EXTENDING SPINNING DISK - REMOTE FOCUSING MICROSCOPY

Michele Gintoli, Sharika Mohanan, Elizabeth Williams, Alexander D. Corbett
Department of Physics and Astronomy, University of Exeter, UK,
Living Systems Institute, University of Exeter, UK,
E-mail: a.corbett@exeter.ac.uk

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FAST 3D IMAGING: A key challenge of fluorescence microscopy is to capture the complex three-dimensional behaviour of living organisms at high spatial and temporal resolution. Confocal microscopes routinely provide 3D images at subcellular resolution, but using a point scanning strategy severely limits the rate at which images can be acquired. To circumvent this limitation, structured illumination devices can be used, providing higher frame rates for a small compromise in axial resolution and/or optical efficiency.

SPINNING DISK - REMOTE FOCUSING: To quickly image multiple planes at high speeds, an increasingly popular choice is remote focusing (RF) [1], which forms a remote three-dimensional image of the sample that can be rapidly scanned without the risk of specimen agitation. In a recent work [2], we presented the combination of an RF platform and an off the shelf spinning disk (SD) module. This SD-RF system used a 0.8 numerical aperture objective, obtaining 5 µm and 490 nm of axial and lateral resolution respectively. Closed loop control of the piezo-electric axial scanner limited acquisition rates to 1 volume per second.

INCREASING SPEED AND RESOLUTION: In this presentation we will show how the addition of an adaptively corrected initialisation routine has allowed the open-loop control of the SD-RF microscope during acquisition, increasing the sampling rate to over 10 volumes/s. An optical path upgrade, together with the use of a 1.15 numerical aperture imaging objective, has improved light collection efficiency and brought axial and lateral resolutions below 2 µm and 350 nm, over a ±100 µm range. We demonstrate the improved signal and spatio-temporal resolution by imaging coordinated neuronal activity in *Platynereis dumerilii* (sea worm) larvae over large axial separations (Figure 1).

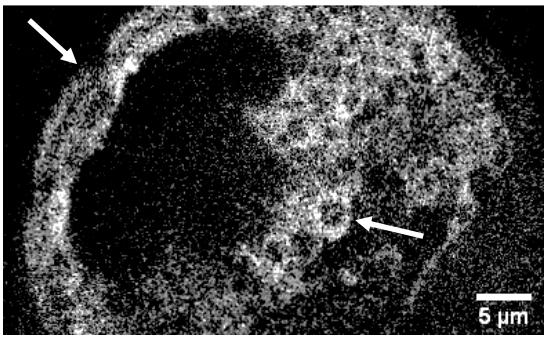

Figure 1. SD-RF image of *Platynereis dumerilii* larva. Correlation between neuronal firing (right arrow) and cilia activation (left arrow) is tracked using GcaMP7s labelled calcium transients.

REFERENCES

[1] E. J. Botcherby, R. Juškaitis, M. J. Booth, and T. Wilson, 'An optical technique for remote focusing in microscopy', *Optics Communications*, vol. 281, no. 4, pp. 880–887, Feb. 2008, doi: 10.1016/j.optcom.2007.10.007.

[2] M. Gintoli *et al.*, 'Spinning disk-remote focusing microscopy', *Biomed. Opt. Express*, vol. 11, no. 6, p. 2874, Jun. 2020, doi: 10.1364/BOE.389904.