OBLIQUE PLANE MICROSCOPY 3D PLATE-READER INVESTIGATION OF CONTROL OF CELL SHAPE IN DISTINCT MICROENVIRONMENTS

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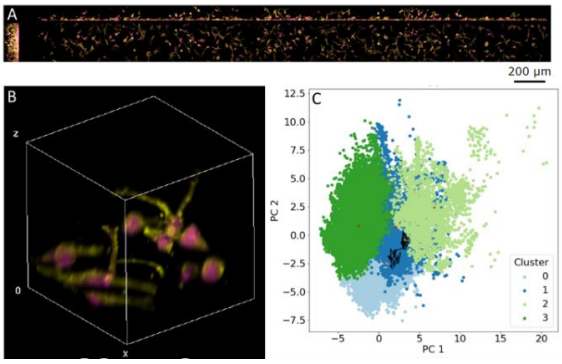
Keywords: OPM, Single objective light sheet, 3D, time lapse, segmentation

Cell shape and migration are essential to metastasis in cancer, and cell shape control by rho-regulators is dysregulated in diseases including cancer. We applied oblique plane microscopy (OPM) to study the physical environments in which different rho-regulators regulate the shape of WM266.4 melanoma cells.

OPM [1] retains many of the advantages of light-sheet microscopy but allows imaging of more conventionally-mounted samples. One such example is the ability to perform light-sheet microscopy in multi-well plates [2] where cells have been cultured in a 3D collagen matrix. OPM therefore allows us to image cells in the same well (chemical environment) but distinct physical environments (coverslip proximal and distal).

In fixed cells OPM allowed imaging of ~200 cells per well across 3 plates, taking 90 minutes to image each plate. The spatial resolution was 0.5x0.5x5 μm^3. To build upon this work we then imaged live cells in a collagen and tracked cell shape changes. This study imaged 30 wells every 5 minutes for 9 hours tracking how drug treatments affect cell morphological plasticity.

To analyse the data, automated 3D cell segmentation approaches were investigated. We verified that our measurements were robust across segmentation method (active contour vs. Otsu's method) and that shape changes were not due to the anisotropic PSF or the inherent spatial variation in the PSF due to the divergence of the Gaussian light sheet. By cluster analysis we identified relevant cell shape parameters and characteristic shape changes that occur between coverslip proximal and distal cells in control conditions. In fixed cells we identified four rho-regulators that change cell shape in either proximal or distal conditions. In live cells we were able to track individual cells moving between shape clusters.



(A) Full volume from one well. (B) 3D render of subvolume from one well. (C) Tracking cells in PCA space based on shape features. Colours correspond to clusters based on k means clustering.

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