Exocytotic event kinetic measurements for molecular target functional characterization

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Introduction

Exocytosis is a common mechanism for excitable cells to release physiologically active substances. Regulated exocytosis is a fast, dynamic and complex process requiring the trafficking of cargo to the plasma membrane, reorganization of the cytoskeleton and actin networks and the fusion of vesicles with the plasma membrane. Many diseases involve perturbations of these processes. Thus understanding of the behavior of secretory vesicles at the plasma membrane and of the regulatory molecules involved is an essential goal of medical sciences. Many key molecules involved in exocytosis have been identified. However, their underlying mechanisms are complex and largely unknown.

To characterize the functions of molecular targets, exocytosis modulating proteins were overexpressed or depleted to the neuroendocrine cells. Live cell total internal reflection fluorescence (TIRF) microscopy was used to image hormone-containing vesicles. Extended from our previous work[1,2,3], SVCell™ recipes were configured to automatically track vesicles and detect exocytosis events. The detected events were interactively reviewed using SVCell Gallery Viewer. The confirmed events were quantitatively characterized by kinetic measurements for different target conditions.

The objective of this study is to assess the effectiveness of our automatic exocytosis event detection recipe and SVCell Gallery Viewer tool. We also perform molecular target functional characterization by exocytotic event kinetic measurements.

Exocytotic Event Kinetic Quantification Tool

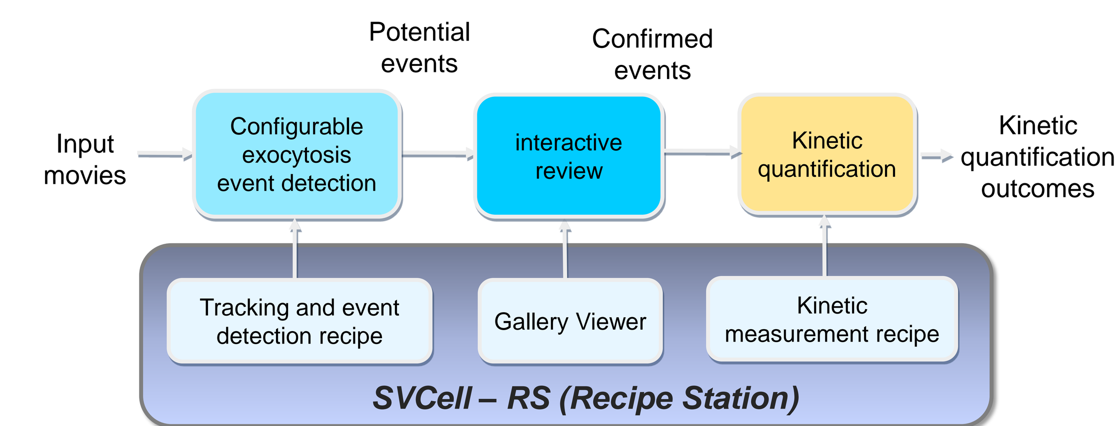

Fig 1. Exocytotic Event Kinetic quantification tool consists of a configurable exocytosis event detection step, an interactive review step and a kinetic quantification step. It is implemented in SVCell™–RS (recipe Station). Input movie(s) are processed by SVCell tracking and event detection recipe to detect potential events. The potential events are interactively reviewed using SVCell gallery viewer to generate confirmed events. The confirmed events and their associated tracks are used by the SVCell kinetic measurement recipe to calculate track and object measurements associated with the confirmed events. The kinetic measurements are used for kinetic quantification to generate kinetic quantification outcomes.

Configurable Exocytosis Event Detection

This step can be configured to detect a variety of exocytotic events such as rapid emerging events, slow emerging events (we call them point type) and large, disperse, cloud like events (we call them gross type). The detailed processing steps are described in reference 3. In addition to the exocytotic event detection, vesicle tracking is performed to generate tracks. This allows the association of events with tracks. The association enables track measurements to be calculated for each events.

