Lecture 5

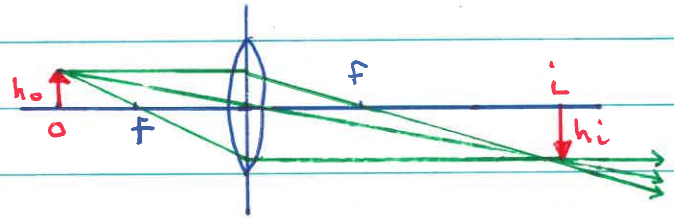
1/30/26

Today < How does a μscope work?
What factors contribute to resolution & contrast (& thus image quality)

There are two ways to get magnification w/ a converging lens

(1) Position object close to but just beyond focal point

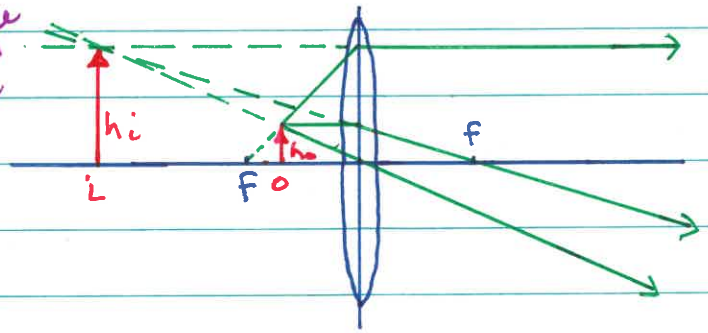
Domain of μscope objective



Form a real inverted magnified image

(2) Position object close to but within focal point

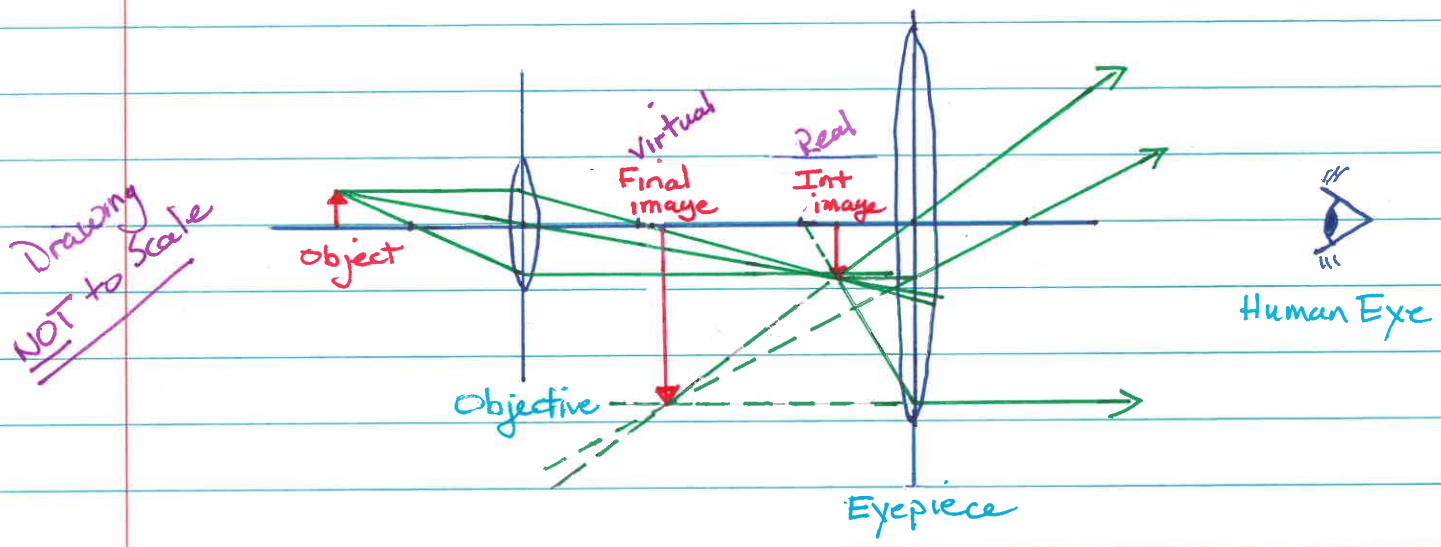
Domain of μscope eyepiece



Form a virtual upright magnified image

Lens overpowered → cannot bring light to focus

Use both approaches in a simple compound μscope



The simple compound μscope uses two lenses to form a magnified image

(1) Objective.

