

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

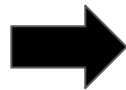
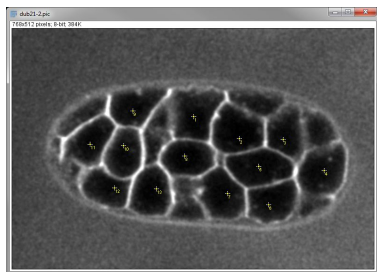
Teaching assistants : Aaron Lin, Aoming Sun, Catherine Chia

Date: 16th June, 2023

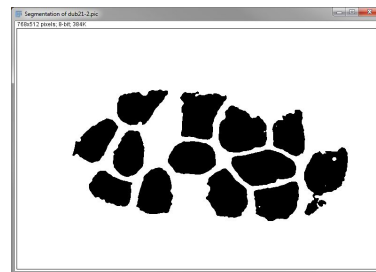
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2D stack

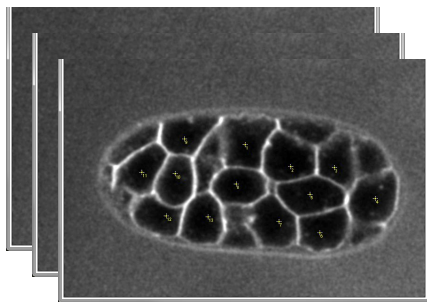


2D mask

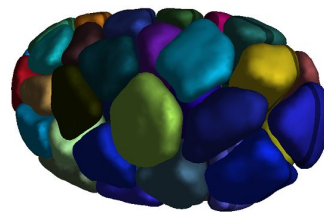


3D stack
(XYZ)

Z = thickness

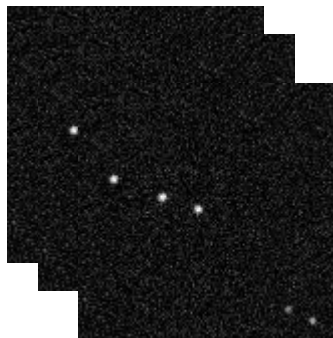


3D mask

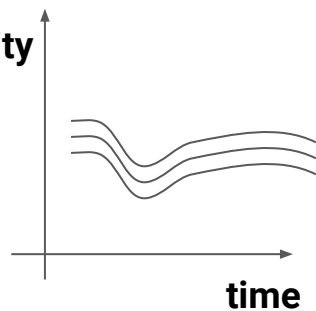


3D stack
(XYT)

T=time



Intensity



2D tracking

Assignment overview

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

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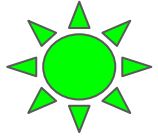
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Kinase Translocation Reporter (KTR)

