RWTH-GE (1)

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Platform: Windows 10 (x64)

Prerequisites: MATLAB 2018b (x64)

RWTH-GE (1): SUMMARY

The presented algorithm for segmentation and tracking follows a three-step approach where we detect, track and finally segment nuclei. In the preprocessing phase, we detect centroids of the cell nuclei using a convolutional neural network (CNN). Tracking is performed in a backward fashion on the predicted seed points, i.e., starting at the last frame and sequentially connecting corresponding objects until the first frame was reached. Correspondences were identified by propagating detections of a frame t to its preceding frame t - 1 and by combining redundant detections using a hierarchical clustering approach. The tracked centroids were then used as input to a seeded watershed algorithm to obtain the final segmentation.

RWTH-GE (1): PREPROCESSING

Centroids of cell nuclei for the 2D datasets **Fluo-N2DH-GOWT1** and **Fluo-N2DL-HeLa** were identified using a U-Net [1]. To avoid that the network automatically learns to suppress detections in the border regions at this early stage, we cropped the original images to the field of interest by *E* pixels as specified in the annotation procedures. The intensity of the raw images was linearly scaled from the 0.4th and the 99.6th percentiles to the 8-bit value range [0, 255], while values above or below were set to the minimum and maximum value of this range. Moreover, the images were resized to 512×512 pixels for the CNN processing. We adapted the network described in [2] to work with single channel 2D images and additionally performed an intensity weighting of the loss, such that cells in low intensity areas contributed more to the loss. We trained a single detection network for both datasets using all available gold truth images. The obtained probability maps were then resized to original resolution, morphologically opened with a disc structuring element of a radius *r* and binarized using a threshold *T*. Remaining connected seed points were separated using a watershed-based clump splitting applied on the Euclidean distance map of the inverted seed image (i.e., intensity minima located in the nucleus centers). Detections touching the image border were removed from further processing.

RWTH-GE (1): SEGMENTATION

Segmentation was performed as the final step of the pipeline. See *RWTH-GE (1): POST-PROCESSING*.

RWTH-GE (1): TRACKING

Tracking was performed in a backward fashion by sequentially linking corresponding objects until the first frame was reached. Correspondences were identified by assigning a new tracking label to all unlabeled detections at frame t and by copying the labeled detections to the preceding frame t - 1. At frame t - 1 we then performed a hierarchical clustering using Ward's linkage criterion and identify clusters that contain detections from time point t and t - 1. The distance-based cut-off is determined based on the average spatial distance of each detection to a set of nearest neighbors (large in less dense frames, smaller in densely occupied frames). We empirically chose to use half the average distance to the 3rd to 8th nearest neighbors of each detection, i.e., excluding potential redundant detections or division events from the computations. There are multiple cases of label presence in the clusters: (1) if a cluster contains no labeled detection, a new track label is introduced, (2) if one of the detections has a valid tracking label assigned, the label is copied for the cluster, (3) if two or more detections in the cluster have a valid tracking label assigned, a merge (i.e., a cell division) is introduced by adding a new tracking label and by correctly assigning the predecessor and successor links, and (4) if only one labeled detection is contained, the track should end. The detections contained in a cluster are averaged and form the set of tracked objects at frame t - 1. The steps above are repeated until the first frame of the sequence is reached. In principle, this tracking approach is similar to a nearest neighbor tracking but additionally handles redundant detections by clustering nearby seed points. Moreover, cell divisions are naturally included in the algorithm design, as daughter cells are clustered together if their spatial distance falls below the cluster cut-off value. A drawback of the clustering-based approach, however, is the required global cut-off value that is used for the entire dataset. This unavoidably leads to fragmented tracks if the datasets exhibit large density variations. Therefore, we additionally used the optical flow algorithm [3] in its MATLAB implementation to propagate detections from one frame to another. This was performed in a forward and backward fashion, i.e., detections of a frame t were copied to t - 1 and t + 1 and transformed using the velocity field proposed by the optical flow estimation step to be closer to the assumed true position. We found that this double-redundant seed propagation slightly improved the detection and tracking scores, potentially by eradicating flickering artifacts of the detection stage, where detections are sporadically missing in a few frames. Moreover, cell division events were more reliably

detected with this approach as daughter cells were mostly correctly shifted on top of their mother cell, i.e., ending up in the same cluster during the hierarchical clustering phase.

RWTH-GE (1): POST-PROCESSING

The segmentation was largely based on a seeded watershed technique [4] with a few improvements to tune the results. The tracking labels were used to generate seed images with positions and labels identical to the tracked centroids. We generated a large background label based on a Euclidean distance map (EDM) of the seed points. The EDM was binarized using the maximally expected object radius as threshold value, such that all detected cells were surrounded by a background label with sufficient margin to allow for a proper segmentation. Moreover, seeds were dilated with the minimum expected object radius, to have a reasonable initialization for the seeded watershed and to prevent degenerate segmentations of only a few pixels. The edges identified by a Sobel edge detection were added to the inverted Gaussian-filtered raw images and then processed with a seeded watershed algorithm. Thus, the raw images were transformed such that intensity minima were located in the nuclei centers with additional boundaries provided by the Sobel operator to prevent leakage of background signal into the interior of the cells. While this approach worked properly for most nuclei, very dim regions were sometimes mis-segmented due to a very low signal to noise ratio. These issues could not be fixed before the result submission and need to be improved in future revisions. The background label as well as segments that clearly exceeded the maximum expected area were set to zero. The final segmentation images were double-checked with the tracking results and in cases where the segmentation algorithm erroneously missed a cell (e.g., if the background label flooded a cell region), we manually added the detections again to provide segmentation images that are consistent with the tracking results.

REFERENCES

1. Ronneberger O, Fischer P, Brox T. U-net: Convolutional networks for biomedical image segmentation. In *Proceedings of Medical Image Computing and Computer-Assisted Intervention*, 234-241 (2015).
2. Eschweiler D, Spina TV, Choudhury RC, Meyerowitz E, Cunha A, Stegmaier J. CNN-based preprocessing to optimize watershed-based cell segmentation in 3D confocal microscopy images. arXiv preprint arXiv:1810.06933 (2018).
3. Farnebäck G. Two-frame motion estimation based on polynomial expansion. In *Proceedings of 13th Scandinavian Conference on Image Analysis*, 363-370 (2003).

4. Beare R, Lehmann G. The watershed transform in ITK – discussion and new developments. *The Insight Journal* **6** (2006).