

# Chapter 1

## Parts

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Figure 1.1 illustrates the parts of an upright compound microscope and indicates the terminology that I use in these notes.

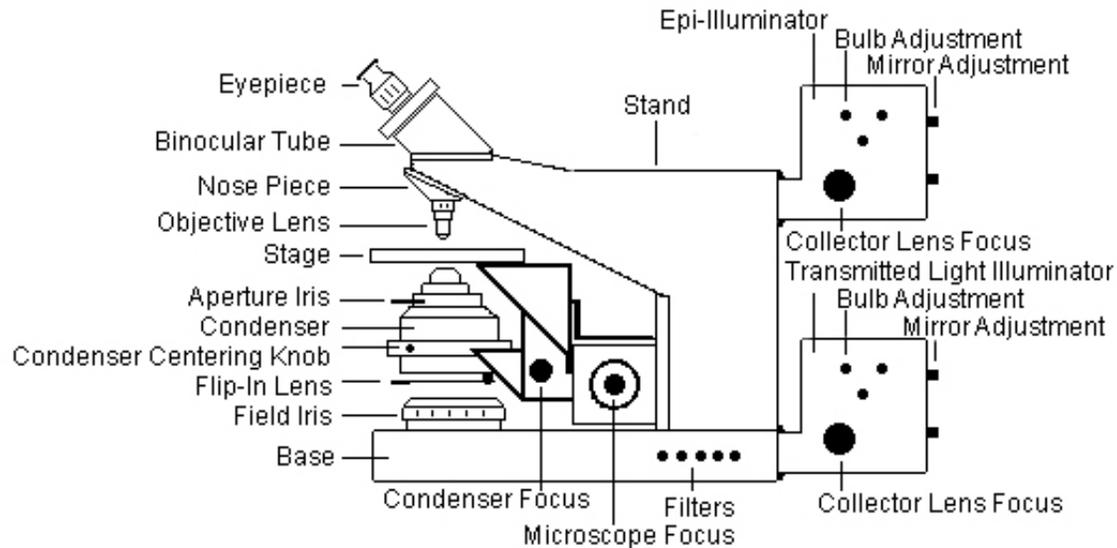


Figure 1.1. – Parts of a Compound Light Microscope

### Stand

The Stand is the solid support for the optics and mechanical parts of the microscope. We used to say that the stand is made of the **Arm** and the **Base**; the base being the bottom of the microscope and the arm the part that supports the objective lenses and eyepieces. Stands have evolved past this simple description. Over the years manufacturers have adopted many different configurations for the stand. Regardless of the exact style, there are two basic types: The **upright stand** has the objective lenses facing downward; the **inverted stand** has the objective lenses facing upward.

### Stage

The stage carries the specimen. On some microscopes it is the stage that moves when focus is adjusted. Stages are often equipped with mechanical devices for holding and moving the specimen. Special rotatable stages are available for polarization microscopy. Special specimen holders are made for stages on inverted microscopes.

### Nosepiece

The nosepiece is a revolving turret that carries the objective lenses. On some microscopes it is the nosepiece that moves when focus is adjusted. The thread size of the nosepiece positions was, but is no longer, an international standard enabling objectives

from any manufacturer to be mounted. It is not, however, a good idea to mix and match objective lenses. This will be discussed in Chapter 7. The nosepiece may be of the reversed type in which the lenses not in use are placed toward the arm rather than toward the microscopist. The arrangement of objective lenses on the nosepiece is traditionally in increasing order of magnification clockwise. There is no harm in breaking tradition.

### **Focus Knobs**

The ability to focus a microscope in very fine increments has been a goal long sought by manufacturers. Today's microscopes have focusing mechanisms that are more precise than the resolution of the microscope itself. Course, fine, and, sometimes, medium focusing knobs are present. Focusing can be achieved by moving either the specimen stage or the objective lens. On most inverted stands, the stage is fixed and the nosepiece moves. A mechanical focus mechanism usually has some means to tighten and loosen it. Mechanical focus mechanisms need periodic servicing. Their lubricant dries out, they become dirty and hard to operate, and they begin to drift. Servicing is best left to a qualified service engineer. Some modern, computer controlled microscopes utilize optical encoders in the focus knobs and a separate motor controlled by this encoder actually does the focusing. This enables automated computer control of focusing.

