ENERGY MINIMIZATION METHODS FOR CELL MOTION CORRECTION AND INTRACELLULAR ANALYSIS IN LIVE-CELL FLUORESCENCE MICROSCOPY

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ABSTRACT

The ultimate aim of many live-cell fluorescence microscopy imaging experiments is the quantitative analysis of the spatial structure and temporal behavior of intracellular objects. This requires finding the precise geometrical correspondence between the time frames for each individual cell and performing intracellular segmentation. In a previous paper we have developed a powerful multi-level-set based algorithm for automated cell segmentation and tracking of many cells in time-lapse images. In this paper, we propose approaches to exploit the output of this algorithm for the subsequent tasks of cell motion correction and intracellular segmentation. Both tasks are formulated as energy minimization problems and are solved efficiently and effectively by distance-transform and graph-cut based algorithms. The potential of the proposed approaches for intracellular analysis is demonstrated by successful experiments on biological image data showing PCNA-foci and nucleoli in HeLa cells.

Index Terms— Fluorescence microscopy, cell segmentation, cell tracking, level sets, intracellular analysis.

1. INTRODUCTION

Many live-cell biological studies using time-lapse fluorescence microscopy imaging aim to investigate the dynamics and (co)localization of subcellular structures [1–4]. Examples of interesting phenomena studied in the literature include the spatiotemporal patterns of specific protein complexes within cells or their nuclei, which may be indicative of cell phase or approaching cell division, or the colocalization of different proteins imaged using multichannel fluorescence microscopy, which may reveal important information about the molecular machinery involved in specific processes. In many such studies it is necessary to analyze and compare cells and their intracellular foci patterns over time. In order to do this quantitatively, using any dedicated pattern analysis algorithm developed for the purpose, a required preprocessing step is to establish the correspondence between (the same or different) cells over time. But even if the analysis is done qualitatively, by visual inspection, the task is greatly facilitated by first applying cell motion correction.

The extraction of cells from multiple-cell time-lapse images, and the creation and analysis of single-cell movies, corrected for global cell motion and showing only the remaining dynamics of intracellular processes, requires at least the following four steps: segmentation, tracking, and local registration (alignment) of the image data, followed by foci segmentation. For the first two steps, a variety of cell segmentation and tracking algorithms have been developed in recent years [5–10]. Model evolution based methods, such as level sets, have been found to be particularly useful for this purpose [11], as they can naturally handle topological changes due to cell division and incorporate prior knowledge about shape and intensity distributions.

In this paper, we propose approaches to perform the subsequent steps of 3D cell alignment and intracellular foci segmentation. Concerning the former, we exploit our previously presented multiple-level-set based cell segmentation and tracking algorithm [5], which generates binary cell masks and cell correspondences over time, and employ a shape-based image registration algorithm based on distance functions [12] of these masks to map subsequent cell patches to a common coordinate framework. As for subsequent intracellular analysis, several approaches have been reported to detect and segment different types of foci [1, 3], but the study of their relation to nucleolar regions still remains a challenging problem due to the large differences in appearance, either within one cell over time or between cells. Here we propose to segment both foci and nucleolar regions with a single algorithm based on graph cut energy minimization [13]. Preliminary experiments on real time-lapse fluorescence microscopy image data showing PCNA-foci and nucleoli in HeLa cells demonstrate the applicability of the proposed approaches.
2. CELL SEGMENTATION AND TRACKING

For the extraction of the image regions occupied by the cells throughout the image sequence, we use our level-set based cell segmentation and tracking algorithm reported earlier [5]. The algorithm is based on the model evolution approach, where level sets are chosen for the model describing each of the objects being tracked. Usage of the level sets as the model ensures high quality of segmentation of cells even if its intensity strongly varies throughout the time span of the sequence. The final state of each of the level set functions at each time point is obtained via energy minimization, where the energy functional being minimized includes both image-based and smoothness-based terms. The evolution equation for each of the level-set functions is obtained by using the Euler-Lagrange equation. In general, the mentioned evolution equation represents a region competition scheme, in which we assume the intensity distributions within both foreground and background to be Gaussian.

3. CELL MOTION CORRECTION

In order to be able to perform analysis on intracellular dynamics we first have to separate its local motion from the global motion of the cell. This can be achieved by performing registration (alignment) of the cell’s region at each time point. In general, either intensity or shape information is used for biological image registration [4]. Since the intracellular structures may change in intensity and position, shape-based registration is clearly a more appropriate approach. The shape of each of the nuclei at all time points is obtained as the output of the tracking algorithm described in the previous section. Here we apply a registration based on distance functions [12], which is especially convenient because each of our objects is already represented by a level-set function.

