

Quantitative Microscopy:  
Bridge between “wet” biology  
and  
computer science

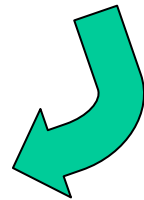
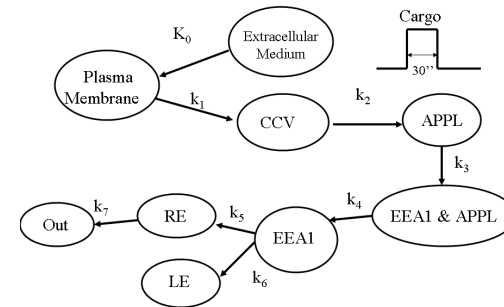
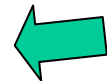
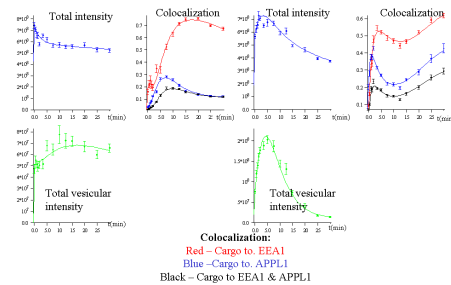
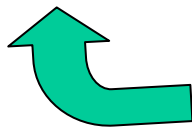
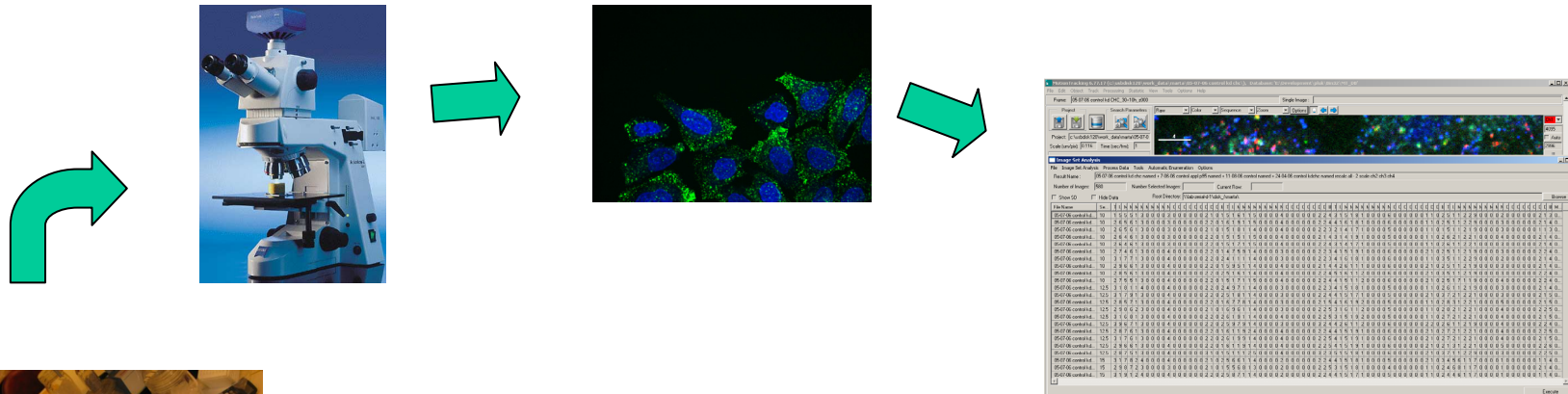
Yannis L. Kalaidzidis

MPI-CBG, Dresden

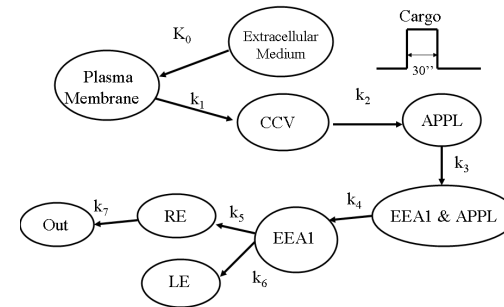
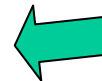
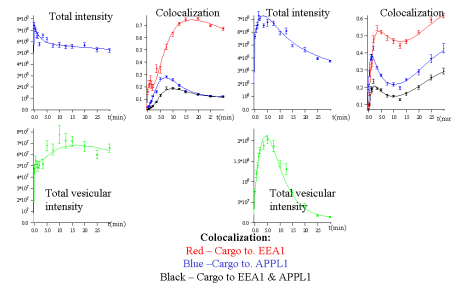
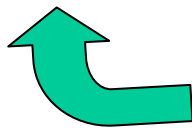
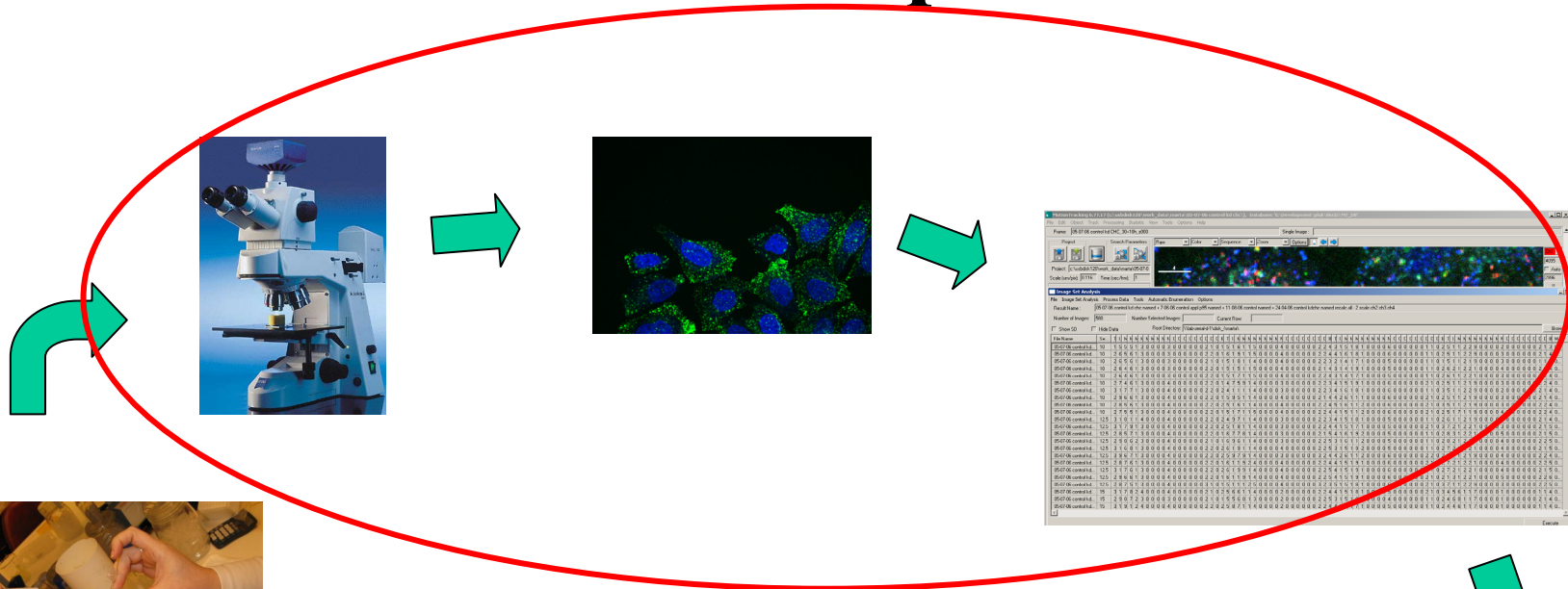
MLSB'09

05 Sept. 2009

# System Biology: Experiment Driven Model and Model Driven Experiment



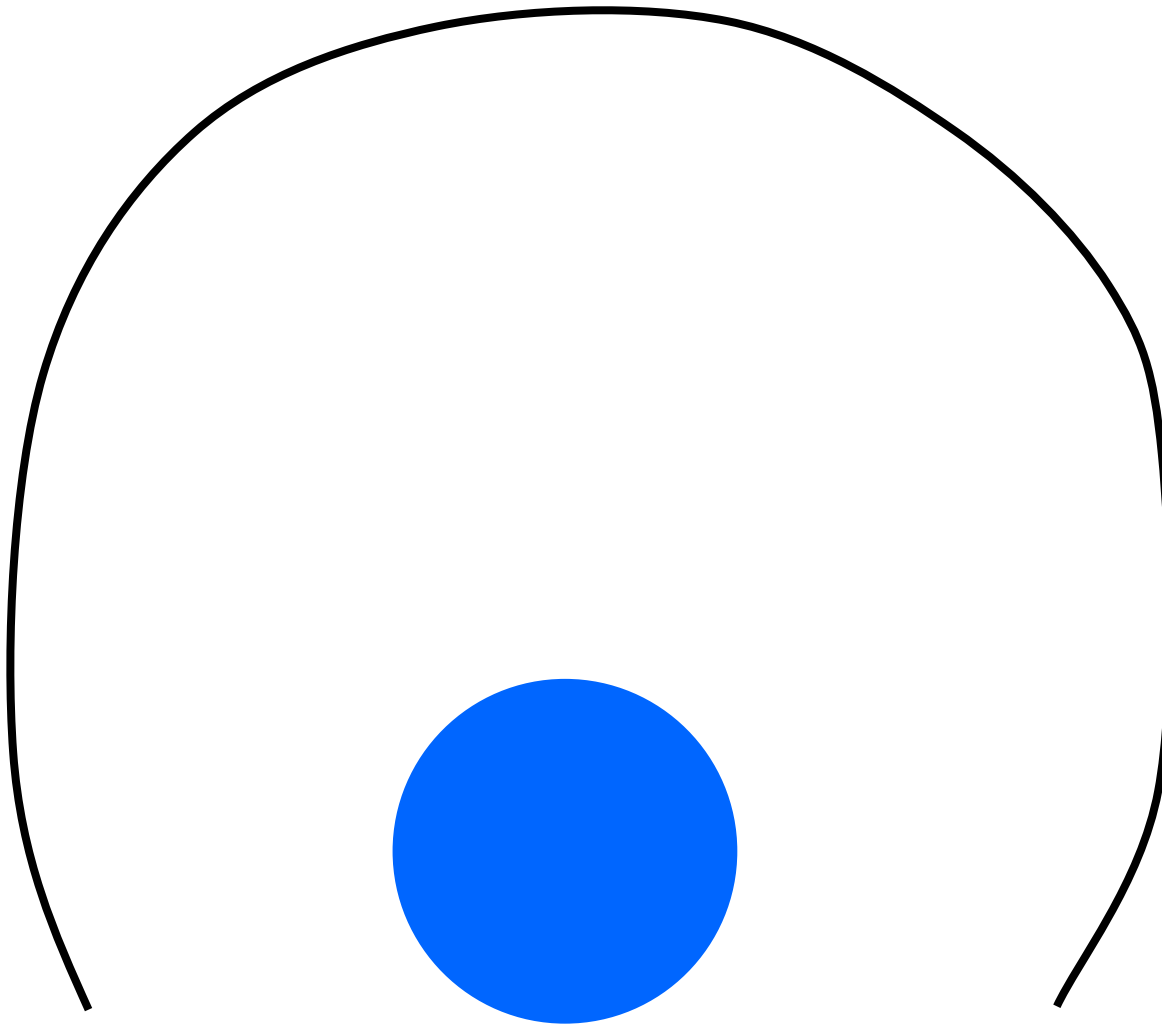
# System Biology: Experiment Driven Model and Model Driven Experiment



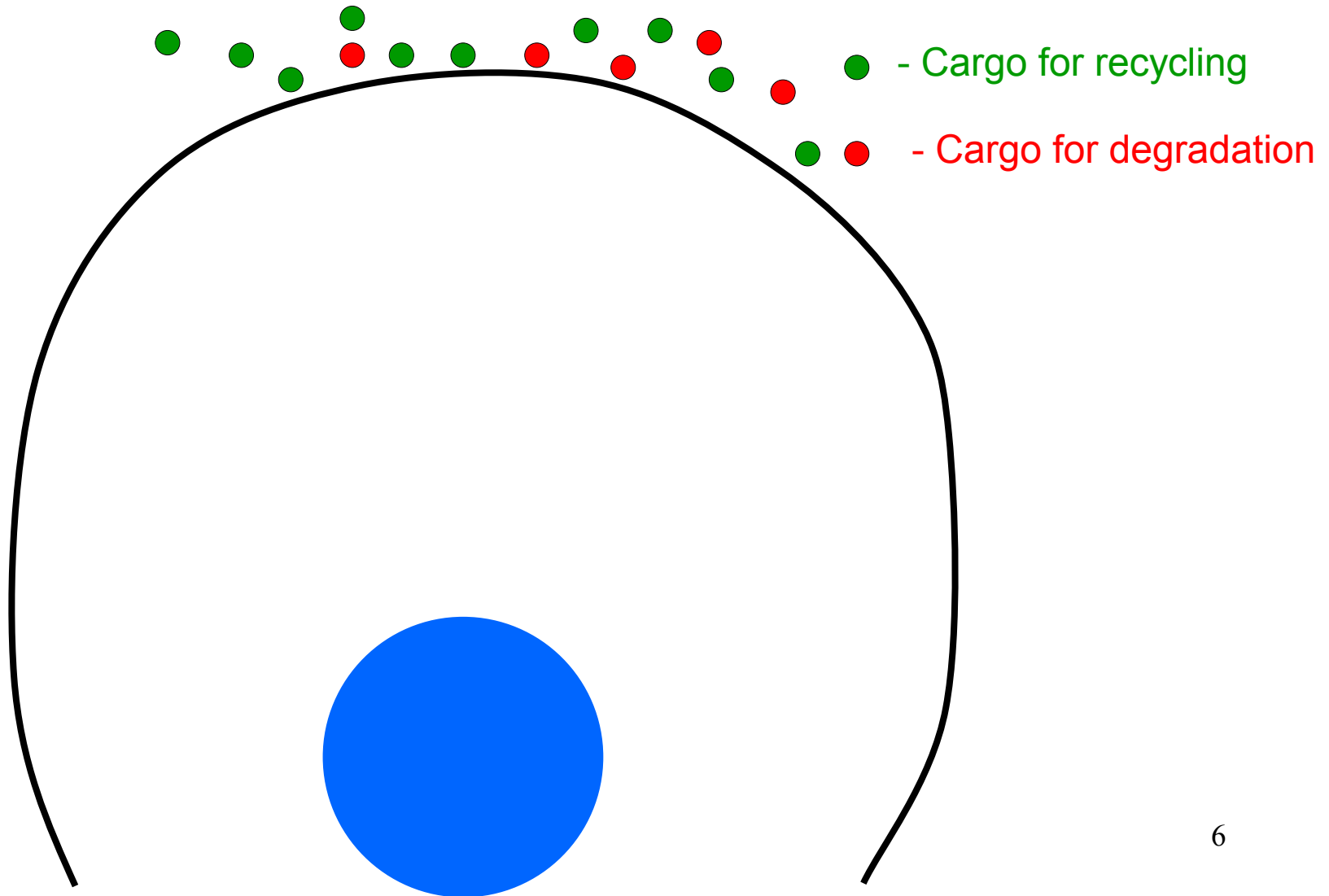
Quantitative Microscopy in wild :  
Learning endocytosis kinetic model  
from pulse-chase experiment



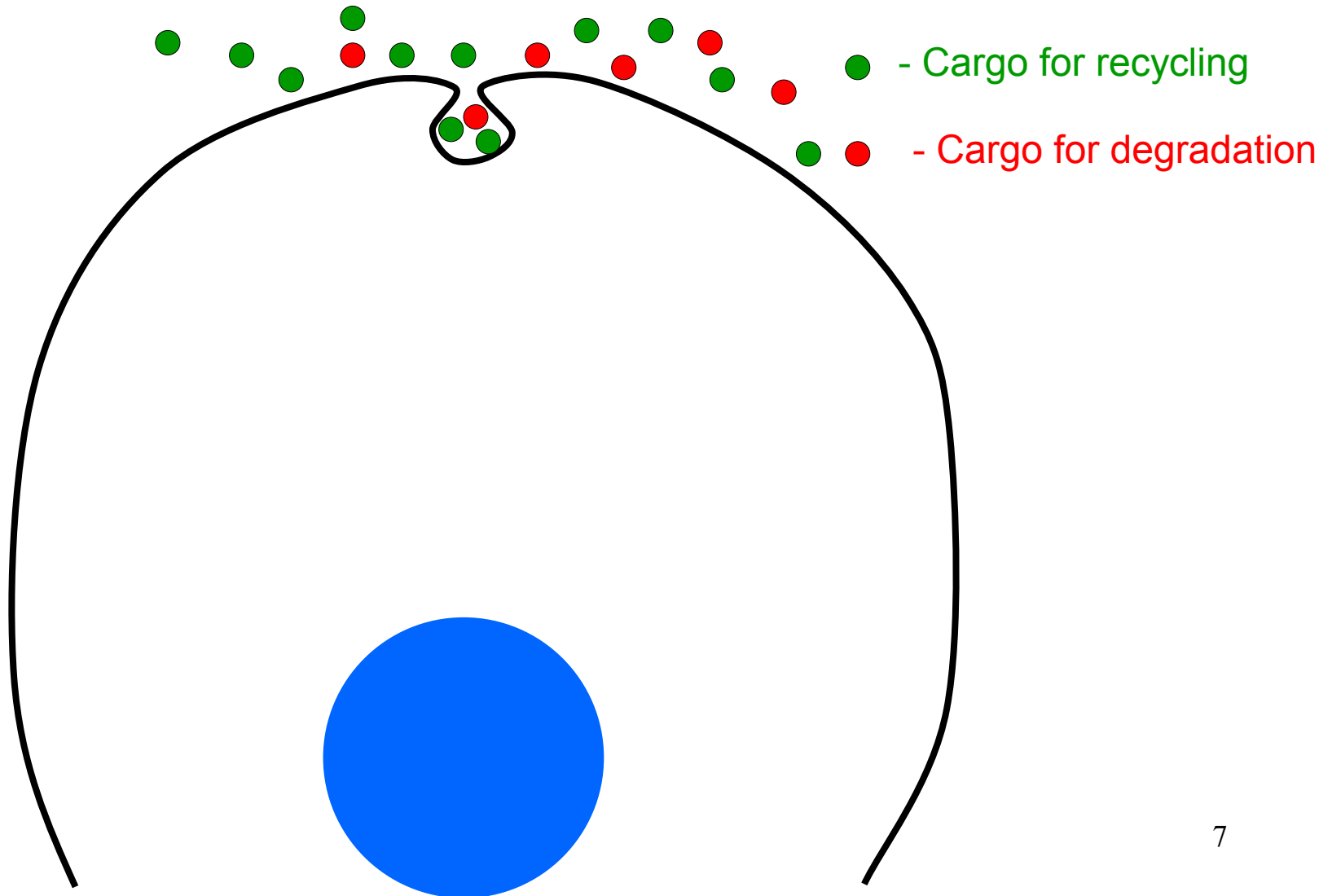
# Endocytosis: Cellular Organelles Transport Cargo



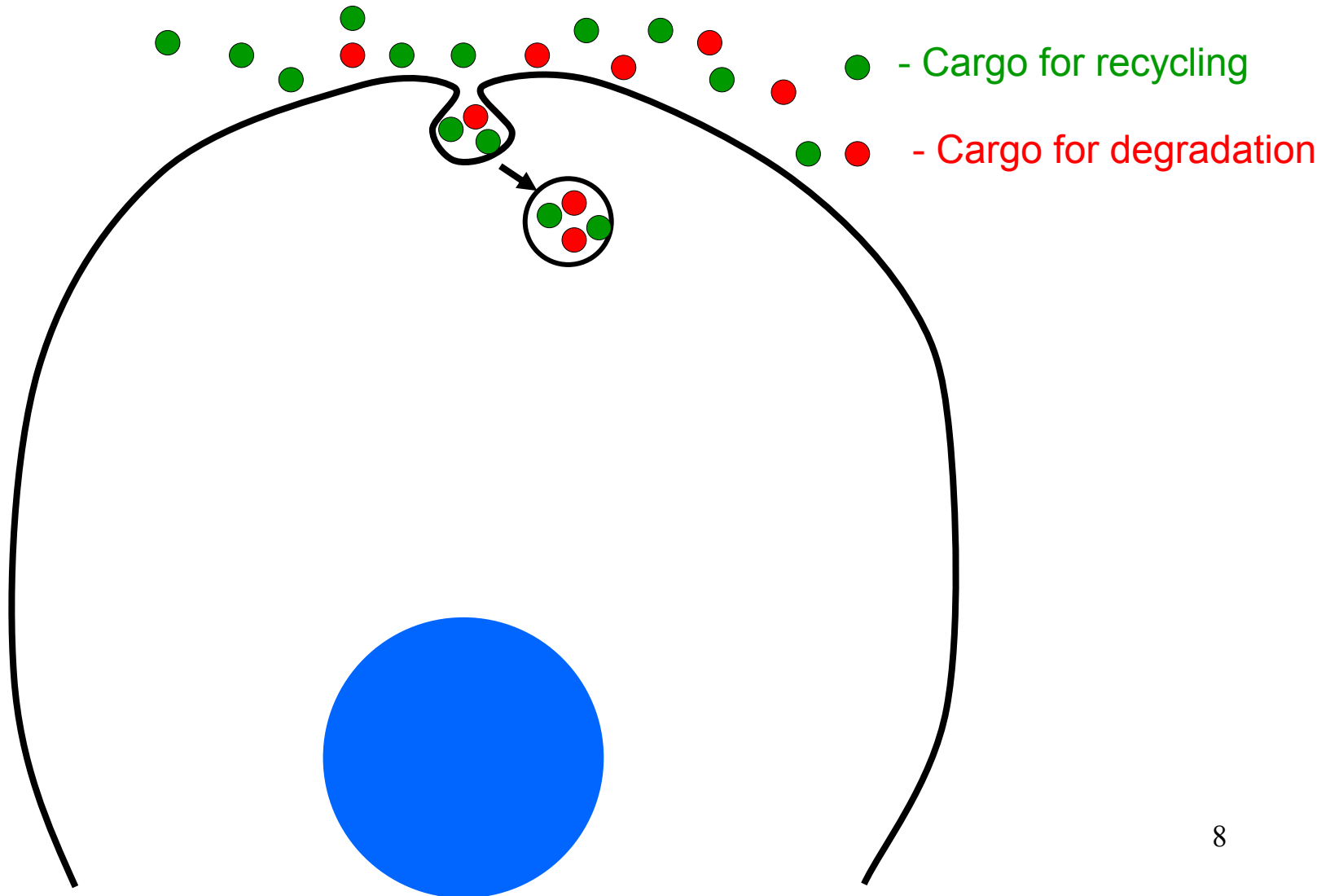
# Endocytosis: Cellular Organelles Transport Cargo



