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Course-MLS 410

ASSIGNMENT

1.Discuss the physics of the light microscope diagrams and illustrations needed

2.Write notes on the biomedical equipment, Add notes on principle, brand, care and maintenance and cost. a) Centrifuge b) Automatic Tissue Processor c) Microtome

ANSWER

1.A light microscope works very much like a refracting telescope, but with some minor differences.

How a telescope works.

A telescope must gather large amounts of light from a dim, distant object; therefore, it needs a large **objective lens** to gather as much light as possible and bring it to a bright focus. Because the objective lens is large, it brings the image of the object to a focus at some distance away, which is why telescopes are much longer than microscopes. The eyepiece of the telescope then magnifies that image as it brings it to your eye. In contrast to a telescope, a microscope must gather light from a tiny area of a thin, well-illuminated specimen that is close-by. So the microscope does not need a large objective lens. Instead, the objective lens of a microscope is small and spherical, which means that it has a much shorter focal length on either side. It brings the image of the object into focus at a short distance within the microscope's tube. The image is then magnified by a second lens, called an **ocular lens** or **eyepiece**, as it is brought to your eye. The other major difference between a telescope and a microscope is that a microscope has a **light source** and a **condenser**. The condenser is a lens system that focuses the light from the source onto a tiny, bright spot of the specimen, which is the same area that the objective lens examines. Also unlike a telescope, which has a fixed objective lens and interchangeable eyepieces, microscopes typically have interchangeable objective lenses and fixed eyepieces. By changing the objective lenses (going from relatively flat, low-magnification objectives to rounder, high-magnification objectives), a microscope can bring increasingly smaller areas into view -- light gathering is not the primary task of a microscope's objective lens, as it is a telescope's.

**Principles of Microscopy**

***Brightfield microscopy***

The microscope that is available to you for general use in this laboratory is a sophisticated optical instrument that can provide you with high-resolution images of a variety of specimens. Image quality is based largely on your ability to use the microscope properly. Below you will find some basic information that you have probably heard before, but information that is rarely presented in a thorough way.

*- Resolution -*

The magnification of small things is a necessary facet of biological research, but the fine detail in cells and in subcellular components requires that any imaging system be capable of providing spatial information across small distances. Resolution is defined as the ability to distinguish two very small and closely-spaced objects as separate entities. Resolution is best when the distance separating the two tiny objects is small. Resolution is determined by certain physical parameters that include the wavelength of light, and the light-gathering power of the objective and condenser lenses. A simple mathematical equation defines the smallest distance (dmin) separating the two very small objects:

**dmin = 1.22 x wavelength / N.A. objective + N.A. condenser**

This is the theoretical resolving power of a light microscope. In practice, specimen quality usually limits dmin to something greater than its theoretical lower limit. N.A. (Numerical Aperture) is a mathematical calculation of the light-gathering capabilities of a lens. The N.A. of each objective lens is inscribed in the metal tube, and ranges from 0.25-1.4. The higher the N.A., the better the light-gathering properties of the lens, and the better the resolution. Higher N.A. values also mean shorter working distances (you have to get the lens closer to the object). N.A. values above 1.0 also indicate that the lens is used with some immersion fluid, such as immersion oil.From the equation above, you should be aware that the N.A. of the condenser is as important as the N.A. of the objective lens in determining resolution. It is for this reason that closure of the condenser diaphragm results in a loss of resolution. In practice, at full aperture and with good oil immersion lenses (N.A. 1.4 for both the condenser and the objective) it is possible to be able to resolve slightly better than 0.2 µm. From the equation above, it should also be clear that shorter wavelength light (bluer light) will provide you with better resolution (smaller dmin values). However, there are practical considerations in how short the wavelength can be. In the early 1950's, a UV microscope was designed, but required quartz objectives and a specialized imaging device. The quartz lenses provided slightly better resolution (dmin = 0.1 µm), but image quality suffered from an inability on the part of the manufacturers to correct for aberrations caused by the quartz. The human eye is best adapted for green light and our ability to see detail may be compromised somewhat with the use of blue or violet. Most manufacturers of microscopes correct their simplest lenses (achromats) for green light.

