POST-PROCESSING, COMPUTATIONAL ADAPTIVE OPTICS FOR 3D SUPER-RESOLUTION MICROSCOPY

Atsushi Matsuda
Advanced ICT Research Institute Kobe, National Institute of Information and Communications Technology, 588-2, Iwaoka, Nishi-ku, Kobe 651-2492, Japan
E-mail: a.matsuda@nict.go.jp

KEY WORDS: Adaptive optics, Deconvolution, Super-resolution microscopy, Deep imaging, Zernike polynomials

Recent development of adaptive optics (AO) microscopy has revealed its significant potential to improve resolution and signal to noise ratio in deep imaging and super-resolution microscopy by correcting sample-induced optical aberration. While AO has been successful in advanced microscopy, it is not yet used widely in biology due to its complex optics, expensive devices, and demanding operations. Specifically, the optics must be precisely aligned to overcome non-common path errors between a wavefront sensor and a science detector. Measuring wavefront requires excess light unsuitable for live imaging. Furthermore, AO is hard to apply to a large field of view due to variation of subregional wavefronts.

Here we report post-processing, computational AO of the 3D fluorescence images already acquired. Our method may seem to be related to parametric blind deconvolution, where optical transfer function (OTF) is created from the parametrized wavefronts and used for iterative deconvolution to improve the image and to optimize OTFs. Our method is, however, much faster than classical parametric blind deconvolution, due to omission of the major iteration cycle. While conventional deconvolution is oriented toward optimizing the OTF as a whole, we focus on optimizing only the phase transfer function (PTF) to modify images. We found that, when optical aberration of the image was corrected only by PTF, Strehl ratio of the image was improved while the total intensity remained unchanged. Thus, we measured Strehl ratio of the image to find out the right PTF. When we know the answer for PTF, we simultaneously know the wavefront that create the PTF, and in turn, we can calculate OTF from the wavefront.

We showed that our method was able to separate two points that were previously recognized as a single point object due to spherical aberration, and the resulting FWHM of a fluorescent bead was close to the diffraction limit. The method was robust enough to process noisy images. Our method worked in any kind of biological images if sampling criterion was fulfilled. Furthermore, we have successfully applied our method to super-resolution microscopy, namely 3D structured illumination microscopy (3D-SIM), resulting in significant improvement of the signal to noise ratio of the reconstructed images.

These data show that our approach can correct optical aberrations of the detection path similarly to the conventional AO. However, our approach has number of advantages over hardware-based AO. Our method works with any conventional fluorescence microscopes, without expensive devices and without careful optical alignment. The process works with the 3D image itself, but no other things are required, including additional images, excess illumination and a guide star. It is also possible to apply to fast live imaging, because it is post-processing. It should be easily extended to subregions. We believe our approach is one of the choices for biological AO.