Near-field optics

3. Near-field optics

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3.5 Scanning near-field optical microscopy : Applications

- *3.5.1 Single molecule imaging*
- *3.5.2 Imaging of single proteins in biological membranes*
- ***3.5.3 Autocorrelation measurements***
- *3.5.4 Fluorescence Correlation Spectroscopy*
- *3.5.5 Observation of single protein transport through a biological membrane*

Autocorrelation and Crosscorrelation

Definitions:

The autocorrelation function of a time-dependent function f(t) is given by

$$ACF(\tau) = \int_{-\infty}^{+\infty} f(t) \cdot f(t+\tau)\, dt$$

with lag time τ.

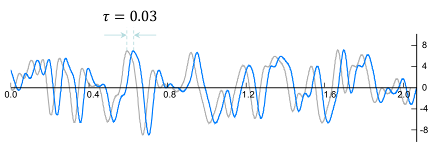
The crosscorrelation function of two different functions f(t) and g(t) is

$$CCF(\tau) = \int_{-\infty}^{+\infty} f(t) \cdot g(t+\tau)\, dt$$

Example

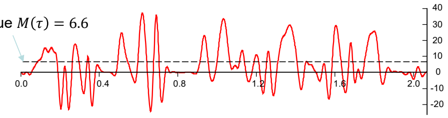
$E_1(t)$

$E_1(t+\tau)$



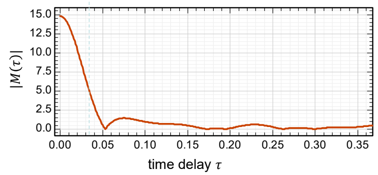
Mean value $M(\tau) = 6.6$

$E_1(t) \cdot E_1(t+\tau)$



$$M(\tau) = \frac{1}{T}\int_0^T E_1(t)\, E_1(t+\tau)\, \mathrm{d}t$$

(„autocorrelation function")



Autocorrelation of the light-electric field

First-order electric field correlation function:

$$\langle E^*(t)\, E(t+\tau)\rangle = \frac{1}{T}\int_T E^*(t) E(t+\tau) dt$$ with measurement time T

Normalized first-order electric field correlation function:

$$g^{(1)}(\tau) = \frac{\langle E^*(t)\, E(t+\tau)\rangle}{\langle E^*(t)\, E(t)\rangle}$$ with $\langle E^*(t)\, E(t)\rangle = |E(t)|^2 \sim \bar{I}$ (average intensity)

- ➢ depends on phase (interference, e.g. Michelson intereferometer)

Normalized second-order electric field correlation function:

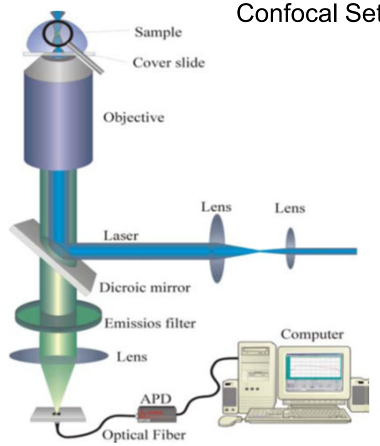
$$g^{(2)}(\tau) = \frac{\langle I(t)\, I(t+\tau)\rangle}{\bar{I}^2}$$

- ➢ depends on photon statistics (quantum optics)

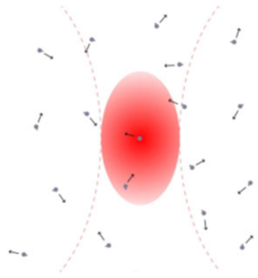
Near-field optics

Fluorescence Correlation Spectroscopy

Confocal Set-up

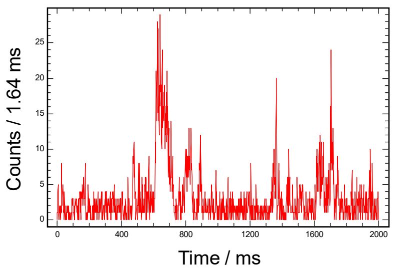


Combined excitation and detection volume



Haustein & Schwille (2004)

FCS



- How long, in average, does a molecule stay in the excitation-detection volume?

