Fundamentals of Optical Microscopy

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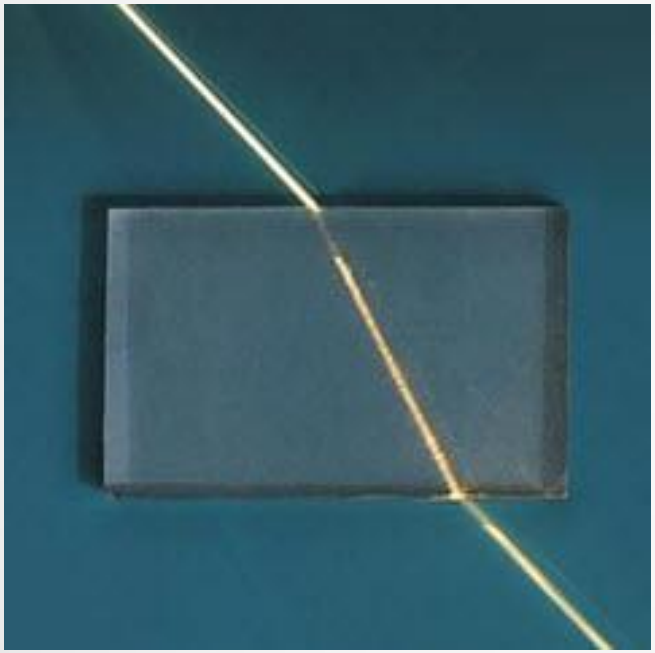
REFRACTION

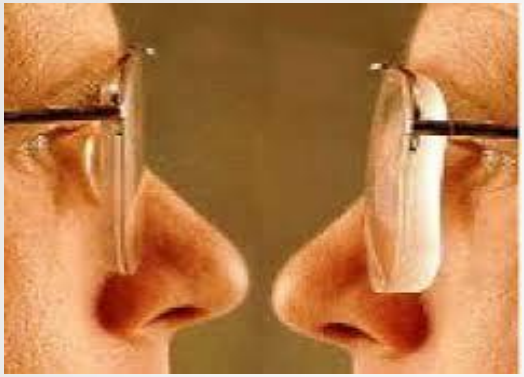
- Bending of light as it passes through different materials

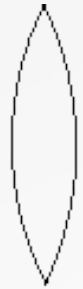


Lenses

- Rounded transparent materials that focus or disperse light by REFRACTION.

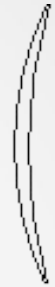
Thin Lenses





bi-convex

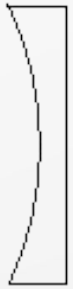
plano-convex



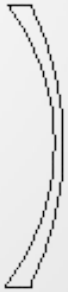
convex-meniscus

Any combination of lenses, thick or thin can be represented as a single thin lens.

