$$

PROJECTOR LENS MAGNIFICATION

$$M2 = (v2 - f2) / f2$$

TOTAL MAGNIFICATION

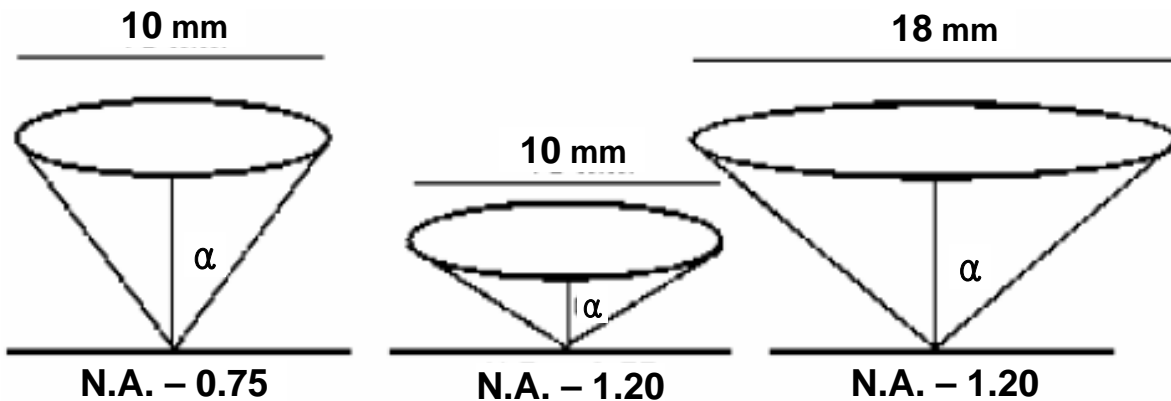
$$M1 \times M2$$

- **Numerical aperture (N.A.)**

**N.A.** - the numerical aperture (N.A.) is basically a value which describes the quality of a lens.

$$N.A. = \mu \sin \alpha$$

depends on size of the lens; working distance; refractive index of medium between object and objective lens ( $\mu$ ).



$\alpha$  - the half acceptance angle of the lens.

# Optical Performance

## Resolution

- Recall: the resolution of a system is the smallest distance between two points or lines that can be observed.
- In the case of optical microscopy it is a function of the wavelength of light and the N.A. of the objective.
- Resolution limits magnification in an optical microscope.

$$d = \frac{0.612\lambda}{\text{N.A.}} = \frac{0.612\lambda}{\mu \cdot \sin \alpha}$$

( Abbe's Equation )

