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Planar light-sheet microscopy with curved Airy beams

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ABSTRACT

Light-sheet microscopy enables rapid 3D imaging of biological samples. Its field-of-view can be extended ten-fold by relying on propagation-invariant Airy beams. However, such beams propagate on a parabolic trajectory. By consequence, a light-sheet formed by Airy beams is not planar, thus warping the images. Here, we demonstrate a planar Airy light-sheet that does not rely on digital image restoration techniques for two-photon microscopy.

Keywords: Fluorescence Light-sheet Microscopy, Beam shaping, Propagation-invariant beams.

1. INTRODUCTION

Fluorescence light-sheet microscopy is rapidly adopted by biologists to non-invasively study intact specimen. 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 autofluorescence. Confocal fluorescence microscopy can improve contrast by blocking out-of-focus light, 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 advent of planar illumination fluorescence microscopy has revolutionised *in vivo* studies of large specimen. This microscopy technique decouples the illumination and the detection optics so that, optical sections can be imaged rapidly with high resolution and contrast. Although autofluorescence must be eliminated through other means [1], the orthogonal excitation completely avoids out-of-focus fluorescence within the Rayleigh length of the light-sheet. However, while tight focussing of the light-sheet can certainly improve the axial resolution beyond that of the wide-field optics, diffraction limits our ability to focus the light-sheet throughout the entire field-of-view.

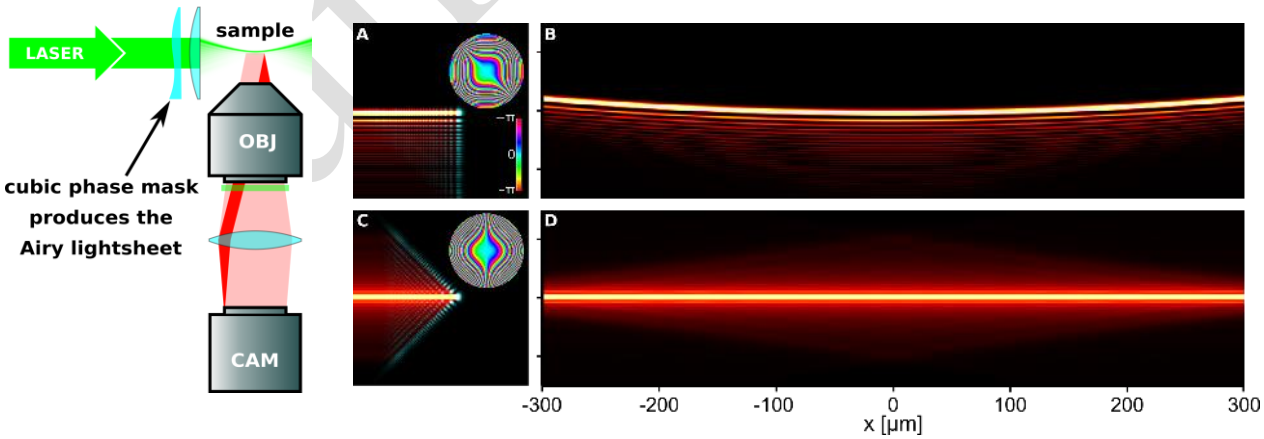

Figure 1 Left: A diagram of the Airy light-sheet fluorescence microscope. A collimated laser beam (green) is modulated by a cubic-polynomial phase mask and focused into the sample to produce a propagation-invariant Airy light sheet. The emitted fluorescence (red) is collected by the objective and reimaged onto the camera (CAM). The asymmetry of its profile enables high-resolution image reconstruction over an order of magnitude larger field-of-view. Right: adapted from Hosny et al. [10] (CC BY 4.0). Scanning an Airy beam with parabolic trajectory to form a virtual light sheet as a curved surface (A-B), or as planar illumination (C-D).

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2. EXTENDING THE IMAGING VOLUME WITH PROPAGATION-INVARIANT BEAMS

To overcome this trade-off, we employed the propagation-invariant Airy beam to form an Airy light-sheet (Fig. 1). As Bessel beams and plane waves, Airy beams have a transverse intensity cross section that is propagation invariant. I.e. the same image quality can be expected throughout the entire field-of-view. However, 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 Gaussian beam waist at the same numerical aperture, thus negating their propagation invariance advantage. Moreover, the Airy beam has a trajectory that curves along a parabola, thus leading to warped images. Several techniques can be used to recover sub-cellular resolution over the significantly larger field-of-view. Techniques such as confocal slit de-scanning or methods from structured illumination can be used to single out the focal plane fluorescence [2-4]. 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 [5]. The ability to digitally deconvolve with high contrast is directly related to the characteristic asymmetry of the Airy light-sheet [6-8].

3. THE PLANAR AIRY BEAM LIGHT-SHEET

With the ability to image larger volumes comes the increased importance of light scattering. Two-photon excitation can penetrate deeper into tissue. However, this non-linearity suppresses the outer lobes of the transversal structure. While this limits the effectiveness of the deconvolution [7,9], it also reduces its need to some extent. Yet, digital correction is still required to correct for the curved trajectory of the Airy beam. Fig. 1C, demonstrates how deconvolution can be completely avoided by rotating the curved Airy beam trajectory 45° into the focal 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 digital post-processing. 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).

