

What do FRAP curves tell us?

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*To proceed, press Page Down or click the mouse button. To go back, Press page Up.
Clicking hyperlink (blue fonts) leads you to a website in the Internet.

Notes

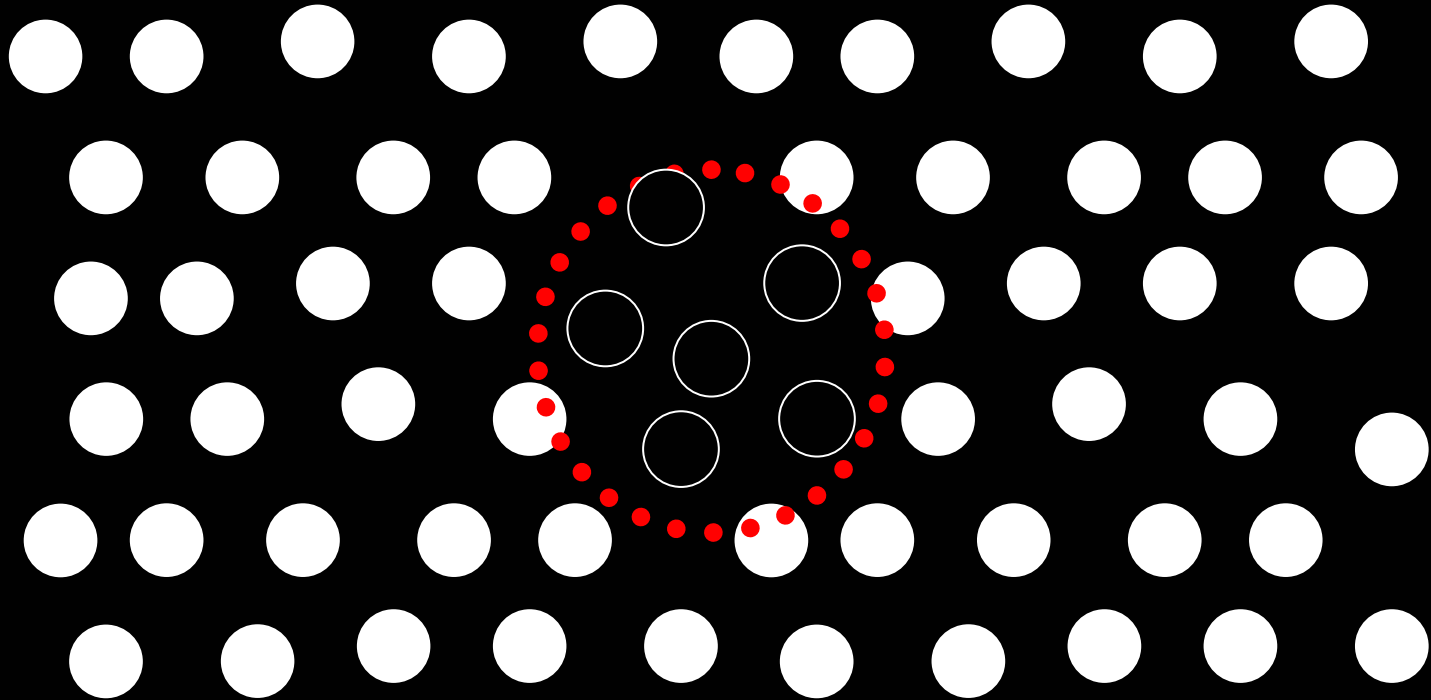
In this presentation, I will give a short overview on what we can know about molecular dynamics by studying the Fluorescence Recovery After Photobleaching (**FRAP**). The presentation is intended for beginners of FRAP.

The slides consists of the following topics.

- What is FRAP?
- Quantitative Analysis of FRAP
- Modeling and FRAP

1. What is FRAP?

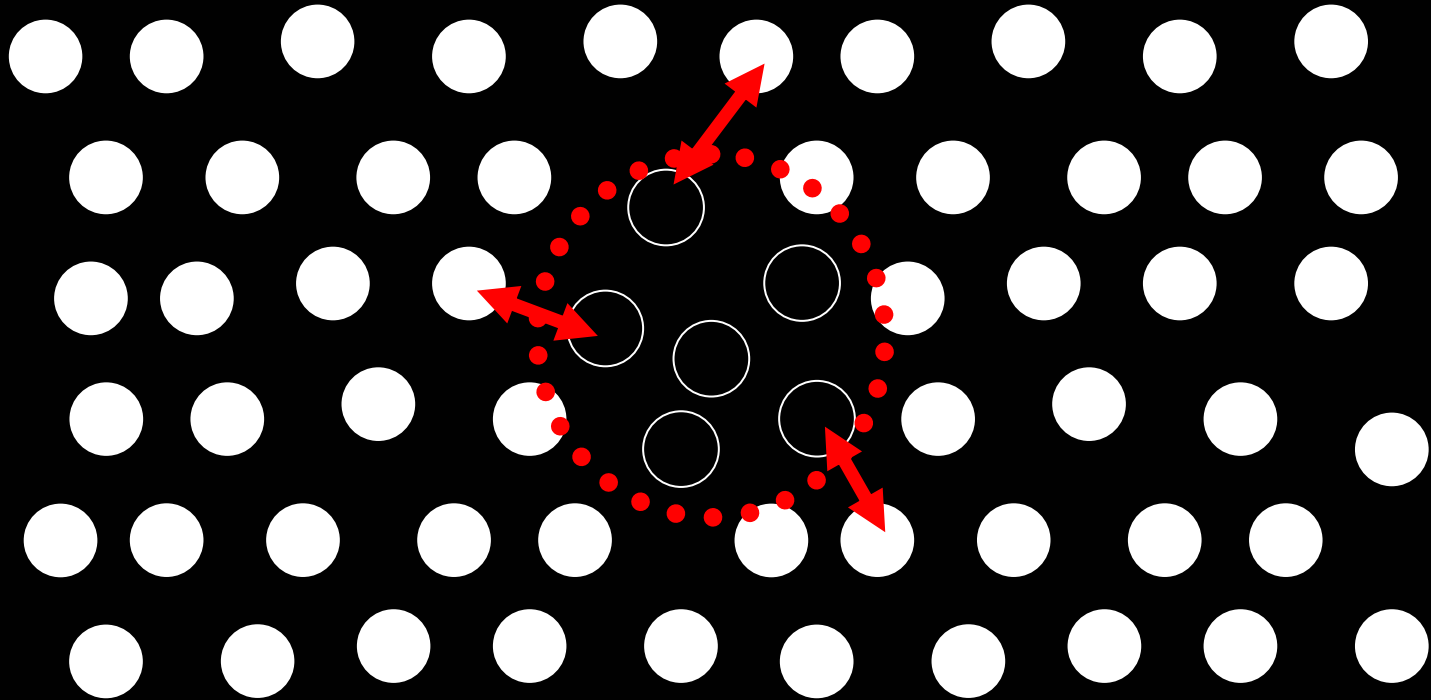
Fluorescence Recovery After Photobleaching (FRAP)



We think about fluorescence as a random walk. Each time a molecule moves, it can be bleached or not. The probability of being bleached is proportional to the intensity of the laser. The probability of not being bleached is proportional to the probability of not being in the laser spot. The probability of being bleached is proportional to the probability of being in the laser spot. The probability of not being bleached is proportional to the probability of not being in the laser spot.

