

Appendix A

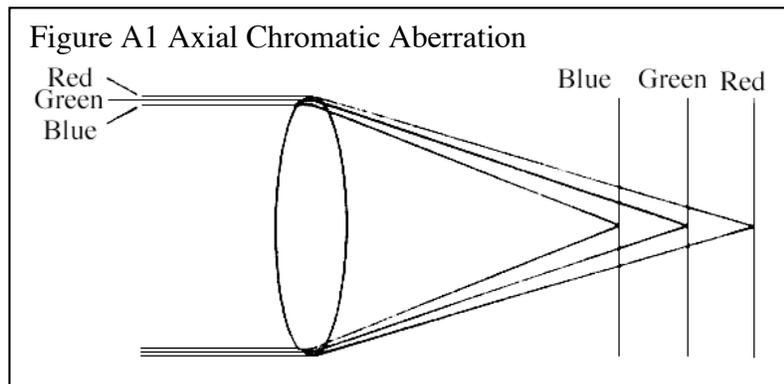
Lens Aberrations And Their Corrections

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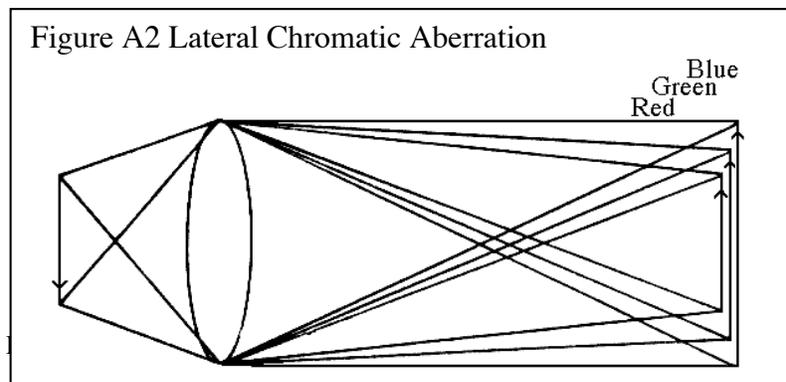
Chromatic aberration, spherical aberration, field curvature, astigmatism, coma, distortion – these are problems created by lenses, and the lens maker's solutions to them have given rise to achromats, plan achromats, apochromats, plan apochromats, fluorites or semi-apochromats, compensating oculars, and cover glass correction collars. In modern light microscopes, lens aberrations have been so greatly reduced that they are no longer a factor in the instruments resolving power; however, some knowledge of these aberrations, how they are corrected, and how to test for them is extremely helpful in properly using the instrument and in purchasing an instrument.

Chromatic Aberration

Chromatic aberration must certainly have been noticed by Antony van Leeuwenhoek as he used his single lens microscopes in the early 1700's. Looking through a van Leeuwenhoek 200 X single lens microscope one sees an image with colored fringes. This, of course, did not prevent van Leeuwenhoek from laying the essential groundwork for all of microbiology. The colored fringes are produced because different frequencies of light are refracted through different angles by the glass of the lens. Another way of saying the same thing: The refractive index of van Leeuwenhoek's glass, or any transparent material, is different for different colors of light. Blue light is brought to a focus closer to the lens than red light with the other colors somewhere in between. This is known as axial chromatic aberration (figure A.1).

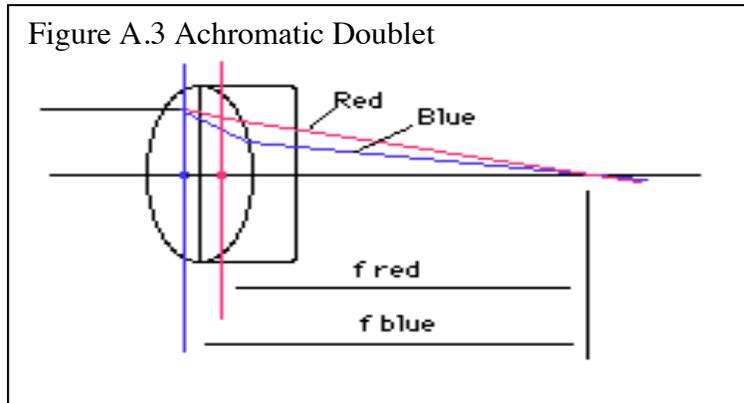


Each color of light forms its own image and since the focal lengths of the images differ, so do their magnifications (figure. A.2). This second type of chromatic aberration is called lateral chromatic aberration and it creates the phenomenon called chromatic magnification