KTR types



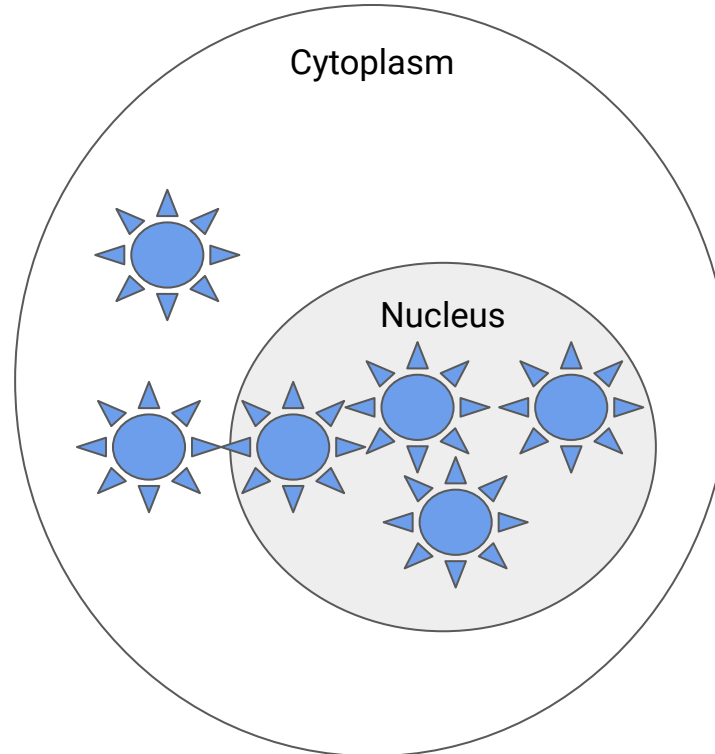
ERK-KTR



Akt-KRR

Not phosphorylated

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



KTR types



ERK-KTR



Akt-KRR

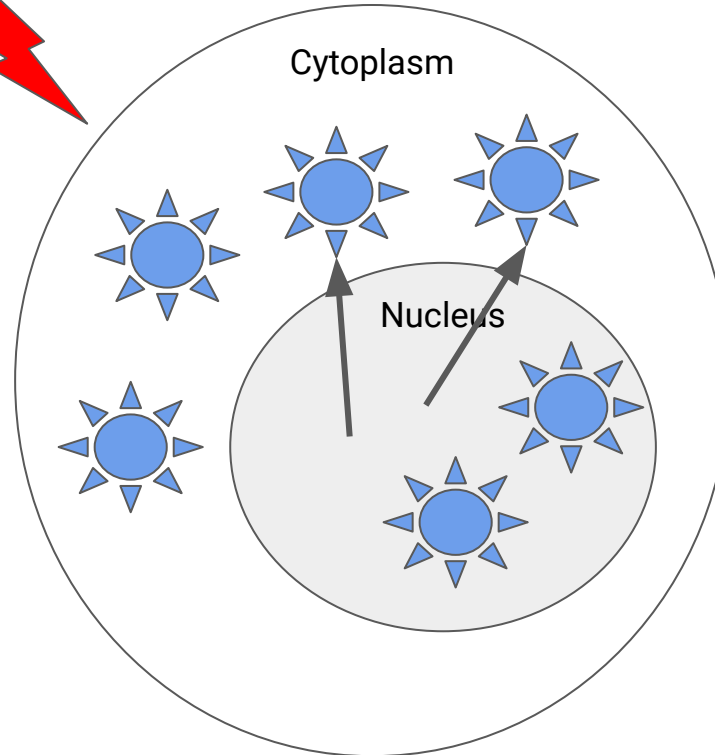
Phosphorylated

Kinase Translocation Reporter (KTR)