### **Condenser Carrier and Condenser Focus Knob**

The condenser must be raised and lowered in order to achieve Köhler illumination. The condenser focus knob does not require the precision of the microscope focus knobs. This focus mechanism may have a means of loosening and tightening it. It should be sufficiently tight to keep the condenser from drifting out of focus. The condenser carrier mechanism must keep the condenser optically aligned to the objective. Any amount of tilt of the condenser relative to the objective will degrade Köhler alignment. You can check for condenser tilt by observing the field iris as you adjust the condenser focus. The iris should come into focus around its entire circumference simultaneously. If it does not, get help from a qualified service engineer.

### **Binocular Tubes**

On microscopes equipped for binocular viewing, the eyepieces are connected to the stand by a binocular tube. There are two types of binocular tubes and they differ in the way they should be used. Figure 1.4 illustrates these rotational and translational types. Each microscopist must adjust the interpupillary distance of the binocular tubes to achieve a unified circular field of view. Then, each tube and/or eyepiece is adjusted to make a diopter correction for the microscopist's vision. Over time the prisms in binocular tubes can become misaligned making it difficult for the microscopist to see one circular field of view. Realignment of the prisms should be done by a qualified service engineer.

#### Translational Binocular Tubes

In the translational type of tube the eyepieces move horizontally when adjusting the interpupillary distance. Without some type of correction this movement would change the microscope's tube length (the distance between the back focal plane of the objective and the front focal plane of the ocular). There are several methods of correction based on the tube mechanism: (1) automatic, (2) both tubes adjustable, (3) one adjustable and one

fixed tube, and (4) neither tube adjustable. The eyepieces themselves are sometimes adjustable along with the binocular tubes. The 0 diopter position may be indicated on a scale on the tubes or oculars or by a line circumscribing the tube. If there is a photographic reticule, focus on it with the eyepiece(s) first. Here are instructions for using these four types of tubes.

#### **Automatic Tubes**

On some microscopes, correction is done automatically – the binocular tubes move in or out as the interpupillary distance is adjusted. One tube or eyepiece will be focusable. Adjust the interpupillary distance to achieve a circular field of view. Focus the microscope carefully on a specimen while viewing through the fixed tube, then adjust the focusable tube or eyepiece for your other eye.

#### **Both Tubes Adjustable**

If both tubes are adjustable, each tube will have a scale and there will be a scale between the tubes. Start by turning the tube scales to the 0 position. Set the interpupillary distance, then adjust the scale on each eyepiece tube to match the reading on the scale located between the tubes. Carefully focus the microscope on a specimen using your dominant eye, then carefully focus the specimen for your other eye using the focusable tube.

#### **One Tube Adjustable**

If only one tube is adjustable, set it to 0 on the scale, set the interpupillary distance, reset the eyepiece tube scale to match the reading on the center scale, fine focus the microscope while viewing through the fixed tube and finally adjust the focusable eyepiece for your other eye.

#### **Neither Tube Adjustable**

If neither tube is focusable then at least one eyepiece should be focusable. Set the interpupillary distance for your eyes. Adjust each focusable eyepiece to its 0 diopter position (if only one eyepiece is focusable, use it for your non-dominant eye). Carefully focus the microscope on a specimen while viewing with your dominant eye and adjust the other eyepiece for your other eye.

#### Rotational Binocular Tubes

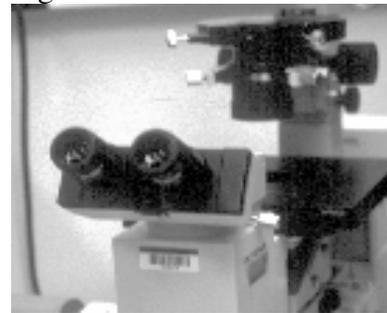
The prisms that create the binocular image in these tubes are rotated together with the oculars. There is no change in tube length and no adjustment is required.

With rotational binocular tubes, the oculars may be independently focusable and their adjustment depends on the type of instrument. In some instruments of both

Figure 1.4 Rotational Tubes



Figure 1.4 Translational Tubes



rotational and translational types, a built in reticule is brought into sharp focus for one or both eyes by adjusting the ocular(s). This assures coincidence of focus on the microscopist's retina and on the film plane of attached cameras. Other microscopes work as follows: Set one or both of the oculars to the 0 diopter position. This may be indicated by scale lines or by a single line encircling the ocular. Adjust the microscope for fine focus using your dominant eye (or using the fixed ocular if only one is focusable). Adjust the other ocular for fine focus for the other eye.