bi-concave



plano-concave

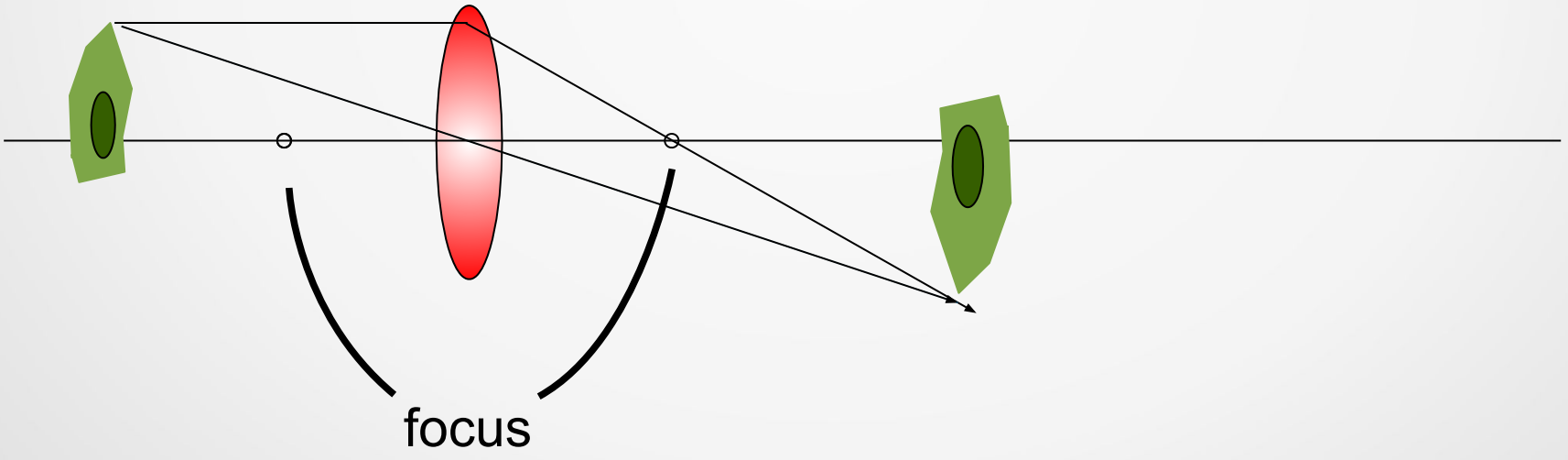


concave-meniscus

Lens Ray Tracing

Thin Lens Rules

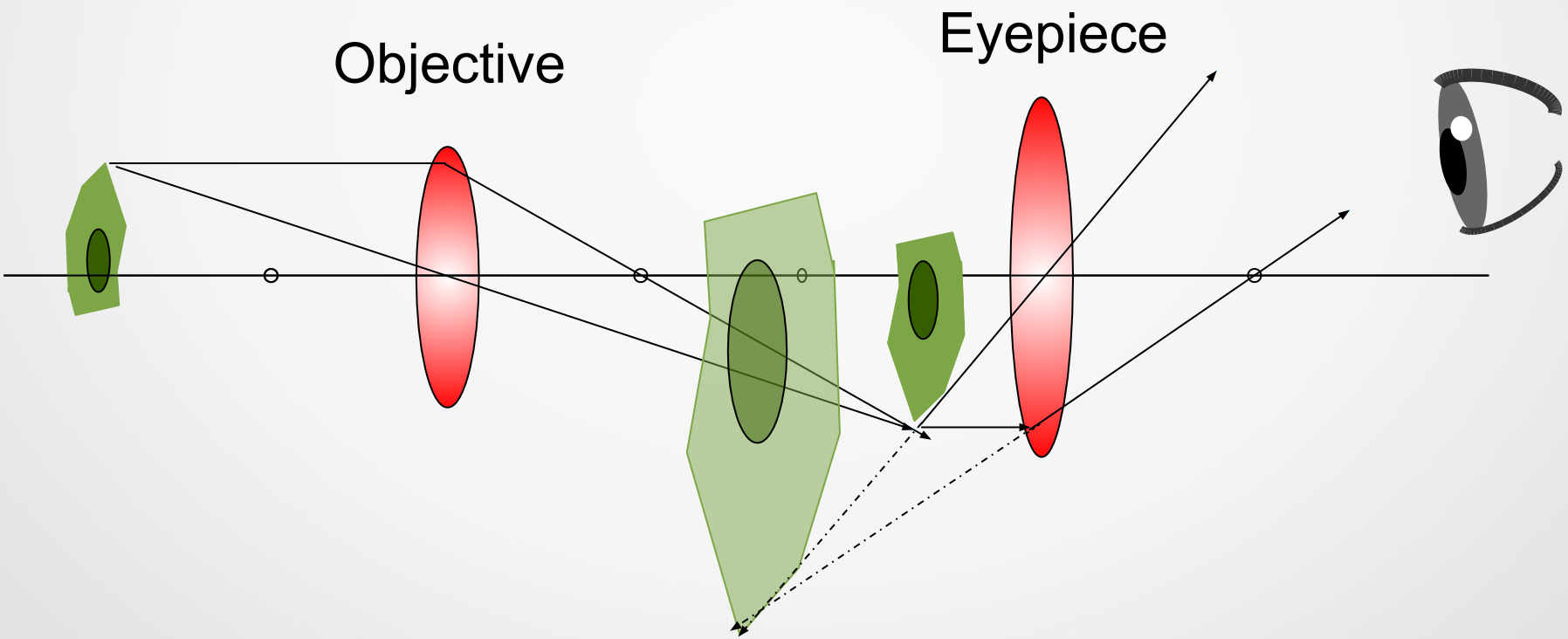
1. Rays passing parallel to the lens axis exit passing through the focus (and vice versa)
2. Rays passing through the center of the lens axis exit along the same direction



