A flexible, adaptable design for sub-cellular light sheet microscopy

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Light sheet microscopy is often used to reduce phototoxicity and photobleaching in large samples, such as Drosophila or zebrafish embryos, allowing for minimally perturbative investigations of functional behaviour and developmental processes [1]. The beneficial aspects of light sheet illumination also apply to smaller samples, but sub-cellular light sheet microscopy is a technique currently accessible to few laboratories.

The OpenSPIM project has successfully democratised large sample light sheet microscopy, providing an open source hardware and software system that can be built and operated for minimal cost while providing performance comparable to, or exceeding, commercial implementations [2]. Our project aims to do the same for sub-cellular light sheet microscopy, with a simple, modular system aimed at high-resolution imaging of single cells and tissues.

By leveraging the recently developed Field Synthesis theorem [3] along with an optimised hardware and software platform, our system is capable of gentle 3D imaging at illumination intensities lower than any commercial or previously published system. A flexible illumination system allows for the production of Gaussian, Bessel, lattice and Lorentzian light sheets tuned to the sample of interest, ensuring maximum resolution and contrast [4, 5, 6, 7].

We introduce the principles of sub-cellular light sheet microscopy and discuss the design decisions leading to our approach. The performance of our system, capable of imaging a $200\,\mu\text{m} \times 200\,\mu\text{m}$ field of view at 100 Hz with truly simultaneous two-colour detection, is described and demonstrated on a variety of biological samples. We give an overview of our software stack, using the open-source Micro-Manager for hardware control and ImageJ for image processing and analysis [8, 9]. Finally, we present alternative hardware choices and directions for future work, particularly with respect to simplified adaptive optics light sheet microscopy.

References

[1] J. Huisken, et al., "Optical Sectioning Deep Inside Live Embryos by Selective Plane Illumination Microscopy", *Science*, **305**, 1007–1009 (2004).

[2] P. G. Pitrone, et al., "OpenSPIM: an open-access light-sheet microscopy platform", *Nature Methods* **10**, 598–599 (2013).

[3] B. J. Chang, et al., "Universal Light-Sheet Generation with Field Synthesis", *bioRxiv* 427468 (2018).

[4] F. O. Fahrbach, et al., "Microscopy with self-reconstructing beams", *Nature Photonics* **4**, 780–785 (2010).

[5] T. A. Planchon, et al., "Rapid three-dimensional isotropic imaging of living cells using Bessel beam plane illumination". *Nature Methods* **8**, 417–423 (2011).

[6] B.-C. Chen, et al., "Lattice light-sheet microscopy: Imaging molecules to embryos at high spatiotemporal resolution", *Science* **346**, 1257998 (2014).

[7] J. D. Manton & N. P. Barry, unpublished work.

[8] A. D. Edelstein, et al., "Advanced methods of microscope control using μManager software", *Journal of Biological Methods* **1**, 2 e10 (2014).

[9] C. T. Rueden, et al., "ImageJ2: ImageJ for the next generation of scientific image data", *BMC Bioinformatics* **18**, 529 (2017).