MICROSCOPES

> ### LEARNING OUTCOMES:
>
> - Investigate different types of microscopes.
> - Learn how image is formed in a compound microscope.

Although the eye is marvelous in its ability to see objects large and small, it obviously has limitations to the smallest details it can detect. Human desire to see beyond what is possible with the naked eye led to the use of optical instruments. In this section we will examine microscopes, instruments for enlarging the detail that we cannot see with the unaided eye. The microscope is a multiple-element system having more than a single lens or mirror. (See [Figure 1]) A microscope can be made from two convex lenses. The image formed by the first element becomes the object for the second element. The second element forms its own image, which is the object for the third element, and so on. Ray tracing helps to visualize the image formed. If the device is composed of thin lenses and mirrors that obey the thin lens equations, then it is not difficult to describe their behavior numerically.

Figure 1: *Multiple lenses and mirrors are used in this microscope. (credit: U.S. Navy photo by Tom Watanabe)*

Microscopes were first developed in the early 1600s by eyeglass makers in The Netherlands and Denmark. The simplest **compound microscope** is constructed from two convex lenses as shown schematically in [Figure 2]. The first lens is called the **objective lens**, and has typical magnification values from $5\times$ to $100\times$. In standard microscopes, the objectives are mounted such that when you switch between objectives, the sample remains in focus. Objectives arranged in this way are described as parfocal. The second, the **eyepiece**, also referred to as the ocular, has several lenses which slide inside a cylindrical barrel. The focusing ability is provided by the movement of both the objective lens and the eyepiece. The purpose of a microscope is to magnify small objects, and both lenses contribute to the final magnification. Additionally, the final enlarged image is produced in a location far enough from the observer to be easily viewed, since the eye cannot focus on objects or images that are too close.
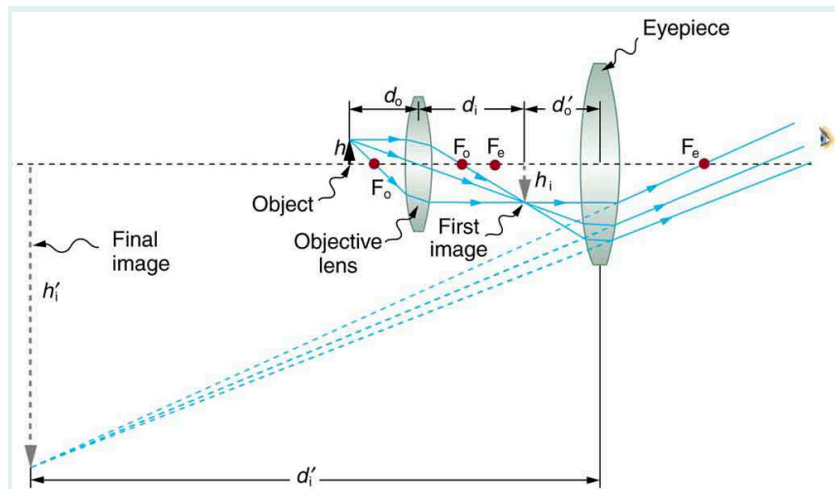
Figure 2: *A compound microscope composed of two lenses, an objective and an eyepiece. The objective forms a case 1 image that is larger than the object. This first image is the object for the eyepiece. The eyepiece forms a case 2 final image that is further magnified.*

To see how the microscope in [Figure 2] forms an image, we consider its two lenses in succession. The object is slightly farther away from the objective lens than its focal length f_o , producing a case 1 image that is larger than the object. This first image is the object for the second lens, or eyepiece. The eyepiece is intentionally located so it can further magnify the image. The eyepiece is placed so that the first image is closer to it than its focal length f_e . Thus the eyepiece acts as a magnifying glass, and the final image is made even larger. The final image remains inverted, but it is farther from the observer, making it easy to view (the eye is most relaxed when viewing distant objects and normally cannot focus closer than 25 cm). Since each lens produces a magnification that multiplies the height of the image, it is apparent that the overall magnification m is the product of the individual magnifications:

$$m = m_\mathrm{o} m_\mathrm{e},$$

Eq.(1)

where m_o is the magnification of the objective and m_e is the magnification of the eyepiece. This equation can be generalized for any combination of thin lenses and mirrors that obey the thin lens equations.

OVERALL MAGNIFICATION

The overall magnification of a multiple-element system is the product of the individual magnifications of its elements.

