Quantitative Microscopy:

Bridge between “wet” biology and computer science

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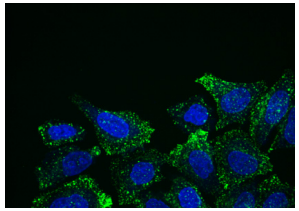
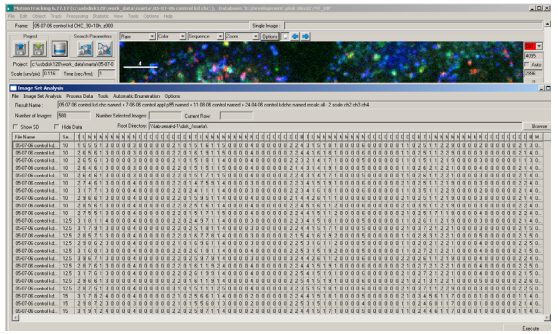
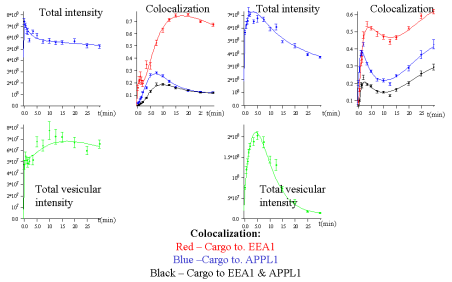
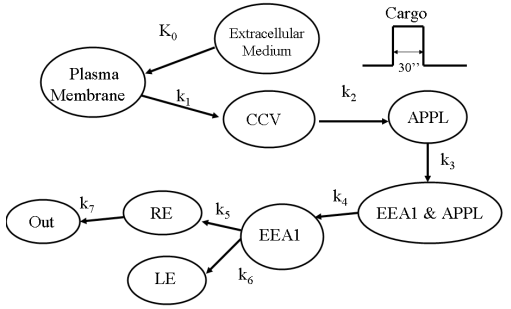
System Biology:
Experiment Driven Model and Model Driven Experiment









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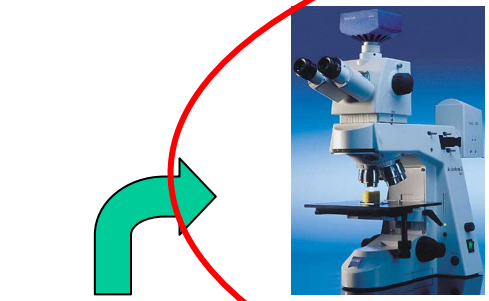
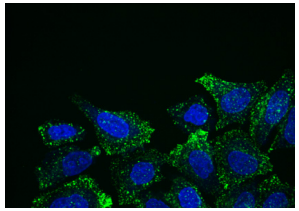
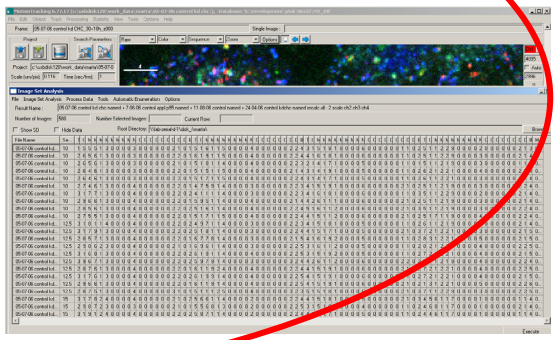
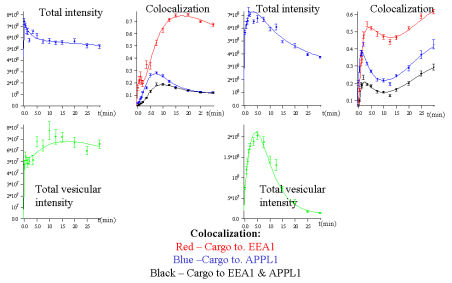
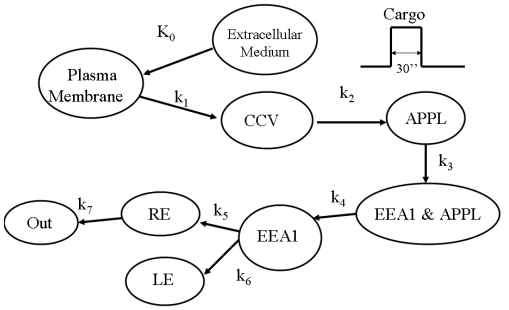




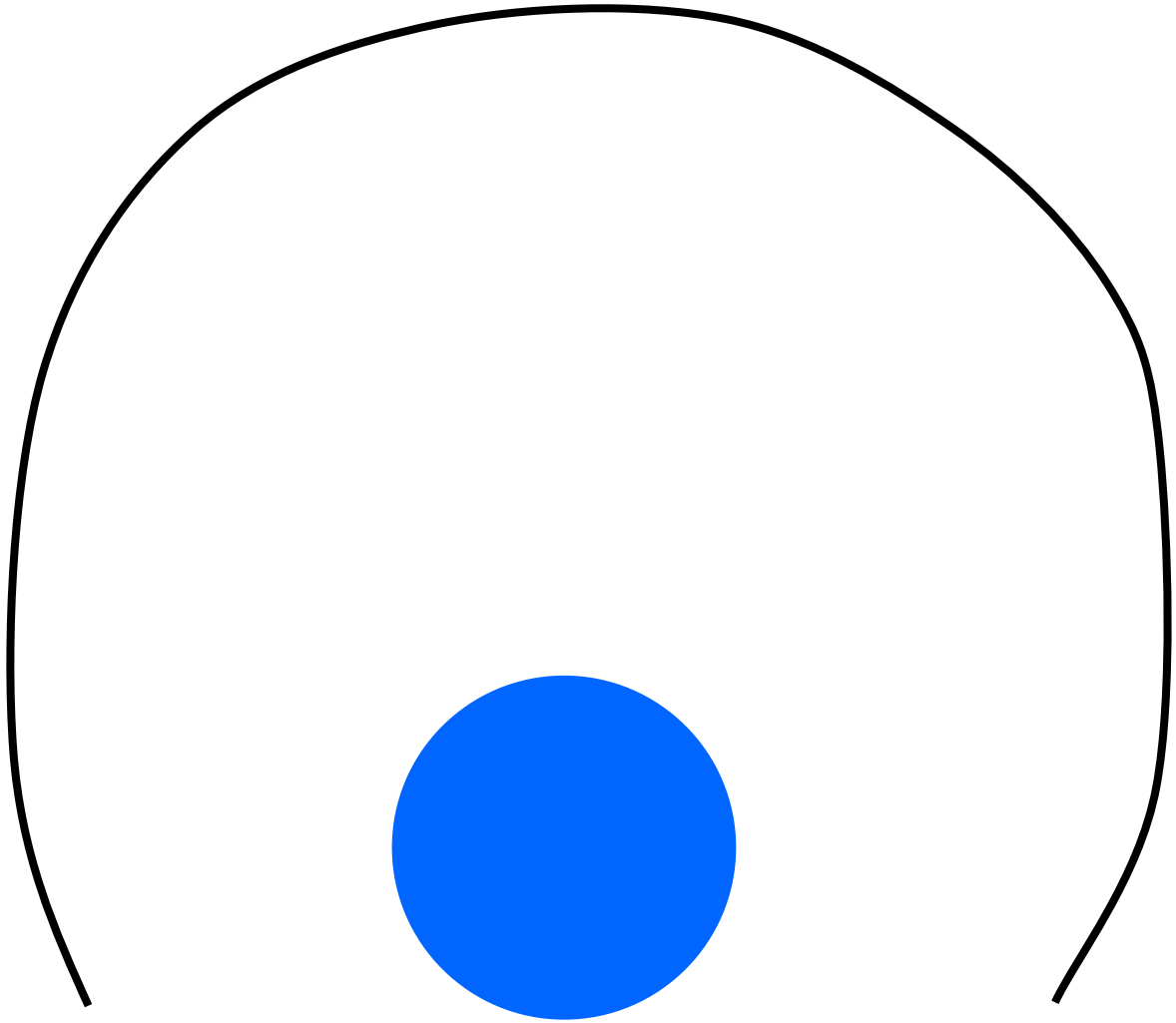






Quantitative Microscopy in wild : Learning endocytosis kinetic model from pulse-chase experiment

Endocytosis: Cellular Organelles Transport Cargo



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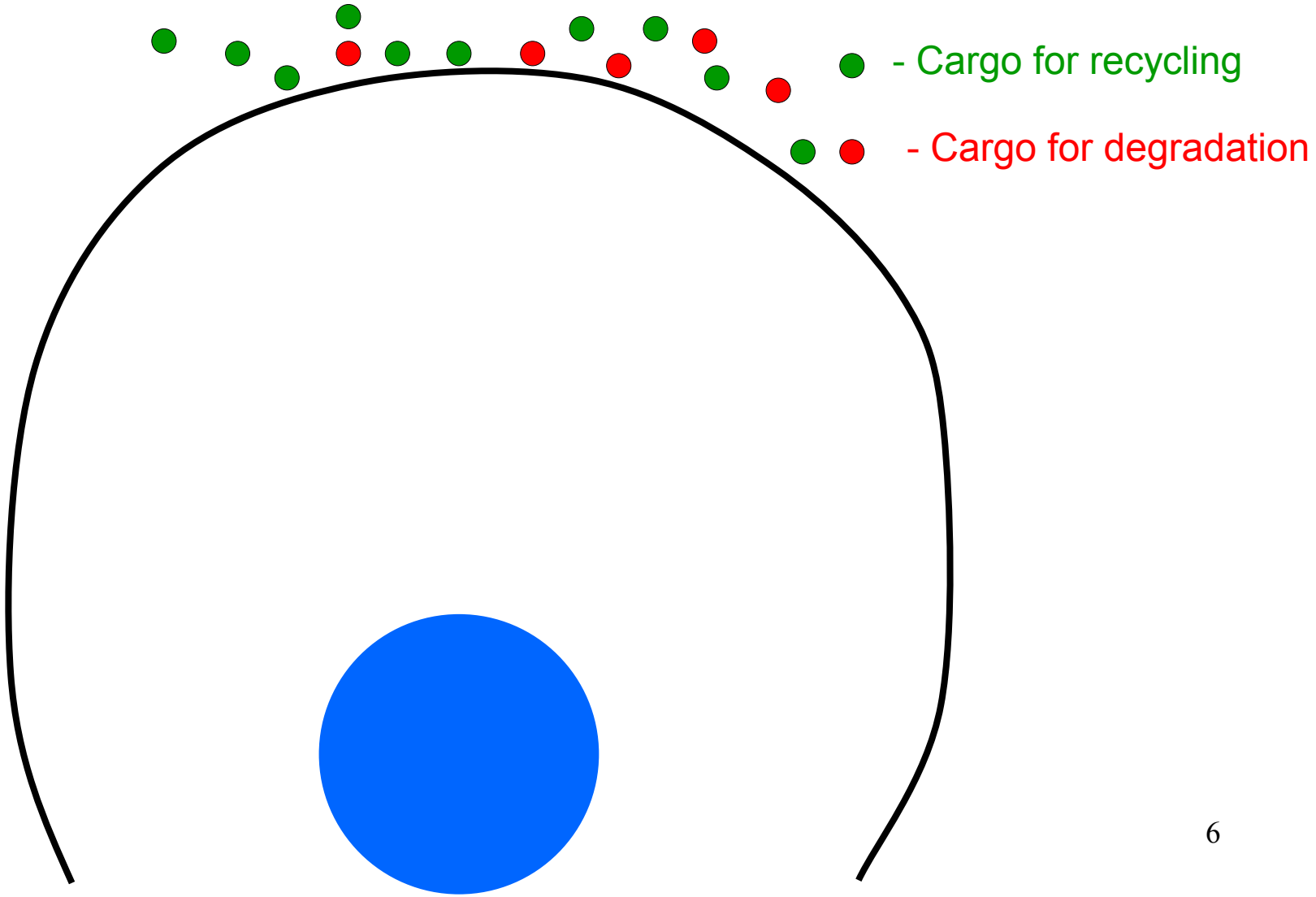

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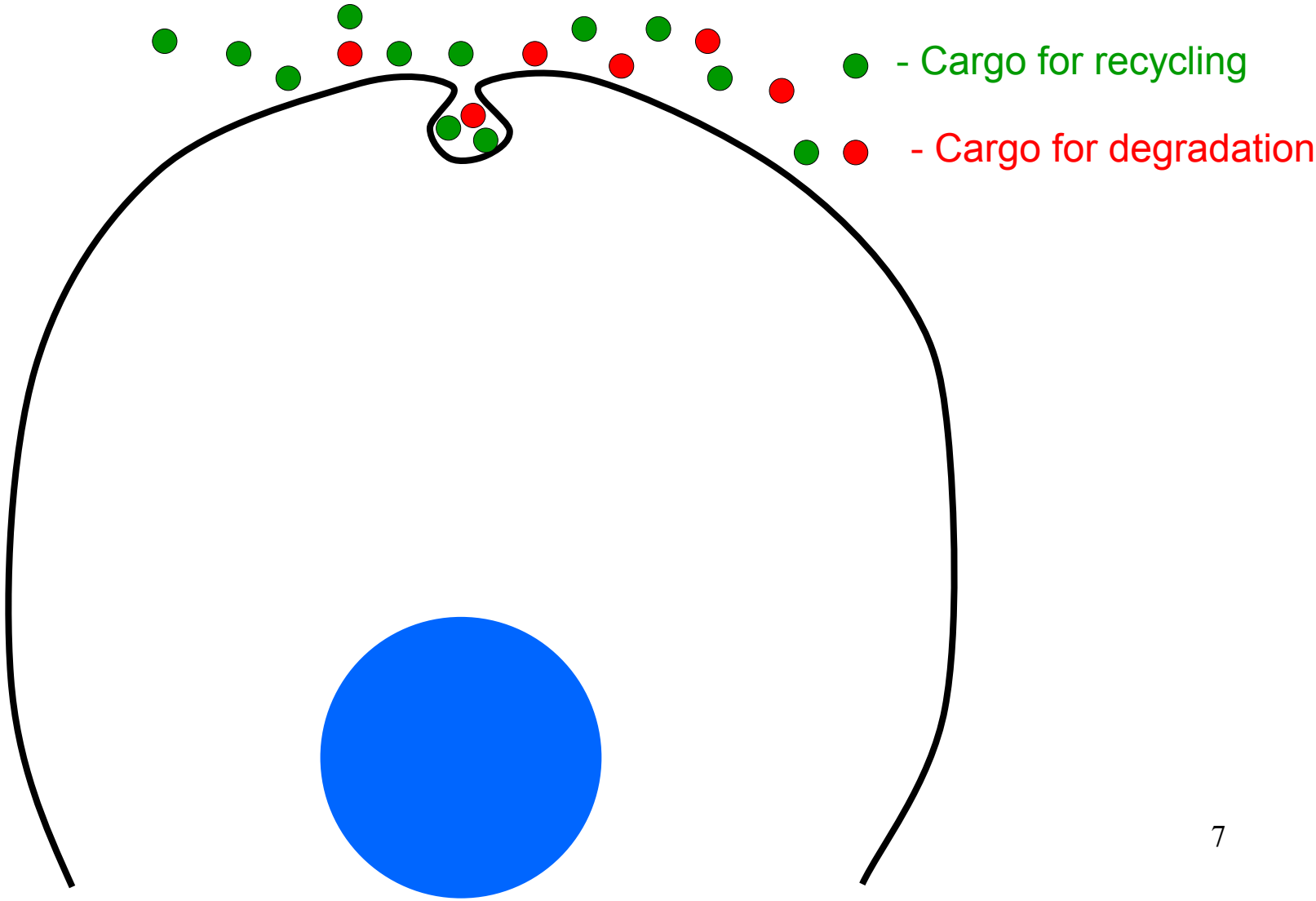

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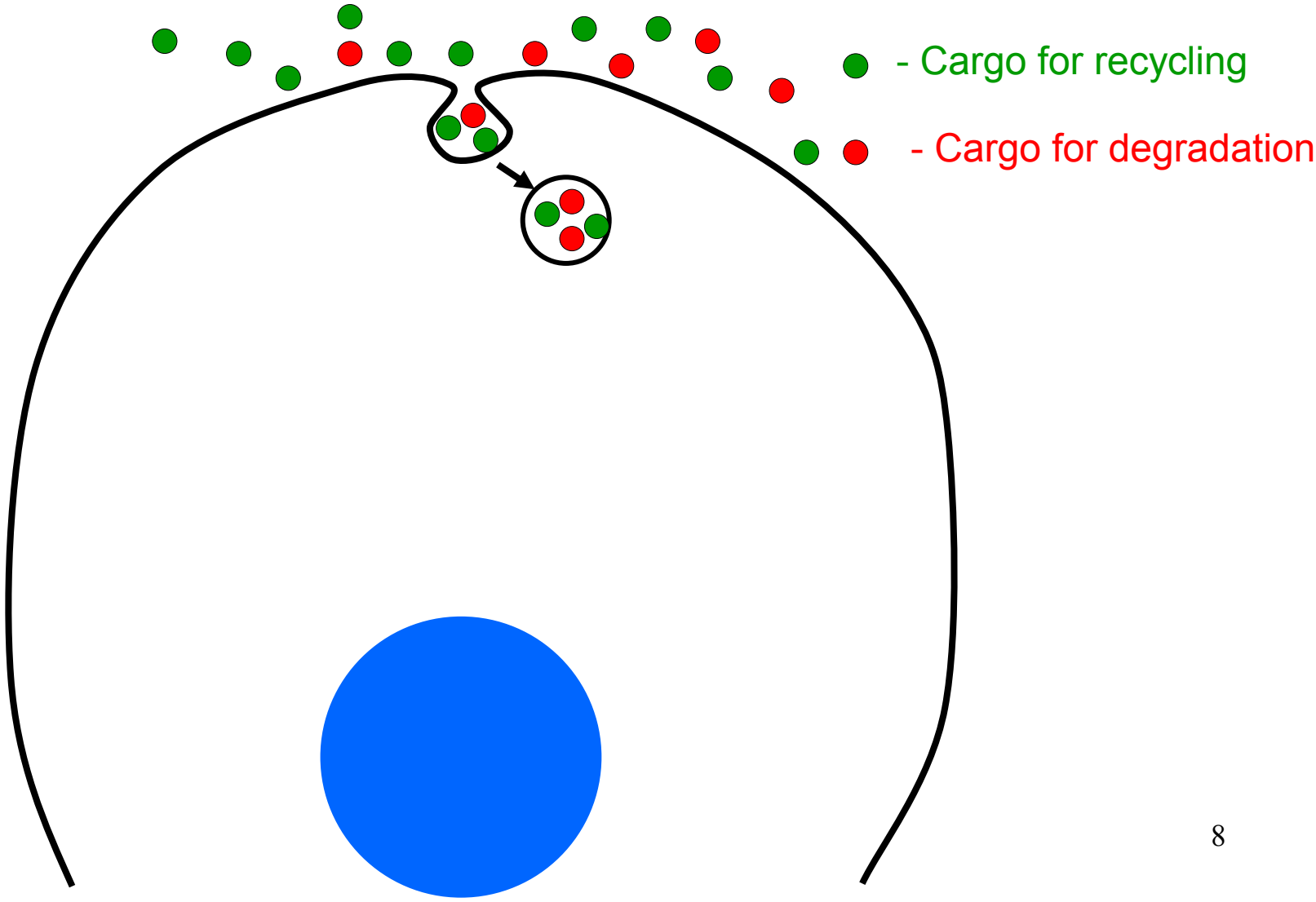

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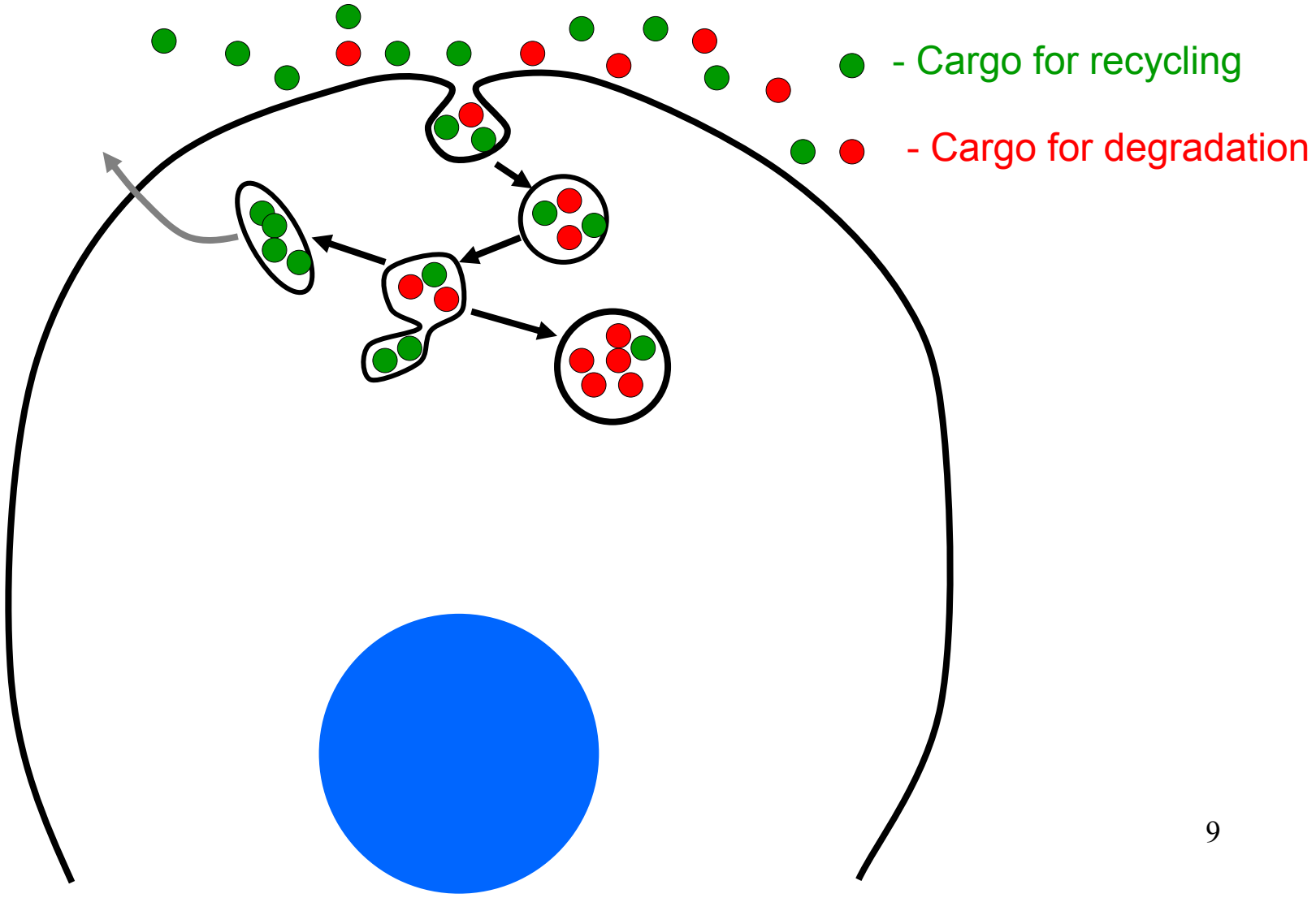

