Optical microscopy

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Numerical aperture
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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

\[ \frac{\sin i}{\sin r} = \frac{\eta_{\text{medium}}}{\eta_{\text{air}}} \]
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Refraction makes lenses possible

Converging or positive lens

$\eta_{\text{air}} \approx 1.000$

$\eta_{\text{medium}} > 1.000$

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

Greater lens curvature $\rightarrow$ shorter focal length
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\[ \frac{1}{u} + \frac{1}{v} = \frac{1}{f} \]

\[ M = \frac{v}{u} \]
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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.

Resolution = $\lambda / 2 \text{N.A.}$
## Resolution and Numerical Aperture by Objective Correction

<table>
<thead>
<tr>
<th>Magnification</th>
<th>Objective Type</th>
<th>Plan Achromat</th>
<th>Plan Fluorite</th>
<th>Plan Apochromat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resolution (µm)</td>
<td>N.A.</td>
<td>Resolution (µm)</td>
<td>N.A.</td>
</tr>
<tr>
<td>4x</td>
<td>2.75</td>
<td>0.10</td>
<td>2.12</td>
<td>0.20</td>
</tr>
<tr>
<td>10x</td>
<td>1.10</td>
<td>0.25</td>
<td>0.92</td>
<td>0.45</td>
</tr>
<tr>
<td>20x</td>
<td>0.69</td>
<td>0.40</td>
<td>0.55</td>
<td>0.75</td>
</tr>
<tr>
<td>40x</td>
<td>0.42</td>
<td>0.65</td>
<td>0.37</td>
<td>0.95</td>
</tr>
<tr>
<td>60x</td>
<td>0.37</td>
<td>0.75</td>
<td>0.32</td>
<td>0.95</td>
</tr>
<tr>
<td>100x</td>
<td>0.22</td>
<td>1.25</td>
<td>0.21</td>
<td>1.40</td>
</tr>
</tbody>
</table>

N.A. = Numerical Aperture

### Optical microscopy
**Optical microscopy**

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

\[ d = \frac{\delta}{\tan \alpha} \]
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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REFLECTED LIGHT MICROSCOPE

- specimen
- objective (5X, 10X, 20X, 50X, 100X)
- field diaphragm
- light source
- condenser lens
- half silvered mirrors
- eyepiece (5X, 10X)
- eye
- image display
- projector lens
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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:

\[ M = \frac{D \cdot M_1 \cdot M_2}{250\, \text{mm}} \]

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.
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.