STRATEGIES FOR THE BLIND DECONVOLUTION OF 3D MICROSCOPY IMAGERY WITH INDEX-MISMATCH INDUCED SPHERICAL ABERRATION

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Spherical aberration is caused by operating the objective lens outside of the design conditions. Ideally, the lens immersion fluid, the cover glass thickness, and the specimen embedding medium must be matched to very precise tolerances. In optical sectioning, a series of images are taken by focusing at increasing depths into the specimen. These images are combined to permit visualization of 3D structures. Unfortunately, the index of refraction of the embedding medium and/or specimen itself can not be carefully controlled. The spherical aberration increases dramatically upon focusing deeply into a specimen; it is especially severe in neurobiology because neural structures extend deeply into tissue. This problem affects both wide-field microscopy as well as confocal microscopy.

Spherical aberration seriously degrades the resolution and quantitative value of the imagery. As the focal plane extends deeper into the specimen, the image intensity diminishes dramatically, significant geometric distortions are induced in the axial direction, and the resolution (especially axial) is strongly reduced. [1] These problems are well illustrated by the confocal image (right) of YFP-labeled motor neuron terminals in an adult mouse hindlimb muscle (Courtesy Lauren Baylor, Harvard University). This XZ image is 212 um wide and extends 291 um into the tissue:

Deconvolution is a widely-used software algorithm that restores a microscopy image using a knowledge of the point-spread function, or PSF. The PSF may be derived from images of sub-resolved fluorescent beads, or deduced from the characteristics of the image itself using the “blind” deconvolution algorithm[2],[3]. We will present recent advances using the “blind” deconvolution method to detect and correct for spherical aberration. Strategies for the accommodating depth-varying spherical aberration will also be addressed.