Example 1: Microscope Magnification

Calculate the magnification of an object placed 6.20 mm from a compound microscope that has a 6.00 mm focal length objective and a 50.0 mm focal length eyepiece. The objective and eyepiece are separated by 23.0 cm.

Strategy and Concept

This situation is similar to that shown in [Figure 2]. To find the overall magnification, we must find the magnification of the objective, then the magnification of the eyepiece. This involves using the thin lens equation.

Solution

The magnification of the objective lens is given as

$$m_{\mathrm{o}} = -\frac{d_{\mathrm{i}}}{d_{\mathrm{o}}},$$

Eq.(2)

where d_{o} and d_{i} are the object and image distances, respectively, for the objective lens as labeled in [Figure 2]. The object distance is given to be $d_{\mathrm{o}} = 6.20\mathrm{mm}$, but the image distance d_{i} is not known. Isolating d_{i} , we have

$$\frac{1}{d_{\mathrm{i}}} = \frac{1}{f_{\mathrm{o}}} - \frac{1}{d_{\mathrm{o}}},$$

Eq.(3)

where f_{o} is the focal length of the objective lens. Substituting known values gives

$$\frac{1}{d_{\mathrm{i}}} = \frac{1}{6.00\mathrm{mm}} - \frac{1}{6.20\mathrm{mm}} = \frac{0.00538}{\mathrm{mm}}.$$

Eq.(4)

We invert this to find d_{i} :

$$d_{\mathrm{i}} = 186\mathrm{mm}.$$

Eq.(5)

Substituting this into the expression for m_{o} gives

$$m_{\mathrm{o}} = -\frac{d_{\mathrm{i}}}{d_{\mathrm{o}}} = -\frac{186\mathrm{mm}}{6.20\mathrm{mm}} = -30.0.$$

Eq.(6)

Now we must find the magnification of the eyepiece, which is given by

$$m_e = -\frac{d_i'}{d_o'},$$ Eq.(7)

where d_i' and d_o' are the image and object distances for the eyepiece (see [Figure 2]). The object distance is the distance of the first image from the eyepiece. Since the first image is 186 mm to the right of the objective and the eyepiece is 230 mm to the right of the objective, the object distance is $d_o' = 230\text{mm} - 186\text{mm} = 44.0\text{mm}$. This places the first image closer to the eyepiece than its focal length, so that the eyepiece will form a case 2 image as shown in the figure. We still need to find the location of the final image d_i' in order to find the magnification. This is done as before to obtain a value for $1/d_i'$:

$$\frac{1}{d_i'} = \frac{1}{f_e} - \frac{1}{d_o'} = \frac{1}{50.0\text{mm}} - \frac{1}{44.0\text{mm}} = -\frac{0.00273}{\text{mm}}.$$ Eq.(8)

Inverting gives

$$d_i' = -\frac{\text{mm}}{0.00273} = -367\text{mm}.$$ Eq.(9)

The eyepiece's magnification is thus

$$m_e = -\frac{d_i'}{d_o'} = -\frac{-367\text{mm}}{44.0\text{mm}} = 8.33.$$ Eq.(10)

So the overall magnification is

$$m = m_o m_e = (-30.0)\,(8.33) = -250.$$ Eq.(11)

Discussion

Both the objective and the eyepiece contribute to the overall magnification, which is large and negative, consistent with [Figure 2], where the image is seen to be large and inverted. In this case, the image is virtual and inverted, which cannot happen for a single element (case 2 and case 3 images for single elements are virtual and upright). The final image is 367 mm (0.367 m) to the left of the eyepiece. Had the eyepiece been placed farther from the objective, it could have formed a case 1 image to the right. Such an image could be projected on a screen, but it would be behind the head of the person in the figure and not appropriate for direct viewing. The procedure used to solve this

example is applicable in any multiple-element system. Each element is treated in turn, with each forming an image that becomes the object for the next element. The process is not more difficult than for single lenses or mirrors, only lengthier.