When electronic cameras are used they should have a method of making them parfocal with the microscope. Even so, the best method is to focus the microscope image on the computer monitor and then adjust each eyepiece.

## **Optics**

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### **Condenser**

The condenser is the bully of the microscope. It ultimately limits the resolution of the entire optical system; it provides the proper kind of light for Köhler illumination; it carries optical elements necessary for dark field, phase contrast, and differential interference contrast. The condenser iris (or aperture iris) controls the amount of contrast and the depth of field seen in bright field. Centering knobs are available for aligning an image of the field iris in the optical path. Movable lenses may be associated with the condenser. These lenses adapt the condenser for different objective lens magnifications. Usually they must be inserted for objective lens magnifications 10 X and above. These lenses also affect the numerical aperture of the condenser.

### **Objective Lens**

The beauty of modern microscopes rests in the objective lenses. The theoretical limits of resolution have been reached largely through the design of objective lenses. These are the jewels of the microscope. Chapter three will tell you how to care for them. Later chapters will cover their characteristics and proper use.

### **Intermediate Lens**

Some stands have one or more intermediate lenses between the objective and the eyepieces. These lenses (sometimes called optivars) provide extra magnification and may include a Bertrand lens that is used to examine the back focal plane of the objective.

### **Eyepieces or Oculars**

Oculars provide the second level of magnification in the microscope. They finish lens aberration corrections begun in the objective lens. They define the diameter of the field of view. Oculars are also used to make diopter corrections for individual microscopists and they can contain reticules for photomicrography or morphometry. Chapter 7 covers the various types of oculars.

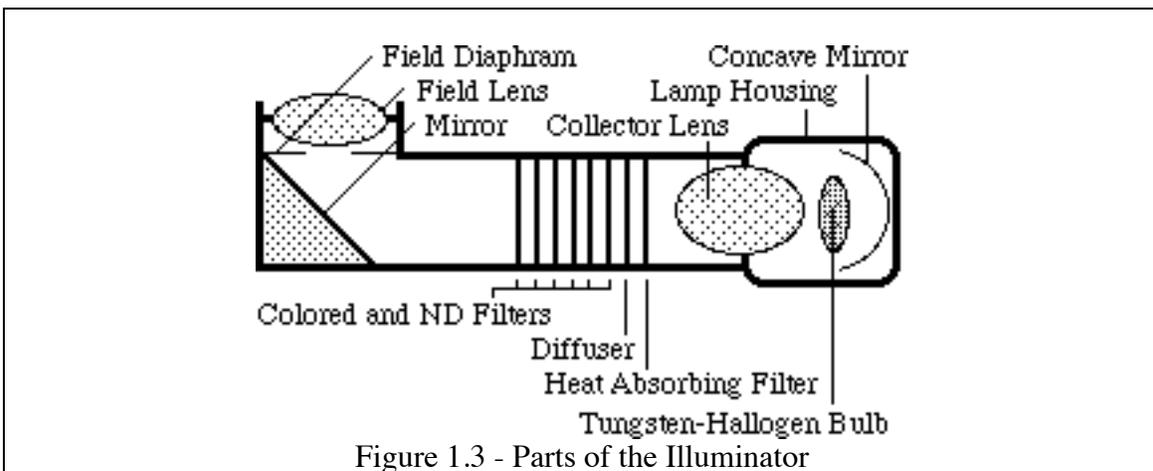
## Illumination System

The old microscopists believed that the best illumination came from the north sky. It was focused on the specimen by use of a concave mirror and it was a beautiful light with a remarkably constant color. It had drawbacks. The light did fluctuate in intensity, especially on cloudy days, and you couldn't work at night. Artificial light sources are more convenient and these are the types I will discuss.

Never-the-less, something is lost when beauty is sacrificed for practicality.

### Parts of the Illuminator

Figure 1.3 illustrates the parts of a modern illuminator as a section through the microscope's base.



### Lamps

There are several types of lamps used in modern microscopes. Table 1.1 lists and compares these. See figure 12.7 for lamp spectra. The tungsten-halogen lamp has become a standard because it maintains its brightness and color temperature throughout its lifetime. Some illuminators use a **concave mirror** behind the lamp to increase the intensity of light.

Voltage to the lamp is provided by a variable transformer of either 6 or 12 volts. Some transformers are continuously variable while others have fixed positions. The microscope's manufacturer will recommend a proper voltage setting for the type of lamp in use. This setting will provide the proper color temperature for photomicrography. Filters such as heat absorbing and color balancing will also affect color temperature of the light. Color temperature is important even for digital cameras.