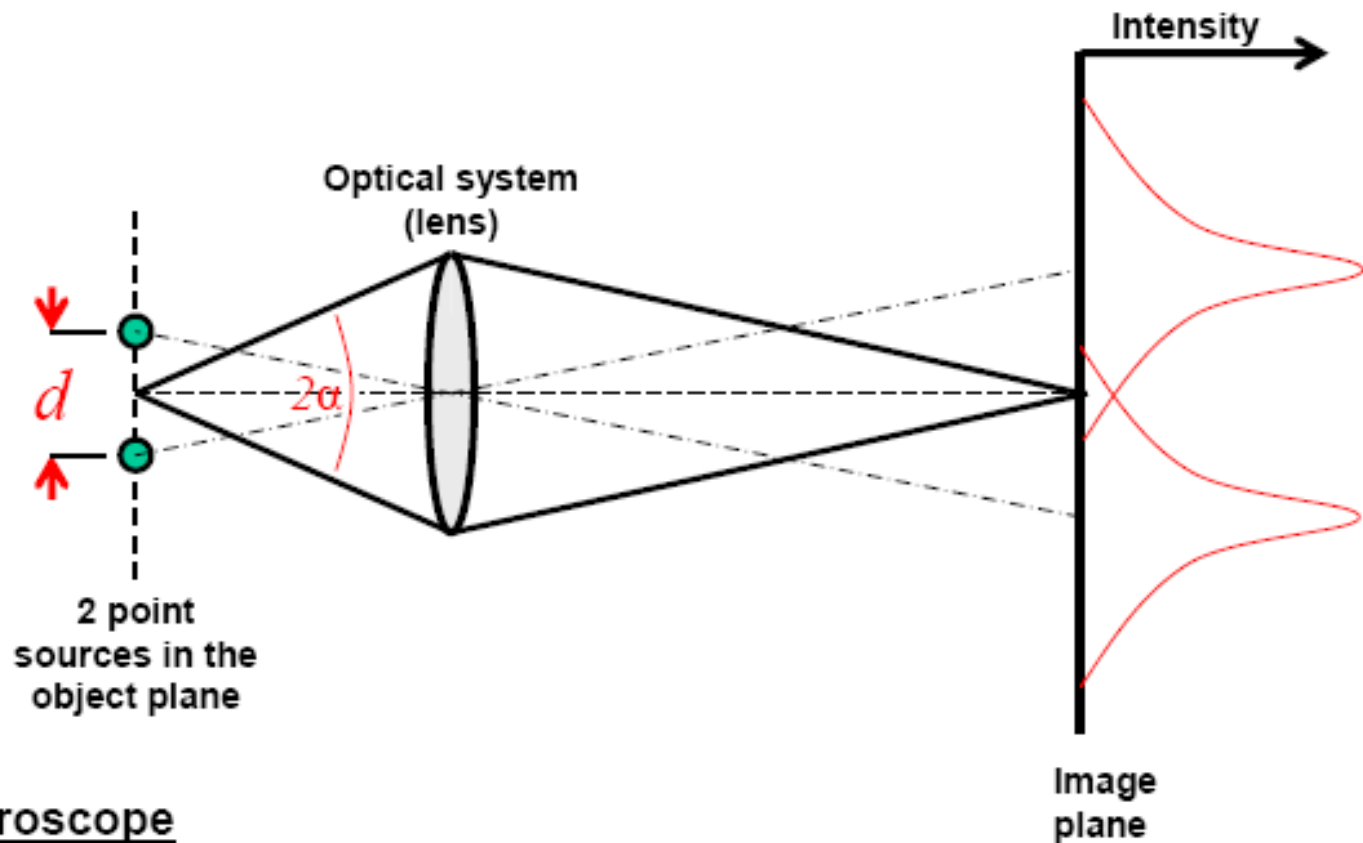
where:  $d$  = resolution

$\lambda$  = wavelength of imaging radiation

$\mu$  = index of refraction of medium between point source and lens, relative to free space

$\alpha$  = half the angle of the cone of light from specimen plane accepted by the objective (half aperture angle in radians)

# RECALL



## Microscope

$$r_1 = \frac{d}{2} \geq \frac{0.612\lambda}{\mu \sin \alpha} = \frac{0.612\lambda}{N.A.}$$

HOW DO WE INCREASE  
MAGNIFICATION?

# Resolution Limit of Light Microscope

- You can decrease  $\lambda$  to 400 nm (green light).
- N.A. is limited to  $\sim 1.6$ .

$$\text{LOM } r_1 = \frac{d}{2} \geq \frac{0.612\lambda}{\mu \sin \alpha} = \frac{0.612\lambda}{N.A.} =$$

- The maximum resolution is around  $\sim 150 \text{ nm}$  ( $0.15 \mu\text{m}$ ).
- For comparison, in an electron microscope  $\lambda$  can decrease to 0.001 nm and N.A. is much smaller (on order of 0.1 radians)

$$\text{EM } r_1 = \frac{d}{2} \geq \frac{0.612\lambda}{\mu \sin \alpha} \approx$$