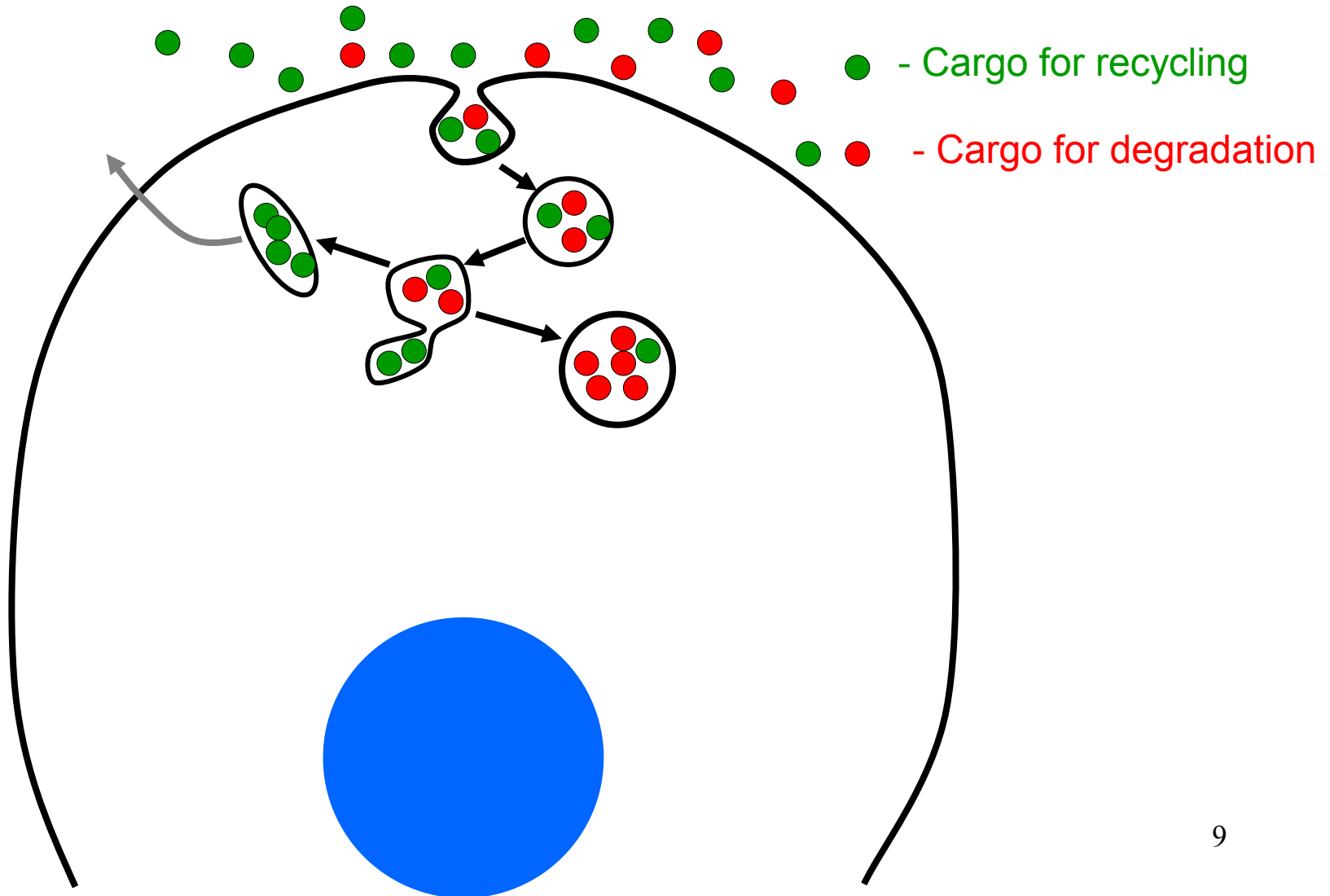
# Endocytosis: Cellular Organelles Transport Cargo



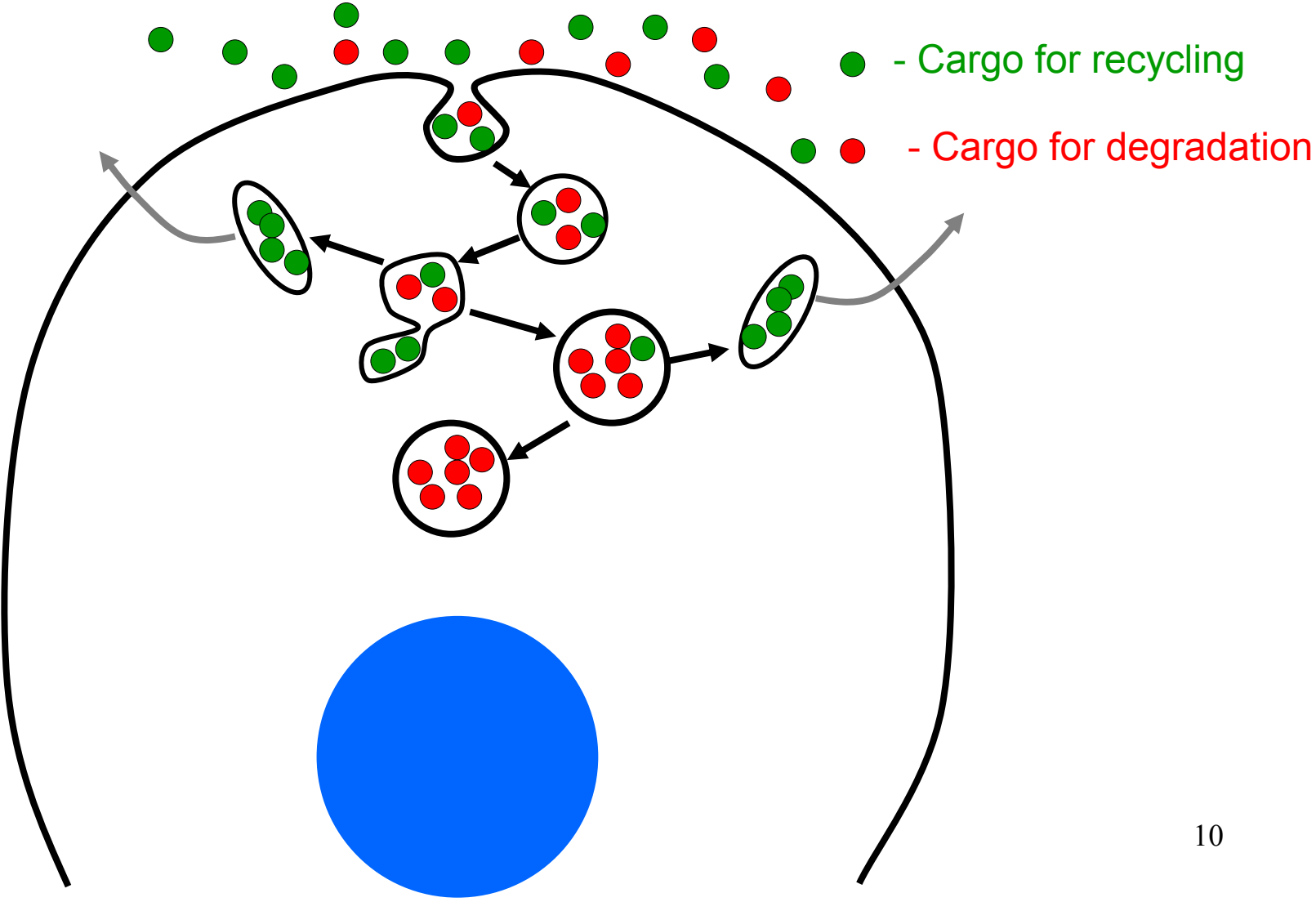
# Endocytosis: Cellular Organelles Transport Cargo



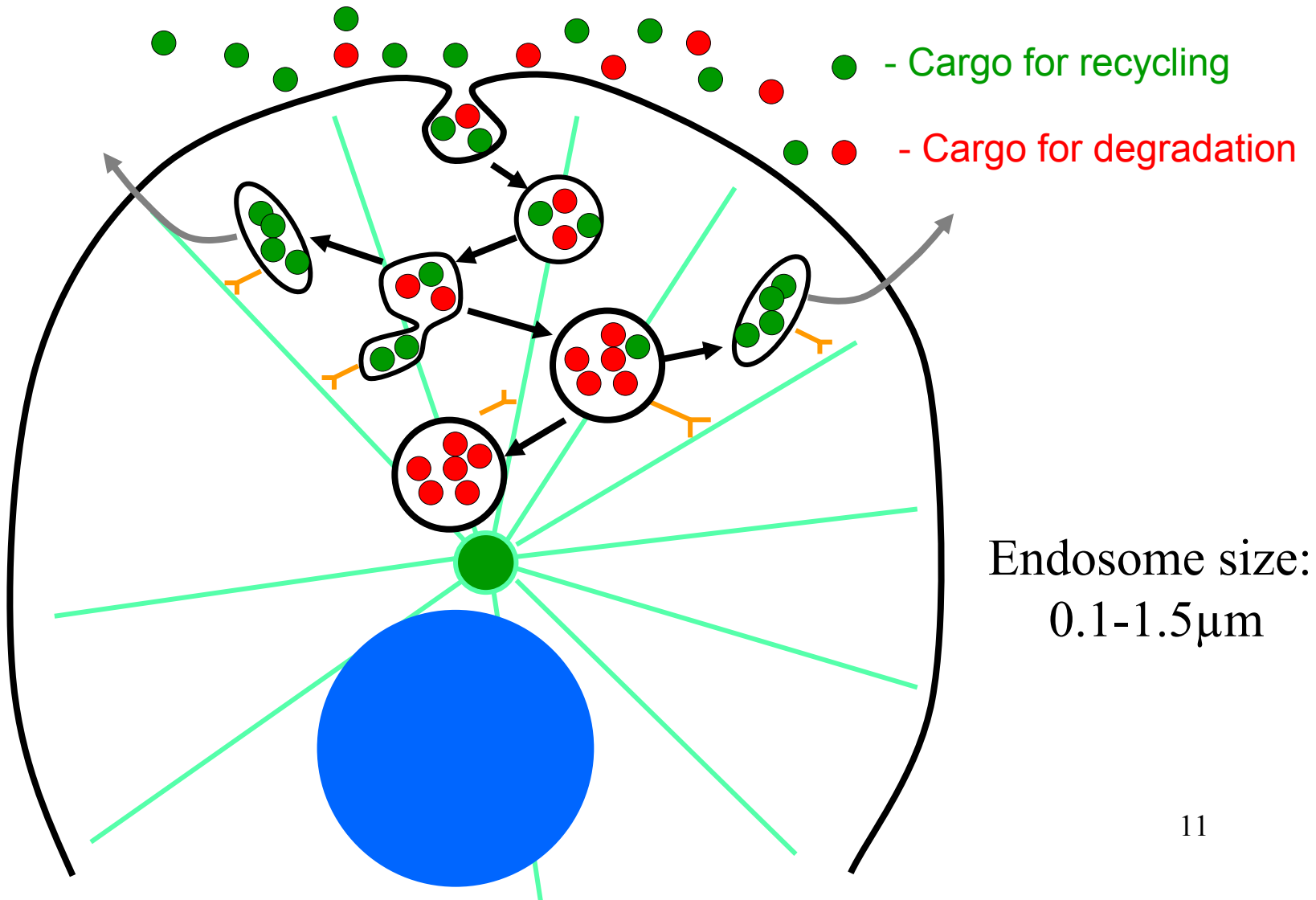
# Endocytosis: Cellular Organelles Transport Cargo



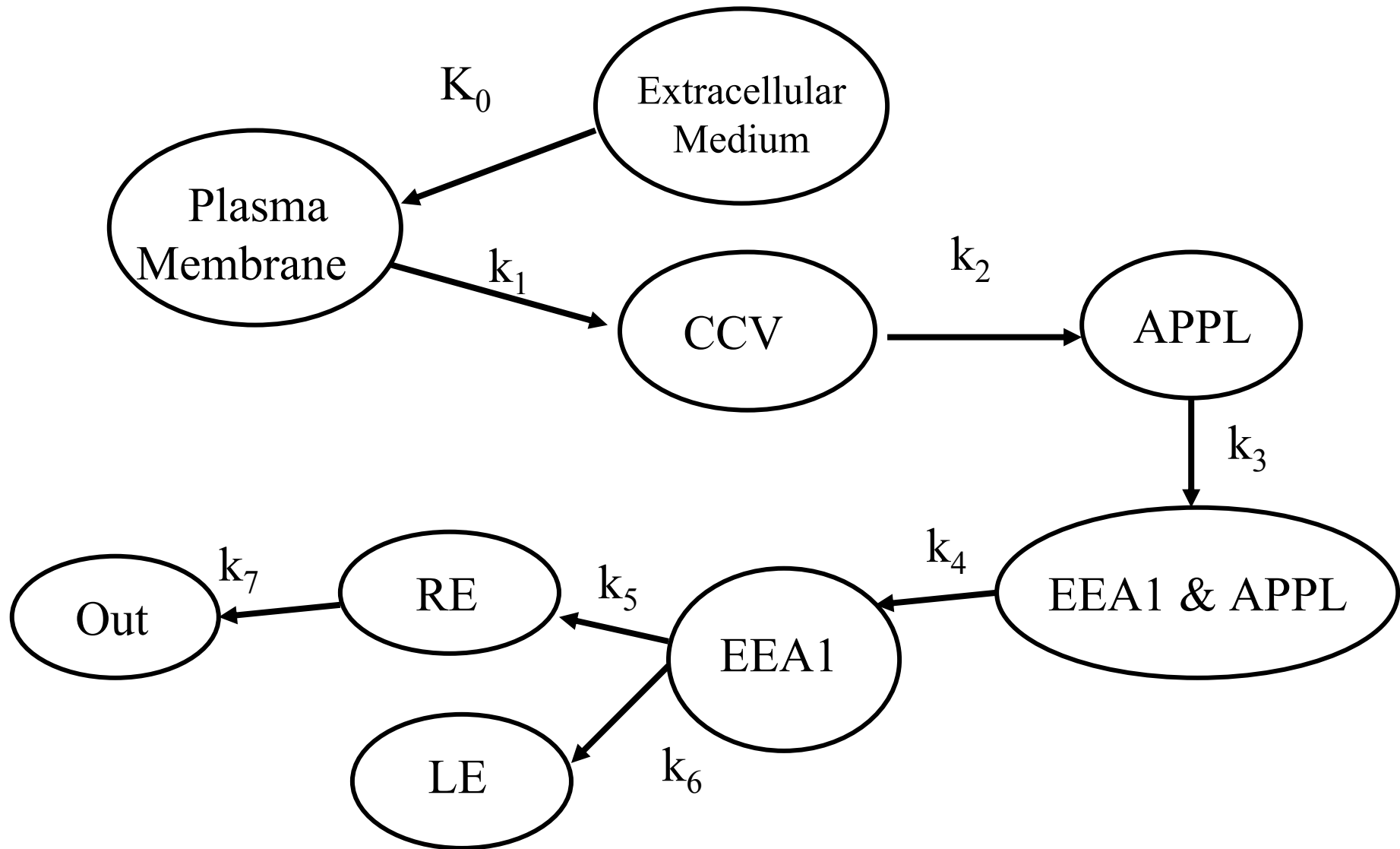
# Endocytosis: Cellular Organelles Transport Cargo



# Endocytosis: Cellular Organelles Transport Cargo

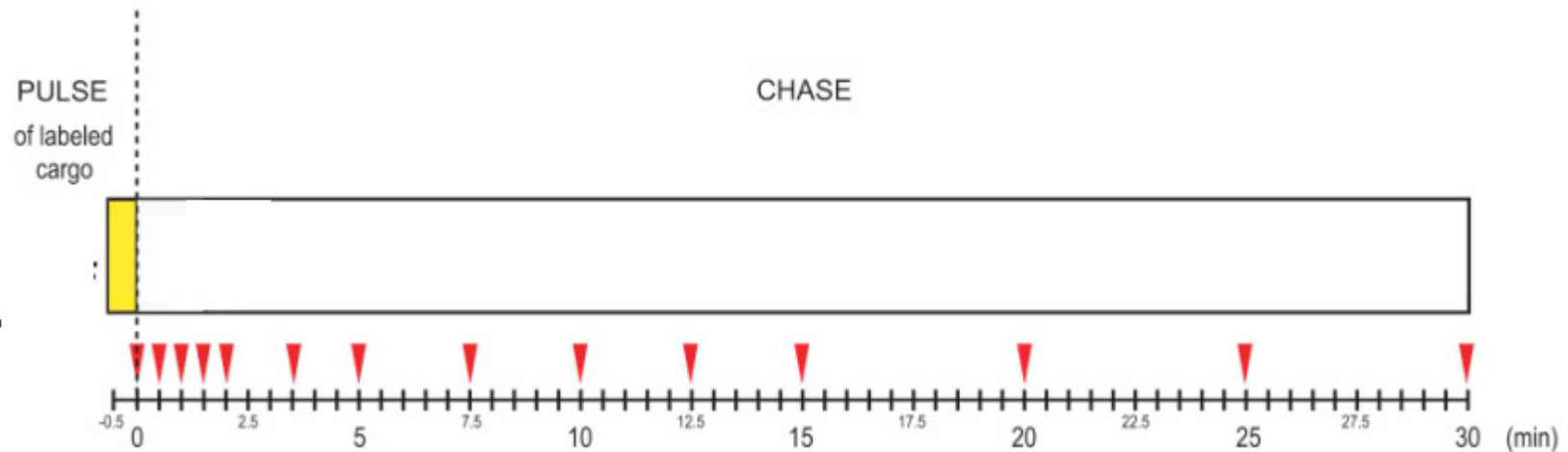


# Simple Cargo Flow Model



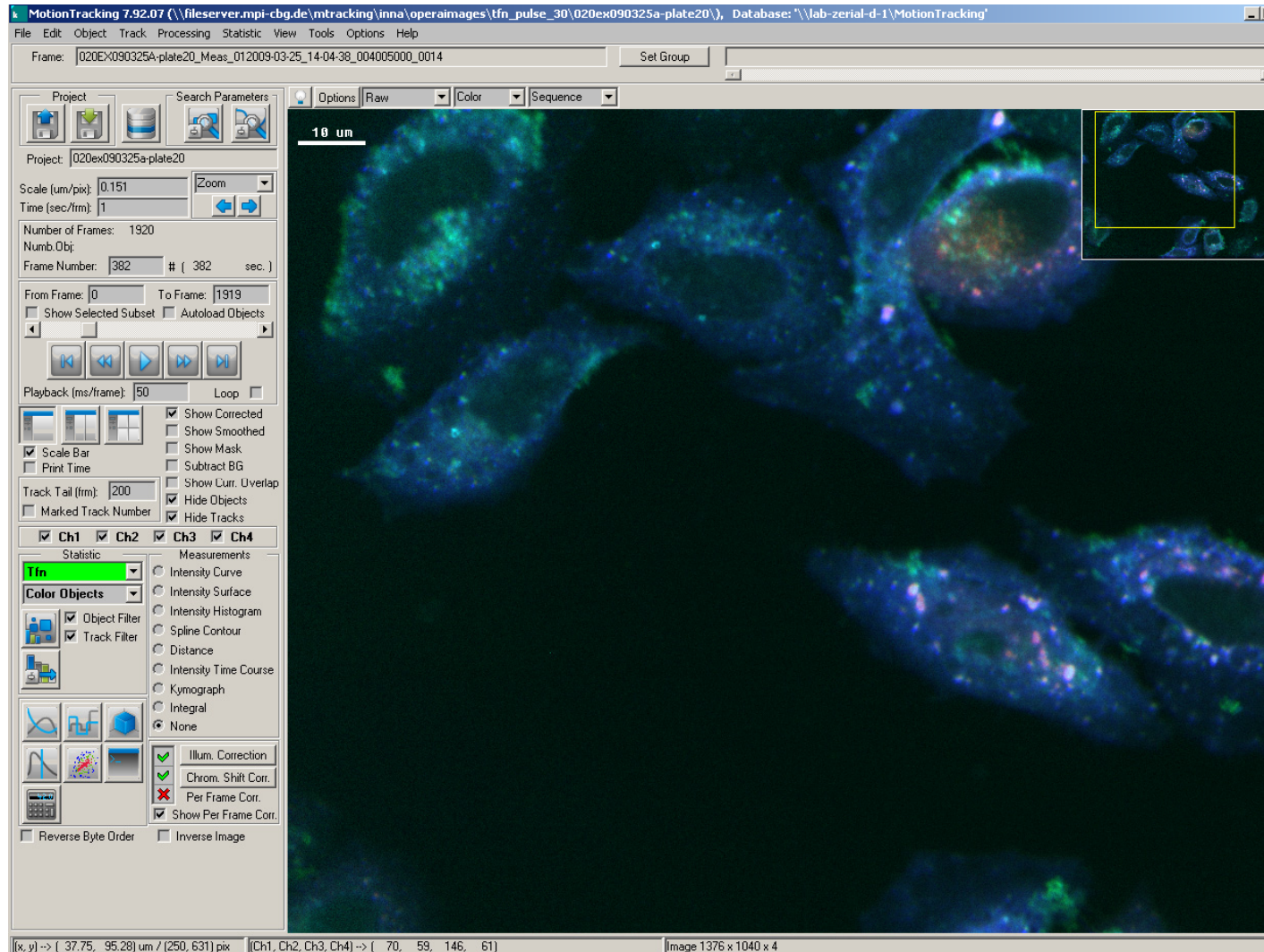


# Pulse-chase experiment

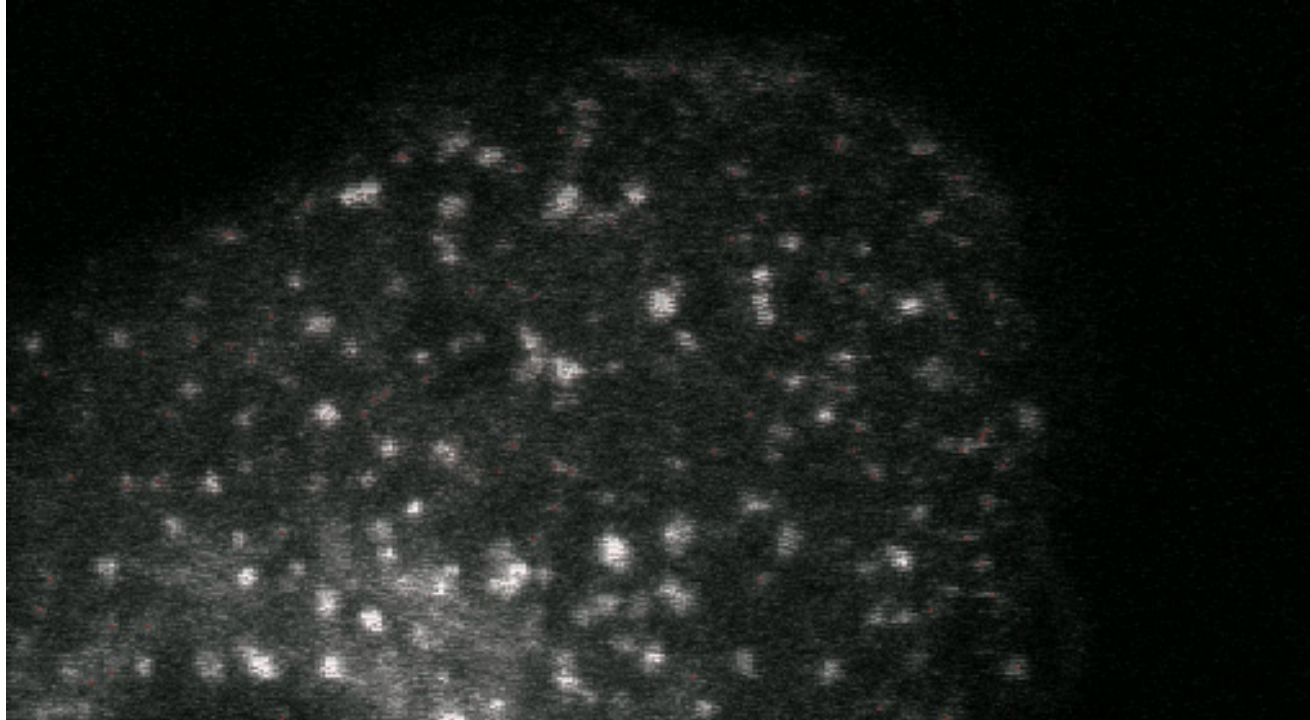


- HeLa cells
- 30'' pulse of fluorescent cargo
- Chasing at set of time points
- Fixing and staining by antibodies endosomal markers
- **Imaging**
- **Endosome identification**
- **Colocalization cargo with endosomal markers**