*- Magnification and Imaging -*

Most microscopes in current use are known as compound microscopes, where a magnified image of an object is produced by the objective lens, and this image is magnified by a second lens system (the ocular or eyepiece) for viewing. Thus, final magnification of the microscope is dependent on the magnifying power of the objective times the magnifying power of the ocular. Objective magnification powers range from 4X to 100X. Lower magnification is impractical on a compound microscope stand because of spatial constraints with image correction and illumination. Higher magnification is impractical because of limitations in light gathering ability and shortness of working distances required for very strong lenses. Ocular magnification ranges are typically 8X-12X though 10X oculars are most common. As a result, a standard microscope will provide you with a final magnification range of ~40X up to ~1000X.Each objective lens consists of six or more pieces of glass that combine to produce a clear image of an object. The six or more lenses in the objective lens are needed to provide corrections that produce image clarity. The interaction of light with the glass in a lens produce aberrations that result in a loss in image quality because light waves will be bent, or refracted, differently in different portions of a lens, and different colors of light will be refracted to different extents by the glass. Spatial aberrations (*e.g.*, spherical aberration) can be corrected by using lenses with different curvature on their surfaces, and chromatic (*i.e.*, color) aberrations can be minimized by using multiple kinds of glass in combination. These corrections increase the cost of the lens to the extent that an apochromatic objective lens exhibiting full color correction and extremely high N.A. can cost several thousand dollars. This objective lens is about the size of your thumb.The objective lenses in most microscopes are achromats, and best suited for imaging with green light. Green filters narrow the bandwidth of the light, and make achromat objectives reasonably effective for most routine uses. The achromat lenses are not suitable for critical high-resolution imaging with white light, because red and blue light do not focus in the same plane as green light. Chromatic aberrations will degrade resolution in color images obtained with achromatic objectives. Color photomicrography aimed at the highest level of resolution and image clarity should be performed with totally corrected apochromatic objective lenses. Fluorite lenses, offer intermediate levels of correction, better than achromats but not as good as apochromats. Fluorite lenses are well suited for fluorescence microscopy because of their high transmittance of shorter wavelength light. Higher levels of correction make objective lenses more expensive; the price range for apochromatic objectives goes from about $3,000 to over $10,000.The oculars in most microscopes are designed to work optimally with the objective lenses from the same manufacturer. Each manufacturer makes some of the color and spatial corrections in the objective and the remainder of the corrections in the ocular. Mixing brands will usually result in a degraded image. In addition, when you look into a microscope, the magnified and corrected image you see through the oculars is actually a virtual image (as opposed to a real image). The ocular, designed to provide a corrected virtual image when viewed by eye, is not suitable for the generation of photographic or video images through the microscope. For photography or video microscopy it is necessary to use a projection lens that generates a corrected real image. Many of the newer microscopes provide total image corrections in the objective lens, thus obviating many of the concerns aboout matching glass components from the same manufacturer. Nevertheless, it is a good practice not to mix parts from one manufacturer with those of another, because unintended image degradation can result.

*- Illumination -*

An essential factor in producing a good image with the light microscope is obtaining adequate levels of light in the specimen, or object plane. It is not only necessary to obtain bright light around the object, but for optimal imaging, the light should be uniform across the field of view. The best way to illuminate the specimen involves the use of yet another lens system, known as a condenser. The front element of the condenser is usually a large, flattened lens that sits directly beneath the specimen. Its placement on a movable rack provides you with the means to focus the light beam coming past the object and maximixe the intensity and control the uniformity of illumination. Two apertures in the illumination system allow you to regulate the diameter of the illumination beam by closing or opening iris diaphragms. One of these diaphragms, housed within the brightfield condenser and known as the condenser diaphragm, allows you to increase contrast, but at the cost of worsening resolution. The second of these diaphragms, known as the field aperture diaphragm, does not affect resolution as dramatically and is regularly adjusted for optimal illumination.

Optimal illumination of a specimen with all microscopes currently manufactured is achieved by using a variation of Kohler Illumination, where (for those of you are technophiles) the filament of the light source is in focus at the rear focal plane of the objective lens. Operationally, it is easy to obtain optimal illumination for brightfield (or phase contrast) by first placing any specimen on the stage and focusing on the object. Next, turn the ring for the field aperture diaphragm (the lowest aperture on the microscope) so that its edges obscure the periphery of the field of view. Next, raise or lower **the condenser** until the edges of the field aperture diaphragm are clearly focused. Do not refocus the objective on the specimen while you are adjusting the condenser. It may be necessary to center the field aperture diaphragm, using the condenser centering screws. When the microscope is properly illuminated, both the object and the edges of the field aperture diaphragm should be in the same plane of focus and the field iris diaphragm should be centered in the field of view.

***Phase Contrast Microscopy***

The human eye can perceive changes in light amplitude (intensity). Unstained biological specimens, such as living cells, are essentially transparent to our eyes, but they interact with light in a fairly uniform way, by retarding (slowing) the passage of a light beam by approximately 1/4 of a wavelength (https://science.umd.edu/CBMG/faculty/wolniak/lambda_bl.jpg). By slowing a light beam this much relative to another light beam that had passed though the surrounding medium, the biological specimen alters the phase of the beams. Intensity (amplitude) is additive and light rays that are 1/2https://science.umd.edu/CBMG/faculty/wolniak/lambda_bl.jpg out of phase are perceived as darkness. Zernicke realized that if he could retard the light passing through biological specimens without affecting the light passing through the surrounding medium, he could generate changes in amplitude within living cells. The phase contrast microscope was invented by Zernicke in the 1930's as a means to generate contrast in biological specimens, changing these invisible phase differences into visible amplitude differences.