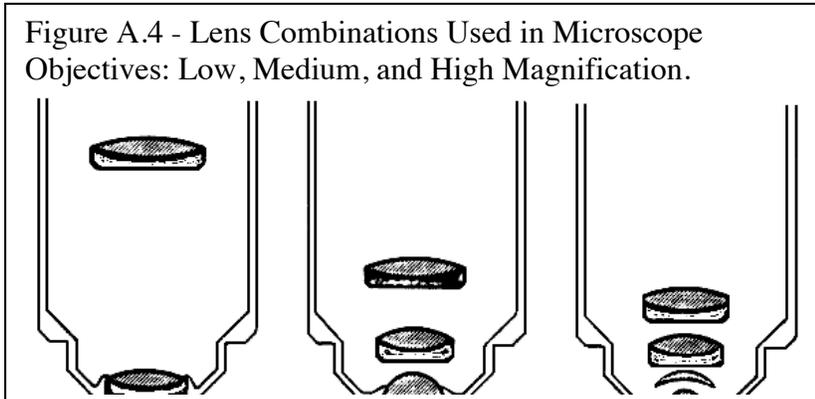
difference. During van Leeuwenhoek's lifetime the cure for axial chromatic aberration was realized in the form of the achromatic doublet (figure. A.3).

The doublet does not solve the problem of chromatic magnification difference for although the different colored images have been superimposed in the image plane, their focal lengths are still different, each having a different principal point in the doublet. The result is that the edge of the field of view



is ringed with colored bands. Achromatic doublets were being produced with trial and error methods by John Dolland in England in the mid 1700's. It wasn't until 1837 that the systematic construction of achromatic lenses began. J.J. Lister (father of the more famous Lister) published a paper in 1830, in the Philosophical Transactions of the Royal Microscopical Society London, concerning the mathematical design of achromatic lenses and in 1837, at Lister's urging, the English lens maker Andrew Ross began producing achromats according to Lister's design. By combining two lenses of different types of glass (i.e., with different refractive indices such as crown and flint) it is possible to bring two colors of light to the same focus. Lenses corrected for two colors of light, usually blue (F line) and red (C line) are called achromats. By adding a third type of glass (e.g., Fluorite) three colors can be brought to the same focus. In the late 1800's Ernst Abbe (working in Germany with C. Zeiss) developed lenses of this type and he called them apochromats.

Now here is an interesting twist. To produce high magnifications, the first lens of a compound objective must be a single, small, nearly spherical (actually hemispherical) lens (figure A.4). This instantly creates axial



and lateral chromatic aberrations that must be corrected by other lens combinations. The axial aberration can be corrected in the objective but the lateral can not be totally eliminated in a high magnification objective. This results in some chromatic magnification difference. Abbe solved this problem by making the final lateral chromatic correction in the eyepieces of the microscope. He called these compensating eyepieces. These eyepieces intentionally introduce the right amount of lateral chromatic aberration

to just neutralize that left over from the objective. Lower magnification objectives can be well corrected for axial and lateral chromatic aberration, but in this case the compensating eyepieces would just add some back. It would be a nuisance to have to change eyepieces every time you went from high magnification to low magnification. In modern microscopes, all the objective lenses are intentionally made to produce just the right amount of lateral chromatic aberration for correction by the compensating eyepieces. You should be careful when combining objectives and eyepieces from different manufacturers or even different lines from the same manufacturer. A given set of compensating eyepieces is designed to work with a given set of objectives.

Spherical Aberration

Van Leeuwenhoek's lenses also suffered from spherical aberration. This aberration gets its name from the spherical shape of a converging lens and from the fact that a spherical surface will refract monochromatic light entering at its edge to a different focus from that light entering at its center – with varying focal lengths for points in between (figure. A.5). More precisely,

Figure A.5 Spherical Aberration.