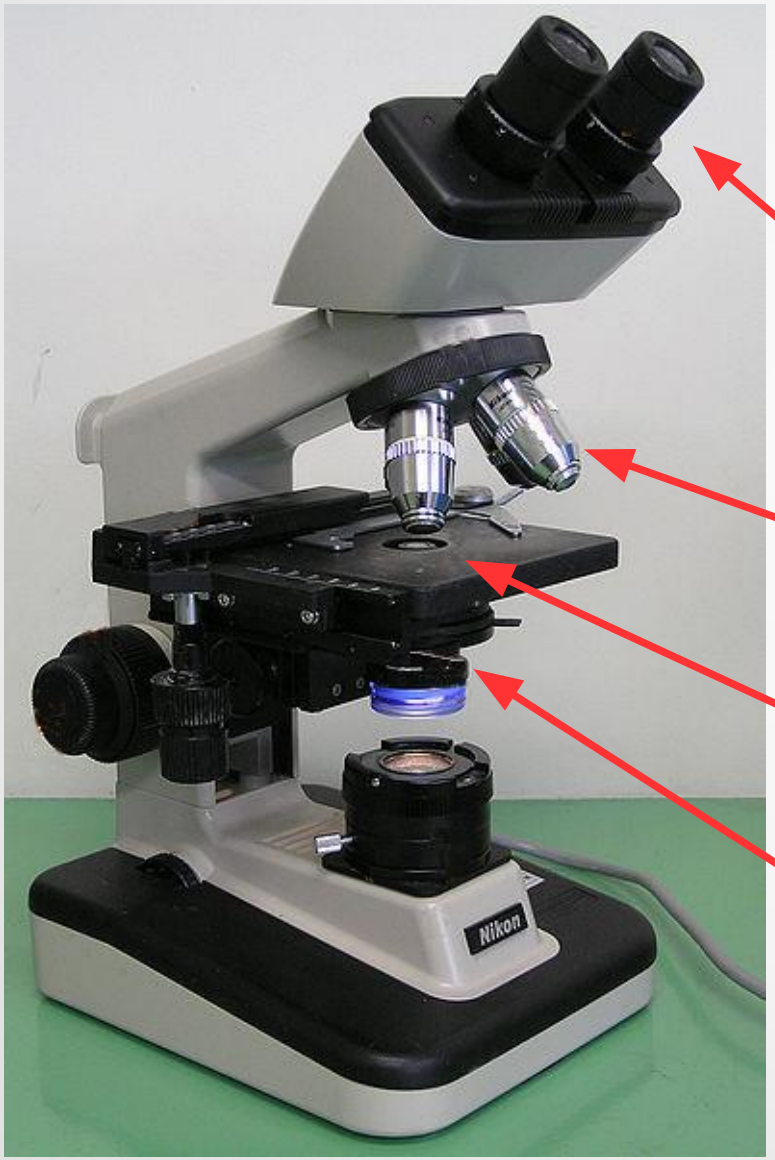
Lens Ray Tracing

Basic Microscope Optics

Microscope has two convex lenses



Basic Microscope

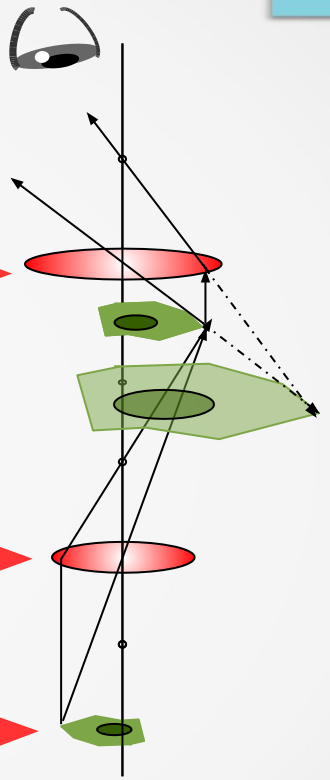


eyepiece

objective lens

specimen

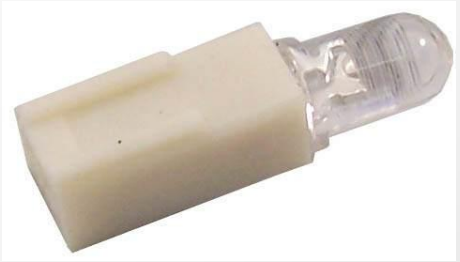
condenser



Illumination for Microscopes (Brightfield)

- **TUNGSTEN-HALOGEN**
  - Tungsten filament in halogen gas
  - color changes with applied voltage
  - gets hot