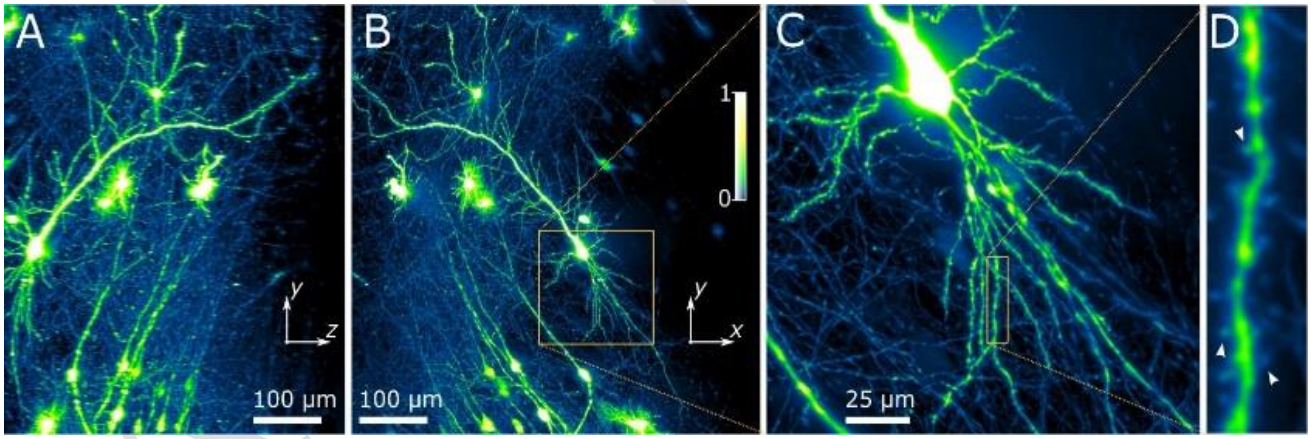

Figure 2: Projections from a 3D volume of neuronal tissue, reproduced from Hosny et al. [10] (CC BY 4.0). Long-range connections between neurons can be seen in (A-B). A single neuron is highlighted in panel (B) and enlarged in panel (C). A single neuron dendrite is highlighted in panel (C) and enlarged in panel (D). Its dendritic spines are visible in panel (D).

4. CONCLUSION

Planar Airy beam light-sheets can be created with a minimal adaptation to a conventional Airy beam microscope. It is sufficient to rotate the phase modulating element by 45 degrees. The planar Airy light-sheet mode is most beneficial for multi-photon excitation, where it eliminates complexity without a loss in imaging volume. The same is not true to the same extent for single-photon excitation. A rotatable phase mask could thus offer the best of both worlds for a microscope with both single and mutli-photon modalities [8].

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REFERENCES

[1] T. Vettenburg et al. “Photoswitching-Enabled Contrast Enhancement in Light Sheet Fluorescence Microscopy” *ACS Photonics* **4** (3), 424-428 (2017), doi:10.1021/acsphotonics.6b00838.

[2] F. O. Fahrbach & A. Rohrbach “Propagation stability of self-reconstructing Bessel beams enables contrast-enhanced imaging in thick media” *Nat. Commun.* **3**, 632 (2012), doi:10.1038/ncomms1646.

[3] T.A. Planchon et al. “Rapid three-dimensional isotropic imaging of living cells using Bessel beam plane illumination” *Nat. Methods* **8**, 417–423 (2011), doi:10.1038/nmeth.1586.

[4] B.-C. Chen et al. “Lattice light-sheet microscopy: Imaging molecules to embryos at high spatiotemporal resolution” *Science* **346** (6208): 1257998 (2014), doi:10.1126/science.1257998.

[5] Z. Yang et al. “A compact Airy beam light sheet microscope with a tilted cylindrical lens” *Biomed. Opt. Express* **5** (10), 3434-3442 (2014), doi:10.1364/BOE.5.003434.

[6] G. Muyo and A.R. Harvey “Decomposition of the optical transfer function: wavefront coding imaging systems” *Opt. Lett.* **30**, 2715-2717 (2005), doi:10.1364/OL.30.002715.

[7] T. Vettenburg et al. “Light-sheet microscopy using an Airy beam” *Nat. Methods* **11** (5), 541-544 (2014), doi:10.1038/nmeth.2922.

[8] Y. Zhou et al. “Twin-Airy Point-Spread Function for Extended-Volume Particle Localization” *Phys. Rev. Lett.* **124**, 198104 (2020), doi:10.1103/PhysRevLett.124.198104.

[9] P. Piksarv et al. “Integrated single- and two-photon light sheet microscopy using accelerating beams” *Sci. Rep.* **7** (1): 1-8 (2017), doi:10.1038/s41598-017-01543-4.

[10] N.A. Hosny et al. “Planar Airy beam light-sheet for two-photon microscopy” *Biomed. Opt. Express* **11**(7): 3927-3935 (2020), doi:10.1364/BOE.395547.

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