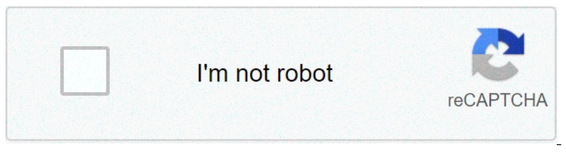

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The compound light microscope can be used to observe

The typical compound light microscope (Fig.1) is capable of increasing our ability to see detail by 1000 times so that objects as small as 0.1 micrometer (um) or 100 nanometers (nm) can be seen. Electron microscopes extend this range further allowing us to see objects as small as 0.5 nm in diameter or roughly 1/200,000th the size we can see with a naked eye. Needless to say, development and use of microscopes has vastly improved our understanding of cells and their structure and function. Figure 1. Binocular compound microscope. B. Magnification, Resolution, and Working Distance Magnification is simply a function of making an object appear bigger, such as when we use a hand lens to enlarge printed word. Merely magnifying an object without a simultaneous increase in the amount of detail seen will not provide the viewer with a good image. The ability of a microscope (or eye) to see detail is a function of its resolving power. Resolving power is defined as the minimum distance between two objects at which the objects can just be distinguished as separate and is a function of the wavelength of light used and the quality of the optics. In general, the shorter the wavelength of the light source, the higher the resolution of the microscope. Working distance is the distance between the objective lens and the specimen. At low magnification the working distance is relatively long. As you increase the magnification the working distance decreases dramatically. Oil immersion lenses pactically touch the specimen. Be aware of this change in working distance with increasing magnification so as to prevent damage to your specimens. Top of page C. Parts of the Monocular Compound Light Microscope: Please take time to familiarize yourself with your microscope and its proper use. The controls of the two makes of microscopes we use in our courses are shown below (Fig. 2). Figure 2. Controls on the Leica and Olympus binocular compound microscopes. 1. Ocular lens or eyepiece: ours are 10x magnification. The scopes we will use are monocular (one eyepiece only.) 2. Body tube: contains mirrors and prisms which direct the image to the ocular lens. 3. Nosepiece: holds the objective lenses, rotates, note the positive stops for each lens. 4. Objective lenses: usually 3-4 on our scopes, 4x, 10x, 43x, 100x oil immersion (red banding). Total magnification = ocular power x objective power. 5. Stage: platform on which slides are mounted for viewing; some scopes have mechanical stages. Learn how to clip the slide in position properly. 6. Diaphragm: the diaphragm controls the amount of light which passes to the specimen and can drastically affect the focus of the image. LEARN TO USE THE DIAPHRAGM AS QUICKLY AS POSSIBLE. MOST PROBLEMS YOU WILL HAVE FOCUSING WILL BE DUE TO INCORRECT ADJUSTMENT OF LIGHT. We have two types: iris diaphragm: Look for a lever just under the stage near the front. dial type: Just below the stage is a rotating dial having different size apertures (holes); this type is useful for creating a pseudo dark field effect. 7. Focusing knobs: Located on side of microscope; outermost is the fine focus and innermost is the coarse focus. 8. Light source: our scopes have built in light sources. The pushbutton switch is located (most often) behind the light lens on the base. Top of page D. Care and Handling of the Compound Microscope There are only a few ABSOLUTE rules to observe in caring for the microscopes you will use. Taken care of, these instruments will last many decades and continue to work well. Please report any malfunctions immediately to your instructor. 1. ALWAYS use two hands to carry the scope - one on the arm and one under the base - NO EXCEPTIONS! NEVER carry the scope upside down, for the ocular can and will fall out. 2. Use lens paper to clean all lenses before each lab session and after using the oil immersion lens. DO NOT EVER, NOT NOW, NOT EVER, USE ANYTHING BUT LENS PAPER TO CLEAN THE LENSES. Other papers are too impure and will scratch the optical coating on the lenses. Also, do not use any liquids when cleaning the lenses - LENS PAPER ONLY! 3. Always use the proper focusing technique to avoid ramming the objective lens into a slide - this can break the objective lens and/or ruin an expensive slide. 4. Always turn off the light when not using the scope. 5. Always carefully place the wire out of harm's way. Wires looped in the leg spaces invite a major microscope disaster. Try sliding the wire down through the drawer handles bside your bench space. 6. Always replace the cover on the microscope when you put it away Top of page E. Focusing Procedure: Monocular Compound Microscopes 1. Turn on the light source. 2. Switch to the 10x objective lens. 3. Back off on the coarse focus to raise the nose piece. 4. Place the specimen slide on the stage and secure in the proper position. Look at the slide and place it so the specimen is over the light aperture in the stage. 5. Lower objective lens to lower limit (close to slide). Raise the lens using the coarse focus knob until you see the image come into focus and then go out again, then focus back until you find center focus. Adjust fine focus similarly. 6. Center the image and adjust the light using the diaphragm. 7. Recenter and adjust focus, first coarse, then fine focus as in step 5. 8. Readjust diaphragm as needed. 9. Now switch objectives to the 43x if a higher magnification is needed. Readjust fine focus and light (diaphragm) as needed. Our scopes are parfocal which means that when you switch from low (100x) to high (430x) power, a focused image at low power will remain more or less in focus at the higher power. Most likely you'll have to readjust the fine focus and diaphragm slightly. Top of page F. Oil Immersion Procedure On some of our monocular, and all of the binocular compound microscopes, we have 100x oil immersion lenses. These can be identified by a red band around the lens housing. At magnifications greater than about 500x light is refracted too much as it passes through air to yield good resolving power. Thus, optics for these higher magnifications are made to use with a high grade mineral oil as the medium for transmitting light. It is imperative that you use only immersion oil and that you clean the lens thoroughly with lens paper after each use. 1. Locate the region of interest on your slide and center it at 430x. 2. Raise the objective lens to its limit (i.e., maximize the distance between stage and objectives) and swing the lens out of the way about half way to the next position. 