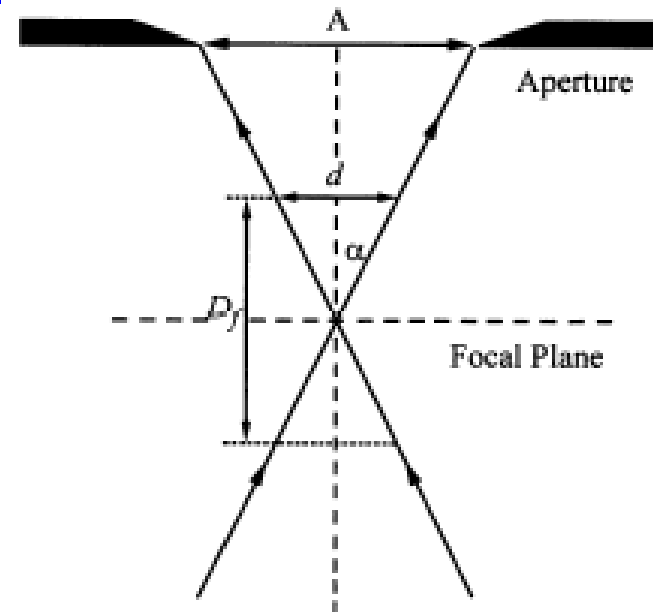
For wavelength of 0.0037 nm and  $\alpha = 0.1$  radians, the resolution is about **0.02 nm**.

# Depth of Field

- The distance along the optic axis over which image details can be observed with acceptable clarity.
- The same factors that effect resolution effect the depth of field but in the opposite way; therefore, a compromise must be reached between these two factors

$$D_f = \frac{d}{\tan \alpha} = \frac{2r}{\tan \alpha} = \frac{1.22\lambda}{\mu \sin \alpha \tan \alpha}$$

- Object will be sharp if it is anywhere within the range  $D_f$





# Optical Performance

## Resolution & Depth of Field

-Resolution

$$r_1 = \frac{d}{2} \geq \frac{0.612\lambda}{\mu \sin \alpha} = \frac{0.612\lambda}{N.A.}$$

-Depth of Field

$$D_f = \frac{1.22\lambda}{\mu \sin \alpha \tan \alpha}$$

- Large  $D_f$  and  $r_1$  cannot be obtained simultaneously.
- Large  $D_f$  means larger  $r_1$  and worse resolution.