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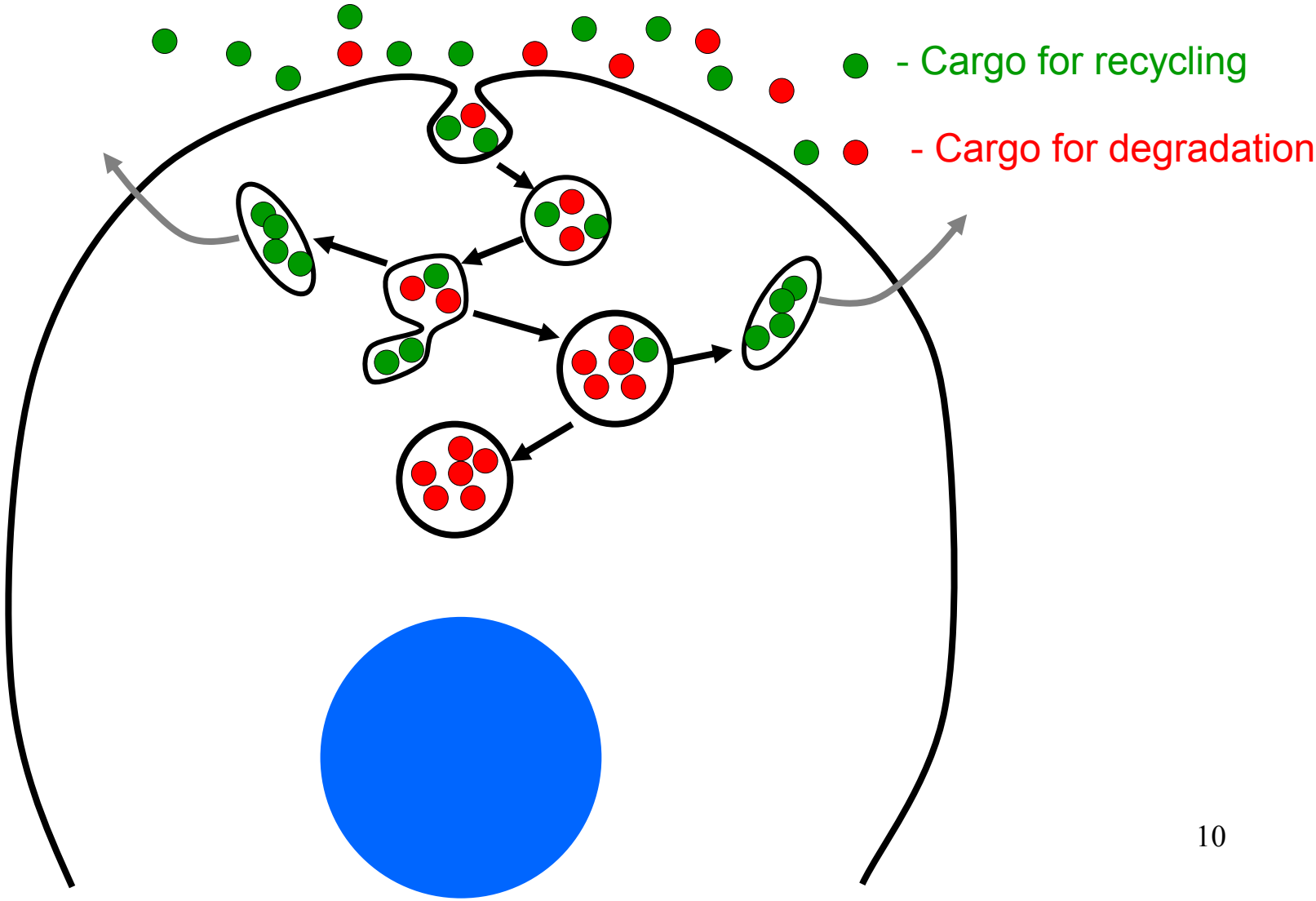
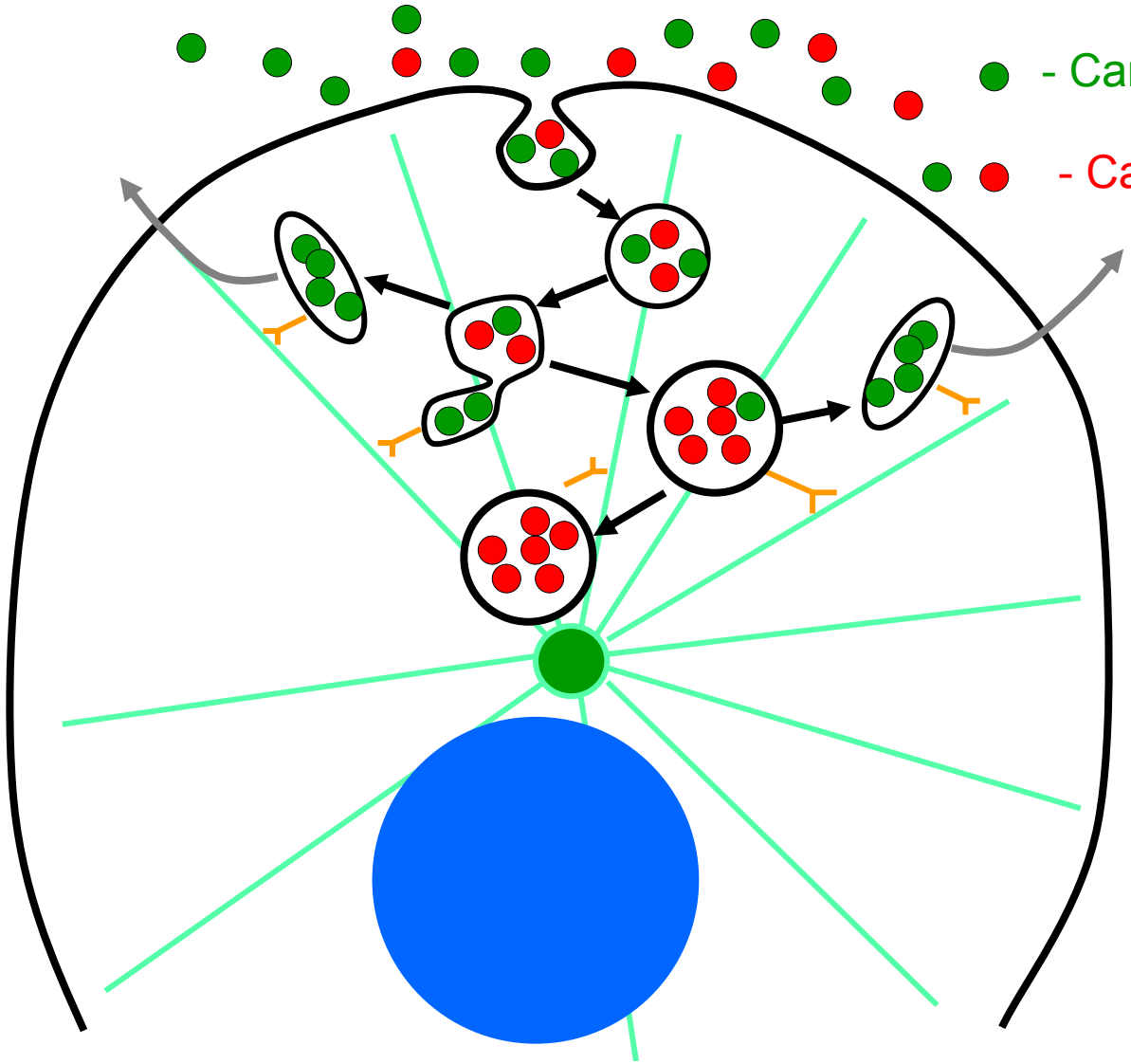
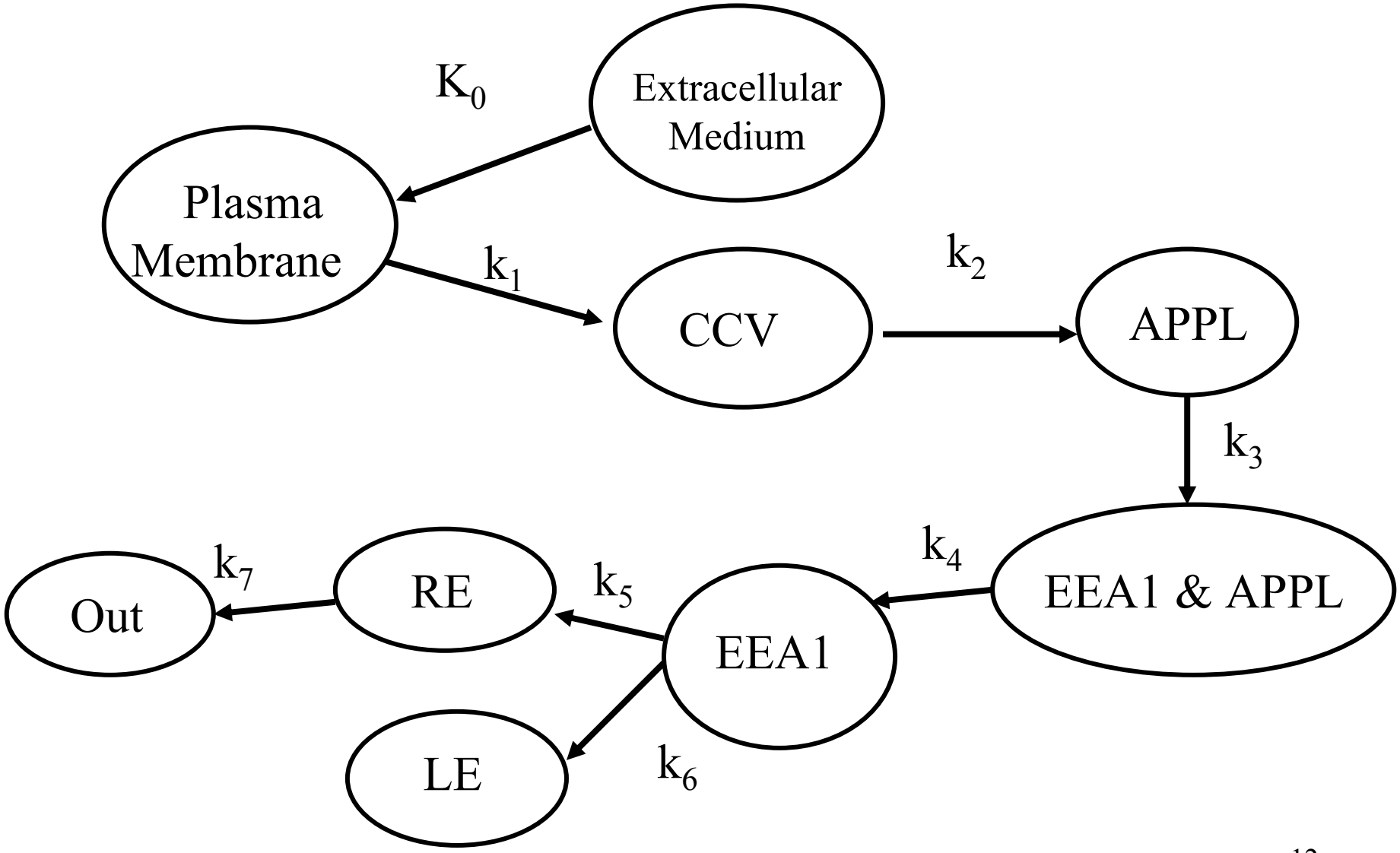


Endocytosis: Cellular Organelles Transport Cargo

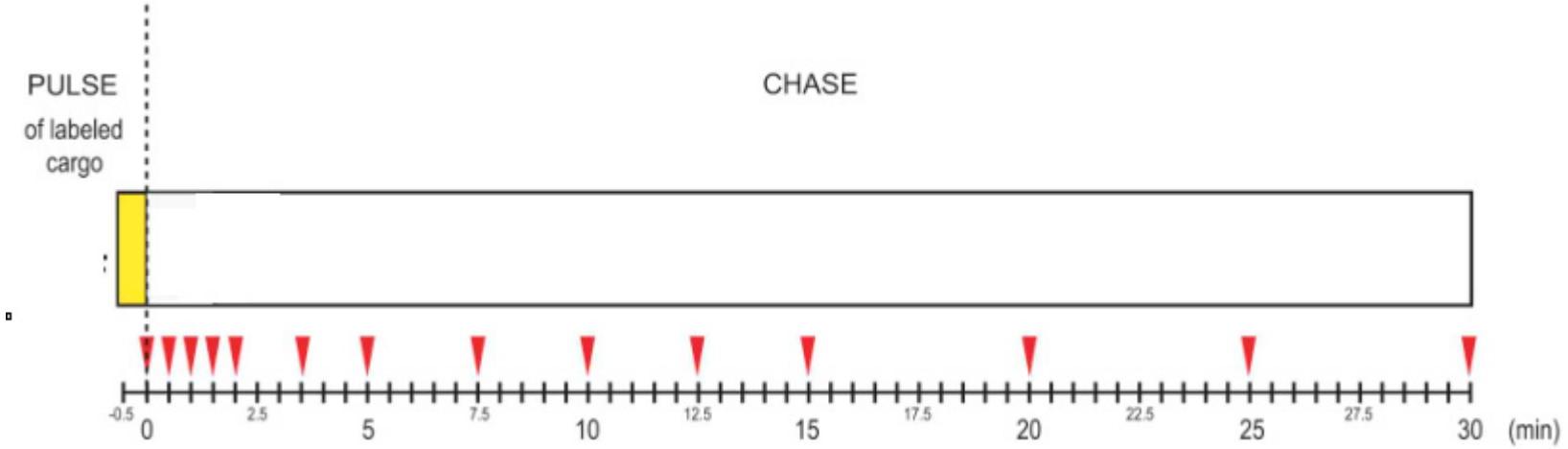


Endosome size:
0.1-1.5μm

Simple Cargo Flow Model

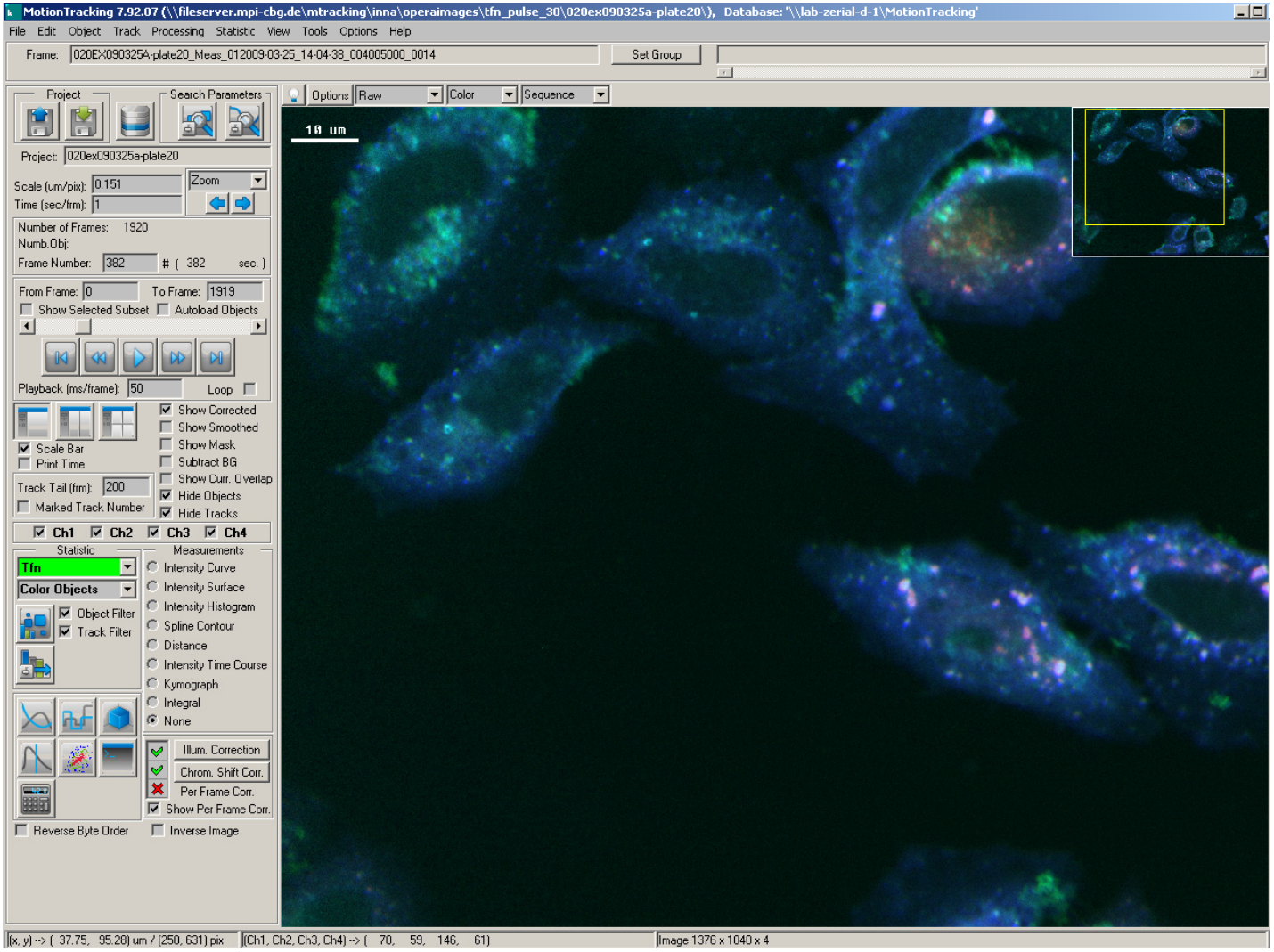


Pulse-chase experiment

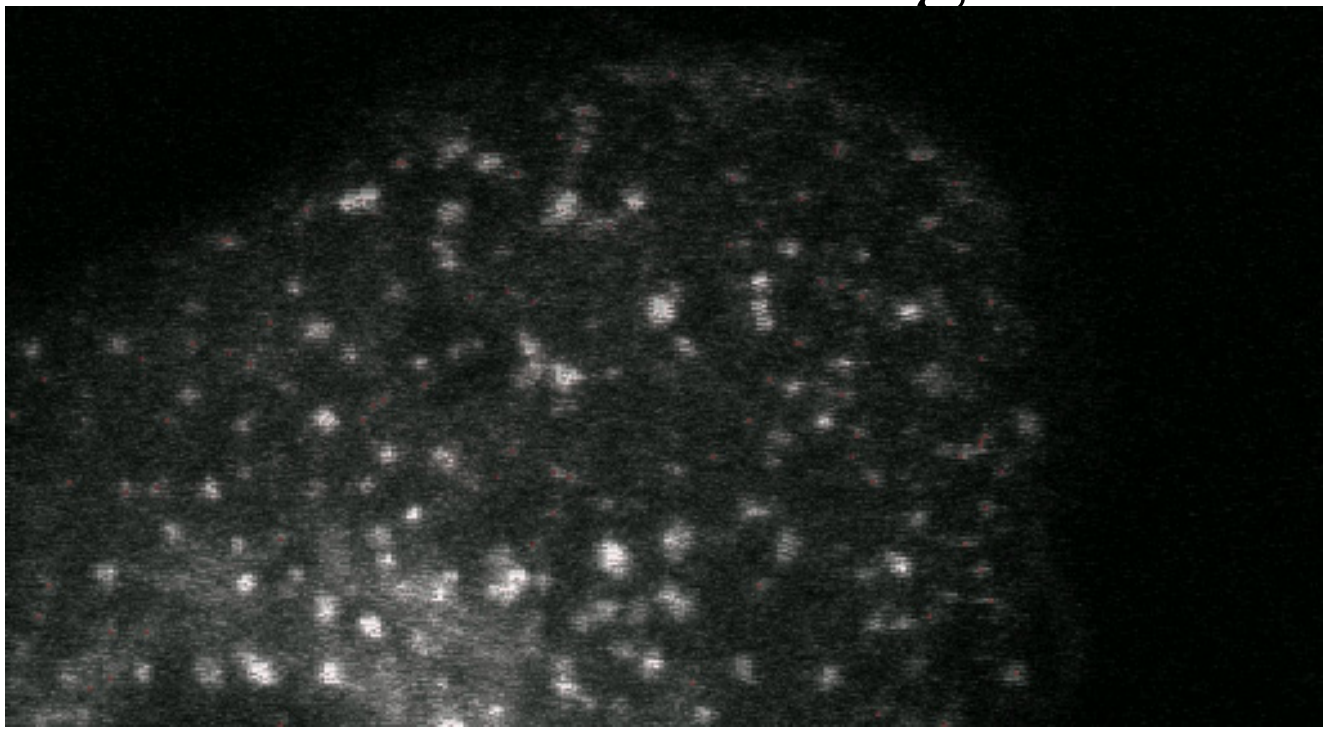


- HeLa cells
- 30'' pulse of fluorescent cargo
- Chasing at set of time points
- Fixing and staining by antibodies endosomal markers
- **Imaging**
- **Endosome identification**
- **Colocalization cargo with endosomal markers**

First step from “wet” experiment to computer: imaging

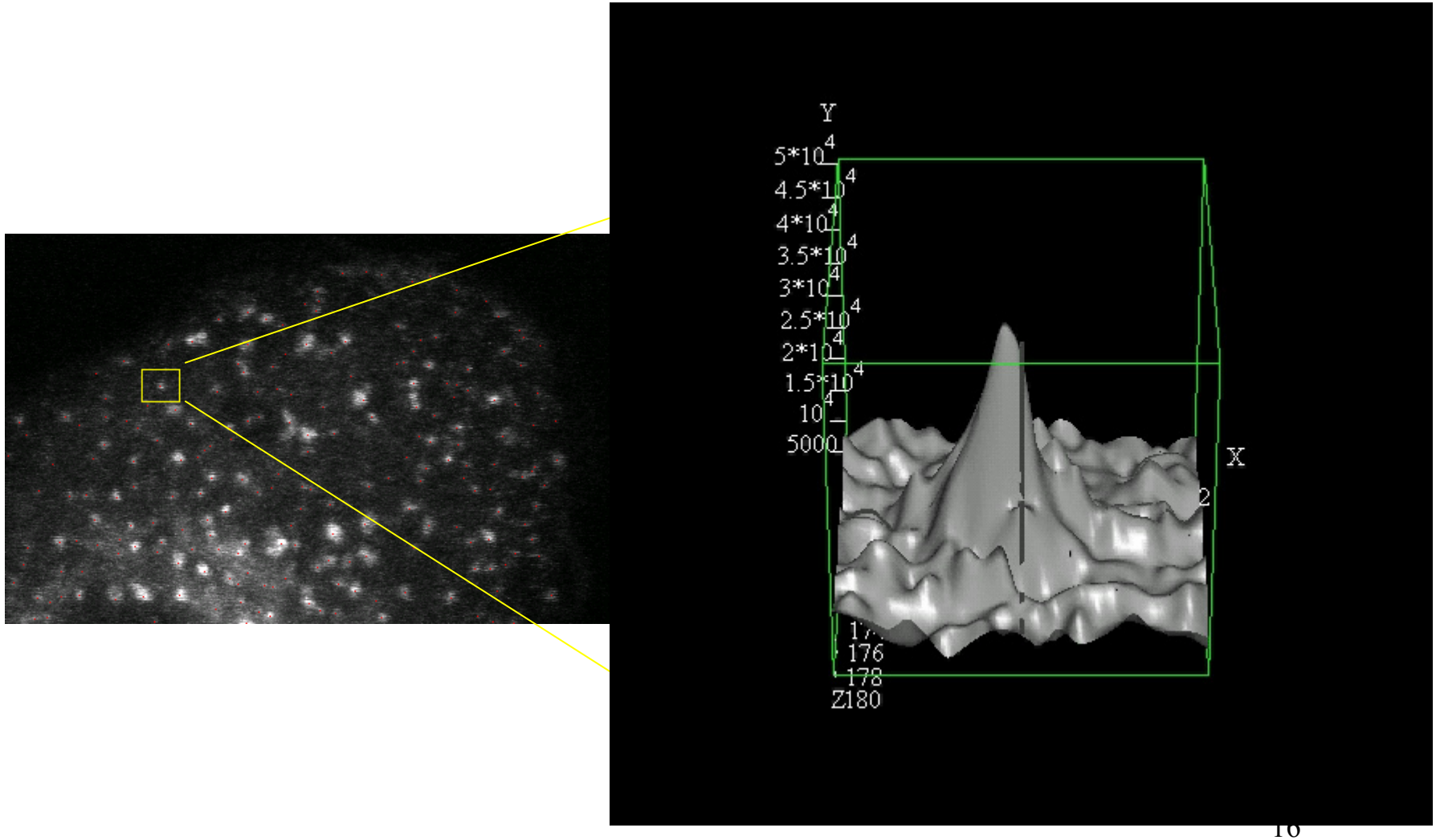


Endosome identification: What is challenge?



