Single-Particle Tracking (SPT)

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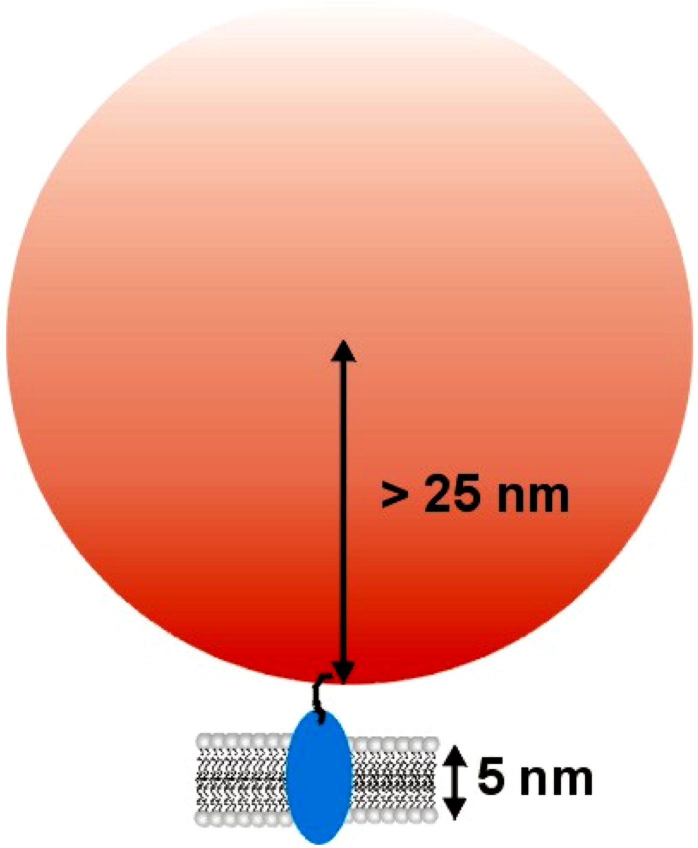
INSERM workshop, 29.6-2.7 2005, La Londe sur Mer, France

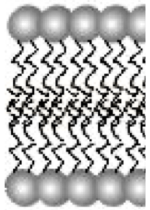
how to visualize a biomolecule

(by fluorescence) ?

- single-particle tracking (SPT)



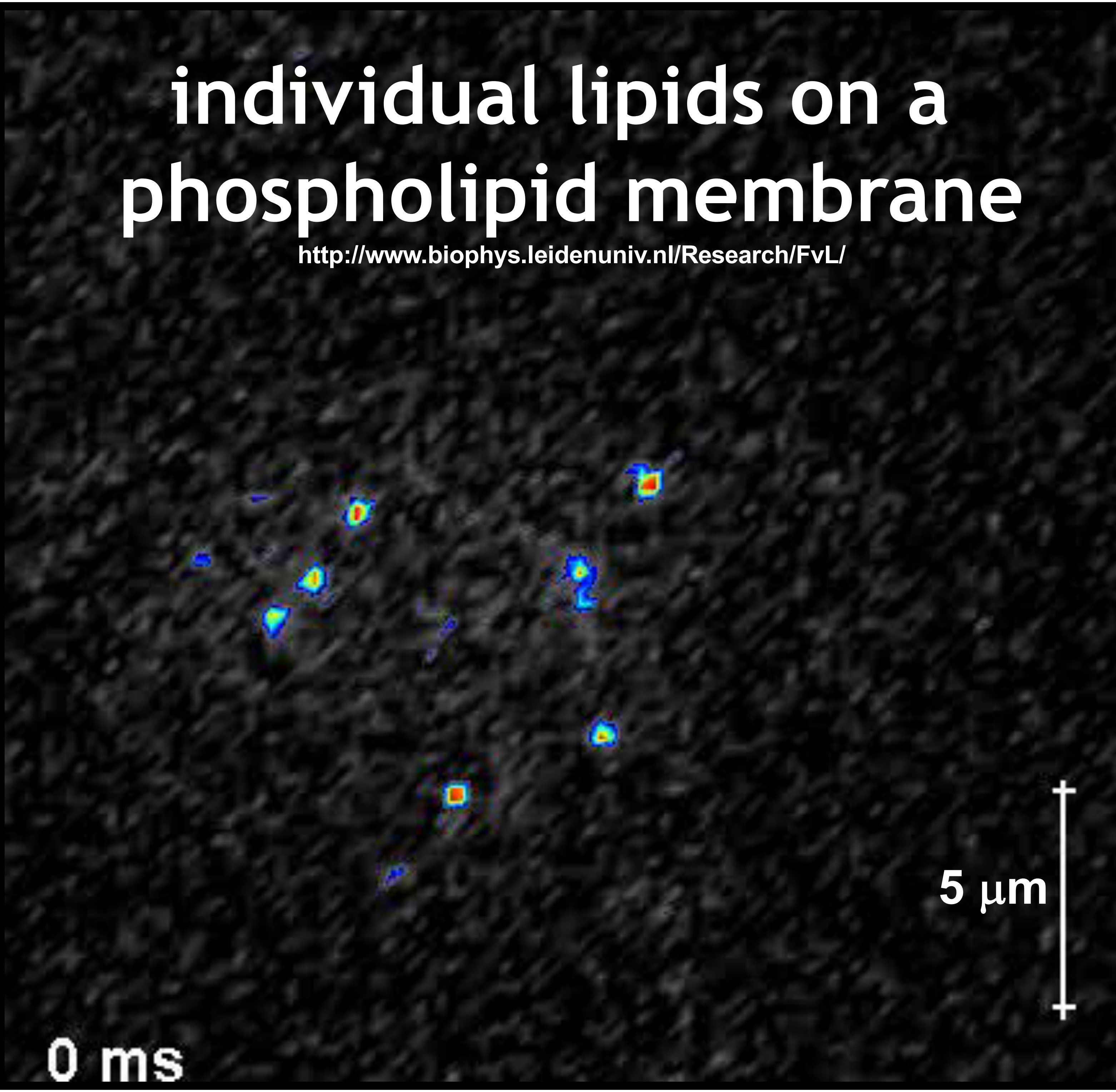
- single-molecule



- Berg et.al., *Tracking of bacteria* (1980's)
- de Brabanter & Geerts, *Nanovid microscopy*, Am J Anat (1989) 185:282
- Qian, Sheetz & Elson, *Single particle tracking*, BJ (1991) 60:910
- Gosh & Webb, *LDL tracking*, BJ (1994) 66:1301
- ... many others
- Kusumi, *10^5 images/s* (2002)
- Gratton, *3D single-particle tracking* (2004)

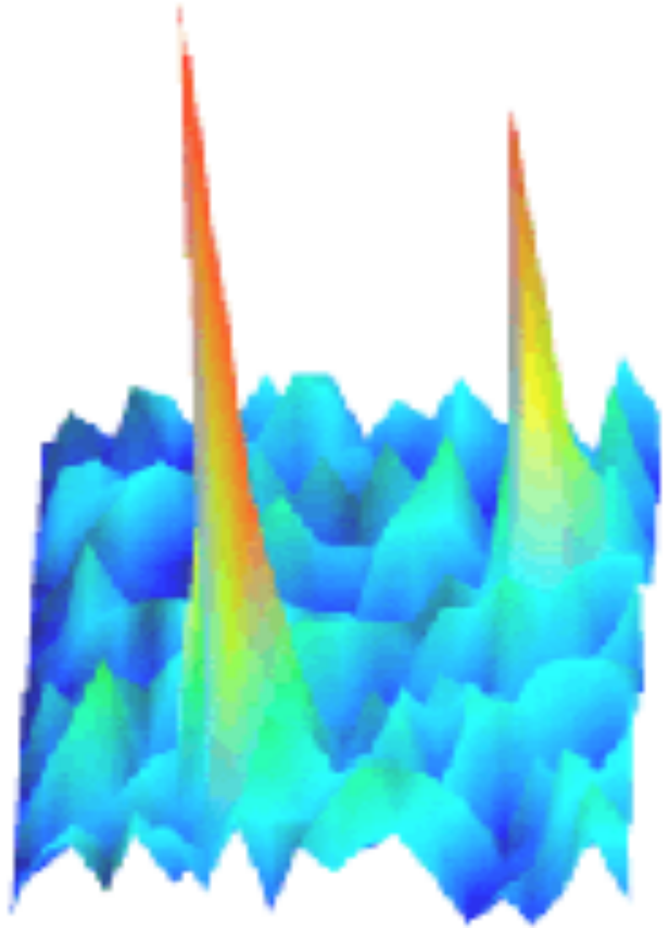
individual lipids on a phospholipid membrane

http://www.biophys.leidenuniv.nl/Research/FvL/



Outline

- Movie analysis
- Biomedical application
  - Aggregation
  - Local environment
  - Mobility



Movie analysis

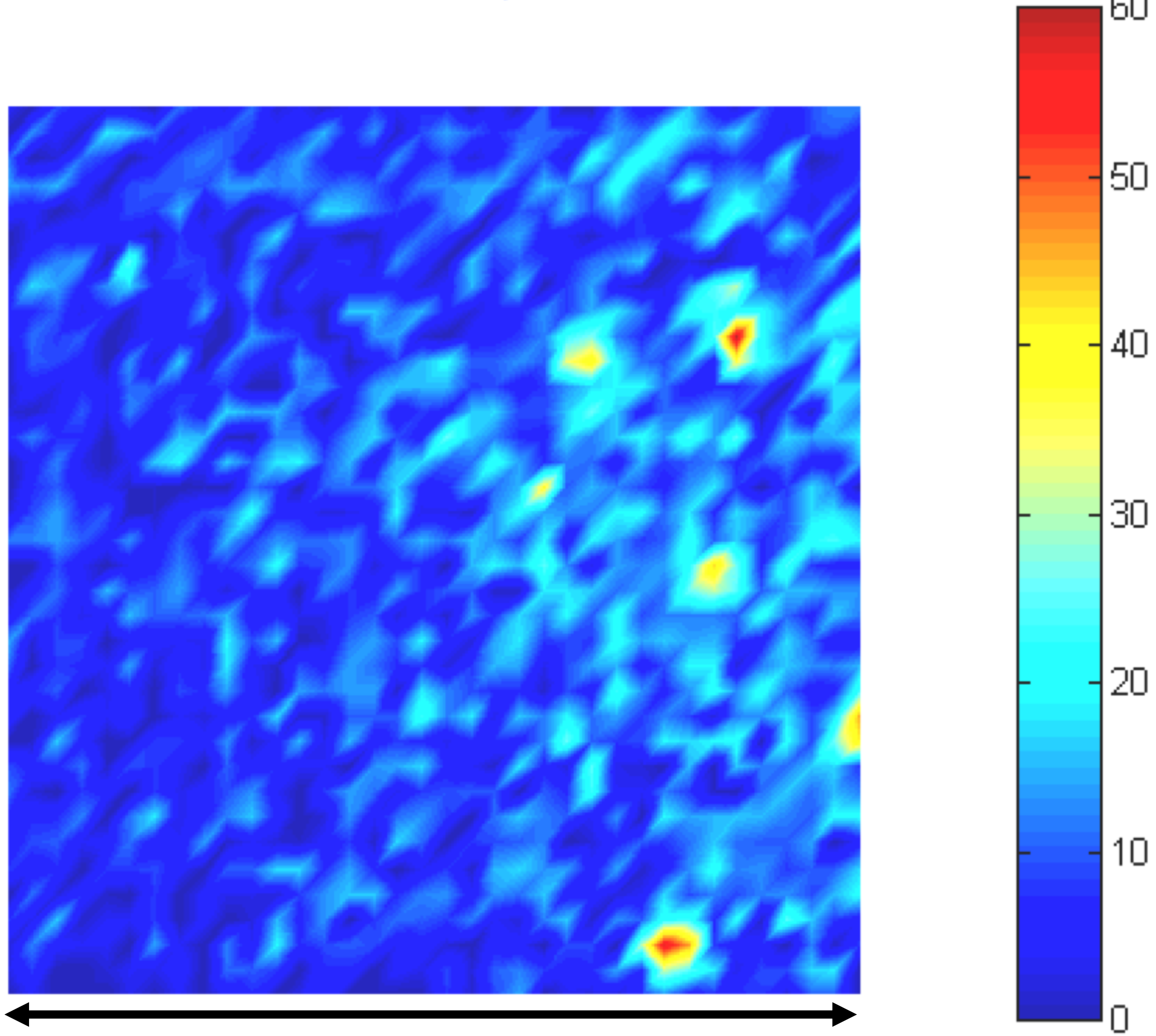
- Identification of possible single-molecule signals

- extract Information: signal level + position

- statistical tests on the result

Identification (1)

of possible single-molecule signals



photon counts

shot-noise

36 pixel = 7.2 µm
200 nm/pxl

pixelation-noise

Identification (2): diffraction

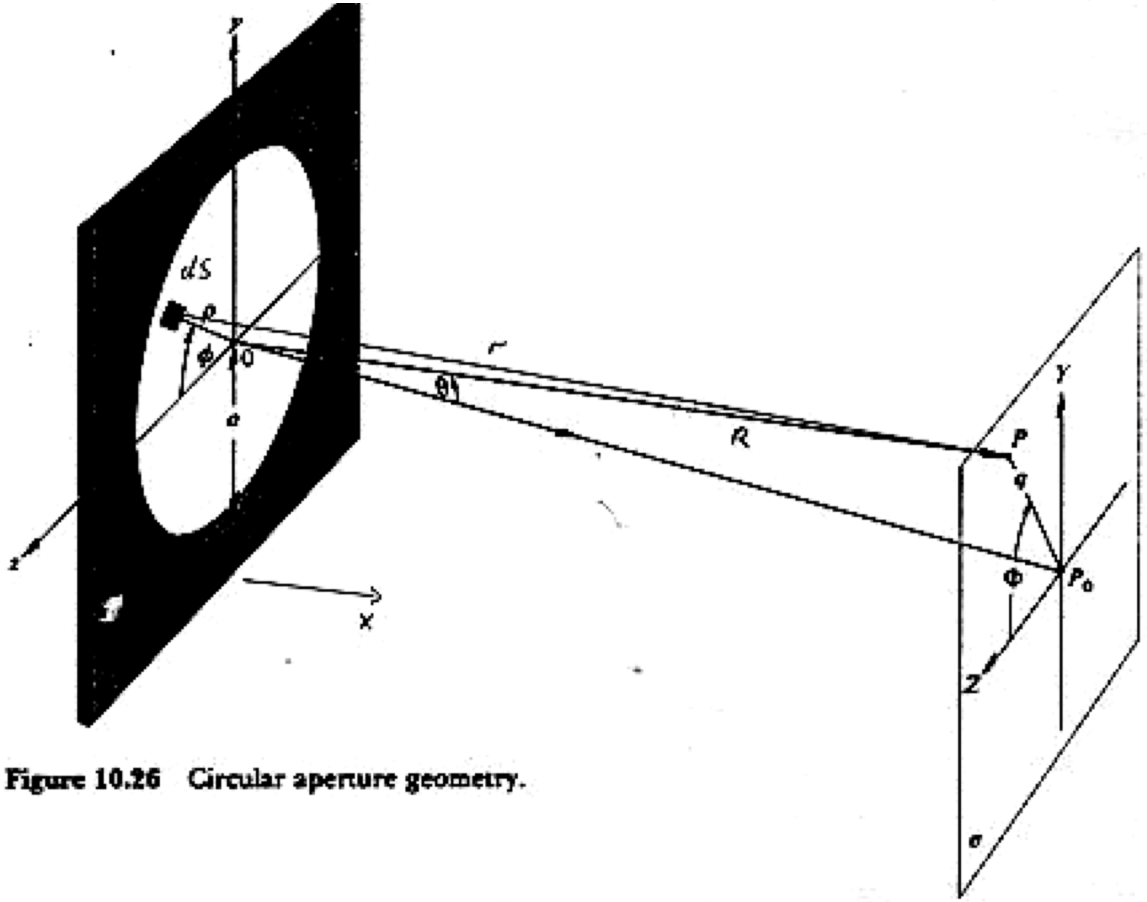
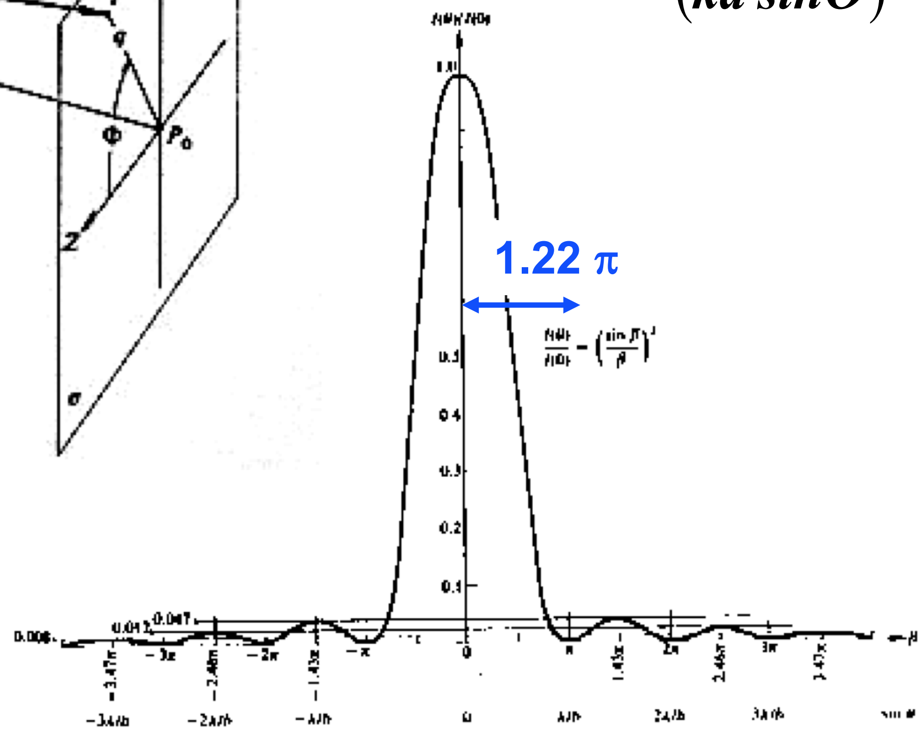


Figure 10.26 Circular aperture geometry.

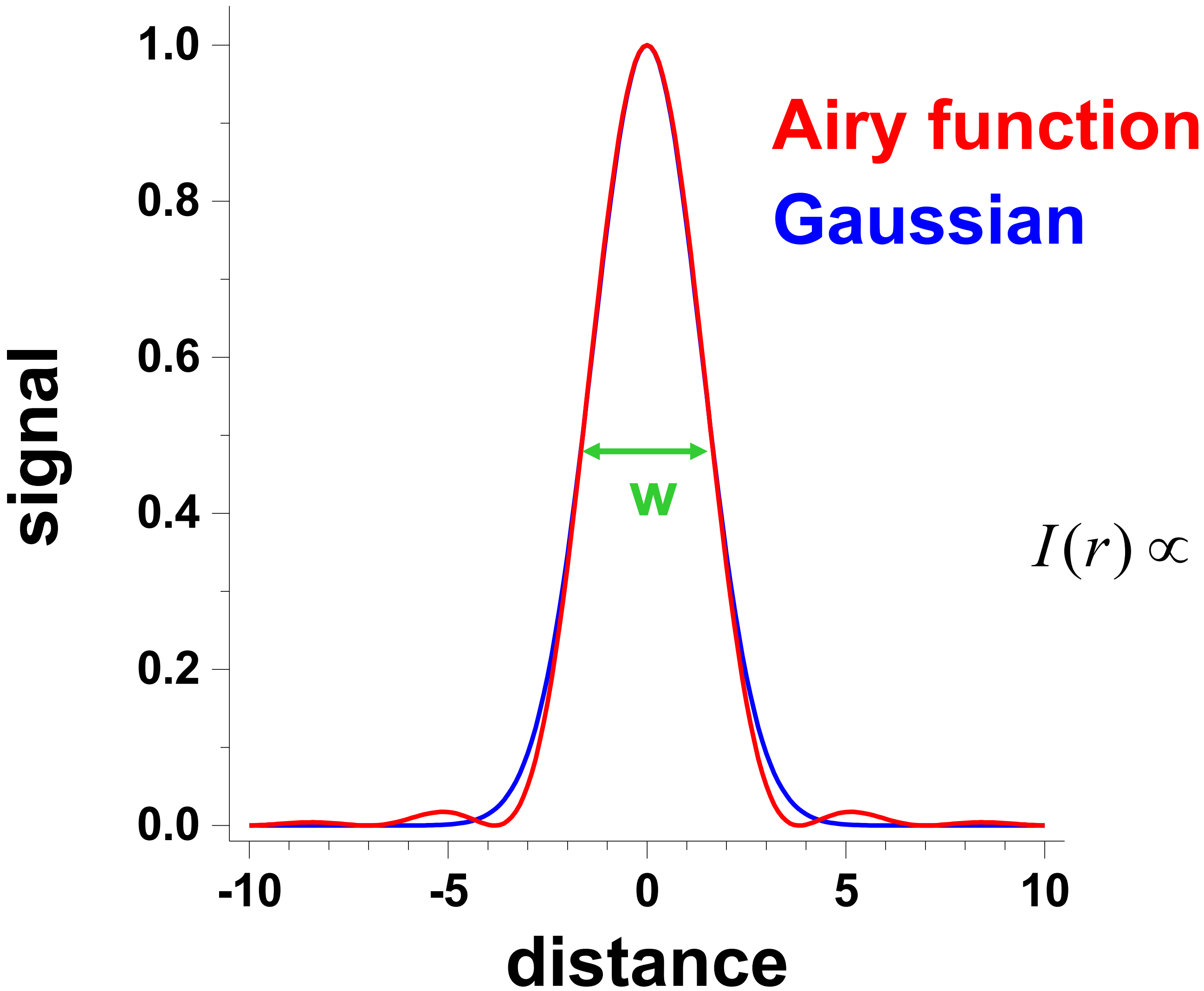
Airy pattern

$$I = I_0 \frac{J_1(kaq/R)^2}{(kaq/R)^2}$$

$$= I_0 \frac{J_1(ka\sin\Theta)^2}{(ka\sin\Theta)^2}$$



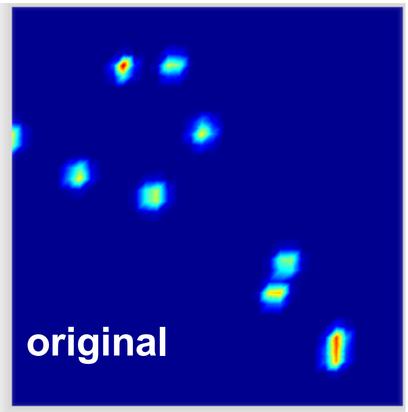
Identification (4): optimal filter



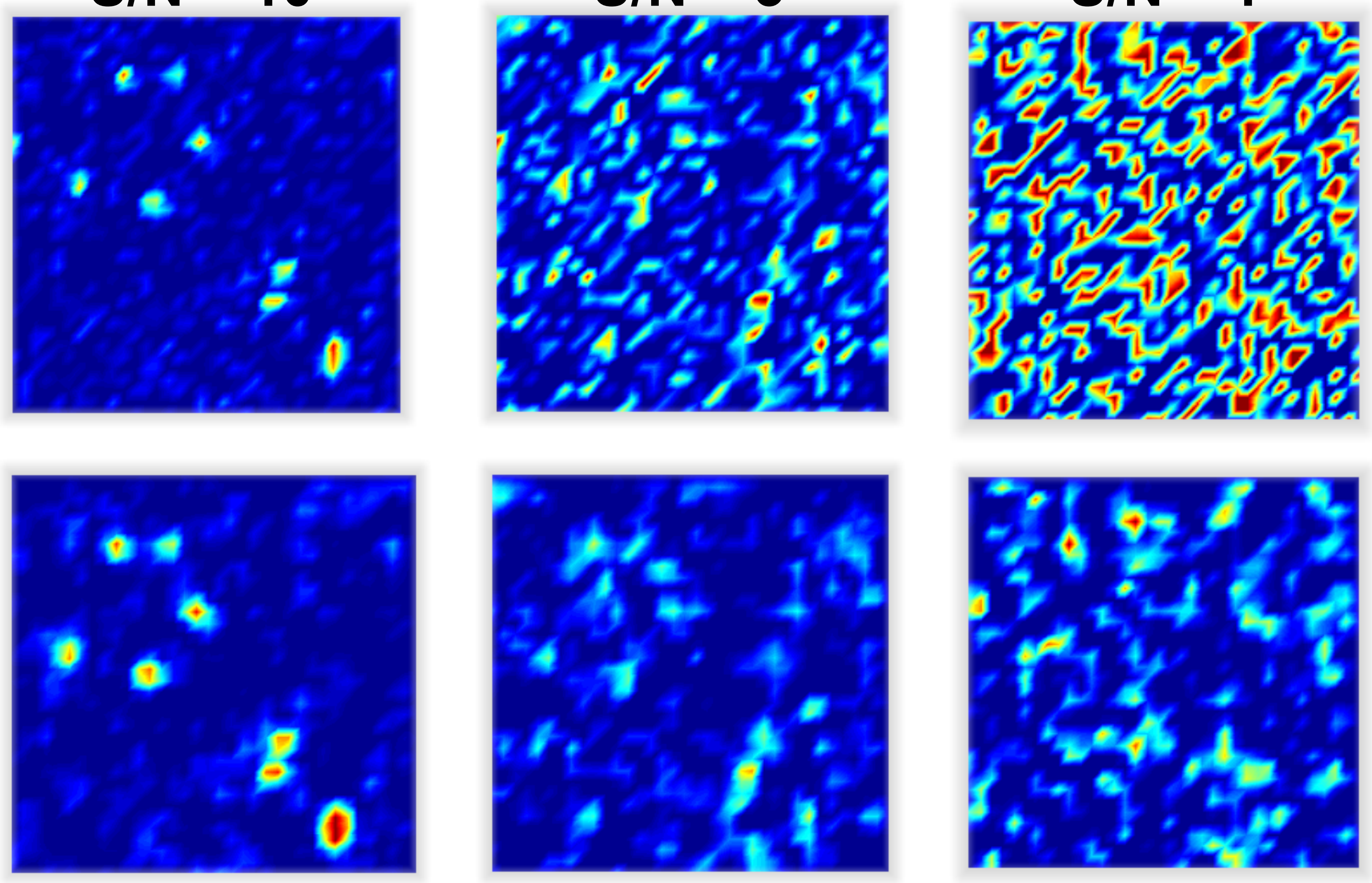
$$I(r) \propto \left(\frac{J_1\left(1.61\, r/w\right)}{1.61\, r/w} \right)^2$$

$$I(r) \propto \exp\left(-\frac{4 \ln 2\, r^2}{w^2} \right)$$

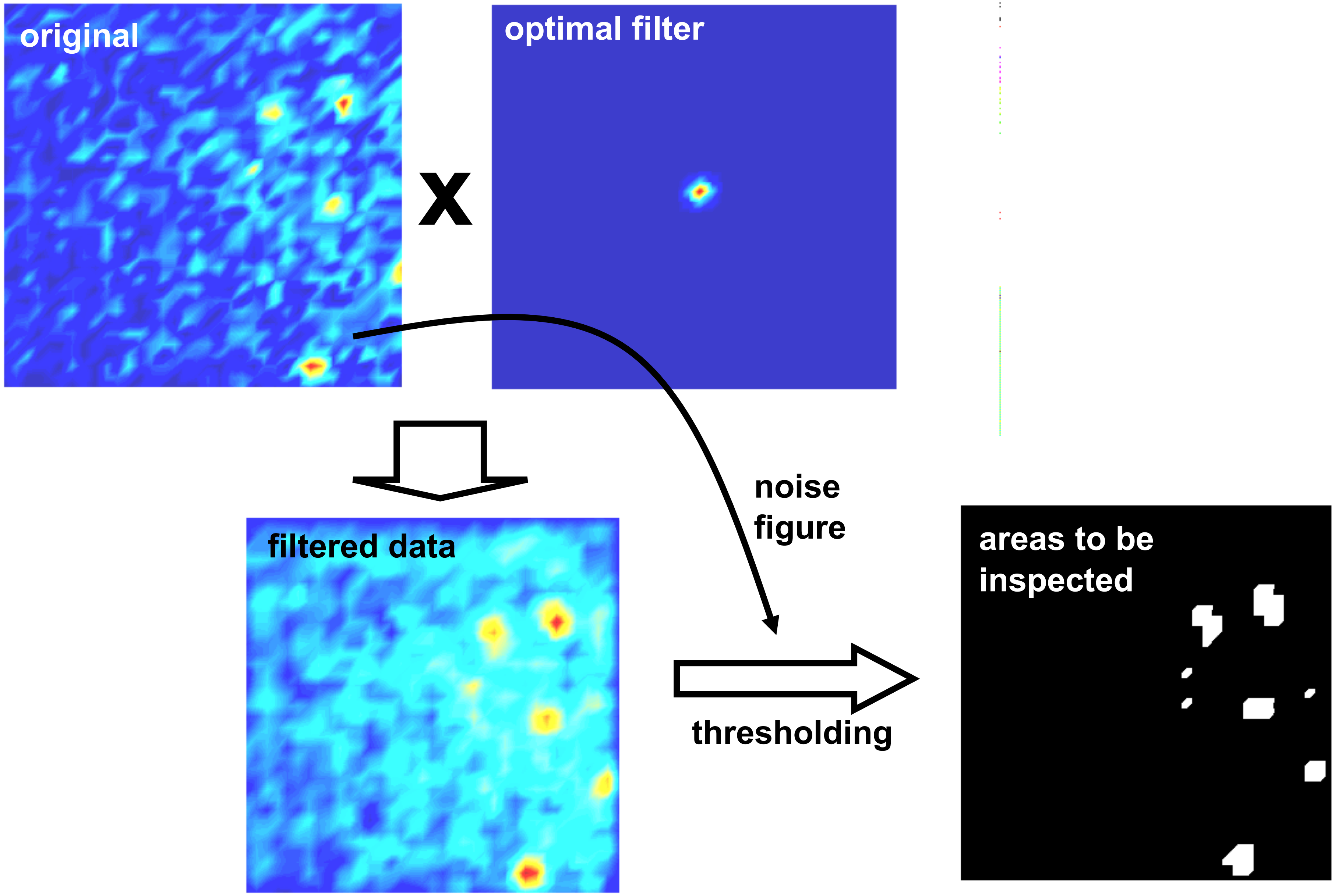
Identification (5):
Gaussian correlation filter



