A quantitative analysis of cell motility

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Through the many high-resolution microscopy techniques that have emerged in experimental biology over the last several decades, it has become possible to capture images containing rich spatiotemporal information at the cellular level. Unfortunately, there exists a bottleneck in extracting biologically relevant information from these images; given image data, microscopists typically spend considerable time and effort annotating, processing, and analyzing micrographs by hand. We have developed an automated image analysis scheme to quantify membrane velocity for time-lapse sequences of cell spreading, polarization, and movement.

We approached the problem as one of statistical image segmentation and velocity inference, where the edge of the cell needs to be identified in each frame from which the normal velocity of the cell edge can be calculated over all space and time. To segment the images, we employed a two-component Gaussian mixture model over pixel intensities, with the low- and high-intensity components corresponding to the regions outside and inside the cell, respectively. We used expectation-maximization [5], an iterative coordinate ascent algorithm that provably converges to a local optimum of the expected log-likelihood function, to learn the model parameters that maximize the probability of the observed image data. We subsequently used the maximum-likelihood parameter estimates to calculate an intensity threshold from which pixels were clustered into regions outside and inside the cell, identifying the cell edge. The resulting boundary was parameterized by arc-length to accommodate arbitrarily complex boundaries and the normal velocity of each point on the contour was calculated from spatial and temporal gradients of the image data using optical flow techniques. This resulted in the normal velocity as a function of arc-length and time, displayed in a color-coded plot in Fig. 1, from which revealing area and velocity statistics were calculated and correlated with experimental conditions. Fig. 1 illustrates experimental validation of the technique, where one can see a decrease in mean protrusive velocity of the cell edge with increase in the concentration of cytochalasin d, a drug known to reduce cellular motility.

The developed analysis has facilitated the formulation of mechanical model of cell spreading and resulting in the submission, acceptance, and publication of several papers on the topic [6, 4, 3, 1, 2]. We have released the current tools as an open-source MATLAB GUI, via http://cellmap.sourceforge.net, and have received positive feedback from several other labs who have adopted the software package for similar analyses.

References

[1] A. S. Ada-Nguema, H. Xenias, J. M. Hofman, C. H. Wiggins, M. P. Sheetz, and P. J. Keely. The small GTPase R-Ras regulates organization of actin and drives membrane protrusions through the activity of PLC-ϵ. *J Cell Sci.*, page 4364.http://jcs.biologists.org/cgi/content/full/119/20/4364, Apr 1 2006.

[2] Y. Cai, N. Biais, G. Giannone, M. Tanase, G. Jiang, J. M. Hofman, C. H. Wiggins, P. Silberzan, A. Buguin, B. Ladoux, and M. P. Sheetz. Nonmuscle myosin IIA-dependent force inhibits cell spreading and drives F-actin flow. *Biophys J.*, 91(10):3907–20. Epub 2006 Aug 18., Nov 15 2006.

[3] H. G. Dobereiner, B. J. Dubin-Thaler, J. M. Hofman, H. S. Xenias, T. N. Sims, G. Giannone, M. L. Dustin, C. H. Wiggins, and M. P. Sheetz. Lateral membrane waves constitute a universal dynamic pattern of motile cells. *Phys Rev Lett.*, 97(3):038102. Epub 2006 Jul 20., Jul 21 2006.

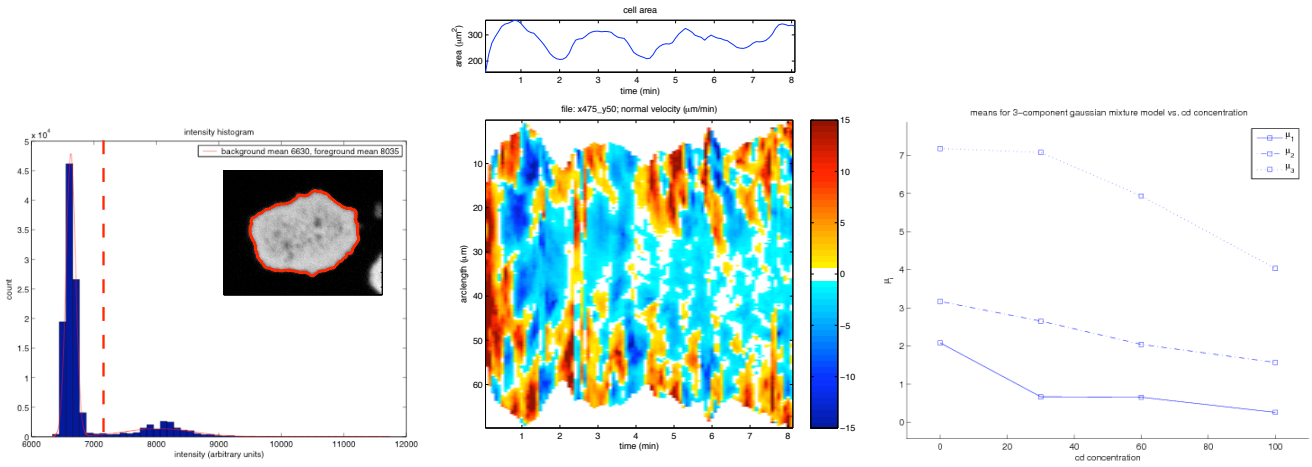

Figure 1: Left: A two-component Gaussian mixture model fit to an intensity histogram using expectation-maximization. The inset shows the segmented image, where the dashed red line shows the automatically calculated threshold determined from the model parameters. Middle: Velocity as a function of arc-length and time and area as a function of time for the entire time-lapse sequence; bright red corresponds to high-velocity protrusions, bright blue to high-velocity retractions. Left: Correlation of mean velocity of the cell edge as a function of cytochalasin d concentration; as expected, mean activity decreases as drug concentration increases.

[4] B. J. Dubin-Thaler, J. M. Hofman, H. Xenias, I. Spielman, A. V. Shneidman, L. A. David, H.-G. Dobereiner, C. H. Wiggins, and M. P. Sheetz. Quantification of cell movement reveals distinct types of edge motility during cell spreading. *Nature Precedings*, 2007. Submitted.

[5] T. Hastie, R. Tibshirani, J. Friedman, et al. *The Elements of Statistical Learning: Data Mining, Inference, and Prediction*. Springer, 2001.

[6] T. N. Sims, T. J. Soos, H. S. Xenias, B. Dubin-Thaler, J. M. Hofman, J. C. Waite, T. O. Cameron, V. K. Thomas, R. Varma, C. H. Wiggins, M. P. Sheetz, D. R. Littman, and M. L. Dustin. Opposing effects of PKCtheta and WASp on symmetry breaking and relocation of the immunological synapse. *Cell.*, 129(4):773–85, May 18 2007.