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Reconstruction of Missing Cells in Fluorescent Microscopy

Nat Leung[1] and Justin W.L. Wan[2]

Abstract— **Fluorescent microscopy is one of the several types of imaging techniques used by biologists to study cell activities. One challenge of tracking cells from fluorescence microscopy is that cells in fluorescent images frequently disappear and reappear. The situation is further complicated by cell divisions, which also occur frequently in an image sequence. In this paper, we apply a level set method to reconstruct cells that disappear in an image sequence and in particular, cells that are undergoing cell division. The image frames are stacked together to form a 3D image volume. The disappearance of a cell leads to a broken cell path. We reconstruct the incomplete cell paths by a level set segmentation of the 3D image volume. If the disappearance happens during cell division, the level set method segments the visible cell paths before and after cell division, and then joins them together by extending the cell paths into the missing gap. We also propose a simple and cost-efficient method similar to inpainting techniques to capture the cell appearance when it disappears by making use of the level set function obtained from the segmentation. The idea is that the intensities of a visible cell on a level set contour are copied to the corresponding contours of a disappeared cell. We will present results for reconstruction of cells undergoing cell division for C2C12 cells in fluorescent images to illustrate the effectiveness of our method.**

I. INTRODUCTION

Light microscopy has been an important tool for cell biologists to study fundamental processes such as cell movement, cell growth, cell metabolism, cell differentiation, and cell death [1]. Images are recorded in a time series, for example, taken every 10-15 minutes over a period of several days. Typical experiments result in hundreds gigabytes of image datasets consisting of hundreds to thousands of frames, each containing many cells undergoing difference behaviors. Robust automated tracking of cellular activities becomes an important issue.

Fluorescence microscopy is one of the most popular types of microscopy that biologists use to study living cells [2]. Cells are tagged with a protein that exhibits fluorescence when exposed to light of different wavelengths. The fluorophore is the component of the protein that causes the molecule to be fluorescent. The amount of energy emitted depends on the environment they are in. It is sometimes possible that the protein does not emit a visible light when the cell undergoes chemical changes during its division cycle. It then leads to the issue that the intensities of the fluorophores may change over time, resulting in cells not observable from the frames. It has been reported in the literature that cells in fluorescent images frequently "disappear" [3]. In this scenario, a cell may initially appear in an image sequence and then disappear for a short period of time. Afterwards, it may reappear again, often at a different location.

While the issue of cell disappearance has been known in the literature, it has not been fully addressed. When a cell becomes not observable, it is not known whether it has died or due to low intensity of fluorophores. Only when the cell reappears can we know what happened. However, how does one know the disappeared cell has reappeared? It would be difficult to predict the time and location where the cell will reappear. When a cell has reappeared, it may be mistakenly considered as a neighboring cell from the previous frame, rather than a missing cell from a number of frames before. Manually tracking these cells can be very time consuming.

The standard cell tracking methods are typically based on frame-by-frame segmentation [4]. The identified cell boundaries in a given frame are used as initial guesses for the position of the cells in the following frame [3]. Since this approach only uses information from the previous image frame, difficulty arises to determine the origin of a cell in the case of a cell disappearing and reappearing. Notice that the history of a cell is known through the image sequence which is often available when the current frame is analyzed. A recent approach [5] takes advantage of the temporal information by performing segmentation to all the image frames at the same time in order to identify the missing cells. However, they do not consider the case when cells undergo cell divisions which occur frequently in an image sequence. Moreover, this approach only estimates the outline but not the inside of the cell in the frames where the cell disappears. In this paper, we will focus on reconstructing cell paths for dividing cells and propose a cost-efficient method based on level set for estimating the cell appearance when the cell disappears. This approach is completely automatic without prior information of where the cells are invisible.

II. METHODOLOGY

The image frames are stacked together to form a 3D image volume [6]. In this way, the path of a moving fluorescent cell forms a "tube" in the image volume. The cell tubes travel from the bottom frame all the way up to the top. In the case of a dividing cell, which is the focus of this paper, the cell tube will bifurcate to form two tubes. A 3D segmentation of the image volume will then capture the cell tubes and hence the locations of the cells at different times. It is important to note that the 3D segmentation would capture cell divisions naturally with no manual intervention.