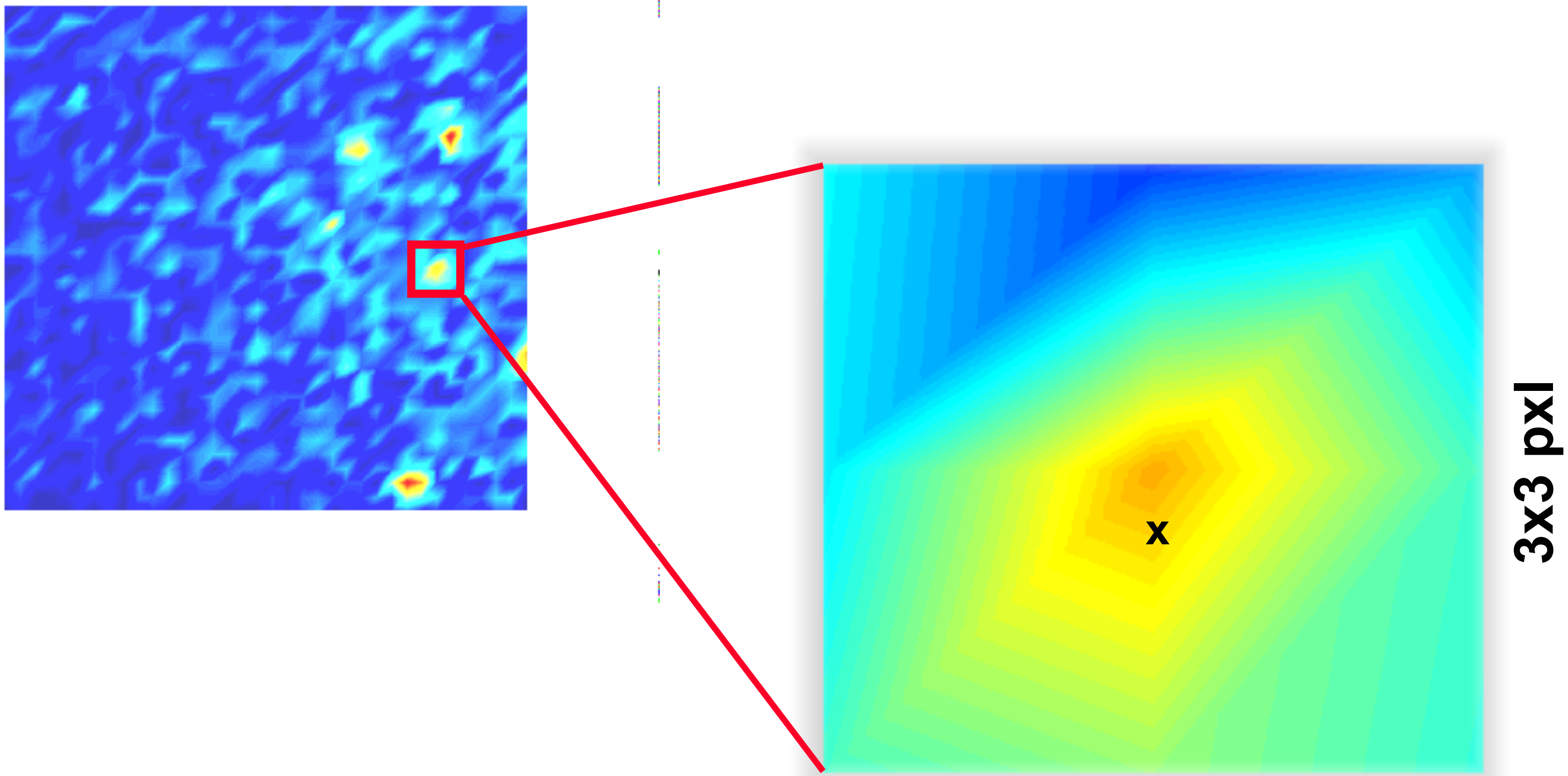
S/N = 10 S/N = 3 S/N = 1



Identification (7): thresholding



Information (1): center of mass



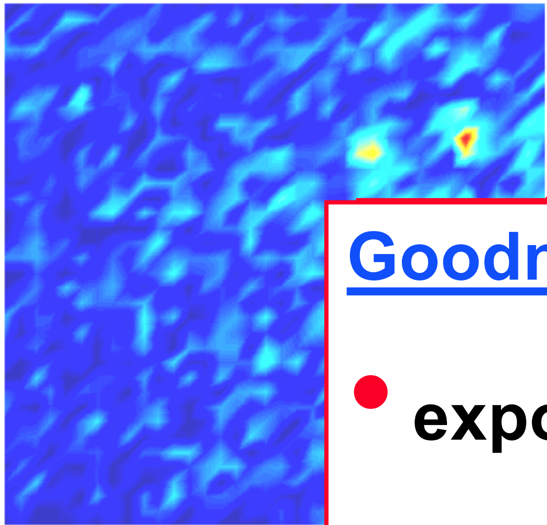
F = 220 cnts
x = 18.0 pxl
y = 29.8 pxl

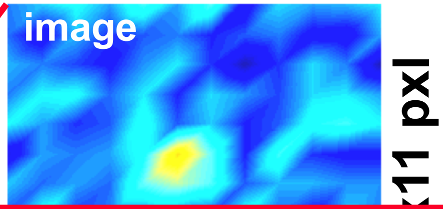
photonconts: $$F = \sum_i \sum_j f_{i,j}$$

position: $$\bar{x} = \frac{1}{F} \sum_i i \sum_j f_{i,j} \quad ; \quad \bar{y} = \ldots$$

Gosh & Webb, BJ (1994) 66:1301

Information (2): Gaussian fit





Goodness of fit: identification

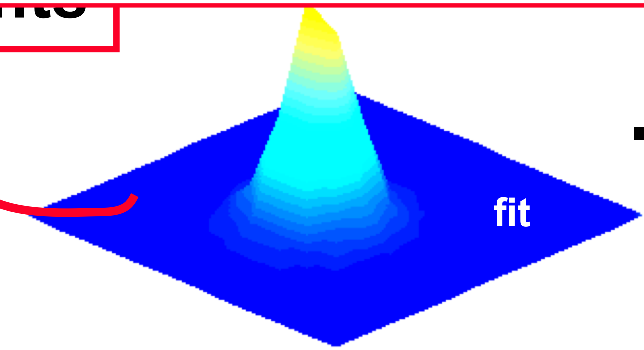
- **exponential test**

  is the image just noise: $\varepsilon = Q(N_i\,|n^*m-1)$
- **chi-square test:**

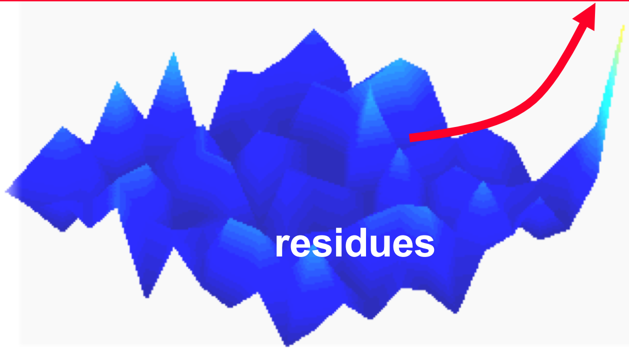
  are the residues not noise: $\chi = 1 - Q(N_r|n^*m-1)$
- **F-test**

  noise of residues = image noise: $F = I(N_r,N_i|n^*m-1)$

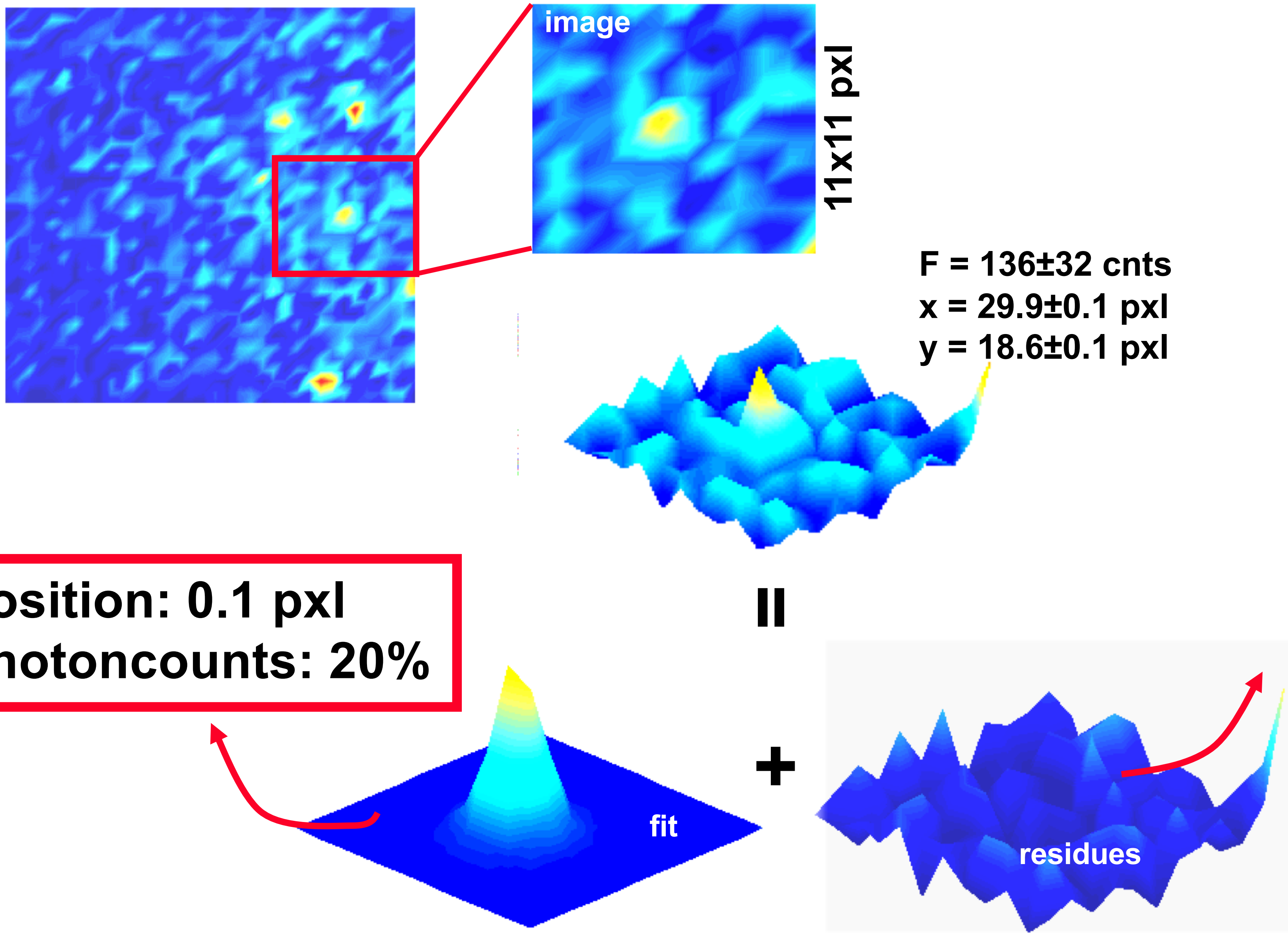
(1) position
(2) photonco



+



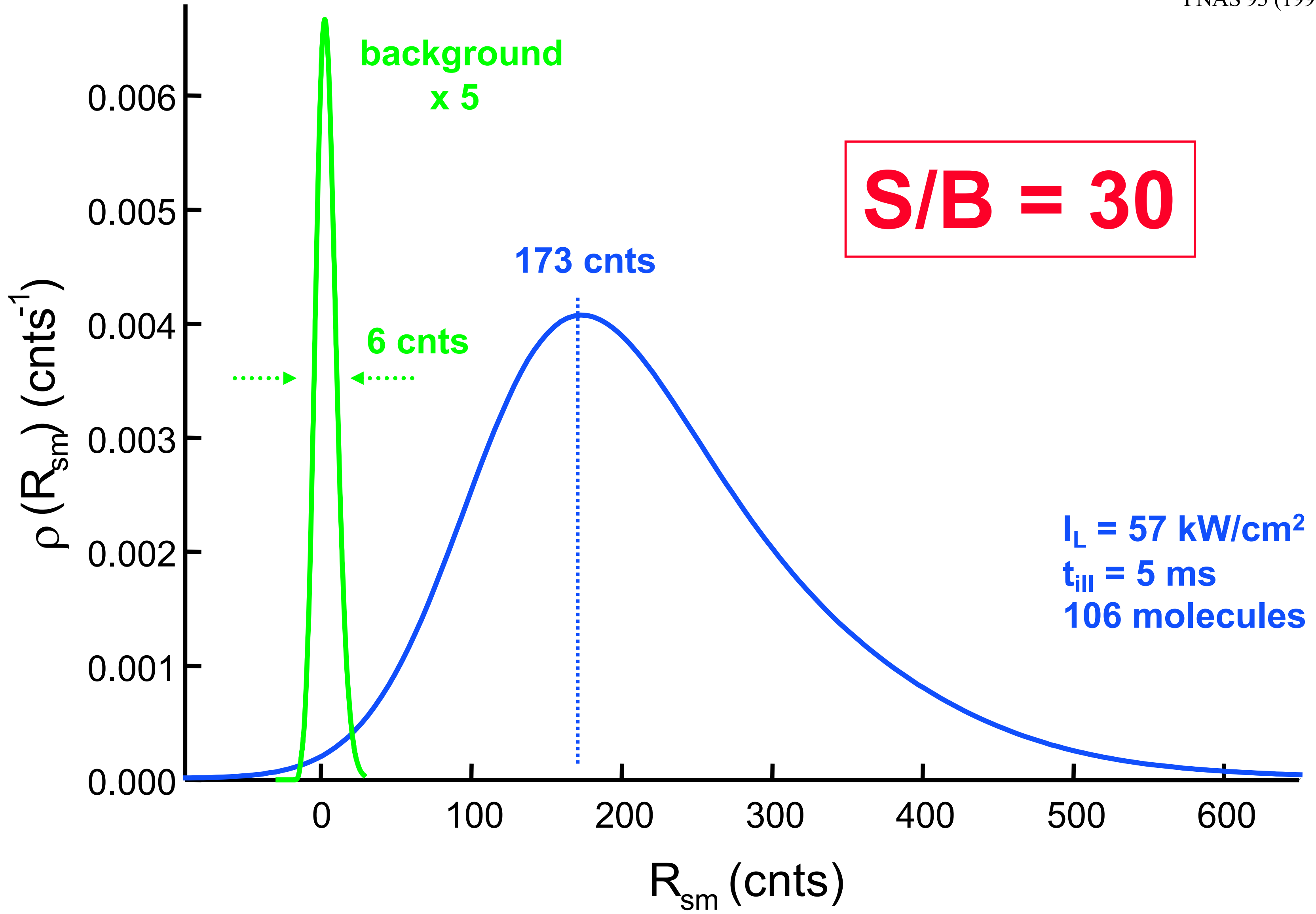
Information (2a): Gaussian fit



position: 0.1 pxl
photoncounts: 20%

signal-to-background

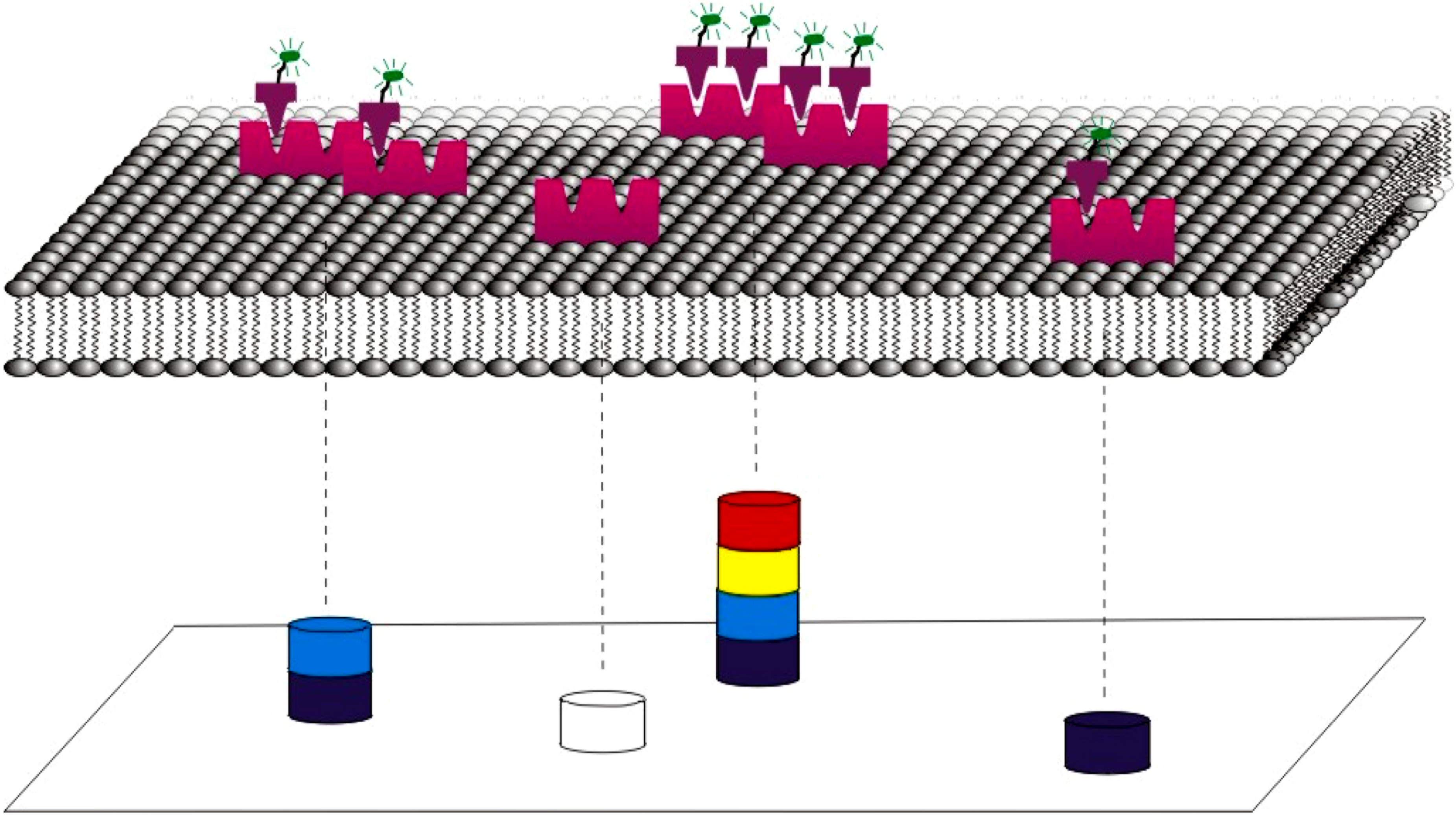
TS, Schütz, Gruber & Schindler
PNAS 93 (1996) 2926.



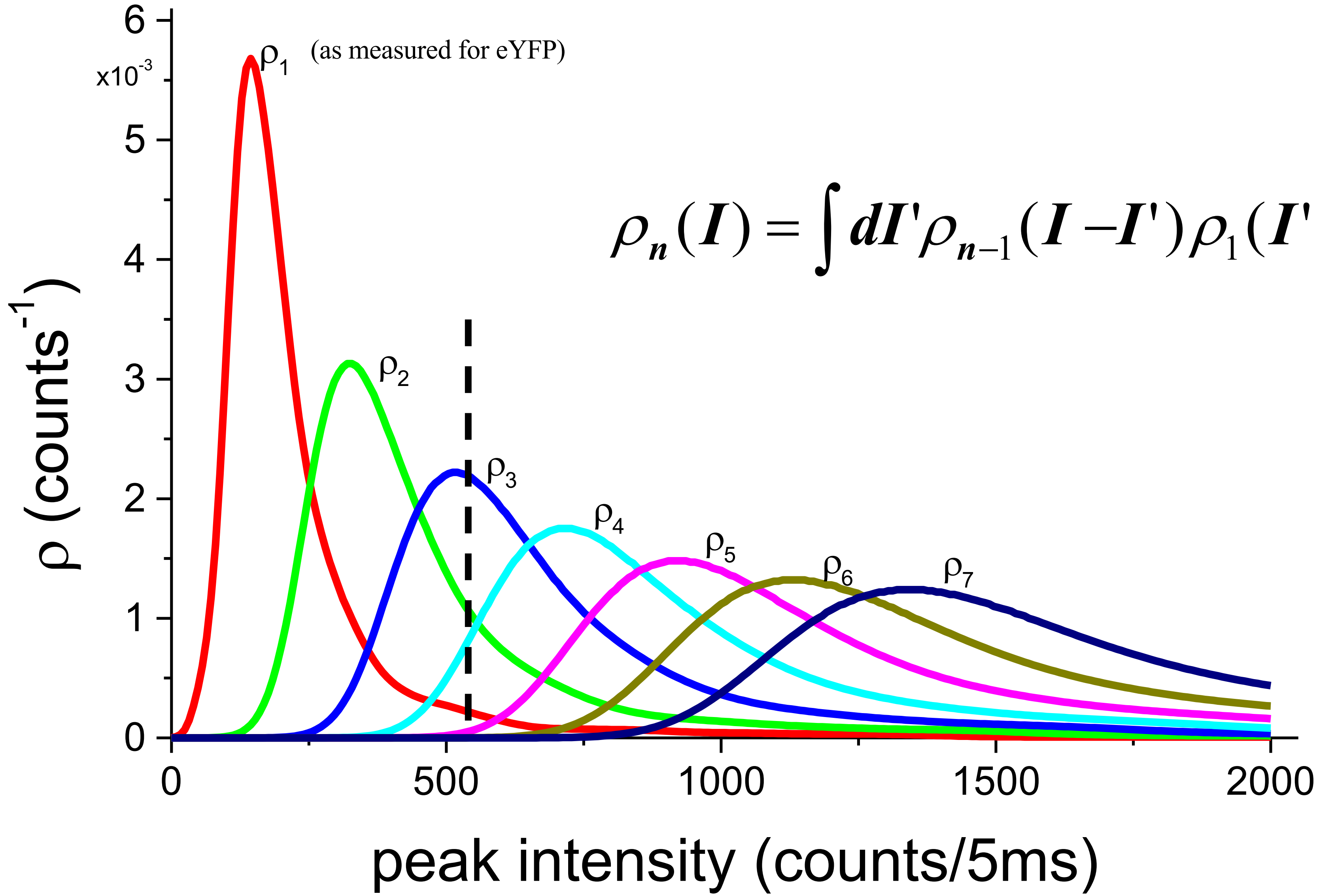
Application (1)

