Frequently Asked Questions Relating to Compound Light Microscopes

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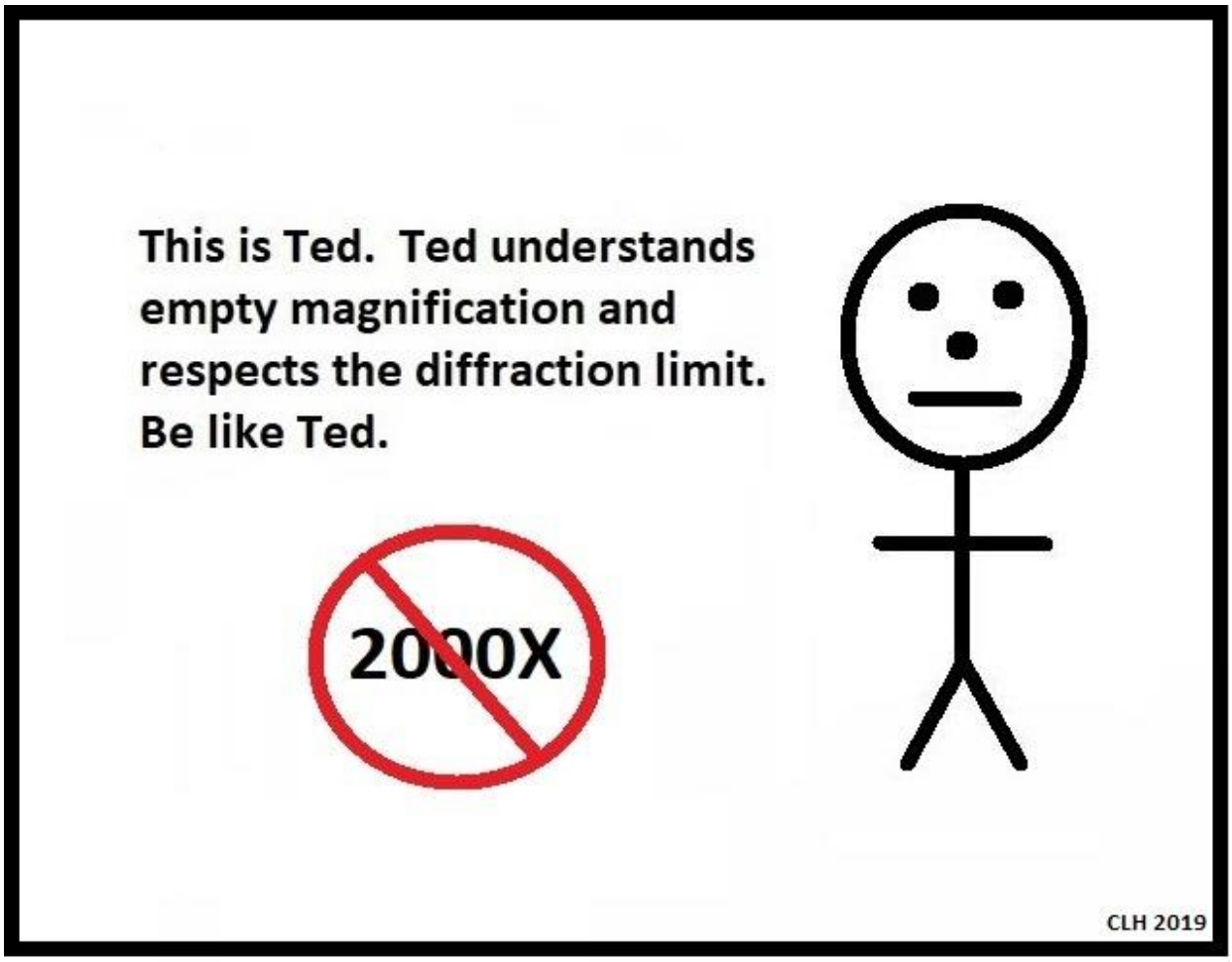

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Questions about Buying a Microscope

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 a quality scope, may need a fair amount of up-front work before it is really nice and usable. It is not at all 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 the manufacturers, leaving only used, surplus, and third-party suppliers.

Although the parts situation for vintage scopes may sound bad, don't let that dissuade you if you really want a vintage scope. 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 the microscopes MADE IN CHINA any good?

There are 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 all or in-part 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, 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 fall 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 significantly more expensive than finite microscopes with 160mm tube-length optics. And except for a very few specific cases, there are no significant performance differences 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 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 or viewing head. 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 or field curvature) and can be picked up for digital imaging with a simple camera that replaces one of the eyepieces. This is a cheap, simple, and elegant solution.

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

The list of potential disadvantages with infinity optics includes 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 being that the proper eyepieces and photo-projection lenses 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 could reasonably be expected to perform poorly in a scope from another manufacturer, since they would quite possibly 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 manufacturers' optical designs. The second reason is that the mechanical design of infinity objectives can make them physically incompatible. While some infinity objectives use the same RMS threads as DIN-compliant finite objectives, many 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, a good recommendation is to 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 1500X. 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 that 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 the wave nature of light and diffraction, preclude you from ever getting *good* or even *decent* images at 2000X magnification, you will nonetheless see an image magnified 2000X in all of its blurry and disappointing glory.

By the latter part of the 19th century, the science of optics had advanced to the point where microscope designers were building scopes that performed right up to the diffraction limit of visible light. The diffraction limit is the limit to the resolving power of a conventional light microscope caused by the diffraction effects of light waves passing through the specimen under observation. 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 improvements 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 15X 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 about 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, 1500X is tops for it (1500X is actually a bit beyond "tops", since 1400X is a more realistic upper limit for this objective). It is important to note that these high-dollar dream optics have the same upper limit of magnification as those made in the 19th century! In comparison to this rock-star objective, the cheaper 100X oil-immersion objectives available today are good to 1250X or so, since they sport a more modest N.A. of 1.25 or so.

Now, just for the fun of it, go ahead and stick a pair of 20X eyepieces on your scope with your new rock-star objective, to raise the total magnification to 2000X (just like the Amscope and Omax 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 that 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 a 100X objective provides the highest objective magnification routinely 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, yes. 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, if 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 damned things just about unusable. So why are these eyepieces included with most microscopes sold today for the low-cost amateur market? The answer of course is so that the manufacturers can claim 2000X, 2500X, or 3000X (!) for their microscopes, to lure naïve and unsuspecting buyers. It is pure snake oil. DO NOT BE DECEIVED! Any claims for magnification above 1500X or so are simply deceptive advertising.

Rather than ask why these eyepieces are included with low-end scopes, perhaps a better question would be to ask 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 have always said that the best use for a pair of 20X eyepieces is to hear the satisfying "clunk-clunk" sound 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, 8X, 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 of 20X, 25X, or God forbid, 30X eyepieces, they will spend their entire lives in a desk drawer somewhere, mocking you every time you run across them. You will wish you could throw them out, but of course you will never do that because they came with your microscope. But that "cluck-clunk" sound would be really satisfying, 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 least interesting living organisms you will ever observe), and you will likely 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 desk drawer, right next to those 20X eyepieces. That way, the 100X objective will be there for you when you 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 (i.e., planar) 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 non-plan objectives. If you already know that microscopy is 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 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 viewing 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 disorder, 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 binocular eyepieces 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 slide-mounted beam splitter to allow the operator to position the 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 will be 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. Halogen lighting operates at higher temperatures than standard incandescent lamps, which shifts the spectrum towards the blue, producing light with a higher color temperature and at a higher power efficiency. Halogen lamps are the only available option for producing black body radiation with a continuous spectrum, similar to that of the sun, making it the most suitable option for the eyes. Today, although there are still halogen-equipped scopes on the market, LED lighting is becoming much more the norm. The pros and cons of both are discussed below.

The filament of a halogen bulb, being a black body radiator, produces a continuous daylight-balanced 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 difficult to see. In this respect, halogen is the clear 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 daylight blue 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 daylight 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. In this respect, LED lighting is the clear winner.

Halogen lighting creates a lot of heat in the housing for the halogen lamp and in the electronics driving the lamp. For halogen-equipped scopes whose lamp is located within the base, below the stage, this can be a real 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, and to some degree, can adversely impact operator comfort. The designers of upper-end clinical and research microscopes generally put the lamp external to the base, in a housing on the rear of the scope, in order to allow for a more comprehensive illumination system than could be placed within the base, and to allow for better thermal management. For these scopes, internal heat rise of the base is not really 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 (i.e., adiabatic glass) in the illumination path, and many higher-cost research or clinical microscopes with halogen lighting contain an integral adiabatic glass 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 in this way.

An additional factor that can cause overheating of the specimen with halogen lighting is the physical configuration of the lighting system. If the halogen lamp is included within the base, directly below the stage, as is true with many low-cost halogen scopes, waste heat from the halogen lamp will heat the stage above, and subsequently will heat the specimen. The better scopes that are equipped with halogen lighting have the lamp in an external lamphouse, typically on the rear of the scope, and these scopes will typically not exhibit specimen-heating problems. The lamphouse on these scopes is far enough away from the stage that the waste heat from the lamp does not affect specimen temperature.

Both halogen and LED lighting can 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 electrical 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 100 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. If you end up with a scope that is equipped for halogen lighting, don't be afraid to really crank up the lighting intensity if the viewing conditions call for it. Many people mistakenly believe that running their halogen scope at high brightness levels will reduce the life of the halogen lamp, when in fact, the opposite is true. Thanks to the halogen cycle, halogen lamps last longer when operated at high output.

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 of course adds some 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 illumination (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 (be that an LED emitter or a 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 direct 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) on the scope, and when using the higher power objectives in these scopes, glare from the light beyond the visible field of the objective (and therefore beyond the field stop of the eyepieces) can cause a noticeable reduction in image contrast.

What kind of microscope should I get to perform LIVE BLOOD ANALYSIS?

You shouldn't. If you are looking to purchase a microscope that will be used to perform *Live Blood Analysis* (aka *LBA*, which is bogus, pseudoscientific crap used to sell dietary supplements and worthless treatments to the unsuspecting public), please go find yourself an honest way to make a living.

The corollary to this of course is that if you are ever approached by someone wanting to show you your blood under a microscope for diagnostic or nutritional purposes, then they are almost certainly a quack trying to hustle you. Microscopic blood observations performed by legitimate medical practitioners will be done in a federal or state-certified medical lab or clinical setting and with few exceptions will be performed using either a hemocytometer slide, for cell counting, or using a dried, heat-fixed and stained (i.e., very dead) peripheral smear on a conventional glass slide. It will not be done in the provider's office with the patient present and watching the blood cells dance around on a computer monitor, nor will it generally be performed using darkfield equipment. There truly is no legitimate reason why your chiropractor or nutritionist should ever need to or want to put your blood under a microscope, or for that matter, for them to use a microscope in their practice at all. If you see a microscope on a table or desk in their office, other than as may be used for decoration, please go elsewhere. The only thing an LBA practitioner can tell by looking at live blood is that the person sitting on the opposite side of their desk and staring at the monitor in wide-eyed amazement is a bona-fide sucker. "Wow", they think to themselves, "this microscope is making me a ton of money."

I once fell for a request from a guy to repair a microscope, whom I thought was using it for his amateur microscopy hobby. I found out after performing the repair (at very low cost) that the guy was actually building a darkfield setup to do LBA for clients in his wholistic, anti-vaxxer, alternative-medicine, pseudoscientific clinic of deceit and quackery. Even now, he is out there using this Olympus microscope that I repaired to hustle money from his clients, and the knowledge that this is happening because of something I did eats away at my soul a little bit more every day. My self-induced penance for falling for this was to become a card-carrying anti-LBA crusader for as many years as I have left on this earth. Maybe I can save as many people as I've damned. Perhaps that will be enough.

Live Blood Analysis cannot find cancer, pre-cancer, inflammation, immune-system problems, Lyme disease, pH imbalances, vitamin deficiencies, viral infections, bacterial infections, fungal or yeast infections, toxins, dehydration, high cholesterol, organ failure, or anything else. Please do not fall for this scam!

Questions about Microscope Basics

What is the APERTURE DIAPAHRAM and how do I properly set it?

The *aperture diaphragm* is an iris diaphragm which is integral to the substage condenser. This diaphragm adjusts the angle of the cone of light that the condenser produces, thereby controlling the numerical aperture (N.A.) of the condenser. This is important because the N.A. of the condenser and the N.A. of the objective lens both contribute to the overall N.A. of the microscope. The aperture diaphragm should not be confused with the *field diaphragm*, which is a separate diaphragm that is only present on Köhler-equipped scopes (the field diaphragm adjusts the diameter of the lighting beam that illuminates the substage condenser).

A good rule of thumb recommended by many microscope manufacturers is to set the aperture diaphragm of the condenser to an N.A. of approximately 75% of the rated N.A. of the objective lens, for routine viewing. This setting will provide a good tradeoff between optical resolving power, image contrast, and depth of field. It is important to understand that resolving power is always directly competing with both image contrast and depth of field. Accordingly, the aperture diaphragm may be reduced from the recommended setting to improve image contrast and depth of field (at the expense of resolving power) or increased to improve resolving power (at the expense of image contrast and depth of field). Be aware

when adjusting the aperture diaphragm that if you close the diaphragm too much, you will get great contrast, but not without introducing severe diffraction artifacts to the image, resulting in a grainy and distorted image. At the other extreme, if you increase the N.A. of the condenser beyond the rated N.A. of the objective lens, you will needlessly degrade image contrast without adding to the resolving power or to the depth of field of the image. There are two ways to set the aperture diaphragm to the manufacturer recommended setting, as described below.

METHOD #1: USE THE N.A. SCALE ON THE CONDENSER: Many substage condensers have a scale engraved or marked on the barrel of the condenser to show the N.A. of the condenser for the various aperture settings. If present, this scale can be used to set the aperture ring or lever to approximately 75% of the N.A. value marked on the objective barrel.

METHOD #2: SIGHT DOWN THE OCULAR TUBE (This method will work whether or not there is an N.A. scale marked on the barrel of the condenser): Start by removing one of the eyepieces and sight down the empty ocular tube. Next, adjust the aperture diaphragm ring or lever on the condenser until the diameter of the visible circle of light in the ocular tube fills approximately 75% of the ocular tube diameter. Then place the eyepiece back into the ocular tube and you should be good to go.

As you experiment with various aperture diaphragm settings, you will very quickly realize that the setting of the aperture diaphragm also affects the image brightness. However (and this point cannot be emphasized enough), *the aperture diaphragm is not an intensity control!* The effects of the aperture diaphragm on image brightness should be seen as a side effect only. You should never use the aperture diaphragm to adjust the brightness of the image[1]. There is a dedicated intensity control on your scope to control the lighting intensity, which allows the brightness of the image to be adjusted without also affecting image contrast, depth of field, or 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 thin-section 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 often be added to the better scopes. 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 observations. 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 ease of use and is the type that a newcomer should generally start with.

What is the CHINESE FINITE MICROSCOPE STANDARD?

The so-call *Chinese Finite Standard* is a de-facto standard for modern short-barrel microscope objectives of finite conjugate design, manufactured primarily in China. Due to their short-barrel construction, these objectives feature a parfocal distance of 35mm, rather than the 45mm defined in the DIN standard, and are therefore not DIN compliant. Although virtually all of the higher-end microscopes manufactured today feature infinite conjugate optics, these short barrel finite objectives are still common in many lower-

[1] There are countless YouTube microscopy videos which tell you to adjust the aperture diaphragm for the desired image brightness. The people who say this are misinformed. When you hear this recommendation, this should be your clue to go elsewhere for microscopy instruction.

tier student grade and amateur microscopes made in China today. The Chinese Finite Standard utilizes objectives of 160mm tube length, a parfocal distance of 35mm, and standard RMS threads (0.7965" diameter, 36 TPI, 55° Whitworth threading). The object-to-image distance (i.e., the conjugate distance, which is the distance from the specimen plane to the focal plane of the real image produced by the objective) is 185mm. Given the 35mm parfocal distance, this leaves 150mm for the position of the eyepiece field lens (where the real image should be focused) from the objective mounting flange. The eyepiece field lens (and hence the real image) is recessed 10mm from the end of the mechanical tube. So, adding the 35mm parfocal distance and the 150mm distance from objective to real image, yields the conjugate distance of 185mm. Similarly, adding the 150mm distance from the objective mounting flange to the real image to the 10mm that the real image is recessed in the optical tube, yields the 160mm tube length. Chinese Finite Standard eyepieces have a 23.2mm diameter barrel.

