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Reflectance microscopy techniques for 3D imaging of the alveolar structure

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Lung disease involving the alveoli and distal bronchioles are poorly understood and most commonly studied indirectly via lung function tests. Available imaging tools for the non-destructive assessment of the alveolar structure include X-ray computed tomography, intra-vital fluorescence microscopy and Optical Coherence Tomography, which are either limited by long acquisition time, inadequate resolution and contrast, or shallow imaging depth.

In this study, we investigated the potential of two high-resolution reflectance microscopy imaging techniques, Spectrally Encoded Confocal Microscopy (SECM; 1μm (x) x 1μm (y) x 5μm (z) resolution) and Full Field Optical Coherence Microscopy (FFOCM; 1μm (x) x 1μm (y) x 1μm (z) resolution), for imaging alveolar microstructural detail. Two mouse lung samples were imaged with both SECM and FFOCM. The specimens were inflation-fixed using a modified Heitzman fixation technique at 20 cm H2O pressure. They were cut in 500mm thick slices and water immersed for imaging. Images were obtained and analyzed to determine whether or not the resolution and contrast of these techniques are sufficient to visualize the fine structures of the alveolar wall.

Alveolar microstructure could be resolved in three dimensions in images obtained by both technologies. Alveolar septal walls from multiple layers could be clearly identified while sub-cellular structures such as nuclei were also visible in the SECM technique. In conclusion, we have demonstrated that two imaging technologies provide important sub-cellular detail that is required to study alveolar microstructure. Future research to develop these imaging modalities further so that they may be used in vivo is merited.

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