

## DUAL-VIEW OBLIQUE PLANE MICROSCOPY

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### ABSTRACT

While OPM [1] has fewer constraints in terms of sample preparation and the ability to easily image large arrays of specimens in multiwell plates, it does not have the benefits of multi-view light-sheet fluorescence microscopy [2]. We present a folded OPM configuration called dual-view OPM (dOPM) that enables two separate orthogonal views of the specimen to be achieved [3]. Only a single mechanical actuator is required in order to scan the light sheet and detection plane through the specimen and to switch between the two orthogonal views. This approach enables the benefits of dual-view SPIM e.g. [4] to be obtained when performing OPM.

To quantify spatial resolution of the dOPM system, volumes of 0.2  $\mu\text{m}$  TetraSpeck™ Microspheres embedded in 1% agarose were recorded and full width half maximum (FWHM) values for line profiles along each axis of the primary microscope Cartesian coordinates are found to determine lateral and axial resolution. While fused bead volumes showed a slight degradation in spatial resolution, deconvolved volumes show an improvement in resolution along each axis and a more isotropic 3D point spread function.

To demonstrate that dOPM can be used to improve spatial resolution and overcome sample-induced artefacts in biologically relevant samples, multi-cellular mammary tumour organoids with size on the order of 100  $\mu\text{m}$  were imaged. Figure 1 shows reconstructed dual-view deconvolved images for a single organoid at three axial positions relative to the sample coverslip.

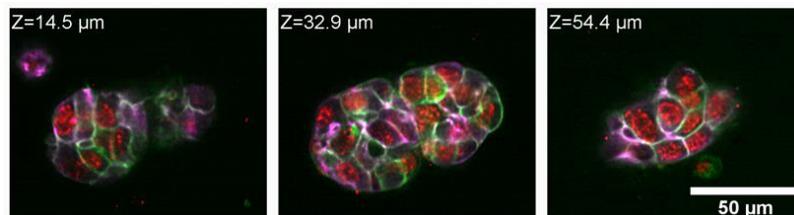


Figure 1: Multi-cellular mammary tumour organoids imaged in three fluorescent channels. Actin in magenta (endogenously expressed GFP), plasma membrane in green (endogenously expressed tdTomato) and nucleus in red (SiR-DNA). The Z value indicates depth into the organoid measured from the coverslip.

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