

Media Cybernetics White Paper

Spherical Aberration

Brian Matsumoto, University of California, Santa Barbara

Introduction

Digital photomicrographers assume that lens aberrations are corrected by the microscope manufacturer and that their instruments are designed for best performance. However, this belief is unwarranted if the specimen is mounted in a medium inappropriate for the objective lens. It is easy to forget that the refractive medium between specimen and objective is critical for maintaining the microscope's optical correction. After all, it is a simple air or liquid medium that does not require the careful polishing, crafting, and alignment of an optician. Nonetheless, cavalier treatment in selecting this component can result in a crucial loss of optical performance. A failure to account for the medium's refractive index or thickness can result in a critical degradation of optical performance by inducing spherical aberration. It should be remembered that the majority of ground lenses cannot focus all the light to a single point. Light rays passing through the periphery of the lens will come to a different focal point than rays passing through its center. (Needham, 1958). This results in blurring of the subject's edges and the formation of an overall haze that reduces contrast. This is especially evident in fluorescence or darkfield illumination whose black background emphasizes the presence of out-of-focus light. (Figures 1A-B).

Considerations

For the majority of objective lenses, the optical engineer assumes that the specimen is embedded and infiltrated in a medium that has the refractive and dispersion index of glass and that 0.17 mm of this material lies between the specimen and the air interface (Delly, 1988). In addition, this interface must be optically flat, lying perpendicular to the microscope's optical axis. Failure to maintain these conditions reduces the lens correction for spherical aberration. Unfortunately, it is easier for designers to specify these parameters than it is for scientists to attain them. Many factors cause a loss of these conditions.

One is the necessity of using an arsenal of objective lenses for viewing a sample at all magnifications. Most microscopes have several objectives designed to work in an air interface (dry lenses) and immersion lenses that require that fluids displace the air between the specimen and the objective lens front element. The former are convenient lenses to use and may be rapidly interchanged while studying the subject. The latter is a high resolution lens that requires that the user take the care and the time to add liquid (usually an oil with defined optical properties) to the top of the coverglass. The last lens is designed with the assumption that the specimen is mounted in a homogenous medium whose optical properties are equivalent to glass. Thus, the mountant (Permout or Canadian Balsam) and the oil have the same index of refraction, 1.515 and a wider cone of light can be used to illuminate the specimen for higher resolution. With such a lens, a coverglass is unnecessary for optimum performance; a thin smear of cells on a glass slide can be viewed directly by simply adding a drop of oil and immersing the front element of the objective into this medium. A simple experiment with an air dried cell suspension can illustrate the importance of properly mounting the specimen. If one takes a stained slide of cells and views them with a series of objective lenses, one observes that the cells appear sharp with the 10X dry objective. As one increases the magnification by changing objective lenses, one notes that using the 20X lens, and then the 40X dry lenses, and a 100X oil immersion lens with oil, there is a progressive deterioration of image quality. However, the image is improved dramatically if a drop of oil is interposed between the oil immersion lens and the specimen. In contrast, if oil is applied to the "dry" lenses, a very poor image is obtained. In order to make the dry lenses perform optimally, a coverglass of the correct thickness needs to be applied to the specimen. When oil immersion is used with a dry lens it is necessary to cover the oil with a coverglass and under these conditions the dry lenses will show a much improved image- assuming, of course, the operator has cleaned the residual oil off the objective's front element. In other words, the coverglass' presence with an oil mountant allows the use of all the lenses of the microscope. But a failure to employ a coverglass will result in

degradation in the performance of all the lenses with the exception of the oil immersion. It should be noted that the deleterious effects of improper mounting is greater for lenses with higher numerical apertures where the microscopist is seeking to attain the highest level of resolution. Thus, the lack of a coverglass is not apparent when one uses objectives of 10X or less (Figures 1A-B).

