

Chapter 1

Parts

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

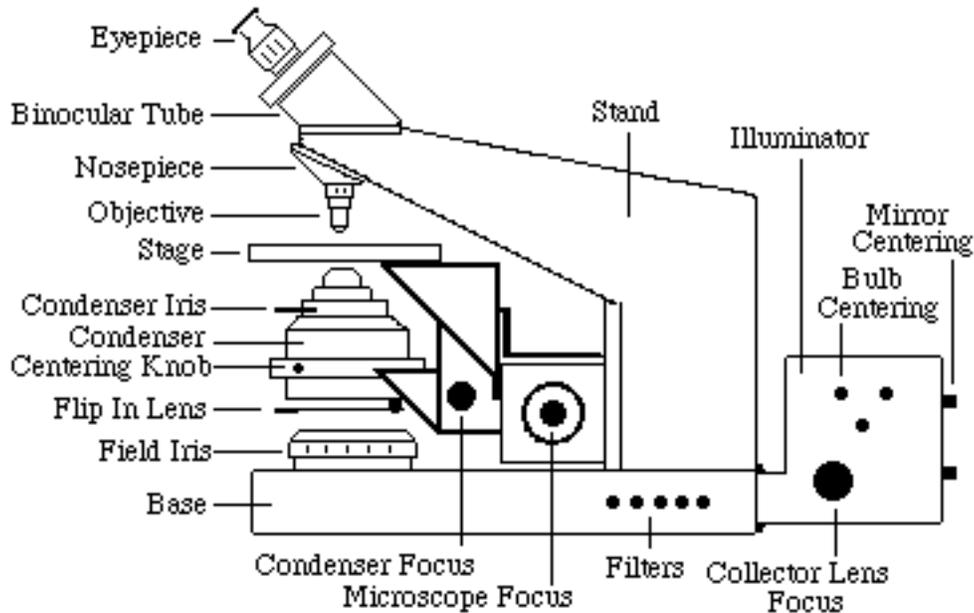


Figure 1.1. – Parts of a Compound Light Microscope

Stand

The Stand is the solid support for the optics and mechanical parts of the microscope. 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.

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. 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.

Illumination Methods

The quality of light, its color and intensity, and the way it is brought to the specimen, whether as diverging, converging, or parallel rays, greatly influences the appearance and resolution of the specimen.

Köhler Illumination

In the late 1800's, August Köhler invented an illumination technique that was so profound it is still the standard method used today. Modern microscopes are designed to use Köhler illumination. Köhler illumination uses optics that place the image of the filament in the back focal plane of the objective lens. This results in the light source being

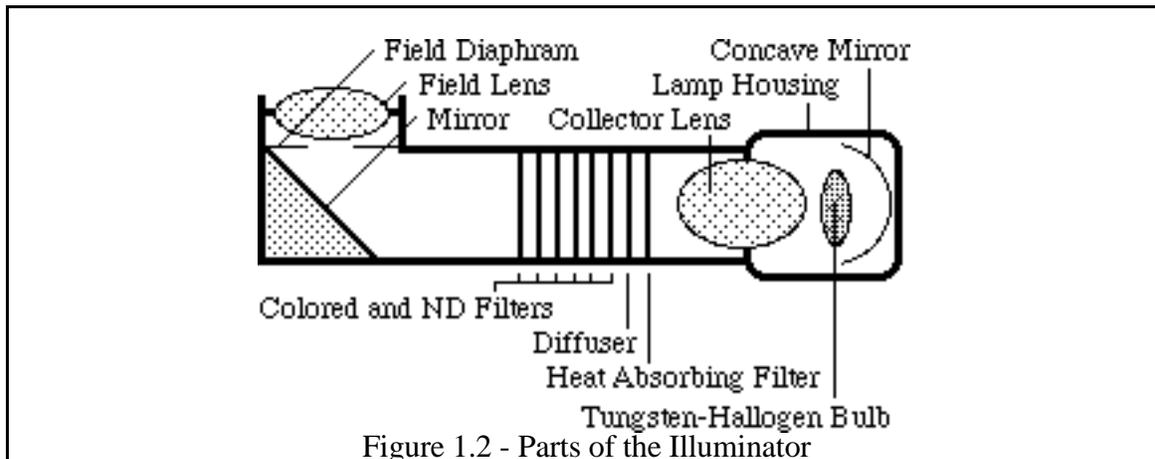
completely out of focus in the observer's eye. A small, inhomogeneous, coiled filament can therefore be used as the light source.