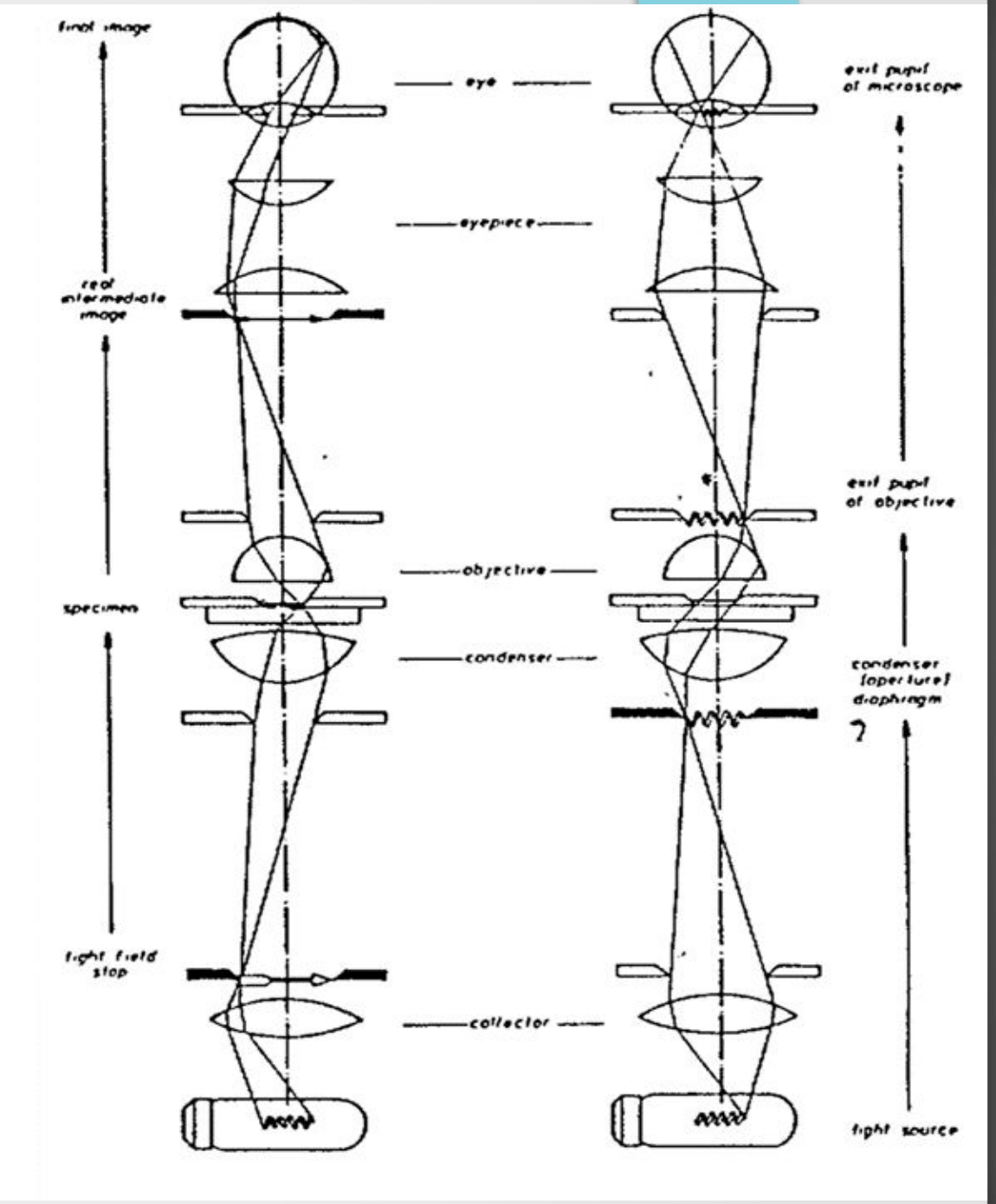
- **LED**
  - Light Emitting Diode
  - color does not change with applied voltage
  - cold light source



Köhler Illumination

- Makes the light shining on the sample very **even**.
- Achieved thru a **condenser**.
- Once the image is focused use the condenser lens to focus the condenser diaphragm.

