Near-field optics

3. Near-field optics

⋮

3.5 Scanning near-field optical microscopy : Applications

3.5.1 Single molecule imaging

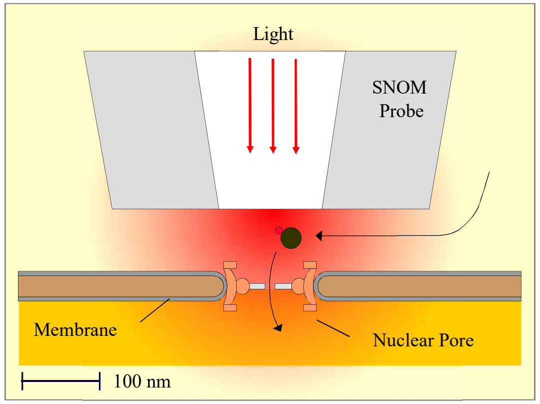
3.5.2 Imaging of single proteins in biological membranes

3.5.3 Autocorrelation measurements

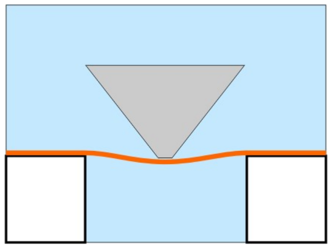
3.5.4 Fluorescence Correlation Spectroscopy

3.5.5 Observation of single protein transport through a biological membrane

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



SNOM on a Freestanding Membrane: Distance Control



Features

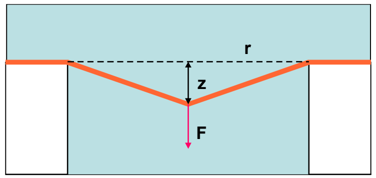
- Imaging at “constant height“ – no active feedback control!
- Probe-sample gap depends on membrane tension (penetration depth of probe in trough)

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

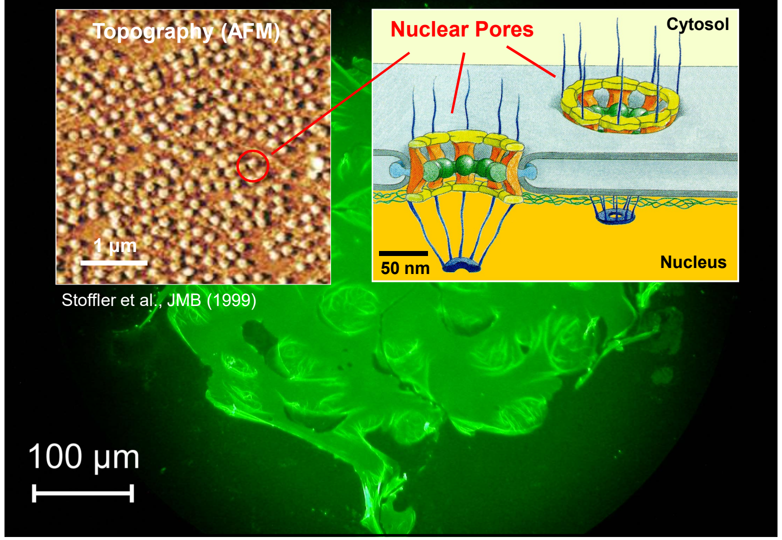


Restoring Force F_R in z-direction for a membrane deformed to a cone:

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

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

Nuclear Membrane of *Xenopus laevis* Oocytes



Estimate of the Elastic Force

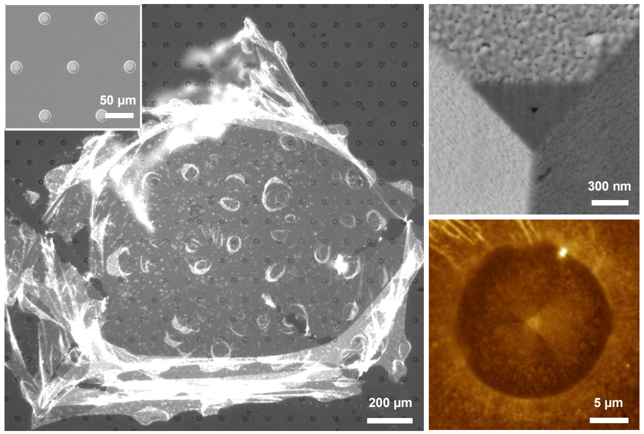
Energy density g_{ext} of an isotropic lateral membrane tension:

$$g = \frac{1}{\ }K\left(\delta A(z)\right)^2$$

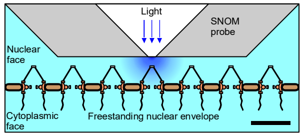
K_A = 1 N/m			
z = 0.2 µm		F_A = 0.13 nN	F_{Pore} = 13 pN
r = 10 µm			
End face of the probe:	A = 0.25 µm²		
Density of pores:	n = 40 NPCs/µm²		

