Deformable modeling for improved calculation of molecular velocities from single-particle tracking

Peter M. Kasson, Mark M. Davis, and Axel T. Brunger
Stanford University School of Medicine, Stanford CA 94305
kasson@slac.stanford.edu, mdavis@cmgm.stanford.edu, brunger@stanford.edu

Abstract

Single-particle tracking provides a powerful technique for measuring dynamic cellular processes on the level of individual molecules. Much recent work has been devoted to using single particle tracking to measure long-range movement of particles on the cell surface, including methods for automated localization and tracking of particles [1-3]. However, most particle tracking studies to date ignore cell surface curvature and dynamic cellular deformation, factors frequently present in physiologically relevant situations. In this report, we perform quantitative evaluation of single-particle tracking on curved and deforming cell surfaces. We also introduce a new hybrid method that uses non-rigid cellular modeling for improved computation of single-particle tracking trajectories on the surfaces of cells undergoing deformation. This method combines single-molecule and bulk fluorescence measurements in an automated manner to enable more accurate and robust characterization of dynamic cell physiology and regulation.

1. Introduction

Single-particle tracking provides a powerful means of analyzing protein movement, as it allows the measurement of individual protein trajectories. Measurement of these trajectories in sufficient numbers yields both population average behavior and the distribution of behaviors within the population, information that cannot be obtained from bulk measurements. Whole-cell single-particle tracking is a relatively recent development, and current analytic methodologies for measuring movement of individual cell-surface proteins ignore the surface-based nature of the movement, instead treating it as purely Euclidean [1-3]. This approximation is accurate only when the movement scale is small relative to the surface curvature. However, errors in Euclidean distance measurement are substantial for long-range cell-surface movements.

Cellular deformation, as occurs when a cell crawls or protrudes filopodia, is another complicating factor in measuring single-particle trajectories of membrane proteins. The observed movement of a labeled protein reflects both the movement of the protein relative to the surrounding membrane—the parameter of interest—and the non-rigid deformation of the cell. Since the goal of many single-particle tracking studies is to measure molecular behavior rather than cellular motion, separating these two types of movement is critical for accurate analysis. Deconvolution of protein movement within the cell membrane from gross cellular movement cannot be performed purely based on single-particle trajectory data. Instead, it is a challenging problem that requires additional information about the movement of the cell surface.

The experimental basis for this surface information can be provided using dual-channel microscopy of a single-particle probe and a bulk membrane probe. One way this can be accomplished is via simultaneous single-particle observation of bead-labeled protein molecules and bulk observation of fluorescently-labeled membrane proteins. We have designed a method for cellular deformation correction that utilizes bulk fluorescence data to generate an ellipsoidal model for the cell surface. This model then enables computation of corrected molecular trajectories from the single-particle data.

Tests of the ellipsoidal correction method on simulated data reveal substantially better performance than the previously published methods [2]. We have applied our analyses to receptor translocation on the surface of a T lymphocyte undergoing antigenic activation. This system provides a relatively well-defined biological frame of reference for measuring molecular motion, and our cellular model is readily generalizable to other systems in which single-particle tracking has been employed, such as the post-synaptic

receptor clustering that occurs in neuronal signaling [3].

2. Methods

2.1. Cellular surface modeling

We use ellipsoidal deformation as a first approximation to model cellular surface changes. The pole of one axis is constrained to lie at the biological reference point for molecular motions (in this case the T cell-antigen-presenting cell interface) to simplify inter-time-point correspondence. We obtain cellular shape data from bulk fluorescence microscopy of cell-surface proteins recorded simultaneously to single-particle measurements. Ellipsoid fitting to the cell image data was performed at each time point by extracting the cell surface using the Moss filter [2] and optimizing axis lengths to minimize the sum of squared distances to the surface data points. The first axis was constrained to the vector from the data center of mass to the biological reference point. Data points were projected onto the plane orthogonal to this vector, and principal components analysis on the projected data yielded the optimal orientations for the two remaining axes. Two-dimensional iterative optimization was used to determine the axis lengths for the best-fit ellipsoid.

