Chromatic Aberration in Digital Photomicrographs from Microscopes Requiring Compensating Eyepieces

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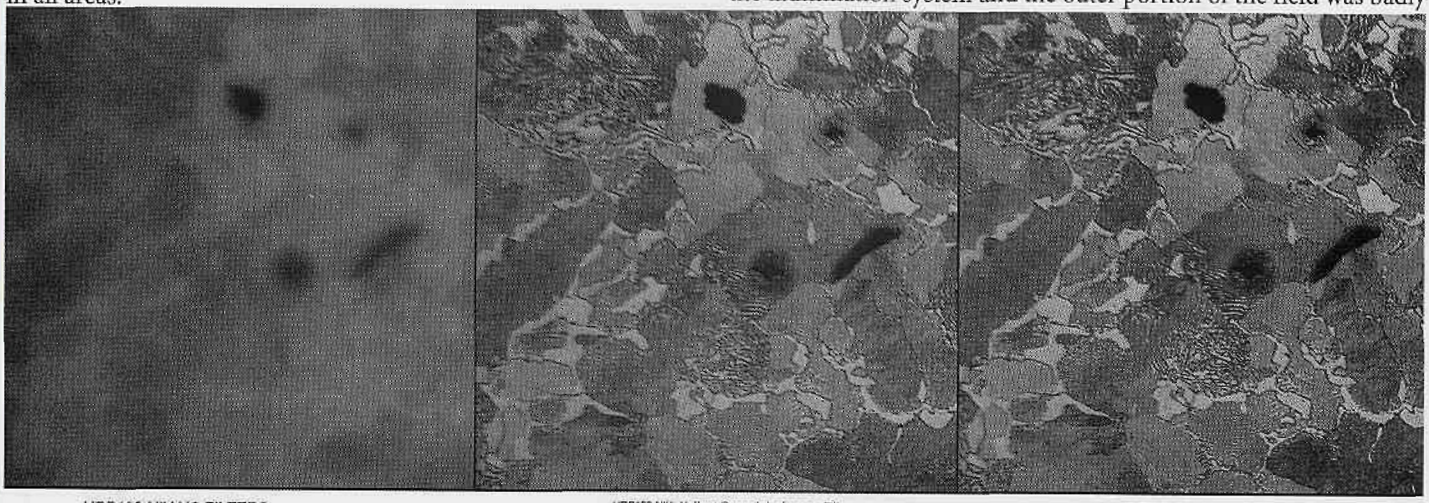
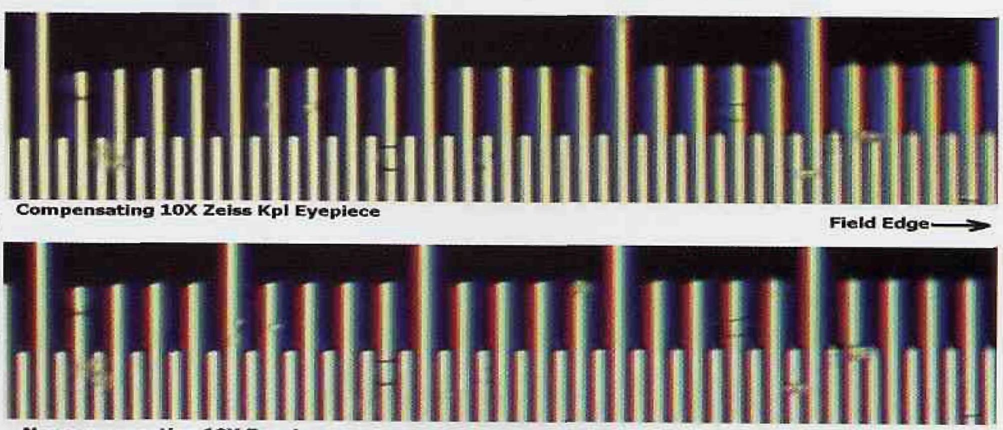
Chromatic aberrations are defects in an imaging system caused by the fact that different wavelengths or colors of light are refracted by different amounts. There are two types of chromatic aberration: longitudinal and lateral. *Longitudinal Chromatic Aberration* arises when a lens fails to focus various colors sharply in the same plane. If white light is used, the resulting image will be unsharp due to the different focal points of its component colors. Some colors will be in focus (and therefore sharp) and other colors will be out of focus. *Lateral Chromatic Aberration* results in a lateral shift of the different color components of an image as a single lens with a fixed refractive index will disperse each color by different amounts. This results in color stripes at slightly different magnifications, much like a rainbow, around hard edges and a general softening or decrease in resolution in all areas.



