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Extended field-of-view light-sheet microscopy

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Abstract. Light-sheet fluorescence microscopy enables rapid 3D imaging of biological samples. Unlike confocal and two-photon microscopes, a light-sheet microscope illuminates the focal plane with an objective orthogonal to the detection axis and images it in a single snapshot. Its combination of high contrast and minimal sample exposure make it ideal to image thick samples with sub-cellular resolution. To uniformly illuminate a wide field-of-view without compromising axial resolution, propagation-invariant light-fields such as Bessel and Airy beams have been put forward. These beams do however irradiate the sample with a relatively broad transversal structure. The fluorescence excited by the side lobes of Bessel beams can be blocked physically during recording; though at the cost of increased sample exposure. In contrast, the Airy beam has a fine transversal structure that is both curved and asymmetric. Its fine structure captures all the high-frequency components that enable high axial resolution without the need to discard useful fluorescence. This advantage does not carry over naturally to two-photon excitation where the fine transversal structure is suppressed. We demonstrate a symmetric and planar Airy light-sheet that can be used with two-photon excitation and that does not rely on deconvolution.

1 Introduction

Light microscopy fulfils a central role in biological research. It can provide direct evidence of processes and cellular or sub-cellular structures, and it can do so non-invasively. Until recently such studies were limited to thin and transparent samples. Beyond a few layers of biological cells, most light is scattered and all contrast is lost due to out-of-focus scattering or fluorescence. Confocal fluorescence microscopy can improve contrast by singling out the light from the focal plane, though at the cost of high irradiation exposure and consequent photo-bleaching and damage. Larger specimen must be chemically fixed and sliced prior to imaging. Naturally, this is an obstacle to the study of the many dynamic processes.

The introduction of planar illumination to fluorescence microscopy has revolutionised how large, dynamic, specimen can be studied. This microscopy technique decouples the illumination and the detection optics so that, optical sections can be imaged rapidly with high resolution and contrast. The orthogonal illumination largely avoids out-of-focus fluorescence. However, while tight focussing of the light-sheet can improve the axial resolution beyond that of wide-field detection optics, diffraction does place a trade-off on our ability to focus the light-sheet throughout the complete field-of-view.

2 Leveraging propagation invariance

To overcome this trade-off, we employed the propagation-invariant Airy beam to form an Airy light-sheet (Fig. 1). Together with Bessel beams and plane waves, Airy beams have a transverse intensity cross section that does not change with propagation distance. In other words, the same image quality can be expected throughout the entire field-of-view. Unfortunately, the axial image-sharpness is severely affected by the large transverse extent of both Bessel and Airy beams. Their width can be tens of times greater than the waist of a Gaussian beam produced with the same numerical aperture, thereby negating the propagation invariance advantage. Moreover, the Airy beam has a parabolically-curved trajectory and thus warped images. Several techniques can be used to recover sub-cellular resolution over the much larger field-of-view. Confocal slit de-scanning or techniques from structured illumination can be used to single out the focal plane fluorescence [1-3]. As with confocal microscopy, this improves contrast in exchange for elevating the sample exposure. It also precludes the use of a simple cylindrical lens to produce the light-sheet [4]. The ability to digitally deconvolve with high contrast is directly related to the characteristic asymmetry of the Airy light-sheet [5-7].

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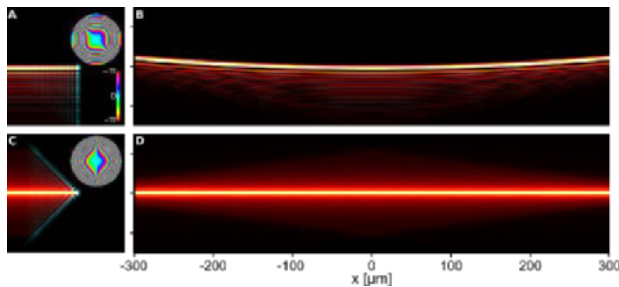


Fig. 1. Scanning an Airy beam to form a virtual light sheet as a curved surface (A-B), or planar illumination (C-D). Adapted from Hosny et al. [9] (CC BY 4.0).

3 Two-photon Airy light-sheet excitation

The impact of light-scattering on image quality grows with our ability to image larger volumes. Two-photon excitation with near-infrared fs-lasers can be used to illuminate deeper into tissue. This requires high peak-powers, hence the light-sheet should be created by scanning a focused beam. However, this non-linearity suppresses the outer lobes of the transversal structure. While this limits the effectiveness of the deconvolution [6,8], it also reduces its need to some extent. Yet, some digital correction is still required to correct for the curved trajectory of the Airy beam. Fig. 1C, shows how digital deconvolution can be completely avoided by rotating the curved Airy beam trajectory 45 degree into the plane, prior to scanning it into a planar virtual light-sheet. Naturally, this light-sheet has the same propagation-invariant profile as the Airy beam that formed it. This blurs the side-lobes into one-another, further reducing the need for deconvolution. We used the two-photon Airy beam light-sheet to image a 0.6mm-thick volume of Wistar rat brain (Fig 2). The high isotropic resolution across the entire field-of-view enabled us to image neuron structures spanning hundreds of microns while simultaneously resolving their microscopic synaptic spines (Fig 2D).

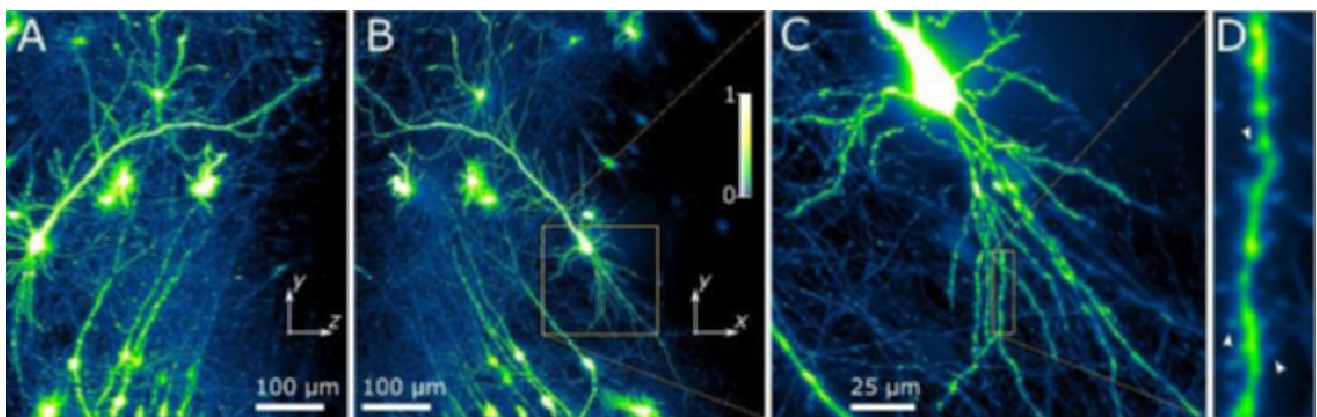


Fig. 2. Projections from a 3D volume of neuronal tissue, reproduced from Hosny et al. [9] (CC BY 4.0). Long-range connections between neurons can be seen in (A-B). A single neuron is highlighted in panel (B) and enlarged in panel (C). A single neuron dendrite is highlighted in panel (C) and enlarged in panel (D). Its dendritic spines are visible in panel (D).

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