Analysis of aggregation

Local Stoichiometry



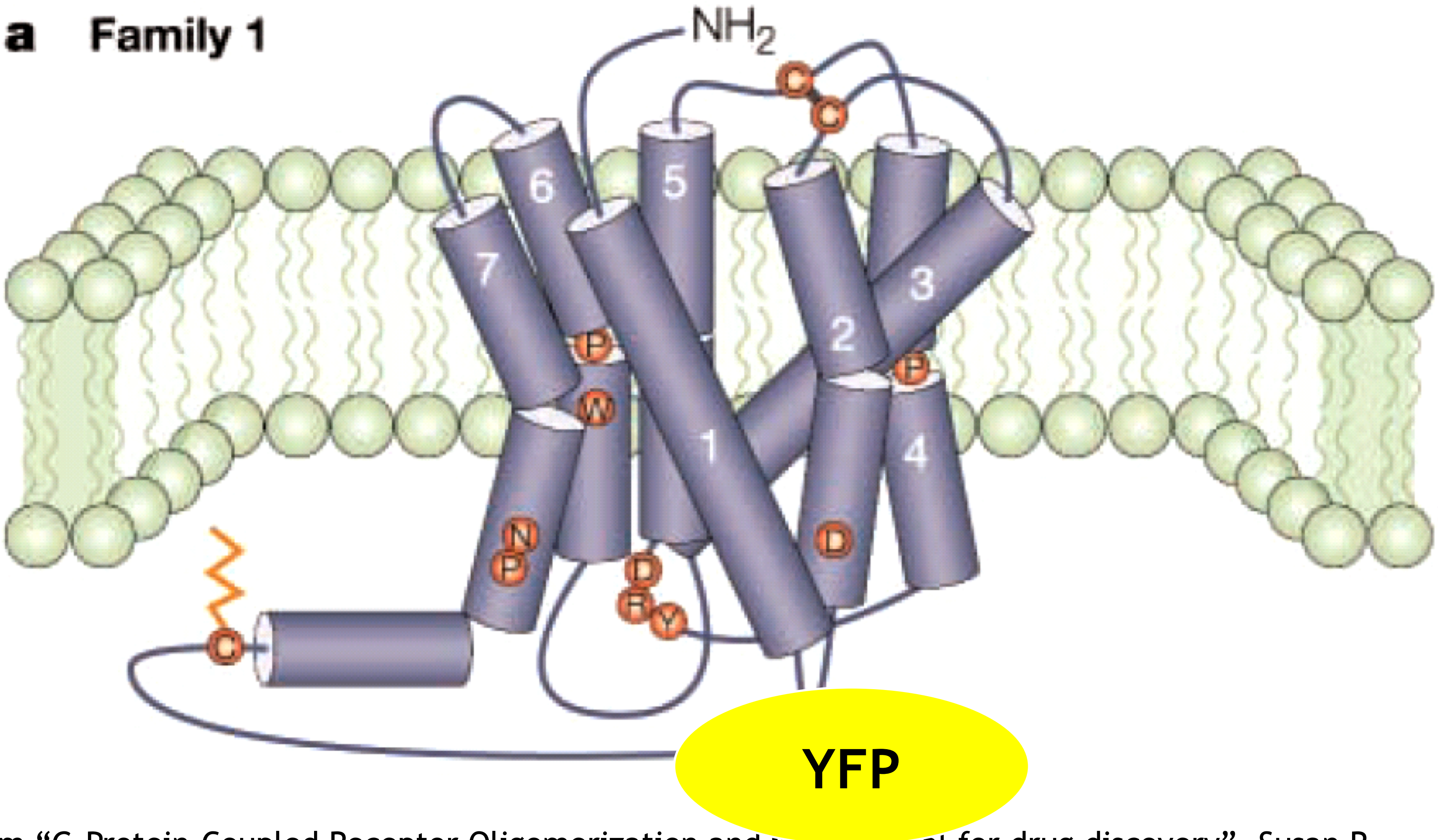
Stoichiometry Algorithm



$$\rho_n(I) = \int dI' \rho_{n-1}(I - I')\rho_1(I')$$

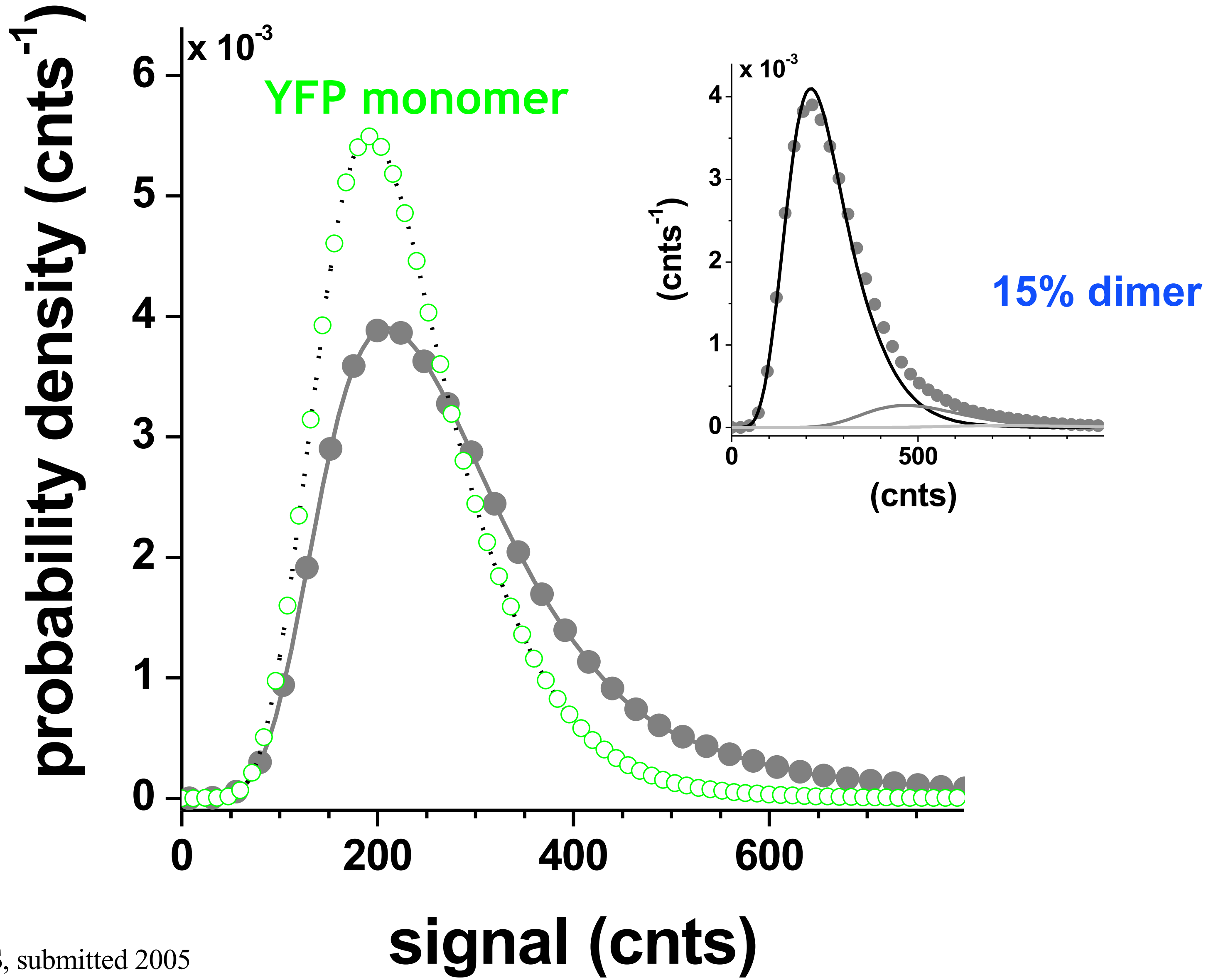
Schütz, **TS**, Gruber, Schindler, Anal.Chem. 68 (1996) 4397

G-Protein-coupled receptor



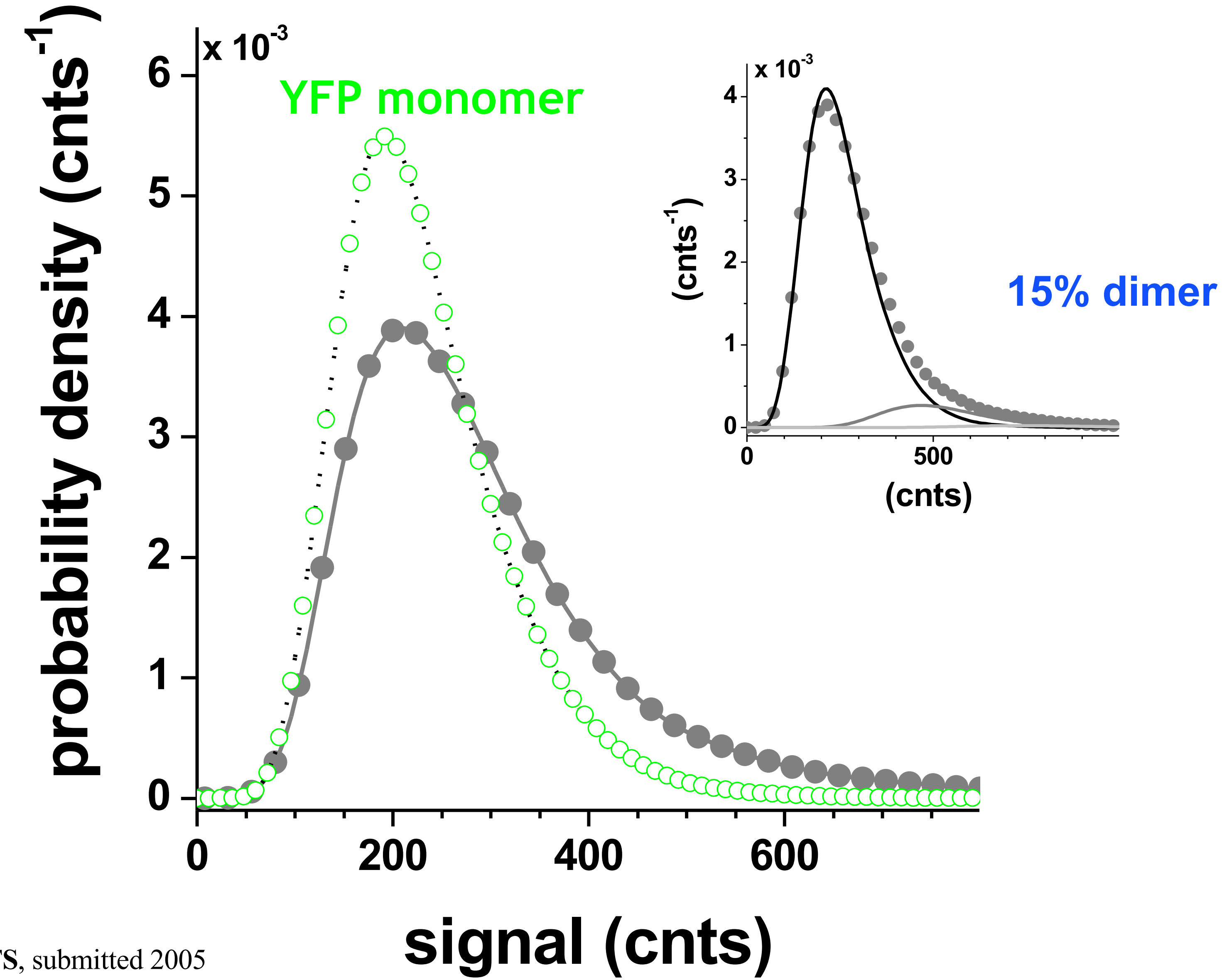
from “G-Protein-Coupled Receptor Oligomerization and its potential for drug discovery”, Susan R. George et al., Nature Reviews 1 (2002), 808-820

dimerization of β2 adrenergic receptors on stimulation



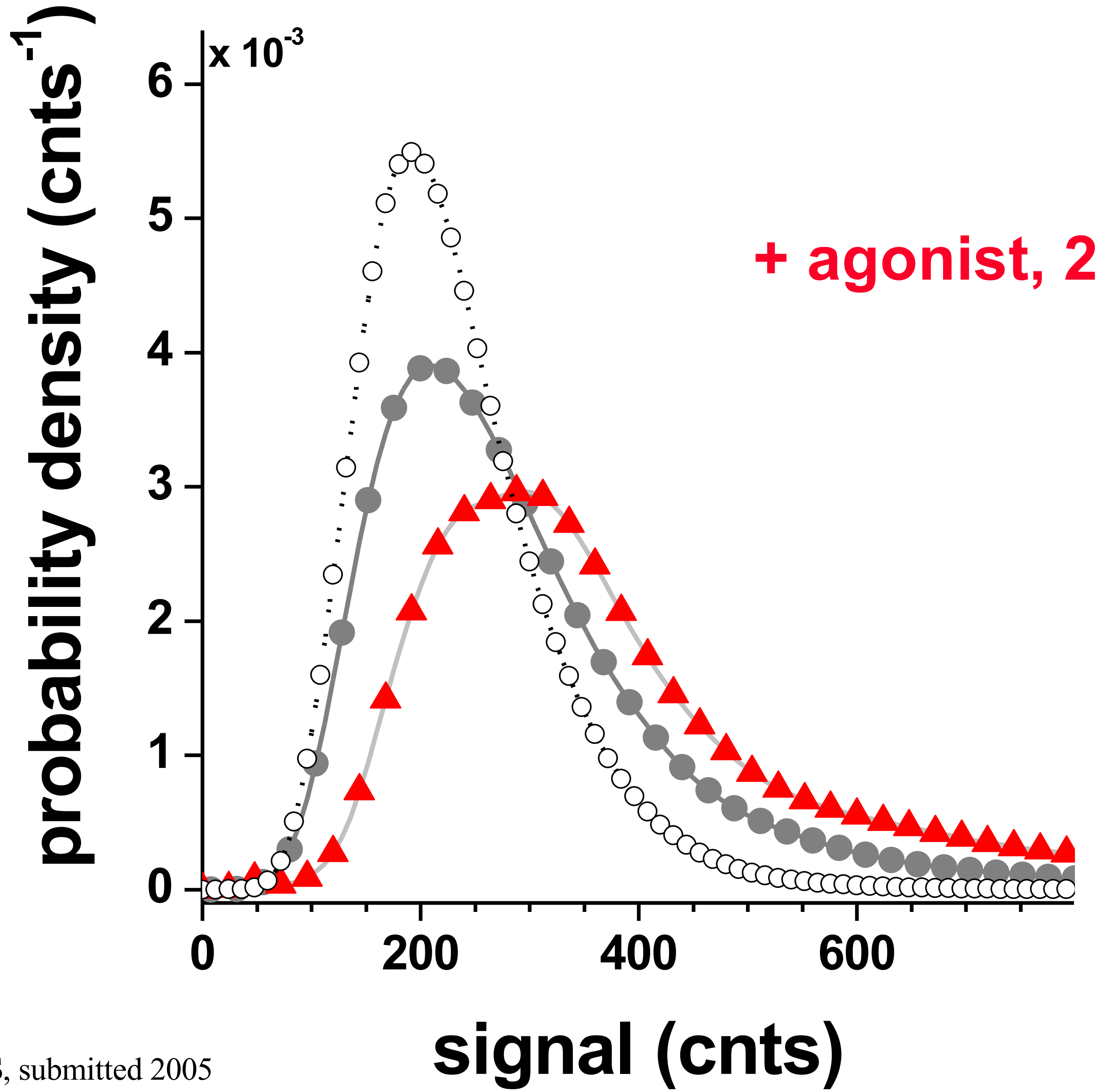
Blab, Bakker, Leurs & **TS**, submitted 2005

dimerization of β2 adrenergic receptors on stimulation



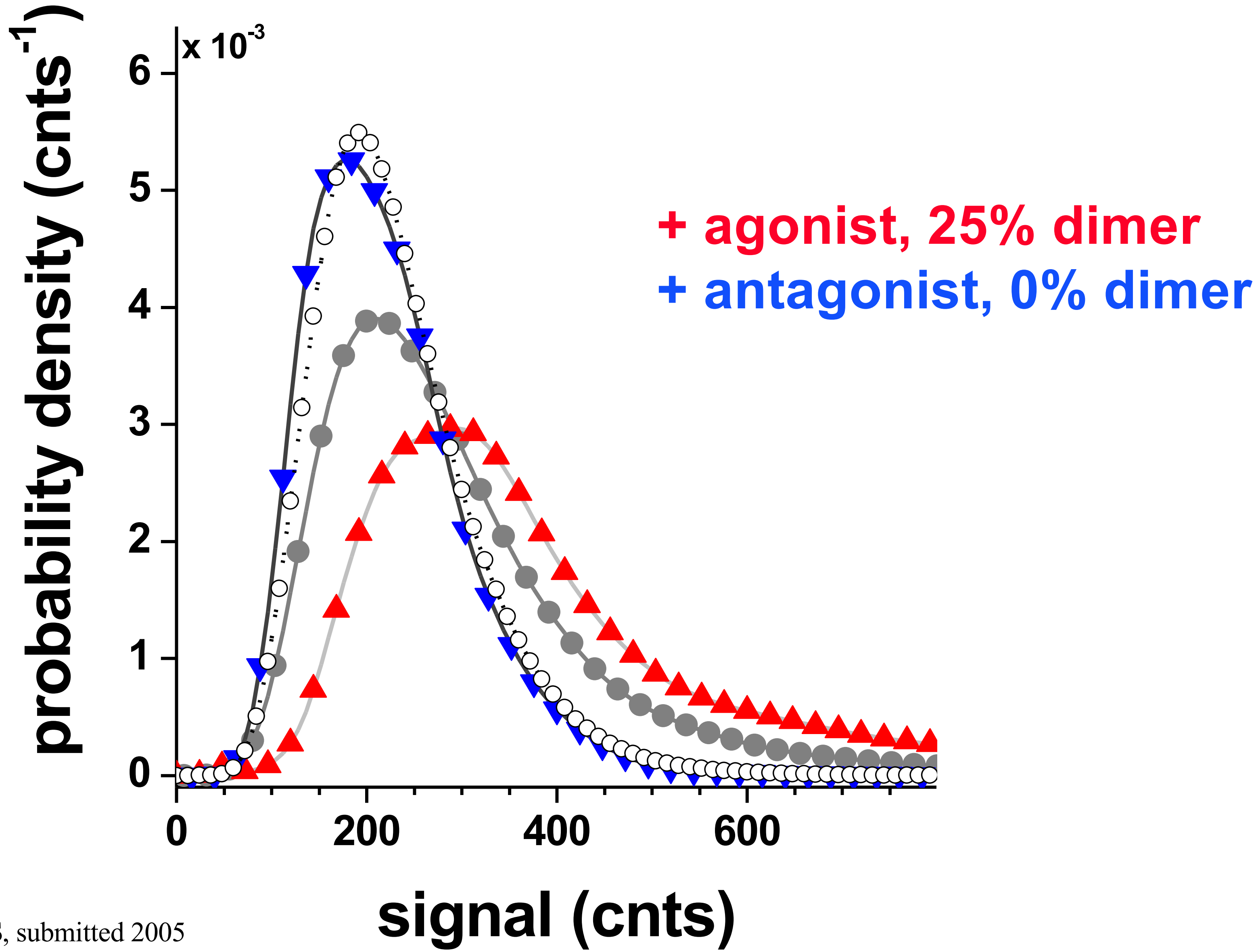
Blab, Bakker, Leurs & **TS**, submitted 2005

dimerization of β2 adrenergic receptors on stimulation



Blab, Bakker, Leurs & **TS**, submitted 2005

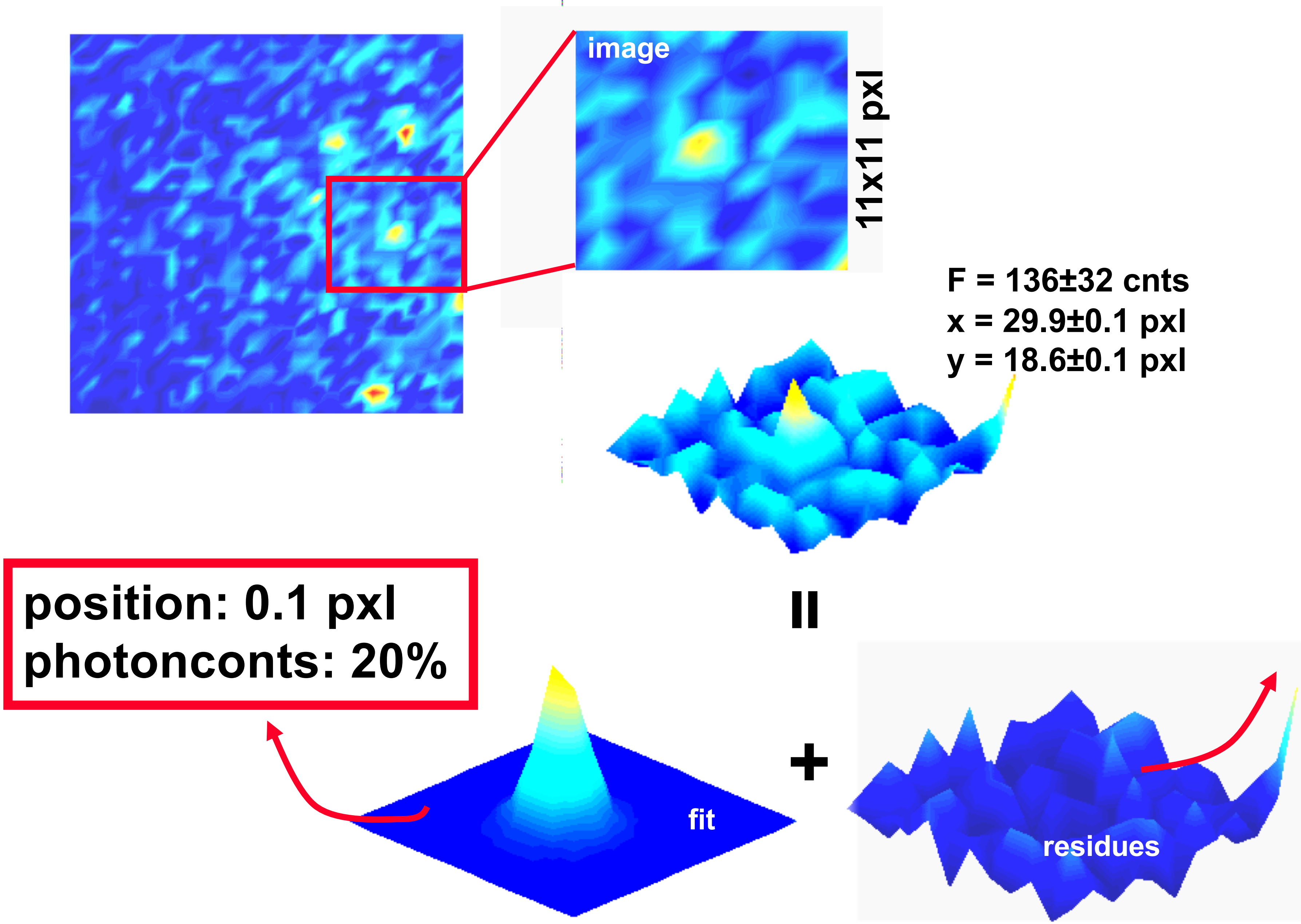
dimerization of β2 adrenergic receptors on stimulation



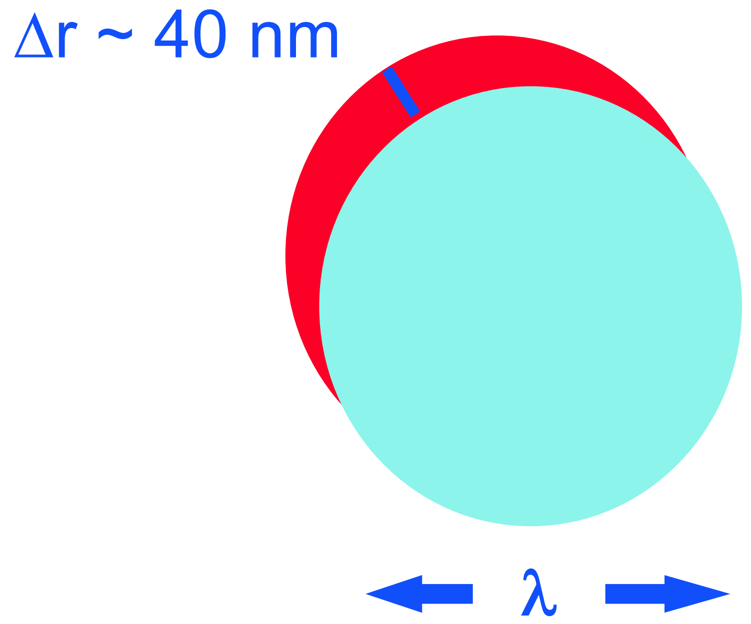
Blab, Bakker, Leurs & **TS**, submitted 2005