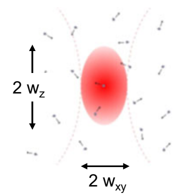
  → residence time τ

- How fast, in average, does a molecule move through the excitation-detection volume?

  → diffusion coefficient *D*

Fluorescence Correlation Spectroscopy

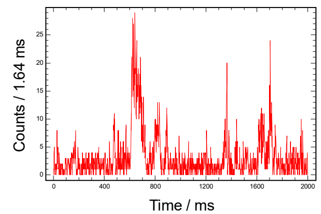
Example: Diffusion of molecules through a Gaussian excitation volume



$$D = \frac{k_B T}{6\pi\, \eta\, R_H}$$

$$\langle \Delta x^2 \rangle = 2 \cdot D \cdot \Delta t$$

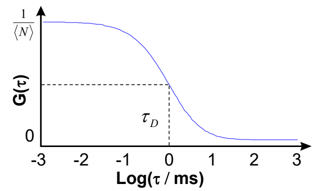
$$\tau_D = \frac{w_{xy}^2}{4\, D}$$



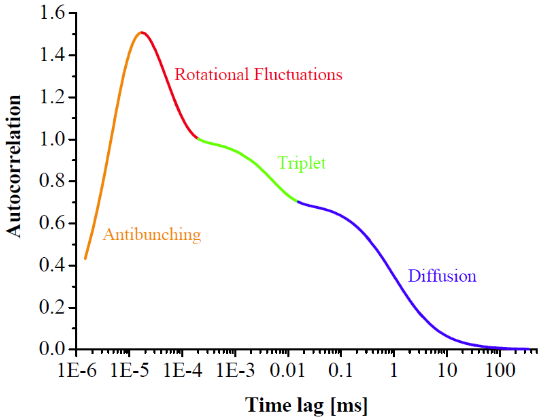
Autocorrelation analysis:

$$G(\tau) = \frac{<\delta F(t+\tau) \cdot \delta F(t)>}{<F(t)>^2}$$

$$= \frac{1}{\langle N \rangle} \cdot \frac{1}{1+\frac{\tau}{\tau_D}} \cdot \frac{1}{\sqrt{1+\frac{w_{xy}^2}{w_z^2} \cdot \frac{\tau}{\tau_D}}}$$

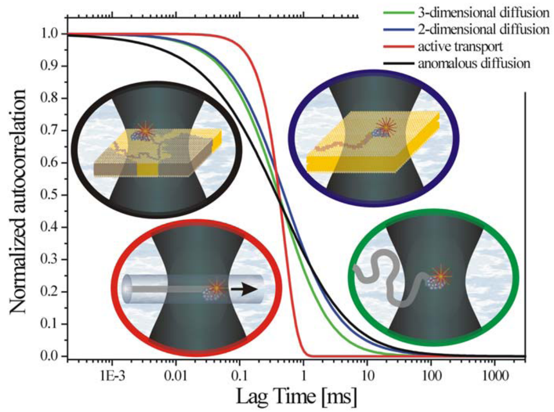


Timescales of Various Processes



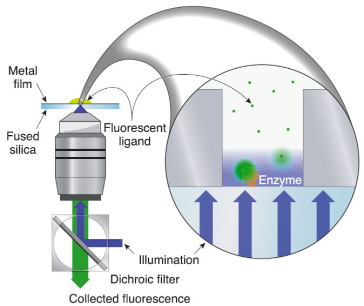
Haustein & Schwille (2004)

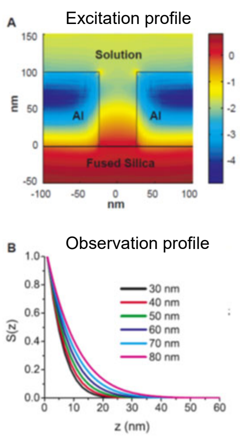
ACFs of different Diffusional Processes



Haustein & Schwille (2004)

