INVESTIGATING THE USE OF STRUCTURED-ILLUMINATION MICROSCOPY AS IMPLEMENTED IN THE ZEISS APOTOME ATTACHEMENT TO IMAGE THICK LUNG TISSUE SLICES

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The focus of this study was to quantify how specimen thickness adversely affects the visualization of information in 3D fluorescence images acquired with the Zeiss ApoTome device that implements structure illumination microscopy (SIM) developed to improve resolution in 3D microscopy [1].

Materials and Methods: Samples of rat lung tissue were sliced using a Stoelting tissue chopper at a thickness of 200 μm. Samples stained with DAPI and Rhodamine were mounted following a protocol. The slides were then imaged using a multimodal wide field (WF) Zeiss Axio Imager microscope. 3D images from the same field of view were acquired with and without the Zeiss ApoTome attachment and raw grid images were also retained. For comparison purposes, images were also acquired with an Olympus Confocal microscope, and with Optical Coherence Tomography (OCT) imaging. In addition, regions of the WF images were processed using the COSMOS Software package [2, 3] for image processing. Isolated nuclei were identified at different Z depths of these 3D images and their size was determined from the FWHM along different axes. For each depth an average nuclei size was computed.

Results: The computed nuclei size was found to remain approximately constant over different depths in images acquired with the ApoTome attachment and it was consistent with the size computed from confocal images. However, nuclei could not be identified in these images after a depth of 90 μm. OCT images confirm that structure is evident through out the thickness of the lung slice. Inspection of ApoTome grid images show that the grid is not projected at focal planes deep into the lung tissue. As expected, light is not properly focused deep in thick samples due to depth-induced aberrations. These aberrations may cause a limitation in the visualization of structures at large imaging depths when using the ApoTome device. COSMOS processed images show potential in the visualization of these deeper structures. Further investigation of both approaches is warranted and it is underway.

References

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