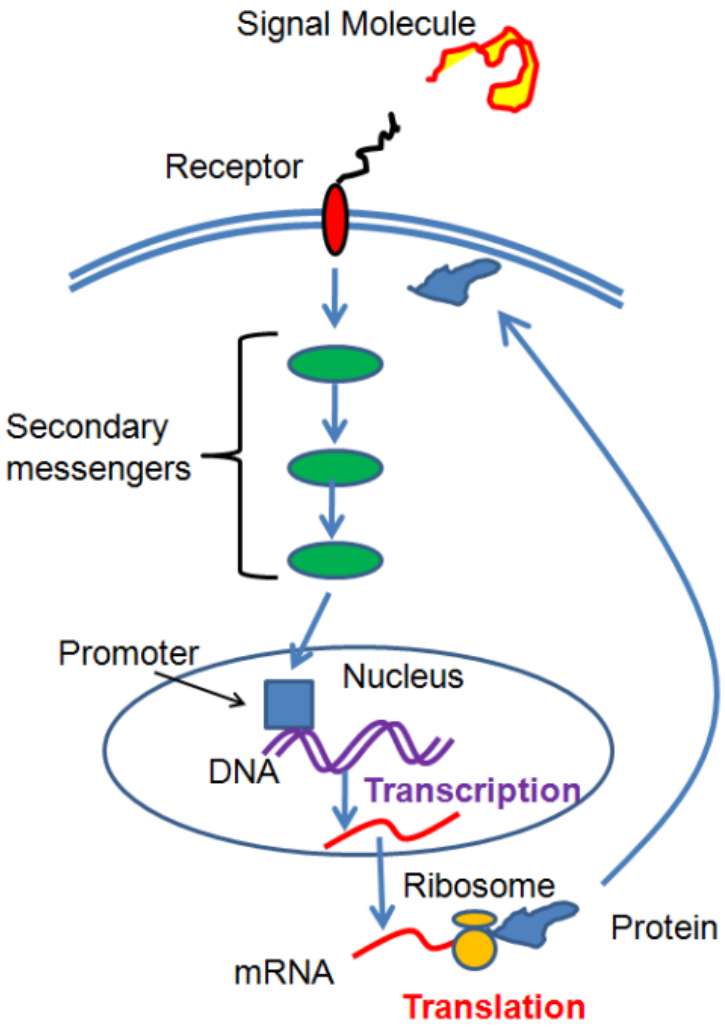
Quantitative Analysis and Visualization of Signaling Networks

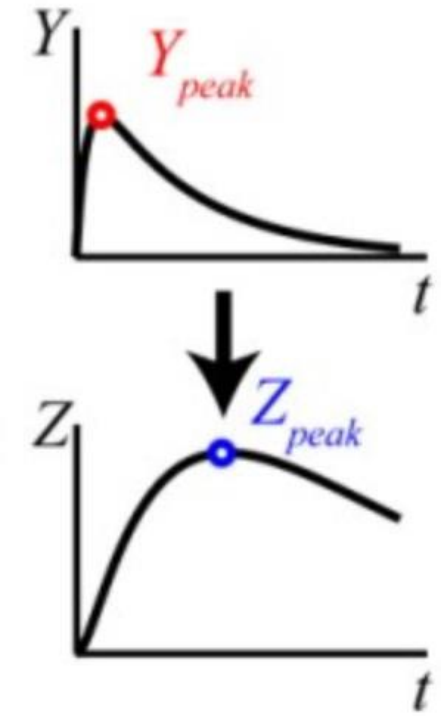
ASCB Workshop

December 14, 2015

1. Dynamics and complexity: Two challenges for modern signal transduction research

Signaling networks regulate cellular processes



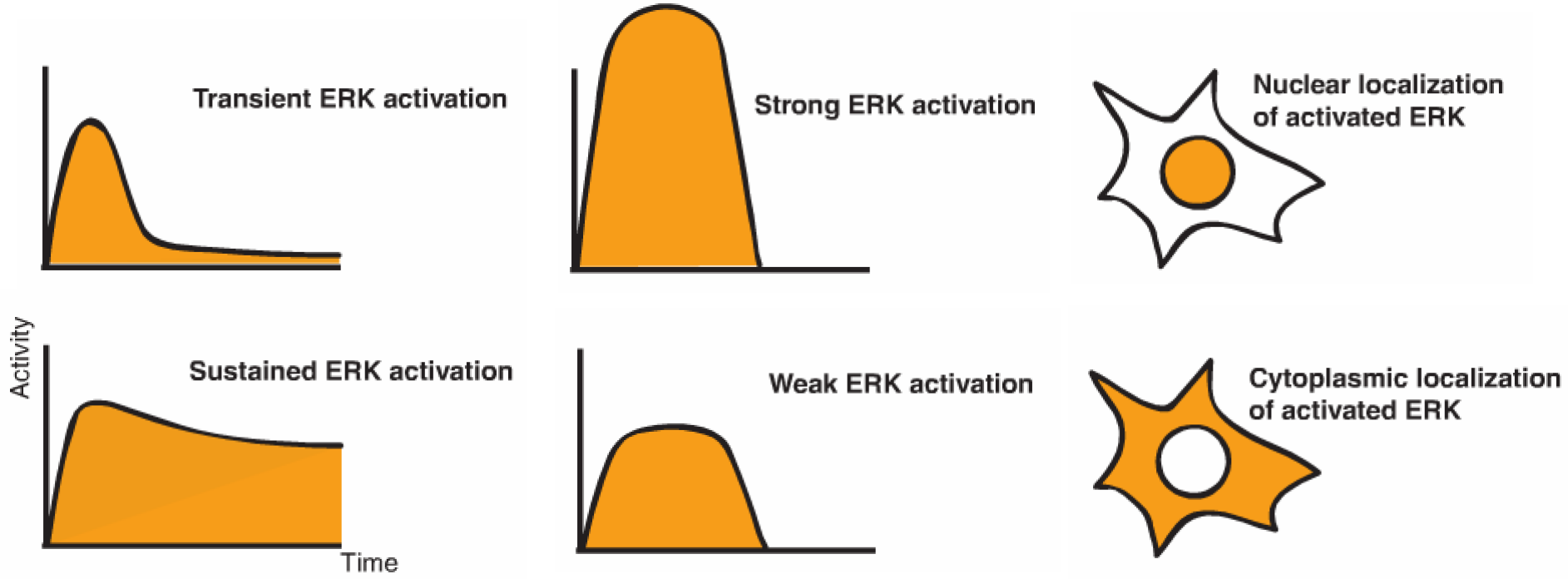


Signal dynamics and localization carry information

Duration

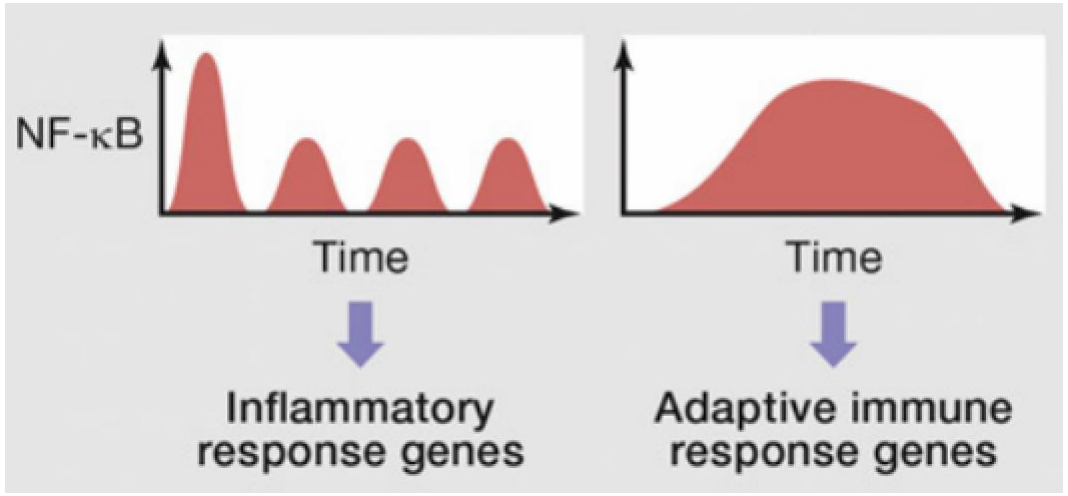
Amplitude

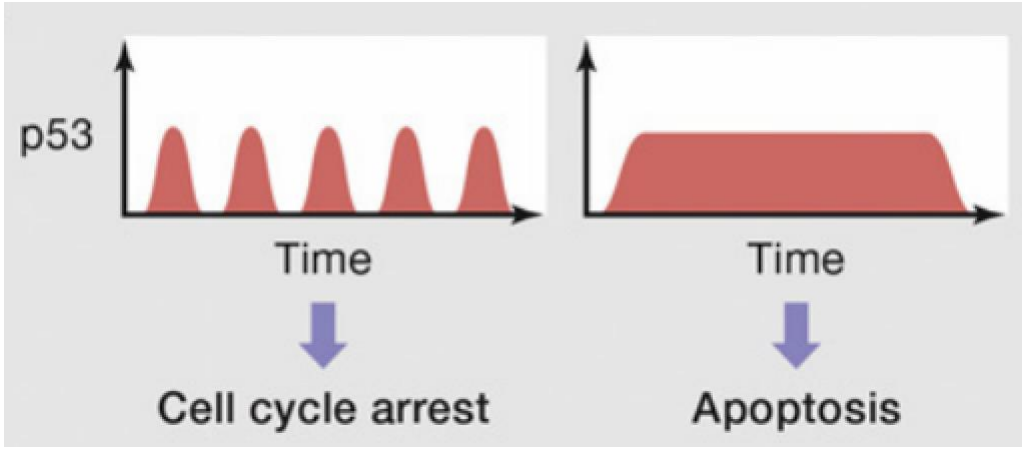
Localization



Ebisuya, Kondoh, and Nishida (2005)

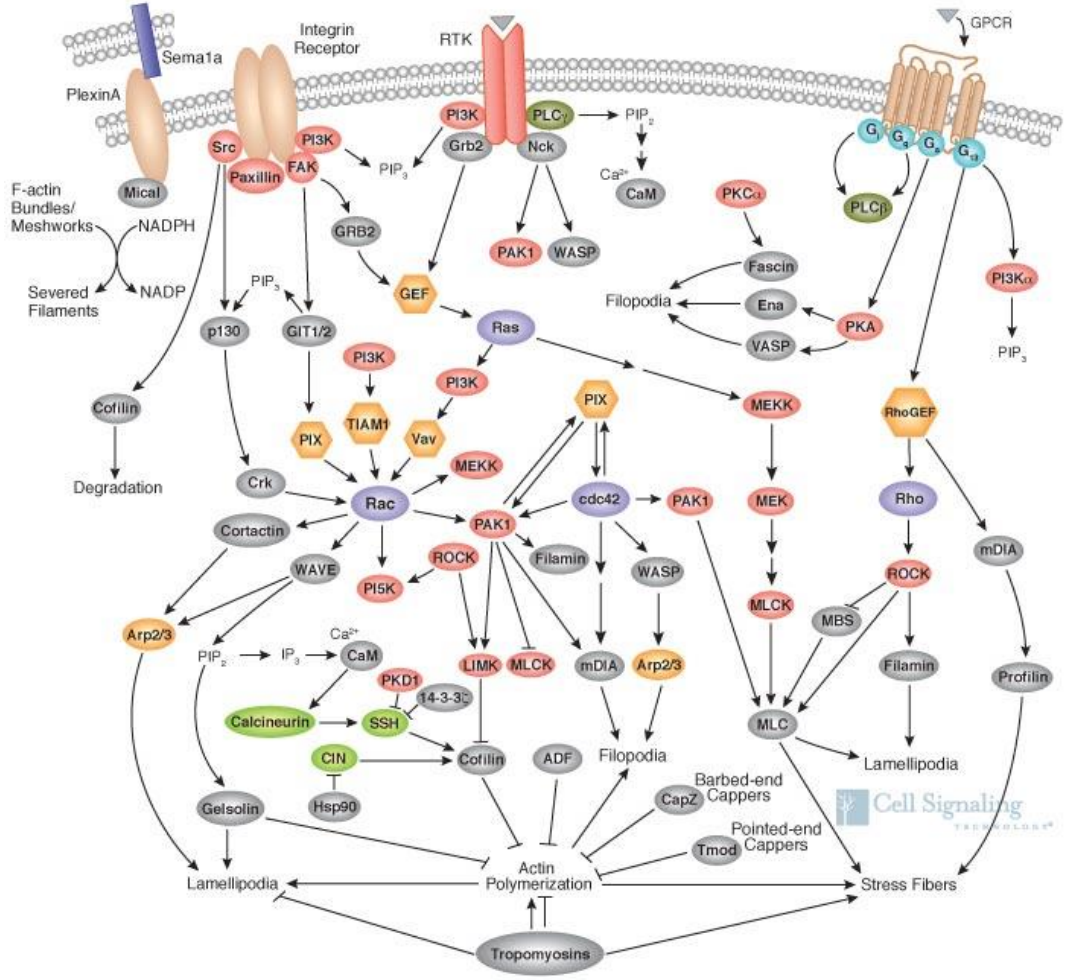
Many signaling pathways oscillate



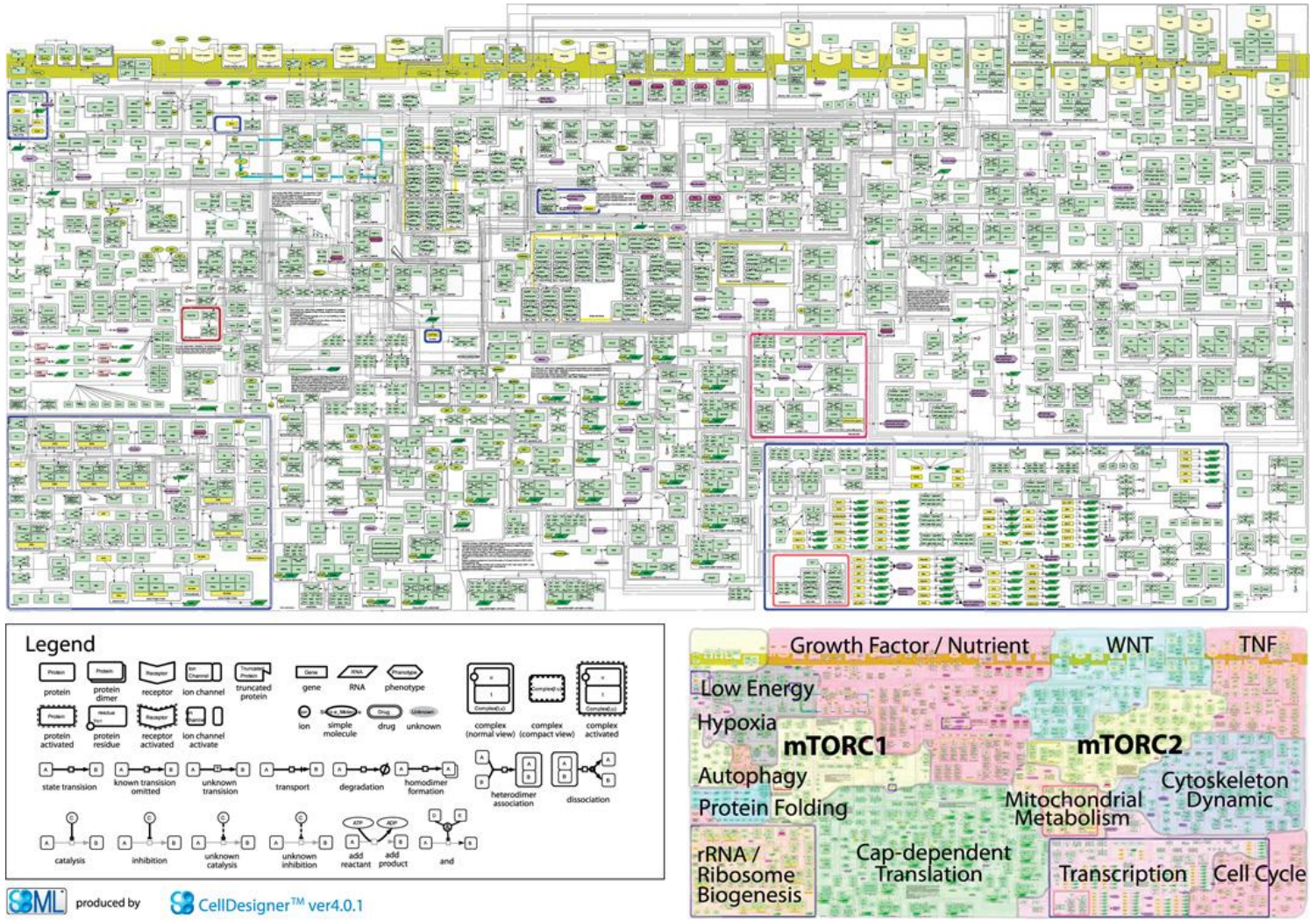


Purvis and Lahav 2013

Challenges in signal transduction: Complexity

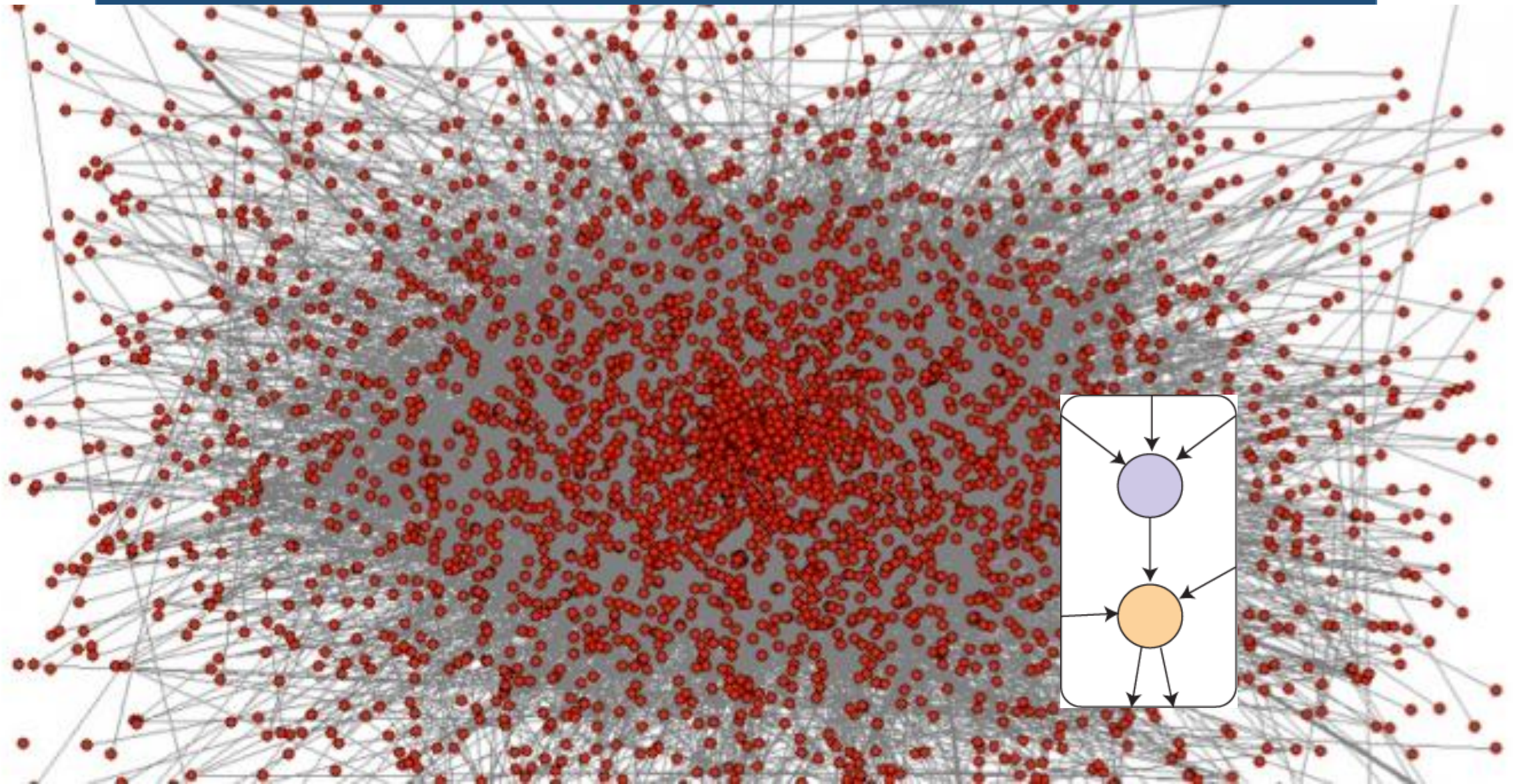


Challenges in signal transduction: Complexity



Caron et al. (2010)

How do we approach the complexity of biological networks?

