HIGH SPEED TIME-LAPSE THREE-DIMENSIONAL FLUORESCENCE IMAGING WITH OBLIQUE PLANE MICROSCOPY

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ABSTRACT:

The oblique plane microscope (OPM) is a light-sheet microscope enabling fast acquisition of 3-D volumes for samples ranging from single cells to tumour spheroids\textsuperscript{1,2}. OPM uses a single high numerical aperture for both light sheet illumination and fluorescence collection and it can therefore be implemented as add-on to a standard microscope frame. Hence OPM is compatible with conventional sample preparation techniques such as microscope slides or standard multi-well plates. The current microscope acquires in two emission channels simultaneously enabling e.g. two separate fluorescent probes to be imaged or ratiometric FRET imaging.

We demonstrate the capabilities of OPM through imaging cardiomyocytes to study the relationship between calcium sparks characteristics and cellular structures (t-tubules). From our high-speed 3D data acquired at 25 volumes per second, we have shown that in cells from hearts with myocardial infarction, calcium waves predominantly originate from well-tubulated regions of the cell. Associated data show that calcium sparks occur most frequently in well-tubulated regions and are larger and longer between two t-tubules.

We will also describe the first results towards a 3-D OPM plate-reader for time-lapse acquisition of live samples, showing data from live spheroids and exemplar ratiometric FRET data. We imaged multiple spheroids over 12 hours, with a typical acquisition time of just 3 s per spheroid for 400x1280x1024 voxels per spheroid, see figure 1.

\textsuperscript{1}C. Dunsby, Opt. Express 16, 20306-20316 (2008).