Commentary: Tracking Biological Cells in Time-Lapse Microscopy

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Introduction

Presented here is an automatic method for tracking and analysing the of cellular structure for various biological processes. This proposed method aims to solve the problem of tracking of large number of cells within multiple frames of a video sequence, which is quite tedious to do manually, with a vision-based method. As with references to previous researches, the automation of cell tracking faces 3 major challenges, namely image acquisition, compund cell topology and jagged motion of cells.

Videos are nothing but an array of images. These images are collected using different image acquisition techniques, and most of these images are captured in a special environment because the biological cells react differently to different types of lights This means there will be a minute disparity between signal and noise, that is the vision-based system is fed a very low threshold image with which it has to identify what is meaningful (signal) and what is noise. Then the large volume of captured data and topological features, also increase the complexity for automation.

Cells of have a complex topology which include shape deformation, close contact, and overlap of cells in the microscopic images. Cells division also occurs during image capturing (helpful in studying cell reproduction) also add to the complexity of the problem, since they change the cell topology over time.

The cells also move unevenly within the captured frame, with some cells entering and leaving the frame during capture, making the detection of mitosis and tracking even harder.

Also, the images may be captured at random time intervals mainly to minimize the ill-effect of phototoxicity; therefore, they are taken every few minutes, which will result in not a smooth video transition. This variation in the image recording, i.e., variation in the stage shift, also increases the complexity of cell detection and tracking.

The method presented here overcomes all these issues and provides a robust solution which should be useful in various applications, such as tissue repair, drug approval, inflammation response, study of neurological diseases(studying myelin sheath), chemotaxis, or in the discovery of new drugs.

Methods

The method proposed here has 3 main modules, namely cell detection then cell tracking and finally recovering any cell trajectories. Nuclei are most well-formed structure within the cell because they retain their shape way better than cytoplasm which is why nuclei are preferred. All processes will focus on nuclei and will work using the local-maximum within a cell.

Cell detection is done by localizing the nuclei of cells (by making it fit an ellipse) in image sequences by first using a top-hat filter and then h-maxima transformation. The result is then made to fit an ellipse with high accuracy. Detection further divided into 3 sub-processes – illumination correction, segmentation of nuclei and nuclei localization.

Illumination Correction is done in the pre-processing step by using a top-hat filter which reduces noise introduced due to nonuniform illumination as seen in fluorescent light, camera sensitivity, or dirt and dust on the lens surface of the image capturing apparatus. This is carried by first inverting the image, then erosion followed by dilation. Erosion and dilation is a technique to get rid of the noise in cytoplasm.

Then the step of *Segmentation of Nuclei* is done because cells are separated from the background but are not distinguishable if they appear within a cluster. To solve this regional maximum of each cell is detected by using Gaussian filtering then performing *h*-maxima transformation. By keeping only one unique local peak, Gaussian filter generates a local maximum within each cell highlighting the nucleus. Then *h*-maxima transformation suppresses any of the regional maxima(within a cluster) whose height is less than *h*. Here *h* is the geodesic height, which is calculated by using contrast within the cells. This a semi-automated process which I believe could be automated using spatial-filtering.

Nuclei Localization helps find the centroid of cells based on the highest peak in a cell whose shape changed during capturing viz. mitosis or cytoplasm blur. An ellipse is then fit with the previous segmentation result which I believe is not really good considering nuclei can be of different shapes other than ellipses. The proposed method uses this approach due to its amazing processing speed and accuracy.

Cell Tracking faces many problems and is without a doubt the toughest segment of this method. A lot of parameters keep changing in a video, thus focusing and using only one feature will not lead to good results therefore cellular motion and cellular topology are used diminish error in tracking. Colour, compatibility and area overlap are employed for cell topology and for domain of motion displacement and skewness are used. Point tracking algorithm cannot ensure high accuracy in its basic form because the cell motion is haphazard. A new approach is needed for the tracking of cells. Ambiguity in finding the correct correspondence is solved by combining the topological features and motion features. A global optimization technique based on weighted bipartite matching [1] is used to match these features, however, bipartite graph only allows for a one-to-one matching among the cells, and generates a single track for every cell in the spatio-temporal domain. Mitosis cannot be detected in the conventional method approach. The proposed method overcomes this problem of mitosis detection in the postprocessing step utilising a template matching-based tracking, which in this method is applied in the backward direction in the spatio-temporal domain. It detects new cell nuclei and when there is break, it connects it with the closest matching track using template matching.

Recovering tracks is needed to solve the broken trajectories. Breaks in the trajectory may happen because a

cell is leaving the frame, or entering of a cell in the capturing field, or just a segmentation error, or cell splitting(mitosis). Within these breaks, a break occurring because cell entering/exiting frame represents the ending, or beginning of a track segment in the field of view, and hence won't be recovered. To handle this problem along with mitosis, a trajectory recovery module that is based on a template matching, is added to the main framework of the proposed model.

