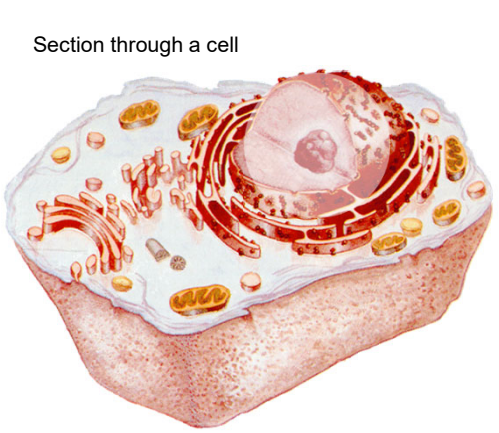
4. Nano-optics using far-field optical techniques

   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

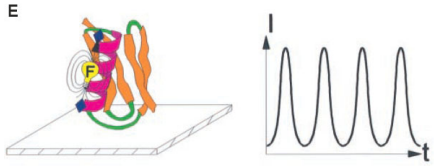
Single Molecule Microscopy in Biology

Section through a cell



Labelling & Physical Observable

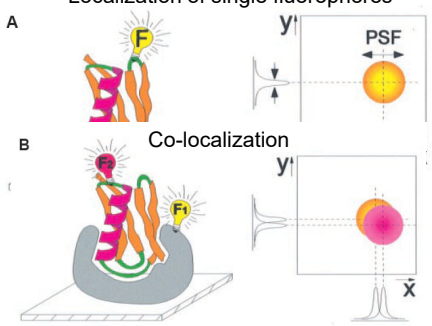
Orientation of a single molecule



S. Weiss, Science 283, 1676 (1999)

Labelling & Physical Observable

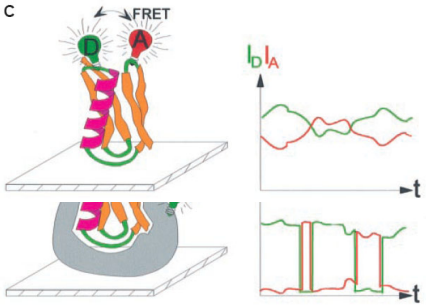
Localization of single fluorophores



S. Weiss, Science 283, 1676 (1999)

Labelling & Physical Observable

Fluorescence-Resonance-Energy-Transfer (FRET)



S. Weiss, Science 283, 1676 (1999)

Resonance Energy Transfer

Comparison of near- and far-field:

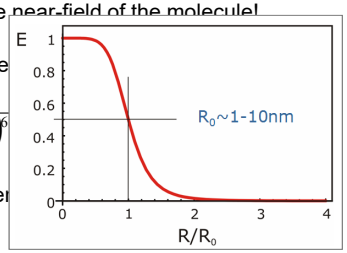
$$\left|\frac{E_{near}}{E_{far}}\right|^2 \sim \left|\frac{kr}{(kr)^3}\right|^2 \sim \frac{1}{(kr)^4}$$

Large field enhancement in the near-field of the molecule!

⇨ Resonance energy transfe

$$E(R) = \frac{1}{1+\left(\frac{R}{R_0}\right)^6}$$

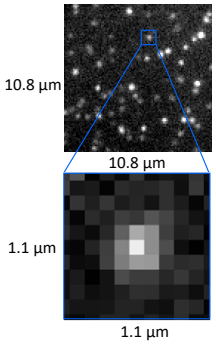
⇨ „Quenching“ of fluoresce

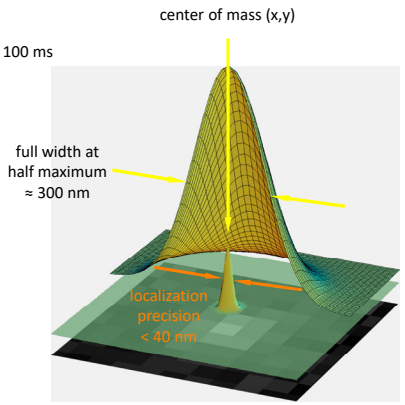


4. Nano-optics using far-field optical techniques

Precise Localization of Submicroscopic Probes

Single, surface-attached
Oyster565 molecules
ex 633 nm, em >650 nm, 2kW/cm^2, 100 ms





U. Kubitscheck, Uni Bonn

Localization Precision and Accuracy

Analysis of the lateral probability density distribution for N photon counts is based on a Gaussian as point spread function (PSF) of the microscope plus a constant background b per pixel area $a \times a$.

$$H = \frac{N}{2\pi\sigma^2}\, e^{-\frac{(x-x_0)^2+(y-y_0)^2}{2\sigma^2}} + \frac{b}{a^2} \qquad \sigma_x = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}\left(x_{0,i} - \bar{x}_0\right)^2}$$

with measured standard deviation σ_x in x-direction, and

n : number of estimates; x_0 : true position of molecule
$x_{0,i}$: i-th estimate of the position; $\bar{x}_0$: mean value of the estimates

As a good approximation for the principle performance limit (Cramer-Rao Lower Bound, CRLB) one gets for the uncertainty of the mean position of the molecule:

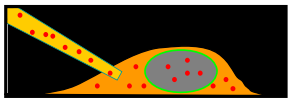
$$(\Delta x_0)^2 \cong \frac{\sigma_x^2 + \frac{a^2}{12}}{N}\,(1 + 4\tau) \quad ; \qquad \tau = \frac{b}{N}\,\frac{2\pi\sigma^2}{a^2}$$

with normalized background τ.

Rieger and Stallinga, ChemPhysChem (2014)

Study of Living Cells

Micro-injection

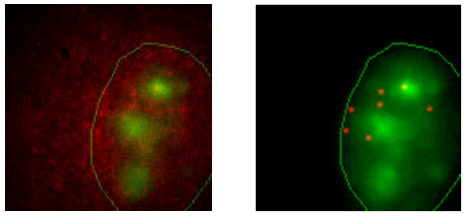


Micro injection of fluorescence-labelled molecules into the cyto-plasma of living cells with subsequent transport into the cell nucleus.

U. Kubitscheck, Uni Bonn

Intra-nuclear Mobility of Micro-injected U1 snRNPs

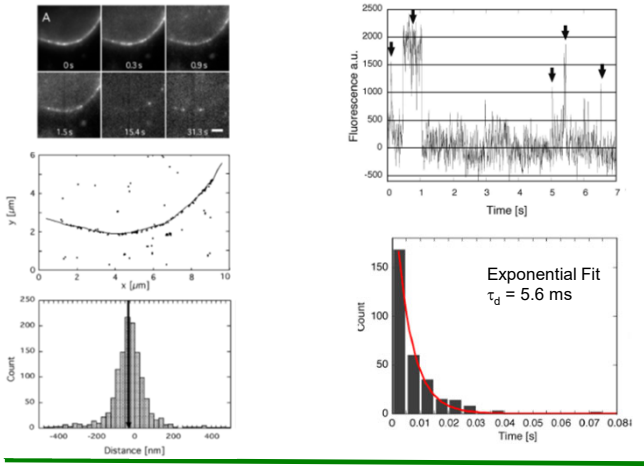
Living HeLa cells
ASF/SF2-GFP temporarily transfected
U1 snRNP-Cy5 micro-injected



10-fold slowdown 10-fold slowdown

U. Kubitscheck, Uni Bonn

Dwell Time of NTF2 at NPCs



U. Kubitscheck et al. (2005)