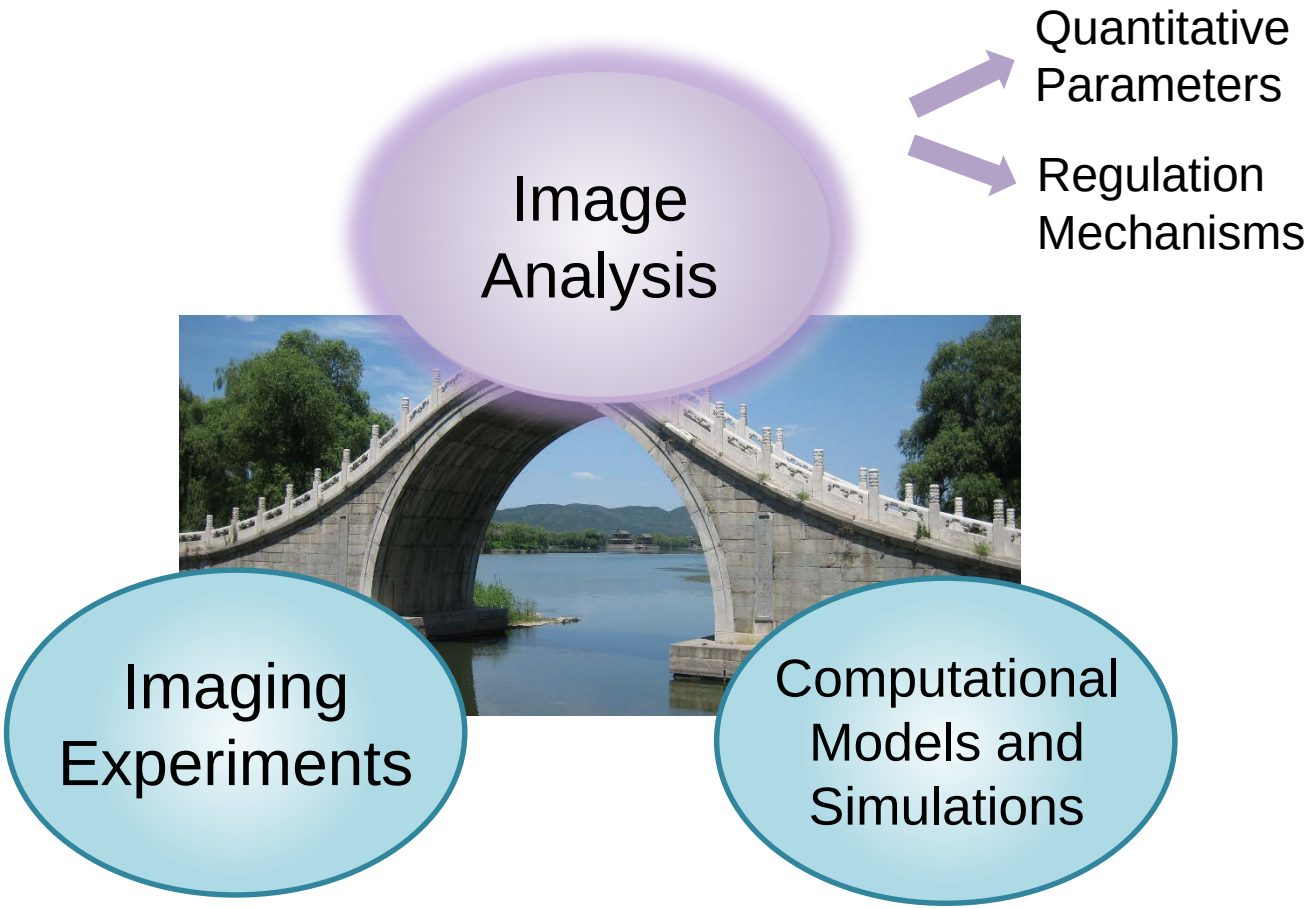
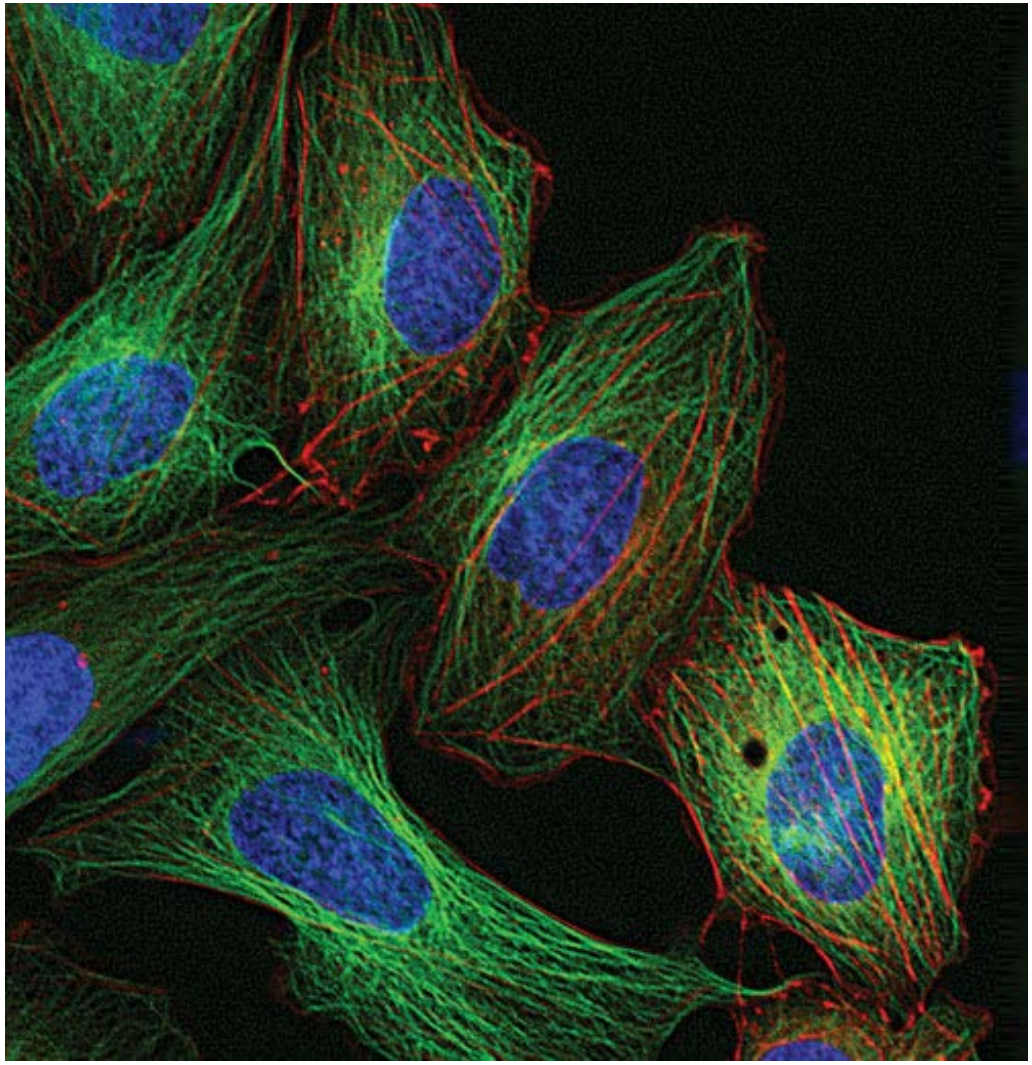
Live-Cell Image Processing, Segmentation, Tracking, and Interpretation

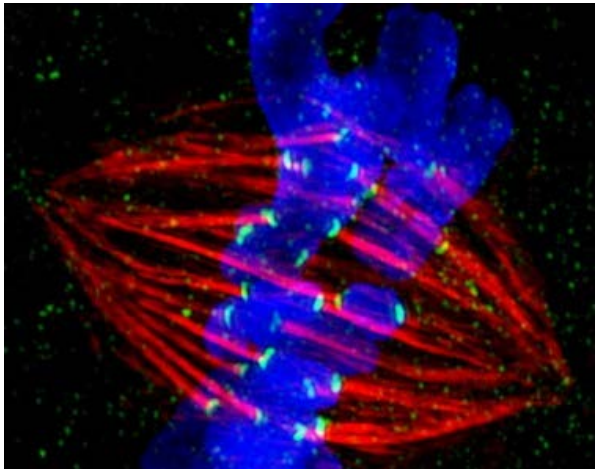
Kathy Lu

Image Analysis Bridges Experiments and Computational Models



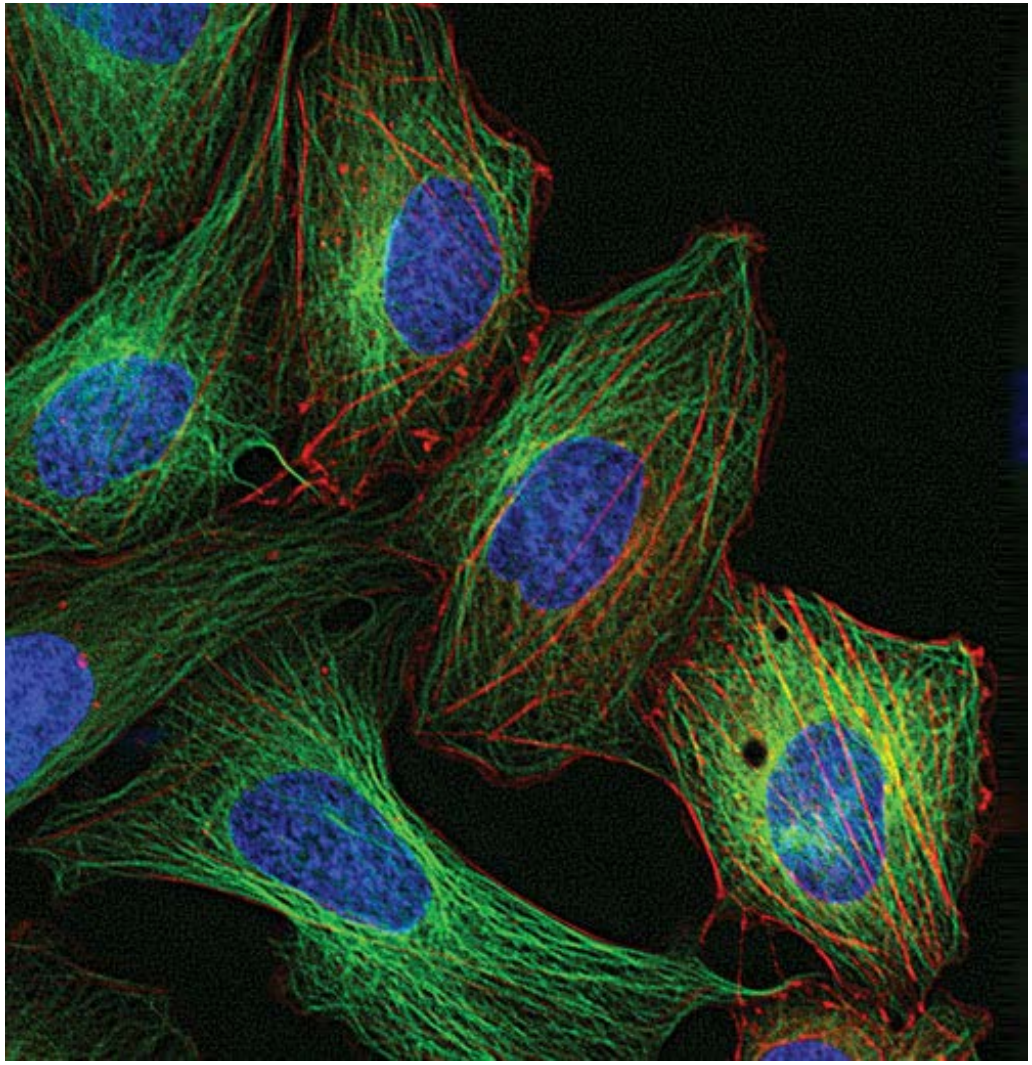
Live-Cell Imaging Provides Tremendous Amount of Data