1. What is FRAP?

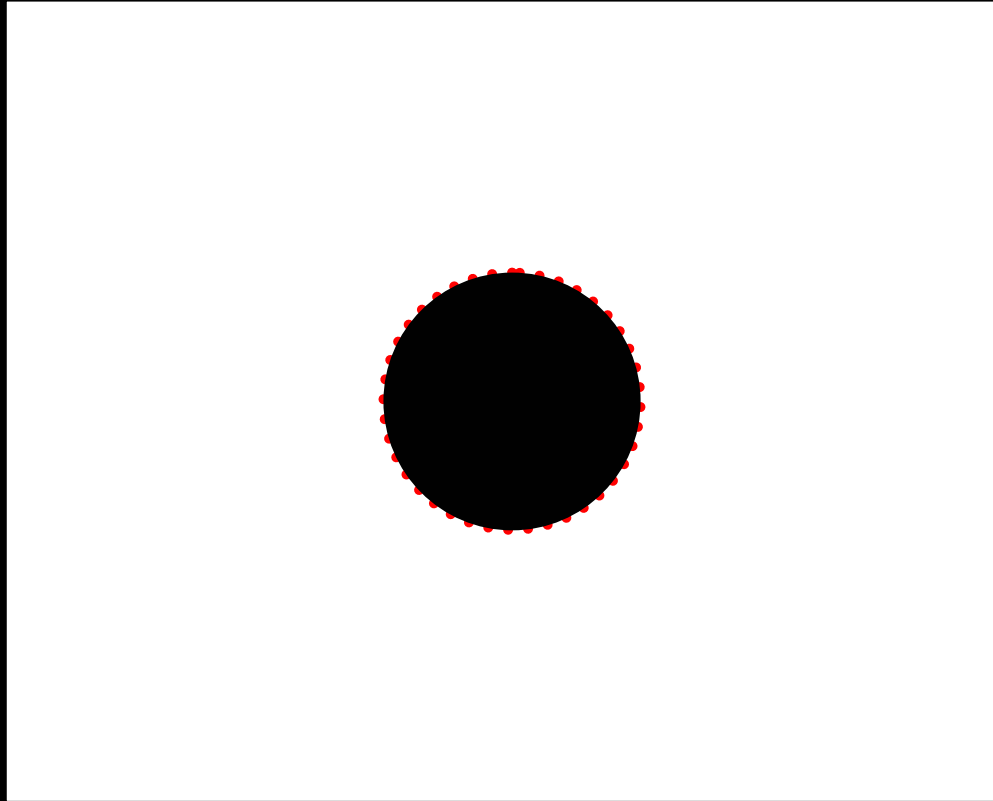
Fluorescence Recovery After Photobleaching (FRAP)



Since molecules are moving driven by diffusion or active transport, bleached molecules exchange their place with un-bleached molecules. Then the average intensity at the bleached spot recovers.

1. What is FRAP?

Fluorescence Recovery After Photobleaching (FRAP)



In practice, the FRAP at a single spot is the following. Above is a microscope field filled with fluorophores.

1. What is FRAP?

Examples

[Drosophila Centrosome](#)
[JORDAN RAFF, Cambridge](#)

[GFP-sialyl transferase in CEF cells](#)
[Banting LAB, MRC](#)

a type 1 integral membrane protein which is localised to the trans face of the Golgi stack in control cells

[FRAP demo](#)
[Federica Brandizzi, University of Saskatchewan](#)

[Filament FRAP](#)
[Leube group, Mainz](#)

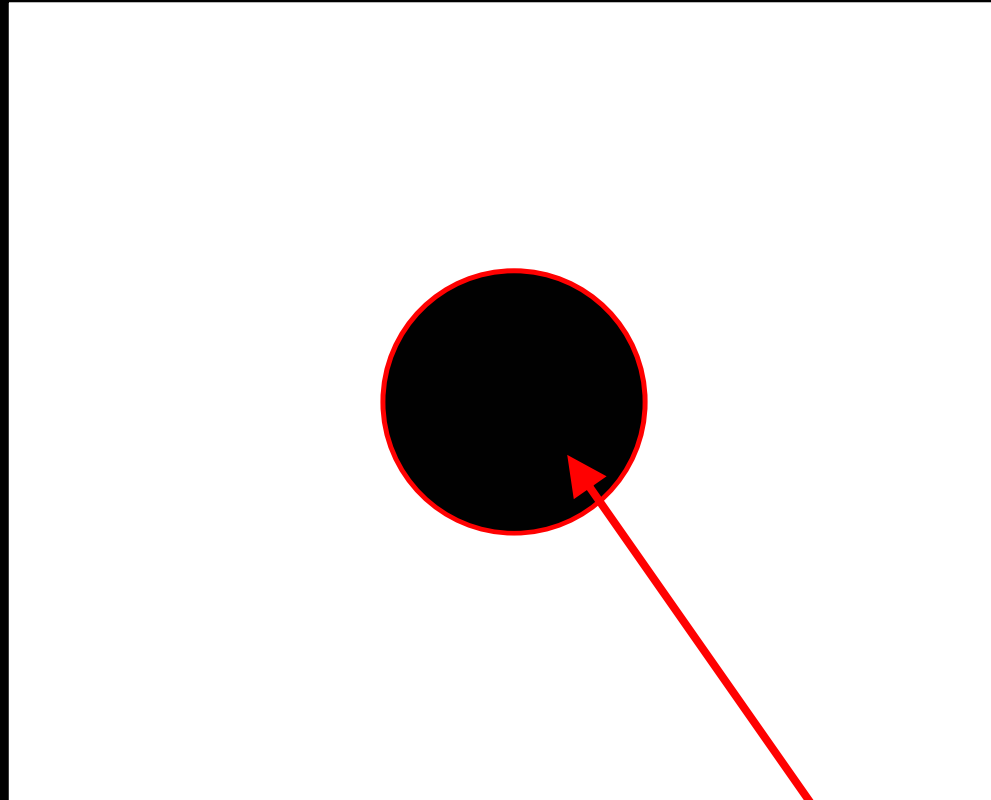
A section of a PK18-5 cell producing HK18-YFP; filamentous fluorescence recovery occurs preferentially in the cell periphery.
dt=2 min.

[Virus \(SV40\) FRAP](#)
[Pelkmans et al.](#)
NATURE CELL BIOLOGY, vol 3 pp473-

Many FRAP movies can be found on the web. Above is a list of examples. You could also find many others by searching keywords “FRAP” and “movie”.

2. Quantitative Analysis of FRAP

Fluorescence Recovery After Photobleaching (FRAP)



To gain information on molecular dynamics, time-course of the fluorescence recovery must be measured.

Measure the temporal changes of the fluorescence Intensity!

