Light sheet imaging the Airy way

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Airy light fields, with their unique transversal structures, offer new possibilities for 3D imaging by light sheet microscopy.

Recent decades have seen an expansion in the breadth of light-based imaging techniques that address problems in biomedicine. Despite perceived limitations in terms of penetration depth and resolution, optical imaging has made significant strides to encroach upon the traditional territory of other modalities, such as electron microscopy and x-ray. At the nanoscale, optical methods exploit light fields in tandem with the properties of fluorescent proteins to answer biological questions in neuroscience and virology that were previously restricted to electron microscopy. At the larger scale, the same methods are making headway to generate biopsies solely with light. A prime example is optical coherence tomography, now a mainstay in ophthalmology and poised to make an impact imaging organs in vivo for colon cancer and atherosclerosis.

At this large scale, the burgeoning need for imaging intact biological specimens has led to the resurrection and refinement of light sheet imaging. Instead of illuminating the whole sample, this technique irradiates a section with a thin ‘sheet’ of light, with the fluorescence or scattered light collected orthogonally to the illumination. The method is appealing for its rapid 3D imaging capability, its low phototoxicity, and its high contrast due to the targeted illumination. Light sheet microscopy is an ideal tool for exploring the function of the brain in model organisms such as zebrafish or C. elegans.

While exceptionally promising, a major challenge for light sheet imaging is creating an optical version of a sheet that is ideally as thin as possible while extending right across the (biological) specimen or sample of interest. According to the laws of Gaussian optics, beam divergence scales with the beam waist, and the same is true for a light sheet. The Rayleigh range—a measure of the distance over which the beam remains uniformly thin—is inversely proportional to its thickness (see Figure 1). Propagation-invariant Bessel beams, generated by passing a laser beam through an axicon or equivalent device, can create an extended propagation-invariant light sheet. Bessel modes are excellent for multiphoton light sheets, where the process naturally limits the contrast reduction due to the outer transverse rings of the mode profile. This is not the case for single photon imaging, where we need to use confocal geometries or structured illumination techniques. Single photon, linear light sheet microscopy is not only less expensive than its multiphoton counterpart, it also lends itself to compact geometries and readily gives access to a large range of fluorophores. A powerful, simple way to achieve this would represent a step change for the field.

Our exploration of the optimum beam profile for single photon light sheet imaging has led to the surprising conclusion that Airy light fields are the beam of choice for such studies. The Airy light field is perhaps best known for the curved trajectory of its asymmetric intensity profile, and at first glance it does not appear particularly convenient for light sheet microscopy. However, as with the Bessel beam, it has a relatively broad transversal structure that is invariant to propagation. Although the main lobe of the Airy beam is several times wider than that of the Bessel and Gaussian modes, its transversal structure has the unique property that it allows the computational

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retrieval of high-resolution information. Instead of illuminating a single slice of the sample with a relatively broad Gaussian light sheet, the Airy light sheet illuminates a small volume with a fine transversal structure. Therefore, each frame of the recorded data contains information for a small volume around it, which we can recombine into the final image using a simple deconvolution. Such a process is simply a multiplication of the Fourier-transformed recorded data and light sheet intensity, which is only possible when the latter has sufficiently large values. In contrast to the Fourier transform of a Bessel beam light sheet, that of the Airy beam has relatively high values. Combined with its propagation invariance, the Airy light sheet gives access to a field of view 10 times larger with high axial resolution (see Figure 2).

The advantage of moving beyond the Gaussian beam is even more pronounced when considering biological tissue with heterogeneities in refractive index. Imaging large volumes at depth necessitates associated aberrations in the illumination and detection path of the light sheet microscope, which are countered using adaptive correction methods. However, the Airy beam appears to be more tolerant of these aberrations, a phenomenon referred to as ‘self-healing.’ Therefore, we expect that Airy light sheet illumination might, to some extent, obviate the need for such adaptive measures.

Beam shaping can dramatically change the abilities of a light sheet microscope, and only requires the introduction of a spatial light modulating element into an existing setup. Our technique exploits the increased flexibility in beam shaping by relying on deconvolution for the image formation. The choice of light sheet is pivotal to the capabilities of the microscope, and the Airy mode is one we see gaining wider adoption and paving the way for further innovation.

So far, we have only considered the Bessel and Airy beams for light sheet microscopes. However, while desirable, propagation invariance is not strictly required, and a large number of beam types remain untested. In the future, we will explore the use of these alternatives to further the development of light sheet microscopy.

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Tom Vettenburg is a research fellow in the group led by Kishan Dholakia. His current research focuses on beam shaping and computational techniques for biophotonics. His work on the Airy light sheet microscope was recently published in *Nature Methods*.

Kishan Dholakia’s research interests cover photonics with particular emphasis on shaping light, trapping of microparticles, and biophotonics. He has around 14,500 career citations, an h-index of 64, and is a Fellow of SPIE, the Optical Society, and the Royal Society of Edinburgh.

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References