Normal optical microscopes can magnify up to $1500\times$ with a theoretical resolution of $-0.2\mu m$. The lenses can be quite complicated and are composed of multiple elements to reduce aberrations. Microscope objective lenses are particularly important as they primarily gather light from the specimen. Three parameters describe microscope objectives: the **numerical aperture ** (NA), the magnification (m), and the working distance. The NA is related to the light gathering ability of a lens and is obtained using the angle of acceptance θ formed by the maximum cone of rays focusing on the specimen (see [Figure 3](a)) and is given by

$$\mathrm{NA} = n\sin\alpha,$$

Eq.(12)

where n is the refractive index of the medium between the lens and the specimen and $\alpha = \theta/2$. As the angle of acceptance given by θ increases, NA becomes larger and more light is gathered from a smaller focal region giving higher resolution. A $0.75\mathrm{NA}$ objective gives more detail than a $0.10\mathrm{NA}$ objective.
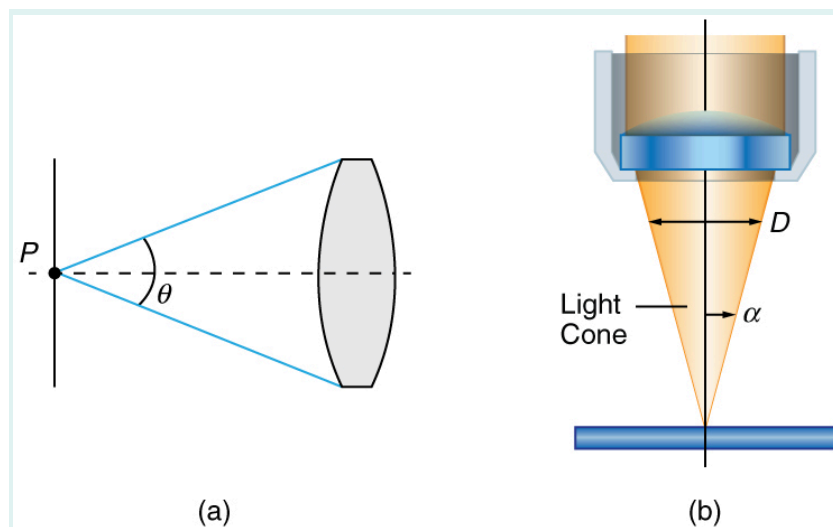

Figure 3: (a) The numerical aperture (NA) of a microscope objective lens refers to the light-gathering ability of the lens and is calculated using half the angle of acceptance θ. (b) Here, α; is half the acceptance angle for light rays from a specimen entering a camera lens, and D is the diameter of the aperture that controls the light entering the lens.

While the numerical aperture can be used to compare resolutions of various objectives, it does not indicate how far the lens could be from the specimen. This is specified by the "working distance," which is the distance (in mm usually) from the front lens element of the objective to the specimen, or cover glass. The higher the NA the closer the lens will be to the specimen and the more chances there are of breaking the cover slip and damaging

both the specimen and the lens. The focal length of an objective lens is different than the working distance. This is because objective lenses are made of a combination of lenses and the focal length is measured from inside the barrel. The working distance is a parameter that microscopists can use more readily as it is measured from the outermost lens. The working distance decreases as the NA and magnification both increase.

The term $f/\#$ in general is called the f -number and is used to denote the light per unit area reaching the image plane. In photography, an image of an object at infinity is formed at the focal point and the f -number is given by the ratio of the focal length f of the lens and the diameter D of the aperture controlling the light into the lens (see [Figure 3](b)) . If the acceptance angle is small the NA of the lens can also be used as given below.

$$f/\# = \frac{f}{D} \approx \frac{1}{2\mathrm{NA}}.$$

Eq.(13)

As the f -number decreases, the camera is able to gather light from a larger angle, giving wide-angle photography. As usual there is a trade-off. A greater $f/\#$ means less light reaches the image plane. A setting of $f/16$ usually allows one to take pictures in bright sunlight as the aperture diameter is small. In optical fibers, light needs to be focused into the fiber. [Figure 4] shows the angle used in calculating the NA of an optical fiber.
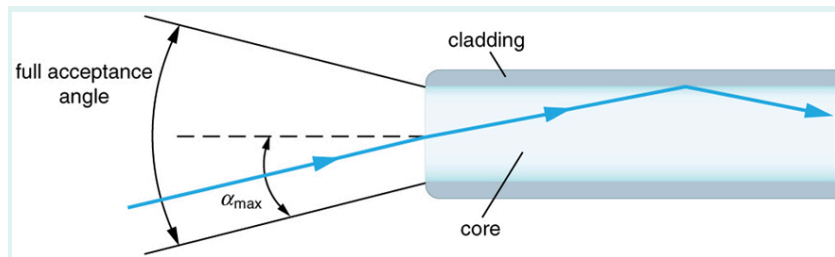