3. Carefully place a small drop of immersion oil directly on the slide over the center of the region of interest. 4. Rotate the oil immersion objective into position and, carefully, while looking from the side, lower it using the coarse focus knob until the lens just makes contact with the oil drop. You will see the drop leap up into a column as the contact is made. 5. Lower the lens a smidgen more and then, using the fine focus and looking through the ocular lens, focus on the specimen. 6. When done, clean lens with lens paper until no more oil comes off and clean slide if it is to be saved. Top of page G. Determining Field-of-View Diameter You may wish to estimate the size of the specimens (e.g., cells) you will see in lab. The best way to do this is with an ocular micrometer, a precision ocular lens insert that has a ruler etched into glass. The monocular scopes we use in the introductory courses are not so equipped, so we will use an alternative method based upon knowing the field-of-view diameter for your particular microscope. To do this, you must determine: the approximate diameter of your low magnification field-of-view for your particular microscope. the total magnification for each of your other objective lenses. Knowing this for each objective lens, you can compare the size of the specimen against the known field diameter and make a reasonable esimate of size. This technique works for any microscope. 1. Obtain a slide scale and position it on your scope. A transparent metric ruler will work as well. 2. Bring it into focus using the 10x objective (100x total). The scale bars are increments of 1mm as shown in the figure below. Thus, a black bar = 0.5mm as does a space. 3. Move the slide such that the edge of an outside black bar is just tangent to the lighted field (see point "A" above). 4. Starting at that edge, estimate how many bars and spaces it takes to cross the field-of-view. You will probably have to estimate the last fraction of a space or bar. For most of our microscopes it is approximately 1.8 -2.0 mm wide. You must check this on any microscope you use that does not have an ocular micrometer. 5. Record your scope's ID number and field diameter at 100x in your lab notebook for future reference. 6. Next, calculate the field width at 430x total magnification using the following formula (we refer to the 100x mag as "low power" and 430x as "high power"): (low power mag/ high power mag) x low power field diameter (in mm) For example, suppose you determine that the 100x field diameter is 1.8 mm, at 430x, the field diameter would be: (100 / 430) x 1.8 mm = 0.418 mm = 418 um (micrometers) Note that the field diameter at high power is proportional to the ratio of the low to high power objectives. That is, as you increase magnification, the actual field of view becomes proportionally smaller. Top of page H. Binocular Compound Light Microscopes Parts of the light Microscope 1. Ocular lens or eyepiece: ours are 10x magnification. The scopes we will use are binocular (two eyepieces). 2. Body tube: contains mirrors and prisms which direct the image to the ocular lenses. 3. Nosepiece: holds the objective lenses, rotates 4. Objective lenses: usually 3-4 on our scopes, 4x, 10x, 43x, 100x oil immersion (red banding). Total magnification = ocular power x objective power. Most of our binocs have fixed position lenses--the stage moves up and down rather then the lens. 5. Stage: Movable platform on which slides are mounted for viewing; all of our scopes have mechanical stages with X,Y vernier scales. Focus knobs move the stage up and down. 6. Condensor: A substage lens which focus the light on the specimen. Our binocs have condensors that move up and down to focus the light beam. 7. Iris Diaphragm: the diaphragm is located just below the stage and controls the amount of light which passes to the specimen and can drastically affect the focus of the image. 8. Focusing knobs: outermost is the fine focus and innermost is the coarse focus. On the binocs these knobs control up/down movement of the stage. 9. Light source: our scopes have built in light sources. The rheostat ON/OFF switch is located either on the scope or on the external power supply and is used to regulate light intensity. Top of page I. Focusing Procedure: Binocular Compound Microscopes 1. Turn on the light source. Binoc scopes have either a built in unit or an external power supply. 2. Switch to the 10x objective lens. 3. Adjust the coarse focus to raise the nose piece (or lower the stage). 4. Clip the specimen slide on the stage in the proper position. 5. Look at the ocular lenses of your scope. One lens is fixed and the other has a focusing ring (like a pair of binoculars). Bring the lens as close to the slide as possible, then, looking only through the fixed ocular lens, back off until the specimen just comes into focus. Adjust fine focus similarly for the fixed lens. 6. Now, looking only through the adjustable ocular, adjust its focus using the focus ring around the lens. Look with both eyes (adjust for interpupillary distance to see a single round lighted field) and make any minor adjustments to focus. 7. Center the image and adjust the light using the condensor lens, iris diaphragm and light source rheostat. 8. Recenter and adjust focus, first coarse, then fine focus as in 5. 9. Readjust diaphragm as needed. 