

Automated Kinetic Characterization of Exocytotic Events in Total Internal reflection microscopy

James SJ Lee¹, Chi-Chou Huang¹, Zakary Kenyon¹, Hirotada Watanabe², Takashi Tsuboi³

¹DRVision Technologies LLC, 15921 NE 8th St. Suite 200, Bellevue, WA 98008, USA

²Nikon Instruments Company, Yokohama-city, Kanagawa Japan

³Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo Japan



Introduction

Total internal reflection fluorescence (TIRF) microscopy is a powerful tool for detecting vesicle movement and trafficking in live cells, and has provided significant advances in understanding the molecular mechanisms of regulated hormone secretion in many types of secretory cells. It is now possible to examine the exocytosis-related proteins and secretory vesicle dynamics in real time down to a single exocytosing vesicle and quantifying and classifying them according to such kinetic dynamics.

To facilitate efficient kinetic characterization of exocytotic events, 1) we applied our automatic subcellular object tracking and characterization technologies called "soft tracking"^{1,2,3} to track the plasma membrane-docked vesicles; 2) we applied our structure motion decomposition and soft matching technologies to detect thunder events; 3) we measure track length, track movement kinetics, and unique fluorescence and thunder confidence values; 4) we use track measurement to classify exocytotic response into nine types: "residents", "visitors", or "passengers" of "Exocytotic", "Partial Exocytotic" or non Exocytotic types.

Standard Framework for Exocytosis Analysis

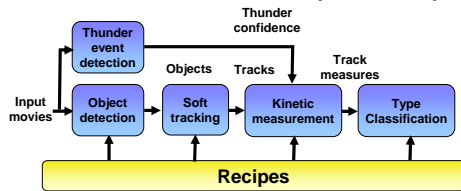


Fig 1. The standard framework for exocytosis analysis consists of an object detection step, a thunder event detection step, a soft tracking step, a kinetic characterization step and a type classification step. It is implemented in SVCeM™ kinetic module.

Object Detection

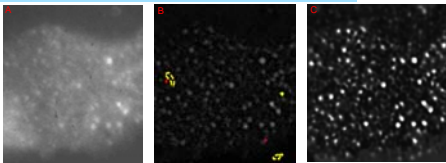


Fig 2. Input image (A); ROI's were drawn on the bright enhanced channel: Green region to enhance, Red region to suppress and Yellow region to ignore (B); and soft matching was used to generate the confidence map (C). The confidence map is thresholded to generate object masks.

Thunder Event Detection

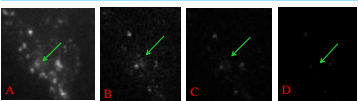


Fig 3. 3 Structure Motion Decomposition (SMD) channels are created to enhance thunder events. All channels are based on fast motion with varying size structures. (A) shows the original image with a green arrow pointing to the thunder event. (B)-(D) show the SMD channels with the smallest structure kernel size being (B) and the largest being (D).

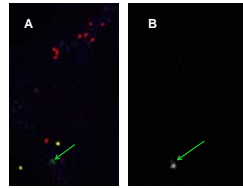


Fig 4. Soft matching was taught on the combined 3 SMD channels seen in Fig. 3. The green arrow shows the thunder event. (A) ROI's were drawn on the combined channel: Green region to enhance, Red region to suppress and Yellow region to ignore; (B) Thunder event confidence map.

Soft Tracking

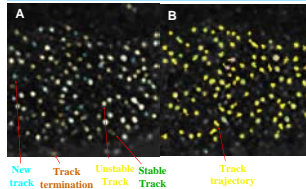
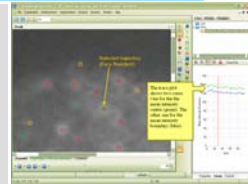


Fig 5. (A) Soft tracking detects new tracks and track objects over time. It automatically create internal states and apply different tracking models adaptively depending on object states. (B) shows tracked object trajectories

Kinetic Measurement

Fig 6. In addition to a comprehensive set of object and track measurements, new object measurements are implemented. The trace plot shows two curves. Mean intensity center (green) and Mean intensity boundary (blue). The intensity can be measured for thunder event confidence as well. The maximum and terminal point intensities and their ratios are also measured.



Teachable Track Type Classification

Fig 7. Six of the nine exocytotic types are illustrated. The vesicles visible before stimulation were named "residents". Vesicles become visible during the stimulation were named "visitors", which remained near the plasma membrane for some time before exocytosis. Vesicles fused without stably docking were named "passengers".

