

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

Course : Image Processing and Quantitative Data Analysis

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1.0 Introduction

Kinases play a key role in regulating cellular biological processes, including in the G-protein-coupled receptor (GPCR)-mediated signaling pathways. In this assignment, we are interested to know the difference in kinase activity using nuclear transport reporters (i.e. kinase reporter molecules moving across the nuclear membrane) between two kinases involved in the GPCR signaling pathways: Akt and ERK, upon extracellular stimuli. These KTRs are fluorescent throughout their fluorescence lifetime (FLT), but move across the nuclear membrane upon phosphorylation by the kinases.

Image data: KTR.tif (Chavez-Abiega et al, 2022)

HeLa cells with nuclei labeled with a histone marker (red channel), kinases Akt tagged with Kinase Translocation Reporters (KTRs) (cyan channel), and kinases ERK also tagged with KTRs (green channel), are stimulated by histamine at the beginning of 7th time frame. After this time frame, the timelapse 2D imaging shows translocation of KTRs from the nuclei to the cytoplasm.

The goal of this assignment is to compute the maximal outflow between the 7th and last time frames (in unit of normalized pixel intensity. See *General Instruction* below) and the rate for this maximal outflow to occur (in unit of normalized pixel intensity over minutes).

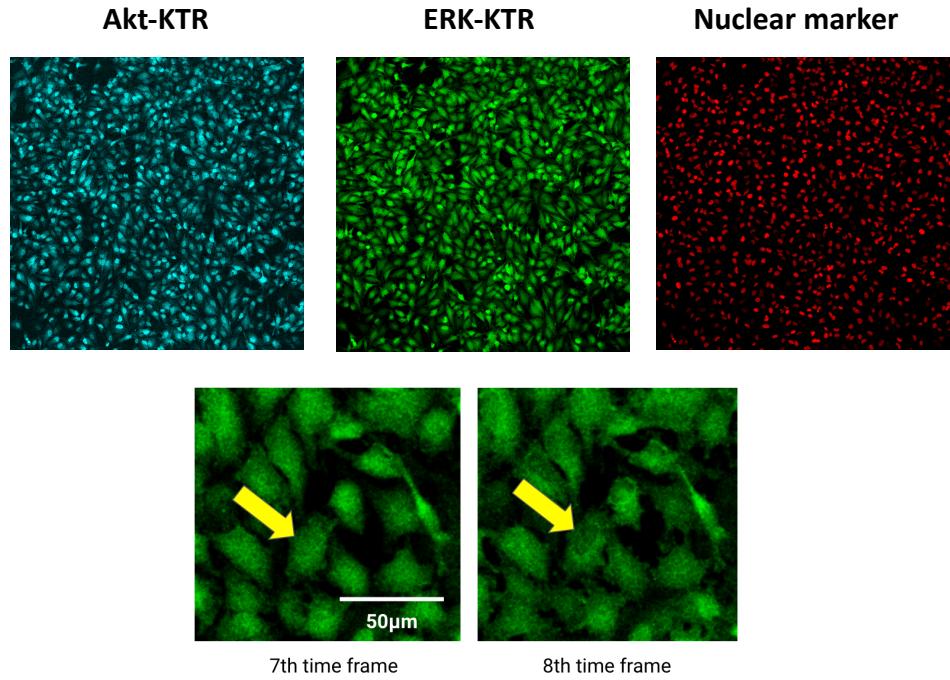


Figure 1 Timelapse dataset used in this assignment. Each image is a 2D image of the cells over time and each dataset has 3 channels of these images: Akt-KTR (cyan channel), ERK-KTR (green) and nuclei (red). Bottom images illustrate the translocation of ERK-phosphorylated KTR from the nucleus to the cytoplasm over two time frames (indicated with arrows).

