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A Gaussian mixture model for automated vesicle fusion detection and classification

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A GAUSSIAN MIXTURE MODEL FOR AUTOMATED VESICLE FUSION DETECTION AND CLASSIFICATION

by

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ABSTRACT

Accurately detecting and classifying vesicle-plasma membrane fusion events in fluorescence microscopy, is of primary interest for studying biological activities in a close proximity to the plasma membrane. In this paper, we present a novel Gaussian mixture model for automated identification of vesicle-plasma membrane fusion and partial fusion events in total internal reflection fluorescence microscopy image sequences. Image patches of fusion event candidates are detected in individual images and linked over consecutive frames. A Gaussian mixture model is fit on each image patch of the patch sequence with outliers rejected for robust Gaussian fitting. The estimated parameters of Gaussian functions over time are catenated into feature vectors for classifier training. Applied on three challenging datasets, our method achieved competitive results on detecting and classifying fusion events compared with two state-of-the-art methods.

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1. INTRODUCTION

Vesicle exocytosis is an essential cellular trafficking process, by which materials (e.g., transporters, receptors and enzymes) are transported from one membrane-bounded organelle to another or to the plasma membrane for growth and secretion. Vesicle exocytosis needs to be highly regulated as the dysregulation of it is related to many human diseases (e.g., neurodegenerative disease, cancer and diabetes, etc. [1]). Total Internal Reflection Fluorescence (TIRF) microscopy is a powerful tool for visualization and analysis of vesicle exocytosis; it selectively records the dynamics of vesicle traffic near the bottom of plasma membrane, with superb spatial resolution. A pH-sensitive mutant of GFP, pHluorin, is widely used in visualization of single vesicle lumen. When a vesicle is exposed to extracellular neutral environment as the vesicle fuses with the plasma membrane, it becomes brightly fluorescent. The vesicle's three-stage movement is shown in Fig.1.1. In stage 1, vesicles move towards the cell membrane. In the stage 2 and stage 3, by the stimulation of insulin, some vesicles fuse on the cell membrane with a visible "explosion" phenomenon (called "puff"), which are considered as a significant feature of full fusion events. Other vesicles undock the cell membrane and suddenly move out of the view, which are considered as partial fusion events.

1.1. RELATED WORK

A typical TIRF image sequence might consist of hundreds of frames with hundreds of vesicles. Manual analysis is very time-consuming, even for experienced biologists. When computer-based microscopy image analysis is used to relieve human from the tedious manual labelling [2, 3], it is unsurprising that the uncontrollable noise interference

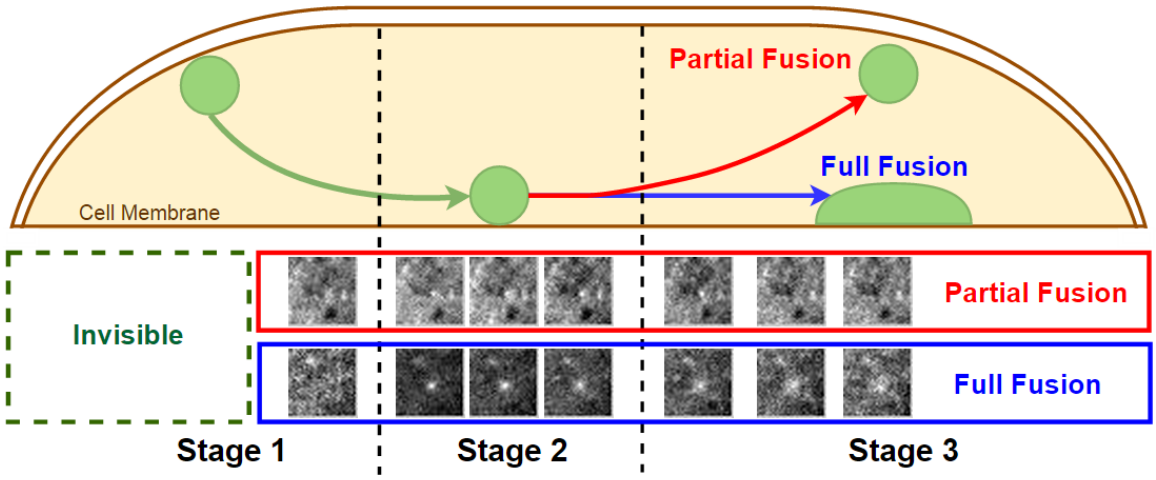

Figure 1.1. The movement process of vesicles and their related fusion events.

