Illumination for the microscope

Köhler illumination: is a Method of specimen illumination used for transmitted and reflected light.
Purpose of Koehler Illumination

1. To obtain even specimen illumination for photomicrography, video microscopy etc.

2. To use field diaphragm alone to control illuminated area of specimen.

3. To control the angle of the cone of illumination (contrast and resolution) by varying condenser diaphragm.
A Lamp Collector Lens and Microscope Condenser Lens are Used to Concentrate Light on the Specimen
Optical Principle

Light emanating from a single point on the optical axis located a distance $f$ from the PPL will emerge parallel to the optical axis. This point $F$ is called the front focal point.

Light emanating from a single point on the object side of the focal plane will emerge as a parallel beam of light oriented at an oblique angle, $\theta$, to the optical axis.
cleaning microscope optics

Sample Preparation

- Before performing an experiment, always consider the information that you want to obtain and the method(s) by which to obtain ALL of it.
- Sample preparation methods vary widely.
- Depends to some degree on the next phase of characterization.
- Particulate: It needs to be mounted in a refractive index liquid for determination of the optical properties. OR Mounted on tape for size and shape analysis.
If the sample is metal, embed in a polymer, section and polish.

Organic samples may be sectioned / processed with a cryomicrotome, among other types to reduce sample prep damage.
Effect of dirty optics.....

Clean (left) and oil contaminated (right) objective front lens. Toad, kidney, stained with Trichrome. Planapo 20/0.80. Bright field

http://www.zeiss.de/courses, The Clean Microscope
Taking care of microscope optics

- Never “dry” clean a lens
- Use a solvent like Windex that will remove most everything.
- Use best quality lens tissue available [e.g. Kodak].
- Clean in spin pattern from center out.
- Remove immersion oil after use to stop leak.
Wipe using a spiral movement – do not use a zig-zag motion!
Diatom Resolution
Test Specimens
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.
Summary

1. All microscopes are similar in the way lenses work and they all suffer from the same limitations and problems.

2. Magnification is a function of the number of lenses. Resolution is a function of the ability of a lens to gather light.

3. Apertures can be used to affect resolution and depth of field if you know how they affect the light that enters the lens.
Contrast and Illumination

- Brightness contrast arises from different degrees of absorption at different points in the specimen.

- Color contrast can also arise from absorption when the degree of absorption depends on the wavelength and varies from point to point in the specimen.

- Phase contrast arises from a shift in the phase of the light as a result of interaction with the specimen.

- Polarization-dependent phase contrast arises when the phase shift depends on the plane of polarization of the incident light.

- Fluorescence contrast arises when the incident light is absorbed and partially reemitted at a different wavelength.
Bright Field Microscopy

Principle

- Light from an incandescent source is aimed toward a lens beneath the stage called the condenser, through the specimen, through an objective lens, and to the eye through a second magnifying lens, the ocular or eyepiece.

- The condenser is used to focus light on the specimen through an opening in the stage.

- After passing through the specimen, the light is displayed to the eye with an apparent field that is much larger than the area illuminated.

- Typically used on thinly sectioned materials
Cheek Cells: Bright Field Image
Dark Field Viewing

Principle

• To view a specimen in dark field, an opaque disc is placed underneath the condenser lens, so that only light that is scattered by objects on the slide can reach the eye.

• Instead of coming up through the specimen, the light is reflected by particles on the slide.

• Everything is visible regardless of color, usually bright white against a dark background.
Dark field microscopy
Mathematical description of the (EM wave)
Light wave that propagates in the z direction:

\[ \hat{E}_x (z, t) = E_{0x} \cos(kz - \omega t) \hat{x} \]
\[ \hat{E}_y (z, t) = E_{0y} \cos(kz - \omega t + \varepsilon) \hat{y} \]

Polarization of light: is the detention of the vibration of the light wave to certain direction.
Vertically polarized light
If there is no amplitude in x ($E_{0x} = 0$), there is only one component, in y (vertical).

\[
\begin{align*}
\vec{E}_x (z, t) &= E_{0x} \cos(kz - \omega t) \, \hat{x} \\
\vec{E}_y (z, t) &= E_{0y} \cos(kz - \omega t + \varepsilon) \, \hat{y}
\end{align*}
\]
Polarization at 45°
If there is no phase difference (\(\varepsilon=0\)) and \(E_{0x} = E_{0y}\), then \(E_x = E_y\)

\[
\begin{align*}
\vec{E}_x (z, t) &= E_{0x} \cos(kz - \omega t) \, \vec{x} \\
\vec{E}_y (z, t) &= E_{0y} \cos(kz - \omega t + \varepsilon) \, \vec{y}
\end{align*}
\]
Polarization at 45°
Circular polarization

If the phase difference is \( \varepsilon = 90^\circ \) and \( E_{0x} = E_{0y} \)
then: \( \frac{E_x}{E_{0x}} = \cos \Theta \), \( \frac{E_y}{E_{0y}} = \sin \Theta \)
and we get the equation of a circle:

\[
\left( \frac{E_x}{E_{0x}} \right)^2 + \left( \frac{E_y}{E_{0y}} \right)^2 = \cos^2 \Theta + \sin^2 \Theta = 1
\]

\[\vec{E}_x (z, t) = E_{0x} \cos(kz - \omega t) \hat{x}\]
\[\vec{E}_y (z, t) = E_{0y} \cos(kz - \omega t + \varepsilon) \hat{y}\]
Circular polarization
Elliptical polarization

Linear + circular polarization = elliptical polarization

Elliptical polarization is the polarization of electromagnetic radiation.
Unpolarized light  
(natural light)

Most sources of electromagnetic radiation contain a large number of atoms or molecules that send out light. The orientation of the electric fields produced by these emitters may not be correlated, in which case the light is said to be unpolarized.
Polarization

Vertical polarization

Horizontal polarization

Spring

Up-down shaking produces vertical polarization

Sideways shaking produces horizontal polarization

45° polarization

Vertical polarization 90°

Horizontal polarization 0°

= +

Waves
Polarization is a vector. A wave with polarization at 45 degrees can be represented as the sum of two waves. Each of the component waves has smaller amplitude.
A polarizer re-emits a fraction of incident light polarized at an angle to the transmission axis. A polarizer is a material that selectively absorbs light depending on polarization.
Applications of Polarizers

1. Polarizing sunglasses are used to reduce the glower of reflected light.
2. The LCD (liquid crystal diode) screen on a laptop computer uses polarized light to make pictures.
**Interference microscopy:**

linking measurements of differences in the path between two beams of light that have been split

The Interference Microscopy or Quantitative Interference Microscopy is one of these techniques that derive from Phase Contrast Microscopy but is more sensitive than this technique and make possible the easy and clarify viewing of living organisms.

This technique is used by taking light from a condenser and using a prism to separate the light into two beams. Thus, one beam (object beam) goes through the specimen and the objective and the other (reference beam) goes through another objective without touching the specimen. These beams allow a specimen to be seen through the difference in the fields caused by the two beams and the differences of the two images allow details to be seen.
There is a variation of interference microscopy called Differential Interference Contrast microscopy (DIC), also known as Nomarski Interference Contrast microscopy (NIC) or simply Nomarski microscopy.

This optical microscopy illumination technique used to improve the contrast in unstained was named after its discoverer and also uses two beams produced by a single polarized light.