Frequently Asked Questions Relating to Compound Light Microscopes

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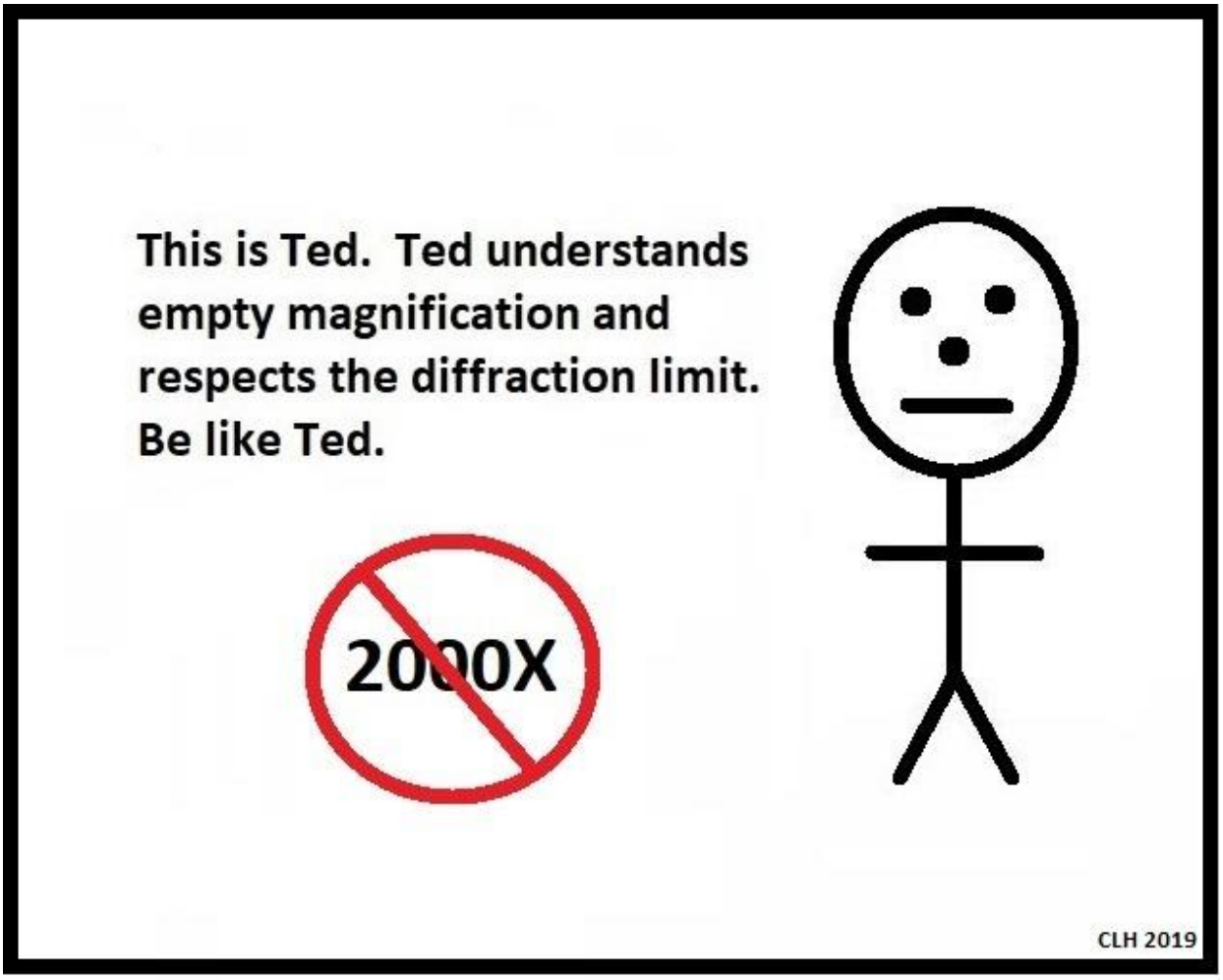

Table of Contents

Questions about Buying a Microscope

Should I buy a new or used microscope?

I know that many dyed-in-the-wool microscopy hobbyists would call the following statement blasphemy, but if you are new to the microscopy hobby and would like to buy a scope that will allow you to dive right in and get started immediately, you should probably buy yourself a new microscope. This is especially true if you are not the type who enjoys tinkering with things to make them work. A new scope is much less likely to give you problems than a used scope, and if it does, it can be returned to the seller for repair or exchange, making it the lowest-risk option for most newcomers. On the other hand, if you are the type who has an appreciation for old-school quality and workmanship that you just can't buy at any price these days, and if you enjoy tinkering with mechanical things, a vintage research microscope from the 1980s could be right up your alley. You can certainly buy a superior microscope for your money this way. But be aware that a scope such as an Olympus or Zeiss research model from the 1980s, while indeed nice, may need a fair amount of up-front work before it is usable. It is not uncommon for the grease in these older scopes to have solidified over the decades, leaving the focus mechanism very stiff or even completely seized, or for various accessory components on these scopes to be incorrect, damaged, or outright missing. Repair services and replacement parts for these vintage scopes are no longer available from their manufacturers, leaving only used, surplus, and third-party suppliers. Although this sounds bad, the situation is not too much better with many re-sellers of low-cost, re-branded Chinese scopes. Outside of a return or exchange during the initial warranty period, good luck finding a knob, an internal gear, or any other mechanical part needed to repair these microscopes. Although you can likely buy eyepieces, objectives, and perhaps even darkfield condensers from the original resellers, most do not offer a bench repair service nor repair parts for their equipment. These scopes are very much a product of our modern throw-away society and as such were designed to a low-cost price point with no provision for long-term serviceability. For these low-cost microscopes, it is simply more economical to replace the entire scope rather than to repair it.

Are microscopes made in China any good?

There are certainly some very high-quality microscopes manufactured in China today. In fact, some of the best microscopes available from the Big Four manufacturers (Leica, Nikon, Olympus, and Zeiss) are now manufactured in China. The flip side of this is that there are also microscopes of abysmal quality manufactured in China. These low-quality scopes tend to be the no-name, rebranded scopes sold by shady dealers who use wildly exaggerated performance claims such as 2000X, 2500X, or even 3000X (!) magnification to sell their microscopes. These scopes are offered at prices that seem too good to be true, and those who take the bait will likely be disappointed. As it turns out, any price is too high for a piece of junk. There are of course many microscopes of mid-range quality made in China as well, so be careful and do your research before buying a microscope, regardless of the country of origin. Whether manufactured in private factories owned by the reseller or in independent factories on contract with the reseller, all microscopes are built to a specific price point to meet the specifications of the reseller. It is not the country of origin that matters, but rather the reseller's dedication to quality and customer support. If you buy a no-name, rebranded scope from a shady dealer who uses wildly exaggerated performance claims such as 2000X, 2500X, or even 3000X (!) magnification to sell their microscopes, then don't be surprised when the scope you receive is horrible, and don't be surprised when the dealer refuses to answer, or cannot answer, any questions you have after your horrible scope arrives.

Should I spend the extra money for an infinity microscope?

The optical designs of compound light microscopes falls into one of two basic types: 1) finite-conjugate optics, and 2) infinity optics. The difference between these two design types lies in how the objective lens produces the intermediate image that is further magnified by the eyepiece lenses. When selecting a

microscope, it is critical to have at least a basic understanding of the differences between finite-conjugate and infinity optics, since each type has its own unique set of advantages and disadvantages, and since the optics of one type cannot be used on microscopes made to accept the other type. In general, microscopes with infinity optics are more expensive than microscopes with 160mm finite tube length, but except for a very few specific cases, there are no inherent differences in optical performance between the two types. The cost difference between finite and infinity optics is irrelevant if you plan to buy a new scope from one of the Big Four manufacturers (i.e., Leica, Nikon, Olympus, or Zeiss), since all they offer these days are infinity scopes and you will therefore have no choice in the matter. But cost aside, there are a few things to consider before making the decision of whether to invest in a microscope with infinity optics or finite DIN-compliant optics.

The primary advantage of an infinity scope over a finite scope is that the length of the infinity space in the optical tube (i.e., the space between the objective lens and the tube lens) is not critical for the performance of the optics. This allows manufacturers of infinity scopes the freedom to provide intermediate optics of modular design, which can easily be installed into the infinity space of the microscope stand, to provide additional functionality without upsetting the optical performance of the scope. Contrast this with a finite scope, wherein the introduction of modular intermediate optics will upset the critical tube length, necessitating the use of supplemental optics in the intermediate attachment to compensate for the increase in tube length. Depending on your specific interests and budget, this may or may not be a significant concern. Unless you plan on adding such things as reflected illumination or differential interference contrast to your scope, this aspect of infinity optics probably doesn't matter much, especially since both of these functions are quite expensive and are not typically available on any but the highest-priced scopes.

A second advantage to infinity optics is photomicrography. Microscopes with infinity optics do not typically require compensating eyepieces (i.e., eyepieces that provide correction for residual chromatic aberration not removed by the objectives), but those with finite optics frequently do. The reason for this is that all of the correction for chromatic aberration in infinity scopes typically occurs either completely within the objectives, or if not, is split between the objectives and the tube lens built into the infinity microscope's frame. Regardless of which way this is accomplished, this means that the intermediate real image presented to the eyepieces is fully compensated (i.e., has no significant chromatic aberration) and can be picked up for digital imaging with a simple digital camera that replaces one of the eyepieces. This is a cheap and simple solution.

Contrast this with the scheme used in many, but not all, finite-tube-length designs. In these finite designs, the intermediate real image contains some degree of uncorrected chromatic aberration, and compensating eyepieces must be used to remove the residual chromatic aberration. Due to the reliance on compensating eyepieces to remove the residual chromatic aberration from the images, the simple technique or replacing one of the eyepieces with a digital camera cannot be used. Instead, either a compensating eyepiece or a photo-projection lens which provides the necessary compensation must always be present in the optical pathway of the camera, making this solution more complex and expensive.

A few potential disadvantages with infinity optics are poor optical and mechanical interchangeability. Finite objectives built to the DIN standard can be freely swapped among DIN-compatible finite scopes from the various manufacturers, with the only caveat that the proper eyepieces will be necessary to provide optimal optical performance. This is not necessarily true for infinity scopes, for a few reasons. First is the matter of optical compensation. Infinity objectives which rely on the tube lens to provide some of the necessary optical compensation would reasonably be expected to perform poorly in a scope from another manufacturer, since it would be used with a tube lens with different characteristics than what was originally intended. Whether or not this difference is objectionable, or whether there even is a visible

difference, is difficult to predict without knowing the specifics of the two manufacturer's optical designs. The second reason is that the mechanical design of the objectives can be incompatible. While some infinity objectives use the same RMS threads as DIN-compliant finite objectives, some do not. Whether or not a given infinity objective would provide acceptable images on another manufacturer's scope is irrelevant if the objectives are not mechanically compatible with that scope.

Which total magnifications do I need?

The correct answer, of course, is that it all depends on what you intend to look at. But without knowing the specifics, I would recommend you just get yourself a microscope with the standard line-up of optics, which includes dry 4X, 10X and 40X objectives, a 100X oil-immersion objective, and a pair of 10X widefield eyepieces. Whatever it is that you intend to look at, it's safe to say you should absolutely ignore any claims made by microscope sellers of magnifications exceeding 1400X. If you look at the marketing brochures from the Big-Four microscope manufacturers (i.e., Olympus, Nikon, Zeiss, and Leica), you will find that none of their brochures even mention the magnification their scopes can provide. Amazingly, even the brochures for their top-of-the-line, obscenely expensive research microscopes say nothing of magnification, and do not include the letters "**2000X!"** (in bold text) anywhere. The reason for this is that any compound light microscope can be fitted with a 100X oil-immersion objective (which provides the highest resolving power of any of the objectives commonly available for routine biological applications) and a pair of ultra-cheap 20X eyepieces. When used together, you will indeed get 2000X magnification. Never mind the fact that the laws of physics, especially those pesky bits concerning diffraction, preclude you from ever getting *good* or even *decent* images at 2000X magnification, you will nonetheless see an image magnified 2000X, in all its blurry and disappointing splendor.

By the latter part of the 19th century, the science of optics had advanced to the point where microscope designers were building microscopes that performed right up to the diffraction limit of visible light. The so-called diffraction limit is the limit to the resolving power of a conventional light microscope caused by the diffraction effects of light. These early microscopes provided useful magnifications of up to 1400X or so. And guess what? The same laws of physics apply today, as then, and because of this you still cannot buy a microscope that can see beyond the diffraction limit. To be sure, there have been many significant improvements made to the design of microscope optics since the 19th century, and there will no doubt be more in the future, but none of these will allow even the best 100X oil-immersion objective to reach beyond the diffraction limit and provide 2000X magnification to any but the most naïve of microscopists.

If you want the highest useful total magnification possible, go out and get yourself a microscope with a top-tier 100X oil-immersion objective boasting an apochromatic design and with a numerical aperture of 1.40, and get yourself a good pair of 14X eyepieces to go along with it. In terms of useful magnification, you'll have the best performing scope available at any price. This rock-star lens with its rock-star price tag will provide a maximum usable magnification of 1000 times its rated N.A., just like the cheapest ones will do. It does a bit better in useful magnification than the cheaper ones, since its rated N.A. is a bit higher, but either way, 1400X is tops for it, just like those in the 19th century! The cheaper 100X oil-immersion objectives available today are good to 1250X or so. Now go ahead and stick a pair of 20X eyepieces on there with your new rock-star objective to raise the total magnification to 2000X, just like the Amscope folks are doing. What do you see? Ignore the annoyingly narrow field of view of these eyepieces. And sure, thanks to the extremely low eyepoint of these eyepieces, your eyelashes will rub the top elements of the eyepieces whenever you blink, which will smudge them with oil in no time, but go ahead and power through. What do you see? A blurry mess of an image that would look better, while showing the same level of detail, when viewed through your comfortable 10X widefield eyepieces is what you will see. So now that you've done this, and now that you've seen how the "other half" lives, treat yourself to the satisfying "cluck-clunk" sound these 20X ergonomic disasters make as they hit the trash can. Now that feels good, doesn't it?

Which eyepiece magnifications do I need?

Many microscopes sold today are advertised as providing 2000X, 2500X, or even 3000X (!) magnification. Since the 100X objective is the highest magnification objective available for biological microscopes, this means that to achieve these stated magnifications, eyepieces of 20X, 25X, or even 30X would be needed, right? Technically speaking, this is true. But the simple truth is that no conventional light microscope can provide usable magnifications of 3000X, 2500X, or even 2000X, so why worry about these eyepieces? All they will do is put you squarely in the camp of empty magnification, where the resulting images will be sadly disappointing. Also, it you've ever peeked into a pair of 20X eyepieces, you know that the visual field is annoyingly narrow, and the eye relief is horrible, making even the best of these damn things just about unusable. Any claims for magnification above 1400X or so are simply bogus, deceptive advertising. So why are these eyepieces included with most microscopes sold today for the amateur low-cost market? The answer of course is so that the manufacturers can claim 2000X, 2500X, or 3000X (!) for their microscopes, to lure unsuspecting buyers. It's pure snake oil. DO NOT BE FOOLED. Rather than ask why these eyepieces are included with low-end scopes, a better question to ask is why they are not included with scopes from the legitimate, high-end manufacturers. Or why do the marketing brochures from legitimate high-end manufacturers not even talk about total magnification? I've always said that the best use for a pair of 20X eyepieces is to hear the satisfying "clunk-clunk" sound that they make when they hit the trash can. All kidding aside, the 10X eyepieces supplied with your microscope are all that you will ever need. In some cases, 12.5X or 15X can be useful, but there is no reason to ever feel eyepiece envy if you "only" have 10X eyepieces. Believe me, if you buy a microscope with a pair 20X, 25X, or 30X eyepieces, they will spend their entire lives in a drawer somewhere, mocking you every time you run across them. You'll wish you could throw them out, but you will never do that because they came with your microscope. But that "cluck-clunk" would sound really good, wouldn't it?

Which objective magnifications do I need?

A mid-range compound light microscope usually comes equipped with four standard achromatic objectives (4X, 10X, 40X, and 100X oil-immersion), which are perfectly fine to get you started in the microscopy hobby. It doesn't take most hobbyists long to figure out that the 100X oil-immersion objective is inconvenient and messy to use. Couple this with the fact that they are used primarily to image bacteria (which are by far the most boring living organisms you will ever observe), and you will find yourself rarely, if ever, using your 100X oil-immersion objective lens. That aside, if you're like most people, as your knowledge and experience in the hobby grows, so will your desire for magnifications other than the basic four. Objectives of 20X and 60X are common additions to many nosepiece turrets, assuming there are open positions to accept them. So do some soul searching, and if you decide you really don't use your 100X oil-immersion objective all that often, take it off the turret, put it back into its protective canister, and tuck it safely away in that drawer, right next to those worthless 20X eyepieces. That way, the 100X objective is there if you ever need it, and more importantly, you've made room on the turret for either a 20X or 60X objective lens, which you will use way more often than that 100X oil-immersion objective in the drawer.

Should I buy Plan objectives for my microscope?

If you do much photomicrography, or if you plan to do so in the future, you will certainly want to upgrade from standard objectives to Plan objectives. Plan objectives will render a much larger area of the visual field in acceptable focus, providing photomicrographic images which are vastly superior than those produced by standard objectives. If you know now that microscopy is really going to be the hobby for you, and if you can afford it, buy a scope with Plan objectives right from the start. Although it will cost you more up-front, it will save you money in the long run.

What is a Mechanical Stage, and do I need one?