# First step from “wet” experiment to computer: imaging

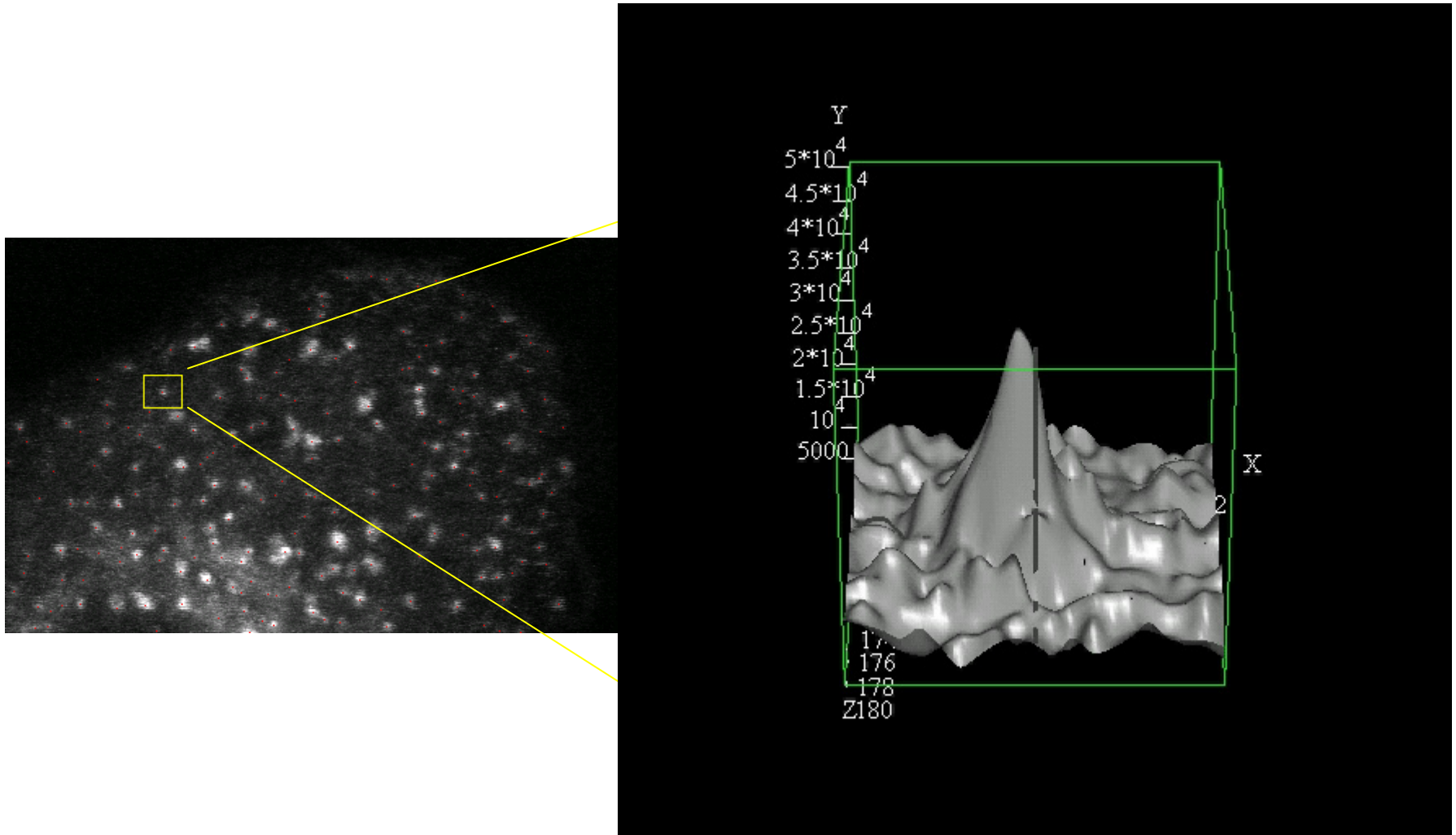


# Endosome identification: What is challenge?



- 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

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

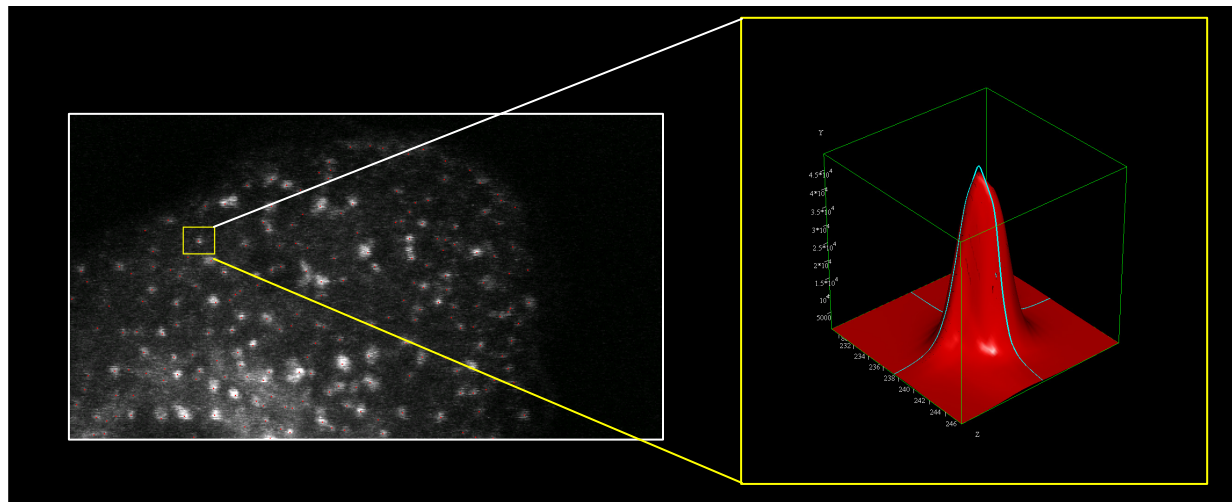
where  $A_0$  - intensity at the center

$x_0, y_0$  - center coordinates

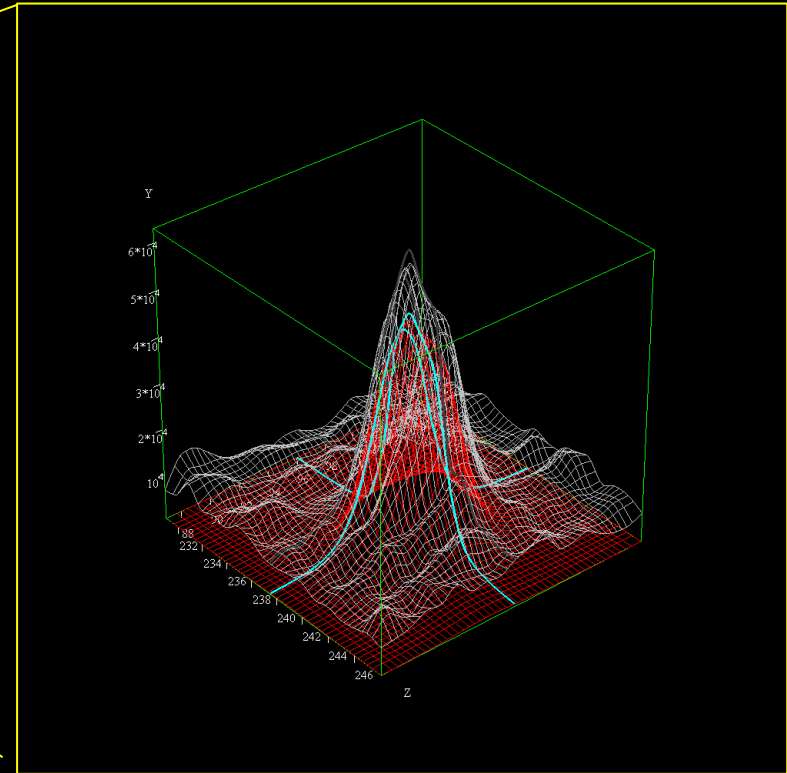
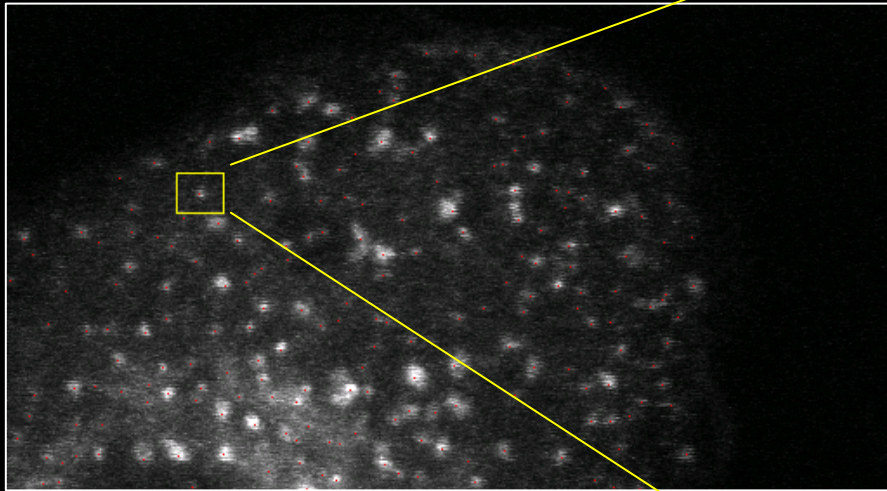
$w, h$  - width by perpendicular dimensions

$\alpha$  - angle between main axis and axis Y

$p$  - power factor

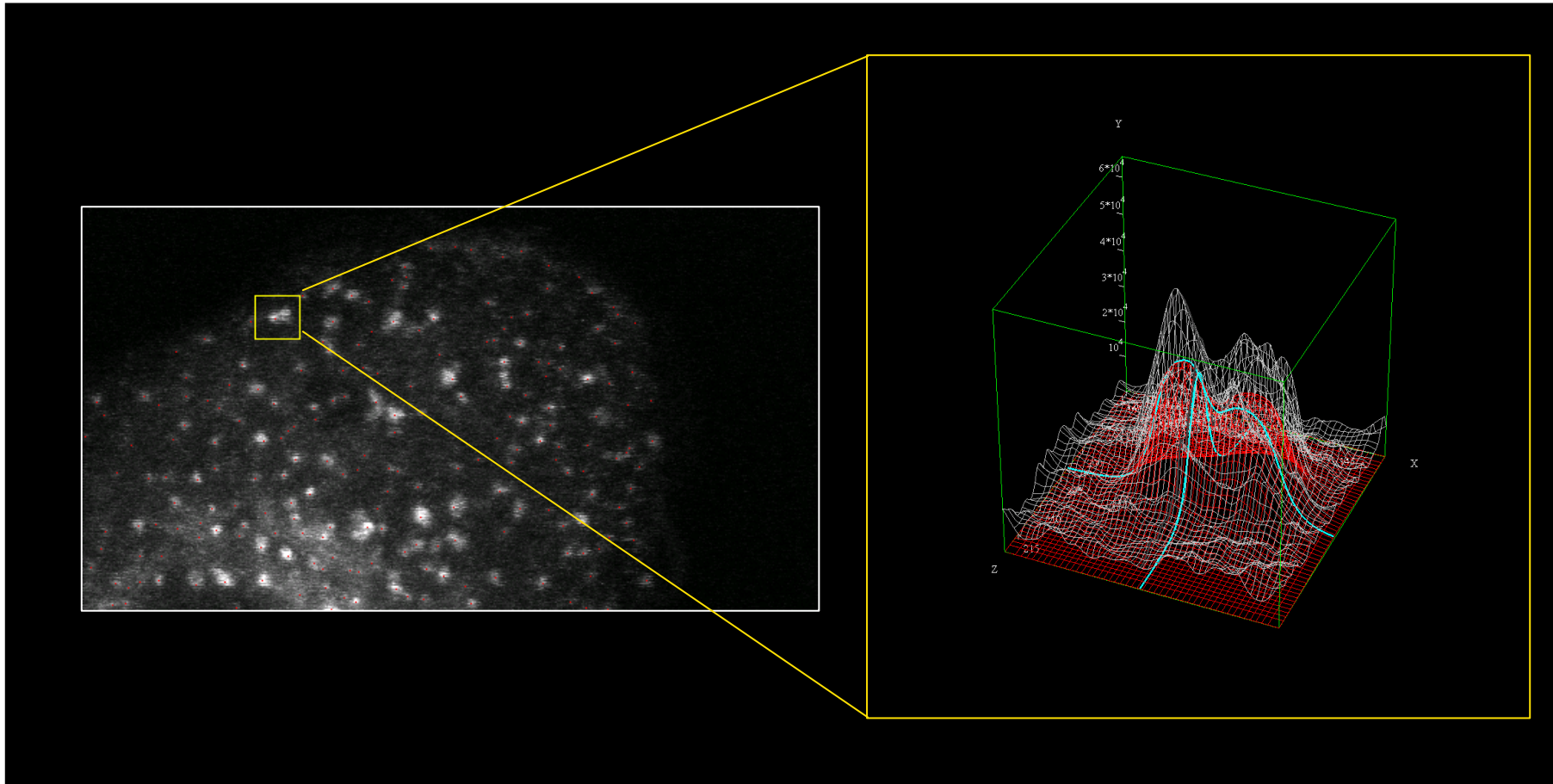


Base function is fitted to the image  
by minimization  $\chi^2$



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

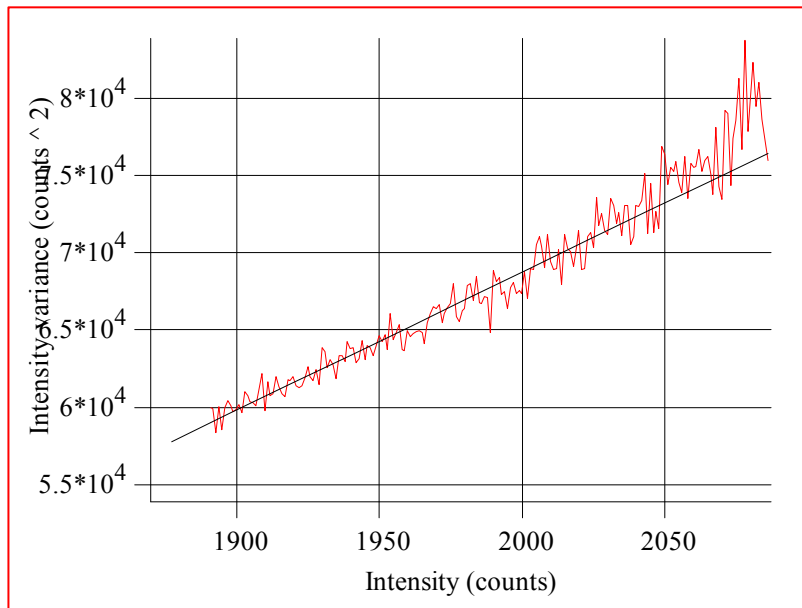
# Endosomes with complex shape can be modeled by sum of base functions



# Fitting requires image noise analysis

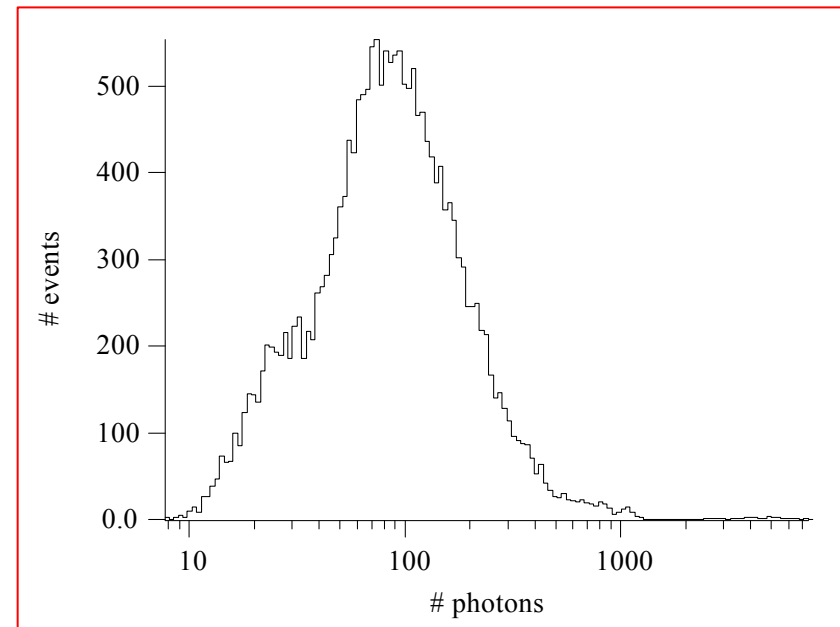
$$\chi^2 = \sum_{i,j} \frac{(I_{i,j} - F_{i,j})^2}{\sigma_{i,j}^2}$$