of TIRF images and the high variability of fusion events' size, duration and maximum intensity hinder the automated image processing.

An automated method to identify full fusion events was proposed in [4], which uses a local maximal detector to search fusion event patches, then connects detected patches in the same position as patch sequences. If the total patch intensity increases within 1 second, while the patch's peak intensity decreases during the same time window, then this patch sequence is considered as a full fusion event. However, local maximal detector is sensitive to intensity variation caused by background fluctuation inside the cell, which may generate many false positive detections (Fig.1.2(a)). The fusion event patch detection may be affected by moving objects too (Fig.1.2(c)). Lorenz et al.[5] proposed a method to detect pixels with local maximal/minimal intensity in each frame. For each detected pixel, a diffusion model is developed to analyze the quantity of local maximal pixels and the local minimal pixels happened at this pixel's position, in a time window. The diffusion model method effectively distinguished full fusion events from non fusion regions, leaving a large amount of partial fusion events unrecognized. A Gaussian model was used to fit fusion events in [6]. The standard deviation in the Gaussian model is used to classify fusion

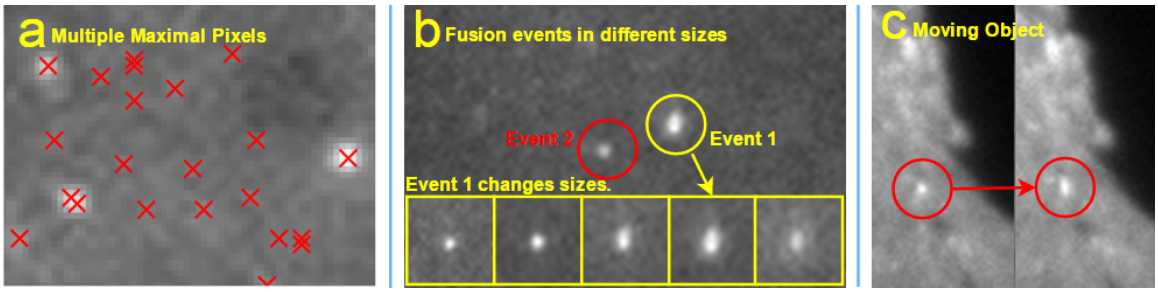

Figure 1.2. Examples of major challenges of fusion event detection.

events, assuming full fusion events have greater standard deviation than the partial fusion events. However, fusion events generally have large variation on its blob sizes spatially and temporally (Fig.1.2(b)).

1.2. OUR PROPOSAL

In our work, an adaptive detection method based on local contrast is proposed first for searching potential fusion event patches in each frame. Then, a tracking method is developed to link detected patches over consecutive frames into patch sequences as the fusion event candidates. Thirdly, a mixture of Gaussian functions are fit on each patch of the patch sequence with Random Sample Consensus algorithm to remove outliers during the Gaussian fitting. Finally, the patch sequences are aligned with the same time length and a feature vector is extracted from a series of Gaussian functions fit on the patch sequence, based on which a Support Vector Machine classifier is trained to classify the patch sequence into one of three classes: full fusion, partial fusion or non-fusion event.

2. DETECTION OF FUSION EVENT CANDIDATES

During the 3-stage vesicle movement in Fig.1.1, vesicles intend to halt at a position near the cell membrane for a few seconds before they either fuse or undock. This visible phenomenon is perceived as a process of intensity changes which suddenly appears in the image and then disappears either gradually (full fusion) or all in a sudden (partial fusion). In this process, the high local contrast is a significant feature to recognize fusion events, which is visible in the stage 2 or 3. According to these observation, we propose a local contrast detector to detect potential fusion event image patches frame-by-frame, and then the detected patches are tracked/linked over consecutive frames into patch sequences as the fusion event candidates.

