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Airy Beams for Light-sheet Microscopy

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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.

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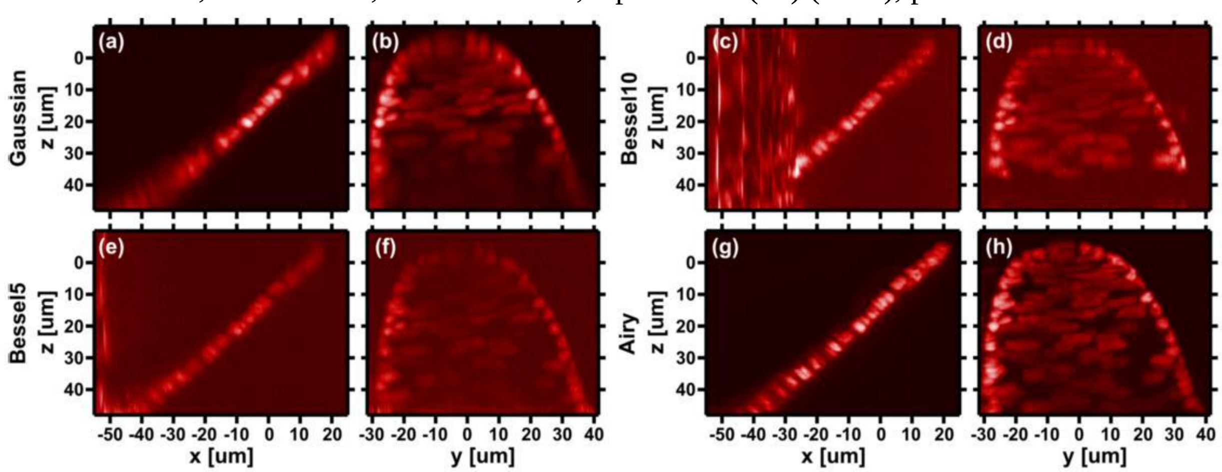

Figure 1. Comparison of various light sheet types imaging the dorsal end of the notochord in a fixed amphioxus (*Branchiostoma lanceolatum*). Nuclei have been fluorescently labelled with propidium iodide. y-axis and x-axis projections, respectively for the case of (a,b) Gaussian, (c,d) Bessel10, (e,f) 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.

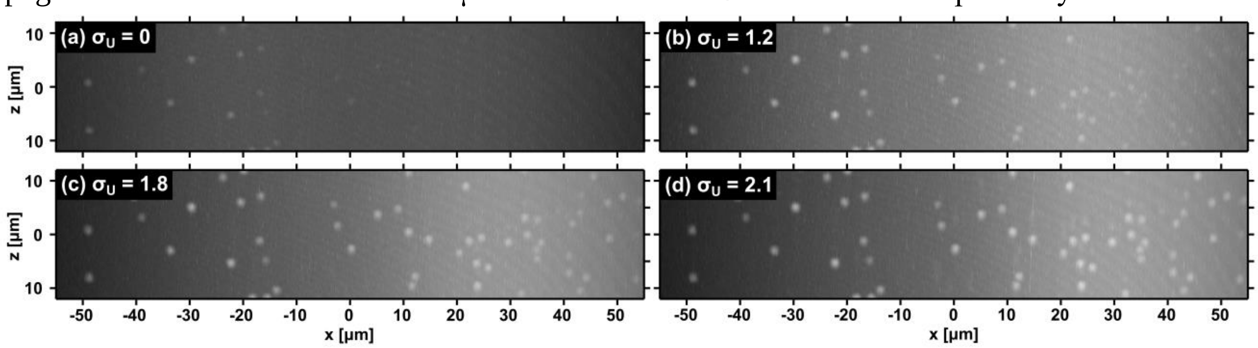

Figure 2. Comparison of attenuation compensated Airy beam light-sheets. y-axis projections are shown for fluorescent microspheres embedded in an absorbing medium.