Configurable tool for automated exocytotic events quantification

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Introduction

Exocytosis is one of the fundamental cellular processes. However, its underlying mechanisms are complex and are still largely unknown. Using TIRF movies to quantify exocytosis event statistics, such as the types, occurrence counts and timings for different assay scenarios could provide a powerful tool in basic research and drug development for new discoveries in exocytosis processes.

The kinetic profiles of exocytosis events vary significantly in TIRF images depending on factors such as cell types, probes, imaging systems, assays, etc. It is challenging to create a universal classifier for automated exocytosis event detection.

Extended from our previous work[1,2], we developed a tool that can be configured to automatically detect a variety of exocytotic events in TIRF images such as rapid emerging events, slow emerging events and gross events. The objective of this study is to assess the effectiveness of our new tool.

Configurable Exocytosis Quantification Tool

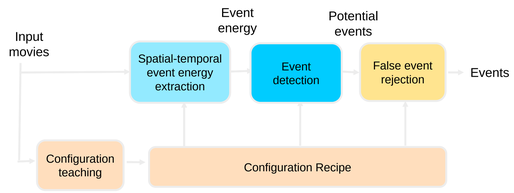

Fig 1. Configurable exocytosis quantification tool consists of a spatial-temporal event energy extraction step, an event detection step and a false event rejection step. It is implemented in SVCell™. Input movie(s) are processed to generate event energy. The high energy regions are then detected as potential events. False potential events are rejected by the false event rejection step. The event energy extraction, detection and rejection steps can be taught by a configuration teaching step to generate configuration recipe that can be applied to multiple input movies for exocytosis quantification

Spatial-temporal Event Energy Extraction

This step can be configured to extract spatial-temporal event energies from 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)

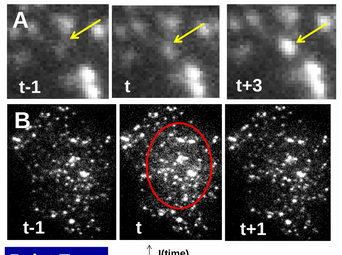

Fig 2. Shows the two types of exocytotic events: (A) shows a point type slow emerging exocytotic event at frames t-1, t and t+3. The event occurs near the position highlighted by the yellow arrow. (B) shows a gross type large, disperse, cloud like exocytotic event at frames t-1, t and t+1. The event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

Point Type

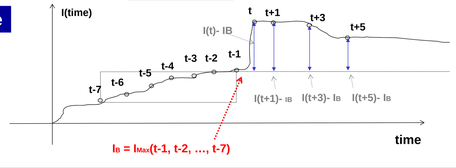

Fig 3. The event energy extraction step for point type exocytosis extracts event signals at multiple configurable durations. Four durations at t, t+1, t+3 and t+5 are used in the illustrative example. The background level I_B is determined by taking the maximum intensity of 7 (configurable) previous frames, t-1 to t-7. The event signal at each duration is calculated using its intensity minus the background level, i.e. $I(t) - I_B$, etc. The event energy is the minimum of event signals at all durations

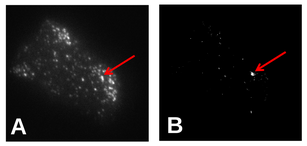

Fig 4. (A) shows a TIRF image having a point type exocytotic event as indicated by the red arrow. (B) shows the event energy response at the exocytotic event position as indicated by the red arrow

GrossType

The event energy extraction step for gross type exocytosis performs the following operations:

1. Detect single time-point impulse event signal
   I_impulse(t) = MIN(I(t)-I(t-1), I(t)-I(t+1))
2. Smooth impulse event signal
   I_smooth(t) = Average (I_impulse(t), 7x7)
3. Integrate event energy
   I _energy(t) = (I_smooth(t) - I_smooth(t-1)) + (I_smooth(t) - I_smooth(t+1))

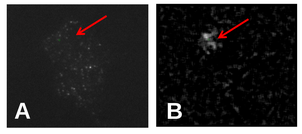

Fig 5. (A) shows a TIRF image having a gross type exocytotic event as indicated by the red arrow. (B) shows the event energy responses at the exocytotic event position

Event Detection

The event detection step performs a threshold to the event energy for both point and gross type exocytotic events. The threshold method and value can be configured by users. The threshold methods include direct or adaptive with or without bimodal offset; and the threshold value could be specified as image intensity or histogram percentile.

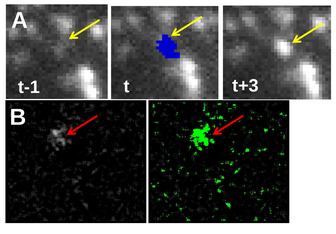

Fig 6. (A) shows event detection result for image in Fig 2 A. The blue overlay shows the detected event mask. (B) shows event detection result for image in Fig 5. The green overlay shows the detected event masks

False Event Rejection

The false alarm rejection step removes small objects. The users can set the minimum object size to be considered as an event. An additional false alarm removal step is applied to point type exocytotic events before the small object removal. This step removes objects not bright enough to be considered events.