The lamp housing can contain centering controls for the lamp and for the concave mirror. Access to these controls is usually through holes in the lamp housing. Not all lamps have these controls.

Light Emitting Diodes (LEDs) are developing into an additional light source for microscopy. Their advantages are high intensity at cool operating temperatures, fixed color temperature and long life. Their small size requires an array of LEDs with the attendant problems of diffusing the lightsource.

**WARNING:** Never touch a lamp with your bare fingers. Many lamps have a quartz envelope that may break due to uneven heating caused by contamination from fingers. If you touch a lamp, wipe it clean with alcohol.

Lamp Type	Spectrum	Intensity	Uses
Tungsten	Continuous Color temp at 3200K but drops with time.	High	General and photography
Tungsten-halogen	Continuous Color temp. at 3200K or 3400K	High	General and photography
Xenon arc	Continuous Color temp is daylight - 5500K	High	General and motion picture photography, Fluorescence
Zirconium arc	Continuous Color temp is 3200K	Moderate	General and photography
Mercury-vapor	Discrete: 546 nm, 436 nm and 365 nm plus others	High	Fluorescence

Table 1.1 - Comparison of Lamps for Microscopy (See Ch 12 Fig 12.7 for spectra)

Collector Lens

The collector lens sits directly in front of the lamp. In some illuminators, such as the mercury-vapor type, this lens is adjustable. It focuses an image of the lamp's filament onto the front focal plane of the field lens or of the condenser if there is no field lens.

Diffuser

The diffuser serves to break up the image of the filament. It may be built in and non-removable. In modern research level instruments, it should be removable. The ability to remove the diffuser is important in aligning the filament for phase contrast microscopy.

Filters

A series of filters may be present in the illumination system. A **heat absorbing filter** is used to reduce the amount of infrared light that passes into the rest of the optical system. This filter usually has a light blue color and can affect color micrographs. Various **colored filters** may be present. These are used to affect contrast and color temperature of the light for photomicrography, and to set the proper wavelength for phase contrast microscopy. **Neutral density filters** may also be present. These filters are used to alter the intensity of light without changing its color. For high precision polarization microscopy, a **light scrambler** may be incorporated into the illuminator. This device,

which consists of a coiled quartz optical fiber, insures that light leaving the illuminator shows no hint of uneven brightness or polarization.

#### 45° Mirror

The 45° mirror directs light into the field lens. This is a flat, first-surface mirror. It is easily damaged. A mirror is used, rather than a prism, because it introduces no refractive artifacts.

#### Field Lens of the Illuminator

Some illuminators have a field lens that serves to focus an image of the lamp's filament onto the front focal plane of the condenser.

#### Field Iris

The field iris, or **radiant field diaphragm**, is also an important item in Köhler illumination. It serves to limit the area on the specimen that is illuminated.

Modern microscopes may have more than one illuminator attached to the stand. In addition to the transmitted light (diascopic) illuminator there may be an epi-illuminator (episcopic) that is used for fluorescence or reflected light microscopy.

### **Exercises**

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- 1) Locate the parts shown in figure 1, or their equivalents, on your microscope.
- 2) Inspect the lamp in your illuminator. What are its type and wattage? Is it discolored? Do you have spare bulbs?
- 3) Observe the frosted end of a slide under low power. What happens to the color of the illumination as you decrease the lamp's voltage from full to minimum? The color of the light is very important in photomicrography.
- 4) If your microscope is a photo-microscope, determine the voltage of the various settings of your illuminator's power supply. Your lamp has specific color temperatures at specific voltage settings (Chapter 13 Photomicrography).
- 5) If you have an arc lamp, note the type of lamp and power supply. Is there a way of timing the number of hours the lamp has burned? Arc lamps degrade over time. Do you have a spare lamp?
- 6) Write down the information printed on each of your condensers, objectives, and oculars. This information will be needed later on.
- 7) Determine your dominant eye: Cut a hole about 2 inches square in a sheet of paper. Hold the paper at arms length directly in front of your face. Look with both eyes through the hole in the paper at some distant object. Close one eye and see if you are looking

through the hole or at the paper. Try the other eye. The eye that is seeing through the hole is your dominant eye. Knowing which eye is dominant is helpful in correctly adjusting the oculars.