- Wide dynamic range of vesicle intensity
- Variety of shapes
- Non-homogenous background
- Multiplicative (Poisson) noise

Consider intensity distribution as a surface in 3D-space



Intensity distribution of individual small endosome can be modeled by hat-like function

$$I(x,y) = \frac{A_0}{1 + \left\{ \left[\frac{((x-x_0)\cos(\alpha) - (y-y_0)\sin(\alpha)}{w} \right]^2 + \left[\frac{(x-x_0)\sin(\alpha) + (y-y_0)\cos(\alpha)}{h} \right]^2 \right\}^p}$$

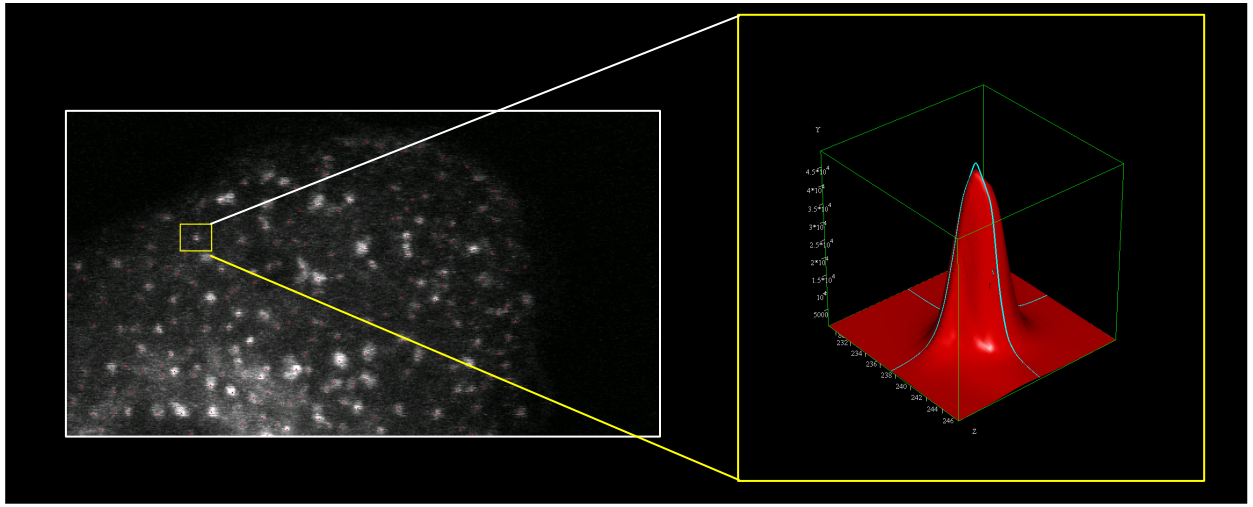
where A_0 - intensity at the center

x_0, y_0 - center coordinates

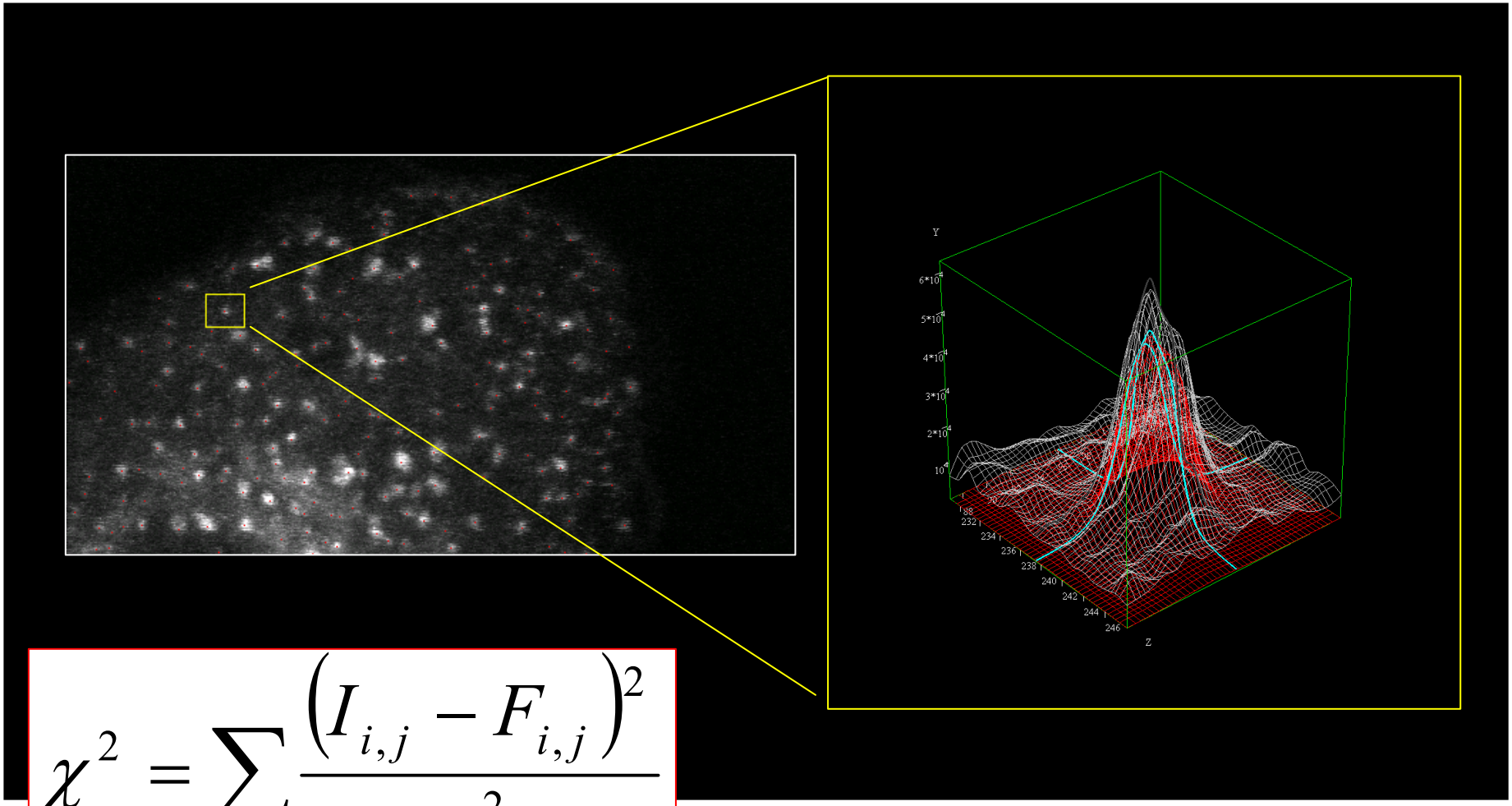
w, h - width by perpendicular dimensions

α - angle between main axis and axis Y

p – power factor

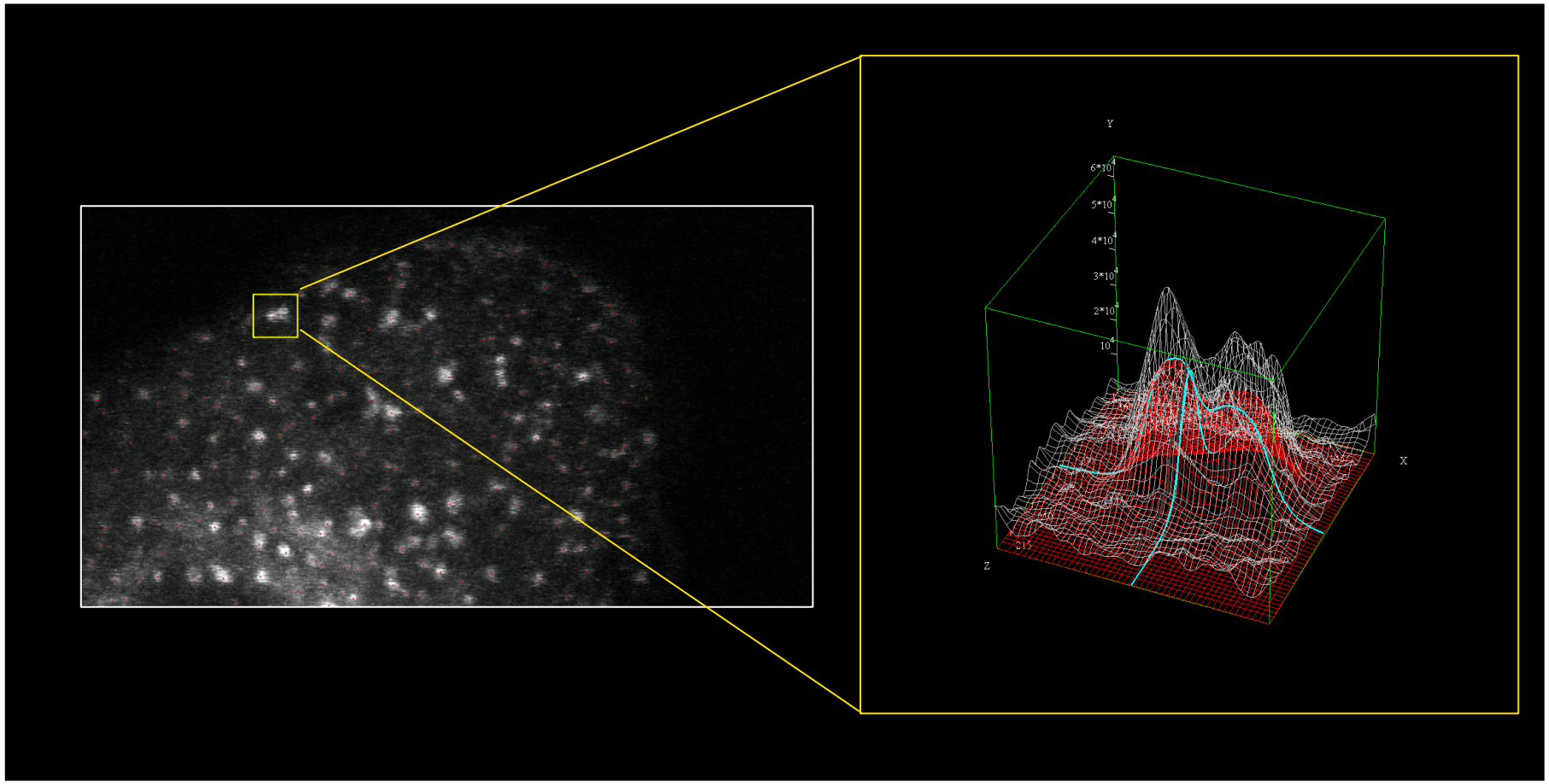


Base function is fitted to the image by minimization χ^2



$$\chi^2 = \sum_{i,j} \frac{\left(I_{i,j} - F_{i,j}\right)^2}{\sigma_{i,j}^2}$$

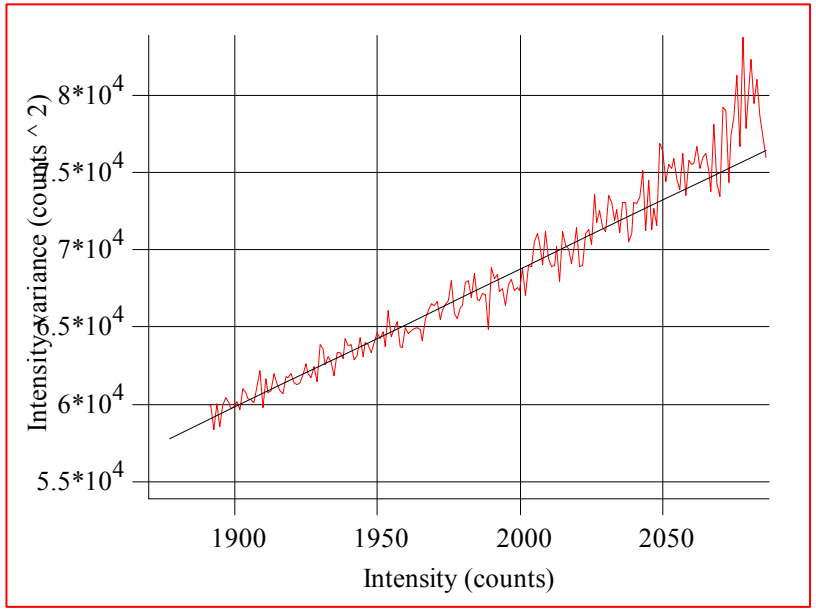
Endosomes with complex shape can be modeled by sum of base functions



Fitting requires image noise analysis

$$\chi^2 = \sum_{i,j} \frac{\left(I_{i,j} - F_{i,j}\right)^2}{\sigma_{i,j}^2}$$

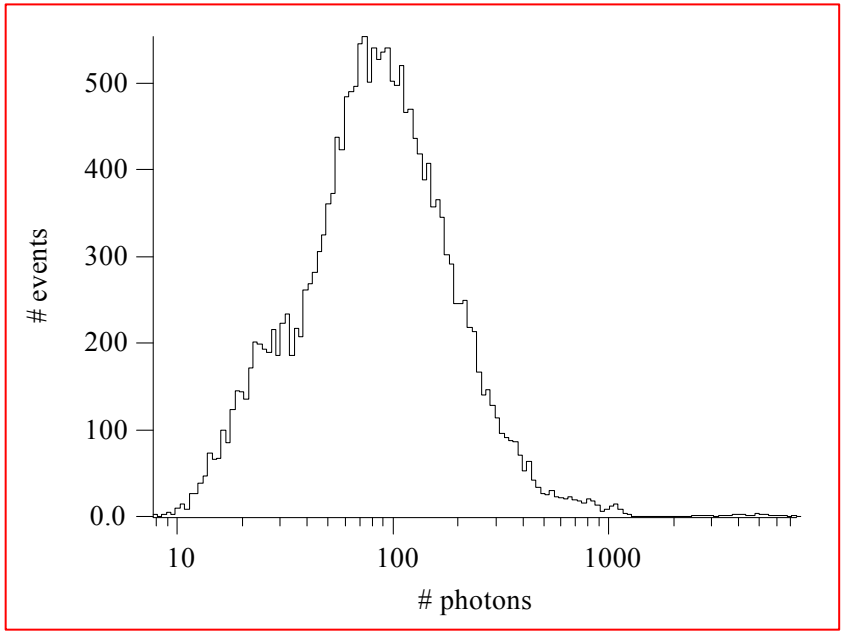
Photon flux calibration



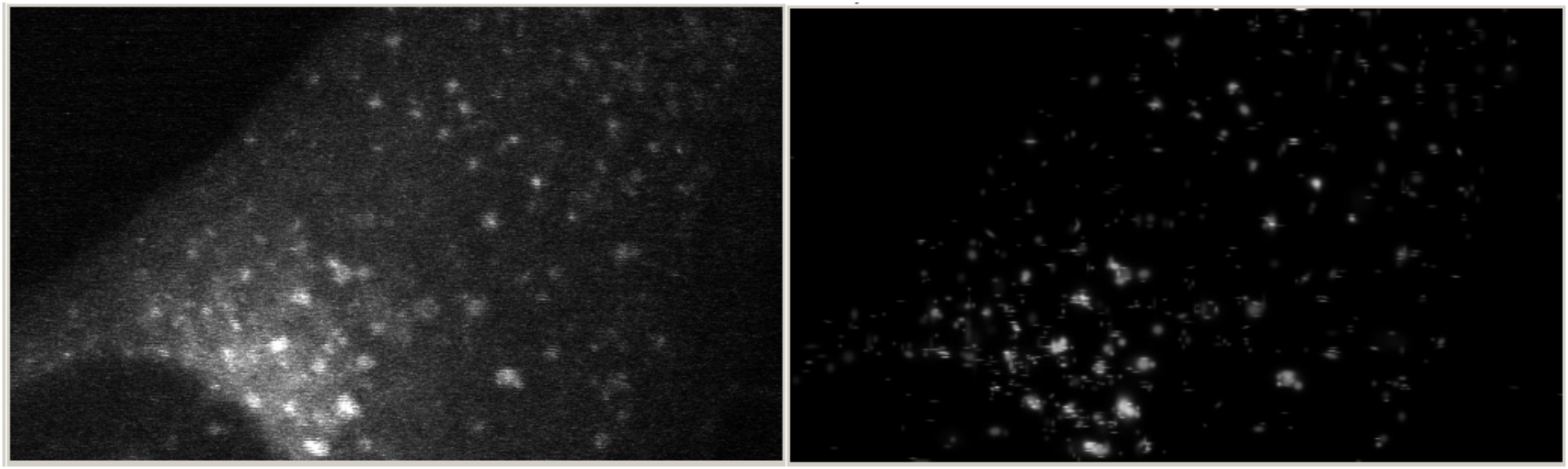
Bonus: Number of photons per object

a) Counting number fluorescent molecules

b) Single molecule experiments



Result of image fitting



Benefits of approach

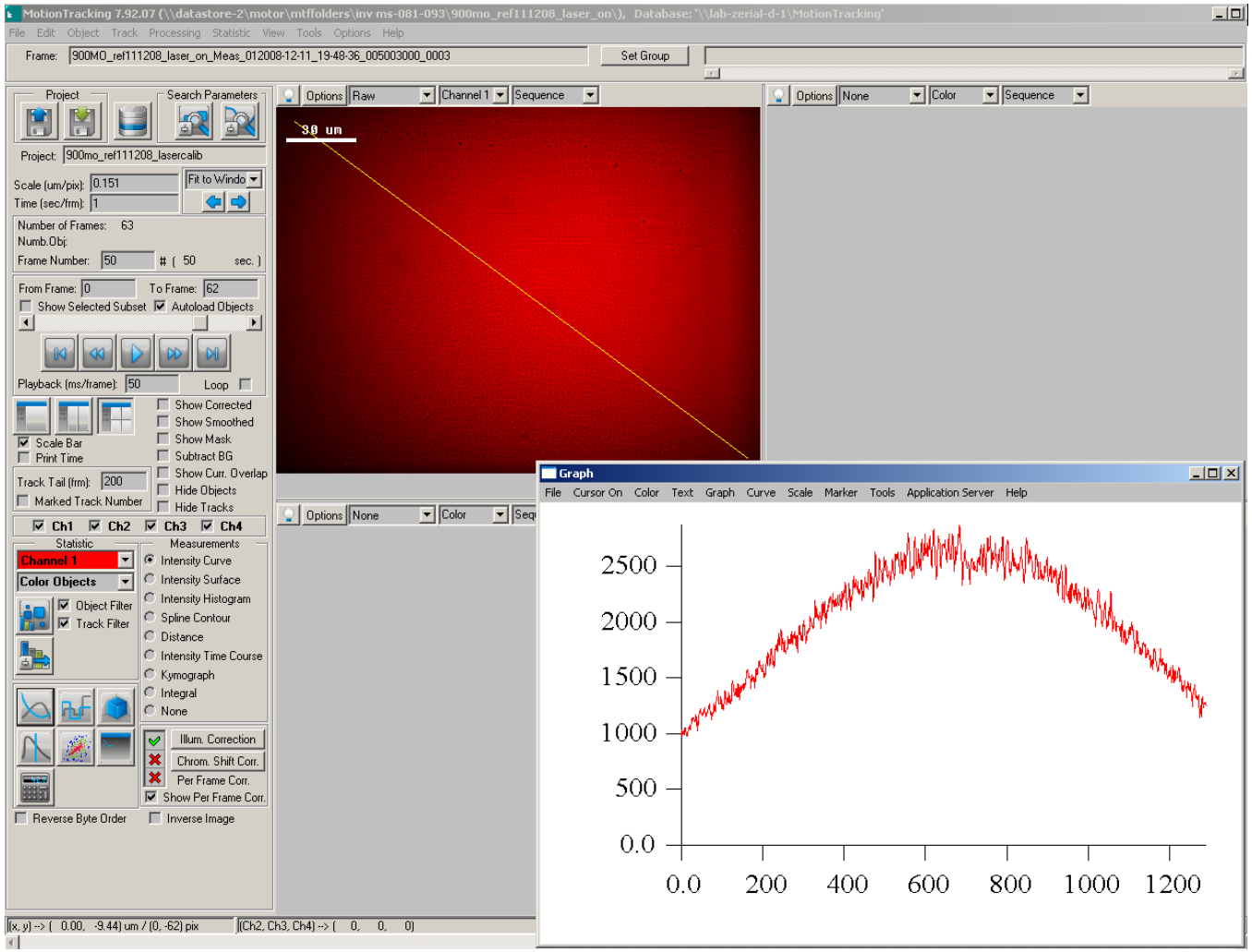
- ✓High dynamic range
- ✓Sub-pixel resolution
- ✓Accurate estimate of mean intensity, integral intensity and endosome size
- ✓Possibility to count number of fluorescent molecules per endosome

Are we ready to model cargo flow?