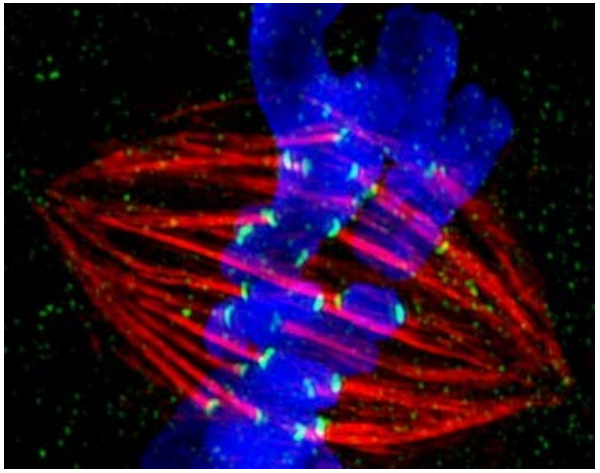


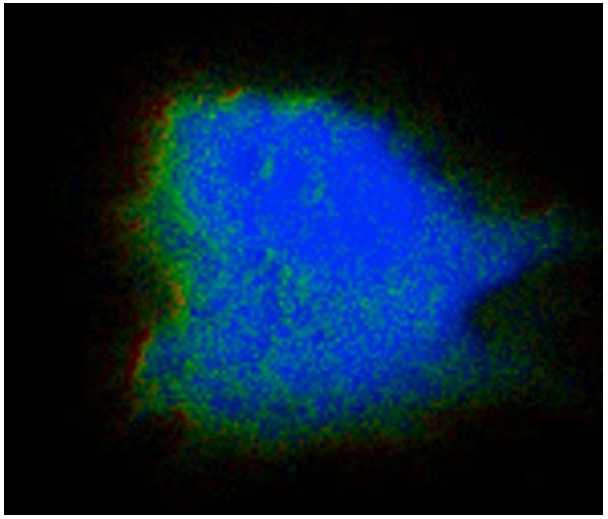


Live-Cell Imaging Provides Tremendous Amount of Data

40,000 images in 1 hour



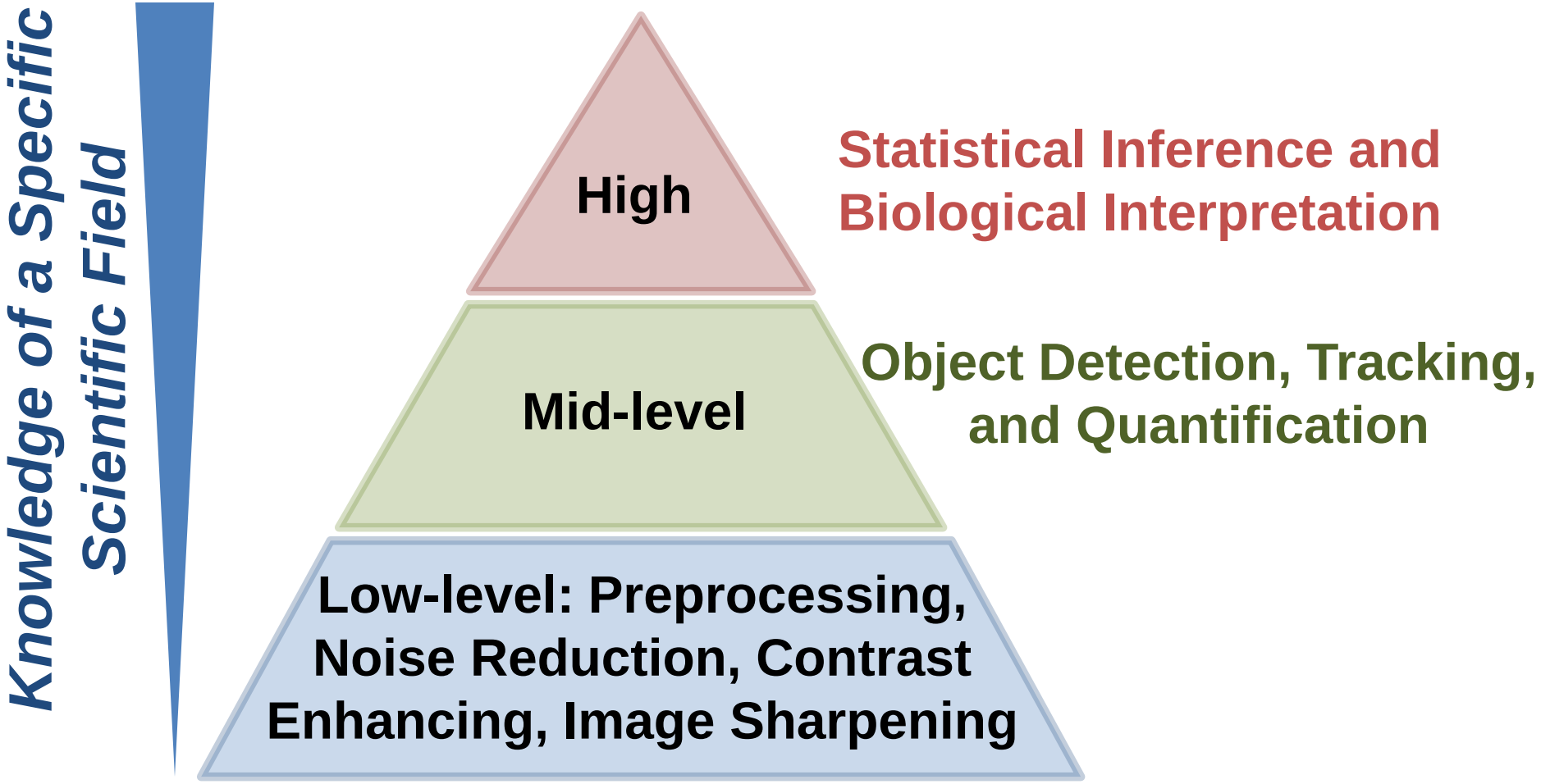




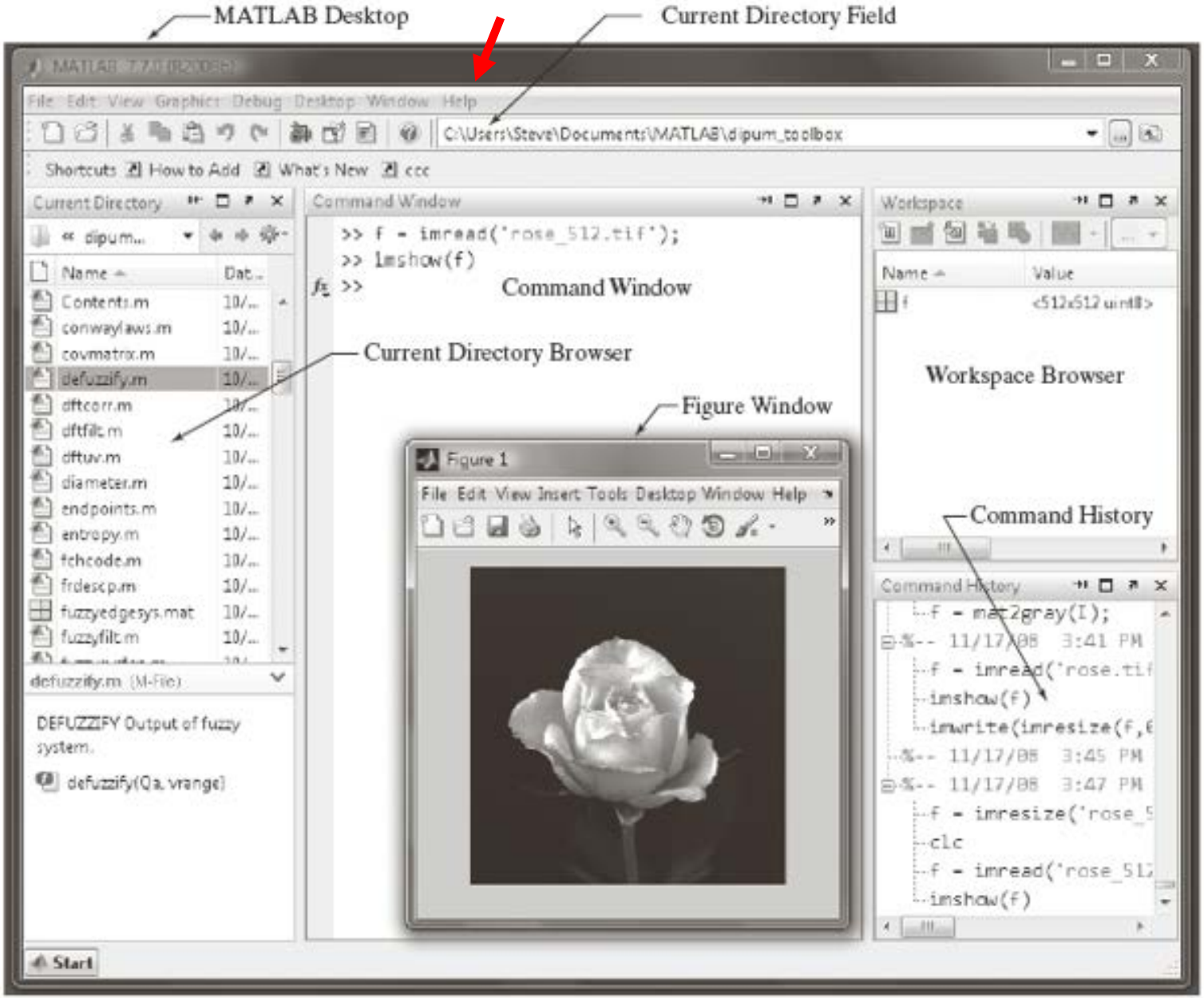
Strength of Automated Analysis

- ***Large Scale, Quantitative, and Objective***
- ***Reveals Non-obvious and Hidden Information***
- ***Systematic Understanding of Dynamic Images***
- ***Multi-channel and Multi-dimensional***

Three levels of Image Analysis

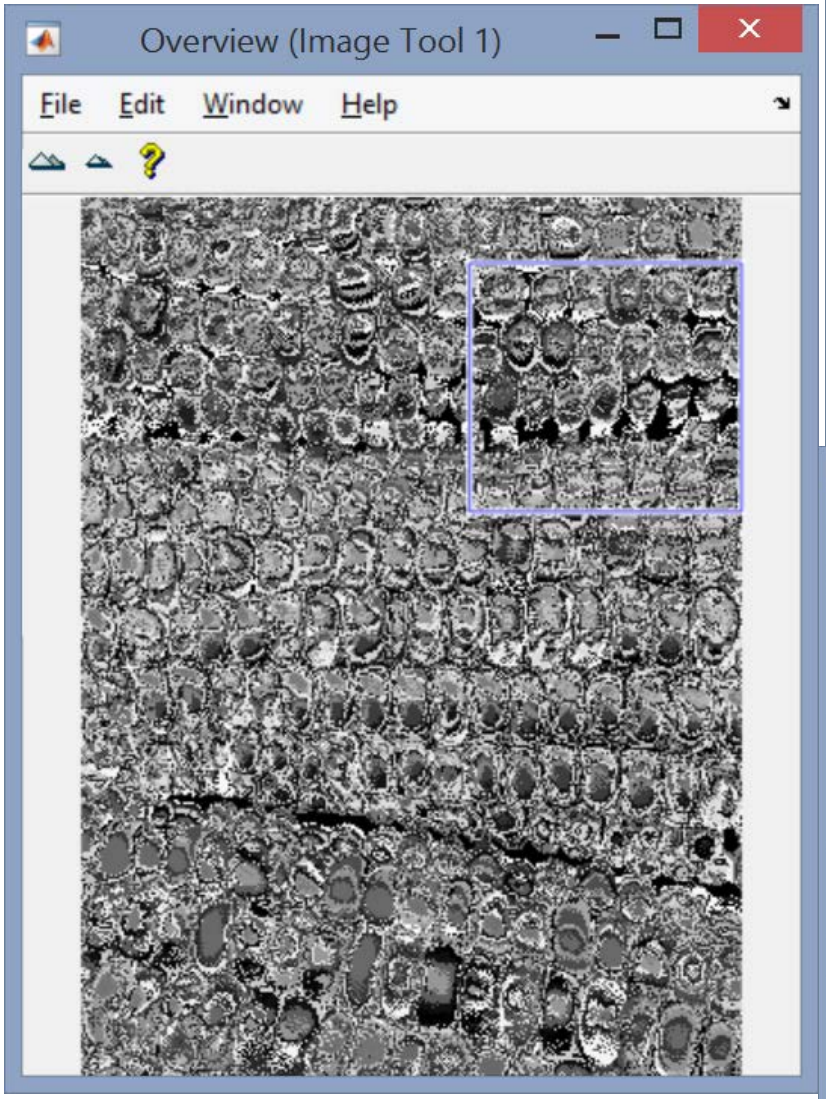


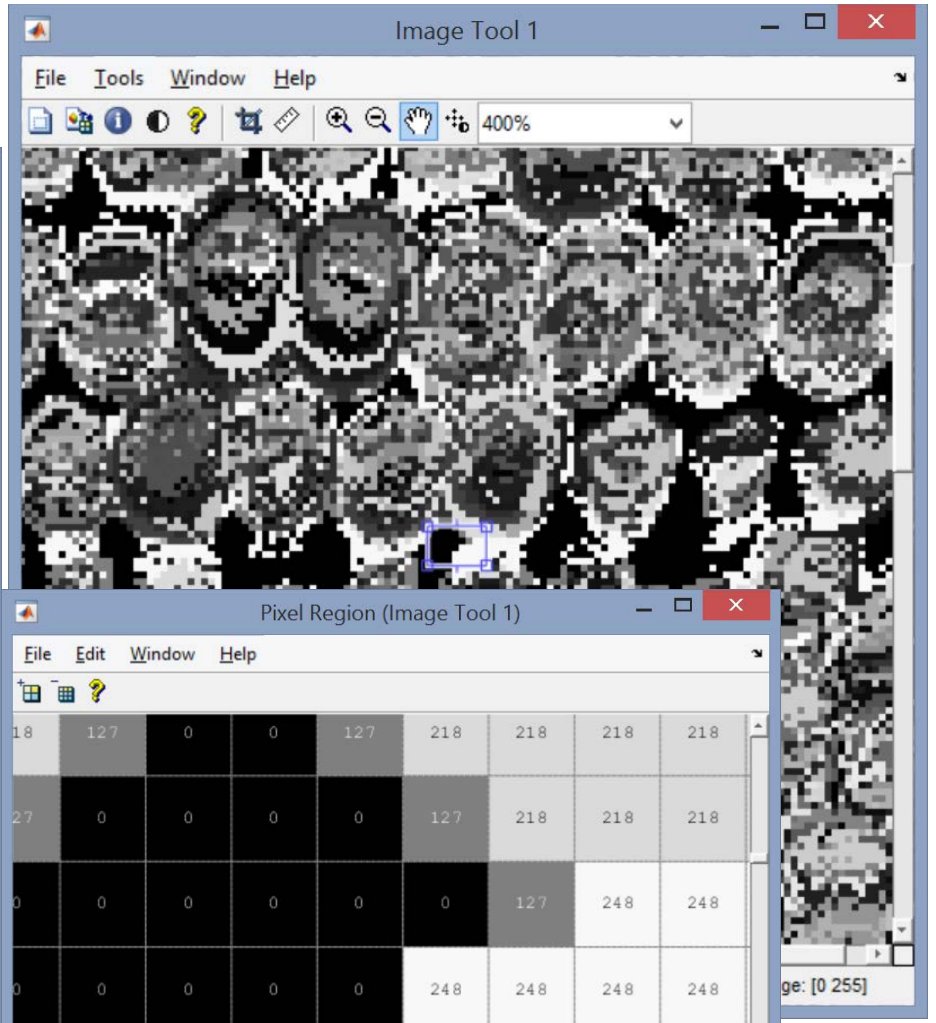
MATLAB for Image Processing



MATLAB Basics – Imagetool

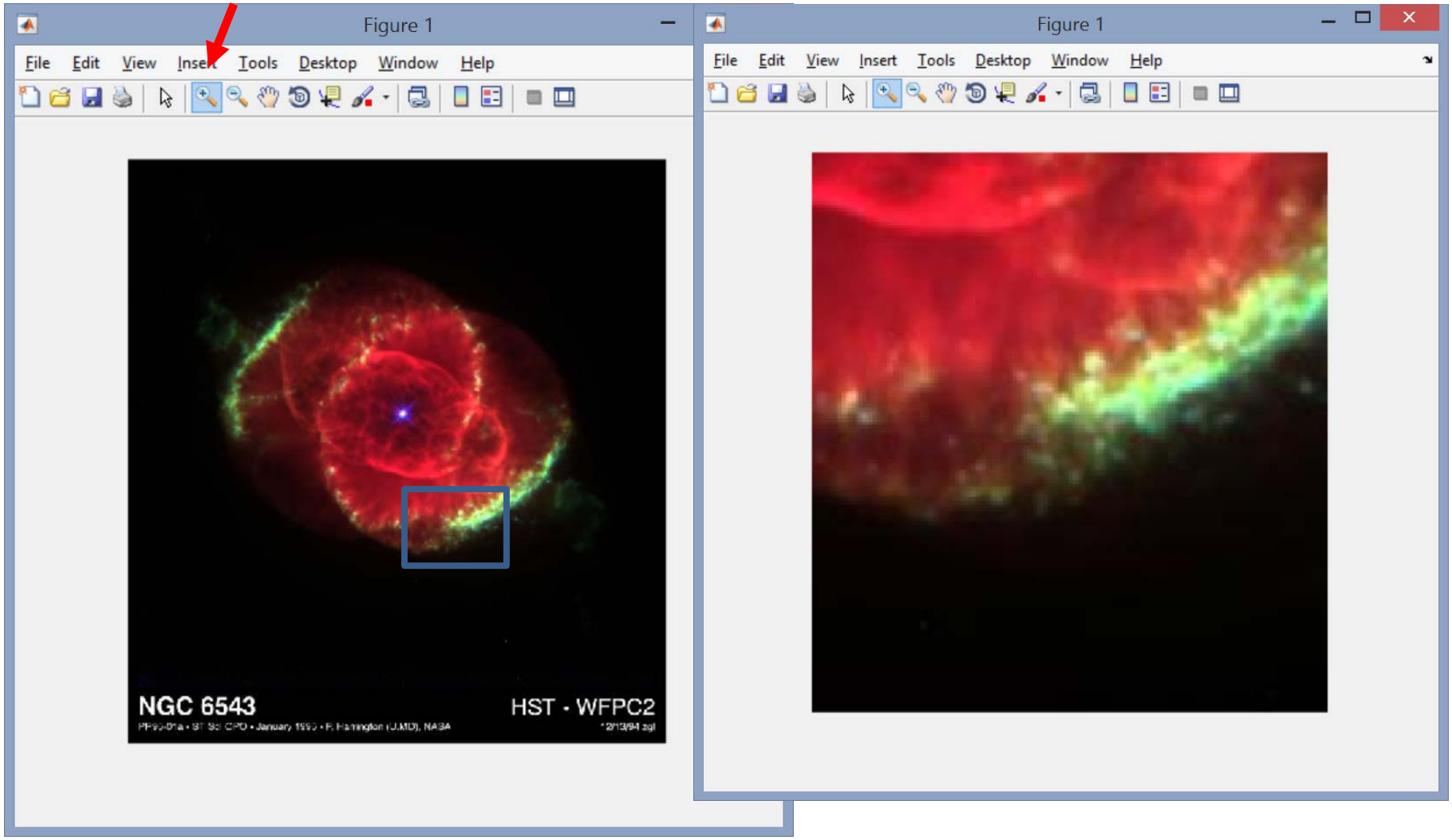
```
>> im = imread('corn.tif');
>> imtool(im);
```



