Abstract 10

TRACKING OF MIGRATING CELLS UNDER PHASE-CONTRAST VIDEO MICROSCOPY WITH COMBINED MEAN-SHIFT PROCESSES

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We propose a new algorithm to analyze cell migration on the basis of sequences of frames automatically recorded from standard (unmarked) cell cultures by means of phase-contrast microscopes equipped with video acquisition systems. This algorithm is able to automatically and simultaneously follow the locations of many cells during sequences covering relatively long periods of time (such as 1 to 3 days), and to compute quantitative features characterizing the cell trajectories. The proposed method is based on the mean-shift principles and introduces the use of adaptive combinations of linked kernels. This approach allows the detection of various gray-level configurations and the transition between them. We demonstrated its ability to track a large number of cells in culture in the presence of cell divisions. More particularly, this algorithm enabled cell trajectories to be connected during mitoses in order to establish the migration potential of a cell through its “descent”, e.g. by measuring the distance covered by a cell and its successive cell generations (if the observation time allows it). As the method does not focus on cell boundary detection, it shows robustness with respect to variability in cell morphologies (between different cell cultures), cell overlaps and dynamical changes in cell shape during cell migration. Furthermore, the running time of the software is very short, allowing improved possibilities in acquisition frequency and, consequently, improved descriptions of complex cell trajectories presenting quick displacements and strong cell shape deformations. Our adaptive model is in fact controlled by several parameters whose values essentially depend on general cell and image features, such as the range of cell sizes encountered in regards to the image resolution used. Comparing the tracking results automatically obtained to those generated manually by a human expert, we tested the stability of the different algorithm parameters and their effects on the tracking results. This evidenced relatively small levels of variability in the case of parameter values chosen in reasonable intervals. Furthermore, the descriptive features extracted from the cell trajectories appeared as being very stable statistically (especially the distance-based features). We also evaluated how the method is resistant to a decrease in image resolution and accidental defocusing (which may occur during long experiments, e.g. dozens of hours), showing that acceptable blur levels did not significantly affect the performances.

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