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 real image produced by the objective lens with the eyepiece lenses. 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 in the 1600s, this is a very uncomfortable way to go. If this were still how microscopy was done, you almost certainly 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?

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?

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 will appear 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, in practice, 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. The term "Depth of Field" should not be confused with the term "Depth of Focus", which is described elsewhere.

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 cell's 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.

Nonetheless, there are many times where an increase in the depth of field would be desirable. This happens less often with direct visual observations, however, for photomicrographic applications the limited depth of field is much more likely to be objectionable. 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 has been 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 and assemble them into a composite image which is wonderfully sharp throughout.

What is DEPTH OF FOCUS?

Depth of Focus is another term that the makers of YouTube microscopy videos never seem to get right, as they tend 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 image sensor where projected images would appear acceptably sharp in the resulting photomicrographs. The depth of focus is generally shallower for low-magnification objectives than for higher-magnification objectives, whereas depth of field is generally shallower for high-magnification objectives than for lower-magnification objectives.

What is the DIN MICROSCOPE STANDARD?

The DIN (Deutsches Institut für Normung) industry standard is an older German standard (or, more properly, a collection of German DIN standards) for microscope objectives and eyepieces using finite conjugate optics. In higher-end microscopes of today, finite conjugate optics have been abandoned in favor of infinite conjugate optics, but microscopes built to the DIN standard are still commonly made and sold as upper-end student and amateur microscopes today. The DIN microscope standard utilizes a 160mm tube length, an objective parfocal distance of 45mm, and standard RMS threads (0.7965" diameter, 36 TPI, 55° Whitworth threading). The object-to-image distance (i.e., the conjugate distance, which is the distance from the specimen plane to the focal plane of the real image produced by the objective) is 195mm. Given a 45mm parfocal distance, this leaves 150mm for the position of the eyepiece field lens (where the real image should be focused) from the objective mounting flange. The eyepiece field lens, and hence the real image, is recessed 10mm from the end of the mechanical tube. So, adding the 45mm parfocal distance and the 150mm distance from objective to real image, yields the conjugate distance of 195mm. Similarly, adding the 150mm distance from the objective mounting flange to the real image to the 10mm that the real image is recessed in the optical tube, yields the 160mm mechanical tube length. DIN standard objectives often have "DIN" marked on the outside barrel. DIN standard eyepieces have a standard 23.2mm diameter barrel.

What is EMPTY MAGNIFICATION?

A compound light microscope has the capability to magnify and resolve a lot of detail hidden 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 the wavelength of the illuminating light itself, impose strict limitations on what can be resolved. This limit is called the *diffraction limit* and is essentially the result of 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 (N.A.) of the objective lens by 1000. If you have a 40X objective with an N.A. 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 to be had. Don't be fooled by vendors who advertise 2000X, 2500X, or God forbid, even 3000X! That is total snake oil intended to dupe beginners. It is interesting to note that high-dollar microscopes made by the Big Four manufacturers (Olympus, Nikon, Zeiss, and Leica) won't include any claims of magnification in their marketing literature. None at all.

What is the FIELD DIAPHRAGM and how do I set it up?

The *field diaphragm* is an iris diaphragm present only in the lighting path of microscopes equipped for Köhler Illumination. This diaphragm controls the diameter of the illuminating beam entering the substage condenser and is used by the operator to control the diameter of the illuminated field (i.e., the diameter of the lighting spot that illuminates the specimen), to match the field size of the selected objective lens. The ability to restrict the diameter of the illuminated field is advantageous, since anything outside the direct field of view of the eyepieces that gets illuminated contributes excess glare to the final image, thereby reducing image contrast. The ability to restrict the diameter of the illuminated field is especially valuable when viewing specimens which exhibit very low inherent contrast. Non-Köhler scopes (i.e., those equipped for critical illumination) do not include a provision for restricting the diameter of the illuminated field, since to do so would require a substage condenser with a variable focal length, and this is considered impractical due to the complexity and subsequent expense of such a condenser.

The field diaphragm in a Köhler-equipped scope should not be confused with the *aperture diaphragm*, which is an iris diaphragm integral to the substage condenser of all scopes (whether or not they include Köhler illumination). These two diaphragms perform entirely separate functions. The aperture diaphragm within the substage condenser controls the angle of the illuminating light cone reaching the specimen, thereby affecting numerical aperture, resolving power, and image brightness, but not affecting the diameter of the illuminated field. In contrast, the field diaphragm affects the diameter of the illuminated field, but does not affect numerical aperture, resolving power, or image brightness.

In addition to allowing the microscopist to optimize 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 properly focus the substage condenser during Köhler setup. To do this, the operator simply closes the field diaphragm to the point where the visual field is no longer fully illuminated, then adjusts the vertical position of the substage condenser until the illuminated area has sharp edges (i.e., so that the leaves comprising the field diaphragm are sharply focused). Once the substage condenser has been properly focused in this manner and has been adjusted laterally such that the illuminated area is centered within the visual field, the operator should then open the field diaphragm just to the point where the entire visual field is illuminated (i.e., where the leaves comprising the field diaphragm are no longer visible).

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 at the intermediate image plane within the optical tube, and this real image is further magnified by the eyepieces to produce the final image for your eyes. 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 things so complicated? If the FN rating is marked on your eyepieces (many, but not all of them, include FN markings), then the approximate diameter, which is sufficiently accurate for most purposes, of the visual 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 = 2.0mm). 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. If you really need a very accurate measurement, then sure, go ahead and bust out the calibration side and reticle eyepiece. But don't waste your time unless you know that they are both of high quality with known accuracy. Don't assume that a cheap calibration slide or reticle is any more accurate than the field number and magnification values stamped onto your optics.

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 or field flatness within the objective. Those who do not provide full compensation rely on compensating eyepieces to provide correction for the residual chromatic aberration or field flatness, and those that do provide full compensation require optically neutral eyepieces, so as to not introduce lateral chromatic aberration or distortions in the field flatness. If either of these types of objective lenses are used with incorrect eyepieces, lateral chromatic aberration and/or distortions in field flatness 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 demanded by professional users. 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 an existing finite scope lacking this feature without changing the optical tube length of the microscope in the process (and 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 plane in the optical tube (which is orthogonal to the optical axis of the system) at which this intermediate image is focused is referred to as the intermediate image plane.

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 curved leaves, placed in a circular arrangement within a stationary base (i.e., the stator) and a control ring (i.e., the rotor) in such a way that when you twist the control ring one direction, relative to the stator, the aperture 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 substage condensers and objectives, as well as to control the size of the illuminated field in microscopes equipped for Köhler Illumination. The iris diaphragm is so named because its appearance mimics that of 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 many 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 on some fixed point where the eyepieces would be situated, but instead focused at optical infinity. Downstream from the objective lens, a second lens (commonly called the tube lens, or Telan 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 intermediate image plane in the optical tube.

In hindsight, the answer seems obvious, but microscope users at the time no doubt wondered why Reichert would ever do such a thing. After all, this scheme requires the addition of a tube lens, which is not needed with finite optics, and this inevitably adds complexity and expense to the scope, 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? The answer of course is that in addition to eliminating the artifact that occurs in reflected lighting, as stated above, 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 of the tube (i.e., the portion of the optical tube wherein the light rays are parallel). 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 downstream. It took 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 the top-tier manufacturers today produce only microscopes that use infinity objectives, which can be readily identified by the infinity "∞" symbol marked on the outer barrel.

Most of the lower-tier manufacturers who offer microscopes 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 the DIN standard 160mm 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 exact 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 is the JIS MICROSCOPE STANDARD?

The JIS (Japanese Industrial Standards) industry standard is an older standard for microscope objectives and eyepieces made in Japan using finite conjugate optics. The JIS standard was later superseded by the more-recent DIN German standard, and in higher-end microscopes of today, finite conjugate optics have been abandoned in favor of infinite conjugate optics. While JIS standard scopes are no longer produced, microscopes built to the DIN standard are still commonly made and sold as upper-end student and amateur microscopes today. The JIS microscope standard utilizes a 170mm tube length, an objective parfocal distance of 36mm, and standard RMS threads (0.7965" diameter, 36 TPI, 55° Whitworth threading). The object-to-image distance (i.e., the conjugate distance, which is the distance from the specimen plane to the focal plane of the real image produced by the objective) is 182.5mm. Given a 36mm parfocal distance, this leaves 146.5mm for the position of the eyepiece field lens (where the real image should be focused) from the objective mounting flange. The eyepiece field lens (and hence the real image) is recessed 23.5mm from the end of the mechanical tube. So, adding the 36mm parfocal distance and the 146.5mm distance from objective to real image, yields the conjugate distance of 182.5mm. Similarly, adding the 146.5mm distance from the objective mounting flange to the real image to the 23.5mm that the real image is recessed in the optical tube, yields the 170mm tube length. JIS standard eyepieces have a 23.2mm diameter barrel.

Are JIS and DIN OPTICS INTERCHANGEABLE?

Strictly speaking, the answer is no. Objectives made to the JIS and DIN standards use the same RMS threads, and as such are mechanically interchangeable in the nosepiece turrets of either scope type. There may or may not be sufficient focus range in the scope stand to bring the foreign objectives to a focus, depending on the particular microscope stand, but they will physically mount onto the scope. Similarly, the diameters of the eyepiece barrels are the same 23.2mm, so the foreign eyepieces should drop into the ocular tubes with no issues. However, the use of optics made to one standard on a scope with a mechanical tube length of the other standard will result in some degree of exaggerated spherical aberration. When either an objective alone, or an objective and its matching eyepiece made for a 170mm mechanical tube length is used on a scope with a 160mm mechanical tube length, the objective will under-compensate for spherical aberration. Conversely, when either an objective alone or an objective and its matching eyepiece made for a 160mm mechanical tube length is used on a scope with a 170mm mechanical tube length, the objective will over-compensate for spherical aberration. These conditions are minimized if only the objective (but not the eyepiece) is foreign to the stand and are worse if both the eyepiece and objective are interchanged as a matched set. But either way, the optical tube length will be adversely affected with the inevitable result of additional spherical aberration.

On a similar note, the common wisdom that the overall magnification of a microscope's optical system is determined by multiplying the objective and eyepiece power markings together is only true when you use objectives, eyepieces, and mechanical tube lengths made to the same standard. If your eyepiece, objective, or tube length do not all conform to the same standard, then a magnification error in the overall optical system will result. Having said all of that, why not try it? You will obtain an image that may or may not meet your standards of acceptability, but the only sure way to know is to try it. When using JIS optics on a DIN stand, an intermediate 10mm extender may be used between the nosepiece turret and objective, to correct the tube length error. This extender will eliminate the excess spherical aberration that would otherwise be present and will bring the parfocal distance of the JIS objective from 36mm to 46mm, which is a much closer match to the 45mm parfocal distance of DIN objectives.

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. In practice, no microscope is perfectly parcentered, and therefore slight positioning adjustments may be needed to re-center the specimen whenever a new objective is selected. The Latin word *par* means equal. Therefore, *parcentered* means "equally centered".

What does PARFOCAL mean?

A microscope that is *parfocal* is one in which an object that is in focus with one objective will remain in focus for all subsequent objectives. In practice, 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. Parfocality can be improved by the addition of shims, of appropriate thickness, onto the mounting surface of the various objective lenses to bring them into parfocality. The Latin word *par* means equal. Therefore, *parfocal* means "equally focused".

What is the RMS MICROSCOPE STANDARD?

The RMS (Royal Microscopical Society) standard for microscope conjugate optics, which dates back to the 19th century, was perhaps the first industry standard for microscope optics. This standard has been long superseded by other industry standards (such as the Japanese JIS standard, and the later German DIN standard) for finite conjugate optics. In higher-end microscopes of today, finite conjugate optics have been abandoned in favor of infinite conjugate optics (*finite conjugate optics* and *infinite conjugate optics* are described elsewhere). However, finite conjugate optics are still used in many amateur-grade scopes made today. The RMS microscope standard utilizes a 170mm tube length, an objective parfocal distance of 31mm, and 0.7965" diameter, 36 TPI, 55° Whitworth threading (i.e., *RMS Threads* or *Society Threads*, as they are known today). RMS standard eyepieces have a 23mm diameter barrel.

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 is positioned for observation. In other words, the surface of the stage is the specimen plane.

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 of course 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), or from the tube lens, in an infinity scope. 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 are the proper distance apart. The earliest compound light microscopes were constructed exactly 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 produced by the mirrors or prisms which provide the inclined or binocular views.

What is a TUBE LENS or TELAN 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 corrected objective lenses and focuses them further downstream in the optical tube to produce a real image at the required intermediate image plane, for the eyepieces. This lens is called a tube lens since it is conceptually integral to the optical tube (in practice, the tube lens may be located within the viewing head). It should be noted that not all tube lenses are created equal. The tube lens in a given scope may or may not include compensation for residual optical aberrations in the objectives and may or may not provide compensation for field flatness. Additionally, the focal length of the tube lens varies from one manufacturer to the next. For instance, Nikon and Leica typically use 200mm tube lenses, whereas Olympus uses 180mm and Zeiss uses 165mm. Using an infinity objective with a tube lens of incorrect focal length will result in the objective not providing the rated magnification. Using an infinity objective with a tube lens with improper corrections for optical aberrations or field flatness will of course cause poor optical performance. It is for these reasons why infinity optics are in general not compatible from one manufacturer to the next. Finite-conjugate microscopes do not utilize tube lenses, since finite-conjugate objectives do not produce parallel rays, but instead produce rays focused directly onto the intermediate image plane in the tube, thereby directly producing the real image needed by the eyepieces.

How is MAGNIFICATION of a microscope 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 1mm 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 $1mm^2$, so a square 400X larger in area would be $400mm^2$, or 20mm 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 400mm per side, and would have an area of $160{,}000mm^2$. 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 described 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”. Note: As a technical clarification, the term *linear magnification* applies only to a real image which can be projected onto a surface. Since the image seen by the microscope operator is not a real image, but is instead a virtual

image, the pedantically correct term to use here is *angular magnification*, rather than *linear magnification*. The reason for this is that in a virtual image, the linear dimensions of an object seen by the operator cannot be directly measured. Instead, *size* relates to the angle subtended by the object at the focal point.

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 belief, modern compound microscopes are not designed to provide stereo imaging. Instead, they use a binocular or trinocular head to improve operator comfort (i.e., to reduce eyestrain) by providing an identical image to both eyes. Stereo imaging in a high-power compound microscope is not technically feasible, since in order to provide stereo images, 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 typical 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 (as in some of the older stereo scopes). Since compound microscopes are designed to provide high magnifications, their objective lenses require very short working distances (meaning that 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 prevents 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 high-power compound scope is not an acceptable tradeoff, since resolving power is everything in high-power microscopy.

Why can't they design MICROSCOPES where the OBJECTIVE CANNOT CONTACT THE SLIDE?

When using low-power objectives, it's relatively straightforward for engineers to design a microscope that can focus properly, yet whose objectives cannot strike the slide under observation. The designers have in fact done this. It is impossible for the 4X or 10X objectives of a properly engineered microscope to contact the slide. The same cannot be said of the higher-power objectives, such as 40x and 100x.