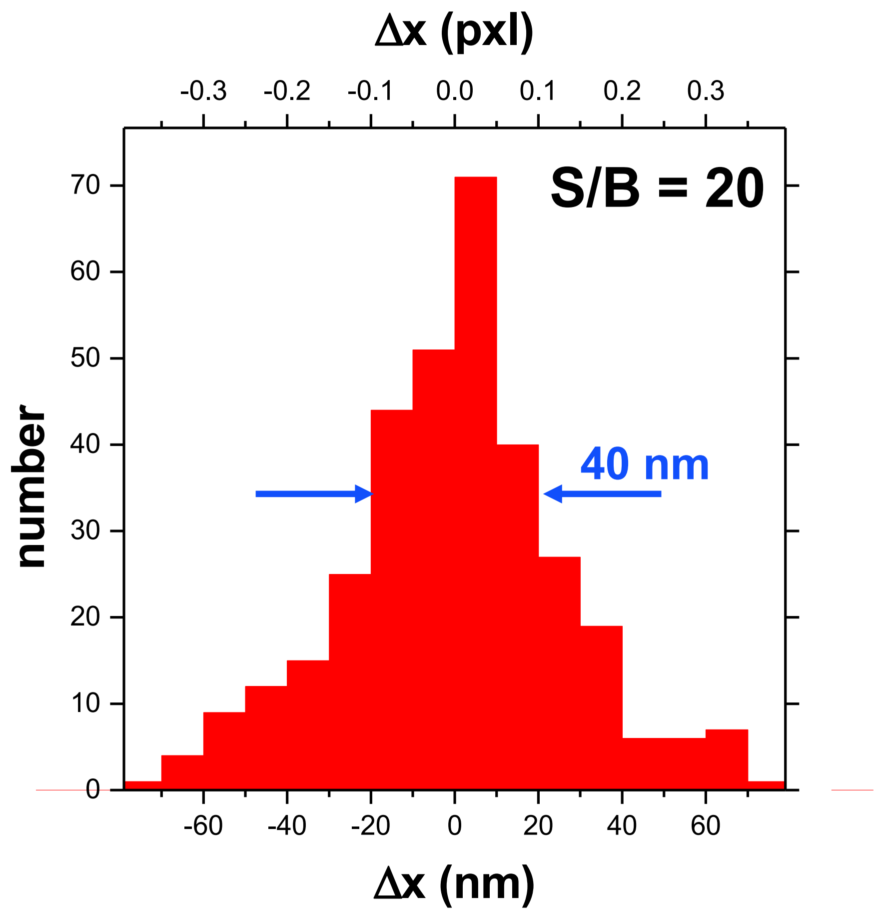
back to movie analysis …

Information (2a): Gaussian fit



Information (4): super-resolution





the accuracy for determination of the position << λ

N. Bobroff, Rev.Sci.Inst. 57 (1986) 1152

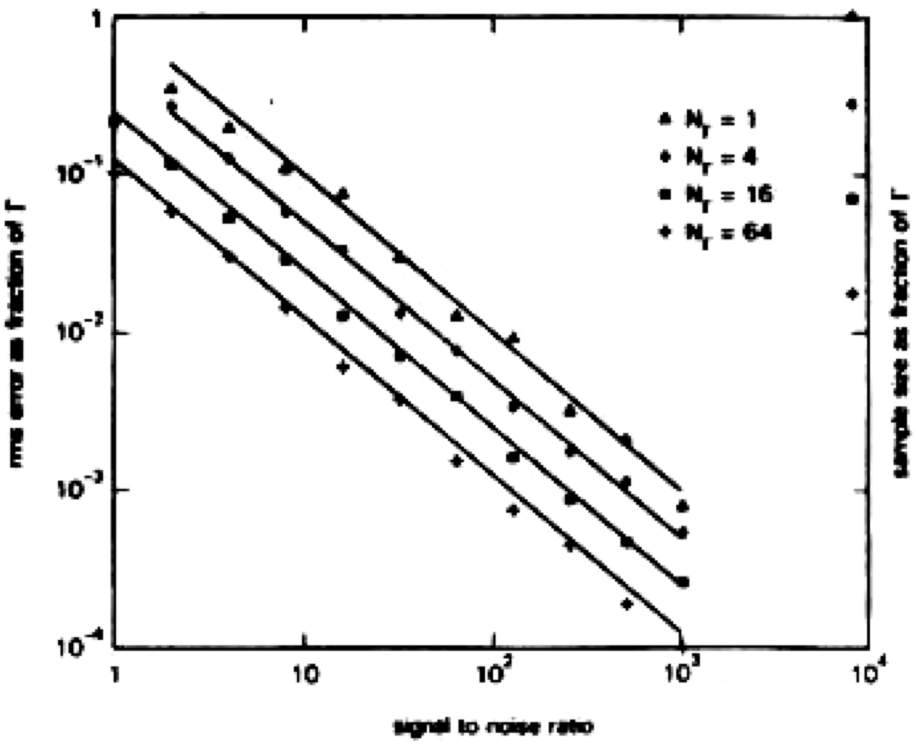
Information (3): Bobroff model

Gaussian object characterized by

- Γ: width (pxl^{-1})
- N_{cnts}: total number of photoncounts
- N_{max}: photoncounts in maximum
- σ_B: background noise

$$\Delta x_{max} = 0.7\,\Gamma \sqrt{\frac{\sigma_B^2/N_{max} + 2/3}{N_{cnts}}}$$

$$= 0.57 \frac{\Gamma}{\sqrt{N_{cnts}}}$$

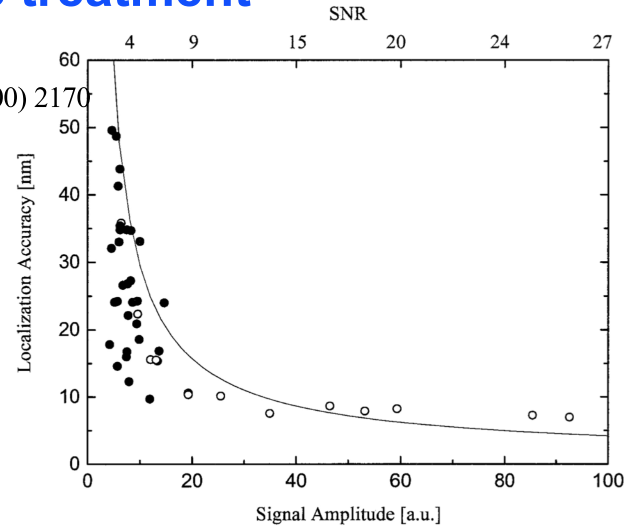


N. Bobroff, Rev.Sci.Inst. 57 (1986) 1152

Information (3):
Bobroff model & beyond

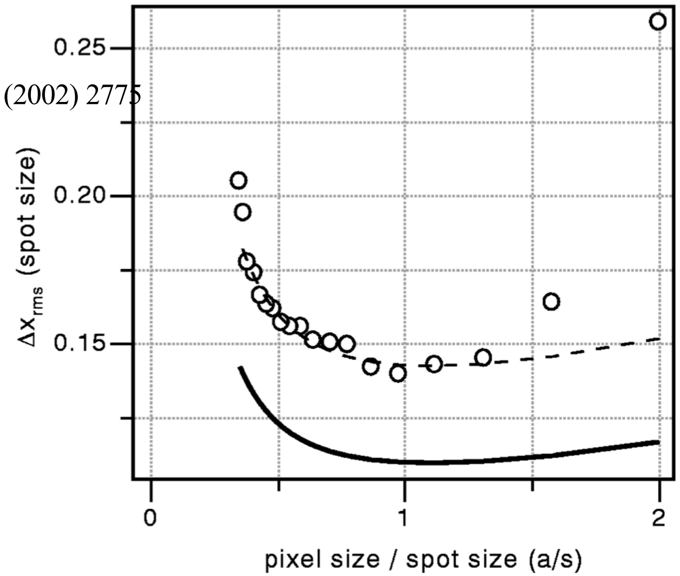
more rigorous treatment of noise

Kubitscheck et al., BJ 78 (2000) 2170



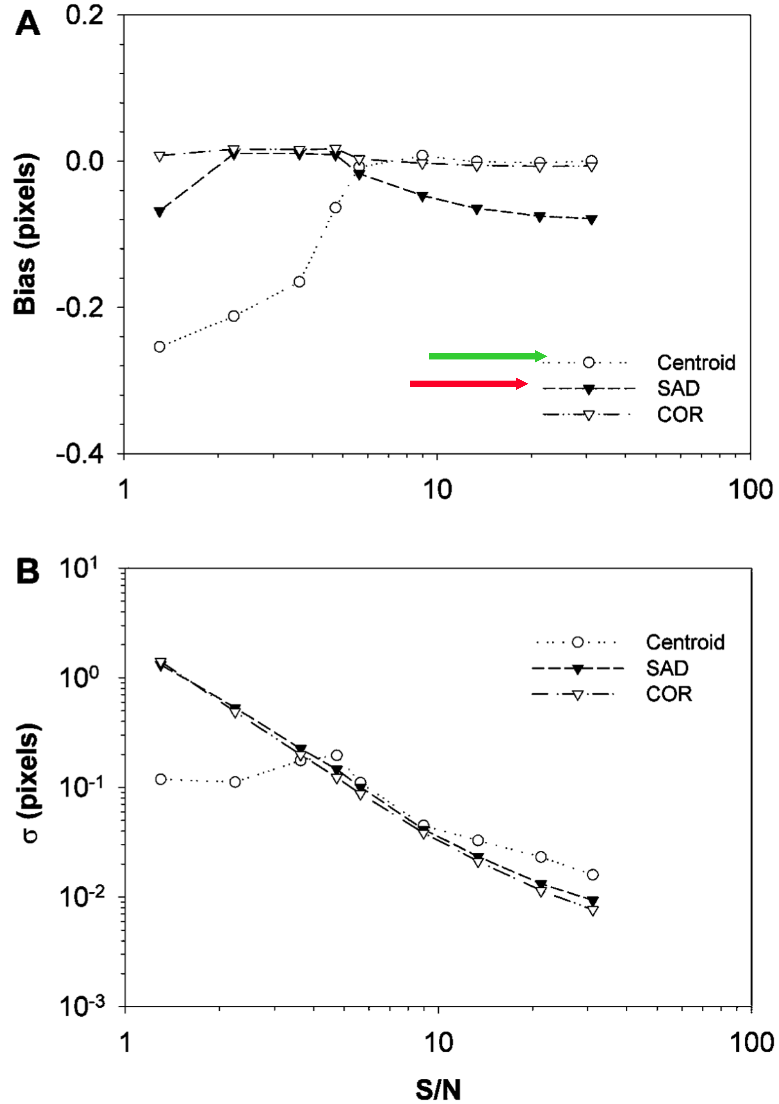
pixelation

Thompson, et al.,BJ 82 (2002) 2775

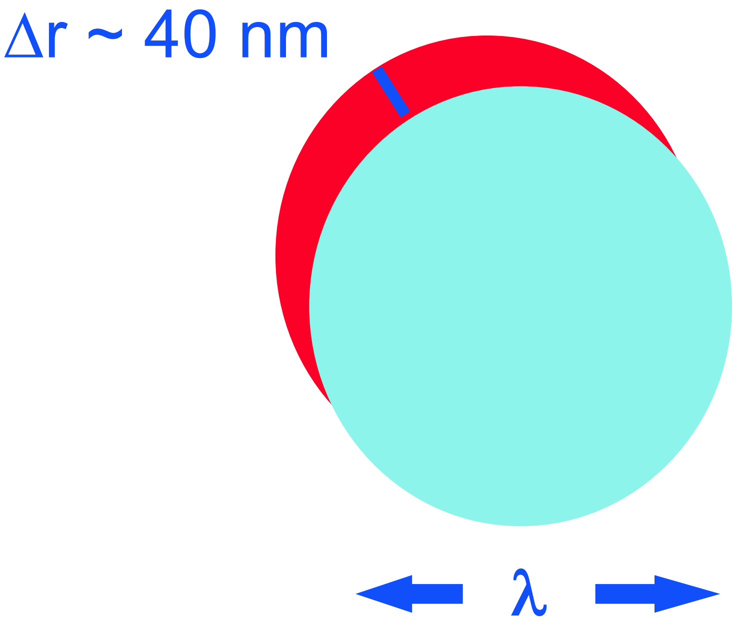


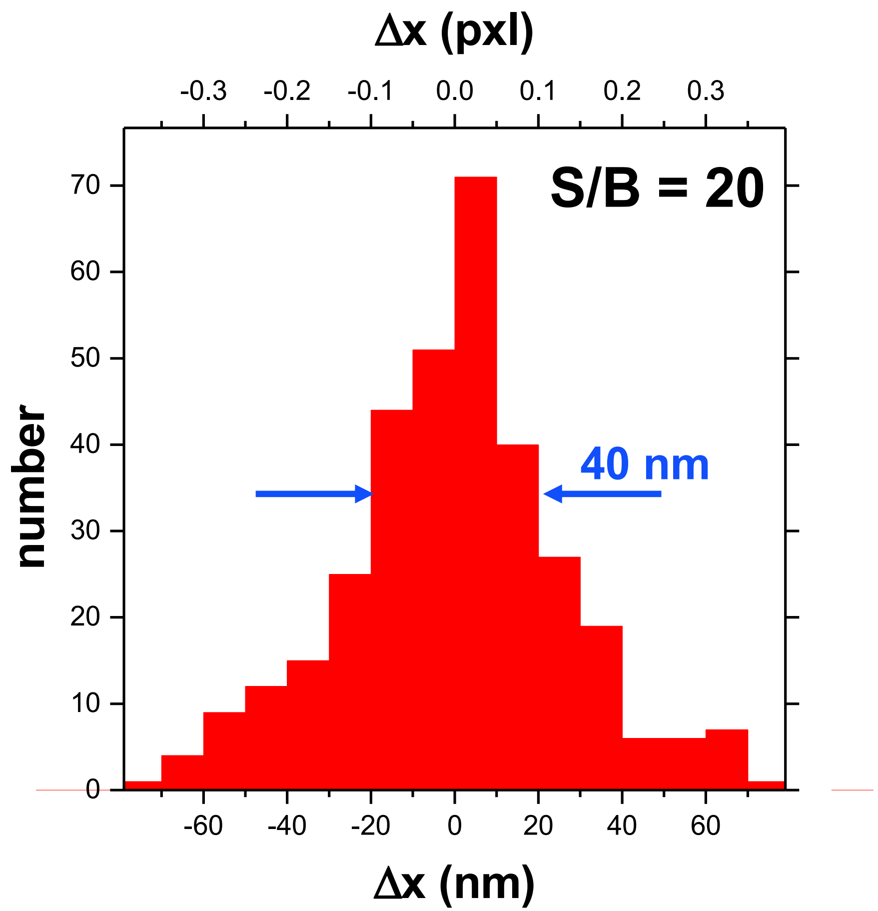
comparison of different methods

Cheezum et al., BJ 81 (2002) 2378



Information (4): super-resolution





the record so-far: 1 Å

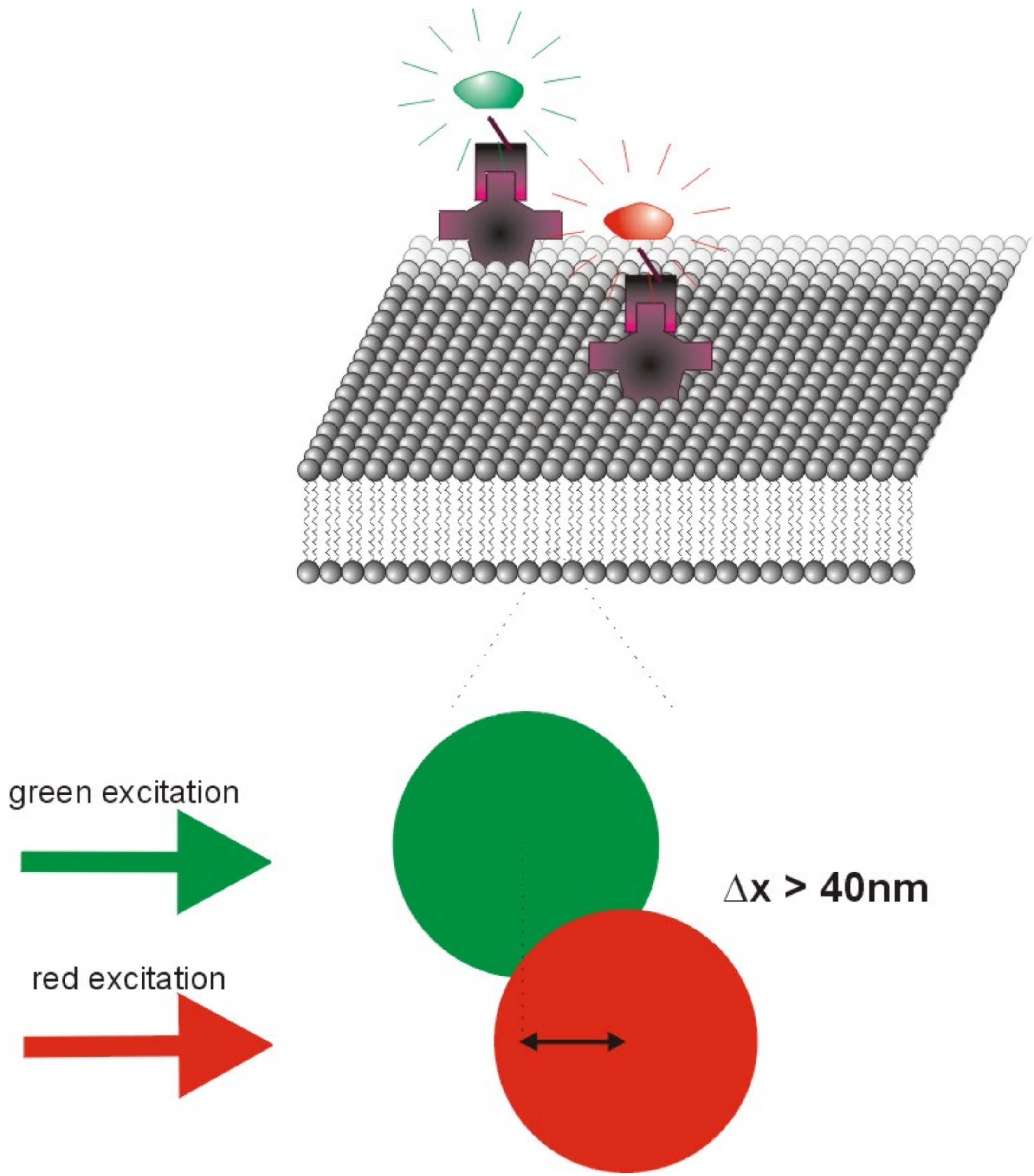
(Block, 2005)

N. Bobroff, Rev.Sci.Inst. 57 (1986) 1152

Application (2)

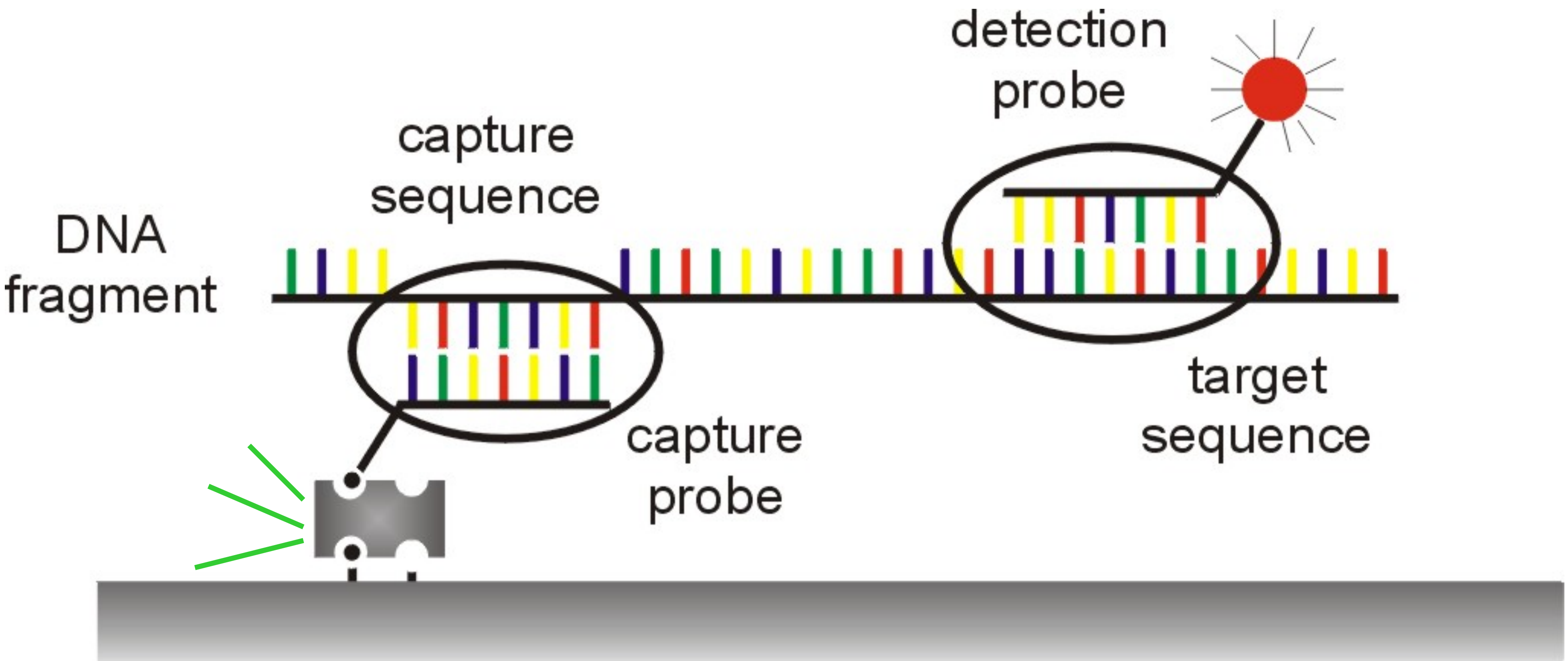
Analysis of distance & colocalization

colocalization

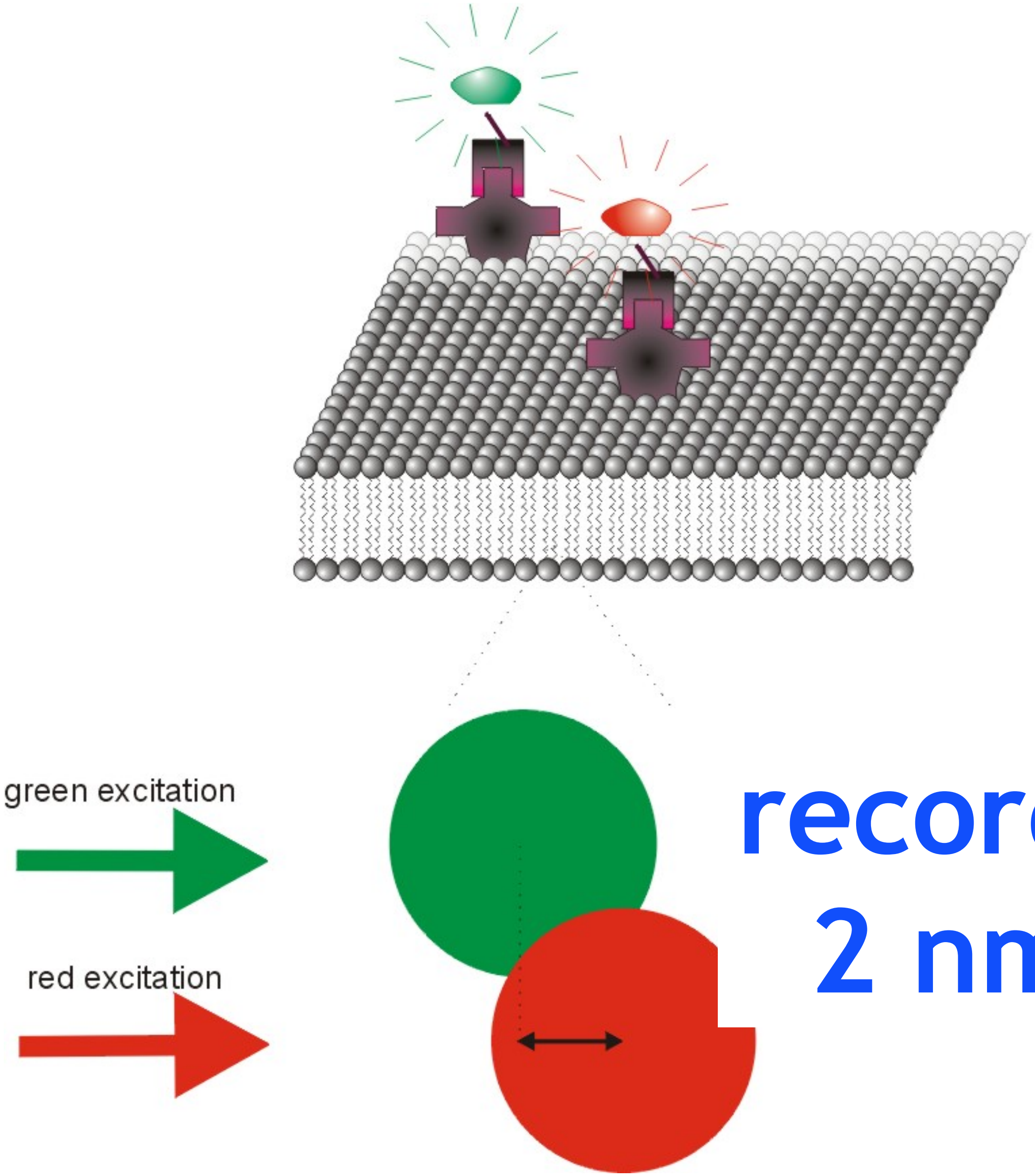


single-molecule DNA assay

Trabesinger, Schütz, Gruber, Schindler, **TS**, Anal. Chem. 71 (1998) 279



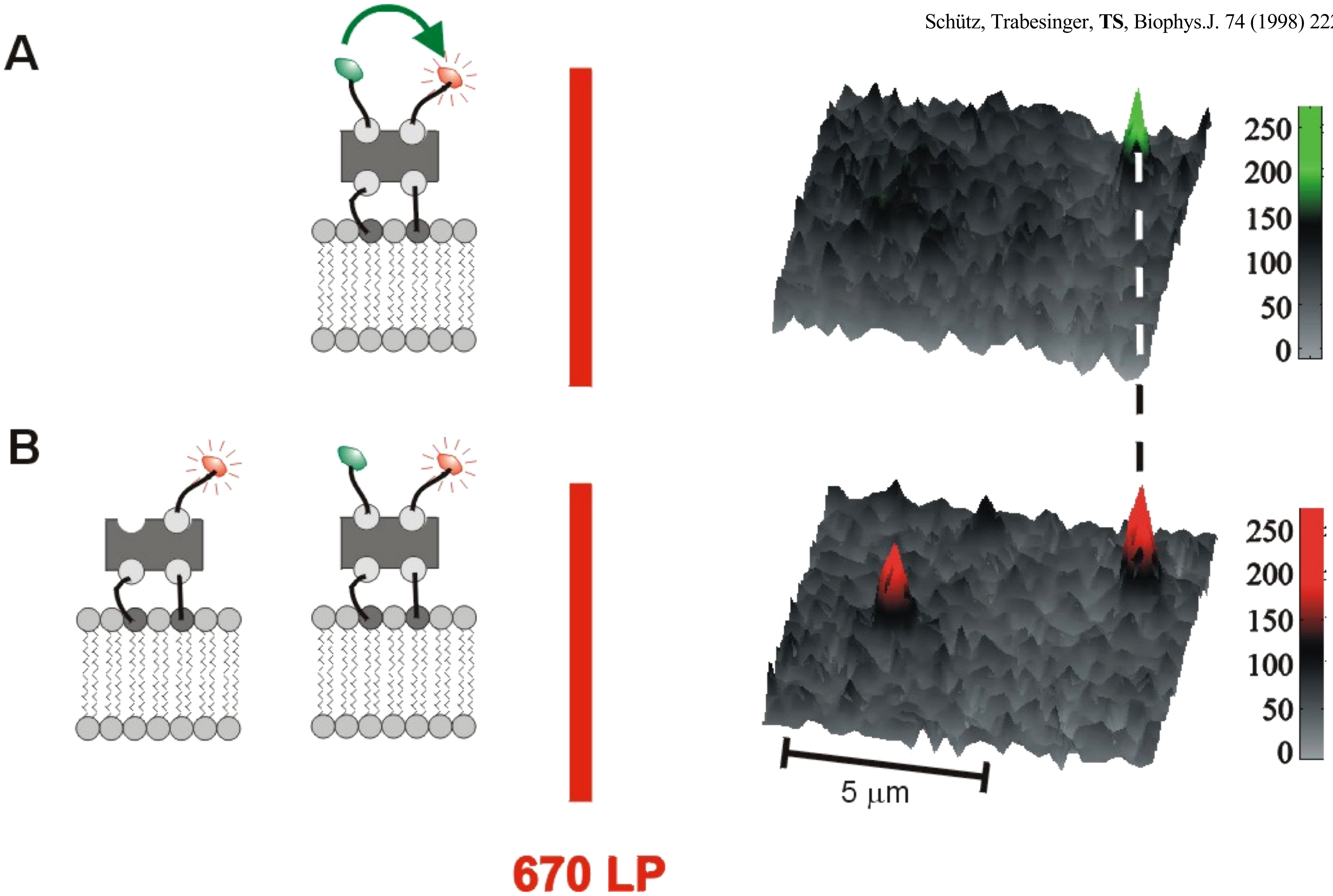
colocalization



record so-far:
2 nm (Ha, 2003)

single-molecule Förster transfer

Schütz, Trabesinger, **TS**, Biophys.J. 74 (1998) 2223

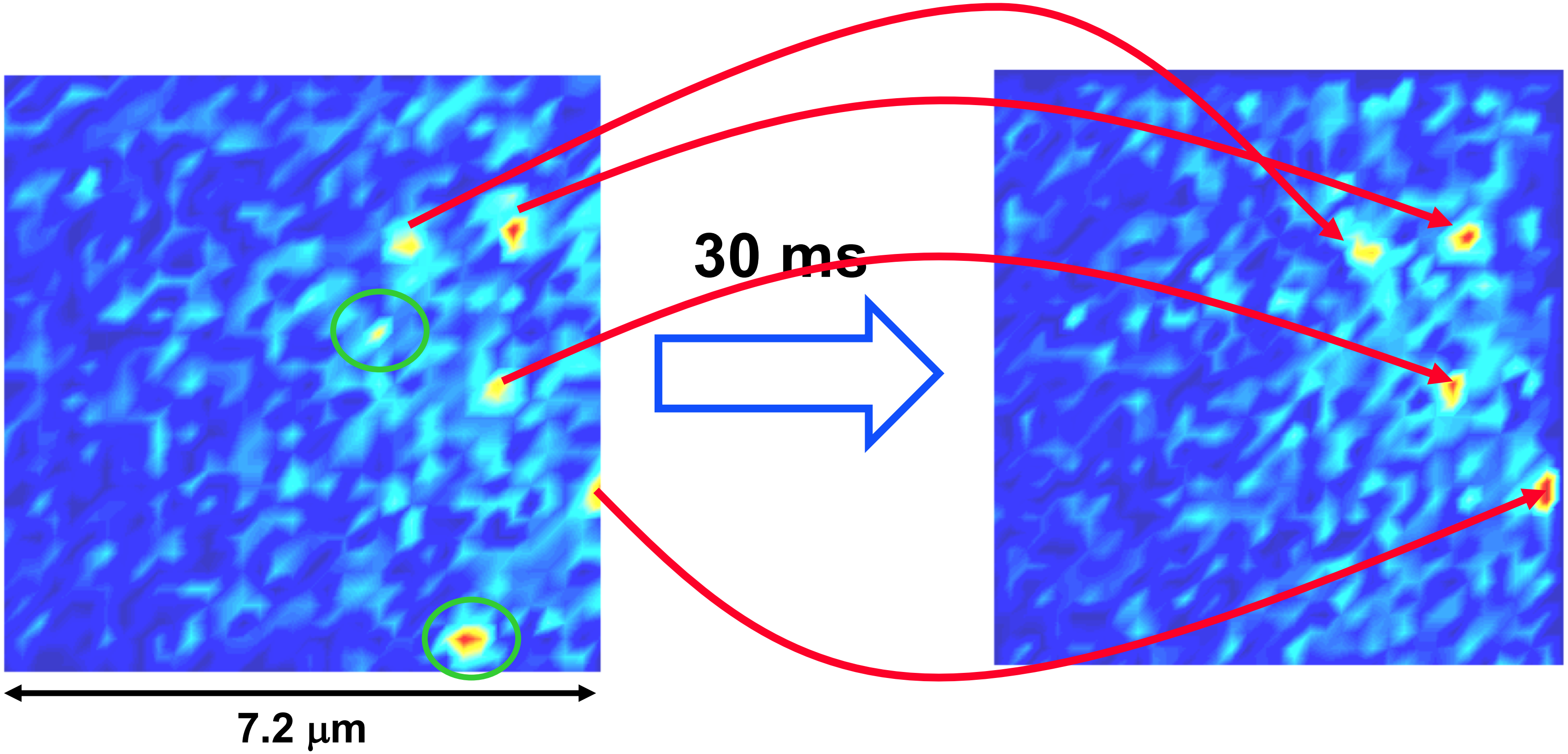


Movie analysis

- Identification of possible single-molecule signals
- extract Information: signal level + position
- statistical tests on the result
- identify Trajectories in subsequent frames
- analyze the mobility in terms of diffusion models
- correlate the observations in relevant biological terms