Image scanned from Olympus Manual



Condenser controls

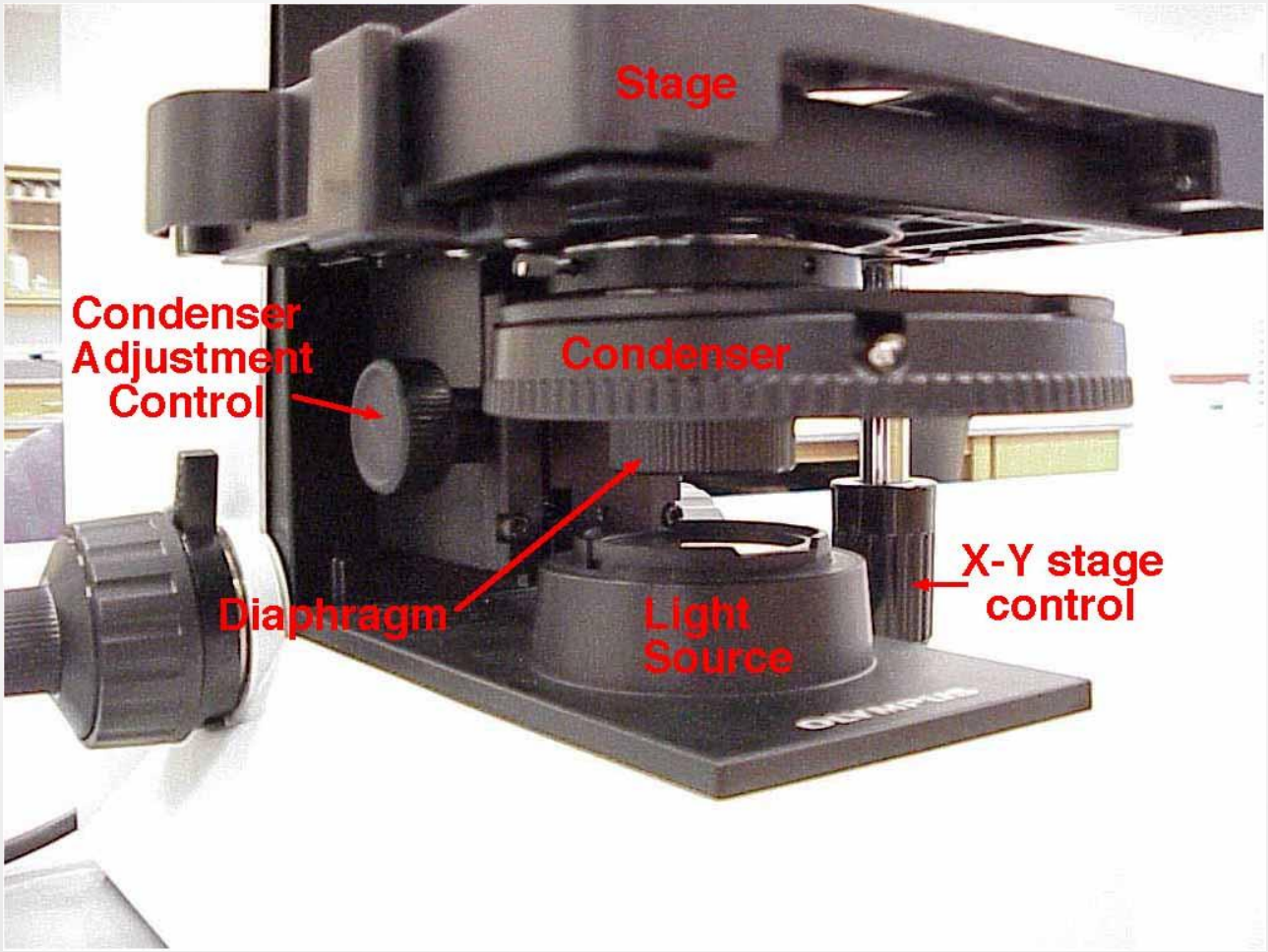
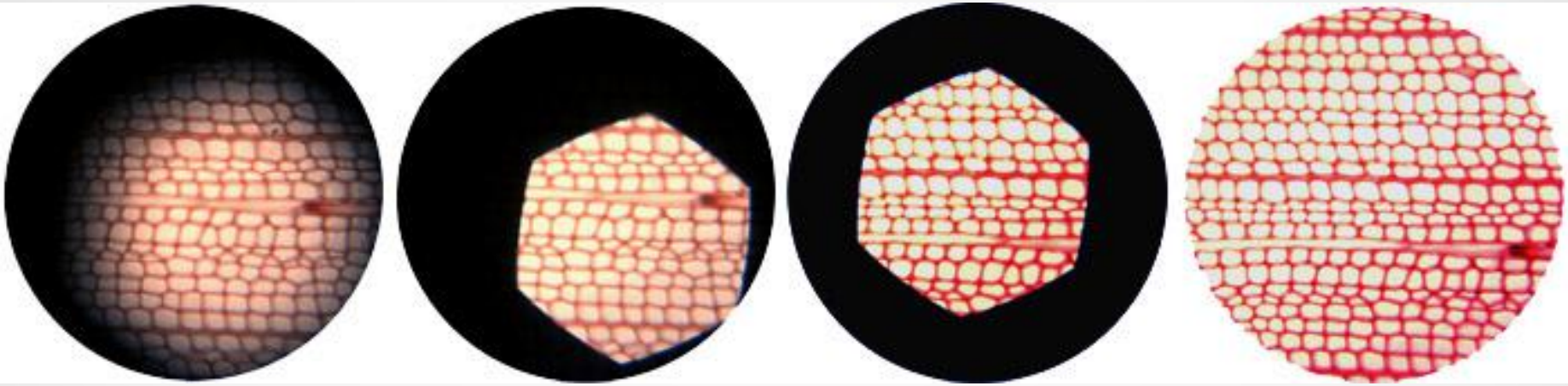


Image from : http://www.bio.davidson.edu/courses/bio111/bio111labman/ili/cond.jpeg

Achieving Köhler Illumination



1. Focus specimen

2. Slightly close condenser diaphragm and focus condenser until sides are sharp

3. Use centering screws to adjust the image of aperture to the center

4. Open condenser diaphragm until it fills the field of view.

Image from: http://web.uvic.ca/ail/techniques/kohler.jpg

Resolution Limit

- The detail that we see depend on
  - The **wavelength** of light
  - The **numerical aperture** of our lens.
- The numerical aperture of the lens is NA = nsinθ where θ is the maximum half angle at which the rays from a focused point can enter the lens with diameter D and n is the index of refraction of the medium between the lens and the sample. f is the focus distance.