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[1]N. Leung is with the Centre for Computational Mathematics in Industry and Commerce, University of Waterloo, Ontario N2L 3G1, Canada. ch5leung@uwaterloo.ca

[2]J. Wan is with the David R. Cheriton School of Computer Science, University of Waterloo, Ontario N2L 3G1, Canada. jwlwan@uwaterloo.ca

When a cell disappears and then reappears, it will create a gap in the cell tube; see Figure 1(a). A standard 3D segmentation would normally capture the broken pieces rather than one complete piece of the cell tube, thus failed in capturing the disappeared cell. The case complicates further if the disappearance occurs when the cell is dividing. If the cell disappears when it is dividing, the gap will be located at where the cell tube bifurcates. Our goal is to connect the two divided cell tubes with the original single cell tube.

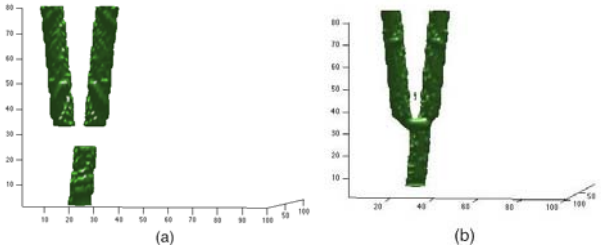

Fig. 1. (a) Disappearance and reappearance of a cell leads to a cell path with a gap in the middle, (b) segmentation of the incomplete cell path bridges the gap in the middle.

A. Level set segmentation

The idea is based on the active contour segmentation approach [5]. Level set methods can segment cells of different shapes and more importantly, capture naturally topological changes when cell division occurs. An initial level set surface, for instance, a large cylinder, is shrunk until the visible parts of the cell tube are captured. The level set surface at the location of the gap gives the reconstruction of the disappeared cell. One may think of the level set surface as an elastic membrane which captures the gap by stretching the membrane from a broken piece of the cell tube to the other. The key is to make sure that the level surface would not split at the gap.

Let ϕ be a level set function where $\phi > 0$ denotes the inside and $\phi < 0$ denotes the outside of the region. Thus, the level set surface is given by the zero level set of ϕ. Given an image function u defined on Ω in 3D, consider the energy functional [7]:

$$E(\phi) \equiv \mu \int_\Omega |\nabla H(\phi)|\, d\vec{x} + \lambda_1 \int_\Omega |u - c_{in}|^2 H(\phi)\, d\vec{x} + \lambda_2 \int_\Omega |u - c_{out}|^2 (1 - H(\phi))\, d\vec{x},$$

where $H(\cdot)$ is the Heaviside function, and μ, λ_1 and λ_2 are parameters. The λ_1 and λ_2 terms are defined such that the energy is minimized when the level set surface is located right on the boundary of the object. The μ term is a regularization term to minimize the surface area. c_{in} and c_{out} are mean intensities of the cell image. They play an important role to evolve the level set surface so that it will capture the visible broken cell tubes and in the mean time prevent the level set surface from shrinking at the gap.

The minimizer ϕ of the energy functional $E(\phi)$ satisfies the steady state of the Euler-Lagrange partial differential equations:

$$\frac{\partial \phi}{\partial t} = \delta(\phi) \left[\mu \nabla \cdot \frac{\nabla \phi}{|\nabla \phi|} - \lambda_1 (u - c_{in})^2 + \lambda_2 (u - c_{out})^2 \right],$$

where $\delta(\cdot)$ is the Dirac delta function and the initial contour is given by $\phi(x, y, z, 0) = \phi_0(x, y, z)$.

Now, we define c_{in} and c_{out}. Consider the voxel at (x, y, z) where z denotes the direction of the image frames. For the image frame corresponding to z, define the projection of ϕ onto this image frame: $\phi^z(x, y) = \phi(x, y, z)$. Then c_{in} is defined as the mean intensity of u in the 2D region given by $\phi^z > 0$ and c_{out} the mean intensity of u in the region given by $\phi^z < 0$. It is important to note that the mean intensities are computed on each image frame so that the segmentation of the visible cells essentially takes place on each 2D slice. The entire segmentation, however, is still in 3D since the μ term is computed in 3D.