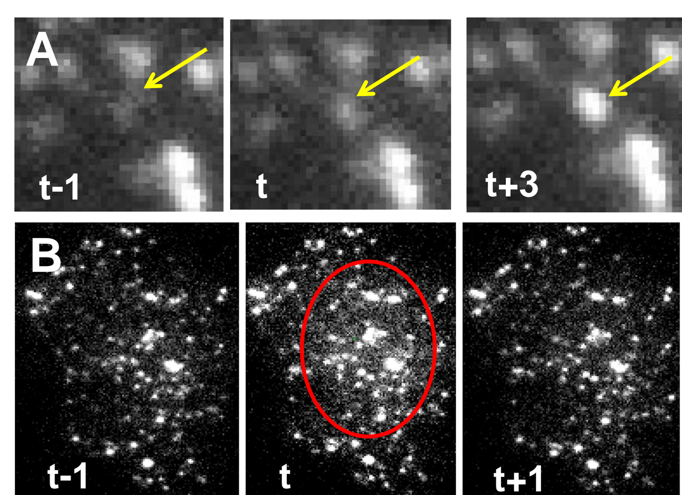

Fig 2. Shows the two types of exocytotic events: (A) shows a point type slow emerging exocytotic event at frames t-1, t and t+3. The event occurs near the position highlighted by the yellow arrow. (B) shows a gross type large, disperse, cloud like exocytotic event at frames t-1, t and t+1. The event occurs at frame t near the region highlighted by a red circle

Event to Track Association

We associate an event to its closest track. However, gross type event areas could cover multiple tracks. Therefore, we allow the association of an event to multiple tracks. Also we allow one track to associate with multiple events at different time points. The greedy association approach may result in extra associations. But users could reject the wrongly associated tracks during gallery reviewer.

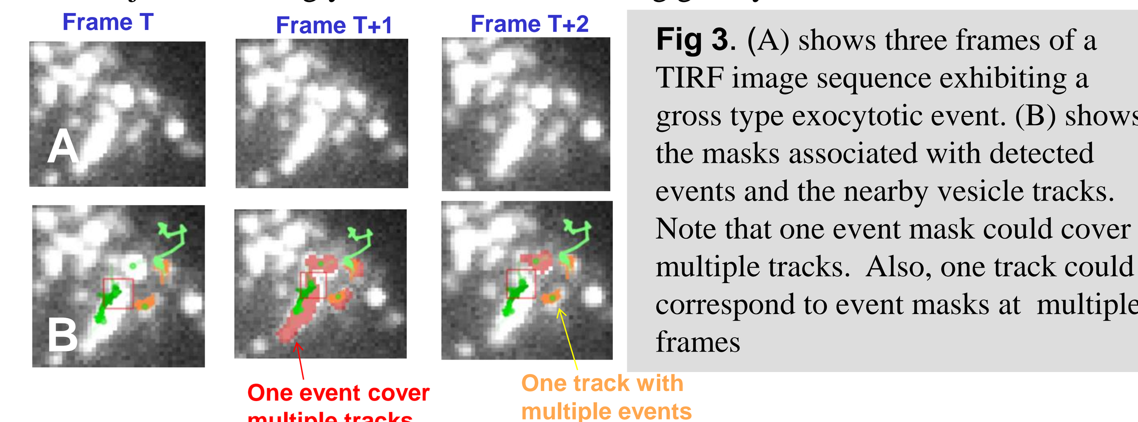

Fig 3. (A) shows three frames of a TIRF image sequence exhibiting a gross type exocytotic event. (B) shows the masks associated with detected events and the nearby vesicle tracks. Note that one event mask could cover multiple tracks. Also, one track could correspond to event masks at multiple frames

Interactive Review Using Gallery Viewer

The potential events are interactively reviewed using SVCell gallery viewer to generate confirmed events.

