CA^{2+} SPARKS DETECTION AND CLASSIFICATION USING GAUSSIAN-MEXICAN HAT WAVELET

Zhi Zhou[1], Yingzi Du[1], George G. Rodney[2], Martin F. Schneider[2]*

[1]Dept. of Electrical and Computer Engineering
Indiana University-Purdue University Indianapolis
Indianapolis, IN 46202, USA

[2]Dept. of Biochemistry & Molecular Biology
University of Maryland School of Medicine
Baltimore, MD 21201, U.S.A

ABSTRACT

It is evidenced that Ca^{2+} plays a critical role in cell activities. Understanding and analysis of a Ca^{2+} sparks event can provide critical information in cell communications. It is known that a Ca^{2+} spark spatial spread in a local area can be modeled by a Gaussian distribution. There are some available methods in Ca^{2+} spark detection, but little research has been done to quantitatively analyze and classify the Ca^{2+} sparks into single or multiple release events. In this paper, a new method based on Gaussian-Mexican Hat Wavelet is proposed to automatically classify Ca^{2+} sparks into single release events or multiple releases events. Then Ca^{2+} sparks from single release events are further categorized into different energy levels. The experiment results using mouse skeletal muscle fiber cells show this method is promising.

Index Terms **−** Ca^{2+} sparks detection, Ca^{2+} spark classification, Gaussian-Mexican Hat Wavelet.

1. INTRODUCTION

It is evidenced that Ca^{2+} plays a critical role in cell activities [1-3]. Understanding and analysis of a Ca^{2+} sparks event can provide critical information in cell communications. It is found that spontaneous Ca^{2+} sparks are discrete, localized elevations of Ca^{2+}, which can be detected using Ca^{2+} indicator dye and a laser-scanning confocal microscope [3]. The spatial spread of Ca^{2+} sparks in a cell can be modeled by Gaussian distribution [4].

Cheng *et. al.* proposed an automatic Ca^{2+} Spark detection method using amplitude distribution [5]. This method was then modified by Chun *et. al.* [3] to identify Ca^{2+} release events in XY confocal images. While the detection process is automatic, the region of interest has to be manually selected. Recently, we [6] proposed a fully automatic approach. This system automatically detects the region of interest and separates it from the background, automatically finds the optimal thresholds for Ca^{2+} Sparks detection, and takes advantage of the correlation between two consecutive frames in detecting the Ca^{2+} sparks.

However, little research has been done in Ca^{2+} sparks classification. In this paper, we propose a method to categorize the Ca^{2+} sparks into two types: Ca^{2+} sparks from single release events or from multiple release events. For a single Ca^{2+} spark release event, we further classify it into different energy release levels. This analysis and classification will help further our understanding of the activities of calcium in the cell. The proposed method first automatically detects Ca^{2+} sparks and then classifies each spark using Mexican Hat wavelet.

2. AUTOMATIC CA^{2+} SPARKS DETECTION

Based on previous related work [3-6], we model the Ca^{2+} spark events in Fig. 1. Let the cell image be $\boldsymbol{f(x,y)}$ and the Ca^{2+} spark events at i^{th} frame to be $c_i(x,y)$. The release process of the Ca^{2+} Spark events in the cell can be modeled as a filter *H*. The obtained cell image from confocal microscope is:

$$\boldsymbol{g_i(x,y) = f(x,y) + c_i(x,y) \otimes H + \eta_i(x,y)} \quad (1)$$

Here $\eta_i(x,y)$ is noise.

The goal of the Ca^{2+} sparks detection is to estimate $c_i(x,y)$ from the observed image $\boldsymbol{g_i(x,y)}$. Here, the cell image $\boldsymbol{f(x,y)}$ is assumed unchanged in the image acquisition process.

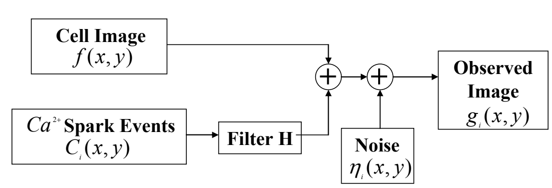


Fig.1. The model of Ca^{2+} sparks in a cell image

To detect the Ca^{2+} sparks from the observed image $\boldsymbol{g_i(x,y)}$, the cell image should be first estimated (Fig. 2). Fig.2 shows the model to estimate the cell image

Each observed image is first enhanced to reduce the noise and then averaged together to detect the background and find the region of interest (ROI). Then the index image is extracted. This index image $\hat{f}(x, y)$ is a close estimation to the cell image $f(x, y)$ [3,5,6].