Object (e.g., cell) is placed close to the focal point of a First lens, called the "objective."

Objective is a high quality lens used to gather light from specimen. Can cost up to $10,000. Good objectives for looking at cells can have magnifications of 60-100X.

Power of objective inversely related to size of field of view.

- Higher power objective → smaller field of view
- Lower power objective → larger field of view

The objective creates a real inverted magnified image

(2) Eyepiece

The uscope is arranged so that the image created by the first (objective) lens is positioned just inside the focal point of a second lens, called the "eyepiece".

Key Point: In multi-lens systems, the image created by one lens, called an "intermediate image," is the object of a second downstream lens

To find the final image of the two-lens system, make the first, intermediate image an object for the second lens, & ray trace as usual.

Here, because the image formed by the objective is within the focal point of the eyepiece, the eyepiece will form a virtual image

The eyepiece creates a virtual upright magnified image.

* Human Eye

The μscope user's eye is a third lens that looks at the virtual image from the eyepiece & brings it to focus as a real image on the retina. This image is minified to fit on the retina.

Magnification. The magnification of the image created by the two-lens objective & eyepiece system is the product of the separate magnifications of each lens.

Example 60x objective
10 x eyepiece

TOTAL MAG = $60_x \times 10_x = 600x$

Total magnifications beyond about 1000X give NO extra information about the specimen.

⇒ "Empty magnification"

Skip discussion of abberations (to save time)

What are the requirements for creating a quality μscope image?

(1) Quality Illumination

Fluorescence - excite w/ illumination & see emission
Brightfield - see illumination directly

(2) Good Resolution

Want to see fine features of specimen & be able to see two closely spaced objects as two things & NOT one blurred blob
This is nontrivial!

(3) Good Contrast

Need good color or grayscale variations in image to distinguish different features

Kohler Illumination

Look first at illumination. The gold standard for generating high-quality μscope illumination is called "Kohler illumination."

Kohler worked w/ Zeiss (of Carl Zeiss μscopes)

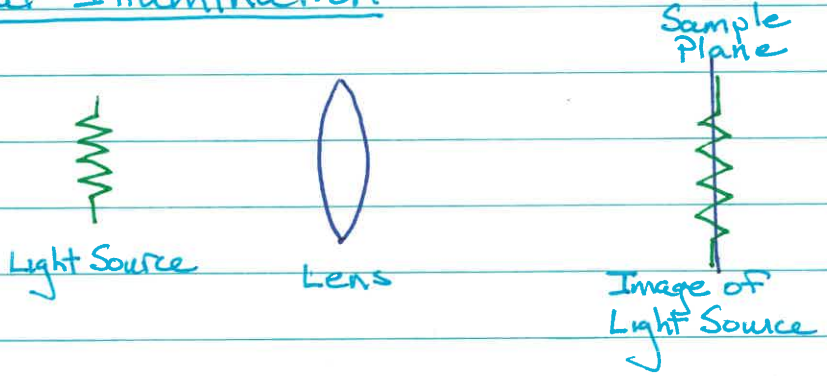
In Kohler's time, the light source for a μscope (the source of the illumination) was typically a filament or an electric arc. Now light sources also include lasers & LEDs.

Filaments typically get hot, so want to keep them away from the specimen.

Use a lens, called a "collector lens," to capture light from light source & direct it toward the specimen.