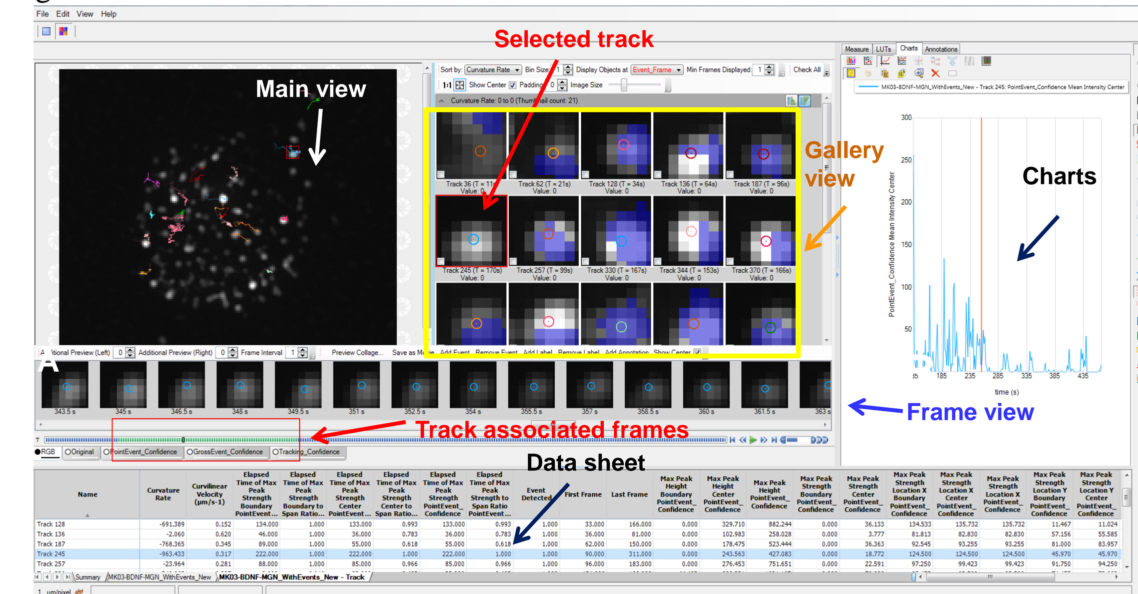

Fig 4. shows SVCell gallery viewer interface. Tracks of potential events are displayed in gallery view format. Gallery view, main view, charts and data sheet are linked. Objects can be selected from either of them and they are highlighted on all. The selected track will also be shown in frame view and its measurements could be plotted in the chart area. Gallery objects can be sorted, zoomed, padded. The time frames associated with a track is highlighted. Selected object can be automatically moved to either its first frame, last frame, middle frame or the event frame. The time frames of a track can be easily advanced, reversed, paused, resumed, etc.

