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High content 3-D light sheet microscopy in 96 and 384-well plate formats applied to study cancer cell size, and invasiveness and morphology in 3D matrices

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Conventional light sheet fluorescence microscopy (LSFM) requires two microscope objective lenses orientated at 90° to one another. However, their proximity to one another and the sample makes high content imaging of samples mounted on conventional 96 and 384-well plates difficult. Oblique plane microscopy (OPM)1 uses a single high numerical aperture microscope objective to provide both fluorescence excitation and detection whilst maintaining the advantages of LSFM.

We present the development and application of a stage scanning OPM (ssOPM)2 approach for high content light sheet fluorescence imaging in commercially available glass and plastic-bottomed 96 and 384-well plates. 3D images of cells were acquired by scanning the sample through the tilted light sheet at a constant velocity. Methods for implementing autofocus during acquisition together with the data acquisition pipeline will be discussed.

The ssOPM system was used to perform functional screens for regulators of cell size and 3D invasiveness. In the screen for regulators of size, melanoma cells were grown as 2D cultures in 384-well plates and genes were systematically knocked down with a library of 120 siRNAs. For 3D invasion assays, 9 siRNAs were used and plates were incubated for 24 hours allowing cells to invade vertically into the gel prior to fixation and staining. For both assays, 100s-1000s cells were quantified per condition to allow 3D cytometric data analysis.

We developed a MATLAB 3D image analysis pipeline for automated segmentation and morphological quantification of the image data. This allowed determination of cell size in 2D and 3D, measurement of cell invasiveness into the collagen matrix, and quantification of cell morphology of invading cells. The ssOPM approach will enable a better understanding of which genes are responsible for cancer cell size determination and invasion in 3D cultures.

1 Dunsby, C. Opt. Express 16.25 (2008): 20306-20316; 2 Maioli, V., et al. Sci Rep 6:37777 (2016).

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