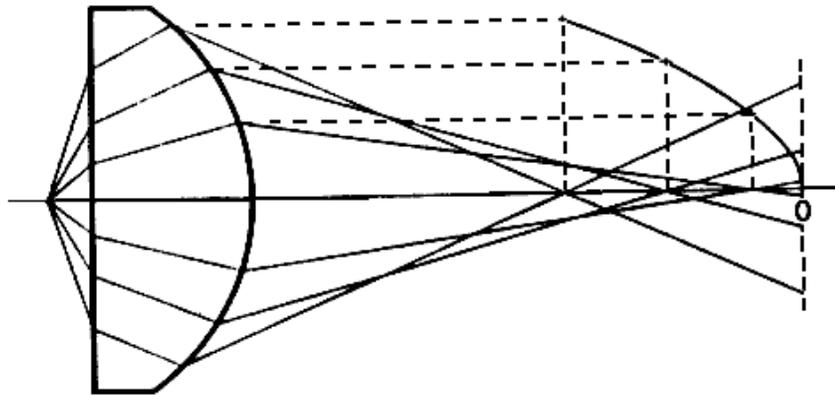
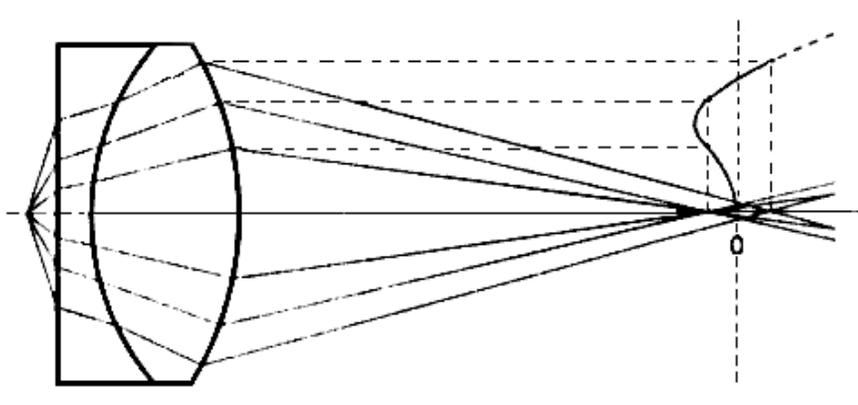


Figure A.6 Corrected Spherical Aberration



when parallel rays of light strike a spherical lens, a ray close to the edge will have a different angle of incidence than a ray that hits the center and will be refracted at a different angle. The situation is even worse when the rays are diverging, as they are when originating in a specimen. This causes the image to look hazy, that is to lack sharpness. A simple way of reducing this effect is to place an aperture or lens stop over the entrance pupil of the lens to block out some of the peripheral rays. Unfortunately this also has the effect of limiting the amount of light that can enter the lens and of reducing the lenses

resolving power. Lister's work had also shown that a series of lens combinations could be made to correct spherical aberration (figure A.6) and again Ross built lenses based on Lister's design. (The two famous Ross lenses of this type were a 1 inch 22 degree triple front, two double backs and a 1/8 inch 63 degree triple front, two double backs. See Bradbury). As with chromatic aberrations, both axial and lateral spherical aberrations exist and the degree of these will be different for different colors of light.

Ross's lenses were spherically corrected for one color and chromatically corrected for two colors. Achromats are today's equivalents being spherically corrected for the sodium D (green-yellow) line and chromatically corrected for the hydrogen F (blue) and hydrogen C (red) lines.

Around the time Ross was creating these lenses it had been discovered that applying a thin slip of glass over the specimen improved the image. Figure A.7 illustrates how diffracted light that might otherwise be lost is brought more toward the lenses aperture by action of the cover glass. Ross's lenses were so well corrected he discovered that this cover glass introduced spherical aberrations of its own. For example in figure A8-B a cover glass that is too thick causes the specimen (black dot) to appear to be too close to the lens. Just the opposite is true in figure A8-C where the cover glass is too thin. Figure A8-A illustrates a cover glass of the correct thickness for the lens.

Lister proposed that this spherical aberration could be corrected by altering the distance between the lens combinations of the objective. Since the amount of correction needed depended on the refractive index and thickness of the cover glass, some type of adjustable correction was necessary. Ross produced the first objective with an adjustable front element. Later, in 1855, F. H. Wenham improved on this by making the back rather than the front element adjustable. There is some debate whether this priority should go to Charles Spencer, an American lens maker of that time. Today, high quality dry objectives of large numerical aperture have a cover glass correction collar. The refractive index of modern cover glasses has been standardized, but there is still a variety of thicknesses.

