HD-Har-GE

Authors: Nathalie Harder, Karl Rohr

Email: k.rohr@dkfz.de

Platform: Linux

Prerequisites: None

HD-Har-GE: SUMMARY

Our approach for cell tracking in different types of 2D and 3D microscopy image sequences combines segmentation and tracking methods. Segmentation comprises filtering for noise reduction, region-adaptive thresholding, and watershed transformation for splitting cell clusters. Tracking is based on local optimization using a cost function within spatio-temporal regions. The tracking algorithm first determines one-to-one correspondences, second, detects mitosis events based on a likelihood measure, and subsequently combines mitotic tracks.

HD-Har-GE: PREPROCESSING

To reduce the image noise, Gaussian filtering (standard deviation σ_F) or median filtering (radius r_{m1}) was applied. In the case of bright speckles within the cell nuclei or strongly varying contrasts of neighboring objects, intensity clipping was performed where the optimal clipping value was determined using Otsu or Renyi entropy thresholding.

HD-Har-GE: SEGMENTATION

After preprocessing, region-adaptive thresholding was applied to obtain an initial segmentation. The approach uses small image regions (radius r_{inner}) and computes local thresholds in enclosing larger regions with a size similar to the average object diameter (r_{outer}). The sensitivity of the approach can be adapted to the image data based on the minimum intensity variance within image regions (σ^2_{min}). Namely, local threshold values are computed if the variance within the respective region exceeds the minimum value, otherwise a global (usually higher) threshold is used. The segmentation result is further enhanced by median filtering (r_{m2}) and hole filling. To split up cell clusters, a watershed transform after Euclidean distance transform is used. This approach is well suited for splitting up clusters of circular or spherical objects, however, for more elongated shapes it tends to result in over-segmentation. Thus, for data sets with elongated objects (e.g., complete cells) we usually skipped this step to avoid wrong splits.

HD-Har-GE: TRACKING

Our tracking approach exploits the information from cell segmentation and consists of three main steps: (1) Determination of one-to-one correspondences, (2) mitosis detection and establishment of one-to-many correspondences [1], and (3) detection and merging of trajectories that do not cover all frames of an image sequence. In the first step, we use a local optimization procedure, where for each object, hypotheses are generated, namely triplets with all potential predecessors and successors within a limited Euclidean distance (expected maximum displacement $d_{\max}$). These triplets are ranked and compared with other possible triplets. The rank of a hypothesis is determined based on a cost function, which includes the Euclidean distance, the morphological similarity, as well as the trajectory smoothness. The weights for these three components (w_1, w_2) are specified based on the properties of the image type. In the second step, appearing objects are investigated to detect mitosis events. Depending on the type of staining we exploit different measures (*mitMeasure*) for mitosis detection: The overlap-distance ratio, an object morphology-based likelihood measure, and a combination of both measures. The overlap-distance ratio (ODR) determines the ratio between the area overlap of potential mother and daughter cell objects and their centroid distances [2]. The object morphology-based likelihood measure (Likelih) takes into account the sizes and the mean intensities of potential mother and daughter cells normalized to the average values of the whole population. The different terms can be weighted and adapted according to the image data, for example, to define whether mitotic cells are darker or brighter than the average object intensity value (extension of the likelihood measure for mitosis detection in [3]). The combined measure (Combi) exploits both the overlap-distance ratio and the object morphology-based likelihood measure. If a mitosis event is detected, the trajectories of mother and new daughter cell are merged.

HD-Har-GE: POST-PROCESSING

In the final step, trajectories that do not cover all frames of an image sequence are considered and merged if they are in close spatio-temporal vicinity.

REFERENCES

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2. Withers JA, Robbins KA. Tracking cell splits and merges. In *Proceedings of IEEE Southwest Symposium on Image Analysis and Interpretation*, 117-122 (1996).
3. Harder N, Mora-Bermúdez F, Godinez WJ, Wünsche A, Eils R, Ellenberg J, Rohr K. Automatic analysis of dividing cells in live cell movies to detect mitotic delays and correlate phenotypes in time. *Genome Research* **19**, 2113-2124 (2009).