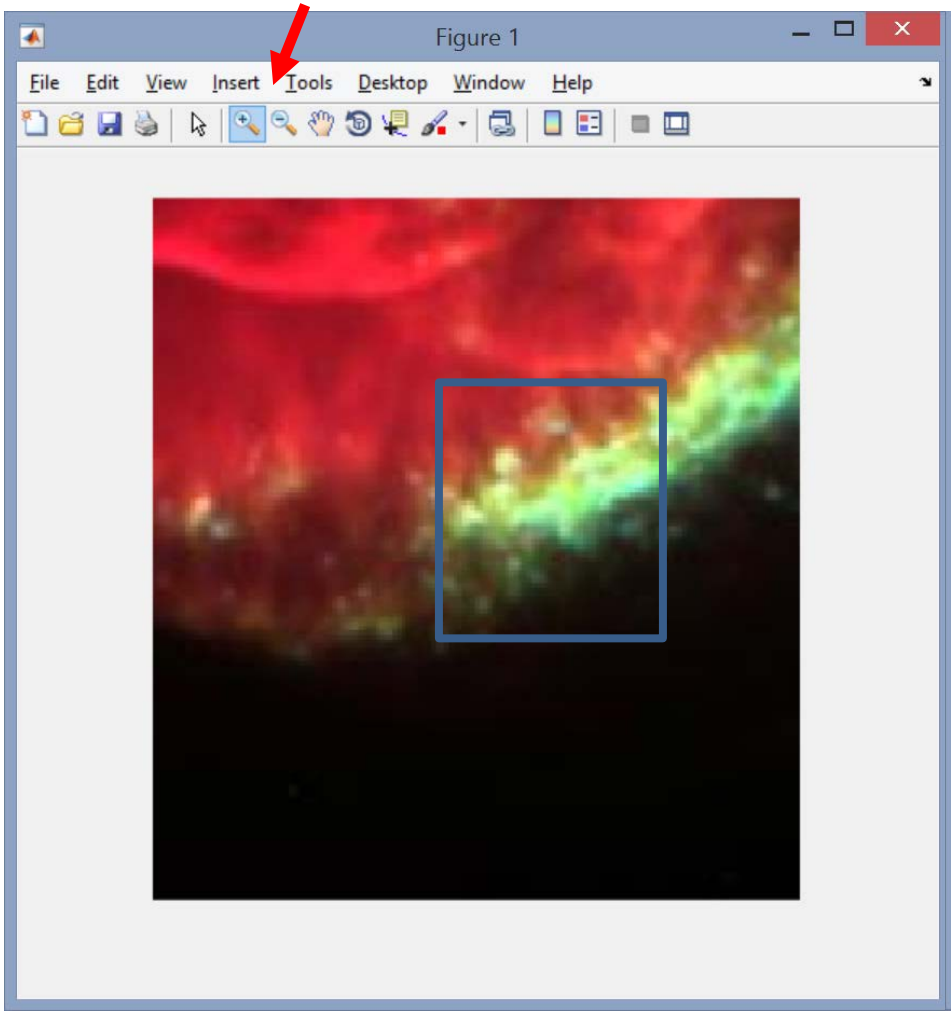


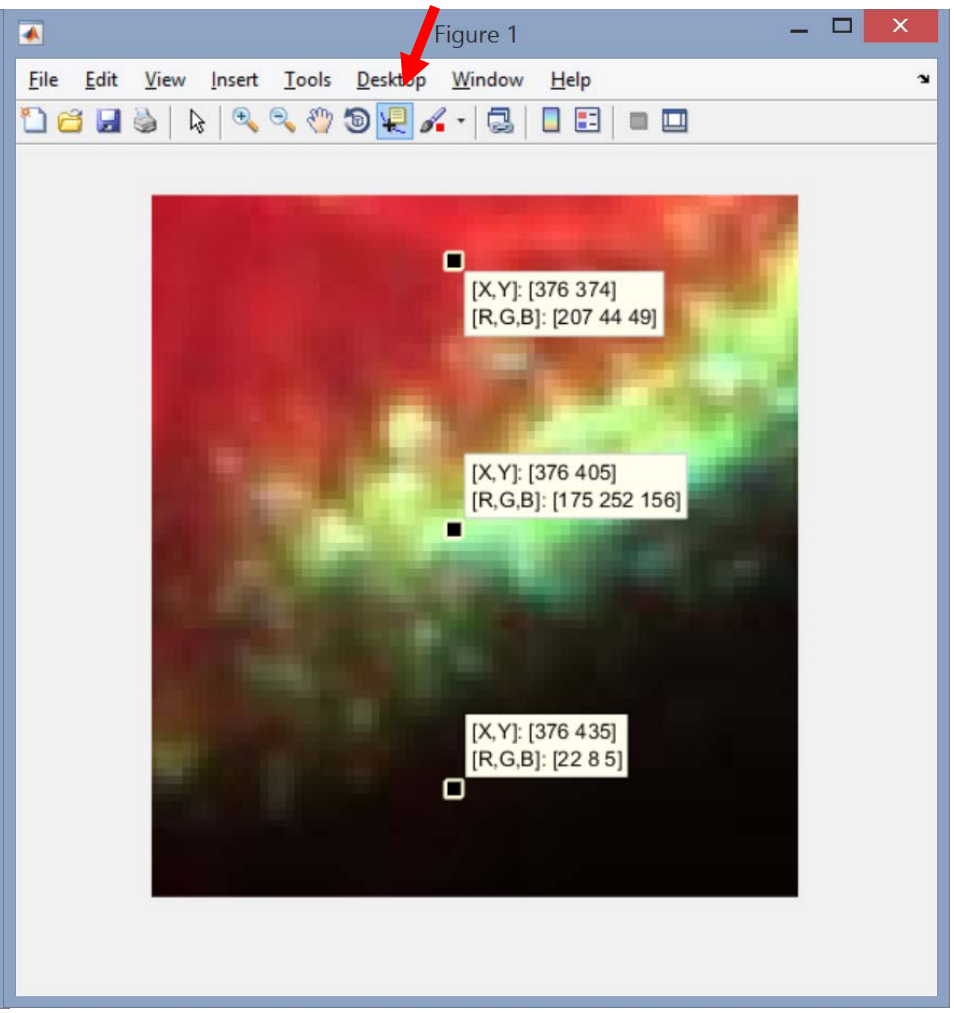
MATLAB Basics – Figure

```
>> im = imread('ngc6543a.jpg');
>> figure; imshow(im);
```



MATLAB Basics – Figure

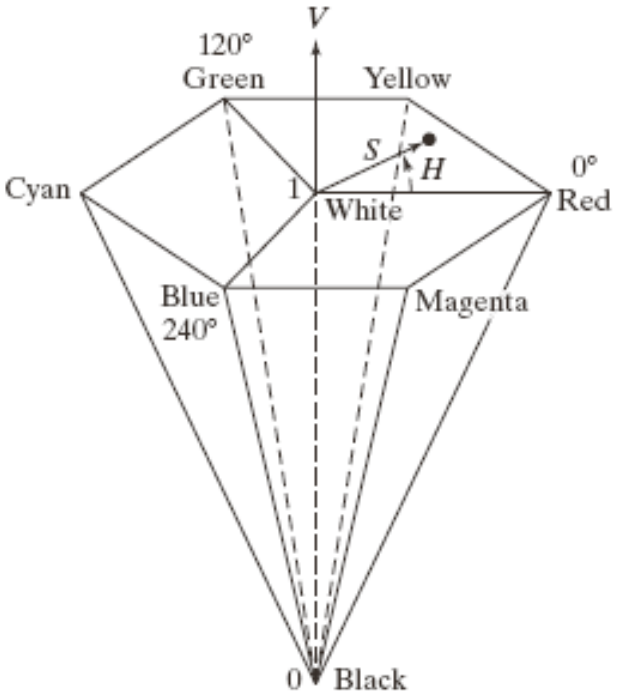




In the matrix im: y --- row number ; x --- column number.

Color Image – Red, Green, and Blue (RGB)

HSV/HSI Color Image
Hue, Saturation, Value/Intensity



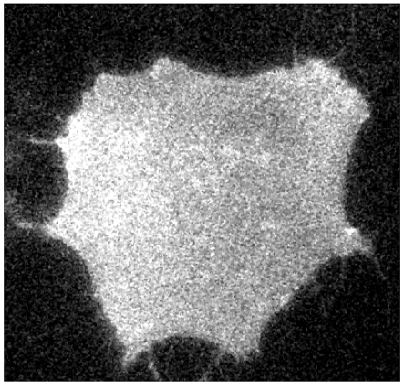
An HSV color system is close to human color interpretation.

- **Hue : a pure color with values in the range of [0 360]**
- **Saturation: the degree to which a pure color is diluted by white light [0 1]**
- **Value/Intensity: brightness [0 1]**

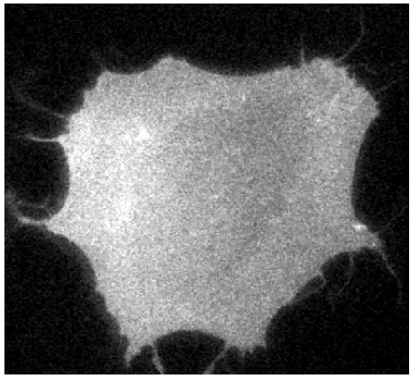
Example: ECFP/FRET/mCherry/DIC Image (Fluocell)

http://code.google.com/p/fluocell/

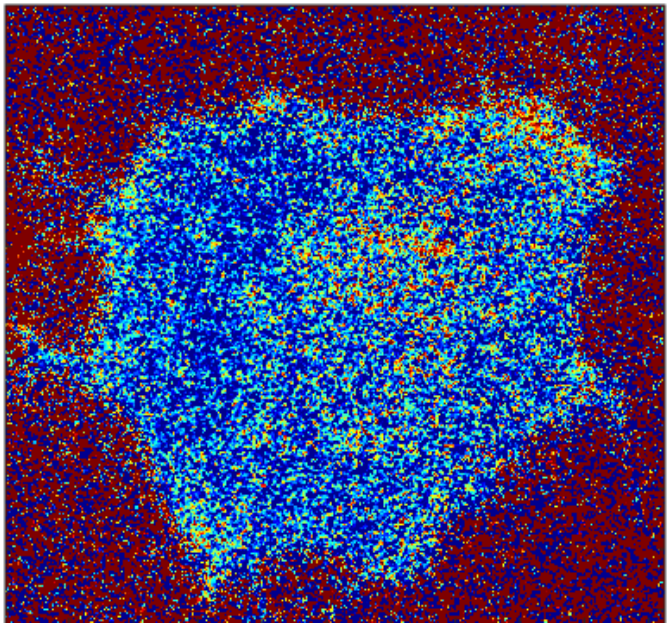
ECFP



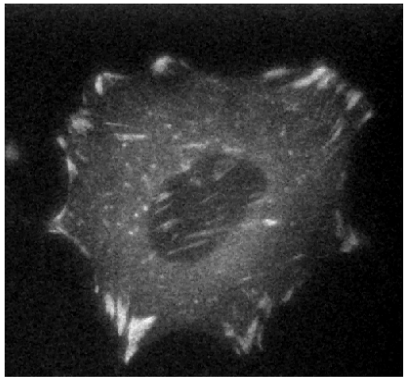
FRET



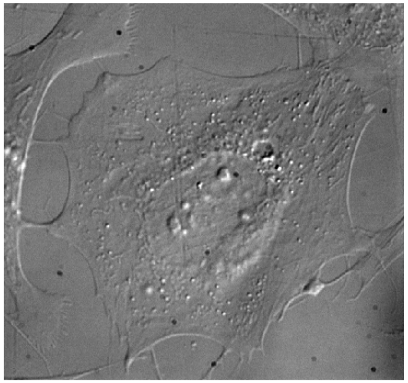
ECFP/FRET Ratio in Pseudo Color



mCherry



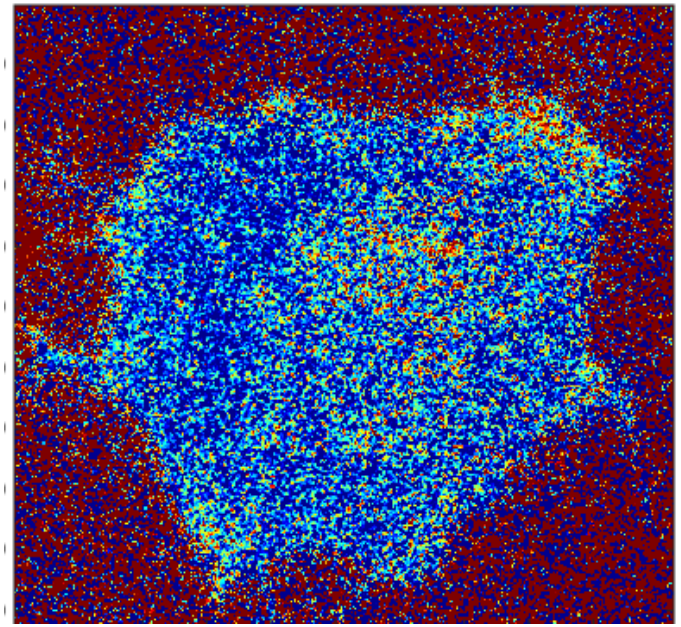
DIC



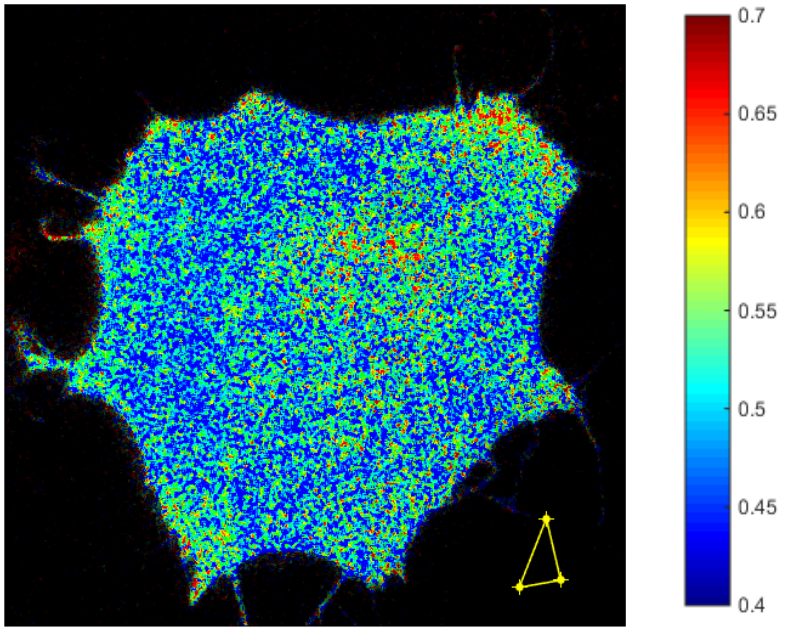
Color Image - Intensity Modified Display

