Optical Spherical Aberration Correction

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Spherical aberration (SA) is the most serious of optical aberrations in a modern microscope. It, along with sample absorbance, is one of two phenomena that make 3-dimensional imaging difficult. Spherical aberration arises from the use of spherical lens elements. Lens designers have been able to correct for spherical aberration in modern high-end objective lenses at a single optical condition. This condition is met at the surface of the indicated cover glass. For modern oil immersion objectives this cover glass must be exactly 0.17 mm.

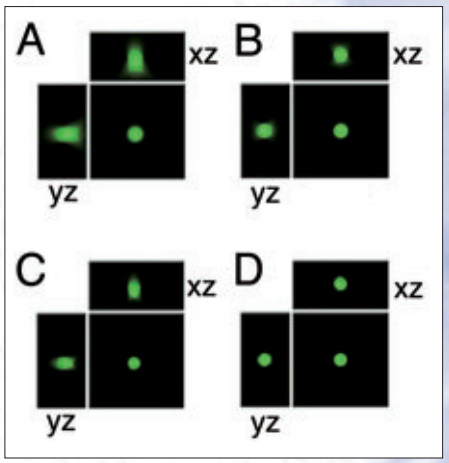

Figure 1: 3-D images of a 4 µm bead collected with and without SA correction. Panel A is with SA. Panel B is corrected via the NTSAC system. Panel C is with SA and deconvolution. Panel D is corrected and deconvolved. Note the restoration of a spherical shape in Z when SA is corrected. This effect is improved with deconvolution.

Figure 2: 3-D images of a fluorescent biological sample collected with different extents of SA. Each xz view of the data is indicated in the figure. The SA-free image is indicated by an arrow. Note the compressed axial distribution and increased intensity of the SA-free image.

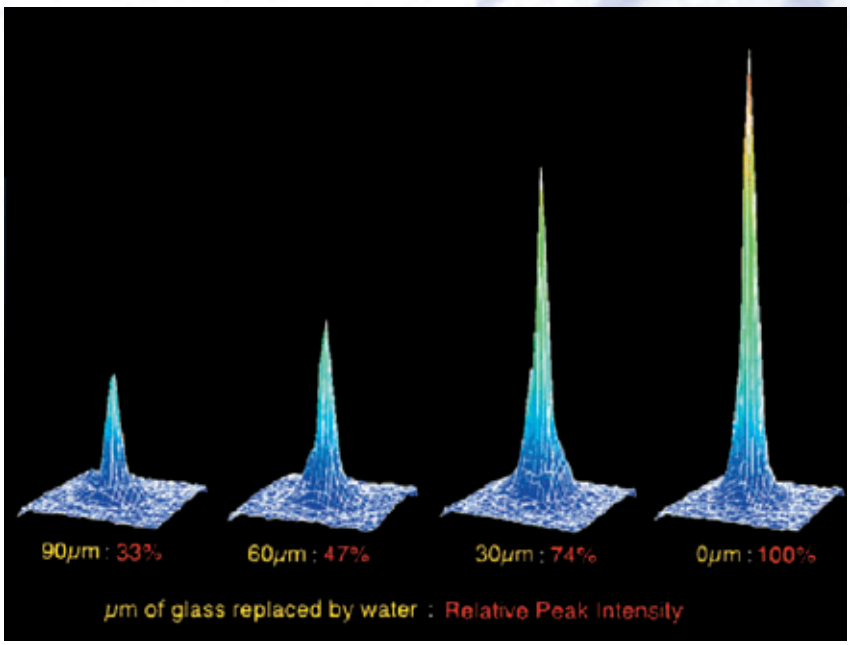

Figure 3: A surface plot of an in-focus point spread function taken under different conditions of SA. Note that the relative peak intensity falls dramatically as glass is replaced by water in the imaging conditions. This indicates increasing amounts of SA.