A mechanical stage is a mechanism on the microscope, either integral to the stage or an attachment to the stage, that allows the operator to easily and precisely position the specimen slide on the stage in order to observe specific areas of interest of the specimen. A typical mechanical stage consists of two knobs, usually coaxial, where one knob moves the specimen slide in the X (east-west) axis, and the other moves the slide in the Y (north-south) axis. A mechanical stage is especially valuable for those scope operators who have difficulty adapting to seeing the image through the eyepieces move in the opposite direction in which the specimen slide is maneuvered by hand. Although a mechanical stage is not a necessity, it is a *very nice* feature to have on your scope. If your scope does not have a mechanical stage, and if for some reason you find yourself someday using somebody else's scope which has one, then you will become immediately inflicted with a severe case of *mechanical stage envy*, and for this there is but one cure. Do yourself a favor and buy a scope with a mechanical stage right from the start.

Which should I get, a microscope with a monocular, binocular, or trinocular head?

A binocular or trinocular head is the best option for most people. The binocular images provided by these viewing heads can significantly reduce eyestrain during extended periods of observation, as compared to using a monocular scope, at least for those people who are able to use a binocular scope. For some people with certain vision disorders (such as strabismus or monofixation syndrome), it can be difficult or even impossible for their brains to fuse the images presented to their eyes by a binocular or trinocular head into a single, discernable image. Do not buy a monocular scope if you are able to successfully use a binocular microscope. But even those whose vision cannot fuse binocular images might want to consider a binocular scope if the scope will be used very often by others. Additionally, even if you cannot fuse binocular images, you may want to consider a trinocular scope, for convenience if the scope will be used for photomicrography, since this allows a camera to remain permanently affixed to the scope while it is also used for visual observations.

A trinocular head is essentially a binocular head fitted with an internal beam splitter, and with a camera port on top which allows you to permanently affix a camera for photomicrographic purposes while retaining full access to the eyepieces for visual observations. There is usually a shaft, with a knob on the end, sticking out the side of the head, which is attached to the internal beam splitter to allow the operator to position the internal beam splitter as necessary to accommodate the task at hand. If the light-path selection shaft is positioned such that the beam splitter is in the optical path, the available light is split into two components, one of which is sent to the eyepieces and the other is sent to the camera port. The light-path selection shaft may also provide positions to send all of the available light to the eyepieces, for light-starved observations, or to send all of the available light to the camera port, for light-starved photomicrography. Some of the less expensive trinocular heads might have only one of the last two light-path selections described above, and some may not even have a light-path selection shaft at all, meaning that operation is only possible with the light split between the eyepieces and the camera port.

Which is better, halogen lighting or LED lighting?

Halogen lighting has enjoyed a long history in microscopy, going back to the 1970s. Today, although there are still halogen-equipped scopes on the market, LED lighting is becoming more common. The pros and cons of both are discussed below.

Halogen lighting produces a continuous spectrum of light that renders all colors present in the specimen visible to the observer. In contrast, LED lighting is to some degree or another discontinuous, meaning that some wavelengths of light will be lacking in the spectrum, making the colors associated with those deficient wavelengths more difficult to see. In this respect, halogen is the winner. But halogen has the inherent disadvantage that the apparent color temperature of the light changes as the lighting intensity is varied. At low levels of illumination, halogen lighting has a distinct yellowish, warm cast, which tends to go away as the intensity is increased. For visual observations, this objectionable yellow cast can be

removed by including a blue-tinted filter in the illuminating path. For photomicrography, this aspect of halogen lighting means that the resulting exposures will have a different color cast, depending on the intensity with which the exposures were made, making it necessary for the operator to perform a custom color balance for each intensity setting to eliminate this effect. LED lighting does not suffer from this issue. The color temperature of LED lighting does not appear yellowish at all, at any intensity, so the blue filter discussed above is not needed. In fact, if anything, LED lighting may appear a bit too cool to the eyes, with a slight bluish tint which some microscopists may find objectionable. However, after using an LED-equipped scope, most operators quickly adapt to the cool appearance of LED lighting. When LED lighting is used for photomicrography, a custom light balance for the camera needs to be performed only once.

Halogen lighting creates a lot of heat in the housing for the halogen lamp. For halogen-equipped scopes whose lamp is located in the base, below the stage, this can be a problem since the heat produced by the lamp causes the entire base to run hot, which can adversely affect the reliability of the electronics in the base. The designers of upper-end clinical and research microscopes generally put the lamp in a housing on the rear of the base, in order to allow for a more comprehensive illumination system than could be placed within the base. For these scopes, internal heat rise of the base is not an issue. However, lower-cost microscopes which contain a halogen lamp integral to the base will have an inherently lower electrical reliability as compared to scopes with an externally mounted lamp, due to heating of the electronics in the base. The significance of this depends on the design of the microscope. A good design which properly addresses these thermal issues will have higher reliability than one which neglects thermal management.

Another issue that can be a problem with halogen lighting is heating of the specimen during extended observation at high light intensities. This can be a problem because of the relatively high level of infrared wavelengths present in the lighting spectrum produced by halogen lamps. This can be alleviated by the inclusion of an IR-blocking filter in the illumination path, and many higher-cost research or clinical microscopes with halogen lighting contain an integral IR-blocking filter or have provisions for placing one in the illuminating pathway. Lower-cost halogen-equipped scopes may not have such a filter, but these scopes tend to be the ones whose lighting is not powerful enough to pose a significant risk to the specimen either way.

Both halogen and LED lighting produce significant amounts of short-wavelength UV radiation. In general, LED sources are worse than halogen, but this is not always the case. Since excessive UV radiation can cause cataracts and other health issues, the design of a microscope should include a UV-blocking filter in the lighting pathway to greatly reduce or eliminate the UV exposure of the operator. If you buy a microscope from one of the Big Four manufacturers (Nikon, Olympus, Zeiss, and Leica) you can be sure that this issue has been addressed and the microscope is safe to use. On the other hand, if you buy a no-name, rebranded commodity scope sourced through China, who knows?

The efficiency of LED lighting is much greater than that of halogen lighting. This means that an LED-equipped scope will run significantly cooler and will require less electrical power to operate than a comparable halogen-equipped scope. This is not a significant issue for most applications, but the improved efficiency of LED lighting makes possible a design that can be operated on batteries, for convenient portable or field use. If ease of portability is important to you, make sure you buy one which includes the capability to operate off batteries, since many LED scopes are not so equipped.

A halogen lamp can be expected to provide anywhere from 200 to 2000 hours of service life (depending on the lamp type) when operated at full brightness. If you have a halogen-equipped scope, be sure to keep a spare bulb or two on hand. In contrast, an LED should provide 20,000 or more hours of service life before failing. This means you probably won't be replacing LEDs. On the other hand, if the LED ever does fail for some reason, the only way to repair the scope will likely be to obtain the necessary parts or service from the manufacturer. This can be an option if you have a scope from some of the better manufacturers,

but be aware that individual parts to repair lower-cost, rebranded commodity microscopes are generally not available.

Should I get a scope with Köhler Illumination?

If you intend to use your scope for photomicrography and are already planning to pay more for an upgraded model with a trinocular head and plan objectives, then do yourself a favor and find one that also includes Köhler illumination. The addition of Köhler illumination to a scope requires hardware not needed for conventional source-focused illumination, and this adds cost, but in most cases the benefits are well worth the additional expense. Köhler illumination provides much better lighting uniformity than can be achieved with conventional source-focused (aka Critical or Nelsonian illumination), and although you might be hard pressed to see the non-uniformity of source-focused illumination during routine visual observations, the difference will be much more noticeable in your photomicrographs. Köhler illumination solves the problem of lighting non-uniformity by projecting perfectly defocused light onto the specimen plane, whereas conventional source-focused illumination projects a perfectly focused (and therefore visible) image of the light source (LED emitter or lamp filament) onto the specimen. Manufacturers of scopes using source-focused illumination almost always include a frosted glass diffuser in the lighting path to improve the lighting uniformity, but the results still do not match those achievable with Köhler illumination. An additional benefit of Köhler illumination is that it provides higher image contrast than conventional source-focused illumination, since the field diaphragm, which is present only in Köhler-equipped scopes, allows you to restrict the illumination to only the area of the specimen visible to the objective in use, thereby preventing glare from light that would otherwise fall outside the field of view of the objective lens. The design of the illumination system of scopes not equipped with a field diaphragm, by necessity, must illuminate the entire field visible to the lowest power objective (i.e. the objective with the widest field), and when using the higher power objectives in these scopes, glare from the light beyond the visible field of the objective can cause a noticeable reduction in apparent image contrast.

Basic Microscope Questions

What is an Aperture Diaphragm?

The aperture diaphragm is an iris diaphragm integral to the substage condenser. The aperture diaphragm should not be confused with the field diaphragm, which is a diaphragm present only in the illumination paths of Köhler-equipped scopes, and not in scopes equipped for Critical illumination. The aperture diaphragm controls the angle of the cone of light that the condenser produces, thereby controlling the numerical aperture of the condenser. A good rule of thumb is that for routine viewing, the aperture diaphragm should be set so that the N.A. of the condenser is approximately 75% of the rated N.A. of the objective lens used for the observation. This setting may be adjusted somewhat to improve image contrast or depth of field, by reducing the aperture, or to improve resolving power, by increasing the aperture. It is important to understand that resolving power is always directly competing with image contrast and depth of field. As you increase the aperture to gain resolving power, you decrease the apparent image contrast and depth of field. Similarly, as you decrease the aperture to gain apparent image contrast or depth of field, you decrease resolving power. You should also be aware that if you close the aperture diaphragm too much, you will get great contrast, but you will introduce undesirable diffraction artifacts to the image. Conversely, if you increase the N.A. of the condenser beyond the rated N.A. of the objective lens, you will degrade image contrast without adding anything to the resolving power.

What is a Biological Microscope?

A biological microscope is a compound light microscope whose design has been optimized for the high-magnification study of biological specimens, such as protists, bacteria, parasites, and histological tissue

samples, using transmitted light (i.e., light that is transmitted through the specimen from a light source on the opposite side of the specimen from the viewing optics). Biological microscopes do not typically provide reflected lighting capabilities (i.e., light that is reflected off the sample from a light source on the same side of the specimen as the viewing optics), although this capability can usually be added to the better ones. Biological microscopes come in two basic varieties: upright and inverted. An upright biological microscope is made to examine specimens mounted on conventional glass slides and is the type best suited for bacterial or histological observation. This is the type that most people imagine when they hear the word "microscope". The light source of an upright microscope is located below the specimen under observation and the viewing optics are located above the specimen. In contrast, an inverted microscope is built the other way around. The light source is located above the specimen under observation and the viewing optics are located below the specimen. Rather than using a conventional glass slide, the inverted microscope is intended to view specimens (typically live) in a glass petri dish or similar container. Of the two types, the upright design provides best optical performance, and is the type that a newcomer should generally start with.

What is a Compound Microscope?

The closer an image is to your eyes, the larger it appears in your field of vision and therefore the better you can resolve the fine details that comprise that item. To read small text, you instinctively know to move the book closer to your eyes to make the text appear larger. But this technique only works to a certain extent, and anybody over the age of 50 will tell you that this doesn't work for them anymore. As a person ages, their eyes lose the ability to focus as close as they once could, and by the time they're in their 50s, most people can't focus close enough to allow them to read small text without glasses. But regardless of your age, and whether you wear glasses or not, there is simply no way your eyes could ever focus on microscopic objects that were held close enough to the eyes to otherwise be visible. This is where the compound light microscope comes in. It provides sufficient magnification to allow the viewer to see microscopic objects and the fine details that comprise them. Obviously, the smaller the item, the higher the magnification needed to see the object. For example, red blood cells have a diameter of around 7µm (that is, seven one-millionths of a meter), and a high magnification is needed to see them. This high degree of magnification is obtained by first magnifying the blood cells with the objective lens of the microscope, and then magnifying the resulting image produced by the objective lens with the eyepieces. It is these two successive stages of magnification that make the microscope a *compound* microscope, and it is the compound microscope that allows small specimens like red blood cells to be comfortably observed. Although it is possible to use a single magnifying lens (i.e., a simple microscope) to view red blood cells, and this is exactly what Antony van Leeuwenhoek did it in the 1600s, this is a very uncomfortable way to go. If this were still how microscopy was done, you wouldn't be reading this, since you would have found a different hobby to pursue.

What are Conjugate Planes?

Conjugate planes are the planes which exist at various points in the optical pathway of a light microscope which are simultaneously in focus. There are two types of conjugate planes of interest: conjugate specimen planes and conjugate lighting planes. The conjugate specimen planes are the various planes that are in focus with the specimen, whereas the conjugate lighting planes are the various planes that are in focus with the illumination source.

What are Conjugate Lighting Planes?

The conjugate lighting planes are the planes which exist at various points in the optical pathway of a light microscope that are simultaneously in focus with the plane of the illuminating source. In a Köhler-equipped microscope, this includes the plane of the lighting element (i.e., lamp filament, LED emitter, or

diffuser), the plane of the condenser aperture diaphragm, the rear focal plane of the objective, as well as the exit pupils of the eyepieces and of the camera port.

What are Conjugate Specimen Planes?

The conjugate specimen planes are the planes which exist at various points in the optical pathway of a light microscope that are simultaneously in focus with the plane of the specimen under observation. In a Köhler-equipped microscope, this includes the plane of the field diaphragm, the intermediate image plane (i.e., the plane of the eyepiece fixed field stop), the plane of the camera film or image sensor, the plane of the observer's retina, and of course the specimen plane. When the scope is properly set up for Köhler illumination (if applicable) and is focused on a specimen, anything in any of these planes will appear to the observer to be in focus and superimposed on the specimen under observation.

What is Depth of Field?

In theory, only an infinitely thin plane of the specimen under observation is in perfect focus at any specific focus setting of the microscope. However, all the planes within a certain depth range, both above and below the plane of perfect focus, will appear to the observer to be acceptably sharp. This range of acceptable sharpness is what is referred to as the *depth of field* of the microscope. Microscopes whose optics are configured to operate with lower numerical apertures will have a greater depth of field, compared to those configured to operate with higher numerical apertures. This means that as a rule, the higher the magnification of the objective you use, and the wider you open the aperture diaphragm of the condenser,
the less the resulting depth of field. The depth of field is the axial (i.e., parallel to the optical axis) analog of resolution. In other words, as the numerical aperture of the optics increases, the resolving power in the lateral axes (i.e., X and Y axes) increase and the depth of field in the axial axis (i.e., Z axis) decreases. Although the limited depth of field of a high-power compound microscope is often seen as a disadvantage, it really is not. Imagine a high power, high N.A. scope that by some magic of optics had unlimited depth of field. Such a scope would have very high resolving power (i.e., resolution) in the X-Y plane of the specimen and would simultaneously render the entire thickness of the specimen in sharp focus. If a sample four cell layers thick were to be observed on such a scope, all four cells layers would appear superimposed and in sharp focus in the visual field, likely preventing the microscopist from making heads or tails of what was presented. The limited depth of field allows the microscopist to focus through the various layers of the specimen to observe specific areas of interest without being totally obscured by details from other layers. The limited depth of field is not too bad of a situation for direct visual observations, however, for photomicrographic applications the limited depth of field is much more problematic. For direct visual observations, the observer can quickly and easily vary the focus point, as necessary, to observe details at any desired depth within the specimen. However, this is not the case for photomicrography. The limited depth of field can be a major problem here, since for a given photographic exposure, only a limited depth range of the specimen will be in acceptable focus, and the rest can be annoyingly out of focus. For digital photomicrography of non-moving specimens, a good solution to this problem is to take an entire series of exposures, the first focused on the deepest plane of interest of the specimen, with each subsequent exposure focused a bit higher, until the upper plane of interest is reached. Dedicated image-processing software can then be used to scan through the resulting image stack, on a point-by-point basis, to pick out the sharpest components from whichever depth they are found in and assemble them into a composite image which is wonderfully sharp throughout.

What is Depth of Focus?

This is another term that the makers of YouTube microscopy videos never seem to get right, as they always seem to confuse this term with *Depth of Field*. I guess that's not too surprising, considering that even some microscope manufacturers make this mistake. For instance, at some places on their website,

Olympus talks about Depth of Focus as if it were the depth of acceptable focus in the specimen place, yet in other places, (i.e., sections credited to Mortimer Abramowitz) they get it correct. So, to be clear, the term *Depth of Field* is the vertical distance around the exact plane of focus of the specimen wherein objects are seen to be acceptably sharp, whereas *Depth of Focus* is the same concept applied to the image sensor or film plane, rather than the specimen plane. In other words, Depth of Focus describes the vertical distance around the exact plane of focus at the film or sensor where projected images would appear acceptably sharp in the resulting photomicrographs. Depth of focus is shallower for low-magnification objectives than for higher-magnification objectives, whereas depth of field is shallower for high-magnification objectives than for lower-magnification objectives.

What is Empty Magnification?

A compound light microscope has the capability to magnify and resolve a lot of detail in microscopic specimens. However, this ability is not without limit. There comes a point where additional magnification of the specimen reveals no further detail, and in fact too much magnification (known as empty magnification) can make the existing detail harder for the viewer to discern. We have all seen this basic phenomenon when looking at digital images on a computer. You double click on a picture to open it up, and there on the screen is Aunt Trudy, in all her glory. But what's that small object on Aunt Trudy's coffee table? No problem. Let's just zoom in a bit to see. As the zoom is increased, everything starts to get bigger, but eventually you reach a point where instead of seeing more detail of the package of Marlboro cigarettes on Aunt Trudy's coffee table, you instead just see a large blocky image. The image on the screen is larger, to be sure, but what good does that do? It's just a blocky, pixelated mess. The additional zoom did not reveal any more detail, since the detail was never there. Instead, it just revealed the individual pixels making up the image. The same thing happens in optical microscopy. The optical parameters of the lenses used, and even the wavelength of the light itself, impose strict limits on what can be resolved. This is called the diffraction limit, which is essentially the laws of physics working against you. A good rule of thumb is that the maximum usable magnification for a given objective lens can be found by multiplying the numerical aperture (NA) of the objective lens by 1000. If you have a 40X objective with an NA of 0.65 (as stamped on the barrel of the objective), then that objective should be usable at 650X total magnification. There really is no point in taking it much further. Anything greater than 650X total magnification will simply put you into the realm of empty magnification, where you'll begin seeing the analog equivalent of the pixels that make up the image, rather than specimen details within the image.