2.1. DETECT POTENTIAL FUSION EVENT IMAGE PATCHES

During the stage 2 and 3 of a vesicle fusion event, the intensities of pixels around the vesicle location increase. Thus, finding local intensity maximums has been used to detect image patches as the candidates of fusion events [4, 6]. However, due to the illumination variation and intensity fluctuation of background pixels, the local maximal method may find many false positives (Fig.2(a)). Instead, we use a local contrast detector to detect image patches of fusion event candidates. If a pixel's intensity value, $I(p)$, is more than γ times of the maximal intensity value among the pixels which are away from pixel p by a radial distance r, then pixel p is detected as a high contrast pixel. After detecting all pixels with high contrast, those pixels which are close to each other are clustered together into a group and an image patch around the group centroid is extracted as a fusion event candidate in the current image I.

2.2. LINK POTENTIAL FUSION EVENT IMAGE PATCHES

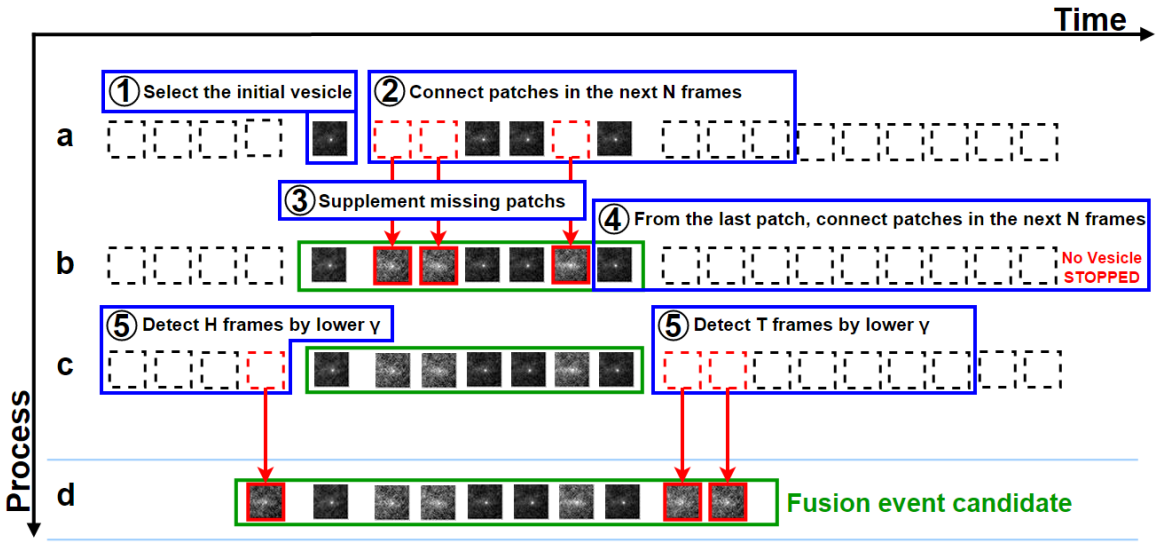

Figure 2.1. The method to link image patches into fusion event candidates.

After the frame-by-frame detection, all detected potential fusion event patches are stored in a matrix $\mathbf{V}$ with the size of $P \times 3$ where P is the number of candidate patches in the image sequence and each row of $\mathbf{V}$ stores the spatial location and timestamp of a patch. $\mathbf{V}$ stores the patches of the 1st frame, then the 2nd frame and so on. We propose a five-step approach to link the patches into patch sequences over time, as illustrated in Fig.2.1. The 1st step starts from picking the first patch in $\mathbf{V}$ as the initial potential vesicle and the frame in which it appears is the initial frame. The 2nd step is to search in the next N frames for other fusion event candidate patches around the initial patch. In the 3rd step, we connect candidate patches from the initial frame to the last frame in the N frames which contains a candidate patch. During this connection, patches missed in some frames can be supplemented by extracting patches around interpolated positions. In the 4th step, the last connected patch will be the new initial vesicle, then the 2nd step and 3rd step are repeated until there is no potential patches in the next N frames. In the 5th step, we search