Critical Illumination

Köhler illumination is not the only method of illumination. A very different method, which was popular in the 1930's and 40's, is called Critical Illumination or Nelsonian Illumination after its inventor. Critical illumination forms a real image of the light source in the specimen plane. In so doing, the specimen acts as if it were a "self luminous" object. This has certain theoretical advantages when the physical optics of the microscope system are considered. I will discuss this further in the chapter on confocal laser scanning microscopy. One problem with critical illumination is that it requires a large light source that is very homogeneous since the image of the light source is in focus along with that of the specimen. A wide, ribbon-like filament was used for this purpose. The practical advantage of a small coiled filament far outweighs any theoretical advantage in image improvement due to critical illumination.

Parts of the Illuminator

Figure 1.2 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. 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.

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.

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.

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
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 microscopy

Table 1.1 - Comparison of Lamps for Microscopy

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.

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. A mechanical focus mechanism usually has some means to tightening 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. Appendix B lists USA contacts for microscope manufacturers.

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.

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.

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 rotateable stages are available for polarization microscopy. Special specimen holders are made for stages on inverted microscopes.

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. This chapter will tell you how to care for them. Later chapters will cover their characteristics and proper use.

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 is 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 arrangement of objective lenses on the nosepiece is

traditionally in increasing order of magnification clockwise. There is no harm in breaking tradition.

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 1.3 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 type of binocular tube: (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

Figure 1.3 Rotational Tubes



Figure 3.1 Translational Tubes



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 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 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.

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.

Exercises

- 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?
- 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.
- 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.

Chapter 2 Alignment

Figure 2.1 is an image of striated muscle taken with a misaligned microscope and figure 2.2 is with a properly aligned microscope. In 2.1 the illumination is uneven and artificial fringes have been added to the image. In 2.2, the illumination is even, there are no artificial fringes, and resolution is higher.