Gallery Review

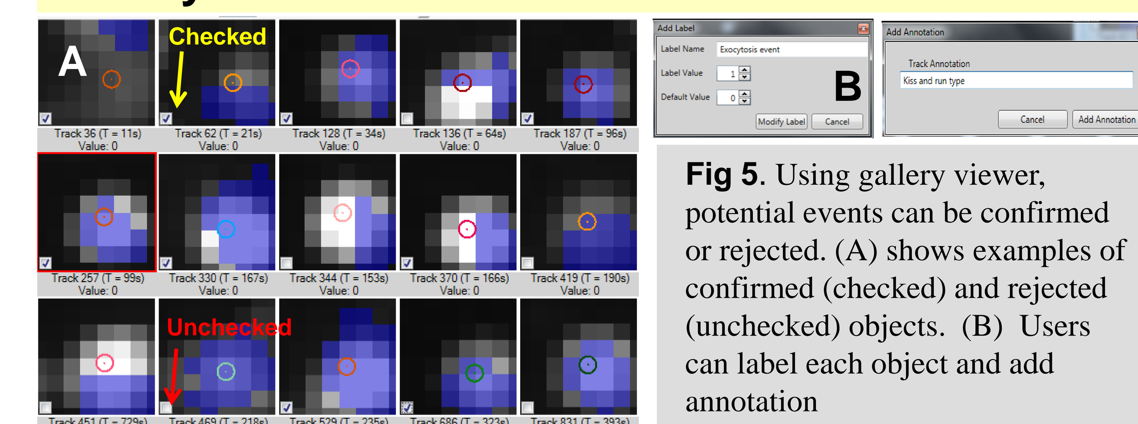

Fig 5. Using gallery viewer, potential events can be confirmed or rejected. (A) shows examples of confirmed (checked) and rejected (unchecked) objects. (B) Users can label each object and add annotation

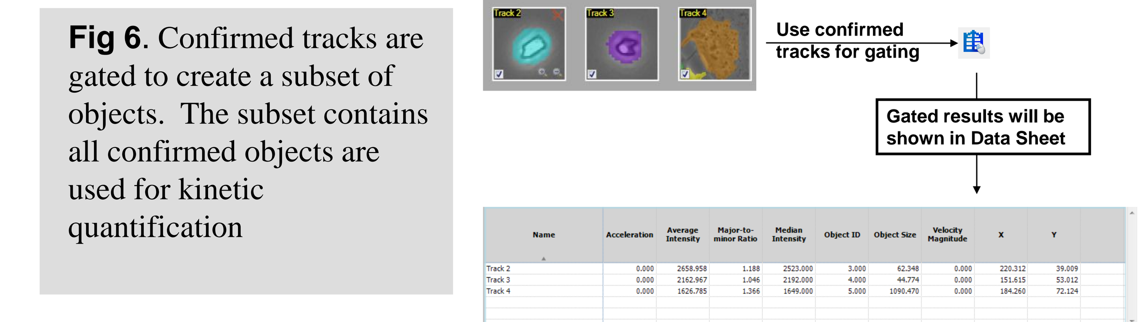

Fig 6. Confirmed tracks are gated to create a subset of objects. The subset contains all confirmed objects are used for kinetic quantification

Kinetic Quantification

After interactive review, the confirmed exocytotic events and their associated tracks are characterized. The same measurement from all exocytotic tracks of the same experimental conditions are combined into a histogram. The cumulative density function (CDF) of histograms from different experimental conditions can be compared to assess phenotypic differences. The phenotypic related measurements include

- ***Exocytosis elapsed time ratio***: a measure of the degree of partial exocytosis
- ***Exoctytosis track length***: a measure of the time duration of exocytosis vesicles
- ***Exoctytosis track mean velocity***: a measure of the exocytotic vesicle movement
- ***Non-exoctytosis track mean velocity***: a measure of the non-exocytotic vesicle movement

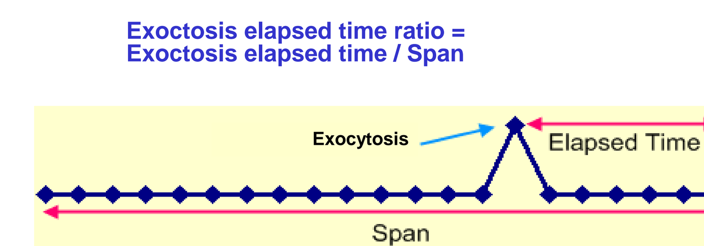

Fig 7. Illustrate an exocytosis track, its span (track length), elapse time and elapse time ratio

Study Materials and Methods

Study Data Set

Study data includes 43 TIRF microscopy movies of PC12 cells whose dense-core granules are labeled with neuropeptide Y-Venus fusion protein. The experimental conditions include

- Cdc42 overexpressed constitutive active (CA) form that are expected to increase fusion events (11 movies);
- Cdc42 overexpressed wild type (WT) that are expected to increase fusion events (13 movies);
- Cdc42 overexpressed dominant negative (inactivated) (DN) form that are expected to decrease fusion events (11 movies);
- Controls (8 movies).