Relation of NA and f-number (f/#)

NA =n sinθ can also be

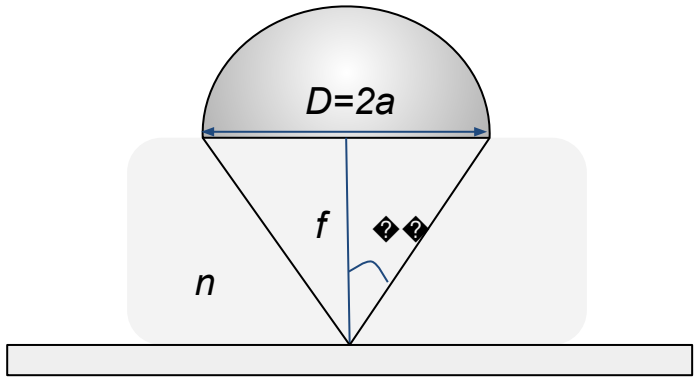
$$NA = n\sin(\arctan\frac{D}{2f}) \approx \frac{nD}{2f}$$

In photography, the f-number or f/# of a lens is the ratio of the focus f of the lens and its diameter or,

$$\mathrm{f}/\# = \frac{f}{D}$$

Thus,

$$NA = \frac{n}{2\mathrm{f}/\#}$$



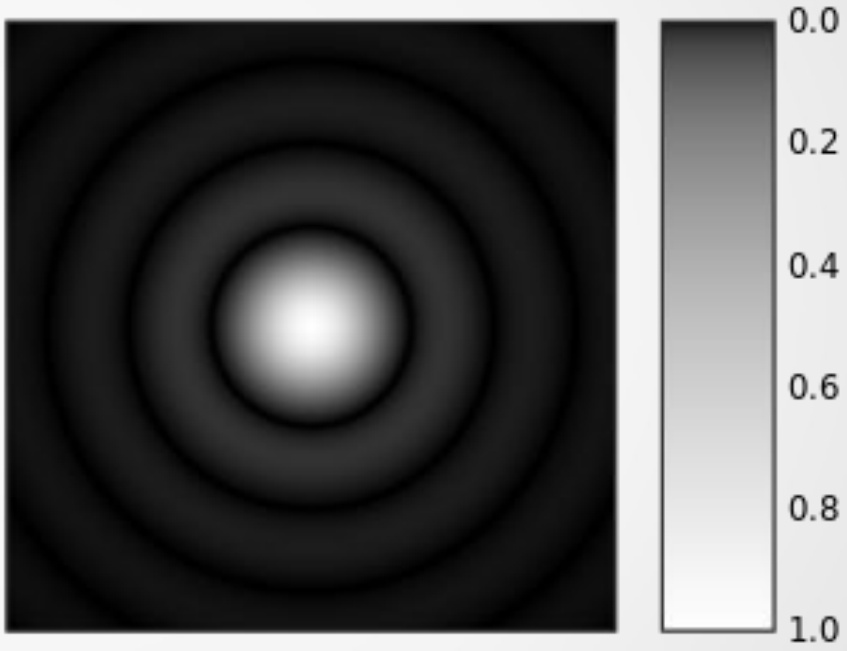
A spot does not look like a spot

Because of the finite extent of the lens not all rays diffracted by the object enter it.

Small spots will appear as circles with concentric rings – the **Airy Pattern.**

The Airy pattern is the **point spread function** of the lens and is due to the diffraction pattern of the circular aperture of the lens.

Therefore, there is a limit on the size of the object which we can image.