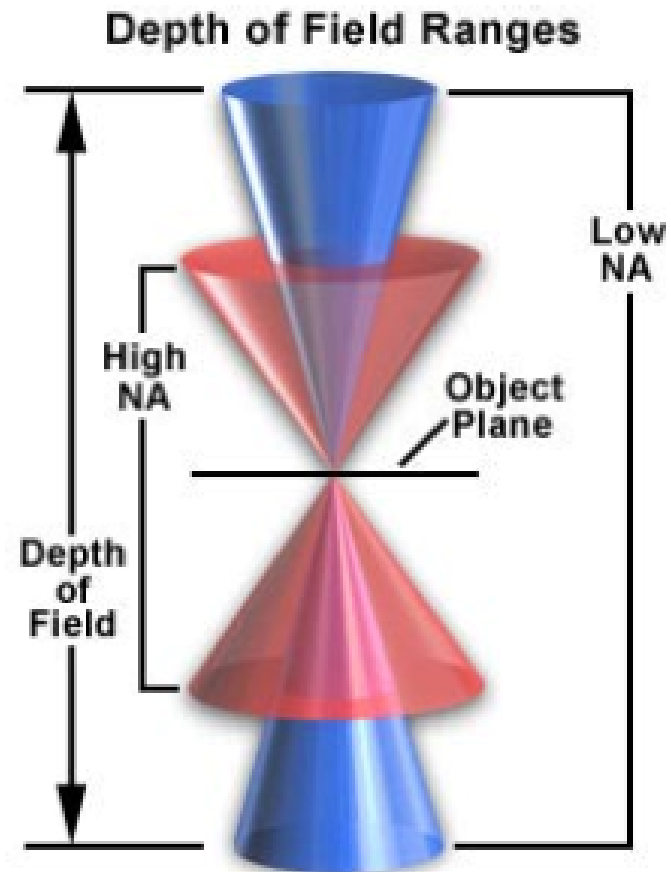
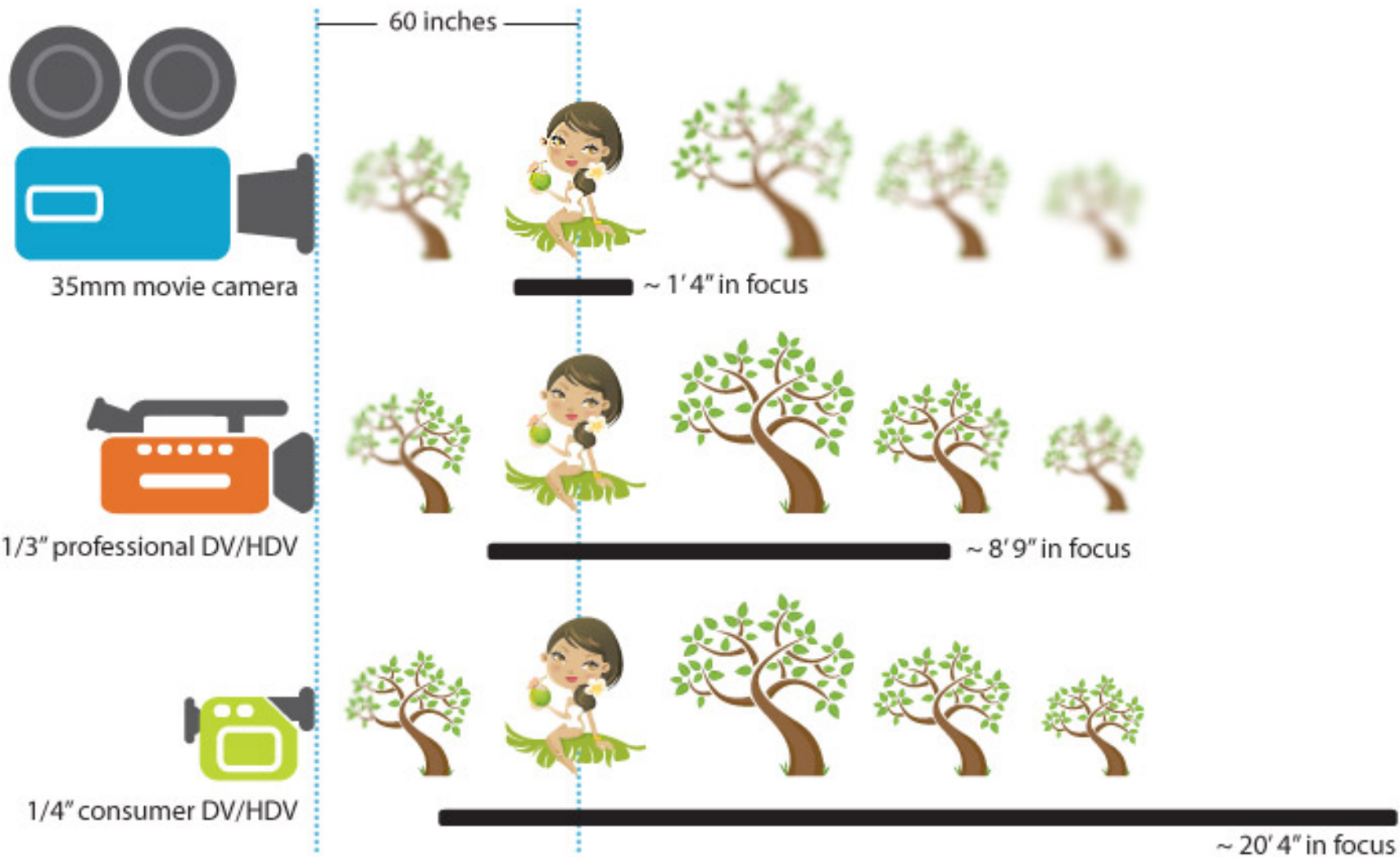


Figure 1

[www.microscopyu.org](http://www.microscopyu.org)



# Optical Performance

## Resolution & Depth of Field - cont'd

$$D_f = \frac{1.22\lambda}{\mu \sin \alpha \tan \alpha}$$

- For a light microscope,  $\alpha$  is around  $45^\circ$ . Thus,  $D_f$  is not much different from resolution.
- In comparison, in an electron microscope,  $\alpha$  and  $\lambda$  are much smaller.

$$D_f = \frac{0.61\lambda}{\alpha^2}$$

- In an electron microscope,  $D_f$  is nearly ten times the resolution.