Most modern objective lenses, whether they have a correction collar or not, are corrected for a cover glass thickness of 0.17 mm (or a number 1.5 cover glass: 0.16 to 0.19 mm thick). It is important to keep this in mind when preparing specimens for microscopy to avoid unintentional introduction of spherical aberrations. A number 1.5 cover glass is correct if the cover glass makes contact or nearly makes contact with the specimen. If there is more than a minimal distance between the coverglass and the specimen then a number 1 cover glass (0.13 to 0.17 mm thick) would be appropriate.

Figure A7 Cover Glass Effect on Light from the Specimen

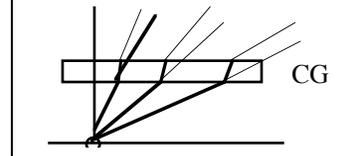
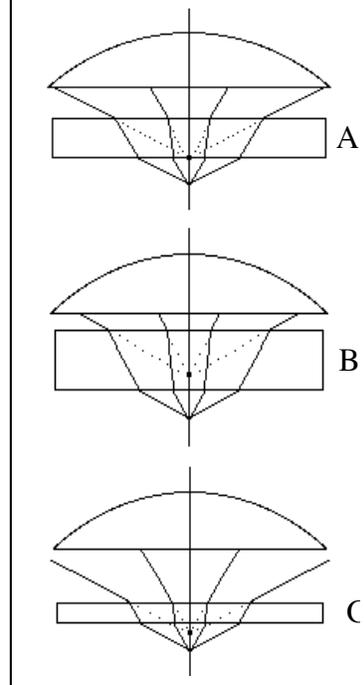


Figure A8

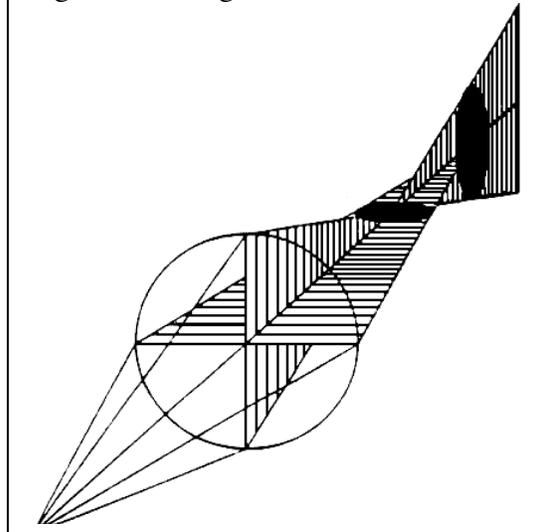


Off Axis Aberrations

The final four aberrations all occur away from the central axis of the lens. These are astigmatism, coma, distortion, and field curvature.

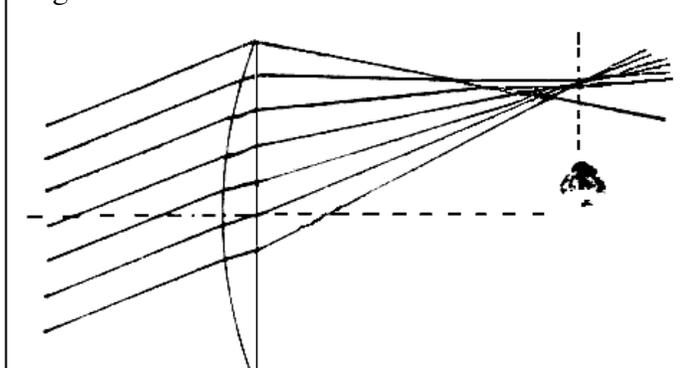
Astigmatism results from asymmetry of a lens. In its presence two rays of light entering at right angles to one another have different focal lengths. In figure A9 the vertical ray focuses closer to the lens than the horizontal ray. The result is an image that appears stretched in one direction when at one plane of focus and stretched in the opposite direction at a different plane of focus. The entire image also appears unsharp.