## Photon flux calibration



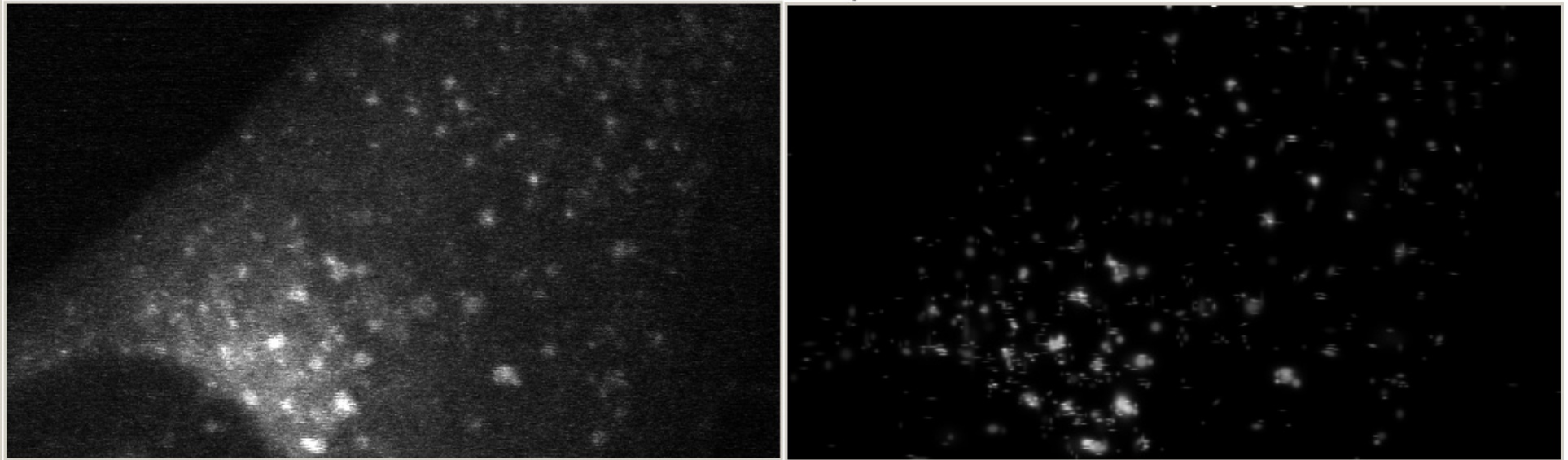
## Bonus: Number of photons per object

- Counting number fluorescent molecules
- Single molecule experiments





# Result of image fitting



# Benefits of approach

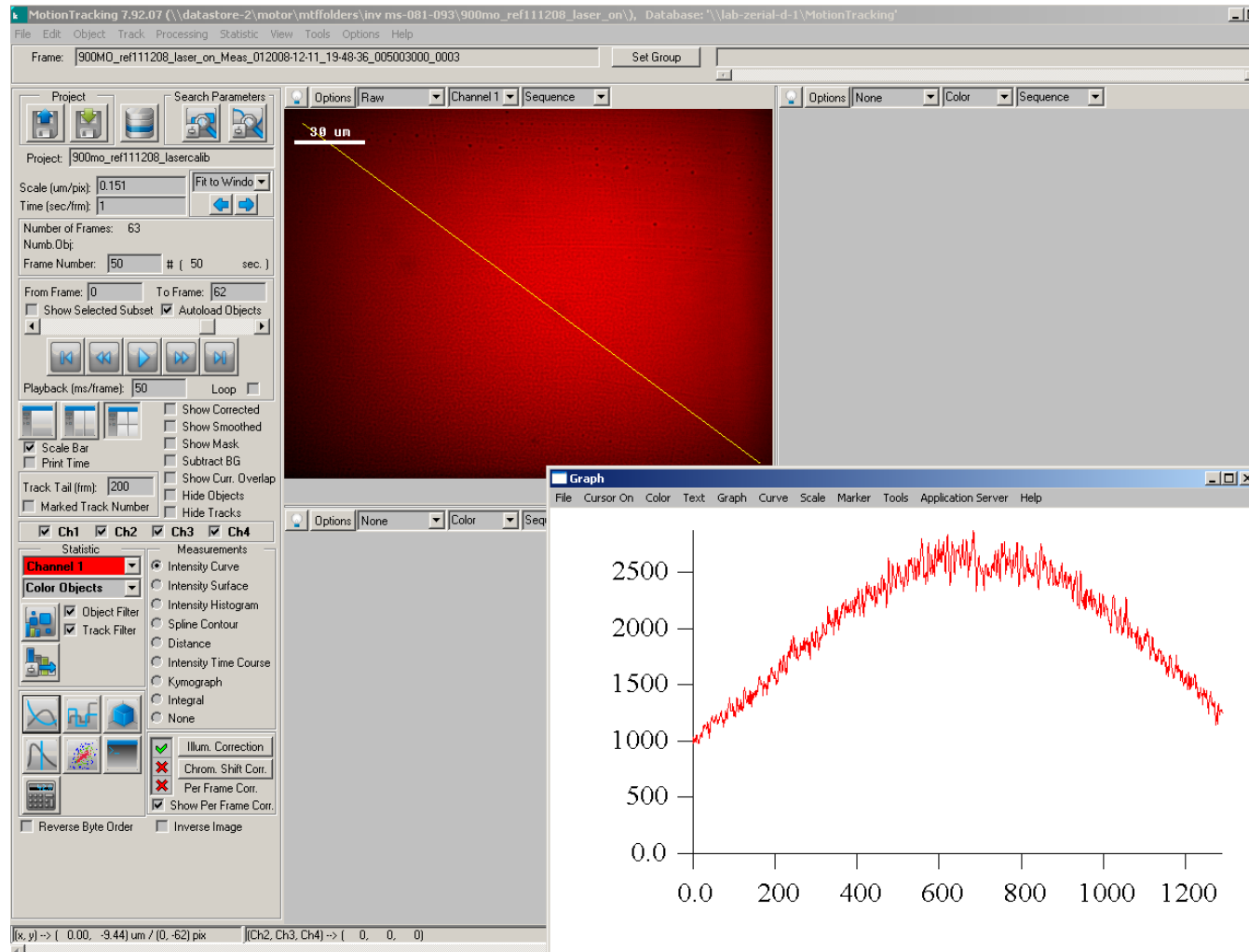
- ✓ High dynamic range
- ✓ Sub-pixel resolution
- ✓ Accurate estimate of mean intensity, integral intensity  
and endosome size
- ✓ Possibility to count number of fluorescent molecules per endosome

# Are we ready to model cargo flow?

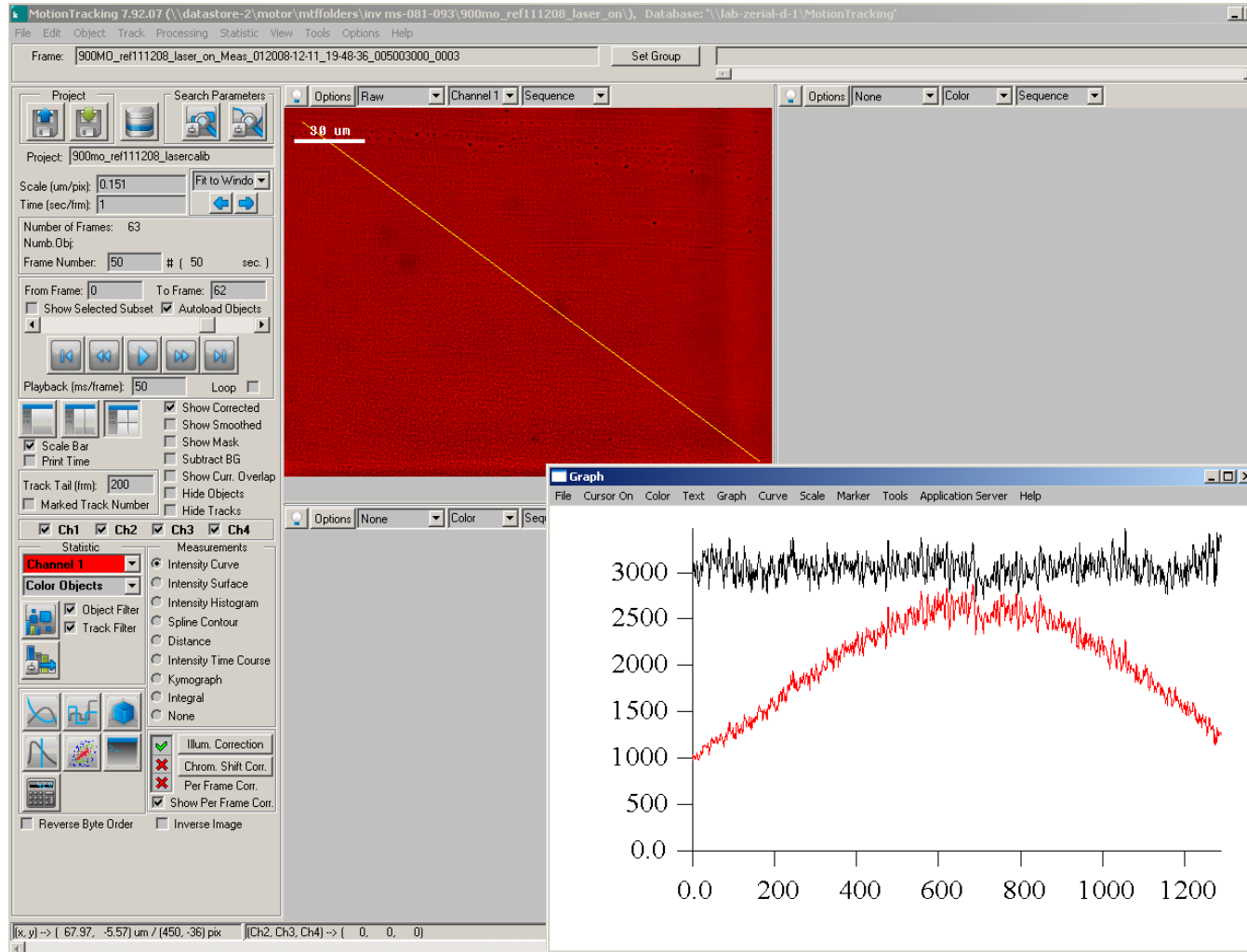
We did not check two major microscope problems:

- Uneven illumination of view field
- Chromatic shift/aberration

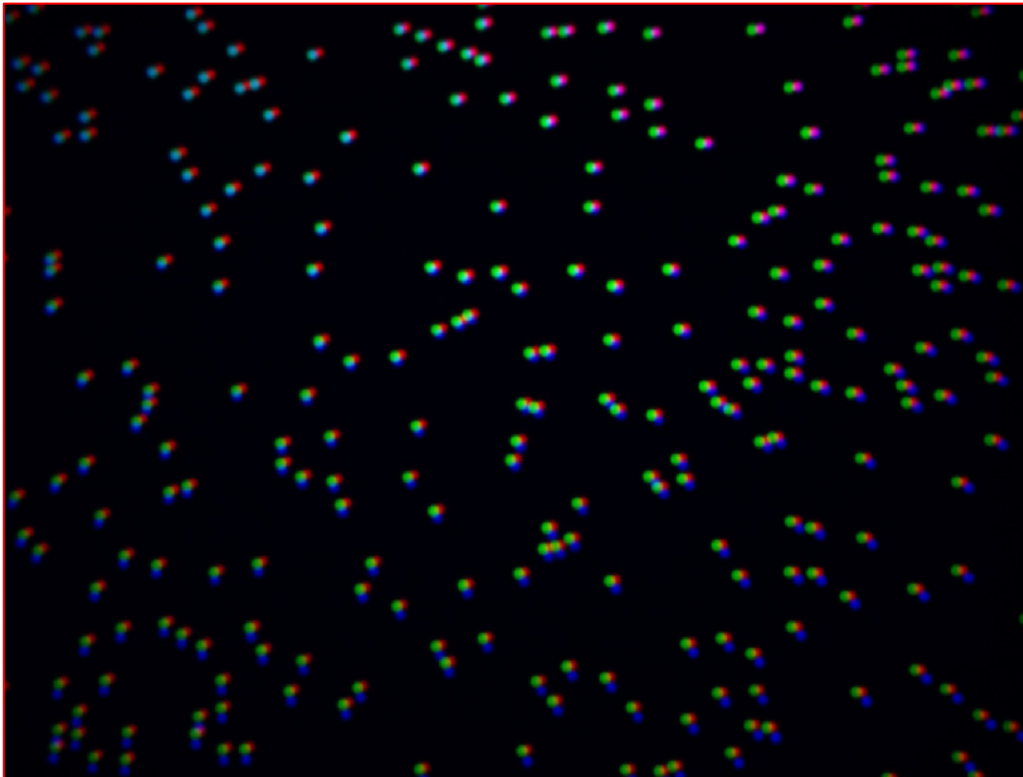
# Quantitative Microscopy: uneven illumination



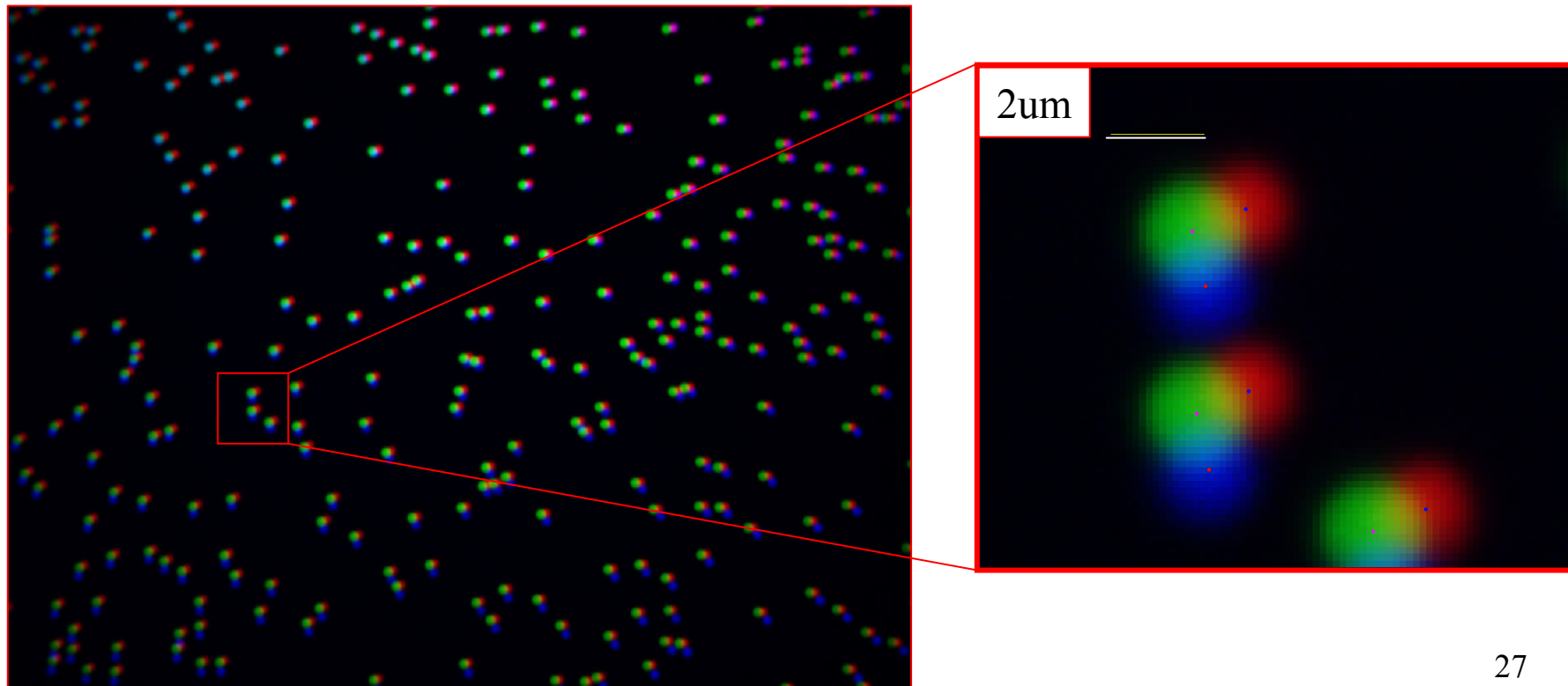
# Quantitative Microscopy: uneven illumination



# Quantitative Microscopy : chromatic shift

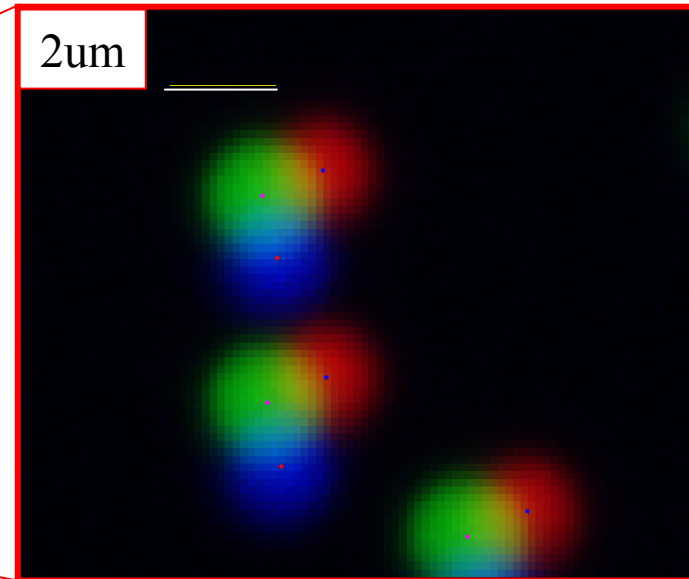
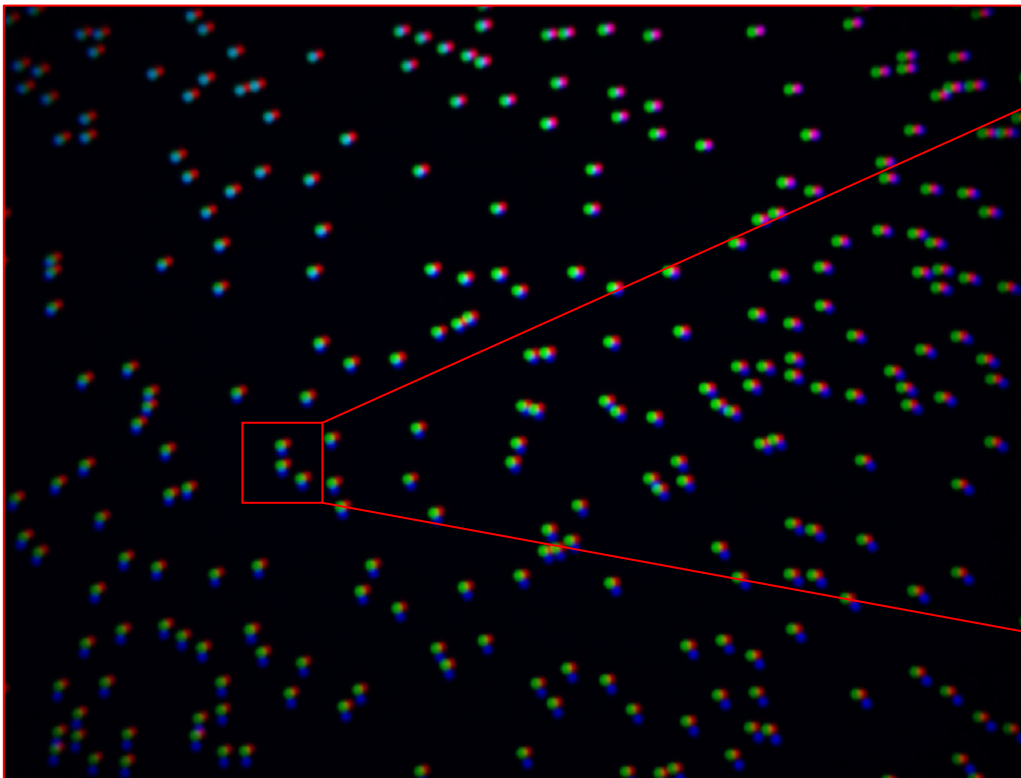
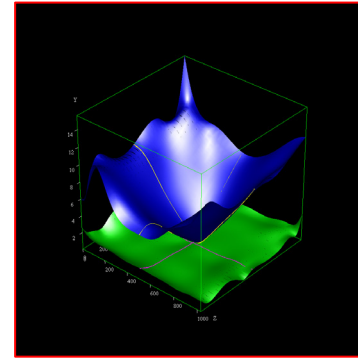


# Quantitative Microscopy : chromatic shift



# Quantitative Microscopy : chromatic shift

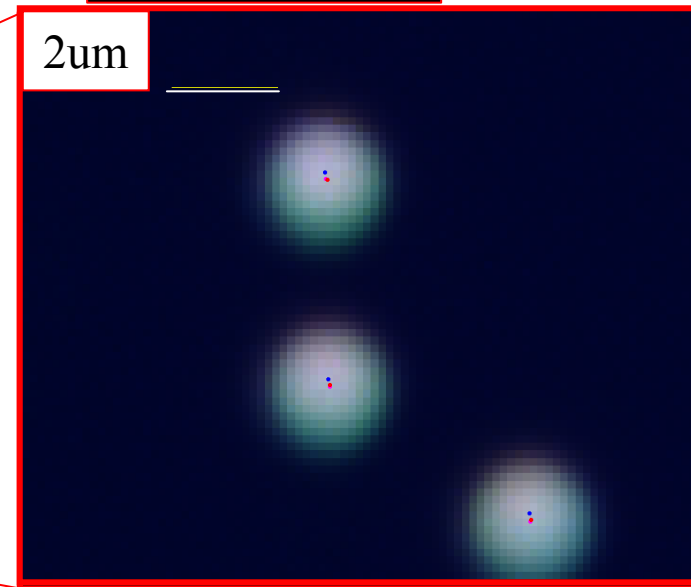
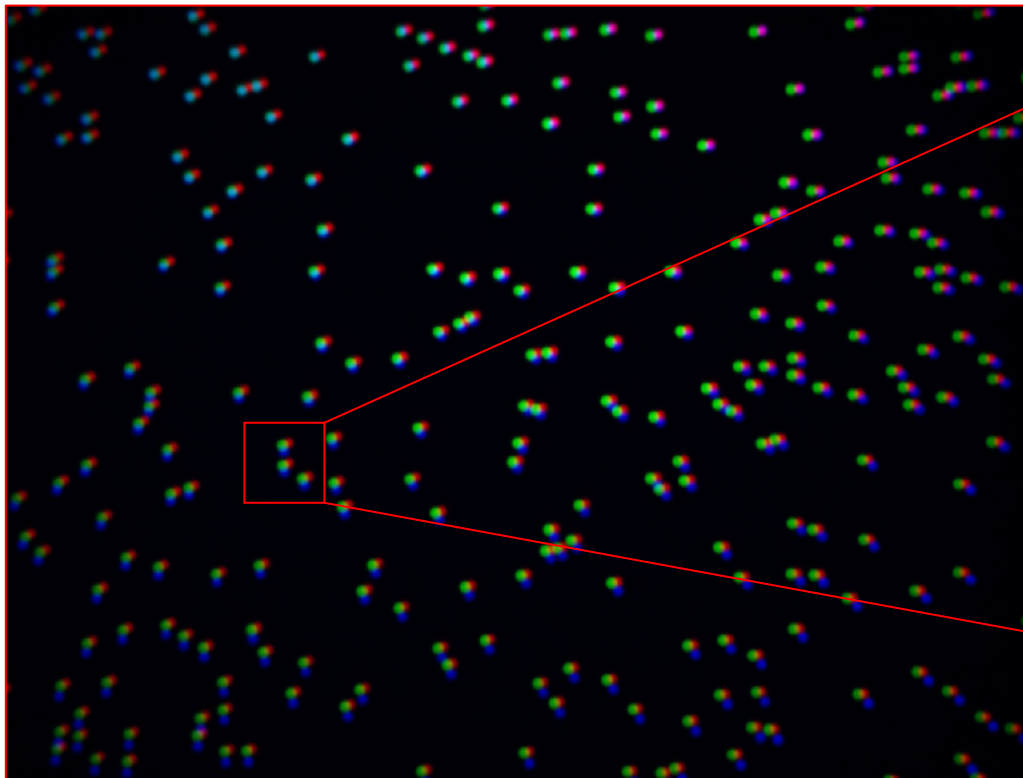
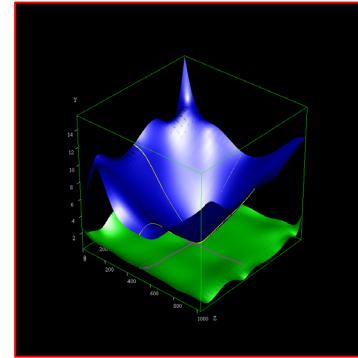
$$\delta x_1 = 7.31, \delta y_1 = 3.37, \alpha_1 = 0.00285$$
$$\delta x_2 = 0.26, \delta y_2 = 10.83, \alpha_2 = 0.0041$$