There are two standard illumination modes:

(1) Critical Illumination

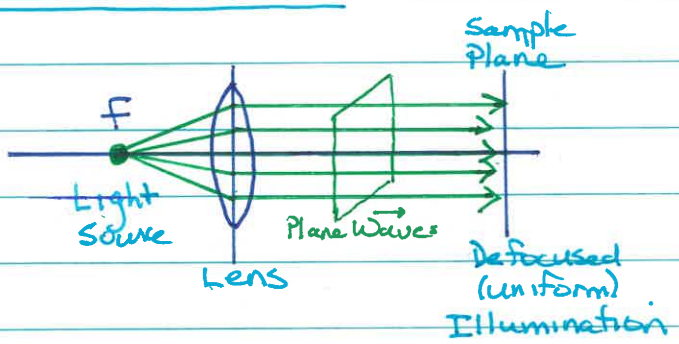


Focus an image of the light source (e.g., filament) on sample. This can provide bright but inhomogeneous illumination. For example, if light source is a filament, illumination will have pattern of filament, because illumination will look like source.

Want grayscale or color variation in sample image to reflect structure of sample, not structure of light source.

Want sample to be illuminated w/ uniform illumination so that all variations in image will reflect sample and not light source.

(2) Kohler Illumination

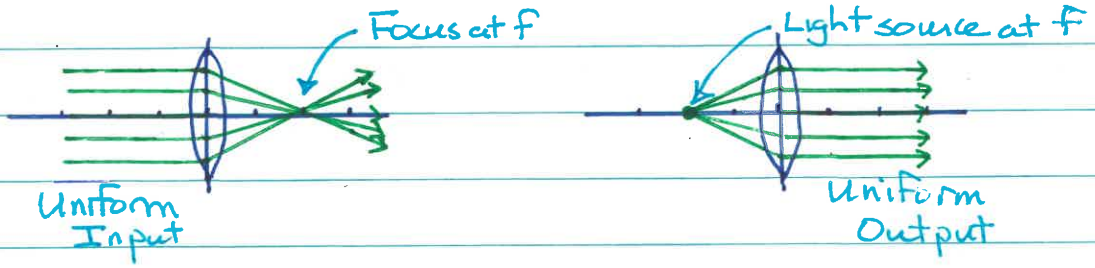


Plane waves - wave value is constant everywhere on plane

Every part of sample sees uniform illumination

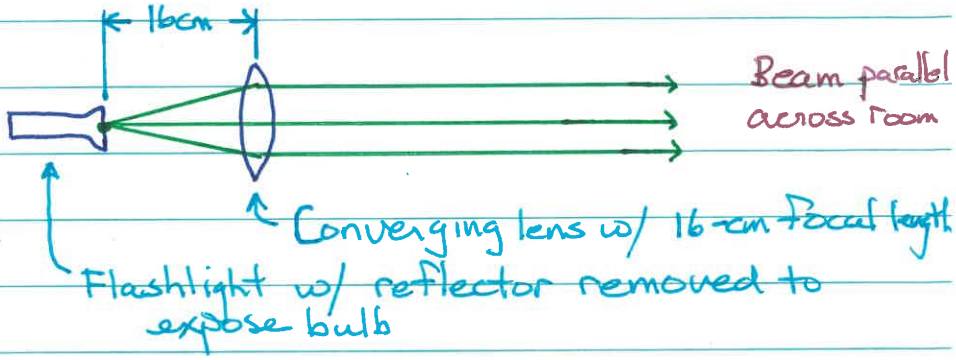
Direct defocused (uniform) illumination on sample by positioning light source at focal point

Definition of focal point is where parallel rays hitting the lens (perpendicular to lens) hit the optical axis



There's nothing sacred about light direction
⇒ light starting at focal point will exit lens parallel

DEMO



Beam diameter varies w/ distance from light source if lens placed at other separations from light source