

# Quantitative Analysis of Membrane Protein Localization and Signaling

Peter M. Kasson<sup>1,2,5</sup>, Johannes B. Huppa<sup>4,6</sup>, Mark M. Davis<sup>4,6</sup>, and Axel T. Brunger<sup>1,3,5,6</sup>  
<sup>1</sup>*Biophysics Program*, <sup>2</sup>*Medical Scientist Training Program*, <sup>3</sup>*Department of Molecular and Cellular Physiology*, <sup>4</sup>*Department of Microbiology and Immunology*, <sup>5</sup>*Stanford Synchrotron Radiation Laboratory, Stanford University* and <sup>6</sup>*Howard Hughes Medical Institute, Stanford CA 94305*. Email: [brunger@stanford.edu](mailto:brunger@stanford.edu)

## Abstract

Fluorescence microscopy of labeled proteins yields a wealth of data on cell signaling processes. However, systems for quantitative analysis of such data have lagged behind the recent progress in data acquisition technology. As cellular protein redistribution plays a key role in proximal signaling and the establishment of cell polarity, quantitative information is critical for understanding many signaling networks. We have developed a robust automated system to analyze membrane protein redistribution based on datasets obtained via fluorescence video microscopy. Our system provides methods for cell surface segmentation and reconstruction, cell shape tracking, cell-surface parameterization, and cluster formation analysis. Our system is novel in both its integration and its surface-based approach, enabling model-free analysis of protein redistribution across the entire cell. We validate our system by measuring receptor clustering in T lymphocytes undergoing activation, obtaining clustering velocities consistent with the previously reported single-particle tracking data that serve as our reference standard. Our methods generalize to many cell-signaling phenomena, allowing quantitative measurement of these cell membrane processes and offering the ability to derive empiric parameters for spatial signaling network models.

## 1. Introduction

Relocalization and clustering of signaling proteins in the plasma membrane drive a diverse array of cellular processes including lymphocyte activation, neuronal synapse formation, and cell motility. Protein localization changes are monitored experimentally using fluorescent probes, which can be visualized in living cells using four-dimensional (x,y,z,t) microscopy. Localization patterns yield information about the regulation of these signaling processes and their failure in disease. Quantitative methods for

protein redistribution analysis are needed to assess these patterns systematically and to enable mechanistic analysis of the underlying signaling networks.

## 2. Methods

Our approach, outlined in Figure 1, utilizes the Moss segmentation filter [1] for identification of membrane voxels. This filter offers increased specificity for membrane structures compared to an edge-detection filter. For accurate distance mapping, we desire a smooth, continuous surface corresponding to the plasma membrane geometry at each time point. To that end, we perform level-set-based surface reconstruction [2] using the membrane points defined by our segmentation filter as input.

We align the surface thus derived at each time point against adjacent time points via a pairwise registration scheme. We optimize a maximum-likelihood search metric over rigid-body transformation space using iterative line searches with descending step size. We thus obtain a set of transformations describing the motion of the cell membrane.

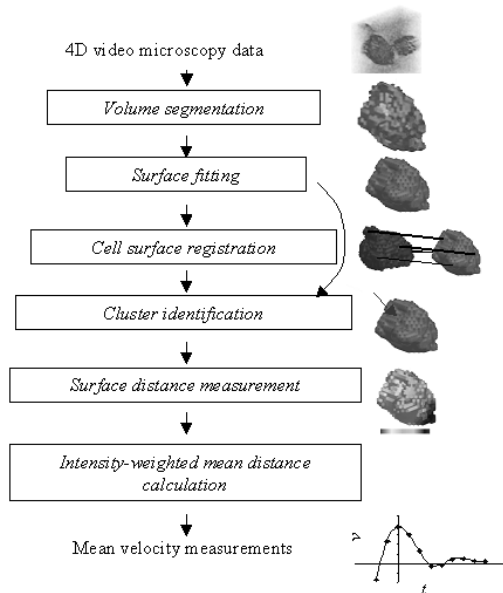
### 2.1 Clustering Analysis

For surfaces isomorphic to a sphere, a continuous two-dimensional parameterization that preserves surface distance is impossible. We therefore identify the biologically relevant reference point for distance measurement (here the center of the cell-cell interface where protein clustering occurs) based on k-nearest-neighbors clustering analysis at a single time point and use surface registration information to map the reference point to all time points.

### 2.1. Distance Measurement and Velocity Analysis

We perform surface distance measurement using a graph labeling strategy. Radial distance-intensity distributions are obtained by mapping the surface

distance information to the original membrane points identified by the segmentation filter. We define the mean intensity-weighted distance at time  $t$  as  $\bar{x}(t) = \sum_{\forall x} F(x,t) \cdot x$ , where  $F$  is the fractional intensity at distance  $x$  and time  $t$ . We assess mean velocity via two measures:  $\Delta x/\Delta t$  and a 5-point linear fit that has lower noise but is temporally smoothed.



**Figure 1. Outline of Analytic Process**

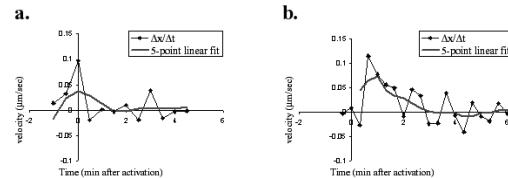
Shown are the sequential stages in our analytic system accompanied by volume renderings of progressive analytic stages from a single time point.

### 3. Results

Using the method described above, we have analyzed CD3- $\zeta$  signaling protein movements during T lymphocyte stimulation. During antigenic stimulation, signaling and adhesion proteins cluster at the interface between the T cell and the antigen-presenting cell in an organized fashion. Among these proteins are the T-cell receptor (TCR) and the TCR-associated signaling protein CD3 $\zeta$ . Despite extensive qualitative study, the precise role of this behavior has yet to be determined. Quantitative data will assist in the development and testing of mechanistic models for signaling.

Our measurements of CD3- $\zeta$  redistribution confirm the qualitatively observed receptor clustering behavior upon T-cell activation (Figure 2). We also compare our mean velocity measurements to previously reported single-particle tracking data for the T-cell receptor [3], a protein that co-localizes with CD3- $\zeta$  (Figure 2b). We measure a peak receptor velocity within 16% of the

single-particle tracking data. Minor discrepancies between our observations and single-particle tracking data illustrate how analyses of bulk movements provide information complementary to single-particle tracking experiments. Bulk analyses can be performed on a broader range of experimental systems and yield population statistics more readily than laborious single-particle analyses, although the latter provide a “gold standard” for movements of individual molecules.



**Figure 2. Receptor velocity in response to lymphocyte stimulation.**

a. shows CD3- $\zeta$  velocity measurement derived from bulk fluorescence measurements via our method. b. shows a single-particle trace from the published data [3]. We have calculated velocity for each trace both using the  $\Delta x/\Delta t$  velocity determination method and using 5-point moving window linear fits.

### 4. Conclusions

We have presented a novel system for measurement of membrane protein movements on the cell surface. Our methods are fully three-dimensional and combine a level-set surface fitting technique with maximum likelihood-based surface registration. Applied to membrane receptor signaling during T lymphocyte activation, our analytic system measures mean CD3- $\zeta$  velocities and yields results consistent with previously reported experiments. Since our analytic framework is generally applicable to membrane protein movements, we anticipate it to be of use to investigations of cell signaling in other systems.

### 5. References

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