MONTE CARLO LIGHT SHEET FLUORESCENCE MICROSCOPY SIMULATOR FOR NEW OPTICAL WINDOWS ASSESMENT

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Optical microscopy techniques are subject to the size, opacity and scattering of the samples. The later reduces notably the resolution of the images due to its strong presence in biological tissues, limiting light penetration to a few millimeters in depth and causing the photons to lose their original directionality. Nowadays, sample clearance is the main technique to obtain optically transparent samples, however, it presents several drawbacks such as variations in their size and shape or a reduction of the fluorescence intensity[1].

The use of wavelengths with reduced scattering in biological tissues has great potential to increase image quality on large samples or even avoid clearance on small ones. The II near-infrared (NIR) is a region of the spectrum within 1300 and 1700 nm that promises to outperform NIR I and visible in terms of depth of penetration with minimum scattering[2]. Light Sheet Fluorescence Microscopy (LSFM) could be greatly benefited from the properties of this window, minimizing the degradation of the plane of light as well as the loss of resolution on the fluorescence image due to scattering. There are several works in the literature using NIR II fluorophores for imaging that take advantage of its properties but so far has never been used in 3D optical microscopy[3].

Before the inclusion of this range of wavelengths in LSFM setups, we believe that a deep evaluation of its performance must be done. For this task, Monte Carlo simulators offer an excellent environment for simulation of light propagation in tissues. In this work, a tool for simulating both illumination and fluorescence in light sheet microscopes has been developed. This software allows to perform exhaustive studies of the depth of penetration of NIR light in LSFM. Also, it can be used to analyze how the fluorescence coming from out of plane excitation due to the degradation of the plane of light affects to the images. The tool has been developed using the well-known and validated package Monte Carlo Extreme (MCX)[4] and will allow to better understand the perspectives and expectations of the use of II NIR light for light sheet microscopy.

[1] D. S. Richardson and J. W. Lichtman, “Clarifying Tissue Clearing,” *Cell*, vol. 162, no. 2, pp. 246–257, 2015.
[2] A. M. Smith, M. C. Mancini, and S. Nie, “Bioimaging: Second window for in vivo imaging,” *Nat. Nanotechnol.*, vol. 4, no. 11, pp. 710–711, 2009.
[3] G. Hong, A. L. Antaris, and H. Dai, “Near-infrared fluorophores for biomedical imaging,” *Nat. Biomed. Eng.*, vol. 1, no. 1, p. 0010, 2017.
[4] Q. Fang and D. R. Kaeli, “Accelerating mesh-based Monte Carlo method on modern CPU architectures,” *Biomed. Opt. Express*, vol. 3, no. 12, p. 3223, 2012.