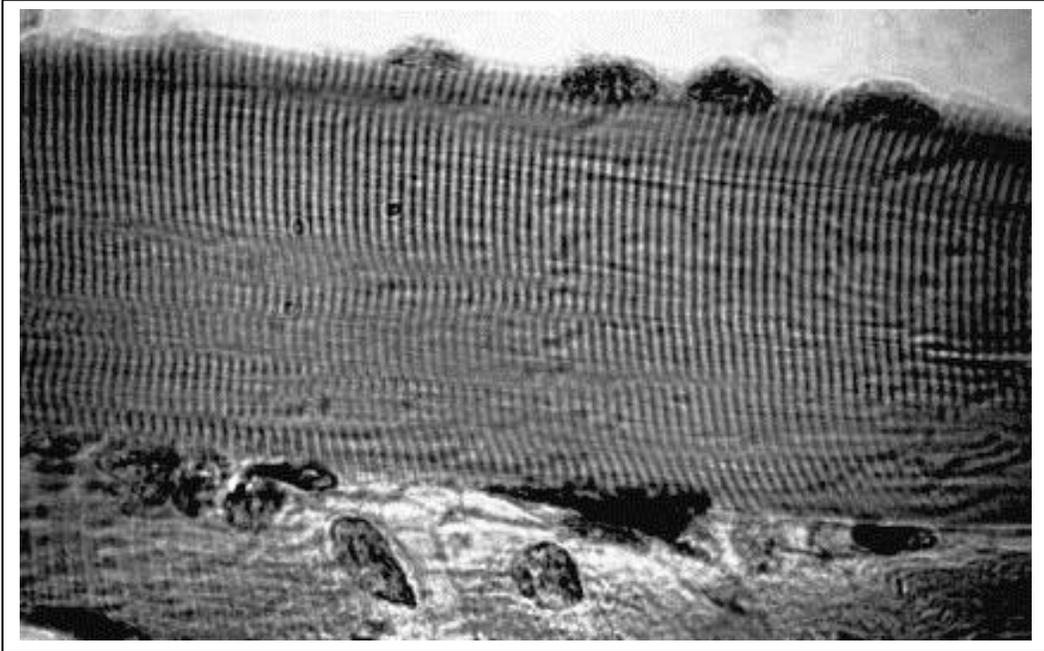
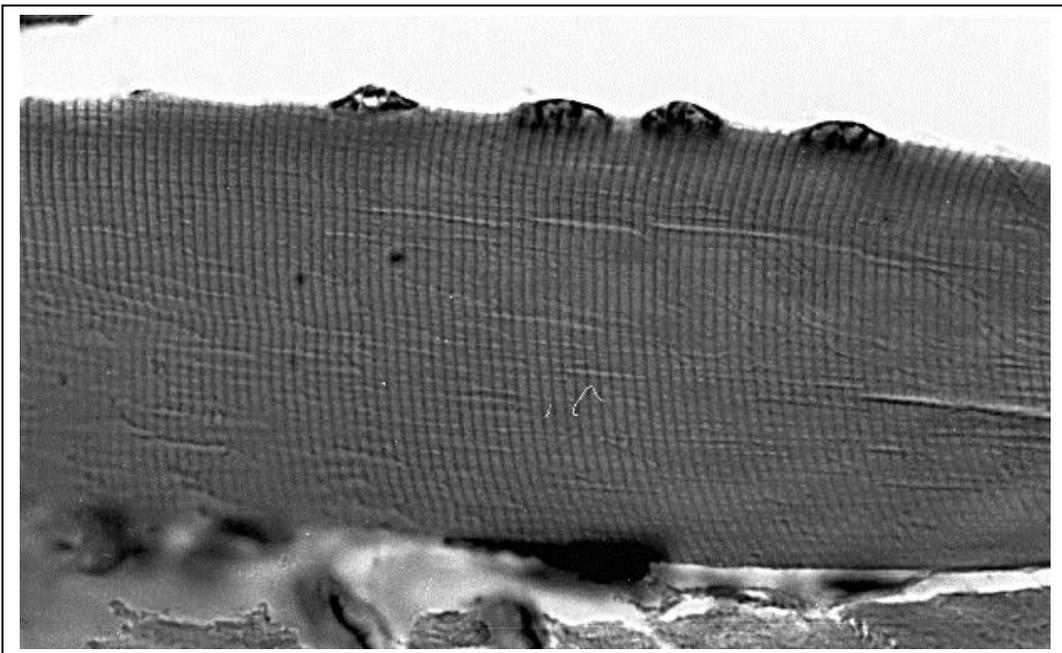


Figure 2.1 Micrograph from a badly aligned microscope.

Figure 2.2 Micrograph from a well-aligned microscope.



There are several methods of aligning a microscope, but the most common one used today was invented by August Köhler (figure 2.3) in 1893 while he was at the Institute of Zoology in Giessen Germany. All modern microscopes are designed for Köhler illumination and will function at their best only when aligned this way. The purpose of Köhler illumination is to provide partially coherent light to the specimen plane.

Figure 2.3



August Köhler

Köhler illumination involves the following sequence of steps. They should be done exactly in order. The sequence includes centration of the illuminator. This step is normally done only after replacing a lamp or after moving the microscope. An abbreviated method is listed later on. Common mistakes are (1) not starting with the field and aperture irises open, and (2) not beginning with a specimen in focus. If the microscope is greatly out of alignment, the specimen may go out of focus as the field iris is brought into focus. In this case the specimen should be refocused and then the field iris refocused. Both specimen and field iris should be in focus together.

- 1) Turn on the illuminator.
- 2) Open the field iris.
- 3) Open the condenser (aperture) iris.
- 4) Select a low power, dry objective lens (10 X or 20 X).
- 5) Put a specimen slide on the stage. A well-stained specimen is best.
- 6) Adjust the binocular tubes for your interpupillary distance. You should see one big circle of light.
- 7) Focus on the specimen as best you can.
- 8) Adjust the eyepieces and / or binocular tube to correct for your vision. See the section on binocular tubes in Chapter 1.
- 9) While observing the specimen close the field iris until its shadow appears.
- 10) Focus the field iris using the condenser focus knob. Try for a thin purple fringe at the edge of the iris. If you can't focus on the field iris, check the condenser for flip-in lenses and find the configuration that lets you focus on the field iris. If the iris moves out of the field of view as it is focused, bring it back into the field of view with the condenser centering screws.
- 11) Center the field iris with the condenser centering screws.
- 12) Open the field iris just to the edge of the field of view.
- 13) Pull out an eyepiece (you could instead insert a phase telescope or Bertrand lens – Chapter 10).
- 14) Remove the diffuser. (Some diffusers are not removable. If this is your case, go to step 17).

