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

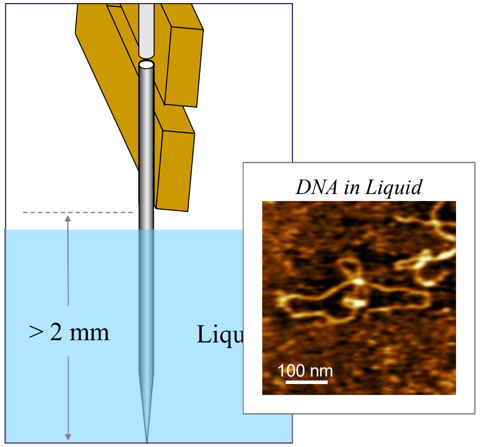
3.5.2 Imaging of single proteins in biological membranes

3.5.3 Autocorrelation measurements

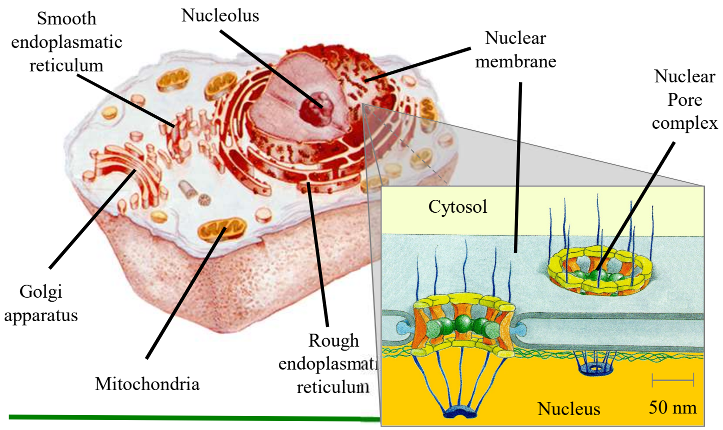
3.5.4 Fluorescence Correlation Spectroscopy

3.5.5 Observation of single protein transport through a biological membrane

SNOM for Biology: Distance Control in Liquid

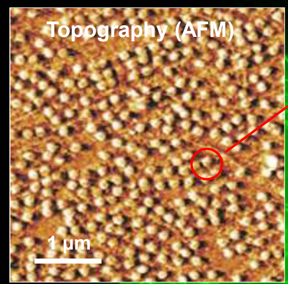


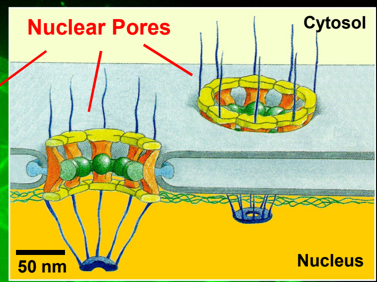
Organels in a biological cell



Alberts et al, VCH, 1995; Lehninger et al, Spektrum, Akad. Verl., 1994

Nuclear Membrane of *Xenopus laevis* Oocytes





Stoffler et al., JMB (1999)

100 µm

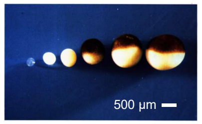
Nuclear Pore Complex (NPC)

Major gateway for passive diffusion of ions and small molecules, active transport of proteins, RNA etc.

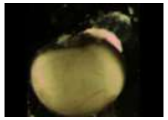
Complex of	> 30 proteins
Molecular mass	~125 MDa
NPC diameter	~120 nm
Central transport channel	~40 nm

Nuclei of *Xenopus Laevis*

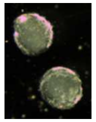
African Clawed Frog
(Xenopus Laevis)