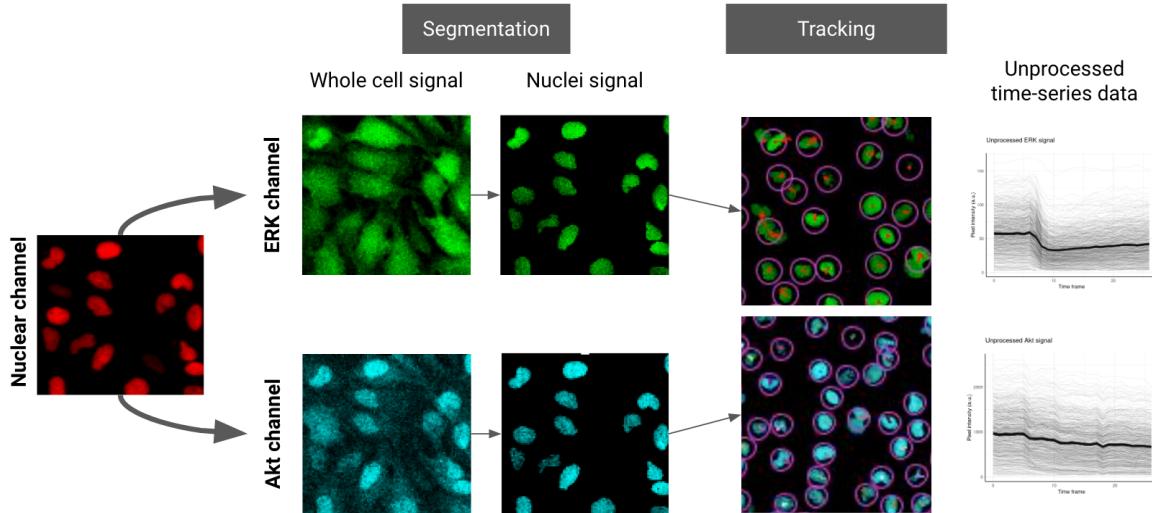


Figure 2 Schematic diagrams of image segmentation and single-cell tracking workflow. Using a mask based on the nuclei channel, extract the nuclear signal for both Akt-KTR and ERK-KTR channels. The individual nuclei on these segmented timelapse images are then tracked over time, so that the pixel intensity over time per nuclei (a.k.a. per cell) can be extracted, forming the unprocessed time-series data. The thick lines in the examples of unprocessed time-series data plots are median of intensity per time frame.

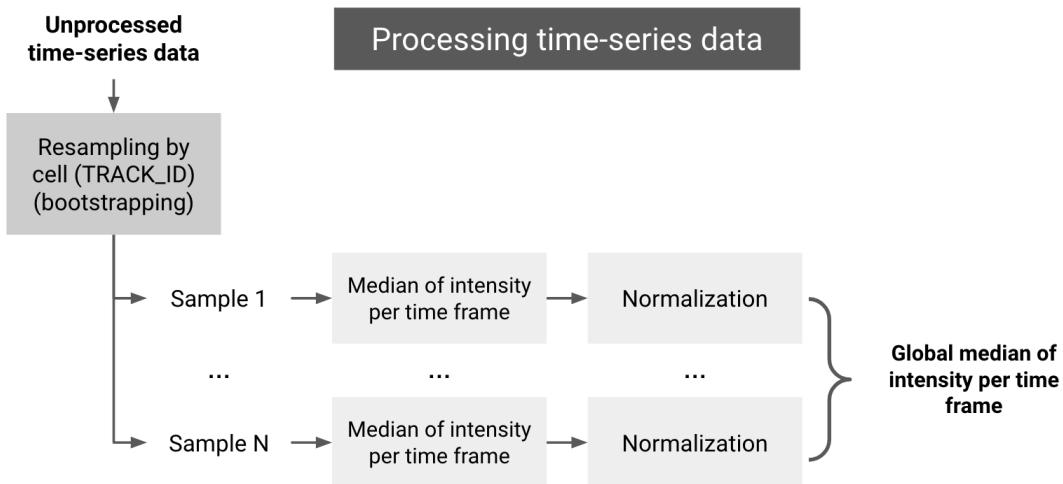


Figure 2 Schematic diagrams of time-series data processing workflow. To process the extracted time-series data, first apply bootstrapping on each TRACK_ID (per cell), generating multiple samples each consisting of multiple TRACK_IDs. In each sample, the median of intensity per time frame is computed, and the resultant median intensity over time is normalized. Finally, a global median of all the normalized median intensity over time is computed to represent the kinase activity upon perturbation with a stimuli.

