Automated exocytotic events detection and quantitative characterization

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Introduction

Total internal reflection fluorescence (TIRF) microscopy enables the examination of the exocytosis-related proteins and secretory vesicle dynamics down to a single exocytosing vesicle. This enables the quantification according to such kinetic dynamics. We have developed an automated tracking tool to accurately track vesicles. However, exocytosis is generally a rare event among the many detected vesicle tracks. In our manual study of 5 movies, the excytosis rate is < 2% for long tracks and < 0.02% for short tracks.

To detect rare exocytotic events with few false alarms, we extended our kinetic characterization tool and implemented a multi-stage classification architecture. To assess the effectiveness of our new approach, a verification study is performed to assess the exocytotic events detection sensitivity and specificity. In addition, a validation study is performed to validate exocytosis vesicle population differences

Automated Exocytotic Quantification

The automated exocytotic quantification performs automated tracking to detect and track vesicles from the input time-lapse movies. The detected tracks (vesicle trajectories over time) are further processed to detect the rare exocytotic events among the many vesicle tracks. The detected exocytotic tracks enable the measurement and assessment of a wealth of track phenotypes for broad experiments. The automated tracking is performed by soft tracking[1,2]. The focus of this poster is on the exocytotic event detection and quantification.

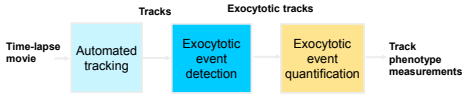


Fig 1. Automated exocytotic quantification framework consists of an automated tracking stage, an exocytotic event detection and an exocytotic event quantification stage

Automated Exocytotic Event Detection

Automated exocytotic event detection performs progressive local-to-global measurements, from track point measurements to track peak measurements to whole track measurements. Spatial-temporal contextual information and model estimation support track measurements. The track measurements are used for exocytosis classification.

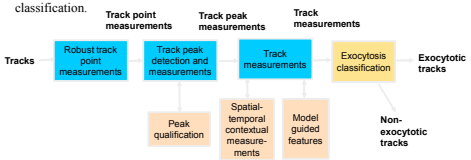


Fig 2. Automated exocytotic event detection performs progressive local-to-global measurements, followed by exocytosis classification

Robust Track Point Measurements

The basic measurements associated with each track point are

- ***Center mean intensity***: center region average intensity (radius adjustable)
- ***Boundary mean intensity***: outside ring average intensity (radius adjustable)

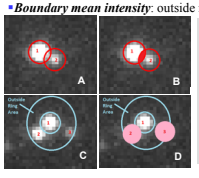


Fig 3. (A) Two vesicles are close to each other, the center regions are overlapped; **(B) robust center mean intensity measurement** excludes intersecting pixels (red area); **(C)** outside ring of a vesicle (1) could cover neighboring vesicles (2 and 3); (D) **robust boundary mean intensity measurement** excludes pixels of neighboring vesicles (pink areas) and excludes top 25% brighter pixels for possible undetected neighboring vesicles

Track Peak Detection and Measurements

Robust peak measurements on track point center and boundary mean intensities are calculated including:

- ***Maximum peak height***: maximum peak height value of entire trajectory
- ***Maximum peak strength***: maximum peak strength value of entire trajectory
- ***Elapsed time of maximum peak height to span ratio (PHR)***: see Fig 4 (B)
- ***Elapsed time of maximum peak strength to span ratio (PSR)***: see Fig 4 (C)

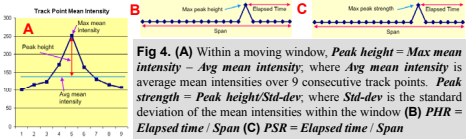


Fig 4. (A) Within a moving window, ***Peak height = Max mean intensity − Avg mean intensity***, where ***Avg mean intensity*** is average mean intensities over 9 consecutive track points. ***Peak strength = Peak height/Std-dev***, where ***Std-dev*** is the standard deviation of the mean intensities within the window **(B)** ***PHR = Elapsed time / Span*** **(C)** ***PSR = Elapsed time / Span***

Peak Qualification

When vesicles are close or crossing, intensity measurements could rise and fall causing false peaks. The peak qualification step rejects the following types of peaks

- Peaks at track points having at least 10% areas intersect with other tracks
- Peaks at track points having at least 25% outside rings intersect with other tracks
- Peaks of track points within ±2 frames of track merging frame

Track Measurements

In addition to the robust peak measurements and basic track measurements (track span, peak time, track elapsed time, etc.), new track measurements are included:

- ***Peak Associated Measurements***: maximum peak associated strength or height
- ***Track Peak Statistics***: self calibration by statistics of all detected peaks in a track
- ***Spatial-temporal contextual measurements***: could reject non-exocytotic vesicles that are brightened by nearby exocytotic vesicles
- ***Model guided features***: fitting peak intensity profiles to a model and deriving features using estimated model parameters