By using combinations of lenses with different refractive indices, index dispersions, and different lens shapes, it is possible to correct for these effects. The early apochromatic objectives corrected for the three primary three colors so that the images in the red, green, and blue were free of spherical aberration,* and would all come to focus on the same plane as the intermediate image at the field stop of the eyepiece, which further magnified the intermediate image. The problem was that the three colored images had different lateral magnifications. Optical engineers knew how to design lens systems giving the reverse order of magnifications for red and blue, so compensating eyepieces were designed to have the same but opposite percent of lateral magnification difference for red and blue giving a final image with the same lateral magnification for red, green, and blue. The less corrected achromatic objectives were commonly designed so they could also be used with the same compensating eyepiece for the apochromat objectives. This was Zeiss practice until they switched to infinity corrected objectives. Zeiss now corrects the chromatic difference of magnification in the telescope (Telan) lens of what is now a three-lens system.

Many laboratories have older microscopes still in use, such as the Zeiss Universal and the Olympus BH2, that require compensating eyepieces. Some laboratories may use modern Lomo microscopes, which all require a compensating eyepiece. Many of these microscopes are being equipped with digital cameras to take advantage of this new image recording technology. We could not afford a modern research microscope at the time we purchased a Kodak MegaPlus 1.6i/AB monochrome digital camera. We had to add the digital camera to our existing Zeiss Universal metallurgical microscope. We were unaware that the 1X relay lens from Diagnostic Instruments, selected by the outside systems integrator, did not have compensation for chromatic difference of magnification (CDM) and field flattening provided by a Zeiss Kpl eyepiece required for the Zeiss Universal. We discovered the consequences in our laboratory when the green filter, normally used on our Zeiss Universal metallurgical microscope for best image resolution with planachromatic objectives, was mistakenly left out of the illumination system and the outer portion of the field was badly blurred, as shown in Figure 1. I gave a presentation using this image at Inter/Micro-98 and subsequently included it in my publication on digital imaging in *The Microscope.*[1] Polaroid Corporation had

Fig. 2 Comparison of Lateral Chromatic Aberration for the Lomo 20X Achromatic Objective Used with Compensating and Noncompensating Eyepieces

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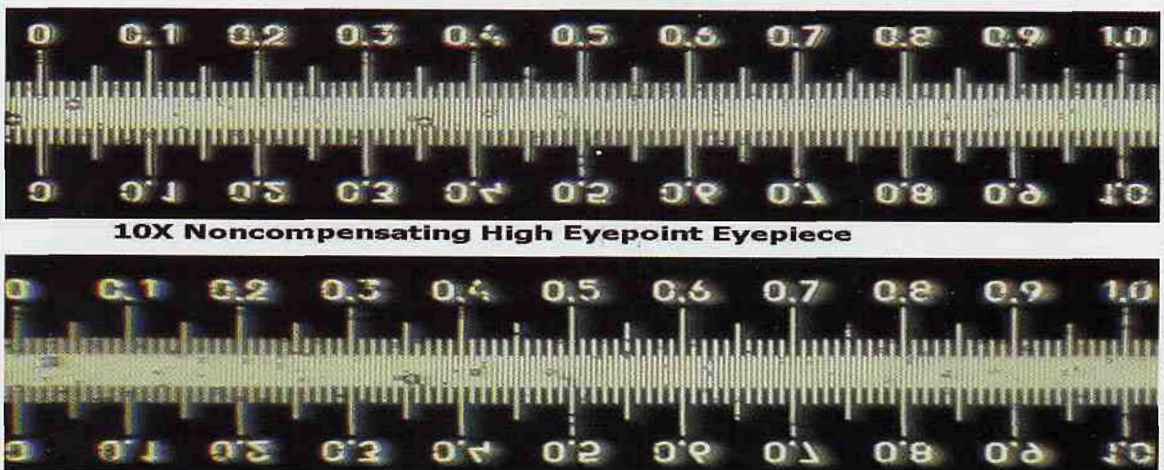
discovered the same problem in testing of their new DMC digital microscopy camera on their laboratory's Zeiss Universal biological microscope. Katherine Macchiarola of Polaroid Corporation gave a presentation on uncorrected CDM in the same session at Inter/Micro-98.[2] There are two types of relay lenses for digital cameras. One type couples to the microscope eyepiece. The other and much more common type replaces the eyepiece. The currently available relay lenses and optical adapters replacing the eyepiece are designed for modern microscopes that do not use compensating eyepieces. The advertising for these relay lenses and adapters normally contains no warnings about being not suitable for use on microscopes requiring a compensating eyepiece. Some adapters are even claimed to be "universal" for all microscopes.