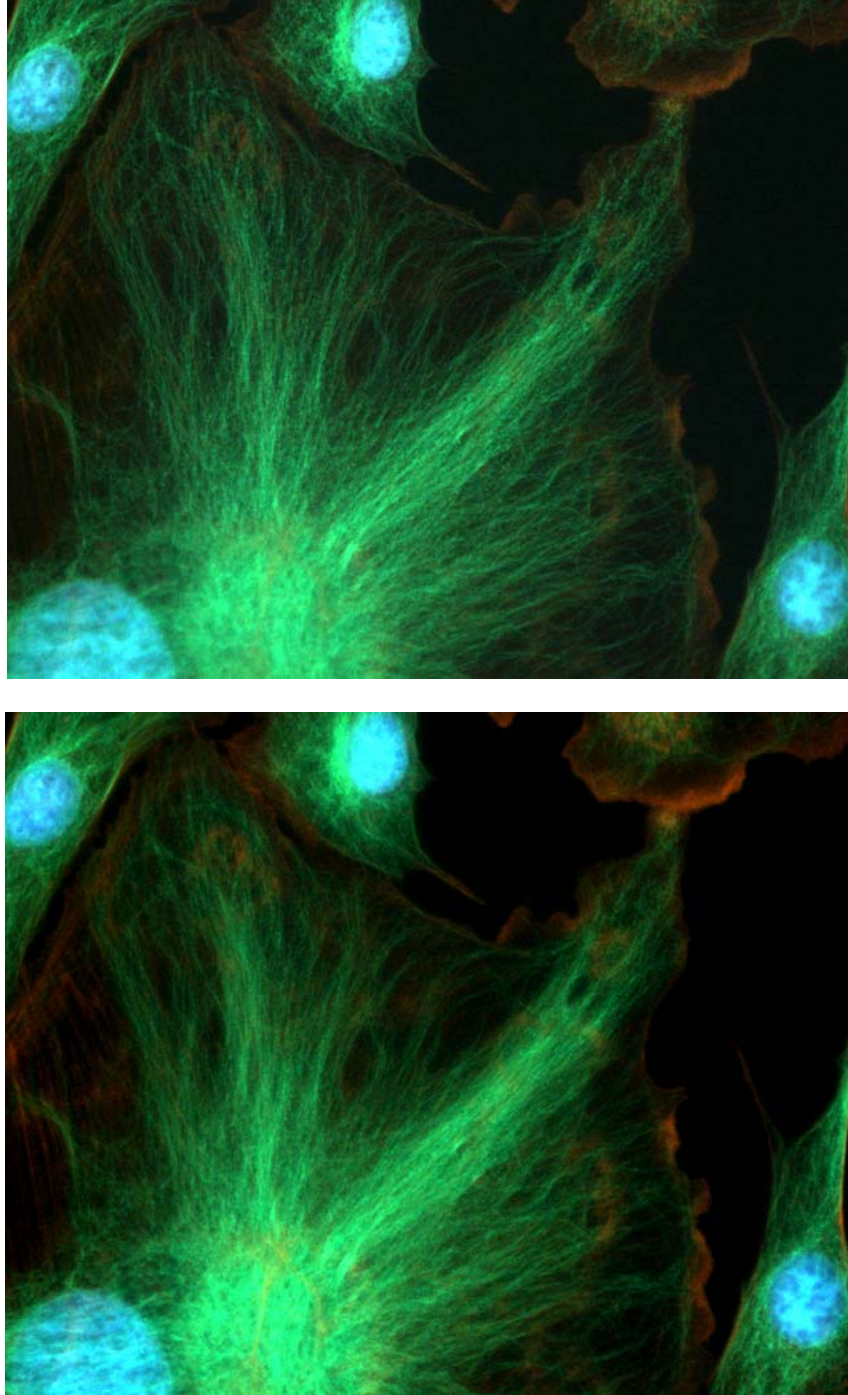


Fig 1A-B. The microtubules fluorescing green in this micrograph of an immunohistochemically stained cell are easily resolved when the coverglass correction of the objection is set properly (1A-Top). However, if the correction color is not adjusted properly (equivalent to a coverglass that is 0.02 mm too thick) there is a noticeable loss of image quality (1B-Bottom). Careful examination of regions where the microtubules converge to form thick bundles reveals a loss of contrast and a reduced capability to discern individual filaments. Micrograph taken with an Optronics Microfire camera mounted on an Olympus BX51 microscope equipped with a Planapo 40X N.A. .9 lens equipped with correction collar.