2.2. Calculation of the corrected surface motion

After calculating a cell surface model in the above manner, we can compute surface distances from the reference point to any location on the cell surface. The intersection of the ellipsoid with the plane specified by the reference point, the model center, and any other surface point forms an ellipse, and arc lengths on this ellipse are geodesics on the ellipsoid. Surface distances on the ellipsoid from the reference point to a given labeled particle are determined by letting the transverse vector $\mathbf{v} = \mathbf{p} - \hat{\mathbf{a}}(\mathbf{p} \cdot \hat{\mathbf{a}})$, where $\mathbf{p}$ is the center-of-mass-subtracted particle position and $\hat{\mathbf{a}}$ is the unit vector along the interface axis. Let the elliptical parameter $\theta = \arg\min(|\cos\theta \cdot \mathbf{a} + \sin\theta \cdot \mathbf{v} - \mathbf{p}|)$, where $\mathbf{a}$ is the axis vector from the center of mass to the interface. The arc length L is then $L = |\mathbf{a}| \cdot E\left(\theta, \sqrt{1 - |\mathbf{v}|^2 / |\mathbf{a}|^2}\right)$, where E is an incomplete elliptic integral of the second kind [4].

Given this means of calculating ellipsoidal arc lengths, deformation corrections are performed as follows. Let arclen(t,θ_t) be the arc length given the ellipsoidal parameters **a** and **v** determined at time t and the particle parameter θ at time t. The surface motion is arclen(t,θ_t) – arclen($t-1,\theta_{t-1}$), and the correction factor for ellipsoidal motion is arclen(t,θ_{t-1}) – arclen($t-1,\theta_{t-1}$), the difference in distance due to change in ellipsoidal axes. The corrected surface motion then becomes arclen(t,θ_t) – arclen(t,θ_{t-1}).

3. Results

3.1. Quantitative evaluation of molecular tracking on deforming cells

To allow evaluation in a situation with known ground-truth values, the ellipsoidal correction method was first tested on simulated data. These data were generated using particle motion on a spheroid as has been described previously [5]. Simulations were performed for two sets of particles: 10,000 "bulk" particles and 10 "single-particle tracking" particles. To simulate the effect of non-motile bulk labeling, the directed velocity of the SPT particles was set 10-fold higher than that of the bulk particles. Deformation was simulated as a change in the major axis length of the spheroid.

To test performance under multiple conditions, three deformation regimes were tested. The initial major and minor axes of the spheroid were 21 and 14 μm respectively. The diffusion constant was set to 9×10^{-12} m^2/s throughout the experiment. For the first 50 second of simulation time, the rate of deformation was set at 0.05 μm/s and the directed velocity of the SPT particles at 0.2 μm/s. For the subsequent 20 seconds, the axial deformation rate was increased to 0.2 μm/s and the particle velocity to 0.3 μm/s. For the final 30 seconds of simulation time, the axial deformation rate was set to zero and the particle velocity was set to 0.2 μm/s. Under these conditions, our method was tested in comparison to purely Euclidean calculations or to the tail-correction velocity correction [2], in which the velocity of the "tail" of the cell with respect to the reference point is subtracted from the velocity of each particle measured.