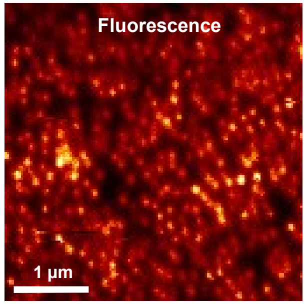
$$F_R = -\frac{\partial W}{\partial z} \cong -\frac{\pi^2}{2}\frac{K_A}{A} z^3 \qquad (z << r)$$

Preparation of Freestanding Membrane Patches



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





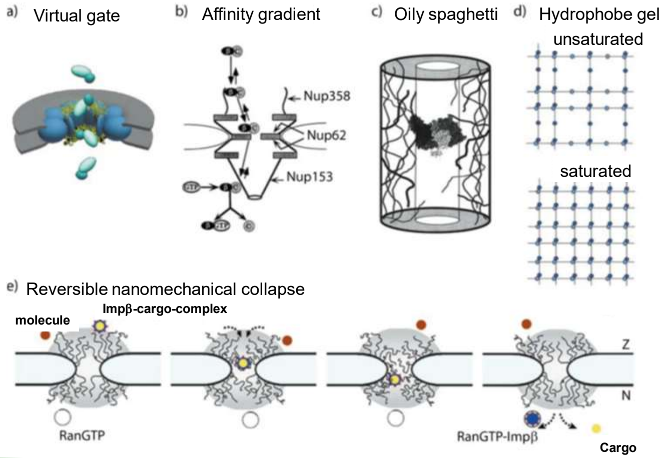
Antibody labeling:
mAb414-Alexa633

Excitation wavelength:
633 nm

Scan-Speed:
4 µm/s

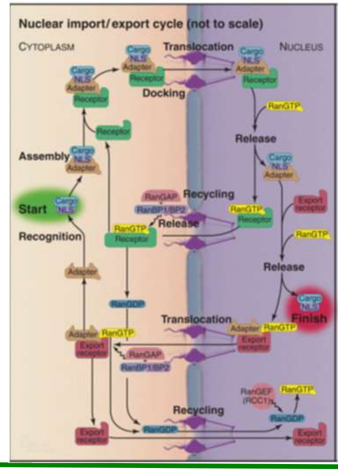
Interaction force:
< 0.1 nN/NPC

Models of Nuclear Transport



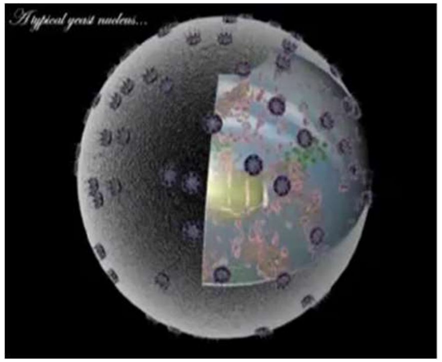
M. Herrmann (2008)

Biochemistry of Nuclear Transport



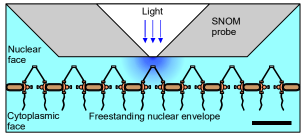
T.D. Pollard & W.C. Earnshaw (2002)

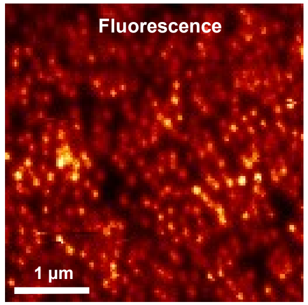
Animation



Samir S. Patel

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





Antibody labeling:
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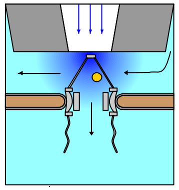
Excitation wavelength:
633 nm

Scan-Speed:
4 µm/s

Interaction force:
< 0.1 nN/NPC

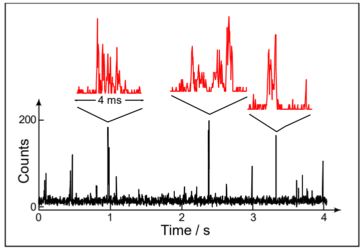
NSOM: Binding and Diffusion of NTF2 at the Nuclear Pore Complex