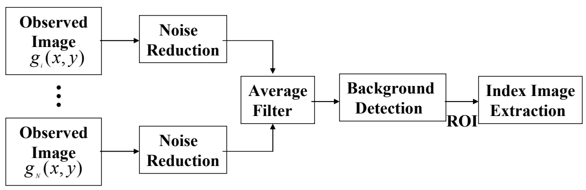


Fig. 2. The model for cell image estimation.

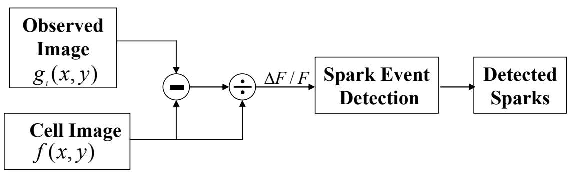


Fig. 3. The model to detect the Ca^{2+} spark events

The model to detect Ca^{2+} sparks is shown in Fig. 3. After estimating the cell image, double thresholds [6] are used to detect the Ca^{2+} sparks. Here the △F/F image $\hat{g}_i(x, y)$ is calculated by:

$$\hat{g}_i(x, y) = [g_i(x, y) - \hat{f}(x, y)] / \hat{f}(x, y) \qquad (2)$$

The diagram of the Ca^{2+} spark events detection is shown in Fig. 4. An example of the Ca^{2+} spark events detection process is shown in Fig. 5.

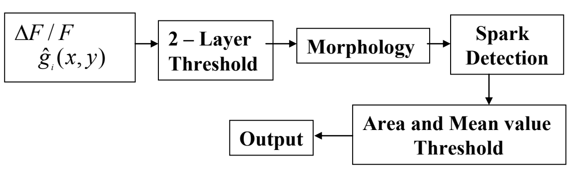


Fig. 4. The diagram of the Ca^{2+} sparks event detection.

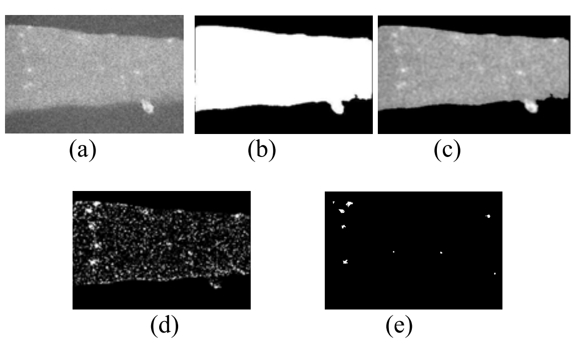


Fig. 5. An example of Ca^{2+} sparks detection process. (a) Original image; (b) ROI; (c) Index image; (d) △F/F image; (e)Detected Ca^{2+} sparks.

3. CA^{2+} SPARKS CLASSIFICATION

Fig. 5 shows that the detected Ca^{2+} sparks have different sizes. How do we model the Ca^{2+} sparks in terms of the energy level? For the large energy level Ca^{2+} sparks, some of them may be from a single release of Ca^{2+}, but others may be a combination of multiple Ca^{2+} releases from close locations.

It is known that a Ca^{2+} spark distribution in a local area can be modeled by a Gaussian distribution [5]. To categorize the Ca^{2+} spark events, it is reasonable to have this assumption: for a single release event, the spatial distribution of the energy of this Ca^{2+} spark is close to one Gaussian distribution; but for a multiple release event from nearby locations, the spatial distribution is a combination of multiple Gaussian distributions. Hence, by classifying the spatial distribution of a Ca^{2+} spark, we can classify if it is a single release event or multiple release event.

Argueso *et. al.* [7] in their experiment with point source detection in cosmic microwave background maps found that the Gaussian-Mexican Hat Wavelet family can amplify the ratio between the point source intensity and the dispersion of the background. If we construct △F/F images into different resolution scales, a Ca^{2+} spark from a single release event can become a point source in a certain scale. But a Ca^{2+} spark from a multiple release event will not be a point source; instead, it will be a small area. Hence, we can use the Gaussian-Mexican Hat Wavelet family to detect the point source (single Ca^{2+} spark event) in each level. The combination of detected point sources in all the levels is a possible Ca^{2+} spark from a single release event. The point source detected can be a false alarm. Only the point sources that are Ca^{2+} sparks detected (using method in Section 2) will be Ca^{2+} sparks.

For those Ca^{2+} spark events detected in Section 2 but not detected using this Gaussian-Mexican Hat Wavelet, these are the Ca^{2+} sparks from multiple events in a close area. In this way, we can classify a Ca^{2+} spark to be two categories: from a single release event or from a multiple release event.