2. Quantitative Analysis of FRAP

Frap Curve

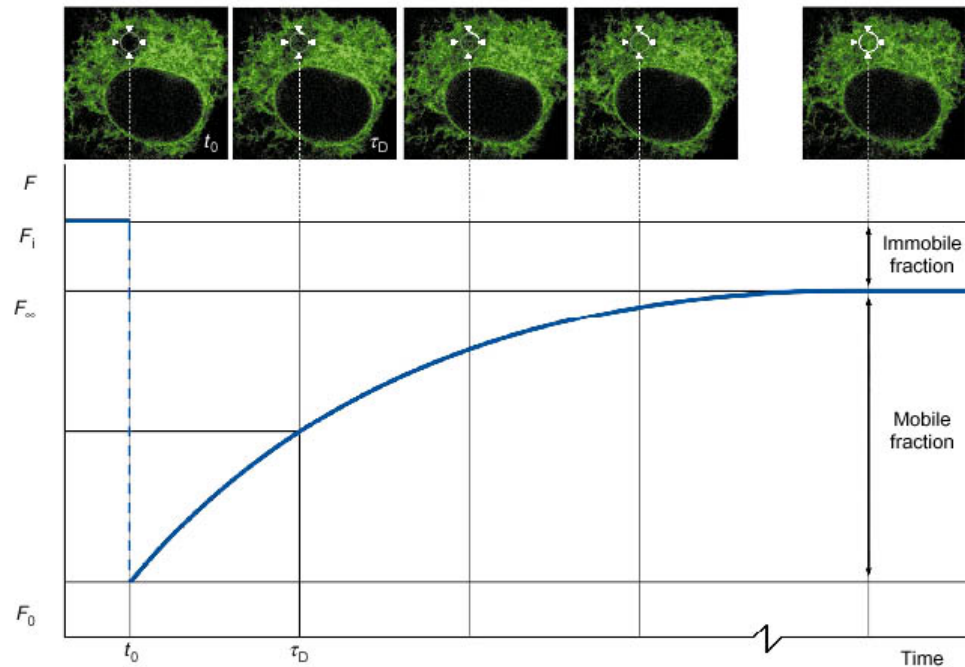


Figure 1 **Fluorescence recovery after photobleaching (FRAP)**. When a region in the fluorescent area (here the endoplasmic reticulum) is bleached at time t_0 the fluorescence decreases from the initial fluorescence F_i to F_0 . The fluorescence recovers over time by diffusion until it has fully recovered (F_∞). The characteristic

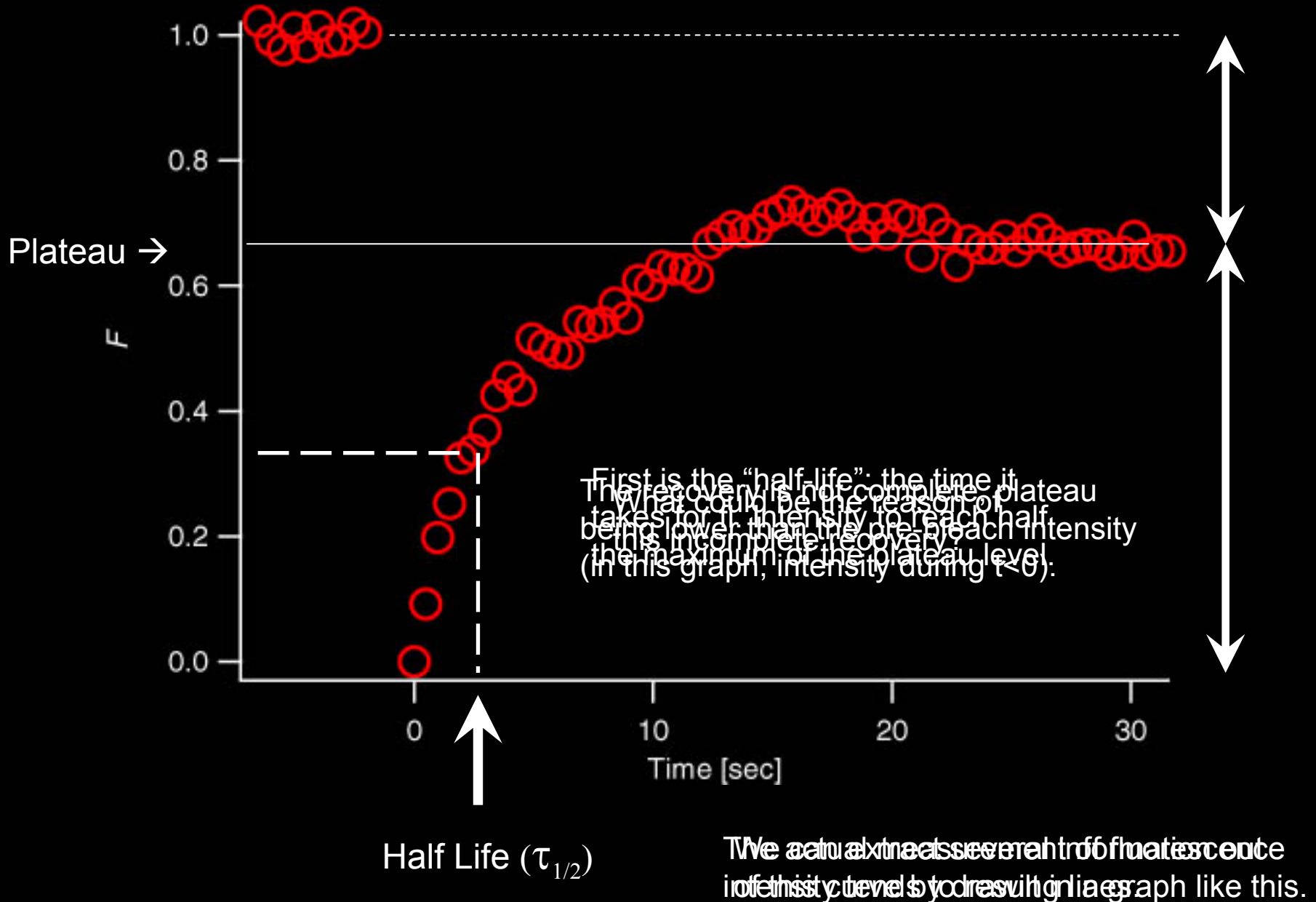
diffusion time τ_D indicates the time at which half of the fluorescence has recovered. The mobile fraction can be calculated by comparing the fluorescence in the bleached region after full recovery (F_∞) with that before bleaching (F_i) and just after bleaching (F_0).

[Reits & Neefjes \(2001\)](#)

This is an example of FRAP curve, a result of measuring the fluorescence intensity at the bleached spot. X-axis is time, Y-axis is the fluorescence intensity.