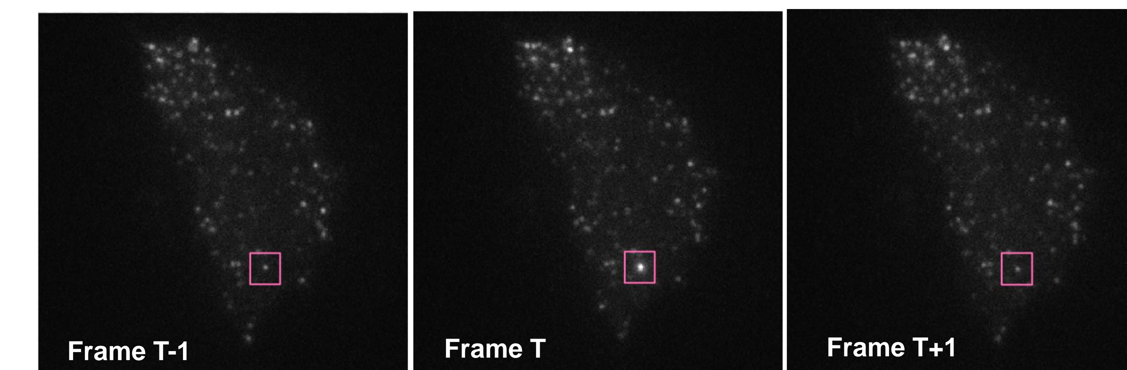

Fig 8. An example study movie having an exocytotic event at Frame T

Kinetic Quantification Metrics

The following characterization metrics are used for the kinetic quantification

Positive Predictive Value (PPV): the proportion of detected events that are confirmed exocytosis events for different experimental conditions.

Mean Confirmed Event Counts (μ_Events): the average exocytosis event counts for different experimental conditions.

Mean Vesicle Counts (μ_Vesicles): the average vesicle counts for different experimental conditions.

Mean Velocity Exocytosis (ν_Exocyt): the average velocity of exocytosis vesicles for different experimental conditions.

Mean Velocity Non-exocytosis (ν_N-exocyt): the average velocity of non-exocytosis vesicles for different experimental conditions.

Mean Repeated Exocytosis Vesicle Counts (μ_R-events): the average counts of exocytosis vesicles having repeated exocytosis for different experimental conditions.

CDF and Kolmogorov–Smirnov Test: cumulative density function and K-S tests of the phenotypic related measurements for different experimental conditions

Results

	PPV	μ_Events	μ_Vesicles	ν_Exocyt	ν_N-exocyt	ν_R-events
Controls	0.738 (107/145) CI (0.666, 0.810)	13.375 (107/8)	4381.875 (35055/8)	0.85304	1.10536	0.875
WT	0.867 (209/241) CI (0.824, 0.910)	16.077 (209/13)	4581.23 (59556/13)	0.88399	1.16301	2.538
CA	0.829 (160/193) CI (0.776, 0.882)	14.545 (160/11)	4299.73 (47297/11)	0.98301	1.35431	1.091
DN	0.797 (98/123) CI (0.726, 0.868)	8.909 (98/11)	4345.909 (47805/11)	0.95002	1.31031	1
Total	0.818 (574/702) CI (0.789, 0.846)	13.349 (574/43)	4411.930 (189713/43)	0.917095383	1.246338765	1.465

Tab 1. Lists the results of the PPV, μ_Events, μ_Vesicles, ν_Exocyt, ν_N-exocyt, and ν_R-events for different experimental conditions