The reason for this is an optical parameter of objectives known as *working distance*, which is the distance between the lower lens of the objective and the cover glass over the specimen under observation, when the specimen is properly focused. In low-power objectives, the working distance is relatively large. For example, the working distance of a typical 4x objective is in the neighborhood of 18mm, whereas a 10x objective is more like 7mm. Either way, this leaves plenty of space below these objectives to prevent collisions with the glass slides.

In higher-magnification objectives, the working distance gets much shorter. For example, the working distance of a typical 100X oil-immersion objective is in the neighborhood of 0.17mm. That's not very far at all! By comparison, the thickness of a typical glass slide is approximately 1.0mm. Older slides were commonly made as thin as 0.7mm and as thick as 1.5mm. So, in order for a microscope to be able to focus on any slide that you are likely to encounter, the travel of the focus mechanism cannot be limited to prevent the 100x objective from striking the slide.

Questions about Microscope Cameras and Photography

What is a microscope EYEPEICE CAMERA?

A microscope *eyepiece camera* is a small digital camera manufactured with a 23mm tube that drops into one of the ocular tubes on a binocular head, replacing one of the eyepieces. The design of the eyepiece camera is such that the digital image sensor of the camera will be located at the intermediate image plane of the optical tube when the camera is installed, allowing the camera to capture the intermediate image produced by the objective lens, without the necessity of any couplers, adapters, or projection optics. In

some scopes, the eyepiece camera may be installed in the camera port of the trinocular head, leaving the eyepieces in place for direct viewing, so long as the mechanical design of the camera port supports this (some trinocular camera ports are not compatible with conventional eyepieces).

Since eyepiece cameras rely on direct projection of the intermediate image (by the objective lens) onto the digital image sensor, only those microscopes whose objectives (or objectives plus the tube lens, in the case of infinity scopes) provide fully corrected intermediate images can utilize an eyepiece camera. Any microscope which does not have a fully corrected intermediate image, and therefore relies on compensating eyepieces to provide final correction, cannot use an eyepiece camera, or very poor images will result.

What is a PHOTO PROJECTION LENS or RELAY LENS?

A *photo-projection lens* (also known as a photo eyepiece or a photo-relay lens) is an optical device that goes into the camera port of a trinocular viewing head and projects the real intermediate image made by the objective (and intended for the viewing eyepiece) onto the film or image sensor of an attached camera, for photomicrography. A photo-projection lens cannot be used in place of an eyepiece for visual imaging. Photo-projection lenses are usually relatively low magnification and must be properly chosen to project the correct image size onto the film or image sensor in the camera, to capture the desired field of view. If the magnification of the photo-projection lens is too high, the visual field captured by the camera will be too narrow, whereas if the magnification is too low, the captured field will be excessively wide and image vignetting may result. It is important that the optical characteristics of the photo-projection lens match the objective in use. If the objective does not provide a "finished" intermediate image (i.e., if the intermediate image contains some degree of uncorrected optical aberrations), then the photo-projection lens must provide correction for these aberrations, otherwise a poor final image will result. If, on the other hand, the objective provides a finished intermediate image with no residual aberrations, the photo-projection lens must be optically neutral, otherwise it will introduce aberrations into the final image.

How do I determine the TOTAL MAGNIFICATION of DIGITAL IMAGES through the microscope?

Everybody knows that the *total magnification* of a compound microscope is equal to the magnification of the objective lens multiplied by the magnification of the eyepieces. That's easy, right? But what about the total magnification when you attach a camera to your trinocular head and shoot digital images? At this point, the eyepieces aren't even part of the optical setup, so then what do you do? The answer is of course similar to the case where the observer is looking into the eyepieces. However, instead of the objective lens and eyepieces producing a magnified image for the observer's eyes, the objective lens and the optics in the camera adapter work together to project an image of the specimen directly onto the digital image sensor of the camera. Beyond this, nothing is fundamentally different. The size of the image that gets projected onto the digital image sensor is determined by the magnification of the objective lens (as before) and by the magnification of the optics in the camera adapter that attaches the camera to the camera port on the trinocular head (instead of the eyepieces).

The size of the projected specimen image on the digital image sensor, and the physical dimensions of the digital image sensor itself, determine the relative size of the specimen as it appears in the field of view of the resulting digital images. If the size of the projected specimen image is half the width of the sensor, then the specimen will fill half the field of view in the resulting digital images. Up to this point, everything is pretty straightforward.

From here on out is where things get a little bit complicated. The size of the specimen seen by the viewer of the digital images will depend not only on the relative size of the specimen in the resulting digital image files (as described above), but also on the size of the image as it's displayed on the viewing monitor. And this of course depends on the monitor size, and on the size of the window of the image-viewing software, and on the zoom factor of the image-viewing software. Or, if you are viewing a hard copy of the digital

image, the answer depends on the size the physical image printed on the page. I think you get the idea. There are a ton of variables here.

So why do we even care about the total magnification? The answer, of course, is so that we can determine the size of the specimen and its various details in the captured images. But take my advice and forget trying to calculate total magnification of the image as seen on the monitor, and all that that entails, and just go at it this way instead. If you know the physical dimensions of the digital image sensor in your camera, and if you know the relative size of the specimen in the image (as compared with the image size), then you can easily find the size of the image of the specimen that was projected onto on the digital image sensor via the objective and camera-adapter optics. For example, the ASP-C image sensor in a Canon DSLR has dimensions of 22.3mm in width and 14.9mm in height. So, if the specimen fills half the width of the resulting image, then that means that the image of the specimen projected onto the sensor was approximately half the width of the sensor, or 11.5mm. You can of course do the same calculation using heights instead of widths, so use whichever is most convenient.

But then how do you then go from here to determining the actual size of the specimen? Just divide the size of the projected image on the digital image sensor by the total magnification of the projecting optics (i.e., the objective lens and the camera-adapter optics) to obtain the specimen size. For example, if you're using a 40X objective and a camera adapter with integral 2.5X projection optics, the total magnification of the image that is projected onto the digital image sensor will be 100X (i.e., 40 X 2.5). Using this value, we can determine the original specimen size by dividing the size of the image that was projected onto the digital image sensor by the total magnification of the projecting optics. In this case, that would be 11.5mm divided by 100, yielding a specimen size of 0.115mm (or 115µm).

If you wish to mark your images with magnification information (rather than scale bars), be sure to not only include the total projection magnification, but also the image sensor type, as knowledge of one is useless without knowledge of the other. The text "25X on ASP-C", for example, provides the viewer with all of the relevant information needed to determine the specimen size, regardless of whether they are viewing the image on a monitor or printed page. Note however that this method of image marking will produce erroneous size estimates if the photograph has been cropped or if the aspect ratio has otherwise been altered from the aspect ratio of the camera sensor. If the image has been cropped or if changes to the aspect ratio have been made, do not use this method of image marking, but instead use scale bars. But whatever you choose to do, don't put "400X" on the image produced using your 40X objective, because this is certainly not the case.

Questions about Microscope Condensers

What is a DRY CONDENSER?

A *dry condenser* is intended to be used without immersion oil between the slide and the condenser. 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 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 or OILED CONDENSER?

A *wet condenser* (or oiled 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 be used dry but be aware that the numerical

aperture of the condenser will drop to approximately 0.95 whenever they are not oiled to the slide. This reduction in condenser N.A. comes with a commensurate reduction in resolving power, thereby producing lower-resolution images.

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.

When observations using objective lenses below 4X are required, microscopists typically turn to one of the following options: 1) Remove the substage condenser. 2) Use a dedicated low-power condenser optimized to provide wide-field illumination for low-power objectives. 3) Unscrew and remove the top lens of the condenser (for condensers which allow this). 4) Use a flip-top or swing-in/out condenser. In terms of performance, Option #1 gives the poorest results, whereas Option #2 gives the best, and can typically be used for objectives all the way down to 1X. In terms of convenience, Option #4 is best, but is typically suitable only for objectives down to 2X.

What is a CONDENSER with REMOVABLE TOP LENS?

Some condensers are built to allow the top lens to be unscrewed and removed from the light path, so as to change the focal length of the condenser and thereby allow the full field of view of low-power objectives to be illuminated. For observations at 4X (typically) and above, the top lens is left in the illuminating path, and for observations below 4X, the top lens is removed from the illumination path. Whenever such a condenser is used with the top lens removed, the aperture diaphragm on the condenser should be opened to its widest position and the field diaphragm (if present), 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 FLIP-TOP or SWING-OUT CONDENSER?

Similar in principle to a condenser with a removable top lens, but far more convenient for the user, is a *flip-top* or *swing*-out condenser. A flip-top or swing-out condenser utilizes a rotating mechanism with an easily accessible knob or lever to allow the operator to quickly flip the upper lens element of the condenser either into or out of the light path, as appropriate, to change the focal length of the condenser and thereby allow the full field of view of low-power objectives (such as 2X) to be illuminated. For observations at 4X (typically) and above, the top lens is left in the illuminating path, and for observations below 4X, the top lens is flipped out of the illumination path. Whenever a flip-top or swing-out condenser is used with the upper lens flipped out of the illuminating path, the condenser aperture diaphragm should be opened to its widest position and the field diaphragm (if present), 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 a flip-top or swing-out condenser, except that it contains an optical lens on a pivot mechanism located below, rather than on top of, the condenser. A swing-in condenser allows the operator to quickly swing the lower lens element of the condenser either into or out of the light path, as appropriate, to change the focal length of the condenser and thereby allow the full field of view of very low-power objectives (such as 2X) to be illuminated. 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 (N.A.) 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 decreasing the overall resolving power 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 (pronounced "ah-bay") 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 mechanism (the aperture 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 commonly used condenser types, it is cheaper to manufacture and provides acceptable performance for objectives of 40X and below. The condenser mounted on your microscope right now is likely an Abbe condenser.

What is an ACHROMATIC CONDENSER?

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

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 corrected for both spherical and 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 portion of the illuminating cone), so that direct, undiffracted light falls outside the acceptance angle of the objective and is therefore not collected and does not contribute to the final image. Darkfield condensers come in two basic types: dry and wet. The design of the most basic 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 illuminating the specimen. In 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 spatially displaced (sheared) 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.

Questions about Microscope Eyepieces

What is a BERTRAND LENS?

A *Bertrand lens* is an accessory lens which can be inserted or rotated into the optical pathway (below the eyepiece) of a suitably equipped light microscope to provide functionality very similar to that of a phase-centering telescope. When used in conjunction with a standard eyepiece, a Bertrand lens allows the operator to visualize various optical planes within a light microscope. Bertrand lenses are commonly used in polarizing microscopy to bring the image of the interference figure at the back focal plane of the objective into focus for conoscopic observations. Additionally, a Bertrand lens can be used to setup a phase-contrast microscope, or to setup Köhler Illumination on a Köhler-equipped microscope.

What is a COMPENSATING EYEPIECE?

Compensating eyepieces provide magnification of the real intermediate image produced by the objective lenses, as is needed in all compound microscopes, and also provide compensation for any optical aberrations (e.g., chromatic difference of magnification) present in this intermediate image as a result of incomplete correction within the objective itself. As such, compensating eyepieces are used with objectives that do not provide fully compensated intermediate images, as a way to clean up the optical aberrations so that the observer sees a complete (fully compensated) image. Compensating eyepieces are traditionally marked with “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).

In contrast, a non-compensating eyepiece is optically neutral and provides no compensation for optical aberrations. Non-compensating eyepieces are used with objectives which fully correct for chromatic aberration, or with objectives that do not fully correct 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.

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 at the extremes of the visual field which are 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 pupil of the observer's eye should be placed to properly see the entire field of view of the microscope (see item 4 in Figure 1). The distance between the upper lens of the eyepiece and the exit pupil is defined as the *eye relief* (see item 3 in Figure 1). In standard-relief eyepieces, the eyepoint is typically in the range of 7 to 13mm above the top lens, although the higher the magnification of the eyepiece, the lower the eyepoint tends to be. Consequently, eyepieces with magnifications of 20X and higher can be very difficult for many people to use, and as a consequence can be quite fatiguing for the operator.

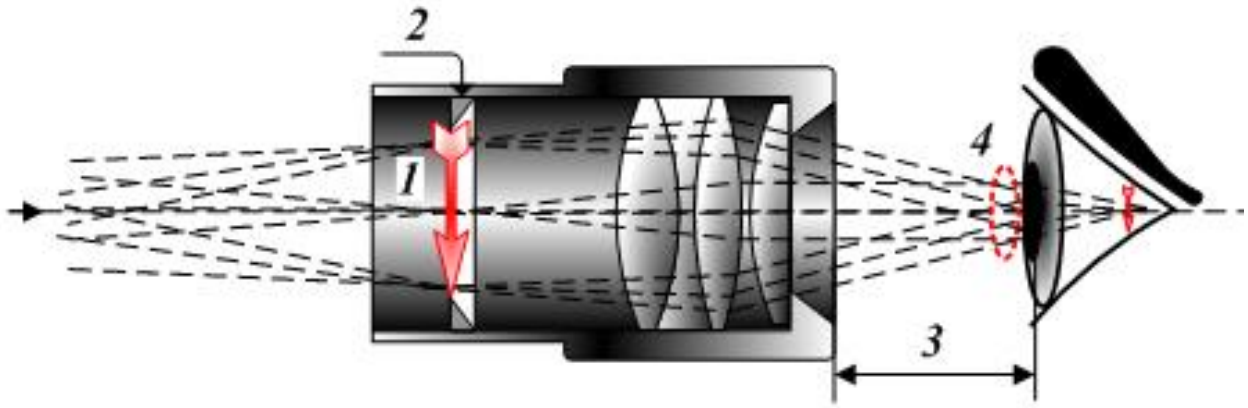

Figure 1 - Eyepiece eye relief (Dimension 3).

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 operators who wear 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 a scope with high-eyepoint eyepieces, 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. Additionally, your scope will then be equipped for other users who may require glasses or will be equipped for you if in the future you require glasses.

It should be noted that if your vision is such that you require eyeglasses and choose to use the microscope without them, the only way to achieve proper focus of the specimen under observation will be to set the objective lens to a distance from the specimen that differs from the specified working distance parameter of the objective lens (in order to compensate for your impaired vision), thereby reducing the optical performance of the microscope. This is especially true when using high-performance objectives with high

numerical aperture. So, if you wear corrective eyeglasses, get yourself a set of good high-eyepoint eyepieces and get used to using them with your glasses on.

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 (i.e., the size of the field stop within the eyepieces). 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 circular specimen needed to completely 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 diameter (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. Widefield eyepieces (i.e., those with a FN of 20-23) often bear the letters "WF". Super widefield eyepieces (i.e., those with a FN > 23) often bear the letters "SWF".

What is a FILAR EYEPIECE?

A *filar eyepiece* (also known as a *Filar Micrometer* or *Filar Reticle*) 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 consists of a reticle with two fine parallel wires or threads, located within 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, to determine the spatial distance between these two points from the readings of the engraved micrometer markings on 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 or EYEPIECE LENS?

The term *ocular lens* is an alternate name for 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 a reticle scale within the eyepiece 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?

In modern usage, the terms *reticle*, *reticule*, and *graticule* are often used interchangeably to describe the same piece of hardware. It should be noted, however, that in historical usage, there were differences between a *graticule* and a *reticle*/*reticule*. Nowadays, as these terms are used, they typically refer to a transparent glass disk, etched with some sort of scale, crosshatch pattern, or some other useful reference pattern, which is placed into an eyepiece and positioned such that the etched image of the reticle is on a conjugate plane of the specimen (i.e. is on the plane of the eyepiece field diaphragm), such that the etched reticle pattern is seen overlaid on the specimen image, allowing for simple measurements of specimen length, diameter, and so forth. 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 fixed ocular field diaphragm, it appears to the observer to be superimposed over the image of the specimen. For optimal results, eyepieces using reticles often contain a helical focusing mechanism to allow the image of the reticle to be brought sharply into focus with the specimen. In addition to measurement purposes, reticles are also commonly used to allow the operator to visualize the section of the visible field that will be captured by a camera, when using the microscope for photomicrographic applications.