10.Now switch objectives to a higher power. Readjust fine focus and light (diaphragm) as needed. Our scopes are parfocal which means that when you switch from low to high power, a focused image at low power will remain more or less in focus at the higher power. Most likely you'll have to readjust the fine focus and diaphragm slightly (increase light at higher powers. Microscope that uses visible light A modern optical microscope with a mercury bulb for fluorescence microscopy. The microscope has a digital camera which is connected to a computer. The optical microscope, also referred to as a light microscope, is a type of microscope that commonly uses visible light and a system of lenses to generate magnified images of small objects. Optical microscopes are the oldest design of microscope and were possibly invented in their present compound form in the 17th century. Basic optical microscopes can be very simple, although many complex designs aim to improve resolution and sample contrast. The object is placed on a stage and may be directly viewed through one or two eyepieces on the microscope. In high-power microscopes, both eyepieces typically show the same image, but with a stereo microscope, slightly different images are used to create a 3-D effect. A camera is typically used to capture the image (micrograph). The sample can be lit in a variety of ways. Transparent objects can be lit from below and solid objects can be lit with light coming through (bright field) or around (dark field) the objective lens. Polarised light may be used to determine crystal orientation of metallic objects. Phase-contrast imaging can be used to increase image contrast by highlighting small details of differing refractive index. A range of objective lenses with different magnification are usually provided mounted on a turret, allowing them to be rotated into place and providing an ability to zoom-in. The maximum magnification power of optical microscopes is typically limited to around 1000x because of the limited resolving power of visible light. The magnification of a compound optical microscope is the product of the magnification of the eyepiece (say 10x) and the objective lens (say 100x), to give a total magnification of 1,000×. Modified environments such as the use of oil or ultraviolet light can increase the magnification. Alternatives to optical microscopy which do not use visible light include scanning electron microscopy and transmission electron microscopy and scanning probe microscopy and as a result, can achieve much greater magnifications. Types Diagram of a simple microscope There are two basic types of optical microscopes: simple microscopes and compound microscopes. A simple microscope uses the optical power of single lens or group of lenses for magnification. A compound microscope uses a system of lenses (one set enlarging the image produced by another) to achieve much higher magnification of an object. The vast majority of modern research microscopes are compound microscopes while some cheaper commercial digital microscopes are simple single lens microscopes. Compound microscopes can be further divided into a variety of other types of microscopes which differ in their optical configurations, cost, and intended purposes. Simple microscope A simple microscope uses a lens or set of lenses to enlarge an object through angular magnification alone, giving the viewer an erect enlarged virtual image.[1][2] The use of a single convex lens or groups of lenses are found in simple magnification devices such as the magnifying glass, loupes, and eyepieces for telescopes and microscopes. Compound microscope Diagram of a compound microscope A compound microscope uses a lens close to the object being viewed to collect light (called the objective lens) which focuses a real image of the object inside the microscope (image 1). That image is then magnified by a second lens or group of lenses (called the eyepiece) that gives the viewer an enlarged inverted virtual image of the object (image 2).[3] The use of a compound objective/eyepiece combination allows for much higher magnification. Common compound microscopes often feature exchangeable objective lenses, allowing the user to quickly adjust the magnification.[3] A compound microscope also enables more advanced illumination setups, such as phase contrast. Other microscope variants There are many variants of the compound optical microscope design for specialized purposes. Some of these are physical design differences allowing specialization for certain purposes: Stereo microscope, a low-powered microscope which provides a stereoscopic view of the sample, commonly used for dissection. Comparison microscope, which has two separate light paths allowing direct comparison of two samples via one image in each eye. Inverted microscope, for studying samples from below; useful for cell cultures in liquid, or for metallography. Fiber optic connector inspection microscope, designed for connector end-face inspection Traveling microscope, for studying samples of high optical resolution. Other microscope variants are designed for different illumination techniques: Petrographic microscope, whose design usually includes a polarizing filter, rotating stage and gypsum plate to facilitate the study of minerals or other crystalline materials whose optical properties can vary with orientation. Polarizing microscope, similar to the petrographic microscope. Phase-contrast microscope, which applies the phase contrast illumination method. Epifluorescence microscope, designed for analysis of samples which include fluorophores. Confocal microscope, a widely used variant of epifluorescent illumination which uses a scanning laser to illuminate a sample for fluorescence. Two-photon microscope, used to image fluorescence deeper in scattering media and reduce photobleaching, especially in living samples. Student microscope – an often low-power microscope with simplified controls and sometimes low quality optics designed for school use or as a starter instrument for children.[4] Ultramicroscope, an adapted light microscope that uses light scattering to allow viewing of tiny particles whose diameter is below or near the wavelength of visible light (around 500 nanometers); mostly obsolete since the advent of electron microscopes Tip-enhanced Raman microscope, is a variant of optical microscope based on tip-enhanced Raman spectroscopy, without traditional wavelength-based resolution limits.