the missing patches from the previous H frames and the next T frames of the fusion event candidate using a lower threshold γ in the local contrast detector.[1] Thurs, a complete set of image patches are linked to form a fusion event candidate (Fig.3(d)). All image patches in this fusion event candidate will be deleted from $\mathbf{V}$, and the five steps are repeated until $\mathbf{V}$ is empty.

[1]We set the detector threshold $\gamma = 1.3$ for the frame-by-frame detection, and γ is lowered to 1.1 for detecting missing patches in the 5th step. Since fusion events tend to have a quick appearance at the end of stage 1, but a relatively longer period to fade away in stage 3, we set $H = 5$ for head frames and $T = 10$ for tail frames. The parameters can also be learned by cross-validation.

3. GAUSSIAN MIXTURE MODEL FOR CLASSIFICATION

The pixel intensity values in a fusion event have been modeled by a 2D Gaussian model in [6, 7]. However, the pixel intensity in a fusion event may be complicated such that a single Gaussian is not accurate enough to model it, thus a mixture of Gaussian might be a good solution. Furthermore, fitting a Gaussian model on observed data is very sensitive to outliers, so a robust mechanism is required to avoid outlier pixels with undesired intensity fluctuation.

In this paper, the region-of-interest of each fusion event is defined by a "peak area" and a "flat area" as shown in Fig.4.1. The "peak area", denoted as $\mathbf{Area}_p$, is a 5×5 neighborhood which is centered by the highest intensity pixel of the impulse. The "flat area", denoted as $\mathbf{Area}_f$ is a 13×13 neighborhood surrounding $\mathbf{Area}_p$. In the Gaussian mixture model, two center-surround 2D Gaussian models will fit the pixel values in $\mathbf{Area}_p$ and $\mathbf{Area}_f$, respectively. Meanwhile, to avoid the outlier effect, a Random Sample Consensus (RANSAC) algorithm is adopted to estimate the parameters of Gaussian models robustly.

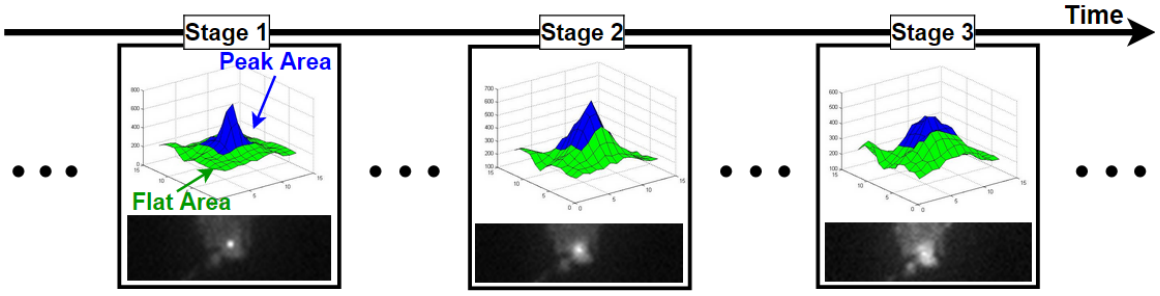

Figure 3.1. The region-of-interest of a fusion event consists of a "peak area" and "flat area". In stage 2 and stage 3, fusion spreads into the "flat area"