- 15) While looking down the empty tube, focus and center the filament image using the adjustment screws on the lamp housing. You should see neither the top nor bottom edge of the coiled filament nor its ends. Some illuminators have no adjustments.
- 16) Insert the diffuser.
- 17) While looking down the empty tube close the condenser iris to cover 10% to 50% of the full bright circle.
- 18) Put back the eyepiece.
- 19) When you change objectives, repeat steps 9 - 12, 17 and 18.

Tip:

When changing a lot between objectives, align the field iris for the lowest magnification and the condenser iris for the highest and forget repeating steps 9 - 18.

Exercises

- 1) Align your microscope for Köhler illumination. Include the bulb centering procedure if you can remove the diffuser. If you do not have a Bertrand lens or phase telescope for observing the objective's back focal plane, make a pinhole in some aluminum foil and use this as a cap over the eyepiece tube to improve this image.

Chapter 11

Differential Interference Contrast Microscopy

Many microscopic specimens are colorless, nearly transparent, and relatively thick, such as whole cells. Thick specimens do not give a clear phase contrast image. This chapter presents a method for producing contrast in this type of specimen. The resulting image is one that has a very topographic appearance. It looks as though you are looking down on the specimen while it is being lit strongly from one side. Figure 11.1 is a DIC image of cheek epithelial cells.

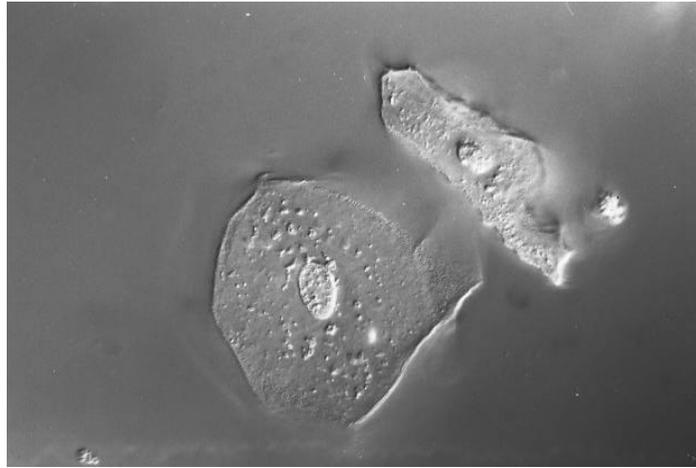


Figure 11.1 DIC of Cheek Epithelial Cells

Differential Interference Contrast (DIC) is optically a rather complicated method requiring several special optical components. These are described and their action in producing this beautiful image is discussed in the following sections.

Types of Specimens Suitable for DIC

Those specimens that would be suitable for phase contrast microscopy are also suitable for DIC. Furthermore, DIC produces clearer images of relatively thick specimens than does phase contrast. Cytological, histological, microbiological, and cell culture specimens are of this type as are chromosome spreads. DIC is especially useful in fluorescence microscopy to indicate the morphological aspect of fluorescent regions. DIC reveals some ultrastructural features of cells such as microtubules and cytoplasmic granules. When coupled with enhanced video techniques, DIC enables the visualization of microtrabecular structures that are actually below the resolution of the microscope. Finally, the use of DIC on normal, stained or unstained histological sections can provide additional useful information.

History of DIC

We owe the current method of DIC to the work of Georges Nomarski (figure 11.1) who developed it in the 1960's. It is based on an older method of interferometry microscopy. Nomarski was able to produce a modified Wollaston prism that he used with high magnification, high NA objectives. He altered the older method by using two of these prisms.