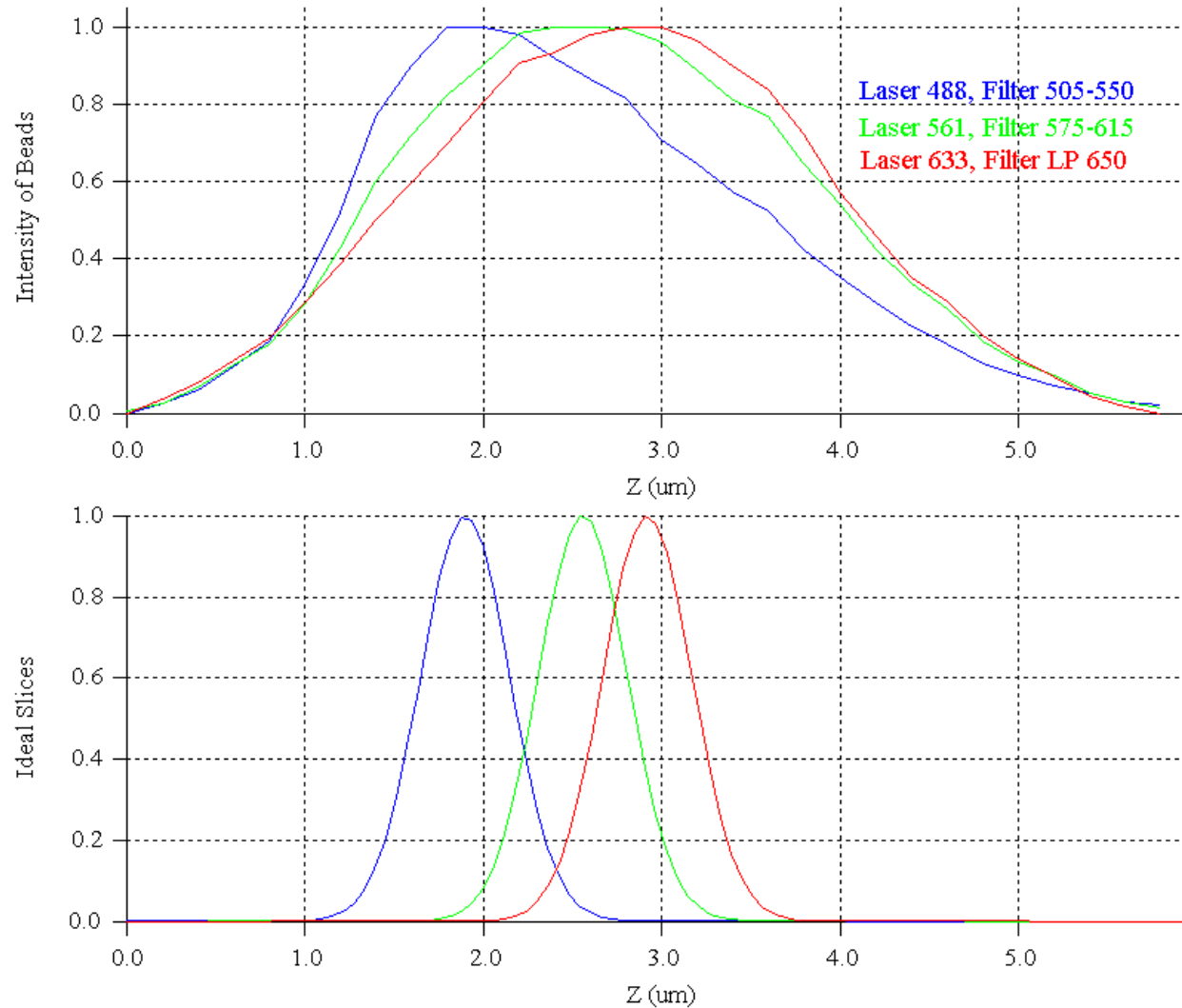


# Quantitative Microscopy : chromatic shift

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



# Colocalization: World is 3D



Is “wet” problem solved?  
Yes, but...

# Is “wet” problem solved?

## Yes, but...

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

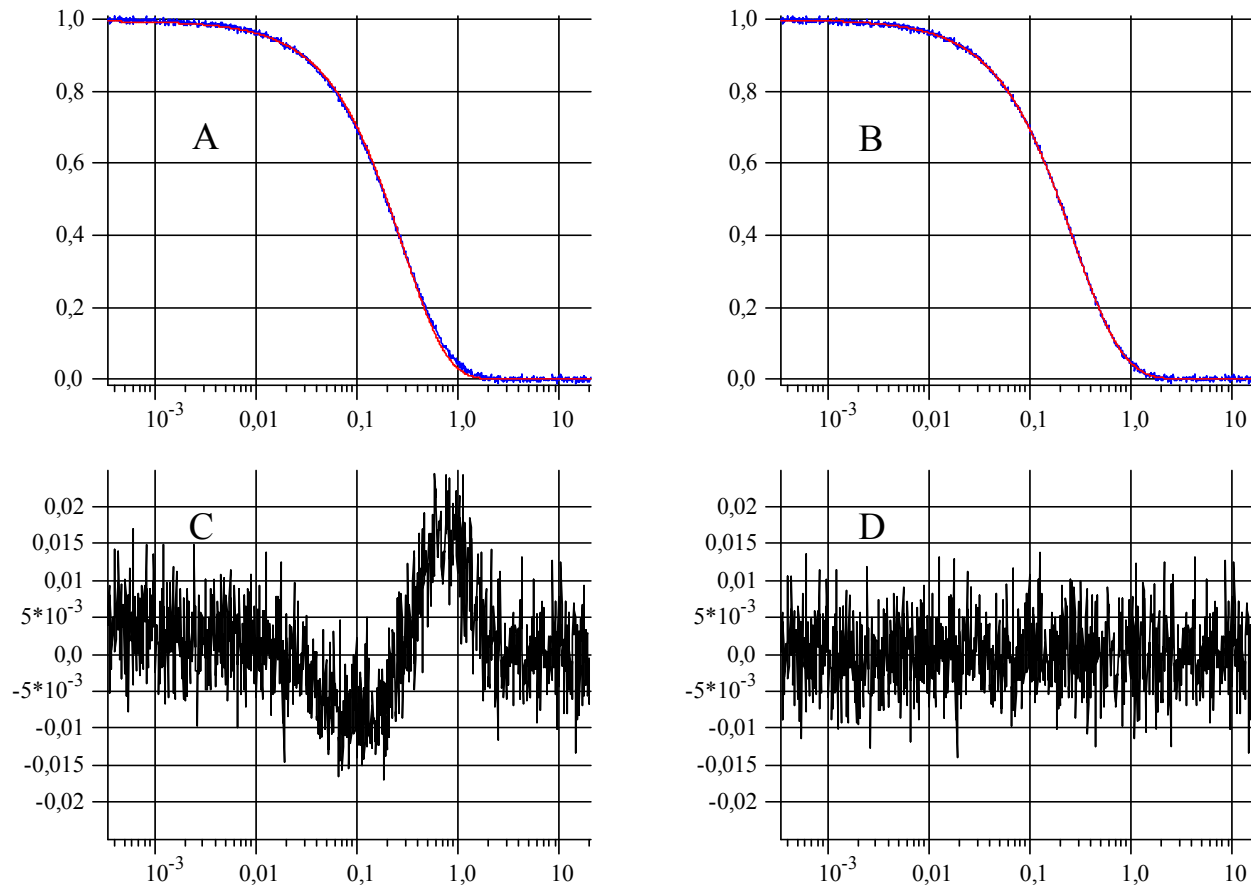
# Is “wet” problem solved?

## Yes, but...

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

## **Does it matter?**

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



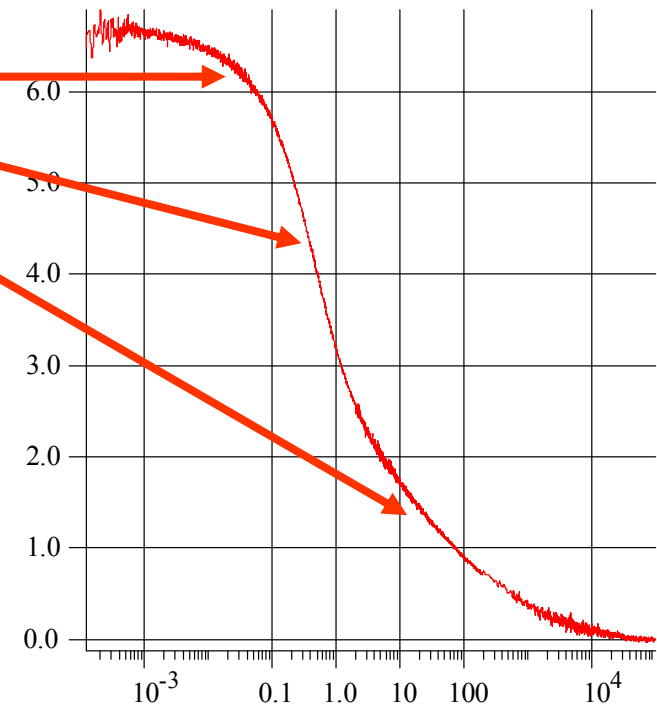
Deconvolution of artificial two-exponential curve ( $\tau_1=0.2$  and  $\tau_2=0.4$ , signal-to-noise ratio = 200) by one exponent ( $\tau=0.286$ ) (panels A, C) and two exponents ( $\tau_1=0.192$  and  $\tau_2=0.385$ ) (panels B, D).

# Model search is $\chi^2$ 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  $\tau_j$ ,

$\sigma_i^2$  - variance in the point  $i$ .



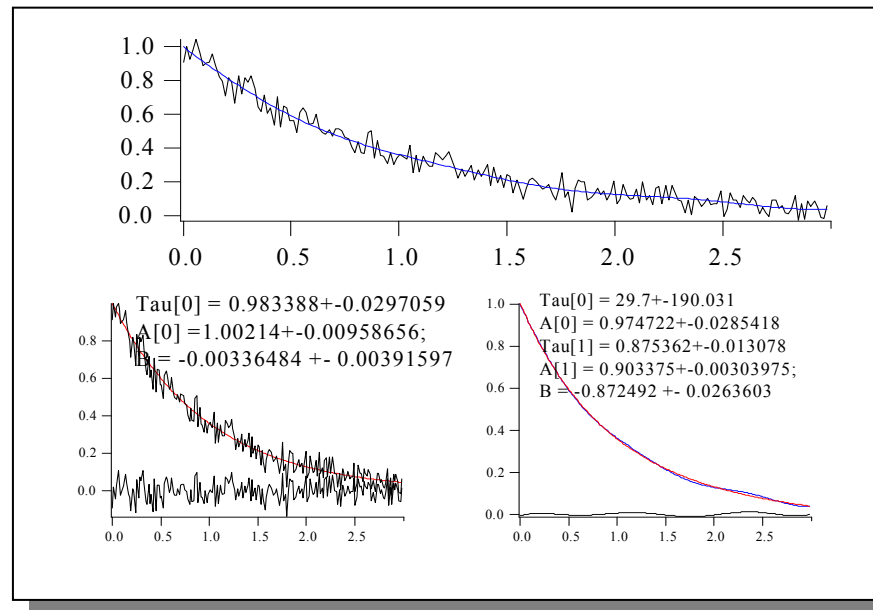
# Model fit and noise correlation

Simulation model:

$$y_i = \exp(-x_i) + \varepsilon, \text{ where } \varepsilon \text{ has distribution } N(0.0, 0.05)$$

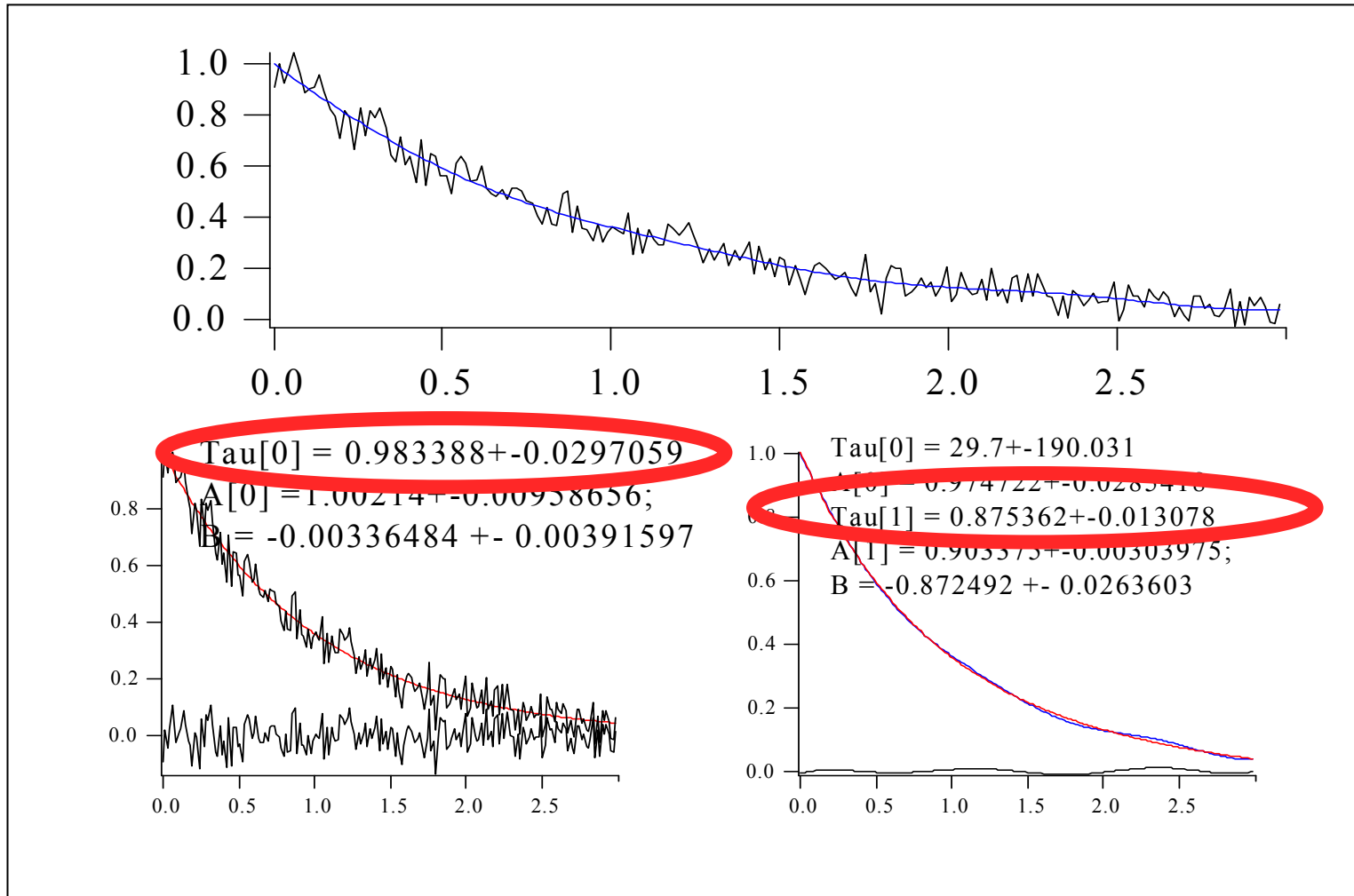
Regression model:

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

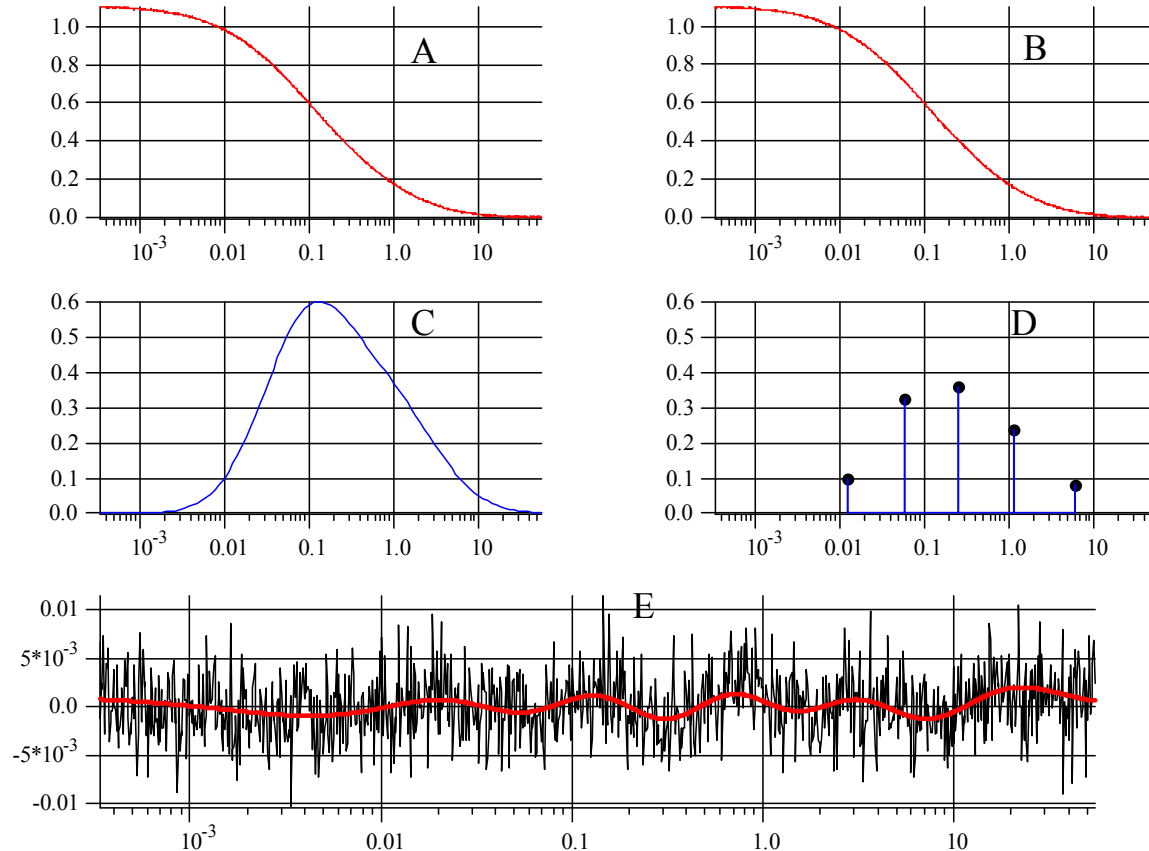




# Model fit and noise correlation



# Model selection: little more complicate example

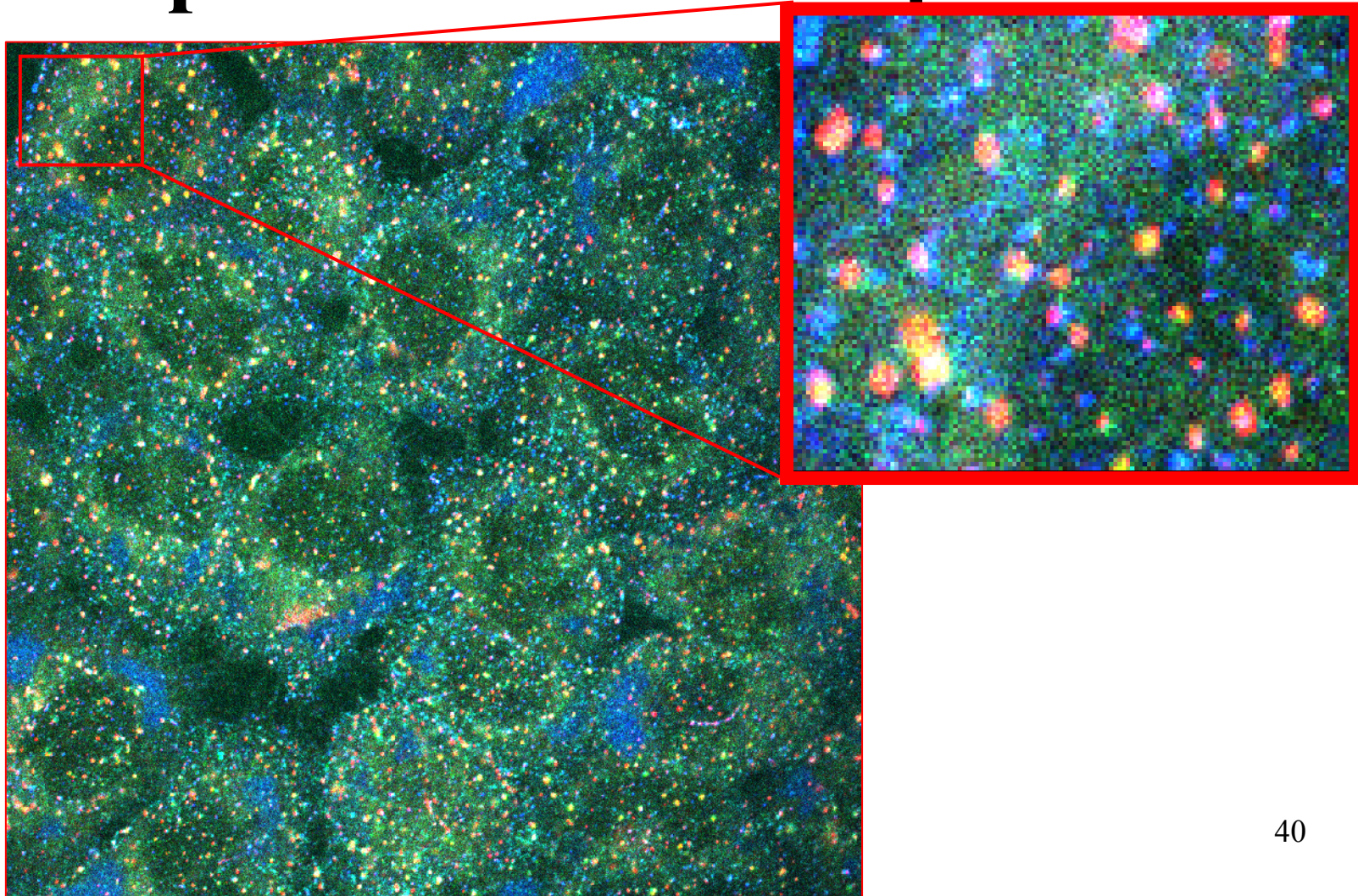


Artificial exponential (panels B, D) and non-exponential (panels A, C) kinetics and spectra. The panel E presents difference between exponential and non-exponential curves without noise (red) and with signal-to-noise = 500 (black).

