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MOTION DETECTION FOR SUBCELLULAR STRUCTURE TRAFFICKING

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ABSTRACT

A detailed understanding of subcellular structure motility is critical to understanding how cells regulate the delivery of specific proteins from the site of synthesis to the site of action. Diverse modalities have been observed for subcellular dynamics, such as directional movement, random movement, tethered movement, object appearing, and disappearing. Motion modality detection is important for efficient subcelluar structure tracking and population study. In this paper, we present a new technique, called "divergence filter," for detecting subcellular structure motion modalities. The plausibility of the efficient technique was applied to caveolar membrane trafficking sequences obtained using confocal microscopy.

Index Terms — subcellular structure, motility, microscopy images, motion detection

1. INTRODUCTION

Spatial and functional compartmentalization of the cell interior is vital to the health of eukaryotic cells, and thus to the health of multi-cellular organisms, including humans. A key mechanism for establishing and maintaining compartmentalization is the cytoskeleton-dependent active transport of organelles, vesicles, and macromolecular complexes to specific subcellular regions. For example, Caveolae—flask-shaped invagination in the plasma membrane—are involved in many cellular tasks such as endothelial transcytosis, cholesterol regulation, signal transduction, and tumor suppression [7]. A detailed understanding of caveloar membrane trafficking remains relatively unknown [3][5].

Inside the highly crowded but organized cell environment, small metabolites, proteins, and mRNA move constantly to maintain cell functions and renew cell constituents. Observations reported on the tendency of sub-cellular particle behavior have suggested that majority of the tagged protein molecules are random walk*, i.e.,* Brownian motion [4]. The most interesting *directed* motion or *transported* motion behaves *anomalous* or *irregular* compared to the large amount of surrounding random walk motions. Features of the trajectories of directed transport reveal interesting biological interactions. Bacher *et al*. have tracked single particles regardless of the type of mobility. Instead the particle motion classification was analyzed at the end of tracking process [2]. To avoid the unwanted computing thus resulted, certain research has been conducted on detection of directed motion [1][6]. Ritchie *et al.* used Monte Carlo simulations to explore the mechanism of anomalous molecule diffusion. Arrio-Dupont *et al.* applied modulated fringe pattern photobleaching directly on the pre-defined translational globular protein tracking.

Unlike simple Brownian motion which is isotropic and homogeneous, anomalous directed motion tends to be anisotropic [8]. Anomalous or irregular motion detection can also find wide applications in broad computer vision domain. One example would be the detection of suspicious behaviors of humans in crowded public environments. The detection can be characterized by irregularity of such humans in terms of movement and behavior while they are monitored by wide-angle cameras.

There are two major difficulties in detecting such irregular motion of particles. Firstly, it is difficult to detect and also to track the particle. We observed that particles in cells change their shapes (deformed) over time, may suddenly disappear, or suddenly appear. Conventional tracking techniques, such as template matching and optical flow, may not work in such cases. Secondly, even if such particles are detected and tracked, it is still difficult to characterize locally and globally the irregularity of particle motion in a region of interest. More specifically, in order to characterize the irregularity of the motion, we need to characterize the regularity with respect to the vicinity of a specific particle within the region of interest. Since such regular motion changes in time, we need to specify the regularity in each time frame, which requires a large computational complexity. For example, one may characterize such regularity by the average of motion vectors obtained from the motion vectors of all particles in its vicinity, and the irregularity of a specific particle may be represented by the discrepancy of its motion vector from the average one. In such cases, we need to update the average of motion vectors in each time frame. At the same time, when all particles are random in motion as is the nature in a stochastic cell environment, the average of the motion vectors may be zero. Therefore, it is technically challenging to distinguish irregular particles from others.

In this paper, we propose a new filtering technique for detecting directed motion of particles named "divergence

filter," utilizing the concept of "divergence" in physics. Divergence is the expansion or spreading out of a vector field, such as the net outflow of air from a given region. We first describe the proposed divergence filter, and then show experimental results.

