ADDRESSING DEPTH-INDUCED SPHERICAL ABERRATION IN COMPUTATIONAL OPTICAL SECTIONING MICROSCOPY USING WAVEFRONT CODING

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Imaging thick biological samples introduces spherical aberration due to refractive index mismatch between specimen and imaging lens immersion medium. Different wavefront coding methods[1] have been studied to test the feasibility of static wavefront correction to reduce depth-induced SA. These methods are found to be effective, but they are not optimized for this application. Inspired by a radially symmetric squared cubic (SQUBIC) phase mask design for reduced depth-induced SA effect in scanning confocal microscopy[2], we used the SQUBIC pupil mask to engineer the point spread function (PSF) of a widefield fluorescence microscope, both in simulation and experimentally. Simulated images of thick test objects were generated using a wavefront encoded engineered PSF (WFE-PSF) and were restored using a space-invariant expectation maximization algorithm (SIEM)[3]. Simulations show that, in the presence of SA, the use of the SIEM algorithm and a single SQUBIC WFE-PSF can yield improved image restoration (Fig. 1e) compared to the result from the conventional system (Fig 1d). Thus, modification of a widefield system with the SQUBIC mask renders the system less sensitive to depth-induced SA and makes it suitable for imaging samples at larger optical depths without an increase in computational complexity. Experimental images were acquired using an upright microscope modified with WFE phase modulation, implemented with a liquid crystal on silicon SLM. Measured WFE-PSFs show agreement with theory[4] and experimental restoration results are underway.

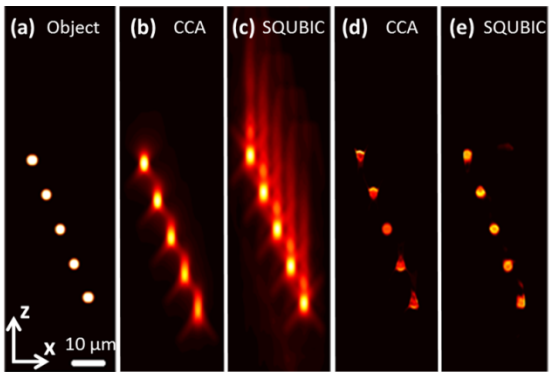

Figure 1: Simulated forward images of an object with five spheres located at 10, 20, 30, 40 and 50 µm depth below the cover slip (a), using a system with: (b) a clear circular aperture (CCA) and (c) a SQUBIC encoded aperture. SIEM restoration using a single PSF at a 30-µm depth for a CCA conventional system (d), and a SQUBIC-encoded system (e). Restorations shown in the same color scale. Lens: 20x/0.8 NA, air.

References

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