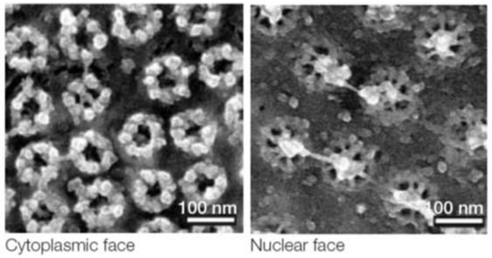
Oocytes –
immature egg cells



extraction
of the nucleus

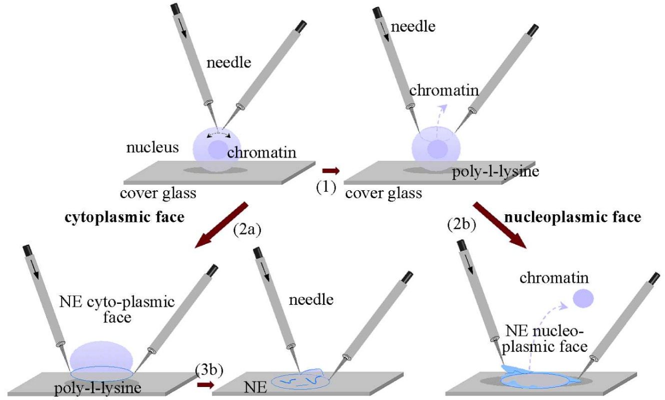


isolated
nuclei



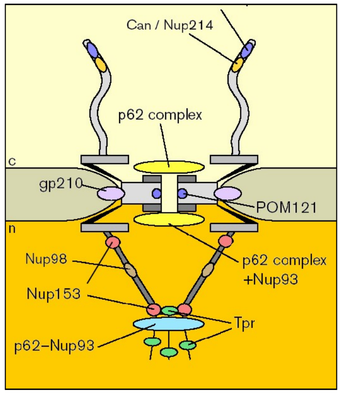
Fahrenkrog & Aebi (2003)

Preparation of the Nuclear Envelope for Scanning Probe Microscopies



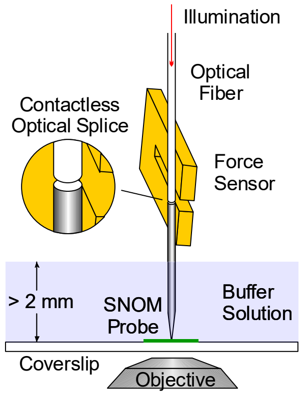
Höppener (2003)

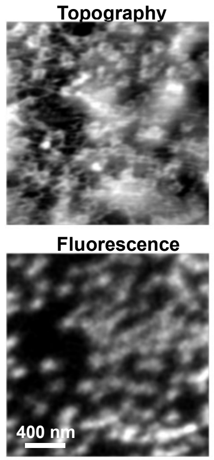
Fluorescence Labeling using Antibodies



D. Stoffler et al. (1999); Fahrenkrog et al. (2002)

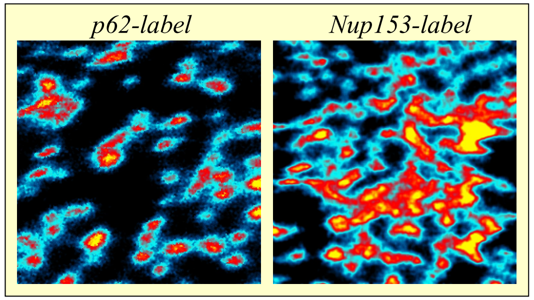
SNOM Imaging of a Supported Nuclear Envelope under Physiological Conditions





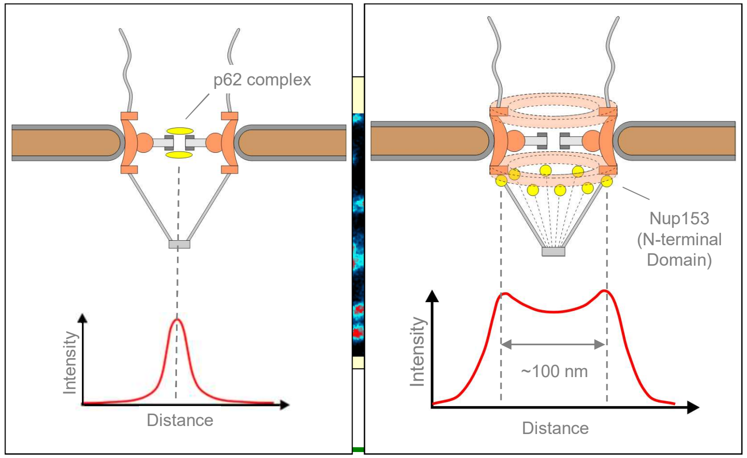
C. Höppener et al., Biophys. J. (2005)

Comparison: p62- and Nup153-Antibody Labeling



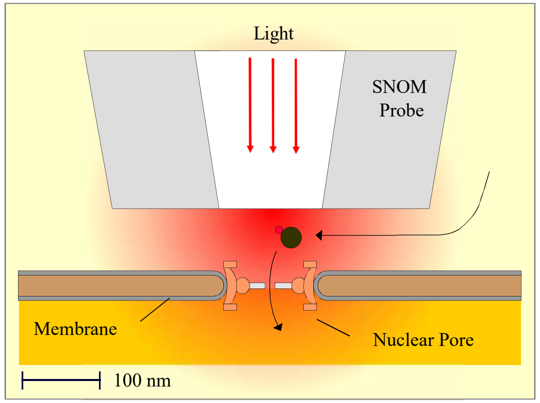
C. Höppener et al., Biophys. J. (2005)

