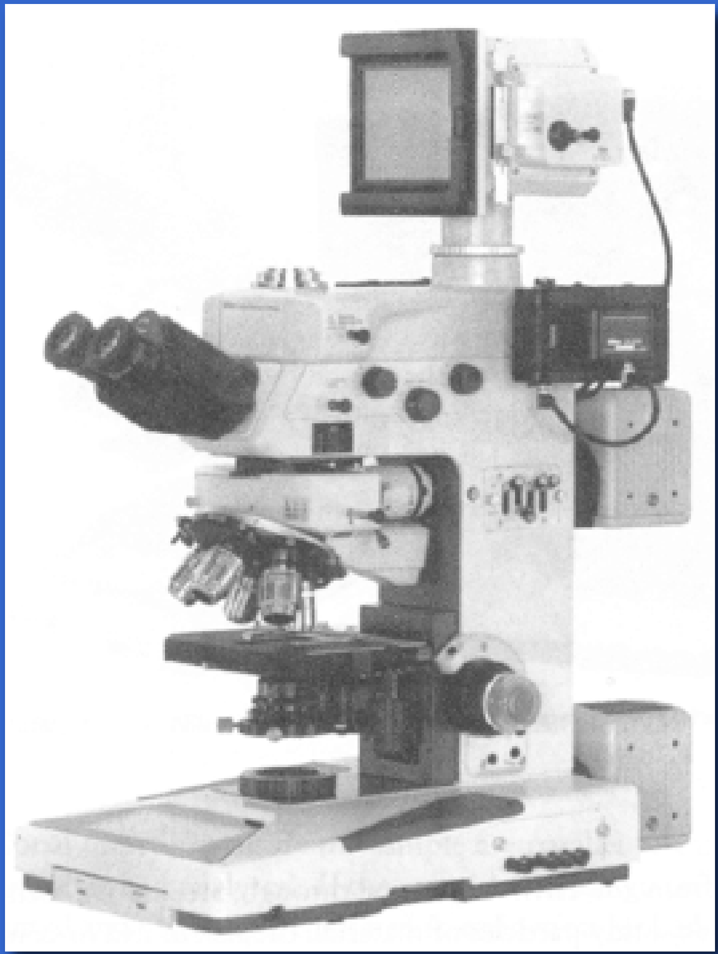
Optical microscopy

Optics
Lenses and image formation
Depth of field
Numerical aperture
Resolution
Instrument
Specimen preparation
Contrast
Examples



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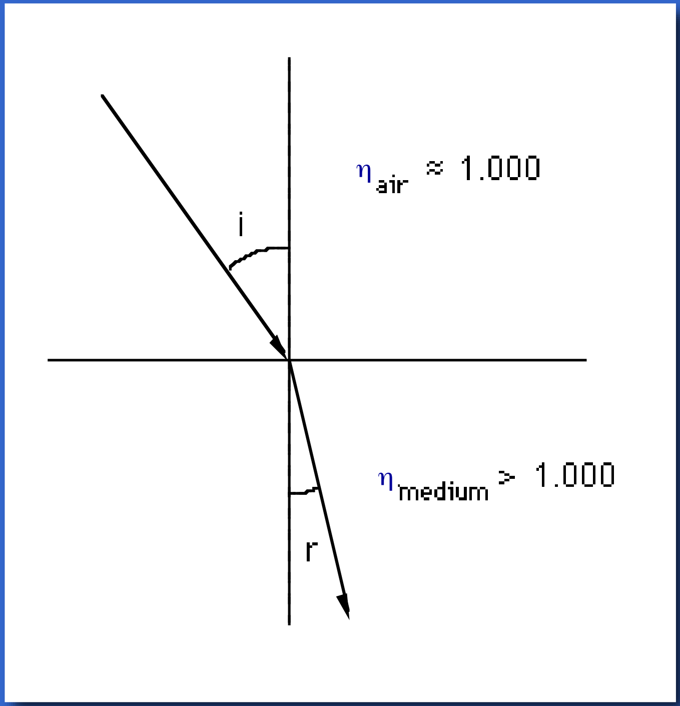
Index of refraction

$$\eta = \frac{\text{velocity of light in air}}{\text{velocity of light in other medium}}$$