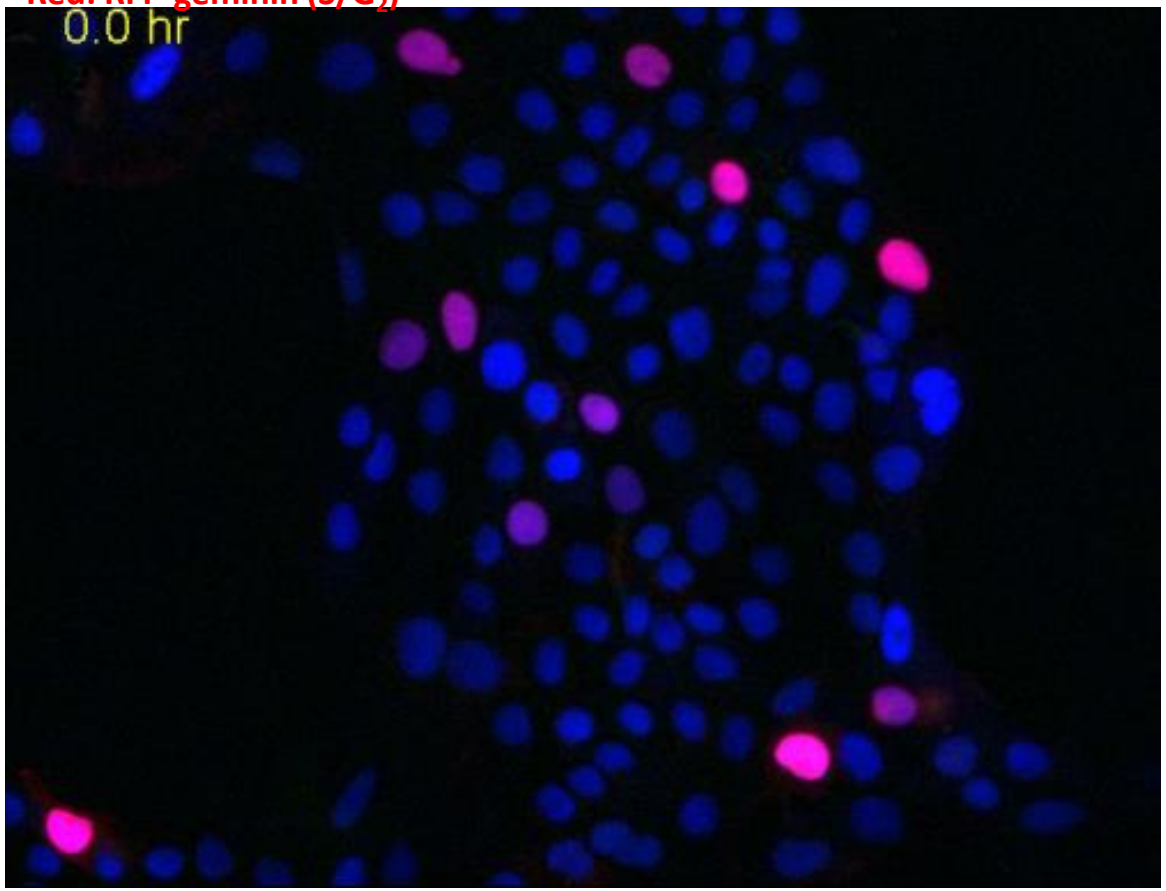


Tracking multiple signaling events with long-term live-cell microscopy

Blue: NLS-CFP (nuclear marker)

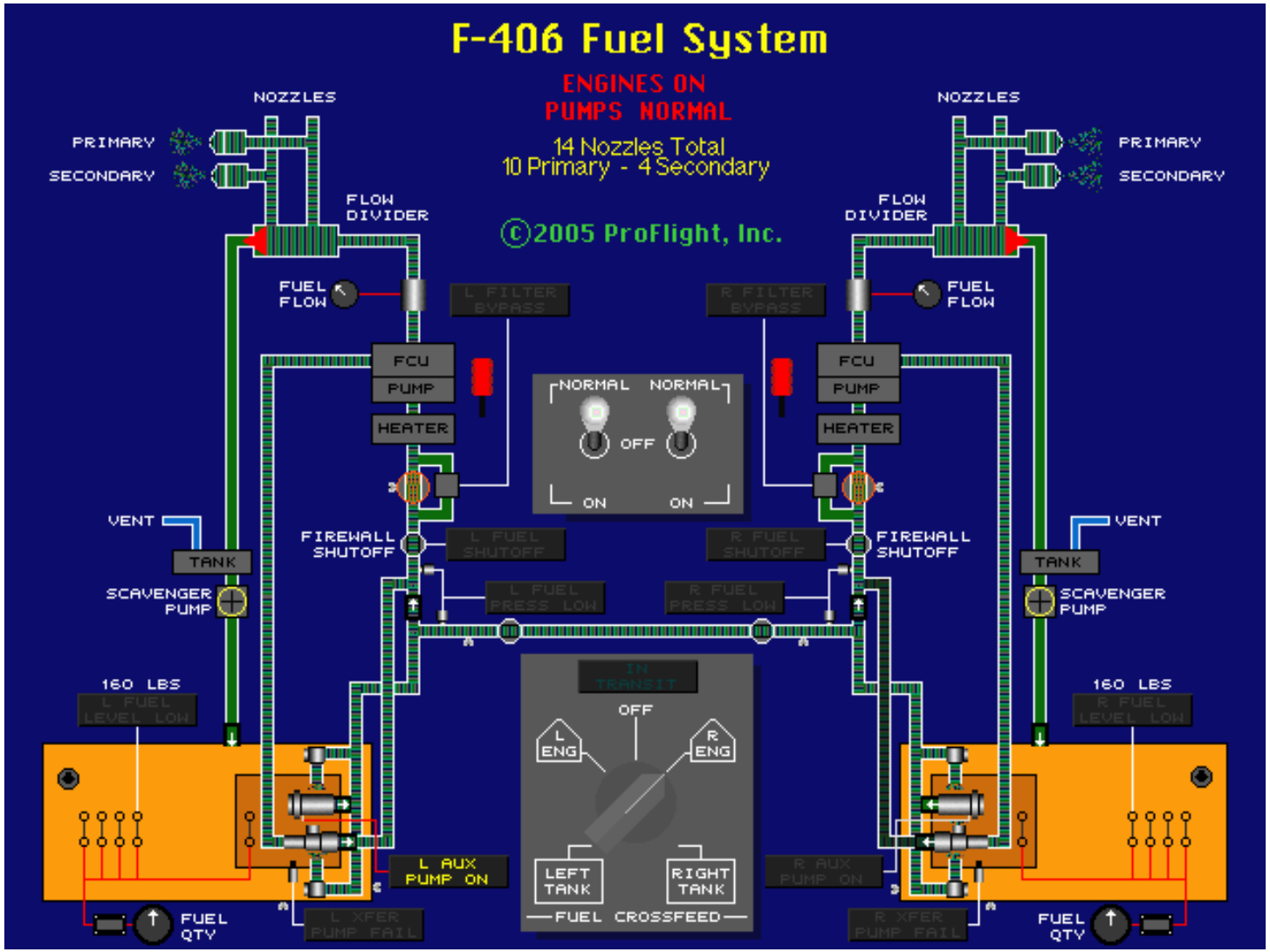
Green: FIRE (ERK)

Red: RFP-geminin (S/G_2)



2. Using tools from the engineering world in signal transduction research

Lessons from other complex systems



Engineering fields that deal with similar challenges

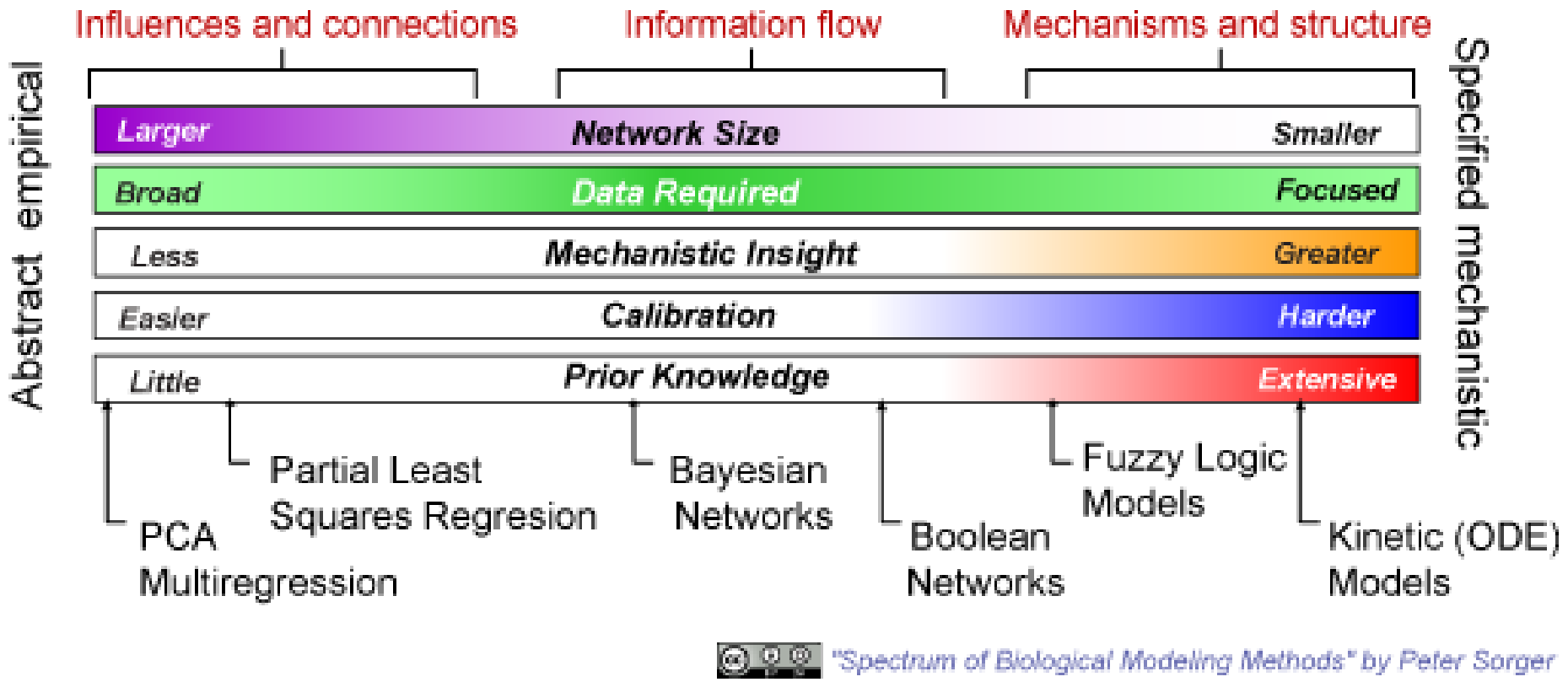
Differential equation modeling

Dynamical systems

Control theory

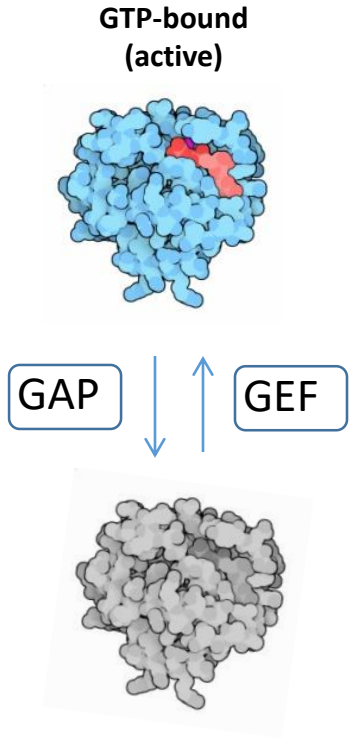
Signal Processing

Computational models can take many different forms



Differential equation models of biochemical dynamics

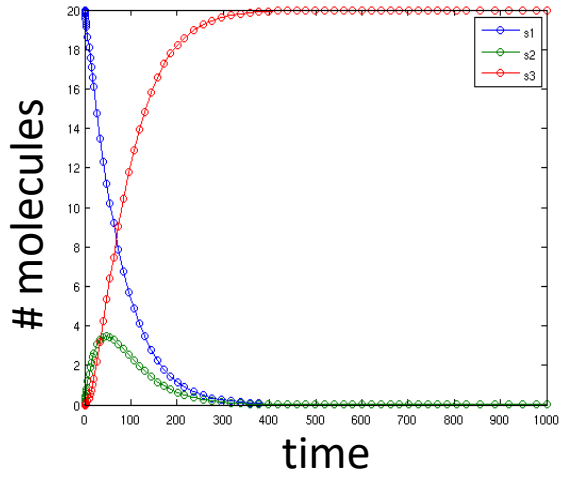
"Figure 7"



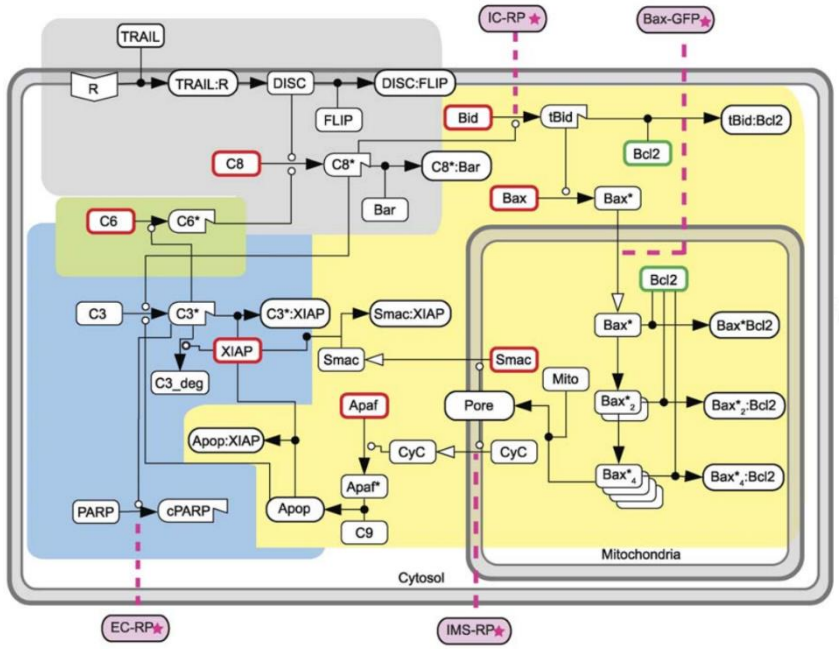
$$\text{Ras-GDP} + \text{GEF} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{Ras-GDP:GEF} \xrightarrow{k_3} \text{Ras-GTP} + \text{GEF}$$

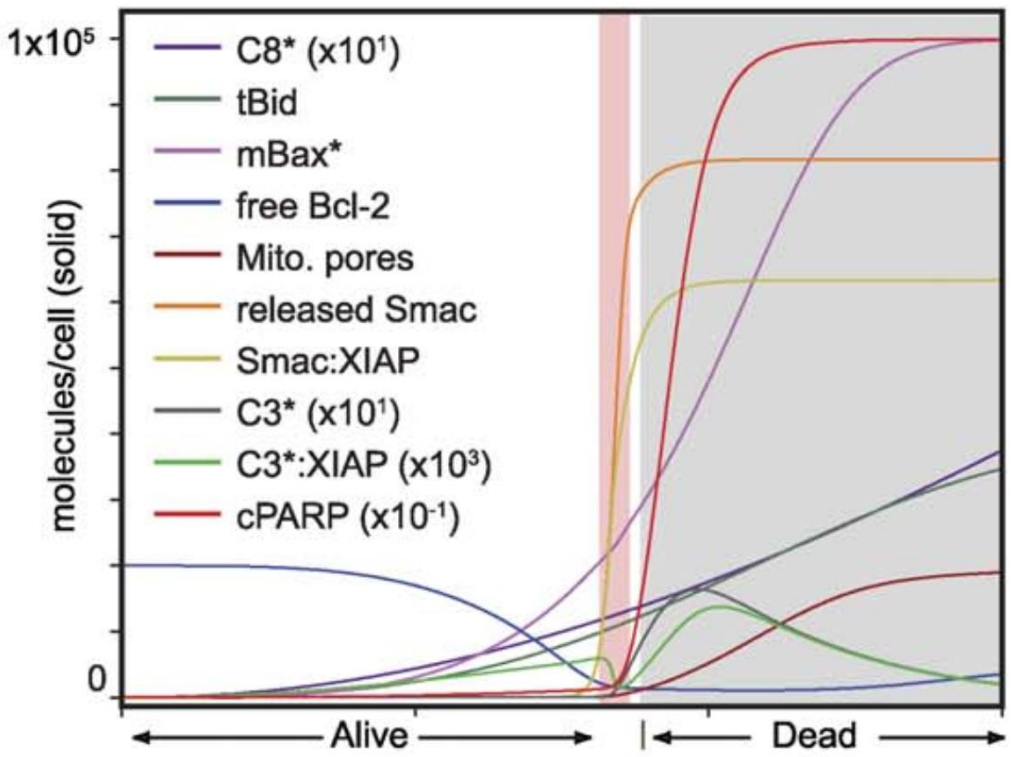
$$\text{Ras-GTP} + \text{GAP} \underset{k_5}{\overset{k_4}{\rightleftharpoons}} \text{Ras-GTP:GAP} \xrightarrow{k_6} \text{Ras-GDP} + \text{GAP}$$

$$\frac{d[\text{Ras-GTP}]}{dt} = k_3[\text{Ras-GDP:GEF}] - k_4[\text{Ras-GTP}][\text{GAP}]$$