Comparison: p62- and Nup153-Antibody Labeling



C. Höppener et al., Biophys. J. (2005)

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



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

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

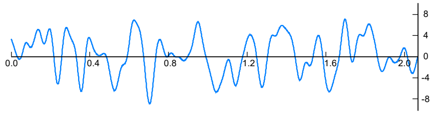
with lag time τ.

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

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

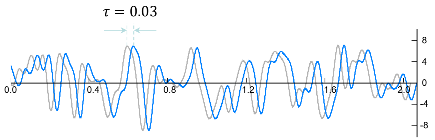
Example

$E_1(t)$



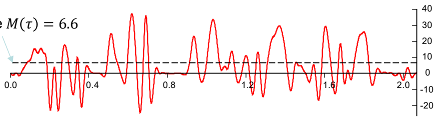
Example

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



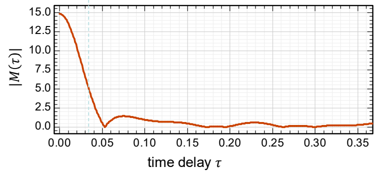
Mean value $M(\tau) = 6.6$

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

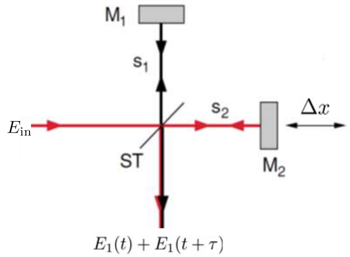


$$M(\tau) = \frac{1}{T} \int_0^T E_1(t)\, E_1(t+\tau)\, \mathrm{d}t$$

(„autocorrelation function")



Example: Michelson-Interferometer

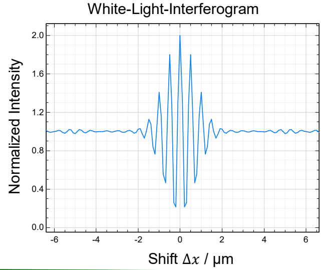


M_1, M_2	Mirror
ST	Beamsplitter
s_1, s_2	Beampath

$$s_2 = s_1 + \Delta x$$

$$\tau = \frac{2\Delta x}{c_0}$$

White-Light-Interferogram



Detector measures

$$I(t) \propto 2\langle E_1^2(t)\rangle_T + \langle E_1(t) \cdot E_1(t+\tau)\rangle_T$$

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:

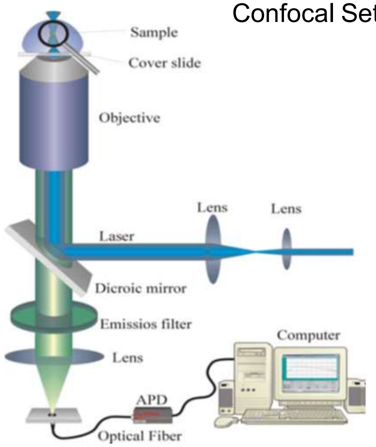
$$g^{(2)}(\tau) = \frac{\langle I(t)\,I(t+\tau)\rangle}{\bar{I}^2}$$

- depends on photon statistics (quantum optics)

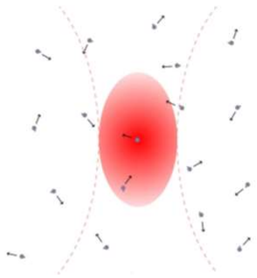
Near-field optics

Fluorescence Correlation Spectroscopy

Confocal Set-up

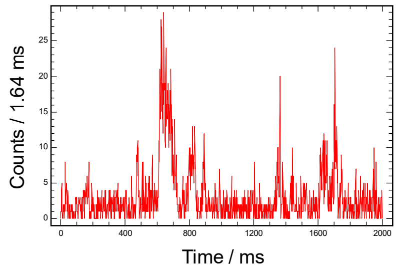


Combined excitation and detection volume



Haustein & Schwille (2004)