Each cell in the specific spatio-temporal domain is given a unique identifier and then they are assigned one of 3 categories - A, B or C. *Category A* means the cell is present in next image sequence, but not in previous frame. *Category B* contains cells present in before and after frames. *Category C* has cells which are visible in previous and present frame but not next frame – cell exiting the frame. Cells near the boundaries are rejected because they may be detected as noise and can cause unnecessary error for tracking. Category A is mainly segmentation error whereas B and C are track breaks due to mitosis. The magnitudes of intensity inside template T are assigned by using Gaussian distribution using intensity values and pixels corresponding to the fitted nuclei. If a break occurs, the frames are the backward propagated to find where the break occurs and a link is made between the closest track to the origin of the mitosis event cell track.

Results

Analysis for the proposed model was done on 4 different kinds of sequences of time-lapsed microscopy, each with different properties, namely image capturing technique, cell size and movement of cells in that sequence. Of the four sequences, three were recordings of murine embryonic development, that had been captured using phase-contrast with a pixel resolution of 0.863 μm/pixel, whereas the fourth sequence containing the Hela cells line has been captured using fluorescence microscopy with a pixel resolution of 1.059 μm/pixel. These sequences were chosen considering because they had different cell densities, were captured in nonuniform illumination, had noticeable variation in cellular motion, and stage shifts.

The images were resized to 640 by 512 pixels and the tests were conducted on an Intel Pentium4 3.4 GHz processor computer having 2 GB of RAM. Experimental results for qualitative and quantitative evaluation are presented in two parts – cell detection and tracking. Cell detection performs better as tracking is the harder of the two. Also, if there is an error in segmentation, it will add onto the error for tracking. Validation of the result of cell detection, is done by measuring the accuracy of detection of the number of nuclei
in the segmentation result. From the 4 video sequences, 700 random frames are chosen and evaluated against manually counted cells of the same frames. Using a top-hat filter followed by the h-maxima transformation gives better results compared to the already existing methods. For cell tracking validation, an illustration of spatio-temporal trajectory of cell proliferation in the murine embryonic sequence is done, showing mitosis at 3 different events. For quantitative evaluation of tracking, both cell tracking and mitosis events are considered. Ground truth for tracking is the track segment which is considered to be valid if it corresponds to the same cell in all the frames. For mitosis, the ground truth is manually calculated mitosis events.

The method presented here has mean accuracy of 91.47% for cell detection and average accuracy for cell tracking is 85.38% and mitosis events is 82.66%, which was better than using, Compactness [2], Hybrid Merging [3] and watershed segmentation [4].

These results will make potential users to adopt the proposed method, but to have a really good acceptance rate, I'd suggest that the weight parameters that prefer either motion features or topological features could be automated. Using parameters $\tau = 0.7$, $k = 3$ and $\alpha i = 0.2$, isn't going to be ideal for all datasets and would require adjustments, something I believe can be automated during pre-processing phase of these image sequences. More robust testing could have been done where there is mitosis and simultaneously those cells leaving the frame which would show the combined correctness of the proposed model. Receiver operating characteristic and False discovery rate could also be used as different evaluation metrics.

Conclusions

The proposed method presented an automatic method for the detection and tracking of biological cells in time-lapse microscopy with good results and robustness. The process to focus on the nuclei rather than the cells themselves is a good aspect since there is little deviation for nucleus compared to the cell cytoplasm which can vary quite a lot. Using only ellipses is a drawback since this method should be made universal, without having to adjust the parameters and hyperparameters for either more focus on topological or motion features, something I believe should be automated based on cell size.

The method addresses all the challenges faced in automating cell tracking. The main techniques used for detection, in order are, top-hat filtering followed by an h-maxima transformation for the purpose of cell detection, this process costs in time but makes up in accuracy of results. It can still be made faster using newer, better more accurate filtering techniques such as spatial filtering to make the entire process a lot quicker.

Earlier models compared in this paper don't handle segmentation error that well which may create problem in tracking, all of that is handled quite well with the proposed method.

For tracking, the introducing a dissimilarity measure that combines the cellular motion characteristics with the topological features for the matching of a cell in consecutive frames gives good results. This makes tracking work well, even in the case the cell shape is deformed, or cells moving unevenly or clusters of cells close to each other. Also, for better acceptance the proposed method has to be faster than the existing methods.

Overall, the method provides quite good results and can be easily adopted, although more tests can be done using different sequences that are captured in different lights with different shape nuclei.

More research could be done in improving the cell trajectory in a forward moving fashion while improving the time and accuracy. Cells events such as mitosis and tracking are fine but other cell events also provide critical information by growing or shrinking in size. Cells might also die, like for example virus cells and detecting if placed in a vaccine kills the cell or not. Future models should look into these issues.

References

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