	Without thunder event	With thunder event
Long track	Previously Docked Vesicles	Exocytotic Resident
Relatively short track	Newly Recruited Vesicles	Exocytotic Visitor
Really short track	Previously Docked Vesicles	Exocytotic Passenger
		Partial Released Resident
		Partial Released Visitor
		Partial Released Passenger

	Without thunder event	With thunder event
Long track	Previously Docked Vesicles	Exocytotic Resident
Relatively short track	Newly Recruited Vesicles	Exocytotic Visitor
Really short track	Previously Docked Vesicles	Exocytotic Passenger

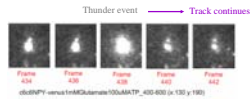


Fig 8. We defined three more types for tracks continue after initial exocytosis and named them partial Exocytotic types

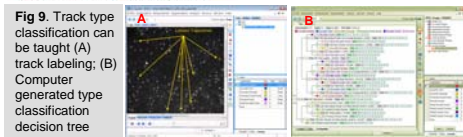


Fig 9. Track type classification can be taught (A) track labeling; (B) Computer generated type classification decision tree

Study Materials and Methods

The objective of this study is to validate the performance of the standard framework for TIRF microscopy capturing exocytotic responses and docking step of dense-core vesicles of NPY-Venus expressing live PC12 cell movies.

Data and truth

Eight movies were included in the study. Significant exocytotic tracks are selected by Tokyo University scientists. Initial truth including start and end points of the track and their types were provided by Tokyo University scientists. DRVision engineers reviewed and confirmed/revise truth for Tokyo University scientists' final decision.

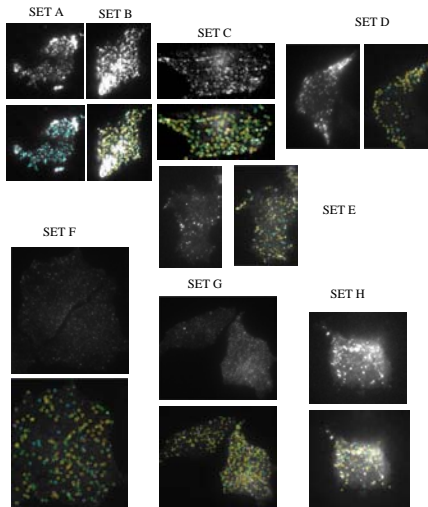


Fig 10. The eight validation movies with their tracking result overlays

Exocytotic Resident	2 tracks
Exocytotic Visitor	2 tracks
Exocytotic Passenger	3 tracks
Previously Docked Vesicle	17 tracks
Recruited Vesicle	15 tracks
Newly Recruited Vesicle	14 tracks
Partial Exocytotic Resident	6 tracks
Partial Exocytotic Visitor	4 tracks
Partial Exocytotic Passenger	9 tracks

Tab. 1. The truth tracks for the validation tests. 63 tracks are included.

Study Methods

We applied the standard framework to the validation data sets. We evaluate the tracking accuracy and track classification accuracy separately. For each of the tracks with truth, if a corresponding track could not be found, it is counted as "Miss-tracked". If a corresponding track could be found, the classified exocytotic type is compared to the exocytotic type specified in the truth. If the exocytotic types are matched, it is counted as "Correct classification". Otherwise it is counted as "Misclassified".

Results

	Total	Correct Tracked
Exocytotic Resident	2	1
Exocytotic Visitor	2	2
Exocytotic Passenger	3	2
Previously Docked Vesicle	17	15
Recruited Vesicle	15	15
Newly Recruited Vesicle	14	12
Partial Exocytotic Resident	6	4
Partial Exocytotic Visitor	4	4
Total	63	55

Tab 2. The tracking result for different exocytosis types are listed on the table. The overall tracking sensitivity is 87.3±8.2% (55/63)

	Correct Tracked	Correct classified
Exocytotic Resident	1	1
Exocytotic Visitor	2	1
Exocytotic Passenger	2	2
Previously Docked Vesicle	15	14
Recruited Vesicle	15	13
Newly Recruited Vesicle	12	9
Partial Exocytotic Resident	4	4
Partial Exocytotic Visitor	4	2
Total	55	46

Tab 3. The track classification result for different exocytosis types are listed on the table. The overall track classification accuracy is 83.6±9.8% (46/55)

Conclusion

Study results show that our standard framework for exocytosis analysis achieves good tracking sensitivity and good track classification accuracy. We believe the technologies could standardize kinetic characterization of exocytotic events, and are working to validate them on additional live cell exocytosis movies.

Future Efforts

- Tracking result directly impacts the measurements relevant to the accuracy of the exocytotic type classification. We intend to further improve the tracking module for better tracking accuracy.
- Using additional training samples to improve the classification module for more accurate classification accuracy.
- Create wizard graphical User Interface to allow easy adjustment of the standard framework recipe parameters for robust and reliable tracking and track classification outcomes.

Literature cited

- Lee JSJ, et al., 2008 Automatic quantitative characterization of rapid protein dynamics in live cell microscopy assays. Poster presentation at the 2008 American Society of Cell Biology conference in San Francisco, CA.
- Lee JSJ. 2002. Structure-guided image processing and image feature enhancement. United States patent number 6,463,175 .
- Lee JSJ. 2009. Automatic quantitative characterization of kinetic events during exocytosis. Poster presented at the 2009 Society for Neuroscience conference in Chicago, IL.

Acknowledgments

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