Zernicke employed an optical trick to separate the light beams interacting with the specimen from those that do not encounter the specimen. To separate the beams of light from each other, he placed a transparent ring (known as an annulus) in an opaque disk and inserted this disk into the optical path of the microscope, within the condenser. He placed a complementary ring inside the objective lens. Nearly all of the light that passes through the sample but misses the specimen then passes through the objective lens through this ring. Most of the light that passes through the specimen is scattered and some of it enters the objective lens in such a way that it will not pass through the objective lens ring, but will pass this plane in some other location. He designed the glass plate holding the ring so that all light missing the ring would encounter an additional 1/4https://science.umd.edu/CBMG/faculty/wolniak/lambda_bl.jpg of retardation relative to the beams of light that had not interacted with the specimen, placing the light rays that had interacted with the specimen out of phase with rays that had not interacted with the specimen by 1/2https://science.umd.edu/CBMG/faculty/wolniak/lambda_bl.jpg. He found that a reduction in intensity of the light that had not passed through the specimen would create a grey background and increase contrast even more, with some parts of the specimen darker and other parts of the specimen brighter than the background.The operation of any microscope in the phase contrast mode requires that you first set up proper brightfield illumination, with a centered field iris diaphragm whose edges are in focus in the specimen plane. Next, rotate the condenser turret cylinder until the number on the condenser turret matches the number engraved on the objective lens. Under this condition, the condenser annulus is matched to the phase ring present in the objective. Next, remove one of the oculars and insert the Bertrand focusing telescope into the ocular hole. This lens enables you to see the rear focal plane of the objective lens, the plane where the ring resides. You will see a bright circle of light (the condenser annulus) and a dark ring (present within the objective). The dark ring is stationary, but the bright annulus is not. You may need to align the annulus with the ring so that the two are superimposed. On the back side of your condenser, you will find two adjustment screws that permit this alignment to be performed. When the ring and the annulus are aligned, place the ocular back into the microscope. The difference between phase contrast and brightfield for the observation of living cells is significant.

***Fluorescence Microscopy***

In certain classes of atoms and molecules, electrons absorb light, become energized, and then rapidly lose this energy in the form of heat and light emission. If the electron keeps its spin, the electron is said to enter a singlet state, and the kind of light that is emitted as the electron returns to ground state is called fluorescence. If the electron changes its spin when excited, it enters the triplet state, and the kind of light that is emitted as the electron returns to ground state is known as phosphorescence. Phosphorescence is much longer-lived than fluorescence. Both fluorescence and phosphorescence emissions are of particular wavelengths for specific excited electrons. Both types of emission are dependent on specific wavelengths of excitation light, and for both types of emission, the energy of excitation is greater than the energy of emission. Described another way,https://science.umd.edu/CBMG/faculty/wolniak/lambda_bl.jpg of excitation light is shorter than https://science.umd.edu/CBMG/faculty/wolniak/lambda_bl.jpgof emission light. In biology, we can utilize fluorescence in localization reactions, to identify particular molecules in complex mixtures or in cells. Fluorescence has the advantage of providing a very high signal-to-noise ratio, which enables us to distinguish spatial distributions of rare molecules. To utilize fluorescence, we need to label the specimen (a cell, a tissue, or a gel) with a suitable molecule (a fluorochrome) whose distribution will become evident after illumination. The fluorescence microscope is ideally suited for the detection of particular fluorochromes in cells and tissues.The fluorescence microscope that is in wide use today follows the basic "incident-light" design of Ploem, who employed a novel arrangement of filters with a chromatic beam splitter (often wrongly called a dichroic filter both by biologists and microscope sales people). With the incident light fluorescence microscope, the object is illuminated with fluorescence excitation light through the objective lens. The object emits longer-l fluorescence in response to the shorter-https://science.umd.edu/CBMG/faculty/wolniak/lambda_bl.jpg excitation light. The objective lens then serves both for illumination and imaging. The chromatic beam splitter transmits or reflects light, depending on its color. For this application, shorter https://science.umd.edu/CBMG/faculty/wolniak/lambda_bl.jpglight is reflected and longerhttps://science.umd.edu/CBMG/faculty/wolniak/lambda_bl.jpg light is transmitted by the splitter. Ploem placed the chromatic splitter in the optical path between the objective lens and the ocular, at a 45° angle, so that it would reflect shorter https://science.umd.edu/CBMG/faculty/wolniak/lambda_bl.jpglight downward toward the objective. The longer-https://science.umd.edu/CBMG/faculty/wolniak/lambda_bl.jpg fluorescence emission light would be transmitted through the chromatic beam splitter toward the ocular.The microscopes that you have utilized in this and other courses all operate in the same general fashion. Light beams pass through a condenser lens system and provide illumination of an object at many points simultaneously. For incident light fluorescence microscopy, the objective lens also acts as a condenser for the excitation light beam. In its interaction with the object, some of this light is absorbed, some of this light is scattered, some of this light is reflected, and some of this light is slowed or retarded (relative to a beam of light that does not pass through the object). A portion of the light that has interacted with the object then passes through the imaging lens system of the microscope where it provides us with visual or pictorial image information about the object. Like the process of illumination, the process of image generation operates in a parallel fashion, where large numbers of light beams contribute to the image simultaneously. Resolution is limited by the closeness of overlapping points of brightness or darkness. In a practical sense, the limit of resolution is 0.18-0.2 µm with the best available objective lenses and a good specimen.To observe cells with the fluorescence microscope, it is important to know the spectral characteristics of the fluorochrome that has been employed. In order to excite the fluorochrome properly and then observe its fluorescence emission, the appropriate filter packages must be present in the microscope. The fluorochrome may not fluoresce at all if the cells are illuminated with the inappropriate filter pack present in the optical path. Finally, for any kind of fluorescence localizations to be performed, it is essential to have the appropriate controls, to be sure that the cells do not exhibit excessive autofluorescence (that is, they do not glow in the absence of the fluorochrome), and that the fluorochrome is responsible for the localization pattern observed. In the laboratory, we have several microscopes equipped for incident light fluorescence microscopy.

