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MLS 410

Biomedical engineering

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QUESTION:

* Discuss the physics of the light microscope diagrams and illustrations needed
* Write notes on the following biomedical equipment. Add notes on principle, brand, care and maintenance and cost.
* Centrifuge
* Automatic tissue processor
* Microtome

 

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. 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 the objective lens (dark field). Polarized 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.

There are two basic types of optical microscopes: simple microscopes and compound microscopes. A simple microscope uses 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. Compound microscopes can be further divided into a variety of other types of microscopes which differ in their optical configurations, cost, and intended purposes.

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.

 A compound microscope uses a lens close to the object being viewed to collect light which focuses a real image of the object inside the microscope. That image is then magnified by a second lens or group of lenses that gives the viewer an enlarged inverted virtual image of the object .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

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 (numbered below according to the image on the right):

**Eyepiece (ocular lens)**

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×.

**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**

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**



Some microscopes make use of oil –immersion objectives or water-immersion objectives for greater resolution at high magnification. 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. 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 nor 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.



A **centrifuge** is a piece of equipment that puts an object in rotation around a fixed axis (spins it in a circle), applying a force perpendicular to the axis of spin (outward) that can be very strong. The centrifuge works using the sedimentation principle, where the centrifugal 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.

 There are three types of centrifuge designed for different applications. Industrial scale centrifuges are commonly used in manufacturing and waste processing to sediment suspended solids, or to separate immiscible liquids. An example is the cream separator found in dairies. Very high speed centrifuges and ultracentrifuges able to provide very high accelerations can separate fine particles down to the nano-scale, and molecules of different masses.

Gas centrifuges are used for isotope separation, such as to enrich nuclear fuel for fissile isotopes.

**Principle of centrifugation**

**CENTRIFUGE**

Centrifugation is a technique used for the separation of particles using a centrifugal field. The particles are suspended in liquid medium and placed in a centrifuge tube.  The tube is then placed in a rotor and spun at a definitive speed. Rotation of the rotor about a central axis generates a centrifugal force upon the particles in the suspension.

Two forces counteract the centrifugal force acting on the suspended particles:

* Buoyant force: This is the force with which the particles must displace the liquid media into which they sediment.
* Frictional force: This is the force generated by the particles as they migrate through the solution.

Particles move away from the axis of rotation in a centrifugal field only when the centrifugal force exceeds the counteracting buoyant and frictional forces resulting in sedimentation of the particles at a constant rate.

Particles which differ in density, size or shape sediment at different rates. The rate of sedimentation depends upon:

*1.*       The applied centrifugal field

*2.*       Density and radius of the particle.

*3.*       Density and viscosity of the suspending medium.

Angular velocity = w radians / second;

since one revolution = 360o = 2p radians,



(r =  radial distance of the particle from the axis of rotation)

As the centrifugal field acting on the particle is much greater than the Earth's gravitational field, CF is generally expressed relative to the Earth's gravitational field as multiples of g, the acceleration due to gravity (g= 980 cm/s2)



**Care of centrifuge**

Before arriving at the details of centrifuge care, some paperwork must be done. First, there are manuals—your centrifuge came with one, and it includes a section on cleaning and maintenance, and quite possibly a list of chemicals/cleaners/disinfectants that are safe to use, and a list of those that are not. If the manual is missing, a duplicate is probably available online, and should be downloaded and printed. It is best to read it, and keep it handy, not only during initial runs but during the initial cleanings too. To come full circle, a maintenance log should be kept so that proper maintenance can be verified. This can be especially important in warranty claims.

Following a regular schedule will ensure that time is set aside and used for cleaning. While sometimes inconvenient, adhering to a regular program is cost-efficient and saves time in the long run.

For hazardous spills, your lab and/or institution will have a protocol that could include everything from kinds of protective gear and products, such as spill kits, to be used, to reporting and safety procedures. It is important that these are known and that they are followed. It is frequently the case that shortcuts, because they skip a step or detail, end up taking more time later. Know the kinds of samples being run in the lab; keep this information up to date, especially in a shared lab. This will help determine if any unique products or protocols are needed for cleaning. It is important to note as well that centrifuges can incorporate many materials, from stainless steel to carbon fiber to membrane switches and touch-sensitive display screens. The manual will be the best guide to the products and processes to use for cleaning each of these.

