The Effects of Refractive Index Mismatch on Multiphoton Fluorescence Excitation Microscopy of Biological Tissue

Young, Pamela Anne

Advisor: Rubart, Michael
Decca, Ricardo S.
Bacallao, Robert L.
Dunn, Kenneth William

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Abstract:

Introduction: Multiphoton fluorescence excitation microscopy (MPM) is an invaluable tool for studying processes in tissue in live animals by enabling biologists to view tissues up to hundreds of microns in depth. Unfortunately, imaging depth in MPM is limited to less than a millimeter in tissue due to spherical aberration, light scattering, and light absorption. Spherical aberration is caused by refractive index mismatch between the objective immersion medium and sample. Refractive index heterogeneities within the sample cause light scattering. We investigate the effects of refractive index mismatch on imaging depth in MPM. Methods: The effects of spherical aberration on signal attenuation and resolution degradation with depth are characterized with minimal light absorption and scattering using sub-resolution microspheres mounted in test sample of agarose with varied refractive index. The effects of light scattering on

signal attenuation and resolution degradation with depth are characterized using sub-resolution microspheres in kidney tissue samples mounted in optical clearing media to alter the refractive index heterogeneities within the tissue. Results: The studies demonstrate that signal levels and axial resolution both rapidly decline with depth into refractive index mismatched samples. Interestingly, studies of optical clearing with a water immersion objective show that reducing scattering increases reach even when it increases refractive index mismatch degrading axial resolution. Scattering, in the absence of spherical aberration, does not degrade axial resolution. The largest improvements in imaging depth are obtained when both scattering and refractive index mismatch are reduced. Conclusions: Spherical aberration, caused by refractive index mismatch between the immersion media and sample, and scattering, caused by refractive index heterogeneity within the sample, both cause signal to rapidly attenuate with depth in MPM. Scattering, however, seems to be the predominant cause of signal attenuation with depth in kidney tissue. Kenneth W. Dunn, Ph.D., Chair

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