1723 Microsc. Microanal. 21 (Suppl 3), 2015
doi:10.1017/S1431927615009393 Paper No. 0860

Airy Beams for Light-sheet Microscopy

Jonathan Nylk[1,2], Zhengyi Yang[1], Miguel Preciado[1], Michael Mazilu[1], Tom Vettenburg[3], Clara Coll-Lladó[2], David E. K. Ferrier[2], Tomáš Čižmár[4], Frank. J. Gunn-Moore[2], Kishan Dholakia[1]

[1.] SUPA, School of Physics and Astronomy, University of St Andrews, St Andrews, UK
[2.] School of Biology, University of St Andrews, St Andrews, UK
[3.] Present address: Department of Bioengineering and Aerospace Engineering, Universidad Carlos III de Madrid, Madrid, Spain
[4.] Present address: School of Engineering, Physics and Mathematics, University of Dundee, Dundee, UK

Light-sheet microscopy (LSM) is a promising technique for live imaging as it facilitates fast, high-contrast imaging of large volumes with minimal phototoxicity [1]. Variations on this imaging method are required to give high-resolution over large fields-of-view and to enable imaging at greater depths into specimens.

A fundamental limitation of LSM with Gaussian beam illumination is the rapid divergence of the beam which prevents a uniformly thin light-sheet, thus impeding high-resolution imaging over a large volume. The use of Bessel beams for light-sheet illumination has grown in popularity because the inherent propagation-invariance of the Bessel beam can be utilized to produce uniformly thin light-sheets over an extended field-of-view [2] however, the extended transverse structure of the Bessel beam lowers the achievable axial resolution and must be combined with additional techniques such as multi-photon excitation, confocal scanning, or structured illumination (see, for example, [3]) to recover high-resolution images.

We present a number of innovations using the Airy beam as an alternative propagation-invariant beam for single-photon excitation LSM. The Airy beam also has an extended transverse structure but, rather than detract from image quality, this transverse structure facilitates high-contrast, high-resolution imaging when combined with a simple, one-dimensional deconvolution algorithm [4]. This technique allows for isotropic high-resolution imaging over a ten-fold larger field-of-view compared to a Gaussian light-sheet, and without additional, unnecessary irradiation of the specimen. Figure 1 compares images obtained using Gaussian, Bessel, and Airy light-sheets. Additionally, the broad distribution of energy across the Airy beam lowers peak power and reduces phototoxicity.

Unlike the Bessel light-sheet, which results from an intrinsically two-dimensional pupil modulation and requires the Bessel beam to be scanned to make a light-sheet, an Airy light-sheet can be formed from a one-dimensional cubic phase modulation with a tilted cylindrical lens. This can lead to an inexpensive and accessible Airy light-sheet microscope [5].

An exponentially decreasing signal across the image can result from absorption and scattering of the light-sheet in large biological samples. Our latest approach shows that an attenuation compensating Airy beam can counteract the effects of absorption [6]. We apply this as a novel single-photon excitation approach to deliver a light-sheet deep into specimens without increasing the unnecessary irradiation of other parts of the specimen. The cylindrical pupil function required to produce an Airy light-sheet which compensates for linear attenuation is $P(u,0)=\exp(\sigma_u u)*\exp(2\pi i\alpha u^3)$, where u is the normalised pupil coordinate orthogonal to the light-sheet, α determines the propagation-invariant length of the Airy light-sheet, and σ_u is the strength of attenuation compensation. Figure 2 compares the image quality obtained in a strongly absorbing medium with and without attenuation compensated Airy light-sheets.

In summary, we have shown the benefits of Airy beams for LSM. Airy beams can enable high-resolution imaging over a large field-of-view and their broad transverse structure ensures low phototoxicity. The Airy beam can be easily generated from a tilted cylindrical lens, thus bringing the benefits of Airy beam LSM to a greater number of end users. The use of attenuation compensated Airy beams is also presented as a novel approach to obviate the effects of absorption and scattering in the sample.

References:

[1] J. Huisken, and D. Y. R. Stainer, Development **136** (2009), p. 1963-1975.
[2] F. O. Fahrbach and A. Rohrbach, Opt. Express **18** (2010), p. 24229-24244.
[3] L. Gao *et al*, Cell **151**(6) (2012), p. 1370-1385.
[4] T. Vettenburg et al, Nat. Methods **11** (2014), p. 541-544.
[5] Z. Yang et al, Biomed. Opt. Express **5**(10) (2014), p. 3434-3442.
[6] M. A. Preciado, K. Dholakia, and M. Mazilu, Opt. Lett **39**(16) (2014), p. 4950-4953.