We did not check two major microscope problems:

- Uneven illumination of view filed
- Chromatic shift/aberration

Quantitative Microscopy: uneven illumination



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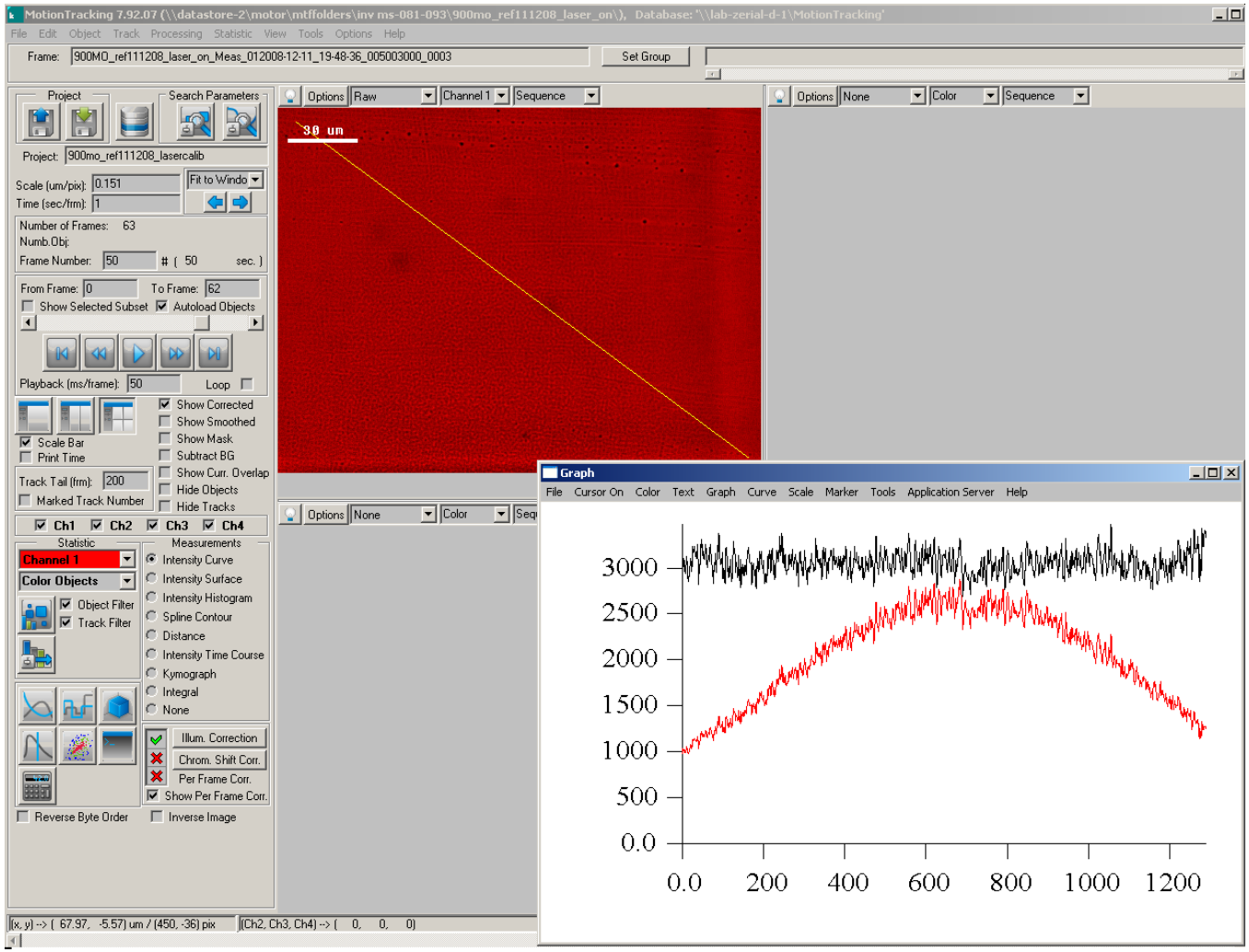
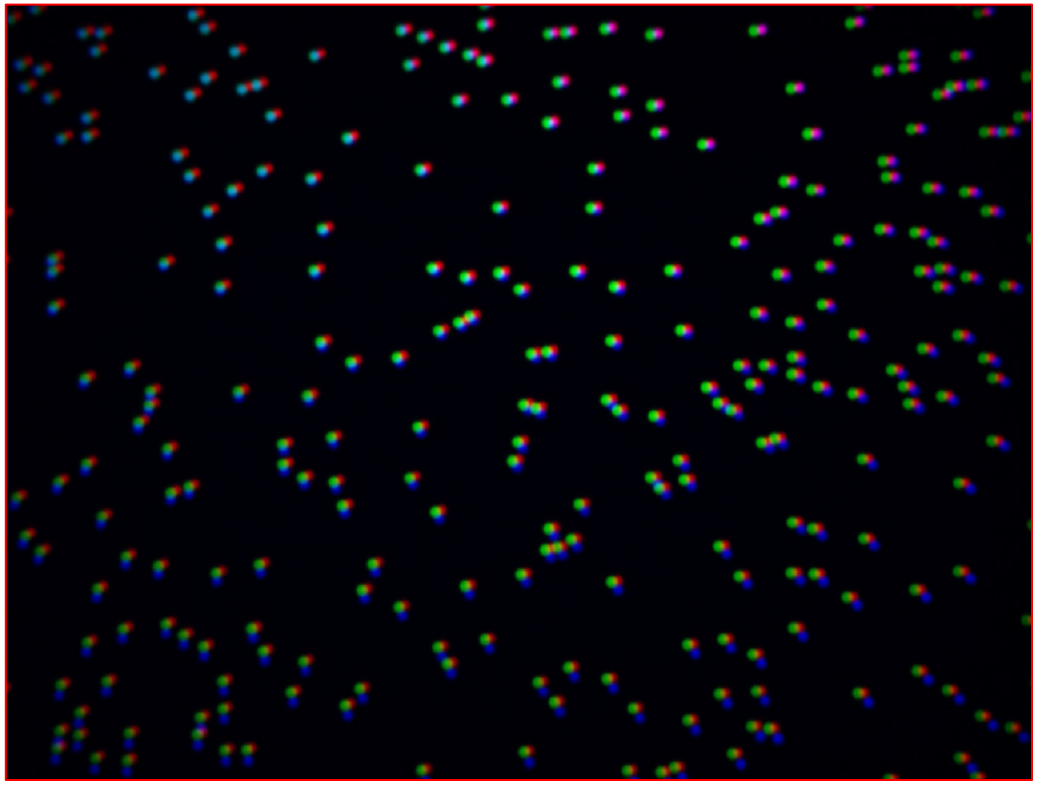


Quantitative Microscopy : chromatic shift



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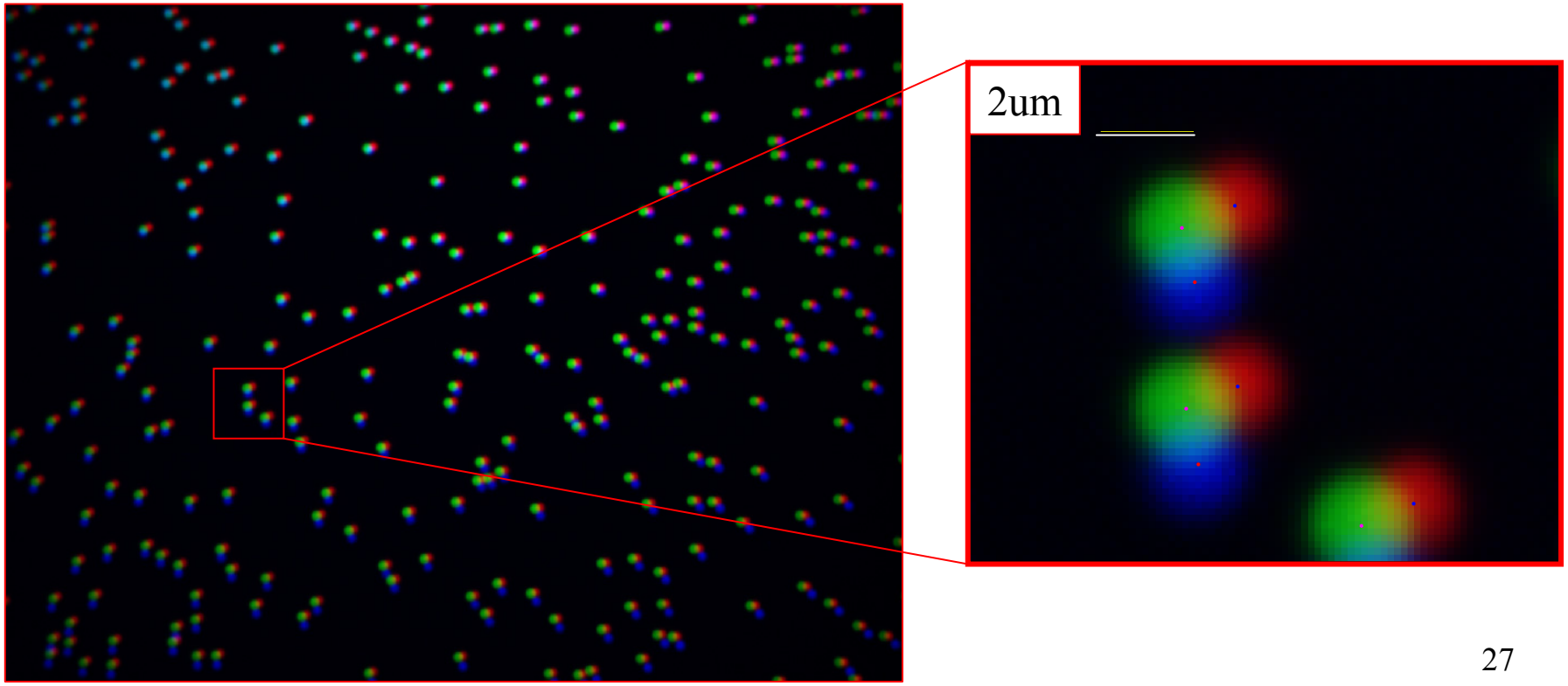
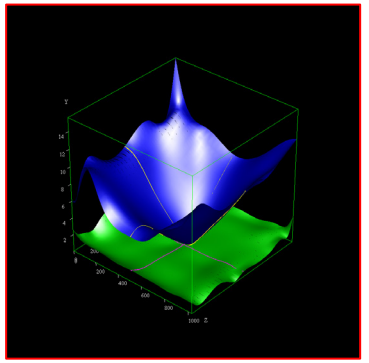
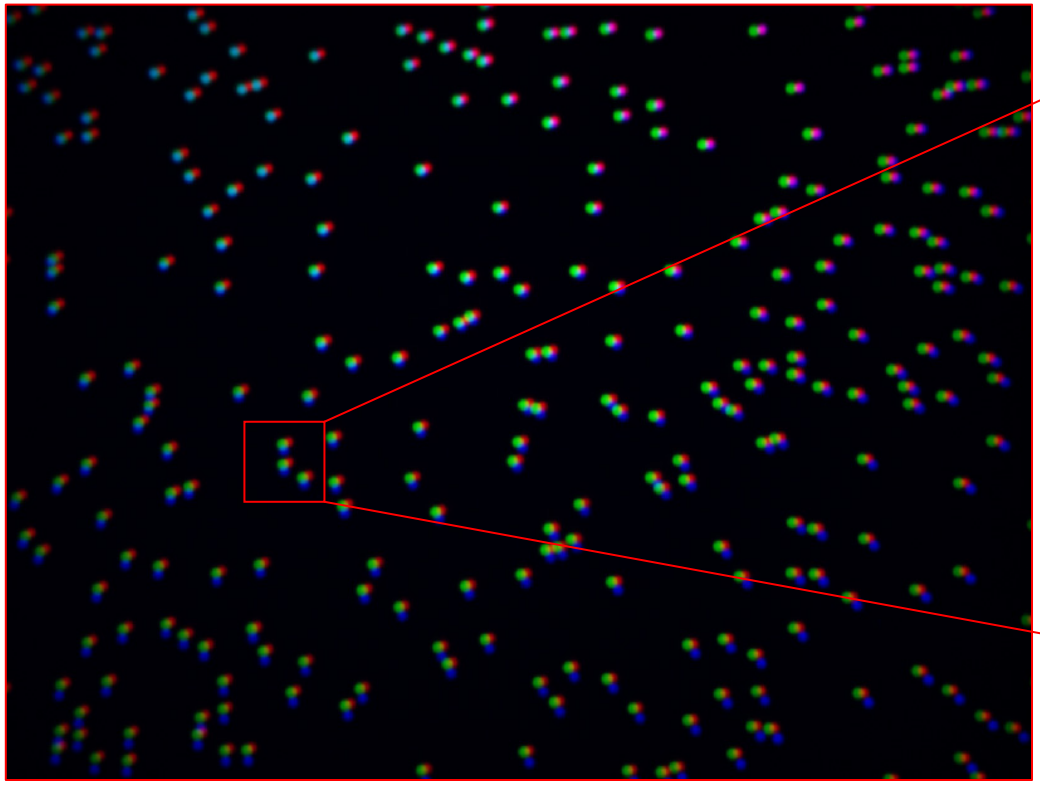
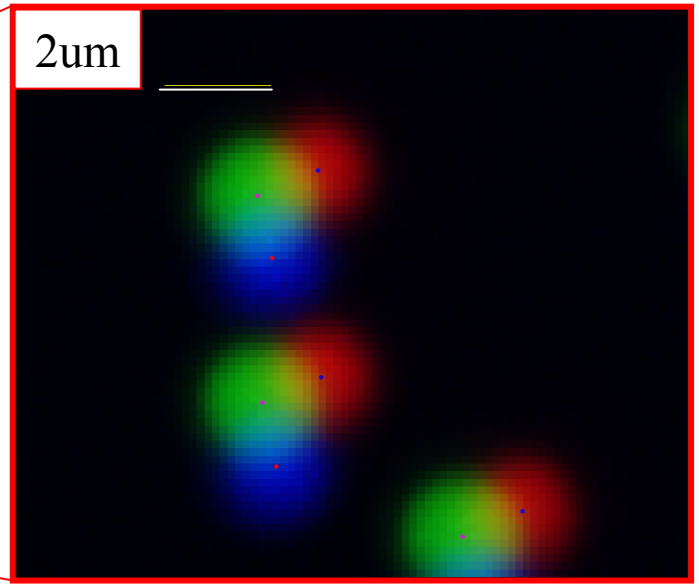


Quantitative Microscopy : chromatic shift

$\delta x_1 = 7.31, \delta y_1 = 3.37, \alpha_1 = 0.00285$
$\delta x_2 = 0.26, \delta y_2 = 10.83, \alpha_2 = 0.0041$







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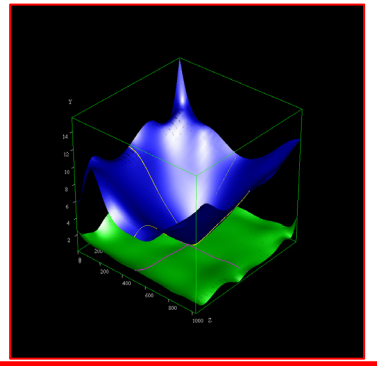
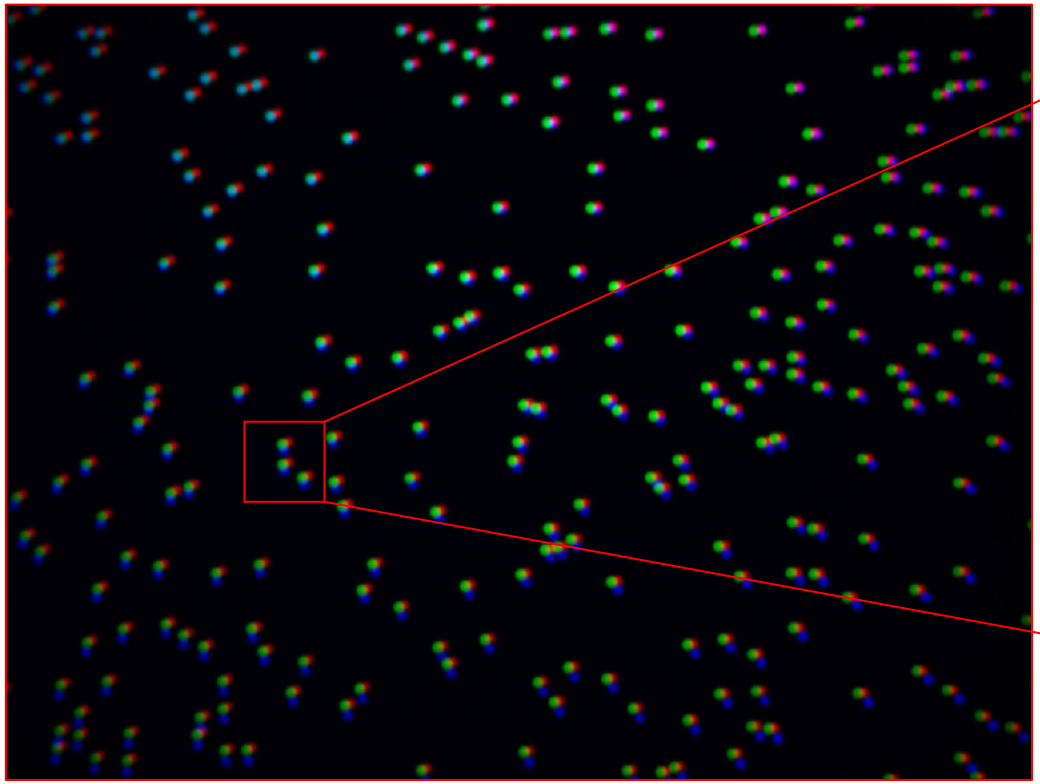
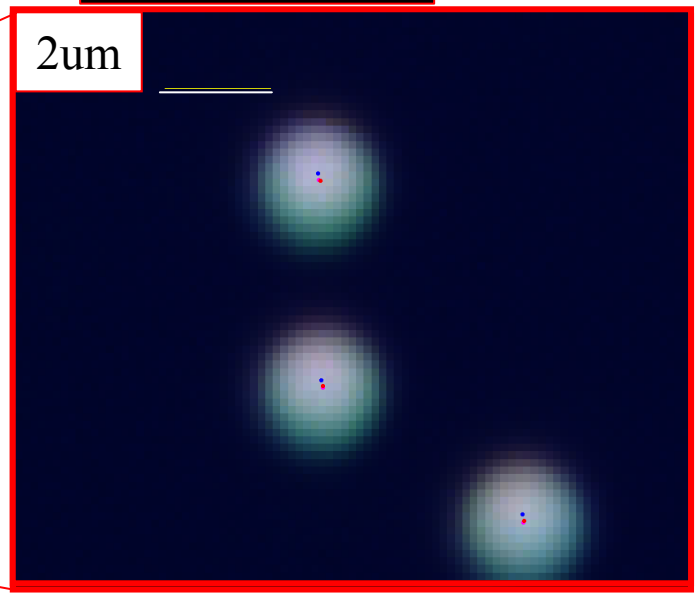
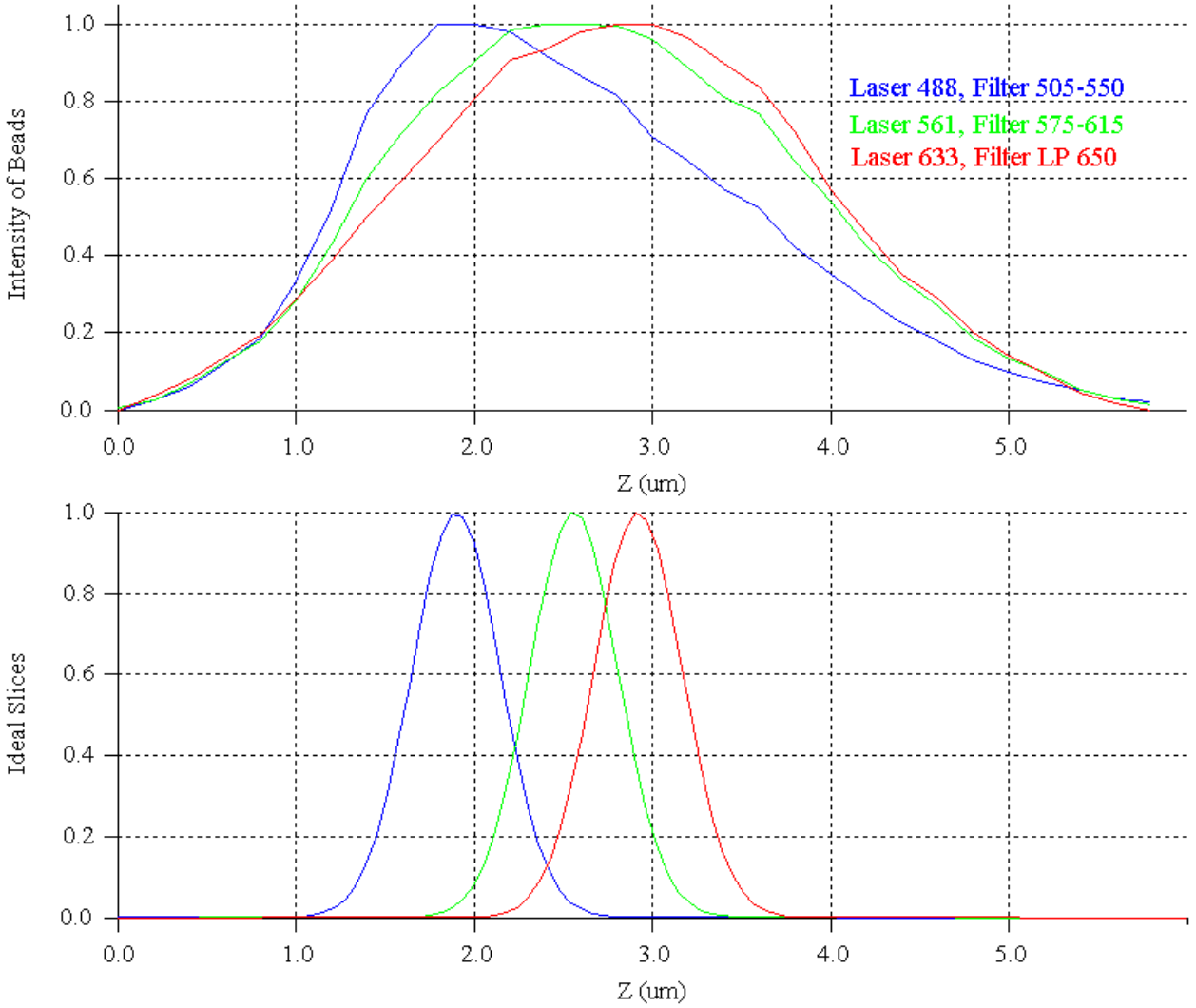