3.1. 2D GAUSSIAN MODEL FITTING

We define our 2D Gaussian function as

$$I(x,y) = \lambda \exp(-\frac{x^2+y^2}{2\sigma^2}) + \beta \tag{3.1}$$

where $I(x,y)$ is the intensity value at the position (x,y). Note that λ is not necessarily to be $\frac{1}{2\pi\sigma^2}$ since we model the pixel intensities by a Gaussian function rather than a Gaussian probability distribution. We further simplify Eq.3.1 by defining $\alpha = -\frac{1}{2\sigma^2}$, thus

$$I(x,y) = \lambda \exp(\alpha(x^2+y^2)) + \beta. \tag{3.2}$$

β is computed as the minimum of pixel values. For example, when fitting the Gaussian function to the peak or flat area,

$$\beta = \min_{m \in \mathbf{Area}_{p/f}} \{I_m\}. \tag{3.3}$$

Suppose M pixels are selected from the peak area to fit its Gaussian function, the following cost function is defined to estimate λ and α for the peak area:

$$L(\lambda,\alpha) = \sum_{m=1}^{M} [log(I_m - \beta) - log(\lambda \exp(\alpha(x_m^2+y_m^2)))]^2 \tag{3.4}$$

Taking the partial derivatives regarding to λ, α and setting them to zero lead to

$$\alpha = \frac{M\sum_{m=1}^{M}(x_m^2+y_m^2)log(I_m-\beta) - (\sum_{m=1}^{M}(x_m^2+y_m^2))(\sum_{m=1}^{M} log(I_m-\beta))}{M\sum_{m=1}^{M}(x_m^2+y_m^2)^2 - (\sum_{m=1}^{M}(x_m^2+y_m^2))^2} \tag{3.5}$$

$$\lambda = exp(\frac{\sum_{m=1}^{M} log(I_m-\beta) - \alpha\sum_{m=1}^{M}(x_m^2+y_m^2)}{M}) \tag{3.6}$$

Similarly, the 2D Gaussian fitting procedure can be applied to the flat area.

3.2. RANSAC ALGORITHM TO AVOID OUTLIERS DURING GAUSSIAN FITTING

When fitting Gaussian functions in the peak and flat areas, the outlier pixels from background may bias the parameter estimation largely. Especially, $\mathbf{Area}_f$ is a relatively larger area in TIRF images compared to $\mathbf{Area}_p$, therefore it may contain more pixels which are interfered by pixel intensity noise. In some cases, the positions of multiple fusion events are very close such that the $\mathbf{Area}_f$ of them may overlap, which will also challenge the compatibility of the Gaussian mixture model we proposed. To avoid these interferences, we apply the Random Sample Consensus (RANSAC) method to estimate the optimal λ, α and β of Eq.3.2 in Algorithm 1.

Algorithm 1 Gaussian fitting with Random Sample Consensus.

Require:
Maximum iterations K, $\mathbf{Area}_{p/f}$, distance threshold T;
Ensure:
1: **while** Iteration less or equal to K **do**
2: Randomly pick 4 pixels in $\mathbf{Area}_{p/f}$;
3: Estimate λ, α and β by Eq.3.6, Eq.3.5 and Eq.3.3, respectively;
4: Compute all pixel intensities in $\mathbf{Area}_{p/f}$ by Eq.3.2: $\hat{I}_m$;
5: Count the number of inliners (those pixels with $(I_m - \hat{I}_m)^2 < T$);
6: **end while**
7: Output the optimal λ, α and β with the maximal number of inliners;

3.3. FEATURE EXTRACTION FROM GAUSSIAN MODELS FOR CLASSIFICATION

Since the fusion event candidates (represented as image patch sequences) may have different time lengths, we need to align the patch sequences and cut/append them with the same time length in order to have comparable feature vectors. The alignment process is illustrated in Fig.3.2. For each fusion event candidate, the maximum intensity value of each image patch is computed and the maximums of all image patches of this fusion

event candidate formulate a time-series signal, whose climax moment (t^*) is selected as the time instant to align the fusion event. We extract features from each image patch in the temporal sliding window $[t^* - F_h, t^* + F_t]$, where $F_h = 10$ and $F_t = 20$ in our experiments. For fusion event candidates whose time lengths are shorter than the sliding window, we will zero-padding them to have the sliding window of $[t^* - F_h, t^* + F_t]$, while the fusion event candidates with longer time lengths will be fit into the time length by dropping frames exceeding the temporal sliding window.