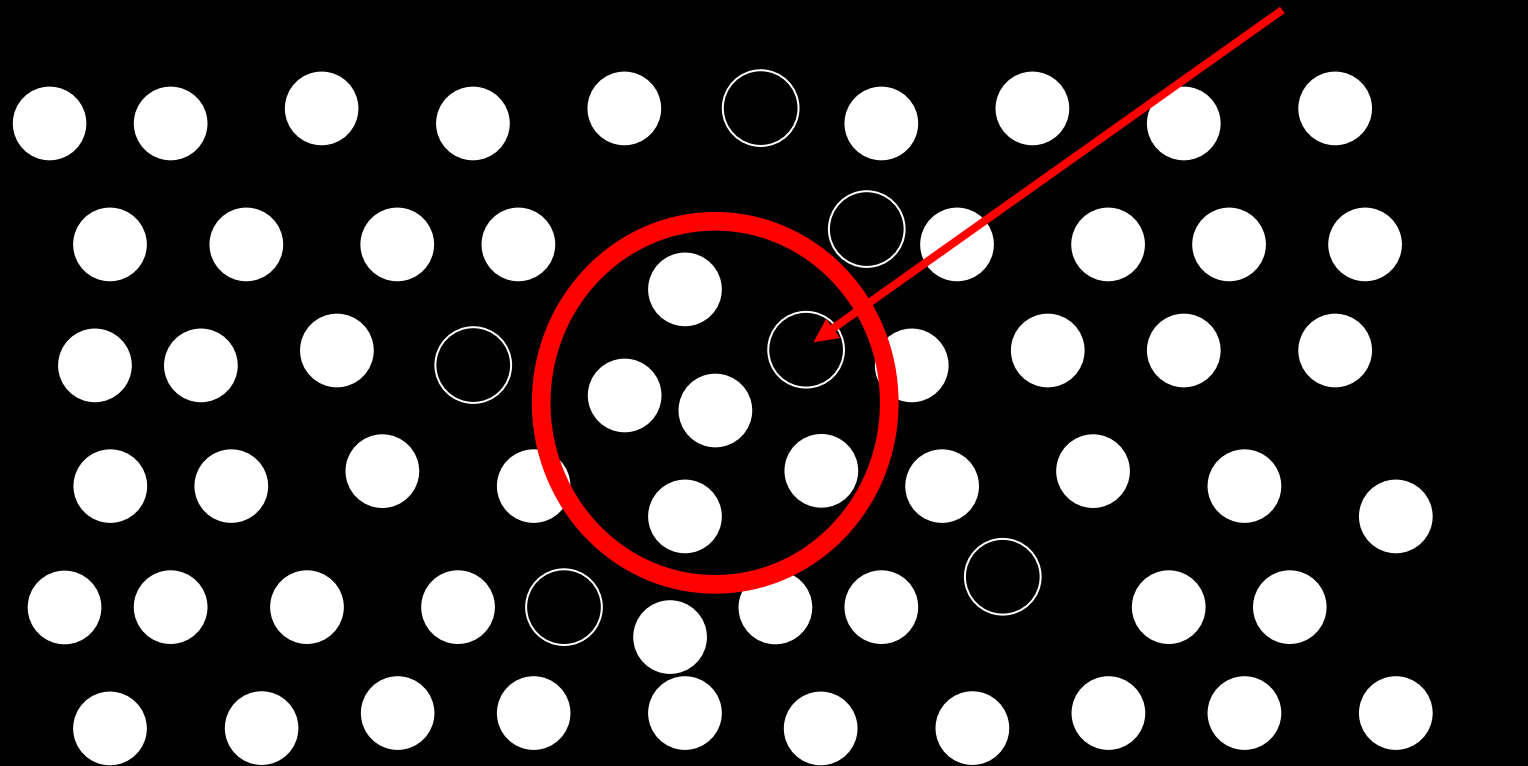
2. Quantitative Analysis of FRAP

Terms Used in FRAP analysis



2. Quantitative Analysis of FRAP

Mobile Fraction and Immobile Fraction

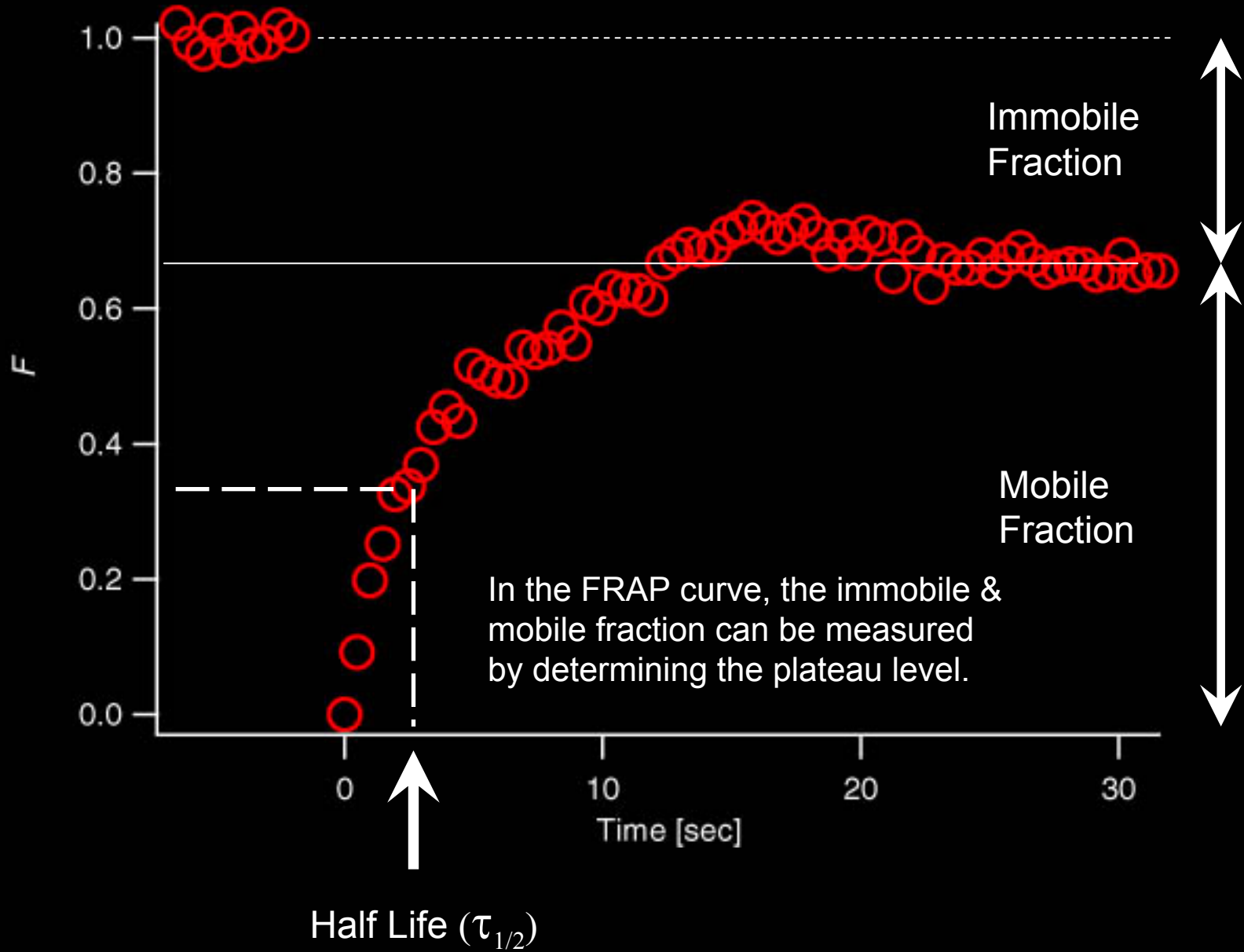


The incomplete recovery is due to some fraction of the molecules that are immobilized at the BLEACHED spot. We call these molecules the “immobile fraction”.

Rest of the molecules are contributing to the fluorescence recovery. We call them the “mobile fraction”.

