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Fusion detection in time-lapse microscopy images : application to lipid droplets coalescence in plant seeds

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Abstract: Detecting fusion events between lipid droplets of *Arabidopsis thaliana* embryos is of significant interest to understand the role of lipid droplet proteins. Lipid droplet proteins, called oleosins, have been shown to influence the size of lipid droplets possibly by preventing coalescence. We propose to detect fusion events in timelapse microscopy images of several oleosin deficient *A. thaliana* embryos. To detect volume preserving fusion events in a dense environment, we propose a procedure based on particle tracking and statistical tests. Using synthetic data, we compare the performances of our method to heuristic decision rules adapted from tracking algorithms from the literature.

Keywords: Event detection, multitarget tracking, image analysis, cell biology, microscopy.

1. INTRODUCTION

Lipid droplets are subcellular organelles which receive an increasing interest due to the growing demand for fossil energy substitutes. These organelles function prominently as a reserve of carbon and metabolic energy. Lipid droplets have been shown to have a very specific structure with a core of neutral lipids surrounded by a phospholipid monolayer, see Yatsu and Jacks (1972) and Tauchi-Sato et al. (2002). The major lipid droplet proteins are called oleosins and are composed of a hydrophobic domain inserted in the neutral lipid matrix, and two hydrophilic domains, exposed to the cytosol.

In *Arabidopsis thaliana*, Miquel et al. (2014) recently showed the influence of lipid droplets surface proteins on the dynamics of lipid storage. In particular, oleosins are believed to influence the size of lipid droplets allowing them to remain in small entities. Thiam et al. (2013) recently proposed emulsion science as a framework to describe the biophysical properties of lipid droplets suggesting that oleosins act as a surfactant and provides kinetic stability to the lipid droplets. However, lipid droplets are only metastable and thus prone to destabilization by coalescence.

The development of confocal microscopy allows the timelapse 3D observation of lipid droplets in early embryos and the precise study of coalescence. Investigation of the role of oleosins can then be carried out through image processing techniques.

In this paper, we tackle the challenge of detecting rare volume-preserving fusion events in dense environment. The use of particle tracking software allows to study the trajectories of lipid droplets in image sequences and detect fusion event triggers. The fusion of lipid droplets is then detected by testing several fusion hypothesis. We propose to use a procedure based on statistical tests to detect the fusion events and compare its performances with heuristic decision rules. We wish to apply this technique to compare coalescence in several oleosin deficient mutants and provide new insights in the role of oleosins.

The paper is organized as follows. Section 2 presents the data and the tracking techniques used for this study. The algorithm to detect fusion event is detailed in section 3. Quantitative evaluations are reported in section 4 and section 5 is dedicated to concluding remarks.

2. TIMELAPSE IMAGES AND PREPROCESSING

2.1 Lipid droplets images

We produced timelapse 3D images of lipid droplets from embryos extracted from seeds of several oleosin deficient mutants, following the protocol from Miquel et al. (2015). The embryos are separated from the seed and neutral lipids are stained with Nile Red and imaged with a confocal microscope (Leica SP5). As shown in fig. 1, lipid droplets appear as blobs of heterogeneous size scattered across the field of view. The field of view is a rectangle of size approximately $64 \times 64 \times 8\ \mu m^3$. We imaged the embryo every 5 minute during approximately 2 hours, resulting in 25-30 stacks of 60 images of size 512×512 pixels.

We know that lipid droplets are quasi-spherical with diameters ranging from $0.1\mu m$ up to $2.5\mu m$ in some embryos. Fusion events have been identified in the images, see fig. 1 and 2, as volume-preserving events that result in the creation of a lipid droplet with a volume equal to the sum of the volumes of the two previous lipid droplets. Fusion can only happen if two lipid droplets are sufficiently close to each other. Obviously, coalescence decreases the number of lipid droplets in the embryo.

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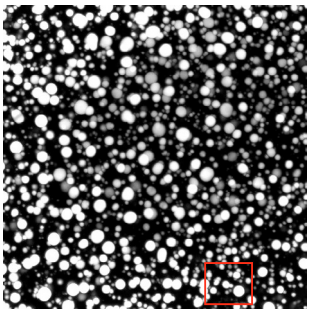

Fig. 1. Fusion event from a timelapse confocal microscopy image sequence.

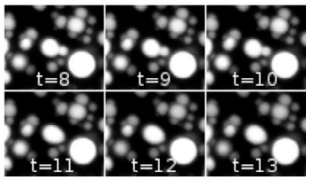

Fig. 2. Zoom-in view of the event with temporal evolution.