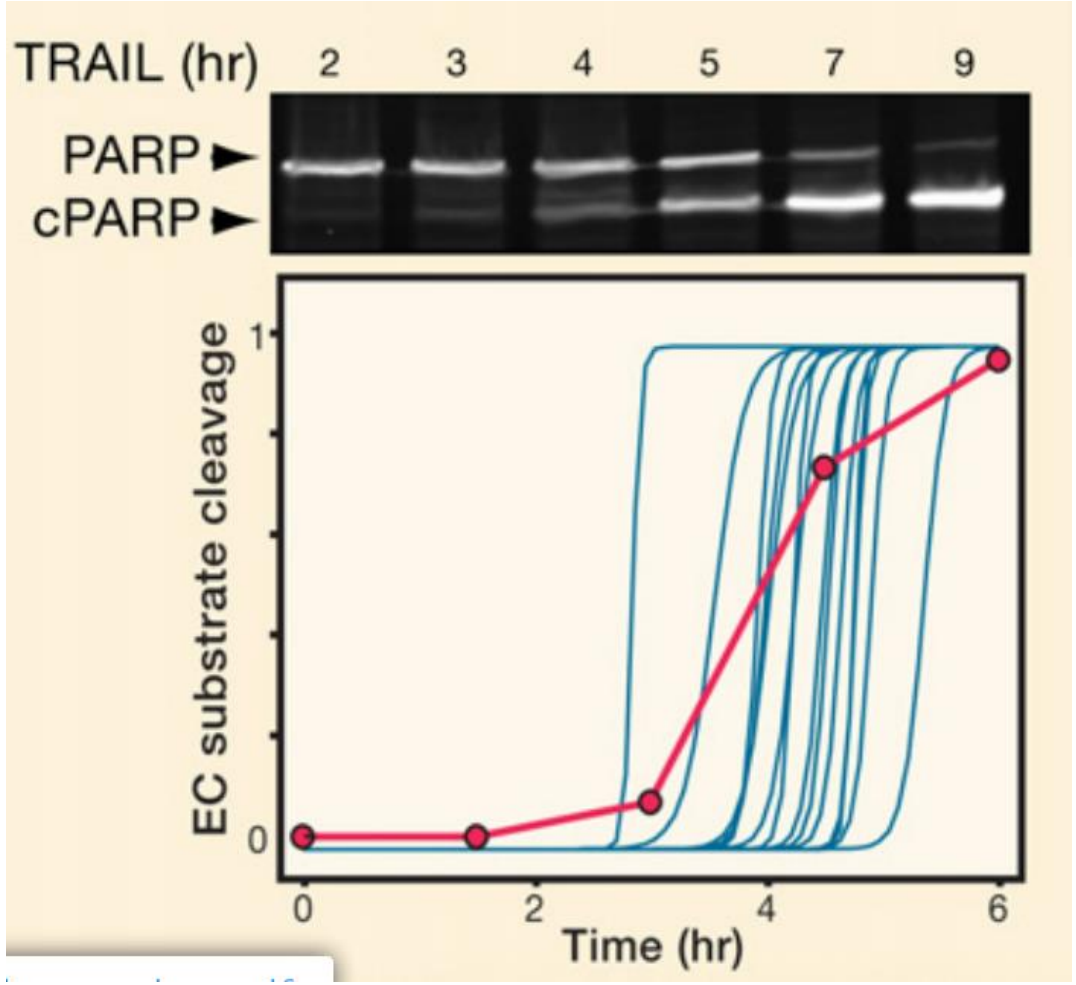


Differential equation models can recapitulate complex biological processes

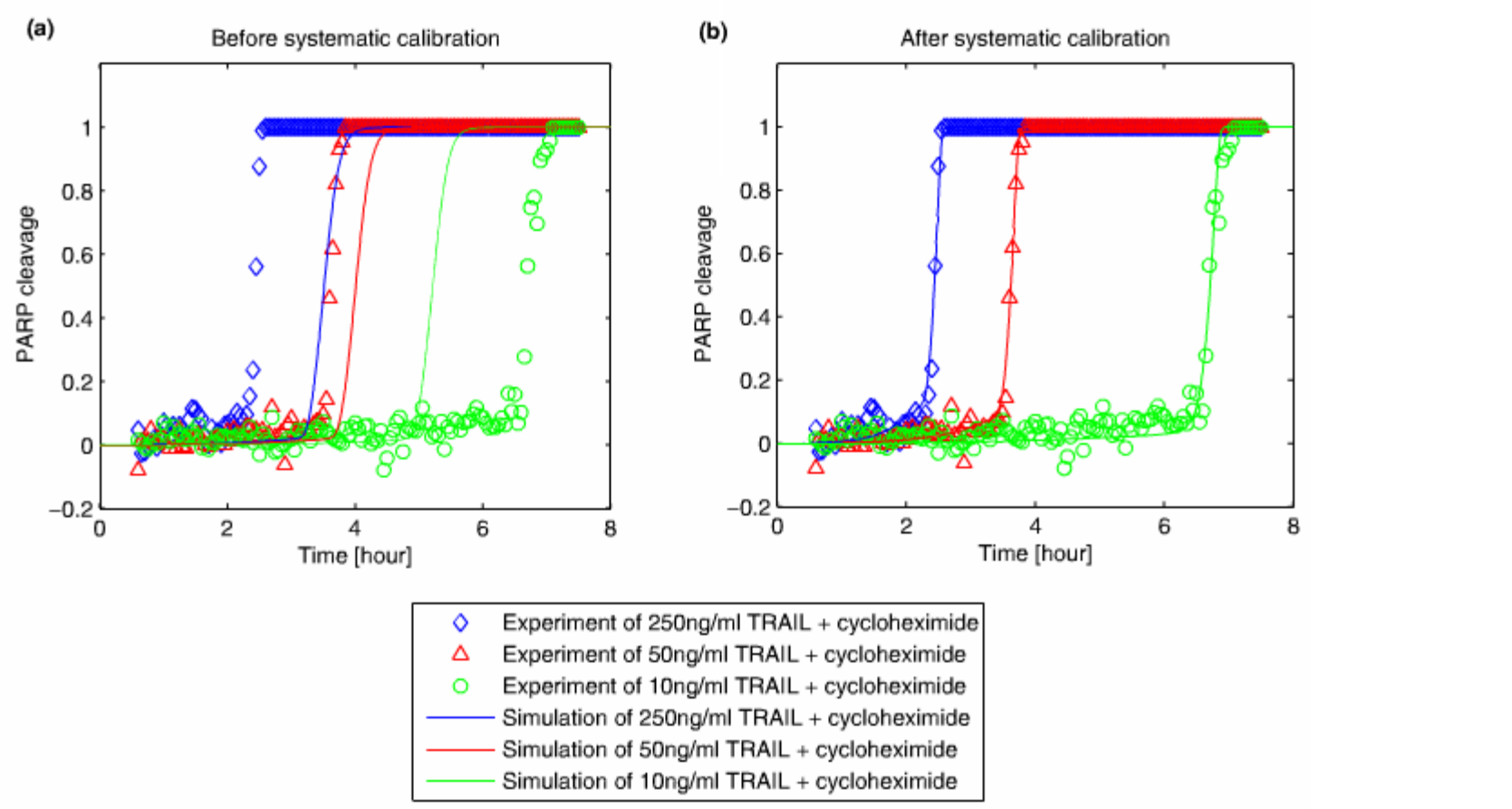




Modeling the right thing: population average data can misrepresent kinetics



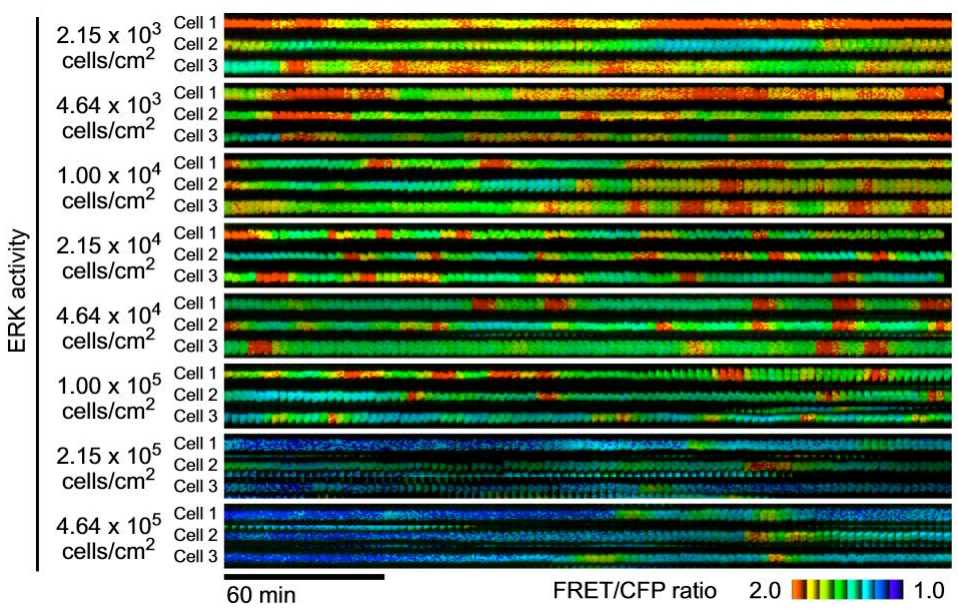
Using single-cell data to fit models



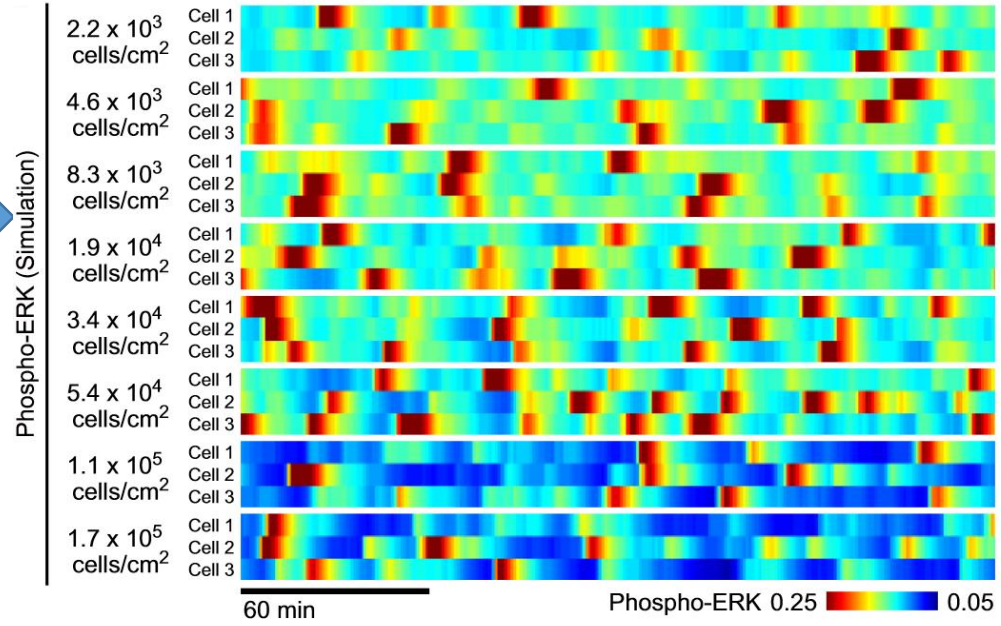
Kim et al., 2010

Using single-cell data to fit models

Population of genetically identical cells

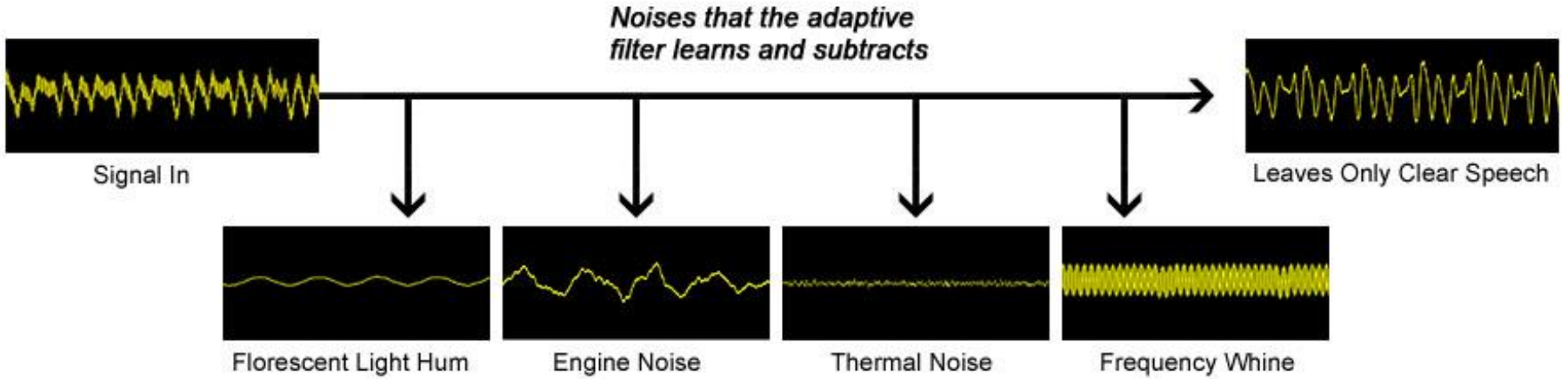


Population of models with varying parameters



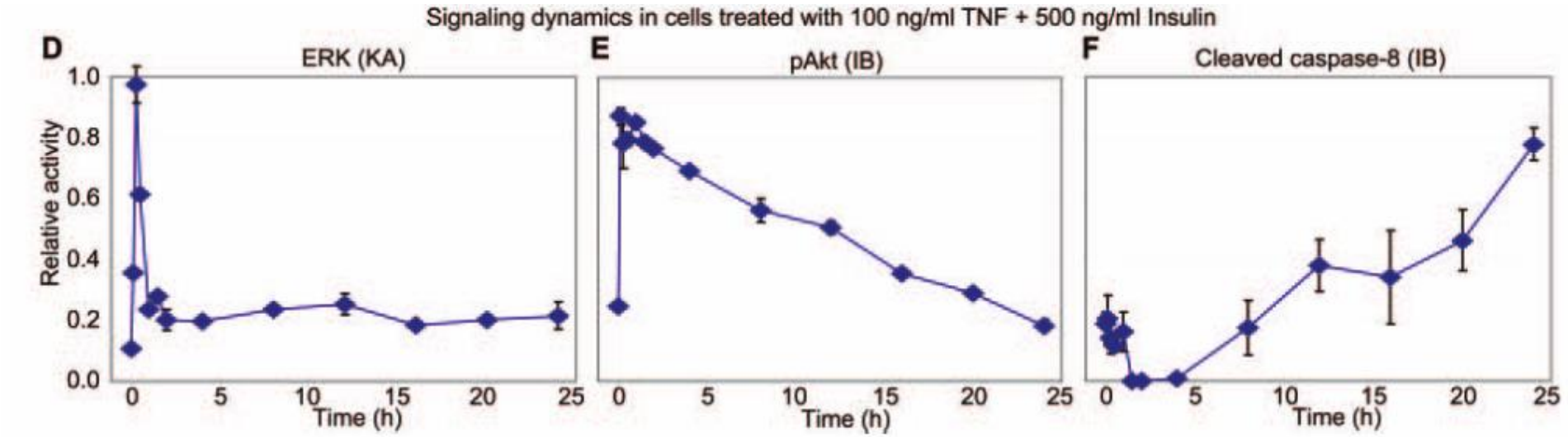
Aoki et al., 2013

Signal processing: methods for separating meaningful signals from “noise”



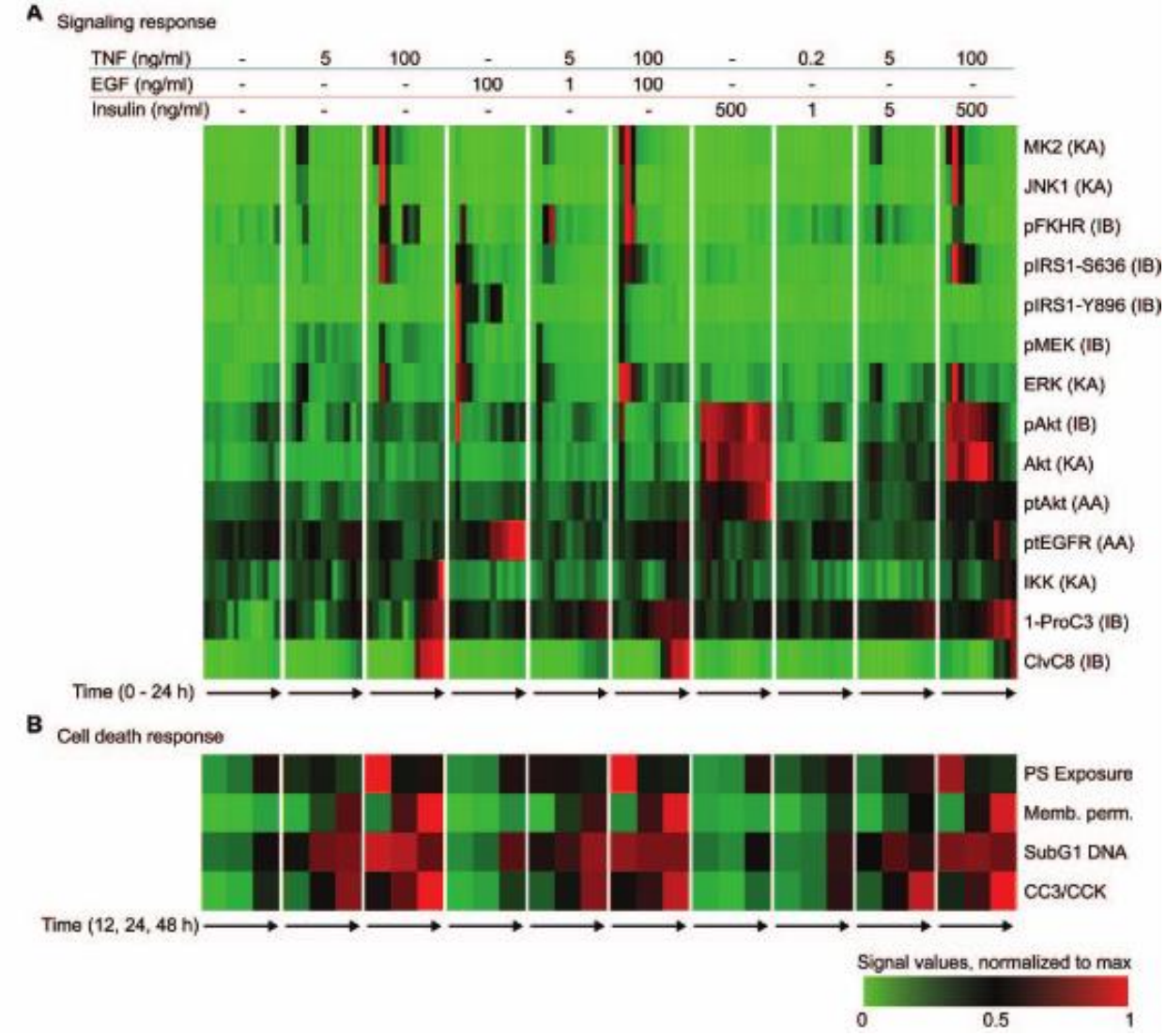
3. Strategies for collecting time-series data