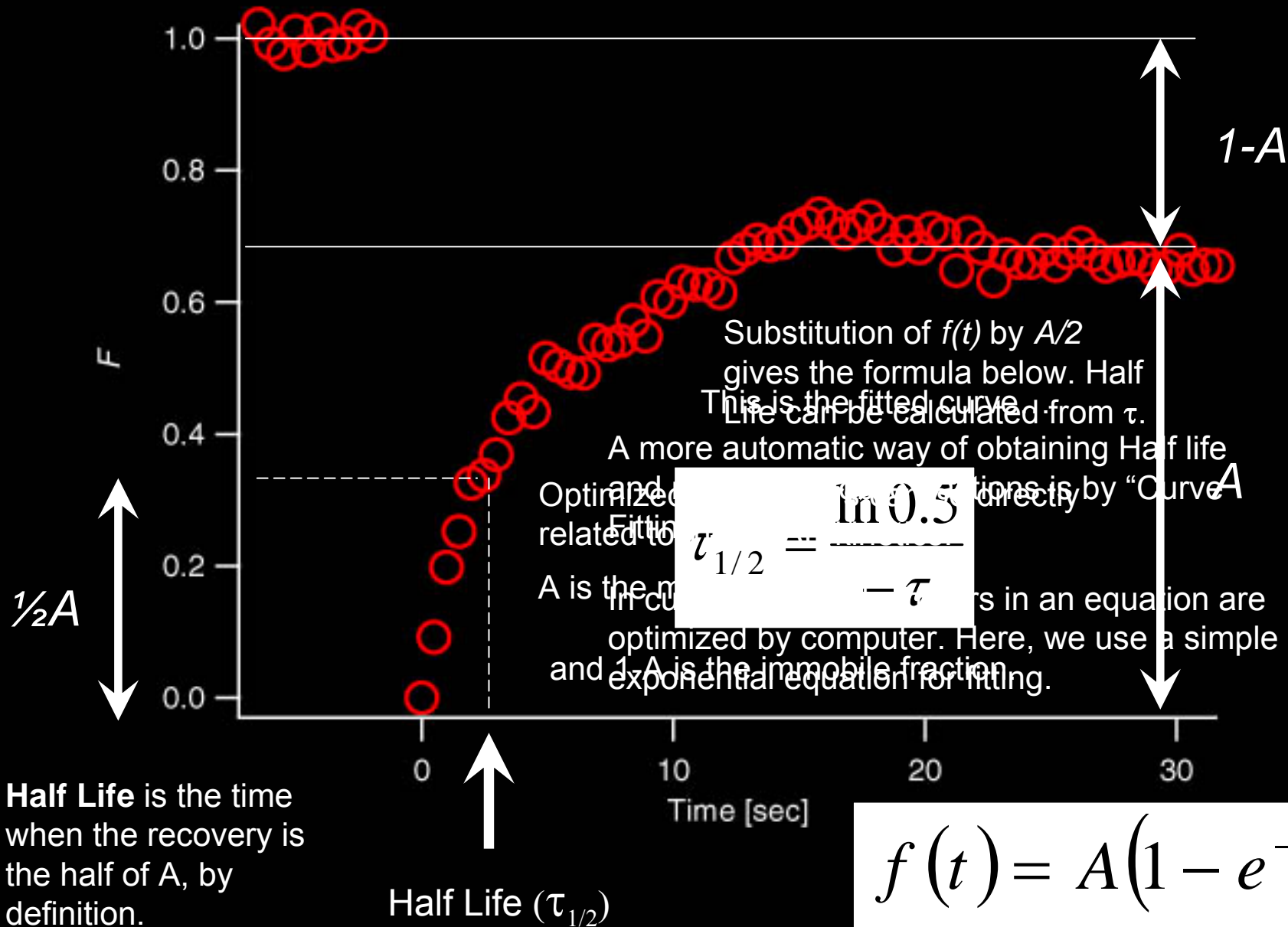
2. Quantitative Analysis of FRAP

The time constant and mobile / immobile fractions



2. Quantitative Analysis of FRAP

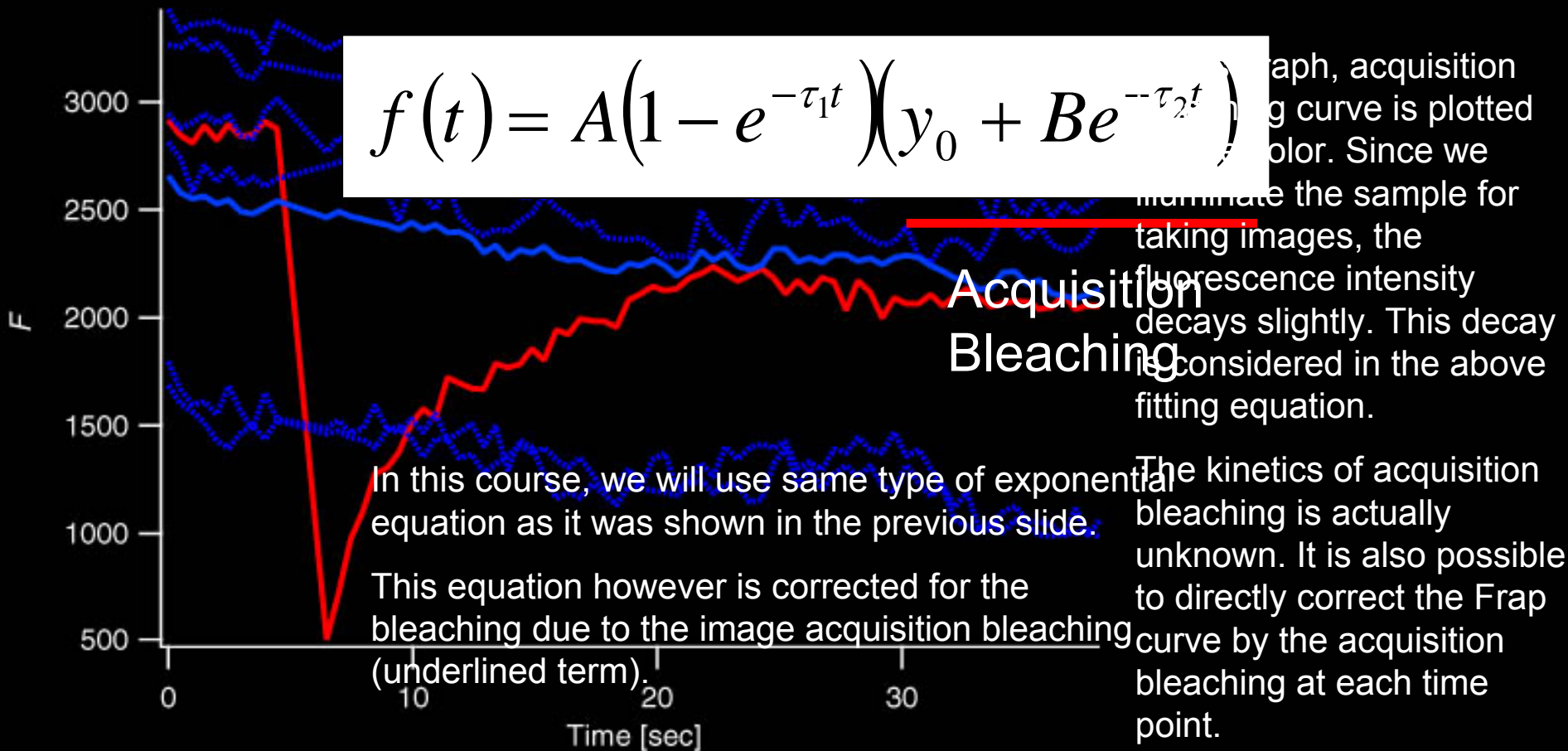
Curve Fitting



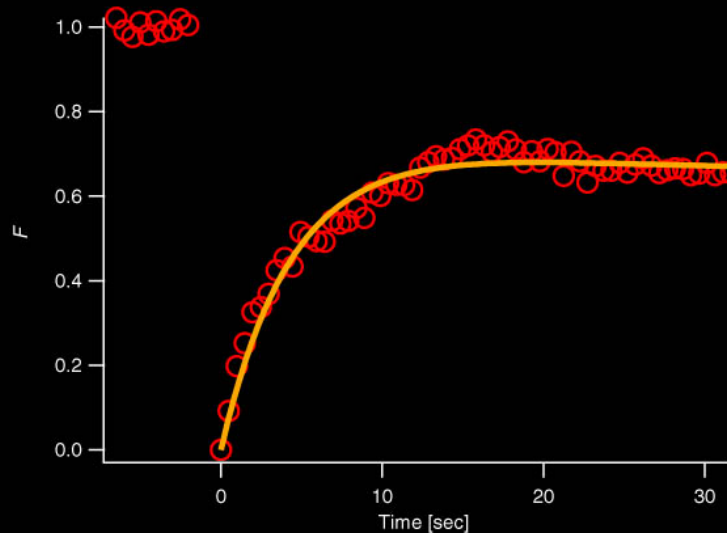
2. Quantitative Analysis of FRAP

The FRAP curve equation we will use....

$$f(t) = A(1 - e^{-\tau_1 t}) \left(y_0 + B e^{-\tau_2 t} \right)$$



3. Modeling & FRAP



Modeling

What does the fitting and obtained parameters actually mean, in terms of **molecular dynamics**?