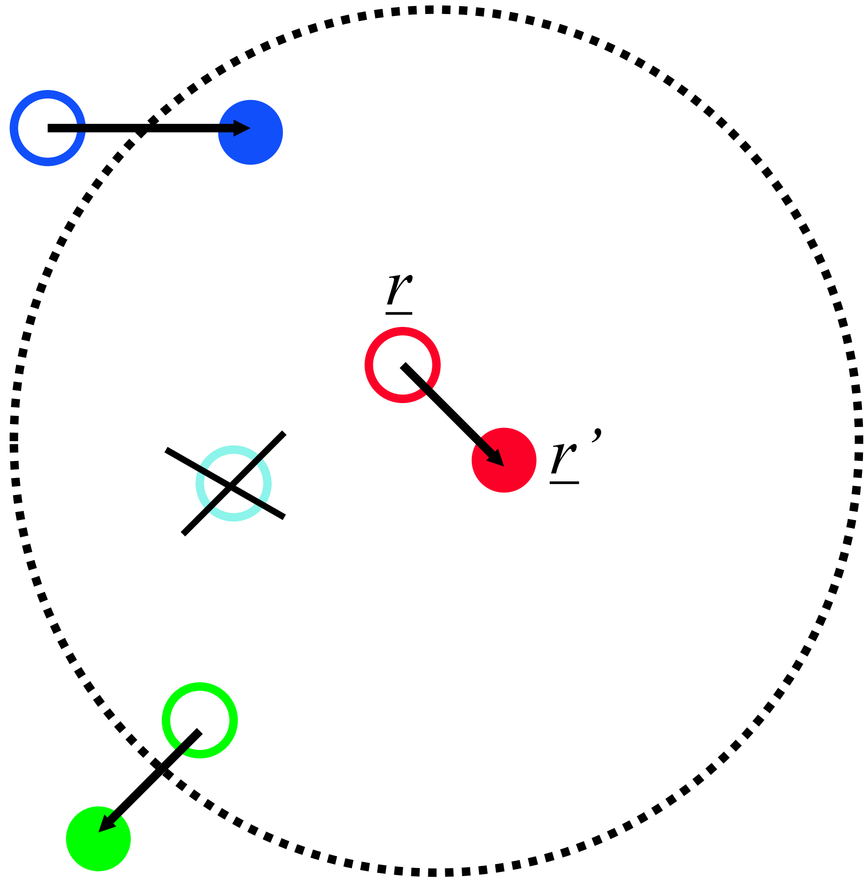
Trajectories (1)

- frame n
- frame n+1



Trajectories (2)

- molecules can
  - move from $\underline{r} \rightarrow \underline{r}'$ due to diffusion
  - disappear due to diffusion
  - disappear due to photobleaching
  - appear due to diffusion



Trajectories (3): *diffusion*

- diffusion

probability to move from $\underline{\boldsymbol{r_0}}$ to $\underline{\boldsymbol{r}}$ in a given time interval, t, and diffusion constant, D

$$p(\vec{r},\vec{r}_0,t)\,d\vec{r} = \frac{1}{4\pi\, Dt}\exp\left(\frac{-\left(\vec{r}-\vec{r}_0\right)^2}{4Dt}\right)d\vec{r}$$

- photobleaching

$$P_{bleach}(t) = 1-\exp\left(\frac{-t}{\tau_{bleach}}\right)$$

- diffusion out of the area

$$P_{out}(\vec{r},t) = 1-erf\left(\frac{-\left(\vec{r}-\vec{b}\right)^2}{2\sqrt{Dt}}\right)$$

- diffusion into area

$$P_{in}(\vec{r},t) = \bar{c}\left(1-erf\left(\frac{-\left(\vec{r}-\vec{b}\right)^2}{2\sqrt{Dt}}\right)\right)$$

Trajectories (4): *traveling salesman*

- generate a probability matrix for all possibilities

p_{11} p_{12} ... p_{1n} p_{21} ... p_{m1}	p_{1a} 0 ... 0 p_{2a} ... p_{ma}
p_{1b} 0 ... 0 p_{2b} ... p_{nb}	correlated disappearing appearing

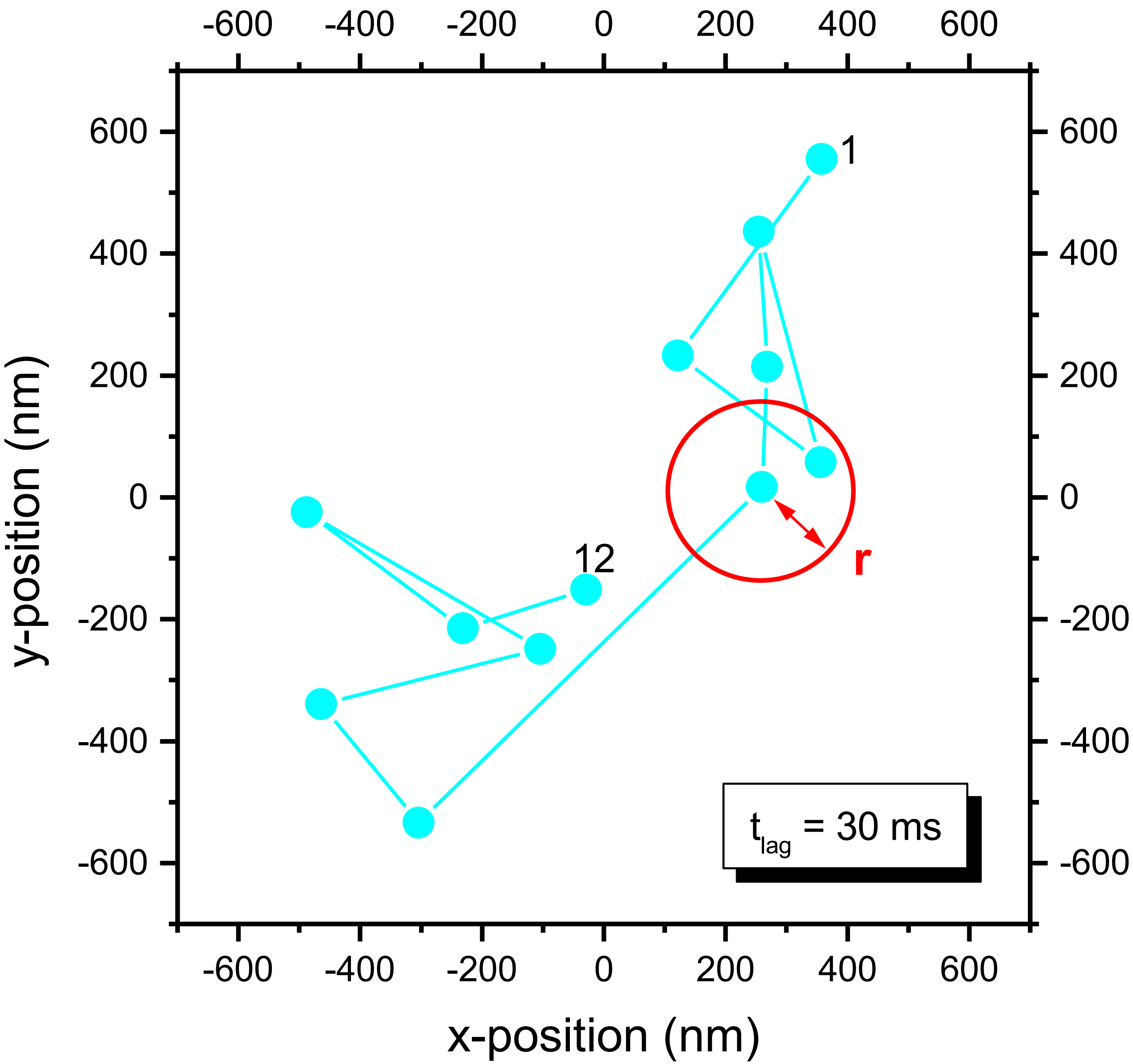
n+m x n+m

- for every molecule in n take the highest probability (Gosh&Webb)
- calculate the highest combined probability (Schmidt et al)

$$P_{tot} = \max_{i,j}\left[\log\left(\sum_{i,j} p_{ij}\right)\right]$$

  - tree: *O(n!)*
  - Vogel algorithm: *O(n)*

single-molecule trajectory



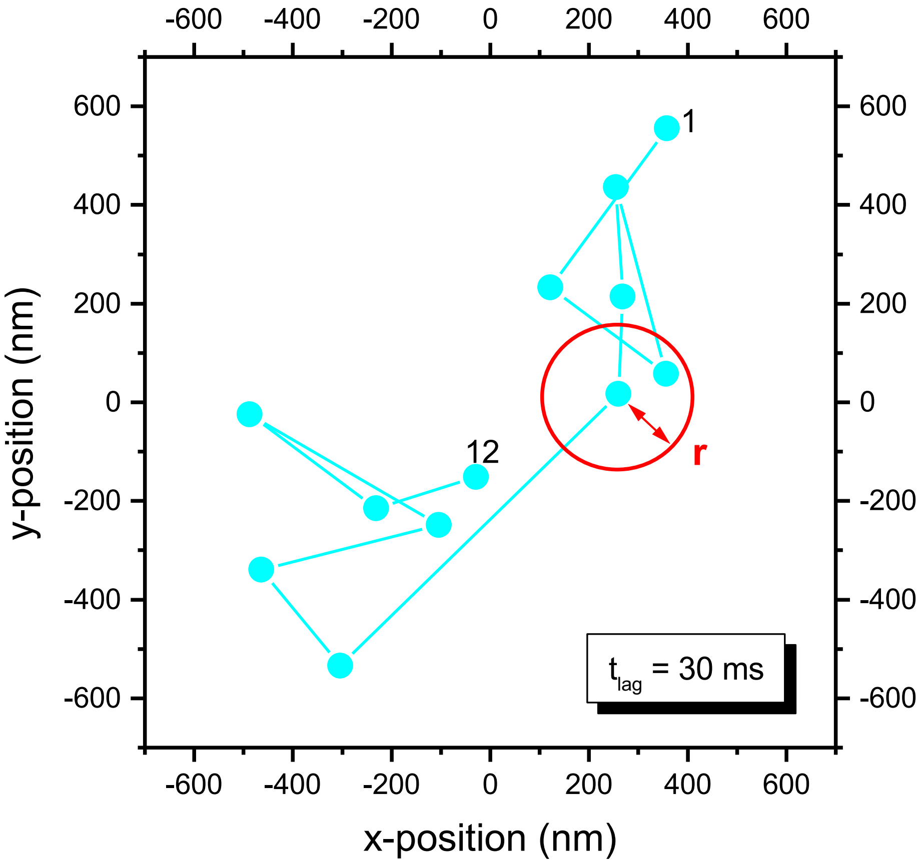
Schütz, Schindler, **TS**, Biophys.J. 73 (1997) 1073

analysis

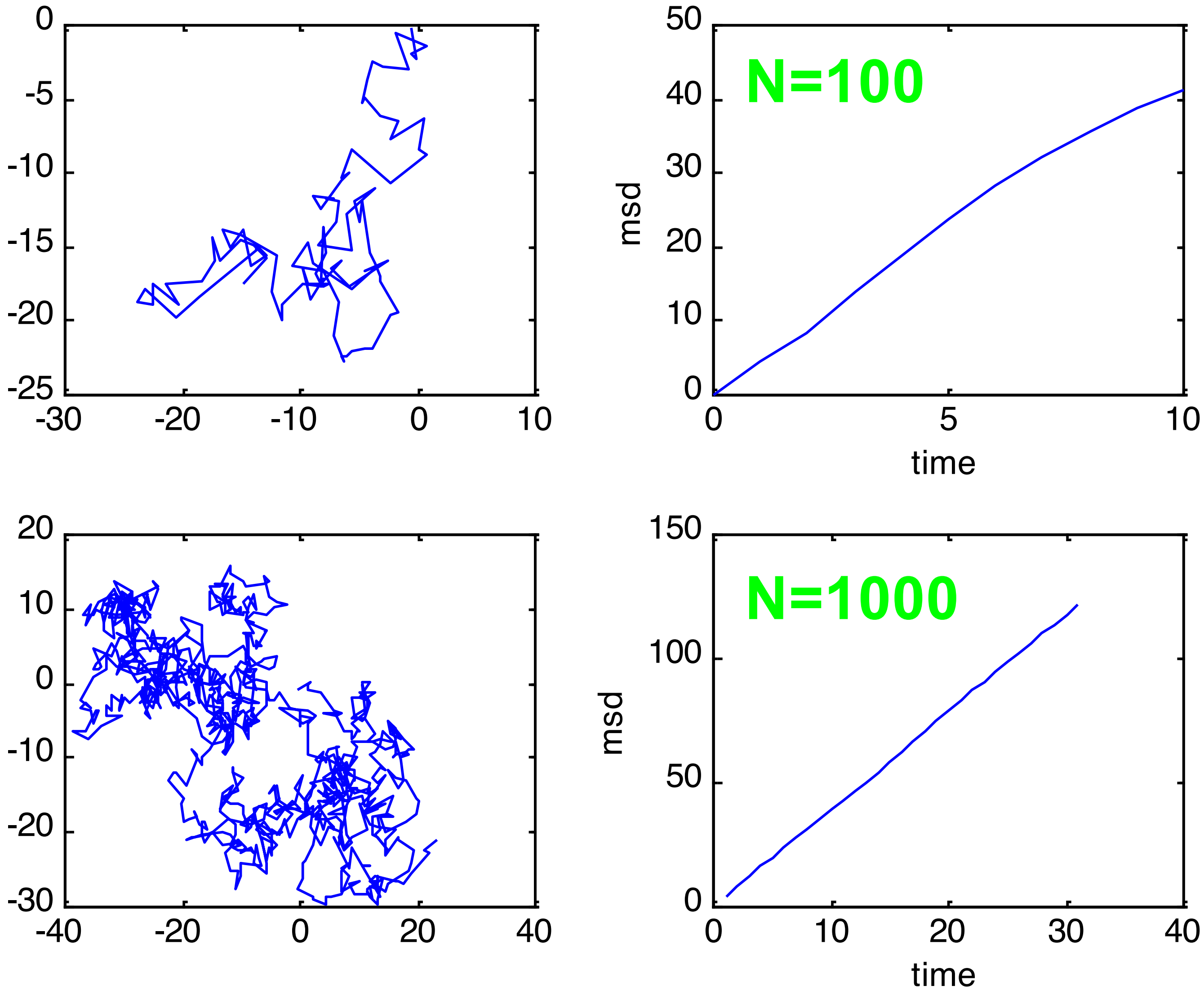
- mean squared-displacements

$$msd(n\Delta t) = \left\langle \frac{1}{N-n} \sum \left(\vec{r}(t) - \vec{r}(t+n\Delta t) \right)^2 \right\rangle$$

$$= \left\langle \frac{1}{N-n} \sum_{i=1}^{N-n} \left(\vec{r}_i - \vec{r}_{i+n} \right)^2 \right\rangle$$



particle tracking



analysis

- diffusion equation

$$\dot{p}(\vec{r},t) = D\,\nabla^2 p(\vec{r},t)$$

$$p(\vec{r},t)\,d\vec{r} = \frac{1}{\sqrt{8\pi\,Dt}}\exp\left(\frac{-r^2}{4Dt}\right)d\vec{r}$$

  - in 2D:

$$p\left(r^2,t\right)dr^2 = \frac{1}{r_0^2}\exp\left(\frac{-r^2}{r_0^2}\right)dr^2$$

$$r_0^2 = 4Dt$$

- mean squared-displacements

$$\boldsymbol{msd}(\boldsymbol{n}\Delta\boldsymbol{t}) = \left\langle \frac{1}{\boldsymbol{N}-\boldsymbol{n}}\sum\left(\vec{\boldsymbol{r}}(\boldsymbol{t})-\vec{\boldsymbol{r}}(\boldsymbol{t}+\boldsymbol{n}\Delta\boldsymbol{t})\right)^2\right\rangle$$

$$= \left\langle \frac{1}{\boldsymbol{N}-\boldsymbol{n}}\sum_{\boldsymbol{i}=1}^{\boldsymbol{N}-\boldsymbol{n}}\left(\vec{\boldsymbol{r}}_{\boldsymbol{i}}-\vec{\boldsymbol{r}}_{\boldsymbol{i}+\boldsymbol{n}}\right)^2\right\rangle$$

$\Rightarrow$ **mean = std**

statistical accuracy of SPT

H.Quian, M.P.Sheetz, E.L.Elson, Biophys.J. 60 (1991) 910.

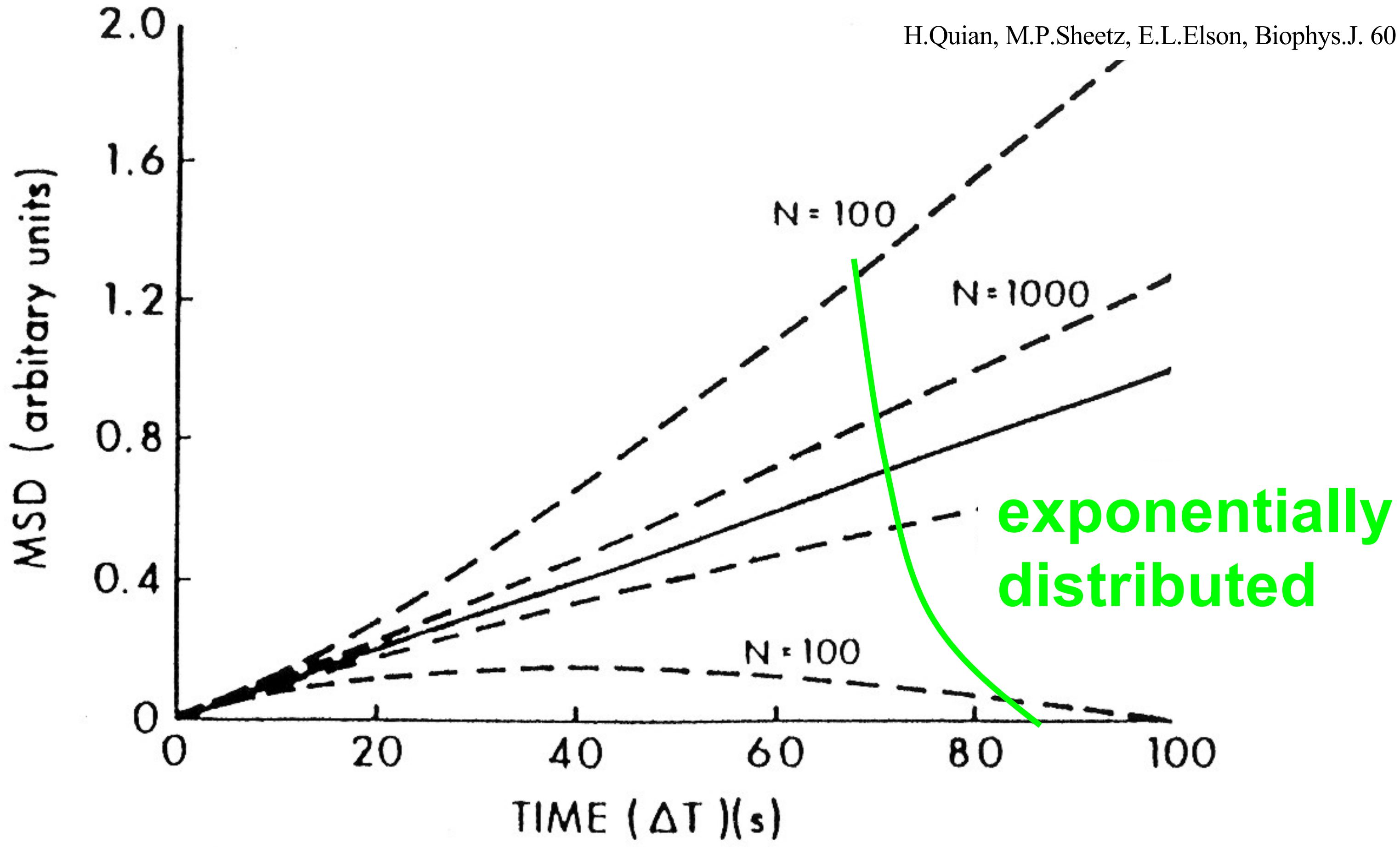


FIGURE 5 The relative statistical error in msd, $\rho(t)$ (——). Upper and lower curve are $\langle\rho_n\rangle \pm (\langle\Delta\rho_n\Delta\rho_n\rangle)^{1/2}$. N is the total number of position measurements.

diffusion analysis

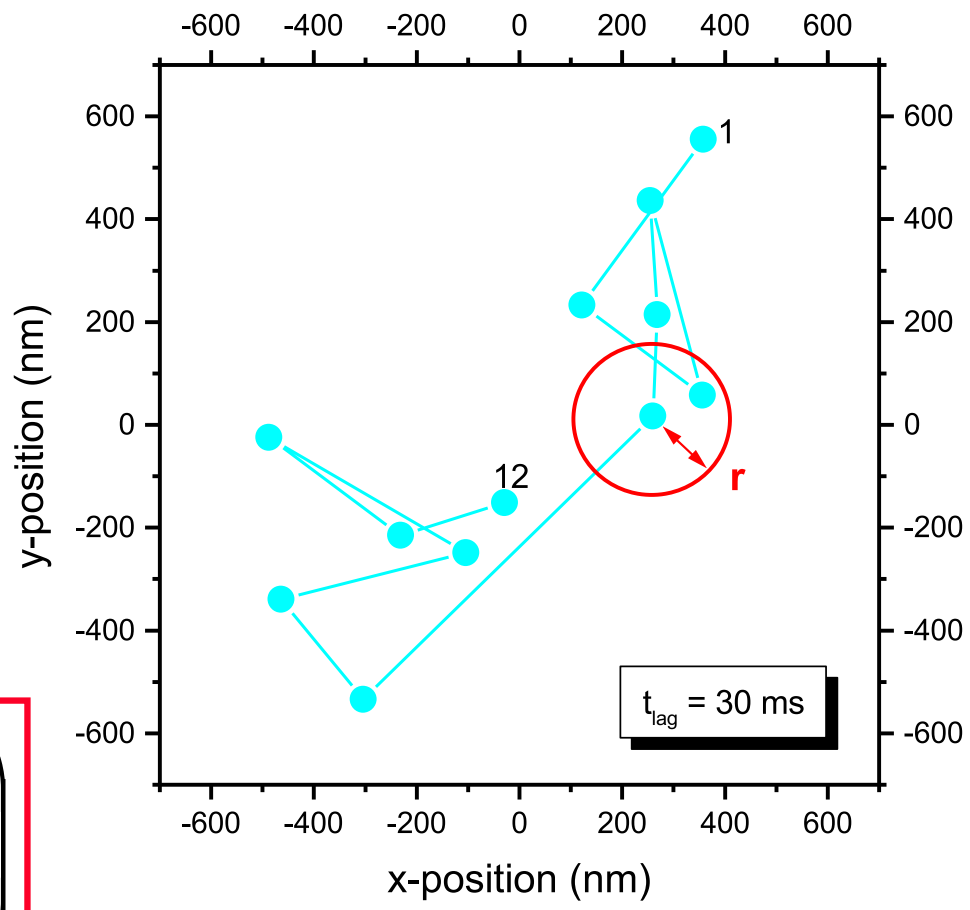
$$\dot{p}(\vec{r},t) = D\,\nabla^2 p(\vec{r},t)$$

$$p(\vec{r},t)\,d\vec{r} = \frac{1}{\sqrt{8\pi\,Dt}}\,exp\left(\frac{-r^2}{4Dt}\right)d\vec{r}$$

$$p\left(r^2,t\right)dr^2 = \frac{1}{r_0^2}\exp\left(\frac{-r^2}{r_0^2}\right)dr^2$$

$$r_0^2 = 4Dt$$

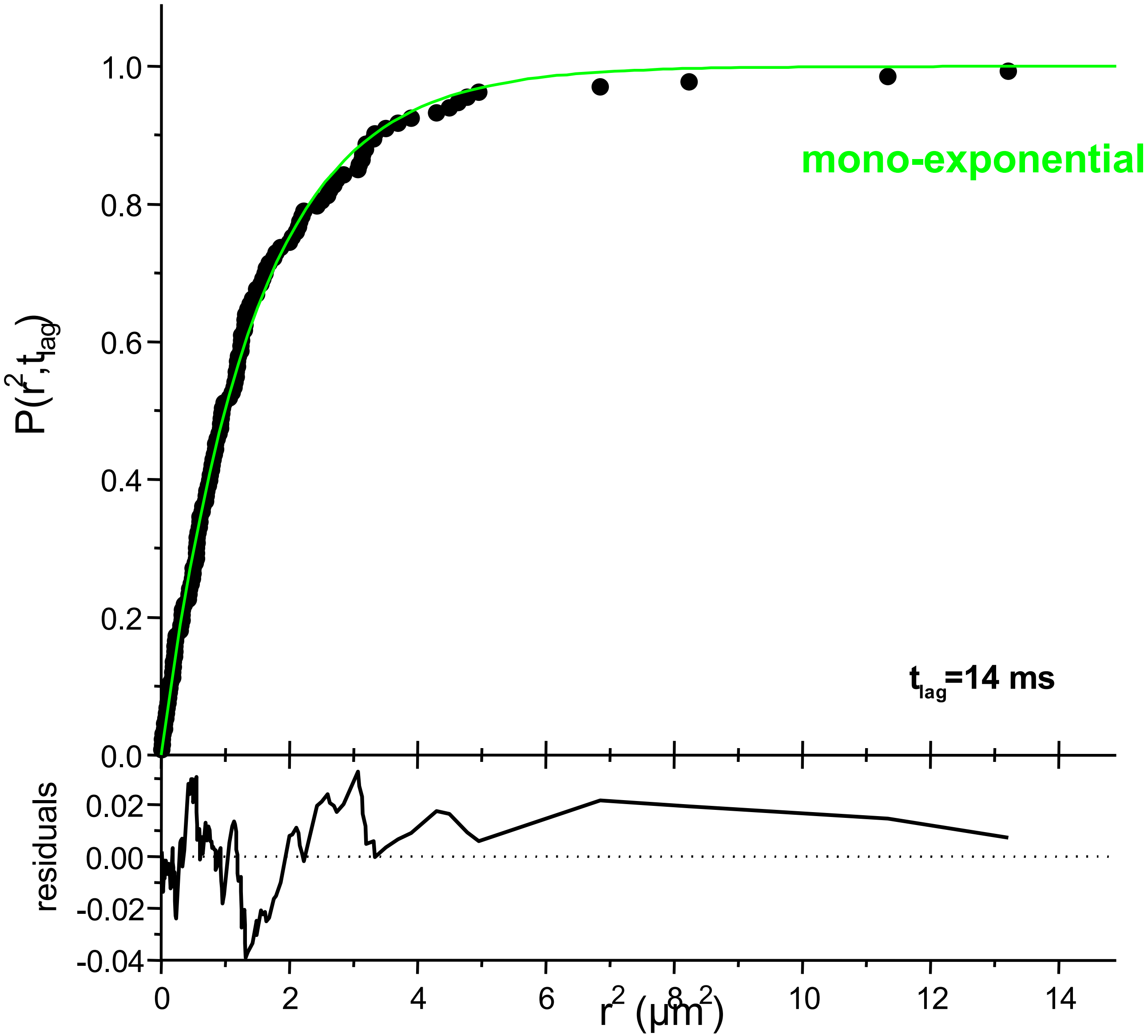
$$P\left(r^2,t\right) = 1 - \exp\left(\frac{-r^2}{r_0^2}\right)$$



