RWTH-GE (3)

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Platform: Windows 10 (x64)

Prerequisites: MATLAB 2019b (x64)

RWTH-GE (3): 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 Laplacian-of-Gaussian Scale Space Maximum Projection approach. Tracking is performed in a forward fashion on a selected subset of identified seed points, i.e., starting at the first frame and sequentially connecting corresponding objects until the last frame was reached. Correspondences were identified by propagating detections of a frame t to its succeeding frame t + 1 and by introducing cell division events if multiple likely candidates are found. The tracked centroids were then used as input to the TWANG segmentation algorithm to obtain the final segmentation.

RWTH-GE (3): PREPROCESSING

We used our nucleus detection algorithm [1]. In brief, the original images were filtered using differently scaled Laplacian-of-Gaussian filters, where the selected scales, σ_{min} and σ_{max}, were matched to the observed cell sizes. The 4D scale-space was reduced to a 3D image by a maximum intensity projection of the individual LoG-filtered images. Local maxima were then identified in the 3D LoG ScaleSpace Maximum Projection (LoGSSMP) and we additionally allowed to detect intensity plateaus to reduce the number of false negative detections in cases where no single maximum pixel was present in the center of a nucleus. To reduce false positive detections in background regions, only detections with an intensity larger than the global mean plus two standard deviations of the LoGSSMP image intensities were considered. Moreover, the raw images were preprocessed using a 3D median filter with the neighborhood radius r_{median}. Redundant detections on intensity plateaus were combined in the subsequent tracking phase.

RWTH-GE (3): SEGMENTATION

Segmentation was performed as the final step of the pipeline. See *RWTH-GE (3): POST-PROCESSING*.

RWTH-GE (3): TRACKING

Tracking was performed in a forward fashion by sequentially linking corresponding objects until the last frame was reached. As only a subset of objects should be tracked for **Fluo-N3DL-TRIF**, the provided gold truth locations were used to initialize the tracked objects in the first frame. For each object of the current frame, we identified the two nearest neighbors in the next frame and linked the neighbors depending on three easy association criteria: (1) if both identified nearest neighbors exceeded a maximum distance of d_{max} = 30, none of them was linked and the previous seed point was simply copied to the next frame. This case can help to prevent occasional misdetections of the seed detection stage; (2) if one of the neighbors was closer than the maximum distance and the other one was not, the track was continued at the closest neighbor; (3) if both neighbors were closer than maximum distance and if none of them had been linked already to another detection, a cell division event was introduced and both of the neighbors were used for track continuation with two new track identifiers. To ensure that the most likely matches are connected first, the linking is performed in the order of ascending distances to the nearest neighbor of all objects, i.e., the closest matches are connected first.

RWTH-GE (3): POST-PROCESSING

The segmentation was based on the TWANG segmentation algorithm as described in [1] that was applied on the median filtered 3D images, with the parameters σ_{kernel}, σ_{grad}, and ω_{kpm}. Instead of detecting the seed points again, we directly supply the TWANG algorithm with the subset of tracked seed points from the previous step. The segmentation algorithm directly uses the labels of the seed points for the final regions. 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 local threshold only detected background instead of a cell region), we manually added the detections again to provide segmentation images that are consistent with the tracking results.

REFERENCES

1. Stegmaier J, Otte J, Kobitski A, Bartschat A, Garcia A, Nienhaus GU, Strähle U, Mikut R. Fast segmentation of stained nuclei in terabyte-scale, time resolved 3D microscopy image stacks. *PLoS ONE* **9,** e90036 (2014).