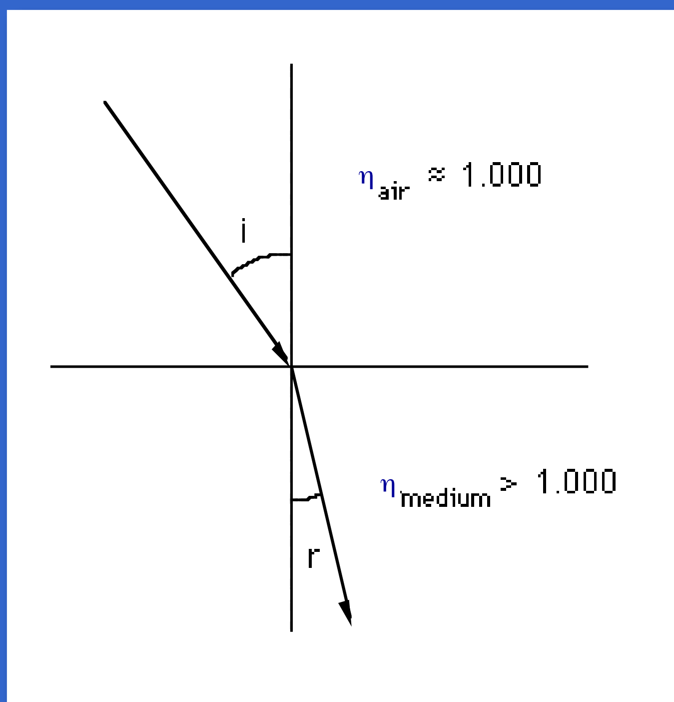


Snell's law

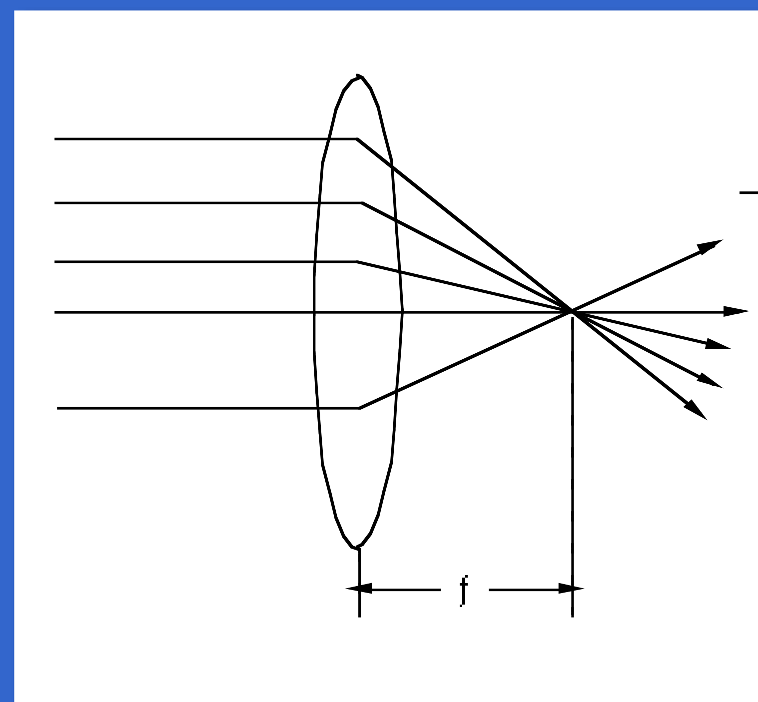
$$\frac{\sin i}{\sin r} = \frac{\eta_{medium}}{\eta_{air}}$$

Optical microscopy

Refraction makes lenses possible



Converging or positive lens



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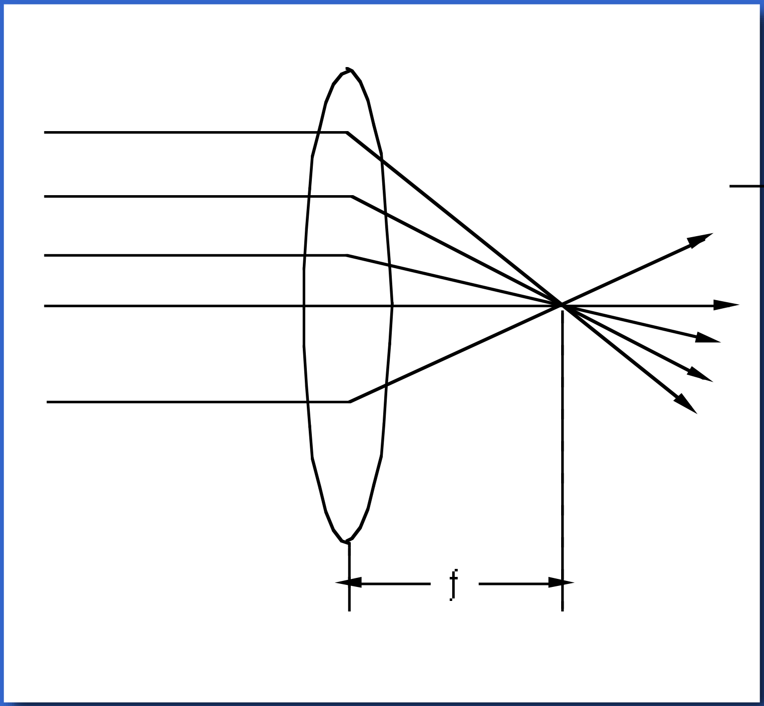
For diverging or negative lens, focal point on other side of lens