On the image frames where the cell is visible, the c_{in} and c_{out} terms will evolve ϕ so that it will capture the cell boundary on those image frames as if it is performing a 2D segmentation. On the frames where the cell is not visible, then $u = c_{in} = c_{out}$ (assuming there is no noise). Hence the last two terms will become zero. The only term that will evolve the level set surface is the μ term. Geometrically, the μ term computes the mean curvature of ϕ in 3D and it has the effect of minimizing the mean curvature of the level set surface. Near the image frame when the cell just disappears (or reappears), if the level set surface suddenly closes the gap, it will generate a large mean curvature. The mean curvature term will extend the level set surface upward (or downward) until the bottom part and top part connects. In the case of cell division, this model will allow the level set surfaces at the top parts to extend and merge, and eventually connect with the bottom part; see Figure 1(b).

B. Inpainting of cells

In the reconstruction of the cell images, we propose a cost-efficient way that preserves the basic appearance structures of the existing cells. After the segmentation stage, for each time slice t, we will obtain a level set function ϕ_t, and we will make further use of it for estimating cell appearance. Before we do so, we note that in general, the function ϕ_t tends to have a drastic behavior away from the zero level set; see Figure 2 (left). The contour lines tend to squeeze to each other. We will apply the reinitialization technique [8] to ϕ_t so that the values of ϕ_t will become more evenly spaced. Specifically, we will solve the following equation:

$$\frac{\partial \phi_t}{\partial \tau} + \text{sign}(\phi)(|\nabla \phi| - 1) = 0,$$

to the steady state where τ is a pseudo time. After reinitialization, the contour lines will be more evenly distributed; see Figure 2 (right).

Now we have obtained contour lines for different level sets in each time slice. Assuming there is no rotational motion in the missing cells, we can readily reconstruct a cell image from an existing one using their corresponding

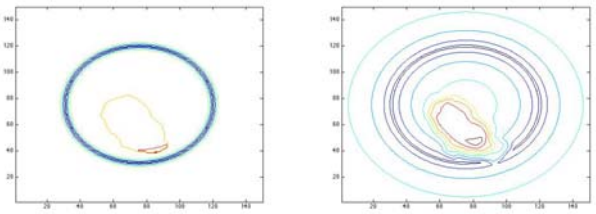

Fig. 2. Contour lines of a level set function (left) before and (right) after the reinitialization process.

ϕ_t. The idea is to copy the intensities on a contour of ϕ_{t_1} to the corresponding contour of ϕ_{t_2}.

We first normalize ϕ_t in each time slice so that $\max \phi_t = 1$ for all t. Assume the center at time t is given by $\arg\max \phi_t$. Let $I(x, y, t_1)$ be the intensity function of an existing cell image with center C_1, and $I(x, y, t_2)$ be the intensity function to be calculated in a missing cell image with center C_2. Let $\theta(x, y, C)$ be the angle of the point (x, y) with respect to a center C. The reconstruction part is then given by $\{(x, y, t_1) : \phi_{t_1}(x, y) \geq 0\}$. Now $I(\cdot, \cdot, t_2)$ can be recovered from $I(\cdot, \cdot, t_1)$ as follows:

$$\begin{aligned} & I(x, y, t_2) = I(u, v, t_1) \\ \Leftrightarrow \quad & \theta(x, y, C_2) = \theta(u, v, C_1) \text{ and } \phi_{t_2}(x, y) = \phi_{t_1}(u, v). \end{aligned}$$

In practice, given a point (x, y) whose intensity is to be found, the second condition $\phi_{t_2}(x, y) = \phi_{t_1}(u, v)$ may not always be attained by some (u, v), so this may be relaxed to $\phi_{t_2}(x, y) \approx \phi_{t_1}(u, v)$. Alternatively, for rotationally symmetric images, we can choose a fine partition $\{0 < a_1 < ... < a_n < 1\}$ of $[0, 1]$, and simplify the reconstruction further to

$$I(x, y, t_2) = \text{average}(I(u, v, t_1)),$$

where $a_{i-1} \leq \phi_{t_2}(x, y), \phi_{t_1}(u, v) < a_i$. The latter approach often works well for images in our context. The cost is low since the main ingredient (contours of ϕ_t) has already been obtained from the segmentation stage, and the reinitialization usually converges quickly.