A compound microscope usually comes equipped with a 100X oil-immersion objective, and this objective would typically have an N.A. of 1.25 to 1.30 when used with oil immersion on both the top and bottom of the specimen slide. This means that you should expect to get a maximum total magnification of 1250X to 1300X out of this microscope, and that's it. There is no more. Don't be fooled by vendors who advertise 2000X, 2500X, or God forbid, even 3000X! That's total snake oil intended to dupe the newbies. It is interesting to note that *real* microscopes made by the Big Four manufacturers (Olympus, Nikon, Zeiss, and Leica) won't include any claims of total magnification in their marketing literature.

What is a Field Diaphragm?

The field diaphragm is an iris diaphragm in the lighting path of Köhler-equipped scopes. The field diaphragm should not be confused with the aperture diaphragm, which is the iris diaphragm integral to the substage condenser of all scopes, whether or not they provide Köhler illumination. The field diaphragm controls the size of the lighting spot that illuminates the specimen. The ability to vary the size of the lighting spot is advantageous since any portion of the specimen outside the field of view of the eyepieces that is illuminated contributes excess glare to the image, thereby needlessly reducing the apparent image contrast. It is important to note that only Köhler-equipped scopes possess the ability to vary the size of the lighting spot on the specimen. Non-Köhler scopes (i.e., those equipped for Nelsonian

or Critical Illumination) cannot do this, since to do so would require a substage condenser with a variable focal length, and this is considered impractical due to the complexity and expense of such a condenser. It is important to understand that the field diaphragm controls only the size of the illuminated portion of the specimen, and not the angle of the illuminating cone (in other words, the field diaphragm does not affect the numerical aperture of the condenser). In contrast, the aperture diaphragm in the condenser controls the angle of the lighting cone, and therefore the numerical aperture of the condenser, but does not affect the size of the lighting spot. In addition to allowing the microscopist to optimize the apparent image contrast by lighting only the portion of the specimen visible through the eyepieces, the field diaphragm can also be used as a convenient way to focus the condenser for proper Köhler setup. To do this, close the field diaphragm to the point where the visual field is no longer fully illuminated, then adjust the position of the condenser until the illuminated area has sharp edges (i.e., so that the leaves comprising the field diaphragm are sharply focused). Once the condenser has been properly focused, open the field diaphragm to the point where the entire visual field is illuminated, but not open it any further.

What is Field of View?

The field of view (FOV) of a microscope is the diameter of the circular area of the specimen plane that can be seen through the eyepieces. The objective lens projects a magnified real image of the specimen onto the intermediate image plane within the optical tube, and this real image is further magnified by the eyepieces to produce the final image. The portion of the intermediate image accepted by the eyepieces and presented to the observer is what determines the field of view, and this is a function of the Field Number (FN) rating of the eyepiece. A scope with high FN eyepieces will have a wider field of view (i.e., will show a larger area of the specimen plane) than will a scope with low FN eyepieces. So how do you determine the field of view of your microscope? If you watch very many YouTube microscopy videos, you will quickly learn that you first need to find a ruler, then place the ruler on the stage, and then… Wait! Why make this so complicated? If the FN rating is marked on your eyepieces, then the diameter of the field of can be quickly and easily determined by simply dividing the FN rating marked on the eyepieces by the magnification factor marked on the objective lens. The result will be the field of view, in millimeters. For example, if the operator of a scope is using the 10X objective to observe a specimen, and the eyepieces are marked with an FN of 20, then the resulting field of view will be 2.0mm (20mm / 10 = 2mm). This means that a 2.0mm diameter disk viewed under these conditions would exactly fill the field of view. It's that simple, and no rulers were harmed in the process of determining the field of view. The table below lists the resulting field diameters of standard objectives when used with eyepieces of various field numbers.

Magnification		Diameter of Field of View (mm)						
Objective	Eyepiece	FN 18	FN 20	FN 22	FN 24	FN 25	FN 26.5	FN 28
1X	10X	18.000	20.000	22.000	24.000	25.000	26.500	28.000
2X	10X	9.000	10.000	11.000	12.000	12.500	13.250	14.000
4X	10X	4.500	5.000	5.500	6.000	6.250	6.625	7.000
10X	10X	1.800	2.000	2.200	2.400	2.500	2.650	2.800
20X	10X	0.900	1.000	1.100	1.200	1.250	1.325	1.400
40X	10X	0.450	0.500	0.550	0.600	0.625	0.663	0.700
60X	10X	0.300	0.333	0.367	0.400	0.417	0.442	0.467
80X	10X	0.225	0.250	0.275	0.300	0.313	0.331	0.350
100X	10X	0.180	0.200	0.220	0.240	0.250	0.265	0.280

What is a Finite Microscope?

All compound microscopes designed prior to the 20th century, and many of those designed well into the 20th century, utilized the basic optical design architecture known as *finite-conjugate* optics (more commonly referred to as *finite optics*). These microscopes use objective lenses designed to produce an intermediate real image a fixed distance away from the objective. By placing the eyepieces at the proper point in the optical path relative to this real image, a virtual image is produced for the observer with a total magnification equal to the magnification of the objective lens multiplied by the magnification of the eyepiece lenses. The distance from the objective lens to the intermediate real image plane is referred to as the optical *tube length* of the microscope. Early microscope manufacturers utilized various optical tube lengths in their designs, making interchangeability of objectives poor, but they eventually settled on a standard tube length of 160mm (as part of the DIN standard), allowing optics to be freely interchanged amongst newer microscope stands. The tube length parameter of a given objective lens can be easily determined, since it is marked on the outer barrel of the objective lens.

It is important to understand that even though DIN-compliant objectives can be freely interchanged between the various microscope stands built for DIN optics, they must be used with the proper eyepieces to achieve maximum performance. The reason for this is that not all objective lenses provide the same degree of compensation for chromatic aberrations within the objective. Those who do not provide full compensation rely on compensating eyepieces to provide correction for the residual chromatic aberration, and those that do provide full compensation require optically neutral eyepieces, so as to not introduce lateral chromatic aberration. If either of these types of objective lenses are used with incorrect eyepieces, lateral chromatic aberration will be present in the final image.

The primary disadvantage of finite-conjugate optics is that since the designs of the finite objective lenses require a specific tube length, it is difficult for optical designers to revise such designs to provide the wide range of options professional users demand. As an example, the addition of polarizing capabilities to a microscope requires, among other things, the addition of a polarizing filter between the objective lens and the viewing head. This cannot be easily accomplished in a finite scope without changing the optical tube length of the microscope, thereby degrading the optical performance of the system. Optical designers have worked around this issue by adding additional lenses in the optical pathway to compensate for the change in tube length introduced with the filter. This is not an ideal solution, since additional lens elements add cost, complexity, and can adversely affect optical performance. The situation gets further compounded with each subsequent addition to the optical pathway, as each one further increases the tube length, and therefore requires the addition of other lens elements to compensate for this effect.

What is the Intermediate Image Plane?

The intermediate image of an optical compound microscope is a real image, produced by the objective lens, in the case of finite optics, or by the combination of the objective lens and tube lens, in the case of infinity optics, that gets picked up and further magnified by the eyepieces, to form the final image. The physical point in the optical tube at which this intermediate image is focused defines the intermediate image plane, which is orthogonal to the optical axis of the system.

What is an Iris Diaphragm?

An iris diaphragm is a physical mechanism used to control the amount of light passing a specific point in an optical system. An iris diaphragm consists of multiple, thin “leaves”, placed in a circular arrangement and attached to a stationary base and a control ring in such a way that when you twist the control ring one direction, the aperture in the center opens up, and when you twist the control ring in the opposite direction, the aperture closes down. Iris diaphragms are used in microscopes to control the numerical aperture of condensers and objectives, as well as to control the size of the illuminated field in Köhler-equipped scopes. The iris diaphragm is so named because it mimics the iris structure of the human eye.

What is an Infinity Microscope?

In order to address certain optical artifacts, such as the ghost images caused by converging light rays passing through beam splitters in reflected illumination, Reichert began working on infinity optics back in the 1930s. At the time Reichert introduced their first infinity scopes, the benefits of this new scheme were not immediately apparent to most microscope users, nor for that matter, to competing microscope manufacturers of the day. In Reichert's new system, the design of the objective lenses was changed so that light leaves the objectives in parallel rays, focused not at some fixed point where the eyepieces would be situated, but instead focused at optical infinity. Downstream from the objective lens, a second lens (known as the tube lens) was added into the optical tube, to accept the parallel rays from the objective lenses and focus them to form a real image for the eyepieces at the desired point in the optical tube.

Microscope users at the time no doubt wondered why Reichert would do such a thing. After all, this required the addition of the tube lens, which is not needed with finite optics, and which adds complexity and expense, to say nothing of the optical penalties inherent in having more air-to-glass interfaces within the optical path. Why would any manufacturer choose to do this? In hindsight, the answer seems obvious. In addition to eliminating the artifact discussed above that occurs in reflected lighting, for the one-time cost of a tube lens, infinity optics gave the Reichert designers a blank check to add anything they wanted into the optical path of the system without having to worry about changing the optical tube length, so long as the addition was placed within the infinity space (i.e., the portion of the optical tube wherein the light rays are parallel) of the tube. In other words, the change from finite-conjugate to infinity optics allowed Reichert to freely embrace design modularity, allowing additional optics to be easily added to the system without any of the problems encountered by manufacturers of competing finite-conjugate scopes. The switch to infinity optics also gave Reichert's designers the flexibility to use the tube lens to provide secondary compensation for any residual aberrations not fully corrected within the objective lenses, thereby allowing optically neutral eyepieces and photo-projection lenses to be used. It took a few decades before market forces conspired against the finite-conjugate manufacturers, forcing them to switch their scopes to infinity optics, but by the late 1980s, this switch was well underway. All of the top-tier manufacturers today produce only scopes that use infinity objectives (infinity objectives can be readily identified since their outer barrel are typically marked with an infinity (∞) symbol).

Most of the lower-tier manufacturers who offer scopes primarily intended for the hobbyist and educational markets (i.e., scopes that are not expected to be upgradeable for such things as polarization, differential interference contrast, or reflected illumination) still manufacture scopes with finite tube length. This is a good thing for the typical hobbyist, since for these markets there are some distinct disadvantages to infinity optics. One such disadvantage is that since the internal tube lens of an infinity scope may contain optical corrections for residual aberrations within the objective lenses, infinity objectives from one manufacturer may not work well on infinity scopes from competing manufacturers, unless of course they happen to have tube lenses with the same optical corrections. Worse still, not all manufacturers of infinity scopes use the same mechanical mounting arrangement, so infinity objectives from one manufacturer may be mechanically incompatible with another manufacturer's infinity scope. This is not a problem at the lower end of the market, since users of finite DIN-compliant scopes may freely interchange optics from the various DIN manufacturers.

What does Parcentered mean?

A microscope that is parcentered is one in which an object in the center of the field of view of one objective will remain centered for all subsequent objectives. No microscope is perfectly parcentered, and therefore slight positioning adjustments may be needed to re-center the specimen whenever a new objective is selected.

What does Parfocal mean?

A microscope that is parfocal is one which an object that is in focus with one objective will remain in focus for all subsequent objectives. No microscope is perfectly parfocal, and therefore slight fine-focus adjustments may be needed to re-focus the specimen whenever a new objective is selected. Poor parfocality can be improved by the addition of shims, as appropriate, onto the mounting threads of the various objective lenses to bring them into parfocality.

What is the Specimen Plane?

The specimen plane is the plane in the optical pathway, orthogonal to the optical axis of the system, where the specimen must be positioned for observation.

What is Total Magnification?

Total magnification is the overall magnification produced by the successive stages of magnification in the optical pathway of a compound microscope. Total magnification is defined as the mathematical product of the various individual stages of magnification in the optical pathway. Since there are two stages of magnification in a standard compound microscope, as provided by the objective and eyepiece lenses, the total magnification for this configuration can be calculated per the following equation.

$$\text{Magnification}_{\text{TOTAL}} = \text{Magnification}_{\text{OBJECTIVE}} \times \text{Magnification}_{\text{EYEPIECES}}$$

This equation applies to the basic configuration of a compound microscope with its two stages of magnification. In some cases, there might be one or more additional stages of magnification in the optical pathway which would need to be considered, such as that which may be present in various accessories for the microscope. An example of such an accessory would be an intermediate attachment for polarizing applications, which might introduce an additional magnification of 1.25X. In this case, the above equation would be extended by the inclusion of the additional 1.25X magnification, providing a total magnification of 500X (40 X 10 X 1.25).

So, that's the theory. Now it's time for an example. Let's once again use red blood cells, for which a total magnification of 400X is sufficient to see their shape. A 40X objective used with 10X eyepieces would provide this level of total magnification and would make red blood cells appear approximately the same size as an object with a 2.8mm diameter (400 X 7µm), positioned the same distance from your eyes. In other words, if you're looking into your microscope and the magnified image of the red blood cells appears to be a comfortable 18" away from your eyes, the red blood cells would appear to you to be approximately the same size as a disk with a 2.8mm diameter viewed at that same 18" distance.

What is Tube Length?

The design of a compound light microscope uses the objective lens to provide one stage of magnification and the eyepiece lenses to provide a second stage. The total magnification of the resulting images is equal to the magnification of the objective lens multiplied by the magnification of the eyepiece lenses. To make this work properly, the eyepiece lenses need to be the correct distance away from the objective lens, as determined by the "tube length" parameter of the objective lens. In the simplest design of the compound microscope, the objective lens is mounted onto the lower end of a straight, hollow tube, and the eyepiece is dropped into the upper end of the tube. The length of the tube is such that the objective lens and the eyepiece lens is the proper distance apart. The earliest compound light microscopes were constructed like this. Eventually, microscope designs were modified to include such things as an inclined eyepiece, binocular heads, and so on, to improve the ergonomics for the operator. In these newer designs, the tube length does not simply include the physical length of a straight optical tube but must account for the total optical length of the light path, including any reflections induced by the mirrors or prisms which provide the inclined or binocular views.

What is a Tube Lens?

The tube lens (also known as a Telan lens) is the optical element of an infinity microscope which accepts the parallel rays from infinity objective lenses and focuses them further downstream in the optical tube to produce a real image at the proper point for the eyepieces. This lens is called a tube lens since it is integral to the optical tube. Finite-conjugate microscopes do not utilize tube lenses, since finite-conjugate objectives do not produce parallel rays, but instead produce rays focused at the proper point in the tube to produce the real image needed for the eyepieces.

How is magnification defined?

What does it mean to say that a compound light microscope magnifies 400X? Common sense would say that specimens observed under such a microscope would appear to the observer to be 400X larger than they actually are, and this is true, but there is still some ambiguity here. What does the word *larger* mean in this context? I know this sounds like a prelude to a bunch of legalese, or even outright gibberish, but hear me out. Suppose an observer is looking at a square specimen 1 mm on each side. How big would a square 400X this size be? If you look at the square in terms of area, the square under observation has an area of 1 mm^2, so a square 400X larger in area would be 400 mm^2, or 20 mm per side. On the other hand, if you look at in terms of linear magnification (i.e., if you look at a single dimension, rather than the area), then a square 400X larger than the specimen would be 400 mm per side, and would have an area of 160,000 mm^2. As you can see, there is a huge difference between these two interpretations! For our purposes, the answer is that the word *magnification* refers to linear magnification. Early in the evolution of microscopy, linear magnification became the de-facto way magnification was specified, and this was often specified in terms of *diameters*. A microscope said to have a magnification of 100 diameters would make a specimen under observation appear to have a diameter 100X that of the actual specimen. In modern terminology, this magnification would simply be called “100X”.

What is the purpose of the blue filter I got with my microscope?

The blue filter included with many microscopes is simply intended to be placed in the light path of the microscope during routine observations, in order to remove the yellow cast that otherwise can be present with halogen lighting, especially when observing at low lamp intensity settings. Although this filter is not strictly needed, it makes the image more pleasant for the observer. Contrary to popular belief, this filter is not the correct filter to use for photomicrographic applications with daylight-balanced film. The proper filter to use in this case would be the Kodak 80A or equivalent.

What is the purpose of the green filter I got with my microscope?

Achromatic objectives are best corrected for spherical aberrations at the green wavelengths of light. Because of this, inserting a green filter into the lighting path produces monochromatic images with minimal spherical aberration and no color fringing due to chromatic difference of magnification. Although perhaps undesirable for routing viewing, this configuration allows the microscopist to produce very high quality photomicrographic images on black-and-white film, or better still, in digital format (the resulting digital images can then be post-processed to convert them into black-and-white images) using basic achromatic objectives. In addition to improving the performance of achromatic objectives, the green filter also provides the best phase-contrast performance possible, since phase-contrast objectives are designed to provide peak phase performance when used with monochromatic green light.

Can I use infinity objectives on a finite microscope?

No. Infinity-corrected objective lenses cannot be used on microscopes designed for finite-conjugate optics, since the finite-conjugate scope will not contain the required tube lens necessary to produce a real image for the eyepieces. You may be able to physically screw an infinity objective onto a finite scope and achieve an image of some sort, the resulting image quality will be quite poor.

Can I use finite objectives on an infinity microscope?

No. Finite-conjugate objective lenses cannot be used on microscopes designed for infinity optics, since the infinity scope will contain a tube lens that will interfere with the ability of the objective lens to produce a real image at the proper point for the eyepieces. You may be able to physically screw a finite-conjugate objective onto an infinity scope and achieve an image of some sort, the resulting image quality will be quite poor.

Will one manufacturer's infinity objectives work on another manufacturer's infinity scope?

In the broadest sense, the answer is no. The reason for this is that the infinity optics from one manufacturer might differ from those of another manufacturer, such that any optical corrections present in a given manufacturer's tube lens may not match those of another manufacturer tube lens, producing images with significant optical aberrations. Additionally, the focus length of the two tube lenses needs to be the exact same in order for the objective lenses to produce their specified magnifications. That said, however, infinity objectives from one manufacturer *might* work acceptably well on another manufacturer's infinity scope, and the only way to know for sure is to try it. Just be aware that although the resulting images might look good to your eyes, they may in fact be performing below their theoretical capabilities.

Why can't I see stereo images through my binocular head or trinocular head?

It is a common misconception that the purpose of binocular imaging in a compound light microscope is to provide stereo imaging capabilities similar to that produced by low-power stereo microscopes. However, contrary to this common belief, modern compound microscopes do not provide stereo imaging at all. Instead, they use a binocular or trinocular head to improve operator comfort by reducing eyestrain. Stereo imaging in a high-power compound microscope is not technically feasible, since in order to do so, the optics would need to provide a separate image for each of the viewers eyes, both with slightly differing perspectives. This means that either dual objective lenses would be needed, as in a low-power stereo microscope, or the output from a single objective lens would need to be geometrically split to obtain the images needed for stereoscopic imaging. Since compound microscopes are designed to provide high magnifications, their objective lenses require very short working distances (i.e., the distance from the objective lens to the specimen is very short), making it impossible to utilize dual objective lenses. Geometrically splitting the optical output of a single objective lens, and routing one half to one eye and the other half to the other, is problematic as well, since such a split would prevent the full aperture of the objective lens from contributing to the images, thereby significantly reducing the resolving power of the microscope. The resulting reduction in resolving power needed to obtain stereo imaging in a compound scope is not an acceptable tradeoff, since resolving power is everything in high-power microscopy.

Questions about Microscope Illumination

What is Afocal Illumination?

The earliest compound microscopes utilized non-focused illumination to light the specimen under observation. This illumination scheme, which is also known as afocal illumination, used a simple mirror beneath the microscope stage to reflect incoming light up onto the specimen. Although this mirror reflected light onto the specimen, it did not focus an image of the light source anywhere in the optical pathway, which is why this technique is called *non-focused* or *afocal* illumination. Non-focused illumination is still used in many toy microscopes manufactured today, but will not be found in any "real" microscopes.

What is Critical Illumination or Nelsonian Illumination?

An early, yet major development, in the evolution of microscopy was the technique known as Critical Illumination (also called *source-focused* or *Nelsonian* illumination). This technique, which was originated by a British microscopist by the name of Edward Miles Nelson, used optical principles first introduced by

Ernst Abbe, and was a huge improvement over non-focused lighting. Nelson's method utilized a sub-stage condenser to project a focused image of the light source directly onto the specimen under observation, thereby providing a much brighter visual field and higher resolving power than could be obtained using non-focused illumination. Critical illumination was the standard illumination method of the late nineteenth century and is still used to this day in most microscopes intended for the educational and hobbyist markets. The primary drawback of critical illumination is that since the image of the light source is focused directly onto the specimen, any non-uniformity in the lighting source (such as the filament of a halogen bulb or the emitter of an LED) is directly visible to the observer as non-uniformity in the brightness of the visual field. Although this is not usually a problem for direct visual observations, such non-uniformity can be very noticeable in photomicrographic images. To be fair, in Nelson's day, either natural daylight or the flame of an oil or gas lamp would have been used as the source of illumination, and the uniformity of these sources was quite good, allowing for acceptable viewing and even photomicrography. It is important to realize that much of the early and important work in the field of microscopy was performed on microscopes equipped for Critical Illumination. Eventually, electric lighting (whether tungsten, halogen, or LED) came to be almost universally used, and these lighting sources exhibit significantly more non-uniformity than the flame and blue skies used in Nelson's day. Because of the inherent non-uniformity of modern electrical lighting sources, designers added a ground glass diffuser between the light source and the substage condenser, which significantly improved the performance of these new electrically illuminated systems, but the ground glass diffusers introduced a new problem. Rather than focusing an image of the non-uniform illumination source onto the specimen plane, the condenser instead focused an image of the ground glass diffuser onto the specimen plane, which appears as a distracting, grainy texture in the observed image. To obscure this graininess, the substage condenser is typically defocused slightly from the critical focus point by raising it slightly.

How do I set up Critical Illumination or Nelsonian Illumination on my microscope?

Start by raising the position of the condenser to the top-most position, using the condenser focus knob, and set the condenser iris diaphragm to the approximate mid-range position. Next, select the low-power (10X) objective and adjust the illumination to a comfortable viewing level. Place a slide on the stage and adjust the focusing, slide position, and condenser iris diaphragm for a good image of the specimen. Using the condenser focus knob, slowly lower the condenser until you see granularity of the diffusion filter come into sharp focus, superimposed on the image of the specimen. This is the critical focus setting of the condenser. Raise the condenser height just enough to obscure the granularity, and you should be good to go.

What is Episcopic Illumination or Reflected Illumination?

Episcopic, or epi-illumination (also called *reflected illumination* or *incident lighting*) is the lighting type used for metallurgical, semiconductor, and fluorescent applications, wherein the illuminating source and the viewing optics are located on the same side of the specimen under observation. Light from the illuminating source strikes the specimen and reflects back into the viewing optics. This differs from convention diascopic or transmitted lighting, commonly used for biological applications, wherein the illuminating source is on one side of the specimen and the viewing optics are on the other, and the image is formed by the transmission of light through the specimen. Episcopic illumination uses a device called a vertical illuminator to send light through the objective lens, down onto the upper surface of the specimen, and then to route the reflected light from the specimen, as collected by the objective lens, to the eyepieces for observation.

What is Köhler Illumination?

The lighting technique known as *Köhler Illumination* was first described by August Köhler in 1893. The Köhler method of illumination uses one or more converging lenses to form an image of the light source at

the front aperture of the substage condenser, allowing light to emerge from the condenser as a parallel beam (i.e., with parallel rays) to provide perfectly defocused illumination of the visual field. Perfectly defocused illumination prevents any non-uniformity in the lighting source from appearing in the specimen plane. In contrast to critical illumination, Köhler illumination provides much more even illumination when using non-uniform illumination sources, and because of this it is found in virtually all upper-tier microscopes manufactured today. It should be noted that the Köhler illumination found in most modern microscopes does not conform to what purists would call true Köhler illumination. Rather, these scopes utilize what is sometimes referred to as modified Köhler illumination, where a ground-glass diffuser is present somewhere in the illumination path such that the image formed at the front aperture of the substage condenser is an image of the diffused light source, rather than the raw lamp filament or LED emitter of the source. The advantage of modified Köhler illumination over true Köhler illumination is that true Köhler relies on the source illuminator being completely planar, so that it can be properly focused onto the front aperture of the substage condenser. Since real-world lighting sources do not meet this total-planarity ideal, some non-uniformity of illumination in the specimen plane subsequently occurs when true Köhler illumination is used with real-world lighting sources. The use of a ground-glass diffuser allows the diffused light source, which appears as a nearly ideal planar source, to be properly focused onto the front aperture of the substage condenser, thereby providing a more uniformly illuminated specimen plane than could be achieved without the diffuser. Some intensity loss invariably results from the scattering of light by the diffuser, but the resulting improvement in lighting uniformity is generally worth the tradeoff of brightness. Some modified Köhler designs utilize a stand-alone ground-glass diffuser, while others use a frosted lens to achieve the same result. An advantage of the stand-alone diffuser over the frosted lens is that the stand-alone diffuser can be removed from the lighting path to allow the lighting source (as visualized by viewing the back focal plane of the objective) to be properly centered and focused at the front aperture of the substage condenser. Once the light source has been properly adjusted, the diffuser can then be reinserted back into the lighting path for observation. Most Köhler-equipped microscopes available today utilize fixed, integral lighting that is permanently positioned within the frame of the scope, and light sources which are manufactured such that the light emitter (i.e., filament or LED junction) is precisely positioned, thereby making focusing and centering provisions unnecessary. In these pre-centered/pre-focused designs, the ground-glass diffuser is typically not removable from the optical path.

How do I set up Köhler Illumination on my microscope?

This procedure assumes you have a Köhler-equipped scope with an integral, pre-centered illumination source (all modern Köhler-equipped scopes will be this configuration). Start by using the condenser focus knob to raise the position of the condenser to the top-most position, then set the condenser iris diaphragm to the approximate mid-range position and set the field diaphragm to the fully open position. Next, select the low-power (10X) objective and adjust the illumination to a comfortable viewing level. Place a slide on the stage and adjust the focusing, slide position, and condenser iris diaphragm for a good image of the specimen. Now close the field diaphragm until the field of view as seen through the eyepieces is noticeably restricted. In other words, until you see a bright circle in the field of view, surrounded by darkness. The bright circle may or may not be centered in the visual field at this point, and that's OK. Now use the condenser focus knob to adjust the condenser height until the edges of the bright circle are sharply defined. If the bright circle is not well centered at this point, use the condenser centering adjustments to center the bright circle in the visual field. Go back once again and tweak the condenser height, to be sure the bright circle is still sharply defined, then open up the field diaphragm until the bright circle just fills the complete field of view (i.e., there is no dark area at the periphery of the field), but don't go beyond this point. Köhler is now properly set up for the 10X objective, but you will need to repeat this setup procedure any time another objective is selected (in most scopes, Köhler cannot be properly set up

with the 4X objective). Note that when adjusting the condenser height to focus the bright circle in the visual field, you might see red fringing on the edges of the bright circle on one side of the focus point, and blue fringing on the other side. This effect is most visible when using a simple (i.e., non-achromatic) Abbe condenser. Just split the difference between the points where blue and red appear and you should be good to go.

What is Diascopic Illumination or Transmitted Illumination?

Diascopic or transmitted illumination, which is the lighting type used in biological compound microscopes, consists of a setup where a condenser on one side of the specimen provides light which transmits through the specimen to the viewing optics on the opposite side of the specimen. This differs from episcopic or reflected illumination, which is the lighting type used in metallurgical, semiconductor, and fluorescent applications, where the illuminating source and viewing optics are on the same side of the specimen, and the image is formed by reflecting light off of the specimen.

What is a Vertical Illuminator?

A vertical illuminator (also known as an epi-illuminator) is a device which mounts below the viewing head and above the nosepiece of an upright microscope, or in the body below the nosepiece of an inverted scope, which sends light through the objective lens and onto the specimen under observation, then routes the light reflected by the specimen, which is collected by the objective lens, to the viewing head for observation. The illuminating light begins in the lamphouse and passes through a collecting lens into the body of the vertical illuminator, where it is controlled by the aperture diaphragm and field diaphragm (if present). A beam splitter consisting of a primary-surface half mirror oriented 45° to the direction of the light reflects the light into the objective lens (which functions as the condenser for the illuminating light) and onto the specimen under observation. Reflected light from the surface of the specimen re-enters the objective lens and is then directed through the beam splitter to the viewing head for observation.

How do I use the substage mirror on my microscope?

I will assume that you do not have a toy microscope, but instead have a real microscope with a true substage condenser that also happens to have a substage mirror. These scopes, which were common well into the 1970s, did not include illumination integral to the scope, but instead relied on an external illuminator to provide specimen illumination. To use this mirror, simply shine a suitable external light source onto the mirror and adjust the mirror so that the visual field is evenly illuminated. Some substage mirrors have only a flat reflecting surface, while some have a second side which contains a concave reflecting surface. The flat side should always be used with the substage condenser, whereas the concave side, if present, should be used only for low power observations with the substage condenser removed from the scope.

Questions about Microscope Eyepieces

What is a Compensating Eyepiece?

So-called *compensating* eyepieces not only provide magnification of the real image produced by the objective lens, as is needed in all compound microscopes, but also provide compensation for any chromatic difference of magnification present in this real image as a result of incomplete compensation within the objective itself. As such, compensating eyepieces are used with objectives that do not provide fully compensated real images, as a way to clean up the lateral chromatic aberration so that the observer sees a correct, fully compensated image. In contrast, a non-compensating eyepiece is optically neutral and provides no compensating for chromatic aberration. Non-compensating eyepieces are used with objectives which fully compensate for chromatic aberration, or with objectives that do not fully compensate for chromatic aberration, but whose optical system includes an accompanying tube lens that provides the necessary correction of the chromatic difference of magnification, such that the observer

sees a correct, fully compensated image. Compensating eyepieces are traditionally marked with the letters "C", "K", or "COMP" (marking with the letter K originated with the early German microscope manufacturers and was used to indicate that the eyepiece was a *Kompensating* type). Note that if an objective that should be used with compensating eyepieces is instead used with non-compensating eyepieces, or if an objective that should be used non-compensating eyepieces is instead used with compensating eyepieces, the resulting image will be improperly corrected, exhibiting significant chromatic aberration visible to the operator. It is therefore critical that objectives always be used with the proper eyepiece types, in order to achieve the best optical performance possible.

What is the Eye Relief of an eyepiece?

Light rays exiting the eyepiece intersect at the so-called exit pupil or eyepoint of the eyepiece (this is also referred to as the Ramsden disk), which is where the pupils of the observer's eyes should be placed to properly see the entire field of view of the microscope. In standard relief eyepieces, the eyepoint is typically in the range of 7 to 13 mm above the top lens, although the higher the magnification of the eyepiece, the lower the eyepoint. Eyepieces with magnifications of 20X and higher can be very difficult for many people to use, as these can be quite fatiguing to the operator. While lower-power eyepieces with standard relief are adequate for many operators, they do not allow the operator to wear corrective eyeglasses while using the scope. To address this, extended relief eyepieces (also known as high-eyepoint eyepieces) were designed to provide an eye relief of 15mm or greater, such that they can be comfortably used by an operator who wears corrective eyeglasses. High-eyepoint eyepieces can usually be identified by either the letter "H" or by an icon showing a pair of eyeglasses marked on the barrel of the eyepiece. If you have a choice, get eyepieces with a high eyepoint, whether or not you wear glasses. If nothing else, you won't have the problem of your eyelashes continually smudging the upper lens that users of low-relief eyepieces tend to have.

What is the Field Number of an eyepiece?

The field number (FN) rating of a microscope eyepiece is the diameter (typically expressed in millimeters) of the circular portion of the real image projected by the objective lenses onto the intermediate image plane that can be accepted by the eyepieces and made visible to the observer. An eyepiece with an FN rating of 20, for example, will allow the observer to see a circle with diameter of 20mm from the intermediate real image, while excluding everything outside of this 20mm circle. The FN rating, which is marked on the barrel of most eyepieces, is useful since it allows the operator to quickly and easily determine the diameter of the visual field for the various objective lenses.

To determine the diameter of the visual field for a given objective lens, simply divide the FN rating of the eyepieces by the magnification rating of the objective lens. The result will be the diameter of the visual field for that specific objective lens (or, said another way, the result will be the diameter of a specimen needed to fill the visual field with that specific objective lens). If your microscope has eyepieces with an FN of 20 and you are using the 10X objective, then a circular specimen of 2.0mm (20mm / 10 = 2.0mm) would exactly fill the visual field. Knowing the diameter of the visual field is important since it allows the operator to quickly estimate the approximate size of specimens within the visual field.

What is a Filar Eyepiece?

A filar eyepiece (also known as a filar micrometer) is a specialized eyepiece used in microscopy for the measurement of specimens when conventional eyepiece reticles do not provide sufficient accuracy. The word *filar* comes from the Latin word *filum*, meaning "thread", referring to the fine wires used in the device. A filar eyepiece is consists of a reticle with two fine parallel wires (or threads), located in the image focal plane of the eyepiece, that can be moved by the observer via a micrometer screw mechanism. Since these parallel wires are in the image focal plane of the eyepiece, they appear sharply focused and superimposed over the specimen under observation. The micrometer screw mechanism moves the wires

across the eyepiece focal plane, allowing the operator to place one wire over one point of interest of the specimen, and then move the second wire to a second point of interest, and then calculate the spatial distance between these two points from the readings of the micrometer markings of the instrument.

What is a Huygenian Eyepiece?

The Huygenian eyepiece (also called a negative, or internal diaphragm eyepiece) was designed by Christiaan Huygens in the late 1660s, and is a simple design consisting of two lenses (usually plano-convex lenses) and a field diaphragm (which determines the field of view of the eyepiece). The two lenses are oriented such that the convex sides both face the specimen, and the field diaphragm is situated somewhere between these two lenses. Although the individual simple lenses in a Huygenian eyepiece are not well corrected, their aberrations tend to cancel due to the geometry involved. Huygenian eyepieces do not fully correct for chromatic aberration and are inferior to the later Ramsden design, and because of this their usage is restricted to microscopes which utilize low-power achromatic objectives, such as educational and low-end hobbyist scopes. Huygenian eyepieces are typically marked with just the magnification factor of the eyepiece and are generally suitable for use with achromatic objectives up to 40X. The achromatized Huygenian eyepiece is an improved version of the basic Huygenian eyepiece, in which the lens closest to the viewer's eye is no longer a simple plano-convex lens but is instead a cemented achromatic doublet. While the achromatized Huygenian design is a definite improvement over the original Huygenian design, it is still best suited for use with low-power achromatic objectives.

What is an Ocular Lens?

The terms *ocular* or *ocular lens* are alternative names for the microscope eyepieces. The ocular lenses are the lenses that the microscopist looks into to see the specimen under observation. Note that throughout this document, the terms *eyepieces* or *eyepiece lenses* will be used instead of *oculars* or *ocular lenses*.

What is a Periplan Eyepiece?

The Periplan eyepiece is significantly more complicated than the basic Huygenian, Ramsden, or their achromatized counterparts. A Periplan eyepeiece contains a cemented doublet, a cemented triplet, and two single lenses (seven individual lens elements in total). The Periplan eyepiece provides improved correction for residual lateral chromatic aberration, improved field flatness, and improved performance when used with higher power objectives.

What is a Ramsden Eyepiece?

The Ramsden eyepiece (also called a positive or external diaphragm eyepiece) was designed by Jesse Ramsden in 1782, and is a simple eyepiece design which, while considered superior to the earlier Huygenian design, still suffers from some degree of chromatic aberration. The Ramsden design consists of two simple lenses (usually plano-convex lenses) and a field diaphragm (which determines the field of view of the eyepiece). The two lenses are oriented such that the convex sides face each other, with the field diaphragm situated below these two lenses. The front focal plane of the Ramsden eyepiece lies just below the lower lens, at the level of the field diaphragm, making this eyepiece suitable for the mounting of measuring reticles. Since the Ramsden design has reduced lateral chromatic aberration, as compared to the Huygenian eyepiece, any distortion of the reticle scale is not readily apparent to the viewer.

The achromatized Ramsden eyepiece (also known as the Kellner eyepiece) was designed by Carl Kellner in 1849 and is an improved version of the original Ramsden eyepiece, wherein the lens closest to the viewer's eye is no longer a simple plano-convex lens but is instead a cemented achromatic doublet, used to correct for residual chromatic aberration. The Kellner design is significantly better than both the Huygenian and Ramsden designs and works well with objectives of low to medium powers. The eye relief of the Kellner is better than that of the Huygenian but is not quite as good as that of the traditional

Ramsden. The main drawback with Kellner's design was internal reflections, but this issue has been largely eliminated thanks to modern anti-reflective coatings, making modern Kellner eyepieces an inexpensive and attractive option.

What is a Reticle, Reticule, or Graticule?

An eyepiece reticle (also known as a reticule or graticule) is a small glass disk with markings etched onto its surface that is placed at the plane of the field diaphragm in the eyepiece, to allow for simple measurements of specimen size. Reticles are commonly marked with simple ruler, crosshatch, or grid patterns, but other types are available for specific measurement purposes. Since the reticle is placed in the plane of the field diaphragm, it appears to the observer to be superimposed over the image of the specimen. For optimal results, eyepieces using reticles should contain a helical focusing mechanism to allow the image of the reticle to be brought sharply into focus for each observer. In addition to measurement purposes, reticles are also commonly used to allow the operator to visualize the field that will be captured by the camera, when using the scope for photomicrographic applications.

What are the standard eyepiece sizes and what are they used for?

Microscope eyepieces come in two standard diameters: 23mm and 30mm. Since the field of view of an eyepiece is determined by the size of the aperture in the field stop inside the eyepiece, an eyepiece with an outer barrel diameter of 23mm can never have a field stop much larger than 22mm, when you consider the inevitable thickness of the barrel walls. This was not a limitation for the early microscope designers, since the performance of the objective lenses in these early scopes was not able to provide good views over a field much larger than 22mm. However, as improvement were made to the optical designs of the objective lenses, it became necessary to go beyond the limiting 23mm eyepiece diameter, and this is why they switched to 30mm eyepieces (30mm eyepieces have been standard on stereo microscopes from way back). With modern optical designs, the field of view has been extended from a maximum of 22mm to as high as 28mm, in some cases. Virtually all microscopes made for the educational and hobbyist markets still use 23mm eyepieces, while many of the higher-end scopes boasting ultra-wide views now use 30mm eyepieces.

How can I tell the various eyepiece types apart?

Simple eyepiece designs, such as the Huygenian and Ramsden types, as well as their more corrected counterparts, will exhibit a blue ring around the eyepiece diaphragm when the eyepiece is held up to a light source. In contrast, the more highly corrected eyepieces (such as compensating eyepieces) will exhibit a yellow, red, or orange ring around the eyepiece diaphragm when held up to a light source.

Questions about Microscope Objectives

What is an Achromatic Objective?

Achromatic objectives are designed to minimize chromatic aberration inherent with glass optics. Rather than using simple lenses, an achromatic objective uses achromatic doublets, which are made of two different types of optical glass which exhibit different amounts of optical dispersion, bonded together such that one optical element counteracts the chromatic aberration produced by the other. Achromatic objectives are not perfect at eliminating chromatic aberration but are fine for all but the most demanding applications. A typical achromatic objective is designed to provide full correction for spherical aberration at green wavelengths, and to also bring red and blue wavelengths to a common focus.

What is an Apochromatic Objective?

Apochromatic objectives are similar to achromatic objectives, but their more complex optical construction allows them to provide significantly better performance than achromats. Apochromatic objectives are designed to correct spherical aberration for deep-blue, blue, and sometimes green wavelengths, and to

bring deep-blue, blue, green, and red wavelengths to a common focus. Of the various objective types, apochromats provide the absolute lowest spherical aberration and lateral chromatic aberration of any and will usually provide a higher numerical aperture to boot. Apochromats are very complicated optical designs which are difficult to manufacture and are therefore horrendously expensive for the typical amateur. Fortunately, while very nice to have, they are not a requirement. A good set of Plan achromats is all most users will ever need.

What is an E Objective or an EA Objective?

The letters "E" or "EA", when printed before the magnification factor on the top line of an objective barrel almost always indicate a low-cost objective intended for the educational or hobbyist market. These basic non-plan achromats are designed to a lower price point than standard achromats, and as such generally have a greater curvature of field as compared to standard achromats, as well as poorer sharpness and contrast at the outer fringes of the field. Educational objectives are generally used with eyepieces which restrict the field of view a bit more than would be used with standard achromats, so that the portions which suffer poor sharpness and contrast are not visible to the observer. Typical educational objectives of today would be used with eyepieces which limit the field of view to somewhere in the neighborhood of 18mm (i.e., FN 18), whereas today's standard achromats would be used with wider eyepieces of FN 20 or greater. These are guidelines and not hard-and-fast rules, and therefore do not apply in all cases. Older optics typically can be expected to perform a bit below modern standards, so for instance mid-range optics designed and built in the 1960s might have a FN of 18, while mid-range optics designed and built in the 1980s would likely have been FN 20 or greater.

What is a Fluorite Objective?

Fluorite objectives (also known as semi-apochromats) are made by including optically clear fluorite crystal, which exhibits very low optical dispersion, to bring red and blue wavelengths to a common focus, with green coming in very close, as well as providing full correction for spherical aberration for the blue and green wavelengths. Relatively speaking, a fluorite lens performs better than an achromatic lens, but not as well as an apochromatic lens.

What is an NC Objective?

Objective lenses marked with *NC* or *NCG* were made to observe specimens that are not covered by a cover glass. For biological applications, NC objectives are frequently used to observe things such as peripheral blood smears made without a cover glass. The lack of a cover glass is a distinct advantage in this case since the observation of blood smears requires high-magnification optics. Because high-power objectives have high numerical apertures, and since objectives with high numerical apertures need a cover-glass correction collar to compensate for variations introduced by the cover glass (otherwise significant spherical aberration can result), omitting the cover glass on a blood smear means that a correction collar is not needed on the objective, thereby allowing for simple observations using relatively inexpensive dry objectives. Metallurgical objectives are almost always NC types, designed for use without a cover glass, as they are made for looking at objects such as semiconductor wafers and polished rocks and metals.

What is the Numerical Aperture of an objective?

The numerical aperture (N.A.) of a microscope objective is an optical property which depends on the maximum angle of incoming light (i.e., the acceptance angle) at which the light will be collected by the objective lens. An objective with a high N.A. value collects light over a wider range of angles than one with a lower N.A. The numerical aperture rating is important since this is what determines the optical resolution, and therefore the maximum useful magnification, that the optical system can provide. The reason that the optical resolution depends on the acceptance angle of the optics is due to a phenomenon known as *refraction*. Without getting too technical, refraction is the scattering of light waves that occur

as the light passes through a specimen under observation. The degree to which this scattering occurs is a function of the physical detail in the specimen. The finer details in the specimen contribute the most scattering (i.e., the widest diffraction angles), while the coarser details contribute the lesser angles of diffraction. Because of this phenomenon, the objective lens must be able to accept incoming light over a wide angle to collect the components of the light that were diffracted by the finer details, otherwise the objective would not be able to resolve these fine details. How does numerical aperture relate to useful magnification? A good rule of thumb is that a given objective lens can provide useful magnifications up to the 1000X the rated N.A. of the objective. So, a good 100X oil-immersion objective with an N.A. of 1.40 can provide useful magnification up to 1400X, and that's all it can do. There is no reason to use such an objective with eyepieces higher than 14X, and any extent to which it is would simply produce empty magnification without revealing additional detail.

What is the Parfocal Distance of an objective?

The parfocal distance of a microscope objective is the distance between the mounting flange of the objective and the specimen under observation. This should not be confused with the working distance of the objective, which is the distance between the specimen and the bottom lens of the objective. It is important that all objectives on a microscope are made to the same parfocal distance, since that allows the operator to focus on the specimen under observation using the lowest-power objective, then freely rotate to the subsequent objectives without risk of slide collision.

What is a Plan Objective?

Plan objectives (also known as a *planar* objective) are designed to provide a flatter field of focus than that of standard, non-planar objectives. In a standard objective, the field of focus is significantly curved, such that when the center of the visual field comes into sharp focus, the periphery of the field will be somewhat out of focus, and vice versa. The further away from the optical center you look, the more significant this effect gets. Any arbitrary point in the visual field can be brought into focus using the focus knobs, but the entire visual field can never be in focus at any single setting of the focus knobs. This is not too much of a problem when a standard objective is used for visual observations, since the operator can easily optimize the focus settings to observe any arbitrary areas of interest in the specimen. For photomicrographic applications, this approach will not work. If the center of the field is in sharp focus, everything in the periphery will be annoyingly out of focus in the resulting photomicrographic images. This is where plan objectives come in, with their much wider area of acceptable focus. Plan objectives produce photographic images wherein the periphery is an acceptable focus when the center of the field is sharply focused. The common definition of a standard achromat is that 65% of the visual field is acceptably sharp when the center is in focus. For a plan achromat, this number rises to 95%. The semi-plan achromat falls between these two, with a flatness covering approximately 80% of the visual field.

What is the Working Distance of an objective?

The working distance of a microscope objective is the distance between the specimen under observation and the bottom lens of the objective where the specimen will be properly focused. In general, the working distance is highest on the lowest-power objectives and is the lowest on the highest-power objectives.

What is the purpose of LWD or ULWD objectives?

The working distance of a microscope objective is the distance between the specimen under observation and the bottom lens of the objective lens (or the top lens, in the case of an inverted scope) where the specimen will be properly focused. The letters "LWD" identify an objective with a long working distance, whereas the letters "ULWD" identify an objective with an ultra-long working distance. LWD and ULWD objectives are useful in inverted microscopes for observing specimens through the thick bottoms of culture vessels or petri dishes.

What is a Dry Objective?

A dry objective is an objective lens intended to be used without homogeneous immersion and should therefore never be used with any sort of immersion medium. Dry objectives are inherently limited to a theoretical maximum numerical aperture of 1.0 (in practice, the N.A. of a dry objective rarely exceeds 0.95). When using immersion oil on wet objectives, do not allow any of the oil to get on the dry objectives, or damage to the dry objective can occur as a result of oil ingress. Be sure to immediately clean off any immersion oil that gets onto the dry objectives, to prevent damage.

What is a Wet Objective?

A wet objective is an objective lens designed for homogeneous immersion, using some sort of immersion medium to obtain the rated numerical aperture. Wet objectives will often be marked "HI", to indicate the requirement for homogeneous immersion, but this will not always be the case. Whether marked as "HI" or not, a wet objective will be marked to identify the proper immersion medium with which it should be used. Oil-immersion objectives, which are by far the most common wet objectives available, will be marked either as "Oil" or "Oel" (if made by a German manufacturer). Although wet objectives often have numerical aperture ratings above the theoretical maximum 1.0 rating of dry objectives, this will not always be the case.

Why do some objectives have spring-loaded tips?

Microscope objectives have a specification called *working distance*, which is the distance between the specimen and the bottom lens of the objective where the specimen will be properly focused. For a low-power objective, such as a 4X, the working distance is sufficiently large, and the mechanical design of the microscope is such that the bottom of the objective cannot ever contact the slide on the stage. As the magnification of the objectives increases, the working distance decreases, and at some point, it becomes possible for the operator (especially those with little to no experience) to crash the slide into the bottom of the objective while focusing the microscope. To reduce the likelihood of the slide or objective being damaged in the event this ever occurs, higher powered objectives often incorporate a spring-loaded design, allowing the tip to safely retract into the barrel of the objective if it collides with the slide. The drawback to spring-loaded objectives is with careless use, the retraction mechanism can become contaminated with immersion oil or other debris to the point where the mechanism gums up and no longer protects the slide and optics, or worse yet will not re-extend following a retraction.

What do the various objective markings mean?

The markings on objective lenses are by no means standardized, but nonetheless, certain conventions are followed by many manufacturers. The most important parameters of objective lenses are typically marked on the barrel, in three lines of text, as described below:

The top line of text identifies the magnification factor, written either as a number or a number followed by the letter "X" (e.g., "10" or "10X"), along with various other optional characters. The letters "E" or "EA" typically indicate an educational achromat, while the letters "A" indicates a standard achromat and "Apo" indicate an apochromat. The letters "LWD" and "ULWD" represent objectives with long or ultra-long working distances. The word "Plan" means just what is says, while semi-plan is often identified by "SP". Other markings which indicate such things as phase contrast, polarization, fluorescence, etc. are much less standardized and are therefore not listed here.

The middle line of text indicates the numerical aperture (N.A.) of the objective, and any immersion medium needed to obtain this N.A. rating. For instance, an objective with the text "0.65" on the middle line has an N.A. of 0.65, and since there is nothing else present on the line, this indicates a dry objective which should not be used with any immersion oil or other media. On the other hand, an objective with "1.25 Oil" on the middle line would mean it has a N.A. of 1.25, when used with oil immersion. Some objectives will use the German word "Oel" to indicate immersion oil is needed. Others will use the letters

"HI" (to indicate Homogeneous Immersion) when oil is needed. Less commonly encountered are the letters "W", "WI", or "Wasser" to indicate water immersion, "SI" or Sil" to indicate silicon immersion oil, or "Gl" or "Gly" to indicate glycerin oil immersion.

The bottom line of text indicates the tube length for which the objective should be used, and any requirements for the specimen cover glass. A few examples are the letters "160/0.17", which indicate that the tube length should be 160mm and that the objective is optimally corrected for viewing through a cover glass 0.17mm in thickness, and the letters "∞/0" which indicate an infinity objective optimized for viewing without a cover slip. Another example is the letters "160/–", which indicates that the tube length should be 160mm and that the cover glass thickness is irrelevant.

What do the various objective colors mean?

The color markings on objective lenses, while by no means universal, are generally consistent between manufacturers of DIN-compliant and infinity scopes. These color rings indicate the magnification of the objective, and when applicable, the type of immersion medium with which the objective should be used.

The objective magnification is marked using a color ring around the upper portion of the objective barrel, as listed below.

Upper Color Ring - Magnification	
Color	**Magnification**
Black or Gray	1.0X, 1.25X, 1.5X
Brown	2.0X, 2.5X
Red	4X, 5X
Yellow	10X
Green	16X, 20X
Turquoise	25X, 32X
Light Blue	40X, 50X
Cobalt Blue	60X, 63X
White or Off-White	100X and higher

Specific types of immersion media s are identified by a second color ring around the lower portion of the objective barrel, as listed below.

Lower Color Ring - Immersion Types	
Color	**Immersion Type**
Black	Oil Immersion
Orange	Glycerol Immersion
White	Water Immersion
Red	Special Immersion
No Lower Ring	No Immersion (Dry)

What is the purpose of the correction collar found on some objectives?

High-power objective lenses have high numerical apertures which makes them inherently sensitive to the cover glass thickness of the sample. When high N.A. objectives are used with a cover glass significantly different from that for which it was designed, significant spherical aberration can result. This is not generally a problem with oil-immersion objectives, since the cover glass thickness simply acts as an extension to the oil bridge, and so as long as the thickness of the oil bridge plus the cover glass equals the correct working distance, the objective performs properly. On the other hand, dry objectives, which do not utilize an oil bridge, may need a correction collar to adjust for errors in the cover glass thickness (in order to prevent excessive spherical aberration) if their N.A. ratings are very high. The correction collar works by changing the spacing of some optical elements internal to the objective lens, thereby allowing the microscopist to compensate for any errors in cover glass thickness. On typical upright scopes, the

correction collars can be adjusted to accommodate cover glass thicknesses over the range of 0.11 mm to 0.22 mm, whereas on inverted scopes, the range of the correction collars are typically from 0 to 2.0 mm, allowing for use with Petri dishes or culture vessels.

What is the purpose of the iris diaphragm found on some objectives?

The iris diaphragm found on some high-N.A. objectives is there to allow for the reduction of the numerical aperture of the objective, so that it can be used for darkfield illumination. For normal brightfield applications, this iris diaphragm should be set wide-open, allowing the objective to provide its rated N.A. For darkfield applications, this diaphragm should be closed just enough to fully exclude all of the un-diffracted (i.e., zero-order) light from the condenser, thereby producing a dark visual field. Do not close this iris diaphragm any further than is necessary to achieve good darkfield, since the more the diaphragm is closed, the more resolving power is lost. Always remember to return the iris diaphragm in the objective to the wide-open position when you are finished using it for darkfield, so that subsequent brightfield will be at full N.A.

How do the N.A. rating and magnification rating of objectives affect image brightness?

In general, the higher the magnification, the dimmer the resulting image. Assuming everything else is equal, the image brightness varies inversely as the square of the magnification of the objective lens. Conversely, the higher the N.A., the brighter the resulting image. Assuming everything else is equal, the image brightness varies directly as the square of the numerical aperture of the objective lens for transmitted light, and as the fourth power of the numerical aperture for reflected light.

Can phase-contrast objectives be used for brightfield observations?

Brightfield images produced by phase-contrast objectives will be almost as good as those produced by conventional brightfield objectives. Many people, in fact, might be hard pressed to see a difference when the two are used for visual observations. The differences are more apparent when they used for photomicrography, as shown in the comparison photos below.

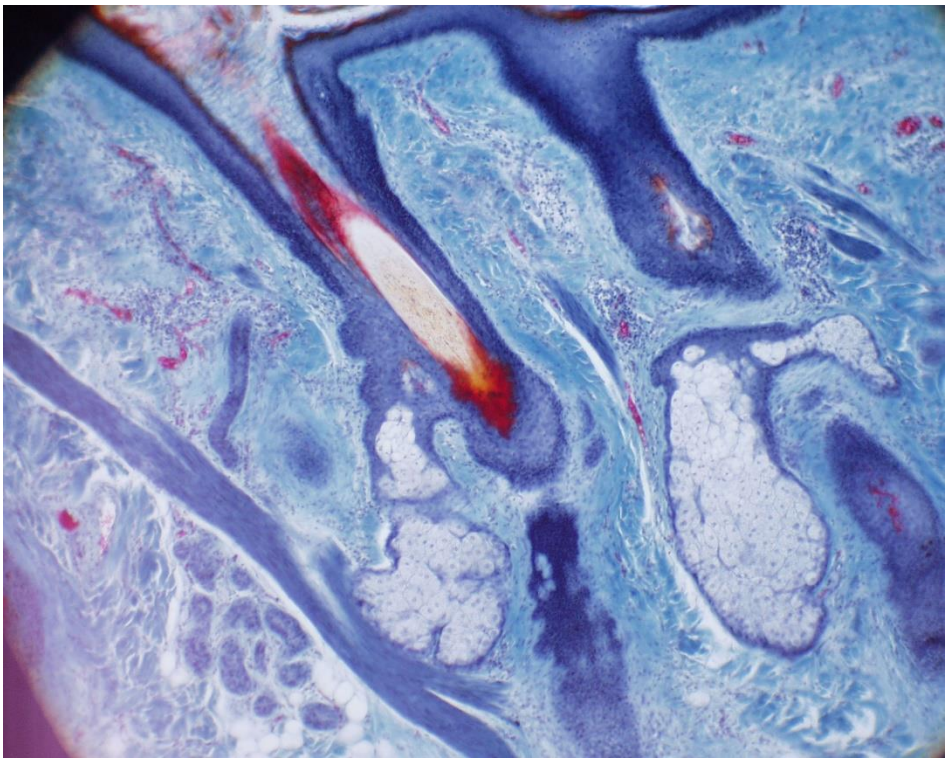

Figure 1 – Olympus A10 Brightfield Objective

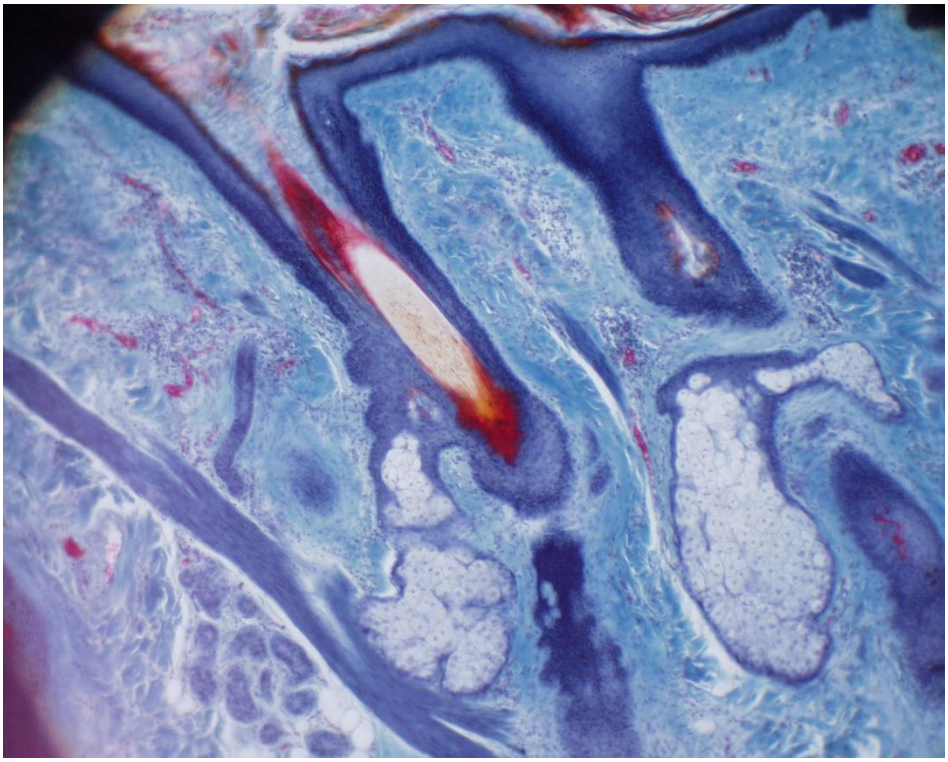

Figure 2 - Olympus A10 PL Phase-Contrast Objective

Why do I get a bad image when using my 40X objective?

This usually comes down to an improperly prepared slide. The dry 40X objective lens has a relatively high numerical aperture (typically 0.65), and because of this, the image quality it produces is very dependent on the thickness of the cover glass placed over the specimen. Any significant variation of the cover glass thickness from the ideal value printed on the objective barrel (typically 0.17 mm), or if there is any significant layer of mounting medium between the cover glass and the specimen under observation, will produce significant spherical aberration in a high-N.A. dry objective. To view a poorly prepared slide without image degradation from spherical aberration, you must either use a dry 40X objective with a correction collar, or switch to an oil-immersion objective.

Questions about Microscope Condensers

What is a Dry Condenser?

A dry condenser is intended to be used without immersion oil. Because dry condensers are inherently limited to a theoretical maximum numerical aperture of 1.0 (in practice, the numerical aperture of a dry condenser rarely exceeds 0.95), they are not the best choice for observations with a 100X oil-immersion objective, whose N.A. often exceeds 1.30. Some types of dry condensers, such as those designed to have a longer working distances, will have significantly lower numerical apertures than 0.95 (i.e., in the range of 0.65). It is important to never apply immersion oil to a dry condenser, since they are not designed for oil contact and the oil can find its way into the condenser, fouling both the optics and the iris diaphragm mechanism. Any immersion oil or other liquids which accidentally contact the dry condenser should be promptly and thoroughly removed.

What is a Wet Condenser?

A wet condenser is designed explicitly to have immersion oil applied to the top optical element, in order to achieve numerical apertures exceeding the theoretical dry limit of 1.0. Wet condensers should be oiled

to the specimen slide whenever maximum resolution is desired when using the 100X oil-immersion objectives. Wet condensers can also be used dry, but be aware that the numerical aperture, and therefore the image resolution, will be limited when used without immersion oil.

What condenser options are there for low-power observations?

For routine microscopic observations, a single condenser with a fixed focal length is often used to provide illumination for routine observations using the 4X, 10X, 40X, and 100X oil-immersion objectives. These fixed-focal-length condensers are easily able to illuminate the visual field of the 10X and higher objective lenses but are often only marginally able to illuminate the visual field of 4X objective lenses and are unsuitable below 4X. Since most microscopists rarely use objectives below 4X, this is not a huge problem. For situations where observations using objective lenses below 4X are required, microscopists typically turn to one of the following options, listed in order of their technical suitability: 1) A dedicated low-power condenser optimized to provide wide-field illumination, 2) A Flip-Top condenser (also known as a Swing-Out condenser) or a Swing-In condenser, or 3) Removal of the substage condenser entirely. Options #1 and #3 are the least convenient for the user, since it takes a significant amount of time to remove, and more still to remove and replace, the substage condenser when low-power observations are desired. The most convenient option is option #2, which is generally adequate for objective lenses with magnifications as low as 2X, but rarely all the way down to 1X.

What is a Flip-Top Condenser or Swing-Out Condenser?

A Flip-Top (Swing-Out) condenser contains an optical lens on a rotating mechanism located on the top of the condenser, such that the condenser can be quickly changed from one focal length to another by simply flipping the upper lens either into or out of the illumination path. For observations at 4X and above, the lens can be flipped into the illuminating path, and for observations below 4X, the lens can be flipped out of the illumination path. A less convenient although technically similar alternative to the Flip-Top condenser is a condenser which allows for the top lens to be unscrewed and removed from the illuminating path for low-power observations and re-installed for higher-power observations. Whenever a Flip-Top condenser or a condenser with a removable top lens is used without its upper lens in the illuminating path, the condenser aperture diaphragm should be opened to its widest position and the field diaphragm, which should then be visible at the back focal plane of the objective, should be used as if it were the condenser aperture diaphragm.

What is a Swing-In Condenser?

A Swing-In condenser is similar to the Flip-Top condenser, except that it contains an optical lens on a rotating mechanism located below, rather than on top, of the condenser. In these condensers, the lens is swung into the illuminating path for low-power observations and swung out for higher-power observations. American Optical used Swing-In condensers on their microscopes for many decades, as it was considered technically superior to the Flip-Top type, since it not only included less glass in the illuminating path during routing viewing, but it also retained the ability to properly focus light onto the specimen when configured for low-power observations.

What is the Numerical Aperture of a condenser?

The numerical aperture of a condenser, in combination with that of the objective, is what determines the overall resolution of the optical system in a compound microscope. In the condenser, the numerical aperture is a function of the geometry of the light cone with which the condenser illuminates the specimen under observation. Specifically, the wider the angle of the illuminating cone, the higher the resulting numerical aperture of the condenser. Most condensers include an aperture diaphragm which is used to vary the angle of the light cone, and hence the numerical aperture of the condenser. Reducing the opening in the aperture diaphragm decreases the angle of the illuminating light cone, thereby decreasing the numerical aperture of the condenser and the overall resolution of the optical system. In

order to achieve the maximum resolution that a given objective lens can provide, the numerical aperture of the condenser must be equal to or greater than that of the objective lens. In practice, there is no reason to set the condenser such that the numerical aperture exceeds that of the objective lens, since no additional resolution will be gained, and image contrast will be reduced.

What is the purpose of the microscope condenser?

The condenser in a compound microscope collects light from the microscope light source and concentrates it (i.e., condenses it) onto the specimen under observation. By concentrating the available light, the specimen is illuminated with an intensity that would otherwise require much greater intensity from the light source. A basic microscope condenser consists of a few lenses to concentrate the incoming light onto the specimen, and an iris diaphragm to control the angle of the light cone striking the specimen (and therefore the numerical aperture of the condenser).

What is an Abbe Condenser?

The Abbe condenser was developed by Ernst Abbe for Zeiss in 1870 and is the most basic condenser type in common usage today. Abbe's design consisted of two simple lenses which project an image of the light source onto the specimen under observation, with no provisions for the correction of chromatic or spherical aberrations of the light cone. An iris diaphragm is present to control the numerical aperture of the condenser, by controlling the angle of the light cone striking the specimen. Although the venerable Abbe design has the poorest performance of the three commonly used condenser types, it is cheaper to manufacture and provides acceptable performance for objective of 40X and below. The condenser mounted on your microscope right now is likely an Abbe condenser.

What is an Aplanatic Condenser?

The aplanatic condenser is similar to the Abbe design, except that its optics have been refined to minimize the spherical aberration of the light cone that illuminates the specimen.

What is a Compound Achromatic Condenser?

The compound achromatic condenser (also known as an aplanatic/achromatic condenser, or AAC) is similar to the aplanatic design, except its optics have been further refined to also minimize chromatic aberration of the light cone that illuminates the specimen.

What is a Darkfield Condenser?

Darkfield condensers are designed such that the light cone illuminating the specimen under observation is modified by blocking the light in the inner part of the illuminating cone, such that the direct (undiffracted) light falls outside the acceptance angle of the objective and is therefore not collected. Darkfield condensers come in two basic types: dry and wet. The design of the typical dry darkfield condenser, which is intended to be used without immersion oil, is based on one of the standard condenser types described above, and includes a simple darkfield stop that has been added to modify the light cone striking the specimen. By contrast, the design of a wet darkfield condenser, which is intended to be used with immersion oil, differs greatly from the basic condenser types, in order to provide the tightly controlled lighting cone needed for proper darkfield with high-power, oil-immersion objectives.

What is a Nomarski (NIC) Condenser?

Nomarski condensers are a vital part of the optical system used in Nomarski Interference Contrast (NIC) microscopy (also known as Differential Interference Contrast, or DIC). A Nomarski condenser creates two mutually coherent, orthogonally polarized, and sheared (displaced) beams of light to illuminate the specimen under observation. To accomplish this, the condenser contains a linear polarizer which creates linearly polarized light from the illumination source, and a Wollaston prism which splits the linearly polarized light into two mutually coherent, orthogonally polarized components with a slight shear.

What is a Hoffman Modulation Condenser?

The Hoffman Modulation condenser is essentially a basic two-lens Abbe condenser with an iris diaphragm that contains an internal slit aperture and a rotating linear polarizer, to provide lighting for Hoffman Modulation Contrast microscopy.

What is a Phase Contrast Condenser?

Phase-contrast condensers are based on one of the basic condenser types described above, to which a provision has been added to allow a phase-contrast annulus to be placed into the optical pathway of the condenser. Many modern general-purpose condensers include a slot which accepts a phase-contrast slider, thereby allowing the user to position the desired phase annulus in the optical pathway by setting the slider to specific positions in the slot. Another common configuration is the *Zernike* style, which is equipped with a rotating disk equipped with the various phase annuli. In the Zernike design, the user selects the desired phase annulus by rotating the disk until the proper annulus clicks into the optical pathway.

How should the Condenser Aperture Diaphragm be adjusted?

The purpose of the aperture diaphragm in the microscope condenser is to control the numerical aperture of the condenser, and not to control the brightness of the image seen through the eyepieces. Although the intensity of the image does vary with the setting of the aperture diaphragm, this should be viewed as simply a side effect, and not as a way to control the lamp intensity. Many online tutorials and YouTube videos will tell you to use the aperture diaphragm to adjust the image brightness, but do not listen to this. If you encounter this incorrect advice, this shows that the person in the video does not understand basic microscopy concepts and you should seek to learn from better-informed sources. The correct way to set up the condenser and lighting on a scope is to first set the intensity control for a comfortable brightness, then focus on a sample specimen. After the specimen is visible with a comfortable lighting intensity, focus the condenser for either Critical or Köhler illumination, as applicable, and then adjust the aperture diaphragm of the condenser to obtain the desired resolving power and depth of field, then use the intensity control to tweak the lamp intensity, if necessary. There are two ways to set the aperture diaphragm to the point of maximum resolving power (and minimum depth of field). The first is to simply adjust the aperture ring or lever on the condenser until the scale indication matches the rated numerical aperture of the objective lens. The second way is to remove one of the eyepieces and look down into the eyepiece tube (which will allow you to see the aperture diaphragm setting) and adjust the aperture ring or lever until the lighted portion just fills the tube, then return the eyepiece to the eyepiece tube and you should be good to go. Regardless of which of these two methods you choose, you will end up with the aperture diaphragm set very close to the point which yields the highest optical resolving power, and you will likely notice that the image appears somewhat washed out at this setting. In order to increase the image contrast and/or improve the depth of field, many microscope manufacturers recommend a setting of 75% of the rated numerical aperture of the objective lens. If using the graduated scale method, just set the aperture ring or lever to 75% of the N.A. printed on the objective. If sighting down the eyepiece tube, set the aperture ring or lever so that 75% of the diameter of the tube is filled with light. Just be aware that by doing this you will be trading off resolving power (i.e., image resolution) for any increase in image contrast or depth of field that this provides. There is no reason to open the aperture diagram beyond the point of maximum resolving power, since doing so will only further wash out the image, while providing no additional resolution. Also, be aware that if you close the condenser aperture too much, not only will you severely reduce the resolving power, but you will also introduce visible artifacts which will make the image appear grainy and distorted. These artifacts, which are caused by diffraction of light passing the edges of the condenser leaves, may appear to the beginner as specimen detail but will be recognized by experienced microscopists as diffraction artifacts. If you set the numerical aperture of the condenser to match that of the objective lens, you will likely notice a significant lack of contrast in the

image, due to glare, and this glare may be so bad as to obscure the fine details to the point where they are not visible. While perhaps not visible, the information comprising the fine details should be present in the image and could potentially be made visible via various image enhancement techniques, thereby producing a good image at maximum resolution.

Questions about Microscope Viewing Heads

What is a Jentzsch Viewing Head?

The Jentzsch style binocular viewing head was developed by German-born Felix Jentzsch in 1913, while Jentzsch was working for the Leitz company. The original Jentzsch design had parallel eyepiece tubes and provided for interpupillary-distance adjustment by allowing the eyepiece tubes and their associated prisms to laterally slide further apart or closer together. The original Jentsch design as produced by Leitz utilized adjustable eyepieces to compensate for the inevitable changes to the optical tube length that happened as a result of adjustments to the interpupillary distance. Subsequent improvements to Jentzsch's original design provided automatic tube-length compensation by coupling the positions of the eyepieces within the eyepiece tubes to the lateral sliding mechanism, such that as the eyepiece tubes were slid further apart, the eyepieces moved deeper into the tubes, and as they were slid together, the eyepieces moved further out, thereby automatically compensating for the tube-length variation.