Schematic



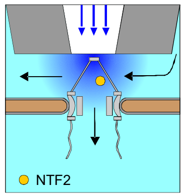
Fluorescently labeled NTF2

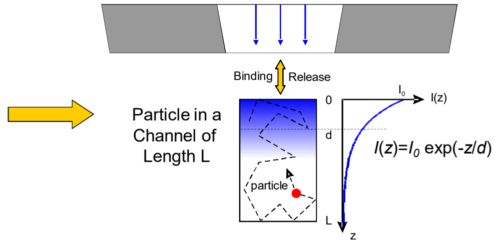
Fluorescence $F(t)$ of NTF2-A633



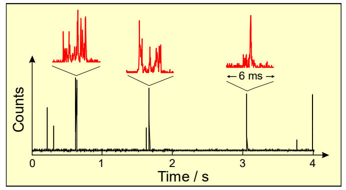
Time bin 1.6 ms
Time bin 25 µs

Confined Diffusion of a Particle in a Nuclear Pore: Model





Monte-Carlo Simulation



Parameters:

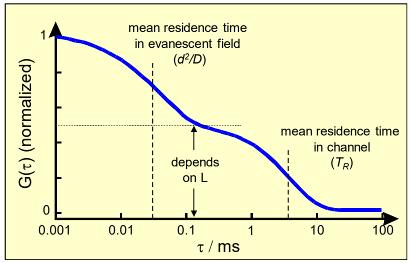
Depth of evanescent field d = 25 nm
Channel length L = 100 nm
Diffusion constant D = 20 µm²/s
Mean residence time T_R = 4 ms

Confined-Diffusion Model: Exact Solution

Fluorescence Autocorrelation Function

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

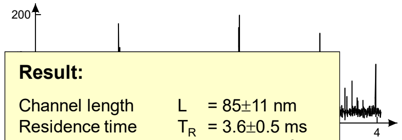
$\langle N \rangle$ mean number of molecules
d depth of evanescent field
L channel length
D diffusion constant
T_R mean residence time



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

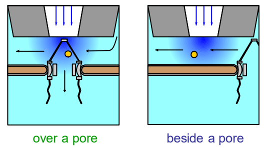
Experiment: Single Molecule Diffusion through Nuclear Pores

Fluorescence intensity $F(t)$

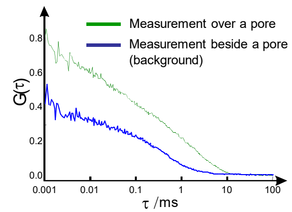


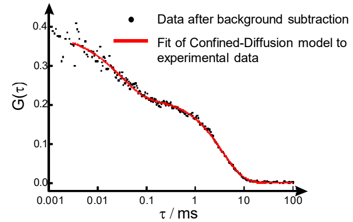
Result:

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



Fluorescence Correlation $G(\tau)$



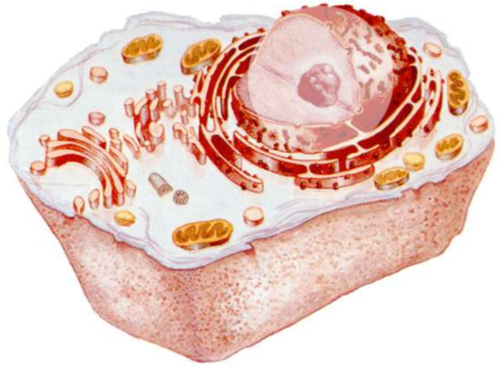


4. Nano-optics using far-field optical techniques

4.1 Introduction: single-molecule methods in biology
4.2 Single-molecule tracking (SMT)
4.3 Stochastic optical reconstruction microscopy (STORM)
4.4 4pi microscopy
4.5 Stimulated emission depletion (STED)
4.6 3D laser lithography using STED

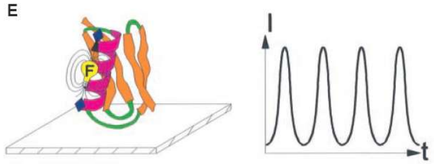
Single Molecule Microscopy in Biology

Section through a cell



Labelling & Physical Observable

Orientation of a single molecule



S. Weiss, Science 283, 1676 (1999)