Figure 4: *Light rays enter an optical fiber. The numerical aperture of the optical fiber can be determined by using the angle \(\alpha \);max.*

Can the NA be larger than 1.00? The answer is 'yes' if we use immersion lenses in which a medium such as oil, glycerine or water is placed between the objective and the microscope cover slip. This minimizes the mismatch in refractive indices as light rays go through different media, generally providing a greater light-gathering ability and an increase in resolution. [Figure 5] shows light rays when using air and immersion lenses.
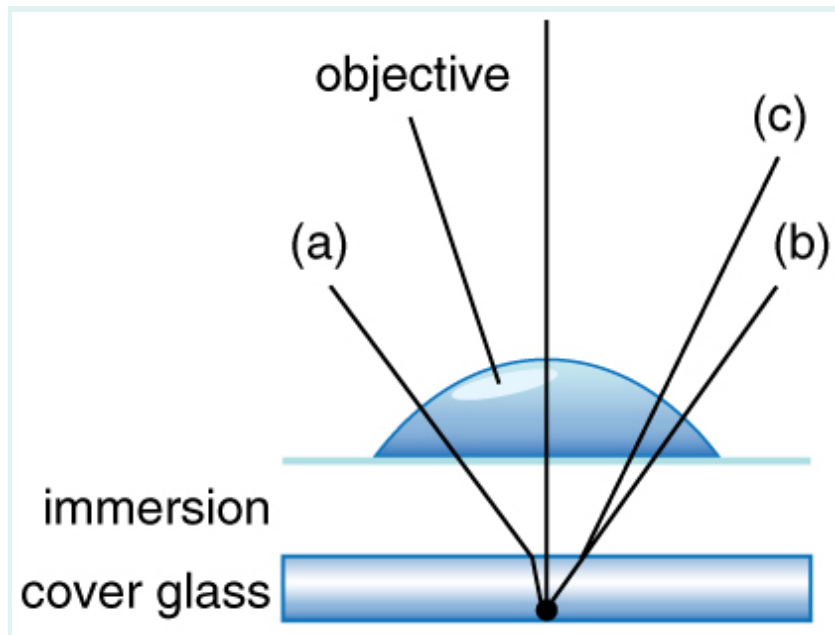
Figure 5: *Light rays from a specimen entering the objective. Paths for immersion medium of air (a), water (b) $((n=1.33))$, and oil (c) $((n=1.51))$ are shown. The water and oil immersions allow more rays to enter the objective, increasing the resolution.*

When using a microscope we do not see the entire extent of the sample. Depending on the eyepiece and objective lens we see a restricted region which we say is the field of view. The objective is then manipulated in two-dimensions above the sample to view other regions of the sample. Electronic scanning of either the objective or the sample is used in scanning microscopy. The image formed at each point during the scanning is combined using a computer to generate an image of a larger region of the sample at a selected magnification.