Greater lens curvature —> shorter focal length



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$$\frac{1}{u}+\frac{1}{v}=\frac{1}{f}$$

$$M = v/u$$

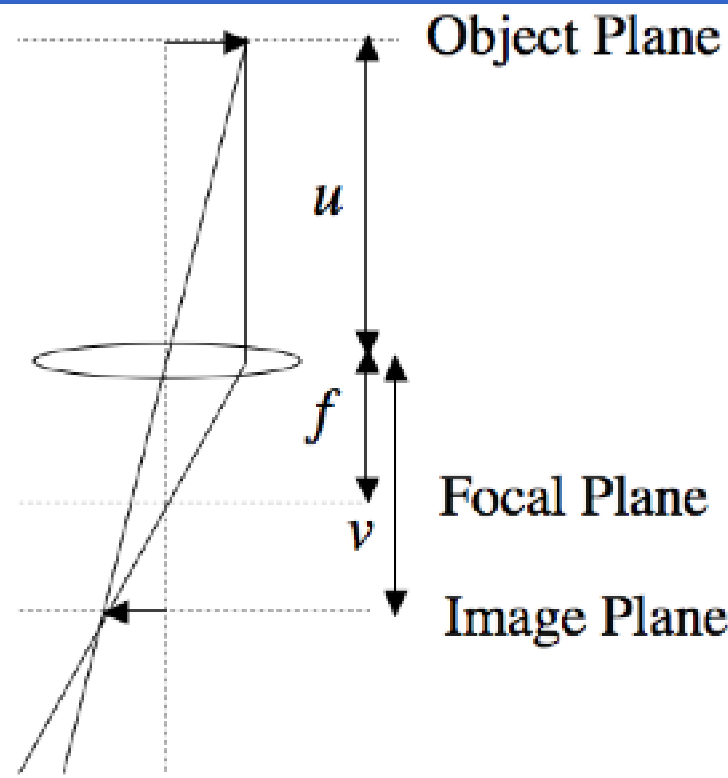

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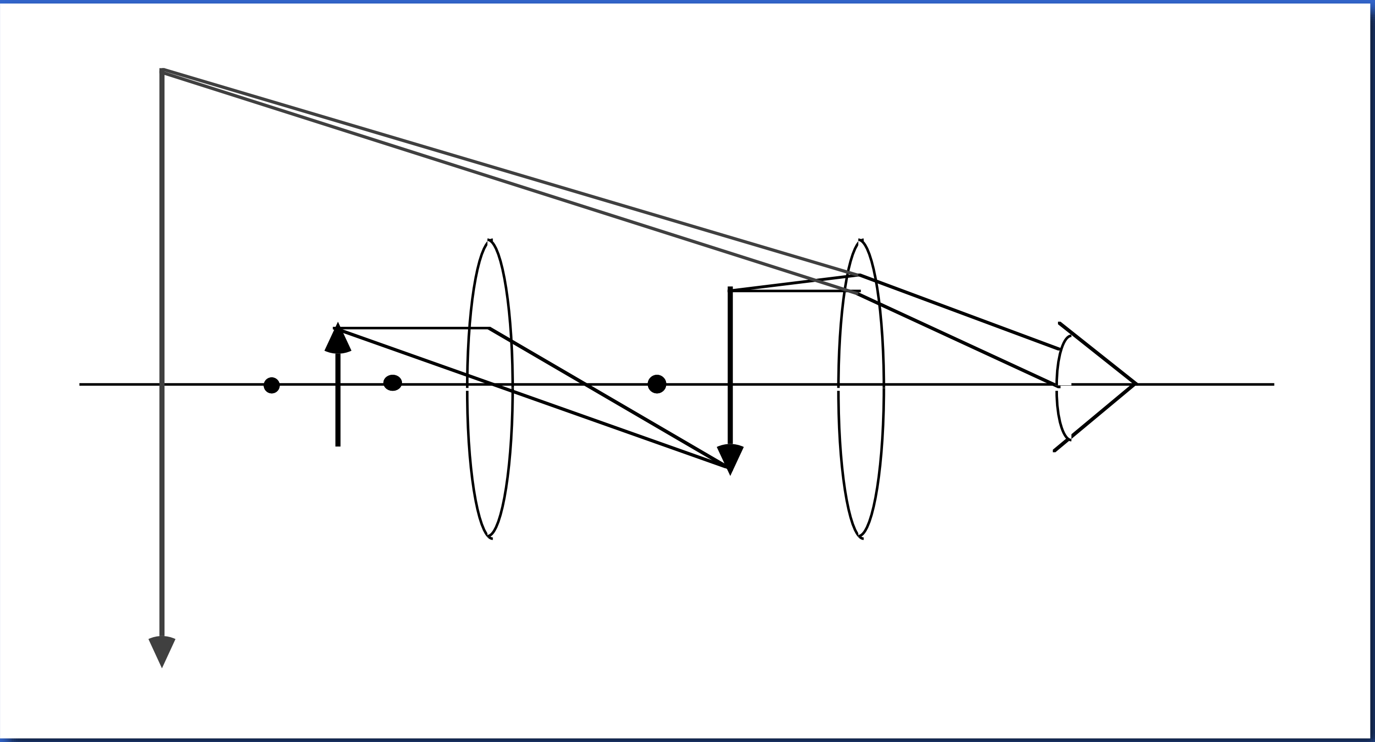
Image formation in lens system