Colocalization: World is 3D



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2. Noise has new, non-Poisson, statistic
3. Noise in neighbour pixels is correlated

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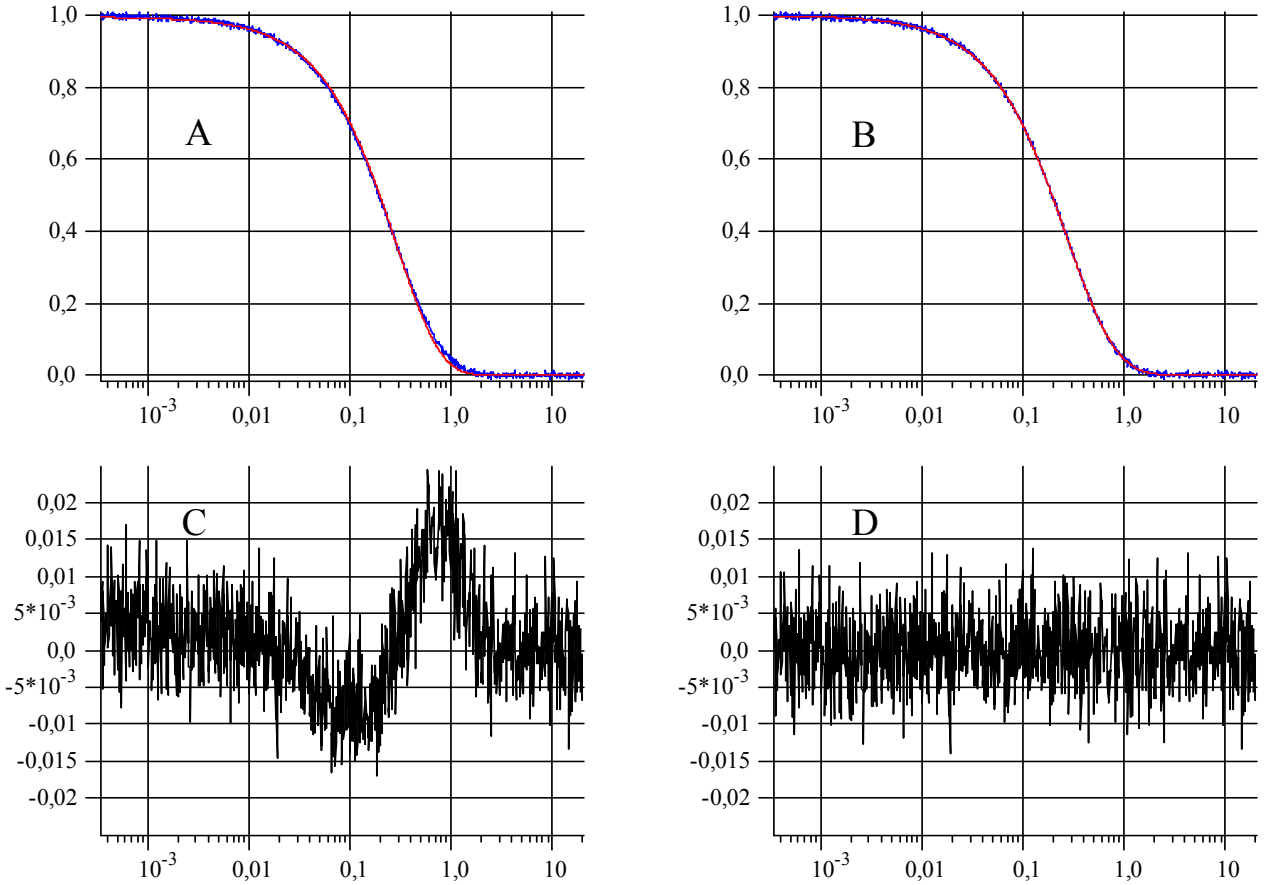
Does it matter?

Correct model choice and noise amplitude: How many components model has?



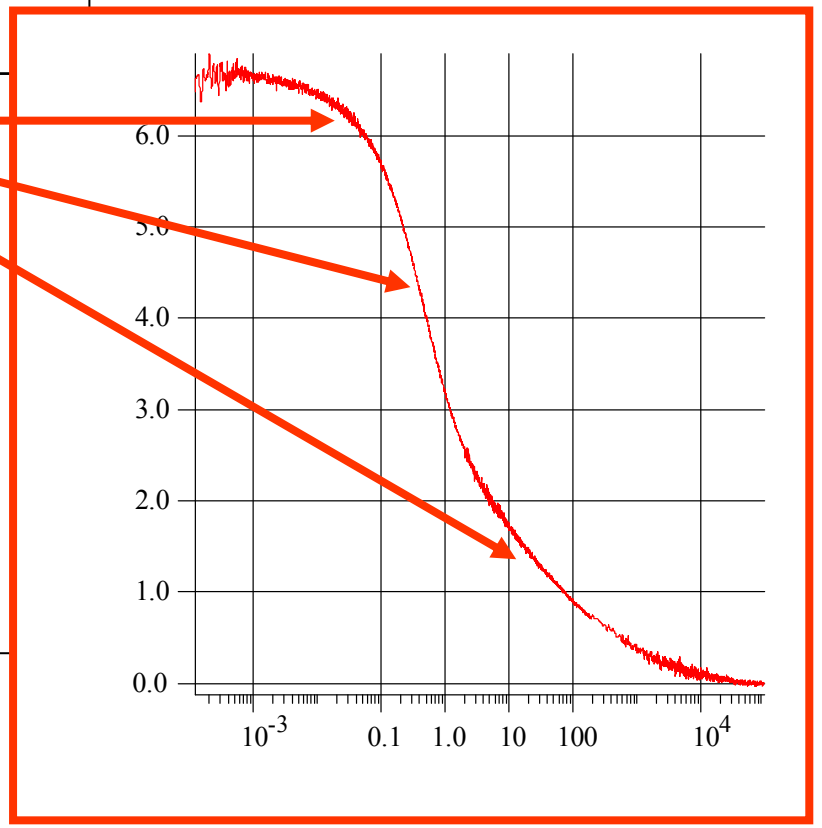
Deconvolution of artificial two-exponential curve (τ_1=0.2 and τ_2=0.4, signal-to-noise ratio = 200) by one exponent (τ=0.286) (panels A, C) and two exponents (τ_1=0.192 and τ_2=0.385) (panels B, D).

Model search is χ^2 minimization problem

$$\frac{\chi^2}{2} = \sum_i \frac{\left[f(t_i) - \sum_j A(\tau_j) \exp\left(-\frac{t_i}{\tau_j} \right) \right]^2}{2\sigma_i^2}$$

where $A(\tau_j)$ - amplitude of exponent τ_j,

σ_i^2 - variance in the point i.



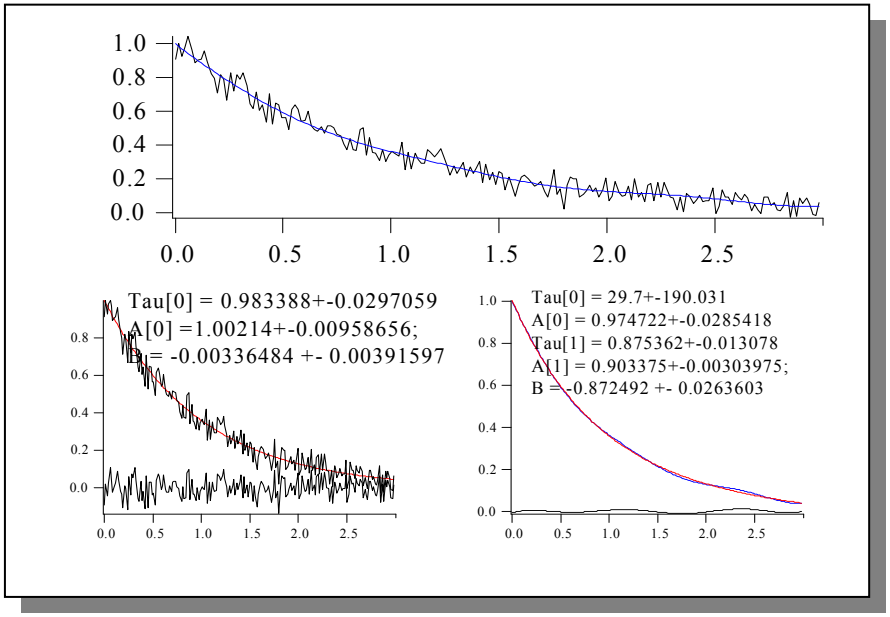
Model fit and noise correlation

Simulation model:

$y_i = \exp(-x_i) + \varepsilon$, where ε has distribution *N(0.0, 0.05)*

Regression model:

$$y_i = \sum_k^K A_k \cdot \exp(-x_i / \tau_k) + B + \varepsilon$$



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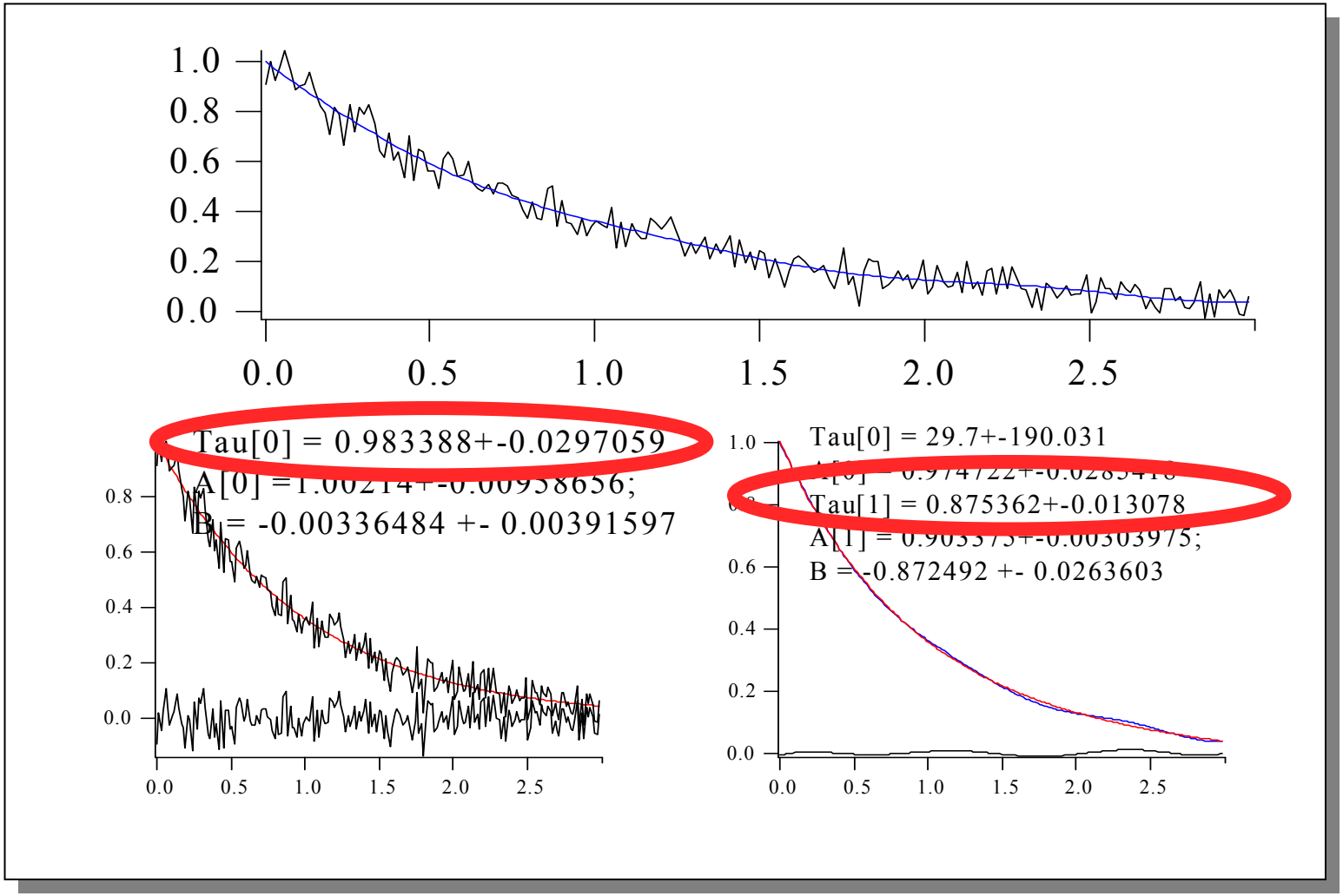
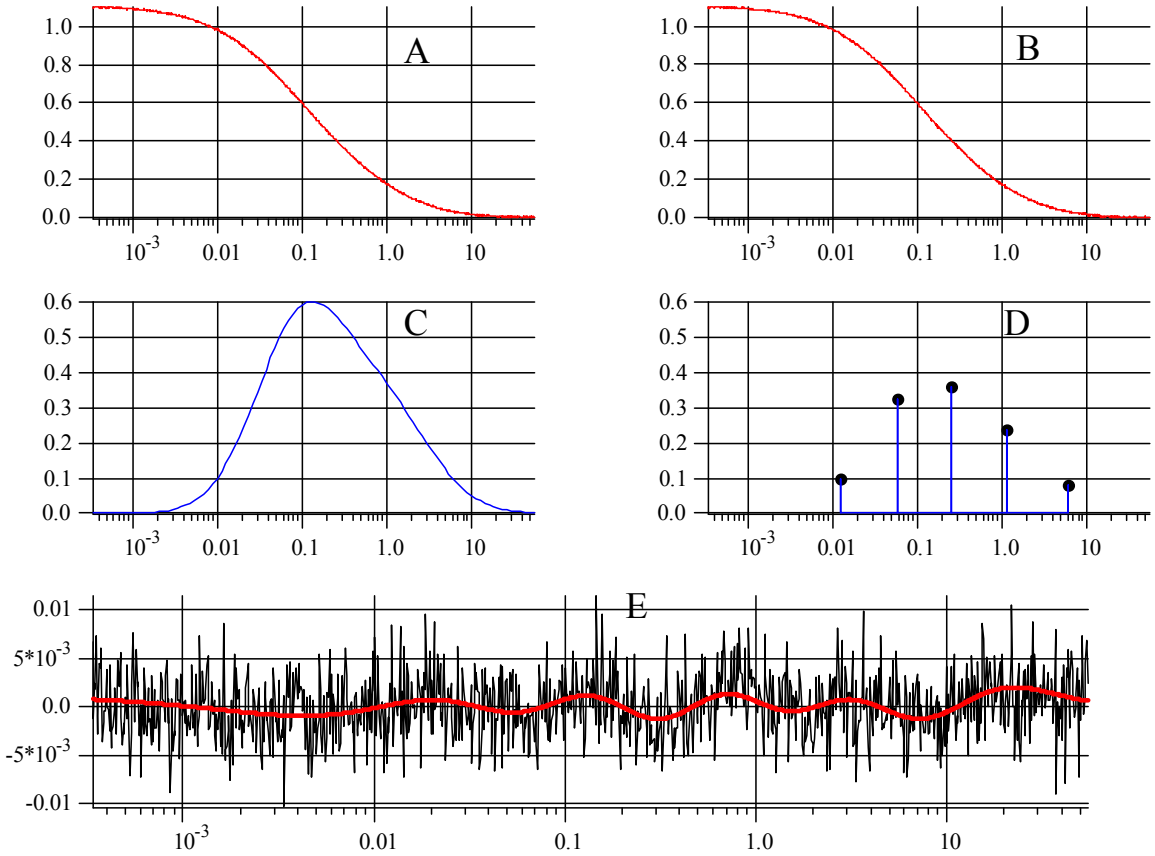


Model selection: little more complicate example

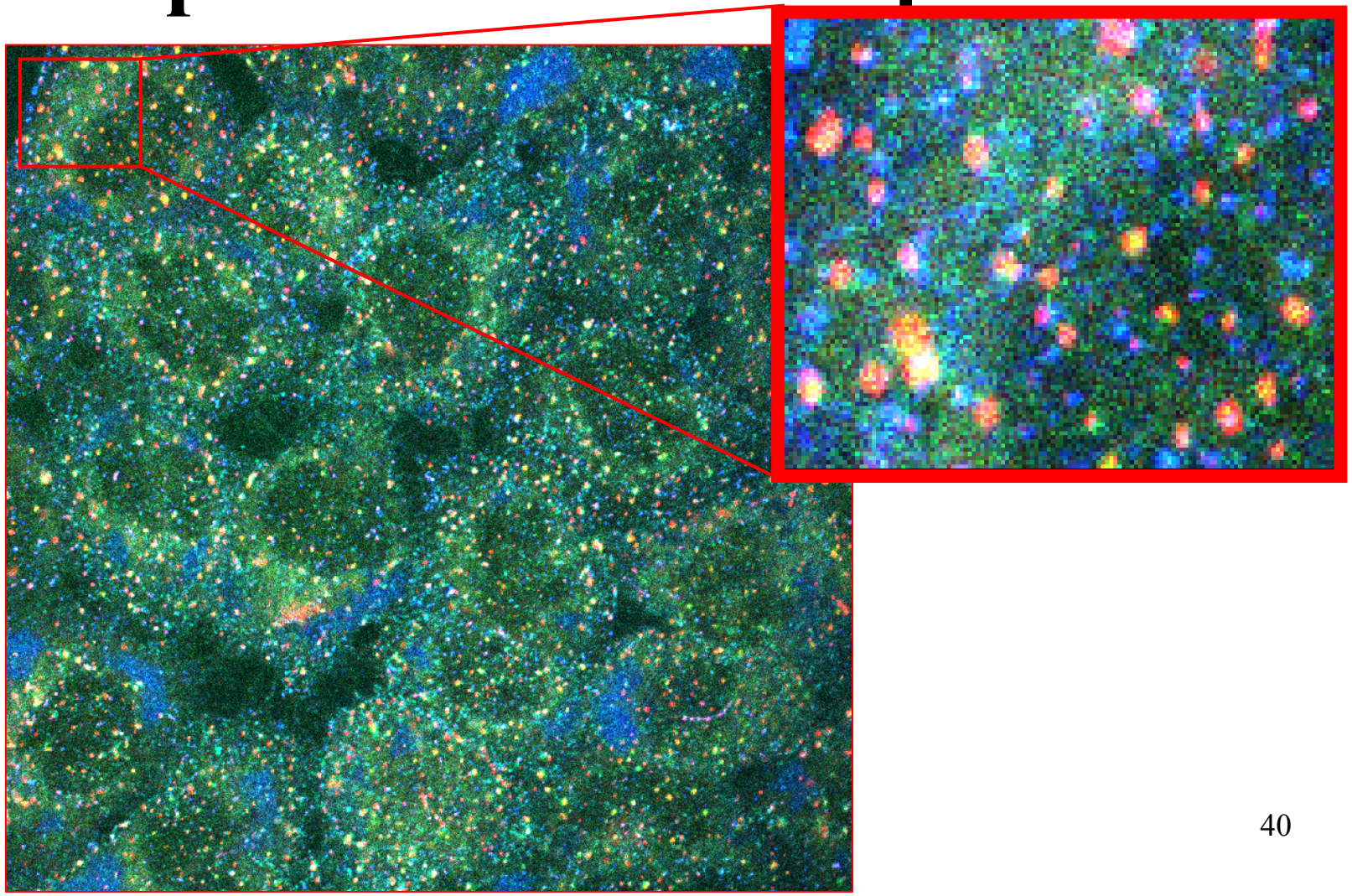


Artificial exponential (panels B, D) and non-exponential (panels A, B) kinetics ands pectra. The panel E presents difference between exponential and non-exponential curves without noise (red) and with signal-to-noise = 500 (black).

