

ANALYSIS OF THE PENETRATION OF NON LINEAR OPTICAL EXCITATION IN THICK SCATTERING SAMPLES.

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Two Photon Excitation (2PE) microscopy provides a powerful tool for deep imaging in biological samples submicron resolution. When imaging is performed in biological tissues, light scattering occurs since different compartments and localized particles produce an unhomogeneous refractive index distribution of the medium. The higher wavelength used in 2PE makes this technique suitable for performing imaging in depth in scattering samples. Notwithstanding scattered light brings to a limited penetration depth inducing a strong reduction in Signal to Noise Ratio (SNR)[1] and this aspect represents a crucial point for non linear imaging in vivo. In some perturbation techniques an high intensity level delivered on the sample is required and this can lead to a significant unwanted excitation of the surface layers of the sample and also photobleaching process can potentially occur. The localization of the maximum 2PE intensity was found to shift closer to the surface[2]and the 2PE imaging depth limit appears strongly limited by near surface fluorescence[3]. In this work we computed the illumination and the photobleaching distribution[4] for different scattering coefficients in order to characterize the effects induced by scattering. Experimental test has been carried out by imaging, with different numerical aperture objectives, thick scattering fluorescent immobile sample (polyelectrolyte gel). Results confirm that under such conditions no photobleaching effects due to scattering occur close to the surface.

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