1 Using the Microscope

Upon arriving in the pathology department, you will most likely be given a microscope of your own. Learning to operate the microscope effectively is the prerequisite to everything else in this book. We will begin with the basics: how not to hurt yourself.

Ergonomics

Many pathology residents have acquired new and painful musculoskeletal complaints after a few months at the microscope. Here are the general principles to avoid injury.

- A neutral neck: When looking through the eyepieces, your neck should be in a neutral position, meaning no active muscle tension is required to maintain the position. Your eyes should be pointed directly forward or slightly downward. Bad positions are those that involve flexing your neck (dropping your chin to your chest), jutting your chin forward, or turning your neck left or right. Tilt-head microscopes are optimal for this positioning, but three-ring binders under the microscope can also adjust the tilt. Your eyepieces should make no more than a 30° angle with the desk surface.
- A straight back: Your mom was right about your posture, a straight back is better than a slouch, but you will need some help in the form of a chair with a supportive back. Your chair should hold you upright so that your head and neck can sit comfortably on top of your spine, without having to crane your neck forward. This can be accomplished by either adjusting your chair back to a more vertical position or adding a support pillow. Always sit directly in front of your microscope; having it off to one side to make more room on your desk will quickly cause back and neck pain.
- Supported elbows: You will be using two hands all the time, one to drive the slide and one to focus. Either job can be done with either hand, but both elbows need to be supported on the desk. Leaving your elbows floating in space while doing fine movements with your hand will lead to a nasty parascapular back spasm. Therefore, your chair should be high enough that you can place your forearms flat on the desk in front of you, with your upper arms perpendicular to the floor and flat against your torso. This may create a new problem for your neck (see the first point) if your microscope is not tall enough to meet your eyes. A good thick book or two under the microscope should fix this problem. Shorter people may also require a footstool to maintain this chair height.
- A padded surface: Your driving hand will probably rest on its elbow, while your focusing hand will lay flat on the desk. For both arms, the point of contact with the table should be padded to avoid a compression neuropathy (often the ulnar nerve). Possible solutions involve pieces of rug or bathmat, sponges, mouse pads, or commercial gel pads designed for desk users.

- Pay attention: When something starts to hurt, take a moment to critically analyze your posture and position. Focus on which muscle group is hurting you and what action relieves it, and jury-rig a way to achieve the more comfortable position. You cannot "push through" the pain; you will only end up with a chronic repetitive motion injury that will be with you for months or years. Once the cycle of pain and muscle spasm has begun, it can be very difficult to reverse it, short of taking a few months away from the microscope.

The Parts of a Microscope

Figure 1.1 shows an Olympus BX40 microscope. The exact positions of the various knobs and rings may vary by microscope, but all of these elements should be present.

1. Light source (Light from the bulb at the back of the microscope is directed upward by a mirror, hidden within the microscope base.)
2. Field diaphragm (The width of this diaphragm is controlled by the knurled ring. Closing this diaphragm reduces the visible circle of light illuminating the image. A neutral density filter, optional and removable, sits atop this diaphragm.)
3. Screws to center condenser (one on each side)
4. Focus knobs (coarse and fine)
5. Knob to raise and lower condenser, focusing the light to achieve Köhler illumination
6. Flip knob to move the condenser out of the light path for viewing at lowest power
7. Eyepieces with diopter adjustment ring
8. Objectives
9. Stage for the slide (The slide holder has been removed from this stage, allowing free movement of the slide, which is preferred by many pathologists.)
10. Aperture diaphragm of the substage condenser (The knurled ring controls the size of the cone of light reaching the specimen, and adjusting it causes changes in image contrast and

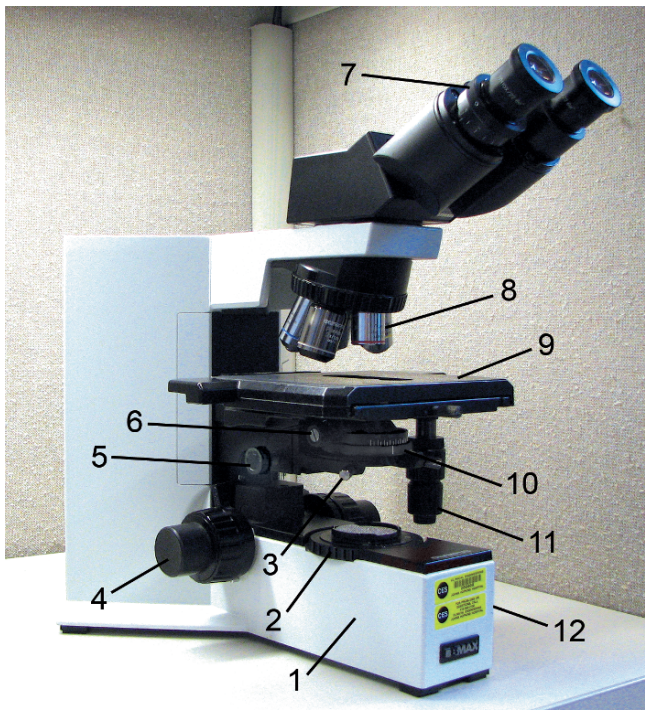

Figure 1.1. Diagram of the parts of a microscope.

