Technical Article

by Katarina Mele, James G. Burchfield, Jamie Lopez, David E. James, William E. Hughes, Adelle Coster, and Pascal Vallotton

TIRF Microscopy Software for Automated Vesicle Fusion Detection

The recent explosion in the use of total internal reflection fluorescence (TIRF) microscopy to learn about cell trafficking means that there is an urgent need for software capable of annotating the corresponding movies automatically. TIRF Explorer software (CSIRO and Garvan, NSW, Australia) speeds up identification of vesicle–plasma membrane fusion events, a notoriously time-consuming task (see *Figure 1*). The authors' results indicate that it is possible to detect the majority of events automatically and that the rate of false positive and missed events compares well to what can be achieved by a human expert. The system is particularly useful to researchers in the areas of diabetes and obesity.

Method

When a fluorescently tagged vesicle fuses with the plasma membrane, a distinctive increase in fluorescence, followed by a characteristic spread of the signal, can be observed in TIRF movies (Figure 1). A TIRF movie can be thought of as a 3-D volume in which time represents the third dimension.

Figure 1 Consecutive time frames showing a very prominent fusion event. Note the rapid increase of intensity followed by a slower diffusion.

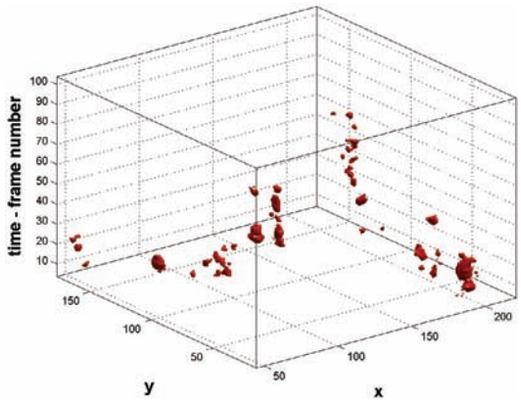

Figure 2 3-D regions represent candidates for fusion events.

TIRF movies usually consist of thousands of frames, and the 3-D volume can be enormous and challenging to analyze, even for very powerful computers. Rather than an extensive search of all the 3-D image space, patches of interest were selected using a very simple and extremely efficient procedure. This significantly reduced the computational time by reducing the search space to about 1% of the original. The process relied on the property that, due to increasing and decreasing intensity inside an event, the variability of the pixels was high where fusions occurred.

Only the patches of interest were then analyzed to identify candidates for fusion events. Regions for fusion events candidates were intersections of higher-intensity regions and highly variable regions. Examples of candidate regions are shown in *Figures 2* and *3*. As shown in Figure 3, not all candidates are fusion events. Bright and variable spots can also represent moving vesicles or simply a noise. Each candidate region was then described by a set of standard (e.g., volume, average intensity) and domain specific descriptors that described typical intensity increase, as shown in *Figure 4*. Principal component analysis (PCA) eigenspace was used to compare the candidates with preidentified genuine fusion events. The likeli-

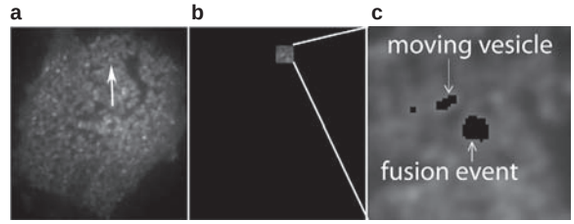

Figure 3 Typical TIRF image. a) The white arrow shows a spot where a fusion event occurred. There are many similar bright spots that could easily be misinterpreted as fusion events. b) Corresponding patch of interest. c) Candidate events, one of which was a genuine fusion event and the second was a moving vesicle.

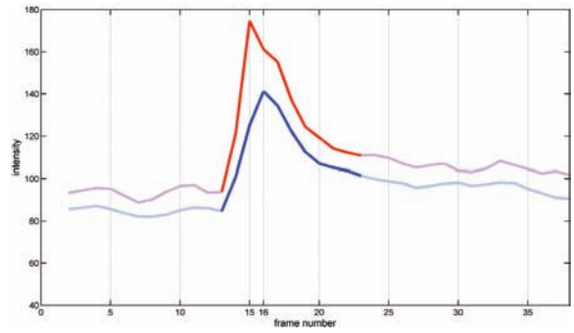

Figure 4 Intensity profile of an example fusion event. The red line is the intensity evolution in the center of an event. The blue line is the intensity evolution of the whole event area. Darker (enhanced) parts of the lines mark the frames where the event occurred.

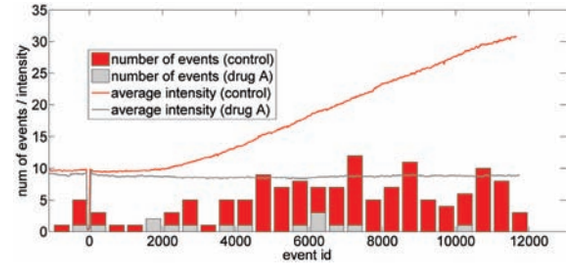

Figure 5 Comparison of the number of fusion events in a cell treated with Drug A and a control cell.

hood that a candidate region is a real fusion event is based on the eigenspace Euclidian distance between a candidate and a set of genuine fusion events, called prototype events. Prototype events were obtained automatically as events with the highest increase of intensity. Alternatively, prototype events can be set manually by an expert.

Application example

GLUT4 is the insulin-regulated glucose transporter found in adipose tissues and striated muscle that is responsible for insulin-regulated glucose disposal. Within these tissues, insulin promotes the exocytosis of GLUT4 molecules to the plasma membrane by a regulated vesicle trafficking event. In this study, the GLUT4 vesicle fusion rate was examined under normal conditions (control) and also under a pharmacologically treated condition (denoted as Drug A below) that has been shown to inhibit GLUT4 vesicle exocytosis. The details of the drug are confidential.

TIRF images were taken with a frame rate of 10 frames per second. There are 13,000 frames per movie. These movies were difficult to analyze because some of the events were very fast and faint. Using TIRF Explorer, the authors were able to demonstrate that pharmacological disruption of GLUT4 exocytosis resulted from a decrease in the GLUT4 vesicle fusion rate. Results are shown in *Figure 5*. Frame number 0 was the time when insulin was added to the cells. Note the increase in average intensity and the number of fusion events after this time point in the control cell, and the absence of response in cell treated with Drug A.

Dr. Mele is a Research Scientist, and Dr. Vallotton is Stream Leader, Biotech Imaging, CSIRO Mathematical and Information Sciences, Locked Bag 17, North Ryde, NSW 1670, Australia; tel.: +61 2 9325 3222; fax: +61 2 9325 3200; e-mail: katarina.mele@csiro.au. Dr. Mele, Dr. Burchfield, and Dr. Lopez are Research Officers; Prof. James is Director of Garvan's Diabetes and Obesity Research Program; and Dr. Hughes is Group Leader, The Garvan Institute of Medical Research, NSW, Australia. Dr. Coster is a Senior Lecturer, School of Mathematics & Statistics, University of New South Wales, Sydney, Australia, and is also affiliated with The Garvan Institute of Medical Research.