UPM-ES

Authors: David Pastor-Escuredo, Daniel Jiménez-Carretero, María J. Ledesma-Carbayo

Email: david.pas@gmail.com

Platform: Linux, Windows

Prerequisites: MATLAB Compiler Runtime 2008a (x64)

UPM-ES: SUMMARY

We propose an algorithm exploiting general and robust features of cell and cell nuclei data of fluorescence time-lapse microscopy. To achieve that we apply spatio-temporal mathematical morphology [1], [2] to exploit the characteristic size range of cells and the redundancy and coherence of trajectories at the same time, just by working with spatio-temporal structuring elements (SEs) in the extended domain –particularly to 2D+t challenge datasets. With this strategy, filtering, segmentation and tracking are intrinsically coupled. Filtering uses the spatial information through grayscale area openings that limit the range of characteristic sizes within the image. On the other hand, the temporal consistency of the trajectories is exploited using a novel proposed stochastic spatio-temporal morphological reconstruction that enhances coherent structures through time to improve the "trajectory-to-noise" ratio. After this filtering, a simple threshold is enough to perform an initial segmentation that is refined through a "merge or split" filtering with binary dilations and erosions that allow us to keep the characteristic size range. Trajectories are found by labeling the binary image in 2D+t with spatio-temporal connectivity. Each trajectory is identified with a label making it possible to analyze different spatial frames of the image to detect divisions and check that the topology is consistent. By isolating each label we can impose constraints and generate the graph. This is an effective method to perform tracking, keeping coherence of trajectories.

UPM-ES: PREPROCESSING

The characteristic noise of microscopy images is a highly non-linear salt and pepper like noise. We apply a non-linear median filter of radius r_M to reduce this effect. The main point of this work is to use spatio-temporal coherence of data to improve the image quality to perform the tracking. Spatial characterization is exploited using a used gray-scale spatial area opening of radius r_{AO} to remove small flat regions that could generate over-segmentation. The temporal coherence is exploited with a novel strategy: "stochastic spatio-temporal morphological reconstruction". This process provides a "trajectory-

to-noise" ratio enhancement as coherent spatio-temporal structures are highlighted over those that do not keep that kind of coherence such as noise:

- Extract regional maxima (already pruned with the residue)
- Perform 2D+t morphological reconstruction having the regional maxima of one frame as markers for the reconstruction
- Sum up the reconstructed images from each step in the 2D+t domain

The resulting images weight the spatio-temporal connectivity of the structures in the image. Thus, trajectories are enhanced over noise. This method is especially effective for images with high temporal resolution or featuring smooth migration dynamics.

UPM-ES: SEGMENTATION

After the trajectory-to-noise ratio enhancement, we simply apply thresholding to binarize obtaining the subsequent candidate segmentations by finding connected components in 2D. Threshold *T* is set at the level that provides a more concentrated histogram of segmentations areas (in 2D). The initial segmentation is polished with a morphological closing of radius r_C and filling holes. Then, we perform a step of "merge or split" using binary dilation and erosion as a hierarchical clustering according to the size. A maximum size (*MSize*) is set for objects to be eroded r_{EP} and a minimum size (*mSize*) for objects to be dilated r_{DP}, a residue size (*rSize*) is used for a final binary area opening to avoid over-detection, so segmentations keep the size constrains in the data while keeping intrinsic consistency temporally.

UPM-ES: TRACKING

As explained, both filtering and segmentation are performed intrinsically contributing to the tracking, as alternative to decoupled segmentation and tracking. We use spatio-temporal *SEs* to find and label connected components in 2D+t as trajectories in the segmented image after erosion for safe labeling r_{SL}. Labels are re-dilated r_{SL} and isolated by thresholding being able to check the 2D objects in each frame and refine the trajectories to create the graph:

- No object found:
    - If no previous detection, the trajectory has not started yet
    - If trajectory was registered before, then it is over and we close the graph
- One object found:
    - If no registered before, the graph branch starts here
    - If registered, the trajectory keeps on and we update the branch

- More than one object found. We perform another 2D+t labeling forward from the current time point. This is done to re-evaluate the topology of the trajectory for the current point:
    - If one trajectory (2D+t object) is detected, it means that it reconnects after this step, so it is not robust to annotate mitosis and we update the branch.
    - If more than one trajectory is detected, it means that indeed there is a division and new branches are opened in the graph. We update the initial 2D+t label images with the new labels got by the forward labeling and keep on the loop.

For now, the method cannot identify over division detection. On the other side, the method is really robust to not generate cross-links or consider false positive detections generating undesired links in the graph. In general, this approach is a straightforward and effective way to perform cell tracking with datasets with a suitable time-step that keeps spatio-temporal coherence of cell trajectories. The extension to 3D+t depends only on computation capabilities and efficient implementation of the algorithm.

UPM-ES: POST-PROCESSING

No post-processing step is performed.

REFERENCES

1. Pastor-Escuredo D, Luengo-Oroz MA, Duloquin L, Lombardot B, Ladesma-Carbayo MJ, Bourgine P, Peyrieras N, Santos A. Spatio-temporal filtering with morphological operators for robust cell migration estimation in "in-vivo" images. In *Proceedings of the 9th IEEE International Symposium on Biomedical Imaging,* 1312-1315 (2012).
2. Luengo-Oroz MA, Pastor-Escuredo D, Castro-Gonzalez C, Faure E, Savy T, Lombardot B, Rubio-Guivernau JL, Duloquin L, Ledesma-Carbayo MJ, Bourgine P, Peyrieras N, Santos A. 3D+t morphological processing: Applications to embryogenesis image analysis. *IEEE Transactions on Image Processing* **21,** 3518-3530 (2012).