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Image Analysis and Quantification of 3D Cancer Cell Migration

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Abstract

Metastatic tumors are known for their ability to migrate toward circulatory apparatus and detach from the primary tumor. Generally, metastasis is quantified *in vitro* using migration assays that are normally measured in two dimensions (2D). Three-dimensional (3D) migration assays can better mimic cancers by providing similar microenvironments to those observed *in vivo*. Imaging 3D cell cultures requires multiple 2D images stacked along a Z-axis; however, imaged cells would be in-focus at varied z-positions at different time points due to the characteristics of cell migration. Our goal in this study was to analyze in-focus cell images and quantify cell migration in 3D in high throughput. Briefly, Hep3B human hepatoma cell line in alginate was printed on top of a layer of chemoattractants in a microwell chip and cultured over time to model hepatocellular carcinoma. Acquired cell images were analyzed using a Fast Fourier Transform (FFT) to create a histogram of pixel brightness variation within an image. We selected a specific frequency range that would correspond to a sharp change in pixel brightness, a spheroid's edge, while the rest was subtracted to delete out-of-focus cells. In-focus cell images were recreated by reverse FFT, and ImageJ macros have been used to calculate the brightness of each corrected image in our 3D culture. By correlating pixel brightness to cell number, it allowed us to calculate the average position of all the cells in our 3D culture, based on brightness and z-position of the cell image. By measuring the change in average position over time, we created a quantifiable method to measure cell migration in 3D.