Collecting a multidimensional signaling dataset



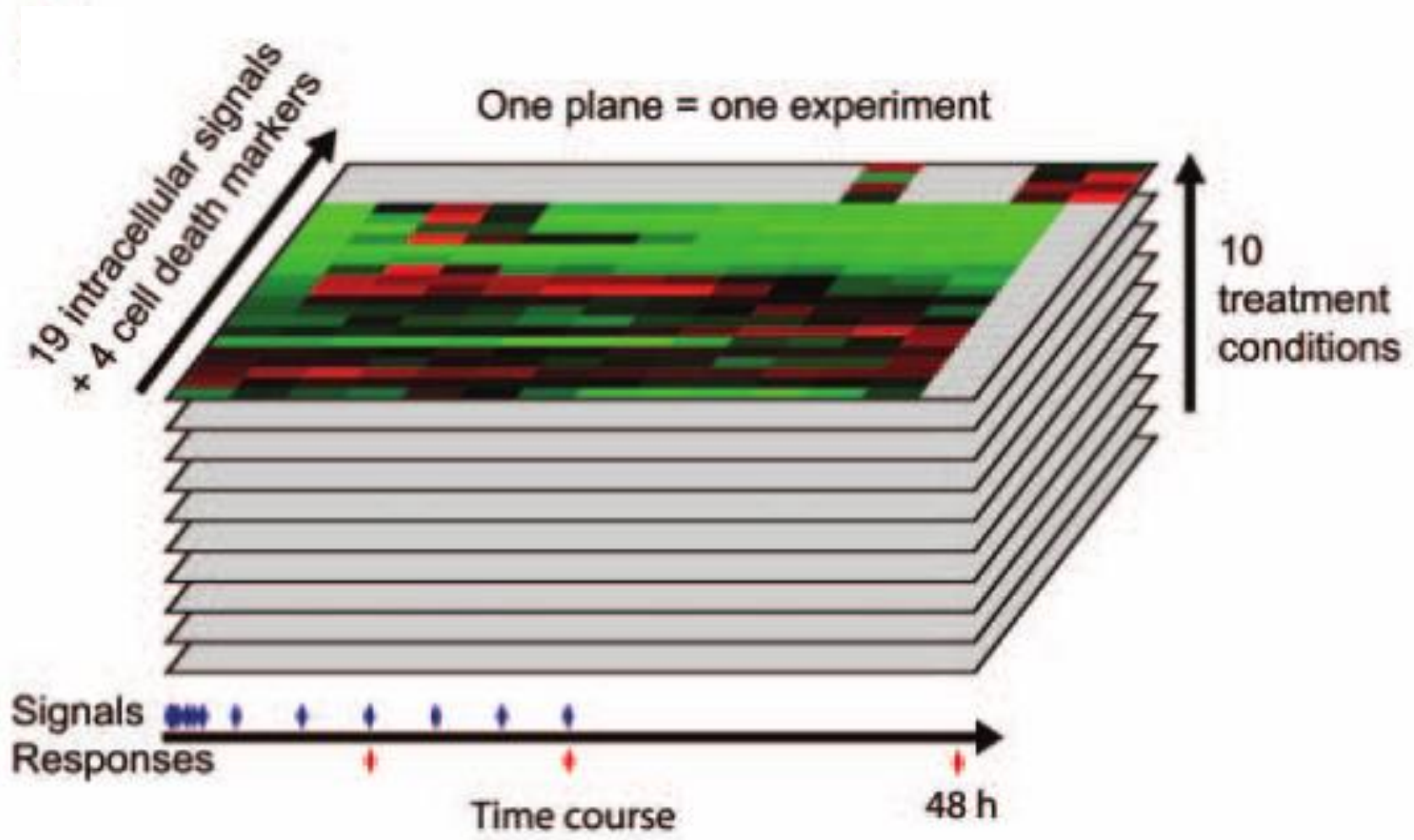
Gaudet et al. 2005

Collecting a multidimensional signaling dataset



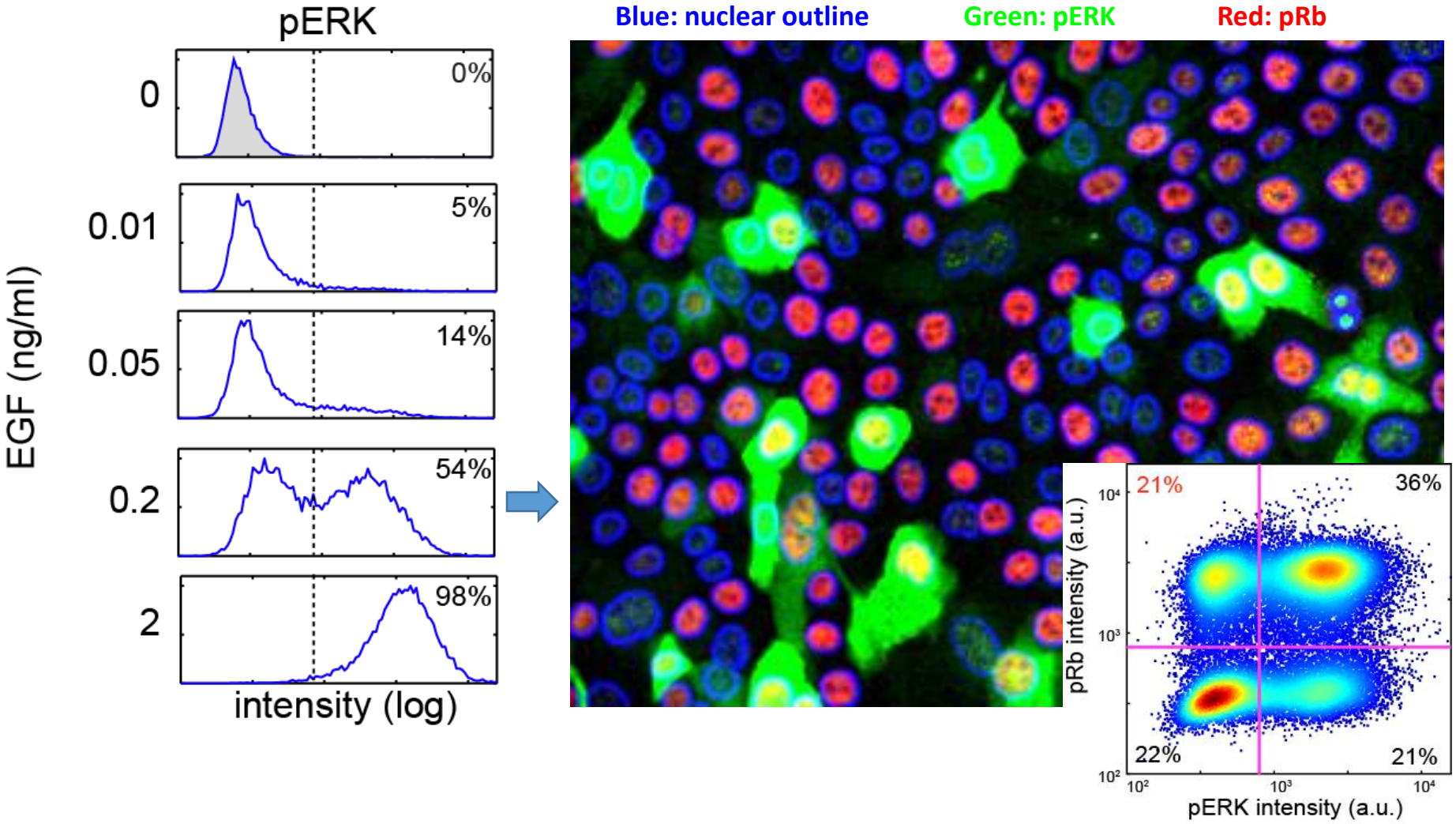
Gaudet et al. 2005

Collecting a multidimensional signaling dataset

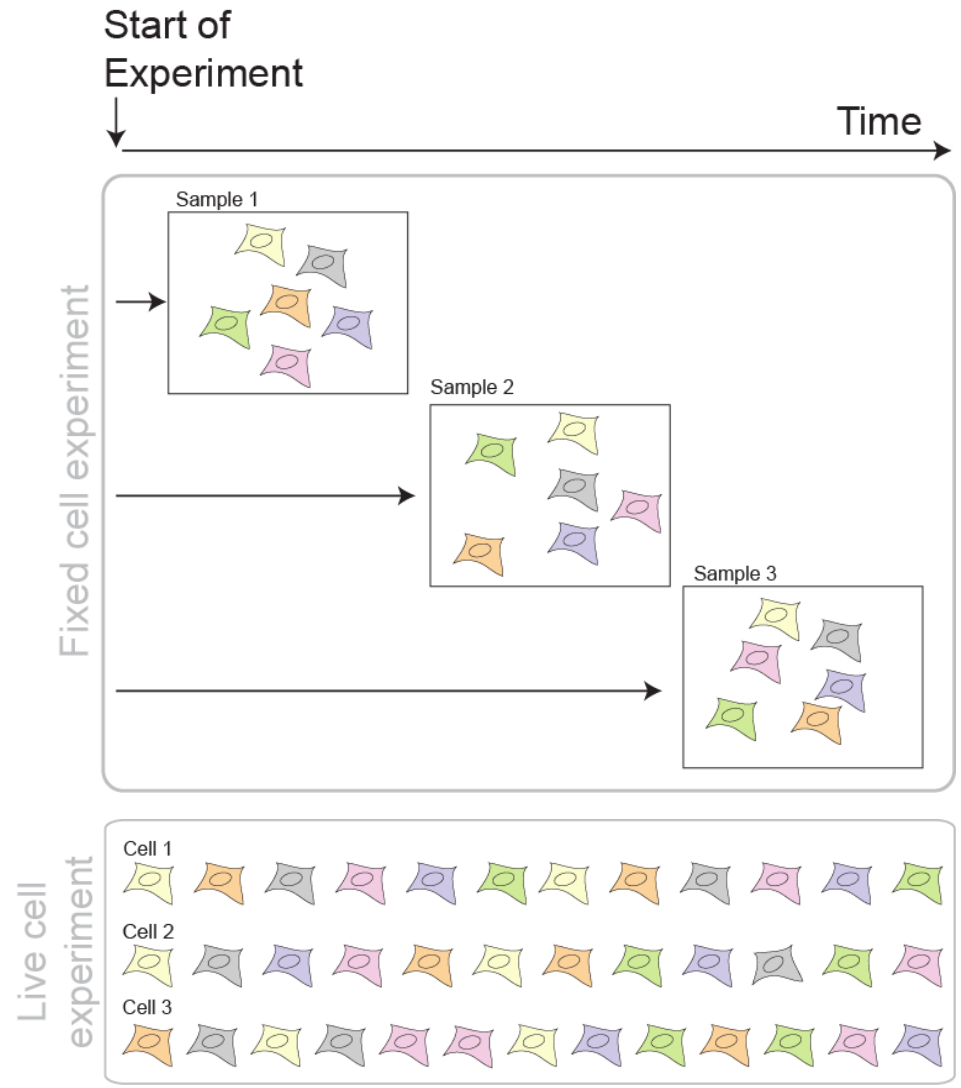


Gaudet et al. 2005

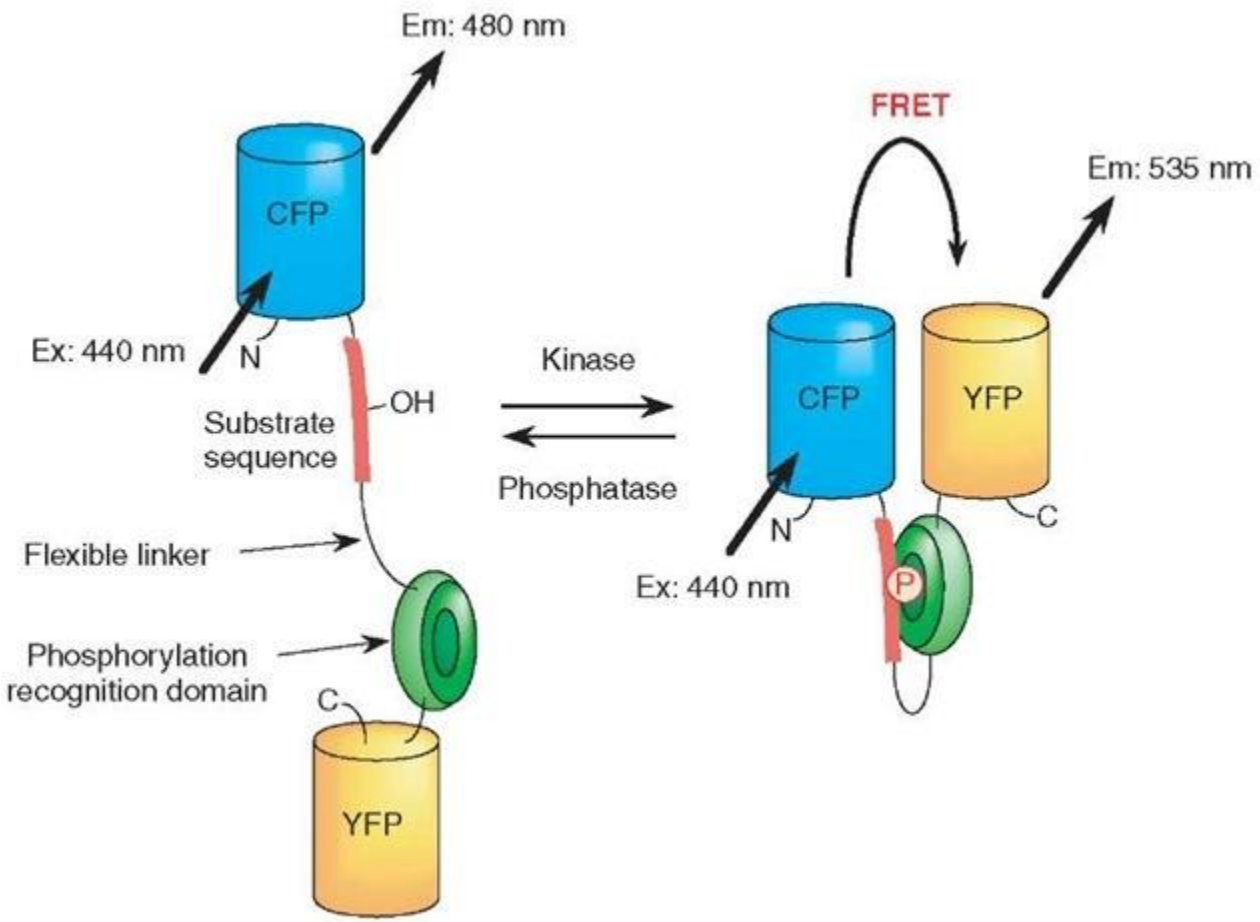
Single-cell imaging shows that signaling pathways rarely follow simple patterns



Destructive vs. real-time experiments



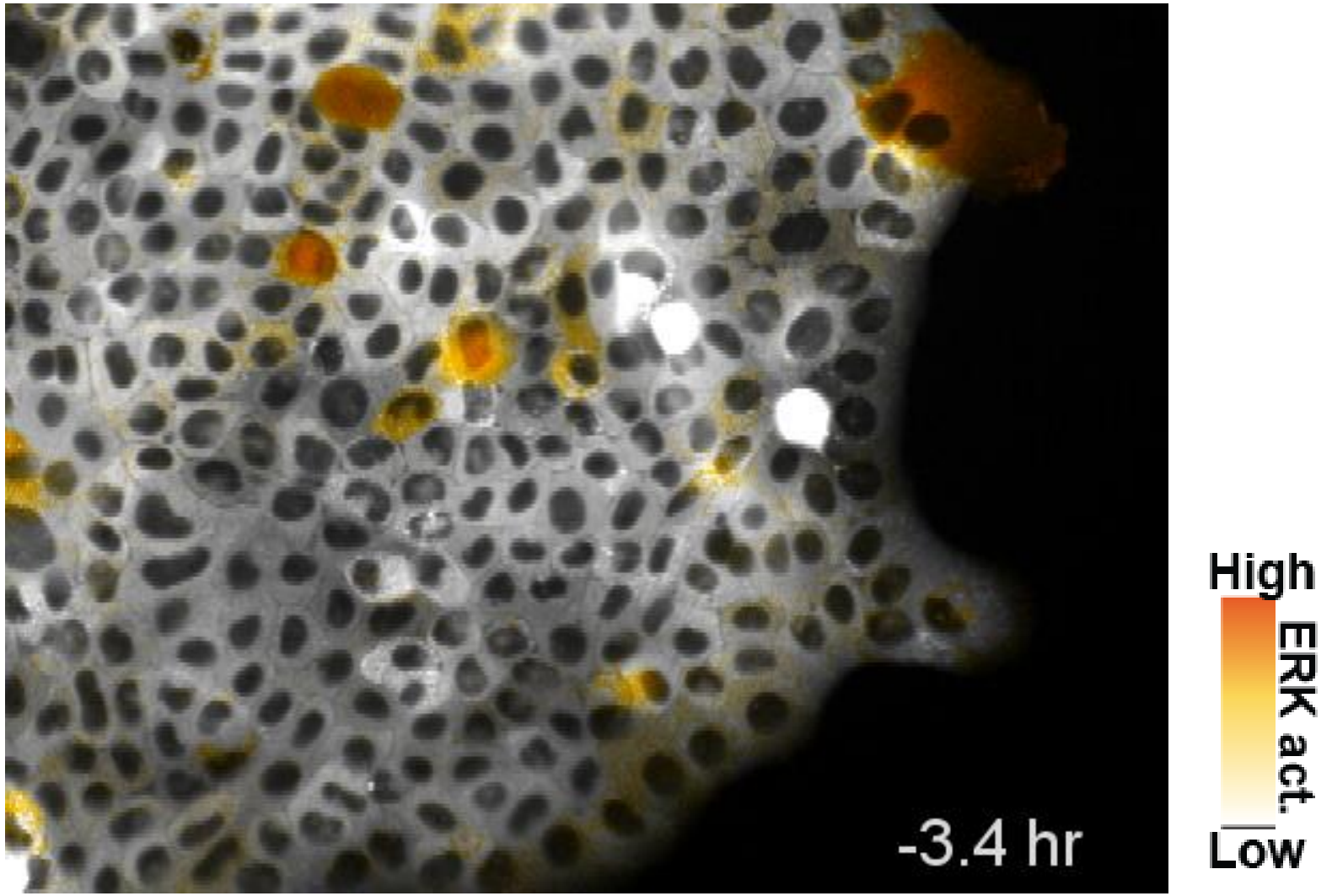
FRET-based kinase reporters



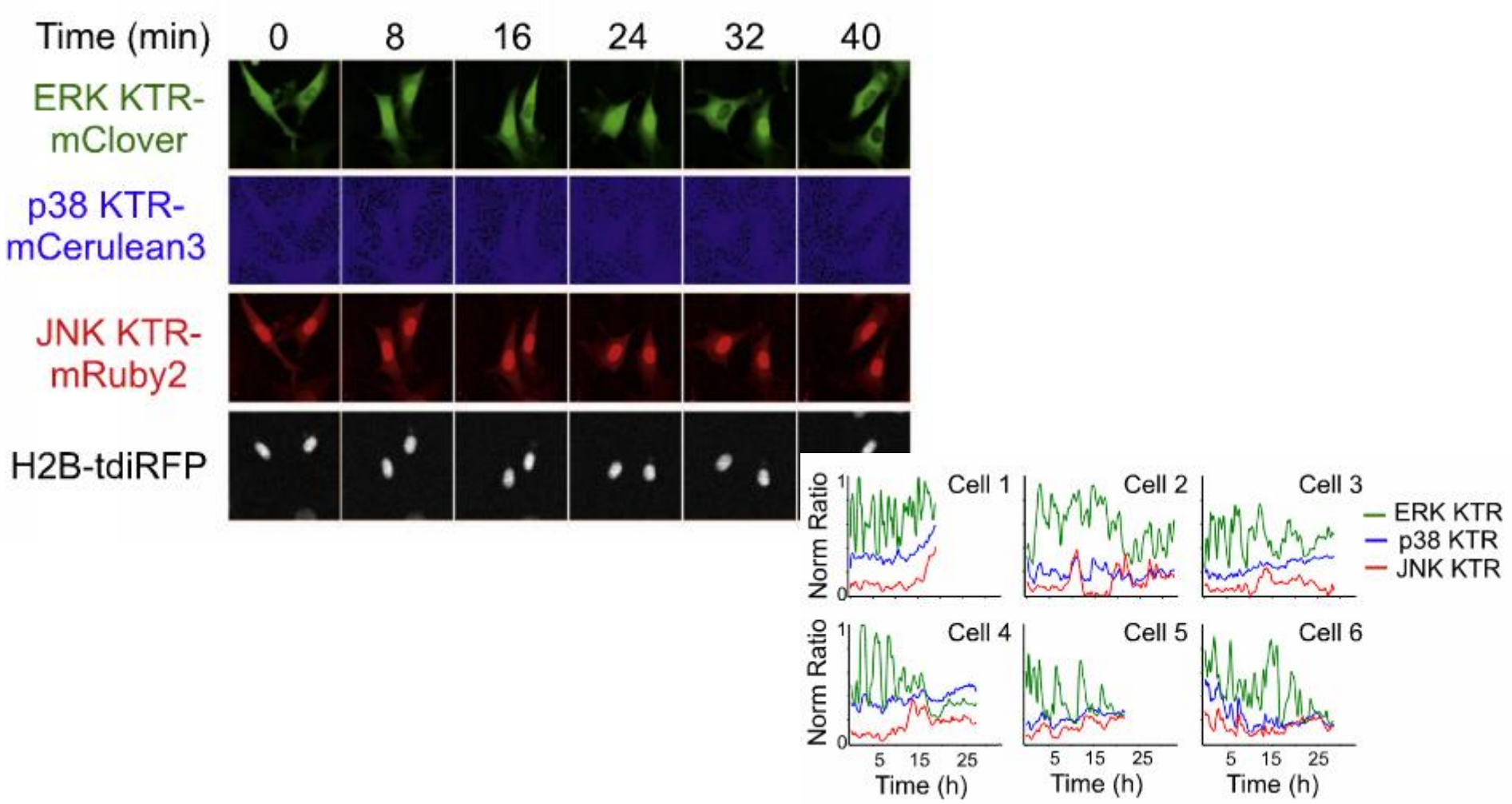
Harvey et al. (2008), Komatsu et al. (2011)

Dynamics of ERK activity in response to EGF

“Physiological” EGF (0.1 ng/ml)

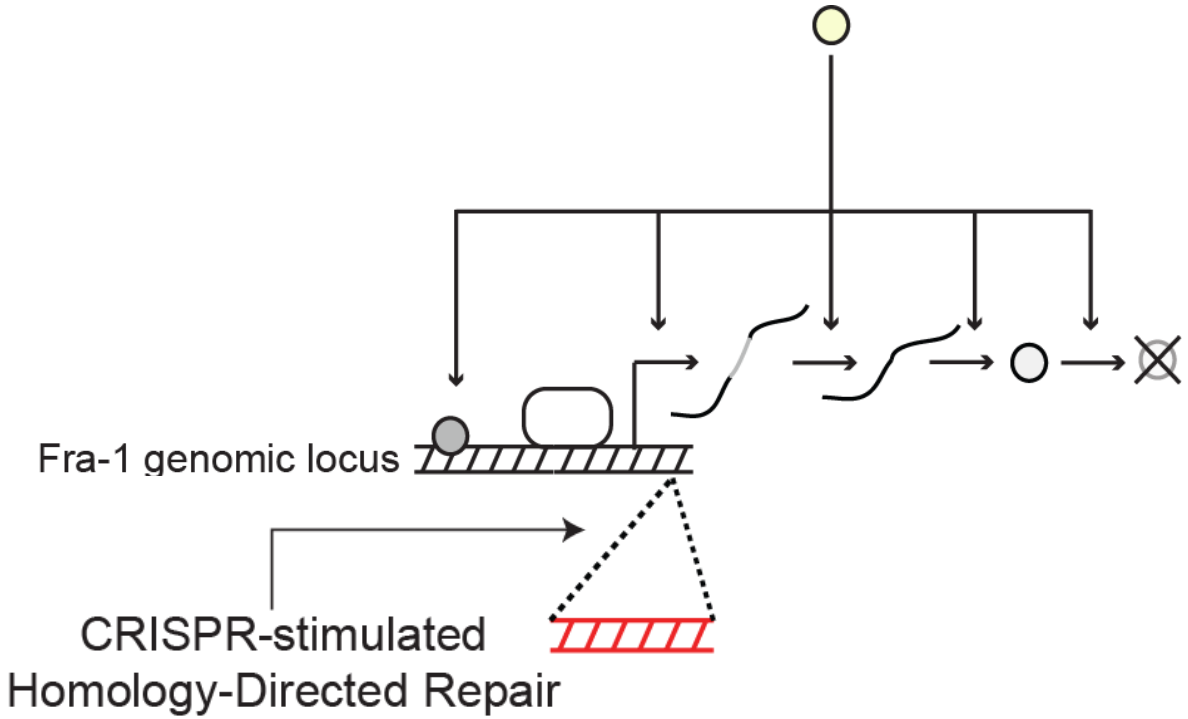


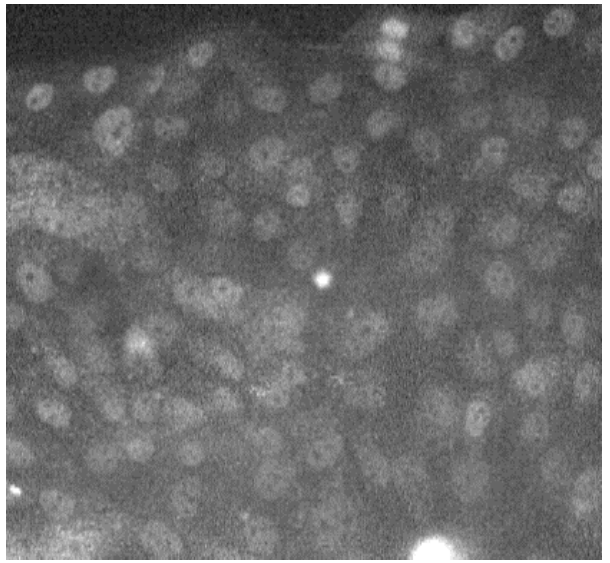
Multiplexing reporters to make network measurements



