Single-cell study of Akt and ERK activities - Assignment D

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1 Image data and tracked data inspection

Question 1: What is the time interval (in minutes) between two time frames? (Hint: it is in the image metadata)

Based on the metadata viewed in ImageJ[2], the time interval is 270,01617 seconds and that equals with 4,5002695 minutes.

Question 2: Do all traces cover the full duration of the timelapse imaging? Why could be the reason if there are incomplete traces in your tracked data?

Our image data is time-series consisting of 27 time-frames. Multiplying the number of the frames, with the frame interval we get the full duration of 7290,43659 seconds. If we briefly look out our exported .csv file including the tracks information, we can easily spot tracks that end before the full time-lapse of the imaging. The main reasoning behind these incomplete traces is that the intensity of some of them falls bellow a specific threshold, meaning they are no more traceable in the nucleus segmented part and probably moved in the cytoplasm or had short lifespan.

2 Methodology

Question 3: Explain your methodology for image processing, segmentation and tracking. Use plots and/or figures generated from your work to support your explanation.

The basic image processing was done through Fiji(ImageJ) app and includes background noise subtraction, smoothing and median filtering for each of the channels(Akt kinase, ERK kinase and nuclear) and time-frames. As a next step, nucleus channel as used to retrieve the positions of the nucleus, we used threshold method to create a binary mask, create a selection (Edit- Selection - Create a selection), and restore the selection in our Akt and ERK channels. That procedure revealed the nucleus positions in the images, while we cropped just that nucleus part in order to use TrackMate[3] effectively. Using TrackMate with Laplassian of Gaussian(LoG) tracker and adjusting the thresholds(1.07) and diameter values(20), we obtained the maximum amount of somehow correct spots and we exported .csv files including further data for their tracks. Akt-KTR methodology workflow is depicted in Fig.1 ,illustrating the steps mentioned above. Then the same methods were used without any individual channel filtering and denoising to observe differences in the rates and maximum changes. Our results showed that these steps of preprocessing affected the values (global intensity median , and the other medians), so it was chosen to not use any of the first steps as we do not know how they affect the trackMate's effectiveness and Max Change, RatesOfChange values.

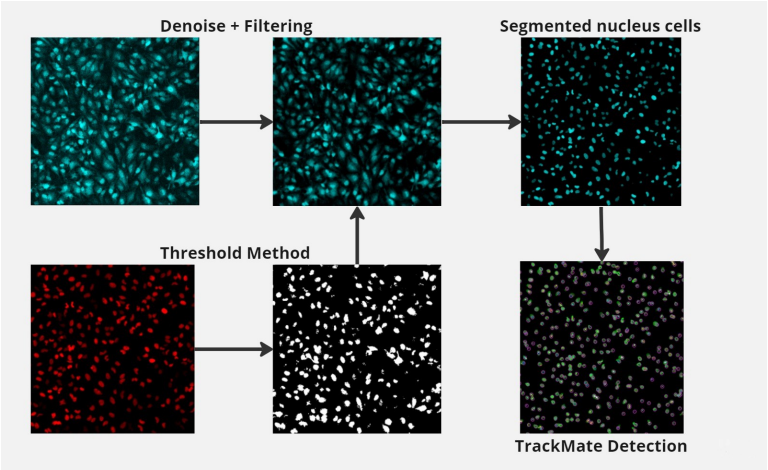

Fig. 1: Workflow outputs of the image analysis and tracking done for Akt-KTR cells.

Bonus question: Validate the segmentation and tracking results via manual inspection and report the accuracy of the segmentation and tracking
Based on the selection and the number of saved ROIS from the selection(Fig.2) done in one of our channels that include the ERK-KTR which number is 29496 segments(ROI), our tracker spotted 22418 spots(ID) and 901 Tracks, using the unique() function in our generated TrackMate CSV file. Counting manually the nucleus i found around 948 cells/nucleus. The detection of the spots was: (22418 / 29496) * 100 = 75.92% accurate, while the segmentation was around 95.04% ((901/ 948)*100).

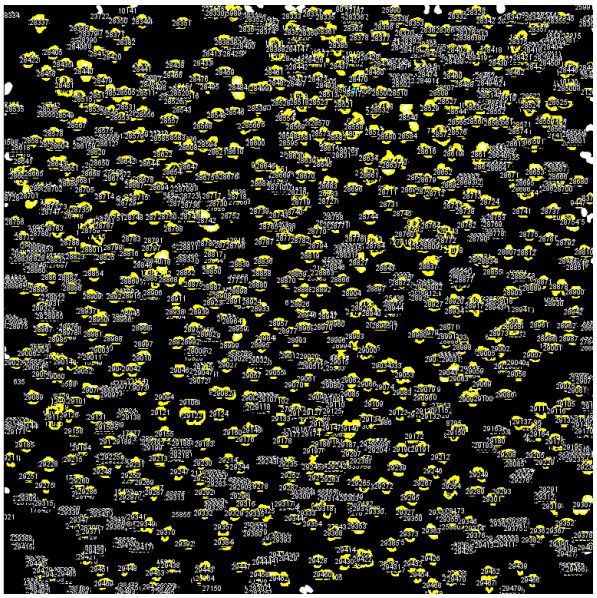

Fig. 2: Nucleus-Selection (ROI) with cropped background areas

Question 4: With code comments in the Python script, briefly describe the workflow used in processing the time-series data and calculation of the maximal changes and rates of change.