# 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**





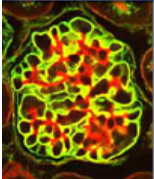
# Pearson & Overlap colocalization criteria

**OLYMPUS**

ADVANCED DIGITAL CAMERA SYSTEMS  
OLYMPUS Color and Monochrome CCD Cameras



Laser Scanning Confocal Microscopy **FluoView Resource Center**  
Unsurpassed Optics



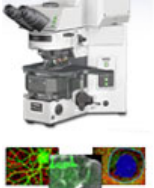
Interactive Java Tutorials

## Colocalization of Fluorophores in Confocal Microscopy

Two or more fluorescence emission signals can often overlap in digital images recorded by confocal microscopy due to their close proximity within the specimen. This effect is known as **colocalization** and usually occurs when fluorescently labeled molecules bind to targets that lie in very close or identical spatial positions. This interactive tutorial explores the quantitative analysis of colocalization in a wide spectrum of specimens that were specifically designed either to demonstrate the phenomenon, or to alternatively provide examples of fluorophore targets that lack any significant degree of colocalization.

- Product Info
- Brochures
- Confocal Theory
- Java Tutorials
- Glossary
- Applications
- Image Gallery
- Resource Links
- Contact Us
- Search
- Home

Olympus Confocal MICROSCOPES



**Specimen Image**

**Colocalization Scatterplot**

Channels:  
 Red  Green  Blue  
 Choose A Specimen

Colocalization Overlay:  
 Full Color  Binary

Region of Interest:  
 Freehand  Rectangle  Ellipse

Colocalization Channels:  
 Red-Green  Red-Blue  Green-Blue

Colocalization Coefficients:  
 Pearson's R(r): 0.19  
 Overlap (R): 0.85  
**Global:**  
 Red: 0.99 Green: 1.0

Large flat rabbit kidney epithelial cells (RK-13 line) were transfected with a DsRed2 (red) fluorescent protein vector localized to the endoplasmic reticulum, fixed, and stained for filamentous actin with Alexa Fluor 488 (green). The cell nuclei were counterstained with Hoechst 33342 (blue). Colocalization is seen in the red and blue channels between the nuclei and endoplasmic reticulum.

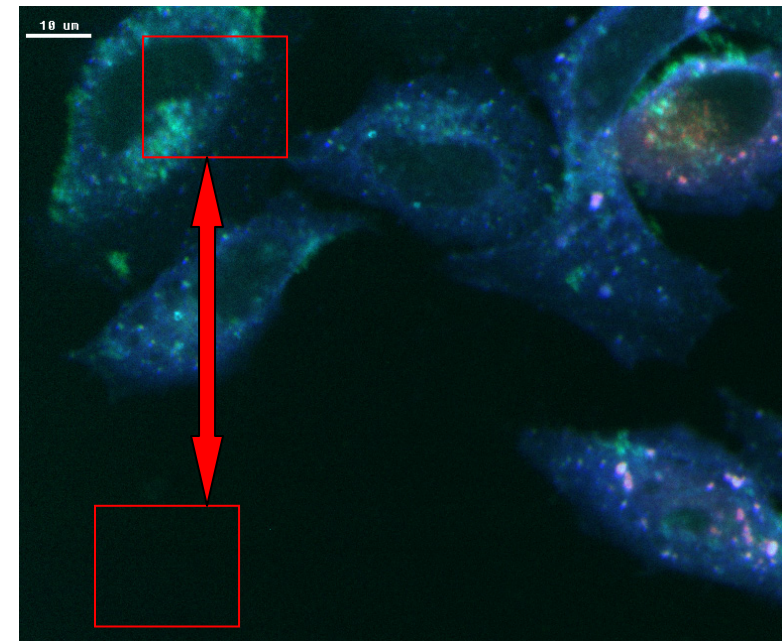
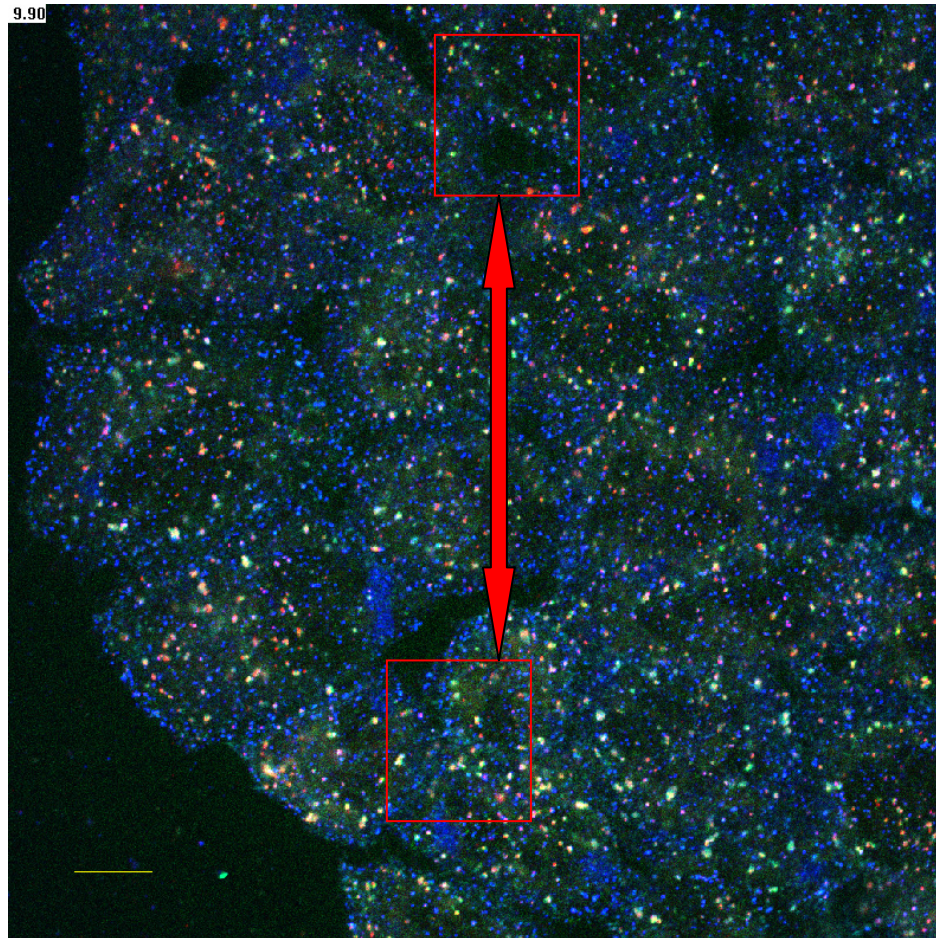
**Pearson's colocalization coefficient:**

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

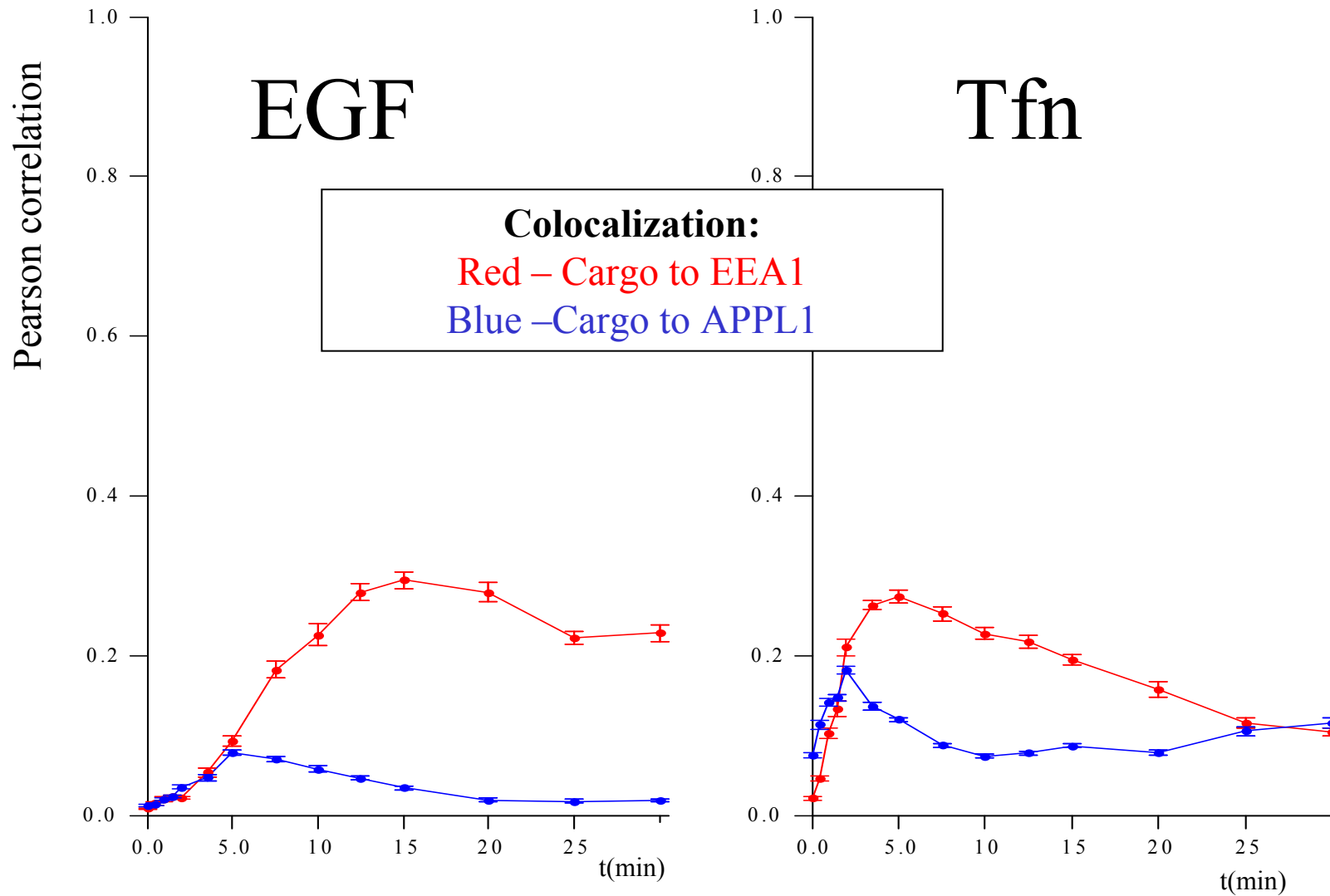
**Overlap coefficient:**

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

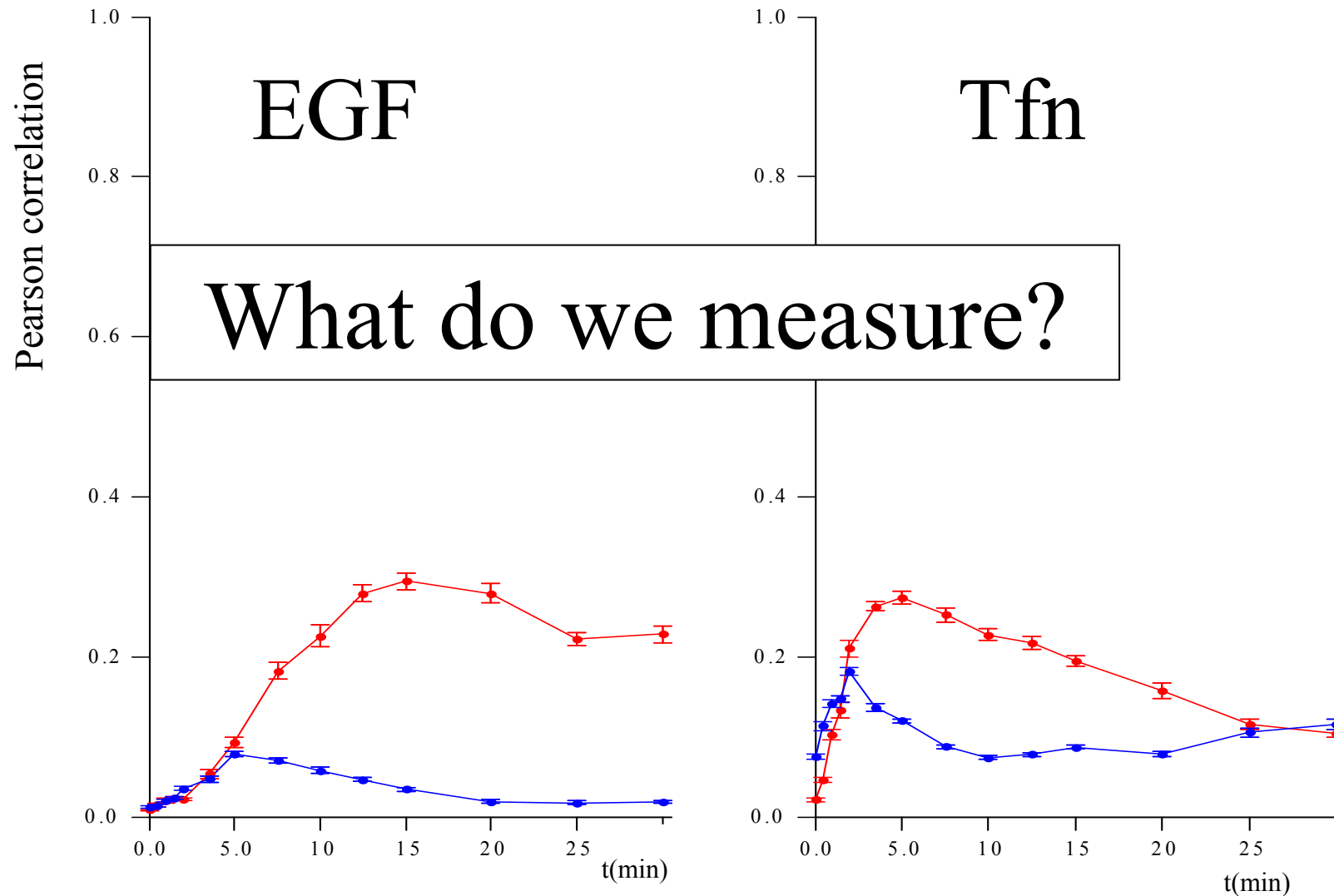
# Estimation of random (apparent) colocalization



# Pearson colocalization time course

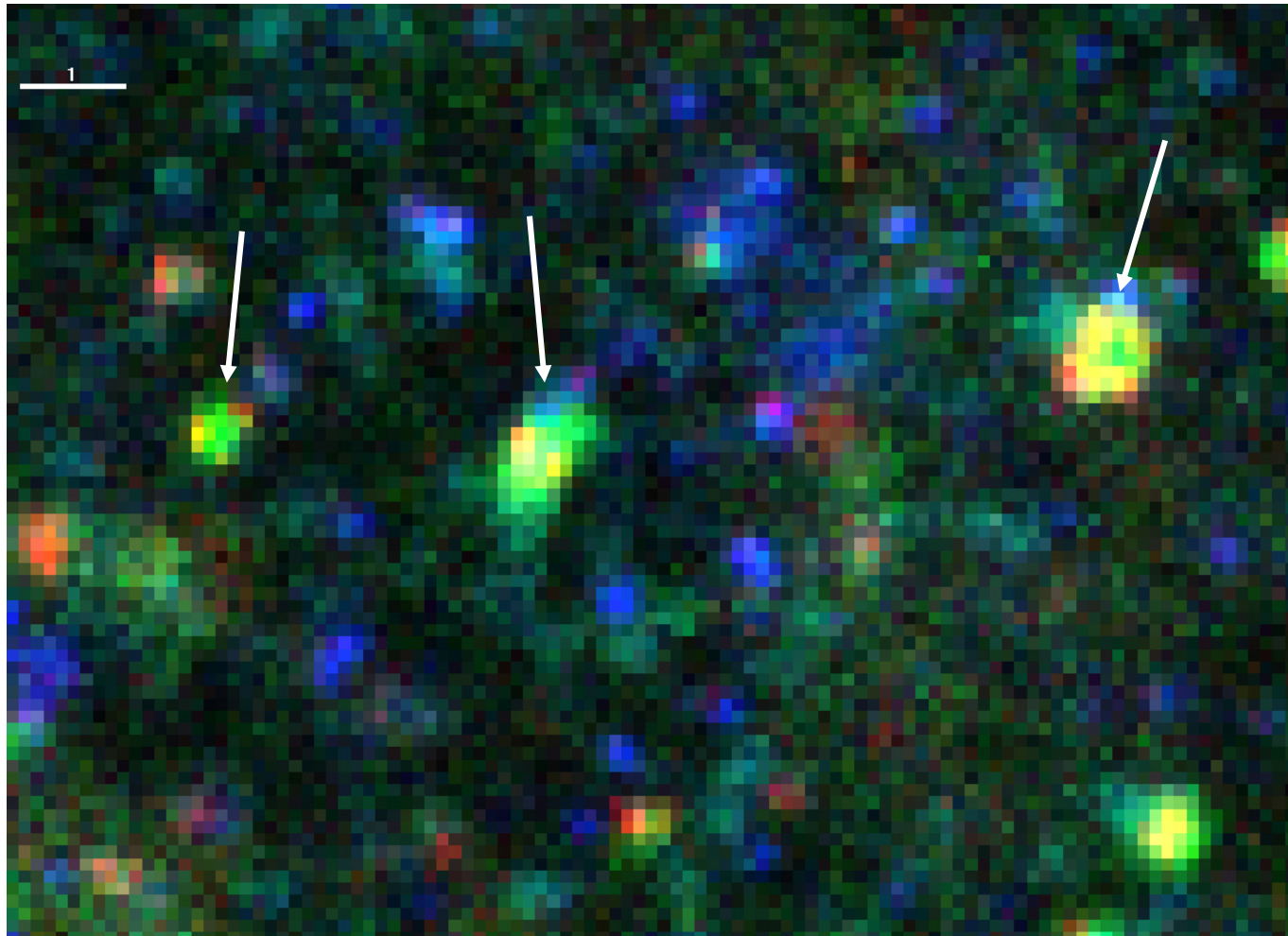


# Pearson colocalization time course



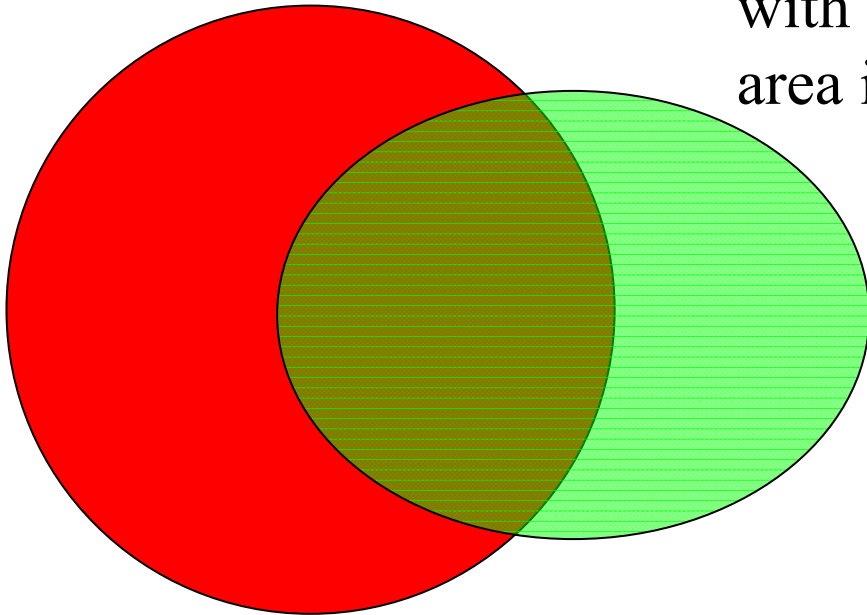


# Are domain structures colocalized?



# Definition of asymmetric colocalization

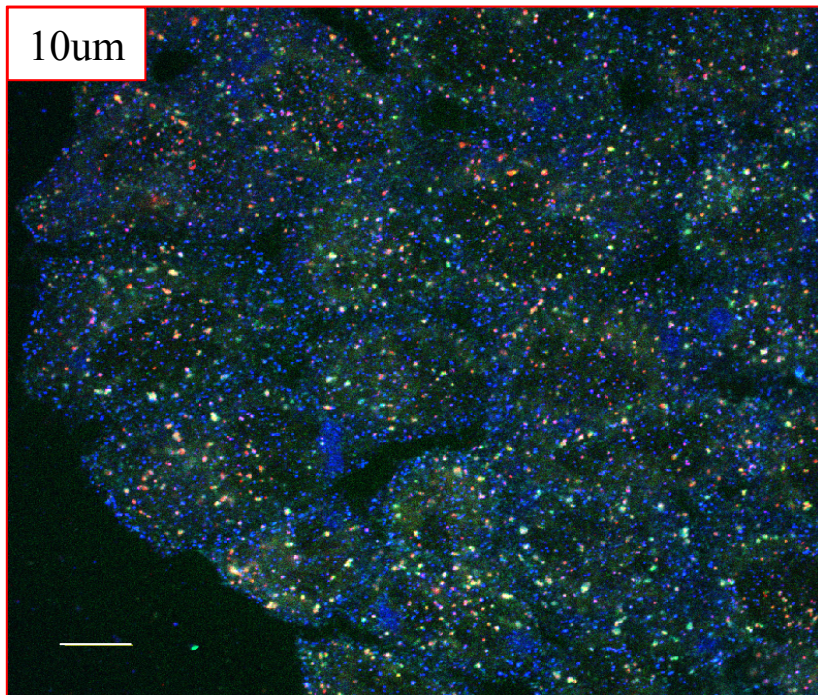
The green vesicle is colocalized with red if more than  $x\%$  of its area is covered by red vesicle(s)