The first inpainting approach is successful in capturing detailed structures between cells that are of similar shapes, because of the presumably similar ϕ and the measurement of θ. The second approach, due to the averaging nature, can retain larger-scale structures. When the averaging is done over all the given cell images, common structures present in most of the given cells can be copied over to the missing cells.

III. Numerical Results

We test our algorithms by cell images showing live C2C12 cells obtained from experiments performed at the Genomic Laboratory, McGill University. The original image size is 512×512, but for illustration purpose, only the portion of a dividing cell is shown with an image size of around 100×100. Due to limited space, we only show a few examples here how the segmentation and inpainting methods perform. All the computation is done on a MAC using MATLAB.

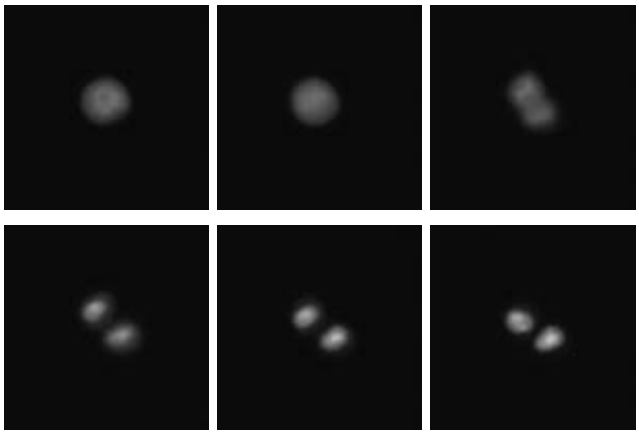

Fig. 3. In a fluorescent cell image sequence, 6 image frames in which a cell was proceeding through a cell division were replaced by blank image frames to simulate the disappearance of the cell.

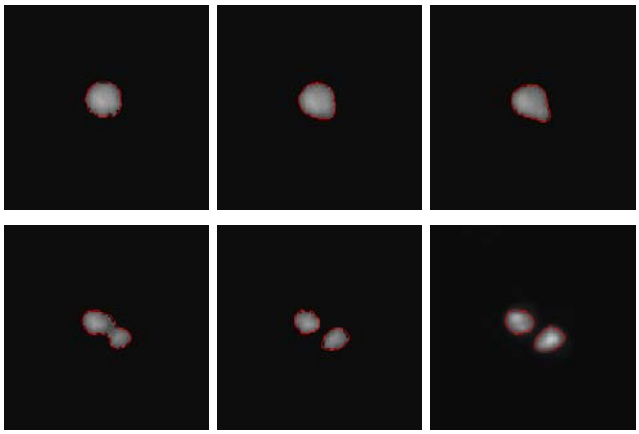

Fig. 4. Reconstruction of the six missing frames shown in Figure 3 using the level set segmentation algorithm. The reconstructed frames reproduce the cell division process.

We demonstrate the reconstruction result given by the level set segmentation described in Section II-A. An image dataset with a cell undergone cell division visible on all image frames is taken as the ground truth. We then manually remove 6 frames from when the cell is just before cell division to when the cell has divided; see Figure 3. The level set segmentation is applied to reconstruct the incomplete cell path. The segmentation results over these 7 frames are shown in Figure 4. As we can see, the segmentation contours generally agree well with the disappeared cell. Notice that the divided cells are recovered in the reconstruction. The result is better near the ends and gets less accurate towards the middle where the prediction becomes difficult farther away from the given visible cells.

Figure 4 also shows the level set inpainting result of the estimated cell appearance. In this example, the intensities are obtained from taking the average intensities on each contour. We note that our cell inpainting method is for simplicity to achieve fast computation.