2.2 Particle tracking

Timelapse 3D images are first preprocessed to extract objects characteristics and trajectories using standard image processing techniques and tracking algorithms.

As stated in Meijering et al. (2012), particle tracking algorithms work in two steps. An object detection step and a trajectory recovering step. We perform the object detection step with a marker-controlled morphological watershed procedure, see for example Soille (2013). We first apply a morphological gradient to retrieve object boundaries. We then detect the minima of the gradient image and apply a minima imposition procedure to the gradient image. Finally we apply a watershed flooding procedure. The result is a label image where each label identifies a specific object. We apply this procedure to all the frames of the image sequence.

For the trajectory recovering step, we choose to represent objects with their mass center, their volume and the frame they come from. The trajectory recovering step will produce a collection of tracks. Each track is a set of objects such that no two objects of the track come from the same frame. Several tracking procedure exist and our method is not specific to any of them. Three of the most popular tracking techniques in cell biology are Nearest Neighbour Search (NNS), Linear Assignment Problem (LAP) and Multiple Hypothesis Tracking (MHT). NNS links each object in a frame with it's nearest neighbour in the next frame. It's simplicity makes it very attractive but it does not perform well with moving objects in dense environments. LAP relies on the global optimisation of association costs between two consecutive frames. This method relies heavily on the correct definition of cost functions. MHT delays the linking decision to the future by building all possible associations for a number of successive frames and selecting the most appropriate one. The heavy cost of this method is its main drawback. However, efficient MHT procedures using parallel computing and pruning strategies have been implemented by Chenouard et al. (2009). Chenouard et al. (2014) performed a comparison of several tracking procedures which revealed that there is no universally best method for particle tracking. Hence, the tracking procedure needs to be selected specifically to solve a given problem. In most algorithm, careful parameter tuning needs to be carried out to get the best possible tracking results.

Some LAP tracking algorithms such as the one proposed in Jaqaman et al. (2008), allow particle merging. A merge is defined as the creation of a link between an ending trajectory with a point from another trajectory. Distance and intensities are used in the cost functions. The cost function for linking objects i and j is defined as follows :

$$c_{ij} = \begin{cases} \delta_{ij}^2 \rho_{ij}, & \rho_{ij} > 1 \\ \delta_{ij}^2 \rho_{ij}^{-2}, & \rho_{ij} < 1 \end{cases} \quad (1)$$

Where δ_{ij} is the distance between objects i and j and ρ_{ij} is a ratio of intensities. Let $L_i(t)$ denote the intensity of particle i at time t, ρ_{ij} is then defined by :

$$\rho_{ij} = L_i(t) / \left[L_i(t-1) + L_j(t-1) \right] \quad (2)$$

The alternative costs for not associating j to i are :

$$c'_i = \begin{cases} \bar{\delta}_i^2 \rho'_i, & \rho'_i > 1 \\ \bar{\delta}_i^2 \rho_i'^{-2}, & \rho'_i < 1 \end{cases} \quad (3)$$

Where $\bar{\delta}_i$ the average displacement of the track of object i and ρ'_i is :

$$\rho'_i = L_i(t) / L_i(t-1) \quad (4)$$

Contrary to this procedure, we propose to use a detection procedure based on statistical tests using the track measurements in several frames. Using several measurements and appropriately correcting the noise for heterogeneous particles will increase the robustness of the detection procedure.

2.3 Track characteristics

The object detection step is performed on the image sequence and provides a collection of objects that are defined by their spatio-temporal position and their volumes in each frame. As mentioned before, the trajectory recovering step, creates a set of tracks from those objects. A track is said to be ending at t if t is the maximum frame on all the objects of the track. Most tracking algorithms allow for gaps between the objects of a track such that the objects of a track might not be on consecutive frames.

Prior to applying our detection procedure, we have gathered some quantitative and qualitative information from our images. The global volume distribution of the objects is inhomogeneous and asymmetrical. In fact, we observe a large amount of rather small lipid droplets and fewer large ones. Volumes are used in the trajectory recovering step in order to get consistent volume measurements for each track. Lipid droplets are isolated in cells and are not supposed to move much so trajectories are not expected to cross thus allowing for realistic particle tracking. Stabilizing the embryo during the observation period is a rather difficult task, therefore the lipid droplets have a general movement that is a composition of translations and rotation in 3D. This general movement can be corrected by registration techniques however the field of view is fixed and objects might disappear as a consequence of the movement of the embryo. Photo-bleaching is the loss of fluorescence due to prolonged exposure to light. It is also a cause of object disappearance and it alters the performances of the object detection step. Misbehaviour of the trajectory recovering step is another cause of track termination.