MODELING OF SCATTERING EFFECTS IN SINGLE AND TWO-PHOTON EXCITATION SELECTIVE PLANE ILLUMINATION MICROSCOPY

Zeno Lavagnino1,2, Francesca Cella Zanacchi1,2, Emiliano Ronzitti2, Alberto Diaspro1,2

1IIT – Italian Institute of Technology, Via Morego 30, 16163 Genoa, Italy
2 LAMBS- MicroSCoBio, Dept. of Physics, University of Genoa, Via Dodecaneso 33, 16146, Genoa, Italy

E-Mail: zeno.lavagnino@iit.it

Scattering of the propagating light in a thick medium is one of the most critical issues while performing optical microscopy experiments. In the last decade two-photon excitation has been found as a suitable tool to limit this problem thanks to its high imaging depth capability. Nevertheless, the intensity of the excitation light decreases exponentially due to the scattering properties of the sample investigated[1,2,3].

The study of the effects induced by scattering is particularly crucial in those optical methods aiming at deep and thick sample observation. Selective plane illumination microscopy (SPIM) is a promising technique which allows to perform three dimensional fluorescence imaging in thick samples with high signal to noise ratio[4] confining the excitation in a thin sheet of light produced by means of a cylindrical lens.

We implemented a theoretical model to estimate the effects of scattering in the excitation intensity distribution provided by single and two-photon excitation regime within a selective plane illumination architecture.

Furthermore, we evaluated the influence of the scattering process in SPIM imaging in single and two photon excitation in terms of experimental measurements on phantom samples created to mimic the behavior of different scattering tissues.

By varying the scattering properties of the phantom sample, we carried out experiments to validate the theoretical model developed.