In older usage, the terms *reticle* and *reticule* were used to describe the arrangement where wires, hairs, spider silks, or other such things were used to produce visual reference marks for the observers, whereas the term *graticule* was reserved for the glass disks etched with reference patterns[2].

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 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 improvements 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 far too 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

[2] Thanks to David Martella for providing a reference clarifying the distinction between *reticle* and *graticule*.

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 just 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 the FIELD CURVATURE of an objective?

All real-world optics suffer from a variety of optical aberrations, making their performance less than ideal. One such aberration which is particularly annoying to microscopists is *Petzval Field Curvature* (named for Joseph Petzval). Petzval field curvature, which is more commonly known to microscopists simply as *field curvature*, is present to some degree or another in all microscope objectives and is especially pronounced in low-magnification objectives. Petzval field curvature causes the images produced by the objective, when observing an extended flattened object such as a flat specimen on a microscope slide, to be projected onto a spherical surface resembling a curved bowl (known as the *Petzval Curvature*) rather than onto a flat plane. This manifests itself to the operator as the inability to focus the microscope such that the entire visual field of a specimen under observation is in sharp focus at the same time. In other words, if you sharply focus the microscope on a specimen detail located in the center of the field of view, specimen details at the edges of the field will not be sharply focused. Similarly, if you sharply focus the microscope on a specimen detail at the edge of the field of view, specimen details in the central portion of the field will not be sharply focused. One simple method manufacturers of low-cost scopes use to mitigate some of the effects of Petzval curvature is to add a simple field stop to the optics (typically in the eyepieces) to limit the visual field to the central portion of the image produced by the objectives, thereby excluding the most out-of-focus portions. When such a reduction of the visual field is undesirable or unacceptable, or when the field of view needs to be especially flat for such applications as photomicrography, manufacturers turn instead to using more complex and expensive *Plan* objectives. Plan objectives, which are made such that the focal length of the optics increases with ray angle, produce far less field curvature than do simple, non-Plan objectives, and are usually included as standard equipment on the better microscopes, or as an upgrade for lower-cost microscopes.

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 that were 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 must necessarily 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 of the objective which depends on the maximum angle of incoming light at which the light will be collected by the lower lens of the objective (i.e., the acceptance angle of the objective lens), as well as the index of refraction of the intervening medium between the specimen and the lower lens of the objective. The equation for numerical aperture essentially uses the acceptance angle as a way to normalize the objective aperture to the working distance of the objective. All things being otherwise equal, and for a given intervening optical medium, an objective with a high N.A. value collects light over a wider range of angles than one with a lower N.A. The N.A. rating of the objective is important since this (along with the N.A. of the condenser) is what determines the optical resolving power, and therefore the maximum useful magnification, that the overall optical system of the microscope can provide.

The reason that the optical resolving power of a microscope depends on the acceptance angle of the objective is because of an optical phenomenon of light, known as *diffraction*. Diffraction occurs as a result of the way light waves interact with the specimen details. Without getting too technical, diffraction is the scattering of light that occurs as the waves of light pass through the specimen under observation. The degree to which this scattering occurs is a function of the physical detail present 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 accept incoming light over a wide angle in order to collect the components of the light that were diffracted by the finer specimen details. Without sufficiently collecting the higher orders of diffraction from the fine details, the objective will not be able to resolve these fine details, since the information for these details resides in the higher orders of diffracted light that come in at the extremes of the collection angle.

For a dry objective (i.e., one which uses air as the intervening medium), the N.A. of the objective is limited in a practical sense to approximately 0.95, regardless of the acceptance angle of the objective. An oil immersion objective is able to achieve a significantly higher N.A. (as high as 1.40) since the index of refraction of the oil is about 50% higher than that of air.

So 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 1000 times the rated N.A. of the objective. So, a really good 100X oil-immersion objective with an N.A. of 1.40 can provide useful magnification up to 1400X (let's say 1500X, since 15X eyepieces are available, whereas 14X are generally not), and that's all it can do. There is no reason to use such an objective with eyepieces higher than 15X, and any extent to which you do will 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 cover glass over 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 *Planar* objectives) are designed to provide a flatter depth of field 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. Plan objectives, which are made such that the focal length of the optics increases with ray angle, produce far less field curvature than do simple, non-Plan objectives, and are usually included as standard equipment on the better microscopes, or as an upgrade for lower-cost microscopes.

What is the WORKING DISTANCE of an objective?

The *working distance* of a microscope objective is the distance between the cover glass over 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 OBJECTIVES and ULWD OBJECTIVES?

The working distance of a microscope objective is the distance between the cover glass over the specimen under observation and the lower lens of the objective lens, when the scope is properly focused on the specimen. 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, or for observing semiconductor wafers without colliding with the wafer.

What is a DRY OBJECTIVE?

A *dry objective* is an objective lens intended to be used without homogeneous (i.e., oil or water) immersion. A dry objective 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 or water on wet objectives, do not allow any of the oil or water to get onto the dry objectives, or damage to the dry objective can occur as a result of fluid ingress. Be sure to immediately clean off any immersion oil or water that gets onto the dry objectives, to prevent such damage.

What is a WET OBJECTIVE or OIL-IMMERSION 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 sometimes 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 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 cover glass over the specimen and the bottom lens of the objective at which 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 minimal 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 that 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 contacts the slide. The drawback to spring-loaded objectives is that with careless use, the retraction mechanism can become contaminated with immersion oil or other debris to the point where the mechanism gums up and can no longer protect the slide and optics, or worse yet will not re-extend to the proper position following a collision.

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 typically 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 typically 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 if 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 typically 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 glass. Another example is the letters "160/–", which indicates that the tube length should be 160mm and that the cover glass thickness is irrelevant.

How can I tell the MAGNIFICATION of OLDER OBJECTIVES that are not marked for magnification?

Older microscope objectives were typically not marked to indicate their magnification but were instead marked to indicate their optical focal length. They were marked this way because the magnification achieved by a given objective on a particular microscope depends not only on the optical properties of the objective lens, but also on the tube length of the microscope upon which the objective is used. Early microscope objectives were typically made by a few lens-makers and were in-turn sold to the various microscope makers. There was no way to predict the actual magnification of an objective since optical tube length had yet to become standardized. However, it is simple to convert the specified focal length

of an objective to magnification, so long as the optical tube length of the microscope is known. Simply divide the microscope's tube length by the objective's focal length, making sure that the same units (inches or millimeters) are used for both. So, for example, an objective marked "4mm" would produce a magnification of 40X when used on a microscope with a 160mm tube length but would produce a magnification of 45X when used on a microscope with a 180mm tube length.

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 of Figure 2.

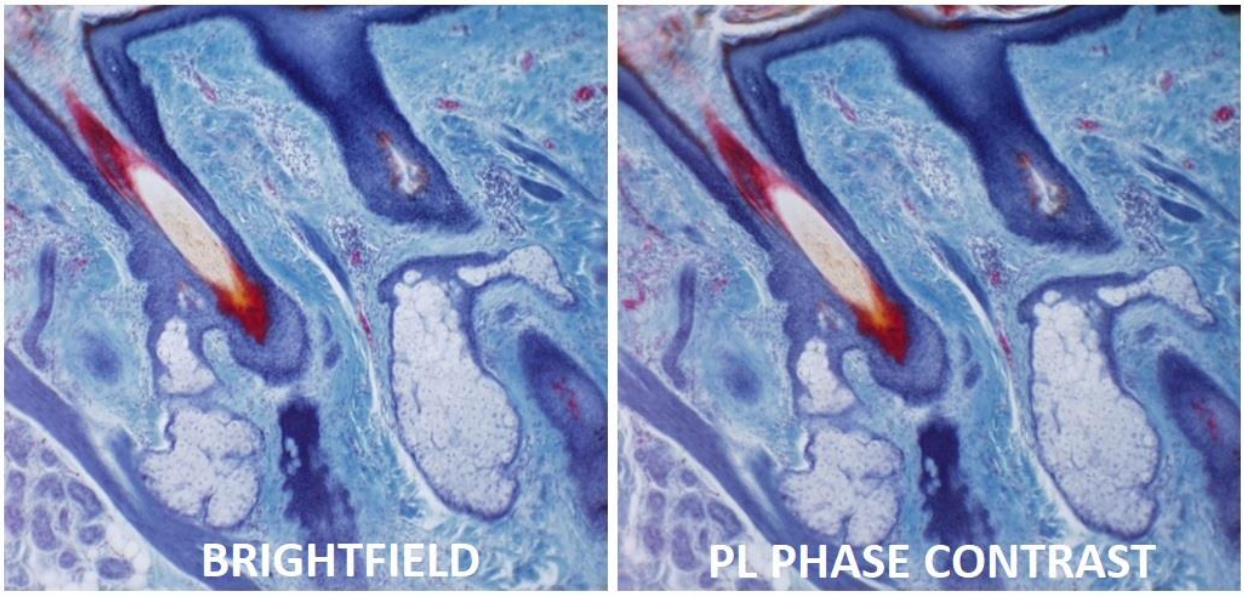

Figure 2 - Comparison of images taken with brightfield and phase-contrast objective.

What do the various OBJECTIVE COLOR MARKINGS 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.

MAGNIFICATION (UPPER BAND): The objective magnification is marked using a color ring around the upper portion of the objective barrel. Black or Gray = 1.0X, 1.25X, or 1.5X. Brown = 2.0X or 2.5X. Red = 4X or 5X. Yellow = 10X. Green = 16X or 20X. Turquoise = 25X or 32X. Light Blue = 40X or 50X. Cobalt Blue = 60X or 63X. White or Off-White = 100X and higher.

IMMERSION (LOWER BAND): Specific types of immersion media are identified by a second color ring around the lower portion of the objective barrel. The lack of a lower ring indicates a non-immersion (i.e., dry) objective. Black = Oil Immersion. Orange = Glycerol Immersion. White = Water Immersion. Red = Special Immersion.

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 whose thickness is 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 errors in cover glass thickness. On typical upright scopes, the correction collars can be adjusted to accommodate cover glass thicknesses over the range of 0.11mm to 0.22mm, whereas on inverted scopes, the range of the correction collars are typically from 0 to 2.0mm, 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 (N.A.) 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.

Why do I get a BAD IMAGE when using my 40X OBJECTIVE?

This often 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 (or 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.

Why CAN'T I FOCUS on a SLIDE with my 40X OBJECTIVE without hitting the glass slide?

This is a fairly common problem seen by people who are new to microscopy. A typical 40x objective has a working distance of approximately 0.5mm, which means that the lower glass element in the objective will be 0.5mm above the cover glass over the specimen when the microscope is properly setup and focused on the slide. So, if your objective is hitting the cover glass and preventing the specimen from coming into focus, this means that there is more than 0.5mm of glass between the specimen and your 40x objective. How can that happen? This is almost always an error on the part of a new, inexperienced microscopist. There are two common mistakes that will cause this to happen: 1) You are attempting to look at a commercially available prepared slide that has been placed on the microscope stage upside-down. A typical slide is around 1.0mm thick, so if the slide is upside-down on the stage, there is no way for the objective to get close enough to the specimen to obtain focus. 2) You are trying to look at a preparation you made (i.e., not a prepared slide), and you are using an improper cover glass over your specimen. A cover glass is a very thin (typically 0.17mm) piece of glass made to cover your specimen on the slide. Many beginners mistakenly believe that a regular old glass slide can be used as a cover glass, but as described above, slides are too thick to be used as a cover glass. You must use a real cover glass!

What is a POLARIZING or STRAIN-FREE OBJECTIVE?

A microscope objective specifically constructed such that the glass elements within the objective are free of mechanical stress are said to be *strain free*. Strain-free objectives are intended for polarizing microscopy, since glass elements under strain can interfere with polarizing microscopy by introducing unintended birefringence. Strain-free objectives are typically indicated by the letters "P", "PO" or SF". Note that best practices state that objectives marked as *strain free* should be used for polarizing microscopy, many simple achromatic objectives which are not specifically marked as strain free are in fact sufficiently strain free that they will work just fine for non-quantitative polarizing purposes. The more complex types, such as apochromatic objectives, should be avoided for polarizing purposes, as residual strain is much more likely in these objectives.

Can I use INFINITY OBJECTIVES on a FINITE MICROSCOPE?

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 proper real image for the eyepieces. An infinity objective used on a stand designed for finite-conjugate optics will produce spherical aberration that severely degrades the image quality. So, while 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 noticeably poor.

Can I use FINITE OBJECTIVES on an INFINITY MICROSCOPE?

Finite-conjugate objective lenses should not be used on microscopes designed for infinity optics, since the infinity scope will contain a tube lens that will degrade the working distance, magnification, and numeric aperture of the finite-conjugate objective. So, while you may be able to physically screw a finite-conjugate objective onto an infinity scope and achieve an image of some sort, the working distance (and therefore parfocality), magnification, and resolving power of the objective will be significantly degraded, as compared to what the objective was designed to provide.

Will one manufacturer's INFINITY OBJECTIVE 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's tube lens, producing images with significant optical aberrations or field curvature if the objectives are interchanged. Additionally, the focal length of the tube lens needs to be an exact match to that needed for the objectives, in order for the objectives to provide their rated magnification. This means that if the two objectives require a different focal length for their matching tube lenses, they will not exhibit the same magnification when used on a stand with a tube lens of the wrong focal length. 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.

Questions about Microscope Filters

What is the purpose of the BLUE FILTER I got with my microscope?

The daylight 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 that 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 digital format (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.

What is the purpose of a DIDYMIUM FILTER?

A *didymium filter* is a pinkish-looking filter made from Schott BG-20 didymium glass. This filter is used to enhance the hues of specimens stained with eosin, fuchsin, and methylene blue. Didymium filters are commonly used to enhance the appearance of histology slides stained by H&E stain, and work by emphasizing the red appearance of eosin, to reduce the washed-out effect otherwise seen in photomicrography of such slides. When coupled with good halogen lighting, a didymium filter provides excellent imaging and photomicrography for histological sections stained with H&E.

What is an INTERFERENCE FILTER or a DICHROIC FILTER?

An *interference filter* (or *dichroic filter*) is made using multiple thin layers of dielectric material of various refractive indices. The configuration is such that the interference (both constructive and destructive) that occurs at the thin-film boundaries provides reflection of certain wavelengths, while transmitting others. The list of advantages of interference filters, as compared to absorptive filters, includes significantly higher optical transmissivity at the wavelengths of interest and much steeper walls of the filter response. Interference filters can be made to provide low-pass, high-pass, band-stop, or band-pass properties.

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*, uses a simple mirror beneath the microscope stage to reflect incoming light up onto the specimen. Although this mirror reflects light onto the specimen, it does 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 on 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 Illumination* 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

quite 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 photomicrography. It should be noted 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. However, the ground-glass diffusers introduced a new problem. Rather than focusing an image of the non-uniform illumination source onto the specimen plane, the substage 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 condenser to its 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 Illumination (or *epi-illumination*, which is 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 surface of the specimen and reflects back into the viewing optics. This differs from convention diascopic or transmitted lighting, which is 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 (which is functioning as the condenser for illumination), 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. Episcopic illumination is commonly used to view optically opaque specimens.

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 collector lens to achieve the same result. An advantage of the stand-alone diffuser over the frosted collector lens is that the stand-alone diffuser can be removed from the lighting path (if provisions exist to do this) to allow for the lighting source, as visualized by viewing the back focal plane of the objective, to be properly centered and focused at the front aperture plane 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 since manual lamp centering is not required.