What Is a Siedentopf Viewing Head?

The Siedentopf style binocular viewing head was developed by German-born Henry Jentzsch in 1924, while Siedentopf was working for the Leitz company. The original Siedentopf design had parallel eyepiece tubes and provided for interpupillary-distance adjustment by allowing the left-most eyepiece tube and associated prism to rotate a fixed distance away from a central axis. The advantage of the Siedentopf design over the Jentzsch design is that changes to the interpupillary distance did not affect the optical tube length, and therefore no tube-length compensation was needed. The 1924 Siedentopf design was first manufactured by Zeiss and was offered as the *Bitumi* attachment for their monocular systems. In 1926, Zeiss released an improvement to the original Siedentopf design in which both eyepiece tubes and their associated prisms rotated, to allow for adjustment of the interpupillary distance.

What is the purpose of the adjustment ring on the left-most eyepiece tube?

The helical adjustment ring on the left eyepiece tube of most microscopes provides a diopter adjustment for the left eyepiece, which is used to compensate for any differences between the left and right eyes of the observer. If this adjustment were not present, and if your eyes were not perfectly matched, then you could only adjust the focus of your microscope for a sharp image in either the left or right eye, but not both at the same time. The correct way to set the diopter adjustment is to look through the microscope in the normal fashion, with the right eye looking into the right-most eyepiece, and with the left eye closed.

Now adjust the focus controls of the microscope until the right eye sees a perfectly sharp image of the specimen. At this point, the focus controls of the microscope are properly set. Now open both eyes, and if necessary, adjust the interocular-distance setting of the microscope so that the images seen by the left and right eyes merge into a single, undistorted image. Now close your right eye. Without touching the focus controls of the microscope, rotate the diopter adjustment ring on the left eyepiece tube until the image in the left eye is sharply focused. Now open both eyes and you should see a good image.

Why is there a notch in the right-most eyepiece tube?

This notch is intended to accept the locator pin on eyepieces which have an internal reticle installed. This locator pin, which protrudes from the eyepiece and fits into the notch, locks the orientation of the eyepiece such that the reticle does not rotate whenever the eyepiece is focused, and also makes sure that the visual orientation of the reticle is always correct. Most microscopes have this notch in only one of the eyepiece tubes (usually on the right-most tube).

Questions about Oil-Immersion Microscopy

What are the benefits of oil-immersion objectives?

The maximum usable magnification of a light microscope depends on an optical parameter of the objective lens, which is known as *numerical aperture*. The higher the numerical aperture (often referred to as *N.A.*) of the objective, the higher the resolving power, and therefore the higher the magnification to which the microscope will be usable. The numerical aperture of an objective is a function of the acceptance angle of the objective (i.e., the maximum angle of incoming light rays that will be collected by the objective) and on the index of refraction of the intervening medium between the specimen under observation and the objective lens. There is a fair amount of physics and mathematics involved in fully describing this, but it shall suffice to say that the wider the acceptance angle of the objective, and the higher the index of refraction of the intervening medium, the higher the resulting numerical aperture, and therefore the resolving power of the objective. Most commonly, the intervening medium for a light microscope is air, which has an index of refraction of 1.0. In order to achieve a sufficient numerical aperture for 1000X observations, the angle of acceptance of an objective would have to be very wide. So wide, in fact, as to not be achievable in actual practice. Once the design of an objective has been optimized for as wide an acceptance angle as can be practically achieved, the only way to increase the numerical aperture further is to increase the index of refraction of the intervening medium. Immersion oil, which is an optically transparent oil with an index of refraction of 1.50 or higher, is used here. By replacing the air gap with immersion oil, the resolving power, resolution, and resulting useful magnification of the optics will be increased by 50% or more, as compared to a dry objective with air as the intervening medium.

What is the benefit of oiling the condenser?

Oiling the condenser is a really good idea, since nobody wants a squeaky condenser! All kidding aside, the condenser-to-slide interface should not be neglected when using oil-immersion to obtain the best possible resolving power. Although this air-to-glass interface is often intentionally left dry as a matter of convenience and as a way to minimize clean-up (with the understanding that it will somewhat limit the maximum resolution of the optical system), if you want to obtain maximum resolution, you will need the highest numerical aperture possible. In order to obtain the highest numerical aperture, you will need to set up true homogenous immersion, wherein the top of the slide is oiled to the objective and the bottom of the slide is oiled to the condenser. And of course, you must set the condenser iris diaphragm to match that of the objective lens, or if that is not possible, set it wide open. The reason that the bottom of the slide must be oiled to the condenser is that an oil-immersion objective with a high numerical aperture, even when properly oiled to the slide, cannot achieve its rated numerical aperture unless used with a condenser of equal or greater numerical aperture. Using for example a 1.40 N.A. oil-immersion objective that is oiled to the slide with a 1.40 N.A. condenser which is left dry, the resulting numerical aperture of the system would be limited by that of the condenser (the N.A. of the condenser would be 1.0 or less, due to the air-to-glass interface below the slide).

How should I use immersion oil with my 100X objective?

The following procedure assumes your microscope is an upright type (not an inverted scope) that has four objectives: a 4X, 10X, and 40X dry objective, as well as a 100X oil-immersion objective. Be very careful throughout this procedure and do not allow any of the dry objectives to contact the immersion oil, as these were not designed for oil contact and can be damaged by immersion oil. If for some reason oil does get onto these dry objectives, be sure to promptly remove it using a suitable optical cleaning tissue and a lens-safe solvent, such as isopropyl alcohol, to prevent subsequent damage to the optics. Also, before using immersion oil on any condenser, make sure the condenser is intended for oil immersion. Check the N.A. marked on the barrel of the condenser to be certain. If the indicated N.A. is less than 1.0, the

condenser is not suitable for oil immersion and could be damaged if oil is applied. If the indicated value is greater than 1.0, then it was designed for the application of oil to the top lens of the condenser.

Oiling the Condenser

Assuming you have the proper condenser type, you should oil the condenser to the bottom of the slide before proceeding to oil the objective to the top of the slide. To set up for homogenous immersion, remove the specimen slide from the stage and lower the condenser such that its top lens is below the stage surface. Next, place a small drop of immersion oil in the center of the top lens of the condenser, and place a second drop of oil on the bottom of the specimen slide, directly over the area to be examined. With the oil drop on the slide facing downwards, place the slide on the stage such that the oil drop on the bottom of the slide is just above the oil drop on the top of the condenser. Adjust the condenser upwards to the proper position, at which point the two oil drops will have merged, leaving no air gap between the slide and condenser. You man now proceed to oil the objective to the slide.

Oiling the Objective

Set the aperture diaphragm of the substage condenser to approximately the mid position. Next, set up the microscope to examine the desired specimen using the 4X objective, adjusting the position of the slide and the coarse and fine focus knobs until the specimen is properly centered within the field of view and is sharply focused. Now rotate the nosepiece until the next higher objective (i.e., 10X) is selected and tweak the slide positioning and fine focus as necessary (do not adjust the coarse focus) to once again center the specimen and sharpen the image. Repeat this procedure once more for the 40X objective, and when the specimen is centered and the image is sharp at 40X, turn the nosepiece such that it is half-way between the 40X and 100X oil objectives. This intermediate setting will allow unrestricted access to the cover glass of the specimen slide. Place a single drop of immersion oil onto the cover glass directly above the specimen and carefully rotate the nosepiece until the bottom of the 100X oil objective contacts the oil drop. The bottom of the 100X oil objective will be very close to the cover glass and this will allow the immersion oil to completely bridge the gap between them, displacing any air between in the process. Tweak the slide position and the fine focus until the image is centered and sharp again. Adjust the aperture diaphragm in the substage condenser to the fully open position, decreasing as desired to improve the image contrast, but be aware that to whatever extent you close the aperture diaphragm to improve contrast, you will be decreasing the image resolution. You are now set up for viewing with the 100X oil objective. After you have finished with your observations, rotate the nosepiece back to the 4X position and lower the stage using the coarse focus knob. Remove the slide from the stage and thoroughly clean all immersion oil from the slide. Next, use a suitable optical cleaning tissue and a lens-safe solvent (such as isopropyl alcohol) to remove the immersion oil from the bottom of the 100X oil objective.

Cleaning Up

Modern immersion oils are synthetic-based and will not gum up or harden over time, which greatly simplifies the clean-up procedure. All that is necessary is to use a suitable optical cleaning tissue to wipe away the excess oil from the condenser and objective lenses. Any remaining oil film need not be removed, since it will not harden or damage the optics. If desired, this film may be removed by moistening the tissue with isopropyl alcohol.

What immersion oil type should I use?

According to the Olympus website, Cargille Laboratories Inc. is the “premiere” manufacturer of immersion oils for microscopy. For conventional upright microscopy, Cargille manufactures Type A and Type B oils, differing in viscosity. These two types may be blended to produce a mixture with any desired intermediate viscosity. Cargille also manufactures Type NVH, which is a very high viscosity oil intended for inverted or inclined microscopes, as well as Type DF and Type FF, which are intended for fluorescence microscopy. Most hobbyists will use either the low viscosity Type A, the higher viscosity Type B, or both. Advantages of the low-viscosity Type A oil are that it produces less resistance to motion of the glass slide on the surface

of the stage (if any immersion oil gets on the stage surface), and when used on temporary wet mounts has less tendency to drag the cover slip when the slide is re-positioned on the stage. An advantage of the higher viscosity Type B oil is that it is better suited for use with optics with longer working distances, since it can bridge wider gaps than lower viscosity oils. Unlike organic immersion oils of the past, modern immersion oils are synthetic-based and will not gum up or harden over time, which greatly simplifies the clean-up procedure. All that is necessary is to use a suitable optical cleaning tissue to wipe away the excess oil. The remaining oil film need not be removed since it will not harden nor damage the optics. If desired, this film may be removed by moistening the tissue with isopropyl alcohol.

Questions about Darkfield Microscopy

What is Darkfield Illumination?

Darkfield illumination is a technique used to provide enhanced contrast to live/unstained specimens that are difficult to see under normal brightfield conditions. In simple terms, darkfield microscopy illuminates the specimen such that the direct light (i.e., the light that is not scattered by the specimen) is not collected by the objective lens, and only light scattered by the specimen is collected, and therefore visible. This exclusion of un-scattered light and collection of scattered light produces a visual field where the specimen appears brightly illuminated against a dark background.

How can I add darkfield illumination to my microscope?

The easiest way to implement simple darkfield in a typical upright compound scope is to place an opaque disk, known as a darkfield stop, in the optical pathway of the substage condenser on your scope. This disk should be placed as near to the iris diaphragm in the condenser as possible and must be centered relative to the iris diaphragm. Additionally, the iris diaphragm should be set to the fully open position. The condenser on many microscopes includes a filter carrier, suitable for a darkfield stop, on the bottom of the condenser. These filter carriers tend to be present on condensers which mount to the scope via a circular collar at the top, making these the easiest types to equip for simple darkfield. Other condenser types, such as those that mount with a circular dovetail on the bottom, must have the darkfield stop inserted up into the condenser, in order to get it close enough to the iris diaphragm for effective darkfield, and subsequently it is much more difficult to get the stop in the correct position on these types of condensers. The presence of the darkfield stop in the optical pathway blocks the center portion of the cone of light that illuminates the specimen, preventing all of the unscattered (i.e., direct) light from entering the objective lens, and allowing only the portions of light that are scattered towards the optical axis of the objective to be collected. This causes the specimen to appear brightly illuminated against a dark background. The size of the darkfield stop is important. If too small, it will not exclude all of the unscattered light and the specimen will not appear against a dark field. If too large, it will be hard to collect sufficient scattered light for an acceptably bright image. The ideal stop size would be such that the dark center portion of the lighting cone is just large enough that the objective does not see it, but not any larger. This means that the ideal stop size is a function of the numerical aperture of the objective, and each objective should, at least in theory, have a specific stop size for optimal darkfield performance. In practice, a single stop is typically used, sized for the objective with the highest numerical aperture for which it will be used. A simple darkfield stop is suitable for objectives ranging from 10X to 20X, and sometimes even 40X, but is never suitable for 100X. For proper darkfield at 40X, a dedicated darkfield condenser is often necessary, and for darkfield at 100X, a dedicated darkfield condenser is required.

Which darkfield condenser type should I use?

There are two types of dedicated darkfield condensers available. The first type, known as a *dry* darkfield condenser, is suitable for lower power objectives and does not use immersion oil. The second type, known as a *wet* darkfield condenser, is suitable for higher power objectives and requires the use of immersion

oil between the top element of the condenser and the bottom of the specimen slide. A dry darkfield condenser can typically be used with 10X through 20X objectives and is much simpler and convenient to use than a wet condenser. A wet darkfield condenser can typically be used with 20X through 100X objectives, but the requirement for immersion oil makes using them messy and much less convenient than a dry condenser. Note that even if you have a wet darkfield condenser, you will not be able to obtain darkfield with a 100X oil-immersion objective unless your objective has an internal iris diaphragm, to allow you to reduce the numerical aperture of the objective. A 100x dry objective will never contain an iris diaphragm, as this is not needed for darkfield with a wet darkfield condenser.

Questions about Phase-Contrast Microscopy

What is Phase-Contrast microscopy?

The technique of phase contrast microscopy was developed in the 1930s by Dutch physicist Frits Zernike and began to be broadly used in 1942. Zernike was awarded the Nobel Prize in Physics for his achievement in 1953. Phase contrast techniques are most useful for studying living, non-stained specimens, since live specimens cannot typically be stained without affecting their behavior or killing them. For these types of specimens, phase contrast provides significantly increased contrast as compared to conventional brightfield microscopy. In a nutshell, phase contrast optics exaggerate the differences in the phase relationships between the light waves in the background illumination and the light waves passing through the specimen, so that they can constructively or de-constructively interfere with each other, thereby converting invisible phase differences into visible image contrast.

Here's the more technical answer: In conventional brightfield microscopy, a visible image is formed by wave interference at the intermediate image plane of the background illumination (the background illumination is the light that does not pass through the specimen, which is also known as the "S", or *surround* wave front) and of the diffracted light (the diffracted light is the light that passes through the specimen under observation, which is also known as the "D", or *diffracted* wave front). The image of the specimen is visible to the observer due to the diffraction, absorption, and phase-shifting that occurs as the D wave front passes through the specimen under observation. Diffraction in the D wave front occurs as a result of detail in the specimen. Absorption occurs as a result of the specimen being not completely transparent. Phase shift occurs as a result of differences in the refractive index of the specimen, as compared to the surrounding mounting medium. When viewing live, unstained specimens, the specimen is often difficult to see since light absorption can be minimal and since the constructive/destructive interference that occurs as a result of the phase-shifted D wave front is minimal as well. Phase contrast microscopy utilizes special optics (both in the condenser and in the objectives) to accomplish two things: 1) The S wave front (i.e., the background illumination) is decreased in amplitude by the phase ring in the objective so that the intensity of the D wave front will not be swamped by the otherwise bright background lighting. 2) The S wave front is phase shifted by a quarter wavelength by the phase ring in the objective, thereby exaggerating the constructive or destructive interference that occurs between the S and D wave fronts. The result of these two things is that images of live, unstained specimens have significantly higher contrast than could otherwise be obtained with convention brightfield microscopy. Phase contrast optics are available in two basic types, *positive* and *negative*. In positive phase contrast, the phase ring in the objective advances the S wave front by a quarter wavelength, relative to the D wave front, and in negative phase contrast, the phase ring retards the S wave front by a quarter wavelength, relative to the D wave front. In both cases, the phase of the D wave front is retarded by areas of the specimen which have a higher refractive index than the surrounding medium, and advanced by the areas of the specimen which have a lower refractive index than the surrounding medium. In positive phase contrast, the advanced S wave and the retarded D wave destructively interfere, resulting in the areas of the specimen with higher refractive index than the surrounding medium appearing darker than the

neutral gray background. In negative phase contrast, the exact opposite occurs. The retarded S wave and the retarded D wave constructively interfere, resulting in the areas of the specimen with higher refractive index than the surrounding medium appearing lighter than the neutral gray background.

How do I set up and use phase contrast?

Assuming you have accumulated all the components necessary for phase contrast, how do you go about setting it all up? The following procedure assumes you will be using either the BH2-PC or BH2-PCD Zernike-style condenser. Start by replacing the condenser and objectives on your microscope with your new phase components. Next, rotate the dial on the phase condenser until the number "0" is visible on the front and is clicked into position. This is the condenser setting for conventional brightfield observations. Set the aperture diaphragm on the condenser to its approximate center position and rotate the nosepiece to select the 10X phase-contrast objective. Place a slide with a suitable specimen on the stage and setup the microscope in the traditional way for Köhler illumination, making sure to carefully center the phase condenser. You should now see a good brightfield image of your specimen. Next, rotate the phase condenser disk to the "10" position, and make sure that it clicks into position. This is the proper setting for 10X phase contrast. Replace the right eyepiece with your phase-centering telescope and peek into the telescope for a look. Assuming the telescope is set for proper focus (and it likely is not), you should see a bright ring of light and a darker ring in the visible field. More than likely, the focus will be off and you will need to rotate the helical focusing ring on the phase telescope until the bright and dark rings are sharply focused. The position of the dark ring is fixed in the visible field, whereas the position of the bright ring can be adjusted via the two orthogonal, spring-loaded centering controls on the back of the phase condenser. To adjust the position of the bright ring, depress the two orthogonal centering controls so that they engage the internal centering screws for the 10X annulus and adjust the two centering controls until the bright ring falls completely within the dark ring. Once this adjustment has been performed, release the two centering controls and allow them to return to their extended positions. Replace the phase-centering telescope with the eyepiece and you should be properly setup for 10X phase contrast. Note that it may be necessary to increase the illumination intensity at this time, since only a portion of the light that is present in brightfield will be available in phase contrast. If you will be taking photographs and wish to achieve the best (monochrome) results possible, place the green filter into the 45mm receptacle, which is located above the field exit lens (below the condenser). The setup for the remaining phase-contrast objectives is done in the exact same way as for the 10X. Just be sure to match the number on the condenser dial with the magnification of the objectives and center the bright ring within the dark ring as described above. Once all the phase-contrast annuli have been properly setup with their respective objectives, do not adjust the condenser-centering or annulus-centering controls, otherwise the quality of the phase-contrast image will be degraded.

