Abstract:

We present a novel approach for deconvolution of 3D image stacks of cortical tissue taken by mosaic/optical-sectioning technology, using a transmitted light brightfield microscope. Mosaic/optical-sectioning offers the possibility of imaging large volumes (e.g. from cortical sections) on a millimetre scale at sub-micrometre resolution. However, a blurred contribution from out-of-focus light results in an image quality that usually prohibits 3D quantitative analysis. Such quantitative analysis is only possible after deblurring by deconvolution. The resulting image quality is strongly dependent on how accurate the point spread function used for deconvolution resembles the properties of the imaging system. Since direct measurement of the true point spread function is laborious and modelled point spread functions usually deviate from measured ones, we present a method of optimizing the microscope until it meets almost ideal imaging conditions. These conditions are validated by measuring the aberration function of the microscope and tissue using a Shack-Hartmann sensor. The analysis shows that cortical tissue from rat brains embedded in Mowiol and imaged by an oil-immersion objective can be regarded as having a homogeneous index of refraction. In addition, the amount of spherical aberration that is caused by the optics or the specimen
is relatively low. Consequently the image formation is simplified to refraction between the embedding and immersion medium and to 3D diffraction at the finite entrance pupil of the objective. The resulting model point spread function is applied to the image stacks by linear or iterative deconvolution algorithms. For the presented dataset of large 3D images the linear approach proves to be superior. The linear deconvolution yields a significant improvement in signal-to-noise ratio and resolution. This novel approach allows a quantitative analysis of the cortical image stacks such as the reconstruction of biocytin-stained neuronal dendrites and axons.