This question leads us to the world of “**Modeling**”. We will consider following three molecular dynamics next slides.

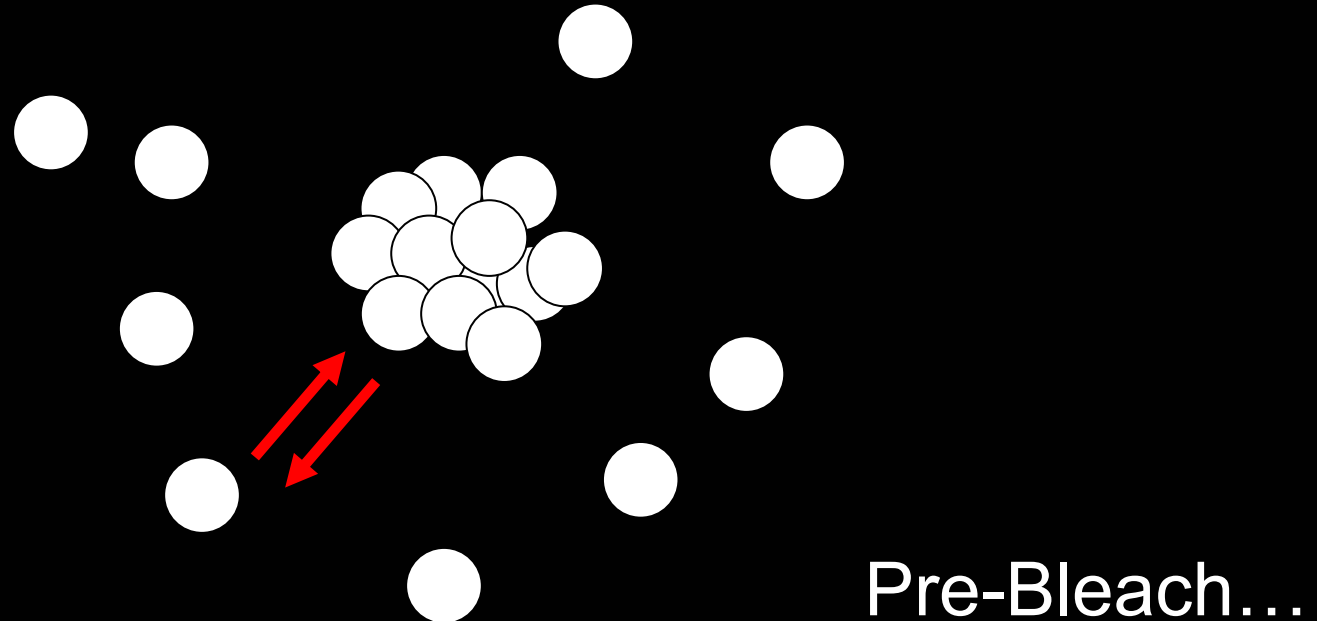
1. Specific Interactions
2. Diffusion
3. Advanced...
 - a. Analytical Approach
 - b. Numerical Approach

$$f(t) = A(1 - e^{-\tau t})$$

Specific interaction is also called chemical interaction. Specific interaction and diffusion are two basic models. In actual research, there are more various and advanced models.

3. Modeling & FRAP

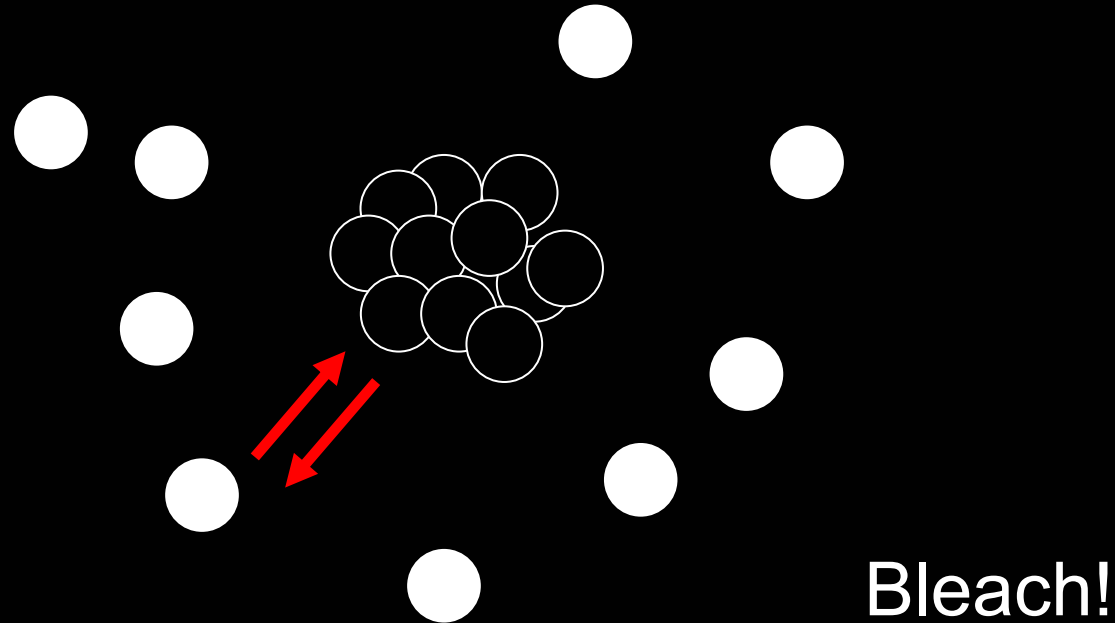
A. Specific Interactions



Let's consider a cluster of molecules, and the same molecular species unbound to the cluster. There are continuous exchange between the bound and the un-bound molecules. The system is in a steady state. The cluster size is not changing.

3. Modeling & FRAP

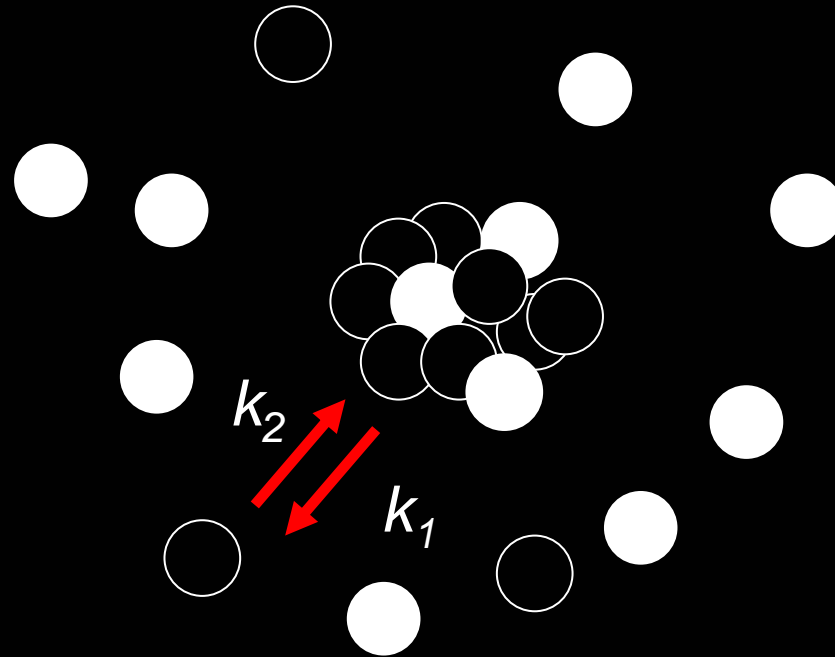
A. Specific Interactions



...then we bleach only the cluster. Immediately after the bleaching, all molecules consisting the cluster loses fluorescence.