Figure A9 Astigmatism



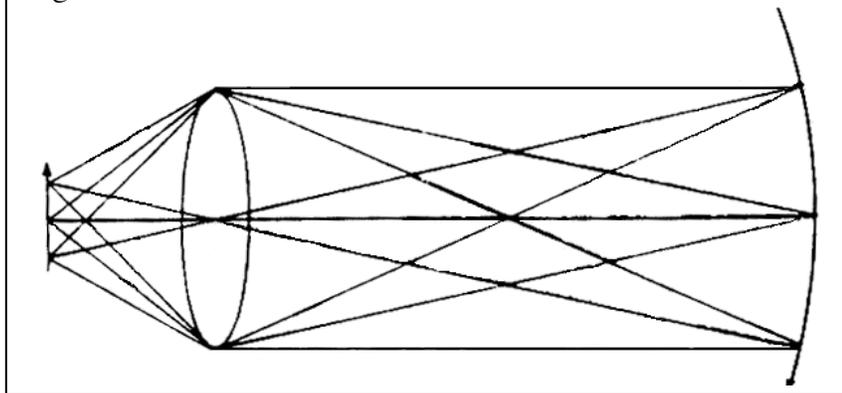
Coma results when a ray of light approaches a lens off axis. The part of the ray that passes through the center of the lens will focus closer to the lens axis than that part that passes through the periphery of the lens. This is actually a combination of astigmatism and spherical aberration. Coma is illustrated in figure A 10. The “hanging blob” represents the pattern of light that would be observed when looking on axis.

Figure A 10 Coma

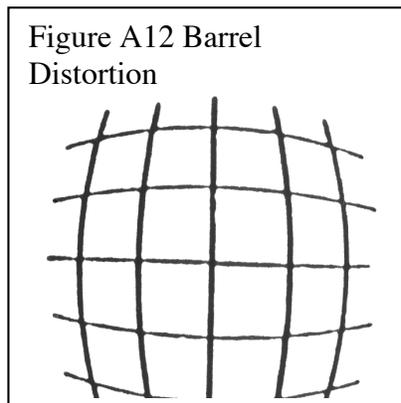
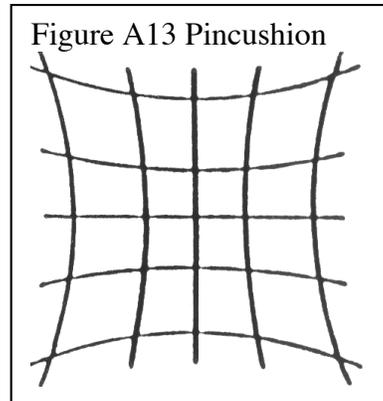


Of particular concern is the distortion called field curvature. It results from the fact that a curved lens produces a curved image plane as illustrated in figure A 11. This aberration causes the image to be out of focus at the edges when in focus at the center and vice versa. This is not so important when observing a specimen but is very important

Figure A 11 Field curvature



when photographing one. Field curvature can be corrected by adding extra elements in the objective, which requires changes in all the other objective corrections, or in the eye piece. Most manufacturers now offer plan objectives that have been corrected for field curvature. It is important to use these objectives for photography.



Two additional types of distortion are illustrated in figure A 12 and A 13. If the specimen consisted of a square grid of lines, figure a 12 illustrates the result if the image has more magnification in the center than at the edge. This is known as Barrel distortion. Figure A 13 illustrates the result if the image is magnified more at the edges than in the center. This is known as Pincushion distortion. Both of these are a result of combined field curvature and spherical aberration. These distortions are sometimes visible when extremely low magnification lenses are used.

Summary

The modern light microscope is the most perfect scientific instrument there is. All of the above aberrations have been corrected. Just imagine the effort you must make to produce a modern objective: You would want it to have a specific magnification and numerical aperture, the various aberration corrections made to a given level of precision, correction for a cover glass (or for no coverglass), a specific working distance, corrections for the type of immersion medium - whether air or water or glycerin or oil,

perhaps strain free optics for DIC and thus with the back focal plane at a specific location for interaction with a Waleston prism, or perhaps with a phase ring engraved on a lens element, or perhaps with a Hoffman prism enclosed, or perhaps with special glass that will pass short wavelength UV light, or perhaps with a cover glass correction collar, or with an enclosed iris. All this, and you would want to make it parfocal with several other lenses to make a matched series. Manufacturers of modern objective lenses use glasses of special formulation to achieve just the refractive properties they need, and they utilize super computers to iteratively calculate the best lens – glass combinations for a given set of lens criteria. And yet, it is so easy to use a microscope.