When using a microscope, we rely on gathering light to form an image. Hence most specimens need to be illuminated, particularly at higher magnifications, when observing details that are so small that they reflect only small amounts of light. To make such objects easily visible, the intensity of light falling on them needs to be increased. Special illuminating systems called condensers are used for this purpose. The type of condenser that is suitable for an application depends on how the specimen is examined, whether by transmission, scattering or reflecting. See [Figure 6] for an example of each. White light sources are common and lasers are often used. Laser light illumination tends to be quite intense, and it is important to ensure that the light does not result in the degradation of the specimen.
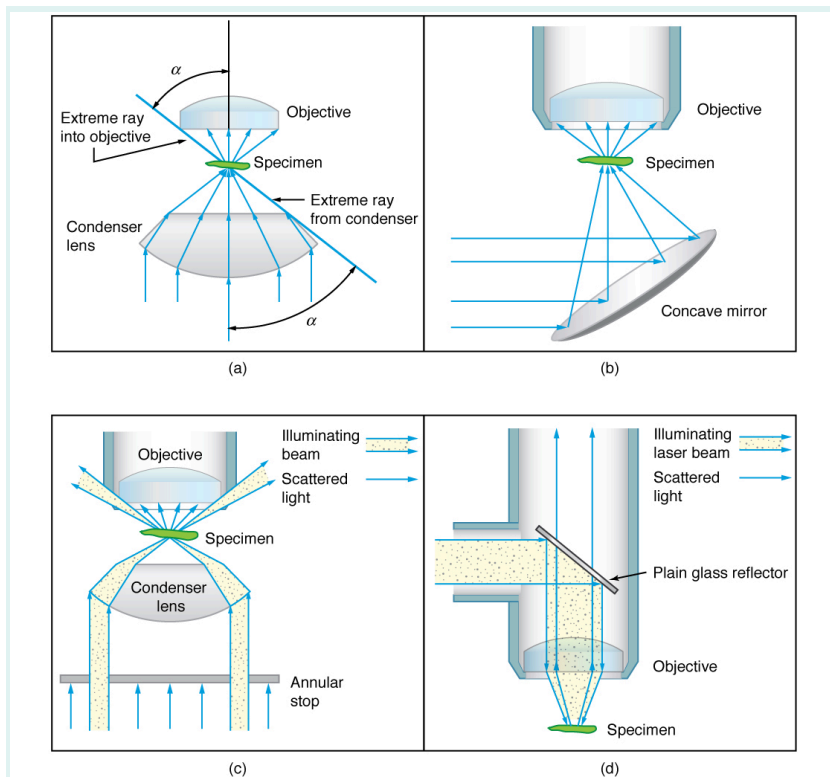
Figure 6: *Illumination of a specimen in a microscope. (a) Transmitted light from a condenser lens. (b) Transmitted light from a mirror condenser. (c) Dark field illumination by scattering (the illuminating beam misses the objective lens). (d) High magnification illumination with reflected light – normally laser light.*

We normally associate microscopes with visible light but X-ray and electron microscopes provide greater resolution. The focusing and basic physics is the same as that just described, even though the lenses require different technology. The electron microscope requires vacuum chambers so that the electrons can proceed unheeded. Magnifications of 50 million times provide the ability to determine positions of individual atoms within materials. An electron microscope is shown in [Figure 7]. We do not use our eyes to form images; rather images are recorded electronically and displayed on computers. In fact observing and saving images formed by optical microscopes on computers is now done routinely. Video recordings of what occurs in a microscope can be made for viewing by many people at later dates. Advances in this powerful technology continue. In the 1990s, Pratibha L. Gai invented the environmental transmission electron microscope (ETEM), which was the first device capable of observing individual atoms in chemical reactions.

Figure 7: *An electron microscope has the capability to image individual atoms on a material. The microscope uses vacuum technology, sophisticated detectors and state of the art image processing software. (credit: Dave Pape)*
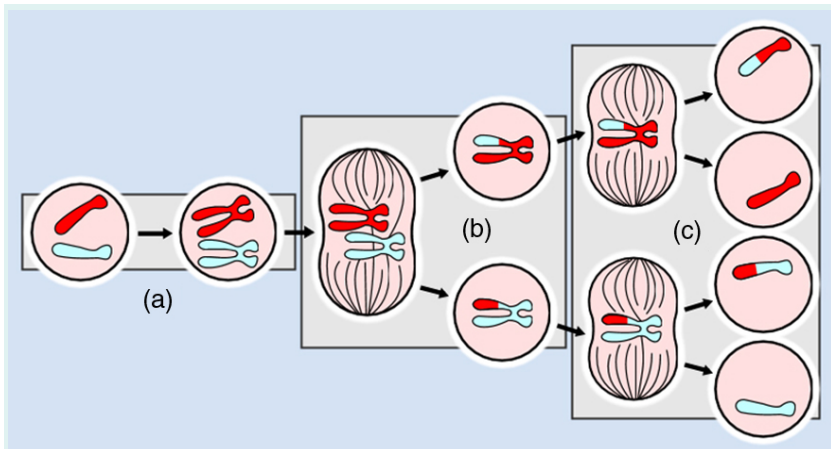

Figure 8: *The image shows a sequence of events that takes place during meiosis. (credit: PatríciaR, Wikimedia Commons; National Center for Biotechnology Information)*

TAKE-HOME EXPERIMENT: MAKE A LENS

Look through a clear glass or plastic bottle and describe what you see. Now fill the bottle with water and describe what you see. Use the water bottle as a lens to produce the image of a bright object and estimate the focal length of the water bottle lens. How is the focal length a function of the depth of water in the bottle?