In a perfect world, a microscopist would be able to select the appropriate coverglass, mount his specimen, and be assured that the conditions for optimum imaging are achieved. Unfortunately, this ideal condition is not obtainable if one uses a dry lens. For example, if one has the properly-sized coverglass, the thickness of the specimen will limit the sharpness to a limited region along the optical axis. Even with an oil immersion lens this can be a problem if the specimen is mounted in an anti-fade mountant whose optical properties differ from glass. In confocal microscopy, it is easy to show that improperly mounted fluorescent specimens provide an optimum image in only a limited region along the optical axis of an oil immersion lens. Specimen sharpness and fluorescent intensity is lost when one attempts to image outside this optical “sweet” spot (Figure 2A-D).

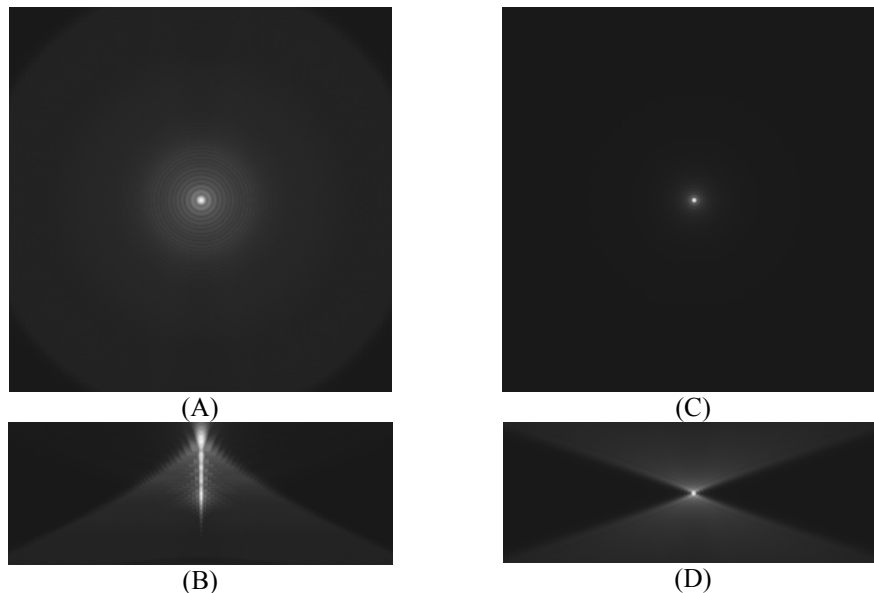


Figure 2A-D. In confocal microscopy, a technique that entices microscopists to view optically thick samples, there is a loss of correction for spherical aberration in the deeper regions of the specimens. In these optical planes, the microscope’s objective cannot delineate the origin of light emanating from a point. In 2A, an examination of the PSF from such depths reveals that significant levels of light are scattered from the point light source. Diffraction rings can be seen surrounding the point. By viewing an XZ projection, the amount of light lost from the originating source can be easily visualized. If one obtains the optical “sweet spot” in the imaging plane, the PSF should look like 2C and 2D. Images and projections courtesy of AutoQuant, Inc.

Unless the operator takes the effort to dehydrate the specimen, place it in an intermediate solvent and then mount it in Permount, only a small portion of the specimen’s three-dimensional architecture can be imaged with an oil immersion objective. To further complicate matters, coverglass thickness is categorized in a traditional fashion that can only be described as bizarre. These critical elements are sorted by manufacturers in a series of sizes, ranging from #0 to #3 whose thickness range within a given number is highly variable. To further complicate matters the ideal thickness range of 0.17 is not found in any of the whole numbers of the categorizing scheme. To obtain a box of coverglasses whose average thickness falls in the 0.17 mm range, one must order 1.5 thickness. In older books on microscopy, authors describe using a micrometer to measure and then sort coverglasses by thickness.

Solving the Problem of Spherical Aberration

The solution to this dilemma is dependent on the design of the objective lens. Many of the high numerical aperture dry lenses have a correction collar for compensating for deviations in coverglass thickness. A variation of mountant thickness of only 0.01 mm can cause a loss in optical performance (Figures 3A-B).