HSV Color Image : hue = ECFP/FRET Ratio, s = 0, value = ECFP intensity

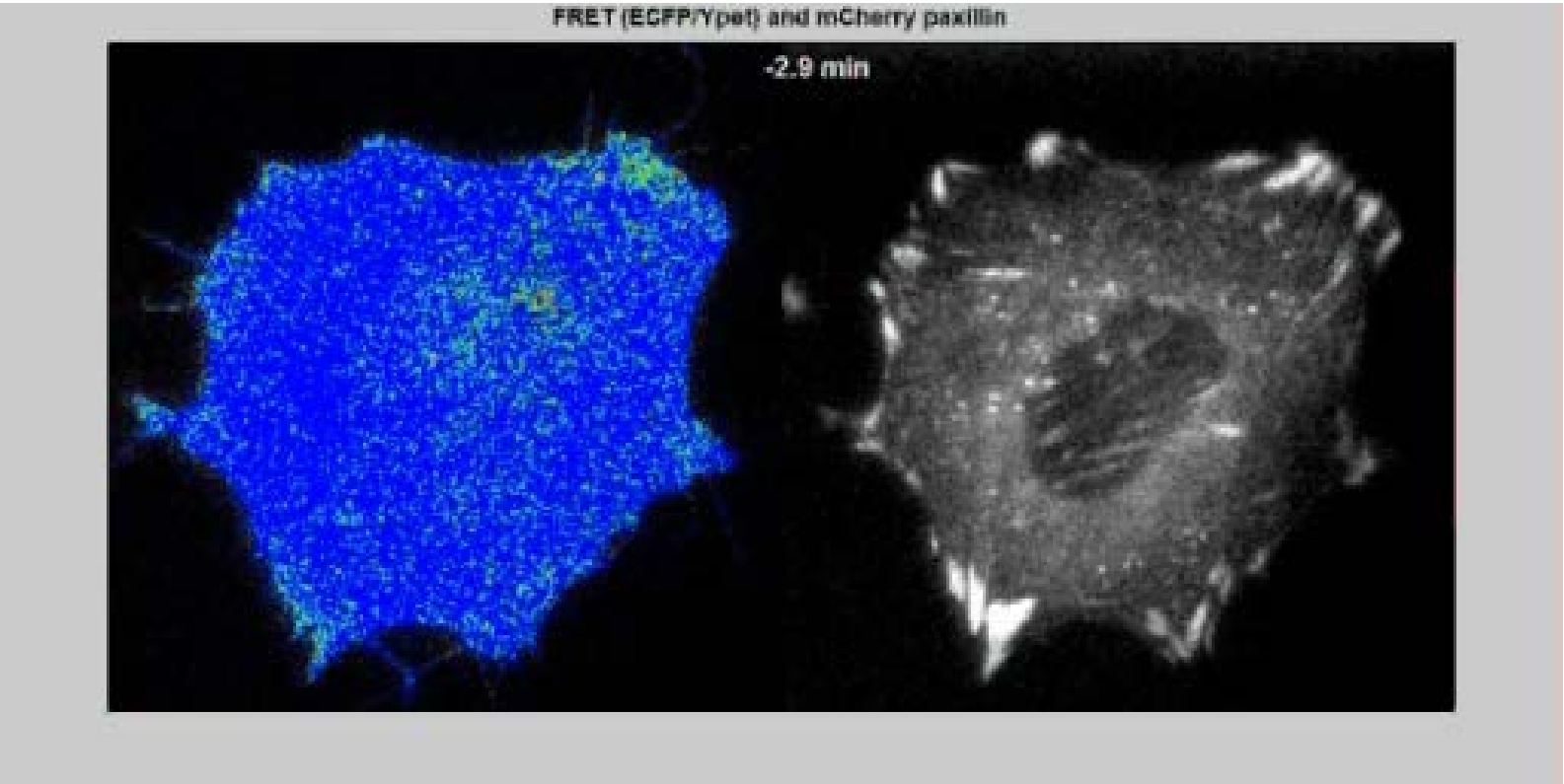
ECFP/FRET Ratio in Pseudo Color



ECFP/FRET Ratio (IMD Display)



Live-cell Video Images of ECFP/FRET Ratio



Quantitative Analysis of Live-cell Images

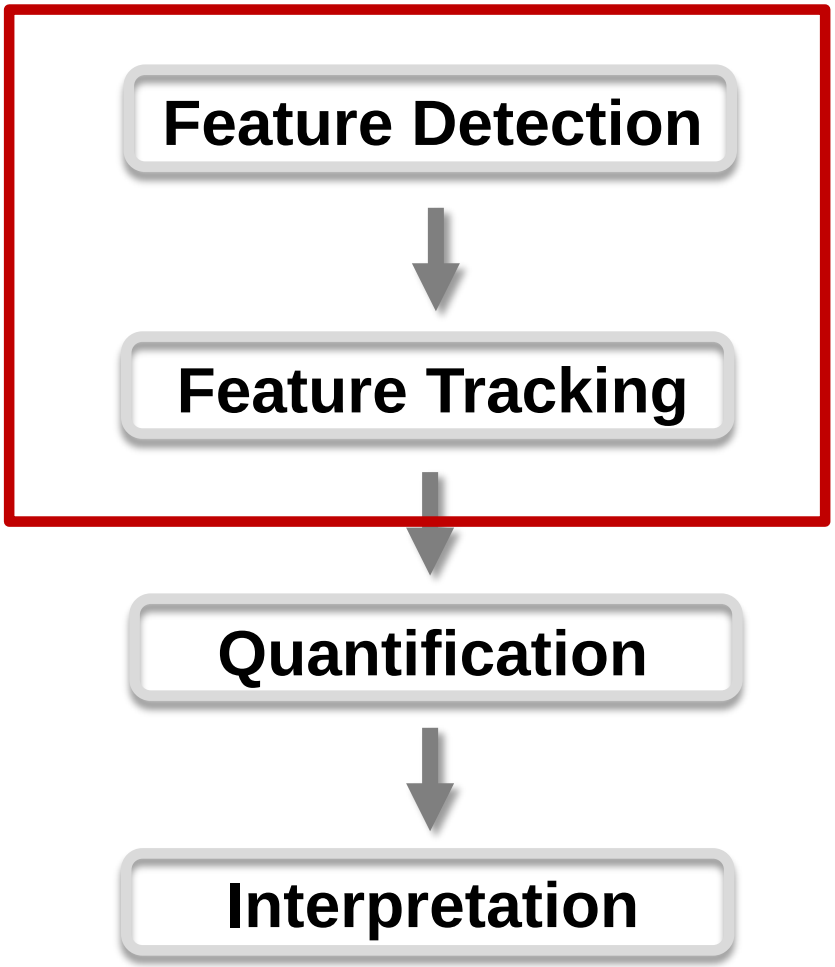
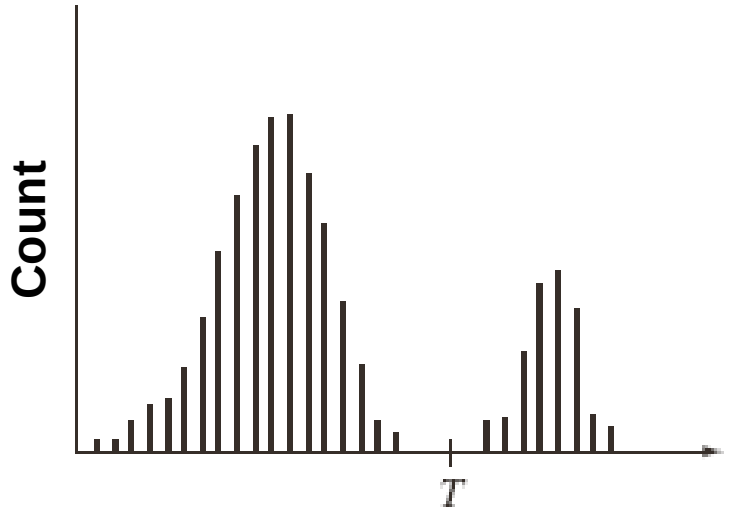


Image Segmentation - Thresholding

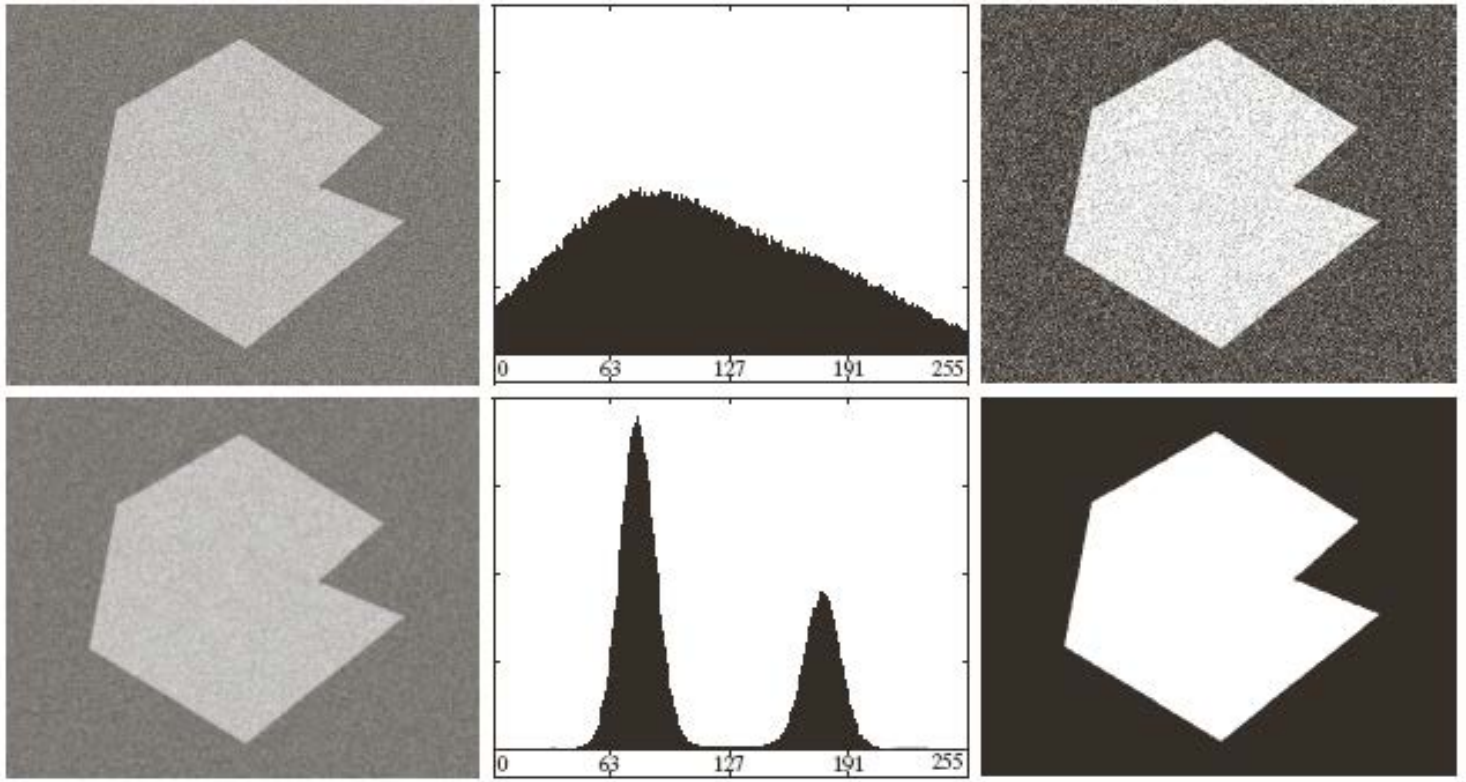
Suppose that the histogram corresponds to the image (matrix) *imA(i, j)*.



The thresholded (binary mask) image *maskA* can be defined as

$$maskA(i,\, j) = \begin{cases} 1, & \text{if } imA(i,\, j) > T \\ 0, & \text{if } imA(i,\, j) \le T \end{cases}$$

Use Image Smoothing to Improve Global Threshold

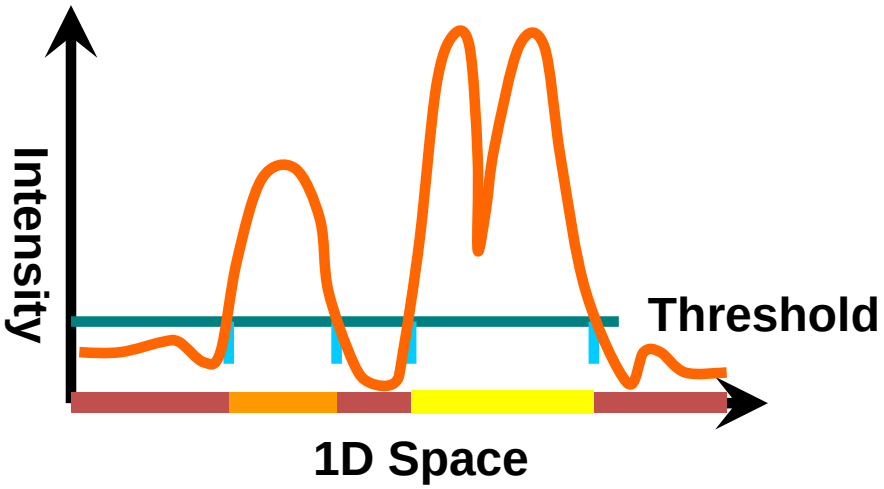


a b c
d e f

FIGURE 11.15 (a) Noisy image, and (b) its histogram. (c) Result obtained using Otsu's method. (d) Noisy image smoothed using a 5×5 averaging mask, and (e) its histogram. (f) Result of thresholding using Otsu's method.