[5][6] This microscope primarily realized on the scanning-probe microscope platforms using all optical tools. Digital microscope A miniature USB microscope. Main article: Digital microscope A digital microscope is a microscope equipped with a digital camera allowing observation of a sample via a computer. Microscopes can also be partly or wholly computer-controlled with various levels of automation. Digital microscopy allows greater analysis of a microscope image, for example, measurements of distances and areas and quantitation of a fluorescent or histological stain. Low-powered digital microscopes, USB microscopes, are also commercially available. These are essentially webcams with a high-powered macro lens and generally do not use transillumination. The camera attached directly to the USB port of a computer so that the images are shown directly on the monitor. They offer modest magnifications (up to about 200×) without the need to use eyepieces, and at very low cost. High power illumination is usually provided by an LED source or sources adjacent to the camera lens. Digital microscopy with very low light levels to avoid damage to vulnerable biological samples is available using sensitive photon-counting digital cameras. It has been demonstrated that a light source providing pairs of entangled photons may minimize the risk of damage to the most light-sensitive samples. In this application of ghost imaging to photon-sparse microscopy, the sample is illuminated with infrared photons, each of which is spatially correlated with an entangled partner in the visible band for efficient imaging by a photon-counting camera.[7] History See also: History of optics and Timeline of microscope technology Invention The earliest microscopes were single lens magnifying glasses with limited magnification which date at least as far back as the widespread use of lenses in eyeglasses in the 13th century.[8] Compound microscopes first appeared in Europe around 1620[9][10] including one demonstrated by Cornelis Drebbel in London (around 1621) and one exhibited in Rome in 1624.[11][12] The actual inventor of the compound microscope is unknown although many claims have been made over the years. These include a claim 35[13] years after they appeared by Dutch spectacle-maker Johannes Zachariassen that his father, Zacharias Janssen, invented the compound microscope and/or the telescope as early as 1590. Johannes' (some claim dubious)[14][15][16] testimony pushes the invention date so far back that Zacharias would have been a child at the time, leading to speculation that, for Johannes' claim to be true, the compound microscope would have to have been invented by Johannes' grandfather, Hans Martens.[17] Another claim is that Janssen's competitor, Hans Lippershey (who applied for the first telescope patent in 1608) also invented the compound microscope.[18] Other historians point to the Dutch innovator Cornelis Drebbel with his 1621 compound microscope.[11][12] Galileo Galilei is also sometimes cited as a compound microscope inventor. After 1610, he found that he could close focus his telescope to view small objects, such as flies, close up[19] and/or could look through the wrong end in reverse to magnify small objects.[20] The only drawback was that his 2 foot long telescope had to be extended out to 6 feet to view objects that close.[21] After seeing the compound microscope built by Drebbel exhibited in Rome in 1624, Galileo built his own improved version.[11][12] In 1625, Giovanni Faber coined the name microscope for the compound microscope Galileo submitted to the Accademia dei Lincei in 1624 [22] (Galileo had called it the "occhiolino" or "little eye"). Faber coined the name from the Greek words μικρόν (micron) meaning "small", and σκοπεῖν (skopein) meaning "to look at", a name meant to be analogous with "telescope", another word coined by the Linceans.[23] Christiaan Huygens, another Dutchman, developed a simple 2-lens ocular system in the late 17th century that was achromatically corrected, and therefore a huge step forward in microscope development. The Huygens ocular is still being produced to this day, but suffers from a small field size, and other minor disadvantages. Popularization The oldest published image known to have been made with a microscope: bees by Francesco Stelluti, 1630[24] Antonie van Leeuwenhoek (1632–1724) is credited with bringing the microscope to the attention of biologists, even though simple magnifying lenses were already being produced in the 16th century. Van Leeuwenhoek's home-made microscopes were simple microscopes, with a single very small, yet strong lens. They were awkward in use, but enabled van Leeuwenhoek to see detailed images. It took about 150 years of optical development before the compound microscope was able to provide the same quality image as van Leeuwenhoek's simple microscopes, due to difficulties in configuring multiple lenses. In the 1850s, John Leonard Riddell, Professor of Chemistry at Tulane University, invented the first practical binocular microscope while carrying out one of the earliest and most extensive American microscopic investigations of cholera.[25][26] Lighting techniques While basic microscope technology and optics have been available for over 400 years it is much more recently that techniques in sample illumination were developed to generate the high quality images seen today. In August 1893, August Köhler developed Köhler illumination. This method of sample illumination gives rise to extremely even lighting and overcomes many limitations of older techniques of sample illumination. Before development of Köhler illumination the image of the light source, for example a lightbulb filament, was always visible in the image of the sample. The Nobel Prize in physics was awarded to Dutch physicist Frits Zernike in 1953 for his development of phase contrast illumination which allows imaging of transparent samples. By using interference rather than absorption of light, extremely transparent samples, such as live mammalian cells, can be imaged without having to use staining techniques. Just two years later, in 1955, Georges Nomarski published the theory for differential interference contrast microscopy, another interference-based imaging technique. Fluorescence microscopy