Image processing before the tracking was done with ImageJ, including background noise subtraction and median filtering, next steps are in code/comments in the python file.

3 Data analysis and discussion

Question 5: Present your results (maximal changes and rates of change) either with a plot or a table. Which kinase (ERK or Akt) exhibited the greatest maximal changes and rates of change?

Based on the global median intensity values generated out our bootstrapping method (Fig.3), the Max Change was spotted on Atk with the value of 0.57142, and highest rate of change on ERK with the value of 0.01113(Table1). Max change was calculated based on the formula:

$$max\_change = \frac{global\_SteadyState - minimal\_intensity}{global\_SteadyState} \tag{1}$$

The rate of change was calculated with the formula:

$$RateOfChange = \frac{Max\_change}{Duration} \tag{2}$$

Duration is calculated accordingly :

$$Duration = \frac{minimal\_intensity \times Frame\_interval}{60} \tag{3}$$

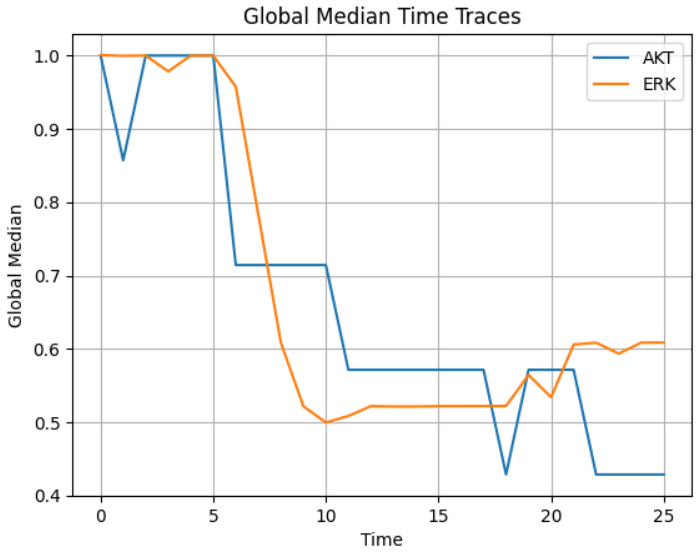

Fig. 3: Global median intensity values of Atk and ERK per time-frame

Table 1: Generated values of Max change and Rate of change of intensity levels on all the bootstrap global median samples of Akt-KTR and ERK-KTR

Kinase	MaxChange	RateOfChange
ERK	0.50045	0.01112
Atk	0.57142	0.00705

Question 6: Identify the limitation(s) of your choice of segmentation methods and propose workarounds.

Apart from the experimental techniques our way of segmenting the cells comes with several limitations. Firstly, it is not entirely clear how the preprocessing of the image affects the TrackMate from detecting the spots. Different ways of filtering and denoising could either increase or deteriorate the performance. For this assignment Median filtering and background noise subtraction was firstly used. On the second step, the threshold method was used to make a binary mask on the nucleus channel and detect the nucleus region of interest, which were used to spot the nucleus in the other two channels and segment the cells. Different thresholds could probably produce different outputs, increase nucleus visibility, cell separability and vise versa. Some of the nucleus were observed to be closely together/overlap, probably affecting the segmentation in dense areas. Other nucleus were difficult to be spotted even after all the preprocessing. TrackMate's threshold and object diameter selection was mostly experimental as we have a heterogeneous set of cells, thus there are no standard values to use, just approximate ones. Lastly, this method was not able to detect the segments close to the edges which were mostly halved. To address these issues, we could try all the combinations of the available preprocessing methods and threshold options by keeping the rest of the variables constant to see how they affect the TrackMate detection. We could also manually separate the dense areas by selecting the edges of the nucleus or apply the distance transform watershed. That operation includes the calculation of the distance transform of the binary mask image, inverting it and then use the watershed algorithm[1].

References

1. Legland, D., Arganda-Carreras, I., and Andrey, P. MorphoLibJ: integrated library and plugins for mathematical morphology with ImageJ. *Bioinformatics 32*, 22 (July 2016), 3532–3534.
2. Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., and Cardona, A. Fiji: an open-source platform for biological-image analysis. *Nat. Methods 9*, 7 (June 2012), 676–682.
3. Tinevez, J.-Y., Perry, N., Schindelin, J., Hoopes, G. M., Reynolds, G. D., Laplantine, E., Bednarek, S. Y., Shorte, S. L., and Eliceiri, K. W. TrackMate: An open and extensible platform for single-particle tracking. *Methods 115* (Feb. 2017), 80–90.