Image Segmentation – The water algorithm

The Water Algorithm for Separating Bright Objects
Zamir E et al. 1999, J Cell Sci 112,1655–1669
Balaban NQ et al. 2001, Nat Cell Biol 3, 466–472



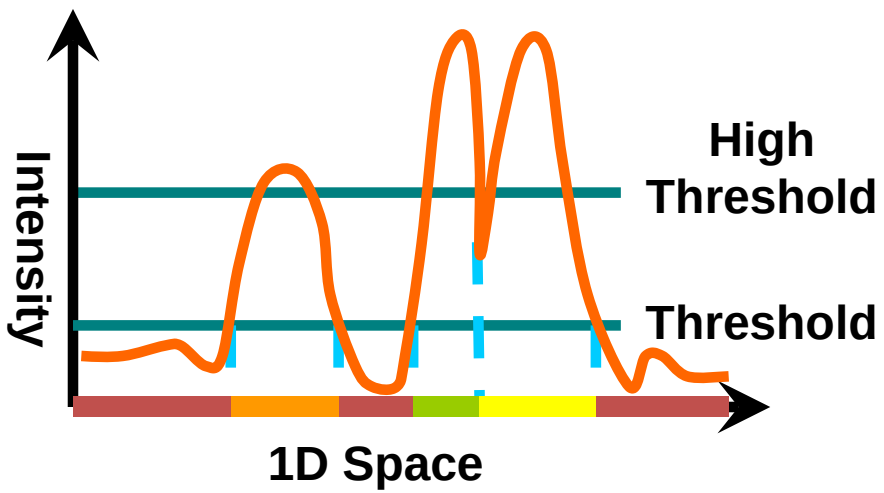
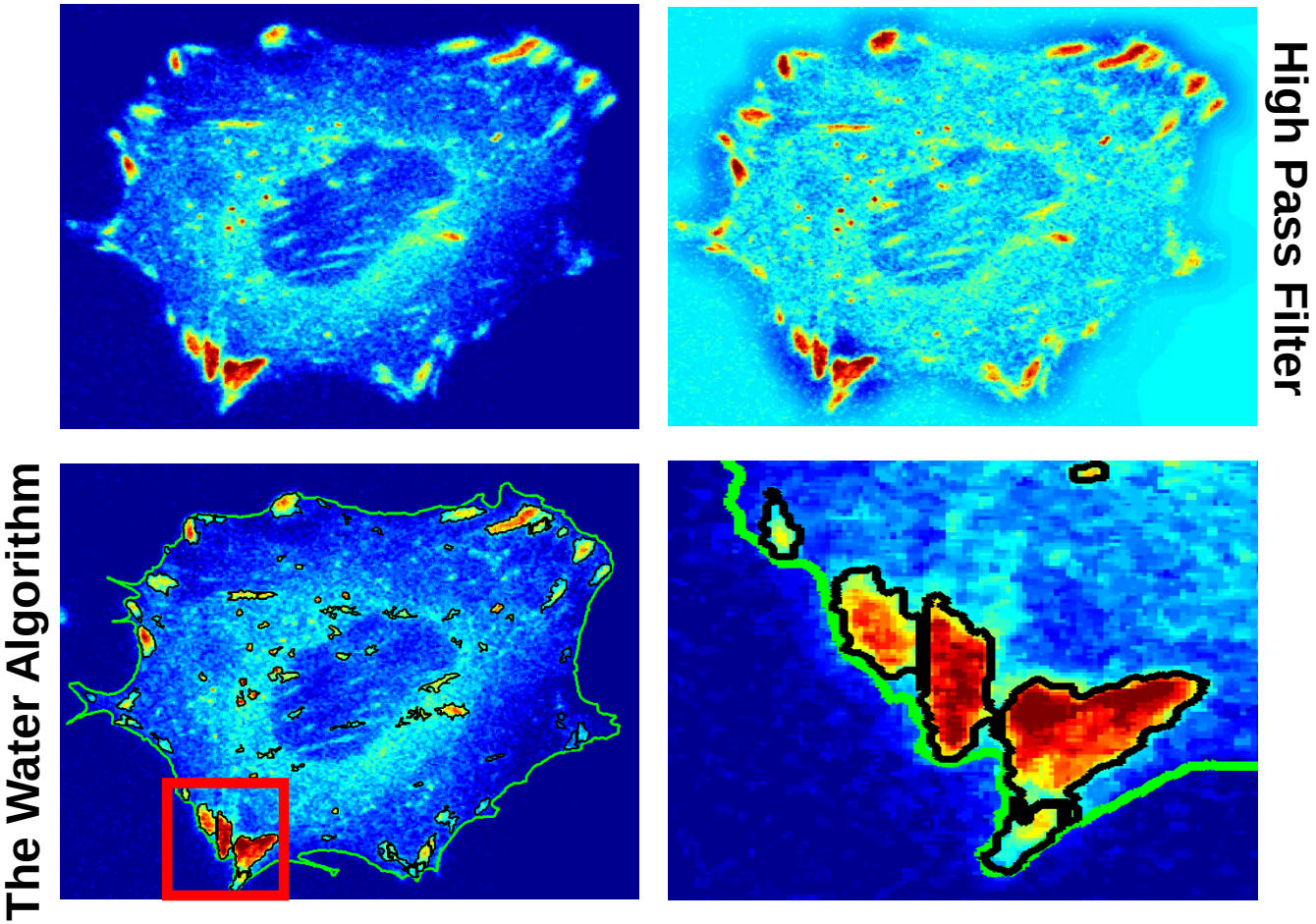


Image Segmentation – Focal Adhesion Detection



<u>Two problems:</u>

1. **The diffusive background is not uniform.**
2. **Bright spots of focal adhesions are connected.**

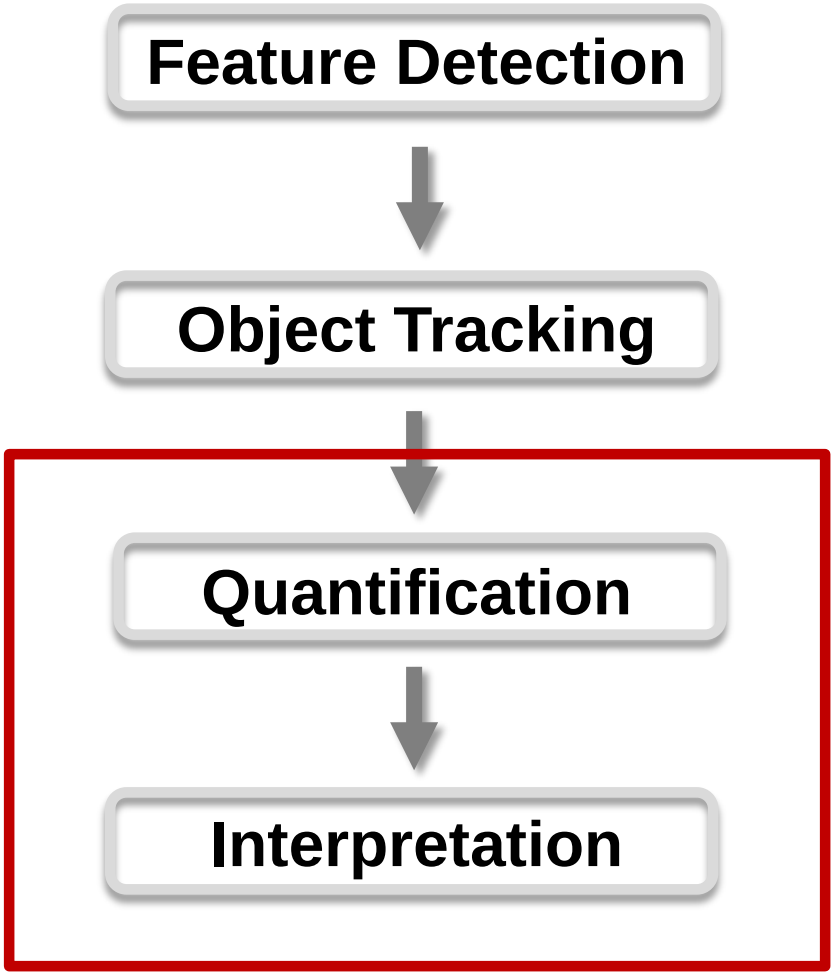
Tracking – Single Particle Tracking

- **Jaqaman et al 2008 Nature Methods 5, 8, 695**
- **Jaqaman et al 2013 Cell, 145, 593-606**
- **Chenouard N et al 2014 Nature Methods 11, 3, 281**

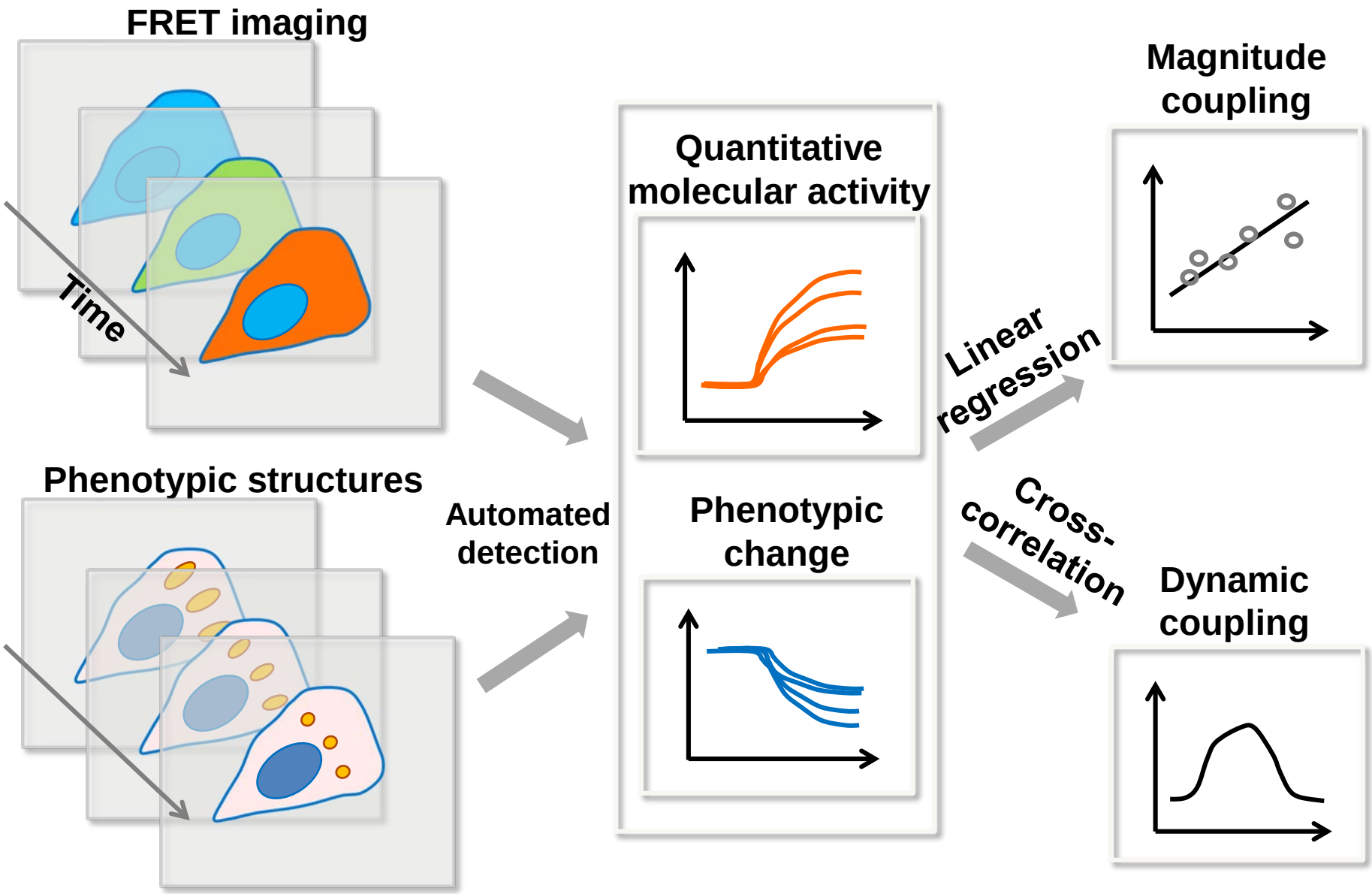
Particle Tracking/Linking

- **An objective comparison of particle tracking methods based on an open competition in 2012.**
- **Research groups world wide were invited to participate.**
- **Registered teams were given 1 month to prepare their methods.**
- **After release of the actual competition data set, without ground truth, the teams submitted tracking results to an independent evaluator.**