How do I SET UP KÖHLER ILLUMINATION on my microscope?

I'm going to make an assumption that you have a relatively modern microscope which you want to set up for Köhler illumination. Older microscopes made before pre-focused/pre-centered Köhler was a thing can be quite complicated to set up, and you should refer to the manufacturer's specific instructions for these older microscopes.

To set up for Köhler illumination in a modern scope, start by raising the substage condenser to the top of its range of travel, adjust the aperture diaphragm in the substage condenser to the approximate center of its range, and adjust the field diaphragm to its fully open setting. Now place a prepared slide on the stage and focus on the specimen using the 10X objective. Set the lighting intensity for a comfortable viewing level and adjust the aperture diaphragm in the condenser for good image contrast.

Once the scope is properly set up to view the slide with the 10X objective, decrease the field diaphragm setting until a bright circle of light appears within the visual field. The light circle may or may not be centered within the visual field at this point. Use the condenser focus knob to adjust the vertical positioning of the substage condenser until the edges of the light spot appear crisp and well-focused. Depending on the type of condenser you have on your scope, the bright spot may now be acceptably sharp, or (more likely) will have either blue or red color fringing around the outer circumference of the light disk. If you do not see color fringing, this means you have a condenser which is well-corrected for achromatic aberration. If you do see color fringing, this likely means that your scope has a standard Abbe condenser, which is uncorrected for chromatic aberration. If color fringing is visible, just set the condenser focus midway between the points where equal amounts of blue and red fringing can be seen.

With the substage condenser properly focused, adjust the two orthogonal condenser-centering screws to move the light spot into the center of the visual field. With the light spot centered, increase the setting of the field diaphragm just to the point where the outer circumference of the light spot can no longer be

seen, but don't go beyond this point (re-adjust the condenser-centering screws if necessary to keep the spot in the center).

Your scope is now properly setup for Köhler illumination on the 10X objective. You will need to repeat this setup procedure anytime you switch to another objective. Note that Köhler illumination cannot be setup on most scopes using the 4X objective.

Will HALOGEN LIGHTING DRY OUT my SLIDES?

Many people state, with some justification, that halogen lighting on a microscope will dry out and kill organisms under observation, due to the heat produced by the halogen bulb. Since many amateur microscopists see this as a major reason to avoid halogen-equipped scopes, let me set the record straight here once and for all: "Halogen lighting does not cause this issue. *Poorly engineered* halogen lighting causes this issue." Heating of the slide on the stage of a halogen-equipped scope can come from two distinct thermal mechanisms. The first of these mechanisms is when the infra-red (IR) portion of lighting spectrum is absorbed by the specimen, directly raising its temperature. The second mechanism is heating of the stage (and subsequently the slide) by the large amount of waste heat produced by the halogen lamp. Both issues can exist in scopes using poorly engineered halogen lighting but are not present to a significant extent in well-engineered halogen-lit scopes.

To eliminate the first issue, the scope manufacturer simply needs to include an IR-absorbing adiabatic glass filter in the lighting pathway of the scope, thereby preventing significant IR from reaching the specimen, protecting against a temperature increase by this mechanism. This is an easy thing to do, but some manufacturers omit this filter from their designs, opting instead to save a few dollars on manufacturing costs.

The solution to the second issue is not quite so simple. The fix for this involves up-front planning in the basic design and layout of the scope, to keep the waste heat well away from the stage, so that it doesn't contribute to specimen warming. A properly designed halogen illumination system will typically have the halogen lamp in an external housing, usually located on the rear of the scope, so that any waste heat from the halogen lamp can safely dissipate without warming the stage. There are other benefits to this basic physical arrangement as well, but regardless of the benefits, many manufacturers opt instead to integrate the halogen lamp into the scope base, directly below (and pointing up at) the stage. While this arrangement may simplify the design, save a few dollars, and streamline the physical appearance of the microscope, it allows the waste heat from the lamp located directly below the stage to directly warm the stage and contribute to drying of the slide preparation.

So be aware that my preference for a halogen-equipped microscope comes with the simple caveat that it needs to be a scope with a properly designed illumination system. The Olympus BH-2 microscope is an example of a microscope with a well-engineered halogen illumination system. Using such a scope, one can observe a wet mount preparation for hours, adding the occasional drop of water now and again to keep up with evaporation that occurs on *any* microscope.

What is DIASCOPIC ILLUMINATION or TRANSMITTED ILLUMINATION?

Diascopic Illumination (also known as *transmitted illumination*) is the lighting type used in biological compound microscopes, wherein 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 the specimen. Diascopic illumination cannot be used to view specimens which are optically opaque.

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 be used with the substage condenser, whereas the concave side, if present, should be used only for low power observations (i.e., when using the 4X objective) with the substage condenser removed from the scope.

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 employed by 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 Jentsch design as first produced by Leitz utilized adjustable eyepieces to compensate for the inevitable changes to the optical tube length that occurred as a result of adjustments to the interpupillary distance. Subsequent improvements 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. The primary disadvantage of the Jentzsch design is the necessity to compensate the tube length with changes to the interpupillary-distance setting, which adds both cost and mechanical complexity. Also, since the eyepiece tubes are mechanically linked to the interpupillary distance slides (in automatically compensated Jentzsch designs), a very loose slide mechanism can allow the interpupillary distance setting to move if pressure is inadvertently applied to the eyepieces by the operator. Advantages to the Jentzsch design are that the height of the eyepieces do not vary, nor do the eyepieces rotate (so measuring reticles within the eyepieces do not rotate within the field of view) with changes in the interpupillary distance setting.

What Is a SIEDENTOPF VIEWING HEAD?

The *Siedentopf* style binocular viewing head was developed by German-born Henry Siedentopf in 1924, while Siedentopf was employed by the Leitz company. The original Siedentopf 1924 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. This was manufactured by Zeiss and offered as the *Bitumi* attachment for their monocular systems. In 1926, Zeiss released an improvement to the Siedentopf design in which both eyepiece tubes and their associated prisms rotated to allow for adjustment of the interpupillary distance. The primary advantage of the Siedentopf design over the Jentzsch design is that changes to the interpupillary distance setting do not affect the optical tube length, and therefore no tube-length compensation mechanism is needed. Disadvantages are that

the height of the eyepieces vary, and the eyepieces rotate (causing any measuring reticles within the eyepieces to rotate within the field of view), as the interpupillary distance setting is changed. Additionally, a loose rotation mechanism can allow the eyepiece tubes to drop under the pull of gravity, or by inadvertent contact by the operator, thereby changing the interpupillary distance setting.

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 interpupillary distance properly set, and with the right eye looking into the right-most eyepiece while the left eye is closed. Now adjust the focus controls of the microscope until the right eye sees a perfectly sharp image of the specimen. Next, open your left eye and 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 small NOTCH in the right-most EYEPIECE TUBE?

This notch is intended to accept the locator pin on eyepieces which have an internal measuring reticle installed. This locator pin, which protrudes from the eyepiece and fits into the notch, locks the orientation of the eyepiece within the tube such that it does not rotate within the tube, making sure that the visual orientation of the measuring 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 is the benefit 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 objective 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 bit 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 higher the resolving power of the objective. Most commonly, the intervening medium for an objective is air, which has an index of refraction of 1.0. In order to achieve sufficient numerical aperture for 1000X observations, the angle of acceptance of the objective must be as large as possible, but this alone is not sufficient. The only way to increase the numerical aperture further is to eliminate the air gap between the slide and the objective. Immersion oil, which is an optically transparent oil with an index of refraction matching that of the cover glass, is used for this purpose. By replacing the air gap with immersion oil, the resolving power, resolution, and resulting useful magnification of the objective will be increased by approximately 50%, as compared to an 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, when using oil immersion to obtain the best possible resolving power, the condenser-to-slide interface should not be neglected. 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 limit the maximum

resolving power of the optical system), if you want to obtain maximum resolving power, then you will need the highest numerical aperture possible, and this means that 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 also 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. When 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 will be limited by the dry condenser (the N.A. of a dry condenser will 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.

Oil 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 merge, leaving no air gap between the slide and condenser. You man now proceed to oil the objective to the slide.

Oil 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 objective and the 100X oil-immersion objective. 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-immersion objective contacts the oil drop. The bottom of the 100X oil-immersion objective will be very close to the cover glass and this will allow the immersion oil to completely bridge the gap between the objective and the cover glass, displacing any air between in the process. Tweak the slide position and the fine focus until the image is centered and sharply focused 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-immersion 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 to remove the immersion oil from the 100X 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 glass 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 Illumination

What is DARKFIELD ILLUMINATION?

Darkfield Illumination is an optical staining technique used to provide enhanced contrast to live/unstained specimens that are difficult to see under normal brightfield conditions. In simple terms, darkfield illumination lights the specimen such that the direct light (i.e., the "zero-order" light that is not scattered by the specimen) is not collected by the objective lens, and only the higher-order light which is scattered by the specimen is collected, and therefore visible. This exclusion of un-scattered zero-order light and collection of scattered light produces a visual field where the specimen appears brightly illuminated against a dark background (see Figure 3). While for most applications in professional microscopy, darkfield has largely been replaced by more modern imaging modes such as Phase Contrast and Differential Interference Contrast, it is still useful for some specific applications. For example, darkfield is used in the diagnosis of certain diseases caused by spirochetes, as is seen in peripheral blood smears, and is also frequently used in the visualization of surface imperfections when using incident-light microscopy.

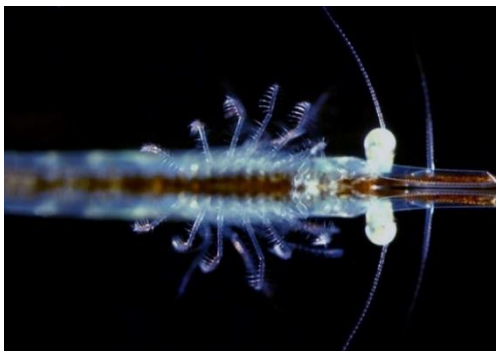

Figure 3 - Mysis zooplankton as seen in darkfield.

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. The iris diaphragm should be set to the fully open position whenever a darkfield stop is used.

The condenser on many microscopes includes a filter carrier, which is 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 simplest 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 body of the condenser, in order to get it close enough to the iris diaphragm for effective darkfield, and subsequently it is 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 the stop is too small, it will not exclude all of the unscattered light and the specimen will not appear against a dark field. If the stop is 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, which is 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 a necessity.

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 more convenient to use than a wet darkfield 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 darkfield 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 Oblique Illumination

What is OBLIQUE ILLIMUNATION?

Oblique Illumination uses off-center lighting to illuminate the specimen under observation. With the specimen obliquely lit (i.e., lit from the side), shadows appear in the specimen which enhance the 3D aspects of the image, often allowing otherwise invisible details to be seen. From a technical standpoint, this occurs because one of the sidebands of diffracted light tends to fall outside the acceptance angle of the objective, and is therefore not collected, whereas collection of the other sideband remains. This sideband suppression increases the contrast that results in the final image from the interference between the zero-order light and the various diffraction orders in the collected sideband.

How can I ADD OBLIQUE ILLUMINATION to my microscope?

Oblique Illumination can be readily obtained by placing a disk with an off-center aperture in the filter holder of the substage condenser of a typical microscope, thus restricting the light reaching the specimen. Experiment with the size, shape, and physical placement of the oblique aperture until you get the results you want.

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, with it becoming broadly used by 1942. Zernike was ultimately awarded the Nobel Prize in Physics for his contribution to microscopy in 1953.

Phase Contrast is a technique of optical staining which uses phase differences in the background illumination and in the waves passing through the specimen to add visible contrast to specimens which otherwise lack sufficient inherent optical contrast, or which are not or cannot be stained, for whatever reason. Phase Contrast microscopy is most often used for studying live, unstained specimens, since live specimens cannot typically be stained without affecting their behavior, vitality, or even killing the specimens outright. However, while Phase Contrast is most often used to observe living specimens, it can also be useful for observing non-living specimens which for whatever reason lack sufficient optical contrast for direct brightfield observation.

For these types of specimens, Phase Contrast microscopy can provide significantly increased contrast as compared to conventional brightfield microscopy. As an example, Figure 4 shows the same cells imaged via conventional brightfield (left) and Phase Contrast (right).

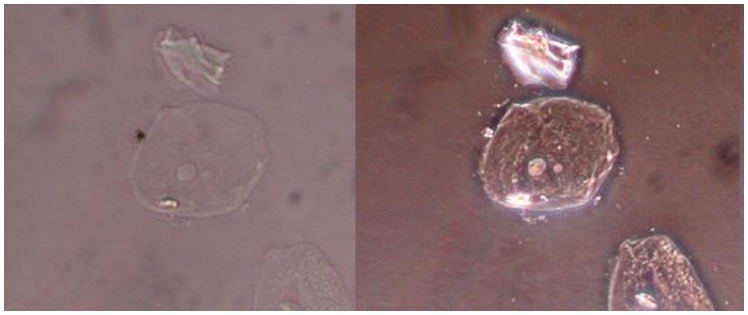

Figure 4 - Brightfield (left) and Phase Contrast (right).

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 slightly 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 not being 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 neutral-density properties of 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-wave 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 methods.

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. 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. Specifically, 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 a Zernike-style condenser, and this should apply to most phase contrast microscopes.

Start by replacing the condenser and objectives on your microscope with your new Phase Contrast 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 set up the microscope in the traditional way for Köhler illumination (if applicable), 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 phase contrast using the 10X objective. Replace the right eyepiece with your phase-centering telescope and peek into the telescope. Assuming the telescope is set for proper focus (and it likely will not be), 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-centering telescope until the bright and dark rings are sharply focused.

The position of the dark ring is fixed within the visual field, whereas the position of the bright ring can be adjusted via the two orthogonal centering controls on 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 set up 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, be sure to place the green filter into the filter receptacle below the condenser (or above the light exit, whichever is appropriate).

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 selector dial with the magnification of the objectives and adjust the centering of the bright ring within the dark ring as described above. Once all the Phase Contrast annuli have been properly set up 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 in the objective. Ideally, only the zeroth-order light (i.e., 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 to 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 in the objective and therefore not experience the desired degree of 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 or POLARIZING 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, in polarized microscopy. During setup, and with no specimen on the stage, the operator first 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 or POLARIZING FILTER?

In general terms, a *polarizer* is an optical *polarizing 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 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 the 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 blocks 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.

Things 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 in the above case is present because the two orthogonal waves did not spend the same of time within the crystal material, due to the different refractive indices they each experienced. Specifically, the emerging wave front aligned with the slow axis will be retarded, as compared to the emerging wave front aligned with the fast axis, due to the effective increase in path length it experienced while propagating through the slow axis of the crystal.

The specific amount of retardation will depend on the thickness of the crystal 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. Information describing the degree of retardation will be 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.

What is a QUARTER-WAVE RETARDATION PLATE?

A *quarter-wave retardation plate* is a wave plate 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.

What is a FULL-WAVE RETARDATION PLATE?

A *full-wave retardation plate* (also known as a first-order plate or a lambda plate) is a wave plate that has been 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 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 somewhat magenta to the observer. This magenta tint 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 POLARIZING CAPABILITY to my microscope?

To add simple polarizing capabilities to just about any compound 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 objective, typically below the viewing head. The actual filters which are used are not too critical here; simple linearly polarized films are usually 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 perpendicularly oriented to each other.

To setup for polarized observations, remove the viewing head and 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 sub-stage 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 like a standard brightfield microscope (albeit with a 50% reduction in brightness). With no slide on the stage, rotate the bottom polarizer 90° until no light (or minimal light) can be seen through the eyepieces. This will be the orientation of maximum extinction. Now place the specimen slide onto 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.

What is a BEREK COMPENSATOR?