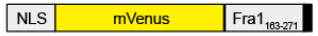
Sergi Regot, Markus Covert

An integrated genomic reporter for Fra-1





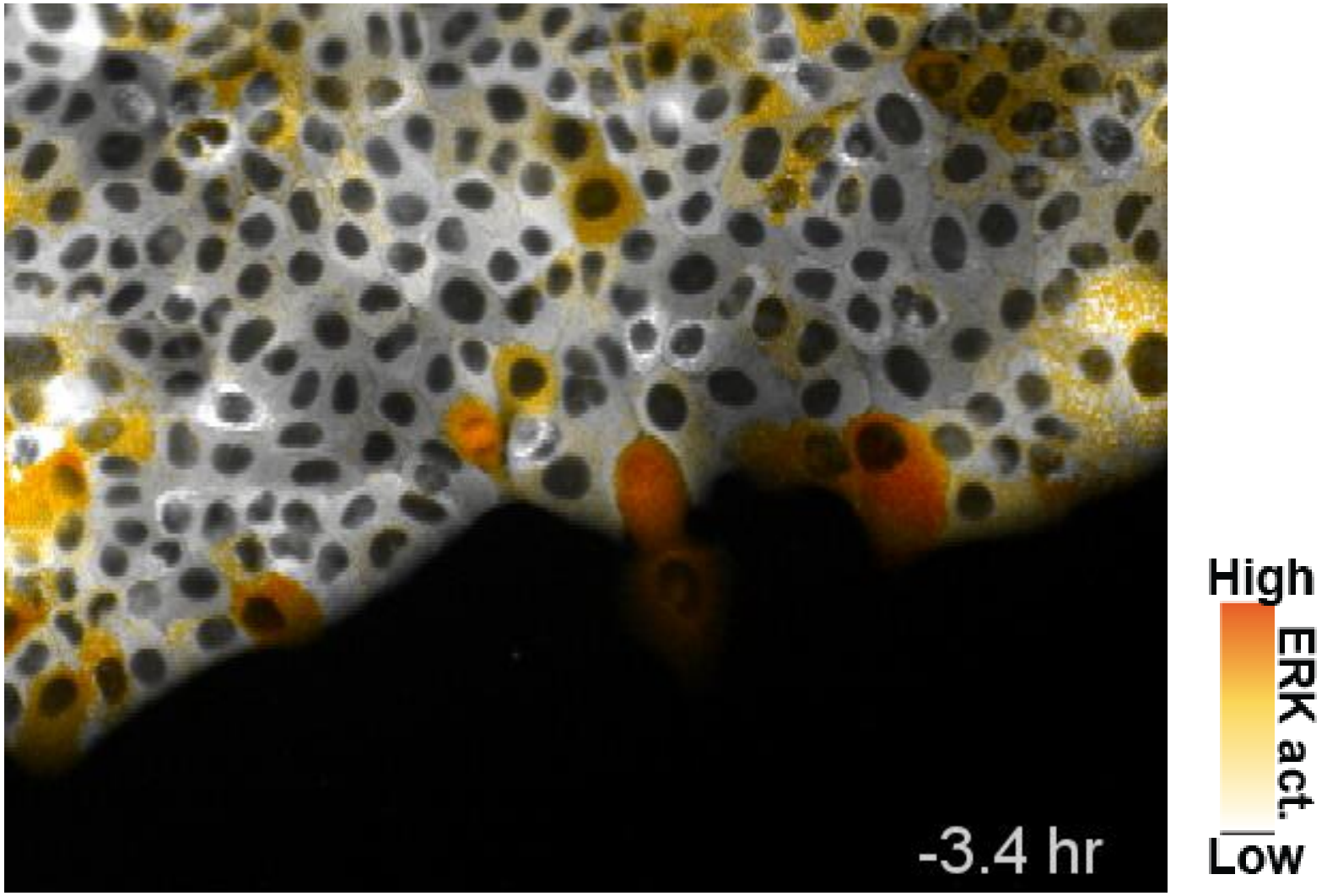
Reporters to track signaling events in long-term live-cell microscopy

Reporter	Target	Modality	Structure
Fucci[1]	S/G_2 phase	INT	
H2B-GFP[2]	M phase	LOC	
d2-CFP[3]	Translation	INT	
IMS-RP[4]	Apoptosis (MOMP)	LOC	
EC-RP, IC-RP[4]	Caspase activities	FRET	
EKAR[5] ,AMPKAR[6],...	Kinase activities	FRET	
FP-FOXO	Akt/SGK activity	LOC	
FIRE	ERK/RSK activity	INT	
FP-Myc	Myc levels	INT	
Perceval[7]	ATP	INT	
Peredox[8]	NADH/NAD^+	INT	
miR-X	miRNA levels	INT	

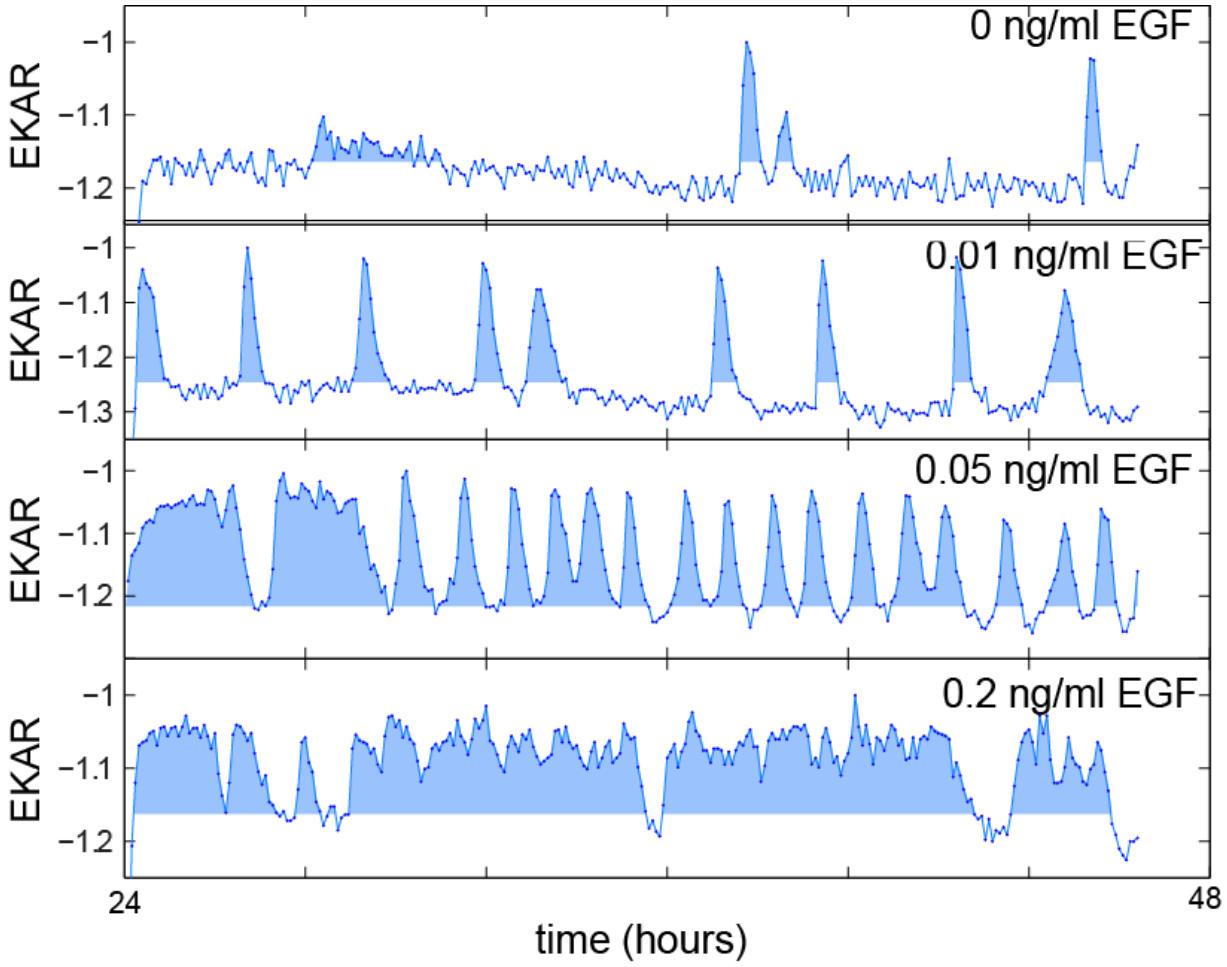
4. Answering questions with large time-series datasets

Dynamics of ERK activity in response to EGF

High EGF (20 ng/ml)

