High-speed computational localisation microscopy in 3D

Author(s): Yongzhuang Zhou, Paul Zammit , Vytautas Zickus, Jonathan M. Taylor and Andrew R. Harvey,
Institution(s): University of Glasgow
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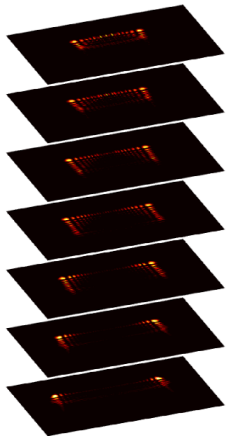
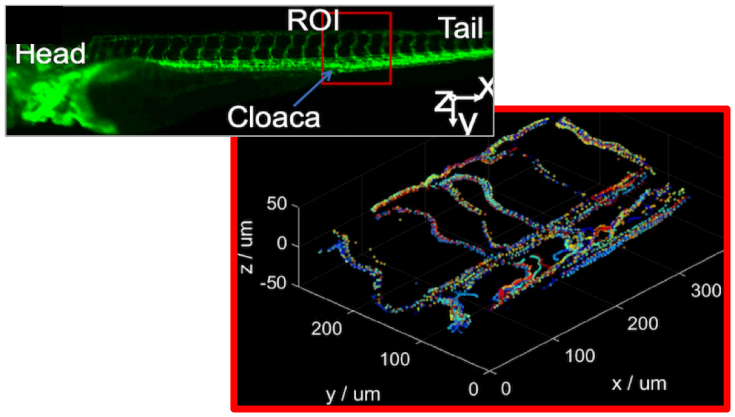
Abstract
We have demonstrated a new capability for high-speed microscopy in 3D, with a precision that is far smaller than a single cell and throughout a volume that can be large enough to encompass a single animal, albeit a small animal. This has a direct application for understanding blood flow, how cells exert forces on each other and for imaging single protein molecules.



twin-Airy PSF variation



Fluorescence image of a zebrafish and a 3D map of blood flow

Project Description
The localization of point sources in optical microscopy enables nm-precision imaging of single-molecules and biological dynamics. We report a new method of localization microscopy using twin Airy beams that yields precise 3D localization with the key advantages of extended depth range, higher optical throughput and potential for imaging higher emitter densities than are possible using other techniques. A precision of better than 30nm was achieved over a depth range in excess of 7um using a 60x, 1.4NA objective. We demonstrate the application of the technique to characterisation of high-speed blood flow throughout the full depth of the body of a zebrafish.

Project outcomes
- Demonstrated high-speed, extended-range localisation microscopy able to resolve more voxels than any other technique – and with higher emitter density
- Applications to blood-flow mapping, traction-force microscopy and single-molecule imaging are in process
- Collaboration welcome

1. Y. Zhou, P. Zammit, V. Zickus, J. M. Taylor, and A. R. Harvey, "Twin-Airy Point-Spread Function for Extended-Volume Particle Localization," Phys. Rev. Lett. **124**, 198104 (2020).
2. Y. Zhou, M. Handley, G. Carles, and A. R. Harvey, "Advances in 3D single particle localization microscopy," APL Photonics **4**, 060901–18 (2019).
3. Y. Zhou, P. Zammit, G. Carles, and A. R. Harvey, "Computational localization microscopy with extended axial range," Opt Express **26**, 7563–7577 (2018).