2. METHODS

Given an image *I(x, y, t)* at time *t*, we define the particle existence probability (PEP) function *P(x, y, t)* as a particle of interest exists at position (*x, y*) of time *t* in the image *P(x, y, t)*. For example, for the fluorescently labeled sub-cellular particle, the intensity at position (*x, y*) represents the probability of existence of the particle at time *t*.

A closed *support region W* is defined around vicinity for each point of interest. We say region *W* is regular when the particle existence probability (PEP) does not change in a certain time frame. This regularity concept implies, in the probabilistic sense, that the region *W* is regular if approximately the same number of particles come into the region *W* and move out from the region. Therefore, isotropic motion like Brownian motion within *W* will be canceled out when we consider the regularity and irregularity motion in a support region.

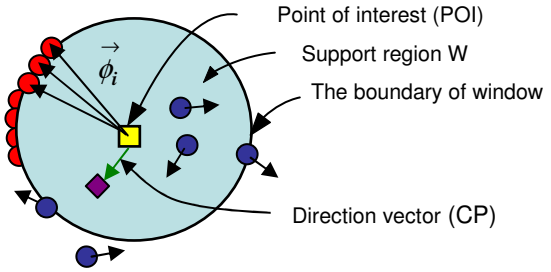

Figure 1 Divergence filter concept.

The analogy to "divergence" comes from the fact that we are paying attention to the increase and decrease of the number of particles in region *W*. A simple example of region *W* associated with each image position (*x, y*) may be a square support region whose size is $(2m+1)^2$. Given probability function *P(x, y, t)*, we define ρ as the rate of particles occupancy in region *W*:

$$\rho(\boldsymbol{x},\boldsymbol{y},\boldsymbol{t}) \equiv \int \boldsymbol{P}(\boldsymbol{x}-\boldsymbol{x}',\boldsymbol{y}-\boldsymbol{y}',\boldsymbol{t})\boldsymbol{W}(\boldsymbol{x}',\boldsymbol{y}')\boldsymbol{dx}'\boldsymbol{dy}' = \boldsymbol{P} * \boldsymbol{W} \quad (1)$$

For example, a uniformly-distributed unit function defined over a square region or a Gaussian-formed function may be considered for ρ.

2.1 Detection of Irregular Motion by a Divergence Filter

Given the above description of particles, we are ready to discuss the formation of particle irregularity. The number of particles existing in region *W* is computed as:

$$\boldsymbol{n}(\boldsymbol{x},\boldsymbol{y},\boldsymbol{t}) = \frac{\rho(\boldsymbol{x},\boldsymbol{y},\boldsymbol{t})}{\rho_0} \quad , \quad (2)$$

where ρ_0 is the average value of the rate of a single particle occupancy in region *W* over the entire image. The time derivative of *n* which measures the increase/decrease rate of number of particles in irregular motion at position *(x, y)* at time *t* is :

$$\boldsymbol{K}(\boldsymbol{x},\boldsymbol{y},\boldsymbol{t}) = \frac{\partial \boldsymbol{n}(\boldsymbol{x},\boldsymbol{y},\boldsymbol{t})}{\partial \boldsymbol{t}}. \quad (3)$$

In Eq. (3), if $\boldsymbol{K}(\mathbf{x},\boldsymbol{y},\boldsymbol{t}) < \tau$, we will say that region *W* associated with center position *(x, y)* becomes *regular*. Here τ is a threshold that controls the sensibility to detect irregular particles. Conversely, if $\boldsymbol{K}(\mathbf{x},\boldsymbol{y},\boldsymbol{t}) \geq \tau$, then $\boldsymbol{K}(\mathbf{x},\boldsymbol{y},\boldsymbol{t})$ represents the irregularity of region *W* associated with the pixel of interest *(x,y)*. In other words, probabilistically there exist *K* many particles that are irregularly moved in or around region *W*. Such irregularity maybe is caused by translational motion which is different from Brownian motion, or maybe is because of sudden appearance or disappearance of particles.