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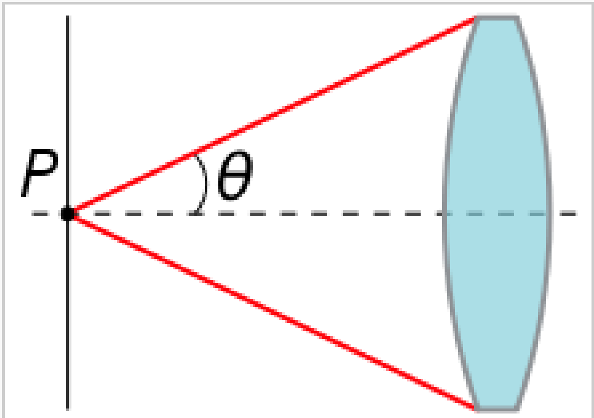
Numerical aperture = N.A. = sine of half the angle over which light enters objective lens.



The numerical aperture with respect to a point P depends on the half-angle θ of the maximum cone of light that can enter or exit the lens.

Resolution = λ/2N.A.

Optical microscopy

Resolution and Numerical Aperture by Objective Correction

	Objective Type					
	Plan Achromat		Plan Fluorite		Plan Apochromat	
Magnification	N.A.	Resolution (µm)	N.A.	Resolution (µm)	N.A.	Resolution (µm)
4x	0.10	2.75	0.13	2.12	0.20	1.375
10x	0.25	1.10	0.30	0.92	0.45	0.61
20x	0.40	0.69	0.50	0.55	0.75	0.37
40x	0.65	0.42	0.75	0.37	0.95	0.29
60x	0.75	0.37	0.85	0.32	0.95	0.29
100x	1.25	0.22	1.30	0.21	1.40	0.20

N.A. = Numerical Aperture

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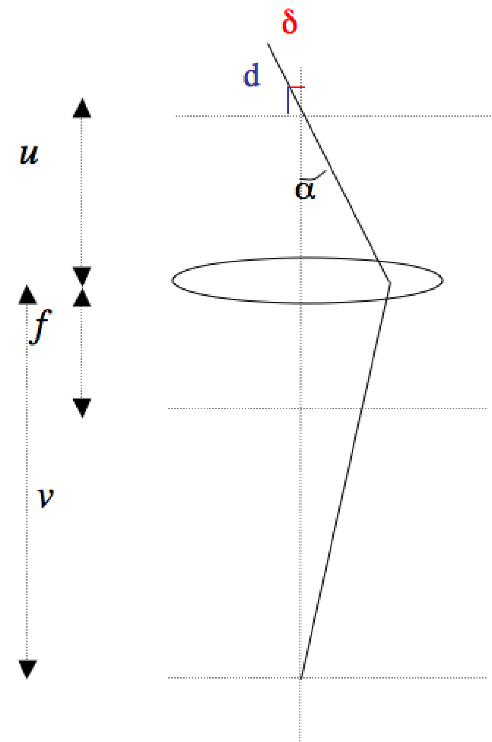
Depth of field: the distance over the **object** that remains in focus

$$d = \delta / \tan \alpha$$



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Depth of Focus

We also need to consider the depth of focus (vertical resolution). This is the ability to produce a sharp image from a non-flat surface.

$$DOF \approx \frac{\lambda}{N.A.}$$

Depth of Focus is increased by inserting the objective aperture (just an iris that cuts down on light entering the objective lens). However, this decreases resolution.

