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Use of objective lens in microscope

Although your microscope may differ in some details from the one described in the web page above, most microscopes will have one or more of the following objective lenses. You should acquaint yourself with each of these objectives and their uses. Scanning Objective (4X): This shortest objective is useful for getting an overview of the slide (especially handy with some of the slides that contain whole organs like a section of the spinal cord, lung, digestive tract, ovary, etc.). Low Power Objective (10X): This next shortest objective is probably the most useful lens for viewing slides. Almost any feature you need to observe in this course can be located with the 100X total magnification this objective provides. As an added benefit, the low power objective is always safe to use as it cannot be lowered to the point of contacting and thus possible breaking a slide. This is also true of the scanning objective mentioned above. High Power Objective (40x): This objective (sometimes called the "high-dry" objective) is useful for observing fine detail such as the striations in skeletal muscle, the arrangement of Haversian systems in compact bone, types of nerve cells in the retina, etc. Oil Immersion Objective (100X): This longest objective is used for observing the detail of individual cells such as white blood cells, the cells involved in spermatogenesis, etc. The lens must be used with a specially formulated oil that creates a bridge between the tip of the objective and the cover slip. Since the refractive indices of air and this lens are different, the lens will not work without this special oil! To use the lens, first make sure the specimen is in focus under the high power (40X) objective. Next, move the high power objective out of position, place a small drop of oil on top of the cover slip above the specimen to be viewed and move the oil immersion lens into place. Use the fine adjustment knob to bring the specimen into focus. When you are finished, make sure to clean the lens and slide with lens paper. Also, it is extremely important that this oil does not contact any of the other objectives. If this should happen, clean the lenses immediately! By the end of this section, you will be able to: 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. 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 × to 100 ×. 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. 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: $m = m_o m_e$, 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. The overall magnification of a multiple-element system is the product of the individual magnifications of its elements. 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 $\frac{d_i}{d_o}$ 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 $d_o = 6.20$ mm, but the image distance d_i is not known. Isolating d_i , we have $d_i = \frac{f_o d_o}{d_o - f_o}$. Substituting known values gives $d_i = \frac{(6.00 \text{ mm})(6.20 \text{ mm})}{6.20 \text{ mm} - 6.00 \text{ mm}} = 30.0 \text{ mm}$. Now we must find the magnification of the eyepiece, which is given by $\frac{d_i}{d_o}$ 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 $d_i' = \frac{f_e d_o}{d_o - f_e} = \frac{(50.0 \text{ mm})(44.0 \text{ mm})}{44.0 \text{ mm} - 50.0 \text{ mm}} = -44.0 \text{ mm}$. Inverting gives $d_i' = 44.0 \text{ mm}$. The eyepiece's magnification is thus $\frac{d_i'}{d_o} = \frac{44.0 \text{ mm}}{44.0 \text{ mm}} = 1.00$. So the overall magnification is $m = m_o m_e = (30.0)(1.00) = 30.0$. 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× with a theoretical resolution of ~0.2 μm. 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 3a) and is given by $NA = n \sin \alpha$, where n is the refractive index of the medium between the lens and the specimen and $\alpha = \frac{\theta}{2}$. 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 NA objective gives more detail than a 0.10 NA objective. Figure 3. (a) The numerical aperture of a microscope objective lens refers to the light-gathering ability of the lens and is calculated using half the angle of acceptance. (b) Here, is half the acceptance angle for light rays from a specimen entering a camera lens, and 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 3b). If the acceptance angle is small the NA of the lens can also be used as given below. $f/\# \approx \frac{f}{D} \approx \frac{1}{2NA}$ 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 α_{max} . 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. 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. 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. 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) 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. Physics provides the science and tools needed to generate the sequence of time-lapse images of meiosis similar to the sequence sketched in Figure 8. Figure 8. The image shows a sequence of events that takes place during meiosis. (credit: PatriciaR Wikimedia Commons; National Center for Biotechnology Information) 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 $m = m_o m_e$, 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 $NA = n \sin \alpha$ 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 through a condenser. The $f/\#$ describes the light gathering ability of a lens. It is given by $f/\# \approx \frac{f}{D} \approx \frac{1}{2NA}$. Conceptual Questions 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? The image produced by the microscope in Figure 2 cannot be projected. Could extra lenses or mirrors project it? Explain. 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.) What advantages do oil immersion objectives offer? How does the NA of a microscope compare with the NA of an optical fiber? Problems & Exercises 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? (c) What magnification is produced by a 0.150 cm focal length microscope objective that is 0.155 cm from the object being viewed? (d) What is the overall magnification if an 8× eyepiece (one that produces a magnification of 8.00) is used? (e) 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? (f) Where should the 5.00 cm focal length eyepiece be placed to produce a further fourfold (4.00) magnification? You switch from a 1.40 NA 60× oil immersion objective to a 1.40 NA 60× 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? 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.) You are using a standard microscope with a 0.10 NA 4× objective and switch to a 0.65 NA 40× 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.) 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? 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 $NA = n \sin \alpha$, where n is the refractive index of the medium between the lens and the specimen and $\alpha = \frac{\theta}{2}$. 1. (a) 4.00; (b) 1600 3. (a) 0.501 cm; (b) Eyepiece should be 204 cm behind the objective lens. 5. (a) +18.3 cm (on the eyepiece side of the objective lens); (b) −60.0; (c) −11.3 cm (on the objective side of the eyepiece); (d) +6.67; (e) −400

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