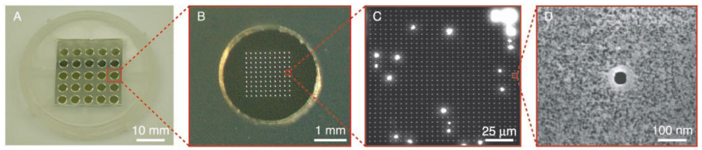
FCS at High Concentrations: Zero Mode Wave Guides (ZMW)

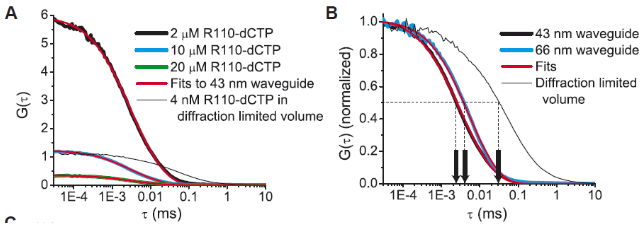




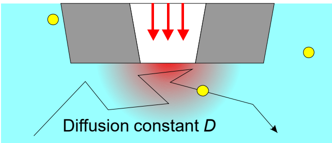
Levene et al. (Science 2003)

FCS using ZMWs





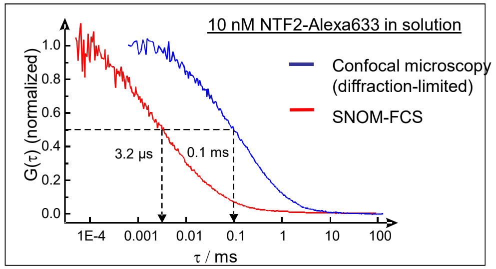
Combination of NSOM with Fluorescence Correlation Spectroscopy (FCS)



Fluorescence intensity: *F*(*t*)

Autocorrelation function:

$$G(\tau) = \frac{< F(t+\tau)\cdot F(t) >}{< F(t) >^2} - 1$$



Near-field optics

3. Near-field optics

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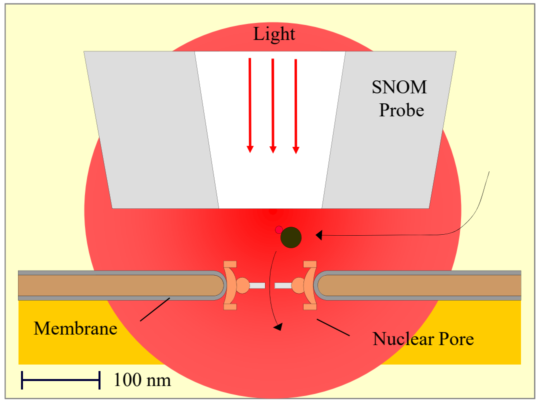
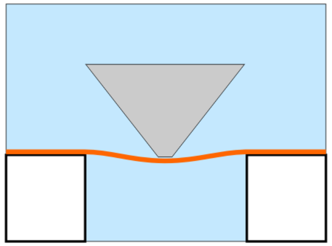
Goal: Observation of Single Transport Events Through a Single Pore in a Biological Membrane



SNOM on a Freestanding Membrane: Distance Control



Features

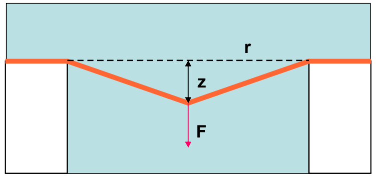
- Imaging at "constant height" – no active feedback control!
- Probe-sample gap depends on membrane tension (penetration depth of probe in trough)

Estimate of the Elastic Force

Energy density g_{ext} of an isotropic lateral membrane tension:

$$g_{ext} = \frac{1}{2} K_A \left(\frac{\delta A(z)}{A} \right)^2$$

K_A	area compressibility module
$\delta A/A$	relative area change of the membrane



Restoring Force F_R in z-direction for a membrane deformed to a cone:

$$W = g_{ext} \cdot A \cong \frac{\pi}{8} K_A \frac{z^4}{r^2}$$

$$F_R = -\frac{\partial W}{\partial z} \cong -\frac{\pi^2}{2} \frac{K_A}{A} z^3 \qquad (z << r)$$

