Single particle tracking: principles and applications

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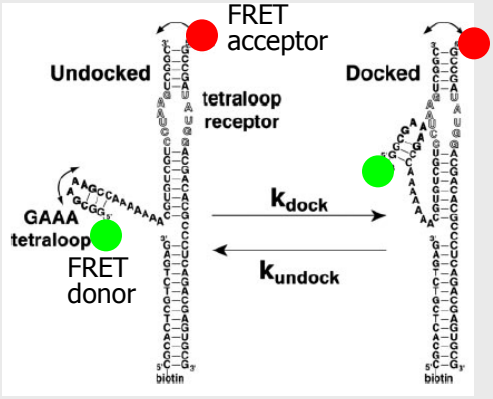
Why single molecule experiments?: An example

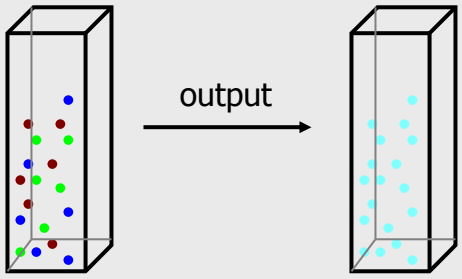
Studying folding of individual structural elements of RNA



1) Main 3D-conformation(s) of the molecule in different conditions
2) Quantification of the docking/undocking dynamics

Hodak J. et al. PNAS (2005)

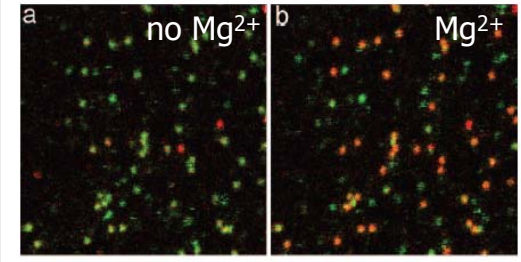
Why single molecule experiments?

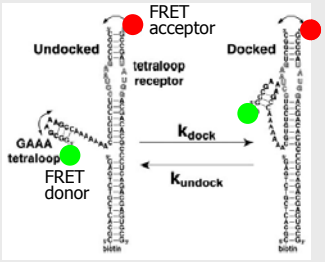


solution 1 μM $\rightarrow$ 10^{15} molecules

What information are we missing in "cuvette" assays?

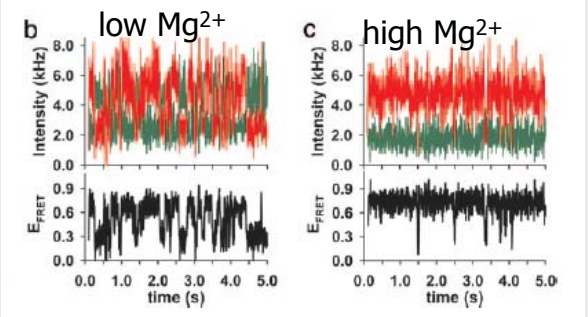
Studying RNA structure and dynamics





FRET trajectories

donor
acceptor



Hodak et al. PNAS (2005)

Bulk vs. single molecule measurements

Limitations of bulk measurements

- molecules are not synchronized
- average properties

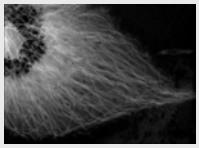
Single molecule/particle assays

- Temporal evolution of each molecule/particle
- No need to synchronize processes
- Detection of different populations

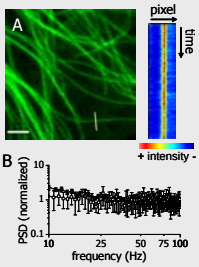
Disadvantages

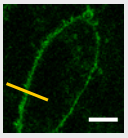
- More expensive equipments
- Statistics!

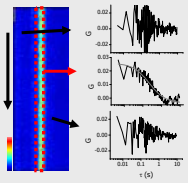
Dynamics of intracellular processes: Tracking

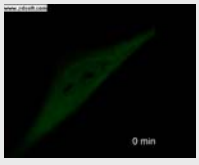


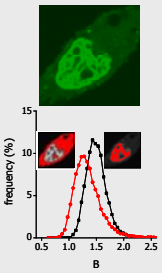
Protein function in the cellular context

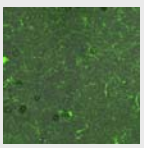






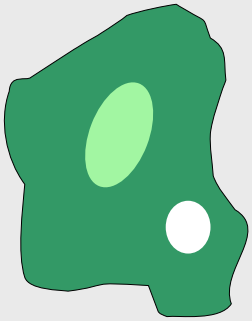


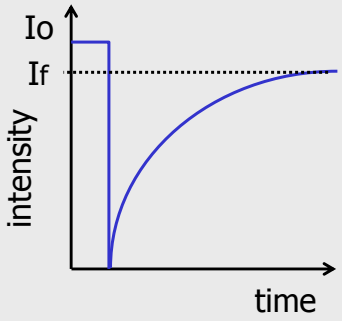




1. Measuring motion in cells: FRAP

FRAP (fluorescence recovery after photobleaching) (Axelrod, 1976) and related techniques (FLIP, etc)