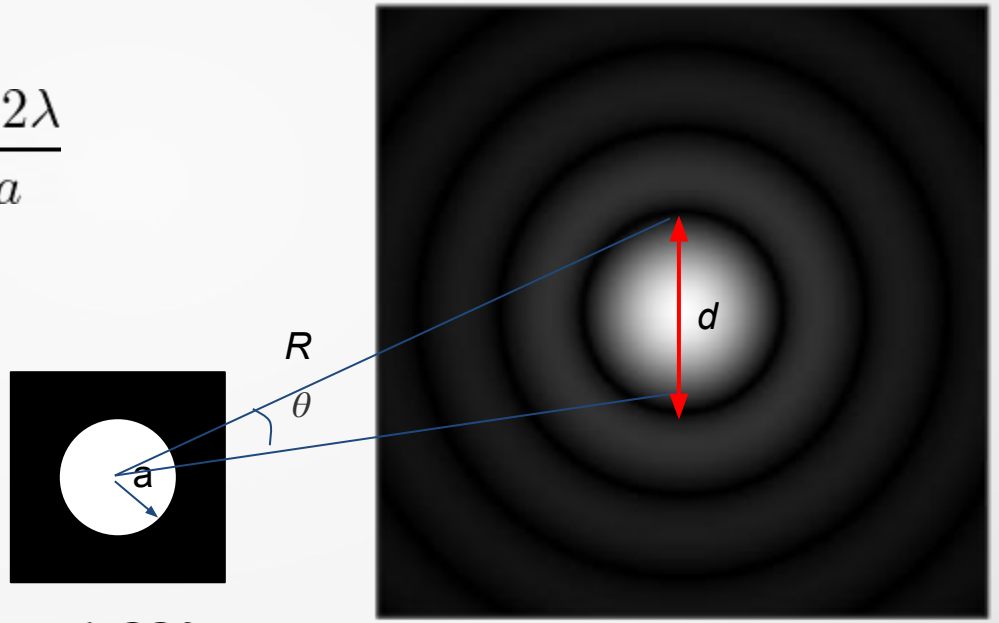
AP 157 Recall: FFT of circle is an Airy pattern.

Resolution

- Analytically, the Airy intensity pattern is given by the square of the Bessel function of the first kind, order 1, $J_1(x)$.
- The first zero of $J_1(x)$ is at $x = ka\sin\theta \sim 3.8317$ where a is the aperture radius and k is the wave number
- Thus

$$sin\theta = \frac{3.8317\lambda}{2\pi a} \approx \frac{1.22\lambda}{2a}$$

Let d be the diameter of the Airy pattern. This is the smallest detail we can see with a microscope. It can be estimated as



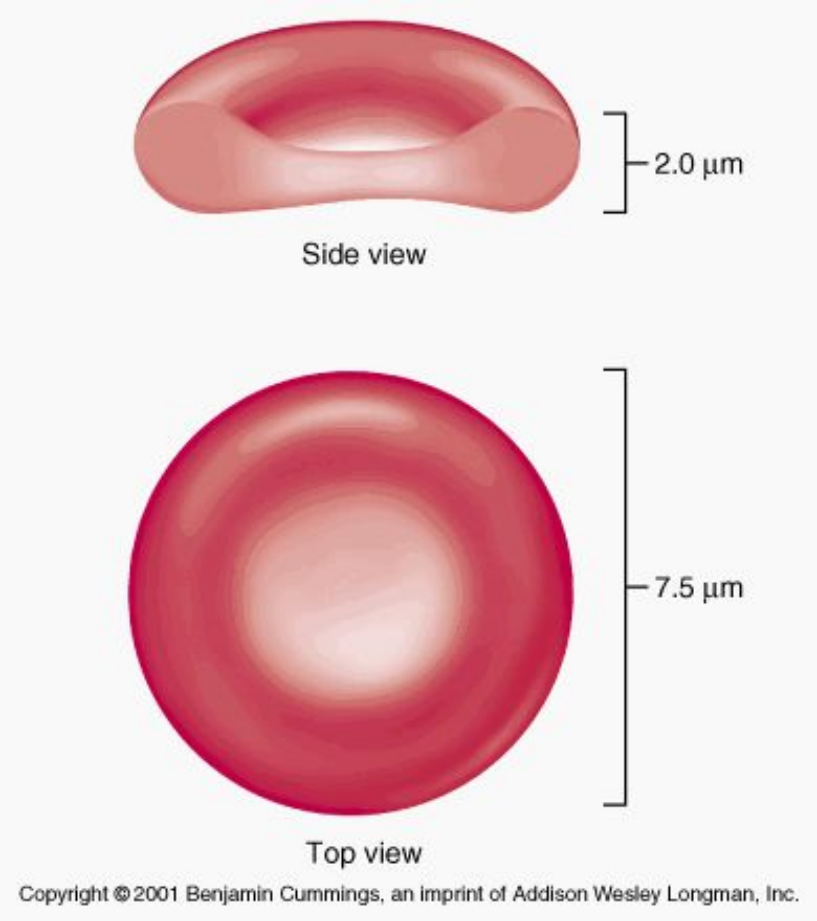
$$d = R\sin\theta = f\frac{1.22\lambda}{2a} = 1.22\lambda\frac{f}{D} = \frac{1.22\lambda}{2NA}$$

With this fundamental limit, a microscope is thus a **diffraction-limited imaging** device.