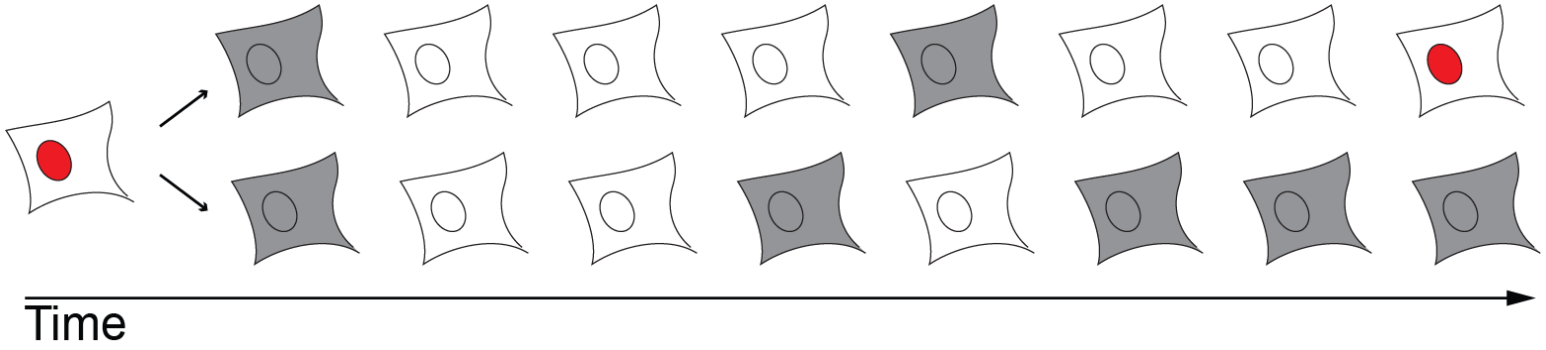


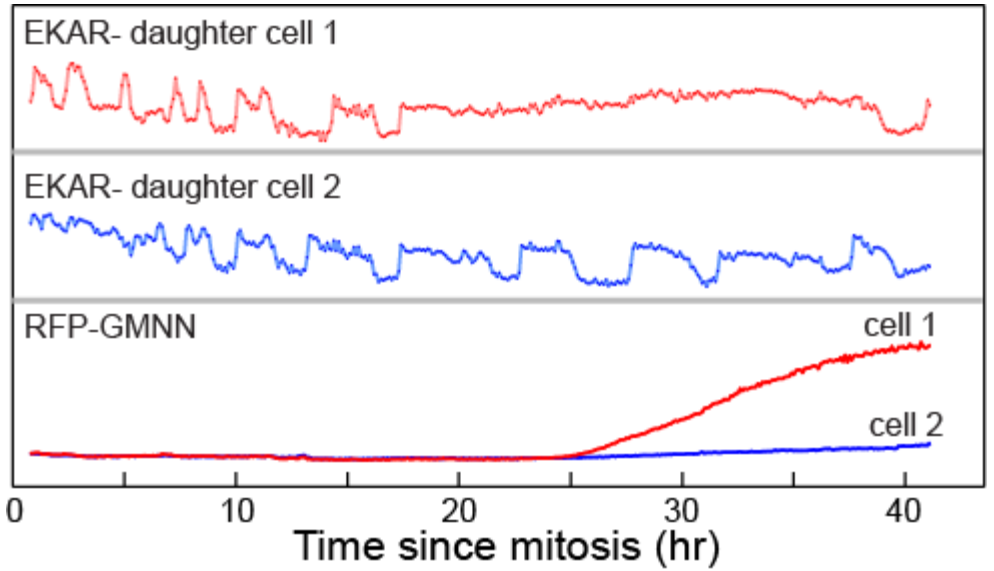
Frequency-modulated ERK pulses



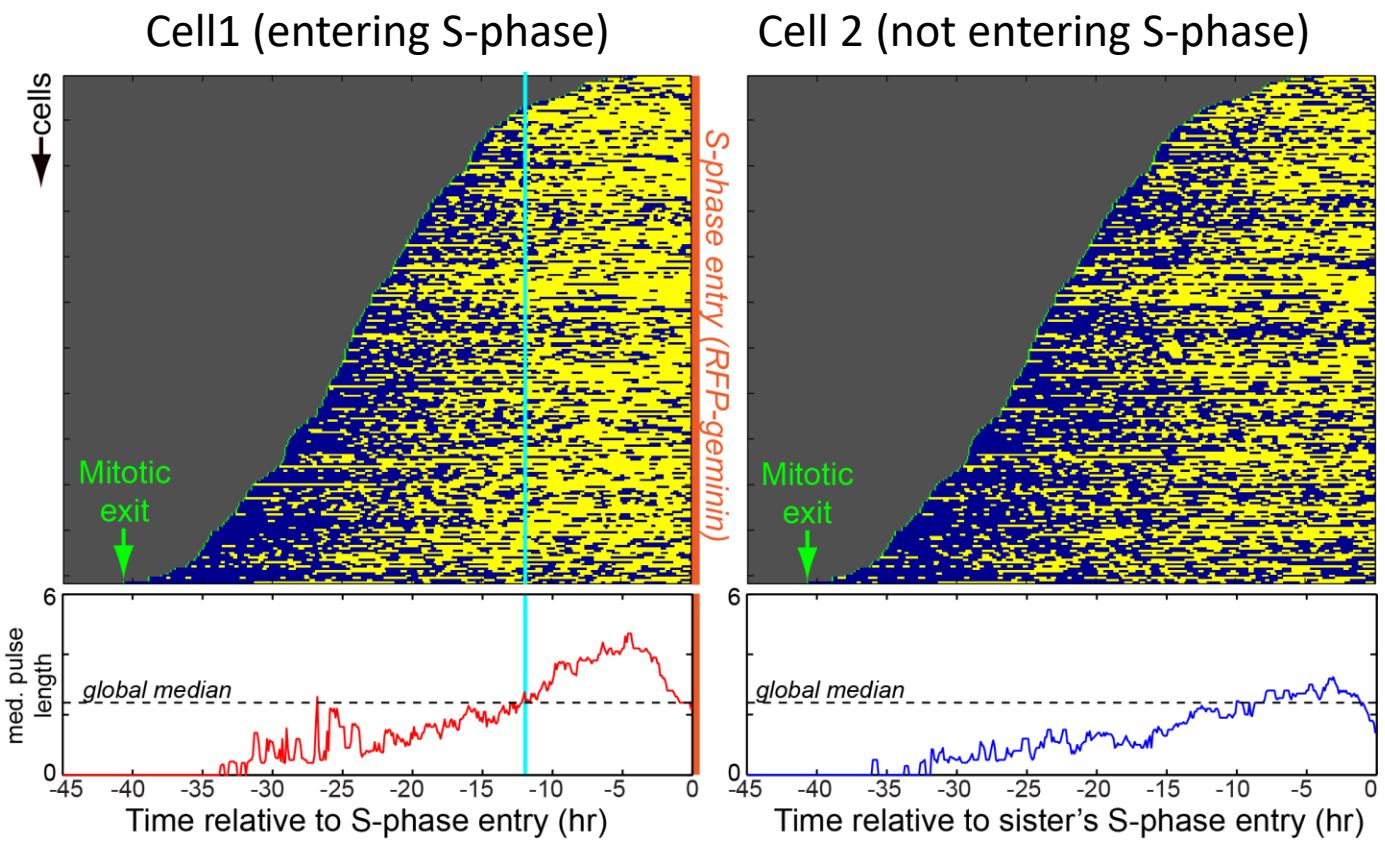
Albeck et al., 2013

Correlating patterns of ERK activity with commitment to S-phase

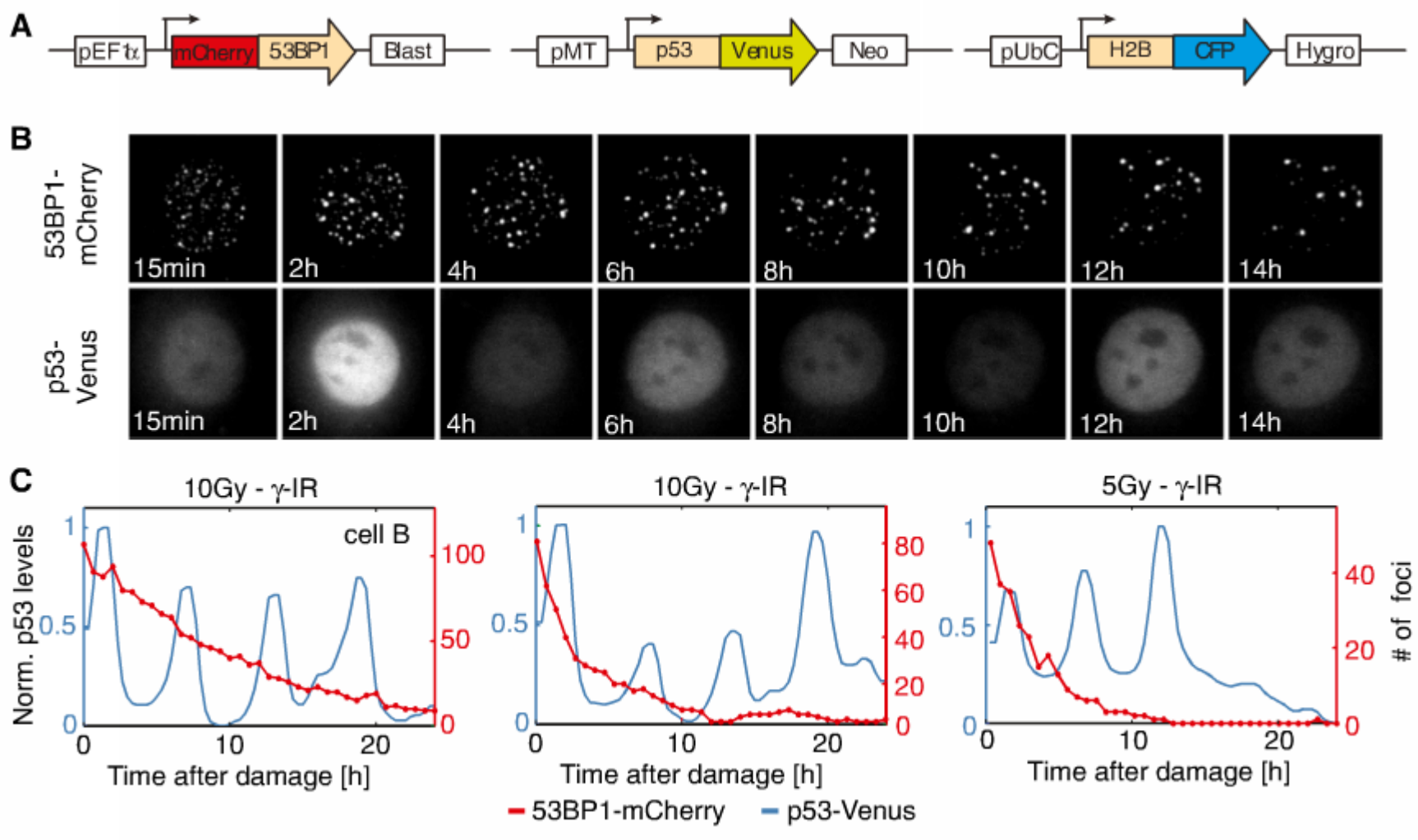




ERK activity increases sharply in the 12 hours preceding S-phase entry

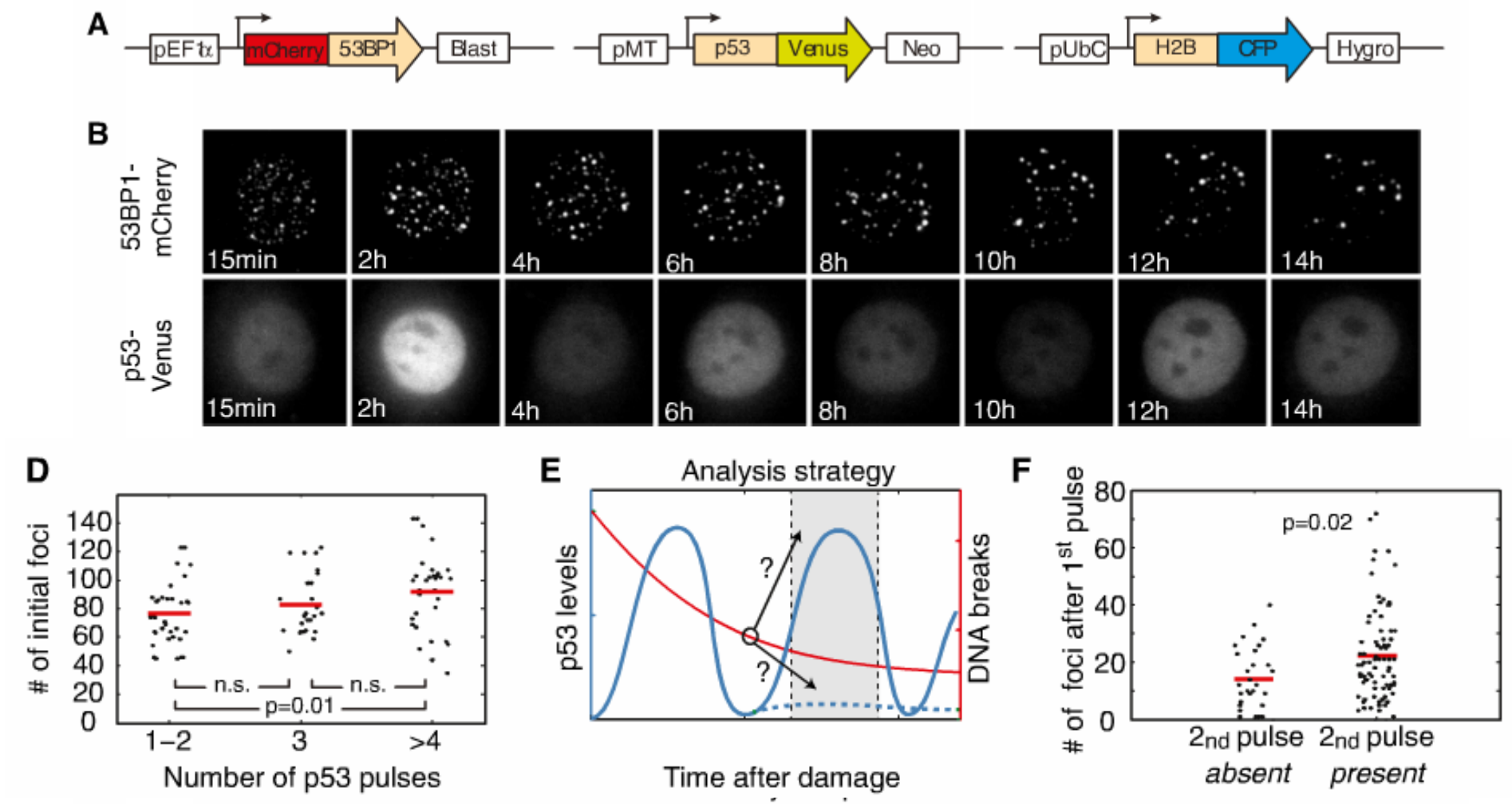


Understanding how the p53 response is triggered quantitatively



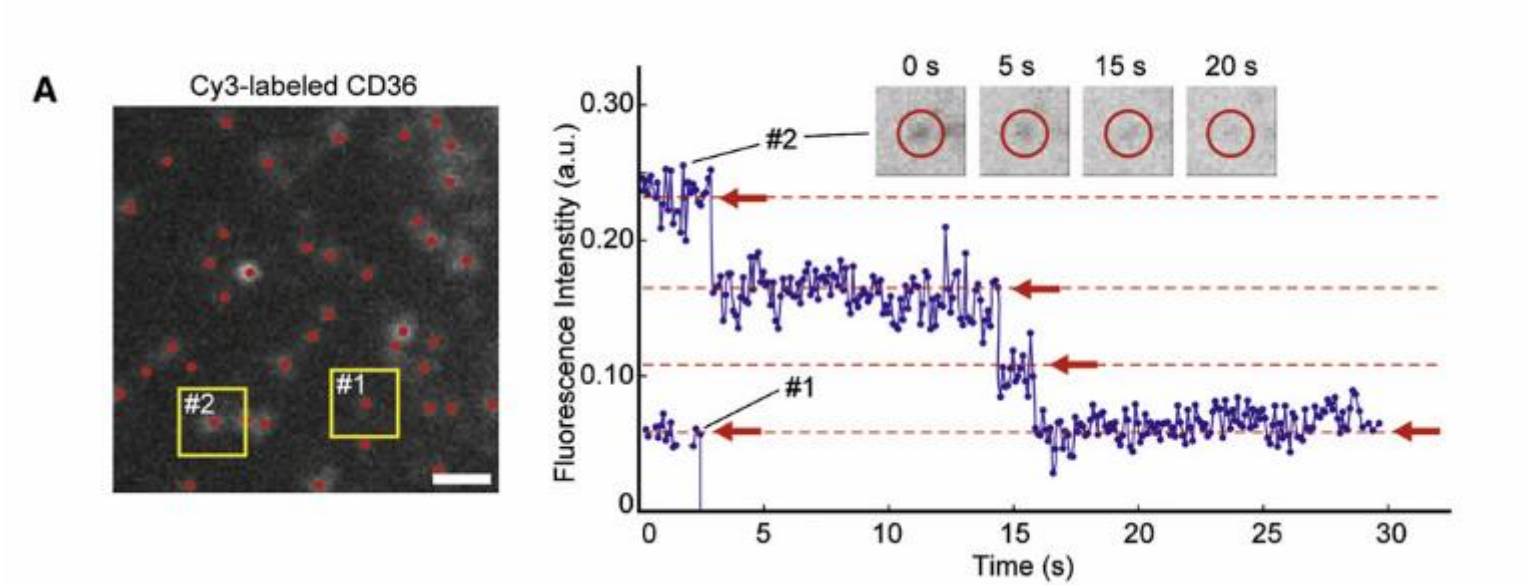
Loewer et al. 2013

Understanding how the p53 response is triggered quantitatively



Loewer et al. 2013

Multiplexing reporters to make network measurements



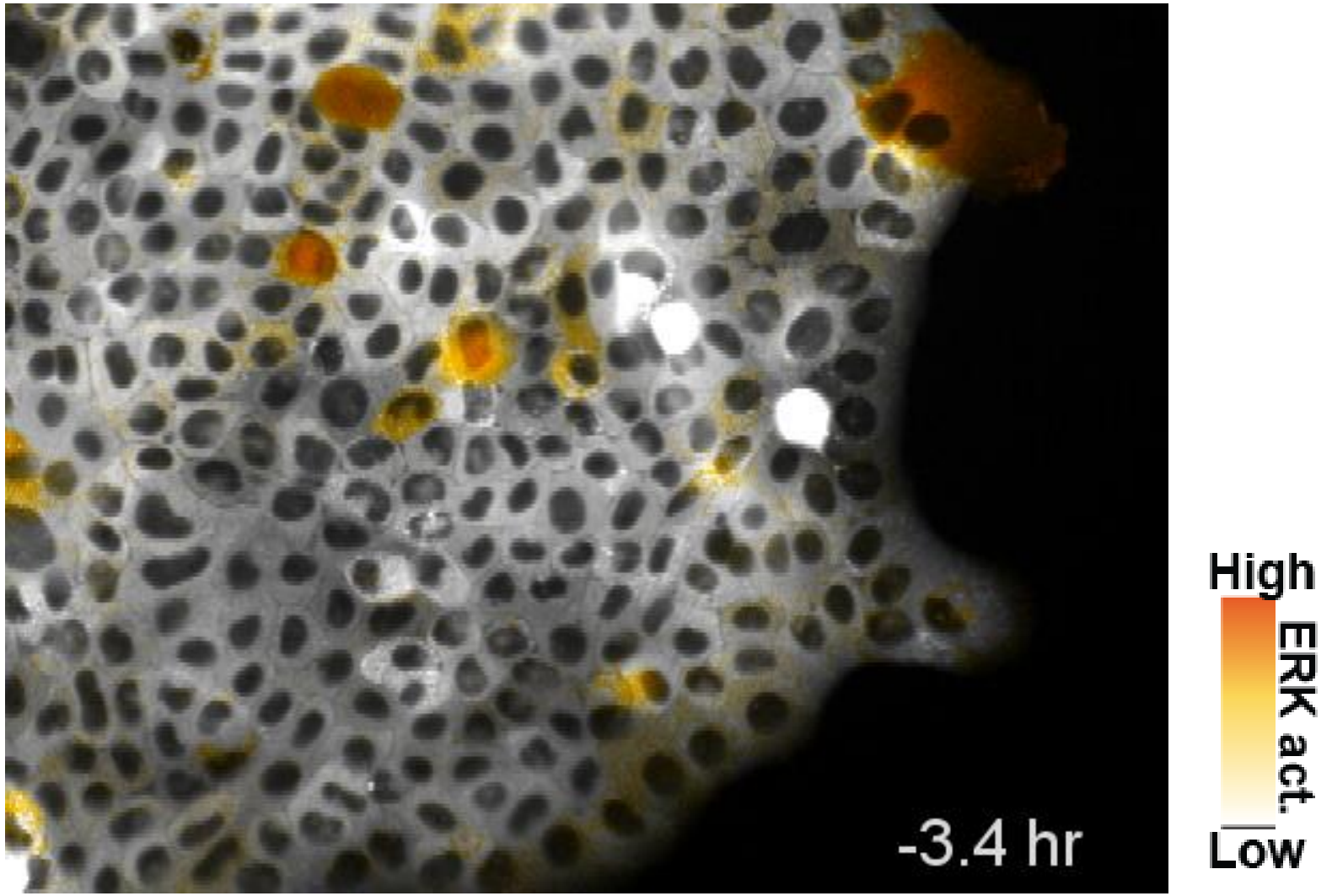
Topics for the hands-on workshop

1. Data visualization
2. Dealing with noise and other problems in your data
3. Identifying dynamic features present in your data
4. Quantifying trends and testing them statistically
5. Making publication-quality figures

Visualization of Data

Dynamics of ERK activity in response to EGF

“Physiological” EGF (0.1 ng/ml)