The microscope I use for home microscopy is a modified Lomo Biolam. I used it with the 20X Lomo achromatic objective to record the Lomo stage micrometer with both a 10X high eyepoint Zeiss Kpl compensating eyepiece and a 10X high eye point, noncompensating eyepiece as the relay lenses for a Nikon Coolpix 995 digital camera.

Comparison of Lateral Chromatic Aberration and Resolution for the Lomo 4X Achromatic Objective Used with Compensating and Noncompensating Eyepieces **Fig. 3**



10X Zeiss Compensating High Eyepoint Kpl Eyepiece

This stage micrometer is a chrome coated glass slide with the scale graduations as transparent openings in the chrome coating. The back of this slide is clear, so it can be used with either reflected or transmitted brightfield illumination. I cemented a cover slip over the scale pattern with Canada balsam to make the stage micrometer suitable for use with high NA transmitted light objectives. Figure 2 shows a comparison of portions of both images after expanding 200% to show the color fringing better. The image taken with the noncompensating eyepiece shows significant color fringing towards the edge of the field. The image taken with the Zeiss compensating eyepiece shows less chromatic aberration, but the colors are reversed on the sides of the clear spaces indicating overcompensation for CDM. I visually checked this surprising result by checking for color fringes with a Lomo 10X wide-field compensating eyepiece and observed the same color fringing as with the Zeiss Kpl eyepiece. The Lomo eyepiece is not high eyepoint; so it would cause significant vignetting if used with the Coolpix camera.

The results with the 20X objective explained the color fringing I had found near the edge of the field in an image of cyanobacteria recorded with this objective and the Zeiss Kpl eyepiece. My previous evaluation of eyepieces was done with the Lomo 40X objective and, more recently, with the 4X objective. I have photomicrographs documenting that the Zeiss Kpl eyepiece with the Lomo 40X objective resolves from end to end *Pleurosigma angulatum*, the classical diatom for verifying the resolution of a 0.65 NA objective. The 4X objective had been found to require a noncompensating eyepiece to resolve the ends of the stage micrometer scale with 5 micrometer spacing. So, my final tests for CDM were conducted with this 4X objective. Figure 3 shows the resulting images confirming my previous visual observations that the 4X objective requires a noncompensating eyepiece to properly resolve the stage micrometer graduations.

I hope that the examples shown have demonstrated the importance of using a digital camera relay lens or eyepiece of the correct compensating type for microscope objectives designed to be used with a compensating eyepiece. The lateral chromatic aberration was shown to seriously degrade off-axis resolution. It is very unfortunate that laboratories in the USA rarely possess resolution test slides and a quality system to verify light microscope system resolution, both on-axis and off-axis. The chrome-on-glass stage micrometer is not commonly used with transmitted light microscopes, but served as a good specimen for checking lateral chromatic aberration. A far superior test slide for this determination would be the Richardson test slide available from Electron Microscopy Systems Sciences. The Richardson test slides are available for all illumination modes and have a finest line pattern with 0.25 micrometer spacing, so the full resolution range of the light microscope can be tested. The Richardson test slides are not currently available with calibration certificates. Laboratories using a Richardson test slide will want to use it as a secondary standard, which they can calibrate against a certified stage micrometer. Many laboratories require certification. The appropriate certification would be ISO-17025, which replaced ISO Guide-25. This is a technical competency standard that ensures the customer that the uncertainty reported in the measurements of each pattern is within the capabilities of the measurer and is justified. An auditor, that is technically competent to evaluate one's process, should visit each year to look over records and procedures. This results in a certificate of accreditation for the scope of one's measurements. The Geller MRS-4 standard is certified in this manner. See http://gellermicro.com/17025-03.pdf. That says, for instance, that the Geller measurement uncertainty for the (0.5µm pitch) 2000 line pair/mm pitch is 0.02µm. ISO-9000 should not be confused with ISO-17025 since it is a management standard and really has nothing to do with the technical competency of the company certified or their capabilities. ■

References:

1. Clarke, T. M. "Digital Imaging in the Materials Engineering Laboratory"; *The Microscope* **1998**, 46:2, 85-100.
2. McCrone, W. C. "Inter/Micro-98"; *The Microscope* **1998**, 46:2, 229.

* Spherical aberration arises when light rays that are parallel to the optic axis but at different distances from the optic axis fail to converge to the same point, off-axis rays are brought to a focus closer to the lens than are on-axis rays.