Resolution

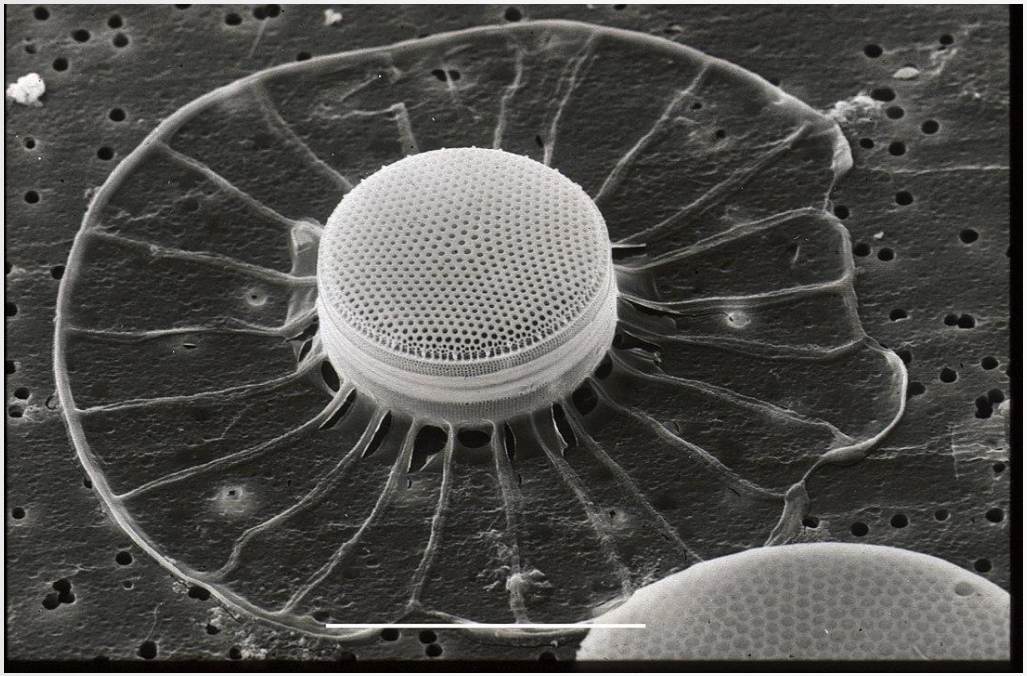
- Consider a 100x oil immersion objective (NA = 1.25) used with white light.
- Since white light has a broad emittance spectrum let us pick the wavelength the human eye is most sensitive to which is around 550nm.
- What is the size of the smallest detail this objective can see?

Resolution vs. Magnification



- A red blood cell is 6-8 μm in diameter and around 2 μm thick
- Can we clearly see the front view detail of the cell? How about the side view? How about the parts of the blood cell?
- A 100x objective and a 10x eyepiece produce a combined magnification of 1000x. An 8μm object appear to be 8mm under the microscope.
- Any additional lens (say from a camera) will only serve to enlarge the image but not the detail. This is **"empty magnification".**

What are the ways to increase resolution?



By CSIRO, CC BY 3.0, https://commons.wikimedia.org/w/index.php?curid=35488101

$$d = \frac{1.22\lambda}{2NA}$$

- Use smaller radiation source with smaller wavelength.
- Electron microscope

$$\lambda_{electron} = \frac{\hbar}{p}$$

- p = mv. With electrons driven with kinetic energy 100V electron wavelength is around 0.12 nm!

Are there ways to “beat” the diffraction limit?

YES!

[The 2014 Chemistry Nobel: Beating the Diffraction Limit | Science | AAAS]

Activity Instructions for Microscopy

For this activity we will be using a stereomicroscope. Stereo because it has two objectives, one for each eyepiece.

The stereo objectives and binoculars allow depth perception. Stereomicroscopes are used to examine tiny 3D objects such as integrated circuits.

The stereomicroscopes we have in the lab were donated by a hard disk company.

Instructions

1. The binocular eyepieces can be moved close or farther apart to match the distance between your eyes. Move until you only see one circular field of view with both eyes open.

2. To focus using the binoculars, cover your right eye with the palm of your hand and look thru the eyepiece with your left eye. Do not close your right eye or press your palm against it.

Place your sample here.

Instructions

3. Use the large knob near the toothed gear to move the lenses up or down until you see a focused image with your left eye.

4. Now cover your left eye and look through the right eyepiece with your right eye. Adjust the focusing ring* on the eyepiece barrel until the image appears focused.

*Only the right eyepiece has a focusing ring. In some cases the focusable eyepiece is on the left. Adjust the procedure accordingly.

5. To increase or decrease the magnification, adjust the magnification knob.

Slide report requirements (individual)

1. Using your cellphone camera, capture the image of your sample through one of the eyepieces. (You can have multiple samples if you're having fun.)

2. Discuss 2 other microscopy modes. Choose among:
   a. Dark field microscope
   b. Phase contrast microscope
   c. Fluorescence microscope
   d. Polarizing microscope
   e. Differential interference contrast microscope
   f. Confocal microscope