KIT-GE

Authors: Johannes Stegmaier, Ralf Mikut

Email: ralf.mikut@kit.edu

Platform: Windows

Prerequisites: MATLAB Compiler Runtime 2014a (x64)

KIT-GE: SUMMARY

Our framework is based on the TWANG segmentation algorithm as described in [1, 2]. In particular, the algorithm performs a seeded segmentation of the provided image data that is capable of extracting fluorescently labeled objects from 2D or 3D images reliably and fast. Temporal associations were identified using a straightforward nearest neighbor matching as implemented in our open-source MATLAB toolbox Gait-CAD [3].

KIT-GE: PREPROCESSING

Noise reduction of the input images was performed using a 2D median filter (*medianRad*) and to smooth the segmentation results, an additional Gaussian filtering was applied (*gaussVar*). In the case of 3D images the median filtering was performed individually for each of the slices. For the **Fluo-N2DH-GOWT1** dataset an additional morphological closing was used to avoid holes in the segmentation (*closingRad*). Using a down-sampled version of the input image (width and height scaled by 0.25, depth unchanged for 3D images), seed points were detected by identifying local maxima in the 8-neighborhood (2D)/26-neighborhood (3D) of each pixel within a Laplacian-of-Gaussian (LoG) space-scale maximum projection, which was iteratively calculated using LoG filtered images of different discrete scales (*LoGMin*, *LoGMax*). We used a non-strict maximum detection to avoid misdetections caused by intensity plateaus and merged redundant seed points based on a minimum expected distance criterion. At each identified location, the mean intensity of a 7x7 window was calculated and used to discard low intensity seed points with a semi-automatically optimized binary threshold (*seedThres*).

KIT-GE: SEGMENTATION

The actual segmentation of the spherical objects was performed on each image individually using the TWANG segmentation algorithm [1]. For every detected seed point, a cube with side lengths proportional to the radius of the respective blob was cropped from the preprocessed images and the regions were processed in parallel [2]. With the goal of a fast approximate segmentation of (hyper-)

spherical objects, the cropped image regions were transformed to a representation that could be segmented by a simple adaptive thresholding. Therefore, a new image was formed based on a Gaussian weighted dot product of the seed point normal (a normalized direction pointing away from the seed point) with the normalized intensity gradient vector at that each pixel (pointing in the direction of the steepest intensity change) as described in [1] (*gradStd*, *kernelSizeMult*, and *kernelStd*). In this transformed image, the transition regions between individual nuclei obtained low intensity values, whereas pixels belonging to the currently considered nucleus obtained high intensity values that could be easily separated from the background using an adaptive binary threshold (Otsu's method).

KIT-GE: TRACKING

The identification of temporal associations of the detected nuclei was derived with the tracking toolbox contained in the open-source MATLAB toolbox Gait-CAD [3]. Essentially, the centroids of identified segments were tracked by identifying nearest neighbors in subsequent frames. Matches were only considered as valid if maximum distance was not exceeded (*maxDist*). If the distance ratio of the closest and the second closest nearest neighbor was sufficiently small, the nucleus was considered a potential cell division candidate (*neighDistRatio*). Post processing routines for cell division detection and the fusion of fragmented tracks, however, are still under development and were disabled for the submitted tracking results. The tracking results were subsequently linked back to the segmentation images, i.e., the intensity values of all segmented regions within each image were set to the assigned tracking ID.

KIT-GE: POST-PROCESSING

No post-processing is carried out after tracking.

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).
2. Kobitski A, Otte JC, Takamiya M, Schäfer B, Mertes J, Stegmaier J, Rastegar S, Rindone F, Hartmann V, Stotzka R, García A, Wezel JV, Mikut R, Strähle U, Nienhaus GU. An ensemble-averaged, cell density-based digital model of zebrafish embryo development derived from light-sheet microscopy data with single-cell resolution. *Scientific Reports* **5**, 1-10 (2015).

3. Stegmaier J, Alshut R, Reischl M, Mikut R. Information fusion of image analysis, video object tracking, and data mining of biological images using the open source MATLAB toolbox Gait-CAD. *Biomedizinische Technik (Biomedical Engineering)* **57,** 458-461 (2012).