Estimate of the Elastic Force

Energy density g_{ext} of an isotropic lateral membrane tension:

K_A = 1 N/m
z = 0.2 µm
r = 10 µm

F_A = 0.13 nN

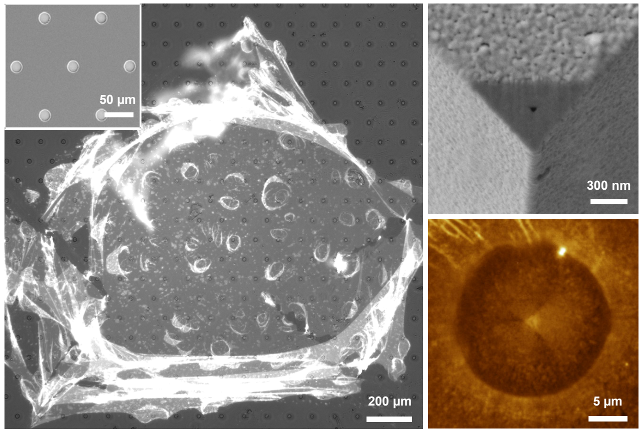
End face of the probe: A = 0.25 µm²

Density of pores: n = 40 NPCs/µm²

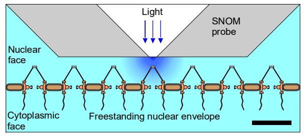
F_{Pore} **= 13 pN**

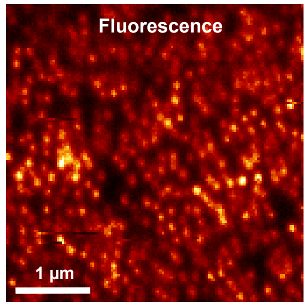
$$F_R = -\frac{\partial W}{\partial z} \cong -\frac{\pi^2}{2} \frac{K_A}{A} z^3 \qquad (z << r)$$

Preparation of Freestanding Membrane Patches



SNOM on a Freestanding Nuclear Membrane (*Xenopus laevis*)





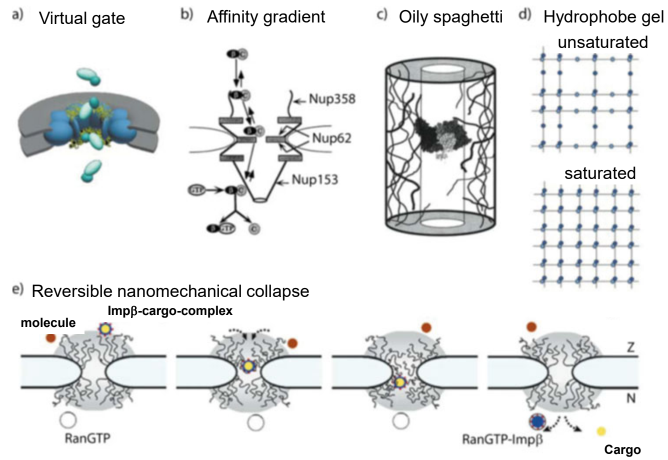
Antibody labeling:
mAb414-Alexa633

Excitation wavelength:
633 nm

Scan-Speed:
4 µm/s

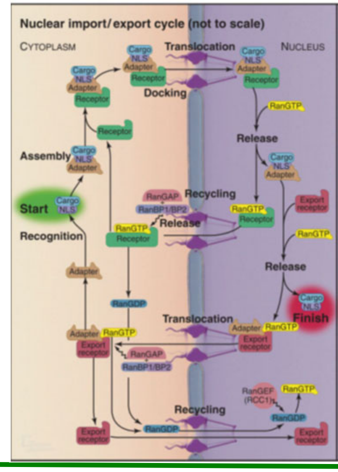
Interaction force:
< 0.1 nN/NPC

Models of Nuclear Transport



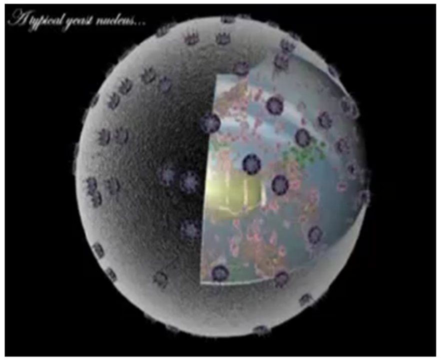
M. Herrmann (2008)