A Berek compensator is an optical device that can quantitatively determine the degree of retardation (as measured in wavelengths) of various birefringent materials. Assuming that the thickness of the birefringent material is known or can be measured, a Berek compensator can then be used to determine the birefringence value of the material. The Berek compensator works by measuring the angle of rotation of a calcite or magnesium fluoride optical plate cut perpendicular to the optical axis of the microscope.

Questions about Rheinberg Illumination

What is RHEINBERG ILLUMINATION?

Rheinberg Illumination, which was derived from darkfield illumination, is another optical-staining 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 zero-order background light that is not scattered by the specimen) is tinted one color, while the higher-order light that is scattered by the specimen is tinted a second, contrasting color. Rheinberg can be easily accomplished by placing a simple bi-color filter (similar to a darkfield stop, but with the center dot being one color, and the outer annulus being a contrasting color) near the aperture diaphragm within the substage condenser of the microscope. Using this simple setup, one can obtain stunning images wherein the specimen appears one color against a background of a contrasting second color (see Figure 5).

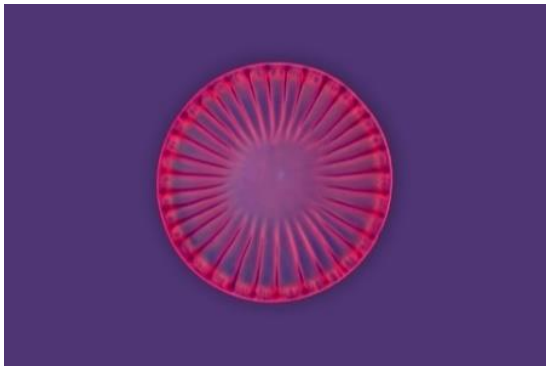

Figure 5 – A diatom under Rheinberg illumination.

How can I ADD RHEINBERG ILLUMINATION CAPABILITY 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 in the Rheinberg filter determines the image background color, while the outer 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 simplest 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 body of the condenser, in order to get it close enough to the iris diaphragm for effective Rheinberg, and subsequently it is 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 diffracted or 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 Relief Phase Contrast Microscopy

What is RELIEF PHASE CONTRAST MICROSCOPY?

Relief Phase Contrast microscopy is a combination of conventional Phase Contrast microscopy with oblique illumination. In conventional Phase Contrast microscopy, the light in the condenser passes through a ring-like aperture (i.e., the phase annulus) to produce a thin, hollow cone of light. The system

is carefully aligned such that this thin cone of light lands within the phase ring in the rear focal plane of the objective lens when there is no specimen in the optical path. In Relief Phase Contrast, rather than using the full ring-like aperture, only a partial segment of this ring is open in the condenser, thereby producing offset, oblique lighting from a partial, hollow cone of light. Additionally, the phase ring in Relief Phase Contrast objectives are a bit different than in conventional phase contrast objectives, to produce images with reduced halos and enhanced shadowing. Olympus offered Relief Phase Contrast on their CK series of inverted microscopes.

Questions about Differential Interference Microscopy

What is NOMARSKI INTERFERENCE CONTRAST or DIFFERENTIAL INTERFERENCE CONTRAST?

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 (i.e., 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.

The two orthogonally polarized lighting 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 from 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 directly visible to the human eye (which is why the two brightfield images carried in the two sheared components would appear identical if you neglect the shear), when the two components are 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 points within the specimen).

Questions about Basic Optical Principles

What is an ACHROMATIC LENS or ACHROMATIC DOUBLET?

An *achromatic doublet* is a composite optical lens made by bonding a concave lens element made from one optical material to a convex lens element made from a different optical material, with the resulting composite lens exhibiting much less chromatic aberration (see Figure 9) and spherical aberration (see Figure 16) than is present in the individual lens elements themselves. The materials from which these lens elements are made are carefully chosen such that the intrinsic chromatic dispersion in the two lens elements differ significantly from each other. A typical convex achromatic doublet contains an optically strong convex element made of crown glass (which has relatively low chromatic dispersion), bonded with a relatively weaker concave element made of flint glass (which has relatively high chromatic dispersion). By carefully choosing the relationship between the focal lengths of the convex and concave elements, the resulting composite lens will function as a convex lens, albeit of reduced strength, which has greatly reduced chromatic and spherical aberration. Since the concave element has more dispersion than the

convex element, a relatively weaker concave element can greatly counteract the effects of chromatic dispersion in the convex element, while not completely negating the optical effects of the convex lens. Additionally, the complementary nature of the convex and concave elements provides cancellation of spherical aberration (see Figure 6). An achromatic doublet is typically constructed so as to bring the red and blue wavelengths of light (i.e., the wavelengths at both ends of the visible spectrum) to a common focal point.

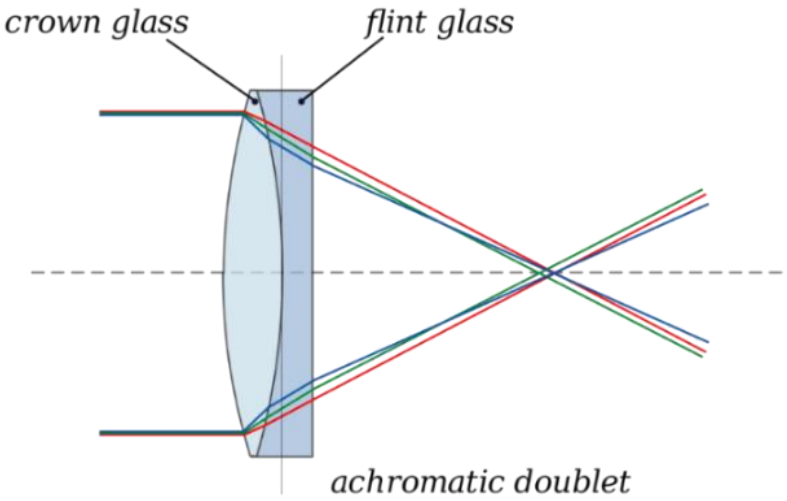

Figure 6 - Achromatic doublet.

The history of the achromatic doublet is very interesting. The performance of early telescopes and compound microscopes was very poor, due to the inherent chromatic aberration of the simple lenses from which they were made. Individually, the performance of a simple lens is poor, but when strung together to make more and more complex optics, the resulting performance quickly degrades from poor to atrocious. In the early 18th century, Sir Isaac Newton (you may have heard of him) decided to look into the issue of dispersion and the resulting chromatic aberration it caused. Due to an unfortunate flaw in experimental technique, Newton erroneously concluded that the problem of chromatic aberration with lenses and prisms was something that could neither be eliminated nor compensated. In deference to this erroneous conclusion, Newton went on to make the world's first achromatic telescope, a reflector scope using mirrors rather than lenses (since primary surface mirrors do not suffer the effects of dispersion that plagues glass lenses). Now, despite this mistake, Mr. Newton was no intellectual slouch. He was in fact, a genius, albeit with a few flaws. This is the same guy who, upon finding that the existing mathematics of his day were incapable of describing the orbits of planets and other astronomical bodies he wished to investigate, developed calculus in order to proceed with his work. And oh yeah, he did this calculus thing when he was 23 years of age! It is because of this mistake made by this undisputed genius that the story of the achromatic doublet is so delicious.

There was a British barrister and amateur optician named Chester Moore Hall who apparently did not get Mr. Newton's memo that the problem of chromatic aberration was intractable and would never be solved. In 1729 or 1733 (accounts vary), Hall hit upon the idea of using a convex and concave element, made from glasses which exhibit differing degrees of dispersion, to make a chrome-free composite. Wishing to keep his idea secret, Mr. Hall contracted with Edward Scarlett to have a crown glass element ground, and he contracted with James Mann to have a flint glass element ground. This way, neither lensmaker would know of the existence of the other critical piece, and his secret would remain safe. Well, as it turns out, Mr. Scarlett and Mr. Mann were both quite busy, so they each in turn sub-contracted Mr. Hall's lenses to another lensmaker, by the name of George Bass. Bass noticed that the two lens elements, which were

for the same customer, fitted together perfectly. While investigating this curious coincidence, he noticed the achromatic properties of the combination of the two lenses.

Having thus defied Sir Isaac Newton, Chester Moore Hall went on construct the world's first achromatic *refracting* telescope, using the very lenses of his own creation that Newton had said could never exist. Not too bad for an amateur optician! Yet for whatever reason, his telescopes never became widely known and his achromatic lenses utterly failed to change the world. In fact, Hall never seemed to promote or commercialize his brainchild much further beyond this, instead giving it away to an instrument maker named John Bird. Bird in turn gave the invention to another instrument maker, James Ayscough, who ultimately went bankrupt, leaving him unable to capitalize on Hall's invention.

This is where George Bass, the original maker of the two lens elements for Hall, comes back into the picture. In the late 1750s, Mr. Bass, who had been selling achromatic lenses based on Hall's design since as early as 1733, mentioned Hall's lenses to another amateur optician, John Dollond. Dollond, who had been working unsuccessfully towards an achromatic lens himself, immediately understood the importance of Hall's discovery, and based on the description provided by Bass, was able to re-create Hall's work and to produce his own achromatic doublets. And by God, if Chester Moore Hall wouldn't do it, John Dollond would: John Dollond filed for and was awarded a patent for his achromatic doublet in 1758. Dolond's patent filing described not only the achromatic properties of the achromatic doublet, but also the cancellation of spherical aberration inherent in the usage of complementary lens types. Ushering in the era of modern optics, the achromatic doublet as created, commercialized, and patented by John Dollond did indeed go on to change the world. As an aside, it should be noted here that John Dollond was not some smarmy character who stole the work of another man for himself. Rather, he put a huge amount of work into the development of the achromatic doublet. In the same year he filed his patent application for the achromatic doublet, he also published a scientific paper pointing out the flaws in Isaac Newton's experimental methods that had led him to his erroneous conclusions regarding chromatic dispersion.

John Dollond successfully commercialized, and made a great deal of money from, his achromatic doublets. However, likely out of respect for Hall's previous work, John Dollond did not actively enforce his patent during his lifetime, allowing other lensmakers to continue to make Hall's doublets. But upon his death in 1761, his son Peter Dollond (who inherited his father's patent) began to actively enforce the patent, suing an instrument maker named James Champness for patent infringement. The word had of course widely spread among the makers of lenses and telescopes that Hall, rather than Dollond, was the original inventor of the achromatic doublet. Armed with this knowledge, Mr. Champness argued in court that since Hall, the original inventor, had declined to seek a patent for his invention, the achromatic doublet should be considered to be in the public domain and therefore be free for all to use.

The court affirmed Hall as the original inventor of the achromatic doublet by acknowledging his prior art, yet went on to uphold Dollond's patent, only because, as Lord Mansfield of the court stated, "It was not the person who locked up his invention in his scrutoire that ought to profit by a patent for such an invention, but he who brought it forth for the benefit of the public." In other words, the court was upholding the patent since John Dollond had actively exploited and commercialized the invention, not only to the benefit of himself, but to the great benefit of the public in general, and since Chester Moore Hall had utterly failed to do so. It was based on this reasoning that the court ruled in Dollond v. Champness that the patent rightfully belonged to John Dollond, and that James Champness had infringed on Dollond's patent.

Due to this infringement, damages in the amount of £250 were levied against James Champness. This was a very large amount of money for the time. The legal costs to Champness, and to the various other lensmakers who had contributed to his defense (including George Bass himself), were very high, thanks to the bitter, protracted litigation that had just played out. In the end, these costs, coupled with the inability of these lensmakers to continue making achromatic doublets, put a few of these lensmakers out

of business. Meanwhile, the victorious and wealthy Peter Dollond carried on in his father's footsteps, eventually developing the apochromatic lens.

What is an APLANATIC LENS?

An *aplanatic* lens is an optical lens that is free of both spherical and coma aberrations. A single-element aplanatic lens is an aspheric lens whose surfaces are surfaces of resolution of a cartesian oval. Aplanatic lenses can also be made by joining two or three individual spherical lens elements.

What is an APOCHROMATIC LENS?

An *apochromatic lens* utilizes three simple lens elements, bonded together in a manner similar to the two elements that comprise an achromatic doublet, to provide correction for both chromatic aberration and spherical aberration better than that which can be achieved with an achromatic doublet. Whereas an achromatic doublet is typically constructed so as to bring red and blue to a common focal point (see Figure 6), an apochromatic lens brings red, blue, and green to a common focus point (see Figure 7). The apochromatic lens was invented by Peter Dollond.

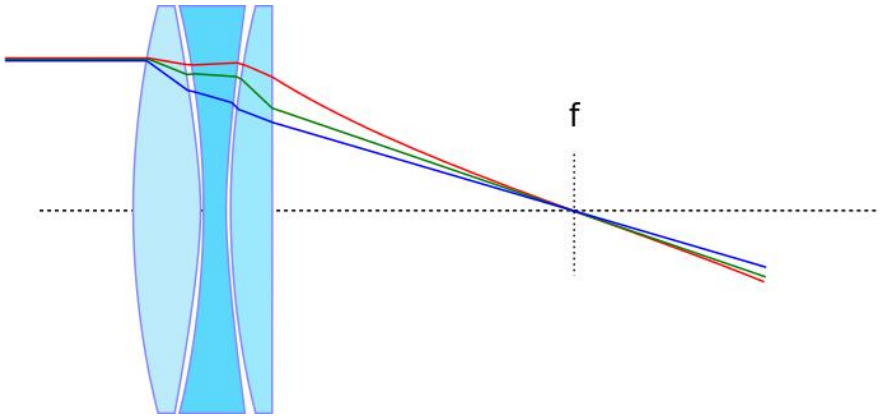

Figure 7 - Apochromatic lens.

What is an ASPHERIC LENS?

An *aspheric* lens is an optical lens whose surface profiles are not portions of a sphere or cylinder. The complex profile of an aspheric lens can reduce or eliminate spherical aberration and can also reduce other optical aberrations such as astigmatism, as compared to simple lenses.

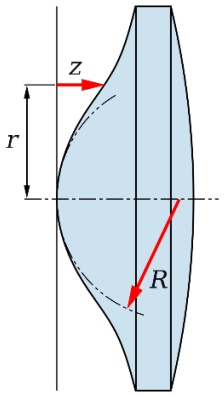

Figure 8 - Aspheric lens.

What are COATED OPTICS?

Light traveling through the air gets partially reflected whenever it strikes a glass window. Everybody intuitively understands this, since if some portion of the light did not get reflected, you would not be able to see your reflection in the window. At the same time, if some portion did not pass through the glass,

you could not see through the window. Intuitive or not, this is a curious phenomenon when you really think about it, since air and glass are both optically transparent. So why should this happen? This happens because air and glass, although both optically transparent, exhibit different refractive indices, and anytime a light wave encounters the interface between two media of different refractive indices (such as air and glass), the wave will be partially reflected by this discontinuity in the refractive index. This phenomenon was discovered centuries ago and is an inherent aspect of physics that cannot be changed.

But what would happen if a transparent coating were to be applied to the surface of the glass, with a refractive index midway between that of air and glass? Well, the light waves would then see two distinct discontinuities in the refractive indices of the three optical media (i.e., the air, the transparent coating, and the glass), and two reflections would therefore result. The first of these discontinuities would occur when the light encounters the interface between air and the transparent coating, and the second would occur when the light encounters the interface between the transparent coating and the glass. So now we have gone from a single reflection to two, so that's even worse, right? Absolutely not. If we make the thickness of the transparent coating exactly one quarter of the wavelength of the incoming light, and if we select a coating with the proper refractive index such that the two resulting reflections are of equal amplitude, then the phase and amplitude of the two reflections will be such that they will destructively interfere and completely cancel, as if neither reflection had ever occurred in the first place! This is the principle of anti-reflection (AR) coatings.