Finally, we show a more complicated example where three

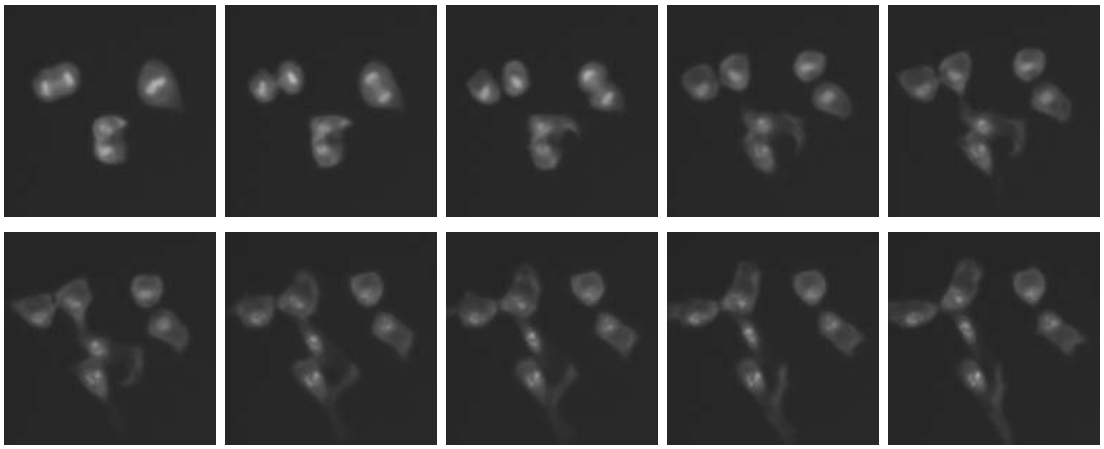

Fig. 5. In this fluorescent cell image sequence, 10 image frames (from 3 locations with gap sizes 3, 3, and 4) in which 3 cells were proceeding through a cell division simultaneously were replaced by blank image frames to simulate the disappearance of the cells.

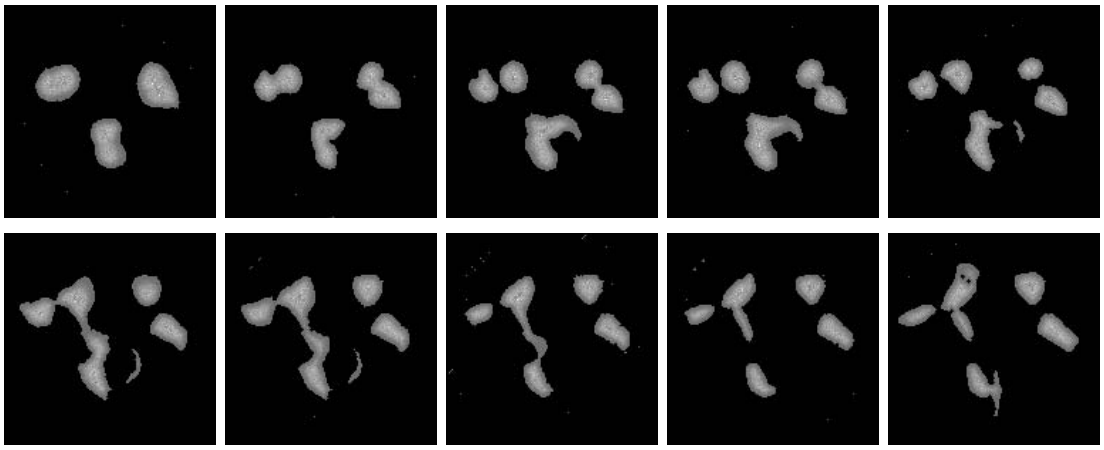

Fig. 6. Reconstruction of the 10 missing frames shown in Figure 5 using the level set segmentation algorithm. The reconstructed frames try to capture the cell division processes of the 3 disappeared cells.

cells are undergoing cell division simultaneously. The original image frames are given in Figure 5 and they are replaced by blank image frames to simulate the disappearance of the three cells. In this example, the three cells divide into six cells and they change shapes continuously. The level set segmentation and inpainting algorithms are then applied to reconstruct these missing frames. The reconstructed image frames are shown in Figure 6. With this difficult example, the reconstruction was able to capture qualitatively the shape variations and the splitting of the three cells.

IV. Conclusion

This paper has demonstrated the challenges of tracking cells in fluorescent images when cells frequently disappear, reappear and divide. A level set segmentation method was applied to capture the disappeared cells. A fast method was proposed to estimate the cell appearance by utilizing the level set function available. Results from cell image sequences show that the segmentation method was able to capture cell division took place when the cell was invisible. A future work is to perform a quantitative analysis of the reconstruction results for the dividing cells.

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