Light sheet microscopy for high content 3-D imaging of 3-D tissue cultures in a 96-well plate format

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Light sheet fluorescence microscopy (LSFM) uses a sheet of laser illumination to selectively excite fluorescence from a single plane within the sample and has the benefits of minimal out-of-plane photobleaching and phototoxicity. However, conventional light sheet microscopes use separate microscope objectives for sample illumination and fluorescence detection and so are not directly compatible with imaging in conventional 96-well plates. Oblique plane microscopy (OPM)[1] is a form of light sheet microscopy that uses a single microscope objective on a commercial microscope frame to illuminate the sample with a tilted illumination sheet and to receive fluorescence from the sample. OPM enabled the first near-video rate time-lapse 3D imaging with sub-cellular resolution[2] and has been applied to study the spatial origin of calcium waves within isolated cardiac myocytes at video volumetric frame rates[3]. We have recently adapted the OPM system to provide light sheet fluorescence microscopy in 96-well plates using a stage scanning approach (ssOPM)[4]. A motorized x-y stage is used to translate the sample laterally through the light sheet and the stage provides electrical synchronisation of image acquisition in two spectral channels.

We demonstrate the application of the ssOPM system to study cells grown in 3D in a collagen gel in a commercially available glass-bottom 96-well plate. In one scenario, cells were seeded onto the bottom a 96-well plate, overlayed with collagen type I gel and then allowed to invade up into the gel for 72 hrs prior to fixing. In a second scenario cells were resuspended in collagen and plated in a 96-well plate. The plate was centrifuged to ensure cells were at the bottom of the wells. Subsequently cells were allowed to invade upwards for 24 hrs prior to fixing. The stage-scanning OPM system enables a large $4\times0.32\times0.15$ mm^3 volume to be imaged in 3D with subcellular resolution, with $2000\times1280\times1000$ pixels per channel giving ~10 GB of data and taking 56 s to acquire. Each field provides data on 100's of cells. The total data acquisition time for 60 wells was 80 minutes and total data volume acquired was 0.6 TB. An automated image analysis pipeline has been developed enabling 3D morphological parameters to be extracted for each individual cell and across a range of siRNA knockdowns. These results pave the way for 3D image-based cytometry and automated morphological quantification of cells grown in 3D cell culture in a 96-well format.

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4 Maioli, V. *et al.* Time-lapse 3-D measurements of a glucose biosensor in multicellular spheroids by light sheet fluorescence microscopy in commercial 96-well plates. *Sci Rep-Uk* **6**, 37777 doi:10.1038/srep37777 (2016).