3D CELL IMAGING WITH COMPUTATIONAL OPTICAL-SECTIONING MICROSCOPY AND STRUCTURED-ILLUMINATION MICROSCOPY

Chrysanthe Preza[1], Amaradri Mukherjee[1], Vimeetha Myneni[1],

Lynn M. Crosby[2], and Christopher M. Waters[2]

[1]Dept. of Electrical & Computer Eng., 206 Engineering Science Bldg.
The University of Memphis, Memphis, TN 38152-3180, USA
[2]Dept. of Physiology, 894 Union Ave., Nash Bldg. 426
The University of Tennessee Health Science Center, Memphis, TN 38163
Email: cpreza@memphis.edu

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Tradeoffs between data-acquisition time, label and specimen viability, resolution and signal-to-noise ratio (SNR), and reduction of possible aberrations exist among different types of fluorescence microscopes. Widefield (WF) microscopy remains the optimal methodology for gathering the most photons efficiently. This property of WF microscopy is exploited in both computational optical-sectioning microscopy (COSM) [1] and in structured-illumination microscopy (SIM) [2], developed to improve resolution in 3D microscopy. The purpose of this study is to investigate achieved resolution and SNR in 3D images acquired with SIM and COSM from samples of human adenoma carcinoma lung cells (Fig. 1) and rodent lung epithelial cells. 3D images from the same field of view were acquired with WF microscopy and with SIM using the Zeiss ApoTome attachment. WF images were processed using the DVEM algorithm which has been shown to provide improvements over deconvolution [3]. Our results show qualitative agreement of COSM and SIM images, with cell features better-resolved in the COSM images (Fig. 1). However, imaging using the ApoTome produced 3D images with reduced SNR compared to the COSM images. Because SIM uses 3 modulated WF images for each optical section, signal attenuation and photobleaching occur. In addition (3-7) images are captured per frame to improve the SNR with averaging, thereby increasing data-acquisition time. COSM avoids these limitations at the expense of increased computational time for off-line processing.

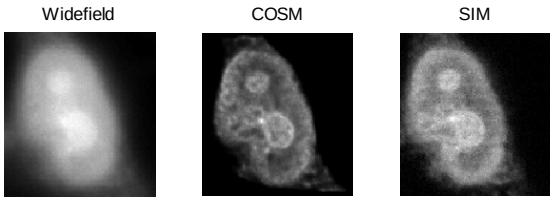

Figure 1: XY section images of a human lung carcinoma cell. The COSM image was computed with the DVEM algorithm [4]. For the SIM image, 3 images / frame were averaged by the ApoTome. Image intensities were scaled for display. Lens: 63X/1.4 NA oil-immersion; Wavelength of 605 nm.

References

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