***Confocal Scanning Optical Microscopy***

In the incident light fluorescence microscope, a light beam passes through a chromatic beam splitter and then the objective lens to illuminate a specimen. This light beam is used to excite electrons in fluorochrome molecules present in the object. As some of those excited electrons return to their ground state, the emission of light is detectable through the oculars of the microscope, or with a camera or video printer. The image is generated continuously, across the entire field of view. A primary problem with the fluorescence images generated in this way is that out-of-focus fluorescence appears as 'flare' in the object, and reduces the signal substantially. In addition, human eyes are not sufficiently sensitive photodetectors for the lowest levels of fluorescence, and most video-based imaging systems are only slightly better than your eyes. Under conditions where there is sufficient signal for you to easily observe fluorochrome distribution patterns, the excitation light can be of sufficient intensity to photooxidize (*i.e.*, burn) your specimen. Much information can be lost with just a few seconds of exposure to the excitation lamp. The Confocal Scanning Optical Microscope, an expensive piece of instrumentation that illuminates the object with a small beam of light in a point-by-point (*i.e.*, serial) fashion, eliminates most of the photoxidation problems, permitting the observation of objects for extended periods at very high resolution with little loss of signal. The placement of a small aperture in the beam path generates a small depth of field, and effectively eliminates out of focus information in image formation. The confocal scanning optical microscope is designed to illuminate an object in a serial fashion, point by point, where a small beam of light (from a LASER) is scanned across the object rapidly in an X-Y raster pattern. The raster pattern can be created in several ways, but in one of the more popular instruments, it occurs as a consequence of the simultaneous rotation and vibration of a polygonal mirror. The vibration is caused by the activity of a servogalvanometer, while the rotation is caused by the activity of a small electric motor. Thus, a bright spot of light scans across an object from top to bottom, line by line. The image is also generated point-by-point. Image formation is translated into intensities at each spot in the X-Y raster by a photomultiplier tube. The intensity information is digitized and stored in a computer. A complex image processing software package permits visualization and manipulation of the images. Resolution is limited by spot size for the LASER and approaches 0.12-0.15 µm for an ideal specimen and with the best available objective lenses. The manufacturers of confocal scanning optical microscopes include a pinhole diaphragm at a very special place in the optical path, near to the site of the photomultiplier tube. This pinhole is situated in a plane where the light from the in-focus part of the image converges to a point. Light from object planes above or below that of the focused image do not converge at the spot in the optical path occupied by the pinhole. Because of this design, out of focus image information is darkened to the extent that it is not detectable. The consequence is that all out of focus information is removed from the image and the confocal image is basically an 'optical section' of what could be a relatively thick object. The 'thickness' of the optical section may approach the limit of resolution, but in practice, the resolution in the Z-direction is somewhat greater, approximately 0.4-0.8 µm. The value of optical sectioning is best realized with fluorescence microscopy, where out-of-focus information alters, distorts, or even degrades the image. Because the confocal images are stored in a computer, it is possible to stack them up and generate three-dimensional reconstructions. The image processing programs also enable us to rotate these images and observe three-dimensional aspects of cellular structure. It may be clear to you that the computer responsible for these image manipulations must be fast and powerful. The biggest problem is one of image storage, where single images can routinely occupy >1,000,000 bytes of space. In rather short periods of use, it is easy to accumulate sufficient numbers of images to fill the largest of hard disks. Two of the three the confocal scanning optical microscopes located on campus were manufactured by Carl Zeiss, located in Germany. The newest instrument (model 510) has three lasers and four photomultipliers and is designed so that we could illuminate with two or three colors of light in rapid succession and detect as many as three superimposed signals (essentially) simultaneously. The signals are separated from each other on the basis of color, using an acoustical optical tunable filter (AOTF). The optical microscope is an inverted stand. The most important operational difference between this microscope and the upright microscope in most laboratories is that with this instrument, the slide is placed in the stage holder upside-down. Like most modern research microscopes, this microscope is equipped for phase contrast, differential interference contrast and fluorescence microscopy and can be used with these imaging techniques for conventional imaging. However, it is equipped with a number of very highly corrected (read expensive) objective lenses attached to the turret, just below the stage. These lenses are necessary for high resolution confocal microscopy. The confocal part of this microscope is contained in a box that is attached to the inverted stand through an access port. As is the case with incident light fluorescence, the laser light passes through the objective lens to illuminate the specimen. An air suspension table is designed to eliminate vibrations present in the building.