How DIC Works

Before tackling the specifics of Nomarski DIC, a quick summary of the technique will help organize the sections that follow.

In DIC, the illumination starts as a polarized beam of light. This is divided into two beams that have different polarization angles and that are separated horizontally by a distance roughly equal to the resolution of the objective lens. As these two beams pass through a specimen, changes in refractive index due to the fine structure of the specimen affect one beam more than the other as shown in figure 11.5. After passing through the specimen and the objective lens, the horizontal separation between the two beams is removed. In the final step, the two beams are converted to the same polarization angle. At this point, in the microscope's intermediate image plane, the two beams can interfere with one another to produce amplitude contrast. This contrast reflects the optical path difference between the two beams that was introduced by the specimen. Because the initial separation or shear between the beams is so small, the technique tends to be very sensitive to changes in the curvature of specimen structures. The differential in DIC is the difference in optical path length of the two beams. This difference results from different horizontal refractive index gradients in the specimen.

Figure 11.1
Georges Nomarski



Properties of Light Involved in DIC

In addition to the properties of light discussed with phase contrast microscopy, DIC also employs the polarization of light from the outset. Two plane polarizers are involved, one before the condenser and one after the objective. Birefringent prisms act on the plane polarized light first to split the beam in two, and later to recombine the two beams.

Polarization Angle

In Nomarski DIC, unpolarized light from the lamp is first plane polarized. Usually a dichroic material such as Polaroid does this. Dichroic materials absorb light. The amount absorbed depends on the light's vibration axis (i.e., polarization angle) relative to the major transmission and absorption axes of the polarizer. Light vibrating in the Polaroid's major absorption axis will be nearly all absorbed whereas light vibrating at 90 degrees to this axis will be nearly all transmitted. Light vibrating at other angles will be partially absorbed and partially transmitted. All transmitted light will have the same vibration orientation as the polarizer's major transmission axis. An important property of polarized light as used in DIC is that interference can not occur between beams of polarized light that have different polarization angles. Figure 11.2 illustrates the action of a polarizer. The polarizer's major transmission axis (vibration axis) is shown vertical; its major absorption axis horizontal.