Quantitative Analysis of Live-cell Images

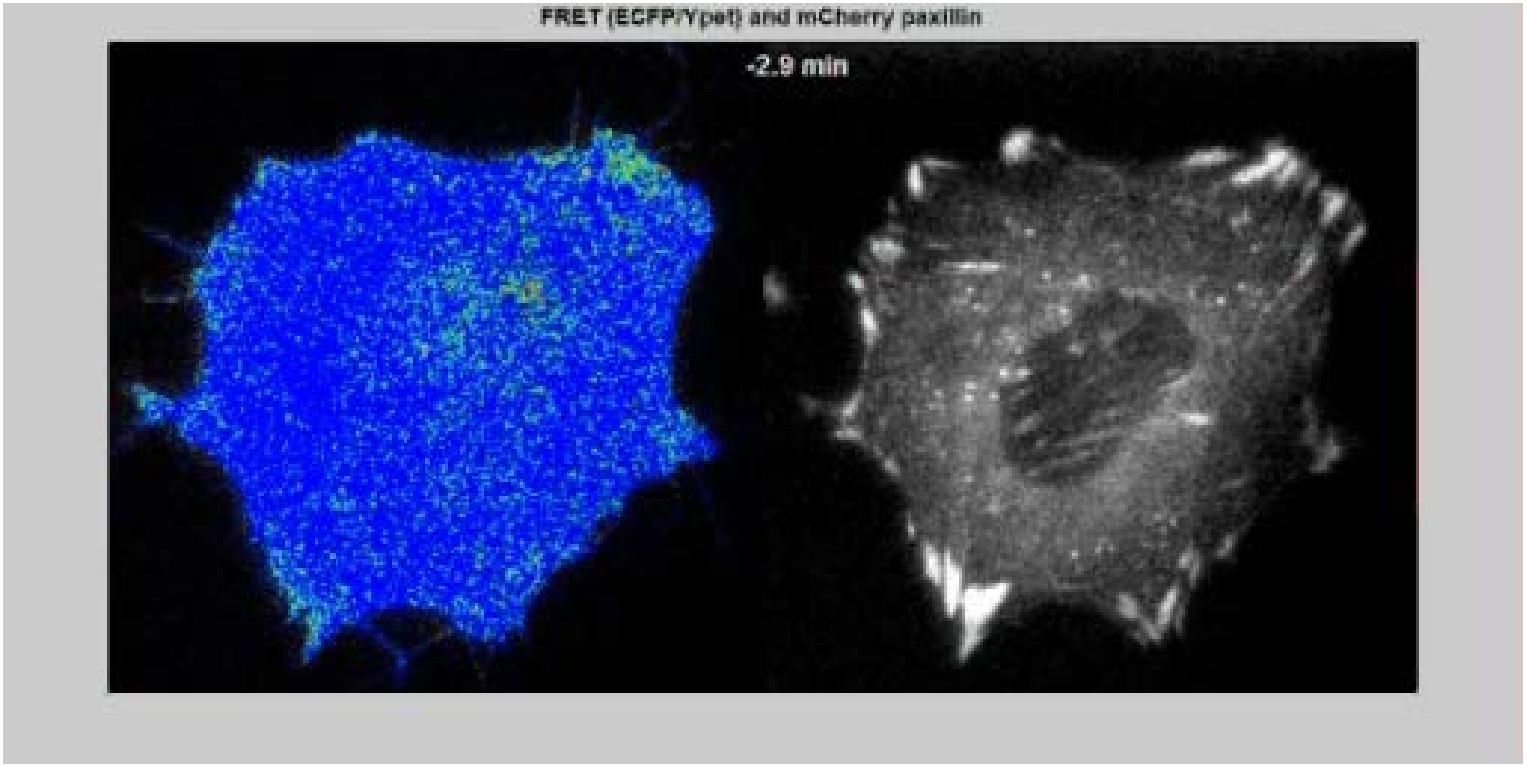


Correlative FRET Imaging Microscopy (CFIM)