**Centrifuge tips**

* Always place the centrifuge on a flat surface first.
* Always unplug the power cord before cleaning.
* Emergency phone numbers and procedures should be posted and kept up to date.
* Wear disposable gloves.
* Follow your facility’s safety procedures when cleaning and disinfecting the centrifuge.
* Before moving the centrifuge to a new location, the exterior and interior surfaces should be cleaned and disinfected.
* Plug in centrifuge only when completely dry.

**Cleaning**

Centrifuge interiors are made from a range of materials—plastic, ceramic, aluminum and stainless steel—that manufacturers use in the search for performance in a high-stress environment. Manufacturers’ recommendations on the cleaners and scrubbers to use and method and frequency of application take this into account and will get the best results.

* Clean the centrifuge daily, or at least weekly.
* Remove the rotor and any sample or container holders.
* Interior cleaning includes the interior bucket, specimen holder, rotor and supports.
* Use a sponge, warm water and a mild detergent such as dishwashing liquid.
* Do not use caustic detergents or any product containing chlorine ions. (Diluted bleach is sometimes used as a disinfectant, but at full strength can attack stainless steel and discolor or damage the bowl (see below). A plastic scrub pad can be used, but products such as steel wool, wire brushes and other abrasives can damage coatings and lead to corrosion.
* Spills should be wiped up immediately.
* Clean both the exterior and the interior.
* Do not pour water directly into the chamber or flood the inside of the centrifuge with cleaner. Sensors, gaskets, seals, wiring and other parts that may be present can be easily damaged. Motors, vacuum pumps, condensers and other expensive parts can also be damaged by exposure to water and cleaning products.
* Scrub tube cavities with a test tube brush with nonmetallic tip. Dry each part with an absorbent towel.

**Disinfecting and Decontamination**

Disinfect on a regular basis. Approved disinfectants and/or “spill kits” should be used. A 10% bleach solution (one part bleach to nine parts water) can be used with some materials: consult manual. After cleaning with a bleach dilution, dispose of any remaining mix.

Many rotors have sealed compartments that provide aerosol containment and confine spills of toxic, pathogenic, infectious or radioactive materials. If breakage occurs, it may be that only the sealed containers require decontamination. It will be necessary to decontaminate the chamber if a sample-holder in an unsealed carrier breaks or if any occurrence allows the sample out of the sealed compartment. Allow sufficient time for the disinfectant to work. If a spill occurs outside a containment device, follow facility rules on procedures and reporting. The protocols for spills outside of containment devices, including centrifuges, almost always differ from those for contained spills, and users of equipment should be aware of the appropriate steps to promote worker and environmental safety. Spill clean-up requires proper personal protective equipment (PPE), including a lab coat or gown, and gloves. A face shield, shoe covers or respirator may be needed as well.

Spills of radioactive substances can often be addressed with a decontamination solution of 70% ethanol and 10% sodium dodecyl sulfate in water. Parts will need to be rinsed with ethanol first, and the following decontamination, with deionized water. Appropriate protective gear should be worn and properly disposed of after use.

**Centrifuge Maintenance and Care**

The centrifugal field which accelerates the separation process also exerts large forces on the rotor material. If a rotor fails, a tube breaks, or any other incident occurs, the centrifuge can be severely damaged, as well as possibly endanger those working in the lab. For this reason, some simple precautions should be observed to improve safety during centrifugation.

**Avoiding Rotor Failures**

The centrifugal field which accelerates the separation process also exerts large forces on the rotor material. If a rotor fails, the centrifuge is severely damaged as well. For this reason, some simple precautions should be observed

Rotors are designed to be run up to their maximum speed with a load of a specific weight. One should never attempt to run a rotor at a speed higher than the one designated by its manufacturer. Also, if high density solutions (greater than 1.2 g/mL, for instance) are used, the run speed must be reduced to prevent undue stress on the rotor. Consult your instruction manual for exact directions.

**Tube Breakage**

Glass tubes can break during centrifugation, due either to improper loading or inherent defects. Any glass fragments must be removed from the buckets, adapters, rubber liners, and rotor chamber before the next run is made. If you find gray dust, which results from sandblasting of the rotor chamber by glass particles, it must be cleaned up too. You should make several dry runs without samples, and clean the chambers between each run to be sure this dust is eliminated from the centrifuge.