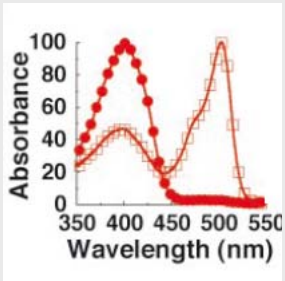




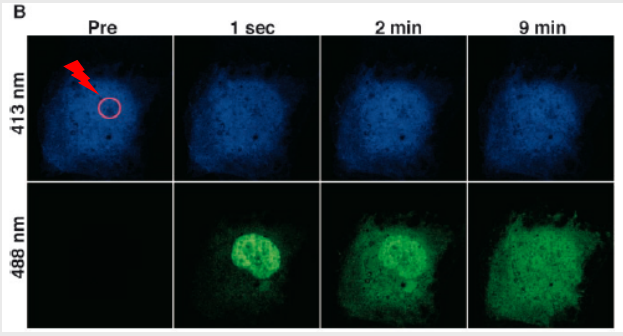
- Photodamage
- Photobleaching is not always irreversible

2. Measuring motion in cells: photoactivation

PA-GFP (photoactivatable GFP) photoconverts to a fluorescent specie after irradiation at λ = 400 nm



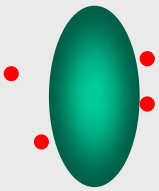
● before photoconversion
□ after photoconversion



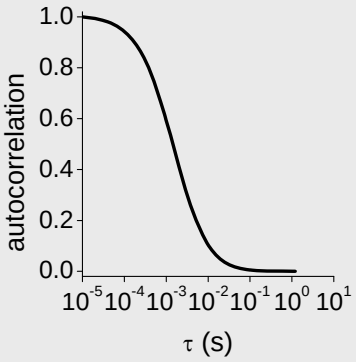
Patterson and Lippincott-Schwartz, Science 2002

3. Measuring motion in cells: FCS

FCS (fluorescence correlation spectroscopy)



confocal volume



- Low laser power
- Low concentration of fluorescent molecules
- Equilibrium

Why single particle tracking experiments?

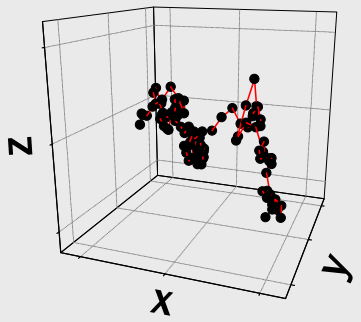
It is hard to study complex processes by "averaging" de behavior of multiple molecules

Single particle tracking (SPT)

Position of the particle as a function of time

mechanism of motion

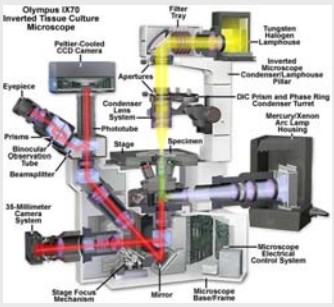
- interactions
- populations
- switches (no synchronization)

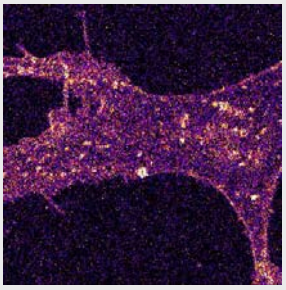


Single particle tracking

1. Acquisition of single particle trajectories
2 .Quantitative analysis of single particle trajectories
3. Applications to intracellular transport
4. 3D particle tracking

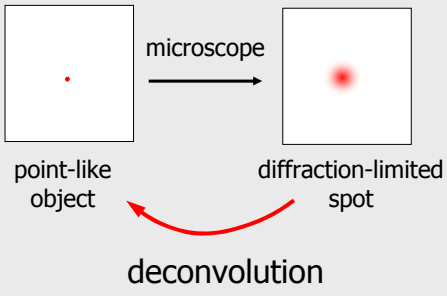
Image-based tracking techniques

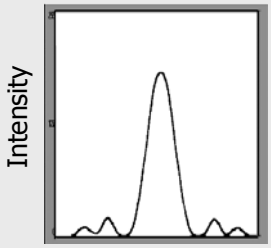




- How can we obtain the trajectory of the particle from the movie?
- What is the accuracy on the position determination?

Locating a particle with nanometer precision





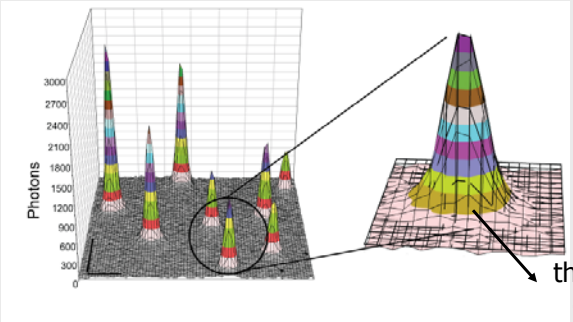
$r \cong \lambda/2NA$

r = resolution
λ = wavelength
NA = numerical aperture of the objective

r~250 nm, visible light

An example of a simple SPT method: FIONA (fluorescence imaging with nanometer accuracy)