***Deconvolution Microscopy and Image Reconstruction***

An alternative approach for eliminating flare from fluorescent image stacks is to perform intensive, iterative image analysis and processing, from objects that have been illuminated and photographed at multiple, adjacent focal planes. The images are obtained with a high-performance CCD camera, operating at very high magnification, using standard incident light fluorescence microscopy. The excitation source is a mercury arc lamp, and bandwidth for excitation and emission are controlled by filters placed in rotating filter wheels. The lamp is stabilized and the beam is randomized for uniform illumination of the specimen. Unlike confocal scanning instruments, the whole field of view is illuminated simultaneously with this microscope. It is possible to perform rapid sequential imaging (4 colors) from multiple fluorochromes with this microscope. At very high magnification, fluorescence from any spot in a cell acts as a point source. By knowing the image spread functions above and below the plane of focus, it is possible to determine points of origin for fluorescence, and spreading beams of light from that point source, above and below the plane of focus. An iterative algorithm, which is essentially a linear combination is performed by a computer on the adjacent pixels within a single image plane, and in successive image planes through the thickness of the object. Spreading light beams are subtracted from reconstructed image stack, and that light is added back to the source, thereby reducing noise and increasing signal, respectively. We have recently acquired a sophisticated DeltaVision microscope from Applied Precision, Inc., which is designed to acquire these images and then perform the computer-intensive operations. This kind of microscope is particularly well suited for generating three-dimensional fluorescence images from small, living cells.

***Polarization Light Microscopy***

*- Birefringence -*

When light passes through an object, it interacts with some or all of the atoms and molecules present in that object. In these interactions, sometimes light of a particular (*i.e*., color) is absorbed by the atoms or molecules, while sometimes light is scattered. The interaction of light with a translucent object often results in a slight reduction in the velocity of the light https://science.umd.edu/CBMG/faculty/wolniak/lambda_bl.jpgbeam. The extent of this reduction in velocity can be measured as the refractive index of the object. For certain kinds of objects, especially those with high order in particular axes of the object, such a crystalline or paracrystalline arrays, the interaction with light beams is vastly different, depending on the orientation of the object relative to the impinging light beam. As a result, the refractive indices are measurably different in different axes of the object. Such an object with multiple refractive indices is termed **birefringent**. Birefringence (multiple refractive indices) results from the alignment of atoms or molecules in particular planes of an object; these atoms or molecules interact strongly with light beams impinging on them from a particular direction, and to a far lesser extent with light beams impinging on them from a different direction. There are two kinds of birefringence, *intrinsic birefringence*, which results from atomic or molecular order in a crystalline or paracrystalline array (*i.e*., calcite crystals, membranes) and *form birefringence*, which results from supramolecular associations in paracrystalline arrays (*i.e*., microtubules in a spindle).

*- Polarized Light and Birefringent Retardation -*

Any light beam shining in a particular direction vibrates in all directions around the axis of travel. Light beams whose vibration has been restricted to a single plane, or to a few planes is known as **polarized light**. Birefringence is directly observable as differences in intensity in different axes of crystalline or paracrystalline objects when they are viewed with polarized light. Since birefringence results from differences in the number of interactions between the light beam and atoms or molecules in the object in different directions, in practice, the object is rotated around the plane of vibration for the polarized light beam to maximize the intensity differences in the object (usually, the dominant object axis is at a 45o angle relative to the plane of polarization). The extent of the difference in refractive indices in different axes of the object is a measurable quantity known as **birefringent retardation (BR)**. BR is measured (as a distance) by placing an object with known birefringent retardation into the light beam, and, by rotating the calibrated object around the optic axis, extinguishing the brightness in the sample. Using this compensation technique, BR has been shown to be directly related to the number of aligned microtubules in mitotic spindles in living cells. This principle and procedure can be of importance in studying microtubule dynamics, where mitotic spindles of developing sea urchins can be visualized in a totally noninvasive way