3. Modeling & FRAP

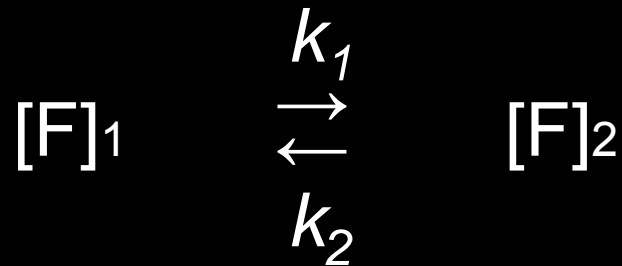
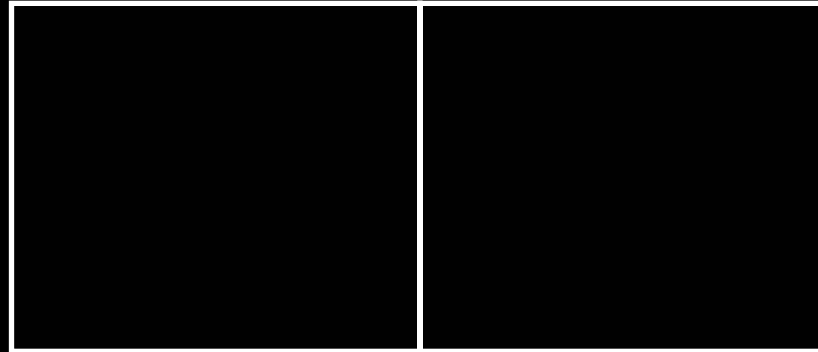
A. Specific Interactions



Recovery...

Since there is a continuous exchange of the molecules, the cluster regains fluorescence by time.

We define the exchange rate k_1 and k_2 to see this event in more detail.

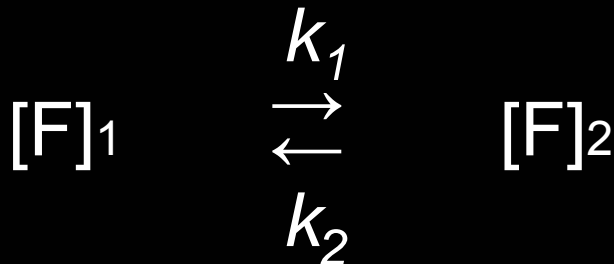
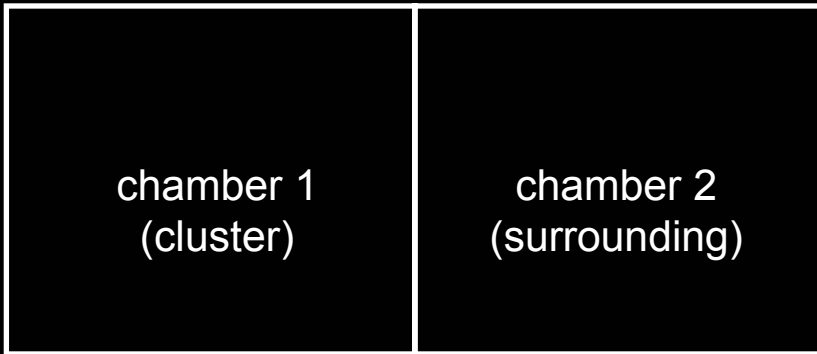


We consider a simplified model. The exchange of molecules between the cluster and the surrounding can be thought as molecule exchange between two chambers as above. The exchange is limited by a wall in between. The transfer rates in two directions are k_1 and k_2 respectively.

We define the number of molecules in the cluster as $[F]_1$. The number of molecules in surrounding environment as $[F]_2$.

3. Modeling & FRAP

Solving the equation (1) and substituting the rate constants as shown in (1a), we get the equation (2). In this differential equation, the speed of the changes in the number of molecules in the chamber 1, which is used for the fitting in the previous slides. We now know the meaning of the parameters we obtained by fitting the FRAP curve.



A. Specific Interactions

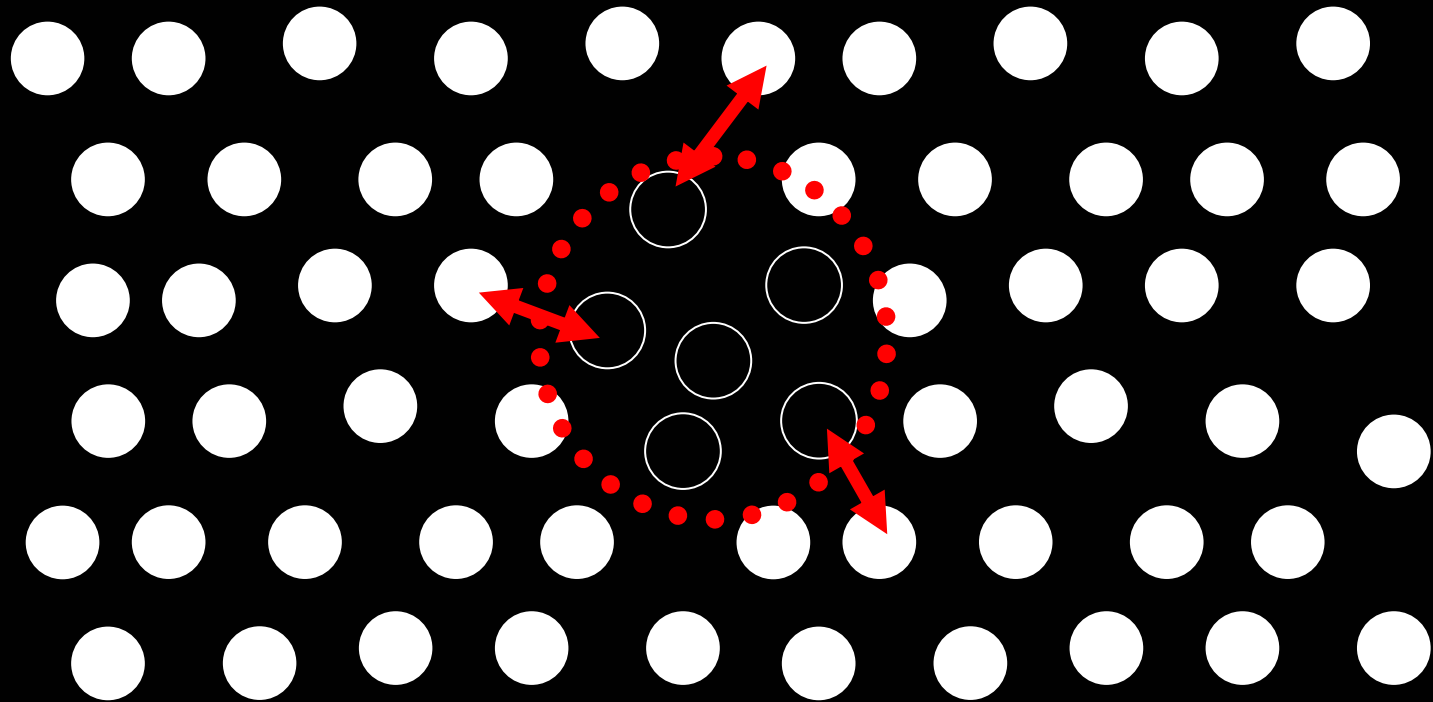
$$\frac{d[F_1]}{dt} = -k_1[F_1] + k_2[F_2] \quad (1)$$

$$\begin{aligned} \tau &= k_1 + k_2 \\ A &= \frac{k_2}{k_1 + k_2} \end{aligned} \quad (1a)$$

$$f(t) = A(1 - e^{-\tau t}) \quad (2)$$

3. Modeling & FRAP

B. Diffusion



FRAP could also occur without the specific interactions. Simply the diffusion drives the exchange of molecules between bleached and non-bleached area.