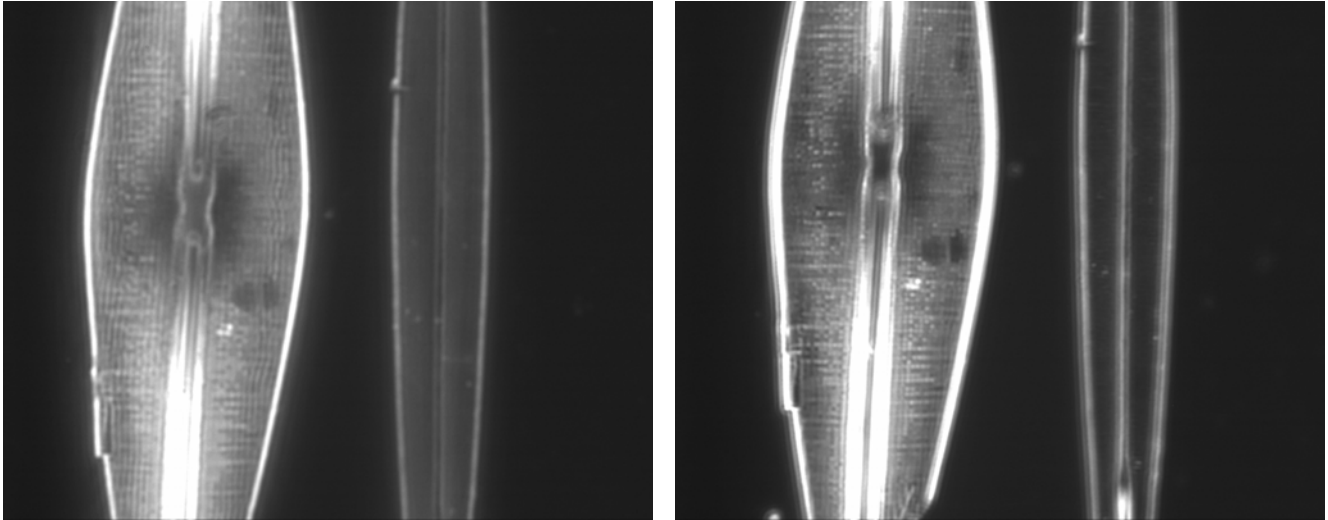


Figure 3A-B. In darkfield microscopy of diatoms, an increase in spherical aberration causes a loss of edge sharpness at the borders of the diatom. An examination of Figure 3A (Left) shows the borders of the silica shell and a comparison to Figure 3B (Right) which has a loss of correction for spherical aberration shows the loss of edge sharpness. Micrograph taken with an Optronics Microfire monochrome camera mounted on an Olympus BX51 microscope equipped with a Planapo 40X N.A. .9 lens equipped with correction color.

Although these collars may be graduated in hundredths of a millimeter, it is difficult to simply set the appropriate number on the objective—the microscopist is in the awkward position of not knowing the thickness of the intervening material between coverglass and specimen. Correcting for these variations is accomplished visually and requires some practice: as one changes the compensation by rotating the correction collar, there is a loss of focus and one must fiddle with the fine focus to evaluate the effects of the collar. With practice this can be accomplished in several seconds while looking through the eyepieces of the microscope. With electronic cameras or confocal microscopes, these adjustments should not, in the former, and cannot, in the latter, be performed on the monitor screen. In the former case, the monitor lacks the ability to display the fine details of the specimen while in the latter; the raster scanning of the instrument will not display the image fast enough for convenient operation.

An easier solution is to define both the optical and the mounting system. As mentioned earlier, this can be accomplished by mounting the specimen in Permount or Canadian Balsam, and by using oil immersion lenses. The refractive index and dispersion characteristic of this oil is matched to glass and the specimen is considered to be embedded in an optically homogenous environment. Thus variations in coverglass or mountant thickness are irrelevant for optical performance. Indeed, in the case of dried smear preparations, cytologists will forgo a coverglass and simply apply oil to the slide and the oil immersion lens. In the case of biological samples, the logical solution is to mount the specimen in an aqueous medium and then use a water immersion lens. With a water immersion lens—where the specimen is mounted in water and the objective front element immersed in distilled water, one can retain optimum imaging along the optical axis of the specimen.

References

Delly, G. (1988) *Photography Through the Microscope*. Publisher Eastman Kodak, New York, NY.

Needham, G.H. (1958) *Practical Use of the Microscope*. Publisher Charles C. Thomas, Springfield, Illinois.

Media Cybernetics, Inc.
8484 Georgia Avenue
Silver Spring, MD 20910 USA
Phone: +1-301-495-3305
Fax: +1-301-495-5964

Email: info@mediacy.com
Web: www.mediacy.com

All products and services mentioned are trademarks of their respective owners.
© 2003 Media Cybernetics, Inc.