General instruction

There are three main components in this assignment:

1. Image segmentation and single-cell tracking (See Tutorial 6)
 - a. Perform image processing step(s) to reduce background noise
 - b. Use the nuclear marker channel as a nuclear reference to extract the nuclear KTR signal in other kinase channels
 - c. Once the nuclear KTR signals are extracted, save the processed image stack/hyperstack.
Image file name:
 - i. **segmented_nuclear_kinases_KTR.tiff** for hyperstack, or
 - ii. **segmented_nuclear_ERK_KTR.tiff** and **segmented_nuclear_Akt_KTR.tiff** for stacks
 - d. Use the processed images for single-nuclei tracking using TrackMate. Extract as many tracks as you can
 - e. Export the tracks as CSV (the *unprocessed time-series data* shown in Figure 2). Name the CSV as:
 - i. **tracked_ERK_KTR.csv**
 - ii. **tracked_Akt_KTR.csv**

2. Processing time-series data
 - a. Each time trace extracted from a cell track (TRACK_ID) represents the kinase activity of a single cell over time. As the unprocessed time-series data is heterogeneous, we apply bootstrapping on the cell traces to sample the heterogeneity.
 - b. In each bootstrapped sample, calculate the **median** intensity per time frame (called **sample median time trace**)
 - c. As the pixel intensity is merely a qualitative representation of the kinase concentration, we **normalize each sample median time trace** to the median of the intensities during the first 6 time frames (also known as the steady-state intensity prior to perturbation)
 - d. Finally, gather all the normalized sample median tracks and calculate a **global median time trace** for each of the kinases

3. Calculate the maximal outflow and the corresponding rates
 - a. Use the global median time traces, calculate the maximal change between the 7th and the last time frames for each kinase. Use the following equation:

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

where,

- the Global Steady State Intensity is the median of the global median time trace during the first 6 time frames
- Minimal Intensity is the lowest intensity during the 7th and the last time frames

- b. Calculate the corresponding rate of change:

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

where,

- the Duration (in minutes) is the duration between the 7th time frame and the time frame when the Minimal Intensity is detected

2.0 Tasks and questions (Total 10 points)

2.1 Image data and tracked data inspection (2 point)

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

2.2 Methodology (5 points)

- Question 3: Explain your methodology for image processing, segmentation and tracking. Use plots and/or figures generated from your work to support your explanation.
- Bonus question: Validate the segmentation and tracking results via manual inspection and report the accuracy of the segmentation and tracking (e.g. select 3 consecutive time frames and estimate the accuracy of the segmentation and tracking)
- 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.

2.3 Data analysis and discussion (3 point)

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

2.4 Submit your work in 3 formats

1. One PDF that contains text-based answers, figures and/or tables. Don't forget about the references.
2. Python script with comments
3. A zipped folder containing:
 - a. segmented_nuclear_kinases_KTR.tiff (hyperstack) **or** segmented_nuclear_ERK_KTR.tiff and segmented_nuclear_Akt_KTR.tiff (stacks)
 - b. tracked_ERK_KTR.csv
 - c. tracked_Akt_KTR.csv

3.0 Reference

Chavez-Abiega, S., Grönloh, M. L. B., Gadella, T. W. J., Bruggeman, F. J., & Goedhart, J. (2022). Single-cell imaging of ERK and Akt activation dynamics and heterogeneity induced by G-protein-coupled receptors. *Journal of Cell Science*, 135(6). <https://doi.org/10.1242/jcs.259685>