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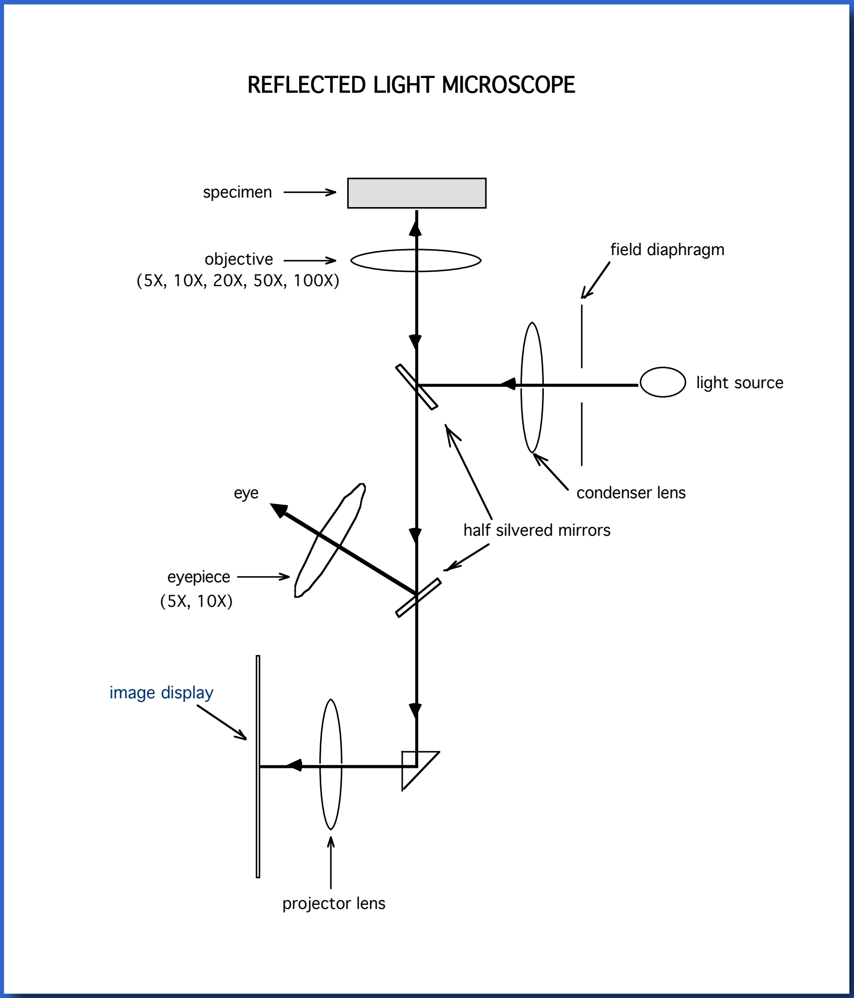

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Magnification

- The overall magnification is given as the product of the lenses and the distance over which the image is projected:

$$M = \frac{D \cdot M_1 \cdot M_2}{250mm}$$

where:

D = projection (tube) length (usually = 250 mm);

M_1, M_2 = magnification of objective and ocular.

250 mm = minimum distance of distinct vision for 20/20 eyes.

Optical microscopy

CONTRAST IN THE REFLECTED LIGHT MICROSCOPE

1. Different phases may be delineated due to differences in reflectivity. Reflectivity varies with wavelength, so contrast may be changed through insertion of a filter.

2. Crystals of different orientations are etched at different rates. Results in contrast due to ledges formed at grain boundaries.

3. Etchants frequently attack grain boundaries preferentially leading to grain boundary grooving.

Optical microscopy

CONTRAST IN THE REFLECTED LIGHT MICROSCOPE

4. Etchants may facet the grains. The faceting is different for differently oriented grains.

5. Etchants often attack different phases at different rates. Leads to surface relief.

6. Some etchants stain the sample surface so that differently oriented grains and different phases take on varied colors.