CUL-UK

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Platform: Linux

Prerequisites: MATLAB Compiler Runtime 2013a (x64)

CUL-UK: SUMMARY

Our algorithm is based on *PhagoSight*, an open-source MATLAB package for the analysis of fluorescent neutrophil and macrophage migration in a zebrafish model [1]. The package consists of pre-processing, segmentation, tracking and, if necessary, post-processing and visualization steps.

CUL-UK: PREPROCESSING

Pre-processing steps are optional and can be determined by the user. The datasets can be reduced in size with a construction of a Gaussian pyramid where neighboring elements are averaged to form a new pixels, this process reduces the uncertainty of the intensity at the expense of the spatial resolution, and in addition can reduce the computational complexity of large datasets. A 3×3 Gaussian filter was applied to those datasets that were not reduced. The datasets which presented an uneven background intensity were applied a retrospective shading correction algorithm [2].

CUL-UK: SEGMENTATION

Segmentation was performed with a hysteresis thresholding that generated three regions: voxels below a lower threshold (*thresL*) were classified as background, and those above a higher threshold (*thresH*) were classified directly as cells. The remaining voxels between these two levels were then classified as cells if they are in contact with voxels above the higher threshold, or as background otherwise. Both thresholds were automatically determined using Otsu's algorithm. A minimum size value (*minSize*) was selected to discard segmented elements that were small and thus considered as noise. It should be noted that the segmentation algorithm was designed to have an automatic value selection without user input, through an optimization of the threshold and minimum size selection. Those parameters are underlined in **Parameter Configurations**. For other datasets, we selected the parameters manually through a particle swarm optimization [3].

CUL-UK: TRACKING

The segmented cells were tracked with a model-based tracking algorithm adapted from the keyhole tracking algorithm [4]. The algorithm links the objects in contiguous time points to form the tracks by means of a keyhole model, which predicts the most probable landing position of a cell at time t+1 ("child"), from the position in times t ("parent"), and t-1 ("grandparent"). The most probable step for a cell that is moving from time t-1 to time t, is to follow the direction of the previous steps with the same velocity to time t+1. Assuming that a child (cell at time t+1) would move with similar direction and velocity as its parent (cell at time t), its landing position can be predicted. To consider for changes in speed, turns or random walk-like movements, two regions of probability where the child cell is most likely to land were defined: a narrow wedge (60° wide) oriented towards the predicted landing position, for straight-moving displacements, and a truncated circle (300°) that complements the wedge, for random-moving displacements, which together resemble a keyhole. The size of the keyhole at t+1 is determined by the distance between times t-1 and t.

CUL-UK: POST-PROCESSING

Temporal variation of intensity was analyzed as cells that disappear from their tracks and then re-appear a few points later. Collisions of cells were analyzed by measuring the volume of cells in time and splitting cells whose volume increased considerably. Finally, as the lack of proofreading and editing tools has been one of the main barriers in adopting automated and semi-automated methods, *PhagoSight* provide such tools, through which users can evaluate the output of algorithms and correct mistakes that can be visually detected.

REFERENCES

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2. Reyes-Aldasoro CC. Retrospective shading correction algorithm based on signal envelope estimation, *Electronics Letters* **45**, 454-456 (2009).
3. Kennedy J, Eberhart R. Particle swarm optimization. In *Proceedings of the IEEE International Conference on Neural Networks*, 1942-1948 (1995).

4. Reyes-Aldasoro CC, Akerman S, Tozer GM. Measuring the velocity of fluorescently labelled red blood cells with a keyhole tracking algorithm. Journal of Microscopy **229**, 162-173 (2008).