Lorentzian light sheets: longer, thinner, more efficient illumination profiles for macroscopic samples

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The use of light sheet illumination in fluorescence microscopy reduces phototoxicity and photobleaching in large and small samples in addition to providing optical sectioning [1]. Whilst almost all light sheet microscopes use conventional Gaussian beams for illumination, a number of alternative illumination profiles, such as Bessel beams and optical lattices, have been introduced and shown to have beneficial characteristics for certain samples [2, 3, 4, 5].

We review the main types of illumination profiles used in light sheet microscopy and present the underlying principles governing their behaviour and performance. Using high-numerical-aperture vectorial diffraction theory we show where the common 'rules of thumb' used to quantify light sheet characteristics break down and why simplified metrics, such as full width at half maximum, fail to adequately characterise 'more exotic' light sheets.

We then use the outlined principles to introduce a new class of illumination profile, Lorentzian light sheets, suitable for high-resolution imaging of mesoscopic samples. These sheets maintain widths less than those of comparably long Gaussian sheets, while also allowing for the extraction of much higher resolution data through deconvolution. Our approach allows for sheets with lengths in excess of a millimetre, with superior excitation confinement and length than one- or two-photon Bessel beams. We present numerical results in support of our theory and detail our progress towards imaging macroscopic biological specimens using a simple optical design.

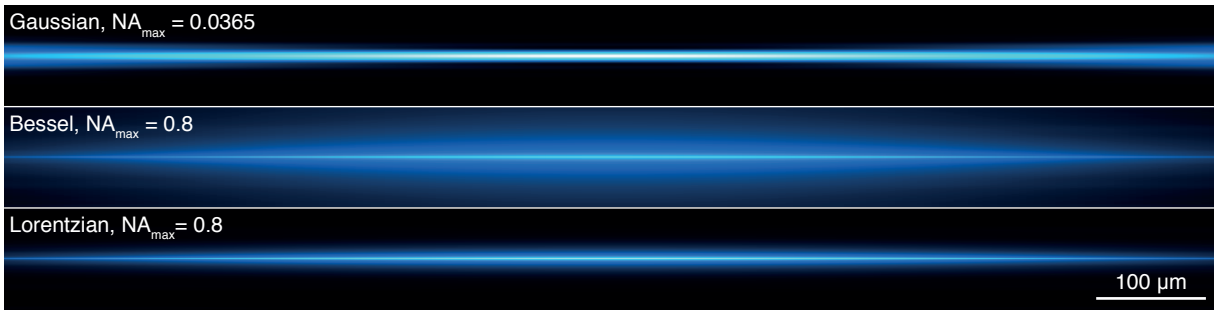

Figure 1: Simulated illumination profiles for different light sheet illumination approaches.

References

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