2.2 Identification of Transported Particles

As discussed in previous paragraphs, if $\boldsymbol{K}(\mathbf{x},\boldsymbol{y},\boldsymbol{t}) \geq \tau$, there exist approximately *K* particles in region *W* that move irregularly from outside. Now we need carefully investigate how likely an irregular particle exists at a specific position (*x, y*). Based on the theorem of divergence, we can only say that something happens through the boundary of region *W*, if $\boldsymbol{K}(\mathbf{x},\boldsymbol{y},\boldsymbol{t}) \geq \tau$.

Our solution to this localization problem is to distribute the value of K to each boundary point that constitutes to region *W* on the basis of the edge magnitude of boundary points. The weight of the distribution *M* is based on the normalized magnitude of the gradient of *W*. (Note as an analogy: In the theoretical sense, Gaussian theorem states that the divergence is computed on the basis of boundary – however in our case, particles may suddenly appear or disappear within the region – not necessarily occur through the boundary points. This case can be handled by distributing the edge magnitude to the boundary.)

$$\boldsymbol{M}(\boldsymbol{x},\boldsymbol{y}) = \frac{\|\nabla \boldsymbol{W}(\boldsymbol{x},\boldsymbol{y})\|}{\int \|\nabla \boldsymbol{W}(\boldsymbol{x},\boldsymbol{y})\| \boldsymbol{dxdy}} \quad (4)$$

In Eq. (4), the higher the magnitude is, the more likely an irregular particle exists at that specific position *(x, y)*. When region *W* becomes larger, *M* becomes smaller.

Finally, irregularity, the averaged number of particles in irregular motion passing through boundary position *(x, y)* of *W* at time *t,* namely *R* in the following equation, is computed by inversely convolving weight parameter *M* and the averaged number along the boundary points of region *W*:

$$\boldsymbol{R}(\boldsymbol{x},\boldsymbol{y},\boldsymbol{z},\boldsymbol{t}) \equiv \int \boldsymbol{K}(\boldsymbol{x}',\boldsymbol{y}',\boldsymbol{t})\boldsymbol{M}(\boldsymbol{x}-\boldsymbol{x}',\boldsymbol{y}-\boldsymbol{y}')\boldsymbol{dx}'\boldsymbol{dy}' = \boldsymbol{K} * \boldsymbol{M}. \quad (5)$$

By considering the irregularities of all the boundary points with respect to the point of interest (POI), the direction vector $\vec{CP}$ shown in Fig. 1can be calculated as

$$\vec{CP} = \sum_i R_i(x, y, t)\vec{\phi}_i , \quad (6)$$

where $R_i(x,y,t)$ is irregularity of boundary point i on region W. When such direction vector $\vec{CP} = \vec{0}$, it indicates the point of interest (x, y) demonstrate *Brownian* motion, which is consistent with the concept of divergence filter. On the other hand, if $\vec{CP} \neq \vec{0}$, but $\sum_i R_i(x, y, t) = 0$, the particle will be classified as *translational* motion; if $\vec{CP} \neq \vec{0}$, with $\sum_i R_i(x, y, t) > 0$, the point of interest demonstrates as newly *appearing*; if $\vec{CP} \neq \vec{0}$, with $\sum_i R_i(x, y, t) < 0$, the point of interest will be classified as *disappearing*.

specific proteins from the site of synthesis to the site of action. Some of the particles appear or disappear as time passes by, which reflects the population change of subcellular structures. The motion modality detection was previously done visually by our collaborators, which is error-prone for a large number of vesicles or organelles in a long time-lapse video sequence [5].