CDF and Kolmogorov–Smirnov Test Results

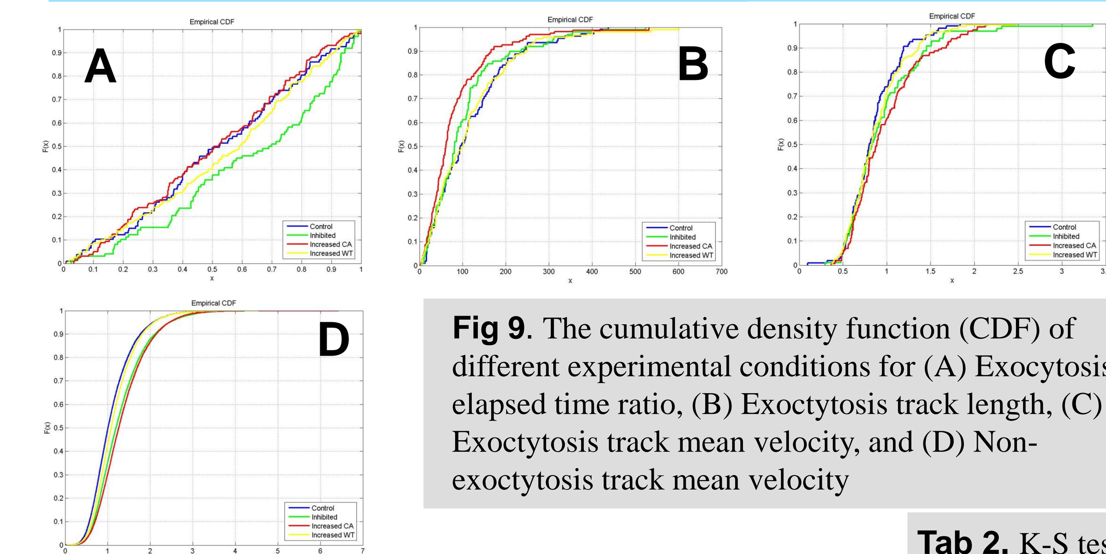

Fig 9. The cumulative density function (CDF) of different experimental conditions for (A) Exocytosis elapsed time ratio, (B) Exocytosis track length, (C) Exoctytosis track mean velocity, and (D) Non-exoctytosis track mean velocity

	ExocyElapsedTimeRatio	ExocyTrackLength	MeanVelocity_Exocy	MeanVelocity_NonExocy
Control to DN	0.012903	0.16755	0.32153	8.32E-260
Control to Increase CA	0.76798	0.00024373	0.045285	0
Control to Increase WT	0.67078	0.93675	0.55196	7.83E-61
DN to Increase CA	0.0040794	0.0047665	0.74963	4.04E-41
DN to Increase WT	0.014776	0.32545	0.7572	9.69E-159
Increase CA to Increase WT	0.45448	2.19E-05	0.053356	0

Tab 2. K-S test results between pairs of the four experimental conditions for the four phenotypic related measurements

Discussions and Conclusion

The high PPV value (0.818) confirms the effectiveness of the SVCell tracking and event detection recipe for accurate detection. The accurate detection enables productive gallery review as 80+% of the detected events are true events. The average exocytosis event count (μ_Events) results indicate that WT and CA have close to 2 times of exocytosis events as compared to DN as expected. The number of vesicles are similar among all conditions but CA vesicles seem to have higher speed than others, yet K-S test only shows marginal statistical significance. K-S test results indicates show that DN has significantly bigger elapsed time ratio when compared with CA. This suggest that DN has more partially released exocytosis events. K-S test results also show that CA vesicles has significantly shorter track length when compared with others. This may suggest that CA exocytosis vesicles are mainly from newly recruited (passenger) type.

The study results confirm the effectiveness of our tool and the findings could open new avenues for molecular target functional understanding leading to new target discoveries.

Literature cited

1. **Lee JSJ,et al.** Automatic quantitative characterization of kinetic events during exocytosis. Poster 2009 Society for Neuroscience conference in Chicago, IL.
2. **Lee JSJ,et al.** Automated Kinetic Characterization of Exocytotic Events in Total Internal reflection microscopy. Poster 2009 ASCB
3. **Lee JSJ,et al.** Configurable tool for automated exocytotic events quantification. Poster 2012 ASCB

Acknowledgments

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