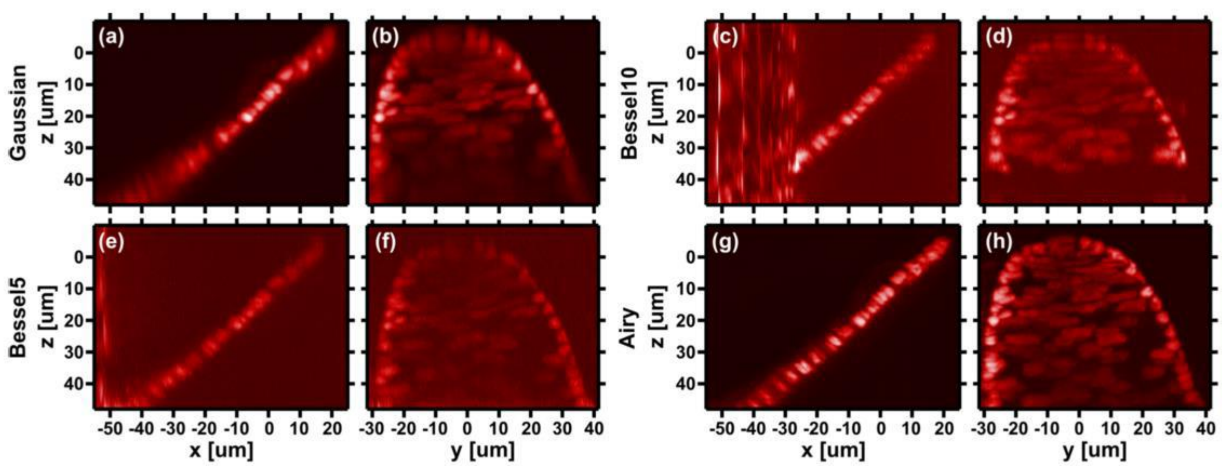

Figure 1. Comparison of various light sheet types imaging the dorsal end of the notochord in a fixed amphioxus (*Branchiostoma lanceolatum*). Nuclei have been fluorescently labelled with propidium iodide. y-axis and x-axis projections, respectively for the case of (a,b) Gaussian, (c,d) Bessel10, (e,f) Bessel5, and (g,h) Airy beam illumination. Bessel10 and Bessel5 denote Bessel beams with a propagation invariance of ±21 and ±42µm from focus of a Gaussian beam respectively.

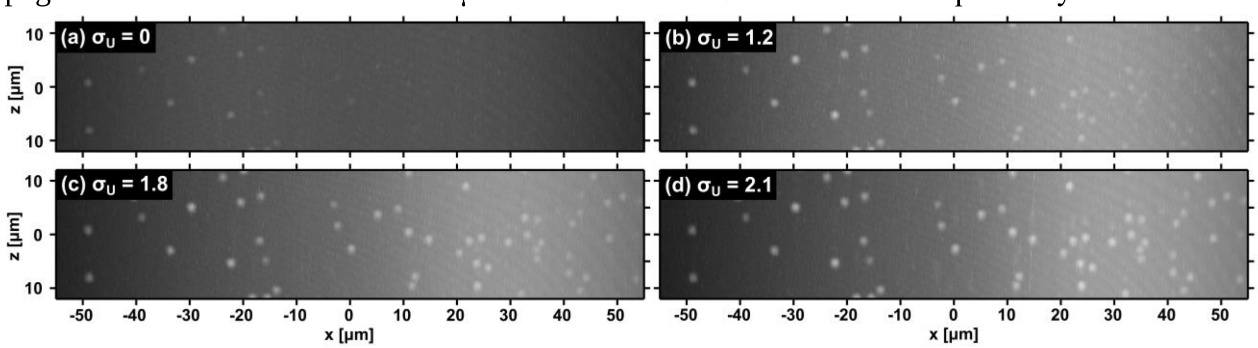

Figure 2. Comparison of attenuation compensated Airy beam light-sheets. y-axis projections are shown for fluorescent microspheres embedded in an absorbing medium.

https://doi.org/10.1017/S1431927615009393 Published online by Cambridge University Press