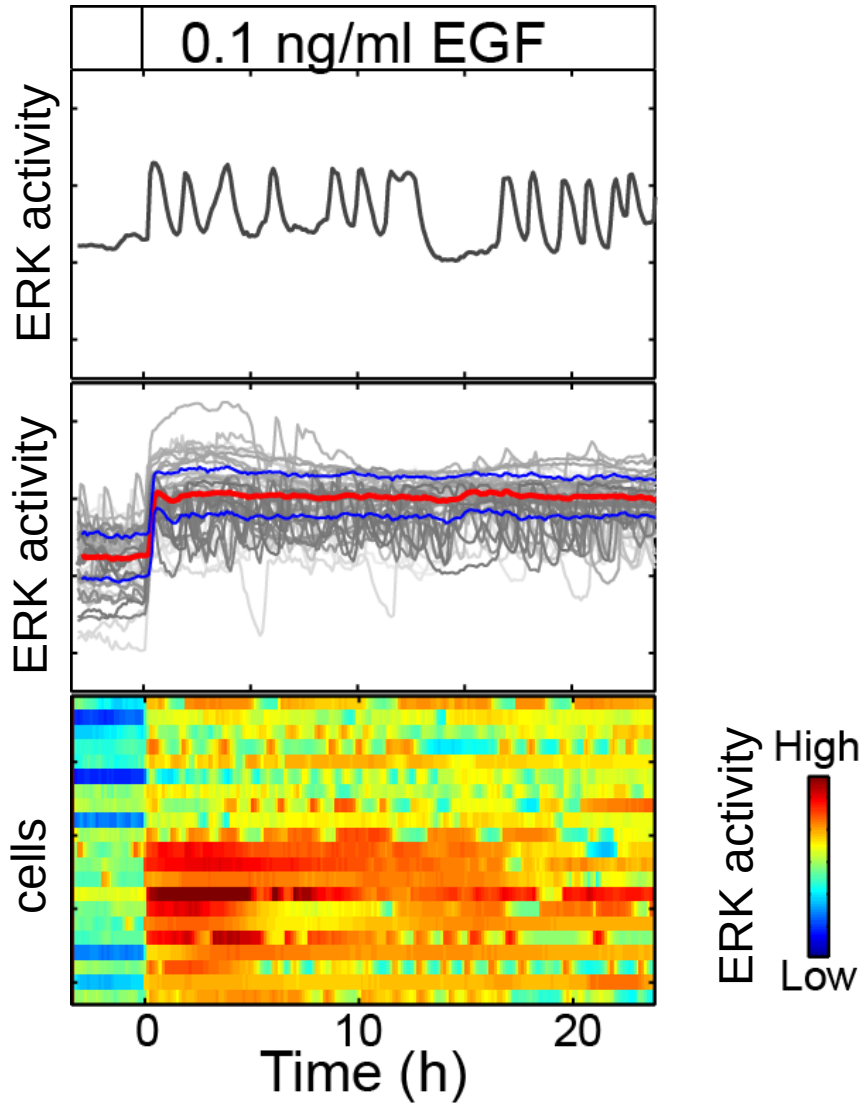


Extracting single cell data from movies

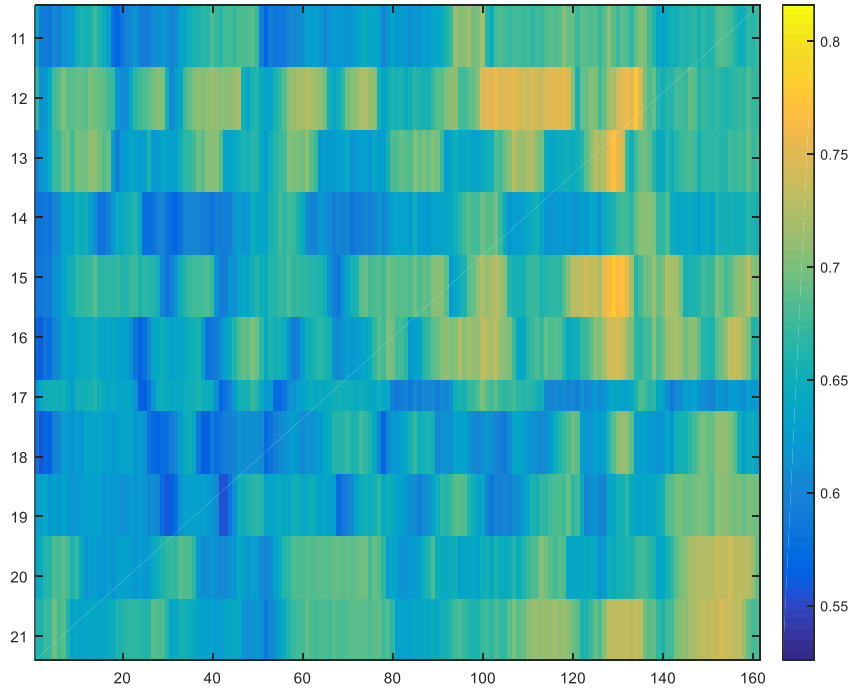
Single cell

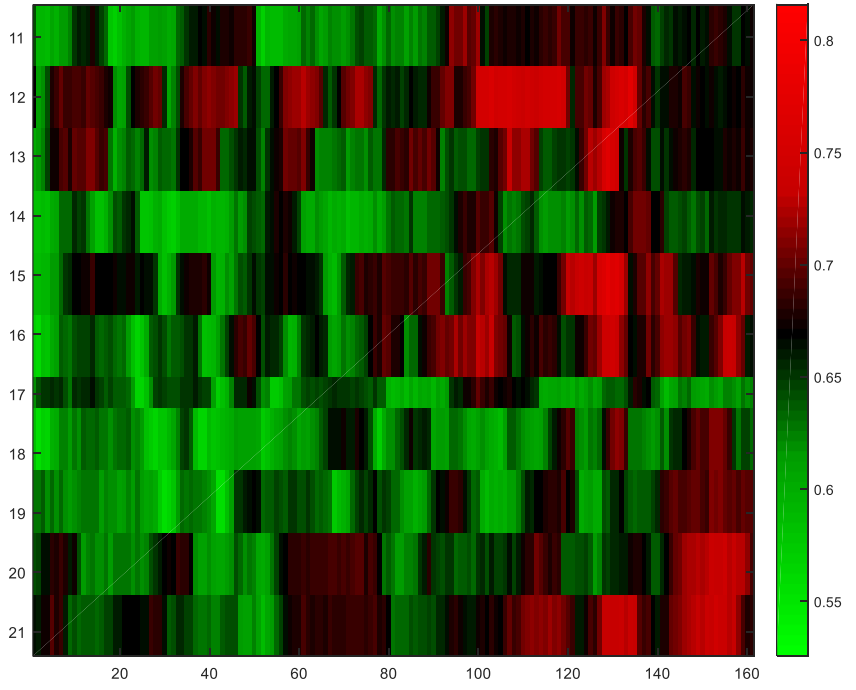
Many cells + median

Many cells as heatmap

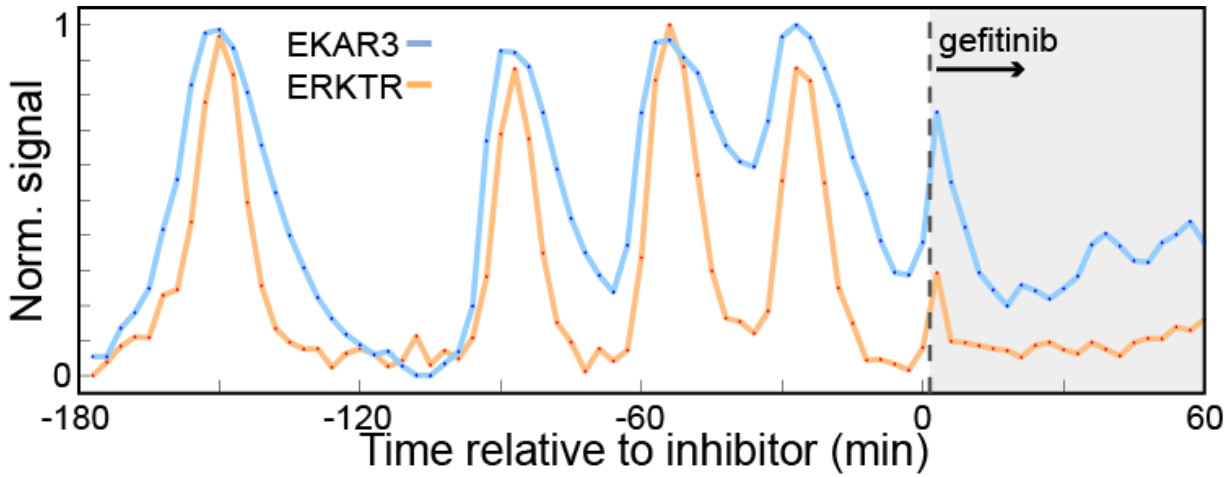


Heatmaps – viewing numbers as colors

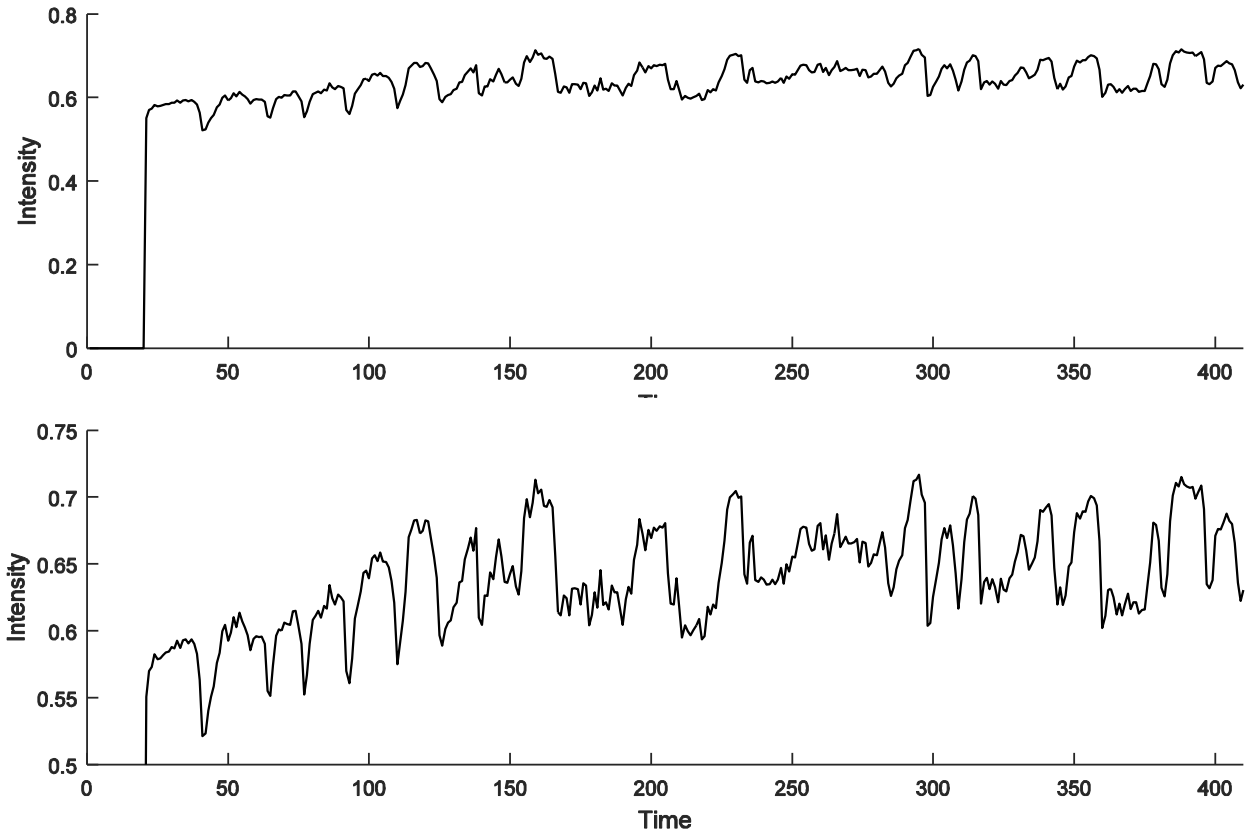


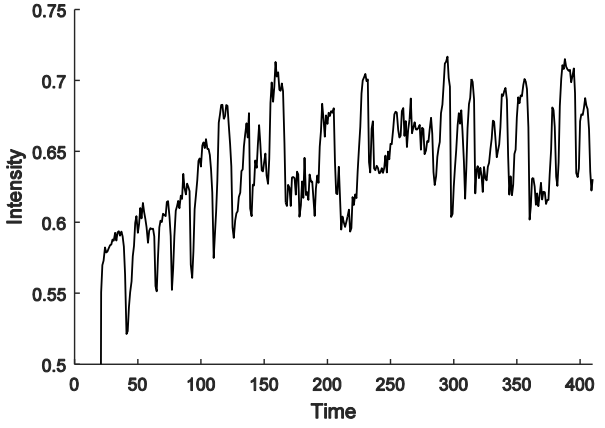


Line plots are ideal for comparing two signals in the same cell

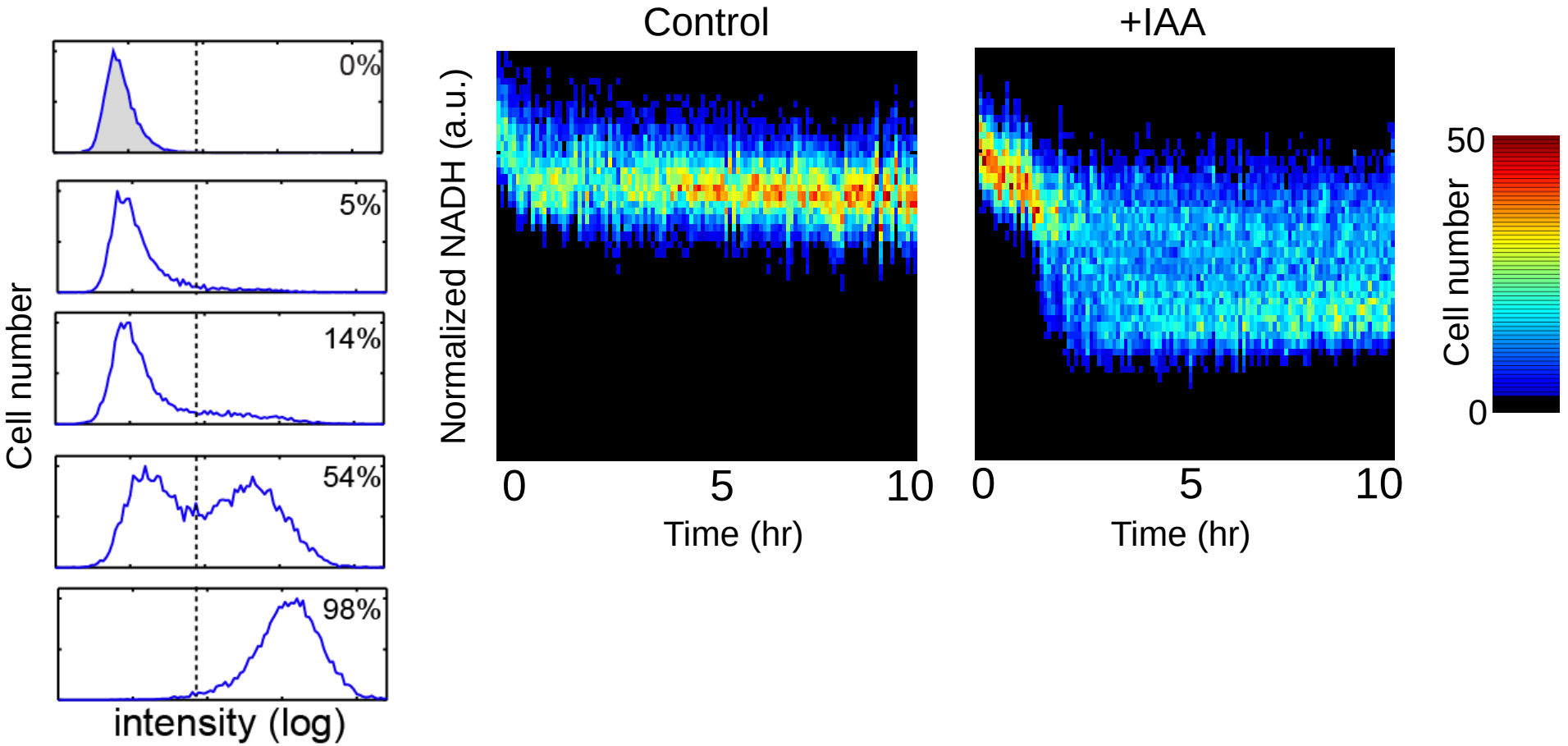


Normalization, scaling, and subjectivity

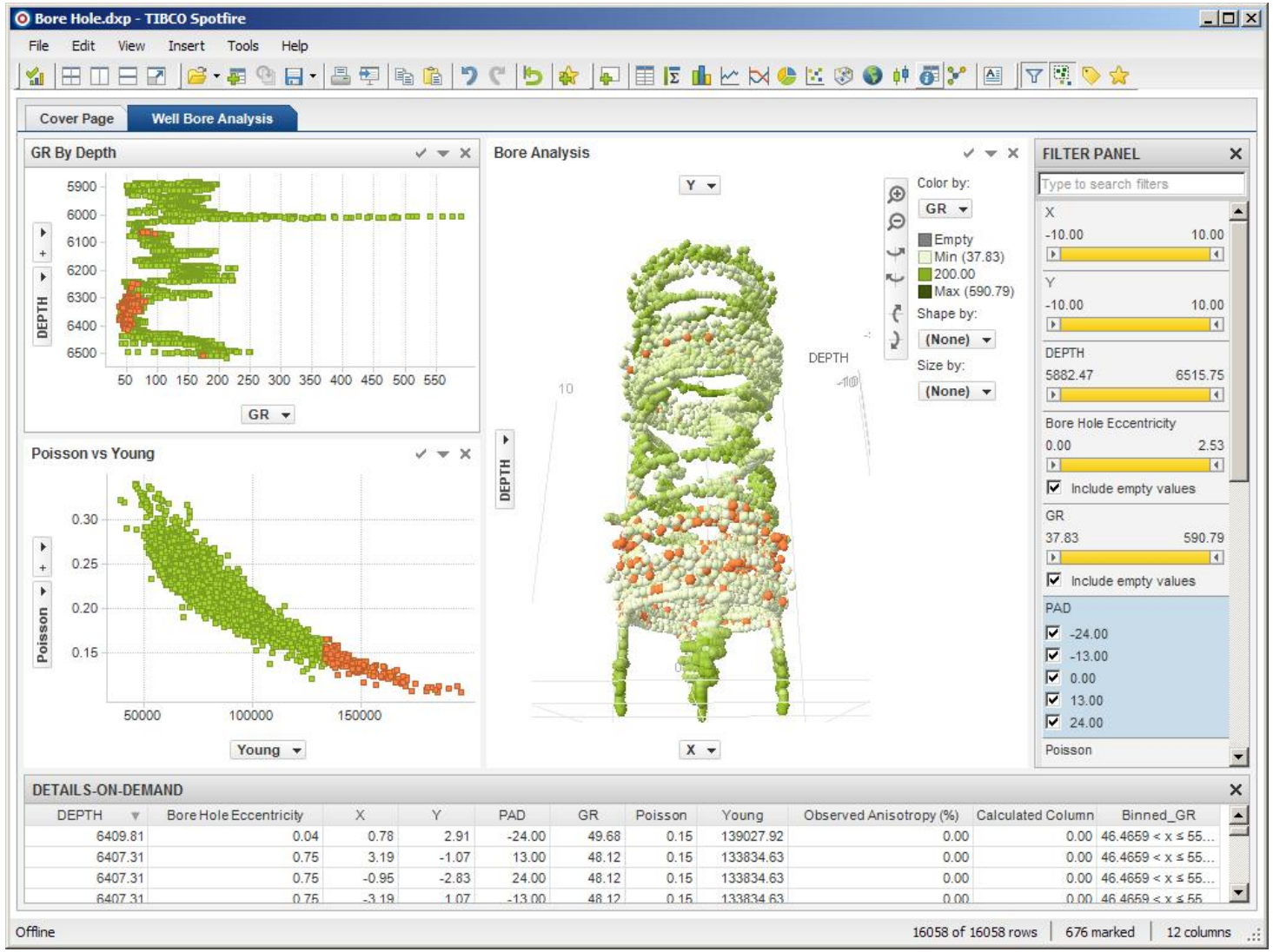




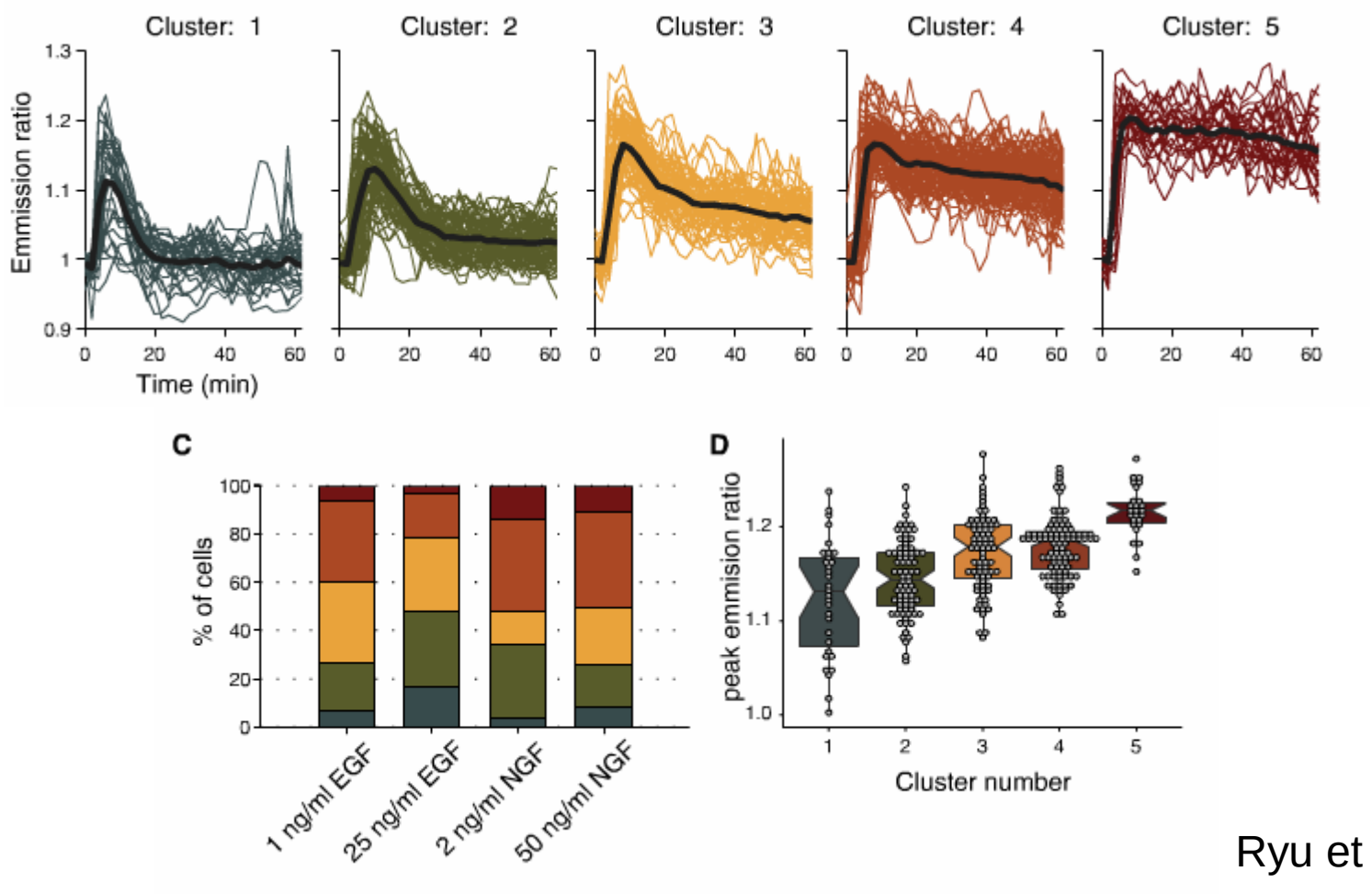
Time-dependent histograms



More advanced options: Spotfire



Separating individual tracks into qualitatively distinct clusters

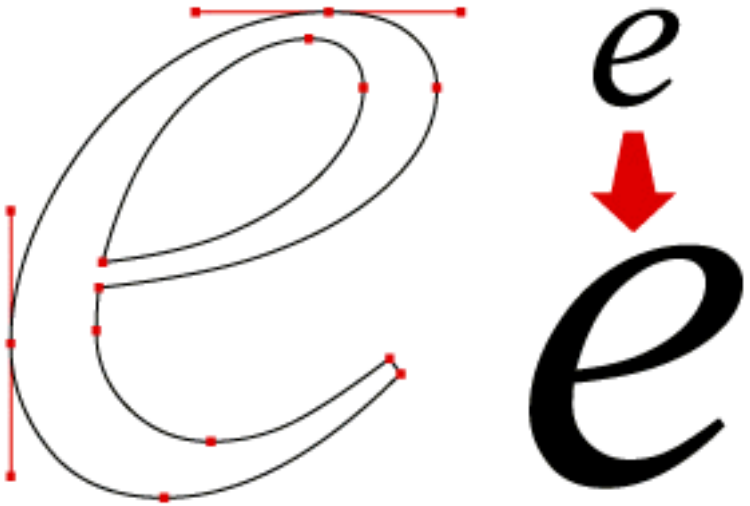


Ryu et al., 2015

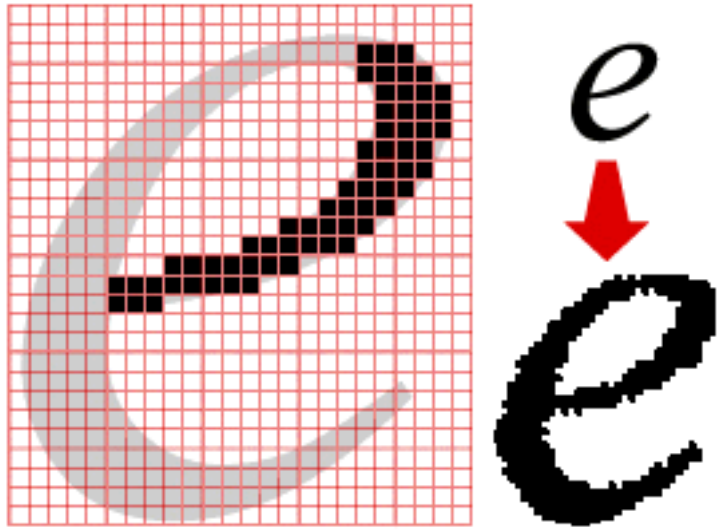
Making publication-quality figures

Vector vs. Raster graphics

VECTOR GRAPHICS



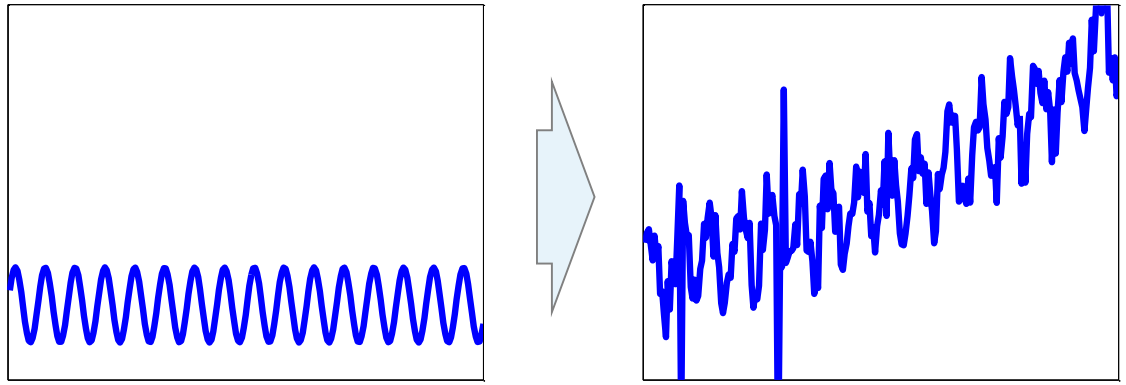
BITMAPPED (RASTER) GRAPHICS



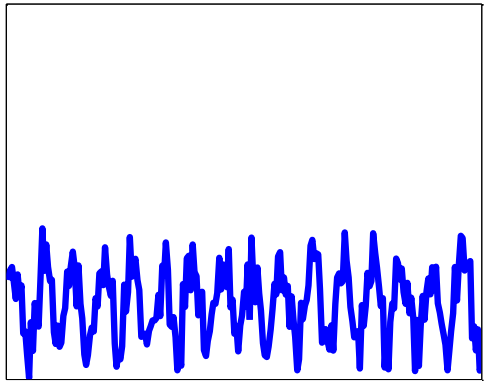
Dealing with noise

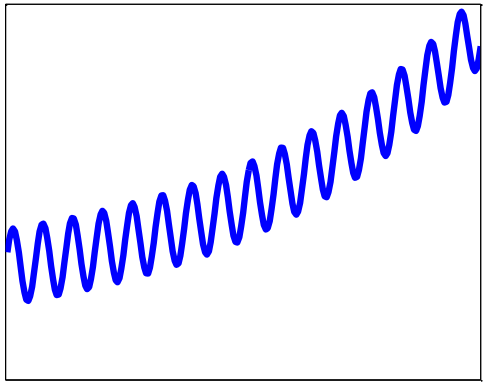
Noise effects in dynamic data

- Random – varies rapidly
  - Many sources throughout measurement
- Bias – ongoing trend
  - Photobleaching, interference
- Outlier – out of range
  - Transient interference
  - Imaging or processing fault



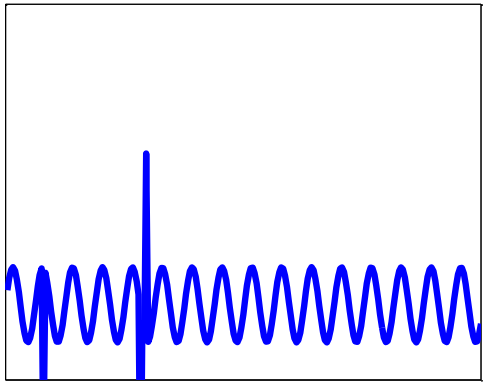
Random noise





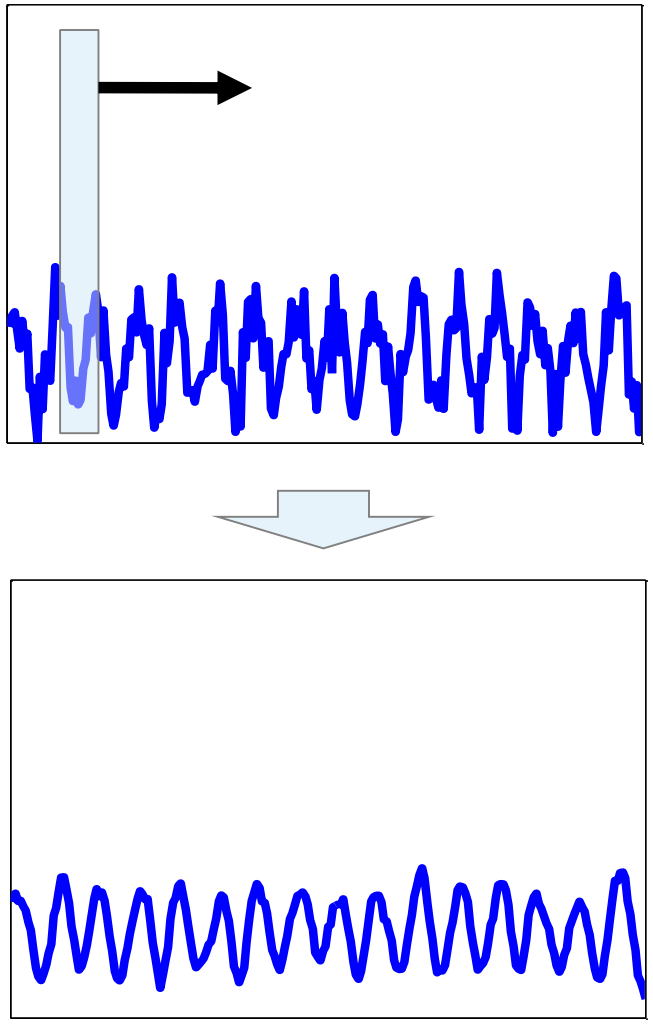
Bias (baseline drift)

