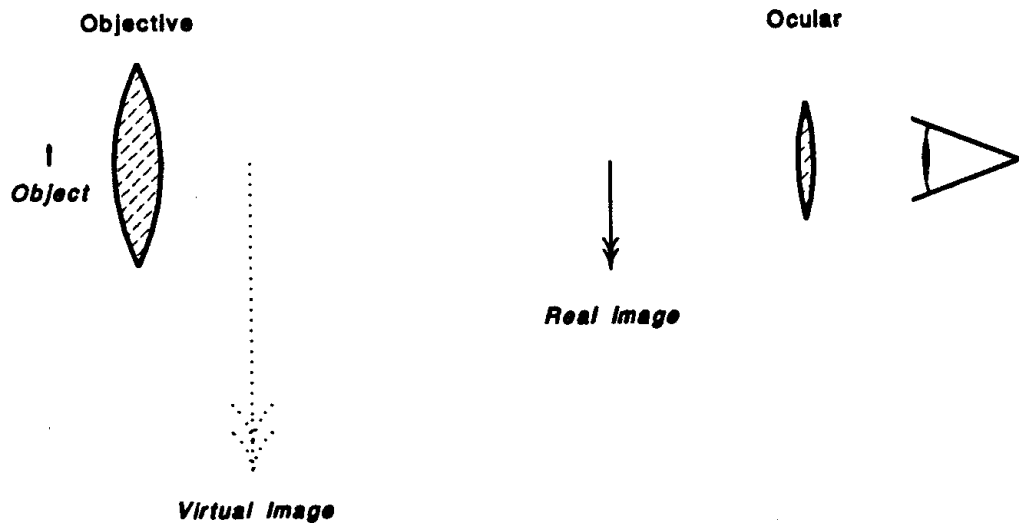


## LAB 9: THE COMPOUND MICROSCOPE

The microscope is a widely used optical instrument. In its simplest form, it consists of two lenses Fig. 9.1. An objective forms a real inverted image of an object, which is a *finite* distance in front of the lens. This image in turn becomes the object for the ocular, or eyepiece. The eyepiece forms the final image which is virtual, and magnified. The overall magnification is the product of the individual magnifications of the objective and the eyepiece.



**Figure 9.1.** Images in a compound microscope.

To illustrate the concept, use a 38 mm focal length lens (KPX079) as the objective, and a 50 mm focal length lens (KBX052) as the eyepiece. Set them up on the optical rail and adjust them until you see an inverted and magnified image of an illuminated object. Note the intermediate real image by inserting a piece of paper between the lenses.

- Q1**
- Can you demonstrate the final image by holding a piece of paper behind the eyepiece? Why or why not?

The eyepiece functions as a magnifying glass, or simple magnifier. In effect, your eye looks into the eyepiece, and in turn the eyepiece looks into the optical system--be it a compound microscope, a spotting scope, telescope, or binocular. In all cases, the eyepiece doesn't view an actual object, but rather some intermediate image formed by the "front" part of the optical system. With telescopes, this intermediate image may be real or virtual. With the compound microscope, this intermediate image is real, formed by the objective lens. In all cases, the function of the eyepiece is to form a virtual, magnified image for your eye to view.

## The Compound Microscope:

For a wealth of information, tutorials, descriptions, and images of early microscopes, point your Internet web browser to:

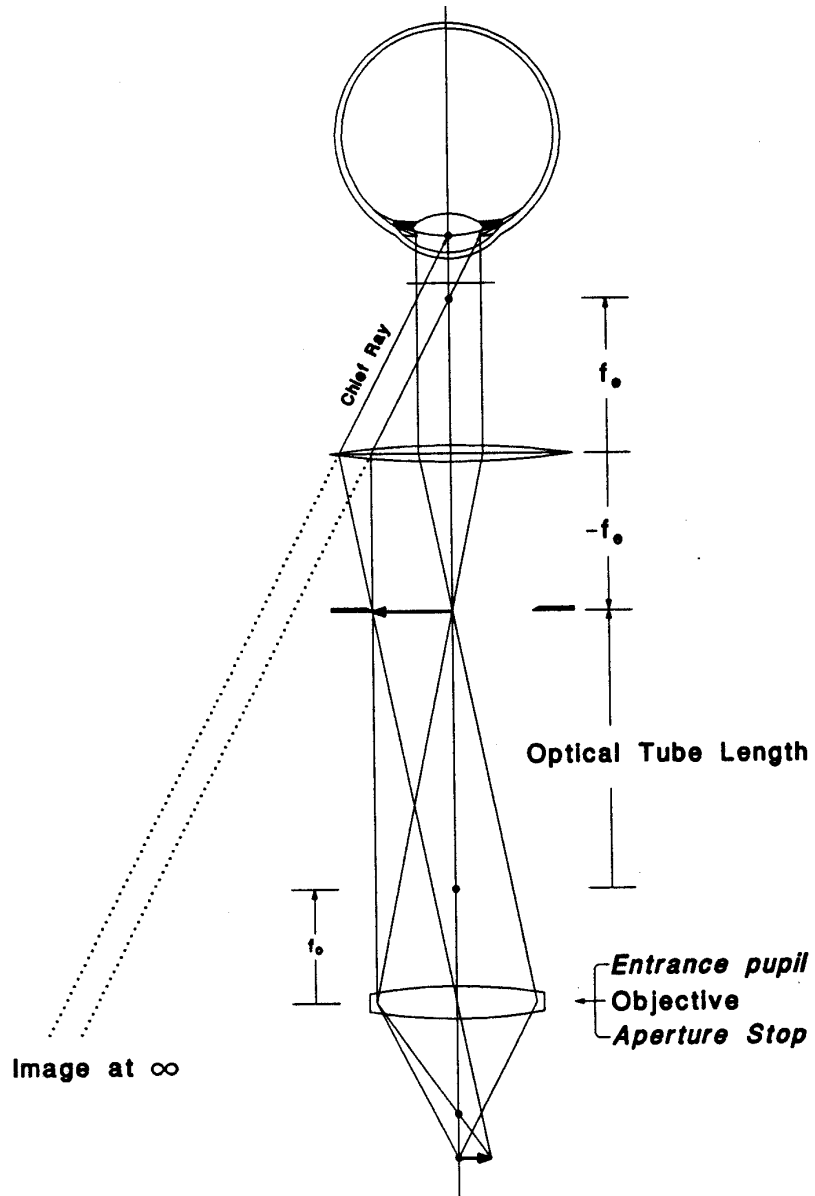
<http://microscopy.fsu.edu/primer/index.html>

The microscope is a combination of an objective lens and a magnifier, or eyepiece. As such it is often called a compound microscope, to distinguish it from the simple magnifier just discussed. Figure 9.2 shows a more detailed drawing of a basic compound microscope. The modern-day version differs only in that the objective and eyepiece are each made up of many lens elements, to correct for aberrations.

The microscope is such a useful device that it has been extensively developed and has been standardized. There are two basic types--**metallurgical** and **biological**. The former are used in reflection (since metals are not transparent), and do not allow for a cover slip. The latter are used in transmission and are compensated for the cover glass.

The **optical tube length (OTL)** is defined as the distance between the back focal point of the objective and the front focal point of the eyepiece. The optical tube length has been standardized to be 160 mm. Even the thread sizes for the mechanical lens mounts have been standardized. The advantage of these standards is that parts become interchangeable from one manufacturer to another.

Evaluating standard components can be difficult since the focal points usually lie within the mechanical housing. We will use the following techniques to measure the focal lengths of the objective and eyepiece of a commercial, low-power handheld microscope. Knowing these values, we will be able to calculate the overall magnifying power of this microscope as a function of the actual tube length.



**Figure 9.2.** A compound microscope.

## Objective Lens

Use the nodal slide to measure the 6 cardinal points for the 10X microscope objective:

- report your values for the "front-side" of the objective relative to the front vertex V
- report your values for the "back-side" of the objective relative to the rear vertex V'

**Q2** • What is your measured value of the effective focal length of the 10X objective?

The magnification of a microscope objective lens (used at finite conjugates, with an optical tube length OTL of 160mm) is given by:

$$M = \frac{OTL}{f_o} = \frac{160\text{mm}}{f_o} \quad (9.1)$$

**Q3** • Based on the objective having 10X magnification, what is the calculated value of the effective focal length?

**Q4** • Based on the Numerical Aperture (N.A.) of this objective, calculate the marginal ray angle  $u$  in object space.

