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Single- and multi-photon shaped illumination for light-sheet fluorescence microscopy

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The use of exotic optical modes is becoming increasingly widespread in microscopy. Particularly, propagation-invariant beams, such as Airy and Bessel beams and optical lattices, have been particularly useful in light-sheet fluorescence microscopy (LSFM) as they enable high-resolution imaging over a large field-of-view (FOV), possess a resistance to the deleterious effects of specimen induced light scattering, and can potentially reduce photo-toxicity (e.g. [1]).

Although these propagation-invariant beams can resist the effects of light scattering to some degree, and there has been some interest in adaptive-optical methods to correct for beam aberrations when they cannot, scattering and absorption of the illuminating light-sheet limit the penetration of LSFM into tissues and results in non-uniform intensity across the FOV.

A new degree of control over the intensity evolution of propagation-invariant beams can overcome beam losses across the FOV, restoring uniform illumination intensity and therefore image quality. This concept is compatible with all types of propagation-invariant beams and is characterised in the context of light-sheet image quality [2].

Another property to control is the wavelength of light used. Optical transmission through tissue is greatly improved at longer wavelengths into the near-infrared due to reduced Rayleigh scattering and two-photon excitation has proved beneficial for imaging at greater depth in LSFM. Three-photon excitation has already been demonstrated as a powerful tool to increase tissue penetration in deep brain confocal microscopy, and when combined with beam shaping can also be a powerful illumination strategy for LSFM [3].

Recent progress in shaping optical fields for LSFM will be presented.

[1] T. Vettenburg et al, Nat. Methods 11, 541-544 (2014), doi:10.1038/nmeth.2922
[2] J. Nylk et al, Sci. Adv. 4, eaar4817 (2018), doi:10.1126/sciadv.aar4817
[3] A. Escobet-Montalbán et al, bioRxiv 323790 (2018), doi: 10.1101/323790

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