HIGH CONTENT 3D AND 4D OBLIQUE PLANE MICROSCOPY IN 96- AND 384-WELL PLATE FORMATS

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We report the development and application of an oblique plane microscopy [1,2] based plate reader, which allows rapid high content 3D imaging in 384-well plates, with sub-cellular resolution. Developments of the system's architecture improve the image acquisition and data storage work flow, which allow the automated 3D time lapse imaging of 100-200 cell volumes per minute, are presented. We then apply the system to automatically quantify multiple cell shape parameters in 3D in fixed plates at a single time point and in live plates over time. We present our approach for 3D cell segmentation and shape analysis and its application to studying differences in cell shape over a panel of 6 melanoma cancer cell lines and over a panel of 175 Si-RNA gene knockdowns in fixed LM2 cells. For time-lapse 3D imaging, we present cell shape measurements over time that are achieved by combining our custom-written MATLAB-based cell shape analysis with cell tracking in 3D provided by the TrackMate [3] software. The aim of this work is to use high-content microscopy to study the effects of different genes on the ability of cancer cells to change their shape and to invade and metastasise. We can currently image 8 volumes (each 884x320x144 μm^3, 819 gigavoxels) at 4.5 minute intervals and present plans to how this can be increased to 12-16 volumes at the same rate.

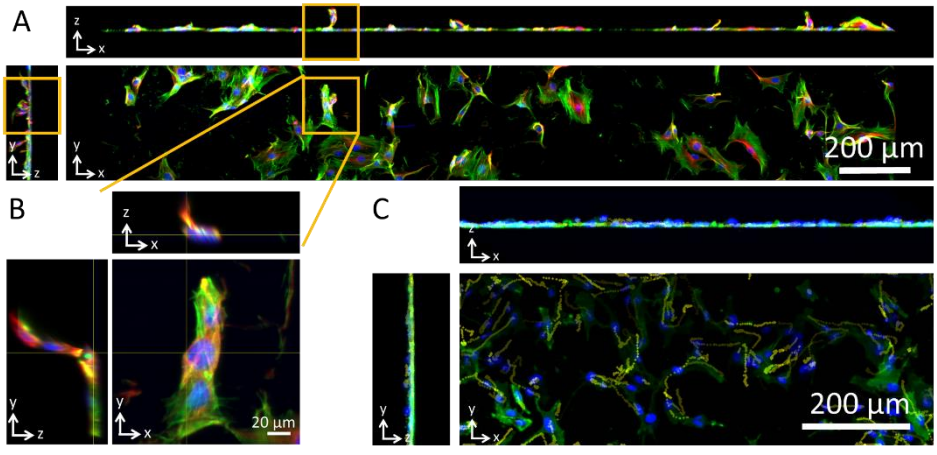

Figure 1: (A) Othogonal views of typical OPM volumes from a 384 plate (green-actin, red-tubulin, blue-nuclei). **(B)** Zoom of the region indicated in (A). **(C)** Cells tracked (yellow) over 6 hours in 3D.

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[2] V. Maioli *et al.*, Sci. Rep. 6:37777 (2016).
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