LEID-NL

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Platform: Windows

Prerequisites: MATLAB Compiler Runtime 2020a (x64)

LEID-NL: SUMMARY

The developed method is based on the model-evolution approach, where the segmentation and tracking tasks are performed simultaneously. The level sets are chosen as the model due to their ability to provide high-quality segmentation on different types of images and natural ability to handle topological changes. In general, the framework follows that described in our earlier publication [1]. The algorithm was tailored to each particular data sequence by selecting a suitable set of parameters. Several additional features were implemented and used in different combinations for each particular data sequence. For example, one of such additional built-in routines helps overcoming the situation when the level set segments two just divided nuclei as one object. Also special attention has been paid to segmenting the nuclei entering the field of view, which were detected by producing a rough estimate of the image foreground at each time step. Several methods, including various thresholds and other fast binary segmentation methods, were added to the algorithm for this purpose. Selection of the most appropriate binary segmentation method for the initial segmentation and detection of nuclei entering the field of view was performed empirically.

LEID-NL: PREPROCESSING

The original image data is preprocessed using the PURE denoising method [2] via the corresponding ImageJ plugin.

LEID-NL: SEGMENTATION AND TRACKING

The presented cell tracking and segmentation algorithm is based on the method described in our earlier publication [1]. Within this approach, segmentation and tracking are encapsulated into a single task by the model-evolution approach. More precisely, each object being tracked is represented by a model, which is fitted to the data at every time point. During such fitting, information of different nature, like image data or regularity information, can be combined into a single energy functional, which is subsequently minimized via the gradient descent method. Level-sets were chosen as the model for their ability to provide high-quality segmentations for wide range of imaging data and natural topology change, which is important for handling cell division events.

In the current version of the algorithm, several modifications have been introduced in comparison with the method presented in [1]. The most important modifications are described below.

Training of neural networks. For some data sets, we incorporated additional training stage using the provided silver truth. The network architecture was ResNet on the backbone of U-Net with five layers. The patch size was equal to the actual image size. The probability maps inferred from the test data were used for performing actual segmentation.

Separation of touching cells. In our method, the position of the optimal separation plane between two touching cells is detected based on the minimum of the Radon transform of the intensity image in the local window containing both objects. For large objects, this operation can be rather computationally expensive. To make the separation more efficient, in this implementation of our method we reduced the space of possible locations of the dividing plane by forcing it to pass through a preselected point. This point is calculated as the one corresponding to the intensity minimum on the segment connecting the markers of the objects being separated.

Initial segmentation and detection of entering cells. In our method, both the initial segmentation and the detection of objects entering the field of view is performed based on a rough estimation of the image foreground. This is achieved by binary segmentation of the image, which can be done in many different ways. To improve the flexibility of the method, we have made this step dependent on the data type being processed. Thus, we have extended our algorithm with a wide range of binary segmentation different methods, in particular various thresholds. The most appropriate method for each data sequence is selected empirically.

Inter-scan cell motion compensation. The model-evolution approach, that our method is based on, assumes that there is sufficient overlap between the two regions occupied by an object at every two consecutive time points. However, in some cases, e.g. when the objects are relatively small and fast moving, and/or the temporal resolution is low, this condition is violated. In order to compensate the inter-scan motion, we have developed a scheme that estimates the new position of each object at the next time point based on its current position. This operation is performed before the evolution of each contour, and consists of two main steps: 1) marker detection, and 2) joint estimation of the new position of all the objects. In a similar way, we deal with the cases when in the data sets with DNA staining (e.g., **Fluo-N2DL-HeLa**), two daughter cells move far away from the final position of the mother cell. For detection of such events, we supply our algorithm with different prior information, in particular with the

observation that the daughter cells typically move in the direction perpendicular to the major axis of the mother cell.

Hybrid algorithm. Our algorithm can combine features of both tracking by detection and tracking by model evolution paradigms. In this modification, image corresponding to each time point (except the first one) is pre-segmented using elliptic features for producing super-voxels. These were consequently merged based on the current configuration of the objects. As result of this procedure, the objects can move, adjust to new shape, divide, disappear, and also new objects can enter the image frame. After that, the algorithm followed the multi-level-set segmentation routine described above.

LEID-NL: POST-PROCESSING

Even though level-sets can naturally change their topology, splitting or not-splitting of the contour in each particular case depends on several factors, in particular image data and energy weights. In some cases, too strong regularization can preclude the contour from splitting. This happens either in the cases when the energy minimum corresponds to the situation when one level-set engulfs two proximate objects, or when the desired energy minimum is not reached due to the contour evolution getting stuck in a local energy minimum. In cell segmentation, such cases are quite often encountered when two daughters of a divided cell or nucleus do not move far apart from each other. To better handle such cases, we have extended our algorithm with an additional post-processing step. The level-sets that might be engulfing two separate cells after the convergence of the contour evolution are detected by low solidity, defined as the ratio of the total volume to the volume of the convex hull, of their shapes. For such objects, the convexity measure is used for making the final decision if the contour has to be split into two separate contours, one for each daughter cell. For split contours, an additional round of contour evolution is performed, starting from the current state.

REFERENCES

1. Dzyubachyk O, van Cappellen WA, Essers J, Niessen WJ, Meijering E. Advanced level-set based cell tracking in time-lapse fluorescence microscopy. *IEEE Transactions on Medical Imaging* **29**, 852-867 (2010).
2. Luisier F, Vonesch C, Blu T, Unser M. Fast interscale wavelet denoising of Poisson-corrupted images. *Signal Processing* **90**, 415-427 (2010).