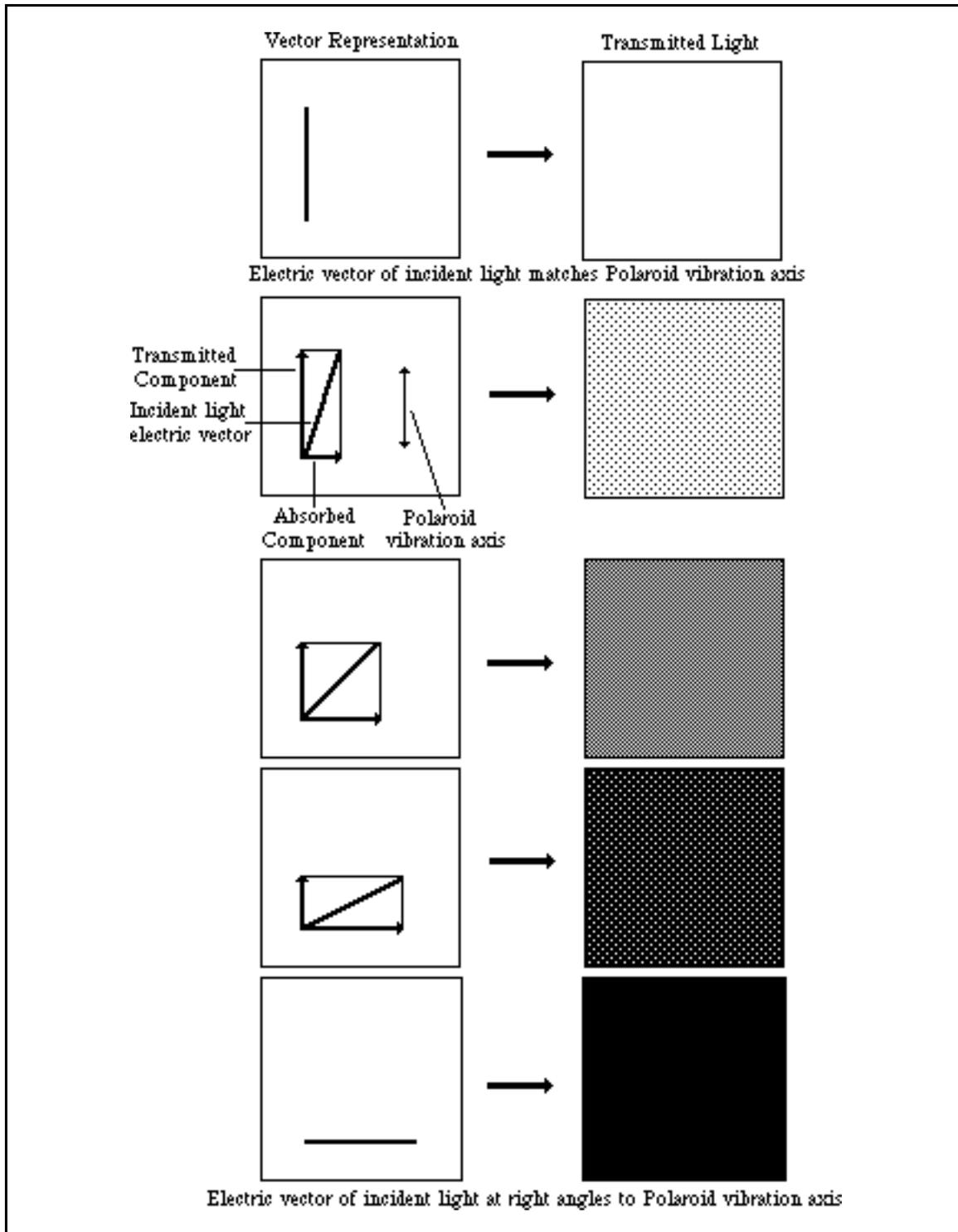
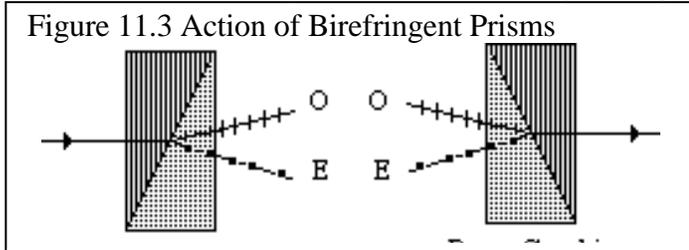


Figure 11.2 - Action of a Polarizer.

Light can enter at any rotational angle. In the left hand panels, the vertical and horizontal lines are vectors representing the amount of light that is transmitted and absorbed for light rays entering at different angles – the middle line. The right hand panels represent the relative amount of light you would see while looking through the polarizer.

Beam Splitting by a Birefringent Prism

Birefringent materials have the capacity to split a beam of light into two beams polarized at right angles to one another. One of these rays is the ordinary ray or O ray. The other ray is the extraordinary ray or E ray. One of these rays travels more slowly through the birefringent material than the other and therefore the two rays emerge with a slight difference in phase. As shown in figure 11.3, a prism can be constructed using birefringent materials in which the two rays emerge traveling in slightly different directions (the divergence is greatly exaggerated for clarity). This difference is referred to as shear between the beams. Nomarski used a modified Wollaston prism to achieve this effect. The emergent beams from the Wollaston prism can not interfere with one another because their polarization angles differ, they are slightly sheared because they are traveling in slightly different directions, and they are slightly out of phase.



Beam Combining by a Birefringent Prism

If an O ray and E ray pass into a Wollaston prism that has the opposite arrangement of the one that made them, the net result will be two beams of light that are polarized at right angles to each other but which are in phase and not sheared (Figure. 11.3).

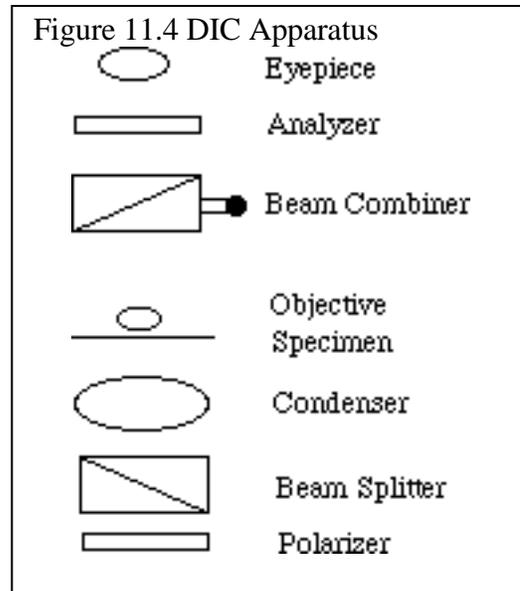
Resolution of two Polarized Beams by an Analyzer

If two beams of light, polarized at different angles, enter a polarizer, light will emerge at a single polarization angle. Interference of the two original beams is now possible. A polarizer used in this capacity is called an analyzer.

Apparatus for Nomarski type DIC

Figure 11.4 illustrates the apparatus commonly used for Nomarski DIC. Starting at the bottom and working up, the apparatus is as follows:

- 1) Polarizer – This produces a single beam of plane polarized light from the lamp.
- 2) Modified Wollaston prism – This produces two beams of light, polarized at right angles to one another, slightly out of phase with one another, and having a very small amount of shear. The amount of shear is on the order of the resolution of the objective lens.
- 3) Condenser – The condenser focuses the two slightly sheared beams on the specimen plane. DIC requires that the microscope be aligned for Köhler illumination.
- 4) Specimen – The specimen causes a change in optical path difference between the two beams. This change is sensitive to very closely spaced variations in the specimen's refractive index and/or thickness since the beams themselves are very closely spaced.



5) Strain free plan achromatic objectives – A DIC objective lens must not affect the polarization of light. This can happen if the glass elements of the lens have been subjected to stresses that cause slight variations in the refractive index. It is difficult to manufacture an apochromatic lens that is strain free because of the large number of lens elements. Thus, most DIC lenses are achromats. Treat your strain free lenses very gently. One jolt can ruin them.

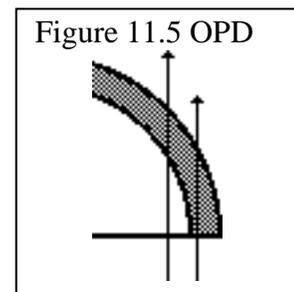
6) Second modified Wollaston prism – A second prism is placed above the objective lenses. The location of the interference fringes of this Wollaston prism must coincide with the back focal plane of the objective. The back focal plane of middle and high power objectives is inside the objective. The Wollaston prism therefore has its interference fringes located outside the prism. Specific prisms are matched to specific lenses. It is therefore unlikely that any objective lens you might have will function in a DIC system. Rather you should buy prisms and objectives that are made for DIC. Manufacturers usually indicate DIC on the barrel of these objectives. The upper modified Wollaston prism(s) will be movable in a lateral direction. Moving this prism horizontally affects the optical path difference between the two beams and therefore the amplitude contrast in the final image. It emphasizes optical path differences introduced by the specimen.

7) Analyzer – This is a second polarizer placed after the second modified Wollaston prism. This polarizer has its major transmission axis at right angles to the lower polarizer.

8) Eyepiece – Interference of the two modified beams occurs in the intermediate image plane forming a real image that is further magnified by the eyepiece.

Effect of the DIC Apparatus

The image that results from all this apparatus is one that is very sensitive to closely spaced changes in refractive index. Figure 11.5 illustrates the affect that a cell membrane would have on the two slightly sheared beams. The right-hand beam is in the cell membrane longer than the left-hand beam. The cell membrane has a higher refractive index than the cytoplasm. The right-hand beam slows down relative to the left-hand beam. The longer the right-hand beam remains in the cell membrane, the greater its optical path difference (opd) will be from the left hand beam. The analyzer recombines these beams. The beams now interfere with one another and the membrane is clearly visible. If the beams in figure 11.5 are shifted to the left, the opd would not be as great and the image would be different.



