04 Tracking, Time resolved microscopy.

Assignment 4, 25 points in total; 16.6% of the total score for the practical part.

In this assignment the problem of feature extraction from a time-series is addressed. Researchers would like to understand about, moving speed, distance trajectory and shape changes of cells. In the experiment presented for this assignment, the cells are cancer cells. The images of the cells are given to you "as is". Let us assume that the time-lapse procedure takes a snapshot of the cells at intervals of 2 minutes. The living conditions of the cells are kept constant (temperature, humidity, oxygen-flow) so that they will survive well over a longer period in culture.

We will use the following series of images:

(A) MTLn3-Ctrl0000.tif – MTLn3-Ctrl0029.tif, and
(B) MTLn3+EGF0000.tif – MTLn3+EGF0029.tif

Series (A) is the control, whereas in the experiment in series (B) a growth factor was added to the cells, and this might resort in an effect on the movement and shape of the cells. We can measure this effect through a readout in the images.
For the first questions we will use series (A), i.e., the Control-experiment, i.e., MTLn3-Ctrl*. We first focus on the method development for feature extraction in these time-series.

Part 4.1

1. (3) In the given sequence (00-29) pick 15 cells. These cells are traced cells over the sequence of the 30 images. Indicate your choices with a label/number in the initial image. These labels are the result from a segmentation operation that successfully find all the relevant, i.e. 15, cells in the image(s). Develop, apply, explain and motivate your segmentation procedure.
2. (2) From the 15 cells that are selected, do a manual check of the tracing for 5 cells. These traces are used to control the outcome of an algorithm that automates this process. Explain your validation procedure – basically, this is a ground truth procedure that provides insight for the success of an algorithm.
3. (3) Develop an algorithm that can trace the cells over the time-lapse sequence. It is important is to make a choice for criteria by which you can determine that a cell in a next frame is the same cell in the current frame. Your algorithm is based on that criterion, or those criteria. This should be clearly stated in the explanation of the algorithm.
4. (4) Apply the algorithm to the control series, i.e. MTLn3 (series A), and the experimental condition, i.e. MTLn3+EGF (series B). This will result in traces of segmented cells that are extracted over the sequence.

In this tracking experiment you should document your results with the intermediate images. These images can then be referred to in the tables that you provide with the measurements. Document your algorithm well with comment lines as you include the **code** in the assignment

report.

Part 4.2

Shape and texture of the cells may vary over time and over the different conditions. For shape you can start by computing the area and perimeter. However, as cells my stretch, in addition, compute the roundness as shape feature. As from the segmentation, a mask for each cell in the tracking is obtained, now compute texture features using the cell mask; i.e., mean, standard deviation, smoothness and uniformity.

5. (4) From the cells obtained from the tracking, compute the aforementioned features for shape and texture. This is for both conditions and presented in two different tables.

Next, given the fact that the tracking is completed, features over the time-line be computed. First compute these features then in order to establish possible differences, do make a graph of distance over time. Again, we assume a time interval of 2 minute between the images.

6. (2) Compute for both conditions, the cell velocity, distance trajectory and present this is in a table.
7. (2) Within the cells that you have followed, identify differences in the trajectories by assessing the trajectories separately for both conditions.

If there are differences within the cells in one condition, these differences need to be formulated in terms of variation within one condition. This need be done for all the measurements in a condition. Next, the two conditions are compared.

8. (2) From the size, shape and texture of the control and experimental condition deduce possible differences between the two conditions.
9. (2) Deduce from the data if a correlation between speed with shape and texture can be established.

From the measurements that are compiled, some conclusions might have been possible. However, given the result, ideas might have developed how to setup a next experiment to get even better measurements and conclusions.

10. (1) Comment on image quality and image resolution in all dimensions studied.