Imaging single fluorophore



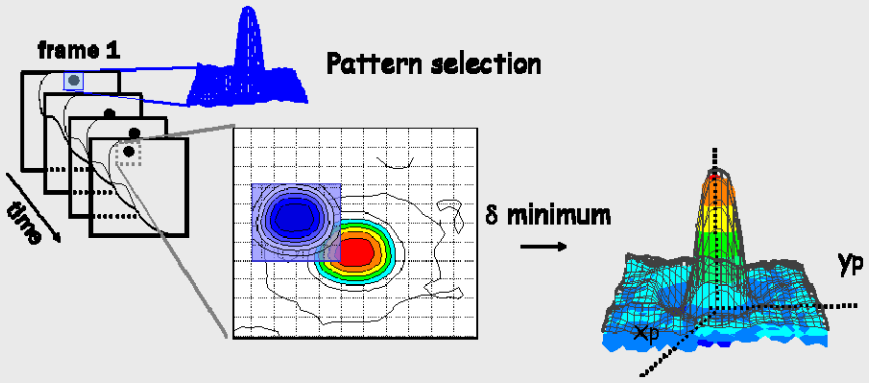
We have to be sure that there is a single molecule/particle !!

the center of this distribution can be found with 1.5 nm precision!

from http://physics.berkeley.edu/research/yildiz/technology.html

The trajectory of the particle is then recovered after locating the particle in every frame of the movie

Pattern-recognition tracking method



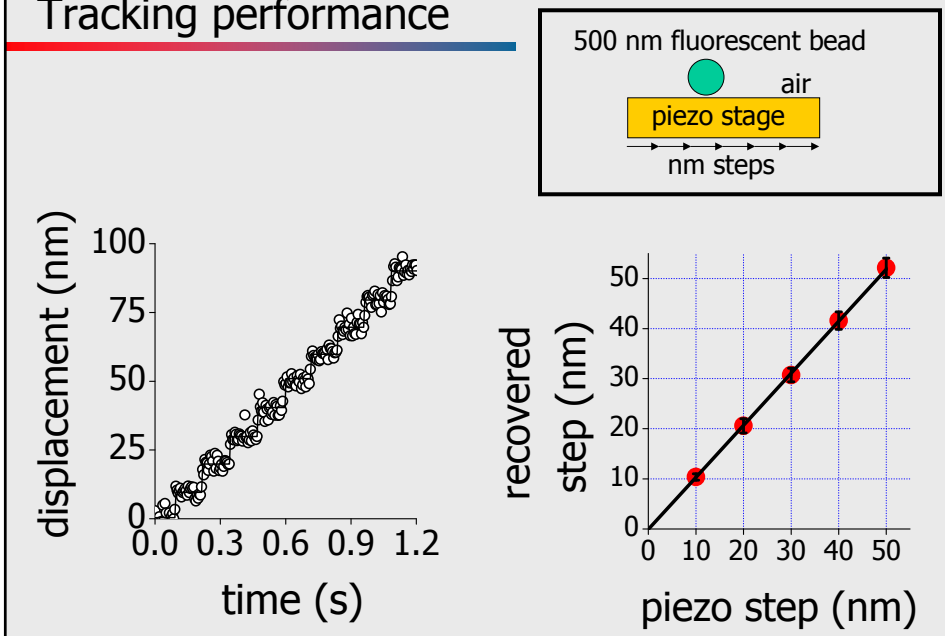
$$\delta = \sum_{i,j} \sqrt{(I_{image}(i,j) - I_{pattern}(i,j) - B)^2} \cdot w(i,j)$$

B = background difference

w(i,j) = weight factor= $\sqrt{(I_{pattern}(i,j) - I_{border})^2}$

Levi et al. Biophys J (2006)

Tracking performance



Levi et al. Biophys J (2006)

Improving SPT: Error in the particle position

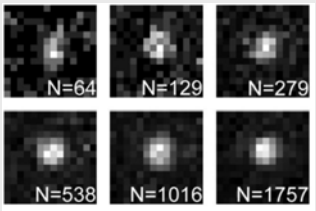
$$\left\langle (\Delta x)^2 \right\rangle = \frac{s^2}{N} + \frac{a^2/12}{N} + \frac{8\pi s^4 B^2}{a^2 N^2}$$

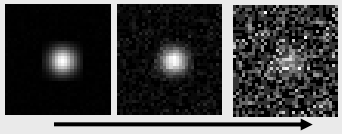
Δx = error in the particle position
s = standard deviation of the PSF
N= number of photons detected
a = pixel size
B = background noise

photon noise

background noise

pixelization noise



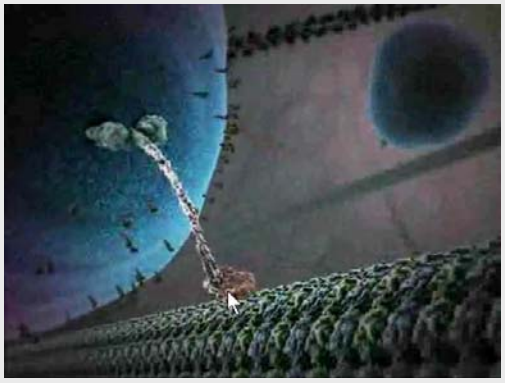


B increases

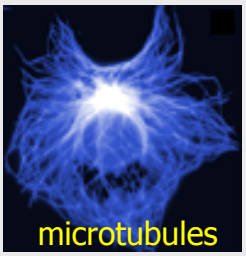
a increases

Thompson et al. Biophys J (2002)

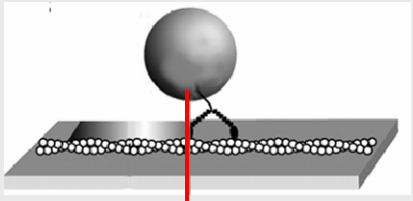
Application: molecular motors



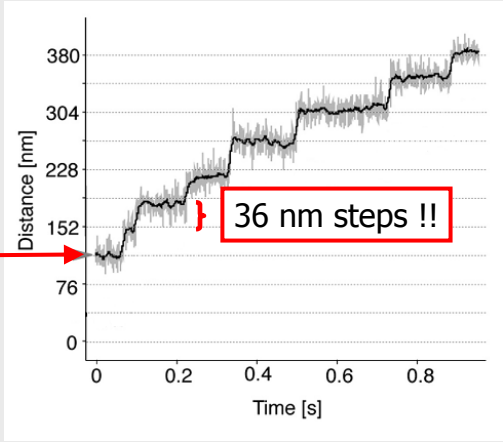
How the motor moves along the filament?