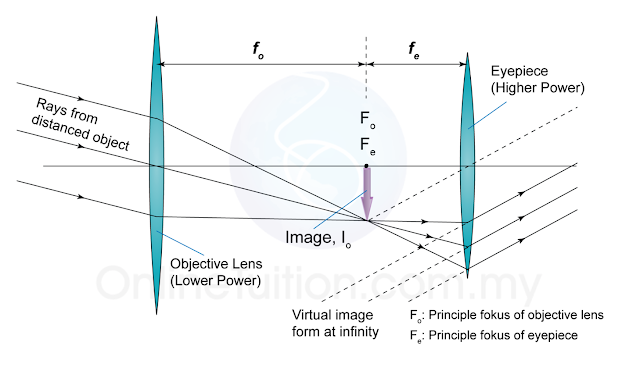
Types of Microscopes

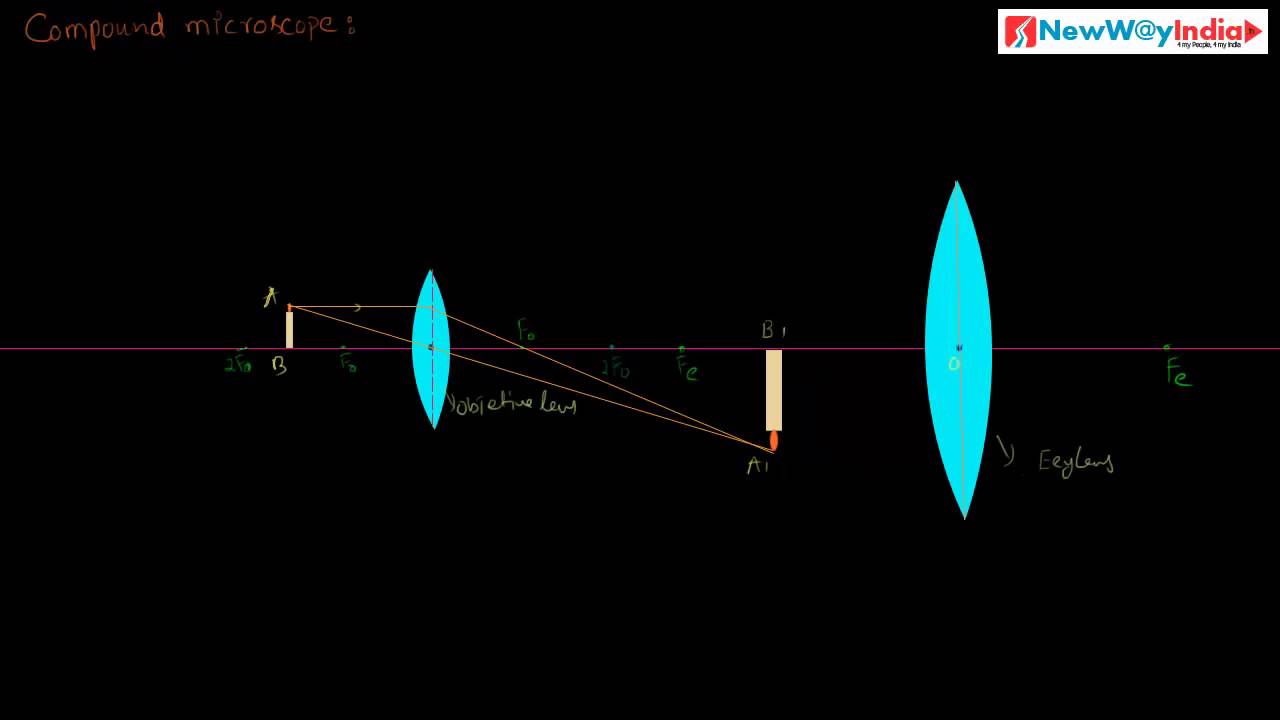
**1)Compound microscope**

### A compound microscope has (at least) two lenses: the objective lens is a strongly converging lens with a short focal length, while the eyepiece is a converging lens with a rather longer focal length. The distance between the two is typically somewhat longer than the sum of the focal lengths. We used two lenses as shown to make a microscope through which the camera could examine the grass. As you can see, stray light reflected from the lenses made it difficult to see the image formed through the two lenses.

### 2) **Simple microscope**

### A simple microscope or magnifier consists of a converging lens with the object placed near to but closer than the focus. It produces an erect, magnified virtual image.

[](http://1.bp.blogspot.com/-42hXN0ywDVU/UeOhtsHn3eI/AAAAAAAACZ4/lq_3pNyPStI/s1600/Picture58.png)



|  |  |
| --- | --- |
|  | Telescope |
| **Objective lens** | Lower power |
| **Eye lens** | Higher power |
| **Position of the object** | At infinity |
| **Nature of the image, I1** | Real, inverted and magnified |
| **Position of the image, I1.** | At the principle focus of object lens, fo. |
| **Nature of the image, I2** | Virtual, inverted and smaller in size. |
| **Distance in between the two lens** | The distance between the object lens and the eye lens in a compound microscope is equal to the sum of the  focal length (fo + fe).  If the distance between both lenses are bigger than (fo + fe), no image can be seen. |
| **Magnification of the** Centrifugation is a technique of separating substances which involves the application of centrifugal force. | m= Focal length of the object lens,  f o Focal length of the eye lens,  f m |

**2.CENTRIFUGE**

Centrifugation is a technique of separating substances which involves the application of centrifugal force.