Section Summary

- The microscope is a multiple-element system having more than a single lens or mirror.
- Many optical devices contain more than a single lens or mirror. These are analysed by considering each element sequentially. The image formed by the first is the object for the second, and so on. The same ray tracing and thin lens techniques apply to each lens element.
- The overall magnification of a multiple-element system is the product of the magnifications of its individual elements. For a two-element system with an objective and an eyepiece, this is

$$m = m_\mathrm{o} m_\mathrm{e},$$ Eq.(14)

where m_o is the magnification of the objective and m_e is the magnification of the eyepiece, such as for a microscope.
- Microscopes are instruments for allowing us to see detail we would not be able to see with the unaided eye and consist of a range of components.
- The eyepiece and objective contribute to the magnification. The numerical aperture (NA) of an objective is given by

$$\mathrm{NA} = n \sin \alpha$$ Eq.(15)

where n is the refractive index and α the angle of acceptance.

- Immersion techniques are often used to improve the light gathering ability of microscopes. The specimen is illuminated by transmitted, scattered or reflected light though a condenser.
- The $f/\#$ describes the light gathering ability of a lens. It is given by

$$f/\# = \frac{f}{D} \approx \frac{1}{2\mathrm{NA}}.$$ Eq.(16)

Conceptual Questions

Exercise 1

Geometric optics describes the interaction of light with macroscopic objects. Why, then, is it correct to use geometric optics to analyse a microscope's image?

Exercise 2

The image produced by the microscope in [Figure 2] cannot be projected. Could extra lenses or mirrors project it? Explain.

Exercise 3

Why not have the objective of a microscope form a case 2 image with a large magnification? (Hint: Consider the location of that image and the difficulty that would pose for using the eyepiece as a magnifier.)

Exercise 4

What advantages do oil immersion objectives offer?

Exercise 5

How does the NA of a microscope compare with the NA of an optical fiber?

Problem Exercises

Exercise 6

A microscope with an overall magnification of 800 has an objective that magnifies by 200. (a) What is the magnification of the eyepiece? (b) If there are two other objectives that can be used, having magnifications of 100 and 400, what other total magnifications are possible?

Exercise 7

(a) What magnification is produced by a 0.150 cm focal length microscope objective that is 0.155 cm from the object being viewed? (b) What is the overall magnification if an $8\times$ eyepiece (one that produces a magnification of 8.00) is used?

Exercise 8

(a) Where does an object need to be placed relative to a microscope for its 0.500 cm focal length objective to produce a magnification of -400 ? (b) Where should the 5.00 cm focal length eyepiece be placed to produce a further fourfold (4.00) magnification? (c) Is this a realistic design for a microscope?

Exercise 9

You switch from a $1.40\mathrm{NA}60\times$ oil immersion objective to a $0.35\mathrm{NA}20\times$ oil immersion objective. What are the acceptance angles for each? Compare and comment on the values. Which would you use first to locate the target area on your specimen?

Exercise 10

An amoeba is 0.305 cm away from the 0.300 cm focal length objective lens of a microscope. (a) Where is the image formed by the objective lens? (b) What is this image's magnification? (c) An eyepiece with a 2.00 cm focal length is placed 20.0 cm from the objective. Where is the final image? (d) What magnification is produced by the eyepiece? (e) What is the overall magnification? (See [Figure 2].)

Exercise 11

You are using a standard microscope with a $0.10 \text{NA} 4\times$ objective and switch to a $0.65 \text{NA} 40\times$ objective. What are the acceptance angles for each? Compare and comment on the values. Which would you use first to locate the target area on of your specimen? (See [Figure 3].)

Exercise 12

Unreasonable Results

Your friends show you an image through a microscope. They tell you that the microscope has an objective with a 0.500 cm focal length and an eyepiece with a 5.00 cm focal length. The resulting overall magnification is 250 000. Are these viable values for a microscope?

GLOSSARY

compound microscope

a microscope constructed from two convex lenses, the first serving as the ocular lens(close to the eye) and the second serving as the objective lens

objective lens

the lens nearest to the object being examined

eyepiece

the lens or combination of lenses in an optical instrument nearest to the eye of the observer

numerical aperture

a number or measure that expresses the ability of a lens to resolve fine detail in an object being observed. Derived by mathematical formula

$$\mathrm{NA} = n \sin \alpha, \qquad\qquad \text{Eq.(17)}$$

where n is the refractive index of the medium between the lens and the specimen and $\alpha = \theta/2$