A simple answer from SPT experiments



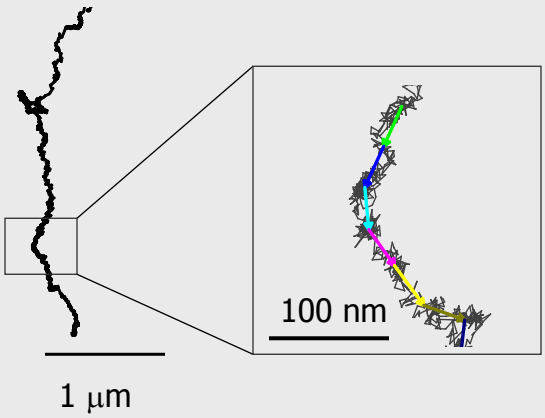
explicar con flechitas



Adapted from Rief et al., PNAS (2000)

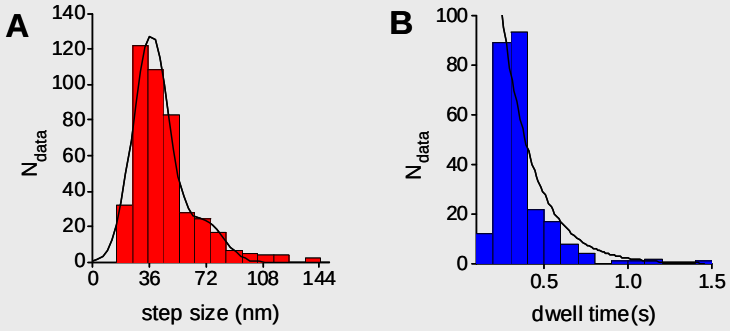
We can observe steps of motors in living cells!

Transport of melanosomes by myosin V



Levi et al, Biophys J (2006b)
Bruno et al, submitted

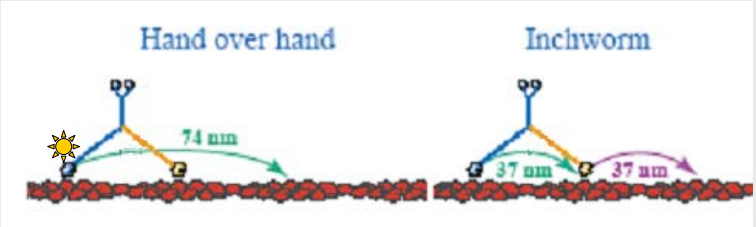
Myosin-V in living cells

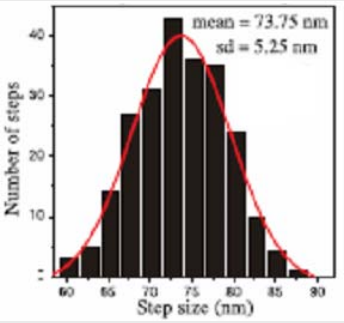


	in vitro	in cells
step size (nm)	37	37.1 ± 0.6
dwell time (ms)	70	200 ± 40

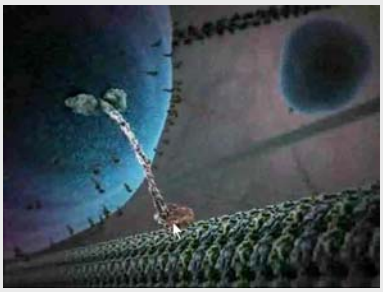
Levi et al, Biophys J (2006b)
Bruno et al, submitted

Understanding the stepping mechanism





Yildiz et al. Science, 2003



www.studiodaily.com

Probes for SPT experiments

In SPT experiments, probes may:

1) delay the motion of the particle
2) change the properties of the tracked molecule

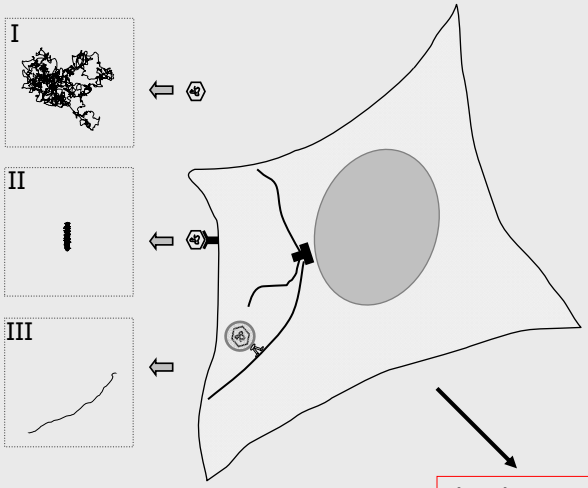
Probe	Size (nm)	Advantage	Disadvantage
fluorescent beads	5-1000	brigthness	size
quantum dots	10-20	brigthness, spectra	blinking
fluorescent proteins	10-20	endogenously expressed	low brightness, high concentration, bleaching
single dye molecules	0.5-10	size	low brightness, bleaching (<10s)
nanoparticles	20-50	last forever	low signal/noise

Single particle tracking

Motor stepping dynamics is just a very specific case..