The keen reader may have already realized that this destructive interference will only completely cancel the two reflected waves at a single wavelength of light, as determined by the thickness of the transparent coating, leaving at least partial reflections for all other wavelengths. By applying multiple coatings of the correct thicknesses and refractive indices, a broad-band AR coating can be made that greatly reduces optical reflections (thereby increasing optical transmission) of the optical elements over the entire visible spectrum. This is referred to as multi-coating.

What is CHROMATIC ABERRATION?

There is a phenomenon in optics known as *dispersion* (see description below), which causes *chromatic aberration* in optical systems. Since all materials exhibit some degree of dispersion, this means that no single-element optical lens can bring all wavelengths of light to a common focus point (and therefore, no single-element lens can bring all colors of an image to a common focal plane), as shown in Figure 9. In layman's terms, a given lens will have a different optical strength (i.e., focal length) for different colors of light. The higher the dispersion of the medium, the worse this disparity will be. The practical upshot to all of this is that an image made by any physical lens, using white light as s source, will have some degree of color fringing (due to dispersion) as only one wavelength of light can ever be perfectly focused at any time. This color fringing is known as chromatic aberration.

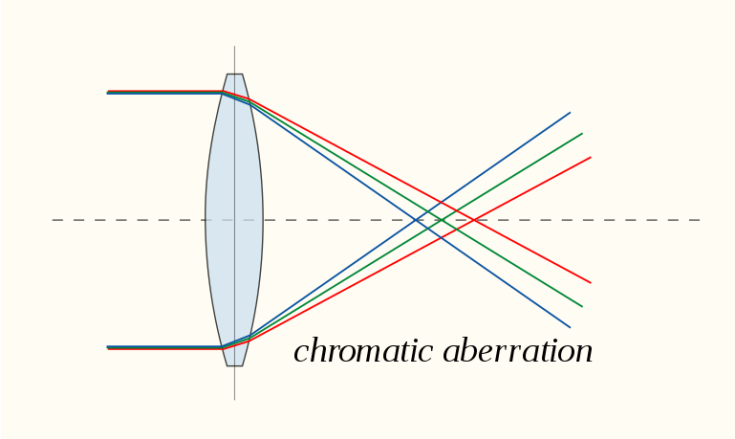

Figure 9 - Chromatic aberration due to optical dispersion.

What is DIFFRACTION and what are its effects?

Diffraction of light is a phenomenon that occurs whenever light waves encounter a physical obstacle or aperture in their path of propagation. Specifically, diffraction refers to the bending of the waves around an object or around the edges of an aperture, into the area where a shadow would otherwise be expected (as predicted by simple geometric ray tracing). This means that diffraction actually causes light rays to bend as they pass an obstructing object or aperture, due to the interaction of the waves with the obstacle or aperture.

To understand diffraction without a lot of math or equations, and in as intuitive a way as possible, let's travel to a fairytale land where diffraction does not exist, and look at how light interacts with an aperture. This is a nice, simple place, where geometric ray tracing is all anybody ever needs to understand optics. At first glance, things look pretty normal here. Without the complicating effects of diffraction, let's examine what happens when light rays from a lightbulb pass through an aperture onto a white wall. If a conventional light bulb is set up to illuminate the wall, and a large piece of cardboard with a hole in the middle is placed between the lightbulb and wall, only the light passing through the hole in the cardboard will make it to the wall. This of course will produce a visible spot of light on the wall. The edges of this spot will be somewhat soft and fuzzy, since the light coming from the lightbulb does not come from a single-point source, but instead comes from multiple points (because the frosted glass envelope of the lightbulb scatters the light rays to make it into a more diffuse light source). This scattering is generally desirable and is why most lightbulbs have a frosted glass envelope in the first place. The shadows cast by a diffuse light source tend to be less stark and jarring, and the diffused bulb is much easier on the eyes.

But stark and jarring be damned. Let's take the frosted bulb out and replace it with one with a clear glass envelope. Now that's better. The edges of the light spot on the wall are now much sharper. Nice! If we could only find a light bulb with a pure single-point emitter, we would see a crisp spot with perfectly sharp edges on the wall before us. Unfortunately, such bulbs do not exist in the real world. To better simulate a single-point source, let's move the bulb further away from the cardboard and turn the intensity up to compensate. Now the spot on the wall looks nice and crisp. This matches our intuitive understanding of how things work in the regular world (where diffraction is a thing) yet is nicely described using only the techniques of simple geometric ray tracing.

So now let's leave fairytale land, where geometric ray tracing is valid, and come back to the real world, where diffraction raises its ugly head. Rather than light traveling in perfectly straight paths from the source to the destination, we will now have to account for the slight bending that arises from real-world diffraction. "Big deal", you say. The light spot looks exactly the same as before when we were in fairytale land. The light rays passing through the hole in the cardboard cast a nice crisp shadow on the wall, just like we saw in fairytale land. What's the difference? To be fair, it's hard to argue with this since the spot on the wall does look deceptively crisp to the naked eye.

So far, what we have discovered is that at the scale of a hole punched into a piece of cardboard, there is really no visible difference, with or without diffraction. But let's zoom in to the world of the microscopic and take a look at what happens there. Let's keep our good, single-point approximation of a light source. Now let's make the aperture *much* smaller. Let's make it microscopic, say something on the order of the wavelength of visible light. And to keep the same relative scale, let's move the aperture closer to the wall. All of this of course means that the illuminated spot on the wall will now be microscopic as well. So, for us to see it properly, we might as well go all the way and become microscopic ourselves.

Things look very different at this scale. The spot is no longer distinct at all. The edges are now very soft and blurry. In fact, annoyingly blurry. We shake our head and blink our eyes a few times, but the spot remains blurry. Also, there are concentric rings around the central spot, and they are blurry as well. What's going on here? At this scale, the light acts in ways that violate our intuitive understanding of the world, where discrete objects are expected to appear distinct. Not coincidentally, the intuitive understanding that drives this expectation was gained *before* we became microscopic. Clearly, simple

geometric ray tracing no longer applies in this world, and to expect otherwise would be a mistake. The wave nature of light is the bully on the block here.

So, what gives? Why is the spot so soft? The answer gets a little complicated. As light passes through the microscopic aperture, the aperture itself, rather than the light source, acts as the source of illumination (i.e., the aperture becomes a secondary illumination source for the wall). So, let's look at the specific point on the wall in the exact center of the blurry central disk, where the light intensity is greatest. Rays of light passing through every single point in the aperture strike this point on the wall and combine to produce the brightness seen in the center. However, the light does not combine through simple addition. No, that would be too easy. Instead, since light propagates as sinusoidal waves, the light will therefore combine by the addition of these sine waves (see Figure 10).

Since the resulting light at the center of the central disk is the sum of the countless *sinusoidal* contributions coming from each point in the illuminating aperture and striking the center point, they add by vector addition, rather than simple addition. Vector addition accounts for the relative phasing of the wave components, and not just the peak amplitudes of the waves. Any time two sinusoidal waves of the same wavelength are added, the result will be a sinusoidal wave of the same wavelength whose amplitude is described by the vector sum of the two contributing waves. As an example, if two waves of equal amplitude and exactly the same relative phasing strike a point, the resulting wave at that point will be twice the amplitude of the constituent components, due to constructive interference of the two waves (see Figure 10, left). Similarly, if two waves of equal amplitude and opposite phasing strike a point, the resulting wave at that point will be zero amplitude, due to destructive interference of the two waves (see Figure 10, right). For all intermediate phase relationships, the amplitude of the resulting waves can be found using basic trigonometry, but we don't need to go through the details of that here.

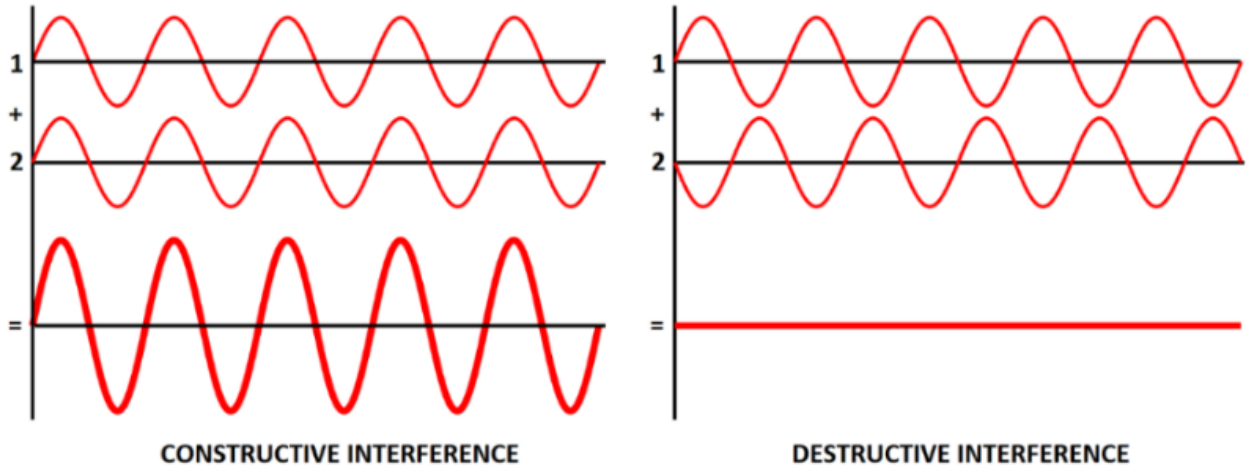

Figure 10 – Constructive interference (left) and destructive interference (right) of waves.

So, what determines the phasing of the various wave contributions striking a given point? The answer is the distances the wave contributions must travel to reach that point. Let's look at the wave contribution from the center point of the illuminating aperture, at the center point of the central disk on the wall. The wave travels some distance to reach this point, and therefore arrives with some arbitrary phase (we will call this the reference phase). This is the shortest path length from this point in the illuminating aperture to any point on the wall (i.e., all paths from any other points in the illuminating aperture to this point on the wall will be longer). So, this means that any wave contribution from any other point in the illuminating aperture will be delayed, with respect to the reference phase, because of the longer path length.

Let's pick another point in the illuminating aperture, other than the center point. As just stated, the wave contribution arriving from this second illumination point will be delayed, with respect to the reference wave, since it must travel a longer path length to reach the center point on the wall. If the distance

between this second point in the aperture and the center point of the aperture is viewed as a radius, the contributions of all the points in the circle around the center point (which includes the second point) will arrive at the center point on the wall with the same relative phase, due to the circular symmetry of the setup. So, the brightness of the center point of the central disk on the wall arises from the vector contributions of wave components from the center point of the aperture and from the countless concentric rings of increasing radius around this center point (from radius zero at the center up to the radius of the aperture). Whew!

So, now that we know how the intensity at the center point of the central disk on the wall is determined, let's pick another spot on the wall to look at. The wave arising at this second point, as a result of the vector sum of the components contributed by the circles of increasing radius of the illuminating aperture (as described above) will have a different amplitude (i.e., intensity) than that of the center point of the central disk, as a result of the constructive/deconstructive interference that results from the various path lengths of the constituent components. If you consider the distance from the center point of the central disk to this second point to be the radius of a circle, all the points on the circle of this radius, around the center point (including the second point), will be the same intensity on the wall, due to the circular symmetry of the setup.

So, the pattern of light on the wall can be determined by analyzing the intensity of a myriad of concentric circles of increasing radius on the wall (these rings are not to be confused with the concentric rings around the central spot in Figure 11), relative to the center point of the central disk. Recall that the intensity of each ring is the vector sum of the contributions from all points in the illuminating aperture, and because of the sinusoidal nature of light, these vector sums will go through alternating conditions of constructive interference (as seen in the center of the disk and in the center of the bright rings in Figure 11) and destructive interference (as seen in the center of the dark rings in Figure 11). This pattern of in-phase and out-of-phase interference repeats itself as you move further and further from the central point of the central disk. The resulting pattern is called an Airy pattern (the central disk is known as the Airy disk), both of which are named after George Biddell Airy (see Figure 11).

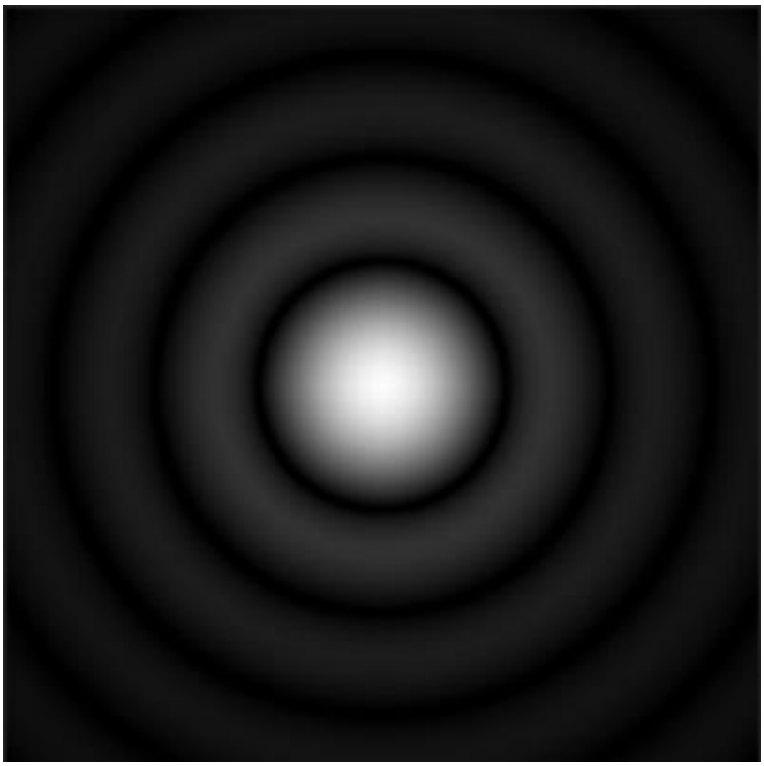

Figure 11 - A typical Airy pattern.

This example uses light passing through a simple aperture to show that the real-world, at the micro scale, behaves in stark contrast to the macro world as we intuitively understand it. Although we may not notice the subtle effects of diffraction in our macro world, we could never begin to miss them in the micro world. Such diffraction happens not only with the simple aperture explored here, but also with light passing through the aperture of the optical lenses used in light microscopes. You may now click your heels three times to return to the world of the huge. Although things look much crisper in our world than they do in the micro world, don't ever forget the lessons you learned in the micro world. Our world is truly not as it appears to be, and our intuitive understanding of the world is fundamentally flawed.

What is the DIFFRACTION LIMIT?

It is the diffraction, and the Airy patterns that diffraction creates, that limits the resolving power of any light microscope. As stated elsewhere, the wave nature of light is the bully on the block in the microscopical world, and the limitation on the resolving power of a light microscope caused by the diffraction of light is known as the *diffraction limit*. No matter how crisp an object really is, it will appear blurry due to the wave nature of the light used to make the observation. And no matter how small an object is, the image it creates will appear no smaller than the Airy disk. Because of this, when two microscopic objects are sufficiently close that their Airy disks significantly overlap, they will start to look like a single object, and at this point you have lost the ability to optically resolve that two separate objects are present (see Figure 12). They will become indistinguishable from a single object, forever safe from prying eyes on the other side of the diffraction limit.

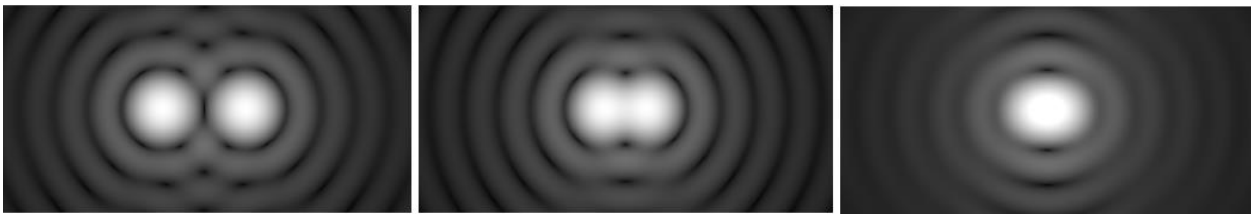

Figure 12 - Limits to resolving power due to Airy Disk overlap.