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

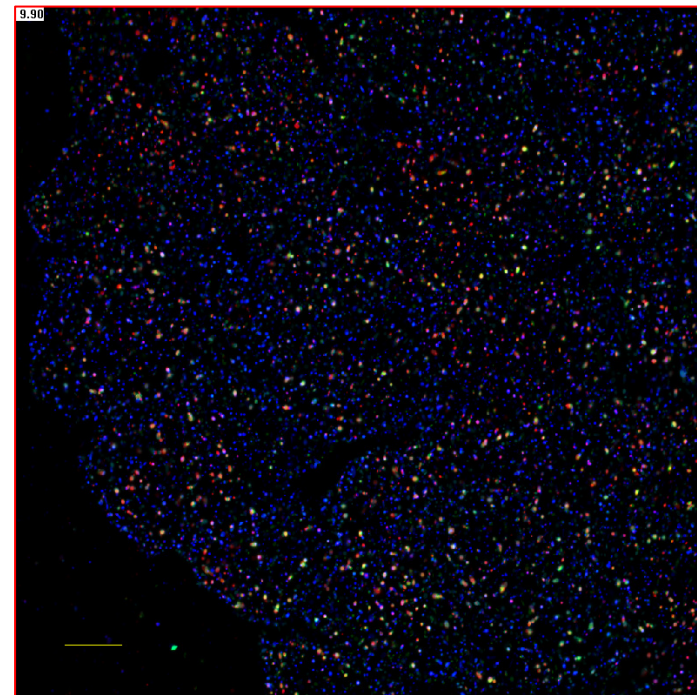
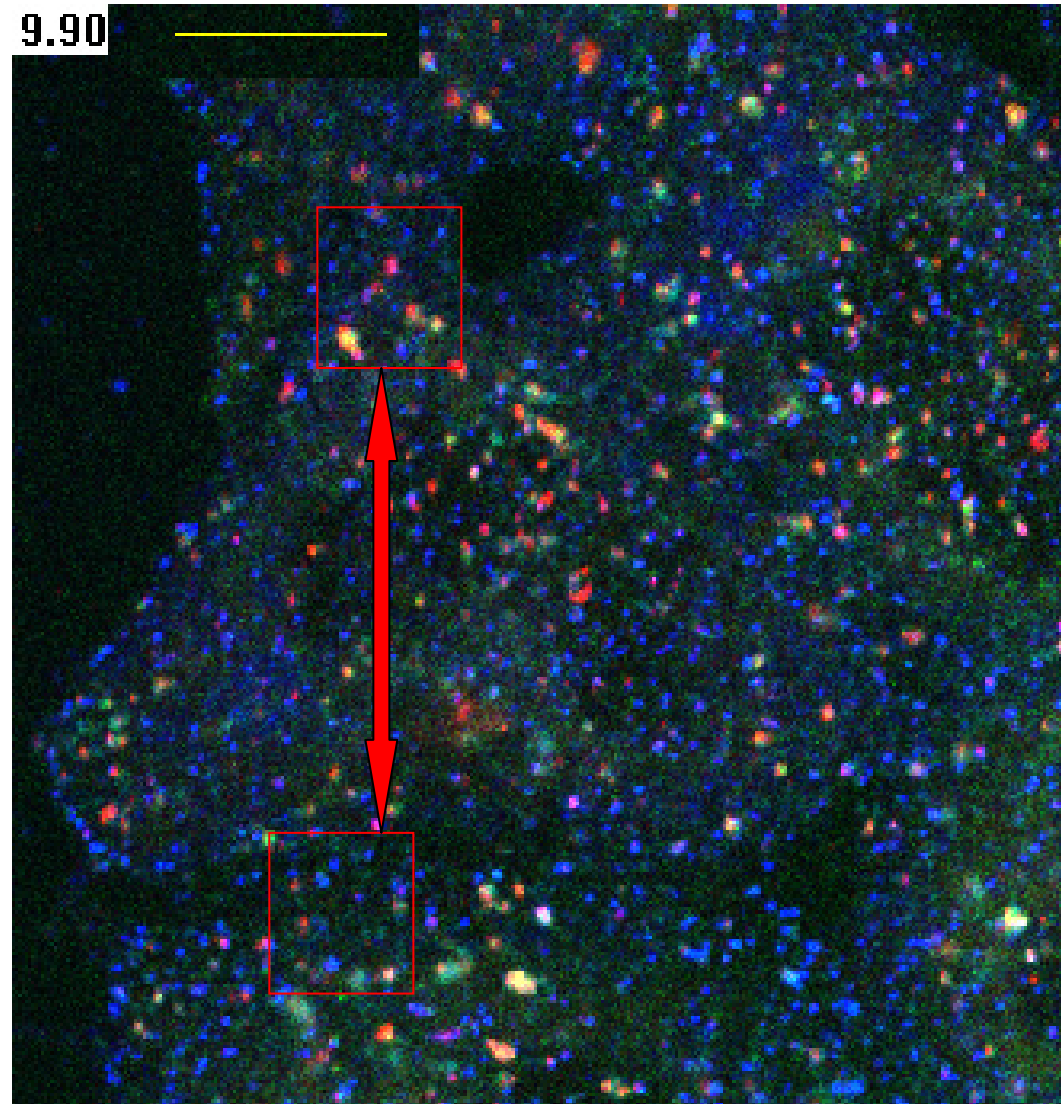


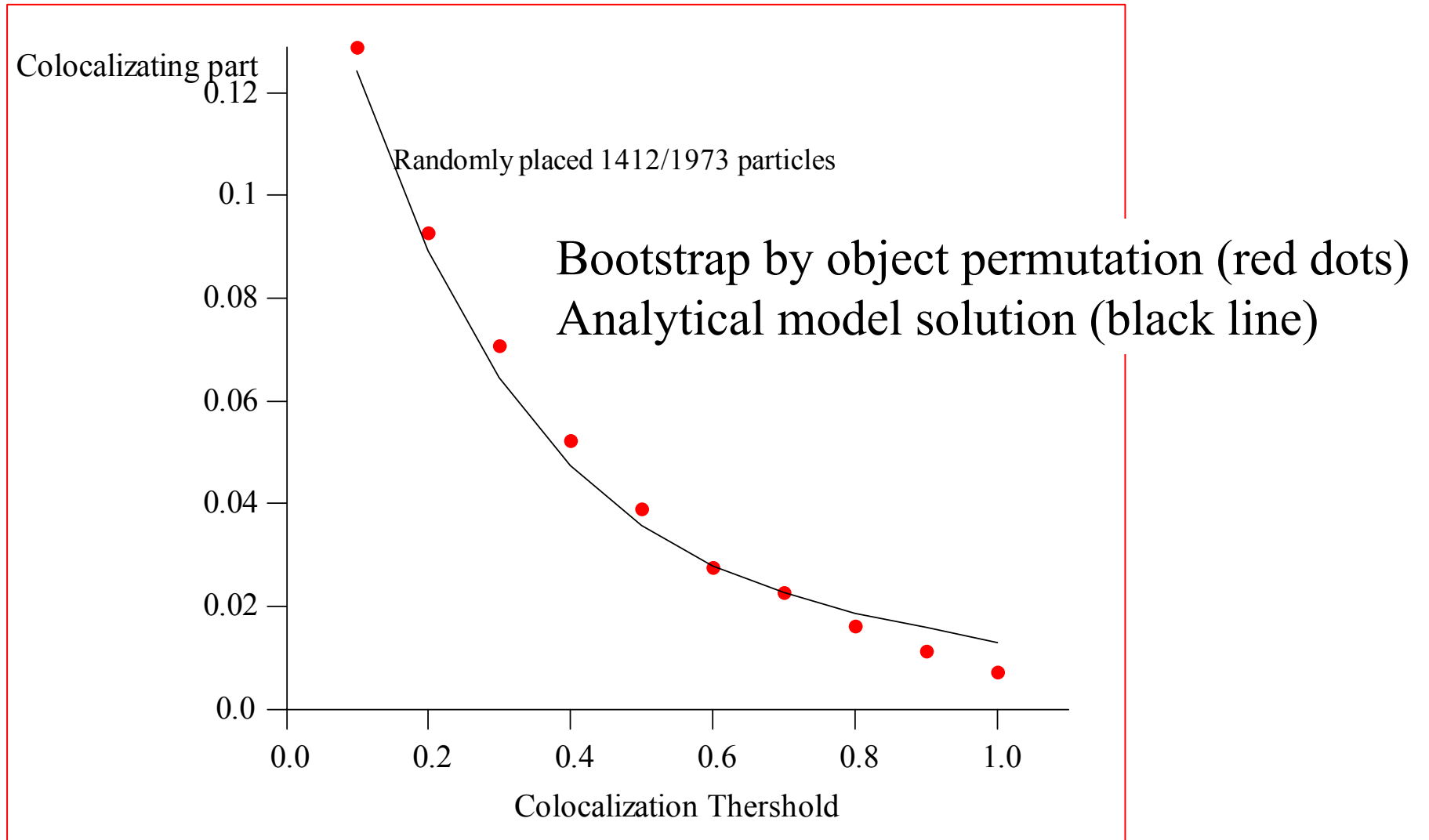
Image reconstructed  
from the fitted functions

# Object-based estimation of random (apparent) colocalization





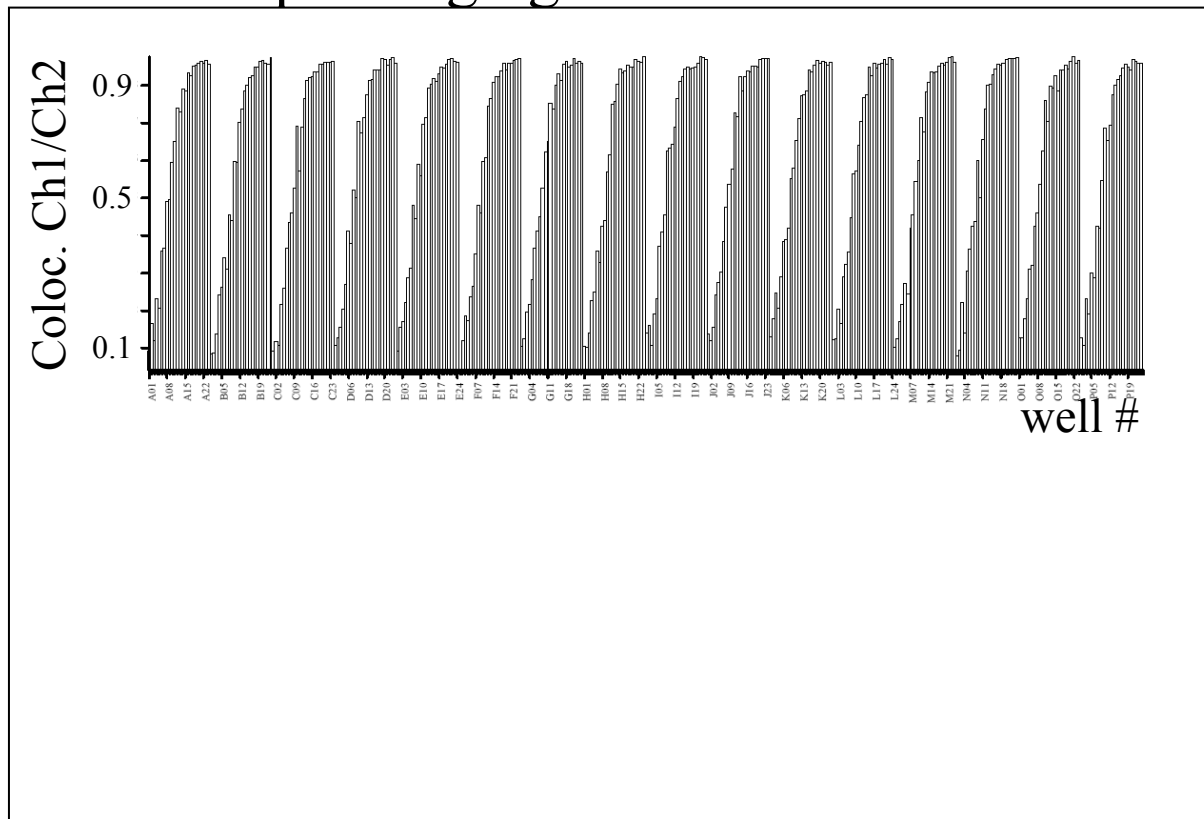
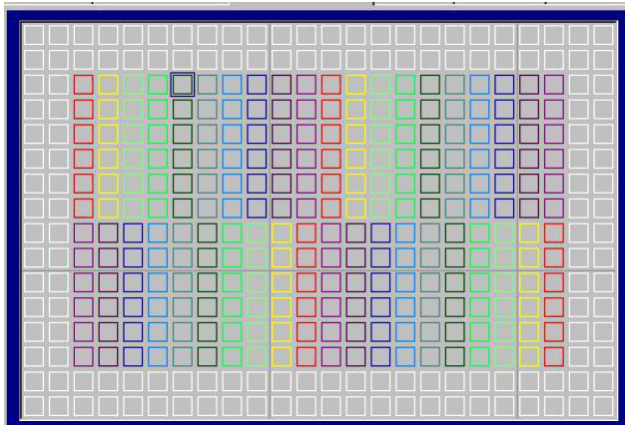
# Random Colocalization



# Unknown microscopy: Is chromatic shift constant over experiment?

(How long did you worm microscope before imaging?)

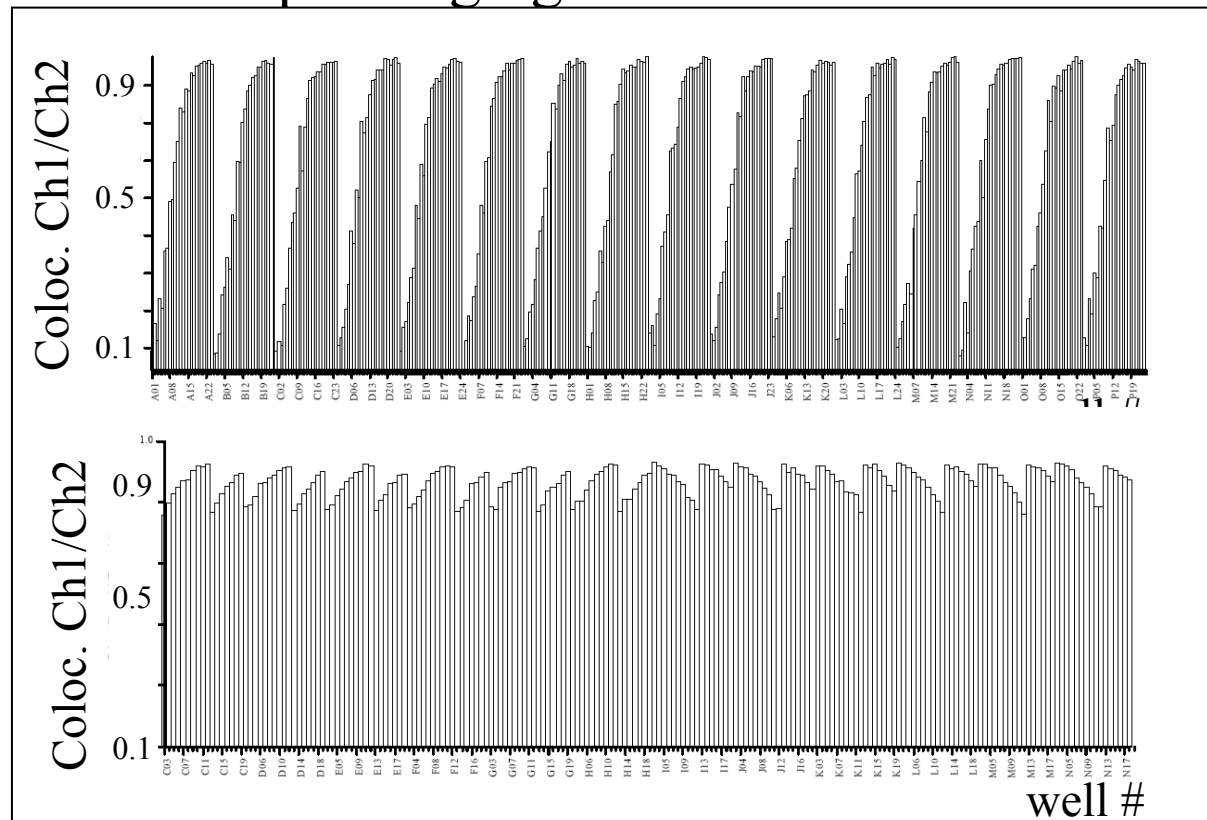
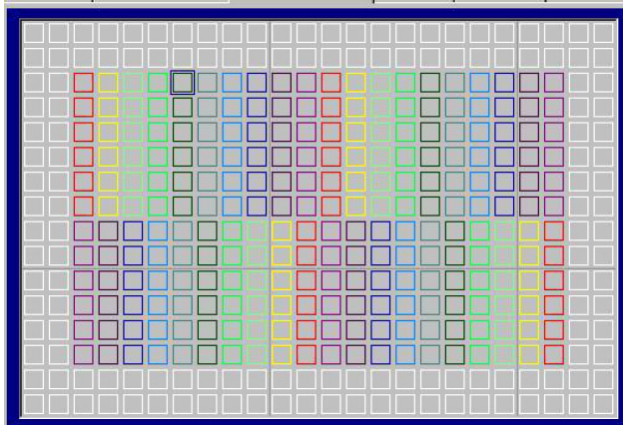
The 10min uptake of Tf-Alexa488 and Tf-Alexa647 mixture by HeLa cells. Automatic microscope imaging (12 images per well)



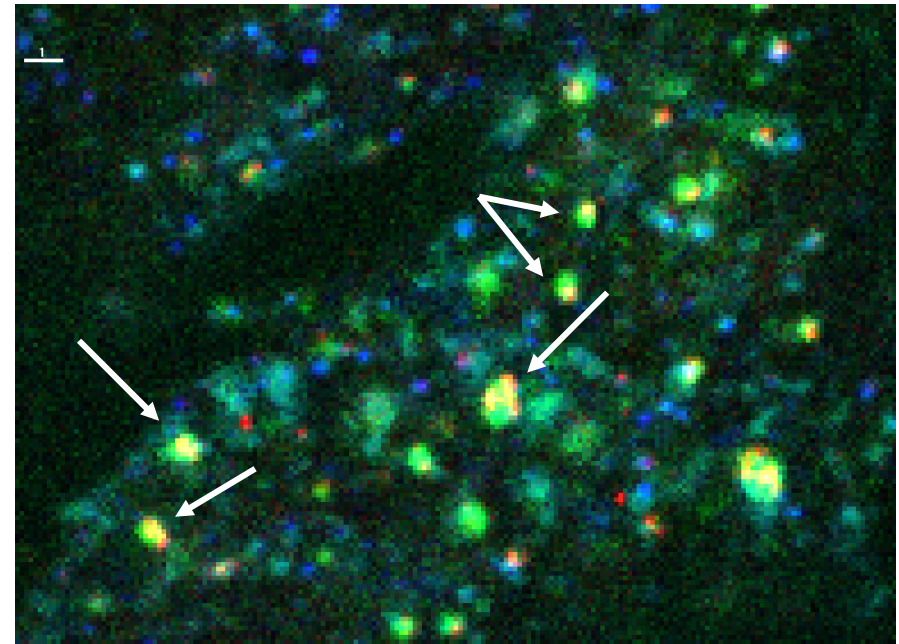
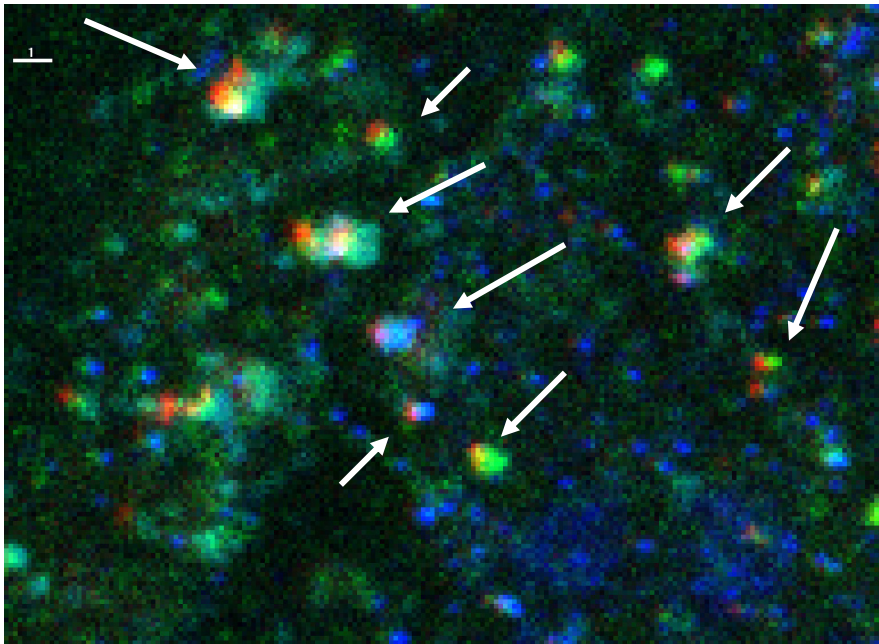
# Unknown microscopy: Is chromatic shift constant over experiment?