Biological imaging conditions tend to not meet the lens designer's condition for SA correction. This is particularly true in the case of 3-D imaging. Biological samples can't be mounted in oil or they will quickly die or, in the case of fixed samples, degrade. Also, the index of refraction of the sample is not known in biology so one necessarily introduces spherical aberration as one focuses into the sample.

Spherical aberration degrades images in a number of ways. Lateral image quality is reduced. However, since SA spreads the rays of a source across the focal axis, it greatly degrades axial resolution. In addition, it degrades intensity by spreading light across several z-sections. This is particularly damaging in modern low intensity imaging of fluorescent proteins (e.g. GFP).

Practically, SA can produce 3-D images that lose detail and signal as you focus into the sample. Additionally, SA can produce misleading geometric distortion that changes as you go deeper into the sample. Even at relatively small focal depths, this can make meaningful changes in sample structures.

A number of solutions have been proposed to this problem. The most common are the correction collar objectives offered by microscope manufactures. Typically, these are low numerical aperture objectives designed to be used under a wide variety of imaging conditions such as imaging through either glass, air or plastic dishes. Microscope manufactures also offer expensive high-performance collared water immersion objectives for live-cell applications. Both of these objective-based correction options require the user to properly configure the collar for each imaging condition.

Some researchers choose to try to compensate for SA by carefully selecting immersion oil that counteracts the SA introduced by the sample. While possible

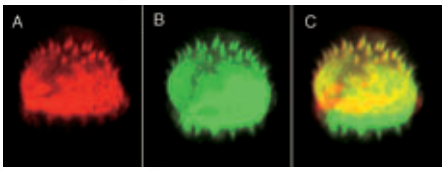

Figure 4: A 3-D, xz rendering of a fluorescent pollen grain collected with and without SA correction using the NTSAC system. Panel A in red represents an uncorrected image. Panel B in green represents a corrected image and Panel C shows an overlay. Note that as you go deeper into the sample (down on the image), detail is lost from the bottom half of the pollen grain. Only the green (SA corrected image) recovers the bottom spikes of the pollen grain.

in theory, this is difficult in practice and requires a great deal of care and experience. As with collared objectives, this oil matching technique can only correct for the SA at a particular z-depth for a particular sample.

In 1997, Z. Kam et. al. describes an alternative optical technique to address SA in the infinity corrected microscope. It involves the use of Infinity Photo-Optical's patented afocal variator technology to create an adjustable draw tube optic in the infinity space. Infinity has recently developed a motorized version of this optical system called the Motorized InFocus Device (MID). Intelligent Imaging Innovations (3i) has integrated MID control into its SlideBook software to create a spherical aberration correction system, the New Technology Spherical Aberration Correction (NTSAC) system, that can automatically set the optics to minimize spherical aberration for any given sample and focus point.

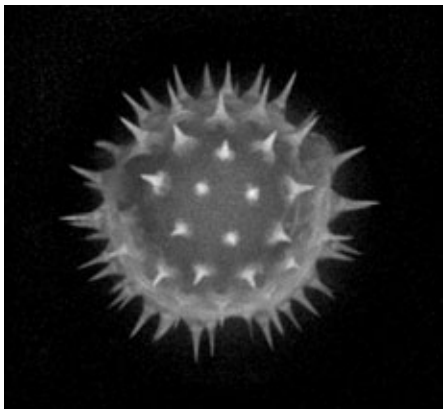

Figure 5: A rendering of an SA corrected, deconvolved pollen grain.

The NTSAC system has a number of distinct advantages over other SA correction strategies. First, because the system is self-setting, it requires significantly less expertise to use. Second, the system can be easily adjusted for different focal planes within the same sample. Third, the system works with any objective lens. And finally, the system can be adjusted within the same z-series collection.

But whatever the approach, today's discriminating microscopists must consider the issue of spherical aberration if they are to make the most of 3-dimensional microscopy. To ignore this artifact is to risk degraded data quantitation and 3-D morphometry.

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