The particles are separated from a solution according to their size, shape, density, the viscosity of the medium and rotor speed.

Brand – Premiere XC-2000, Bench-Top Centrifuge, 4000 RPM

COST-$268.9

**Product description**

The XC-2000 offers a variable speed selection from 1000 to 4000 rpm, which is adjustable in 500 RPM increments, achieving a maximum RCF of 1790 g’s. This reliable bench-top centrifuge features a fixed angle rotor that holds up to six test tubes ranging from 10 ml to 15 ml in fluid capacity. The XC-2000 also provides you with a 60 minute timer, a continuous hold-spin function and a last-spin memory feature that remembers the previous operation, reducing the time it takes to reconfigure your desired setting. Tired of noisy centrifuges? Anyone that has experienced having multiple centrifuges running simultaneously can attest to fact that this noise brings both distraction and discomfort to the workplace. As such, the XC-2000 features one of our proprietary quiet motors generating an operating noise level of under 70 dB, in the hopes of helping you maintain a quiet and comfortable work environment. It is common knowledge that work areas are considered prime real estate in most labs and it is with this in mind that the XC-2000’s compact frame was specially designed to maximize your workspace. Now with your safety in mind, the XC-2000 centrifuge has been designed with an auto-stop feature that is triggered when the centrifuge lid is opened before they cycle is completed. This feature is intended to help avoid injury and reduce instances of tube breakage and contamination of the unit. Along with this, are three suctioned feet that will hold the unit in place, reducing vibrations and avoiding any slipping and shifting of the unit. For over 25 years Premiere has been making dependable laboratory centrifuges that stand the test of time. The Premiere XC-2000 is CE certified and is manufactured at our ISO 9001 certified factory; so whether you are running PRP, PRF, cell washing or simple fluid separation protocols, the XC-2000 is the ideal choice for medical, scientific, clinical and industrial applications and is backed by our comprehensive 1 year warranty against manufacturers defects. If a variable speed model is not required, please check out our more economical XC-1000 fixed-speed centrifuge ASIN # B00EP0ST90, or better yet, please refer to the chart below which illustrates the key points which

**PRINCIPLE OF CENTRIFUGATION**

In a solution, particles whose density is higher than that of the solvent sink (sediment), and particles that are lighter than it floats to the top.

The greater the difference in density, the faster they move. If there is no difference in density (isopycnic conditions), the particles stay steady.

To take advantage of even tiny differences in density to separate various particles in a solution, gravity can be replaced with the much more powerful “centrifugal force” provided by a centrifuge.

A centrifuge is a piece of equipment that puts an object in rotation around a fixed axis (spins it in a circle), applying a potentially strong force perpendicular to the axis of spin (outward).

The centrifuge works using the sedimentation principle, where the centripetal acceleration causes denser substances and particles to move outward in the radial direction.

At the same time, objects that are less dense are displaced and move to the center.

In a laboratory centrifuge that uses sample tubes, the radial acceleration causes denser particles to settle to the bottom of the tube, while low- density substances rise to the top.

**Centrifuge care and maintenance**

A few simple steps can keep a centrifuge functioning properly and reduce the risk of damage or injury.

Keep the centrifuge properly lubricated. O-rings are the main source of protection against sample leakage, and must be lubricated prior to installation of a new rotor or following cleaning. Any threaded components should also be cleaned regularly and lubricated with an approved grease to ensure proper operation and to prevent cross-threading and corrosion.

Ensure all users are aware of how to properly operate the centrifuge, including ensuring buckets are properly seated in their pins, balancing tubes in the rotor, operating rotors within stated guidelines for speed and maximum compartment mass, and avoiding scratching the rotor.

Inspect critical components, and look for signs of wear including scratches, or effects of chemical exposure on the rotor.

Pay close attention to noise, vibration, shaking, or grinding and stop the unit immediately if this occurs.

**Centrifuge cleaning**

Regularly clean the centrifuge with neutral cleaning solutions (alcohol or alcohol-based disinfectant) applied with a soft cloth to rotors and accessories. Daily cleaning should include the interior portion of the centrifuge, the rotor chamber, and surfaces with electronic components, such as touchscreens and keypads.

It is important to be aware of the different types of samples used with the centrifuge and any specific products or protocols necessary for cleaning spills.