# How to improve depth of field

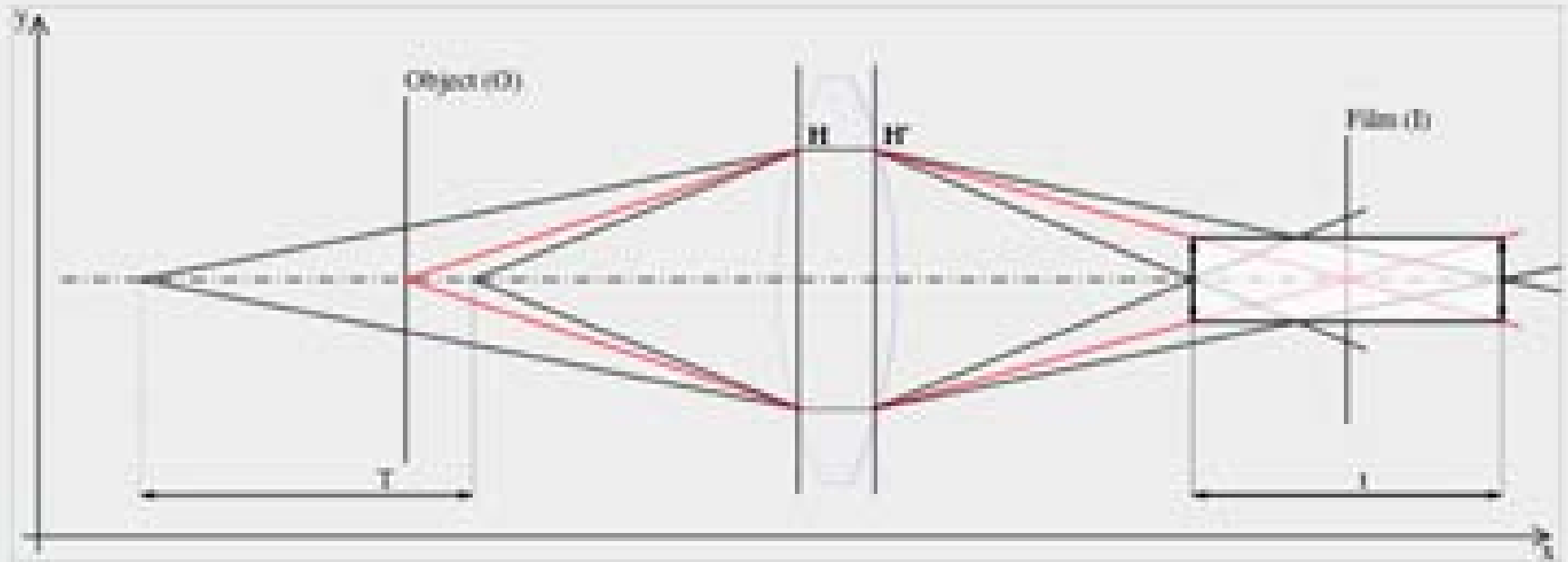
1. Reduce  $N.A.$  by closing down aperture diaphragm, or use a lower  $N.A.$  objective lens.
2. Lower the magnification for a given  $N.A.$
3. Use a high-power eyepiece with a low-power, high- $N.A.$  objective lens.
4. Reduce zoom factor
5. Use longest possible wavelength light.

# Depth of Focus

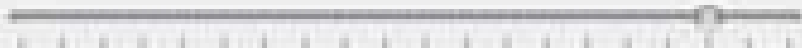
- The range of image plane position at which the image can be viewed without appearing out of focus for a fixed position of object.
  - Often confused with depth of field
  - Not as important as depth of field
  - Depth of focus is  $M^2$  times depth of field

$$D_{focus} = D_f \times M^2 = \frac{1.22\lambda}{\mu \sin \alpha \tan \alpha} \times M^2$$

# Depth of Field and Focus



Conjugate relationship between depth of field (T) and depth of focus (t)



Zoom in

Quit