Image Processing and Quantitative Image Analysis
Assignment D

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1 Image data inspection

1.1 Question 1

The time interval between two time frames is 270.02 seconds which corresponds to 4.5003333333 minutes.

1.2 Question 2

Not all traces cover the full duration of the timelapse imaging. There are 327 traces that have fewer than 27 frames, as we can see in Figure 1.There are multiple reasons why this could happen. For instance, it could be because of a cell dying in this period of time, or a cell moving out of the picture, or because of two cells overlapping each other.

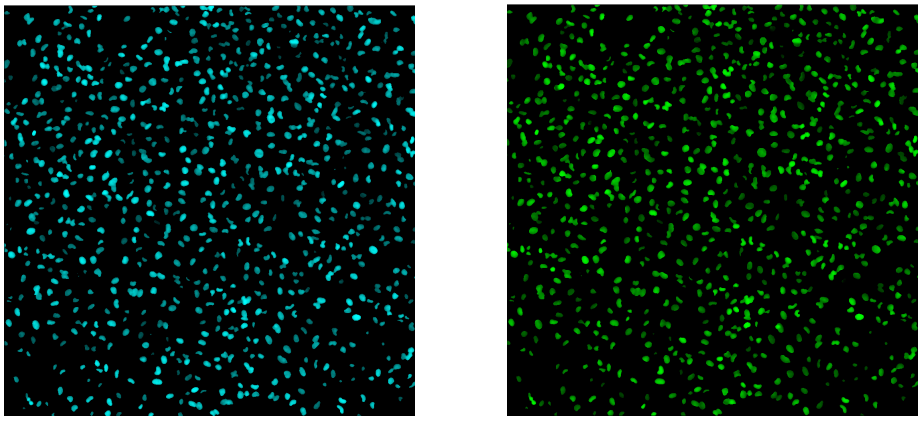
```
TRACK_ID
1000    8
1001    9
1002    7
1003    8
1004    8
       ..
995     6
996     7
997     8
998     9
999     7
Name: FRAME, Length: 327, dtype: int64
```

Fig. 1: The amount of incomplete tracks.

2 Image processing workflow

2.1 Question 3

First of all, we performed image processing. More specifically, we removed the background noise and smoothed the picture. After that, we split the three channels into three different images. Each new image represented a different channel (Akt-KTR, EKR-KTR and nuclear marker). After that, We performed image segmentation, creating a threshold for distinguishing the cells, which can be seen in Figure 2. Tracking was performed for each single cell with a plugin called "TrackMate".



(a) Final image of Akt-KTR after segmentation

(b) Final image of EKR-KTR after segmentation

Fig. 2: Segmentation results.

2.2 Bonus Question

2.3 Question 4

Bootstrapping We performed bootstrapping in our data because our data is heterogeneous, smoothing the heterogenicity in the data to get a more accurate estimate of the trends.

Median Intensity For each bootstrapped sample, we calculated the sample median time trace. This was achieved by calculating the median of the mean intensity for each combination of track ID and frame.

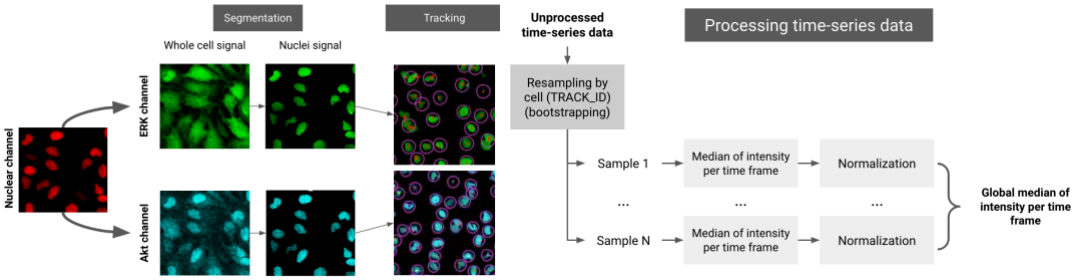


Fig. 3: Complete workflow of segmentation, tracking and processing time-series data.

Normalization The normalization was calculated by dividing the median intensity of each sample with the steady state intensity, which represents the normalized median intensity trace for each track.

Global Median Time Trace The global median time trace was calculated by taking the median of the normalized intensities across all cells at every time point.

Maximal Change The maximal change was calculated by taking the difference between the global steady state intensity and the minimal intensity for each kinase.

$$MaximalChange = \frac{GlobalSteadyStateIntensity - MinimalIntensity}{GlobalSteadyStateIntensity}$$

Rate of Change The rate of change was calculated by dividing the maximal change by the duration for each kinase.

$$RateOfChange = \frac{MaximalChange}{Duration}$$

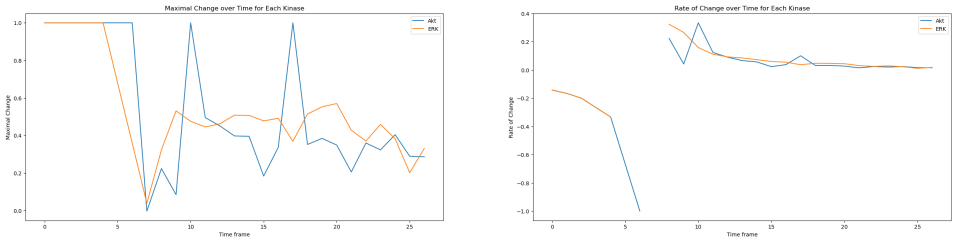
3 Data analysis and discussion

3.1 Question 5

From Figure 4 and Table 1, we can clearly see that EKR kinase achieved greater maximal change and rate of change.

Kinase	Median Rate of Change	Median Maximal Change
Akt	0.020520846919699146	0.36236933797909404
ERK	0.07093490826840915	0.486303091228952

Table 1: Rate of change and maximal change for Akt and ERK kinases.



(a) Maximal change

(b) Rate of change

Fig. 4: Results of the reserach

3.2 Question 6

The limitations we were faced with was mostly the cells that were overlapping each other. Unfortunately, the threshold we chose, in order to pick up on all the cells we could see with our eyes, and also pick the most cells possible, made it very difficult to distinguish the cells that were overlapping each other, as usually both of them would have very high intensities.

References