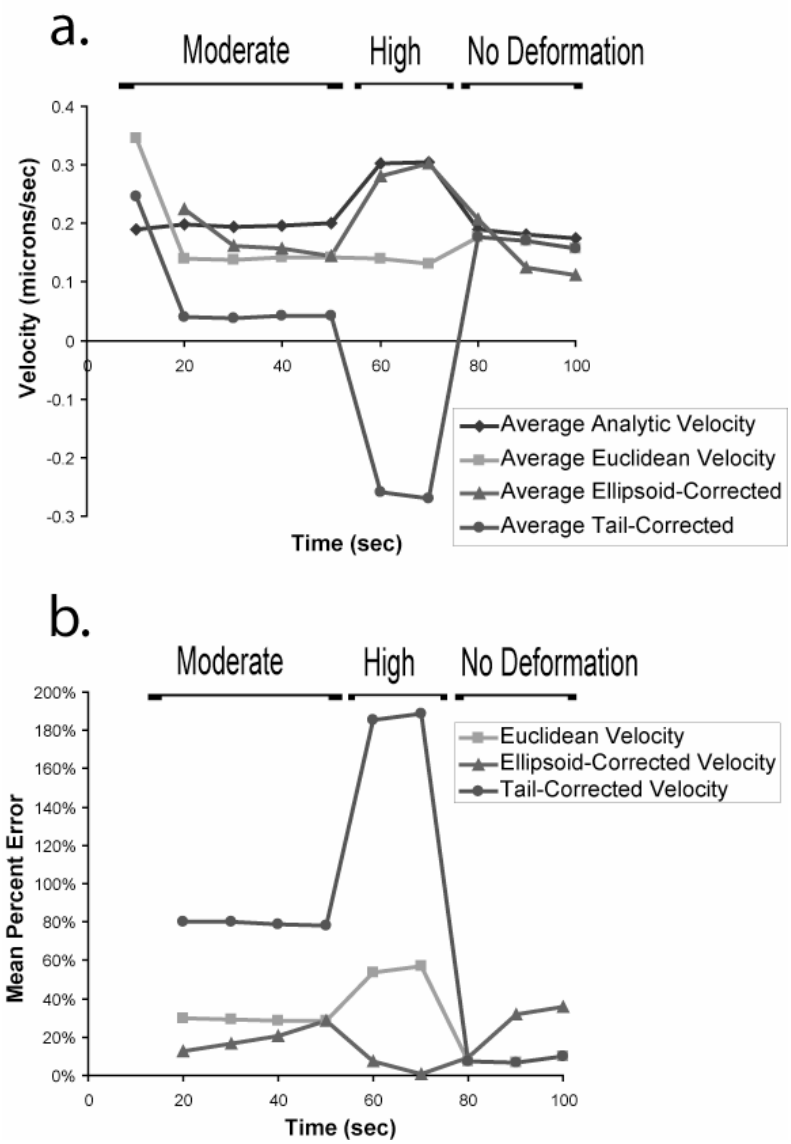

Figure 1. Comparison of single-particle calculation methods

Protein movement was simulated as particle motion on the surface of a spheroid. Plotted in (**a**) are the results of velocity calculations via the Euclidean distance method, the tail-corrected velocity method, and the ellipsoid-corrected velocity method. Velocities are compared to those calculated via direct analysis on the particles and simulation parameters. Three different particle motion and cellular deformation regimes were simulated: times 0-50 seconds show a moderate rate of cellular deformation (.05 μm/s), times 50-70 show a high rate of cellular deformation (.2 μm/s), and times 70-100 show no deformation at all. The particle velocity was .2 μm/s for times 0-50 and 70-100 and .3 μm/s for times 50-70. Plotted in (**b**) is the mean percent deviation from the analytic velocity.

Results from the simulation are plotted in Figure 1. As can be seen from the figure, the ellipsoidal correction method was most accurate in cases of severe deformation, where it performed substantially better than either Euclidean or tail-subtracted velocity calculations (3.9% mean percent deviation from the analytic velocity versus 55% and 190% respectively). In cases of moderate deformation, the ellipsoidal

correction method was slightly better than Euclidean calculations and substantially better than tail-corrected calculation (20% versus 29% and 80% mean percent deviation). In the case of no deformation, the ellipsoid correction method showed some error while the Euclidean and tail-corrected methods were more accurate (26% versus 8% and 8%). This increased error of the ellipsoidal correction method at late times results from clustering of the "bulk" particles that are used for shape determination by the ellipsoidal method. Since the Euclidean and tail-corrected methods do not use this information, they are not affected by this source of error. In contrast, the tail-corrected method greatly overcorrects for any cellular deformation. Overall, ellipsoidal velocity correction outperformed other available methods for both high and low axial deformation rates and worse than other methods when the cell did not undergo deformation. Our non-rigid corrections are substantially more accurate in measuring molecular velocities on deforming cells and can easily be combined with Euclidean calculations in hybrid methods to handle non-deforming cells with high accuracy.

4. Conclusions

Cellular deformation can be a substantial confounding factor in the analysis of whole-cell single particle tracking data. Previously published methods for analysis of these data display substantially reduced accuracy in cases of high cellular deformation. We have developed a correction method that computes an ellipsoidal deformation model on bulk fluorescence data and corrects particle velocities using this model. Quantitative comparison of single-particle velocity calculation methods show a dramatic improvement in accuracy for our method in cases of substantial cellular deformation.

5. Acknowledgements

The authors wish to thank D. Irvine for access to unpublished experimental data and O. Troyanskaya and M. Vrljic for many helpful discussions.

6. References

[1] A. Genovesio, B. Zhang, J.C. Olivo-Marin. Tracking of multiple fluorescent biological objects in three dimensional video microscopy; 2003. p 1105-1108.

[2] W.C. Moss, D.J. Irvine, M.M. Davis, M.F. Krummel. 2002. Quantifying signaling-induced reorientation of T cell receptors during immunological synapse formation. Proceedings of the National Academy of Sciences of the United States of America. 99(23):15024-9.

[3] A. Serge, L. Fourgeaud, A. Hemar, D. Choquet, 2003. Active surface transport of metabotropic glutamate receptors through binding to microtubules and actin flow. J Cell Sci 116(Pt 24):5015-22.

[4] E.W. Weisstein. "Ellipse." From MathWorld--A Wolfram Web Resource.

[5] P.M. Kasson, J.B. Huppa, M. Krogsgaard M.M. Davis, A.T. Brunger. 2005. Quantitative Imaging of Lymphocyte Membrane Protein Reorganization and Signaling. Biophys J 88(1):579-589.