3. Modeling & FRAP

B. Diffusion

Theoretical approach

$$F(t) = C_0 \sum_{n=0}^{\infty} \left[\frac{(-K)^n}{n!} \right] \left[1 + n \left(1 + \frac{2t}{\tau_D} \right) \right]^{-1}$$

C_0 : plateau fluorescence

τ_D : the characteristic diffusion time.

$$\tau_D = w^2/4D$$

$C_u(r)$: unbleached fluorophore concentration

r : radial distance

K : bleach constant

$I(r)$ is the Gaussian intensity profile of the laser

w : half width at e^{-2} intensity.

[Phair & Misteli \(2000\)](#)

$$C_u(r) = e^{-K \frac{I(r)}{I(0)}}$$

FRAP kinetics based on diffusion was examined theoretically many years ago by [Axelrod et al. \(1976\)](#). The formula they proposed are still in used by many researchers with small modifications.

The equation shown in the left needs a parameter before fitting. Bleaching constant K is obtained by measuring the laser intensity distribution $C_u(r)$.

3. Modeling & FRAP

B. Diffusion

Empirical approach

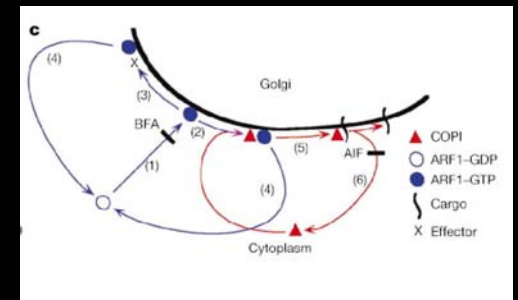
$$f(t) = I_{final} \left(1 - \frac{w^2}{w^2 + 4\pi Dt}\right)^{1/2}$$

[Ellenberg et al. 1997](#)

There are also empirical formulas for fitting the FRAP curves. Above equation is an example. w is the width of bleached spot. I_{final} is the plateau level after recovery. The diffusion coefficient D can be directly obtained by fitting this equation.

3. Modeling & FRAP

C. More Advanced... Analytical Approach



$$\frac{dfARF_g}{dt} = k_{gef} fARF_{cyto} - k_{ae} fARF \cdot effector - k_{ac} fARF_g \cdot COP_{cyto} - k_{bleachARF} \cdot fARF_g$$

$$\frac{dfARF_{effector}}{dt} = k_{ae} fARF_g \cdot effector - k_{gtpase1} fARF_{effector} - k_{bleachARF} fARF_{effector}$$

$$\frac{dfARF_{cop}}{dt} = k_{ac} fARF_g \cdot COP_{cyto} - k_{gtpase2} fARF_{cop} - k_{bleachARF} fARF_{cop}$$

$$\frac{dfARF_{cyto}}{dt} = k_{gtpase1} fARF_{effector} + k_{gtpase2} fARF_{cop} - k_{gef} fARF_{cyto}$$

$$\frac{dfARF_{exch}}{dt} = k_{fxarf} fARF_g + k_{rxarf} fARF_{exch} - k_{bleachARF} fARF_{exch}$$

$$\frac{dfARF_{cyto}}{dt} = k_{uncoat} fARF_{Xg} + k_{ac} fARF_g \cdot fCOP_{cyto}$$

$$\frac{dfCOP_{Xg}}{dt} = k_{gtpase2} ARF_{fCOP} - k_{uncoat} fCOP_{Xg} - k_{bleachCOP} fCOP_{Xg} - k_{fexch} fCOP_{Xg} + k_{rexch} fCOP_{exch}$$

$$\frac{dfARF_{fCOP}}{dt} = k_{ac} ARF_g \cdot fCOP_{cyto} - k_{gtpase2} ARF_{fCOP} - k_{bleachCOP} ARF_{fCOP}$$

$$\frac{dfCOP_{exch}}{dt} = k_{fexch} fCOP_{Xg} - k_{rexch} fCOP_{exch} - k_{bleachCOP} fCOP_{exch}$$

Presley et

Presley et al. (2002)

Advanced techniques for the modeling deal with complex molecular interactions such as those shown above. This is a diagram of molecular interactions involved in the vesicle trafficking system. These equations can be solved and fitted to the FRAP curves from the experiment to study their dynamics.

3. Modeling & FRAP

C. More Advanced...

Numerical Approach

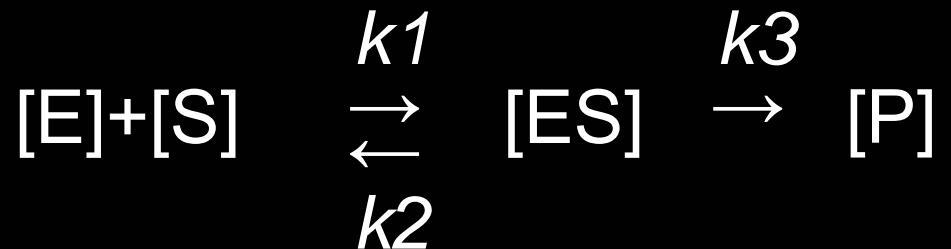
The example shown in the previous slide was a modeling by analytical approach – which is to compose differential equations and solve them for the fitting.

There is another approach for an advanced modeling of biological system. That is to derive the estimated FRAP curve by simulation and compare it with the FRAP curve from the experiments. Simulation has potential in including many more parameters than the analytical approach. This approach is powerful but not yet widely used and still in progress.

Simulations in this sense can be viewed on the web, such as [Adriaan Houtsmuller Lab's web site](#).

Summary

Biochemistry  solution 



$B([E], [S], [ES], [P], t, x, y, z)$

$B([E], [S], [ES], [P], t)$

Now, we can measure the molecular interaction directly inside cells through microscope. This enables us to consider the spatial information that

was disregarded in biochemistry. Maybe it would be more suited to call this technology as "topo-biochemistry". Kinetics is measured and characterized.

Since the site of interaction does matter within cell, these technology are now inevitable in understanding the cell as a system.

Thanks to:

Heiko Runz (EMBL Heidelberg)

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EAMNET

The **European Advanced Light Microscopy Network** (EAMNET) is a EU funded network of eight European laboratories and two industrial partners working in the field of light microscopy. The aim of EAMNET is to assist scientists in exploiting the power of imaging by organizing practical teaching courses, creating online teaching modules and offering software packages for microscopy. All EAMNET partners are also members of the European Light Microscopy Initiative ([ELMI](#)).

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For Suggestions, Comments and Questions, please e-mail
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