Our strategy for extracting and eliminating a cell’s global motion is as follows. For calculation of the registration parameters (shift $T$, rotation $\theta$, and scaling $s$), we adopt the nonrigid registration method for binary images representing object regions in consecutive time steps as described in [12]. The shape alignment (in 2D case) is achieved by minimizing the following energy functional:

$$E(s, \theta, T, (U, V)) = \alpha \int_{\Omega} N_{\delta_1}(s\Phi_D - \Phi_S(A))^2 d\Omega + (1 - \alpha)\beta \int_{\Omega} N_{\delta_2}(s\Phi_D - \Phi_S(A - (U, V)))^2 d\Omega + (1 - \alpha)(1 - \beta) \int_{\Omega} N_{\delta_2}(U^2_x + U^2_y + V^2_x + V^2_y) d\Omega,$$

where $\Phi_D$ and $\Phi_S$ are the distance functions corresponding to the source and the target shapes, $\Omega$ is the image region, $N_{\delta_1} = N_{\delta_1}(\Phi_D, \Phi_S)$ and $N_{\delta_2} = N_{\delta_2}(\Phi_D, \Phi_S)$ are the narrow bands, $\alpha, \beta \in [0, 1]$ are balancing weights,

$$A(x, y) = \begin{pmatrix} A_x \\ A_y \end{pmatrix} = s \begin{pmatrix} \cos \theta & \sin \theta \\ -\sin \theta & \cos \theta \end{pmatrix} \begin{pmatrix} x \\ y \end{pmatrix} + \begin{pmatrix} T_x \\ T_y \end{pmatrix} \tag{2}$$

is the image transformation, and $(x, y)$ are Cartesian coordinates on $\Omega$. The nonrigid deformation field $(U, V)$ is used only as a complement for the rigid deformation to ensure good fit and convergence. Since in most of the cases cells do not undergo dramatic changes of the shape between consecutive time frames, this process tends to converge fast.

Next, we transform the original image by applying the obtained registration transformation to all its channels. For this purpose we use only the parameters describing the rigid motion: rotation $\theta$ and shift $T$. As for cell registration in the 3D image stacks, since most of our data sets acquired for intracellular studies do not cover the full height of the cells, but only a limited part of it, it is hardly possible to apply full 3D registration. Therefore, we use here the following approximation instead. First, we calculate the 2D transformation parameters on the maximum projection of the shape in the axial direction. Second, we apply the obtained transformation to the image stack slice-by-slice. An example of cell motion correction is shown in Figure 1 and Figure 2.

4. INTRACELLULAR SEGMENTATION

The final processing step towards intracellular analysis is the segmentation of the foci and nucleolar regions. Both types of objects can be distinguished from each other as well as the remainder of the nucleus by their appearance: foci typically appear in the images as small and relatively bright blobs while the nucleoli are larger and relatively darker regions. The number, size, and appearance of both structures within one nucleus varies depending on cell phase. At one time the foci may be small in size and large in number while at other phases they may be much larger and irregularly shaped regions having much higher intensities [1] (see figures). In our data the number of nucleolar regions within one nucleus varies from zero up to five, and they may also appear, disappear, or merge as time passes (see figures). However, despite the pronounced characteristic features of both structures of interest, there may still be considerable differences in appearance of these regions between different data sets, within the same data set, and even within the same image. While foci segmentation is a rather well-studied subject, and some of the classical image processing algorithms (top-hats, wavelet transforms, filter banks) can be applied to it, the problem of outlining the nucleolar regions has not been addressed.

Here we choose to use the same algorithm for segmenting both the foci and the nucleoli. Our approach is to consider segmentation as an energy minimization problem, where we assume the intensity distributions within both regions to be Gaussian. The target means and variances of these regions
The top row shows an example of the motion of one cell extracted from one time-lapse fluorescence microscopy image data set. One slice \((z = 1)\) for time steps 1, 18, 35, 43, and 69 (the last before cell division) are shown. Clearly, the cell does not only migrate within the image, it also changes orientation, shape, and appearance. The bottom row shows the same cell (magnified) over time after the proposed motion correction algorithm has been applied.

6. CONCLUSIONS

In this paper we have proposed approaches for the correction of cell motion and the segmentation of intracellular foci and nucleolar regions from time-lapse fluorescence microscopy image data sets showing many proliferating cells. For the extraction of individual cells from the image sequences we used our previously presented level-set based cell segmentation and tracking algorithm. Next, similar to segmentation and tracking, both the cell motion correction and the intracellular segmentation tasks were formulated as energy minimization problems, and were solved using, respectively, distance-transform and graph-cut based algorithms. Finally, we demonstrated the potential of the approaches by applying them successfully to real biological image data.
Fig. 2. Example of motion correction and intracellular segmentation using the proposed approaches. The two top rows show the motion of one cell extracted from a time-lapse fluorescence microscopy image data set. One slice \((z = 1)\) is shown for time steps 1, 11, 21, 31, 41, 51, 61, 71, 81, and 92. The third row shows (magnified) the result of cell motion correction after segmentation and tracking. Finally, the result of segmentation of PCNA-foci and nucleolar regions is shown in the bottom row, illustrating the potential of our algorithms for analysis of these intracellular structures.