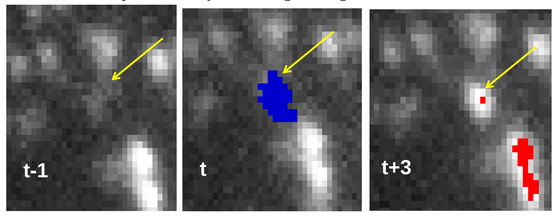

Fig 7. Shows the false alarm removal step. A user specified threshold is applied to the image in t+3 to generate a conditioning mask (red). Only event masks (blue) at t that overlaps with the conditioning mask are retained. Non-overlapping event candidates are removed

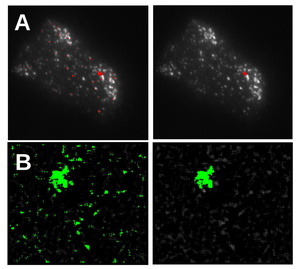

Fig 8. (A) shows the before (left) and after (right) false alarm rejection for a point type exocytotic event (overlaid in red). (B) shows the before (left) and after (right) false alarm rejection for a gross type exocytotic event (overlaid in green)

View Results

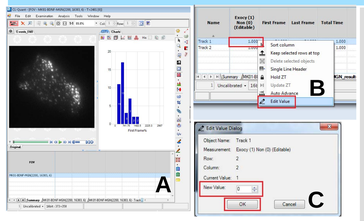

Fig 9. User can view event detection results using SVCell. (A) Select an event from Rframe chart or data sheet will automatically navigate to the frame where the event occurs. (B) Edit mode could be enabled. (C) The event detection result could be modified after image review

Study Materials and Methods

Study Data Set

Study data includes 10 TIRF microscopy movies of endocrine cells (MIN6 and GLUTag) that were provoked with various secretagogues, resulting in rapid emerging, slow emerging, and gross exocytotic events. These represent broad variations: 5 point types and 5 gross types.

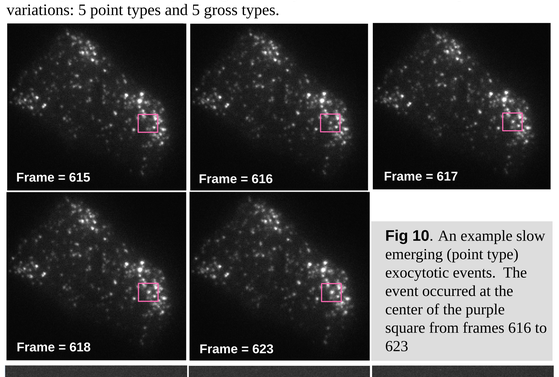

Fig 10. An example slow emerging (point type) exocytotic events. The event occurred at the center of the purple square from frames 616 to 623

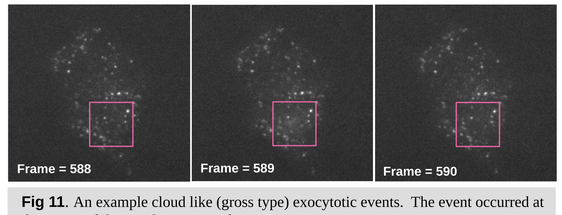

Fig 11. An example cloud like (gross type) exocytotic events. The event occurred at the center of the purple square at frame 589

Study Truth

Study truth is created manually with potential event frame and time recorded and then independently verified by Dr. Tsubi and/or Dr. Numano.

Test Metrics

Test metrics include event detection Sensitivity and Positive Predictive Value.

Sensitivity: number of exocytosis events correctly detected, divided by the total number of true exocytosis events.

Positive Predictive Value (PPV): the proportion of detected events that are true exocytosis events.

Results

Validation Study Results

A

Movie	Correctly detected	Missed	False alarm	Sensitivity	PPV
Gross 1	14	0	0	1	1
Gross 2	20	0	0	1	1
Gross 3	12	0	0	1	1
Gross 4	19	0	0	1	1
Gross 5	18	1	0	0.9473684	1
Total	**83**	**1**	**0**	**0.9880952**	**1**

B

Movie	Correctly detected	Missed	False alarm	Sensitivity	PPV
Point 1	22	2	2	0.9166667	0.9166667
Point 2	20	0	2	1	0.9090909
Point 3	16	0	0	1	1
Point 4	34	3	1	0.9189189	0.9714286
Point 5	20	1	8	0.952381	0.7142857
Total	**112**	**6**	**13**	**0.9491525**	**0.896**

Tab 1. (A) Shows the results for the gross type exocytotic events including the sensitivity and PPV for each movie. The counts of correctly detected events, missed events and false alarms are included. The total sensitivity is 0.988±0.12, and the total PPV is 1.0-0.0006. (B) Shows the results for the point type exocytotic events including the sensitivity and PPV for each movie. Also the counts of correctly detected events, missed events and false alarms are included. The total sensitivity is 0.949±0.039, and the total PPV is 0.896±0.054

Conclusion

Study results show that the configurable automated tracking has high event detection sensitivity and PPV that could support the quantification of broad exocytosis events.

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

Acknowledgments

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