Set Up of the DIC Apparatus

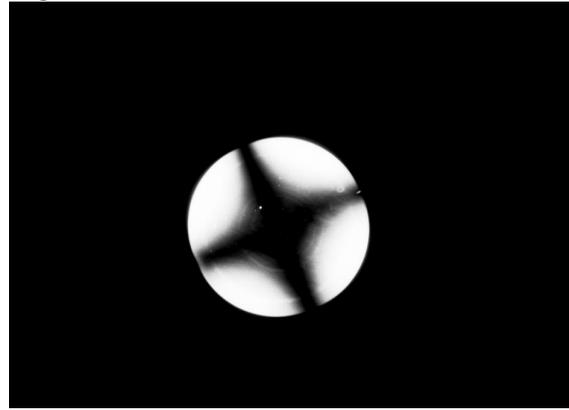
Correct placement and orientation of the various parts of a DIC system are crucial. The polarizer and analyzer must have their major transmission axes at right angles to one another and the modified Wollaston prisms must be arranged optically opposite one another and oriented at 45 degrees to the polarizers. Figure 11.4 illustrates the optical arrangement of these components.

Use the following steps in setting up a DIC system:

- 1) Using a 40 X objective, align the microscope for bright field Köhler illumination.

- 2) Move the specimen to obtain an empty field of view on the slide.
- 3) Fully open the condenser's iris diaphragm.
- 4) Insert the polarizer and the analyzer – insure that they are right side up. Usually this is indicated on the mount. If the polarizer and / or analyzer are rotateable, make sure the polarizer is set to the 0 or 90 degree position and that the analyzer is set to the opposite position. Usually the polarizer's major transmission axis is set right to left across the microscope and the analyzer's is set front to back.
- 5) Make sure neither Wollaston prism is in position.
- 6) Focus on the objective's back focal plane. Use a phase telescope, or a Bertrand lens to do this.
- 7) You should see a dark cross in an otherwise bright field (figure 11.6). If you do not, loosen the analyzer housing and rotate it slightly until the cross appears. Then, lock the housing in place. This insures that the polarizer and analyzer are exactly at right angles to one another.
- 8) Return to normal viewing and insert the Wollaston prisms. On some systems, the condenser contains only a single prism and each objective has a prism placed just above it. In this case it is a good idea to see that the prisms over the objectives are the correct ones. These prisms will be marked with the magnification of the objective for which they are made. On other systems, the condenser contains the objective-matched prisms. In this case the condenser turret positions will be labeled with the magnification of the matching objectives.
- 9) Insert your specimen and adjust the illumination. Move the adjustable Wollaston prism to achieve the desired DIC effect.

Figure 11.6 Maltese Cross



Comparison of DIC with Phase Contrast Microscopy

The annoying phase contrast artifacts of phase halo and shading off are absent in the DIC image. The image has a very realistic three-dimensional appearance. This appearance is not an entirely true representation of the specimen. You only have to adjust the movable Wollaston prism and observe the change in appearance of the specimen to prove this. It is therefore a good idea to compare the appearance of the specimen by phase contrast and DIC to achieve a better understanding of specimen structure.

Because the DIC technique is designed to work at the limits of resolution of the objective lens, higher image resolution can be achieved than with phase contrast. A significant advantage of DIC is its ability to produce very clean optical sections compared to phase contrast.

Although phase contrast requires additional optical equipment, the DIC apparatus is more complex and generally more expensive.

Problems with DIC

DIC is a relatively expensive technique. The microscope stand must be able to accept the DIC apparatus. Special, strain free, objective lenses are recommended, and there is the expense of the associated filtering systems.

Specimens that alter polarized light, such as birefringent specimens and plastic culture dishes, are not suitable for DIC. Phase contrast is still the best method for examining cells in plastic culture dishes. A method called Hoffman Modulation Contrast can be used to produce an image similar to DIC for specimens in plastic dishes, but this method is also expensive.

Exercises

- 1) Set your microscope up for DIC. Note where the following components are located: polarizer, first Wollaston prism, second Wollaston prism, and analyzer. Make a rough sketch of their positions.
- 2) Check the alignment of your DIC system. What method is available for adjusting the system for crossed polars?
- 3) Observe cheek cells using DIC. Describe how the image compares to a phase image?
- 4) Move the adjustable Wollaston prism from one extreme to the other while observing cheek cells. What happens to the image?
- 5) Remove one of the DIC optical components while looking at cheek cells. What is the effect on the image?