Modern biological microscopy depends heavily on the development of fluorescent probes for specific structures within a cell. In contrast to normal transilluminated light microscopy, in fluorescence microscopy the sample is illuminated through the objective lens with a narrow set of wavelengths of light. This light interacts with fluorophores in the sample which then emit light of a longer wavelength. It is this emitted light which makes up the image. Since the mid-20th century chemical fluorescent stains, such as DAPI which binds to DNA, have been used to label specific structures within the cell. More recent developments include immunofluorescence, which uses fluorescently labelled antibodies to recognise specific proteins within a sample, and fluorescent proteins like GFP which a live cell can express making it fluorescent. Components Basic optical transmission microscope elements (1990s) All modern optical microscopes designed for viewing samples by transmitted light share the same basic components of the light path. In addition, the vast majority of microscopes have the same 'structural' components[27] (numbered below according to the image on the right): Eyepiece (ocular lens) (1) Objective turret, revolver, or revolving nose piece (to hold multiple objective lenses) (2) Objective lenses (3) Focus knobs (to move the stage) Coarse adjustment (4) Fine adjustment (5) Stage (to hold the specimen) (6) Light source (a light or a mirror) (7) Diaphragm and condenser (8) Mechanical stage (9) Eyepiece (ocular lens) Main article: Eyepiece The eyepiece, or ocular lens, is a cylinder containing two or more lenses; its function is to bring the image into focus for the eye. The eyepiece is inserted into the top end of the body tube. Eyepieces are interchangeable and many different eyepieces can be inserted with different degrees of magnification. Typical magnification values for eyepieces include 5×, 10× (the most common), 15× and 20×. In some high performance microscopes, the optical configuration of the objective lens and eyepiece are matched to give the best possible optical performance. This occurs most commonly with apochromatic objectives. Objective turret (revolver or revolving nose piece) Objective turret, revolver, or revolving nose piece is the part that holds the set of objective lenses. It allows the user to switch between objective lenses. Objective lens Main article: Objective (optics) At the lower end of a typical compound optical microscope, there are one or more objective lenses that collect light from the sample. The objective is usually in a cylinder housing containing a glass single or multi-element compound lens. Typically there will be around three objective lenses screwed into a circular nose piece which may be rotated to select the required objective lens. These arrangements are designed to be parfocal, which means that when one changes from one lens to another on a microscope, the sample stays in focus. Microscope objectives are characterized by two parameters, namely, magnification and numerical aperture. The former typically ranges from 5× to 100× while the latter ranges from 0.14 to 0.7, corresponding to focal lengths of about 40 to 2 mm, respectively. Objective lenses with higher magnifications normally have a higher numerical aperture and a shorter depth of field in the resulting image. Some high performance objective lenses may require matched eyepieces to deliver the best optical performance. Oil immersion objective Two Leica oil immersion microscope objective lenses: 100× (left) and 40× (right) Main article: Oil immersion Some microscopes make use of oil-immersion objectives or water-immersion objectives for greater resolution at high magnification. These are used with index-matching material such as immersion oil or water and a matched cover slip between the objective lens and the sample. The refractive index of the index-matching material is higher than air allowing the objective lens to have a larger numerical aperture (greater than 1) so that the light is transmitted from the specimen to the outer face of the objective lens with minimal refraction. Numerical apertures as high as 1.6 can be achieved.[28] The larger numerical aperture allows collection of more light making detailed observation of smaller details possible. An oil immersion lens usually has a magnification of 40 to 100×. Focus knobs Adjustment knobs move the stage up and down with separate adjustment for coarse and fine focusing. The same controls enable the microscope to adjust to specimens of different thickness. In older designs of microscopes, the focus adjustment wheels move the microscope tube up or down relative to the stand and had a fixed stage. Frame The whole of the optical assembly is traditionally attached to a rigid arm, which in turn is attached to a robust U-shaped foot to provide the necessary rigidity. The arm angle may be adjustable to allow the viewing angle to be adjusted. The frame provides a mounting point for various microscope controls. Normally this will include controls for focusing, typically a large knurled wheel to adjust coarse focus, together with a smaller knurled wheel to control fine focus. Other features may be lamp controls and/or controls for adjusting the condenser. Stage The stage is a platform below the objective lens which supports the specimen being viewed. In the center of the stage is a hole through which light passes to illuminate the specimen. The stage usually has arms to hold slides (rectangular glass plates with typical dimensions of 25×75 mm, on which the specimen is mounted). At magnifications higher than 100× moving a slide by hand is not practical. A mechanical stage, typical of medium and higher priced microscopes, allows tiny movements of the slide via control knobs that reposition the sample/slide as desired. If a microscope did not originally have a mechanical stage it may be possible to add one. All stages move up and down for focus. With a mechanical stage slides move on two horizontal axes for positioning the specimen to examine specimen details. Focusing starts at lower magnification in order to center the specimen by the user on the stage. Moving to a