quality. The substage condenser itself is the conical lens housing that sits on top of the diaphragm, hidden by the stage in this view.)
11. Knobs to move the stage (which allow for controlled X- and Y-axis movement when the slide holder is in place)
12. Light intensity adjustment (not seen; the voltage, or brightness, of the light is controlled by a knob or sliding bar)

A review of optics

There are excellent Web sites and books out there for a thorough technical review of Köhler illumination in microscopes. This is not one of them. However, the essence is that light is passed up through the microscope and focused down to a point image or spread into a wide cone through the use of lenses and diaphragms. The light originates at the light bulb at the back of the microscope, is redirected upward by a mirror, and is first shaped by the field diaphragm at the base of the microscope. Like a spotlight, this diaphragm directs a column of light up toward the slide. This column of light is concentrated into a tighter beam of light by the condenser, which results in illumination of the specimen with an even, bright, flat light.

When an image or beam of light is sent through a lens, there is a point on the other side of the lens at which the light rays converge to a point and the image is in sharp focus. In the eye, ideally, this point is at the retina, but if the eye is too long or too short relative to the lens, corrective lenses are required. In the modern microscope, there are many lenses and diaphragms in series, but there are essentially two light paths, and each one is in focus (converging to a point) at multiple different levels of the microscope.

One path is the image of the tissue. There are four points within the microscope where, if you placed a tiny projector screen, you would see a focused image of your tissue; these are called the *conjugate planes*. The conjugate planes of the image path are (1) the field diaphragm, (2) the slide or specimen, (3) the fixed diaphragm within the eyepiece (at the bottom of the removable eyepiece), and (4) a point above the microscope where you put your retina or the film of your camera.

The second path is the image of the light bulb filament. At certain points along this path, a tiny projector screen would show an image of the light source; this path is designed to have different conjugate planes than your tissue image, because, at the level of your tissue, you want a wide *unfocused* source of light. The conjugate planes of the light source are (1) the light bulb itself, (2) the condenser's aperture diaphragm, (3) the back focal plane of the objective, and (4) the "eye point" immediately above the microscope that corresponds to about where your cornea should be.

To achieve Köhler illumination is to align all of these lenses and diaphragms such that the conjugate planes are exactly where they should be, creating the best image your microscope is capable of. Fortunately, it is possible to learn this technique without fully understanding the physics behind it. You can certainly use the microscope without knowing how to do this, but the image quality will not be great, and neither will your diagnoses.

Achieving Köhler Illumination

- Place a slide on the stage. Adjust the eyepieces so that they are the correct distance apart for your eyes.
- Focus on a slide using your 10× objective. For microscopes with only one adjustable eyepiece, close the adjustable eye, and focus using the regular focusing knob. For microscopes with two adjustable eyepieces, either eye can be used first.
- Once the fixed eyepiece is in focus, shut that eye and focus the other eye with the eyepiece ring. The scale on the eyepiece ring shows the diopter adjustment; the positive direction is analogous to reading glasses, so it is easier on the eye.

- Make sure your aperture diaphragm on the substage condenser is completely open (this may be clockwise or counterclockwise, depending on the microscope).
- Close down the field diaphragm until you see a small circle or octagon of light. It should be in the center of your field of view and have a crisply focused edge. If not, you can center it using the small screws on the condenser and focus it by raising or lowering the condenser.
- Open the field diaphragm back up so that light completely fills your field of view.
- For most work, this is sufficient to give optimal viewing conditions. However, for viewing translucent (unstained) structures, or for photography, you also need to optimize the aperture diaphragm. Notice that closing it down dims the light and creates a three-dimensional quality to the image, whereas opening it up creates a flatter, brighter image. The optimal diaphragm size closes down the light path to match the diameter of the objective so that the light rays coming up from below make a straight, parallel column of light into the objective, minimizing scatter. This size is different for each objective. To find it, you must remove an eyepiece and look down into the eye tube. You will see a circle of light; close the aperture diaphragm (the ring on the condenser) until the outer one fourth of the field is black. Replace the eyepiece.

Becoming Parfocal

Parfocality means that if an image is focused at 40×, you should be able to switch to 4× and still be in focus. It is not the same as Köhler illumination. You can achieve parfocality only on a microscope with two adjustable eyepieces; it is most important on multiheaded microscopes, when the observers at the additional heads need to be in sync with the person controlling the focus. The beginning of a session with multiple users on a multihead microscope should always start with this focusing ritual.