How can we obtain information of complex intracellular processes?

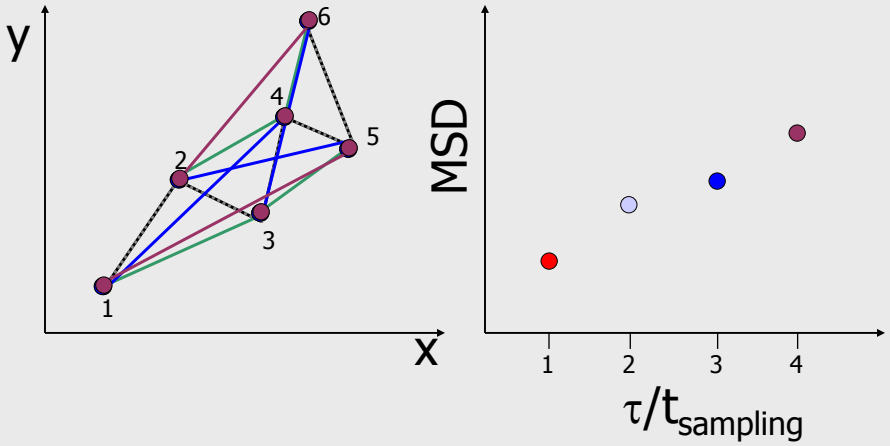
Obtaining quantitative information from trajectories



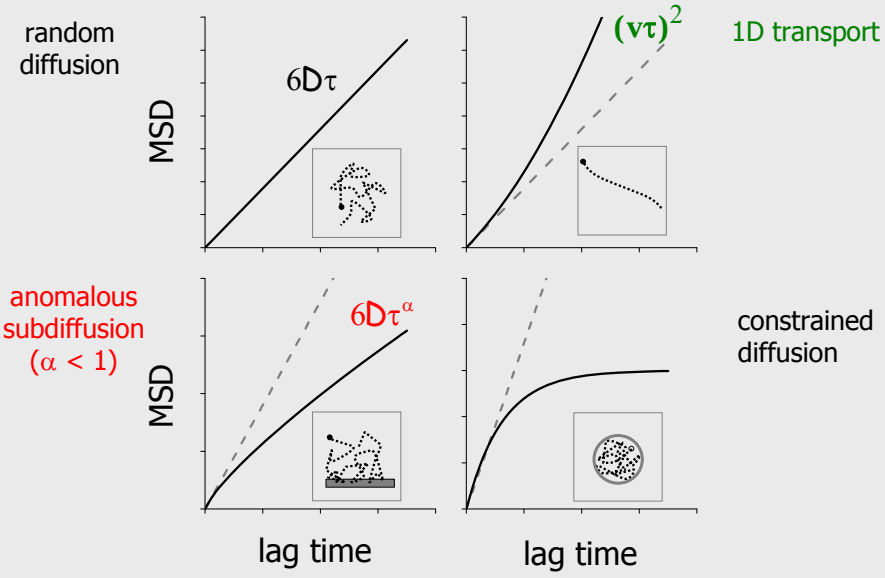
the distance traveled by the particle after a given time period is different for the different motion regimes !

Calculation of the mean square displacement (MSD)

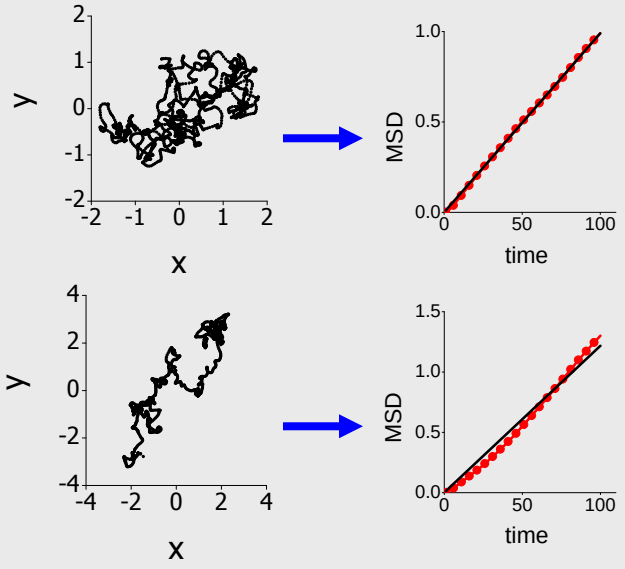
$$MSD(\tau) = \left\langle \left(x(t) - x(t+\tau)\right)^2 + \left(y(t) - y(t+\tau)\right)^2 \right\rangle$$



What information can we obtain from MSD analysis?