Time lapse sequences of cells expressing caveolin 1-GFP were taken with a Leica TCS-SP1 laser scanning confocal microscope with a 100x objective lens. The video images are of size 512x512 with a time interval of 1.2 sec between two frames. The total number of frames is 221, which is 3 min 2 sec. We observed the average size of tagged protein compound is less than 10x10 pixels which indicates challenges caused by small size and homogeneous object regions. The majority of the particles move randomly and locally. A small percent of particles demonstrate fast translational motion while certain ones appear and disappear as time goes on.

In the implementation, support region $\boldsymbol{W}$ is set as 5x5. Figure 2 shows portion of a video sequence where an

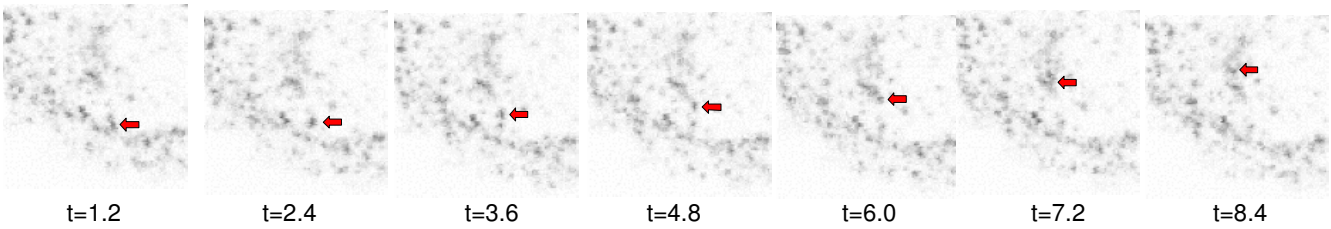

Figure 2 Image sequence where red arrows indicate irregular (translational here) motion of a particle.

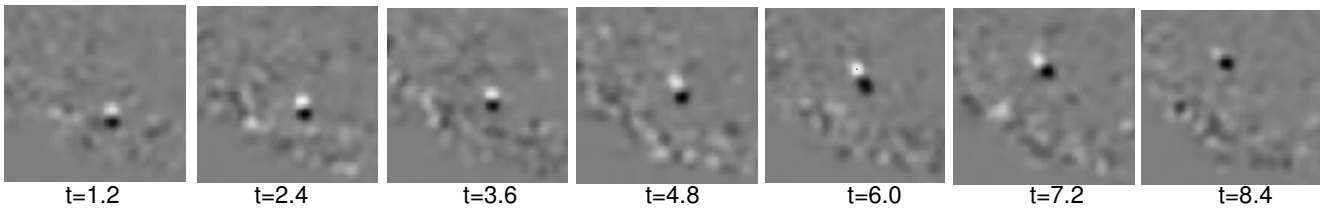

Figure 3 Middle results of divergence filter: value of irregularity *R*.

3. EXPERIMENTAL RESULTS

In this study, live cell, time-lapse confocal microscopy was used to study the trafficking of cavelolin-1-GFP (green fluorescent protein) in CHO (Chinese hamster ovary) cells. Cavelolin-1 is a well accepted marker protein whose C-terminus was attached to GFP. The trafficking of caveolae-derived membrane movement was studied under the dual control factors of microtubules and actin cytoskeleton [5].

The caveolar membrane trafficking has exhibited different modalities under visual inspection. The majority of the subcellular particles display tethered motion and move locally. A small percent of them demonstrate fast directional motion. Particles with obvious spatial movement are more of interest since this information reflects the delivery of *irregular* or *transported* particle is observed in time frames from t=1.2 to t=8.4. This particle indicated by red arrows is moving from bottom to top. Figure 3 demonstrates the result of divergence filter where gray-value 128 is set as zero for the derivative output so that both positive and negative values can be seen. Figure 4 shows the directed trajectory of the detected particle.

Computational analysis was also carried out on the whole image. Figure 5 shows the results of four frames from the recorded video sequence. A pair of yellow-green circles represents a translational particle. A single yellow circle shows a particle that suddenly appear in the images, while a single green circle shows a particle that suddenly disappears. The computational time to process the image sequence is approximately 0.5 seconds per frame for a 1.1Ghz Intel Pentium CPU.

