Week 2, Practical 6

Image segmentation, tracking, processing time-series data

Tutorial 6

Image segmentation and tracking using ImageJ

Assignment D

Single-cell study of Akt and ERK activities

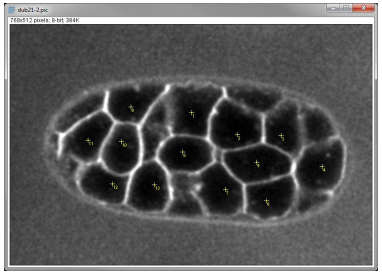
Tutorial overview

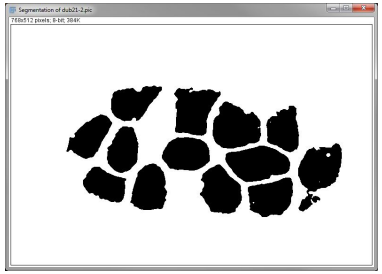
Tutorial 6 : Image segmentation and tracking using ImageJ

Course : Image Processing and Quantitative Data Analysis
Instructor : Marten Postma
Teaching assistants : Aaron Lin, Aoming Sun, Catherine Chia
Date: 16th June, 2023

Table of content

2D stack

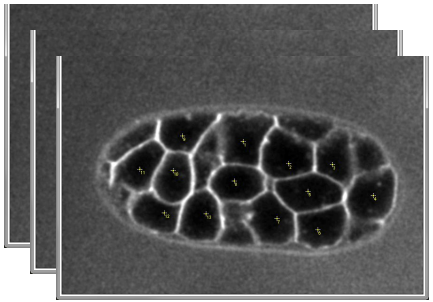


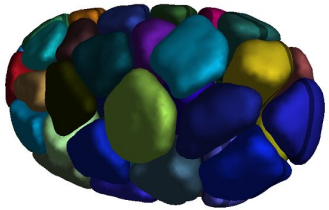


2D mask

3D stack
(XYZ)

Z = thickness

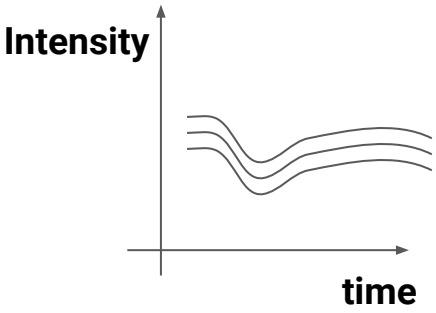




3D mask

3D stack
(XYT)

T=time



2D tracking

Assignment overview

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

Course : Image Processing and Quantitative Data Analysis
Instructor : Marten Postma
Teaching assistants : Aaron Lin, Aoming Sun, Catherine Chia
Date: 16th June, 2023

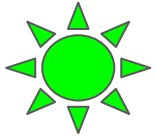
Table of content

Kinase Translocation Reporter (KTR)

- Always shines (fluorescent)
- Moves upon phosphorylation by kinases (ERK or Akt)

