COMPUTATIONAL IMAGING FOR DEPTH-VARIANT FLUORESCENCE MICROSCOPY

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A key challenge in live-cell microscopy is three-dimensional (3D) low-light imaging with improved accuracy. Photon efficiency is of critical importance for live-cell imaging, where low-light imaging is often crucial for specimen viability and fast data-acquisition. Wide-field microscopy remains the optimal methodology for harvesting the most photons in a given optical configuration efficiently. This property of wide-field microscopy is exploited in computational optical-sectioning microscopy (COSM) which has become a viable alternative to confocal microscopy for 3D fluorescence imaging [1]. However, a limiting assumption common to most COSM methods makes them unsuitable for processing data acquired from "thick" biological specimens. This assumption ignores aberrations that arise when imaging deep into a thick biological specimen. Such aberrations are due in part to a refractive index mismatch between imaging layers that has been well-characterized in point-spread function (PSF) models [2].

The previously developed Depth-Variant Expectation Maximization (DVEM) algorithm [3] addresses depth-variant imaging due to spherical aberration only. The algorithm is based on an imaging model that approximates space-variant imaging by modeling only depth variability using a small number of depths (strata) within the sample which is represented by non-overlapping strata. Interpolation of PSFs defined at depths bounding a stratum characterizes the PSF associated with each stratum which is then utilized to predict the image of a stratum. The choice for the number of strata used provides a tradeoff between accuracy of the model and the computational complexity of the algorithm. The performance of the DVEM algorithm is affected by this tradeoff. Physical parameters such as the lens used, the size of the object and its refractive index contribute to the amount of spherical aberration present in the PSF. In this presentation, we will show results obtained from studies using different parameters. The main conclusion from these studies is that a small number of PSFs can be used for the estimation without a significant loss in algorithm performance. The DVEM algorithm does not address specimen-induced aberrations, rendering it best suited for only imaging homogeneous samples.

References

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