## Eyepiece Focal Length

Used as a "simple" magnifier, the magnification of an eyepiece depends on whether or not the final image is formed at infinity or at your Near Point:

$$M_{\infty} = \frac{25\text{cm}}{f_E} \quad ; \quad M_{NP} = \frac{25\text{cm}}{f_E} + 1 \quad (9.2)$$

**Q5** • What is the magnification of a 10X eyepiece?

**Q6** • What is the magnification of a 15X eyepiece?

## Total Magnification of a Microscope

The overall magnification is the product of the magnifications of the objective and the eyepiece. Depending if the final image is formed at infinity or the near point, the equations are:

$$M(\infty) = \frac{OTL}{f_o} \left( \frac{25\text{cm}}{f_E} \right) ; M(NP) = \frac{OTL}{f_o} \left( \frac{25\text{cm}}{f_E} + 1 \right) \quad (9.3)$$

- Q7** • If the 10X objective is used with a 10X eyepiece, calculate the overall magnification of the microscope.
- Q8** • If the 10X objective is used with a 15X eyepiece, calculate the overall magnification of the microscope.

### Model Microscope – Thin Lenses

While hardly a commercial instrument, much can be learned by constructing a microscope from the lens set. Figure 9.4 shows the layout. Use the 50-mm focal length lens (KBX052) as an objective. Use a reticle as an object, and illuminate it with the diffuser and light source as before. This will provide helpful fiducial markings. Use the 100-mm focal length lens (KBX064) as the eyepiece. Assume an OTL of 160 mm, and place the eyepiece the proper distance in back of the objective. Place a reticle (millimeter scale on the clear ruler) at the intermediate image plane.

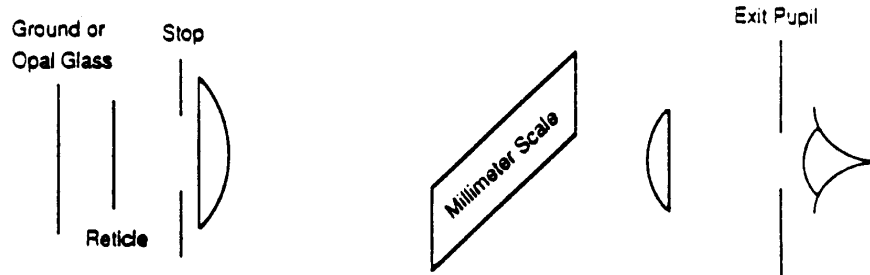
- Q9** • Calculate the position of the exit pupil and locate your eye there.

While looking through the microscope, move the source reticle until it appears in focus. At this point, the final image in the exit pupil should fill your field of view through the instrument. If not, move your head back and forth until your eye is positioned in the exit pupil. As with a telescope, this is the proper location for your eye when looking through the instrument.

Finally, place a beamsplitter between the exit pupil and eyepiece so that you can simultaneously view the image through the microscope and a reticle seen in reflection from the beamsplitter. The beamsplitter is a microscope slide standing along its long side, rotated 45° so that you can see “off to the side” of the microscope. The reticle is positioned “off to the side,” at the Near Point of your eye (25 cm from the exit pupil, in reflection from the beamsplitter).

With your eye in the exit pupil, center the reticle across the diameter of the exit pupil. Focus the microscope until you can see the microscope image and the reticle ‘in focus’ at the same time.

- Q10** • Look through the microscope and describe what you see.
- Q11** • With the reticles in place, describe a procedure to measure:  
 --the magnification of the objective  $M_O$   
 --the magnification of the eyepiece  $M_E$   
 --the overall magnification of the microscope  $M_O \times M_E$   
 (measured experimentally, not just calculated as this product!!)
- Q12** • Measure and report these magnifications.



**Figure 9.3** Model microscope.

### Model Microscope – Thick Lenses

Construct a microscope using the 10X objective lens and the 10X eyepiece. In the following steps, use the milk-glass diffuser on the light source:

- (1) Use the technique of autocollimation, the 1mm dia. "pinhole aperture" and the Xerox copy lens to produce a collimated beam.
- (2) Hold the 10X microscope objective lens in the lens holder by the knurled ring, so that the front end of the objective sticks as far to the left as possible. Position the objective lens around the 600mm mark on the optical rail.
- (3) Use the microscope to focus on the image of the "pinhole" formed by the objective lens. Note the position of the microscope on the optical rail.
- (4) Move the microscope back 160mm. Place the white card on the rail so that the front (white!) side of it is in focus in the microscope.