higher magnification requires the stage to be moved higher vertically for re-focus at the higher magnification and may also require slight horizontal specimen position adjustment. Horizontal specimen position adjustments are the reason for having a mechanical stage. Due to the difficulty in preparing specimens and mounting them on slides, for children it's best to begin with prepared slides that are centered and focus easily regardless of the focus level used. Light source Many sources of light can be used. At its simplest, daylight is directed via a mirror. Most microscopes, however, have their own adjustable and controllable light source – often a halogen lamp, although illumination using LEDs and lasers are becoming a more common provision. Köhler illumination is often provided on more expensive instruments. Condenser The condenser is a lens designed to focus light from the illumination source onto the sample. The condenser may also include other features, such as a diaphragm and/or filters, to manage the quality and intensity of the illumination. For illumination techniques like dark field, phase contrast and differential interference contrast microscopy additional optical components must be precisely aligned in the light path. Magnification The actual power or magnification of a compound optical microscope is the product of the powers of the ocular (eyepiece) and the objective lens. The maximum normal magnifications of the ocular and objective are 10× and 100× respectively, giving a final magnification of 1,000×. Magnification and micrographs When using a camera to capture a micrograph the effective magnification of the image must take into account the size of the image. This is independent of whether it is on a print from a film negative or displayed digitally on a computer screen. In the case of photographic film cameras the calculation is simple; the final magnification is the product of: the objective lens magnification, the camera optics magnification and the enlargement factor of the film print relative to the negative. A typical value of the enlargement factor is around 5× (for the case of 35 mm film and a 15 × 10 cm (6 × 4 inch) print). In the case of digital cameras the size of the pixels in the CMOS or CCD detector and the size of the pixels on the screen have to be known. The enlargement factor from the detector to the pixels on screen can then be calculated. As with a film camera the final magnification is the product of: the objective lens magnification, the camera optics magnification and the enlargement factor. Operation U.S. CBP Office of Field Operations agent checking the authenticity of a travel document at an international airport using a stereo microscope Illumination techniques Main article: Microscopy Many techniques are available which modify the light path to generate an improved contrast image from a sample. Major techniques for generating increased contrast from the sample include cross-polarized light, dark field, phase contrast and differential interference contrast illumination. A recent technique (Sarfus) combines cross-polarized light and specific contrast-enhanced slides for the visualization of nanometric samples. Four examples of transilumination techniques used to generate contrast in a sample of tissue paper. 1.559 μm/pixel. Bright field illumination, sample contrast comes from absorbance of light in the sample. Cross-polarized light illumination, sample contrast comes from rotation of polarized light through the sample. Dark field illumination, sample contrast comes from light scattered by the sample. Phase contrast illumination, sample contrast comes from interference of different path lengths of light through the sample. Other techniques Modern microscopes allow more than just observation of transmitted light image of a sample; there are many techniques which can be used to extract other kinds of data. Most of these require additional equipment in addition to a basic compound microscope. Reflected light, or incident, illumination (for analysis of surface structures) Fluorescence microscopy, both: Epifluorescence microscopy Confocal microscopy Microspectroscopy (where a UV-visible spectrophotometer is integrated with an optical microscope) Ultraviolet microscopy Near-Infrared microscopy Multiple transmission microscopy[29] for contrast enhancement and aberration reduction. Automation (for automatic scanning of a large sample or image capture) Applications A 40x magnification image of cells in a medical smear test taken through an optical microscope using a wet mount technique, placing the specimen on a glass slide and mixing with a salt solution Optical microscopy is used extensively in microelectronics, nanophysics, biotechnology, pharmaceutic research, mineralogy and microbiology.[30] Optical microscopy is used for medical diagnosis, the field being termed histopathology when dealing with tissues, or in smear tests on free cells or tissue fragments. In industrial use, binocular microscopes are common. Aside from applications needing true depth perception, the use of dual eyepieces reduces eye strain associated with long workdays at a microscopy station. In certain applications, long-working-distance or long-focus microscopes[31] are beneficial. An item may need to be examined behind a window, or industrial subjects may be a hazard to the objective. Such optics resemble telescopes with close-focus capabilities.[32][33] Measuring microscopes are used for precision measurement. There are two basic types. One has a reticle graduated to allow measuring distances in the focal plane.[34] The other (and older) type has simple crosshairs and a micrometer mechanism for moving the subject relative to the microscope.[35] Very small, portable microscopes have found some usage in places where a laboratory microscope would be a burden.