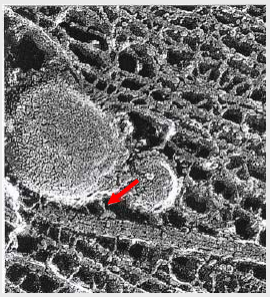


"Single things" analysis means that we have to be extremely careful with the statistics



Applications: MSD analysis helps us to understand the mechanisms involved in organelle transport

in vitro vs. in cells

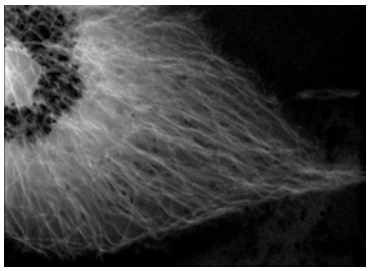


Electron micrograph of mouse axon

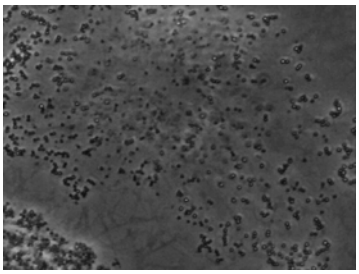
Hirokawa, Science 1998

The conditions in which transport develops in cells are completely different from those of in vitro assays

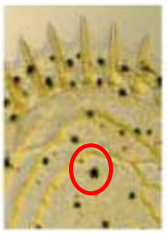
Melanophores: a beautiful system to study transport



MSH
dispersion

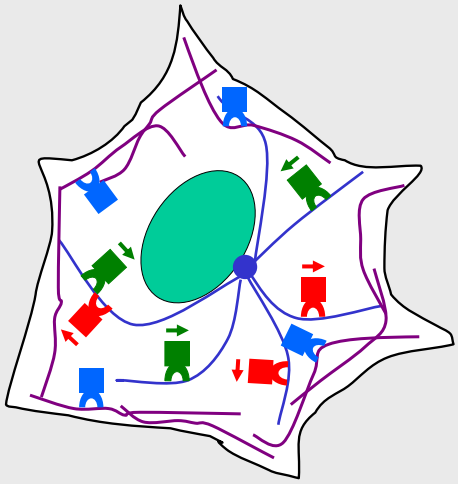


melatonin
aggregation



Gelfand´s lab - Wallin, bioscience-explained.org

Organelle transport in living cells



3 families of motors

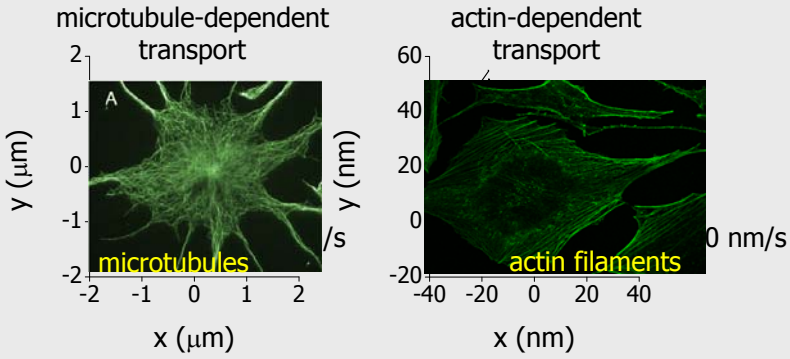
- kinesin
- dynein
- myosin

2 different tracks

- microtubules
- actin filaments

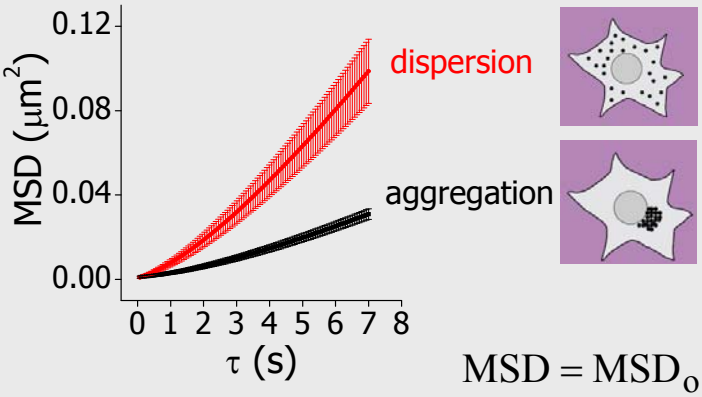
How do these transport systems work together to target organelles to their final destination?

Properties of myosin-V dependent transport



Why trajectories along actin filaments are not curvilinear?