FCS



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

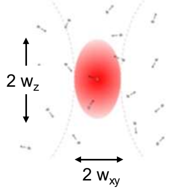
  → residence time τ

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

  → diffusion coefficient *D*

Fluorescence Correlation Spectroscopy

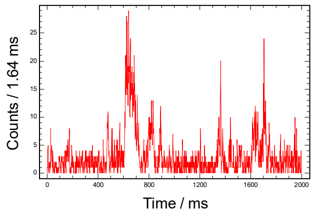
Example: Diffusion of molecules through a Gaussian excitation volume



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

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

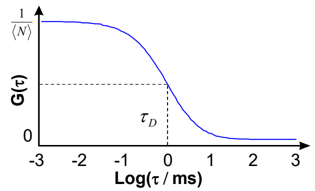
$$\tau_D = \frac{w_{xy}^2}{4\,D}$$



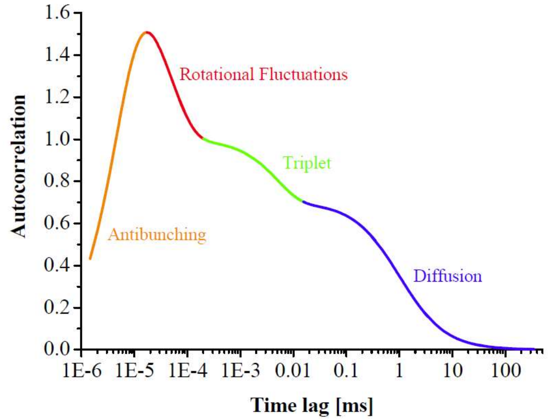
Autocorrelation analysis:

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

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

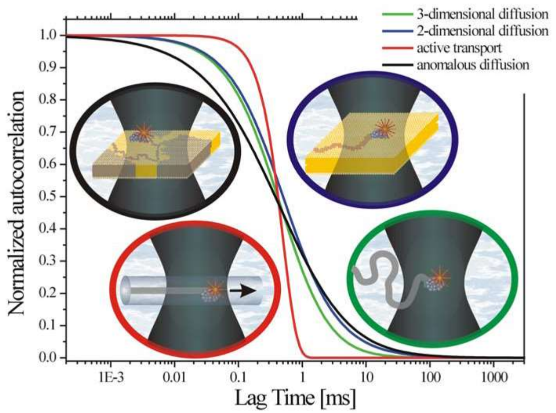


Timescales of Various Processes



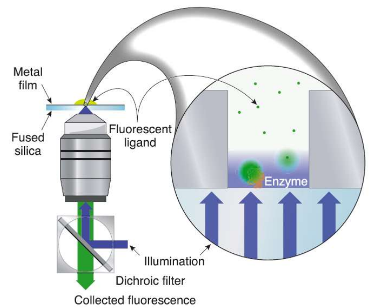
Haustein & Schwille (2004)

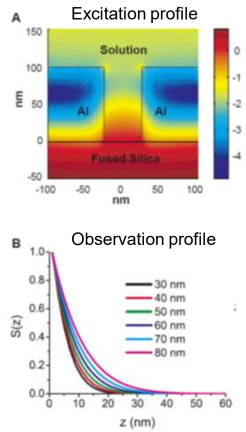
ACFs of different Diffusional Processes



Haustein & Schwille (2004)

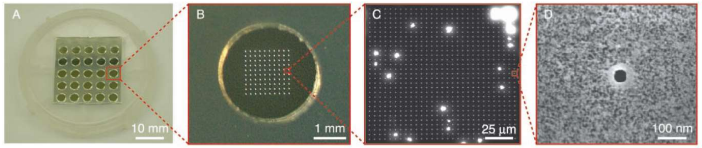
FCS at High Concentrations: Zero Mode Wave Guides (ZMW)

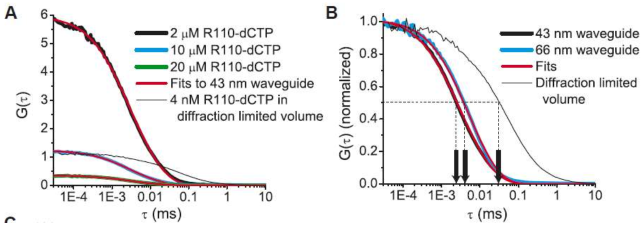




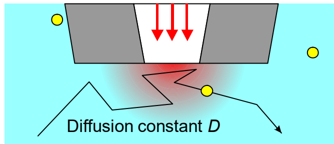
Levene et al. (Science 2003)

FCS using ZMWs





Combination of NSOM with Fluorescence Correlation Spectroscopy (FCS)



Fluorescence intensity: $F(t)$

Autocorrelation function:

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