For a Ca^{2+} spark from a single event, we then categorize it into different energy levels based on the resolution level that the Ca^{2+} spark is detected. This gives meaning for further understanding of the Ca^{2+} sparks released by the cell: it can help to identify if it is from one Ca^{2+} molecule or multiple Ca^{2+} molecules. Currently, we still need biology experiments to understand the energy level of each Ca^{2+} molecular release. Here, we theoretically model and categorize the Ca^{2+} energy release.

Below is the introduction of our proposed method for Ca^{2+} sparks classification.

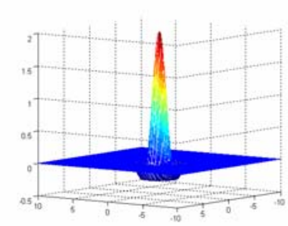
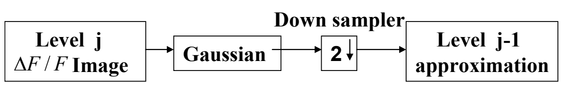


Fig. 6. 2D Mexican Hat Wavelet

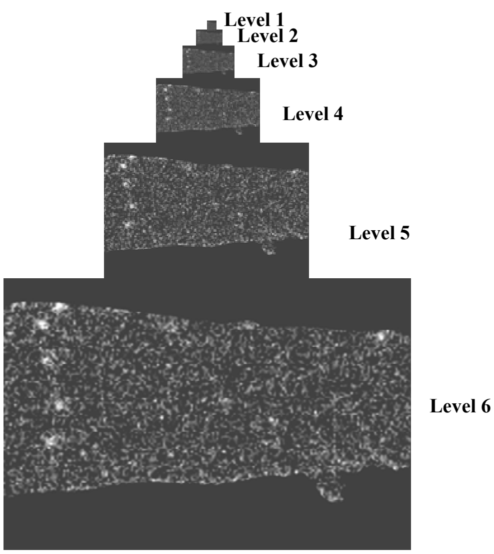
2D Mexican Hat wavelet in the time domain (Fig. 6) is:

$$\Psi(x,y) = (x^2 + y^2 - 2)e^{-\frac{1}{2}(x^2+y^2)} \quad (3)$$

The calculated △F/F images are reconstructed into different resolutions using Gaussian pyramids. And the Mexican Hat Wavelet is applied to each resolution level. In this way, a simple Gaussian-Mexican Hat Wavelet is constructed. The diagram of the Gaussian pyramids is shown in Fig. 7(a) and an example of the constructed △F/F image pyramid is shown in Fig. 7(b).



(a) j-1th level Gaussian Pyramid Construction



(b) An example of the constructed Pyramid.

Fig. 7. Gaussian pyramids construction.

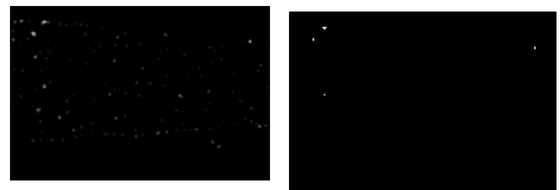
For each level, Mexican Hat Wavelet is applied to the reconstructed △F/F image $\hat{g}_i^j(x,y)$:

$$s_i^j(x,y) = \hat{g}_i^j(x,y) \otimes \Psi^j(x,y) \quad (4)$$

Here $\Psi^j(x,y)$ is the 2D Mexican Hat Wavelet in Eq. (3) applied in j^{th} level of the image. $s_i^j(x,y)$ is then thresholded to detect the Ca^{2+} sparks in this level:

$$P_i^j(x,y) = \begin{cases} 1, & s_i^j(x,y) > T^j \\ 0, & else \end{cases} \quad (5)$$

Here T^j is the threshold.



(a) After Mexican Hat (b) Ca^{2+} sparks detection

Fig. 8. An example of Ca^{2+} sparks detection in the 4th level of the image in Fig. 7(b).

If a Ca^{2+} spark has been detected in a lower level, then it will be eliminated from the rest of the levels. Fig. 8 shows an example of the Ca^{2+} spark detection in the 4th level of the pyramid in Fig. 7(b). Fig. 8(a) is after applying of Mexican Hat Wavelet, and Fig. 8(b) is after thresholding to detect the Ca^{2+} sparks in this level.

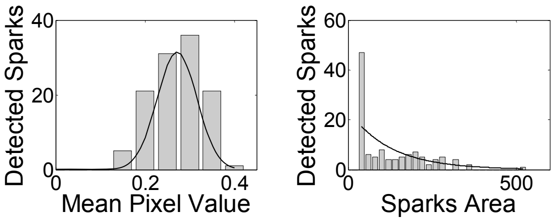
4. EXPERIMENTAL RESULTS AND ANALYSIS

We used muscle fiber cells from a four-to-five week old CD-1 mouse for this study [8]. The observed image sequence includes 40 image frames. Figs. 5,7 and 8 are obtained from experimental results from this image sequence.