Properties of transport driven by myosin-V

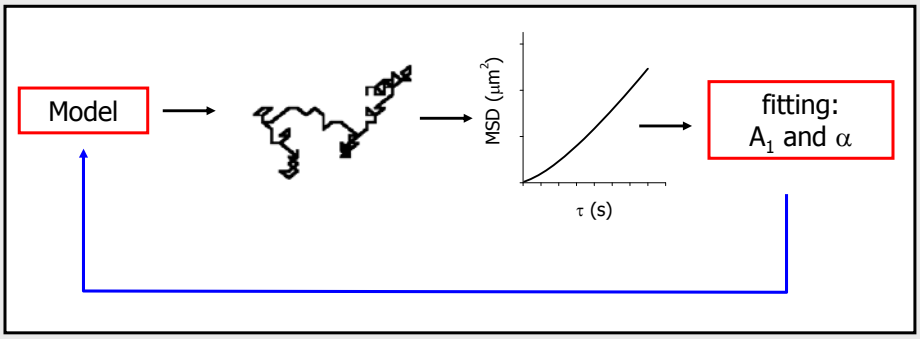


$$MSD = MSD_0 + A1 \cdot \tau^{\alpha}$$

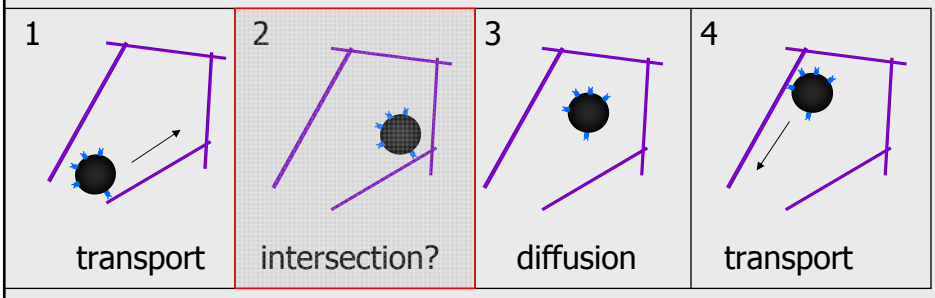
Condition	MSD_0 [nm²]	A_1 [nm²]	α
aggregation	560 ± 30	1500 ± 50	1.37 ± 0.03
dispersion	600 ± 30	4100 ± 1000	1.33 ± 0.02

Numerical simulations

How are these parameters related to the transport mechanism?



The transport-diffusion model



Intersection: switching and detaching probabilities (Ali et al, PNAS 2007)
Lower number myosin motor/melanosome in aggregation (Gross et al. JCB, 2004)

Experimental

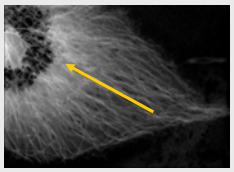
Condition	A_1 [nm²]
aggregation	1500 ± 50
dispersion	4100 ± 1000

Model

diffusion time (s)	A_1 [nm²]
90	1700 ± 300
30	4000 ± 600

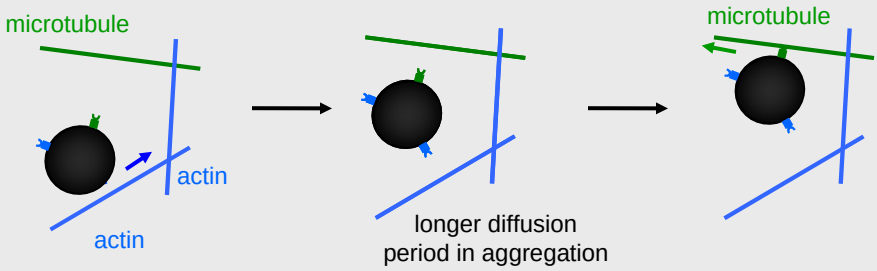
Brunstein et al. Biophys J 2009; Bruno et al. Phys Rev E 2009

Diffusion increases the probability of switching to the microtubule network during aggregation



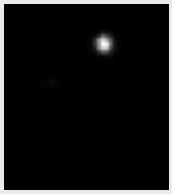
aggregation

During aggregation, organelles need to be transferred from the actin to the microtubule networks



3D Particle tracking

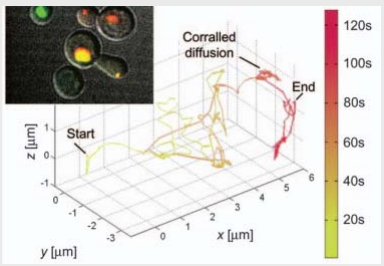
Making use of the z-sectioning capability of confocal and two-photon microscopes



1 z-plane ~ 0.1-1 s

Problems: low temporal resolution
photobleaching

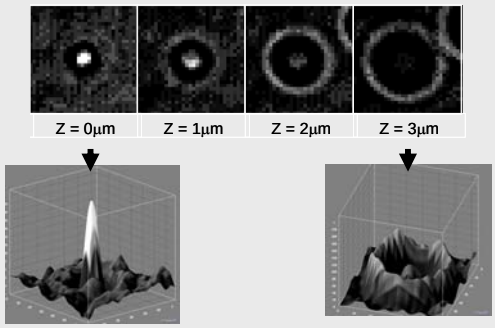
Alternative: spinning disk confocal microscopy

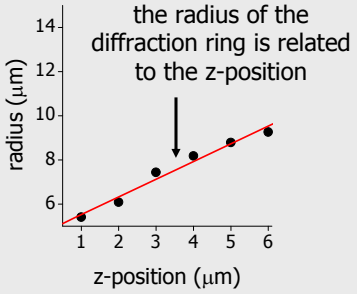


Eg: tracking ribonucleoprotein (RNP)
time resolution: 0.3 s

Lange et al, Traffic, 2008

Image-based 3D methods



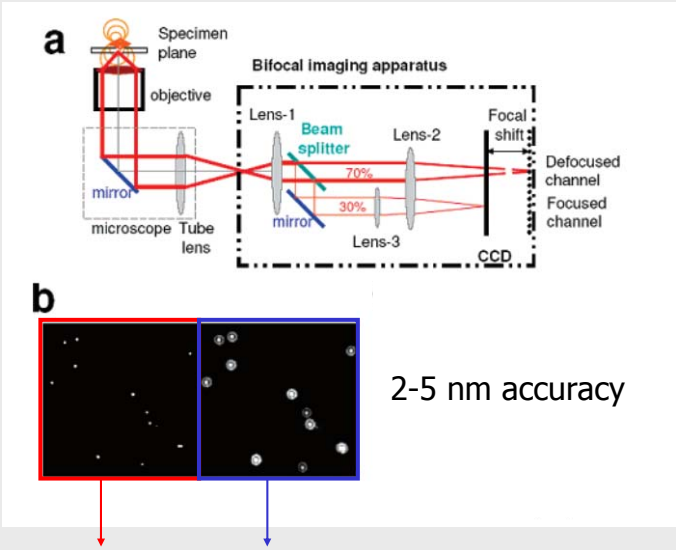


(Tilli and Massaccesi)