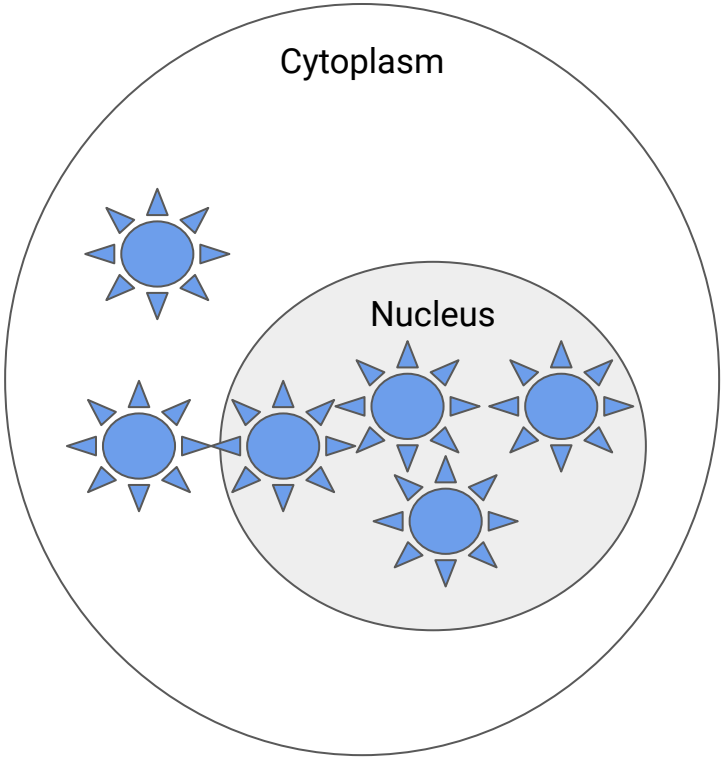
KTR types

ERK-KTR



Akt-KRR

Not phosphorylated



Kinase Translocation Reporter (KTR)

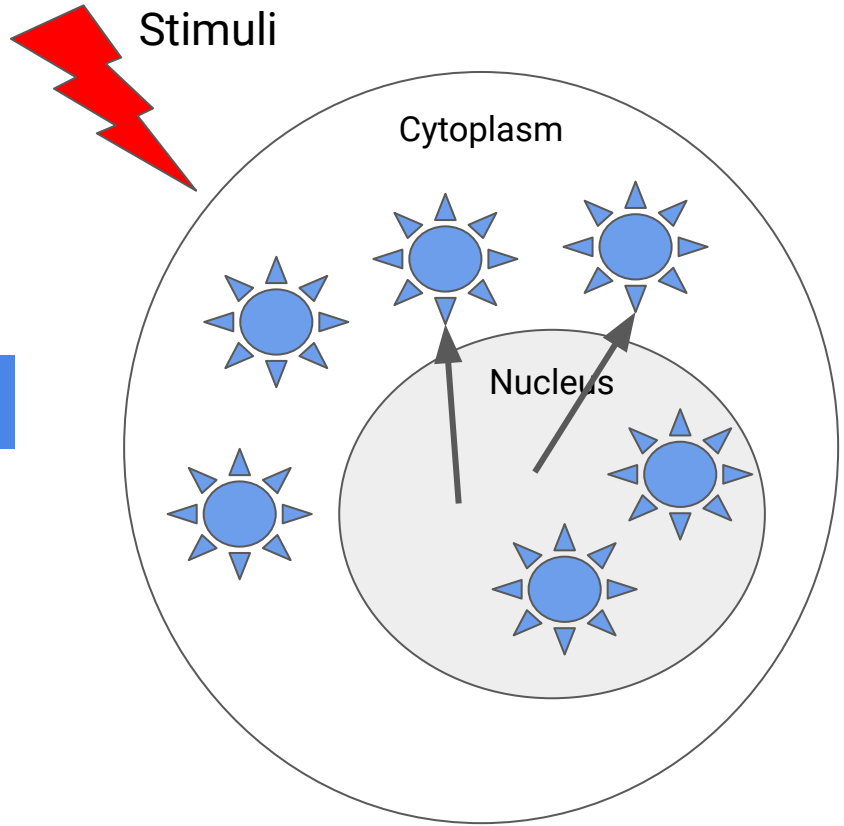
- Always shines (fluorescent)
- Moves upon phosphorylation by kinases (ERK or Akt)