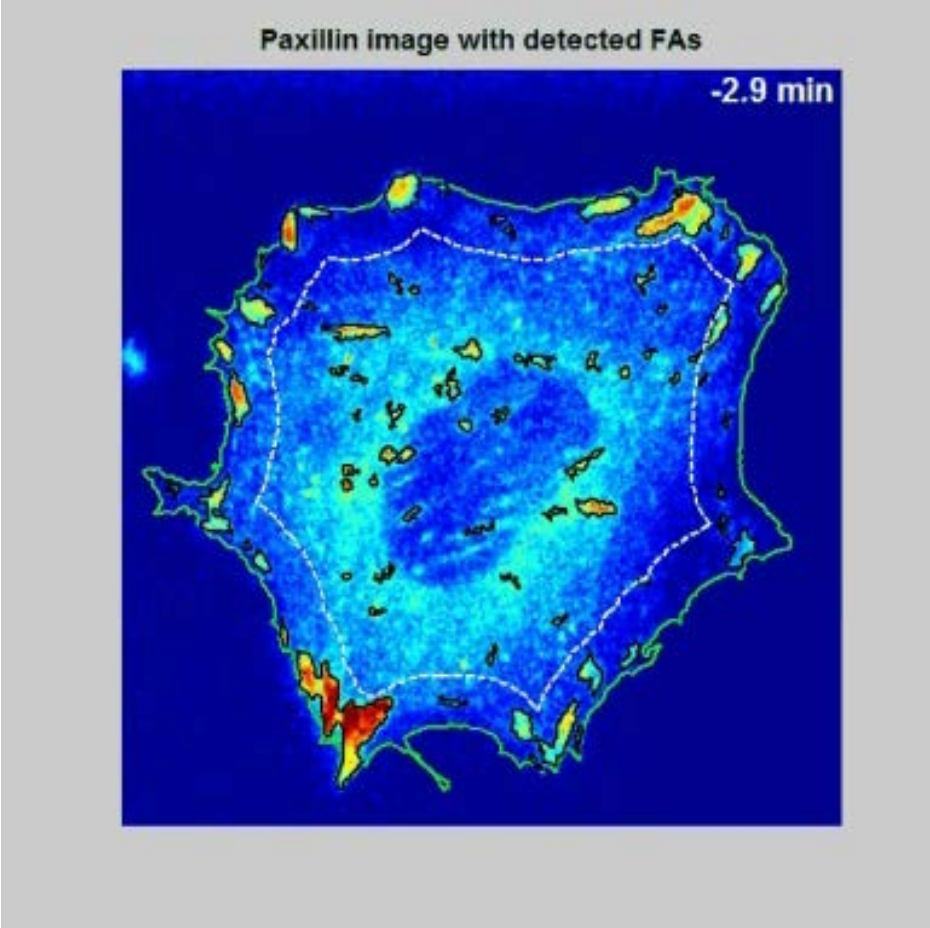


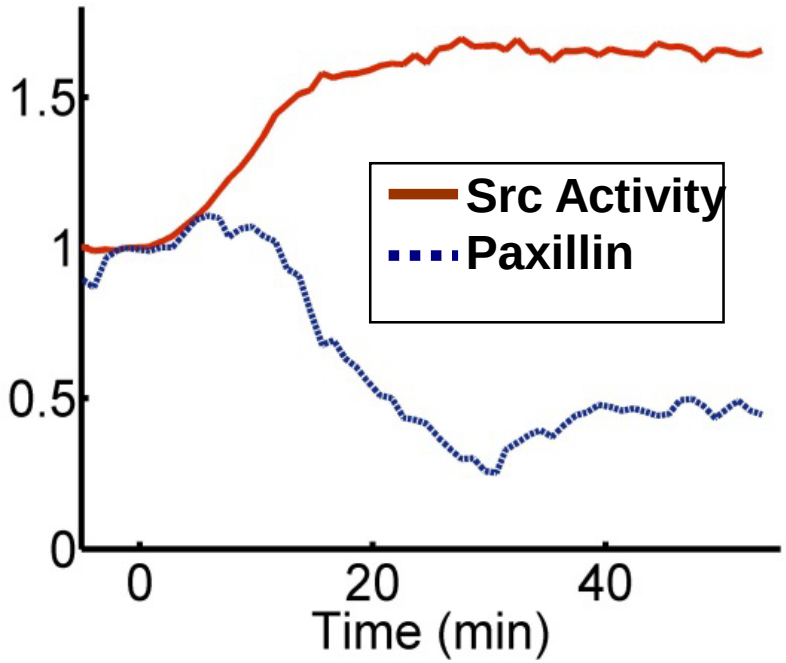
Lu S. et al, Scientific Reports, 2014

Growth Factor Induced Src Activation and Focal Adhesion Disassembly

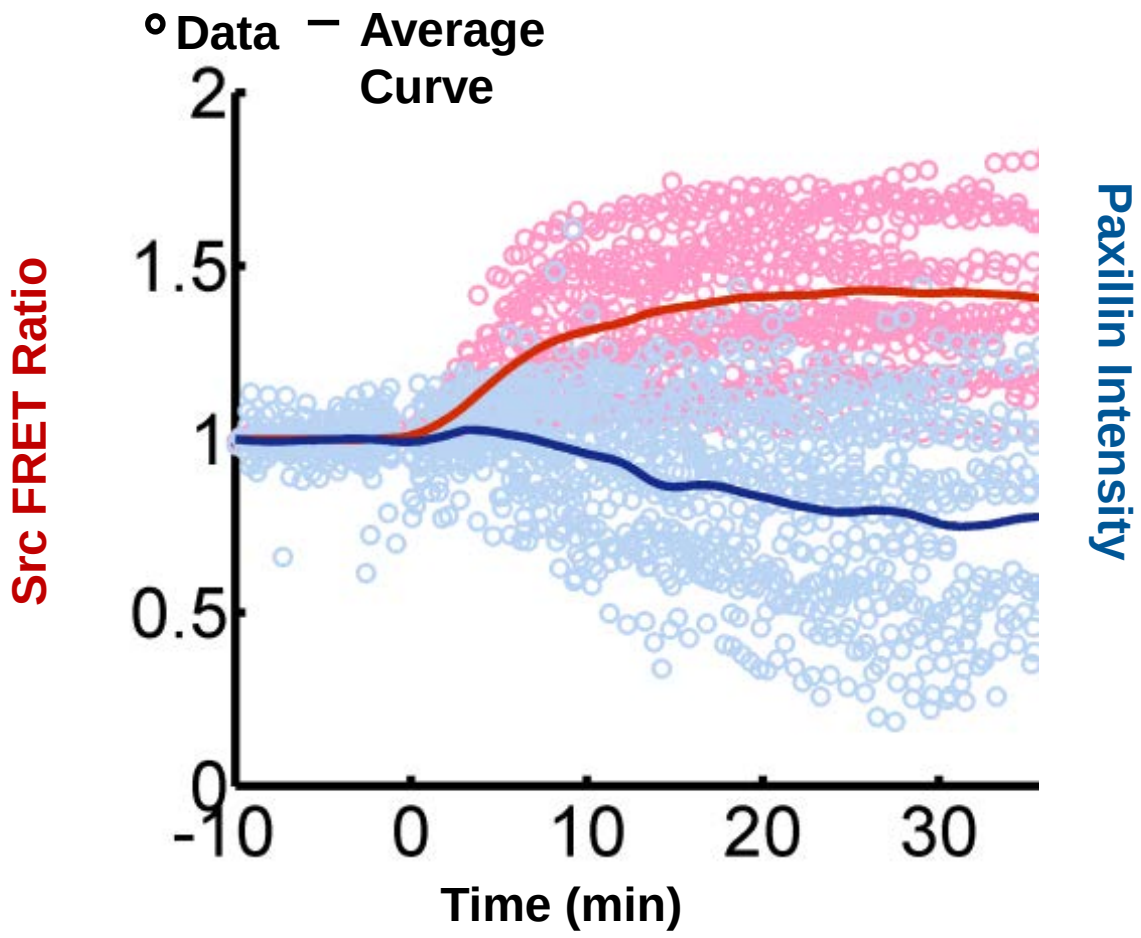


FA Detection and Quantification

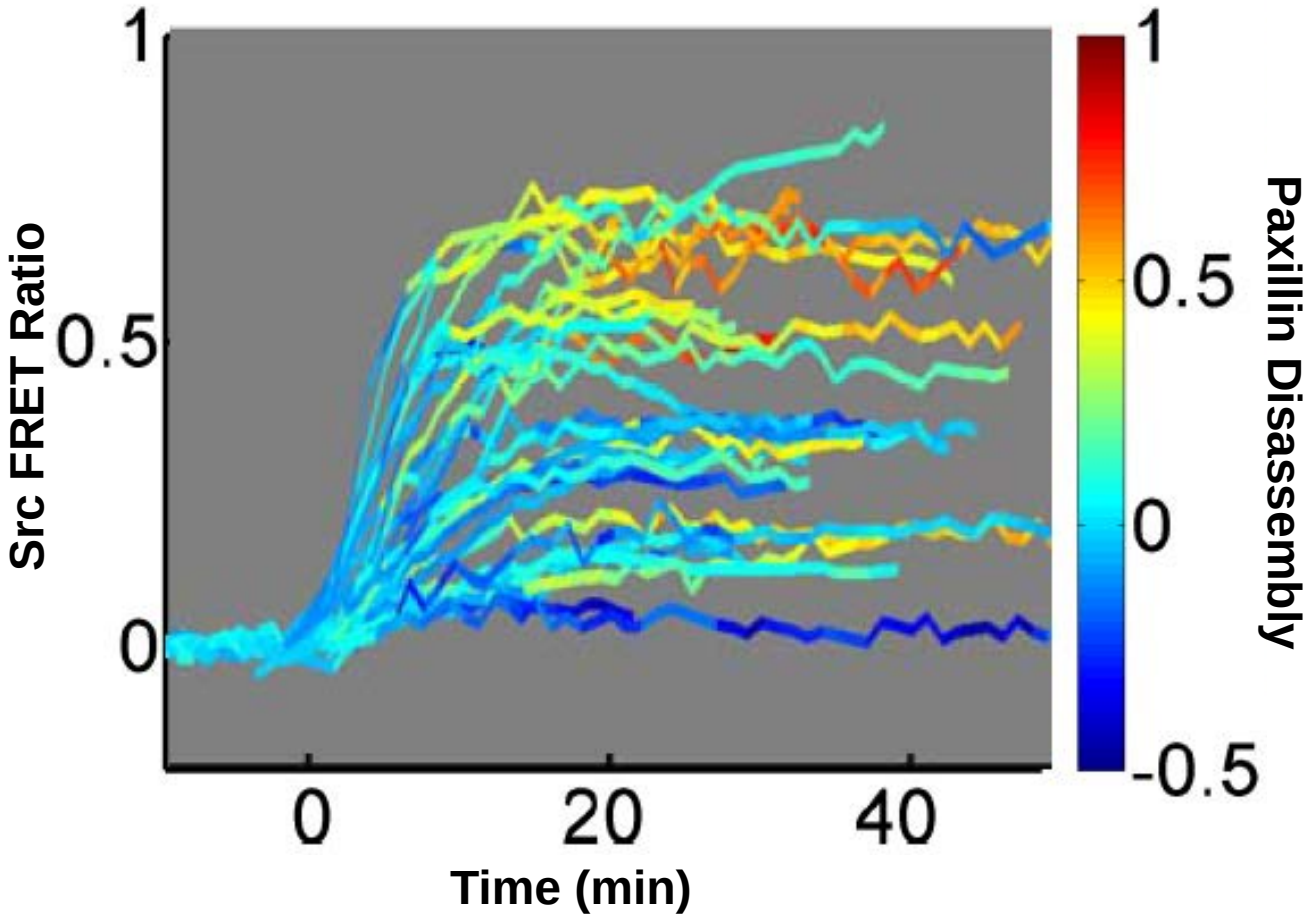




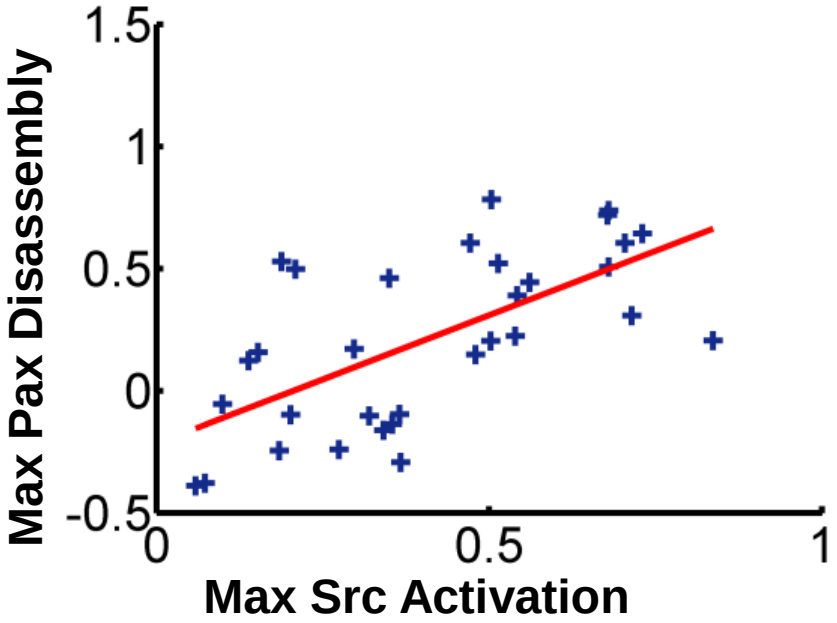
Putting Single Cell Data Together



Putting Different Signals Together



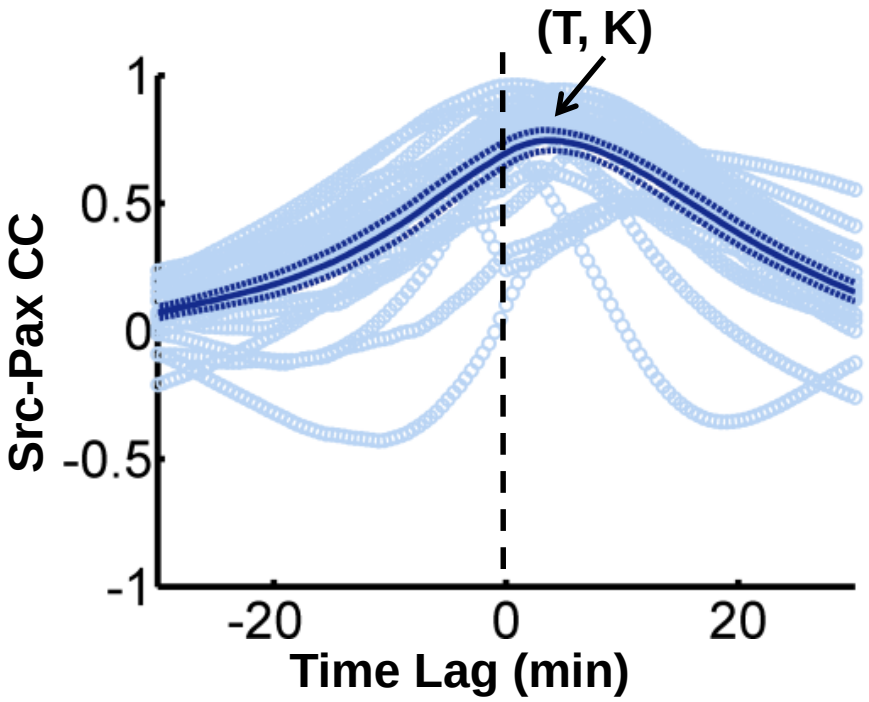
Magnitude Coupling



- Linear Correlation Coefficient – R: measures the degree of Src-paxillin magnitude coupling.

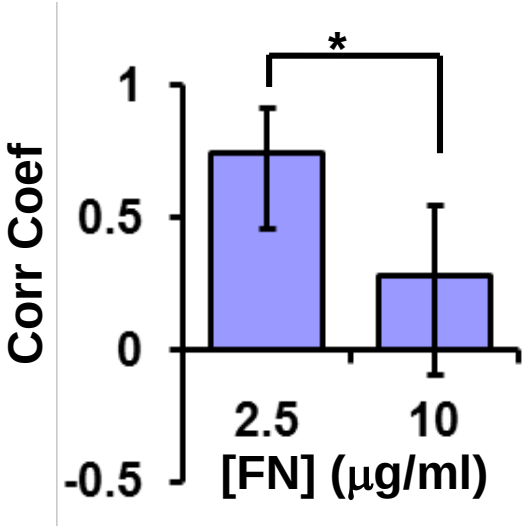
- Slope - *S*: measures the capacity of Src activity in causing paxillin disassembly, or the amount of paxillin disassembly per unit of Src activation.

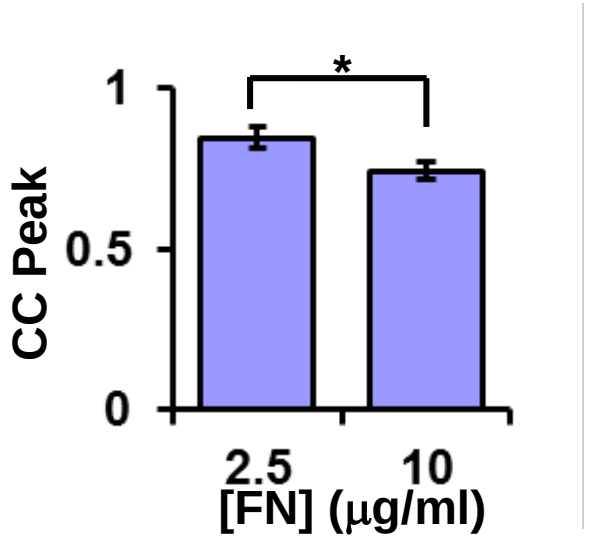
Dynamic Coupling

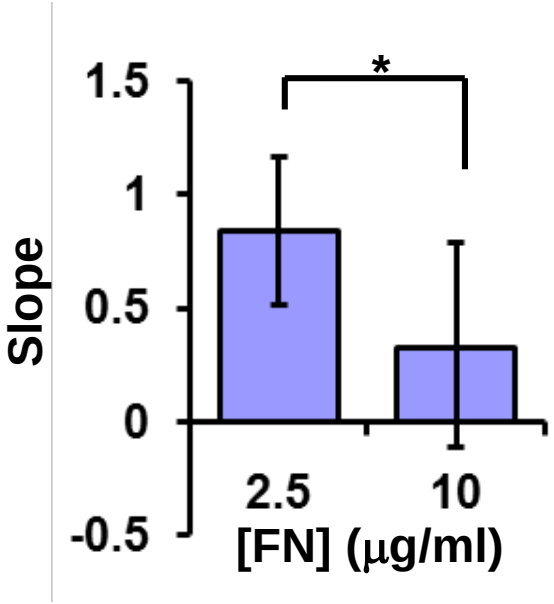


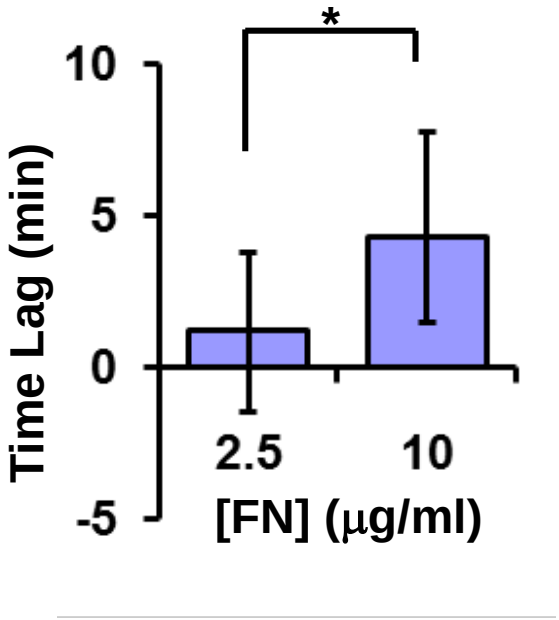
- Cross-correlation Peak– K: measures the similarity between the time courses of Src activation and paxillin disassembly.
- Time Lag – T : The time lag between Src activation and paxillin disassembly.

Effect of Matrix Protein Concentration

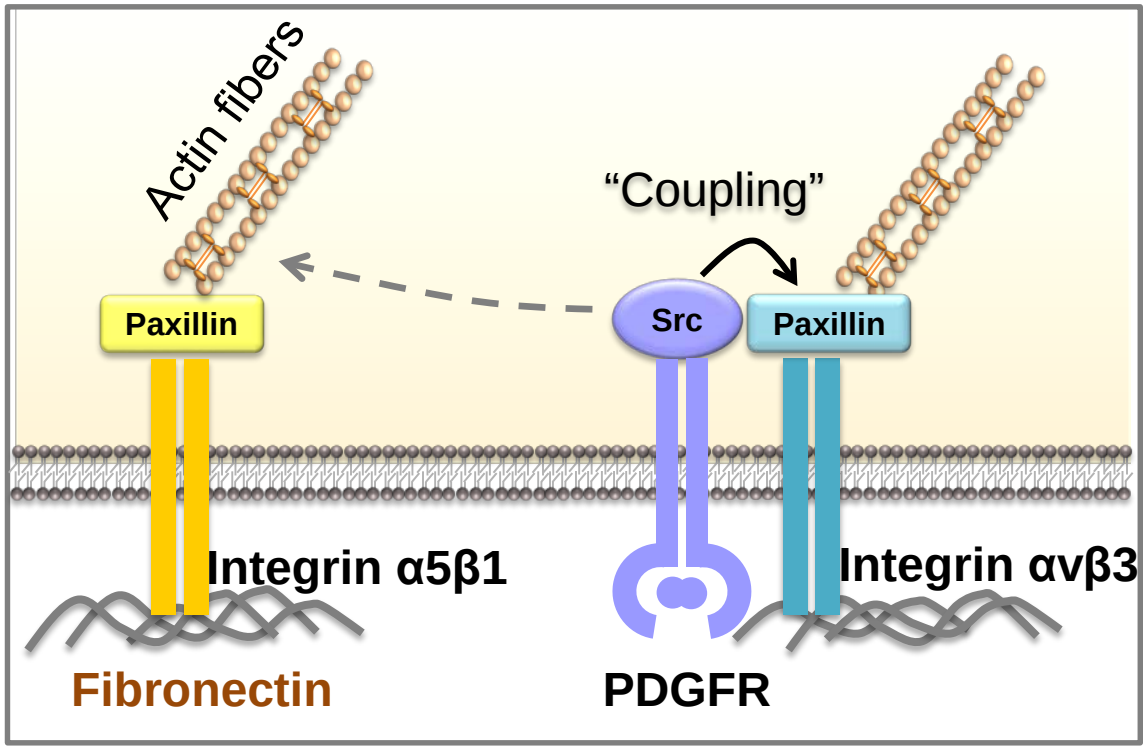








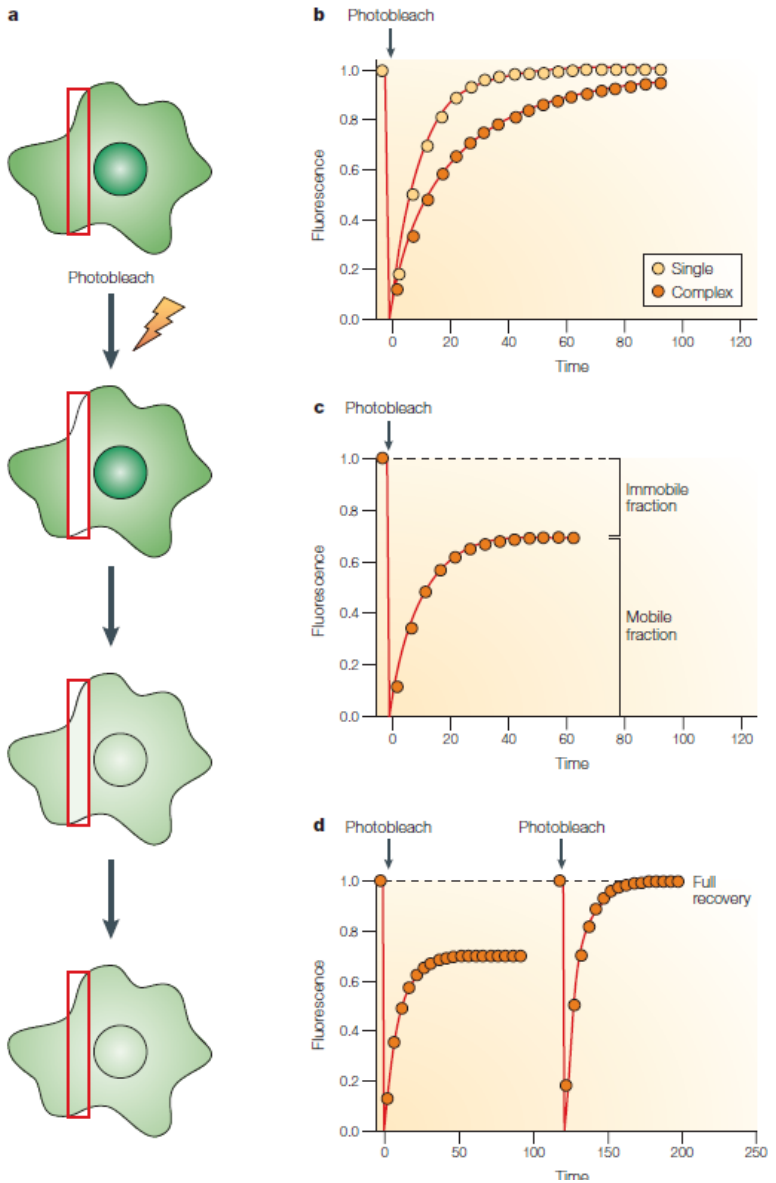
Molecular Model of coupling between Src activity and FA disassembly