(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)



# Problems of 'wet' experiment: Analysis of pulse-chase experiments (chromatic shift)



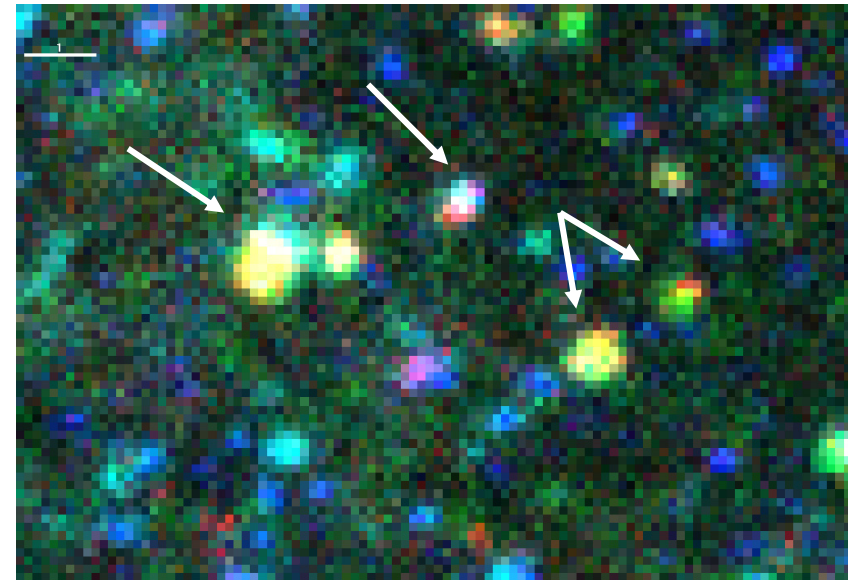
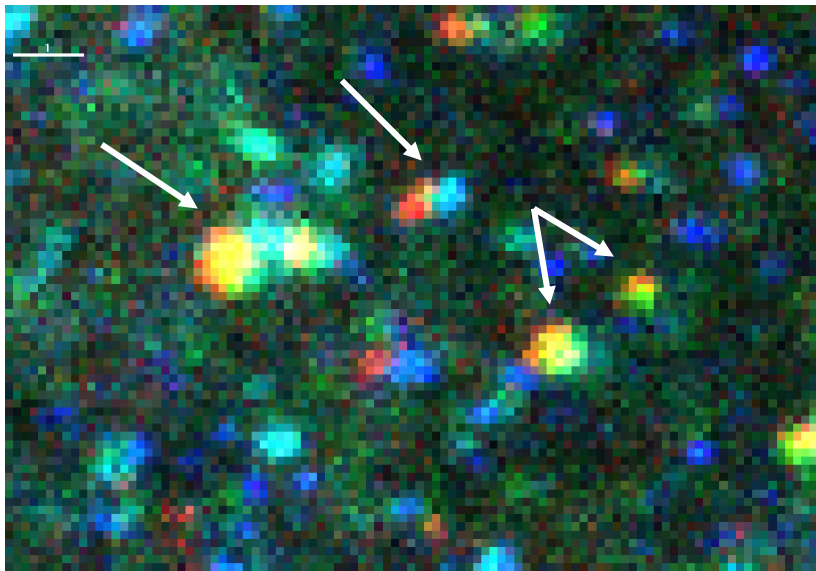
Left and right images have indexes 'a' and 'e' in same imaging session.

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)

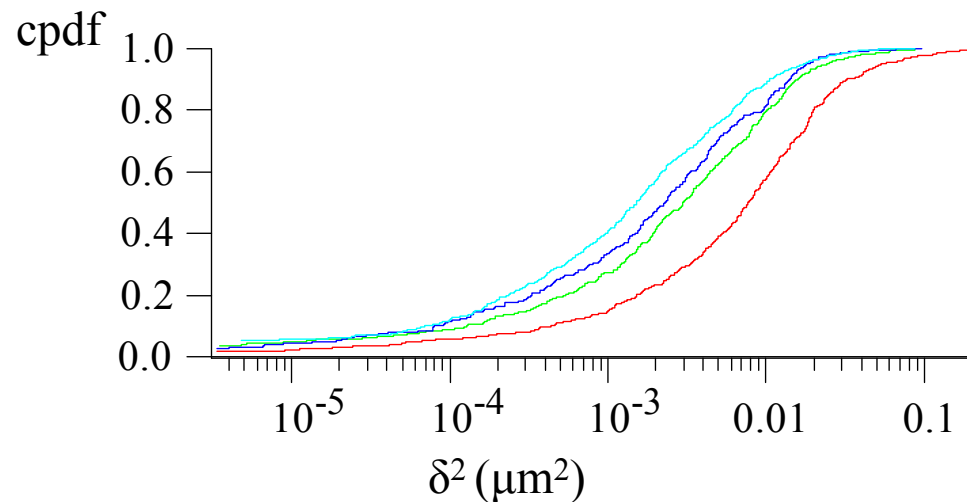
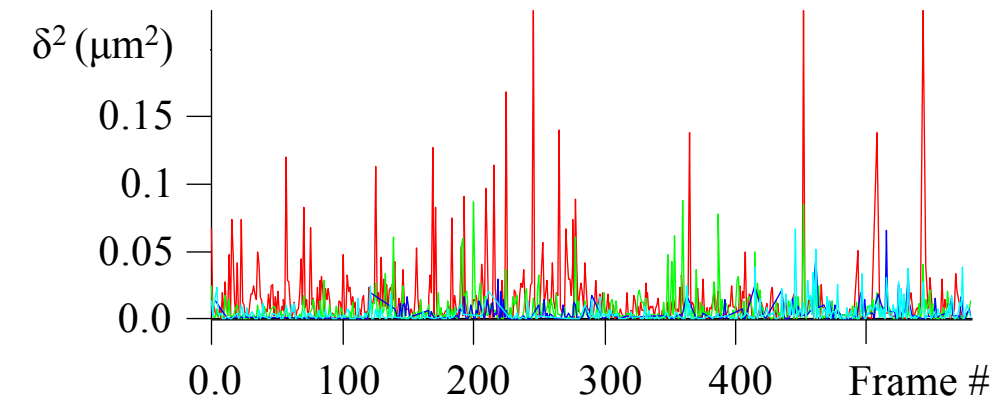


Shift correction on 'per-frame' basis.

HeLa: 30 sec pulse EGF & Tfn, chase 10 min.

Red: EEA1; Green: EGF; Blue:APPL1; Cyan: Tfn

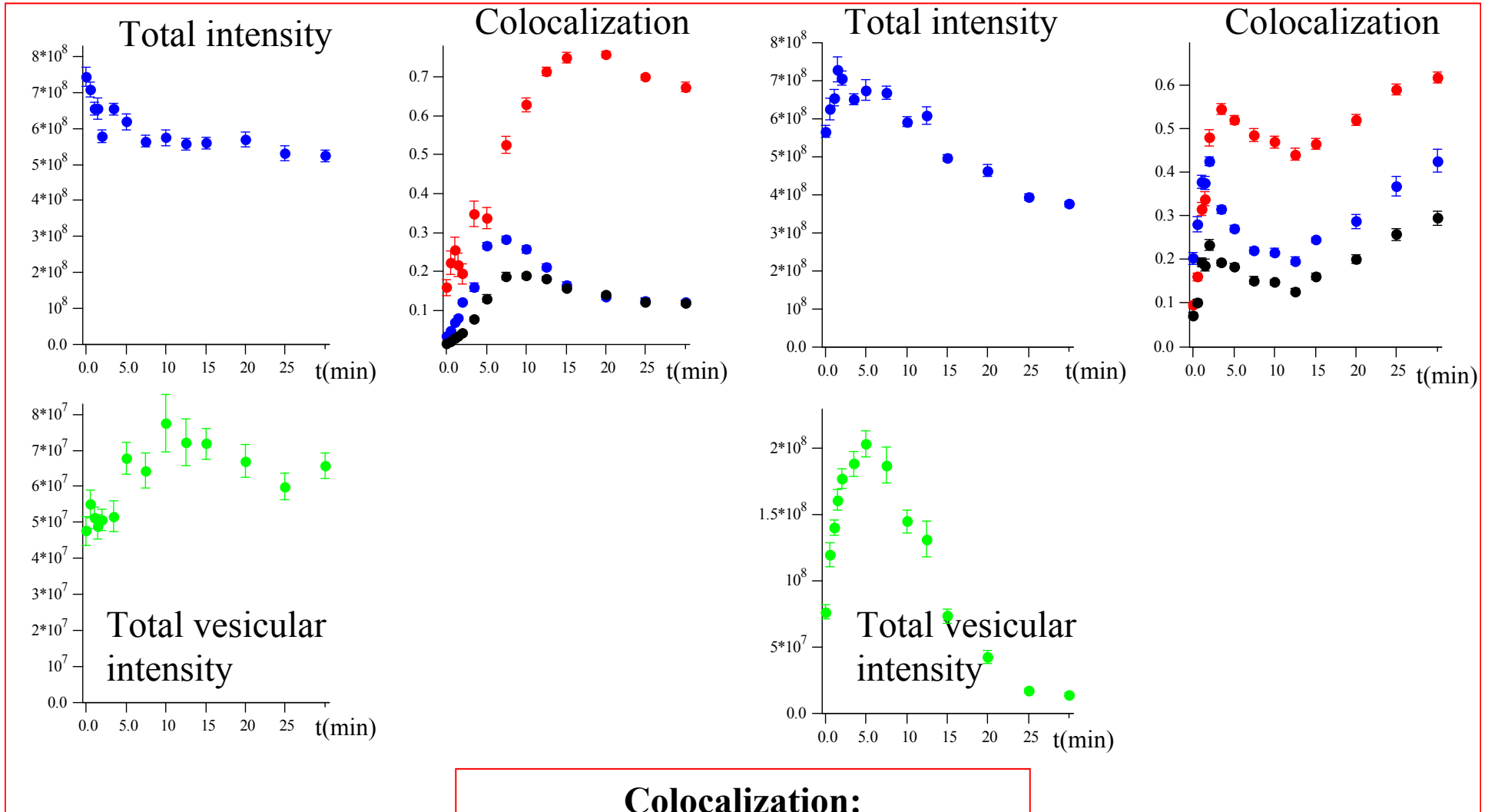
# Problems of 'wet' experiment: Analysis of pulse-chase experiments (chromatic shift)



1 pix = 0.116  $\mu\text{m}$

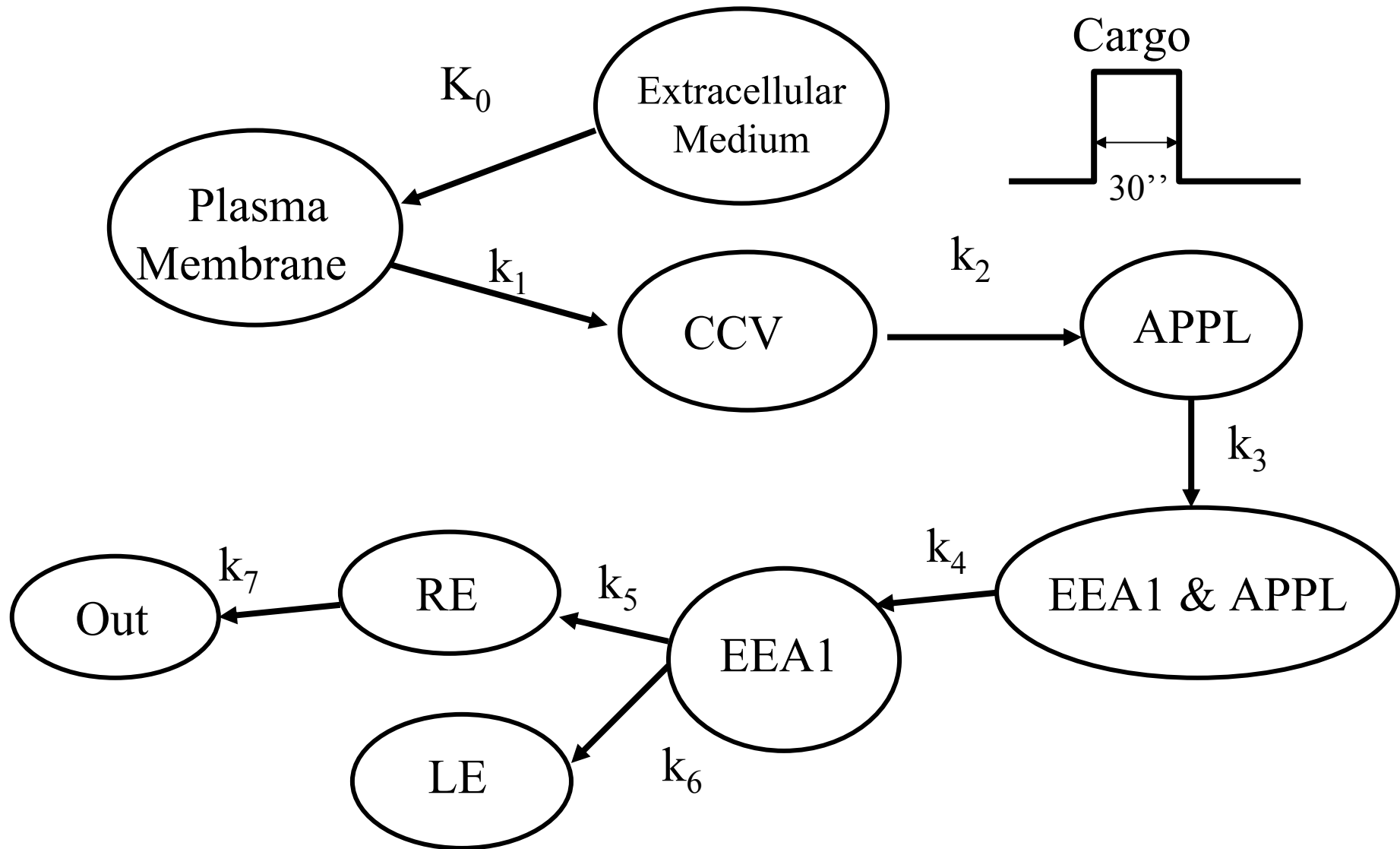
# Cargo for degradation (EGF)

# 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

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

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

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

$$\frac{dC_{APPL - EEA 1}}{dt} = k_3 C_{APPL} - k_4 C_{APPL - EEA 1}$$

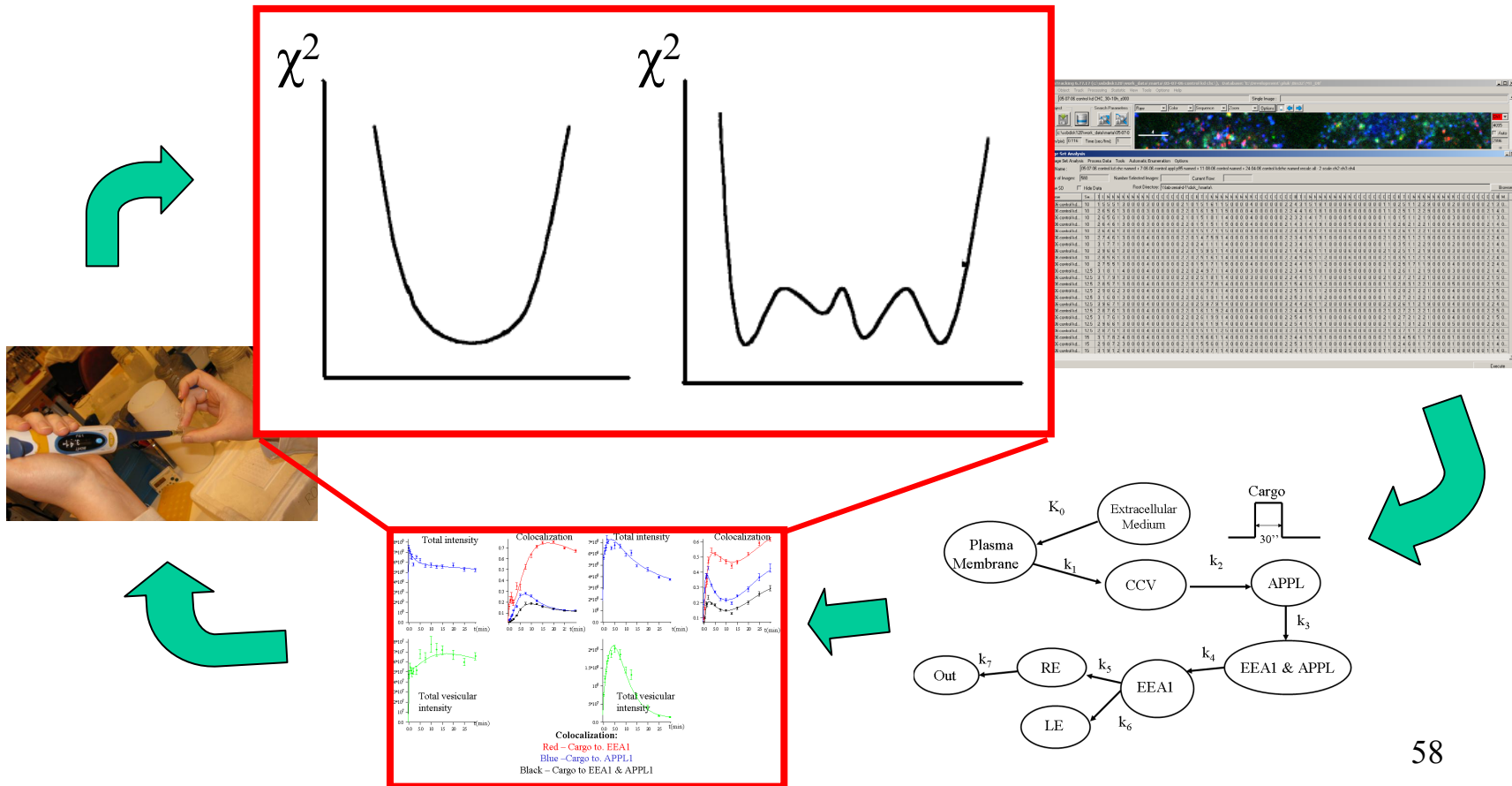
$$\frac{dC_{EEA 1}}{dt} = k_4 C_{APPL - EEA 1} - (k_5 + k_6) C_{EEA 1}$$

$$\frac{dC_{RE}}{dt} = k_5 C_{EEA 1} - k_7 C_{RE}$$

$$\frac{dC_{LE}}{dt} = k_6 C_{EEA 1}$$

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

# 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**

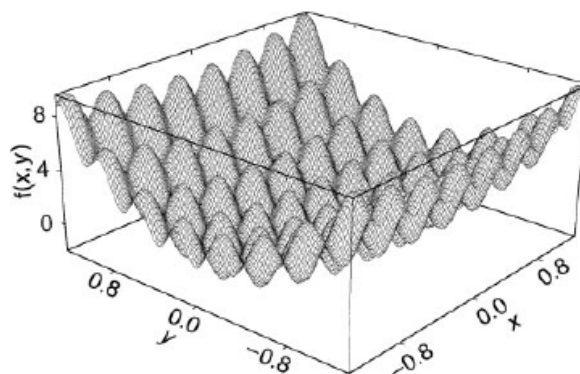
---

Trevor R. Griffiths,<sup>\*a</sup> Dmitry A. Nerukh<sup>\*a†</sup> and Sergey A. Eremenko<sup>b</sup>

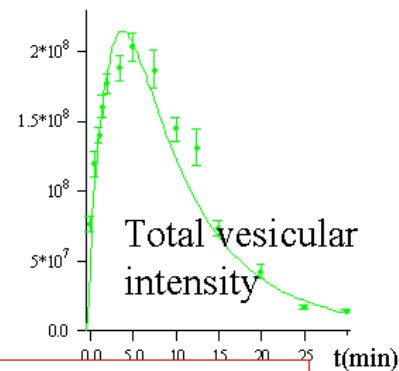
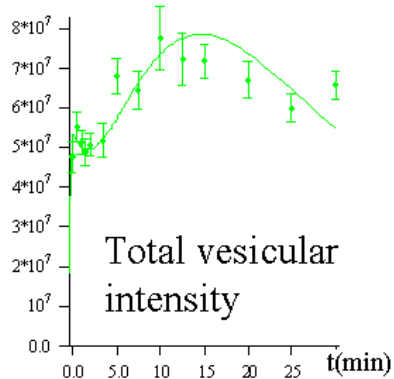
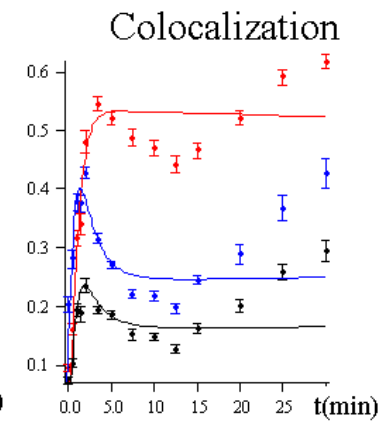
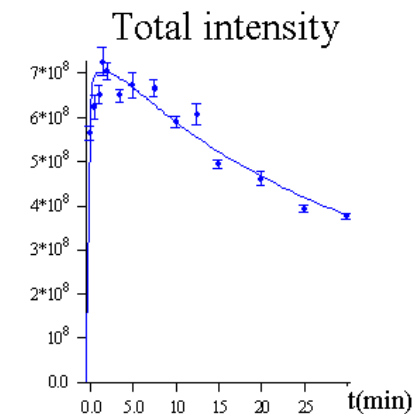
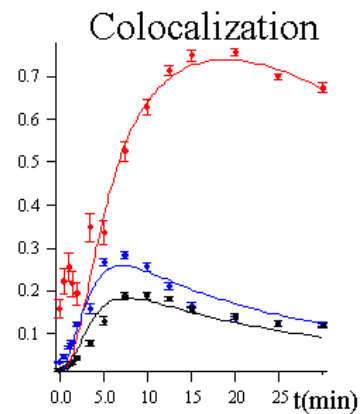
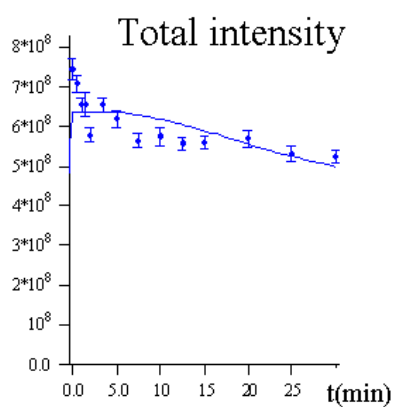
<sup>a</sup> *School of Chemistry, University of Leeds, Leeds, UK LS2 9JT*

<sup>b</sup> *Inorganic Chemistry Department, Kharkov State University, Svobody Sq. 4, Kharkov-77, 310077, Ukraine*

*Received 30th March 1999, Accepted 19th May 1999*



# 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

# Acknowledgements



*Experimental (“wet”) work:*

Jochen Rink

Claudio Collinet

Thierry Galvez

Akhila Chandrashaker

Inna Kalaidzidis

MPI-CBG (Dresden)



## Marino Zerial

*Experimental (“wet”) work:*

**Marta Miaczynska,**

Marta Brewinska

MCB (Warsaw)



*Software development:*

Anton Parusnikov

Igor Kulikov

Plavel Zaitsev

MSU (Moscow)

Alexander Kalaidzidis

(Greece)