KTR types

ERK-KTR

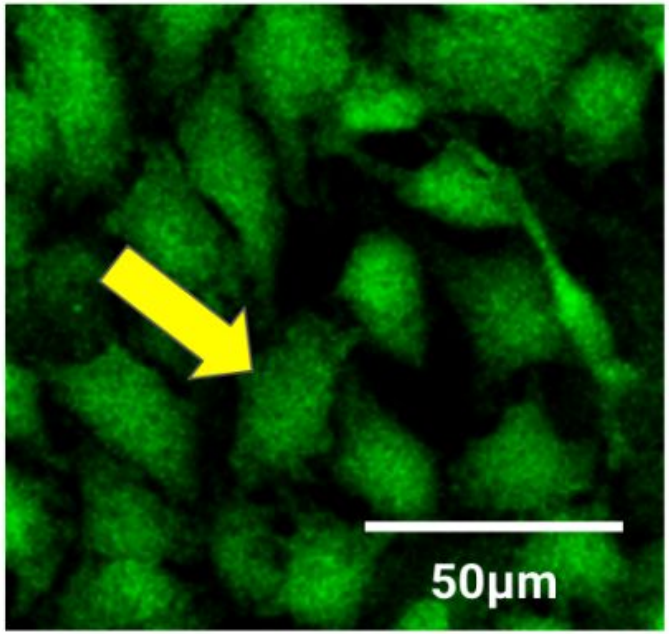
Akt-KRR

Phosphorylated

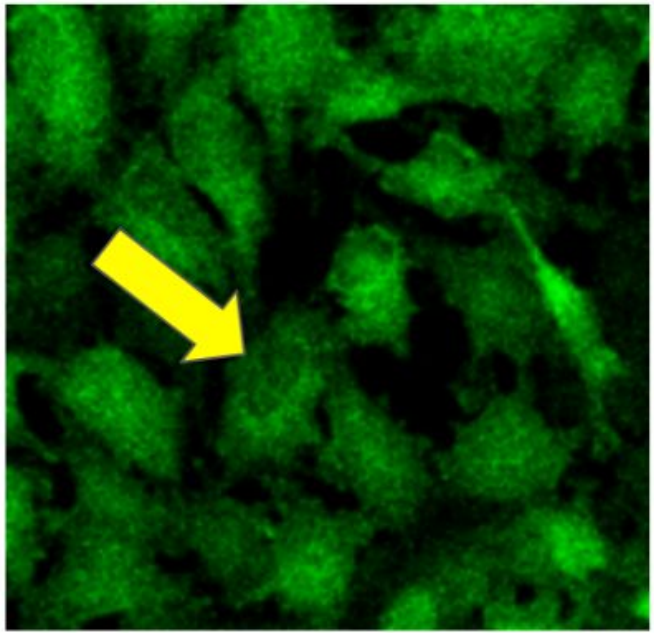


Stimuli

KTRs translocate out of the nuclei

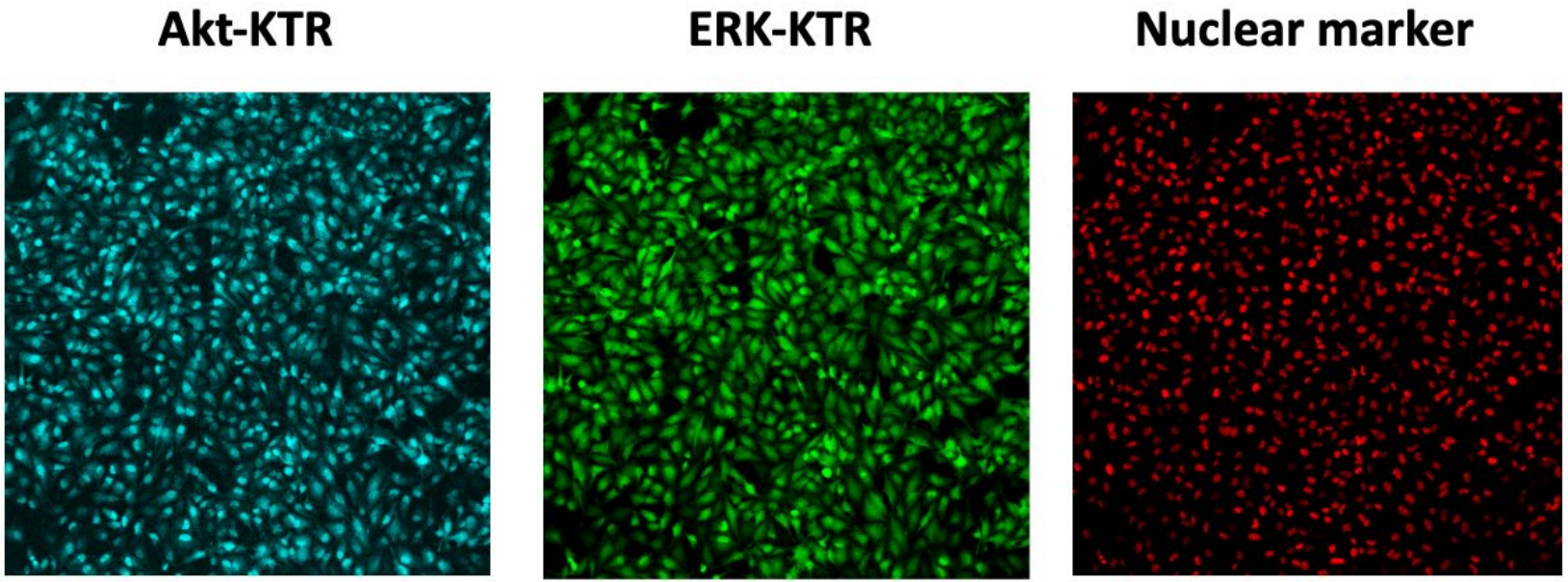


7th time frame



8th time frame

Image dataset is a hyperstack (XYT), T = time



Segmentation

Tracking

Use the nuclear mask to extract the nuclear KTR signals

Whole cell signal

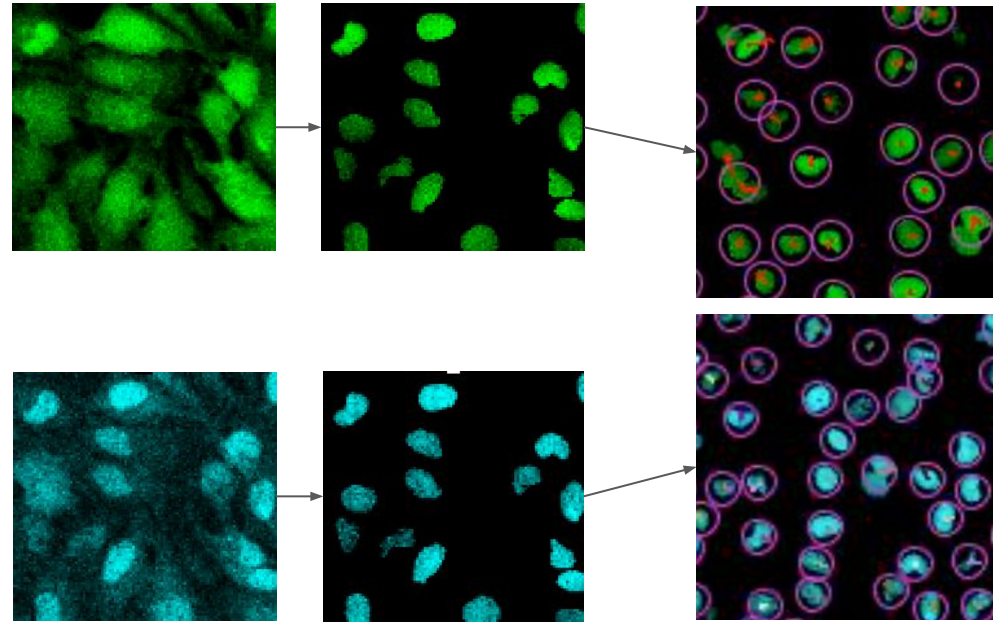
Nuclei signal

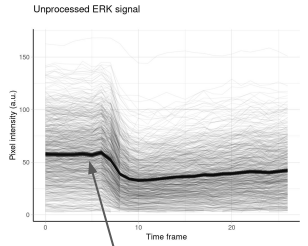
Unprocessed time-series data

Nuclear channel

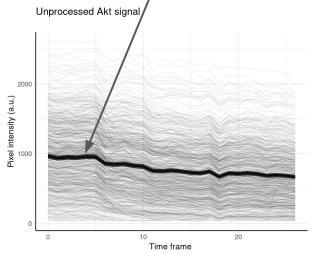
ERK channel

Akt channel





Median per frame



Processing time-series data

Unprocessed time-series data

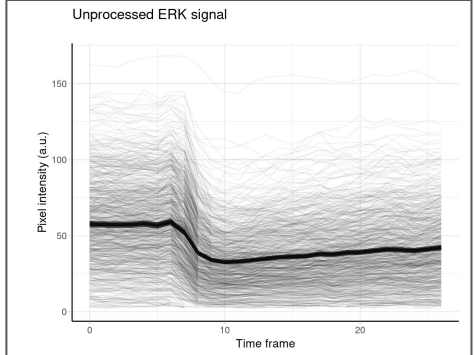
Resampling by cell (TRACK_ID) (**bootstrapping**)

Sample 1 → Median of intensity per time frame → Normalization

...

Sample N → Median of intensity per time frame → Normalization

Global median of intensity per time frame



Each thin line is a track.
Tracks are heterogeneous.
Apply bootstrapping

Calculate: **Maximal changes** and **rate of change**

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

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