**Q13** • Relative to the rear vertex of the microscope objective lens, where is the white card now located? (HINT: Refer to Figure 92)

- (5) Rotate the white card so that the front side is now facing the light source. Remove the Xerox copy lens, and replace the "pinhole" with the clear ruler.
- (6) Move the light source/ruler-object until it forms an image on the white card.

**Q14** • Measure the transverse magnification of the objective.

- Q15** • Compare your measured value to the value you calculated in *Q3*.
- (7) Remove the white card/carrier and place the 10X eyepiece/carrier on the optical rail. Move the eyepiece until you see an in-focus image of the object.
- Q16** • What is the magnification of this microscope?
- (8) Replace the 10X eyepiece with the 15X eyepiece.
- Q17** • What is the magnification of this microscope?
- Q18** • As the magnification of the eyepiece increased, what happened to the field of view (in object space)?
- (8) Replace the ruler with a \$1 bill. Look at the right eye of George Washington using the 10X eyepiece!
- (9) Remove the \$1 bill from the microscope. Use the optical comparator to measure the width of the pupil in George's right eye.
- Q19** • What is the width of the pupil George's right eye, in  $\mu\text{m}$ ?

## “Real-World” Microscope

Study the binocular head from a “real-world” microscope.

- Q20** • Make a drawing of what you see.
- Q21** • Identify the various prisms:  
 - common to both eye-paths.  
 - common to the “right-eye” path.  
 - common to the “left-eye” path.

In Fig. 4.32, two binocular eyepiece prism systems are sketched. Both serve the same function, namely splitting the light beam from an objective lens into two parts. The two beams are displaced sufficiently so that they can be presented to two eyepieces and both eyes may simultaneously view the same subject. Notice that in both systems, extra glass has been added to the left hand path so that the amount of glass in each path is identical; in this way the aberrations introduced by the glass are the same for each path. Most of the glass in these systems could be dispensed with if desired, since each of them is equivalent to a beam splitting cube plus three reflectors. In System 4.32b, the two halves can be rotated about the objective axis to vary the spacing between the eyepieces as shown in Fig. 4.32c. Notice that the image is not rotated by this procedure, but retains its original orientation.

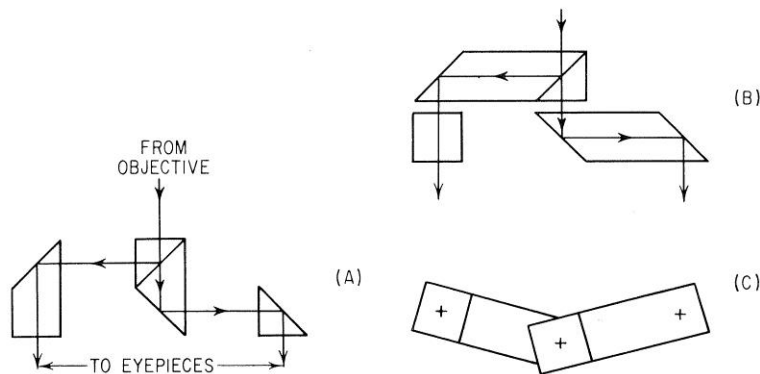


FIG. 4.32. Prism systems for binocular eyepiece instruments. System a) can be adjusted to match the user's eye separation by sliding both outer prisms in or out; this defocuses the instrument. Sketch c) shows how the halves of b) can be rotated about the objective axis to make this adjustment.

From “Modern Optical Engineering”, 1966 Warren J. Smith

NOTE: Smith points out that the amount of glass in each path (the “Right-eye path” vs. the “Left-eye path” as I call it) is the same. Therefore, the optical path length is the same for each eye. The unfolded tunnel diagram for each path is equivalent to a plane-parallel plate, having the same thickness in each path. Also, as Smith points out, the aberrations introduced by the equivalent plane-parallel plates are therefore the same in each path.