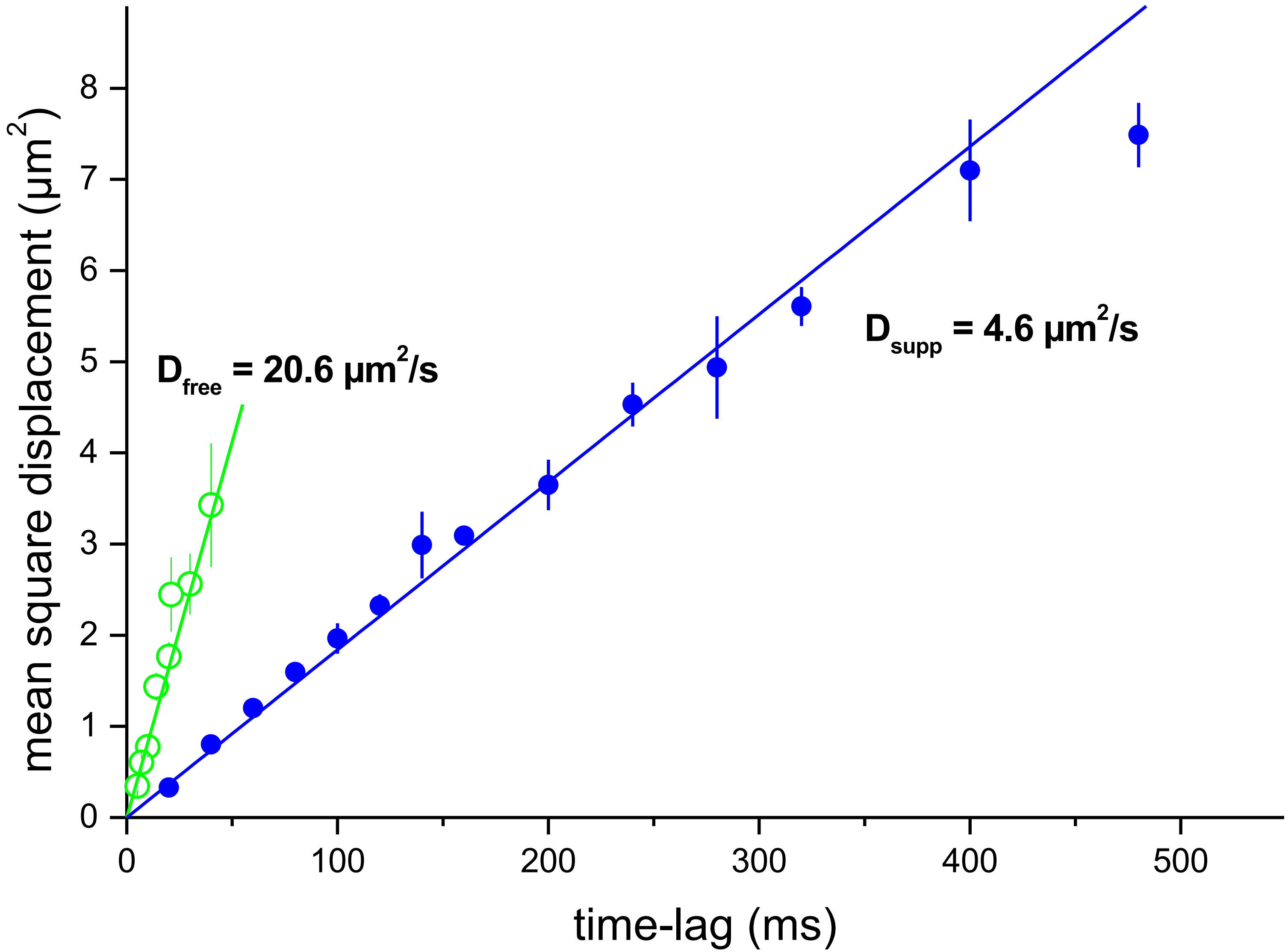
Schütz, Schindler, **TS**, Biophys.J. 73 (1997) 1073

diffusion in a black lipid membrane

A.Sonnleitner, G.J.Schütz & **TS**, Biophy.J. 77 (1999)



diffusion in artificial membranes



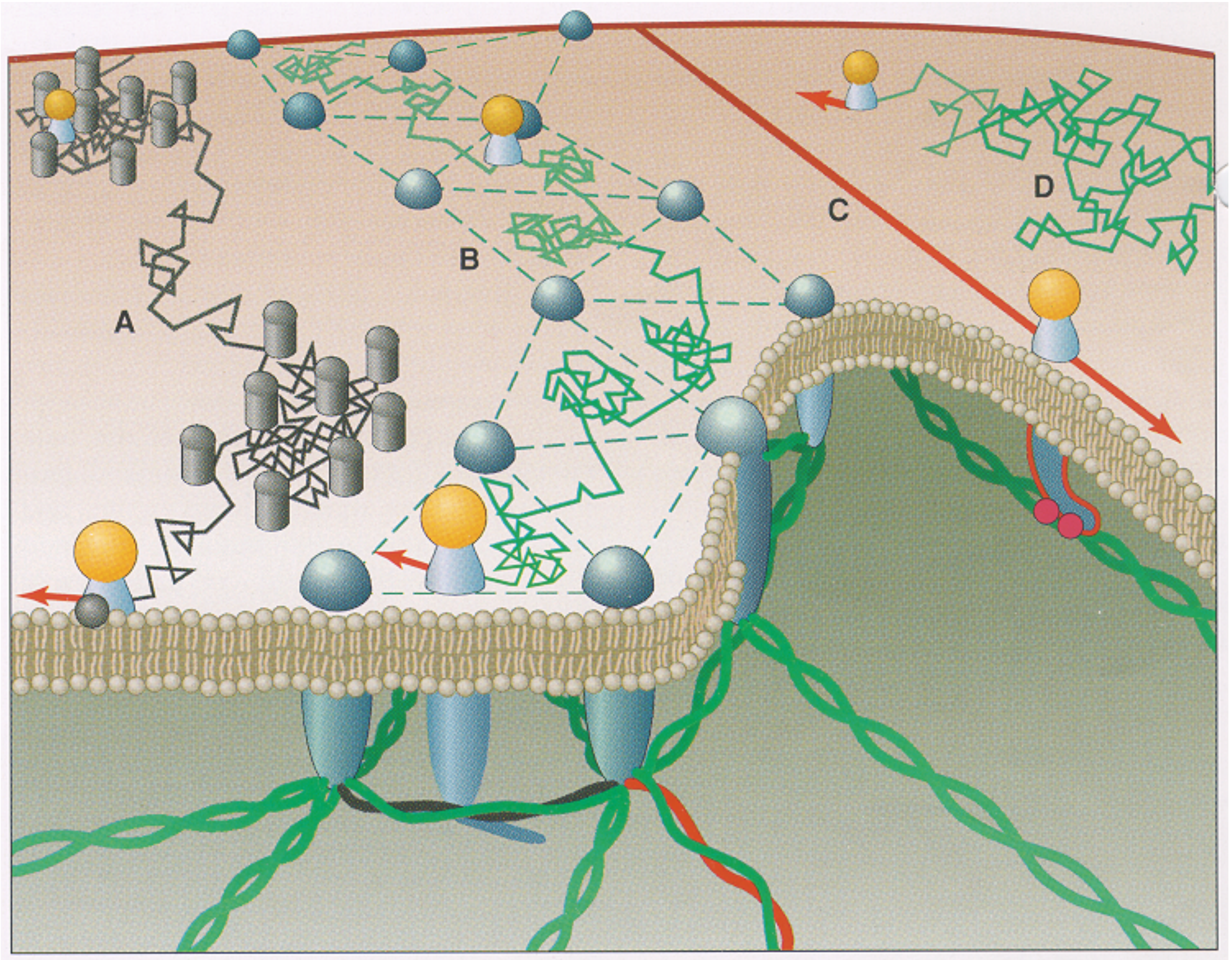
A.Sonnleitner, G.J.Schütz & **TS**, Biophy.J. 77 (1999)

Application (3)

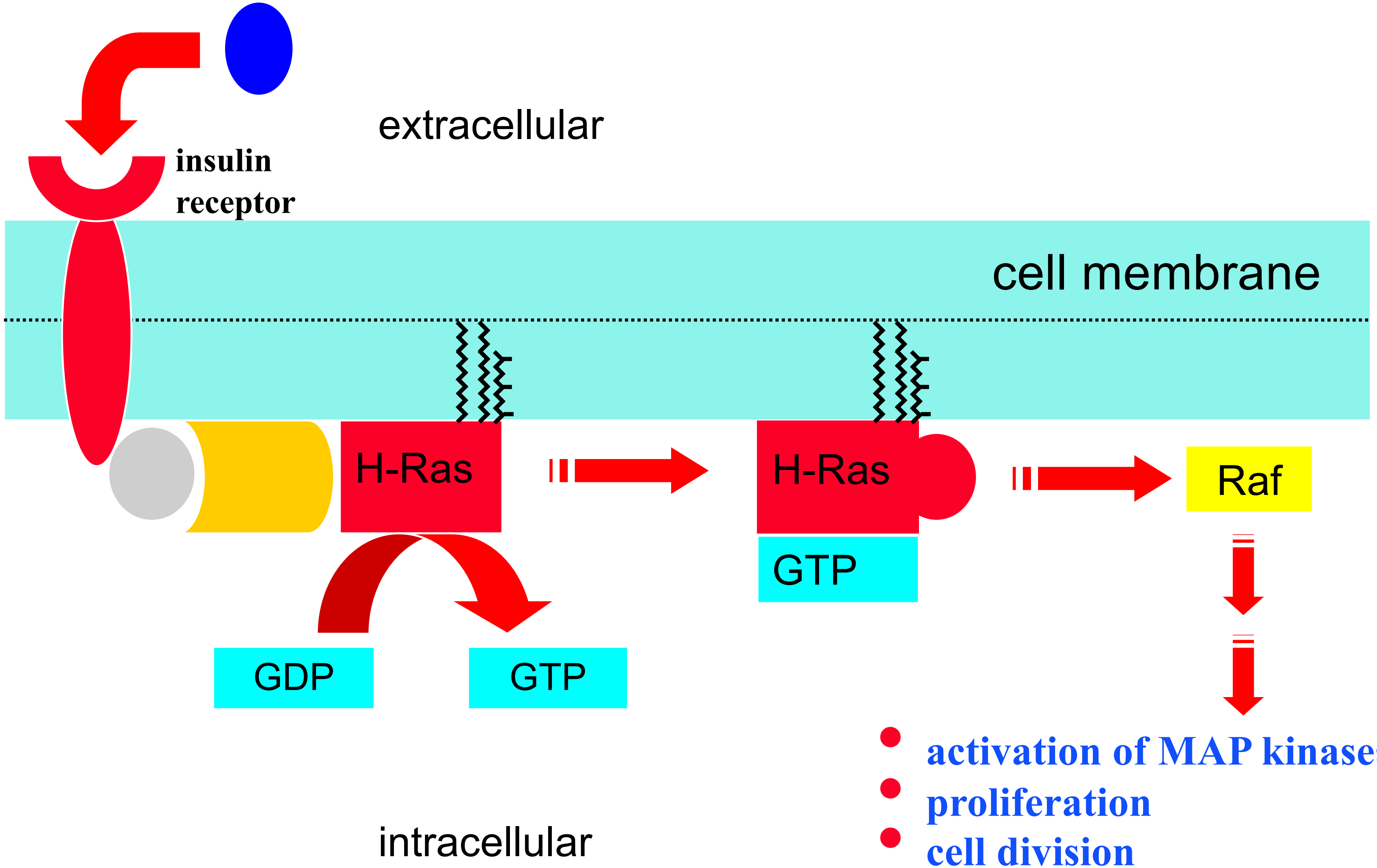
Biomolecular mobility

mobility on the plasma membrane

K.Jacobson, E.D.Sheets, R.Simson, Science 268 (1995) 5216

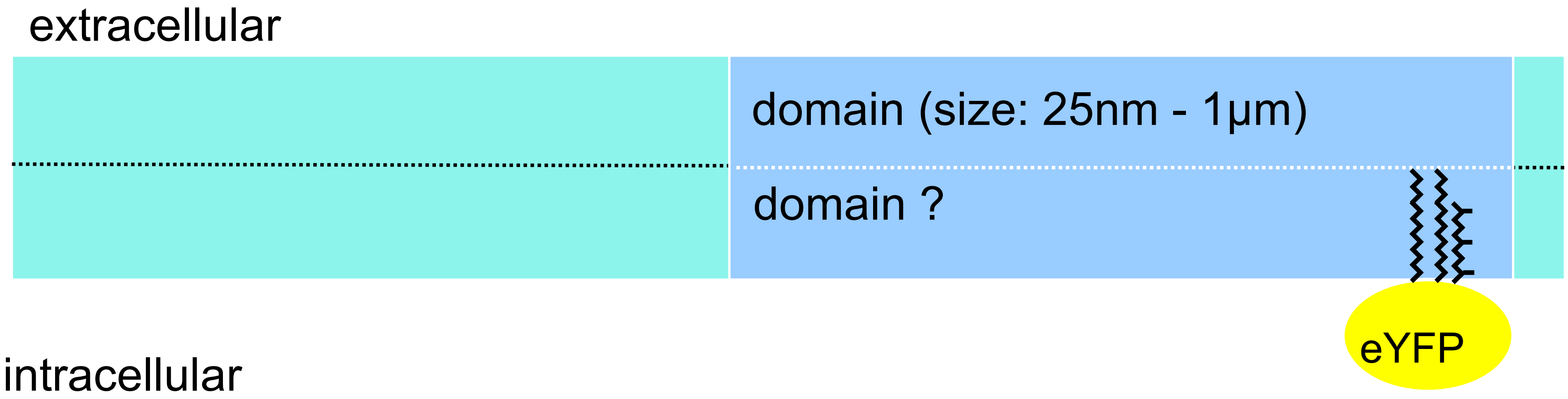


H-Ras: its role in signal transduction



- **activation of MAP kinases**
- **proliferation**
- **cell division**

H-Ras activation: domains involved?



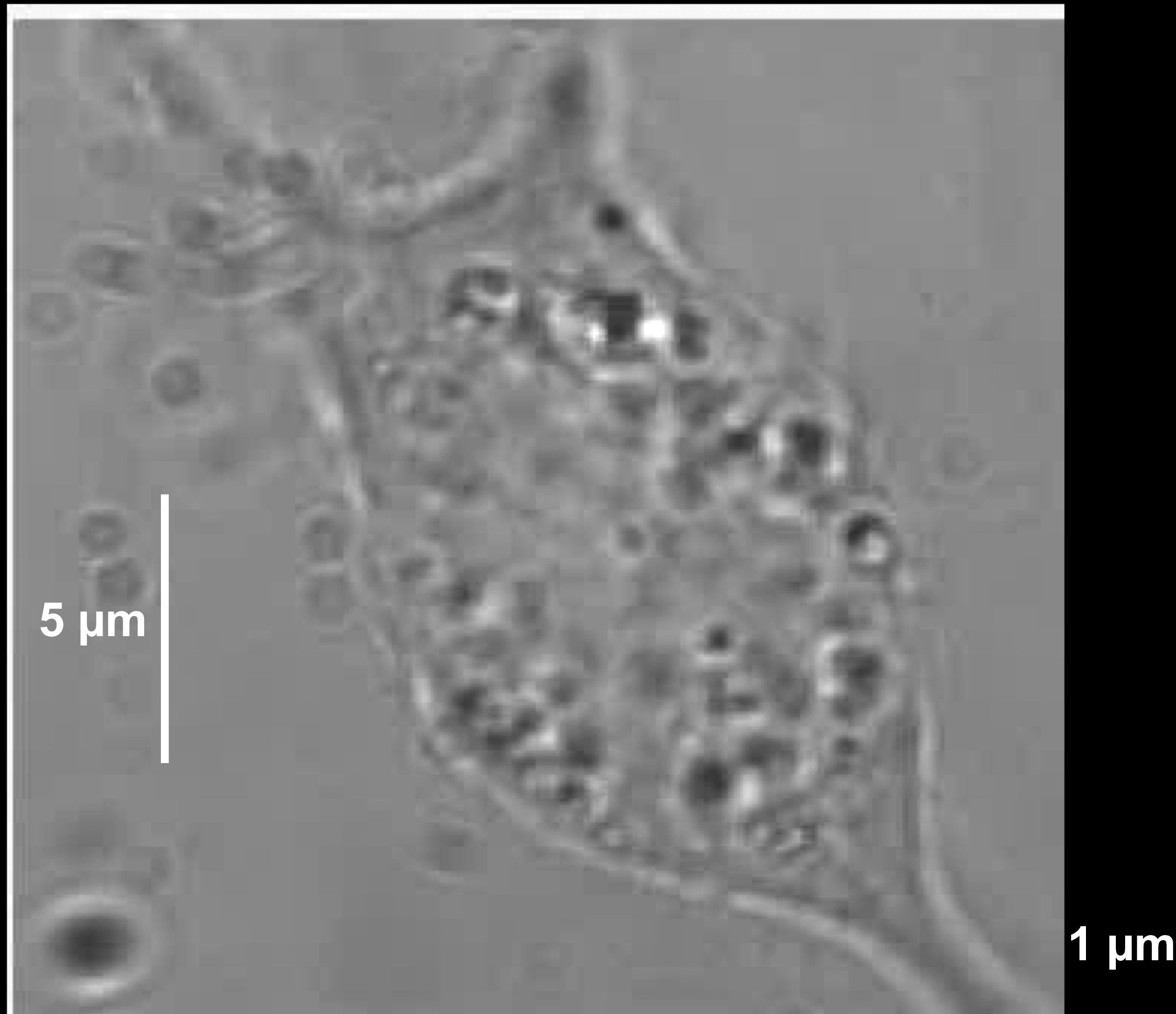
eYFP-C10HRas

membrane anchor

are there domains in the intracellular leaflet ?

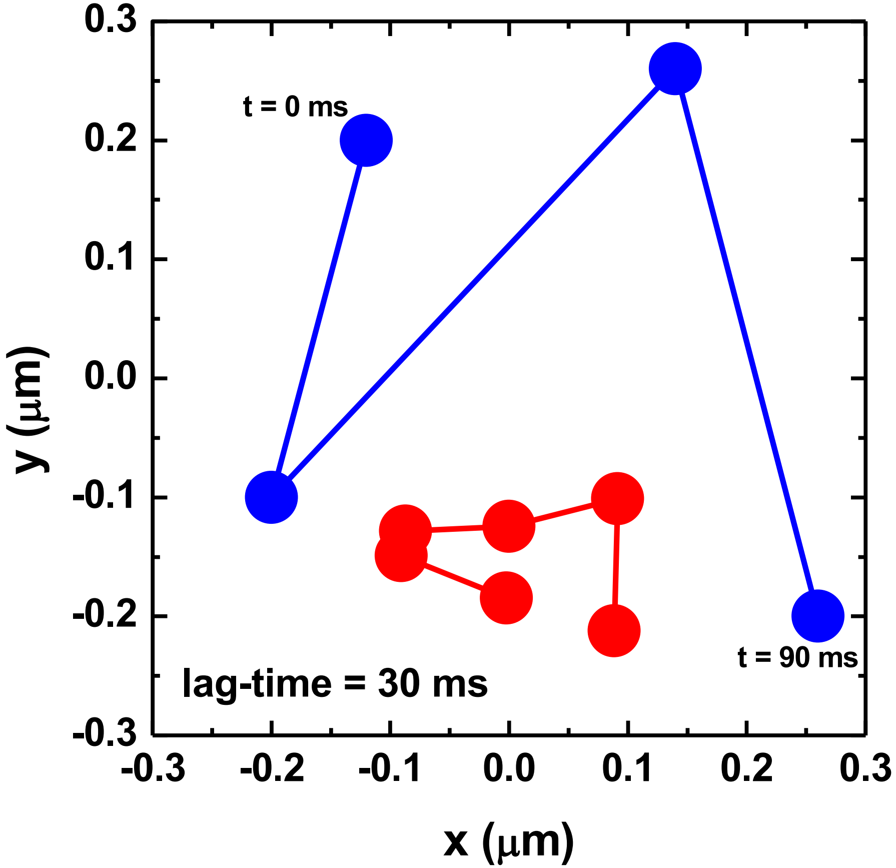
individual eYFP-C10HRas on the apical membrane of HEK293

http://www.biophys.leidenuniv.nl/Research/FvL/

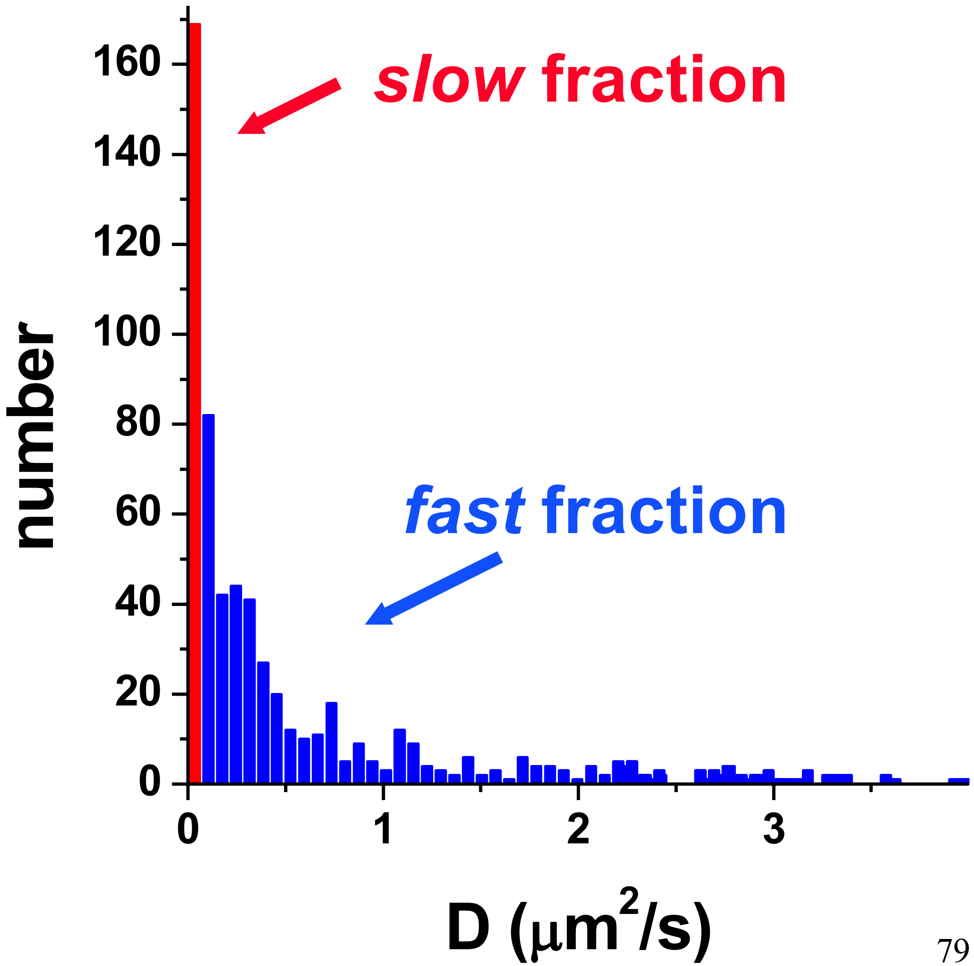


analysis of single-molecule trajectories

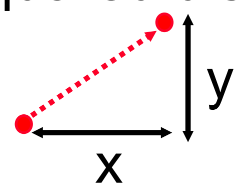
Lommerse, Cognet, Blab, Harms, Snaar-Jagalska, Spaink & **TS,** Biophys.J. 86 (2004)



- statistics of diffusion constants



squared displacement:



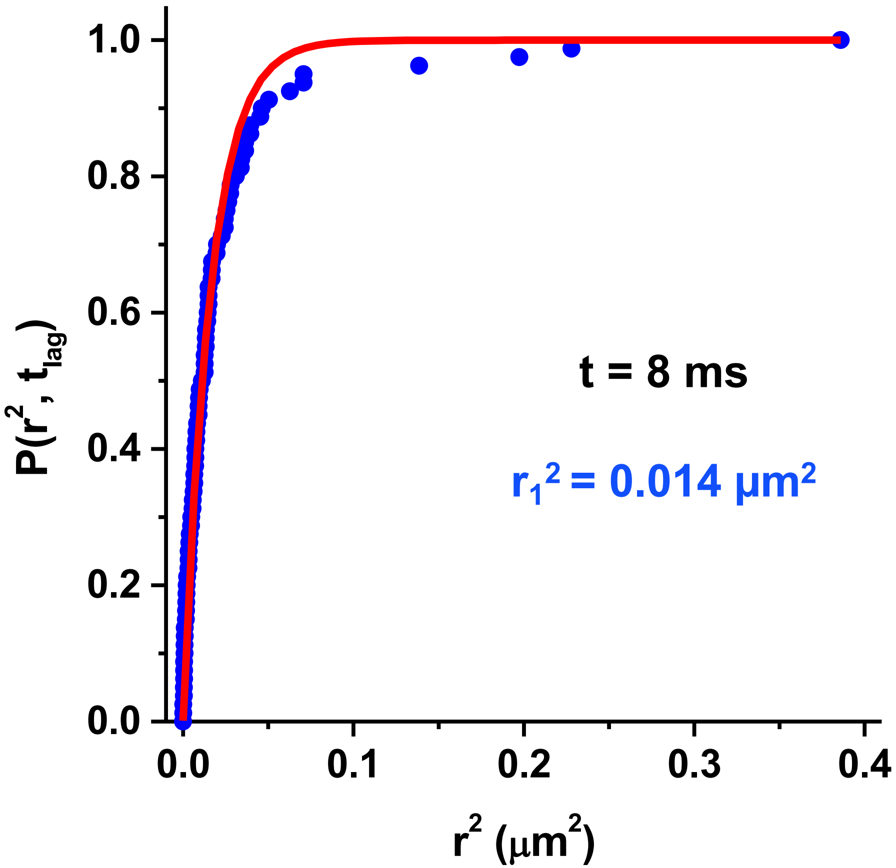
$r^2 = x^2 + y^2$

results: two fractions

Lommerse, Cognet, Blab, Harms, Snaar-Jagalska, Spaink & **TS,** Biophys.J. 86 (2004)

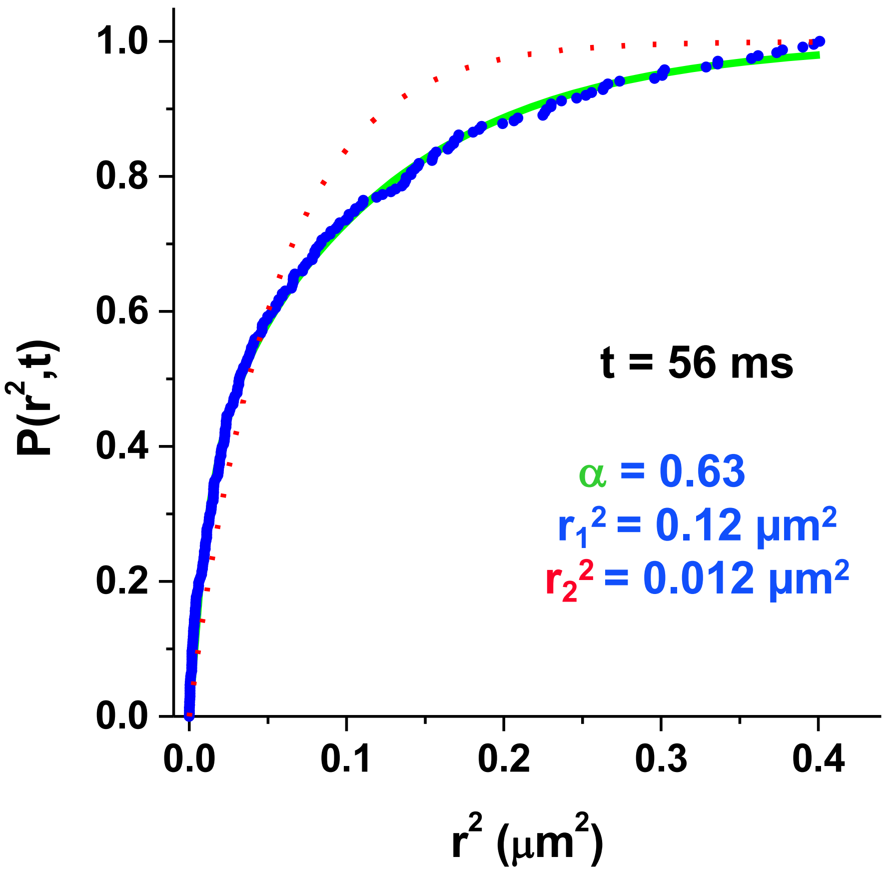
- timelags < 15 ms

$$P(r^2,t) = 1 - \exp\left(\frac{-r^2}{r_1^2}\right)$$



- timelags > 15 ms

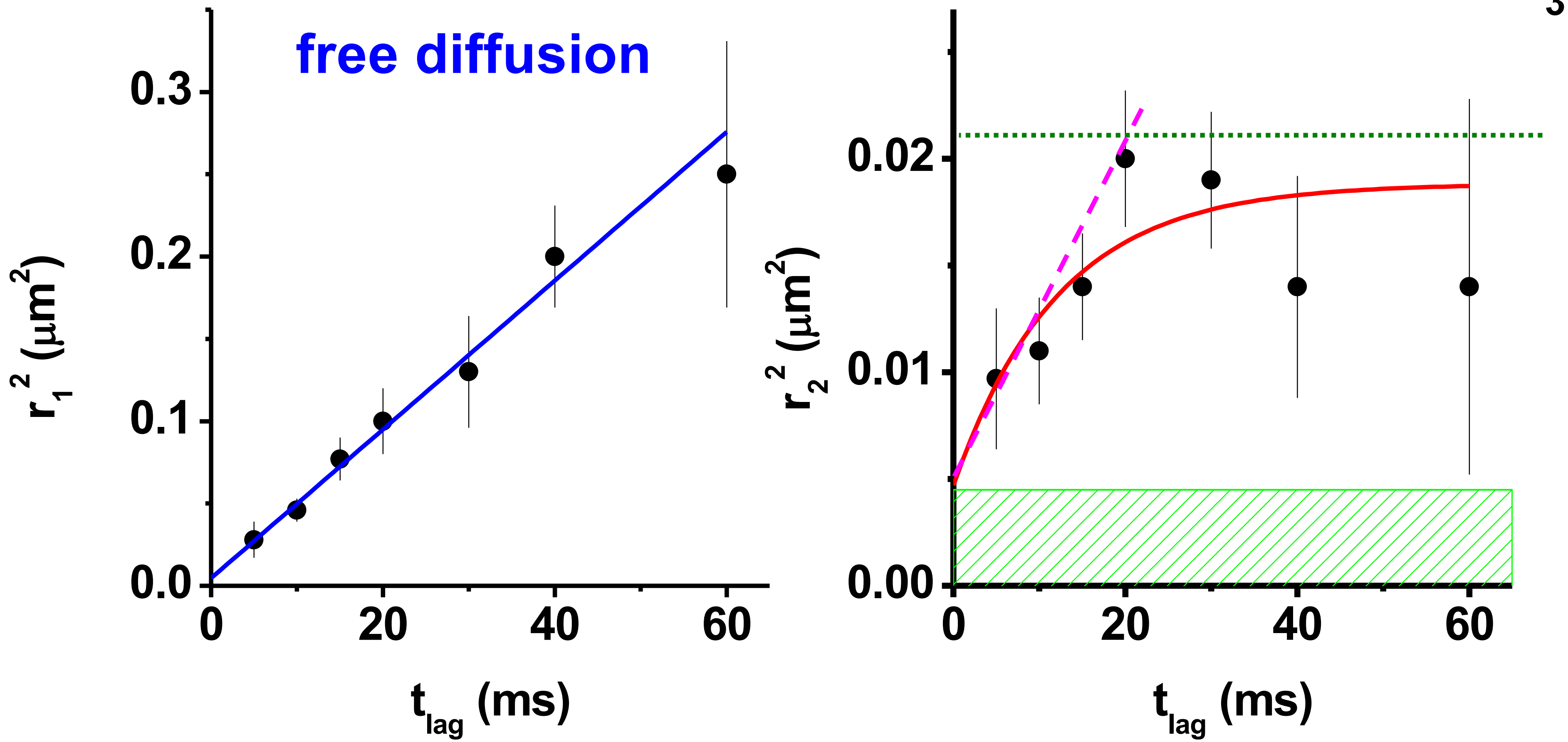
$$P(r^2,t) = 1 - \left[\alpha \exp\left(\frac{-r^2}{r_1^2}\right) + (1-\alpha)\exp\left(\frac{-r^2}{r_2^2}\right)\right]$$



diffusion of H-Ras membrane anchor

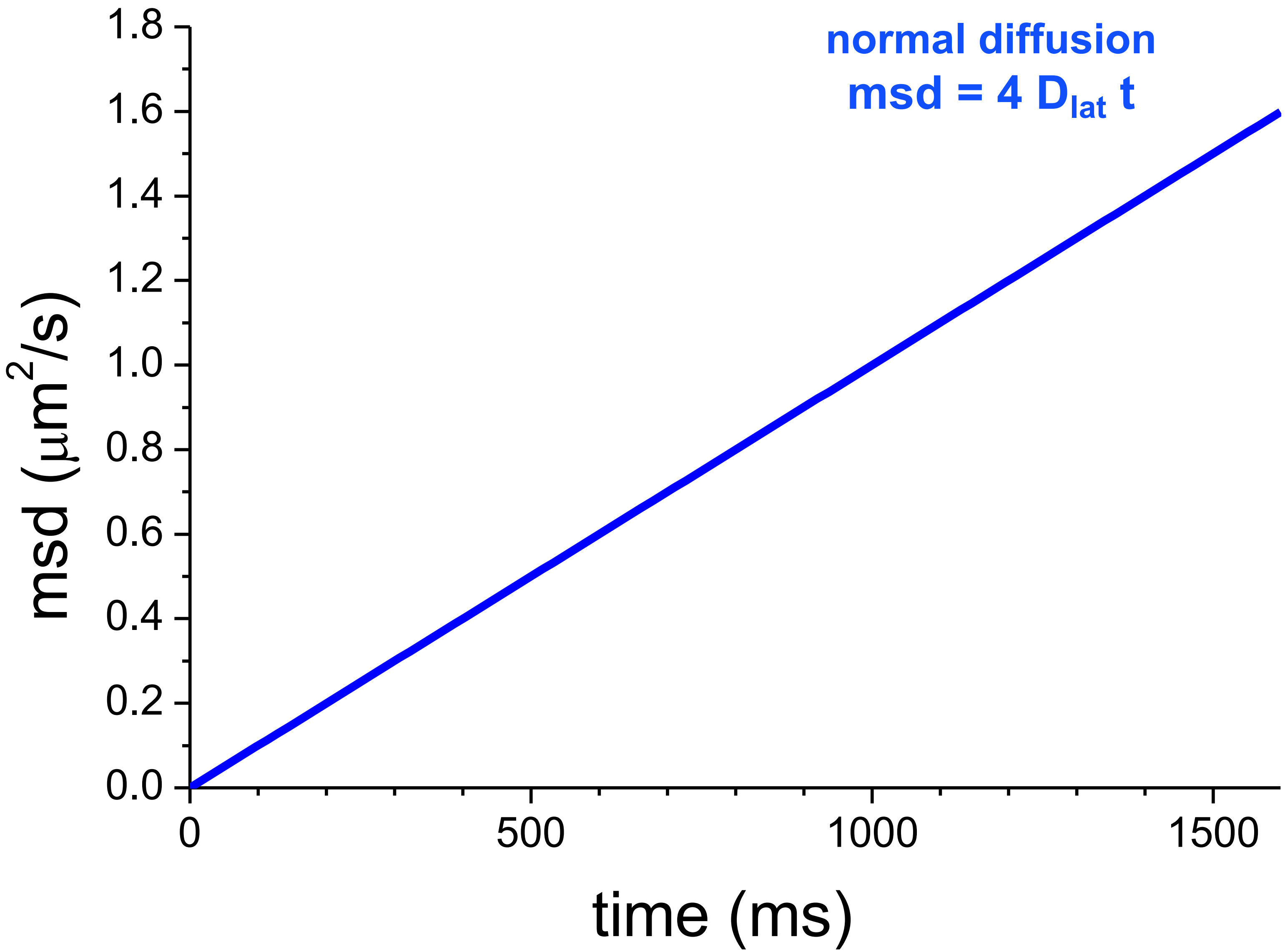
Lommerse, Cognet, Blab, Harms, Snaar-Jagalska,
Spaink & **TS,** Biophys.J. 86 (2004)

cell: 3T3-A14
37 °C

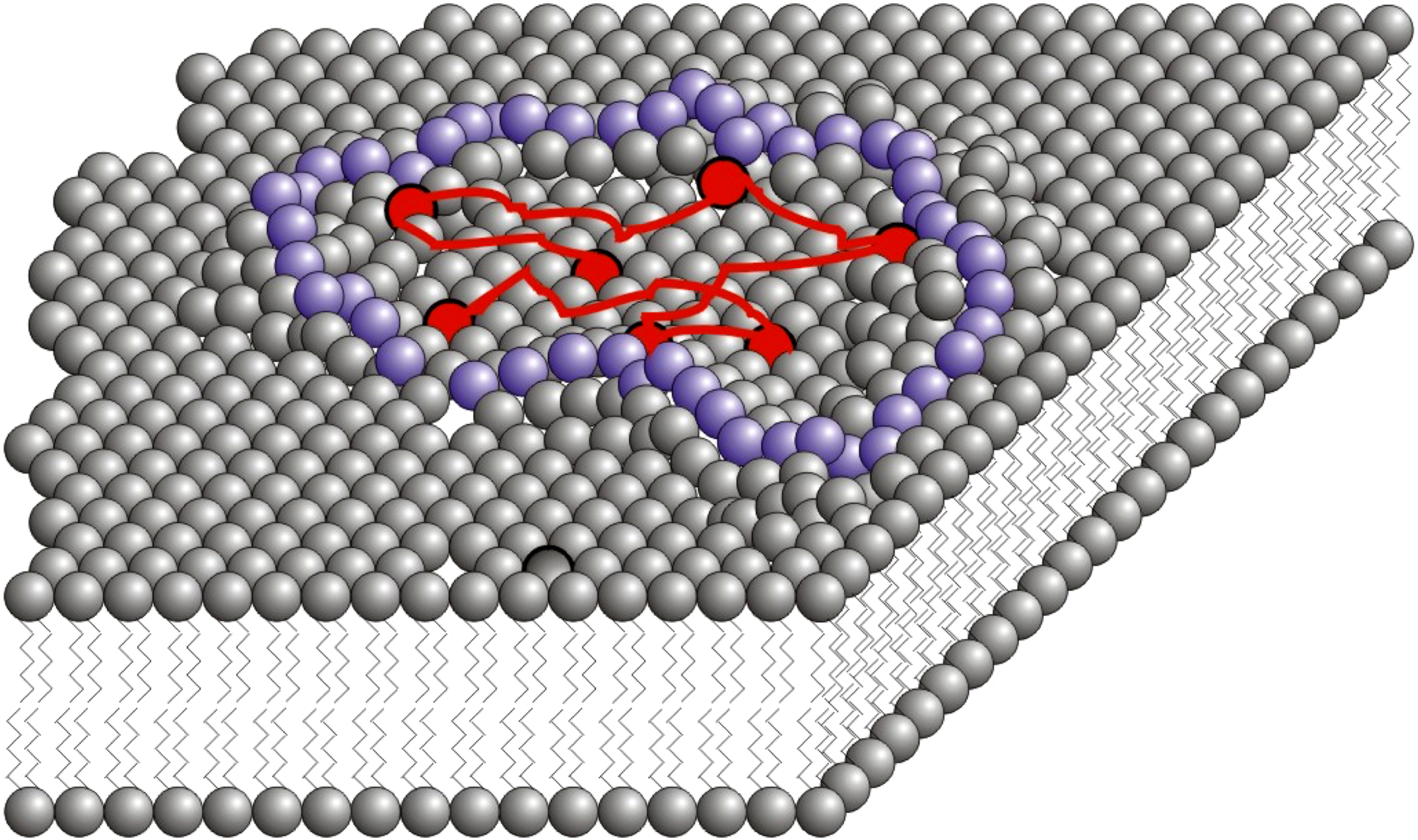


- $D = 1.13 \pm 0.09$ μm²/s

diffusion: summary

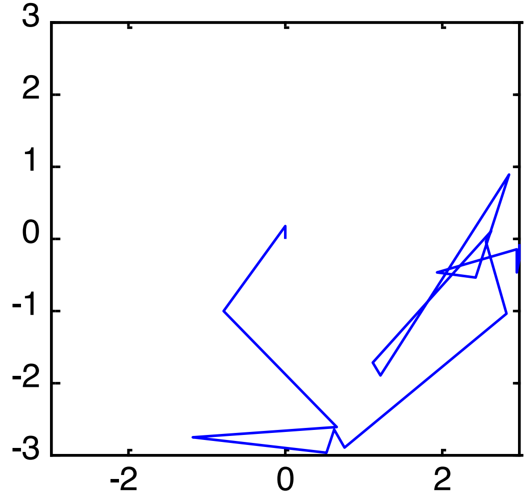


coralled diffusion

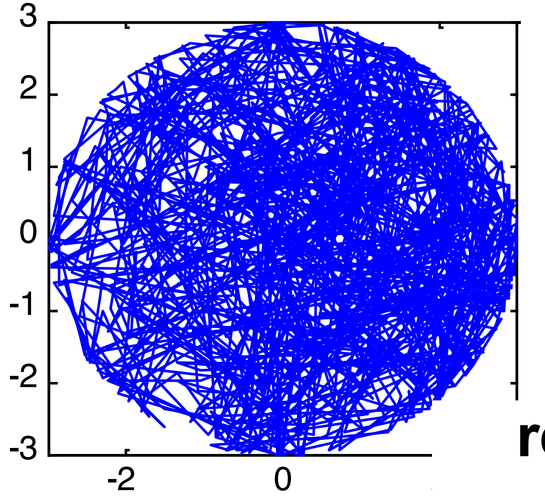


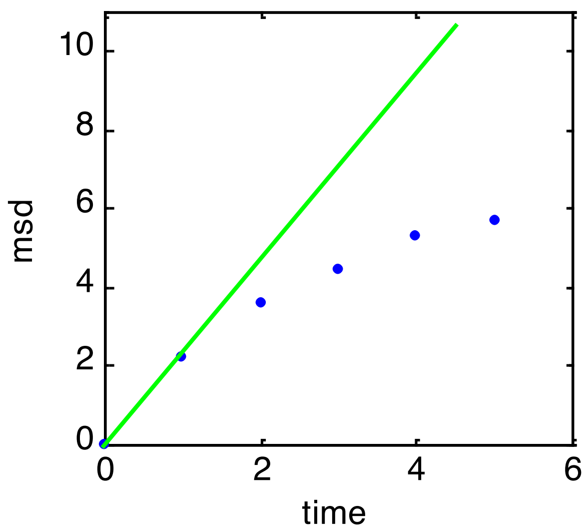
corralled diffusion

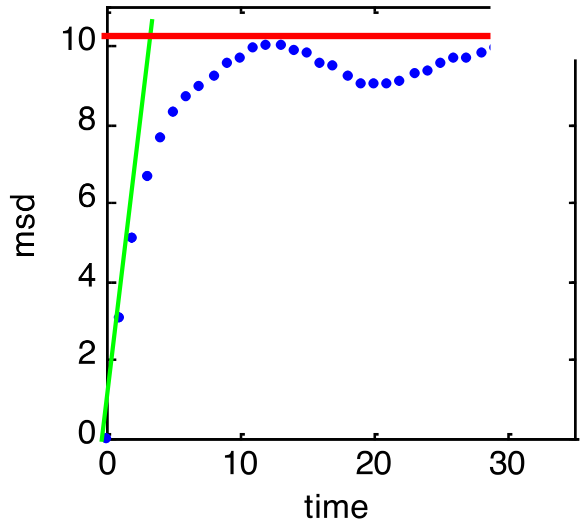
N=20



N=1000





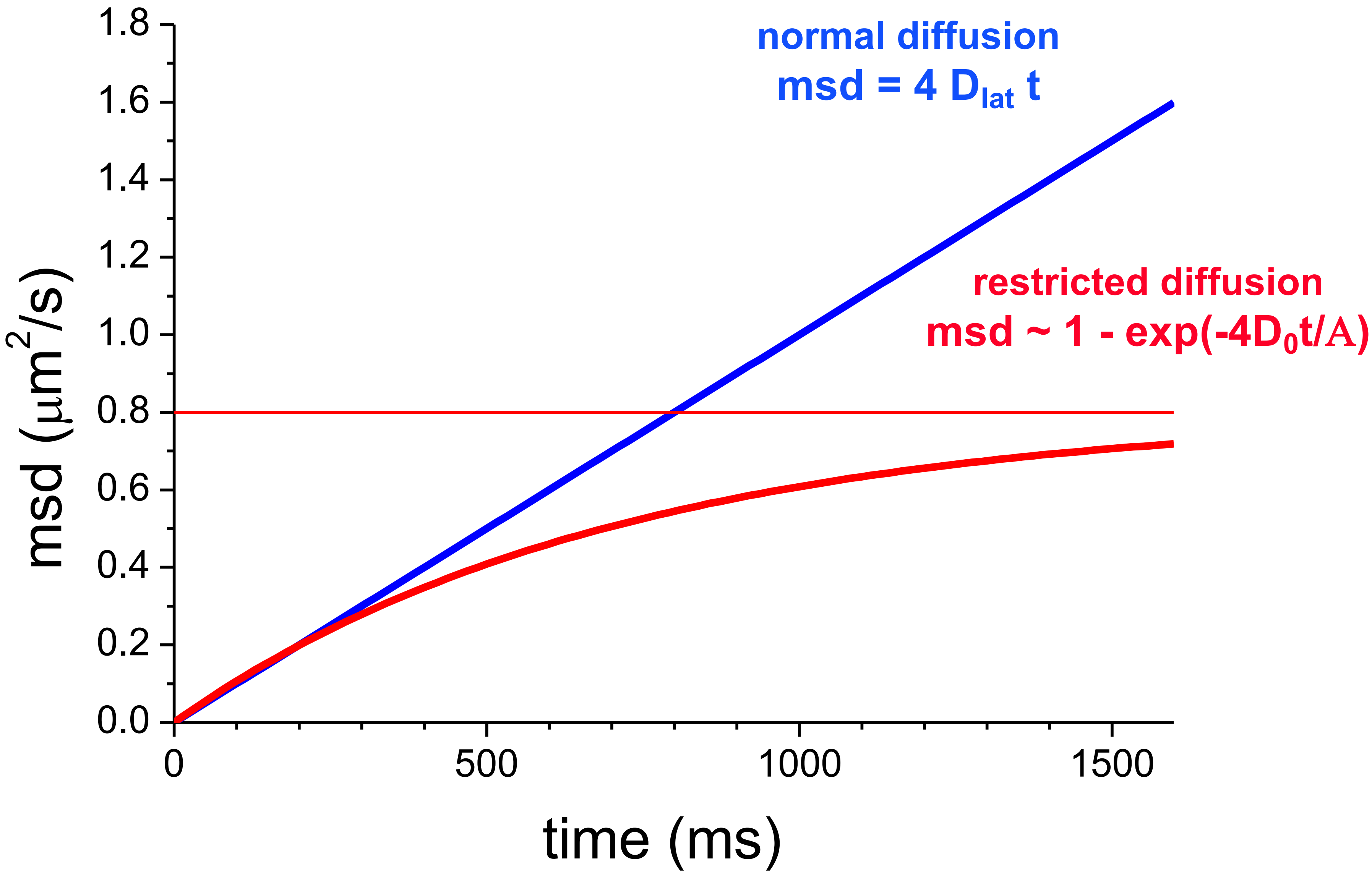


restricted diffusion

$$\mathbf{msd} = \mathbf{A}\left[\mathbf{1} - \mathbf{exp}\left(\frac{-\mathbf{4D_0t}}{\mathbf{A}}\right)\right]$$

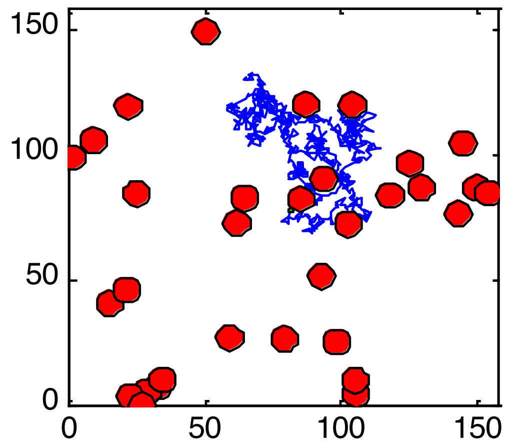
Kusumi, et al. Biophys.J. (1986)

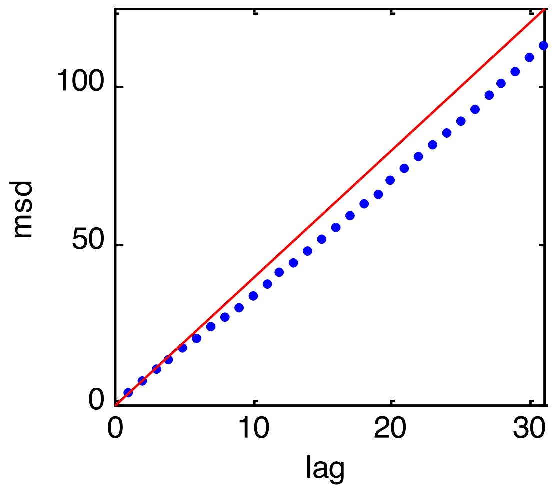
diffusion: summary



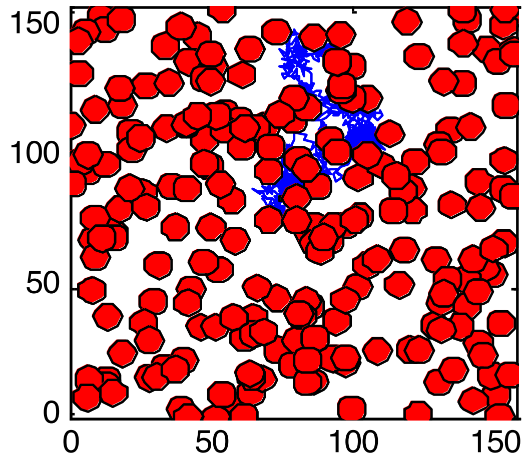
diffusion with obstacles

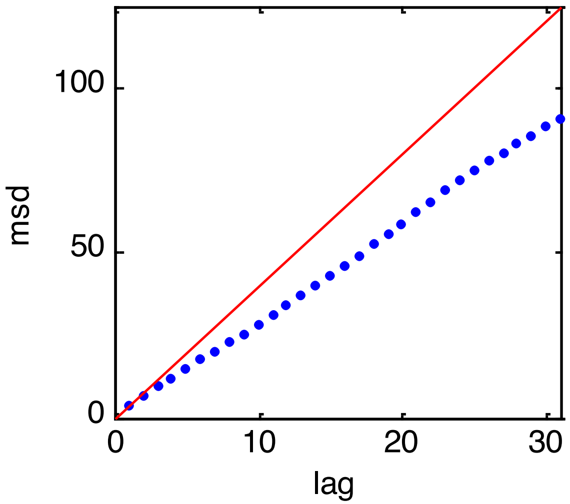
c = 0.1





c = 0.7





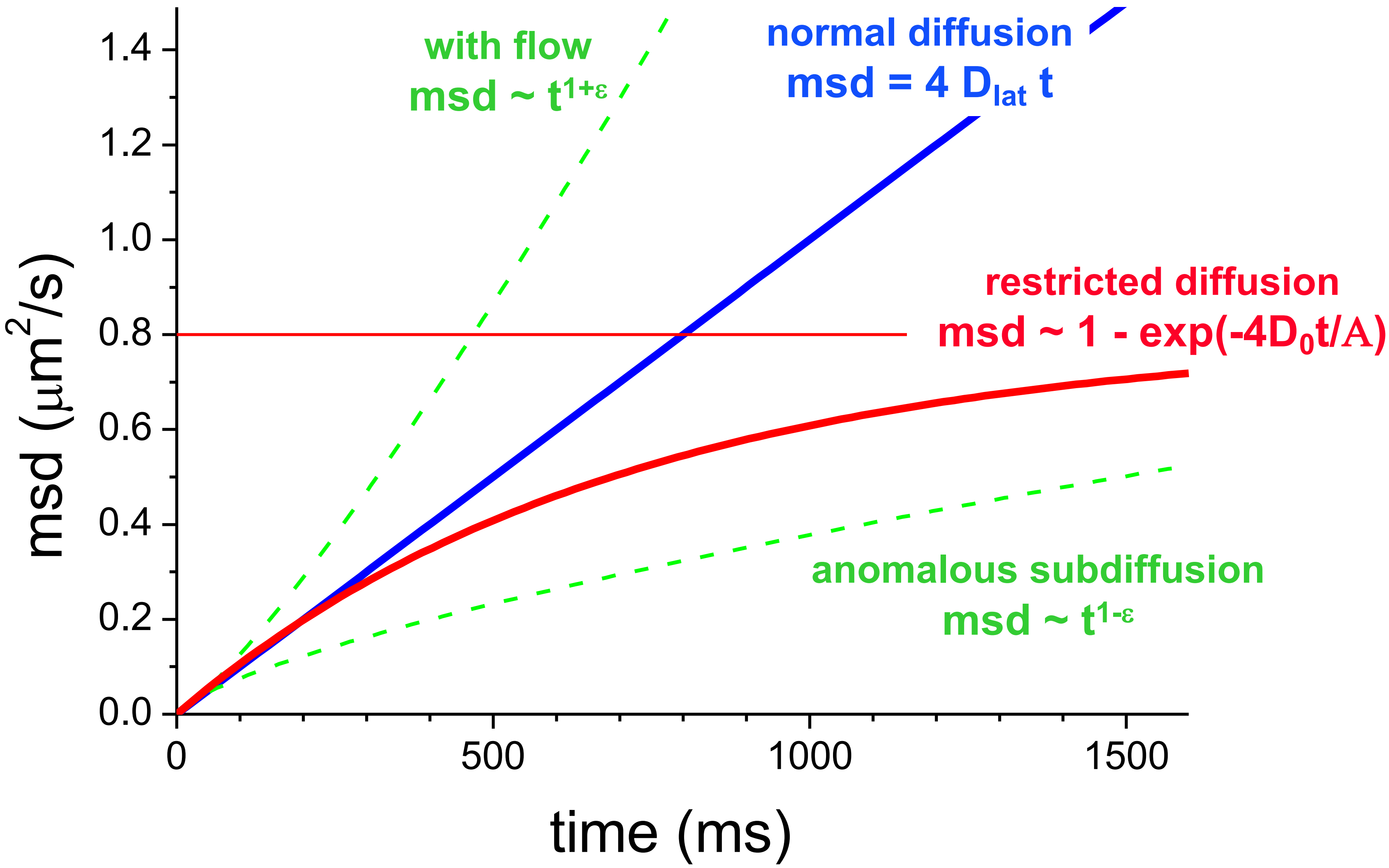
diffusion in an archipelago

$$msd = 4D_0 t^{1-\varepsilon}$$

M.P.Saxton, Biophys.J. 64 (1993) 1766.