Biochemistry of Nuclear Transport



T.D. Pollard & W.C. Earnshaw (2002)

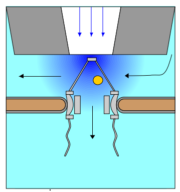
Animation



Samir S. Patel

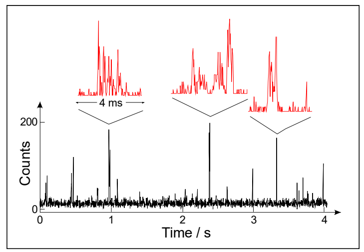
NSOM: Binding and Diffusion of NTF2 at the Nuclear Pore Complex

Schematic



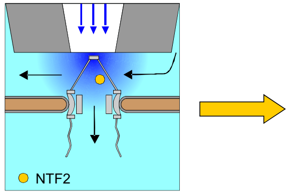
Fluorescently labeled NTF2

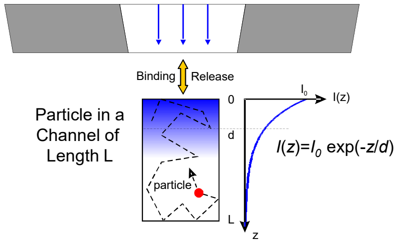
Fluorescence *F*(*t*) of NTF2-A633



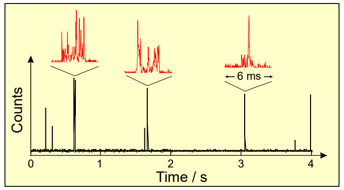
Time bin 1.6 ms
Time bin 25 µs

Confined Diffusion of a Particle in a Nuclear Pore: Model





Monte-Carlo Simulation



Parameters:

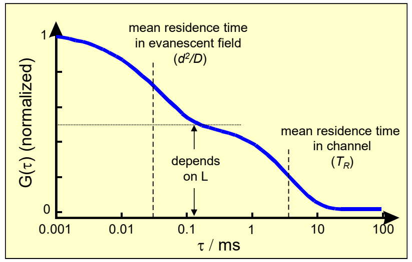
Depth of evanescent field	d	= 25 nm
Channel length	L	= 100 nm
Diffusion constant	D	= 20 µm²/s
Mean residence time	T_R	= 4 ms

Confined-Diffusion Model: Exact Solution

Fluorescence Autocorrelation Function

$$G(\tau) \propto \frac{1}{\langle N \rangle} \cdot \left(1 + 2\sum_{m=1}^{\infty} \frac{\exp\left(-(m\pi/L)^2 D\,\tau\right)}{\left(1+(m\pi\, d/L)^2\right)^2} \cdot \left(\frac{1-(-1)^m \exp(-L/d)}{1-\exp(-L/d)} \right)^2 \right) \cdot \exp(-\tau/T_R)$$

$\langle N \rangle$	mean number of molecules	D	diffusion constant
d	depth of evanescent field	T_R	mean residence time
L	channel length		

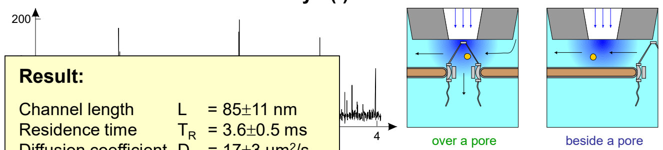


Parameters:

- $\langle N \rangle$ = 1
- d = 25 nm
- L = 100 nm
- D = 20µm²/s
- T_R = 4ms

Experiment: Single Molecule Diffusion through Nuclear Pores

Fluorescence intensity $F(t)$



Result:

Channel length	L	= 85±11 nm
Residence time	T_R	= 3.6±0.5 ms
Diffusion coefficient	D	= 17±3 µm²/s

Fluorescence Correlation $G(\tau)$