4. DISSCUSIONS AND CONCLUSIONS

Intracellular trafficking is a key step in a host of important biological processes such as nerve transmission, growth factor signaling, antigen presentation, viral infection, and drug uptake. An innovative and efficient technique "divergence filter" in analogous to physics concept is presented in this paper to detect subcellular structure

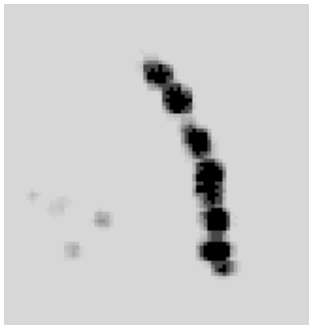

Figure 4 2D Trajectory of detected transported particle.

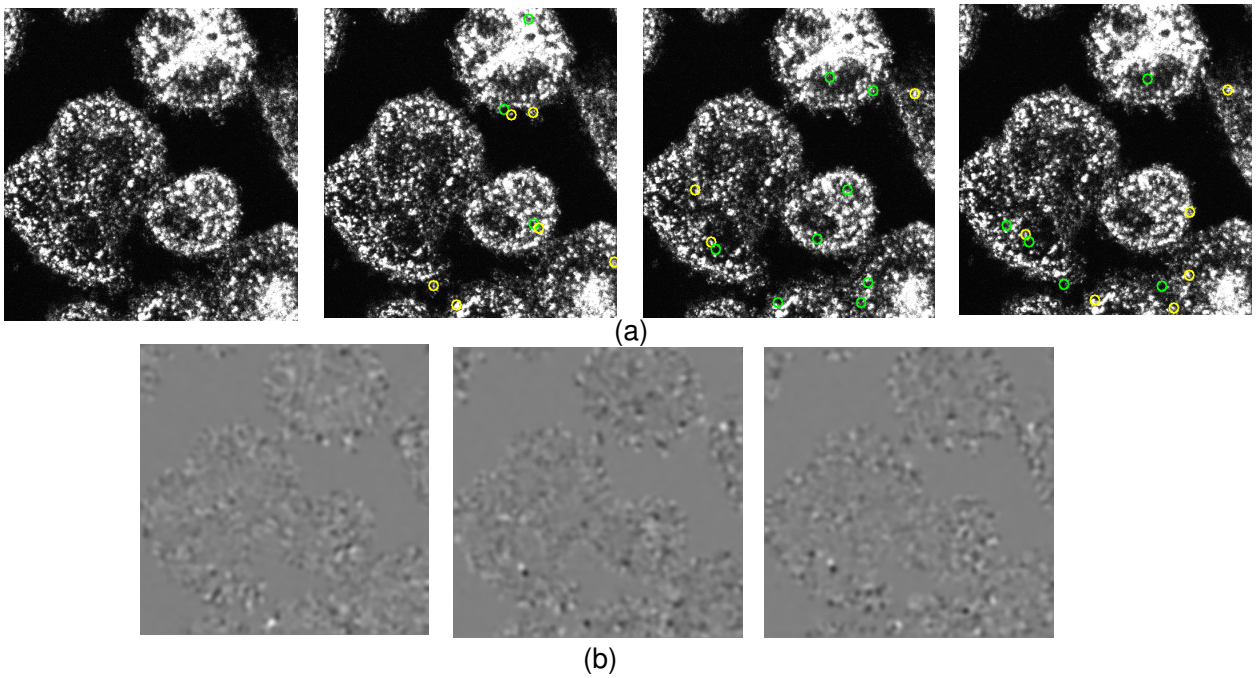

Figure 5 Top Row: results for particle moving pattern detection: translational, appearing, and disappearing. (b) Bottom Row: Irregularity calculation at particle of interest.

motility patterns including translational, appearing, or disappearing. Future work will be carried out on object tracking [9] in corporation with the presented motility modality detection.

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