J.P.Bouchaud & A.Georges
"The physical mechanisms of anomalous diffusion."
Disorder & Mixing (1988) 19

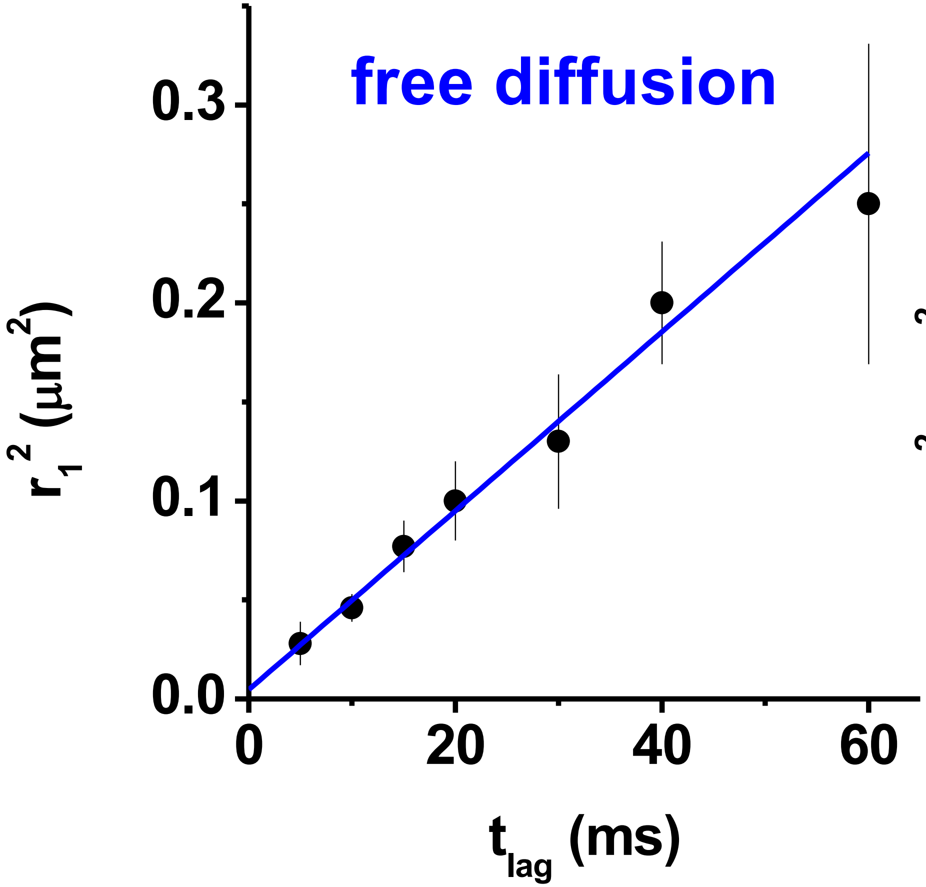
diffusion: summary

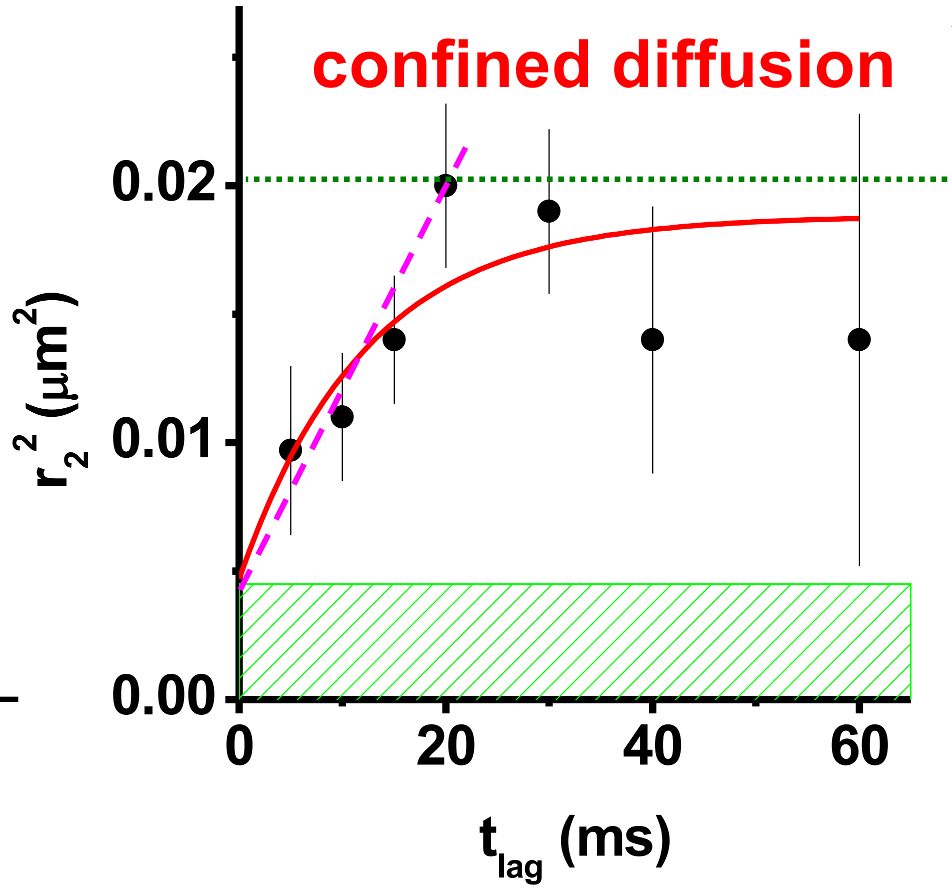


diffusion of H-Ras membrane anchor

Lommerse, Cognet, Blab, Harms, Snaar-Jagalska,
Spaink & **TS,** Biophys.J. 86 (2004)

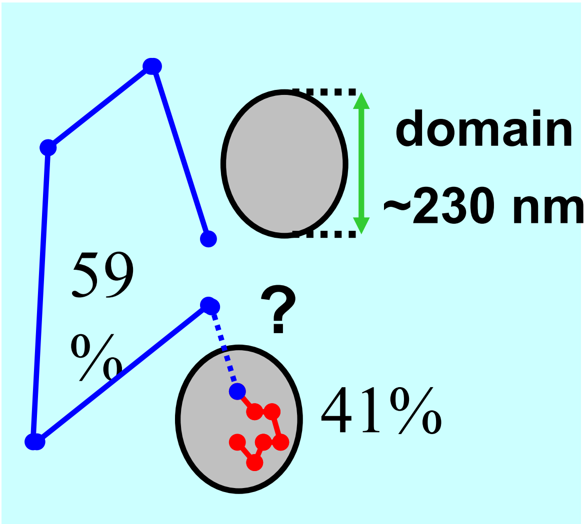
cell: 3T3-A14
37 °C





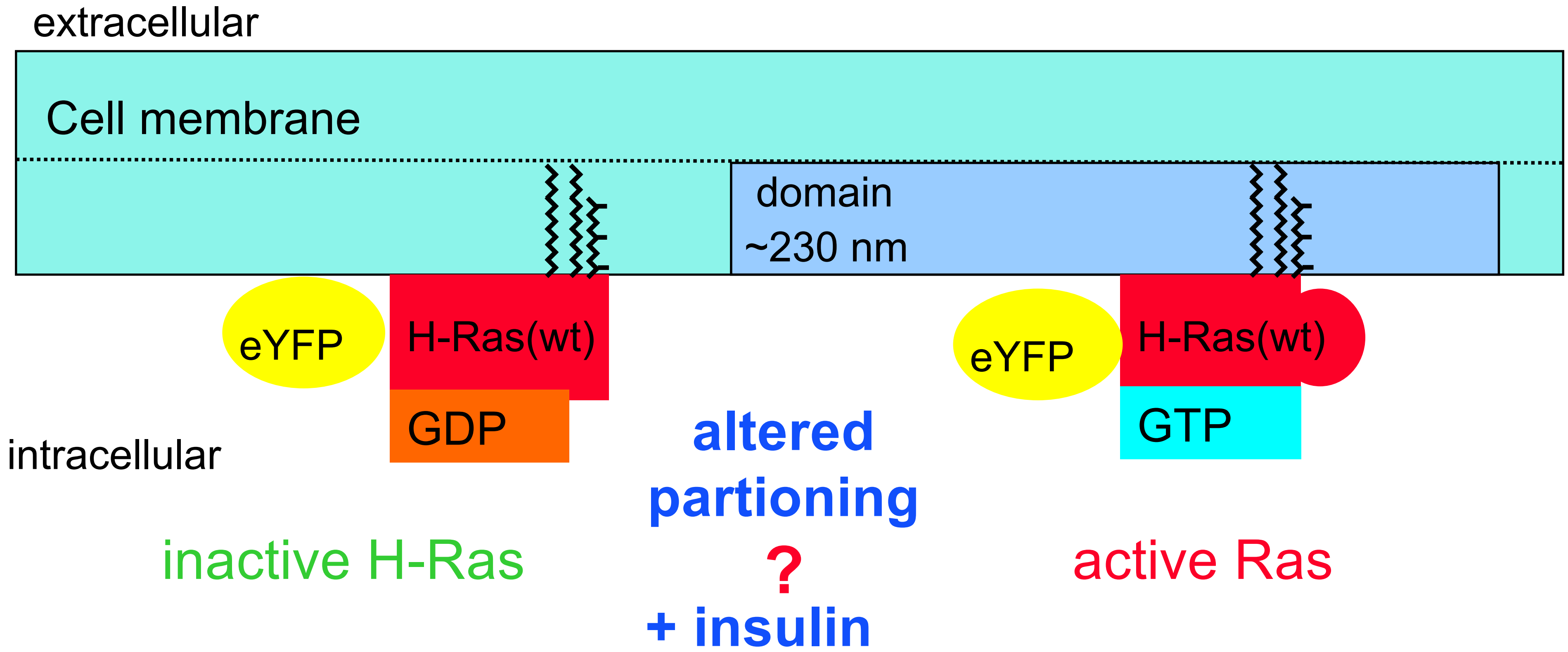
- $D = 1.13 \pm 0.09\ \mu m^2/s$
- $D_0 = 0.29 \pm 0.10\ \mu m^2/s$
- domains: 232 ± 31 nm

diffusion of the anchor: domains



- observed domains in cytoplasmic leaflet of plasma membrane

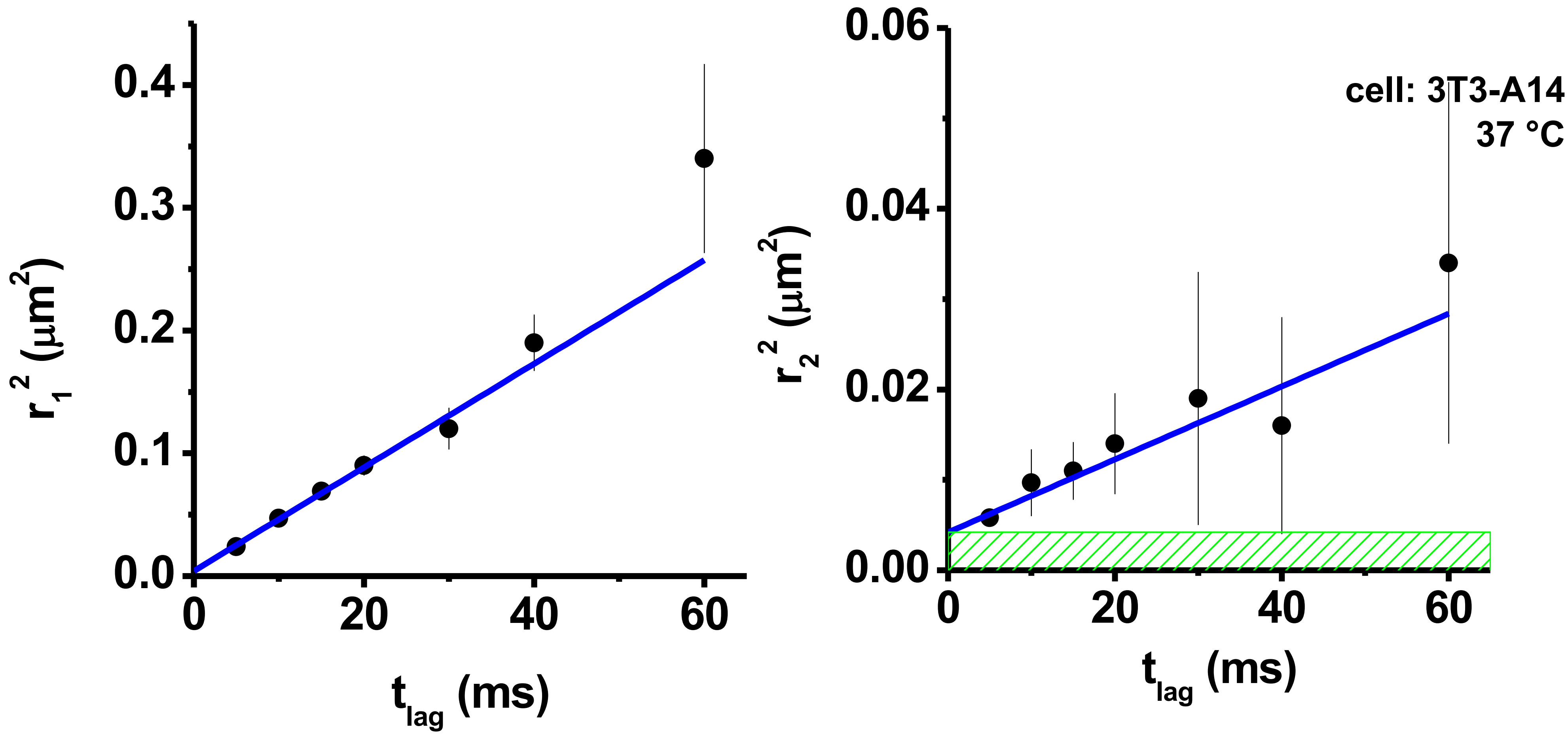
H-Ras(wt) activation: domains involved?



after addition of insulin we expect:

- increase in slow diffusing fraction ~20%
- reduction of diffusion coefficient of fast fraction

eYFP-H-Ras(wt) unstimulated

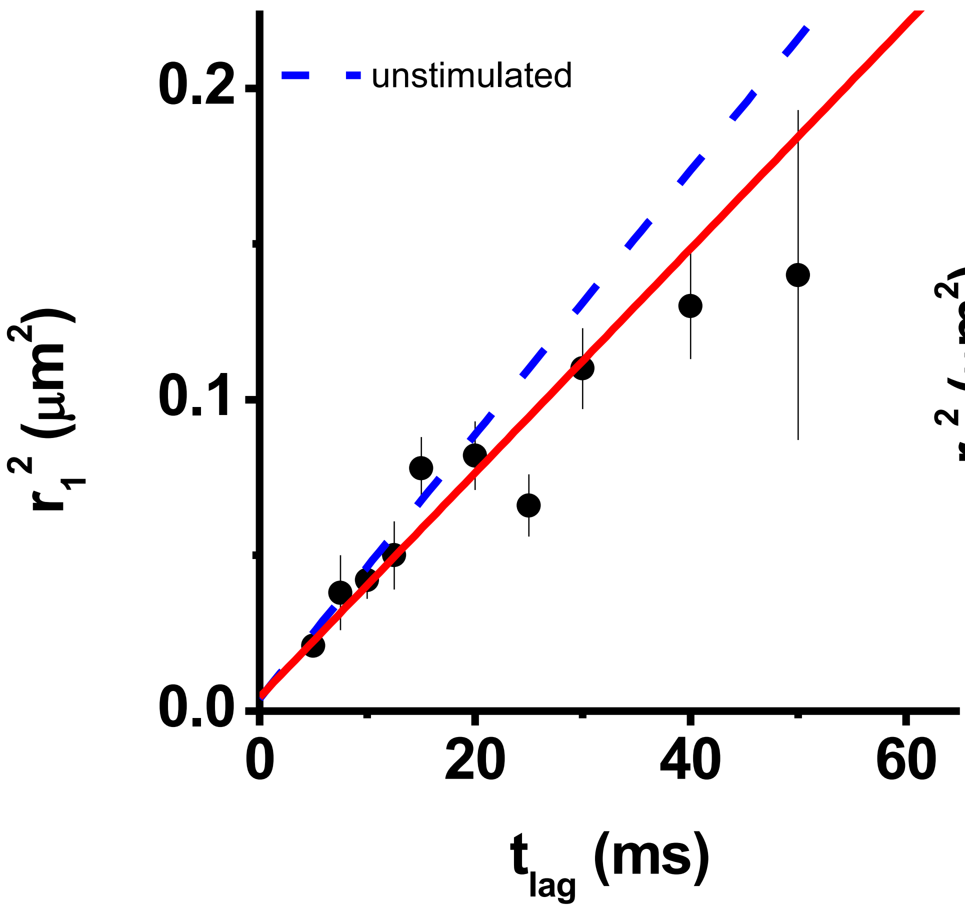


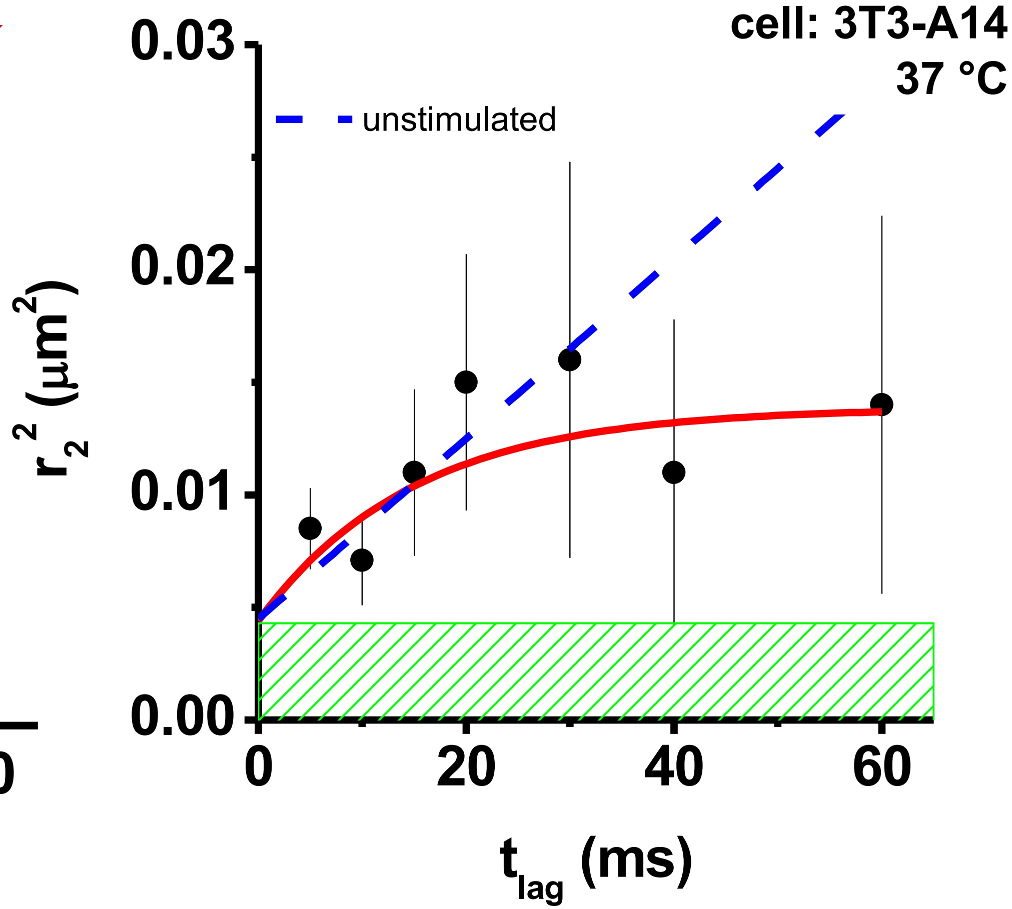
- 78 ± 4 % of population
- D = 1.06 ± 0.04 µm^2/s
- no domains observed

- 22 ± 4 % of population
- D = 0.10 ± 0.03 µm^2/s
- no domains observed

Lommerse, Snaar-Jagalska,
Spaink & **TS,** JCS (2005)

eYFP-H-Ras(wt) 5 min stimulated



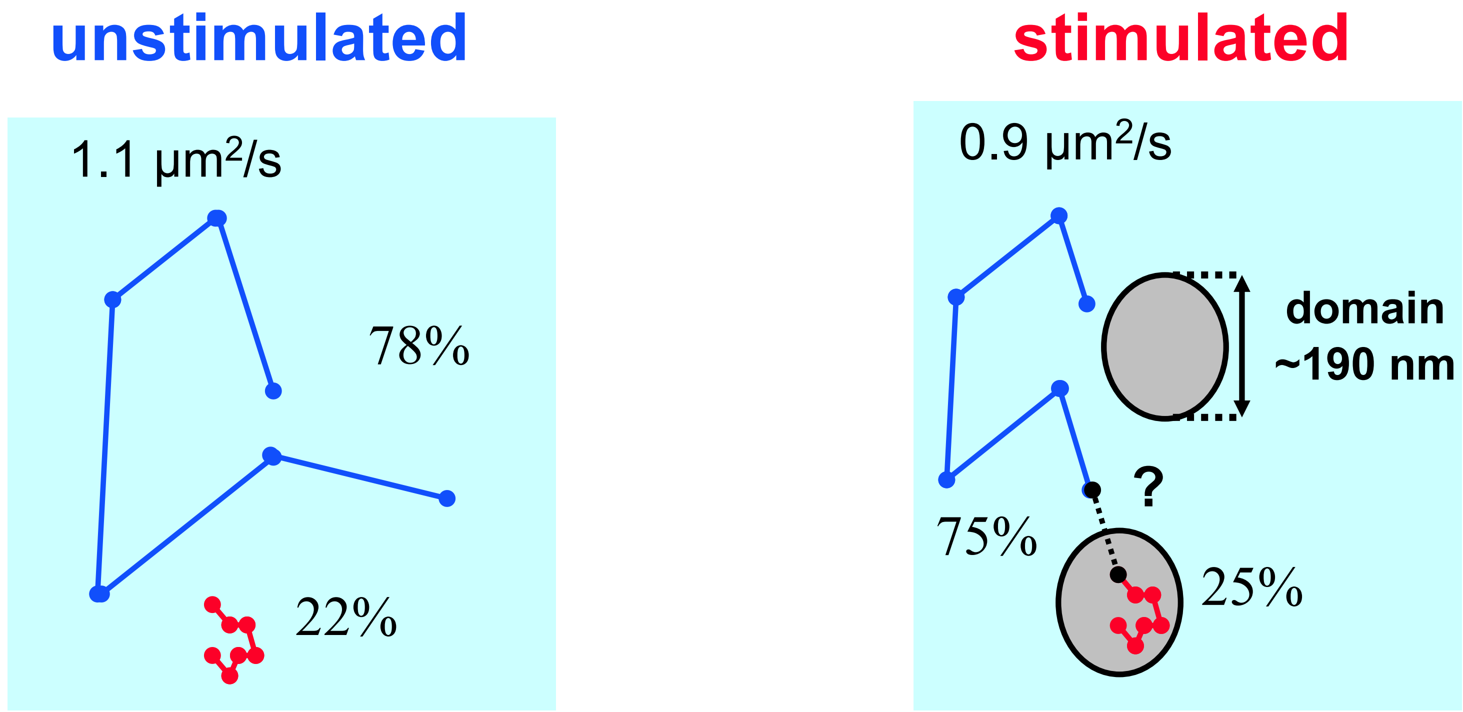


- 75 ± 6 % of population
- D = 0.93 ± 0.04 μm2/s
- no domains observed

- 25 ± 6 % of population
- D_0 = 0.16 ± 0.09 μm2/s
- domains (190 ± 54 nm)

Lommerse, Snaar-Jagalska, Spaink & **TS,** JCS (2005)

eYFP-HRas(wt) activation



after activation HRas is captured in domains of 200 nm size

the team @ Leiden

http://www.biophys.leidenuniv.nl/Research/FvL

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