Outliers



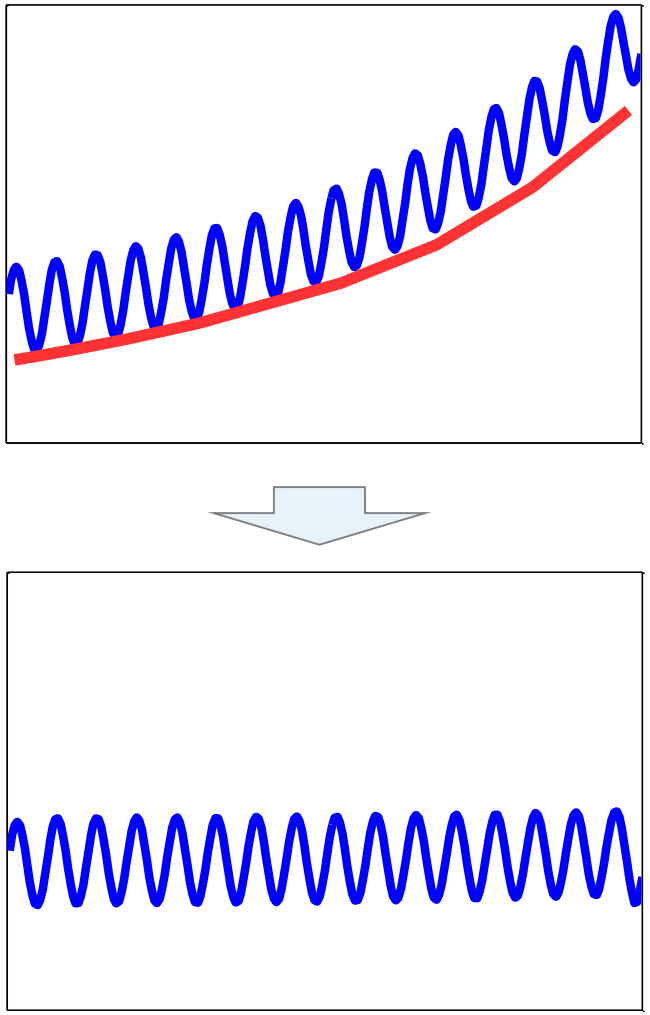
For random noise: average

- Typical of most measurements
  - Real signal is mean value
- Our signals are dynamic
  - Change with time
  - Difficult to sample noise
- Average over time
  - Moving average
  - Filter by frequency



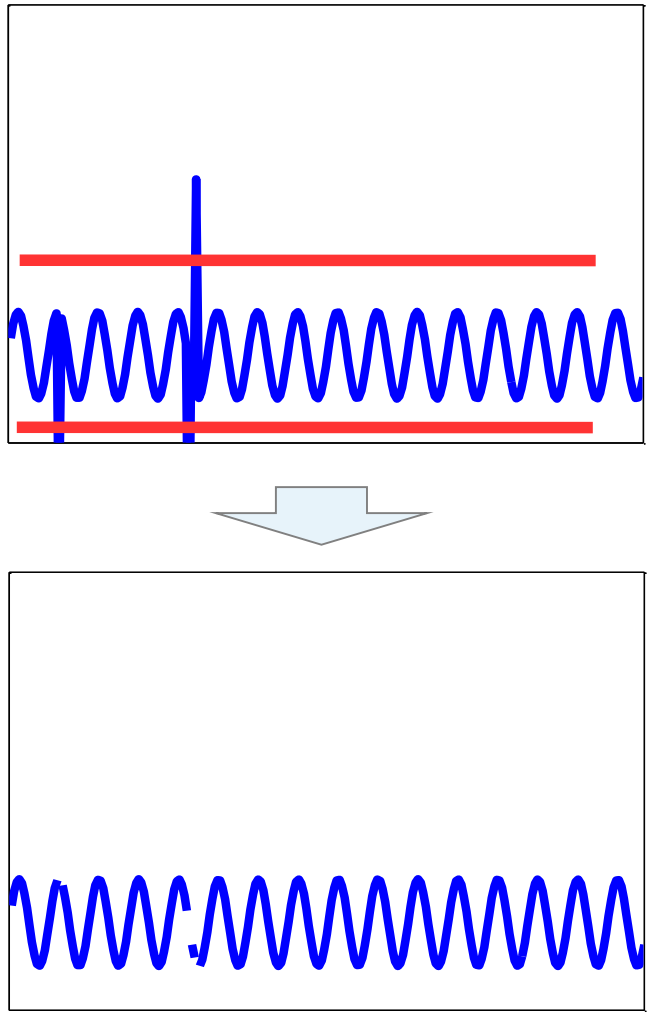
For bias: identify and remove

- Changes in time (or space)
  - Can be removed if identified
  - With caution!
- Fit to a model
  - Exponential for photobleach
- Generic
  - Filter (subtract mean)
  - Subtract local baseline
  - Caution: Risk of new bias



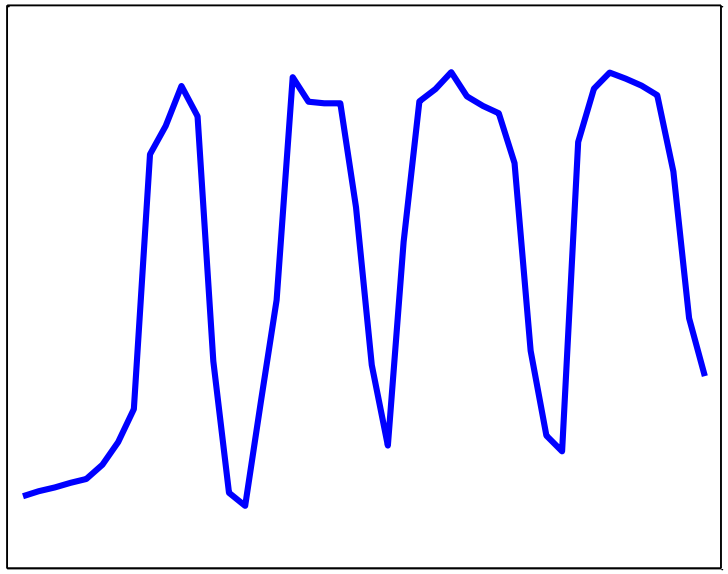
For outliers: identify and remove

- Expect hiccups
  - Ideal if tracked separately
- Identify
  - Out of range
  - Sudden jump
- Remove/replace
  - Interpolate from nearby data
  - If many gaps, or sensitive data, fit model or use Kalman filter

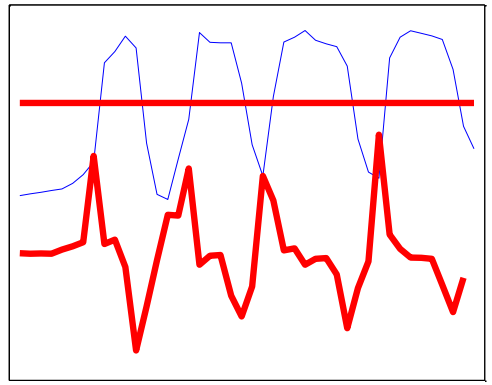


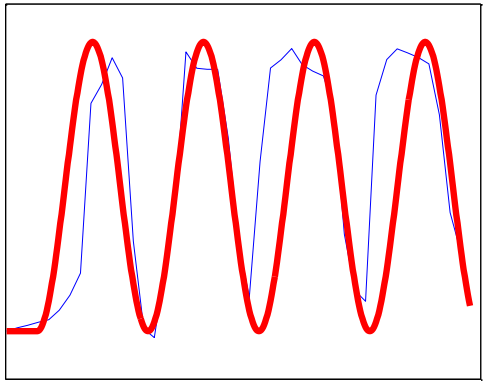
Identifying dynamic features

Dividing dynamics into parts



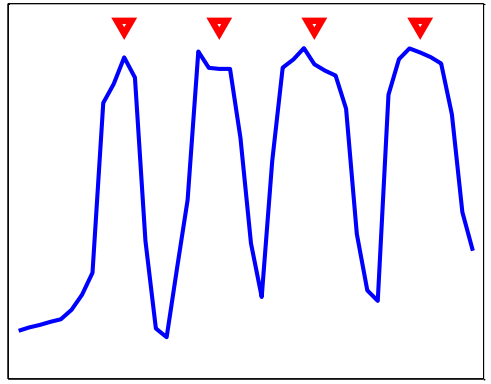
- Mean value
- Derivative





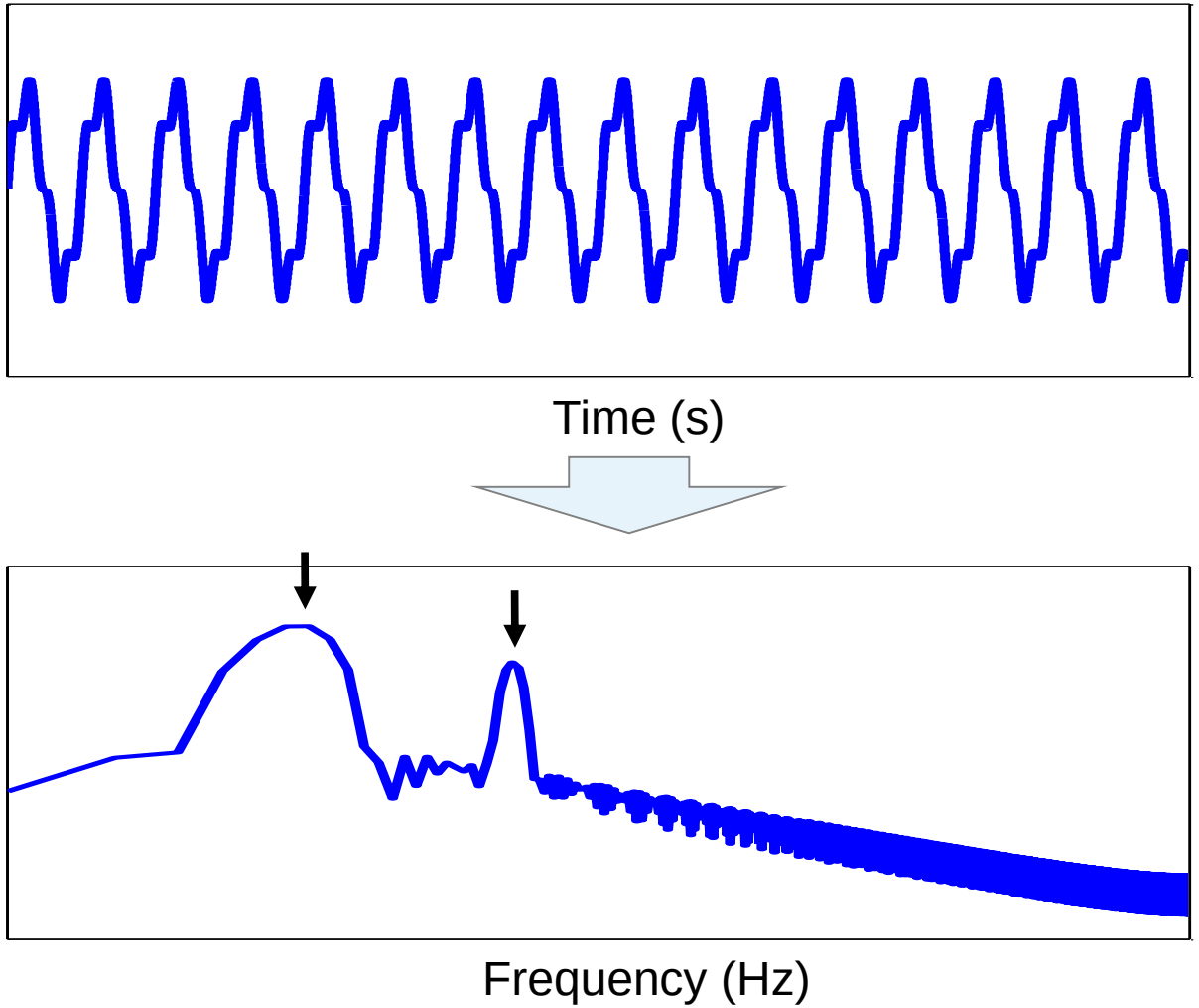
- Oscillations

- Pulses/peaks
  - Peak features



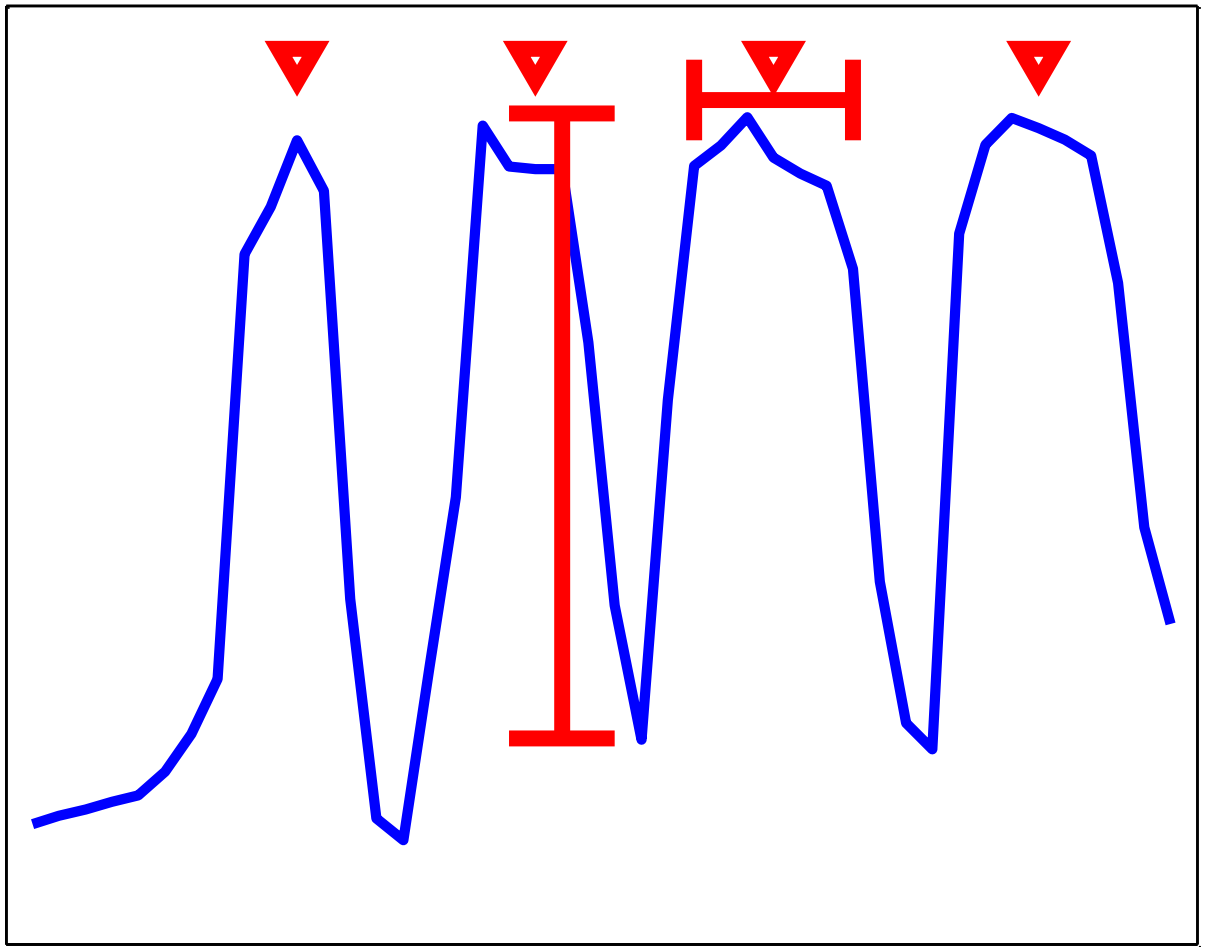
Characterizing oscillations

- Frequency
- Amplitude
- Frequency analysis
  - Fourier transform
  - Power spectral density
  - Phase



Characterizing pulses

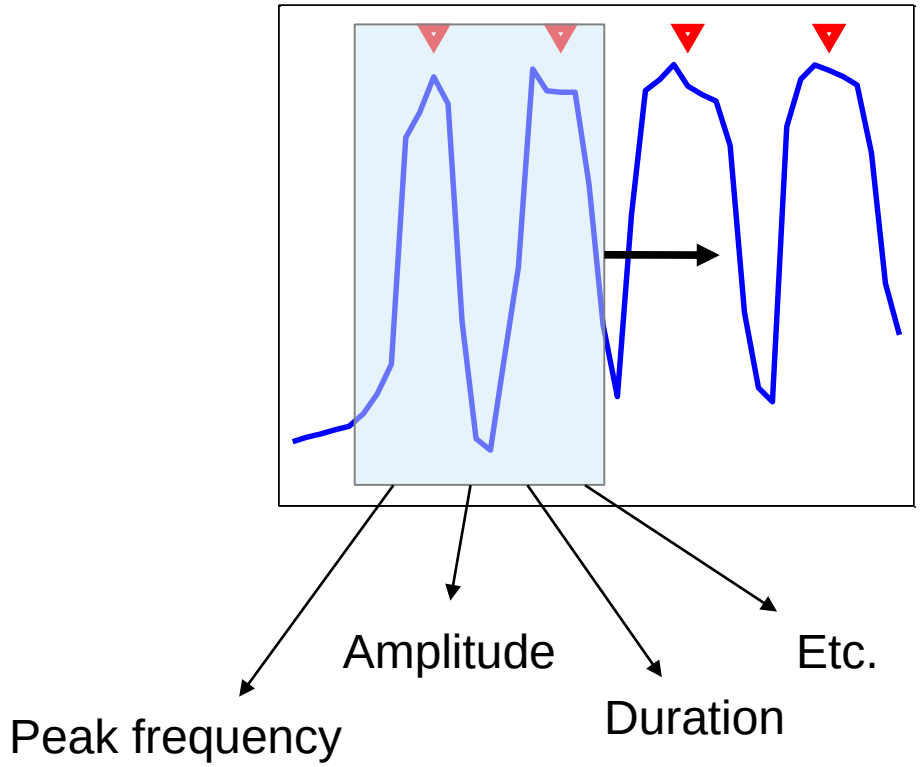
- Timing
  - Pulse duration
  - Spacing/frequency
- Amplitude
- Kinetics
  - Rise/fall time
  - Shape of rise/decay curves, if high res.



Quantifying trends

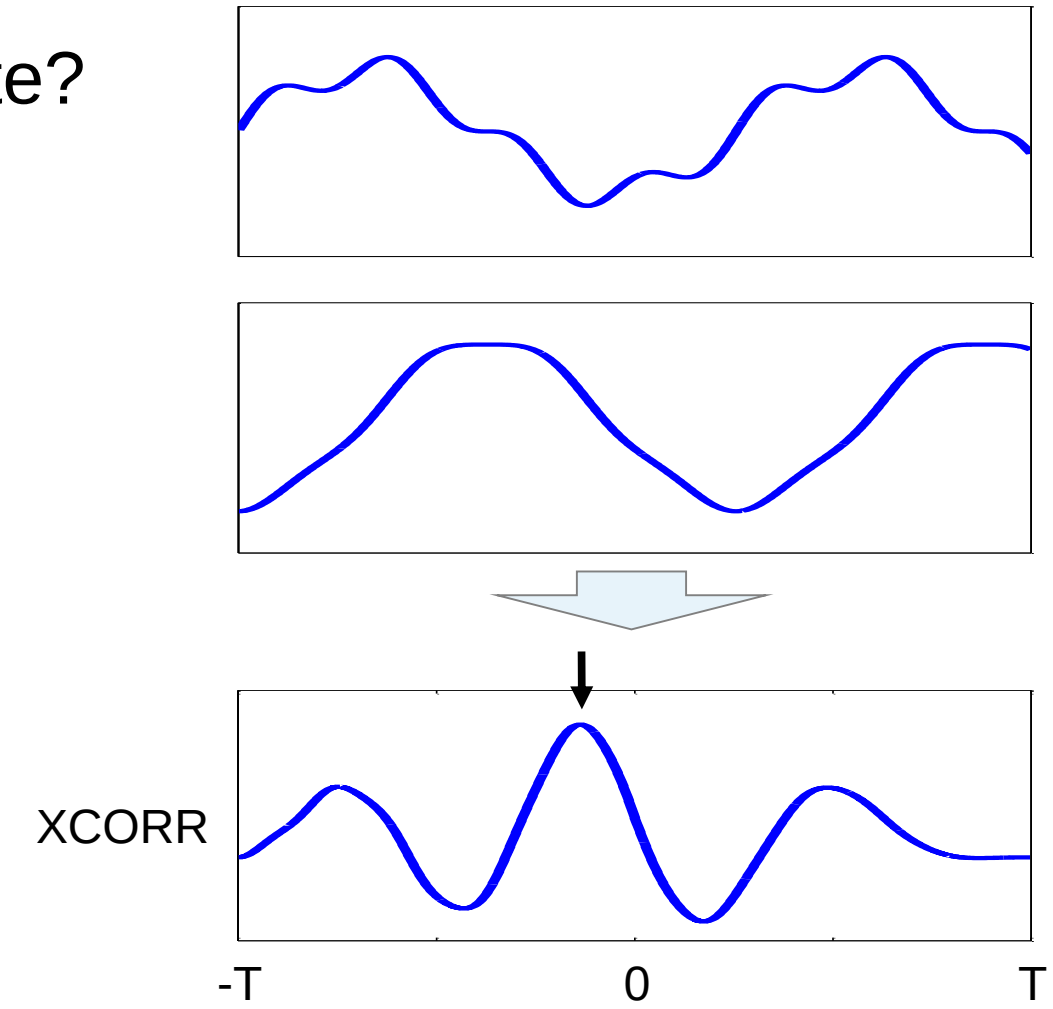
Trends in features over time

- How do the dynamics change over time?
  - Evaluate within windows
- Correlation with events
  - Stimulation
  - Cell cycle
  - Death
  - Movement



Correlating dynamics

- How do the dynamics relate?
  - Direct relationships
  - Can fit/test models
- Correlation
  - Direct relationships
- Cross-correlation
  - Time-shifted correlation
  - Find delays



Hands-on portion