**ii)Automatic Tissue Processor**

Tissue processing describes the steps required to take animal or human tissue from fixation to the state where it is completely infiltrated with a suitable histological wax and can be embedded ready for section cutting on the microtome. Tissue processing can be performed manually (hand processing), but where multiple specimens must be dealt with it is more convenient and much more efficient to use an automated tissue processing machine (a “tissue processor”). These devices have been available since the 1940’s1 and have slowly evolved to be safer in use, handle larger specimen numbers, process more quickly and to produce better quality outcomes. There are two main types of processors, the tissue-transfer (or “dip and dunk”) machines where specimens are transferred from container to container to be processed, or the fluid-transfer (or “enclosed”) types where specimens are held in a single process chamber or retort and fluids are pumped in and out as required. Most modern fluid-transfer processors employ raised temperatures, effective fluid circulation and incorporate vacuum/pressure cycles to enhance processing and reduce processing times.

**Steps involved in tissue processing**

1}Identification

ii)labelling of the specimen with numbering

iii)fixation

iv)Dehydration

v)Clearing

vi)Impregnation

vii)Embedding

viii)Section cutting

ix)Staining

x)Mounting

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| **https://image.slidesharecdn.com/16histotechniques2-121125061150-phpapp01/95/16-histotechniques-2-30-638.jpg?cb=1353823981**  **Technical specifications:**  Main Performance Characteristic  Adopting computer to control whole work process, operate brief work stabilization.  Having exchange jar and change place control draw perch, operate agility brief.  If lose electric, alarm sounds to protect tissue in liquid.  Adopting agitation dehydrate mode enhance reagent and paraffin penetration into tissues.  Wide time range: delay time 59hrs59min. The longest working hours for each container is 59hr59min, Time design least interval is 1 min.  Wax jars adopt constant temperature control system. Temperature precision high: defend pollution, defend canker.  Adopting 9 slices 1.2L medicine aquarium, at any moment observe tissue change at work.  According to user’s need to tailor standby electrical source, after power cut it can also work in great, working hours can reach 4-16 hours.  The brand is KD-TS6B, AUTOMATIC VACUUM TISSUE PROCESSOR  COST-$14,800.00  **MAINTANANCE AND CARE**  1. Caution when handling reagents.  .2. Use disposable Nitrile gloves when handling carcinogens or toxic materials.  3. Do not smoke, eat or d rink in areas where specimens or reagents are handled.  4. Avoid contact of reagents with eyes and or mucous membranes. If contact with sensitive areas, wash with water.  5. Patient’s specimens should be handled as biohazard materials and disposed of with Precautions.  6. Review the Safety Data Sheet (SDS) before handling reagents and solutions.  7. Each open automated tissue processor is operated at least 5 feet from the storage of combustible materials and from the paraffin dispenser  **3.MICROTOME**  A microtome is a specialized precision cutting instrument, which accurately and repeatedly slices sections from a block of embedded tissue. Different kinds of microtomes are used to section paraffin and plastic embedded tissues as well as the specialized microtomes used to section frozen tissues. In any microtome a sharp knife and the tissue block are held in a fixed relation to each other. With each pass of the tissue past the knife it advances the tissue block a preset amount—the section thickness. For frozen sections the section thickness typically ranges from 8 to 15 µm, for wax sections 4–10 µm, and for plastic histological sections 0.5–3 µm. In electron microscopy sections must be extremely thin, about 200× thinner than wax sections. Typically plastic sections used in transmission electron microscopy (TEM) are cut in the range 60–100 nm.  **MAINTAINANCE AND CARE**  Working with microtomes, whether preparing or cutting a sample, is a true art. You need specific hands-on training from your supervisor before using a microtome. Because of the associated hazards, safety must be incorporated into every step of the process to keep fingers and hands protected. Follow the safety tips provided below to keep your fingers, hands, and your artistic touch in perfect condition and to prevent exposure to solvents and biologicals.   * Handle blades very carefully when installing or removing. Follow the manufacturer’s guidelines explicitly. * Tungsten-Carbide knives can cut through your shoes if dropped. Be careful where your feet are positioned when installing or removing blades. * Store blades in a covered container. Use a container that has guides to hold the blades rigid. * Never leave blades on countertops. Lacerations can occur when reaching across the countertop and inadvertently contacting an unprotected blade. * When setting up the microtome, position the sample first then put in the blade. **Never** the other way around. * When applying the brake, ensure that it is tight. Most accidents occur when the brake slips and the operator’s hand is drawn into the blade. * When leaving the microtome, even for a short time, ensure that the blade guard is in place. * When preparing a paraffin sample for the Microtome, remember to clamp the sample down tight. The movement allowed by a loose clamp increases your risks of cuts. * Use forceps to retrieve slices from the boat and to retrieve ribbons, thereby keeping your hands free from the moving parts of the microtome. * To avoid compression or knife marks, ensure that your blade is clean. Follow the manufacturer’s guidelines for cleaning. A high-density polystyrene rod can be used to clean the blade, freeing your hands from potential contact. * Prions are not deactivated by the standard microtome preparation steps. You must wear gloves and use appropriate decontamination procedures when samples may contain prions. * BRAND- Weswox Histology manual rotary microtome * COST- : Rs 2.9 Lakh |