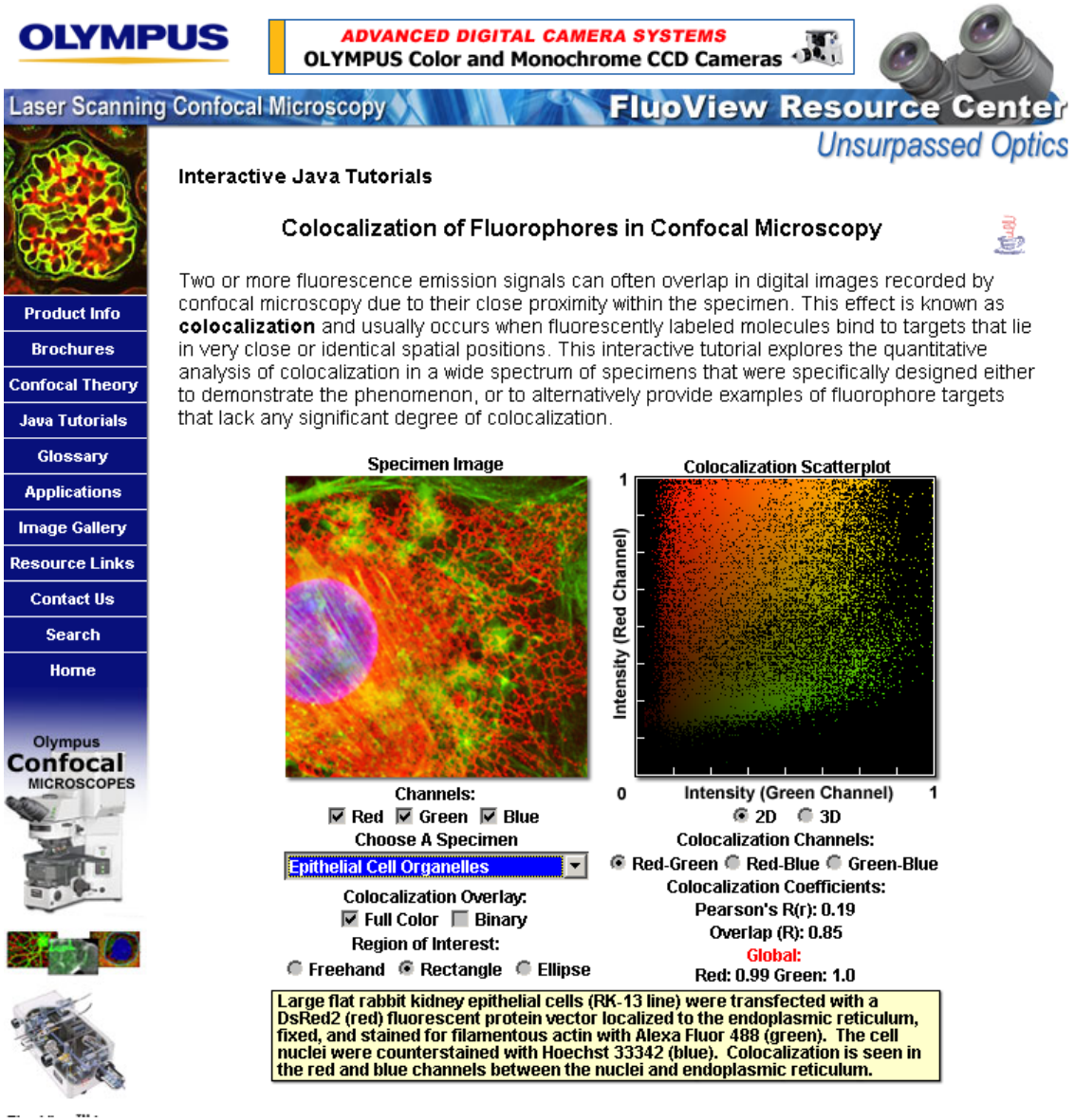
Take home message:

- Analyze image (if it is possible) before any correction and apply correct to the result of analysis latter.
- Avoid automatic (hidden) correction of images by microscope software with unknown characteristics.
- Check actual image noise on consistence with noise model, which is assumed by analyzing software.

Next step: We need ‘just’ quantitative colocalization to compare model with experiment



Pearson & Overlap colocalization criteria



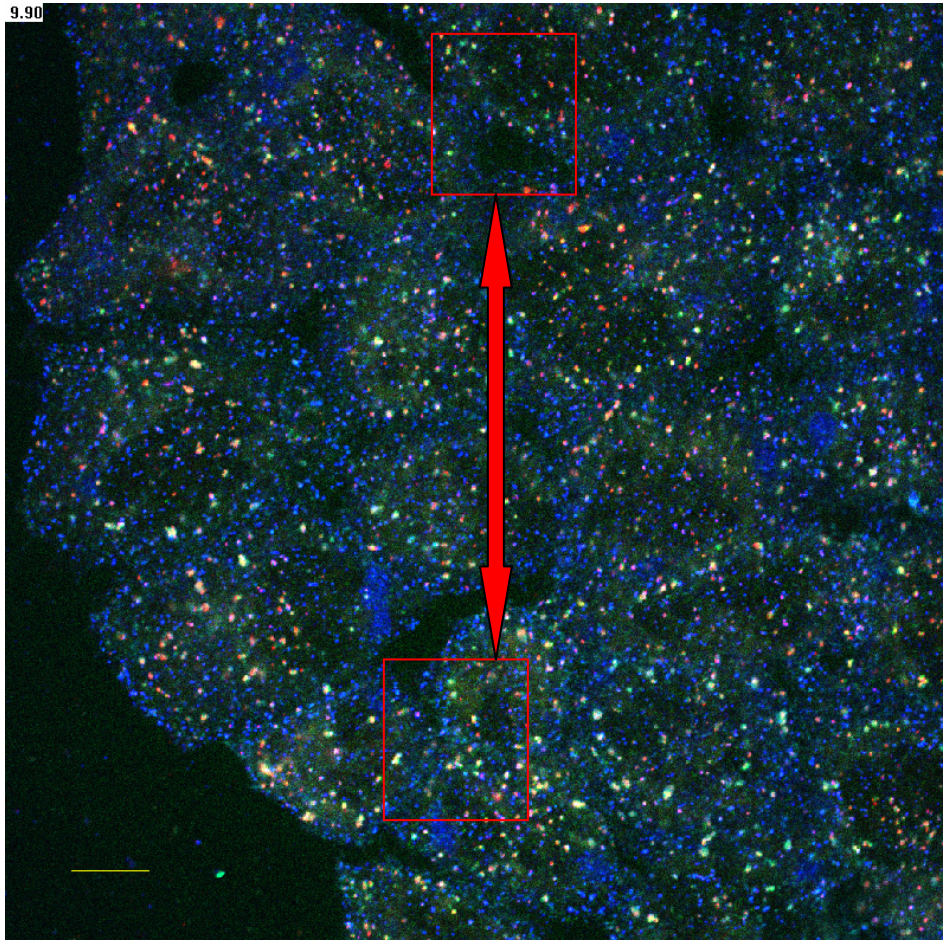
Pearson's colocalization coefficient:

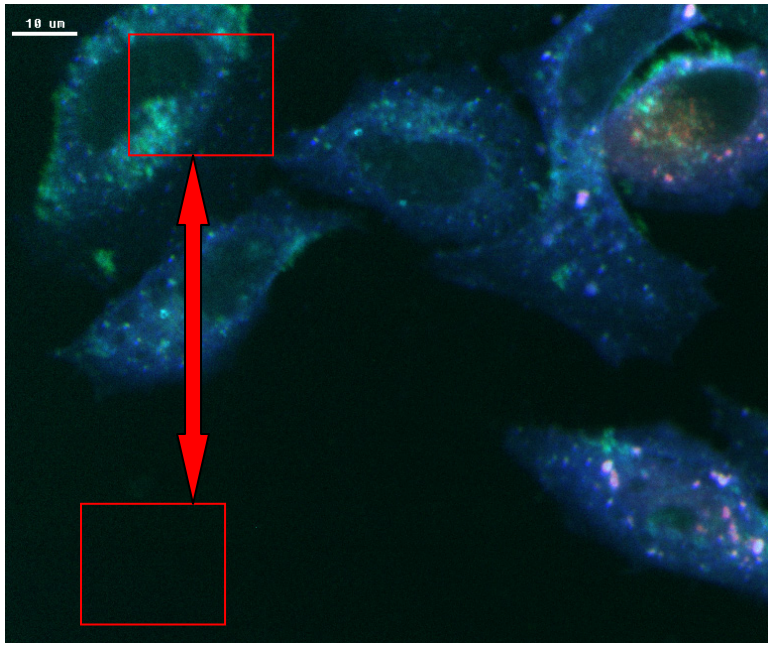
$$r_p = \frac{\sum((R_i - R_{avg})(G_i - G_{avg}))}{\sqrt{\sum(R_i - R_{avg})^2 \sum(G_i - G_{avg})^2}}$$

Overlap coefficient:

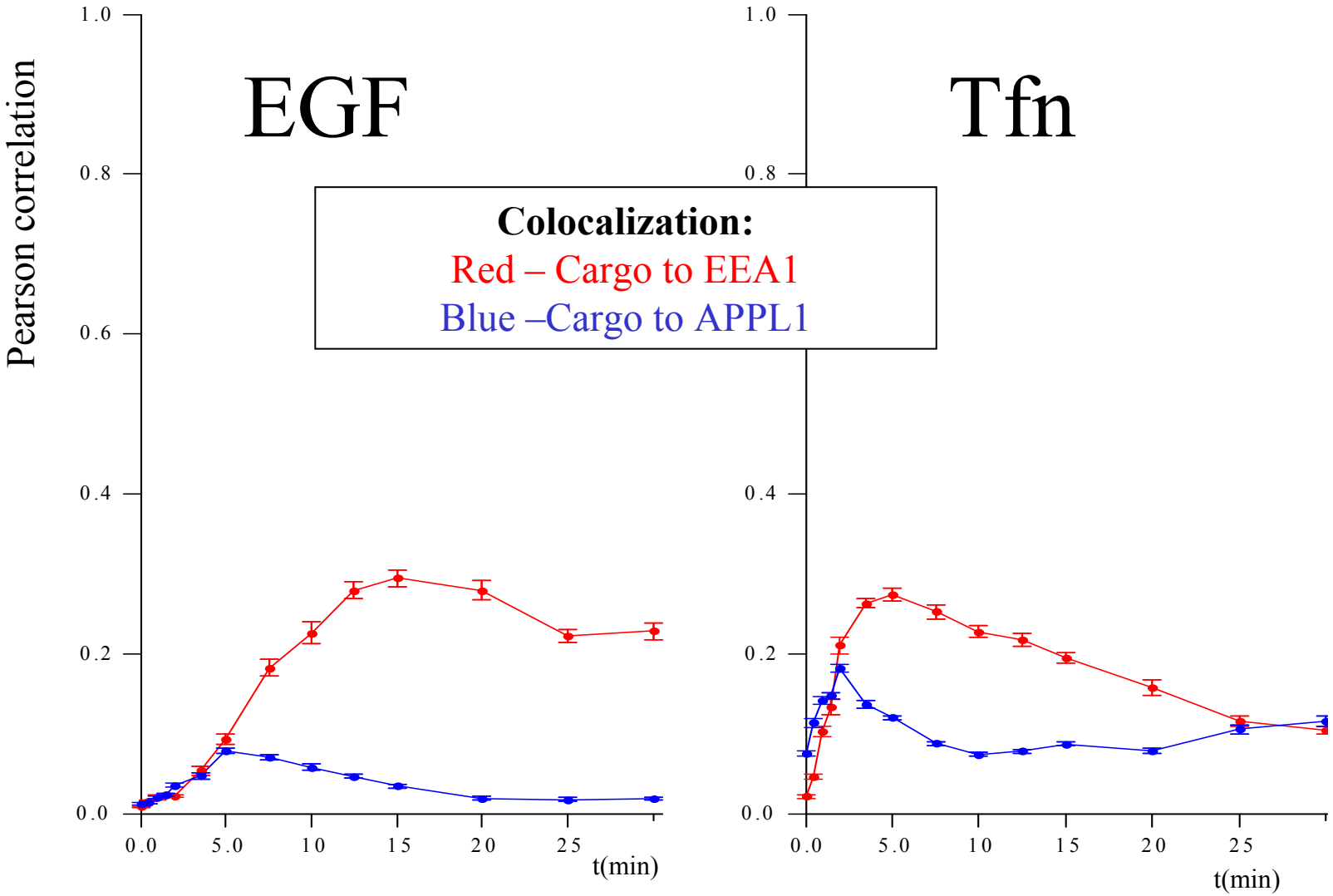
$$r_o = \frac{\sum(R_i G_i)}{\sqrt{\sum R_i^2 \sum G_i^2}}$$

Estimation of random (apparent) colocalization

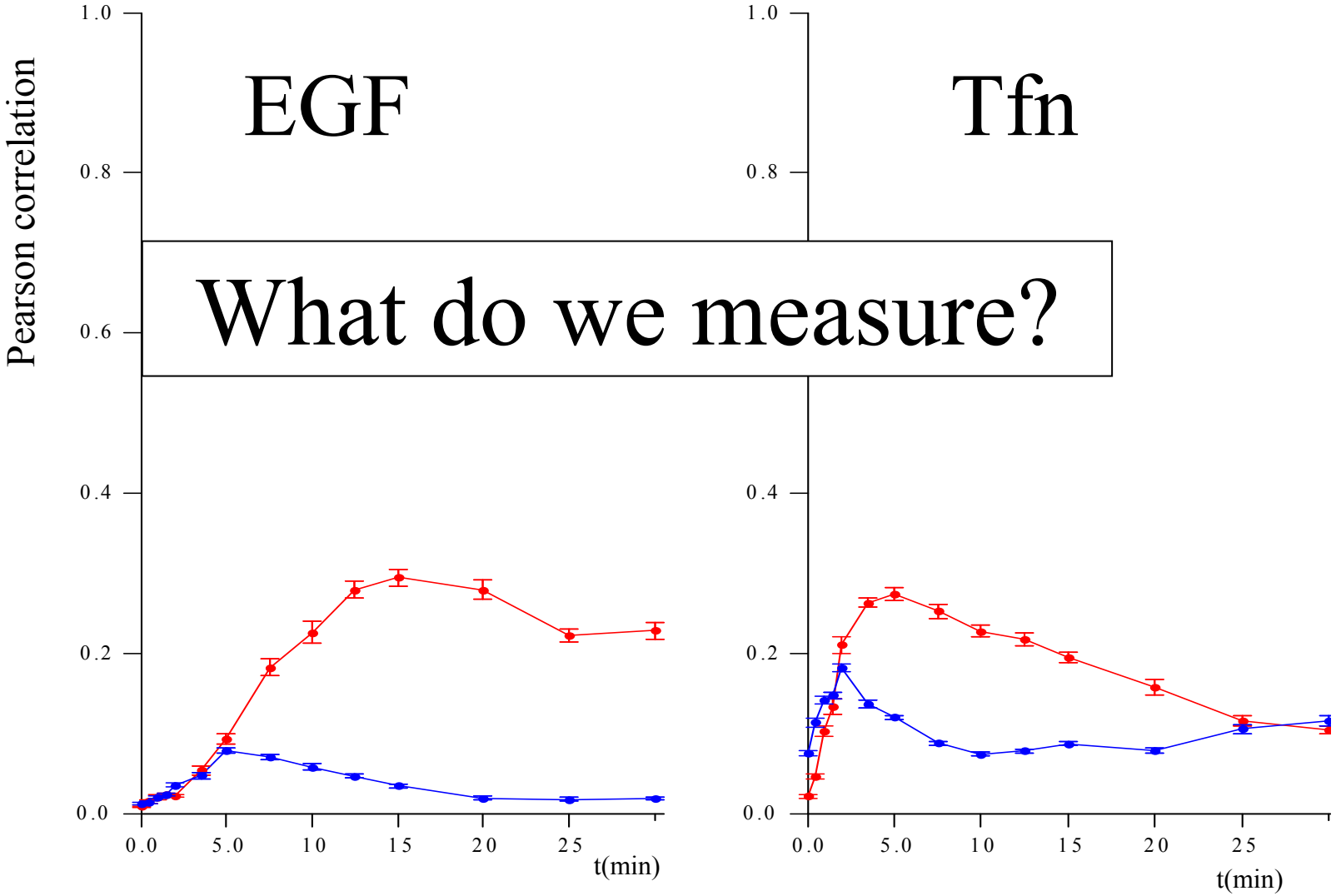




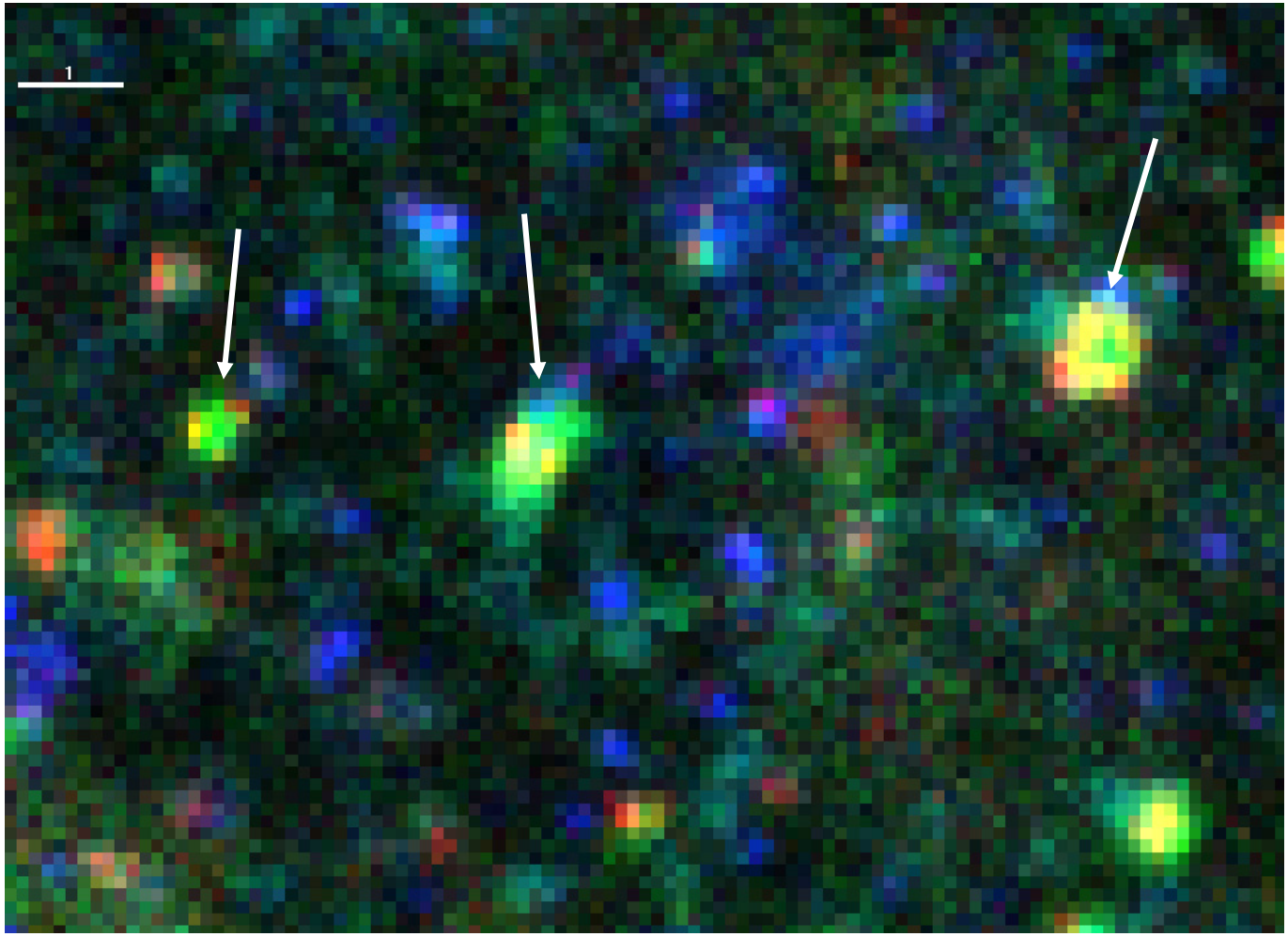
Pearson colocalization time course



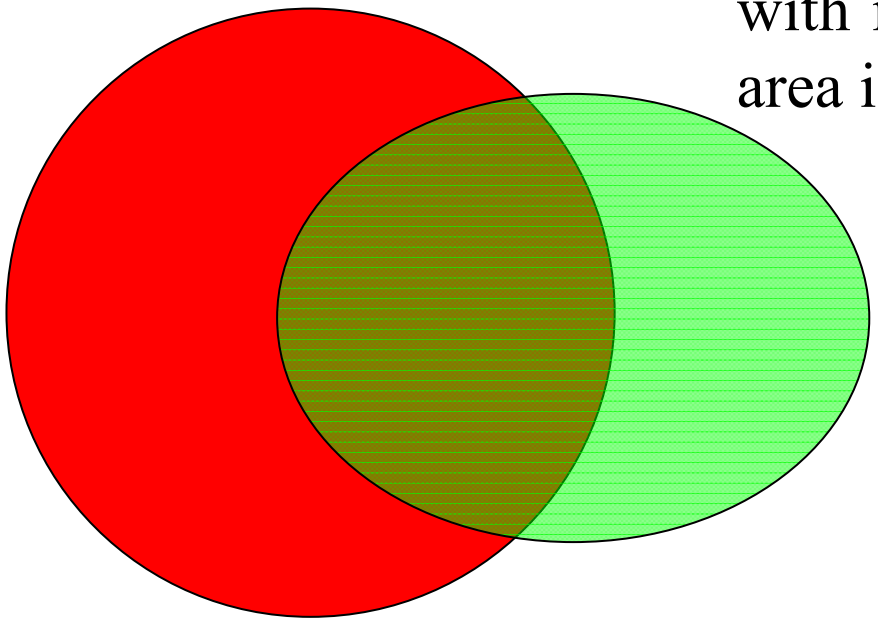
Pearson colocalization time course



Are domain structures colocalized?



Definition of asymmetric colocalization

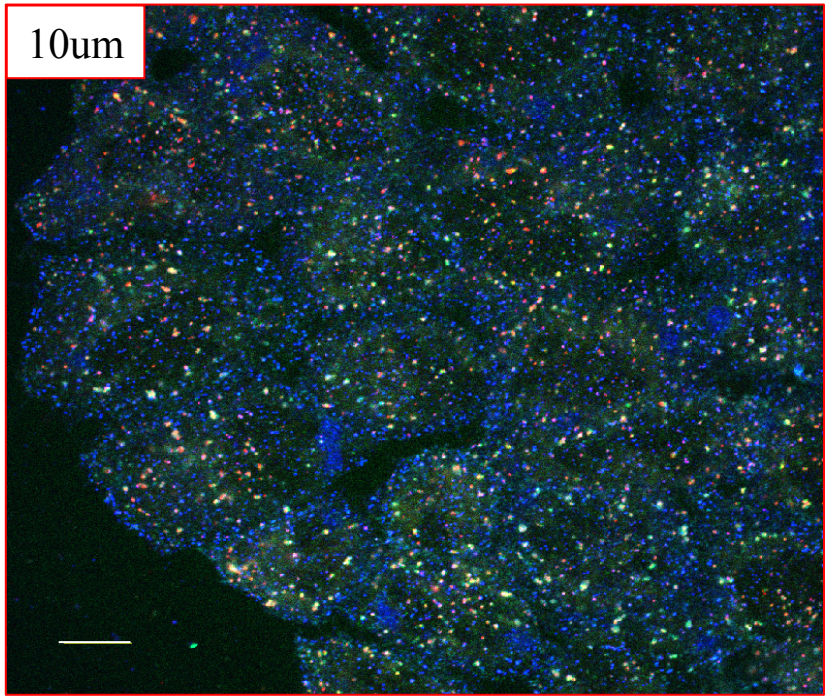


The green vesicle is colocalized with red if more then **x%** of its area is covered by red vesicle(s)

Lets threshold = 50%, then green is colocalized with red, but red is not colocalized with green.

This definition of colocalization is asymmetric, but… better fit our intuition

We don’t have deal with pixels anymore. Now we are working with objects



Original image

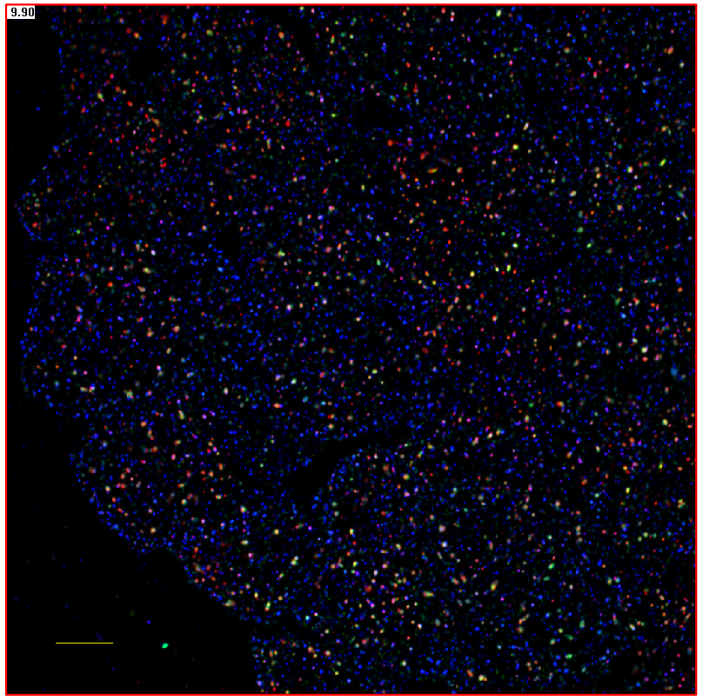
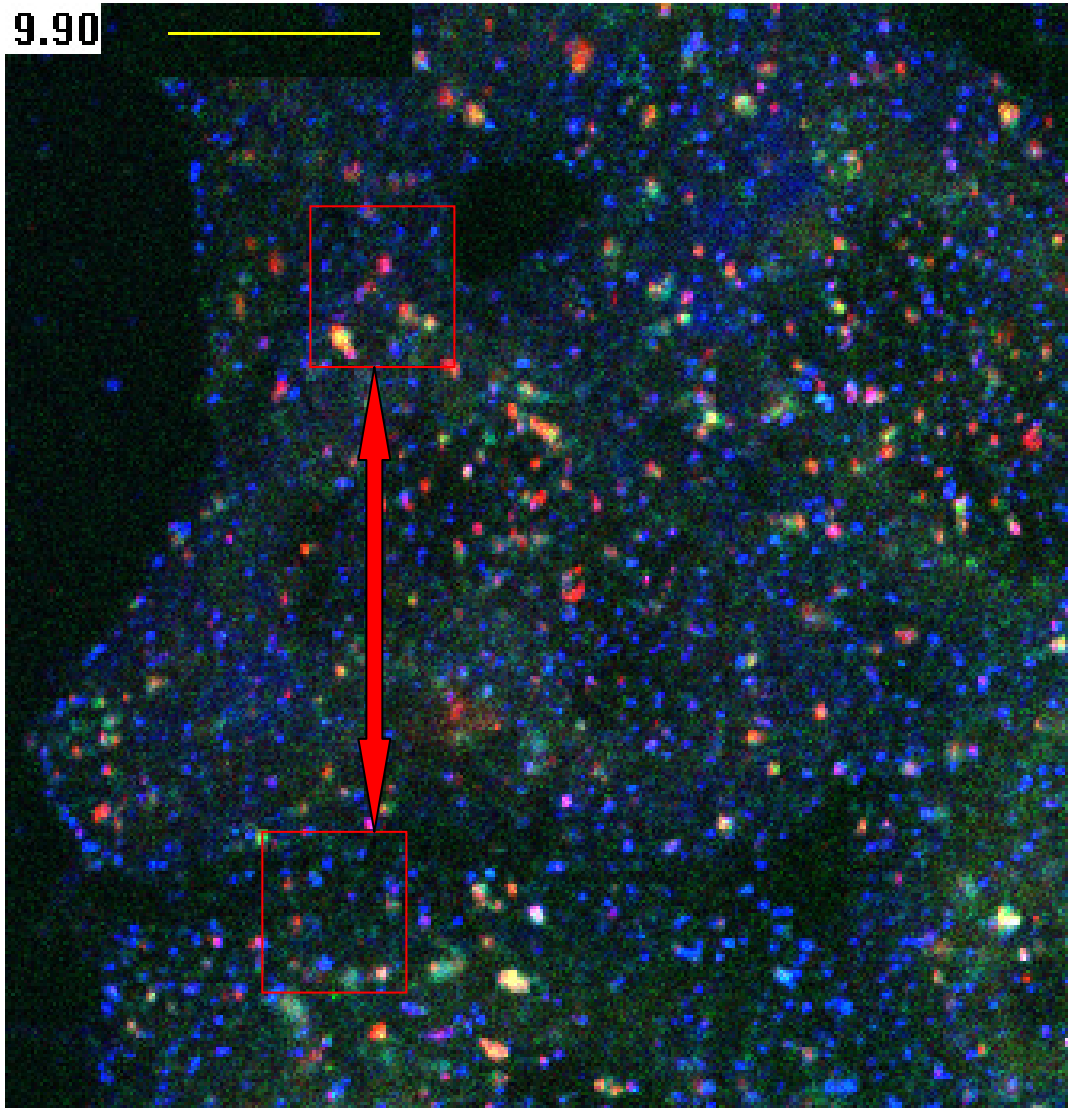
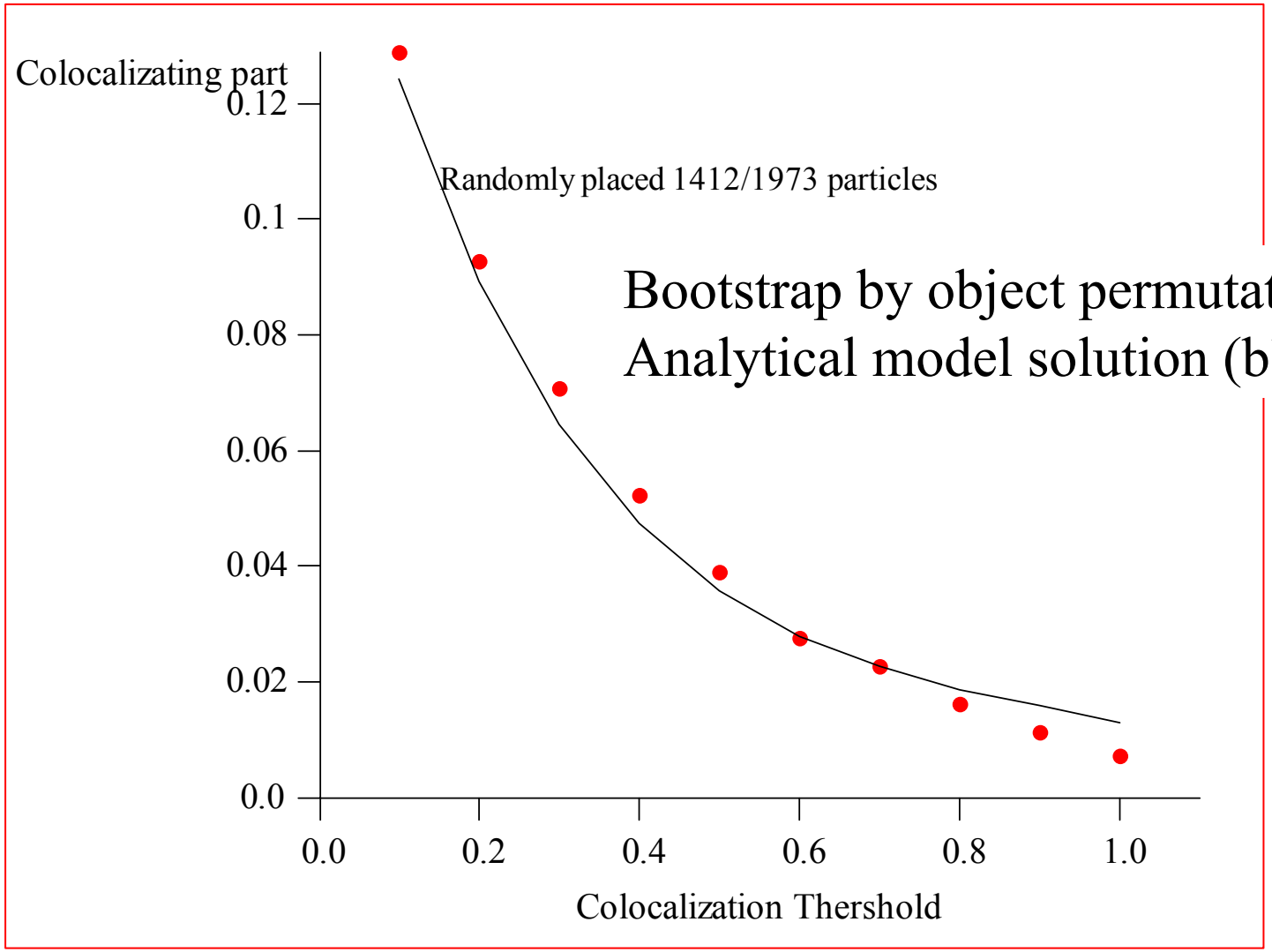


Image reconstructed
from the fitted functions

Object-based estimation of random (apparent) colocalization



Random Colocalization

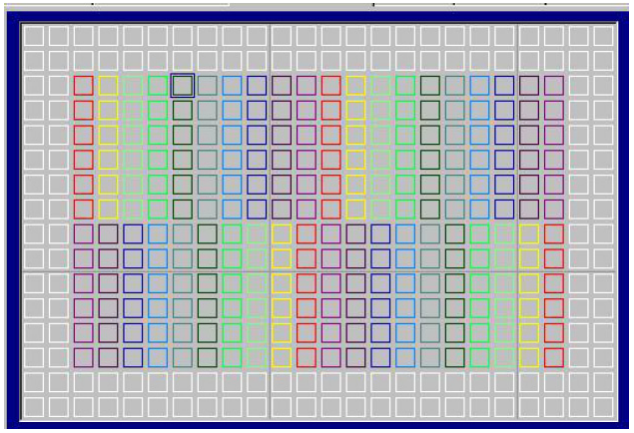


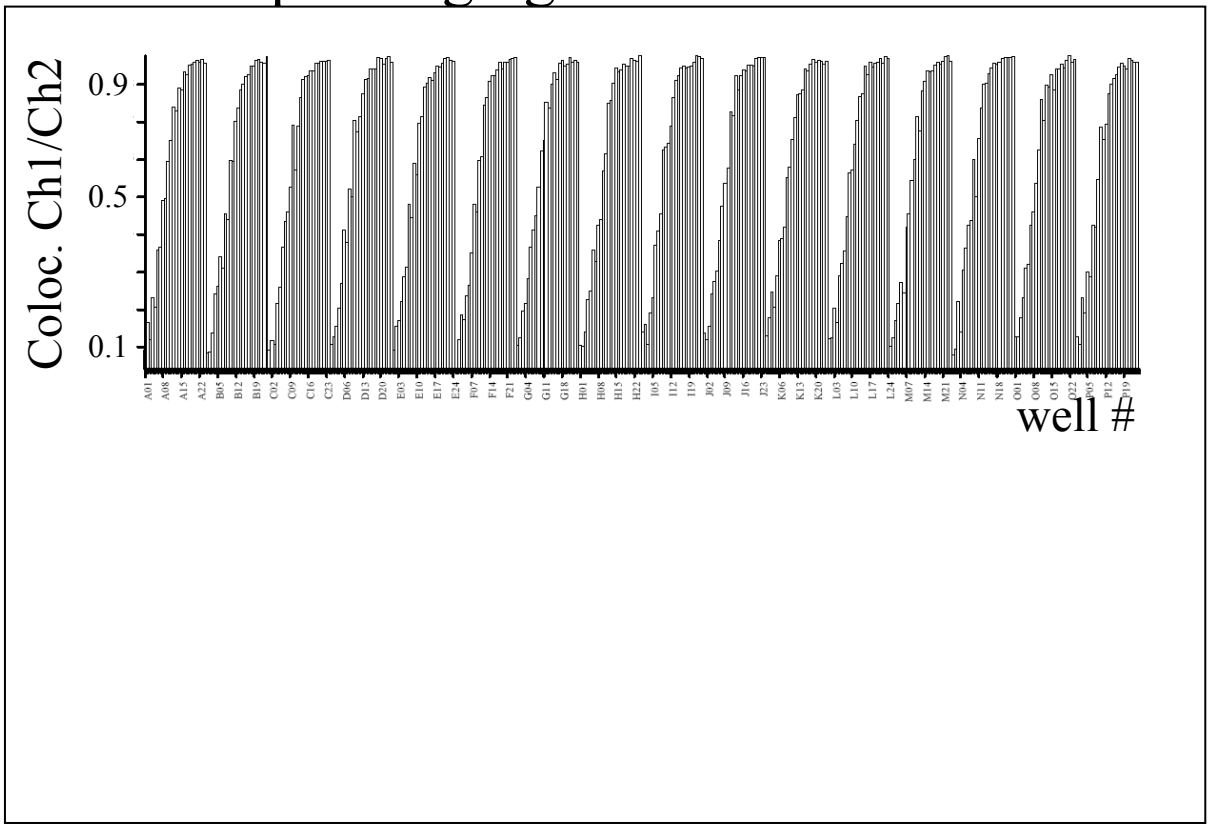
Bootstrap by object permutation (red dots)
Analytical model solution (black line)

Unknown microscopy: Is chromatic shift constant over experiment?

(How long did you worm microscope before imaging?)

The 10min uptake of Tf-Alexa488 and Tf-Alexa647 mixture by HeLa cells. Automatic microscope imaging (12 images per well)





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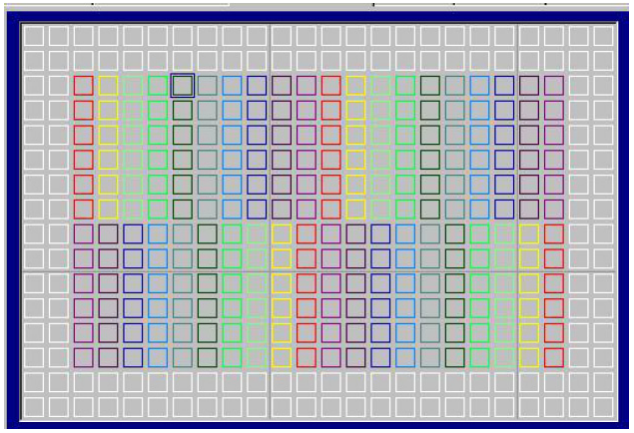
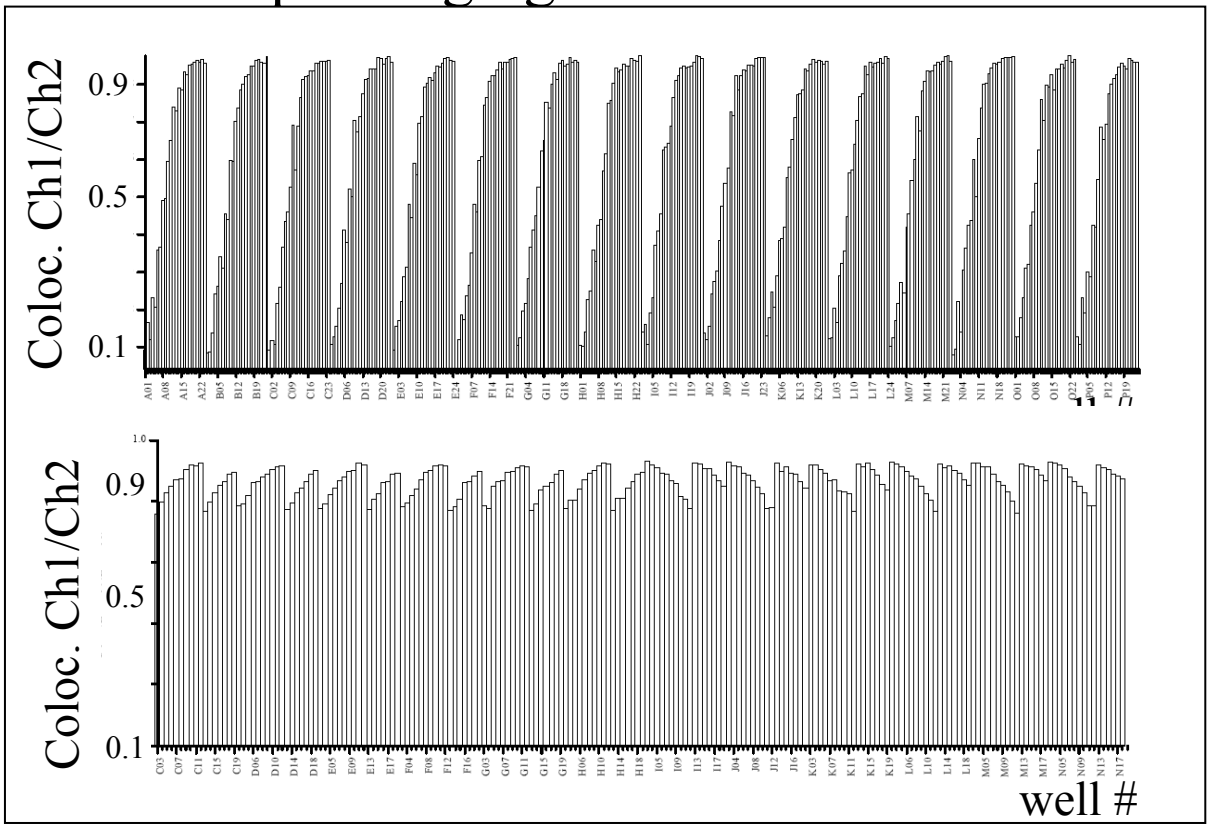
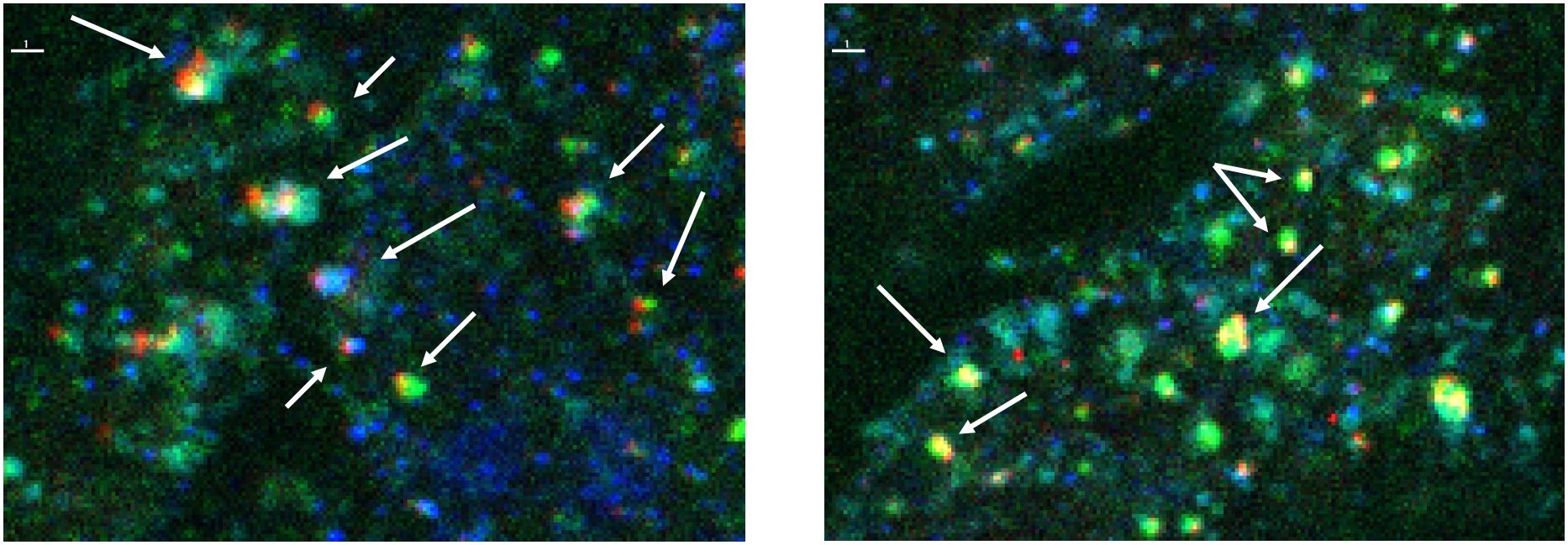




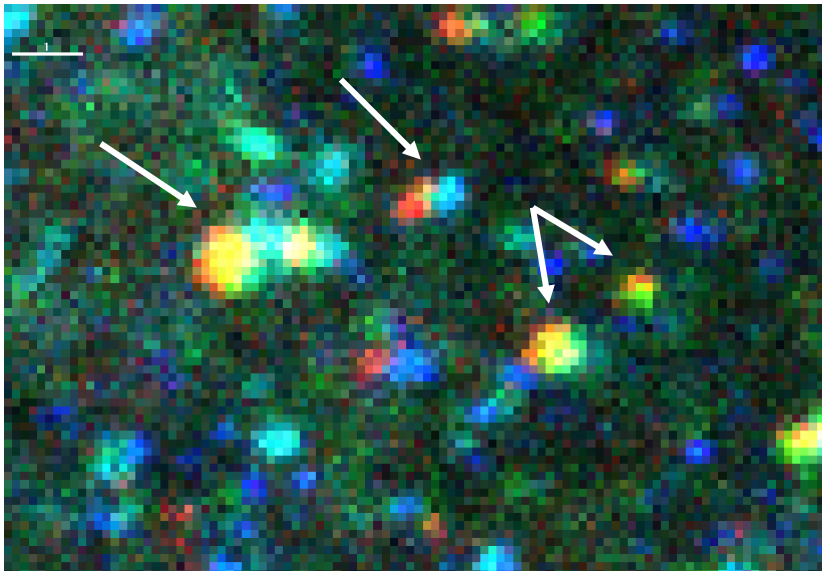
Problems of ‘wet’ experiment: Analysis of pulse-chase experiments (chromatic shift)

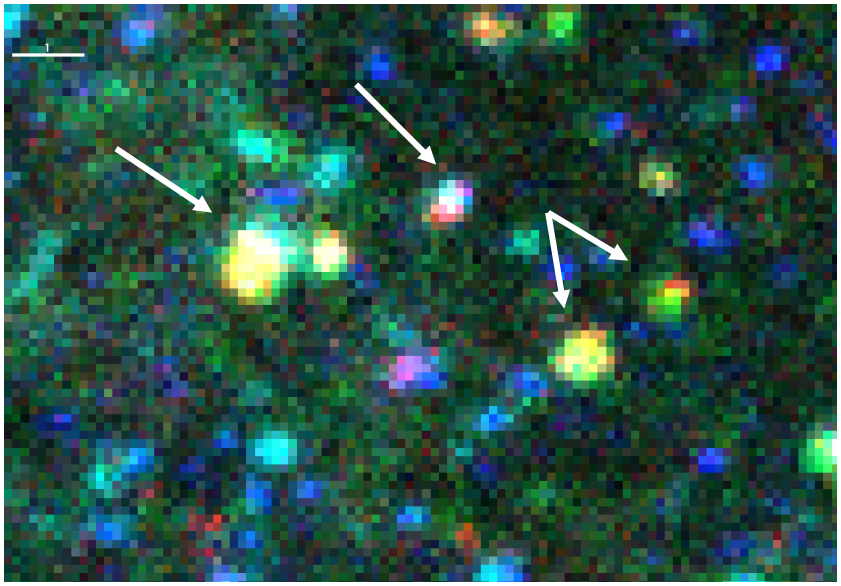


Left and right images have indexes ‘a’ and ‘e’ in same imaging session.