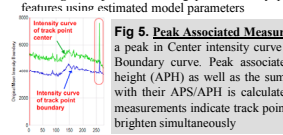


Fig 5. Peak Associated Measurements: an exocytotic track having a peak in Center intensity curve should have an associated peak in Boundary curve. Peak associated peak strength (APS) and peak height (APH) as well as the sum of maximum peak strength/height with their APS/APH is calculated. High values of peak associated measurements indicate track point center area and boundary area are brighten simultaneously

Fig 6. Track Peak Statistics: exocytosis track should have a strong global peak compared with other local peaks of the same track. To reject false exocytosis by self calibration, we calculate statistics of all detected peaks in a peak strength curve, derived from the intensity curve (either track point center or boundary):

- Max peak value
- Average peak value
- Standard deviation of peak values
- Max peak value / 2nd max peak value
- Max peak value / avg peak value

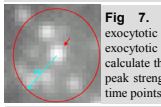
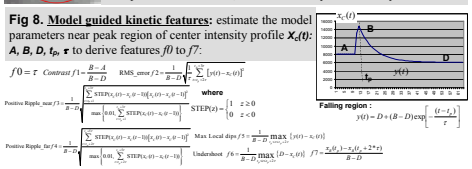


Fig 7. Spatial-temporal contextual measurements: during exocytotic events, neighboring vesicles are brightened, but the true exocytotic vesicle should have the highest peak strength/height. We calculate the ratio between the maximum peak strength/height and the peak strength/height of its neighbors within 20 pixels and within ± 4 time points. If ratio is <1, a vesicle is likely a false exocytotic vesicle.

Fig 8. Model guided kinetic features: estimate the model parameters near peak region of center intensity profile $\boldsymbol{x}_c(t)$: ***A, B, D, t_0, τ*** to derive features *f0* to *f7*:



$$f0 = \tau \quad Contrast\ f1 = \frac{B-A}{B-D} \quad RMS\_error\ f2 = \frac{1}{B-D}\sqrt{\frac{1}{\tau}\sum_{t=t_0}^{t_0+\tau}\left[y(t)-x_c(t)\right]^2}$$

$$Positive\ Ripple\_near\ f3 = \frac{1}{B-D}\left(\frac{\sum_{t=t_0}^{t_0+\tau}\mathrm{STEP}(x_c(t)-x_c(t-1))\left(x_c(t)-x_c(t-1)\right)}{\max\left\{0.01,\sum_{t=t_0}^{t_0+\tau}\mathrm{STEP}(x_c(t)-x_c(t-1))\right\}}\right) \quad \text{where} \quad \mathrm{STEP}(z)=\begin{cases}1 & z\geq 0\\ 0 & z<0\end{cases}$$

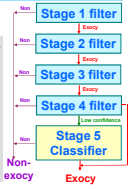
$$Positive\ Ripple\_far\ f4 = \frac{1}{B-D}\left(\frac{\sum_{t=t_0}^{t_0+\tau}\mathrm{STEP}(x_c(t)-x_c(t-1))\left(x_c(t)-x_c(t-1)\right)}{\max\left\{0.01,\sum_{t=t_0}^{t_0+\tau}\mathrm{STEP}(x_c(t)-x_c(t-1))\right\}}\right) \quad Max\ Local\ dips\ f5 = \frac{1}{B-D}\max_{t_0<t_{peak}<t_0+\tau}\left\{y(t)-x_c(t)\right\}$$

$$Undershoot\ f6 = \frac{1}{B-D}\max_{t_{peak}<t<t_0+\tau}\left\{D-x_c(t)\right\} \quad f7 = \frac{x_c(t_0)-x_c(t_0+2*\tau)}{B-D}$$

Exocytosis Classification

We defined medium/long tracks (> 20 frames) and short tracks and performed multi-stage classifications for them separately.

Fig 9. Medium/long track classification: a cascade of SVCell™ decision recipes implements multi-stage classification. The classified non-exocytotic tracks at each stage are final and are excluded from further classification. Stage 1 to stage 3 are classification filters to reject obvious non-exocytotic tracks and retain most of the exocytosis tracks. Stage 4 filter classifies obvious non-exocytotic and exocytosis tracks as final. The remaining difficult ("Low confidence") tracks are sent to stage 5 for model guided feature fine classification.



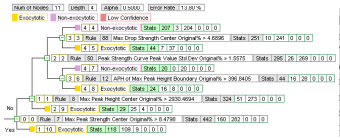


Fig 10. stage 1 decision recipe plot from SVCell™. The decision rules for false exocytosis rejection are reasonable; 224 out of 282 non exocytotic teaching samples are rejected. Other filter stages are similar.