Why are there halos around everything when I use phase contrast?

Ah yes, the inevitable artifacts of phase-contrast optics. These artifacts can make it difficult to accurately interpret the results of phase contrast images, and worse, you cannot get rid of them.

The halos you are seeing are caused by some of the diffracted light from the specimen passing through the phase ring. Ideally, only the background illumination should pass through the phase ring, and only diffracted light should pass through the areas inside and outside the phase ring. In positive phase contrast (PL or PLL), this effect causes the edges of large objects have a bright edge, whereas in negative phase contrast (NM or NH), this effect causes them to have a dark edge.

Another phase contrast artifact is known as the *shade-off effect*. In this case, the homogeneous parts of the image show up at the same brightness level as the background (i.e., the surrounding medium). This occurs because although the light experiences a phase shift as it passes through these regions of the

specimen, only minimal diffraction occurs and the angle it scatters is therefore limited, causing it to pass through the phase ring and therefore not experience interference.

A third phase contrast artifact is known as *contrast inversion*. In the case of positive phase contrast, objects with a high index of refraction situated next to objects with a low index of refraction will appear brighter than the background, instead of darker. This happens because in these cases the phase shift is not the usual quarter wave that should occur, and instead of the expected destructive interference occurring, constructive interference occurs instead. The opposite of this is true for negative phase contrast.

Questions about Polarizing Microscopy

What is an Analyzer?

An *analyzer* is a linear polarizing filter that is located above the specimen, typically in an intermediate attachment located just below the viewing head. During setup, and with no specimen on the stage, the operator sets the analyzer to the zero position (if it is variable) and then rotates the substage polarizer until the visual field totally blacks out. When this occurs, the axis of transmission of the substage polarizer is perpendicular to the axis of transmission of the analyzer, and all of the linearly polarized light produced by the polarizer will be absorbed in the analyzer. This is called *extinction* and produces a black background for the visual field. When a specimen with some degree of birefringence is then placed on the stage, the linearly polarized light passing through the specimen experiences changes in polarization, making it become elliptically polarized to some degree or another. The purpose of the analyzer is to exclude the linearly polarized component of the specimen lighting, which is perpendicular to the axis of transmission of the analyzer, and to transmit the components that are parallel to the axis of transmission. So, when a birefringent specimen upsets the strict linear polarization of the light striking the analyzer, the components parallel with the axis of transmission will pass through and become visible to the operator, against the black background.

What is a Polarizer?

In general terms, a polarizer is an optical filter which allows light waves with a specific axis of polarization (that is to say, the light whose electric field aligns with the so-called *axis of transmission* of the filter) to pass while absorbing waves of different polarizations. There are two basic types of polarizing filters available: linear and circular. A linear polarizing filter operates as described above and produces so-called linearly polarized light, in which the transmitted light experiences no phase shift within the filter, and which therefore has a single axis of polarization. A circular polarizing filter includes a linear polarizer, as well as an additional component to which the linearly polarized light passes which creates two orthogonally polarized wave fronts from the linearly polarized light which are one-quarter wavelength out of phase with each other. The combination of the orthogonal polarization and the quarter-wavelength phase relationship produces a wave that propagates through space with a helical, corkscrew-like pattern to its electric field. Looking at this corkscrew in two dimensions (i.e., neglecting the axis of propagation), a circular plot emerges, and this is where the circular polarizing filter gets its name. In the context of polarization microscopy, the *polarizer* is a linear polarizing filter located below the stage (often located in the condenser or simply placed on top of the field lens) whose job is to produce linearly polarized light from the non-polarized illumination source.

What is Polarized Light?

Light propagates through space as transverse electromagnetic waves, in which there are cross-coupled electric and magnetic field components, each of which are oriented perpendicular to the other, and both of which are also oriented perpendicular to the axis of wave propagation. The electric and magnetic field components of a transverse electromagnetic wave can be visualized as two in-phase sine waves plotted

along the Z axis, (which is the axis of wave propagation) with the amplitude of one wave oriented vertically (i.e., parallel to the Y axis) and the other wave rotated 90° about the Z axis such that its amplitude is oriented horizontally (i.e., parallel to the X axis). The behavior of transverse electromagnetic waves is fully described by a set of vector-calculus equations known as Maxwell's Equations. Fortunately, one does need to understand vector calculus to understand the concept of polarization. The polarization of a given light wave is defined simply, by convention, to be the orientation of the electric-field component of the transverse wave, in space. So, in the example above, if the electric-field component of the transverse electromagnetic wave were the one whose amplitude was oriented vertically, then that wave could be said to be *vertically polarized.*

So, that explains the polarization of light, but what is *polarized light*? To answer that, let's first look at non-polarized light. Consider non-polarized light that exists at a specific location. This light is a collection of many light waves, coming from many different sources, emitted with many different polarizations, coming from many different distances and directions, all composed of many different wavelengths. Some of these waves traveled directly from the source, while others were reflected, perhaps multiple times, before arriving at point under consideration. I think you get the idea. By the time these waves arrive at this point, the polarization of these waves is totally random with respect to each other, so that waves of all polarizations are present in this light. If that unpolarized light is sent through an optical filter that only transmits the waves whose electric field are aligned in a given polarization, and absorbs the rest, then what emerges from that filter is linear polarized light.

What is a Wave Plate or a Retardation Plate?

A retardation plate (also known as a wave plate) is an optically transparent plate made of a birefringent mineral or crystal (such as quartz, mica, calcite, or selenite), or other material (such as organic polymers sandwiched between two glass plates) installed in a metal or plastic support frame to provide protection for, and proper orientation of, the birefringent plate. The word *birefringent* means that the refractive index of the material depends on the polarization and direction of propagation of light within the material. A wave plate will have two axes of interest, which are orthogonal to each other. The axis which exhibits the lowest refractive index is known as the *fast* axis, since light propagates through the material fastest when its polarization aligns with this axis. Conversely, the axis which exhibits the highest refractive index is known as the *slow* axis, since light propagates through the material slowest when its polarization aligns with this axis. These two axes will be identified on the wave plate support frame in some manner. Sometimes only the slow axis will be explicitly marked, with the implicit understanding that the slow axis is orthogonal to the fast axis, and other times both axes will be explicitly marked. If linearly polarized light is sent through a wave plate with its polarization aligned parallel with either the fast or slow axis, it will emerge from the wave plate with the same polarization, just delayed somewhat according to the propagation speed of the axis with which the polarized light was aligned. It gets a bit more interesting if the linearly polarized light is incident on the wave plate such that both the fast and slow axes are illuminated by the same wave front. For instance, if linearly polarized light strikes the wave plate at a 45° angle relative to both the fast and slow axes, both axes will see an equal vector component of the illuminating light, which will then propagate through the wave plate at two separate velocities, since there are two distinct refractive indices involved, and two equal-amplitude wave fronts will emerge from the crystal with mutually orthogonal polarizations that differ in phase. The phase difference is because the two wave fronts did not spend the same of time in the crystal material, due to the different refractive indices they experienced. Specifically, the emerging wave front aligned with the slow axis will be retarded (also known as optical path distance), as compared to the emerging wave front aligned with the fast axis. The specific amount of retardation will depend on the thickness of and on the type of the birefringent crystal from which the wave plate was made. Wave plate manufacturers carefully control the material selection, thickness, and orientation of the chosen material to produce specific amounts of retardation at

a particular wavelength of light. The amount of retardation information is marked on the frame of the wave plate. For polarizing microscopy applications, wave plates are usually constructed so that they provide their specified retardation with a green wavelength of approximately 550 nm. There are a few standard types of retardation plates used for polarizing microscopy, as described below.

Quarter-Wave Plate

A quarter-wave retardation plate for polarizing microscopy is constructed such that the two wave fronts emerging from the birefringent material will differ in phase by one-quarter wavelength (i.e., 90°) when illuminated with green light at (typically) 550 nm. Since the polarization of these two emerging wave fronts will be orthogonal to each other, and since one will be retarded by one-quarter wavelength relative to the other, the emerging light will no longer be linearly polarized, but will instead have elliptical or circular polarization. In polarizing microscopy, the quarter-wave retardation plate is inserted into the optical pathway with a 45° orientation of the fast and slow axes relative to the transmission axis of the polarizer, such that the fast and slow axes are equally illuminated by linearly polarized light produced by the polarizer. In this specific case, the two emerging wave fronts will have equal amplitude and the resulting polarization will be circular. A vector component of this elliptically polarized light will pass through the analyzer. Quarter-wave retardation plates are used to determine optical path differences of birefringent specimens as well as for the qualitative analysis of orthoscopic and conoscopic images.

Full-Wave Plate

A full-wave retardation plate for polarizing microscopy (also known as a first-order plate or a lambda plate) is constructed such that the two wave fronts emerging from the birefringent material will differ in phase by one full wavelength when illuminated with green light at (typically) 550 nm. A phase shift of one full wavelength, or 360°, means that the two emerging orthogonal wave fronts will be in-phase, and because of this the emerging light will maintain the same linear polarization as produced by the polarizer. It is important to note that the emerging light will only be linearly polarized at this single wavelength where the relative phase shift is exactly 360°, and at all other visible wavelengths the emerging wave fronts will experience some degree of relative phase shift which will therefore produce some degree of elliptical polarization. The degree to which the resulting light is elliptically polarized depends on the how far the specific wavelength differs from the linearly polarized green wavelength.

In polarizing microscopy, the full-wave retardation plate is inserted into the optical pathway with a 45° orientation of the fast and slow axes relative to transmission axis of the polarizer, such that the fast and slow axes are equally illuminated by the linearly polarized light produced by the polarizer. Without a birefringent specimen in the optical path, the green light emerging from the wave plate will be linearly polarized and will have an orientation orthogonal to the transmission axis of the analyzer, thereby causing it to be absorbed by the analyzer and not visible to the observer. Since all other wavelengths will exhibit elliptical polarization to some degree or another, and since some vector component of all elliptically polarized waves will pass through the analyzer and become visible to the observer, all wavelengths other than the linearly polarized wavelength will be visible to the observer to some degree or another. This phenomenon wherein the analyzer subtracts the green light from the visible spectrum and leaves behind the red and blue wavelengths makes the remaining light appear magenta to the observer, and is the reason for many of the common names for this device, such as red plate, red-1 plate, red tint plate, sensitive violet plate, or color tint plate.

When a birefringent specimen is placed into the optical pathway and aligned such that the fast and slow axes of the specimen are parallel to those of the full-wave retardation plate, the relative retardation produced by the birefringent specimen adds to that produced by the wave plate, shifting the point where the two orthogonal components are equal (and therefore linearly polarized) from green towards red. So rather than the green light being blocked by the analyzer, the redder wavelengths tend to become blocked instead, while the green wavelengths tend to become visible. This phenomenon shifts the perceived color

of the light that travels through the specimen away from magenta towards cyan (which is a combination of green and blue light), with the degree to which this happens being a function of the amount of phase shift produced by the birefringent specimen. If the birefringent specimen is rotated by 90° from the orientation described above, this will change the relationship between the slow and fast axes of the specimen and those of the full-wave retardation plate until they are now perpendicular, instead of parallel. In this case, the relative retardation produced by the birefringent specimen subtracts from that produced by the wave plate, shifting the point where the two orthogonal components are equal, and therefore linearly polarized, from green towards blue. So rather than green light being blocked by the analyzer, the bluer wavelengths tend to become blocked while the green wavelengths tend to become visible. This shifts the perceived color of the light that travels through the specimen away from magenta towards yellow (which is a combination of red and green light), with the degree to which this happens being a function of the amount of phase shift produced by the birefringent specimen. Full-wave retardation plates can be used to enhance contrast in weakly birefringent specimens, as well as to determine the optical sign of birefringent specimens or to estimate the optical path differences in birefringent specimens ranging from a fraction of a wavelength to several wavelengths.

How can I add simple polarization capabilities to my microscope?

To add simple polarizing capabilities to a microscope, you will need two linear polarizers of some sort. One of these polarizers is placed over the illumination source, typically beneath the condenser, to provide linearly polarized light with which to illuminate the specimen. The other polarizer is placed above the objectives, typically below the viewing head. The actual filters are not too critical here; simple polarizing films are sufficient. Just try to find a pair that provides good extinction (i.e., full exclusion of light) when the polarizers are arranged with their axes of transmission oriented perpendicular to each other. To set up for polarized observations, remove the viewing head, place one of the polarizers in the mounting recess for the head, and reinstall the viewing head. Next place the remaining filter under the condenser, over the light source. Now if you rotate the bottom polarizer such that its axis of transmissions aligns with that of the upper polarizer, the scope should work similar to a standard brightfield scope, but with a reduction in brightness. With no slide on the stage, rotate the bottom polarizer until no light can be seen through the eyepieces. Place the specimen slide on the stage and you are set up for polarizing observations. Any birefringent specimens will upset the polarization of light passing through the specimen, so that it will no longer be completely excluded by the upper polarizer, making the birefringent portions of the specimen visible against a dark background.

Questions about Rheinberg Illumination

What is Rheinberg Illumination?

Rheinberg illumination, which was derived from darkfield illumination, is another technique used to provide enhanced contrast to live/unstained specimens that are difficult to see under normal brightfield conditions. In simple terms, Rheinberg Illumination modifies the light cone such that the direct light (i.e., the light that is not scattered by the specimen) is tinted one color, while the light scattered by the specimen is tinted a second, contrasting color. This can produce stunning images wherein the specimen appears one color against a background of a second color.

How can I add Rheinberg Illumination capabilities to my microscope?

The easiest way to implement Rheinberg Illumination in a typical upright compound scope is to place a special optical disk, known as a Rheinberg filter, in the optical pathway of the substage condenser on your scope, exactly as is done for simple darkfield illumination. A Rheinberg filter is composed of two differing colors of optical filter material, an inner disk of one color surrounded by an outer ring of a second color. The inner disk determines the image background color, while the outer ring in combination with the inner

ring determines the resulting specimen color. As in darkfield illumination, the Rheinberg filter should be placed as near to the iris diaphragm in the condenser as possible and must be centered relative to the iris diaphragm. The condenser on many microscopes includes a filter carrier, suitable for a Rheinberg filter, on the bottom of the condenser. These filter carriers tend to be present on condensers which mount to the scope via a circular collar at the top, making these the easiest types to equip for Rheinberg Illumination. Other condenser types, such as those that mount with a circular dovetail on the bottom, must have the Rheinberg filter inserted up into the condenser, in order to get it close enough to the iris diaphragm for effective Rheinberg, and subsequently it is much more difficult to get the filter in the correct position on these types of condensers. The presence of the Rheinberg filter in the optical pathway tints the center portion of the light cone the color of the inner disk and the outer-most portion the color of the outer ring. The colored light from the inner portion of the cone, when direct and unscattered, enters the objective lens to form the image background, which will therefore be the color of the inner disk. The colored light from the outer-most portion of the cone does not get directly collected by the objective, but instead gets collected only to the extent that it is scattered by the specimen. The size of the inner disk is important. If too small, it will not color all of the unscattered light and the specimen will not appear against a properly colored field. If too large, the scattered light will be a mixture of the two colors. The ideal stop size would be such that the colored center portion of the lighting cone is just large enough that the objective does not directly see any of the outer color, but not any larger. As in darkfield illumination, this means that the ideal inner disk size is a function of the numerical aperture of the objective, and each objective should, at least in theory, have a specific inner disk size for optimal Rheinberg performance. In practice, a single Rheinberg filter is often used, sized for the objective with the highest numerical aperture for which it will be used. A Rheinberg filter is suitable for objectives ranging from 10X to 20X, and sometimes even 40X, but is never suitable for 100X.

Questions about Differential Interference Contrast

What is Normarski Interference Contrast (NIC) or Differential Interference Contrast (DIC)?

Differential Interference Contrast (DIC) is also known as Nomarski Interference Contrast (NIC) or simply Nomarski microscopy. Nomarski Interference Contrast, which was developed by Polish physicist Georges Nomarski, uses a relatively complex optical system to generate visible contrast in unstained specimens from differences in the refractive index of the various points in the specimen. A DIC image not only includes the detail that would be visible in a standard brightfield image of the specimen, but also includes contrast which represents gradients in the refractive index of the specimen.

The condenser in a typical Nomarski-equipped microscope contains a linear polarizer, which produces linearly polarized light from the illumination source, and a Wollaston prism, which splits the linearly polarized light into two mutually coherent yet orthogonally polarized components that are spatially displaced (sheared) by a slight amount. These two spatially displaced components pass through the specimen and the objective lens in the normal fashion and are then converted to the same polarization and recombined, with the original shear removed, by a second Wollaston prism located between the objectives and the oculars. While separate, the two components reaching the second Wollaston prism each contain what is essentially a standard brightfield image, with one spatially displaced from the other. Although these brightfield images, if viewed separately, would appear the same (neglecting the slight shear), each differs in the other in relative phasing, on a point-by-point basis, due to the point-by-point differences in the refractive index of the specimen experienced by the two sheared components. Although phase differences are not visible, which is why the two brightfield images carried in the two sheared components would appear identical (neglecting shear), when recombined by the second Wollaston prism, constructive and deconstructive interference between the two sheared components creates visible contrast in the resulting image representing the path-length differences seen by the two

sheared components, which were caused by differences in the refractive index of various point in the specimen.