[36] Limitations The diffraction limit set in stone on a monument for Ernst Abbe. At very high magnifications with transmitted light, point objects are seen as fuzzy discs surrounded by diffraction rings. These are called Airy disks. The resolving power of a microscope is taken as the ability to distinguish between two closely spaced Airy disks (or, in other words the ability of the microscope to reveal adjacent structural detail as distinct and separate). It is these impacts of diffraction that limit the ability to resolve fine details. The extent and magnitude of the diffraction patterns are affected by both the wavelength of light (λ), the refractive materials used to manufacture the objective lens and the numerical aperture (NA) of the objective lens. There is therefore a finite limit beyond which it is impossible to resolve separate points in the objective field, known as the diffraction limit. Assuming that optical aberrations in the whole optical set-up are negligible, the resolution d, can be stated as: d = λ 2 N A {\displaystyle d={\frac {\lambda }{2NA}}} Usually a wavelength of 550 nm is assumed, which corresponds to green light. With air as the external medium, the highest practical NA is 0.95, and with oil, up to 1.5. In practice the lowest value of d obtainable with conventional lenses is about 200 nm. A new type of lens using multiple scattering of light allowed to improve the resolution to below 100 nm.[37] Surpassing the resolution limit Multiple techniques are available for reaching resolutions higher than the transmitted light limit described above. Holographic techniques, as described by Courjon and Bulabois in 1979, are also capable of breaking this resolution limit, although resolution was restricted in their experimental analysis.[38] Using fluorescent samples more techniques are available. Examples include Vertico SMI, near field scanning optical microscopy which uses evanescent waves, and stimulated emission depletion. In 2005, a microscope capable of detecting a single molecule was described as a teaching tool.[39] Despite significant progress in the last decade, techniques for surpassing the diffraction limit remain limited and specialized. While most techniques focus on increases in lateral resolution there are also some techniques which aim to allow analysis of extremely thin samples. For example, sarfus methods place the thin sample on a contrast-enhancing surface and thereby allows to directly visualize films as thin as 0.3 nanometers. On 8 October 2014, the Nobel Prize in Chemistry was awarded to Eric Betzig, William Moerner and Stefan Hell for the development of super-resolved fluorescence microscopy.[40][41] Structured illumination SMI SMI (spatially modulated illumination microscopy) is a light optical process of the so-called point spread function (PSF) engineering. These are processes which modify the PSF of a microscope in a suitable manner to either increase the optical resolution, to maximize the precision of distance measurements of fluorescent objects that are small relative to the wavelength of the illuminating light, or to extract other structural parameters in the nanometer range.[42][43] Localization microscopy SPDMphymod 3D dual color super resolution microscopy with Her2 and Her3 in breast cells, standard dyes: Alexa 488, Alexa 568 LIMON SPDM (spectral precision distance microscopy), the basic localization microscopy technology is a light optical process of fluorescence microscopy which allows position, distance and angle measurements on "optically isolated" particles (e.g. molecules) well below the theoretical limit of resolution for light microscopy. "Optically isolated" means that at a given point in time, only a single particle/molecule within a region of a size determined by conventional optical resolution (typically approx. 200–250 nm diameter) is being registered. This is possible when molecules within such a region all carry different spectral markers (e.g. different colors or other usable differences in the light emission of different particles).[44][45][46][47] Many standard fluorescent dyes like GFP, Alexa dyes, Atto dyes, Cy2/Cy3 and fluorescein molecules can be used for localization microscopy, provided certain photo-physical conditions are present. Using this so-called SPDMphymod (physically modifiable fluorophores) technology a single laser wavelength of suitable intensity is sufficient for nanoimaging.[48] 3D super resolution microscopy 3D super resolution microscopy with standard fluorescent dyes can be achieved by combination of localization microscopy for standard fluorescent dyes SPDMphymod and structured illumination SMI.[49] STED Stimulated emission depletion (STED) microscopy image of actin filaments within a cell. Stimulated emission depletion is a simple example of how higher resolution surpassing the diffraction limit is possible, but it has major limitations. STED is a fluorescence microscopy technique which uses a combination of light pulses to induce fluorescence in a small sub-population of fluorescent molecules in a sample. Each molecule produces a diffraction-limited spot of light in the image, and the centre of each of these spots corresponds to the location of the molecule. As the number of fluorescing molecules is low the spots of light are unlikely to overlap and therefore can be placed accurately. This process is then repeated many times to generate the image. Stefan Hell of the Max Planck Institute for Biophysical Chemistry was awarded the 10th German Future Prize in 2006 and Nobel Prize for Chemistry in 2014 for his development of the STED microscope and associated methodologies.