Lu S. et al, Scientific Reports, 2014

Photobleaching and Photoactivation Following Intracellular Protein Dynamics

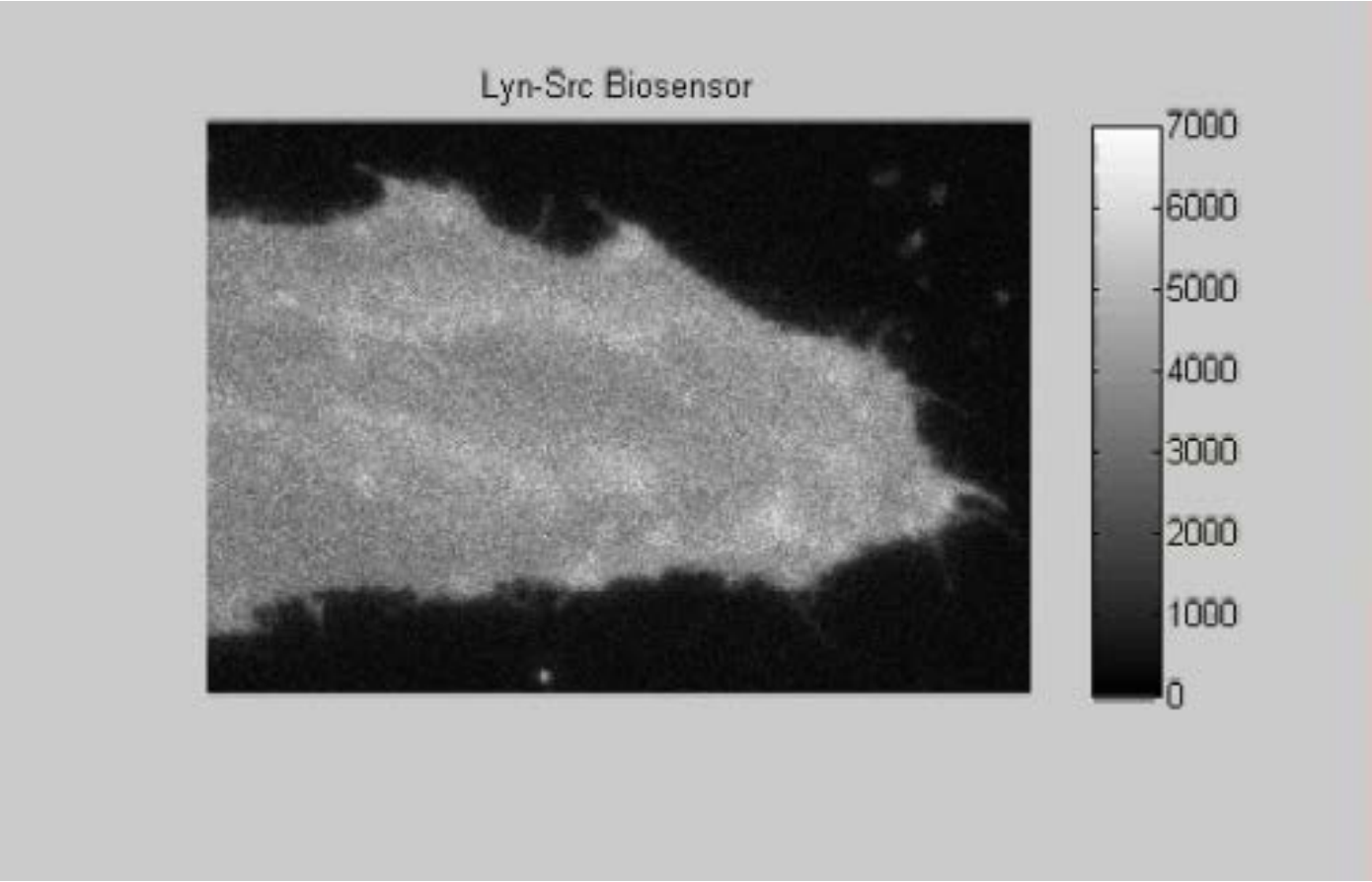
- **Lippincott-Schwartz J et al. 2003 Nat Cell Biol.**
- **Klonis N et al. 2002 European Biophys J.**
- **Lu S et al. 2008 PLoS Comput Biol.**
- **Capoulade J et al. 2011 Nat Biotech.**



Photobleach Analysis

	Classical Analysis Based on Recovery	Finite Element Model (FEM) Based Analysis	Fluorescence Correlation Spectroscopy (FCS)
Experimental Duration	Long	Short	Short
Signal to Noise Ratio	High	High	Low
Flexible PB Pattern	No	Yes	N/A
Flexible Cell Geometry	No	Yes	Yes

Fluorescence Recovery After Photobleaching (FRAP)



Lu S. et al, Scientific Reports, 2014

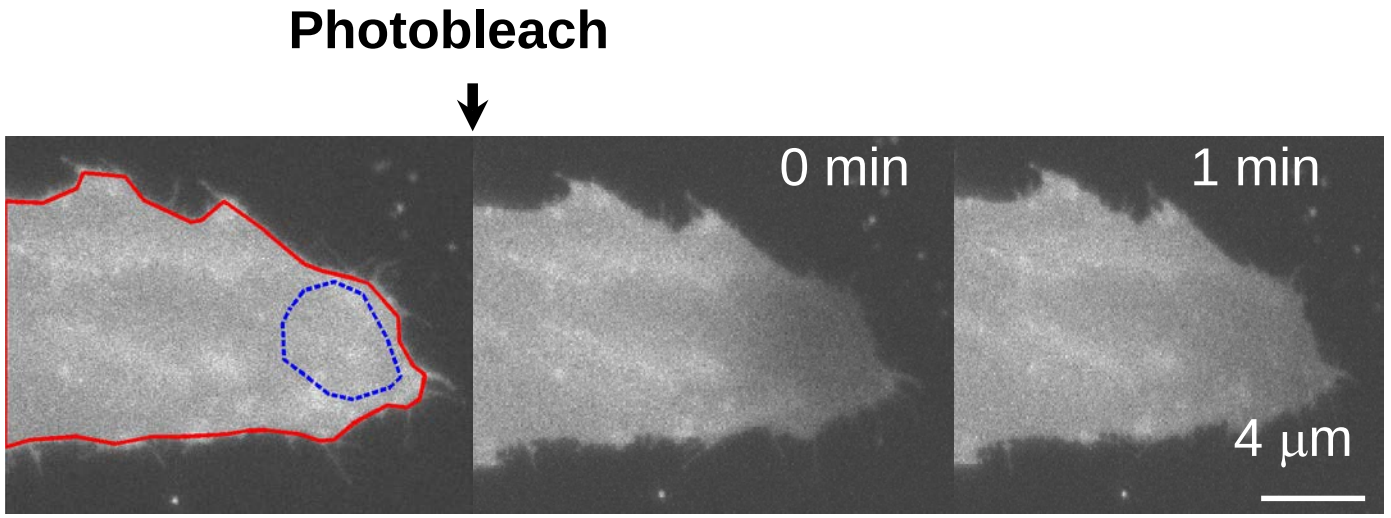
Diffusion Model (2D) and FE-discretization

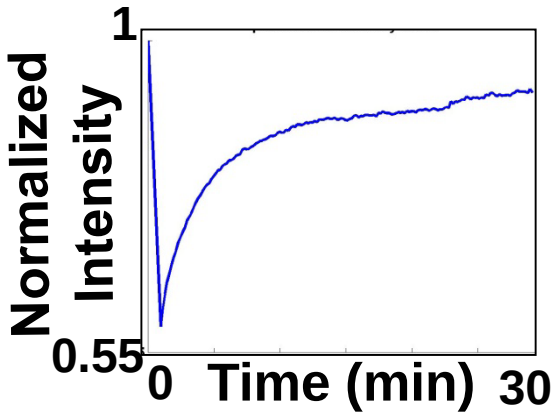
$$\frac{\partial u(x,y,t)}{\partial t} = D \bullet \Delta u(x,y,t)$$

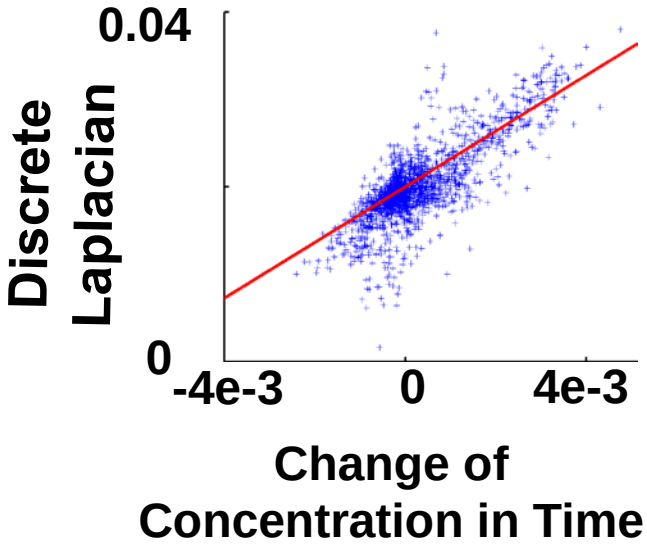
Finite Element Discretization (Crank-Nicholson Scheme in Time)

$$\frac{M(u^{n+1} - u^n)}{dt} = D \bullet \frac{-K(u^{n+1} + u^n)}{2} + r$$

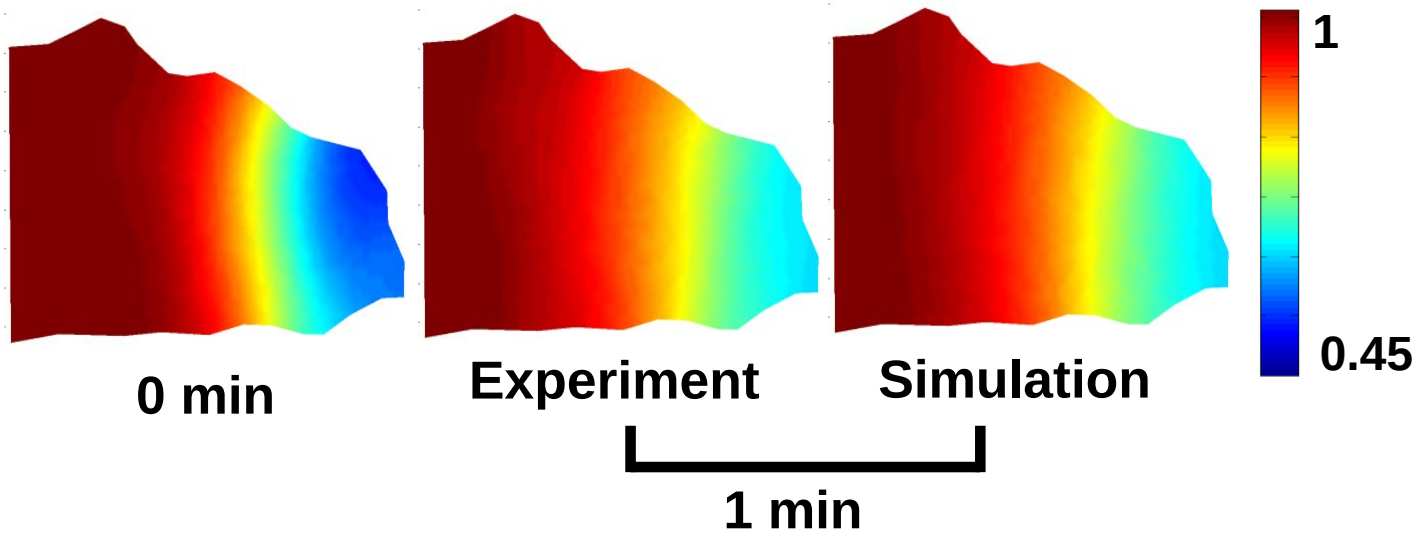
Comparison of Experiment and Simulation





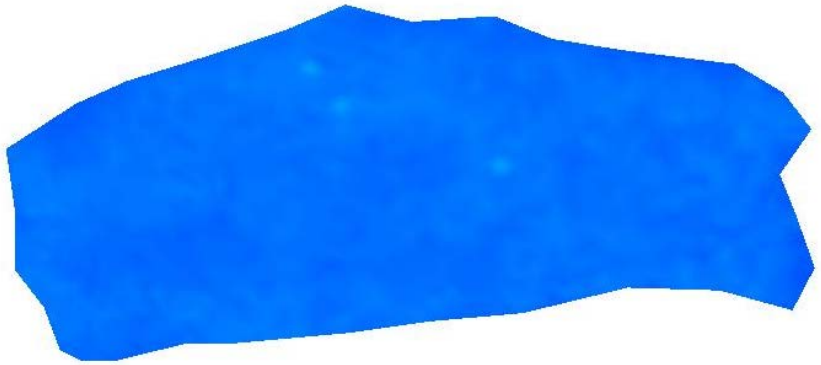


Comparison of Experiment and Simulation

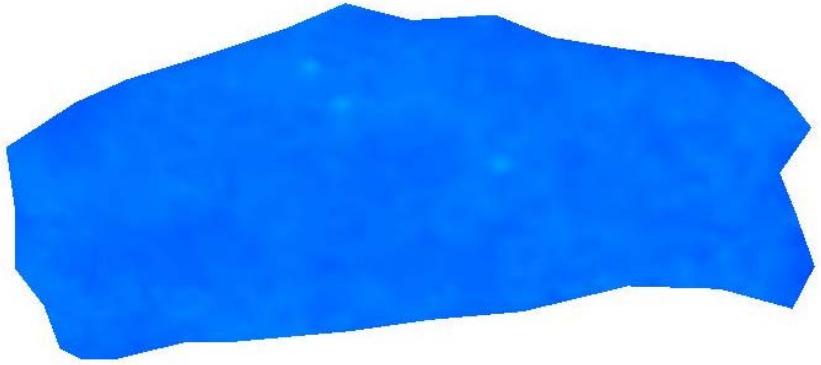


Subtraction of Diffusion in FRET Imaging

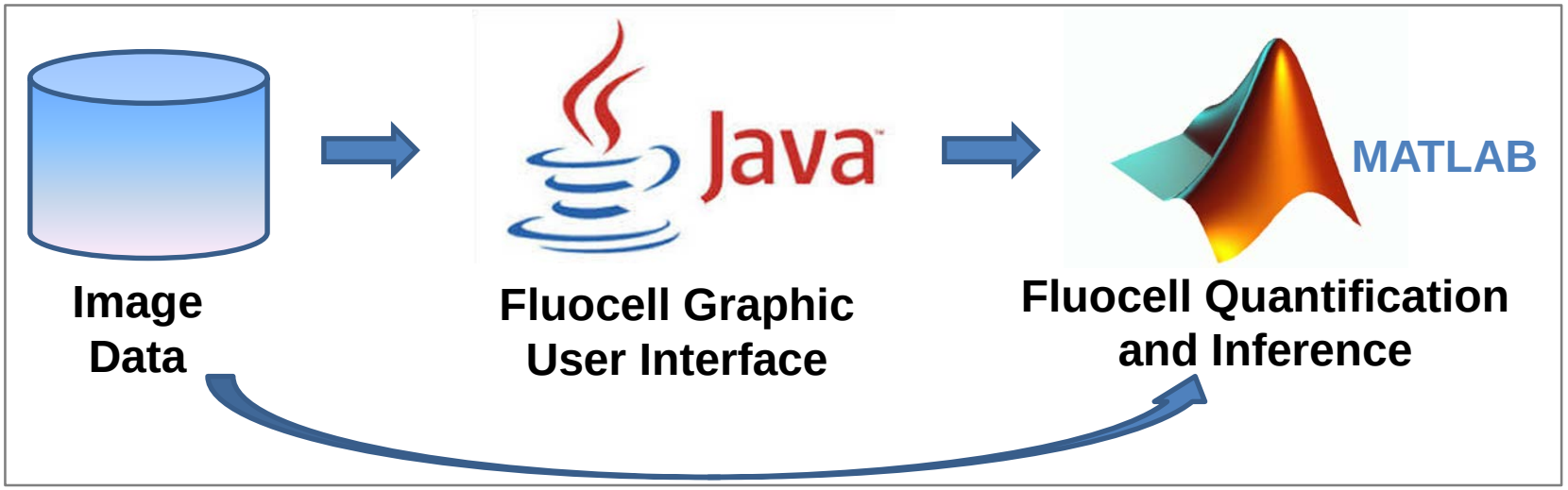
FRET



Subtract Diffusion



Fluocell Image Analysis Software Package



Download: http://code.google.com/p/fluocell

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