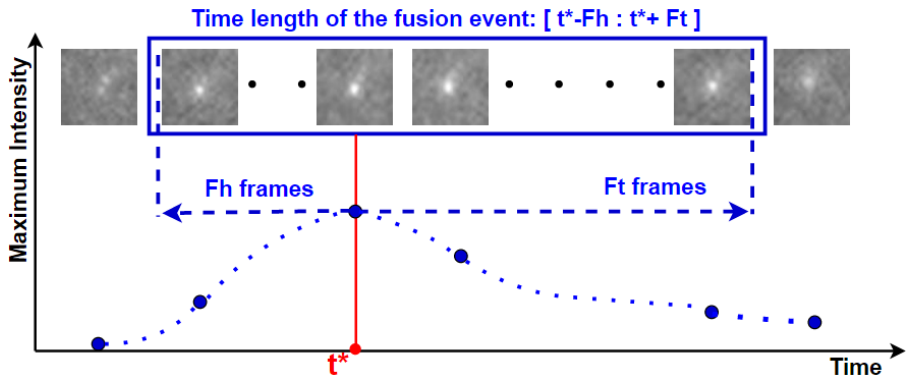

Figure 3.2. Feature vector alignment process.

For each patch, we obtain a 1×6 feature vector, including the λ, α and β from both peak area and flat area. The feature vectors of all image patches in a fusion event candidate are catenated sequentially, so the length of our feature vector for a fusion event candidate is $6 * (F_h + F_t + 1)$, based on which we train a Support Vector Machine classifier to classify the fusion event candidate into one of three classes: full fusion, partial fusion, or non-fusion.

4. RESULT

Three datasets were captured for the experiments, each of which consists of 300 images. The ground truth was provided by an experienced cell biologist working in the field of vesicle trafficking analysis in TIRF microscopy. The comparison between our method, denoted as G.M.M, with RANSAC and without RANSAC, denoted as G.M.M w/o RANSAC, was tested in Dataset 1, with the results shown in Table 1. By avoiding outliners during the Gaussian fitting, our method effectively improve the classification accuracy on both full and partial fusion events.

Table 4.1. The comparison of four methods on dataset 1.

Dataset 1	Full Fusion			Partial Fusion		
	Precision	Recall	F Score	Precision	Recall	F Score
G.M.M	89.2%	91.7%	90.4%	89.3%	83.3%	86.2%
G.M.M w/o RANSAC	85.3%	80.6%	82.9%	78.1%	83.3%	80.6%
S.G.M.[3]	79.5%	86.1%	82.7%	78.6%	73.3%	75.9%
Int.V.[2]	64.4%	80.6%	71.6%	n/a	n/a	n/a

We also compare our method with the single Gaussian model method [6] (denoted as S.G.M.) and intensity-variance-based method [4] (denoted as Int.V.) in all three datasets,

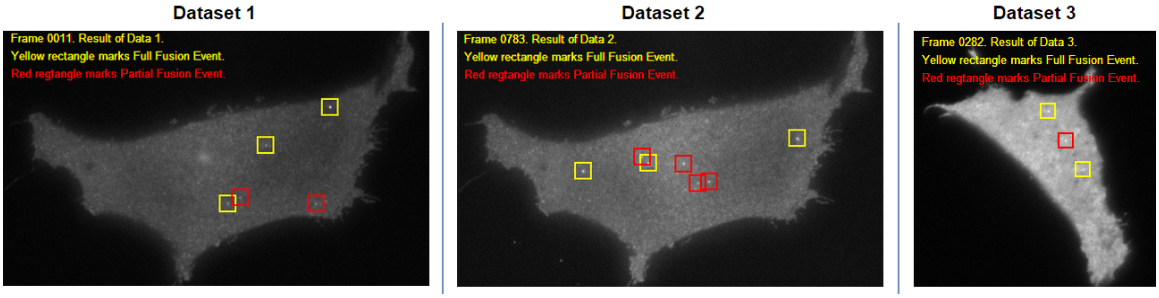

Figure 4.1. Classification examples of our method on three datasets (yellow: partial fusion; red: full fusion).