HeLa: 30 sec pulse EGF & Tf, chase 10 min.
Red: EEA1; Green: EGF; Blue:APPL1; Cyan: Tfn

Problems of ‘wet’ experiment: Analysis of pulse-chase experiments (chromatic shift)

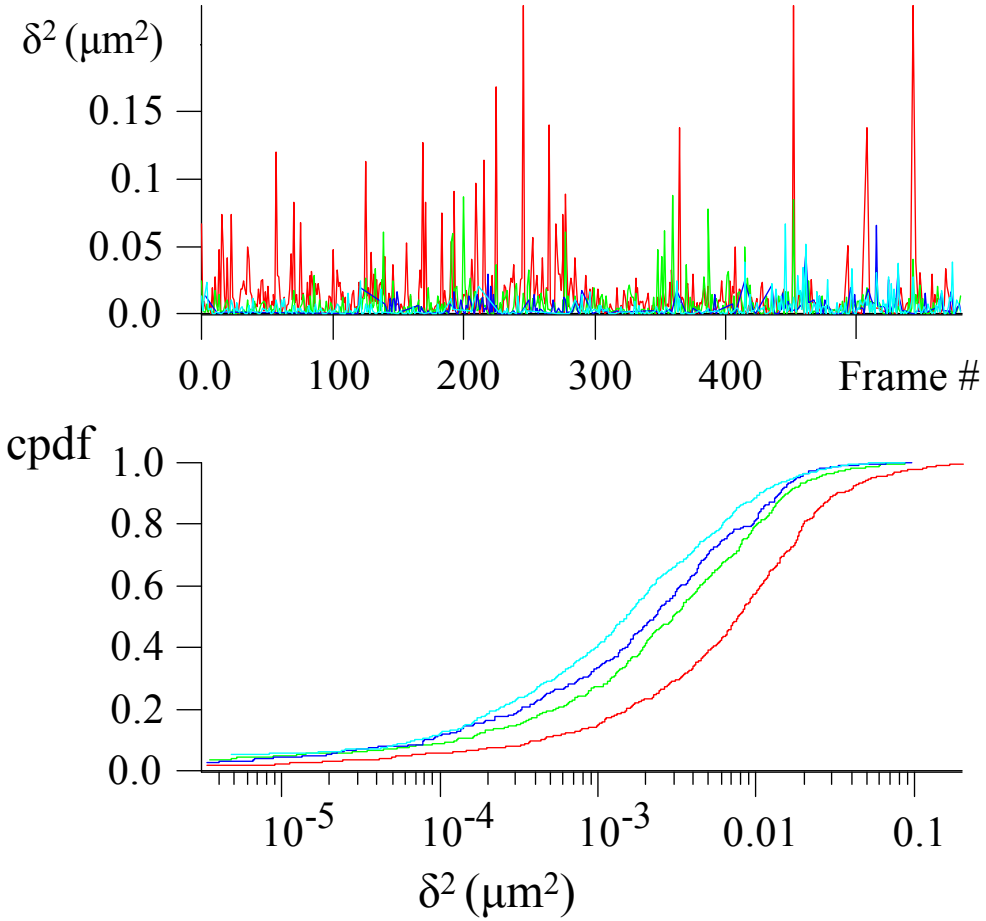




Shift correction on ‘per-frame’ basis.

HeLa: 30 sec pulse EGF & Tfn, chase 10 min.
Red: EEA1; Green: EGF; Blue:APPL1; Cyan: Tfn

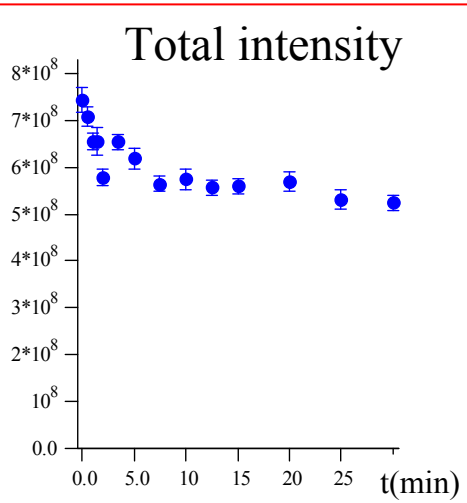
Problems of ‘wet’ experiment: Analysis of pulse-chase experiments (chromatic shift)

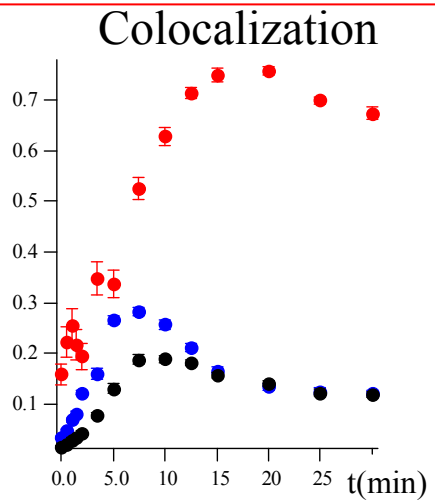


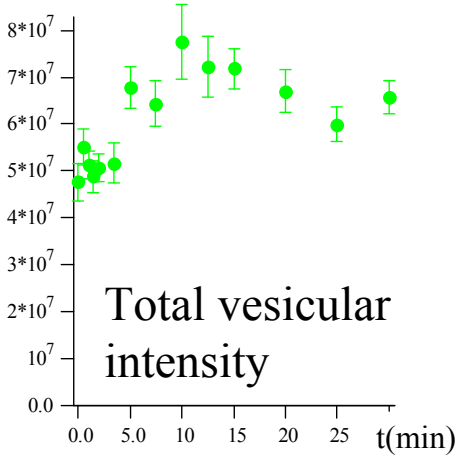
1 pix = 0.116 μm

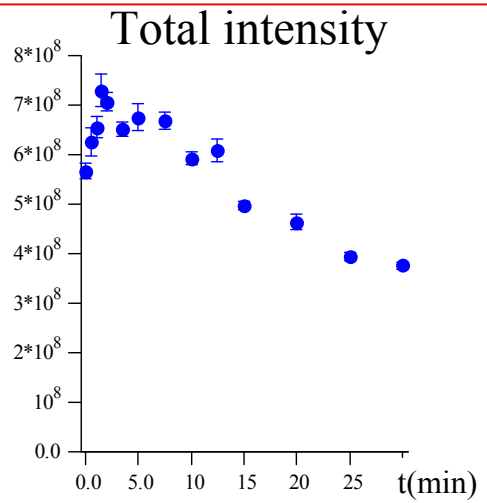
Cargo for degradation (EGF)

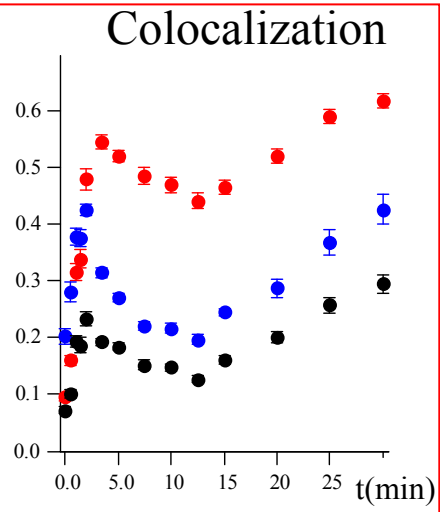
Cargo for recycling (Tfn)

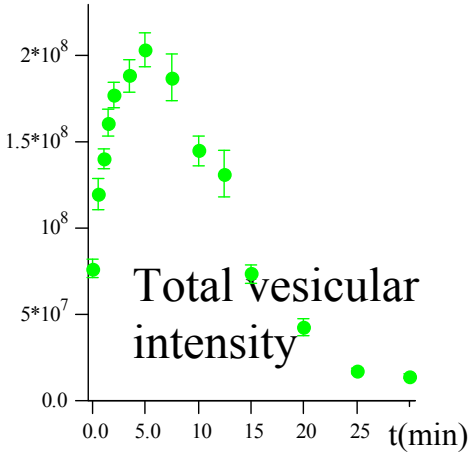












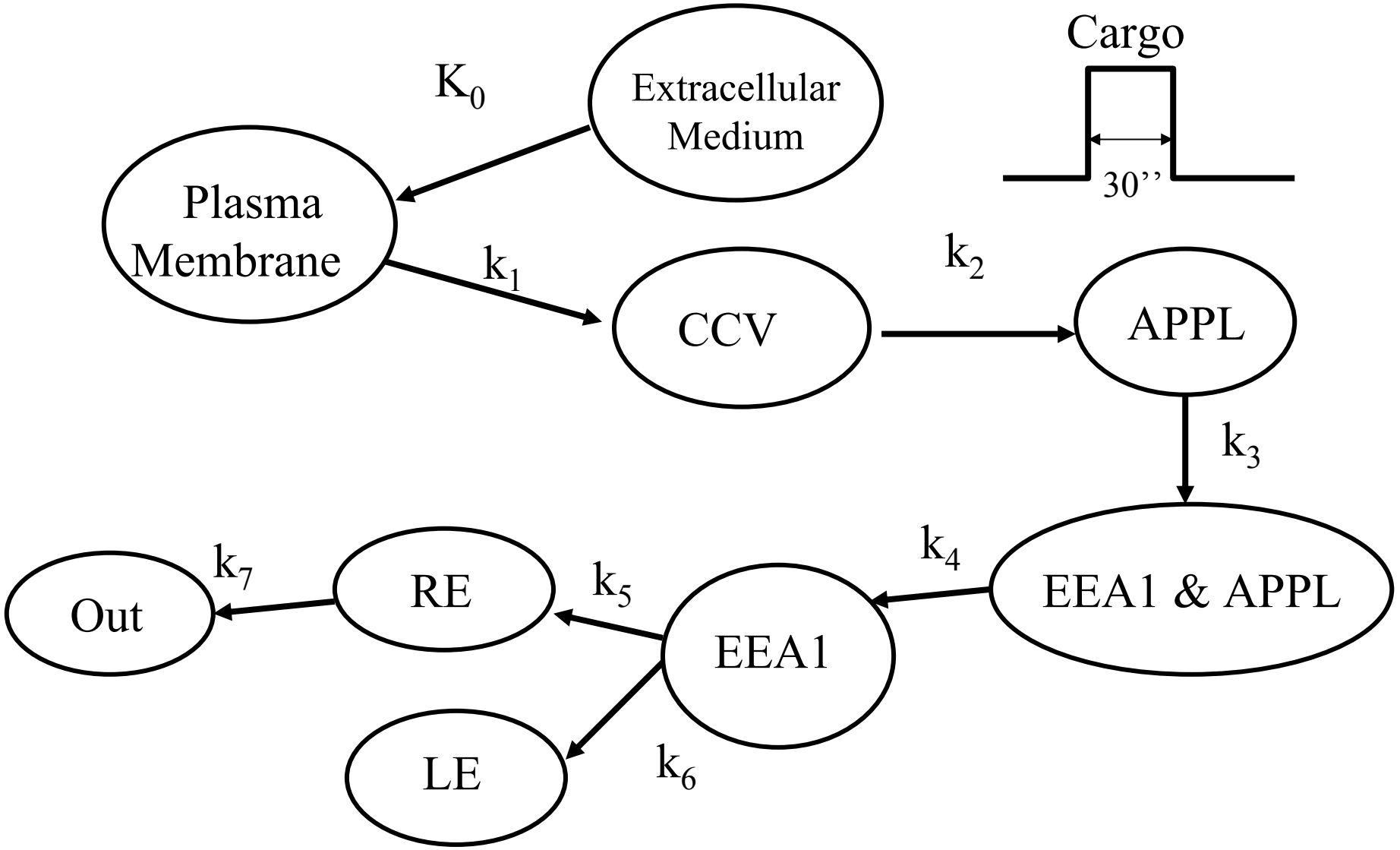
Colocalization:

Red – Cargo to EEA1

Blue –Cargo to APPL1

Black – Cargo to EEA1 & APPL1

Simple Cargo Flow Model



Simple Cargo Flow Model

$$\frac{dC_{pm}}{dt} = \Phi_{in}(t) - k_1 C_{pm}$$

$$\frac{dC_{ccv}}{dt} = k_1 C_{pm} - k_2 C_{ccv}$$

$$\frac{dC_{APPL}}{dt} = k_2 C_{ccv} - k_3 C_{APPL}$$

$$\frac{dC_{APPL-EEA1}}{dt} = k_3 C_{APPL} - k_4 C_{APPL-EEA1}$$

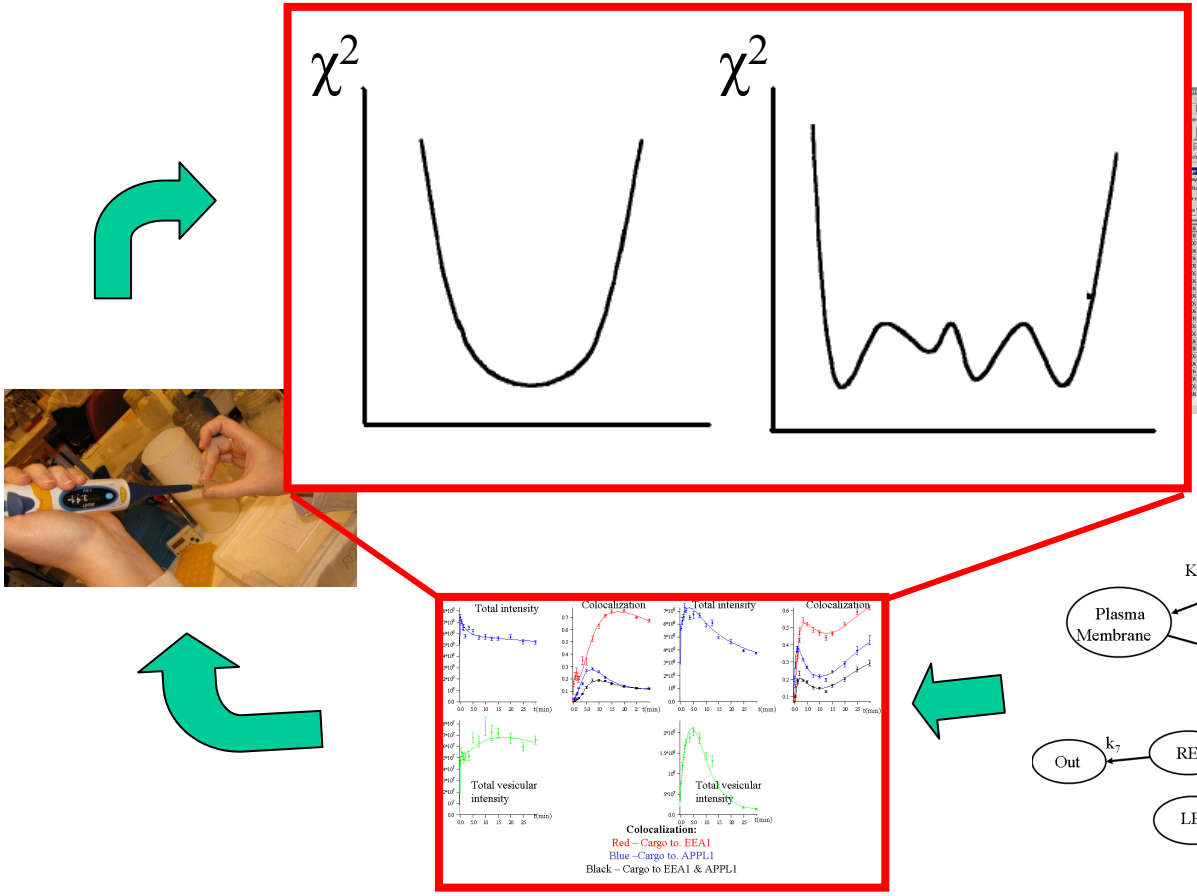
$$\frac{dC_{EEA1}}{dt} = k_4 C_{APPL-EEA1} - (k_5 + k_6) C_{EEA1}$$

$$\frac{dC_{RE}}{dt} = k_5 C_{EEA1} - k_7 C_{RE}$$

$$\frac{dC_{LE}}{dt} = k_6 C_{EEA1}$$

$$\frac{dC_{out}}{dt} = k_7 C_{RE}$$

Model meet Experiment:
Do we get global optimum?



Local minimum traps in the model fitting procedure

The application of theoretical models of complex shape to the fitting of experimental spectra having closely overlapping bands

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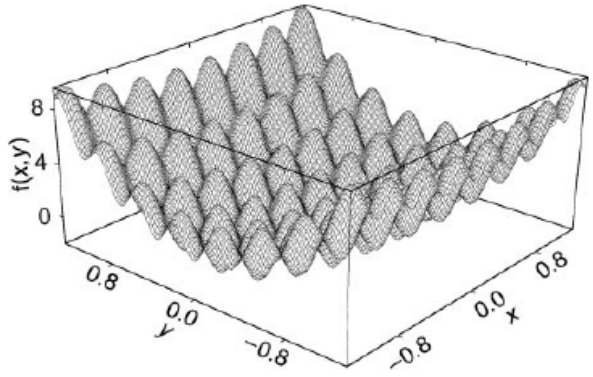
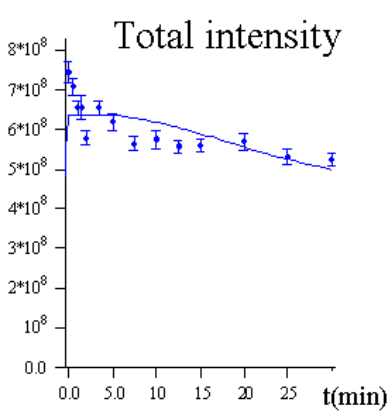
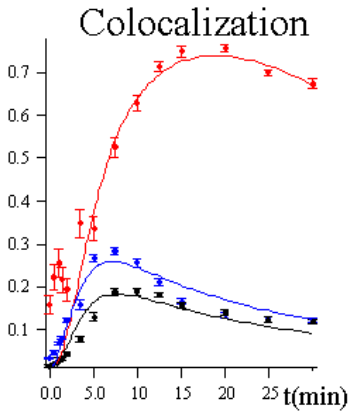
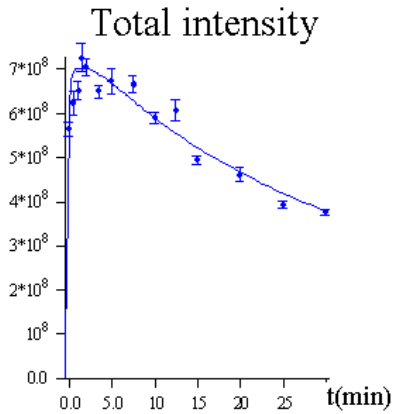
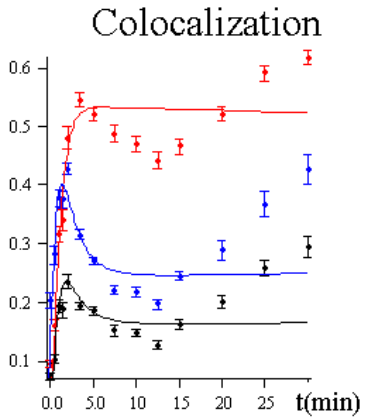


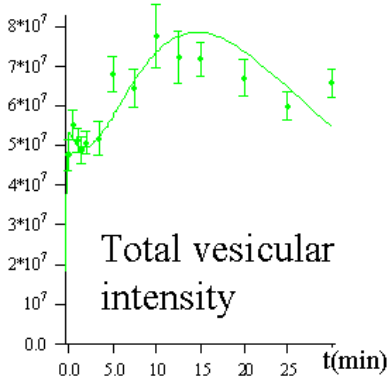
The real life is more complicate than our simple model (to be continued…)

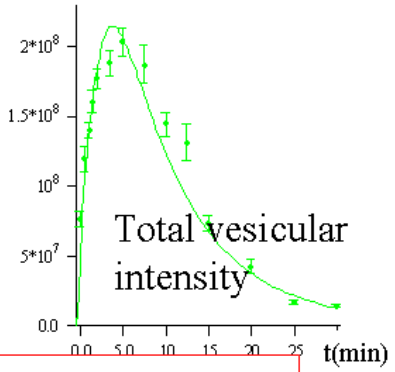












Colocalization:

Red – Cargo to EEA1

Blue –Cargo to APPL1

Black – Cargo to EEA1 & APPL1

Conclusion

- Quantitative comparison model and experiment requires quantitative analysis of all steps, which connects “wet” experiment and “digital” data
- “Small” imperfections of experimental equipment could lead to wrong model selection

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