Automatic quantitative characterization of rapid protein dynamics in live cell microscopy assays

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Introduction

Biological reactions depend largely on the diffusion and localization of biomolecules and intracellular organelles. A new generation of microscope and fluorescent probe technologies has enabled the visualization of rapid protein dynamics and molecular and subcellular events. However, quantitative characterization of biomolecular and intracellular organelle mobility is challenging since the dynamics of objects are complex and may be obscured over time. Therefore, much of the kinetic characterization is based on manual tracking and interpretation, which is tedious, subjective and irreproducible.

We have developed automatic subcellular object tracking and characterization technologies including 1) a highly robust and flexible tracking method called “soft tracking”; 2) track refinement and state detection; and 3) kinetic characterization. We include new kinetic measurements such as track zones of influence, counts of fast-slow objects, counts of “Association” and “Disassociation” states. The objectives of this study is to validate the performance of the technologies using live cell images of photo-convertible, fluorescent protein Phamret (PHotoactivation-Mediated Resonance Energy Transfer) fused to SKL tripeptide for peroxisome localization.

Track Zone of Influence Measures

Fig 6. Track trajectory are fitted to an ellipse. The major and minor axes of the ellipse is estimated for each track; (A) Example track trajectories and (B) their track zone of influence measures. Track zone of influence reflects tracks (C) Remove short trajectories (D) Append new object to near by trajectory that terminated at previous frame (E) Detect Association and Disassociation states.

Average object tracking error: 

\[ \alpha \]

\[ \beta \]

\[ \gamma \]

\[ \delta \]

\[ \epsilon \]

\[ \zeta \]

Average object tracking error is 0.0333 ± 0.003 and the Average matching tracking sensitivity is 0.9792 ± 0.04.

Results

We used manual tracks as the truth and evaluated the tracking performance using the tracking accuracy metrics. Since the manual tracks are subject to human drawing errors and the automatic detection also introduce additional errors, we used “radial limit” from 1 to 5 pixels in both x and y locations for applying tracking accuracy metrics. We believe the results at “radial limit” of 5 pixels can appropriately reflect the tracking performance.

Tracking Accuracy Metrics

Average track error: No. of tracks having ≥10% incorrect tracking time points over the entire time divided by the total number of tracks

Average object tracking error: No. of incorrect tracking time points over the entire time divided by the total number of time points

Average matching tracking sensitivity: For each truth trajectories, no. of objects in the detected tracks having ≥10% overlap with the truth trajectories divided by all objects in the truth trajectories

Conclusion

Study results show that the tracking results are closely aligned with the manual tracks. We conclude that our tracking technologies support the hypothesis with statistical significance as the 95% confidence levels have little error and high sensitivity. We believe the technologies have broad applications, and are working to validate them on a number of live cell assays.

Literature cited


Future Efforts

- We will continue to test these methods using different types of experimental images from additional assays

- We will further incorporate motion energy to improve the tracking

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Pre-processing

Fig 2. Pre-processing is performed by adaptive processing to generate a high confidence map using teachable structure guided processing. (A) shows a representative image frame from the study movie. (B) shows the confidence map.

Soft Tracking Teaching

Fig 7. Soft tracking teaching is performed interactively by SVCall Flash using a ‘teach by example’ point-and-click interface. Single or multiple objects can be used to teach the software typical kinetic behaviors under different tracking states.

Study Materials and Methods

Our hypothesis is that our methods achieve similar performance to the best manual method. The manual tracks are created independently by two analysts and discrepancies are resolved through review with the group. We test the hypothesis using tracking accuracy metrics.

Fig 9. (A) The plots of tracking accuracy metrics for different radial limits. (B) Table of the numerical values of the metrics. Note that at radial limit of 5 pixels, the Average track error is 0.1042 ± 0.086, the Average object tracking error is 0.9167 ± 0.078 and the Average matching tracking sensitivity is 0.9375 ± 0.068.