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



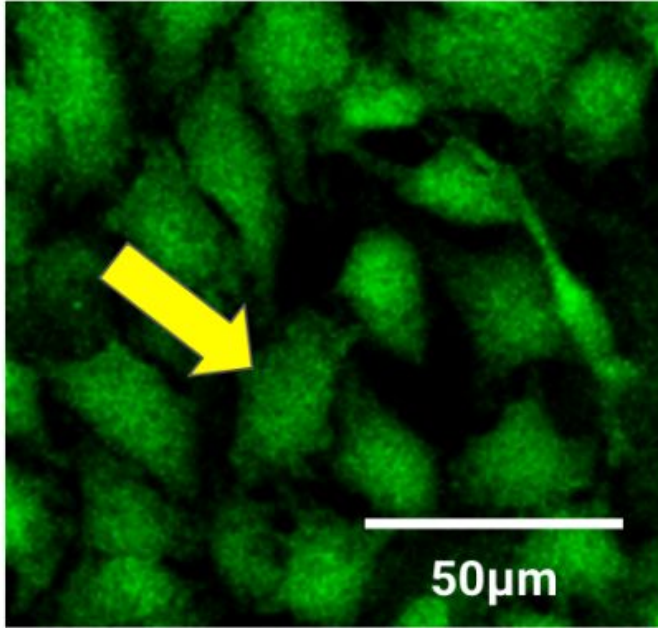
Stimuli





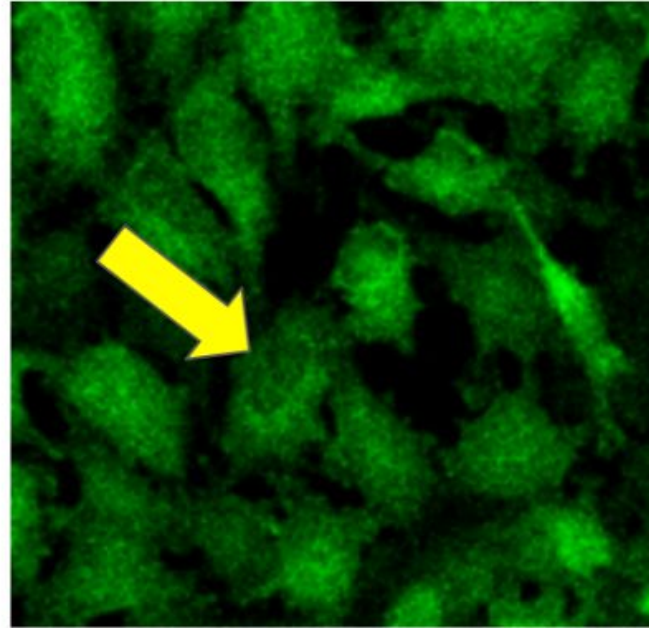
Stimuli

KTRs translocate out of the nuclei



50 μ m

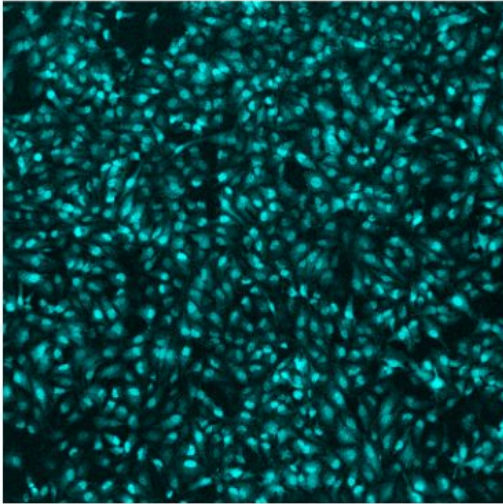
7th time frame



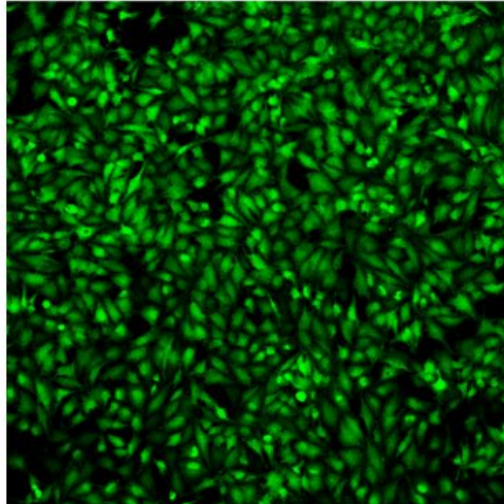
8th time frame

Image dataset is a hyperstack (XYT), $T = \text{time}$

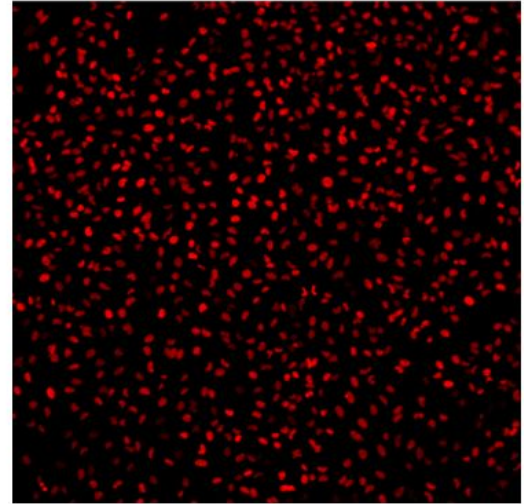
Akt-KTR



ERK-KTR

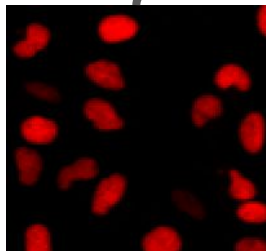


Nuclear marker



Nuclear channel

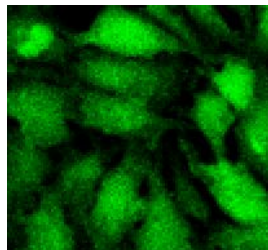
Use the nuclear mask to extract the nuclear KTR signals



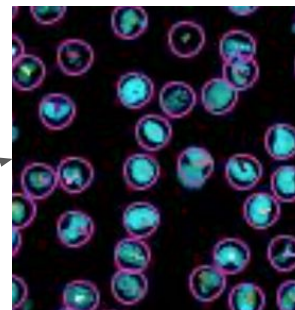
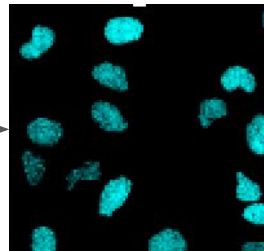
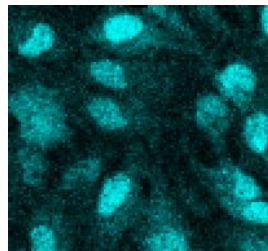
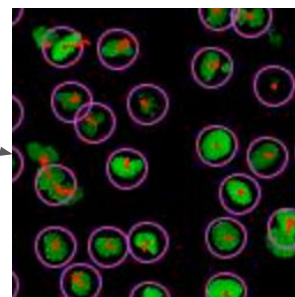
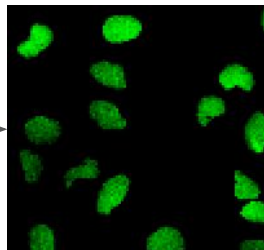
ERK channel

Akt channel

Whole cell signal



Nuclei signal

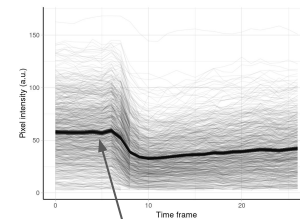


Segmentation

Tracking

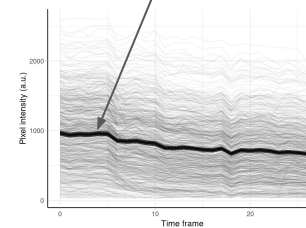
Unprocessed time-series data

Unprocessed ERK signal



Median per frame

Unprocessed Akt signal



**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

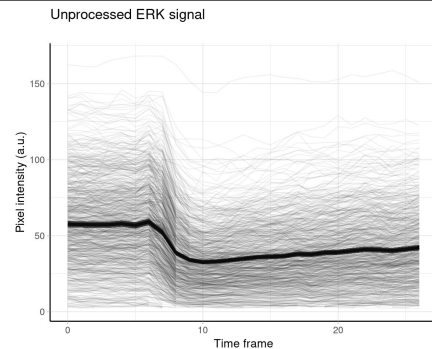


Normalization



**Global median of
intensity per time
frame**

Processing time-series data



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

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