**Chemical Resistance**

If you plan to centrifuge any uncommon solvents or solutions, consult your manual to be sure they are compatible with the various plastics and metals comprising the centrifuge, the rotor, the tubes, and other accessories. These same precautions must be observed with any solvents used for sterilization purposes. A table of 19 chemical resistances for common centrifuge materials is available from Beckman Coulter.

**Aerosol Generation**

If any liquid is spilled on a rotor, it will be dispersed as a particulate mist when the centrifuge is run. Part of this mist will be fine enough to form a relatively stable aerosol which will tend to be dispersed throughout the laboratory. Such spills should be thoroughly cleaned up before running the centrifuge.

**Handling Human Samples**

Human blood or blood components can transmit an infectious disease or virus if the patient or donor carries these. Blood should be handled with respect for this possibility during all laboratory manipulations, including centrifugation.

**Microtome**

A **microtome** (from the Greek *mikros*, meaning "small", and *temnein*, meaning "to cut") is a tool used to cut  extremely thin slices of material, known as sections. Important in science, microtomes are used in microscopy, allowing for the preparation of samples for observation under transmitted light or electron radiation.

Microtomes use steel, glass, or diamond blades depending upon the specimen being sliced and the desired thickness of the sections being cut. Steel blades are used to prepare sections of animal or plant tissues for light microscopy histology. Glass knives are used to slice sections for light microscopy and to slice very thin sections for electron microscopy . Industrial grade diamond knives are used to slice hard materials such as bone, teeth and plant matter for both light microscopy and for electron microscopy. Gem quality diamond knives are used for slicing thin sections for electron microscopy.

Microtomy is a method for the preparation of thin sections for materials such as bones, minerals and teeth, and an alternative to electropolishing and ion milling. Microtome sections can be made thin enough to section a human hair across its breadth, with section thickness between 50 nm and 100 μm.

**Applications**

The most common applications of microtomes are:

* Traditional Histology Technique: tissues are fixed, dehydrated, cleared, and embedded in melted paraffin, which when cooled forms a solid block. The tissue is then cut in the microtome at thicknesses varying from 2 to 50 μm .
* Frozen section procedure: water-rich tissues are hardened by freezing and cut in the frozen state with a freezing microtome or microtome-cryostat; sections are stained and examined with a light microscope. This technique is much faster than traditional histology (5 minutes vs 16 hours) and is used in conjunction with medical procedures to achieve a quick diagnosis. Cryosections can also be used in immunohistochemistry as freezing tissue stops degradation of tissue faster than using a fixative and does not alter or mask its chemical composition as much.
* Electron Microscopy Technique: after embedding tissues in epoxy resin, a microtome equipped with a glass or gem grade diamond knife is used to cut very thin sections (typically 60 to 100 nanometer). Sections are stained with an aqueous solution of an appropriate heavy metal salt and examined with a transmission electron microscope. This instrument is often called an *ultramicrotome*. The ultramicrotome is also used with its glass knife or an industrial grade diamond knife to cut survey sections prior to thin sectioning. These survey sections are generally 0.5 to 1 μm thick and are mounted on a glass slide and stained to locate areas of interest under a light microscope prior to thin sectioning for the TEM. Thin sectioning for the TEM is often done with a gem quality diamond knife. Complementing traditional TEM techniques ultramicrotomes are increasingly found mounted inside an SEM chamber so the surface of the block face can be imaged and then removed with the microtome to uncover the next surface for imaging. This technique is called Serial Block-Face Scanning Electron Microscopy SBFSEM).
* Botanical Microtomy Technique: hard materials like wood, bone and leather require a sledge microtome. These microtomes have heavier blades and cannot cut as thin as a regular microtome.
* Spectroscopy (especially FTIR or Infrared spectroscopy) Technique: thin polymer sections are needed in order that the infra-red beam will penetrate the sample under examination. It is normal to cut samples to between 20 and 100 μm in thickness. For more detailed analysis of much smaller areas in a thin section, FTIR microscopy can be used for sample inspection.

A recent development is the laser microtome, which cuts the target specimen with a femtosecond laser instead of a mechanical knife. This method is contact-free and does not require sample preparation techniques. The laser microtome has the ability to slice almost every tissue in its native state. Depending on the material being processed, slice thicknesses of 10 to 100 μm are feasible.

# Microtome Safety

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.

**Automated 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.