Two-Photon lightsheet: how to preserve the excitation volume in scattering samples in a selective plane illumination microscope.

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In the last years light sheet microscopy has been demonstrated to be an optimal tool for high resolution imaging of large samples such as embryos and tissues. Still, scattering in thick samples could introduce aberrations and distortions of the excitation volume that can decrease the imaging quality. Recently, two-photon excitation microscopy has been coupled with light sheet fluorescence microscopy in order to increase the deep imaging capability of the light-sheet based imaging system(1). Even if two-photon excitation allows the enhancement of the penetration depth capabilities thanks to the use of a higher wavelengths, imaging may still be affected by scattering effects. In fact, scattering produces out-of-focus fluorescence generation, resulting in a shift of the real intensity excitation distribution (2). In this framework, the characterization of scattering based distortions of the excitation volume represent a useful investigation. To this end, we performed measurements of the real light sheet excitation distribution on calibrated phantom samples with tunable optical properties. A comparison between single photon and two photon imaging has been pointed out and results show how effectively two-photon excitation is able to preserve the shape of the excitation light sheet, compared to the single photon case, thus preserving the optical sectioning and the contrast capabilities of the system(3). 3D reconstruction of mammmary cell spheroid is performed using two-photon SPIM in order to show the improved imaging capability.