Table 4.2. The comparison of four methods on dataset 2.

Dataset 2	Full Fusion			Partial Fusion		
	Precision	Recall	F Score	Precision	Recall	F Score
G.M.M	93.3%	89.4%	91.3%	80.0%	91.0%	85.1%
S.G.M[3]	80.4%	78.8%	79.5%	70.8%	77.3%	73.9%
Int.V.[2]	71.4%	74.5%	72.9%	n/a	n/a	n/a

Table 4.3. The comparison of four methods on dataset 3.

Dataset 3	Full Fusion			Partial Fusion		
	Precision	Recall	F Score	Precision	Recall	F Score
G.M.M.	68.2%	71.4%	69.8%	62.5%	71.4%	66.7%
S.G.M.[3]	60.9%	66.7%	63.7%	50%	57.1%	53.3%
Int.V.[2]	53.8%	66.7%	59.6%	n/a	n/a	n/a

with the results shown in Tables (1-3), respectively. All parameters in algorithms of [4] and [6] are optimized to ensure they can achieve the best results in our datasets. Compared to the single Gaussian model method [6], our method achieves better classification results for both full fusion events and partial fusion events in three tested datasets, which validates that the Gaussian mixture model has a better compatibility to fusion events than the single Gaussian model. The algorithm in [4] was developed for full fusion event identification based on intensity thresholds on multiple low level features, e.g., maximum intensity variance and total intensity variance. Compared to the algorithm in [4], our proposed method has a better full fusion classification result, which proves that the feature we extract from proposed Gaussian mixture model is more effective than low level intensity features. Note that dataset 3 has very low Signal-Noise-Ratio and the frequent background fluctuation generates a strong interference on the performance of all methods. The short fusion duration in dataset 3, which is as short as 3 frames, makes the feature extraction difficult.

5. CONCLUSION

Accurately detecting and classifying vesicle-plasma membrane fusion events from TIRF microscopy images is an important research problem on cellular trafficking processes. We proposed an adaptive detection method based on local contrast to detect image patches of fusion event candidates in individual frames and developed a tracking method to link image patches as candidate patch sequences. A center-surround Gaussian mixture model was proposed to fit the image patch intensity with outliers rejected for robust Gaussian fitting. A feature vector is extracted from parameters of the series of Gaussian functions fit on the aligned patch sequence, based on which a SVM classifier is trained. Compared on three challenging datasets, our method showed promising performance and outperformed two state-of-the-arts.

BIBLIOGRAPHY

[1] J. Hou. Ins (endocytosis) and outs(exocytosis) of glut4 trafficking. *Current Opinion in Cell Biology*, 19:466–473, 1997.

[2] W. Godinez. Identifying fusion events in fluorescence microscopy images. *International Symposium on BIOMEDICAL IMAGING*, pages 1170–1173, 2009.

[3] A. Basset. Localization and classificaiton of membrane dynamic in tirf microscopy image sequences. *International Symposium on BIOMEDICAL IMAGING*, pages 830–833, 2014.

[4] S. Huang. Insulin stimulates membrane fusion and glut4 accumulation in clathrin coats on adipocyte plasma membranes. *Molecular and Cellular Biology*, 27:3456–3469, 2007.

[5] B. Lorenz. A diffusion model for detecting and classifying vesicle fusion and undocking events. *The Medical Image Computing and Computer Assisted Intervention*, pages 329–336, 2010.

[6] L. Bai. Dissecting multiple steps of glut4 trafficking and identifying the sites of insulin action. *Cell Metabolism*, 5:47–57, 2007.

[7] Y. Xu. Dual-mode of insulin action controls glut4 vesicle exocytosis. *The Journal of General Physiology*, 193:643–653, 2011.

VITA

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