What is DISPERSION?

Dispersion is the effect wherein the refractive index of an optical medium (and hence the velocity of light traveling through the medium) varies as a function of the wavelength of the light. This means that the degree to which light is refracted by a lens or prism is not the same for all colors of light. This effect is commonly seen in the spread-out rainbow pattern created as white light is sent through a glass prism (think Pink Floyd, Dark Side of the Moon), or as seen in rainbows present in the daytime sky, in the presence of precipitation droplets (see Figure 13).

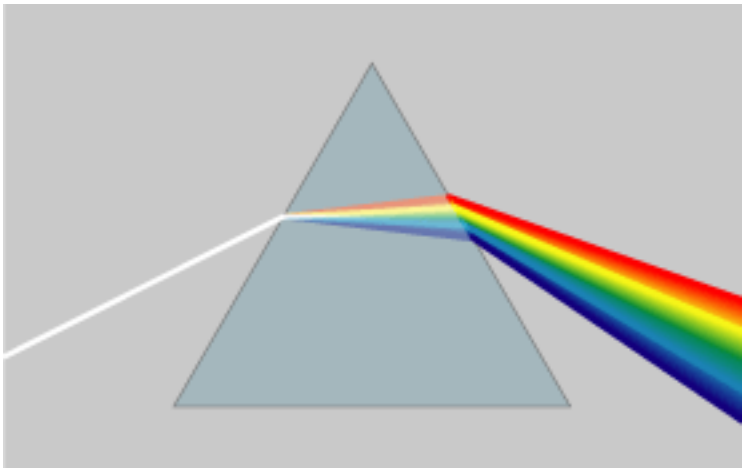

Figure 13 - A rainbow created by dispersion in a glass prism.

The higher the dispersion of the optical medium (i.e., the lower the dispersion number), the more pronounced or spread-out the rainbow effect will be. Although both of the optical phenomena described above are commonly regarded as beautiful by most lay people, dispersion has been a thorn in the side of optical designers, going all the way back to when man first began making glass lenses. So, an optical designer could perhaps be forgiven for thinking that dispersion is anything but beautiful.

What is REFRACTION and SNELL'S LAW OF REFRACTION?

The speed at which light propagates is a function of the density of the medium through which it is traveling. The denser the medium, the slower light travels. Light travels fastest in a vacuum (i.e., 300,000,000 meter per second), since a vacuum is the least dense optical medium there is, and travels slower in all other media, which are denser than a vacuum. The relationship between the velocity of light in a given medium, compared to the velocity in a vacuum, is given by its *refractive index* (also known as *index of refraction*), which is defined as below.

$$n = c / v$$

In the above equation, n is the refractive index of the optical medium, c is the speed of light in a vacuum, and v is the speed of light in the optical medium. The refractive index is a ratio of two velocities, and therefore has no units.

So, that's what the term *refractive index* means. But what exactly is refraction? Refraction is simply the change in the velocity of light and the accompanying change in the direction of wave propagation that occurs as light moves from one optical medium to another (see Figure 14). The change in velocity is closely coupled with the angle at which the wave propagates. The specific details will not be presented here, but any change in velocity of propagation induced by a change in refractive index also comes with a change in the direction of propagation. This change in direction is necessary such that the wave fronts at the interface of the two media will not become discontinuous. The only way to preserve the continuous boundary conditions as the velocity changes at the boundary is for the wave front to also bend at the boundary. So, refraction is both the bending and change in velocity that occurs as light travels through the boundary of two optical media of differing refractive indices.

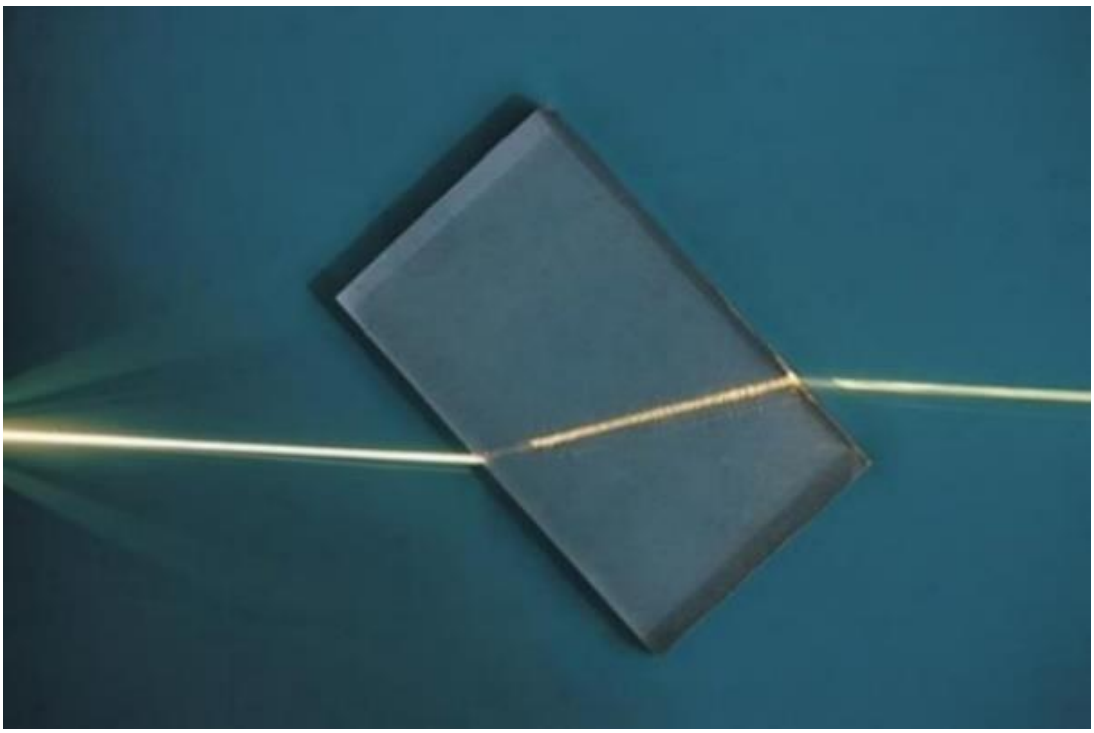

Figure 14 - Bending of light in a glass medium.

Refraction is what gives lenses and prisms their optical properties. Without refraction, a magnifying glass would not magnify, and a glass prism would not bend light. All lenses and prisms are inherently imperfect with regard to refraction. Specifically, the refractive indices of all optical media vary as a function of the wavelength of light. This very troublesome aspect of optics is known as *dispersion* and is described in detail elsewhere in this document.

By definition, the refractive index of a vacuum is unity (since in a vacuum, v = c). The refractive index of air is slightly higher, at 1.000293. The refractive index of optical glasses ranges between approximately 1.5 and 2.0, depending on the specific glass type.

Snell's Law of Refraction (named after Dutch astronomer Willebrord Snellius) describes the inter-relationship between velocities and the angles of propagation that occur whenever light travels through a boundary of two optical media of different refractive indices. To understand Snell's law, you must first understand the term *normal*, as it relates to a plane surface. If you have had many physics or mathematics classes, this term will be familiar to you. On the other hand, if you have not, it will likely seem weird to you. In geometry, a *normal* is a line, ray, or vector that is perpendicular to a given object. In other words, the normal to a plane is simply an imaginary line which is perpendicular to the plane surface (imagine a fence post, sticking up perpendicular to the ground). That's all there is to it. Don't let the terminology wrap you around the axle. The dotted line in Figure 15 is the *normal* to the interface of the two optical media.

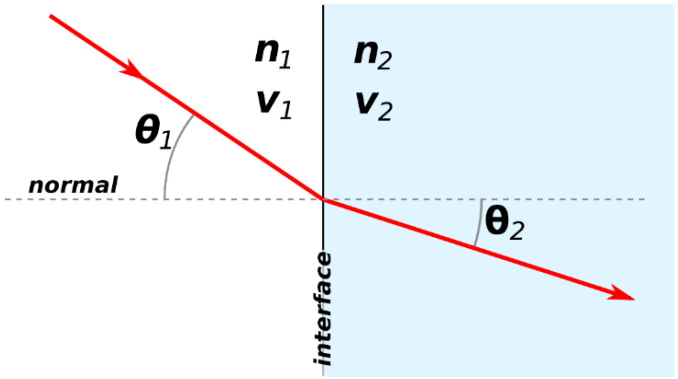

Figure 15 - Snell's Law of Refraction.

Snell's Law of Refraction states that for light traveling through any two optical media, the ratio of the sine of the angle of incidence on the second medium (i.e., the angle at which the light strikes the second media) and the sine of the angle of refraction within the second medium (i.e., the angle at which the light travels within the second medium) is equal to the ratio of the wave velocities in the two media, or to the inverse ratio of the refractive indices of the two media, as shown in the equations below. Note that the angles of incidence and refraction are both measured with respect to the surface normal of the interface of the two optical media (see Figure 15).

$$\sin(\Theta_2) / \sin(\Theta_1) = v_2 / v_1$$

$$\sin(\Theta_2) / \sin(\Theta_1) = n_1 / n_2$$

A slight bit of algebraic manipulation on the above equations yields the two more commonly used forms of Snell's Law, as listed below.

$$v_1 \sin(\Theta_2) = v_2 \sin(\Theta_1)$$

$$n_2 \sin(\Theta_2) = n_1 \sin(\Theta_1)$$

Θ_1 is the angle of incidence of light rays traveling in medium #1, onto the plane surface of medium #2. More specifically, this is the angle between the light rays impinging on the plane surface of medium #2 and the surface normal of medium #2 (at the boundary between the two optical media).

Θ_2 is the angle of refraction, which is simply the angle between the direction of travel of the light rays within medium #2, and the surface normal of the plane surface of medium #2 (at the boundary between the two optical media).

The terms v_1 and v_2 are the velocity of wave propagation within media #1 and media #2, respectively. The terms n_1 and n_2 are the refractive indices of media #1 and media #2, respectively.

So, Snell's Law shows that when light moves from an optical medium of one density to a second, denser medium (i.e., $n_2 > n_1$), the resulting velocity of propagation will be slower in the second medium than it was in the first ($v_2 < v_1$), and the light will bend toward the surface normal within the second medium. Conversely, per Snell's Law, the opposite is true when light moves from on optical medium of one density, to a second, less-dense medium (i.e., $n_2 < n_1$). In this case, the resulting velocity of propagation will be faster in the second medium than it was in the first ($v_2 > v_1$), and the light will bend away from the surface normal within the second medium.

In both of the above cases, Snell's Law ties the ratios of (v_2/v_1) and (n_1/n_2) to the ratio of the sines of the angle of incidence and the angle of refraction. If you know any three of the terms in Snell's Law, the fourth can easily be determined.

In the special case where the angle of incidence is zero (i.e., normal incidence), the angle of refraction will also be zero (i.e., normal refraction), and the light will travel straight through the two optical media, with velocity changing as predicted by the ratio of the refractive indices, but with no accompanying bending.

What is RESOLVING POWER?

Resolving power is the ability of an optical instrument to resolve two closely spaced objects as visually distinct structures. Given that the ability to visualize ever decreasing objects is limited by diffraction and the resulting Airy disk produced by the optics, a point is reached below which no matter how small an object is, it appears to the observer to be the size of the Airy disk produced by the optics. This means that as the spacing between two infinitesimal objects decreases, a point is reached wherein the Airy disks of the two closely spaced objects will begin to overlap. At some point, the overlap will become such that it will no longer be possible to visually resolve the two separate objects, and they will instead appear to the observer as a single object (see Figure 12). The equation for the Rayleigh Criterion that is most applicable to microscopy is shown below.

$$R = 0.61 \lambda / NA$$

In the equation above, R is resolution, λ is the wavelength of the illuminating light, and NA is the numerical aperture of the objective. Note that this equation is directly applicable when the NA of the objective matches the NA of the condenser.

What is SPHERICAL ABERRATION?

In optics, *spherical aberration* is a type of optical distortion found in optical systems that utilize spherically ground lenses (i.e., lenses whose shape is some portion of the surface of a sphere). Most optical lenses and mirrors are spherically ground since it is far easier (and therefore much less expensive) to grind glass in this shape. Light rays that strike a purely spherical surface off-center are refracted to a greater degree than those striking the center. This difference in refraction prevents light striking the entire surface of the spherical segment from converging to a common point (even when disregarding the chromatic effects caused by dispersion). The further the rays are from the optical axis of the lens (i.e., from the center point), the closer to the lens they will be focused. The left image of Figure 16 shows an ideal lens without spherical aberration, whereas the right image shows a spherical lens with spherical aberration. It should be noted that there is an aspherical (non-spherical) lens shape that will not exhibit spherical aberration (i.e., a segment of a hyperboloid), but such lenses are costly to manufacture, and are therefore not technically feasible for most applications. Rather than incur the expense of producing hyperboloid lenses, most manufacturers manage spherical aberration by restricting the surface area of spherical lenses that

are used, and by employing multiple optical elements in configurations such as achromatic doublets to cancel the effects of spherical aberration.

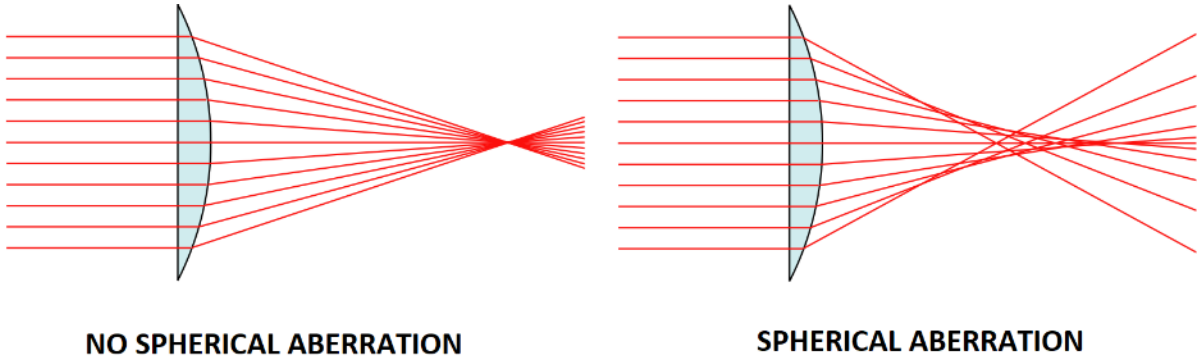

Figure 16 – No spherical aberration (left) and spherical aberration(right).

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Figure 2 - Comparison of images taken with brightfield and phase-contrast objective
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Figure 3 - Mysis zooplankton as seen in darkfield.
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Figure 4 - Brightfield (left) and Phase Contrast (right)
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Figure 5 – A diatom under Rheinberg illumination
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Figure 6 - Achromatic doublet
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Figure 7 - Apochromatic lens
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Figure 8 - Aspheric lens.
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Figure 9 - Chromatic aberration due to optical dispersion. Figure 9 - Chromatic aberration due to optical dispersion
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Figure 10 – Constructive interference (left) and destructive interference (right) of waves
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Figure 11 - A typical Airy pattern.
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Figure 12 - Limits to resolving power due to Airy Disk overlap.
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Figure 13 - A rainbow created by dispersion in a glass prism.
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Figure 14 - Bending of light in a glass medium.
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Figure 15 - Snell's Law of Refraction.
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Figure 16 – No spherical aberration (left) and spherical aberration.
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