Stage 5 classifier applies simple If-Then-Else rules for classification as follows:

If [(f0 < 0.1) **OR** (f6 > 0.0) **OR** (f3 > 0.14) **OR** (f4 > 0.17)] **non-exocytotic**
Else if (f1 > 0.8)
If [(f7 < 0.01) **OR** (f7 > 0.2)] **non- exocytotic**
Else exocytotic
Else
If [(f2 >0.12) **OR** (f5 > 0.31)] **non- exocytotic**
Else exocytotic

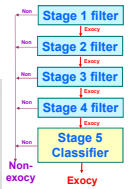


Fig 11. Short tracks classification: a cascade of SVCell decision recipes implements multi-stage classification. The classified non-exocytotic tracks at each stage are final and excluded from further classification. Stage 1 to stage 4 are classification filters to reject obvious non-exocytotic tracks. Stage 5 is a decision tree with more complicated rules to classify potential exocytosis tracks survived from the four filtering stages

Exocytotic Quantification

After exocytosis classification, the detected exocytotic events are characterized. Phenotypic related measurements are calculated for each exocytotic track. 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
- ***Exocytosis time point***: a measure of the kinetic behavior of exocytosis
- ***Exocytosis track length***: a measure of the time duration before exocytosis
- ***Exocytosis track mean velocity***: a measure of the exocytotic vesicle movement
- ***Exocytosis track SD velocity***: a measure of exocytotic vesicle movement variation
- ***Post- pre exocytosis velocity ratio***: a measure of near exocytotic event dynamics

Study Materials and Methods

Two studies were performed. A verification study assesses the exocytotic event detection sensitivity and specificity using five TIRF movies of PC12 and GLUTag cells. All tracks of the movies were manually characterized as truth for evaluation. A validation study tests exocytosis vesicle population differences among four cell types PC12 (12 movies), C6 (5 movies) GLUTag (6 movies) and Neuro-2A (5 movies) using phenotypic measurements. Kolmogorov-Smirnov (K-S) test is used for statistical test.

Tab 1. Medim/long tracks of 5 verification movies. True exocytosis tracks were classified into the following categories

- ***Rank A***: Obvious exocytosis
- ***Rank B***: Non-obvious exocytosis
- ***Rank D***: Subtle exocytosis
- ***Rank Q***: Subsequently detected exocytosis, not in the original truth

Movie	Exocy Truth	Rank A	Rank B	Rank D	Rank Q	Non Exocy
GLUTag1	6	5	0	1	0	260
GLUTag2	7	0	6	1	0	732
GLUTag3	7	2	4	1	0	843
pc12-1	38	8	7	1	22	1669
pc12-2	35	8	5	0	22	1534
TOTAL	93	23	22	4	44	5038

Results

Verification Study Results

The sensitivity for Rank A medium/long exocytosis tracks is 86.96 ±13.8% (20/23) and the specificity is 99.58 ±0.178% (5017/5,038). The sensitivity for short exocytosis tracks is 83.33 ±29.8% (5/6) and the specificity is 99.97 ±0.018% (36,564/36,575). The overall result is listed in Tab 2. It has high Rank A sensitivity of 88.89±11% (24/27) and high specificity 99.88±0.03% (41565/41613)

	Exocytosis Truth			Exocytosis Truth Detected			Rank Q Exocytosis Detected	Extra Exocytosis Detected
	AB	A	B	AB	A	B		
GLUTag1	5	5	0	4	4	0	0	0
GLUTag2	6	0	6	3	0	3	0	0
GLUTag3	6	2	4	3	1	2	0	3
pc12-1	16	9	7	12	8	4	15	23
pc12-2	17	11	6	15	11	4	14	24
TOTAL	50	27	23	37	24	13	29	48

Tab 2. verification study results showing the detection result for different truth categories of each study movie and their combined results

Validation Study Results

The CDFs of the phenotypic measurements for the 4 cell types are shown in Fig. 12. Their K-S test p-values are listed in Tab 3. The p-values associated with the differences are in line with the expected cell phenotype differences by visual examination and some new insights are discovered for velocity related phenotypes that cannot be easily observed.

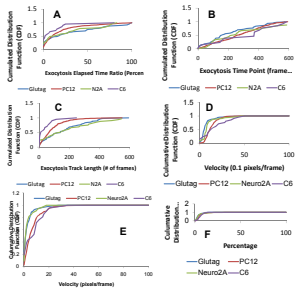
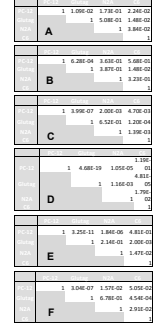


Fig 12. CDFs for (A) Elapsed time ratio, (B) Time point, (C) Track length, (D) Mean velocity, (E) SD velocity and (F) Post-pre exocytosis velocity ratio



Tab 3. K-S test p-values for phenotypic measurements comparing the 4 cell types

Conclusion

Study results show that our automated exocytotic event detection and quantitative characterization tool could provide accurate and reliable results for broad exocytotic events quantification.

Future Efforts

We will make the tool available on SVCell to assist scientists on quantitative characterization. We will also further investigate to confirm or reject the new insights discovered for velocity related phenotypes

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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