Problems:

- limited z range
- error in (x,y) increases when the particle moves far from the focal plane

Image-based 3D methods: Bifocal imaging



2-5 nm accuracy

in-focus (x,y position)

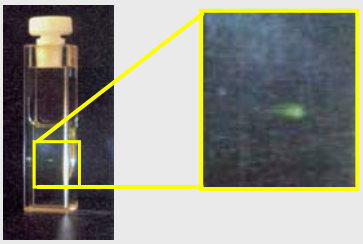
out-of-focus (z-position)

Toprak et al, Nano lett 2007

Particle tracking in a 2-photon microscope

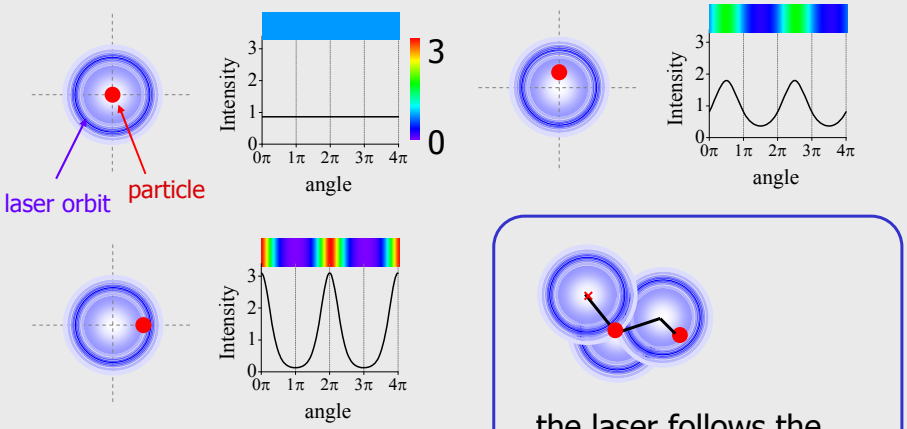
Motivations:

- particles could move far from the field of view
- widefield microscopy involves photobleaching
- Raster imaging is slow



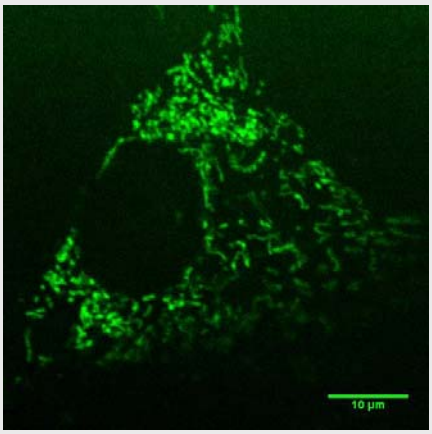
2-photon

Particle tracking in a 2-photon microscope



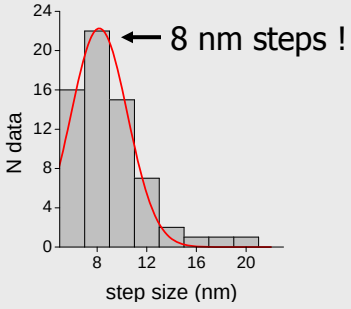
Levi et al Biophys J 2005a, 2005b

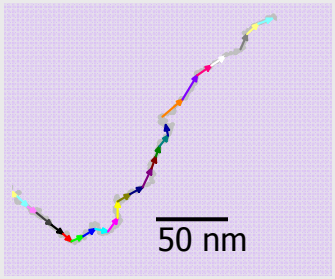
Mitochondria transport in NIH3T3 cells



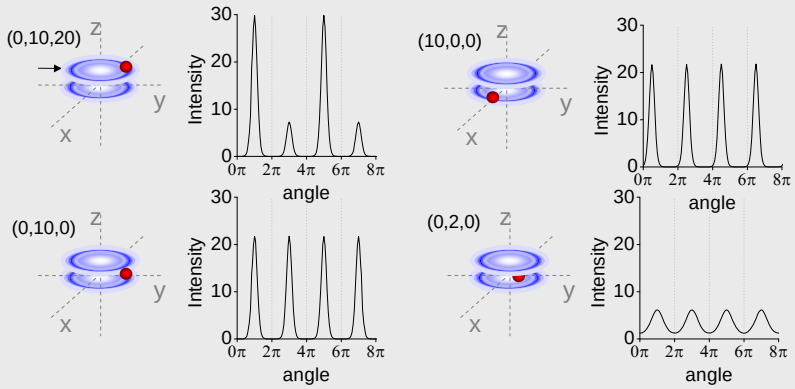
data from Diana Wetzler

time resolution = 4.1 ms
accuracy = 2 nm





3D-Particle tracking in a 2-photon microscope



more on 3D tracking at the Weber Conference

Levi et al Biophys J 2005a

More SPT talks in the Gregorio Weber Conference

Thursday, December 15

12:00-12:20	S2-1) LUCIANA BRUNO (Universidad de Buenos Aires, Argentina)
12:20-12:40	S2-2) ENRICO GRATTON (LFD, University of California Irvine, USA)
12:40-13:00	S2-3) ANDRE GOMES (Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil)