Exercise 1
The Compound Light Microscope

Introduction:

A microscope is an instrument that magnifies an image and allows visualization of greater detail than is possible with the unaided eye. Microscopes are usually classified according to their light source: visible light (LM – light microscope); polarization, phase contrast, interference, ultraviolet, and x-ray microscopes and electron beam (EM – electron microscope).

The basic instrument in this course is the compound light microscope. Early microscopes, for example the first ones made by Anton Van Leeuwenhoek in the 17th century, had only one lens and were “simple” ones. All modern instruments have at least two lenses, and thus are “compound”. The basic operating principle of the compound microscope is that the image formed by the first lens is magnified by the second, thus providing a much greater magnification than could be attained using only one lens.

Objectives:

After completing this exercise you should be able to
- Identify the parts of the microscope and name their function
- Learn to use a microscope correctly
- Differentiate between resolution and magnification
- Describe how tissue sections are prepared and why they are stained for observation

Parts of the Microscope:

Refer to the Figure 1 in the next page and to the following text to locate the different parts of your microscope:

The compound microscope has several major parts, and these are common to all instruments, regardless of make or model. The body (sometimes called the stand) hold the rest of the parts, and in some cases it incorporates filters and/or photographic equipment. The stage is the movable part that sticks out from the body, and into which the slide is placed. In most modern instruments the stage moves up and down to achieve focus, and the lenses remain fixed in place, though this is not always the case; in some (especially older scopes) the stage is fixed and lenses move. Below the stage is the illumination system, which consists of a light source and a sub-stage condenser. The latter is a set of lenses which take the beam from the light source and refract it into a coherent and relatively narrow beam. If the specimen is to be uniformly illuminated, and to be given maximum brightness, the rather diffuse light from the source must be narrowed; otherwise much of its intensity will be wasted by lighting the bottom of the stage and not the specimen.

Incorporated into the sub-stage condenser is an iris diaphragm. There is a great tendency on the part of the beginning microscope student to close the diaphragm down, in order to limit the light entering the condenser, but this should be avoided. BOTH THE CONDENSER AND DIAPHRAGM SHOULD BE FULLY OPEN WHEN USING THE MICROSCOPE. Stopping them down will result in greatly decreased resolution and you may not be able to see your subject.

A modern microscope usually mounts anywhere from three to five lenses in a rotating turret called the nosepiece. These are the lenses which form the primary image of the object, and which provide most of the magnification. More than any other consideration, the quality of the lenses determines the quality of the microscope, and the more expensive instruments put most of their price into the glass. The objectives will be inscribed with a set of numbers, including their magnification, and a number (typically less than 1.0, but occasionally 1.2 or 1.3) called the numerical aperture (NA). The NA is of great importance in the overall resolution of the lens. The higher the NA, the better the resolution, as we shall see later.
Setting the Microscope

To set up the microscope, obtain a slide and perform the following steps:

1. Visualize the tissue on the slide, by holding the slide in front of light, prior to placing the slide on the stage.
2. Place the tissue over the hole in the center of the stage.
3. With the 4X objective in place, raise the stage to a point approximately ½ inch from the slide.
4. Manipulate the coarse adjustment knob until the tissue is roughly in focus.
5. Use the fine adjustment knob only to sharpen the focus.
6. Shift the objective turret to the next highest power, ensuring that the objective does not touch the slide.
7. Repeat steps 5 and 6 until the 40X objective is in place.

**WARNING:**

**DO NOT:** go directly to the 40X objective.

**DO NOT:** move the stage UPWARD when using the 40X objective.

**ALWAYS:** use great care when you focus. The objectives and the slides are expensive.

8. To use the oil immersion lens, place a small drop of immersion oil on the slide directly over the tissue you wish to observe. (Be careful not to get the oil on your skin, if possible). Oil must be used on the
slide with great caution. The oil immersion lens is constructed so that oil cannot leak into the lens and coat its inner surface. However, the other lenses are not protected against oil.

WARNING: Be extremely careful not to get the 40X objective into the oil when you are changing powers.

(This lens will not be used very frequently in this course)

Resolution and Magnification

The primary image formed when the specimen is placed at the front focal point of the objective lens passes through the tube of the microscope. In older instruments, this was quite literally a tube, and straight up and down. Nowadays, the tube is in part a prism which reflects light internally and allows the use of angled eyepieces, which are far more comfortable to use. Nevertheless, the older term is still used to denote the optical path of the primary image, and the “tube length” has been standardized on modern instruments to a total of 160 mm.

The primary image is secondarily magnified by the ocular lenses, which sit in the eyepieces. THE FINAL MAGNIFICATION OF AN IMAGE IS THE PRODUCT OF THE INDIVIDUAL MAGNIFICATIONS OF THE OBJECTIVE and THE OCULAR LENSES:

\[ \text{MAG}_{\text{final}} = \text{MAG}_{\text{obj}} \times \text{MAG}_{\text{ocl}} \]

The most important consideration in selecting a microscope is its resolution. Resolution is the ability of an instrument to discriminate between two points on a specimen. The resolving power of the human eye is determined by the spacing of the photoreceptor cells in the retina. The role of the microscope is then, to magnify an image to a level at which the retina can resolve the information that would otherwise be below its limit of resolution. Table 1 compares the resolution of the eye to various instruments.

<table>
<thead>
<tr>
<th>Table 1: Eye Versus Instrument Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Eye</td>
</tr>
<tr>
<td>Bright Field (light) Microscope</td>
</tr>
<tr>
<td>SEM</td>
</tr>
<tr>
<td>TEM</td>
</tr>
<tr>
<td>Theoretical</td>
</tr>
<tr>
<td>Tissue Section</td>
</tr>
</tbody>
</table>

The formula for the resolution of a microscope is:

\[ R = \frac{\lambda}{2 \text{ NA}} \]

Where \( \lambda \) is the wavelength of the illumination and NA is the numerical aperture of the objective lens. You should note several things about this equation. First of all, it should be obvious that the higher the NA, the better the resolution will be, because \( R \) will be smaller. Secondly, it should also be obvious that the shorter the wavelength the better the resolution. Achieving high resolution is a matter of getting the NA as high as possible and the illumination wavelength as low as possible. Note also that magnification has nothing to do with resolution. You do not get better resolution at high power, unless the NA of the lens is higher.

Improper illumination of a microscope generally causes the greatest amount of difficulty encountered in the use of this instrument. (Dirty lenses run a close second!) In order to properly illuminate a microscope, it is necessary to understand that the function of a condenser is to focus the light on the specimen in such a manner that the specimen
acts as the source of illumination. The iris diaphragm of the condenser serves to eliminate stray light, not to reduce intensity.

To properly adjust the condenser and condenser diaphragm, the following procedure should prove useful.

1. Focus on a specimen with the 4X, 10X or 40X objective
2. Focus the condenser up and down until you see the sharpest possible image of the ground glass diffusing plate (in the light source) superimposed on your focused specimen. You should now see a grainy field over your specimen. This will be easy to see if you close the condenser diaphragm as far as it goes. While this is the ideal condenser focus, you will want to eliminate the grainy field by defocusing your condenser above its focal point until the grain disappears.
3. When you are seeking the greatest possible resolution, open the condenser diaphragm fully. From its open position, slowly close the condenser diaphragm until it just begins to darken the field. This will increase the contrast. You might want to check your adjustment by removing one of the eyepieces; look down the tube and insure that the diaphragm cuts into the disk of light at the back of the objective by about 9/10 of its diameter.
4. To insure maximum resolution, this procedure should be repeated for each objective as the required adjustments are slightly different, especially for the condenser diaphragm setting. For special purposes you may wish to close the condenser diaphragm more than usual to increase contrast. This adjustment will result in some loss of resolution but can actually help to visualize low contrast features of the specimen, such as fine fibers. To appreciate this effect, examine your slide of heart muscle with the condenser diaphragm wide open, producing light flare that degrades specimen contrast, making it difficult to see the striations and intercalated disks of this tissue. Now slowly close the diaphragm. You will notice an increase in contrast that makes the muscle striations and intercalated disks quite easy to see.
5. The light intensity is controlled at the light source by adjusting the rheostat.

Cleaning the Microscope Lens

A dirty objective lens is a common cause of lack of resolution in microscopes. If you suspect a dirty objective, unscrew it from the lens turret and examine the surface of the lens with an eyepiece from your scope. If you invert the eyepiece and look through the eyepiece the "wrong way" it can be used as a fine quality loupe. This will reveal any deposit on the lens.

The most common way of dirtying an objective lens (especially the 40X one) is to accidentally drag it through oil. This can be avoided by never rotating the lens turret after you have the oil in place and by using only the oil lens (100X objective) on oiled slides. This lens, by the way, has a black ring around the lens barrel to make it easily distinguishable from the high dry lens (40X). If you must look at an oiled slide with the high dry lens, then lower (focus) the slide away from the lens, blot up the bulk of the oil from the slide with lens tissue and then carefully rotate the lens turret to bring in the 40X lens. The 4X and 10X objectives should clear any puddle of oil.

You must clean the oil off the oil immersion objective after each use. If you do not remove the oil, it will collect dust in the oil making a grinding compound when you finally do decide to wipe it off. The 100X oil immersion objective is easy to clean:

1. Turn the lens turret so that it is accessible.
2. Wipe off the oil as well as possible with dry lens tissue (lens tissue only, please).
3. Slightly dampen a clean piece of lens tissue with lens cleaning fluid and gently wipe lens with this. You might finish with another dry wipe. It is exceedingly important that you do not use an excess of lens cleaning fluid as this can dissolve the cement between the lens elements and thus ruin the objective.

We have found that the dry lenses (especially 10X and 40X) are best cleaned with styrofoam. We will provide each lab with styrofoam "peanuts," the kind used by shippers to pack around fragile equipment. You simply break the styrofoam in two and use the freshly exposed fracture face to entirely clean your objectives. These surfaces will absorb oil and clean the lens quite nicely if you use a few clean areas of styrofoam. Do not use lens cleaner with this method.
Finally, you should never have to clean the lenses or prisms in the interior of your microscope. If you think you have a problem here, call it to the attention of your lab instructor. It shouldn't even be necessary to clean the inner surface of the lens on your eyepiece, though you may have an occasion to clean the outer surface of your eyepiece lens.

Histochemistry

Tissues are composed of various chemicals such as proteins, carbohydrates, lipids, inorganic salts and miscellaneous substances. Thus, in some cases, special stains are used to illustrate the presence of some of these tissue components. For example:

1. **enzymes**: there are numerous histochemical procedures for identifying specific enzymes in phosphatases, lipases, oxidases, esterases, and dehydrogenases.
2. **carbohydrates**: periodic acid Schiff test (PAS) is used to demonstrate the presence of glycogen, resulting in a purple color; basement membranes and reticular fibers are also PAS-positive and are demonstrated using the stain on some of your slides.
3. **lipids**: Sudan red or black dyes. This dye is especially good for membranes, cytoplasmic, lipophilic inclusions and myelin.

Most of the prepared slides in your sets are stained with hematoxylin and eosin (H & E) using the procedures previously described. Below is a list of commonly used stains, their abbreviations and the significance of their use.

- **Alcian blue** stains complex carbohydrates (glycosaminoglycans rich in free acidic groups, such as connective tissue mucins and most epithelial mucins, mast cell granules and pneumococcal capsules. Color is turquoise blue.
- **Aldehyde fuchsin** stains elastic fibers violet to purple. Secretory granules in mast cells, gastric chief cells, pancreatic B cells, beta cells of the pituitary, and mucins also stain purple.
- **Bodian silver** is a technique for impregnating nerve fibers (black) in paraffin sections. The nuclei and Golgi of cells also stain with silver. Osteocyte canaliculi also appear black with this stain.
- **Carmine** is a basic dye used as a counterstain for vital dyes, in the Mucicarmine technique, and for coloring gelatin used to inject blood vessels. Stains nuclei and other basophilic substances red (purple).
- **Copper-chrome hematoxylin** (CCH) stains mitochondria deep blue against a yellowish background.
- **Elastic stain** stains elastic fibers black or dark blue.
- **Fast green** is an acid dye used as a counterstain (rarely used alone). Stains connective tissue a light green.
- **Giemsa** is used for study of morphology of blood and marrow cells.
- **Gomon** allows distinction of different cell types in pancreatic islets.
- **Hematoxylin and eosin** (H and E) is the most common histologic stain used for routine study of general morphology. Stains nuclei blue and practically all cytoplasmic structures red. Those constituents staining blue are commonly called basophilic and those staining red, acidophilic. Pronounced basophilia in the cytoplasm of cells usually indicates a high level of RNA and protein synthesis such as is observed in developing organs in the embryo or in cells of the adult organism which are very actively engaged in protein synthesis (e.g., the pancreatic acinar cells). As a general rule, the basic components of a tissue stain with acidic dyes and so are called acidophilic, whereas the acidic components stain with basic dyes and are called basophilic.
- **Hematoxylin and orange G-erythrosin** (H and OGE) is a general purpose stain for morphology. The hematoxylin primarily stains nuclei and other basophilic constituents of the cell, if any. Orange G is a rather strongly acid dye.
which stains acidophilic components such as the cytoplasm, an orange-red color. The erythrosin is also an acid dye, but stains some structures such as smooth muscle a light pink.

**Iron hematoxylin** (IH;FeH) is particularly useful for secretory granules, but in addition stains chromatin, nuclei, mitochondria and cross striations of voluntary muscle an intense blue or black. The stain is not specific for anything other than dense protein in any tissue. The iron (iron ammonium sulfate) actually serves as a mordant to which the hematoxylin can bind.

**Mallory trichrome** (M) consists of three dyes: acid fuchsin, aniline blue and orange G. Stains collagen an intense blue; mucus and amyloid various shades of blue; and nuclei, cytoplasm, elastic fibers, neuroglia, microglia and fibrin red. Erythrocytes and myelin sheaths stain yellow to orange.

**Metachromasia** is a shift in the absorption spectrum of a basic dye, such as thionin or toluidine blue. This shift results from interaction between dye molecules when they are bound close enough together (within 5-7 Å) to a series of uniformly spaced negative sites such as exist in heparin, other acid mucopolysaccharides and nucleoproteins. Tissue substances that are stained metachromatically do not appear to be the same color as the dye used but rather acquire a reddish shade.

**Methlene blue, Toluidine Blue** is a common basic dye and counterstain, which shows metachromasia in combining with certain sulfated mucopolysaccharides.

**Mucicarmine** (Muci) is an aluminum carmine compound that stains mucins a bright red.

**Neutral Red** demonstrates mast cell granules.

**Osmium** (osmic acid) demonstrates myelinated nerve fibers in peripheral nerves.

**Papanicolaou** demonstrates pituitary cell types.

**PAS-PTH** is a combination stain of periodic acid Schiff and phosphotungstic acid hematoxylin. It allows the simultaneous staining of mitochondria, muscle fibers, etc. with basement membrane, glycogen, etc.

**Periodic acid Schiff** (PAS) stains glycosylated structures rich in polysaccharides, mucopolysaccharides, glycoproteins and glycolipids. The periodic acid selectively oxidizes 1, 2 glycols and 1, 2-amino alcohols, thus splitting off free aldehydes which are then detected in situ by forming a stable complex with---the Schiff reagent, appearing as a reddish-purple. Tissue constituents stained various shades of red by this technique include glycogen, basement membranes of epithelia, mucins (intestinal and salivary gland mucoids), and colloids of thyroid and anterior pituitary. It is most frequently used in your slide collection to show basement membranes and the glycocalyx.

**Phosphotungstic acid hematoxylin** (PTAH) stains mitochondria a deep blue. Collagen and ground substance of cartilage and bone stain yellow to brownish-red. Many fibrous elements of tissue, such as fibroelastic fibroglial, myoglial and neuroglial fibrils, striated muscle fibers and fibrin, stain blue. Coarse elastic fibers may stain purple as may the intercalated disks of cardiac muscle.

**Resorcin-fuchsin** (RF: Weigert's resorcin fuchsin) stains elastic fibers deep purple to violet. Nuclei stain paler red, cartilage matrix violet and cytoplasm yellow.

**Silver methods** entail the impregnation of blocks or sections of tissue with silver compounds (AgN03) and a subsequent reduction of the so-called argyrophilic tissue elements to free silver. The various techniques are used to demonstrate nerve axons, reticular fibers and melanin.

**Tetrachrome** is a mixture of hematoxylin, orange G, fast green, and chromotrope 2R, an acid dye that stains the cytoplasm mildly pinkish. The orange G stains the erythrocytes orange in this preparation.

**Trypan blue** is a colloidal dye, injected intravally to demonstrate phagocytosis.
Verhoeff’s hematoxylin (VH), an empirical stain, stains elastic fibers and nuclei a clear blue-black or black. Fibroglia, myoglia, neuroglia, collagen, RBCs and fibrin may stain pink (orange-red).

Wright’s stain contains eosin and a series of methylene blue derivatives, especially azure B. Used almost exclusively for blood smears and marrow smears. Erythrocytes, pink; nuclei, deep blue; basophilic granules, deep purple; eosinophilic granules, red to red-orange with bluish cytoplasm; neutrophilic granules, reddish brown with pale pink cytoplasm; granules of monocytes, azure; lymphocyte granules, more reddish than monocyte granules with sky blue cytoplasm; platelets, violet to purple.

As you can readily appreciate, the tissues you study under the microscope have undergone a considerable number of alterations during their preparation for visualization in the microscope. Water soluble components are lost, and lipides are readily removed in the lipid solvents. During sectioning artifacts may be introduced in the tissue. You will learn to recognize these during your study of microscopic slides and to distinguish between artifacts and pathological changes.

Specimen Preparation for Light Microscopy

What you’re actually looking at on the stage of your microscope is a section. Tissue selected for microscopy is removed from an animal and fixed by immersion in (usually) formaldehyde. Fixation is necessary to prevent deterioration from microbial attack and autolysis. Following this step, the fixed tissue is washed, dehydrated in a series of increasingly concentrated solutions of ethanol, and embedded in wax. The wax impregnated block of tissue is chucked into the holder of a microtome, a device which (as the name implies) cuts very thin slices off the block. "Very thin" in this case means somewhere on the order of 2 to 7 mm thick. You can see that this is, for all intents and purposes, a two dimensional slice.

The section is collected on a glass slide, and the wax removed by dissolving it in xylene. Water is then replaced by rehydration (back down through the alcohol series) and the tissue is stained. Staining is needed for contrast, because unstained tissue has much the same refractive index as glass, and would otherwise be invisible. The stain imparts colors to the tissue, permitting differentiation of structures. The most common stain is hematoxylin/eosin, which is really a combination of two dyes. Hematoxylin comes from the Peruvian logwood tree (Haemotoxylon campechianum), and has an affinity for acidic materials. It binds to nucleic acids, and stains nuclei well. Eosin, the other component, is a dye derived from coal tar, and originally used as a fabric dye. Its name comes from "eos," the Greek word for "dawn," and the dye has a rosy hue like the sun at dawn on a clear morning (Homer sings of the "...rosy-fingered dawn..." in the Iliad and the Odyssey). There are thousands of other stains, but only a few dozen are commonly used. In the course of lab exercises, as new stains are mentioned, their properties and staining affinities will be discussed.

After staining has been completed, the now colored section is again dehydrated (back up through the alcohol series, like a party-goer on a Saturday night) and finally covered with a glass coverslip cemented in place with a clear material such as Canada Balsam or "Permount." It's dried and ready to examine in the microscope.

Appendix: Electron Microscopes

The microscope you are using is a brightfield light microscope, the most common type. There are many others, and their operating peculiarities, advantages, and disadvantages, are too long to go into detail. If your interest has been piqued, I suggest reading any one of the numerous books available on microscopy.

There are, however, two other microscopes you should have at least a nodding familiarity with since it’s likely that in the course of your studies and/or practice you will encounter images made with either the transmission electron microscope or the scanning electron microscope. Aside from size and cost, one major difference between these and the ordinary light microscope is that the specimen must be examined under a vacuum; electron beams are stopped by gas molecules.
The Transmission EM

The transmission electron microscope (TEM) dates from 1931, and it operates very much like the brightfield light microscope does. However, in the TEM the "light" is actually a beam of electrons, produced by energizing a tungsten filament with high voltage, so that electrons come off it. The condenser lens focuses the beam and maintains it in a stable position.

Lenses

The TEM's lenses are not made of glass: they are, rather, electromagnetic coils. The charged particles composing electron beams can easily be focused using magnetic fields, and much of the circuitry in a TEM is dedicated to producing stable and reproducible lens currents.

A TEM has condenser and objective lenses, just as the light microscope does; but instead of an ocular, the primary image is passed to an imaging system which is the functional equivalent.

The imaging system consists of another lens, the projector, which casts the image onto a flat metal plate coated with a phosphorescent material, much like the coating on a TV screen. When an electron hits this coating, it causes the coating to glow. In those areas of the viewing screen where many electrons hit, the glow is bright; in others it's dim to blank, in proportion. The image created on the plate is really a highly detailed shadow of the specimen that is in the beam. It's sometimes called a "mass density map" because it reflects the different densities in different parts of the specimen. Below the viewing screen is a camera chamber, holding a sheet of film. The screen can be moved out of the way to expose the film to make a permanent record of the image (photographic emulsions are sensitive to electrons).

Contrast in the TEM

In a light microscope, the image is seen in color, and contrast is achieved by staining the specimen with different dyes. In the TEM, since there is no light, and since the electron beam has only a single wavelength, color has no meaning. Contrast is achieved instead by introducing variable density in the different parts of the specimen. Instead of dyes, "staining" for the TEM depends on the impregnation of the tissue with heavy metal atoms, typically uranium and lead. These serve to scatter the electrons passing through the specimen, so that areas with low levels of impregnation produce bright regions on the screen, and heavily impregnated areas produce dark ones (the electrons can't get through). All shades of gray are represented by partial transmission.

The Scanning EM

The scanning electron microscope (SEM) is a relatively recent invention. Although the theoretical principles were quite well understood more than 50 years ago, the realization of the SEM as a practical device required advances in electronics far beyond the frontiers of the early 1930's. The Second World War gave a considerable impetus to the development of related technology, as did the massive expansion of the television industry in the post-war period. Nevertheless, it was not until the early 1960's that SEMs were commercially available, and even then their application to the life sciences was limited: the first commercially supplied SEM was sold to a paper company! Today they find their greatest use in the manufacture of microchips.

The SEM is unmatchable in its utility for examining surface structure, which is why chip producers use them. All SEMs are constructed with a specimen stage that permits the object under scan to be rotated, tilted, and traversed from side to side and back and forth. Thus, unlike a conventional microscope, it's possible to view almost all parts of the specimen by twisting it around in various ways to get a different vantage point. This permits an "observer" to examine any point on a surface, so long as a straight line can be drawn between that point and the "observer," whose virtual position is at the top of the microscope, looking "down" at the specimen.
Parts of an SEM

The SEM has three principal components: the control console contains circuitry to maintain stable lens currents, evacuate the specimen chamber, produce a beam of electrons, and so on; the display output produces the image in the form of a television type picture on a cathode ray tube (CRT); and the column contains the specimen and the means of observing it.

During observation, the column is under vacuum, and the specimen is at the bottom. At the top is the electron source, a hair-thin, V-shaped filament of fine tungsten wire. This is heated to incandescence in the evacuated column by passing a high voltage, low amperage current through it. It glows, exactly as does the filament in a light bulb, and electrons boiling off it are channeled down the column as a tightly coherent beam, aimed directly at the specimen. The cohesiveness and direction of the beam is controlled by electromagnetic lenses, hollow coils of fine wire whose magnetic fields can be controlled from the console to provide for focusing and magnification changes.

As the name of the instrument implies, the beam is not static: it "scans" the specimen. The beam moves over the specimen from one side to the other, drops down one line, then returns and completes another sweep at the lower level. When the last sweep is completed, the beam returns to the start point, and begins again to scan, back and forth, down, and repeat. The scan rate is controlled by the console circuitry.

When a beam of electrons impinges on an object, it knocks other electrons loose from the object itself. These secondary electrons scatter in directions determined by the angle of incidence of the beam, and by the surface topography of the specimen. Secondary electrons are detected by inserting a detector near the specimen. The detector produces a variable voltage output; the more secondary electrons it detects, the greater will be the voltage generated. This signal, in the form of a fluctuating voltage, is used to modulate the output of an electron gun on a cathode ray tube (CRT). When the signal is high, the display CRT’s electron gun produces large numbers of electrons, and when it’s low, small numbers. The beam generated in the CRT thus corresponds in its output to the secondary electron emissions of the specimen.

The SEM Image Display

The CRT beam is not static, either. It sweeps back and forth across the face of the tube (exactly as in a TV set, which is what a display CRT actually is) and its sweep is synchronized with that of the electron beam in the column. When the column beam knocks off large numbers of secondary electrons from some point on the specimen, the CRT beam produces a bright spot on the viewing screen. Conversely, small numbers of electrons produce low CRT beam output, and a dark point on the viewing screen. Thus the SEM image is really an analog of what is going on inside the column; it’s constructed, point-by-point and line-by-line, based on the information supplied to the CRT by the detector circuitry. The same process is used to produce a television image from a transmitted signal, and an SEM can be considered as a sort of gigantic light bulb hooked into a TV set.

Similarities and Differences in EMs

The two types of electron microscope have some similarities and some differences. They are similar in that they both examine specimens under vacuum; but they differ in how the images are produced. The TEM, as the name implies, actually relies on the transmission of the beam through the specimen: the direct physical interposition of the specimen into the beam is necessary to produce the image. The SEM, on the other hand, can examine specimens much too thick for the beam to pass through, and its image is produced electronically by modulation of the CRT output based on the detector signal. Therefore, no physical connection exists between the object and its image; the column can be in one room and the display CRT in another. So long as the electronics are intact, the image can be produced. Furthermore, contrast and brightness can be adjusted, as on a TV set, with console controls. Photographic records are made by actually making images of the CRT screen. Alternatively, the output can be fed to a videotape recorder.
Appendix

Maintenance of the Omega 1200

- Always cover your microscope with the dust cover when not in use. When cleaning the lenses, use lens paper or a cotton swab dipped in lens cleaning solution.
- Excess oil should be cleaned off at once. An alcohol pad is best for removing oil from the stage mid from the other metal parts, buds not recommended for use on die lenses.
- Dust in the nosepiece or ocular tubes should be blown out using only filtered air. Canned air dusters work well for this job.
- Whenever you remove an objective, we recommend that you place it back into the original plastic shipping vial until ready to be placed back on the microscope. This will keep the objective safe from dust and other foreign matter.
- To keep your microscope in top condition for years, we recommend that you have the microscope professionally serviced oil once a year.

Operation of the Omega 1200

1. Turn the light on using the black rocker switch on the side of the base. Next, adjust the light intensity using the side switch located on the same side but further back on the base. **Note**: Rapid, repeated changes in light intensity will dramatically shorten the life of the quartz halogen lamp.
2. In order to expedite your familiarity with the controls, choose a specimen slide you're familiar with, such as an old hematology slide or a commercially prepared slide. Place the slide into the slide holder by pushing back on the black plastic thumb guard and laying the slide flat. Allow the slide holder "finger" to **gently** hold the slide in place. **Note**: Do not allow the slide holder to "snap-back" against the slide as this could cause the slide to chip or shatter.
3. Move the slide to the center of the stage, by turning the stage and slide control knobs, just below the stage. These knobs allow you to move the slide in the X-Y axis (forward/backward and left/right).
4. Open the aperture diaphragm on the Abbe condenser (controlled by the small black lever on the condenser). Insert the filter of your choice in the filter holder (flip out holder located just below the condenser).
5. Once you are comfortably seated, look into the oculars and move the eyepiece tubes together or apart until you see only one complete circle of light. You have now adjusted your inter-pupillary distance.
6. Using the 10x objective and the coarse and fine adjustment knobs, bring the field of view into focus. Now move the 40x objective into place. You will feel a "clicking" action when the objective is seated properly. Again, focus for the best image. You will now be in the middle of the focus range. You may have to adjust the aperture diaphragm (on the condenser) for the best contrast.
7. **Diopter Adjustment**. Since you are using a binocular microscope, you have to adjust for the normal difference in vision between your two eyes. This is a simple but critical adjustment! The Omega 1200 has dual diopter adjustment rings (on the eye tubes). You can choose to adjust for either your left or right eye. Typically, you adjust the left diopter ring. To begin, set the right diopter ring to the zero (0) mark. Close your left eye and look into the right ocular with your right eye. Adjust the coarse and fine focus to give you the best image. Next, close your right eye and look into the left ocular with your left eye. Adjust the left diopter ring until you see a clear, focused field. Your diopter adjustments are set.
8. **Stage Locking Lever**. Omega 1200 has a special "Locking Lever" to prevent the accidental breaking of a slide by raising the stage too high. The locking lever is on the left side of the microscope, between the coarse adjustment and the body of the microscope. To engage this feature, adjust the stage to a maximum height for your normal use. Next, move the lever to the lock position. The stage will no longer move beyond the point you have established. To disengage, move the lever back to the original position.
9. **Tension Control**. With repeated use and wear, the stage may drift out of focus. If this happens, you need only to **Tighten** the tension control ring (located on the right side of the microscope between the coarse adjustment and the body of the microscope)