- Start by adjusting the eyepieces on the main microscope head to the neutral position, or zero diopters. The person driving the microscope should first adjust for Köhler illumination, as above, and then go to 40× and focus on the slide. (If using a camera that projects to a TV or screen, focus the microscope such that the TV is in focus.) While the driver adjusts his or her own eyepieces, all observers should also adjust their own eyepieces to optimal focus.
- Now go to 4× without moving the slide or touching the main focus knob. While at 4×, the driver and all observers should readjust their eyepieces to be in focus. Now the screen and each individual should be in focus at each objective, or parfocal.
- If one objective is slightly "out," make sure it is tightly screwed in to the objective carriage. Sometimes one objective just can not be made perfectly parfocal, but if the above procedure is followed, at least the observers will be in sync with the driver, who can make corrections using the main focus knob.

Cool Microscope Tricks

Some things on slides do not pick up stain and therefore appear transparent or translucent on the slide. Good examples are calcium oxalate and suture material. They can be essentially invisible during normal viewing but will glow under polarized light. However, most residents' microscopes do not have polarizers. A quick and easy substitute is to flip the condenser out of the light path, just like you do when viewing at 2×. This will cause refractile material to "pop out" and be easily visible.

The knowledge of different paths of light being focused at different planes can be useful. For example, if you are looking at a slide and see debris or dust in sharp focus, that debris must be located in one of the same planes in which the image path is focused: on the surface of the field diaphragm, on the slide itself, or at the fixed eyepiece diaphragm. This diaphragm is located at the bottom of the eyepiece, in the tube, and is not usually exposed to dirt. The eyepiece diaphragm is the position where an ocular micrometer sits to superimpose a tiny ruler

on your image. On the other hand, if the debris is out of focus when the image is focused, it is more likely to be on the condenser or the top of the eyepiece.

Sometimes, at a multihead microscope session, you would like to give everyone a very low-power view of a slide, even lower than the 2× objective. The slide itself can be placed directly on the field diaphragm at the base of the microscope. This focal plane is in sync with that of a slide on the stage, so you will actually get a reasonably focused image of the entire slide. This trick also works with Kodachrome slides.

If your slide stubbornly refuses to come into sharp focus at high power, it is probably one of two problems: either the slide is upside down (coverslip on the bottom), or the objective is dirty (either a fingerprint or oil from the 100×). A dirty 40× is hard to clean, so many residents avoid ever using oil immersion on their own microscopes. If you do have a rotation that requires use of the 100×, consider arranging the objectives so that the 100× and the 40× are not next to each other, reducing the chances that you will drag the 40× through a puddle of oil. The lower power objectives are usually far enough from the slide that they pass above the oil slick.

Eyeglasses

For your average moderately nearsighted scholar, it is better to use the microscope without corrective lenses (glasses or contacts) in place. The microscope eyepieces can correct for mild to moderate vision problems, and it is easier on your eyes without an additional lens in the way. However, for more severe vision problems, or for those with astigmatism, it may be necessary to work with corrective lenses on. If you must wear glasses, there are special "high eye point" eyepieces that can be purchased. These account for the fact that because of the glasses on your face, your retina is farther from the eyepiece than if you were not wearing glasses. They may be a good investment for residents who must spend long hours looking through the microscope.

Motion Sickness

There are some unfortunate individuals out there who are very sensitive to vestibular–ocular mismatches. If you are not one of them, you may disregard this section. For some reason, having a moving image that fills most of your field of view while your body is motionless can trigger, essentially, car sickness. This phenomenon is usually only a problem when someone else is "driving," or moving the slide around, but as a resident you do quite a bit of observing while the attending drives. Some drivers are better than others; the habit of constantly moving the slide, as opposed to quick movements with long pauses, is particularly nauseating for the susceptible. Here are some suggestions to get through this unpleasant experience:

- Be reassured that you will quickly get your sea legs. Most people have to battle with this for only a few weeks before their vestibular systems adjust.
- If the experience is really bad, consider medication. There are over-the-counter medicines for this. Meclizine, sold as Bonine, does not cause as much drowsiness as Dramamine.
- If you have an unexpected episode and you are stuck at the microscope for an indefinite period of time, you need to reduce the amount of moving images hitting your eyes. If you are in a conference with the microscope hooked to a TV monitor, watch the monitor instead. Another option is to let your head sink down just enough that the images hit your eyelids, not your eyes; this is subtle, and you can straighten back up when the attending asks, "What do you think of this?" You can also close your eyes while the slide is moving, but this is a little more obvious. Studying your paperwork, looking up the patient history on the computer, answering a page, or going to get the old biopsy material can all give you momentary breaks. In desperate times, you just do what you can.