We detected 114 Ca^{2+} spark events in this image sequence. Using Gaussian-Mexican Hat Wavelet, 71 Ca^{2+} sparks were detected by the Gaussian-Mexican Hat Wavelet, which are classified as Ca^{2+} sparks from single release events. The rest of 43 Ca^{2+} sparks are then classified as Ca^{2+} sparks from multiple release events.

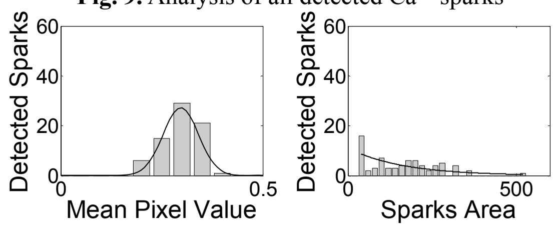
The mean pixel value distribution in △F/F images of all detected Ca^{2+} sparks, Ca^{2+} sparks from single release events, and Ca^{2+} sparks from multiple releases events are shown in Fig. 9(a), Fig. 10(a), and Fig. 11(a) respectively. The bins are from real data, and the smooth lines are the result of curve fitting. It shows from these figures that the distribution of the mean pixel value of the sparks is close to Gaussian. The average mean pixel value from single release events is higher than that from multiple release events. This occurs because the Ca^{2+} sparks from multiple release events are generally more spatially spread.

The spark area distribution in △F/F images of all detected Ca^{2+} sparks, Ca^{2+} spark from single release events, and Ca^{2+} sparks from multiple releases events are shown in Fig. 9(b), Fig. 10(b), and Fig. 11(b) respectively. The bins are from real data, and the smooth lines are the result of curve fitting. It shows from these figures that the distribution of the spark area is an exponential distribution.



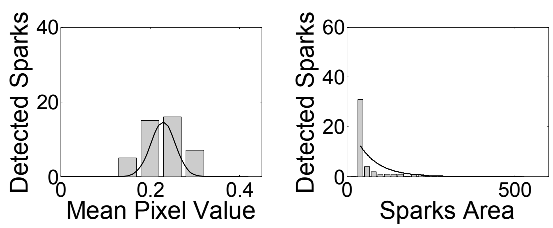
(a) (b)

Fig. 9. Analysis of all detected Ca^{2+} sparks



(a) (b)

Fig. 10. Analysis of detected Ca^{2+} sparks from single release events.



(a) (b)

Fig. 11. Analysis of Ca^{2+} sparks from multiple release events

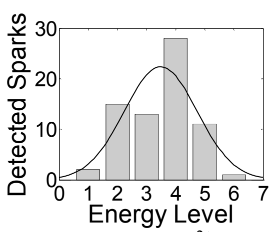


Fig. 12. The distribution of the Ca^{2+} sparks in different energy levels

For Ca^{2+} sparks from single release events, we further classify them into different energy levels. Fig. 12 shows the distribution of the Ca^{2+} sparks in different energy levels and it follows a Gaussian distribution. Most Ca^{2+} sparks are detected in the 4th level.

5. CONCLUSIONS

In this paper, we model Ca^{2+} spark events in a cell. We proposed a method to detect and classify the Ca^{2+} spark events. Gaussian-Mexican Hat wavelet is used to classify the Ca^{2+} sparks into single event or multiple events. The Gaussian-Mexican Hat Wavelet family can amplify the ratio between the point source intensity and the dispersion of the background. A Ca^{2+} spark from a single release event can be modeled by one Gaussian distribution while a Ca^{2+} spark from a multiple release event is modeled by a combination of multiple Gaussian distributions. Using multiple resolution approach, each single Ca^{2+} spark event can become a point source in a certain resolution scale. But a Ca^{2+} spark from a multiple release event will not be a point source, instead, it will be a small area, which will not be detected by the Gaussian-Mexican Hat Wavelet. In this way, we can classify if a Ca^{2+} spark is from a single release event or multiple release event. Furthermore, in this paper, we use the resolution scale to classify each single Ca^{2+} spark event into different energy levels. We applied this proposed method to mouse skeletal muscle fiber cells for Ca^{2+} spark event detection and classification. Our experimental results show this method can be used for Ca^{2+} spark classification and analysis. Further research using other kinds of cell images and further understanding of Ca^{2+} spark events will be necessary.

6. REFERENCES

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