[50] Alternatives In order to overcome the limitations set by the diffraction limit of visible light other microscopes have been designed which use other waves. Atomic force microscope (AFM) Scanning electron microscope (SEM) Scanning ion-conductance microscopy (SICM) Scanning tunneling microscope (STM) Transmission electron microscopy (TEM) Ultraviolet microscope X-ray microscope It is important to note that higher frequency waves have limited interaction with matter, for example soft tissues are relatively transparent to X-rays resulting in distinct sources of contrast and different target applications. The use of electrons and X-rays in place of light allows much higher resolution – the wavelength of the radiation is shorter so the diffraction limit is lower. To make the short-wavelength probe non-destructive, the atomic beam imaging system (atomic nanoscope) has been proposed and widely discussed in the literature, but it is not yet competitive with conventional imaging systems. STM and AFM are scanning probe techniques using a small probe which is scanned over the sample surface. Resolution in these cases is limited by the size of the probe; micromachining techniques can produce probes with tip radii of 5–10 nm. Additionally, methods such as electron or X-ray microscopy use a vacuum or partial vacuum, which limits their use for live and biological samples (with the exception of an environmental scanning electron microscope). The specimen chambers needed for all such instruments also limits sample size, and sample manipulation is more difficult. Color cannot be seen in images made by these methods, so some information is lost. They are however, essential when investigating molecular or atomic effects, such as age hardening in aluminium alloys, or the microstructure of polymers. See also Digital microscope Köhler illumination Microscope slide References

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External links Antique Microscopes.com A collection of early microscopes Historical microscopes, an illustrated collection with more than 3000 photos of scientific microscopes by European makers (in German) The Golub Collection, A collection of 17th through 19th century microscopes, including extensive descriptions Molecular Expressions, concepts in optical microscopy Online tutorial of practical optical microscopy OpenWetWare Cell Centered Database Retrieved from " 2A 4Pi microscope is a laser scanning fluorescence microscope with an improved axial resolution. With it the typical range of the axial resolution of 500–700 nm can be improved to 100–150 nm, which corresponds to an almost spherical focal spot with 5–7 times less volume than that of standard confocal microscopy.[1] Working principle The improvement in resolution is achieved by using two opposing objective lenses, which both are focused to the same geometrical location. Also the difference in optical path length through each of the two objective lenses is carefully aligned to be minimal. By this method, molecules residing in the common focal area of both objectives can be illuminated coherently from both sides and the reflected or emitted light can also be collected coherently, i.e. coherent superposition of emitted light on the detector is possible. The solid angle Ω {\displaystyle \Omega } that is used for illumination and detection is increased and approaches its maximum. In this case the sample is illuminated and detected from all sides simultaneously. Optical Scheme of 4Pi Microscope The operation mode of a 4Pi microscope is shown in the figure. The laser light is divided by a beam splitter and directed by mirrors towards the two opposing objective lenses. At the common focal point superposition of both focused light beams occurs. Excited molecules at this position emit fluorescence light, which is collected by both objective lenses, combined by the same beam splitter and deflected by a dichroic mirror onto a detector. There superposition of both emitted light pathways can take place again. In the ideal case each objective lens can collect light from a solid angle of Ω = 2 π {\displaystyle \Omega =2\pi } . With two objective lenses one can collect from every direction (solid angle Ω = 4 π {\displaystyle \Omega =4\pi }). The name of this type of microscopy is derived from the maximal possible solid angle for excitation and detection. Practically, one can achieve only aperture angles of about 140° for an objective lens, which corresponds to Ω ≈ 1.3 π {\displaystyle \Omega \approx 1.3\pi } . The microscope can be operated in three different ways: In a 4Pi microscope of type A, the coherent superposition of excitation light is used to generate the increased resolution. The emission light is either detected from one side only or in an incoherent superposition from both sides. In a 4Pi microscope of type B, only the emission light is interfering. When operated in the type C mode, both excitation and emission light are allowed to interfere, leading to the highest possible resolution increase (~7-fold along the optical axis as compared to confocal microscopy). In a real 4Pi microscope light cannot be applied or collected from all directions equally, leading to so-called side lobes in the point spread function. Typically (but not always) two-photon excitation microscopy is used in a 4Pi microscope in combination with an emission pinhole to lower these side lobes to a tolerable level. History In 1971, Christoph Cremer and Thomas Cremer proposed the creation of a perfect hologram, i.e. one that carries the whole field information of the emission of a point source in all directions, a so-called 4 π {\displaystyle 4\pi } hologram.[2][3] The first description of a practicable system of 4Pi microscopy, i.e. the setup with two opposing, interfering lenses, was invented by Stefan Hell in 1991.[4] He demonstrated it experimentally in 1994.[5] In the following years, the number of applications for this microscope has grown. For example, parallel excitation and detection with 64 spots in the sample simultaneously combined with the improved spatial resolution resulted in the successful recording of the dynamics of mitochondria in yeast cells with a 4Pi microscope in 2002.[6] A commercial version was launched by microscope manufacturer Leica Microsystems in 2004[7] and later discontinued. Up to now, the best quality in a 4Pi microscope was reached in conjunction with super-resolution techniques like the stimulated emission depletion (STED) principle.[8] Using a 4Pi microscope with appropriate excitation and de-excitation beams, it was possible to create a uniformly 50 nm sized spot, which corresponds to a decreased focal volume compared to confocal microscopy by a factor of 150–200 in fixed cells. With the combination of 4Pi microscopy and RESOLFT microscopy with switchable proteins, it is now possible to take images of living cells at low light levels with isotropic resolutions below 40 nm.[9] See also Stimulated emission depletion microscope (STED) Multifocal plane microscopy (MUM) References

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