
ICFO
Institut
de Ciències
Fotòniques
Optical nanotools & mechanical manipulation
for subcellular studies
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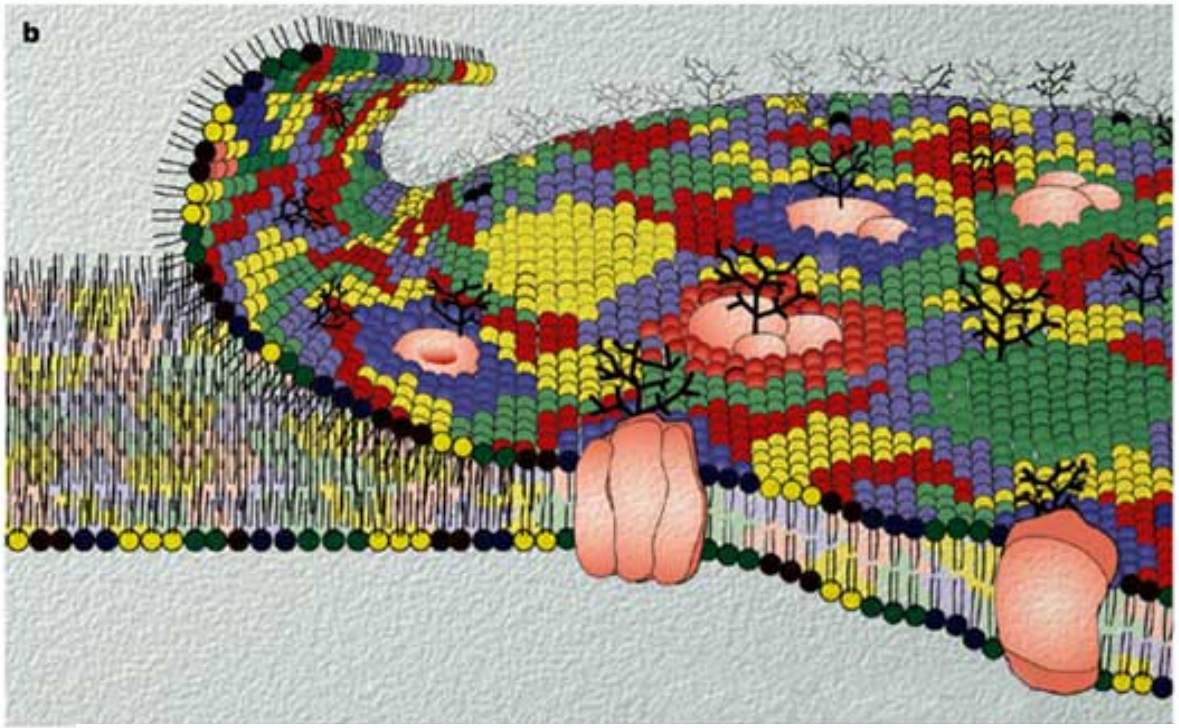
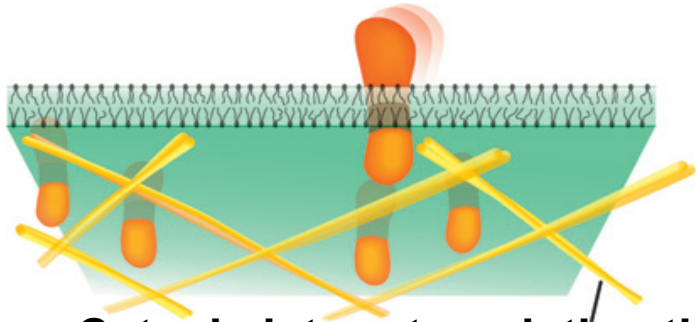
The Barcelona
Super-resolution
Nanoscopy Alliance

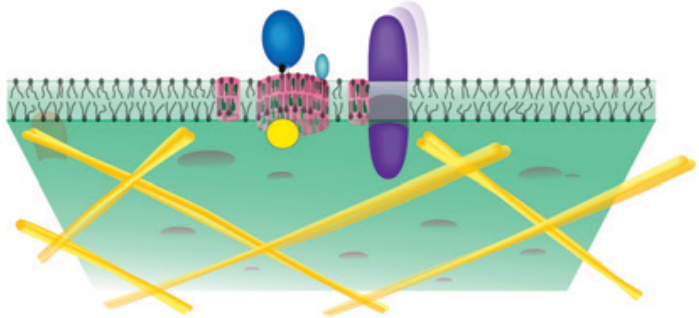
Key property of cell membranes: Compartmentalization



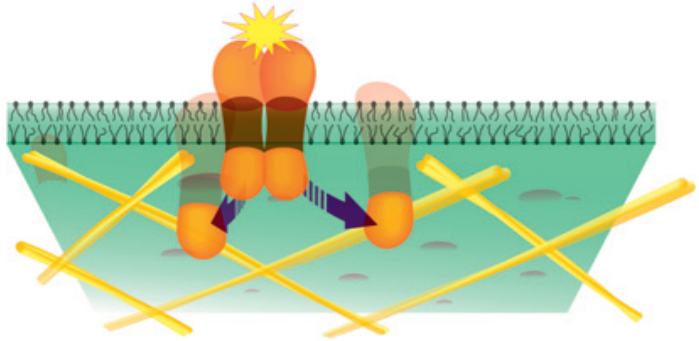
M. Edidin, *Nature Rev. Mol. Cell. Biol, 2003*



Cytoskeleton: templating the membrane



Lipids: favoring interaction



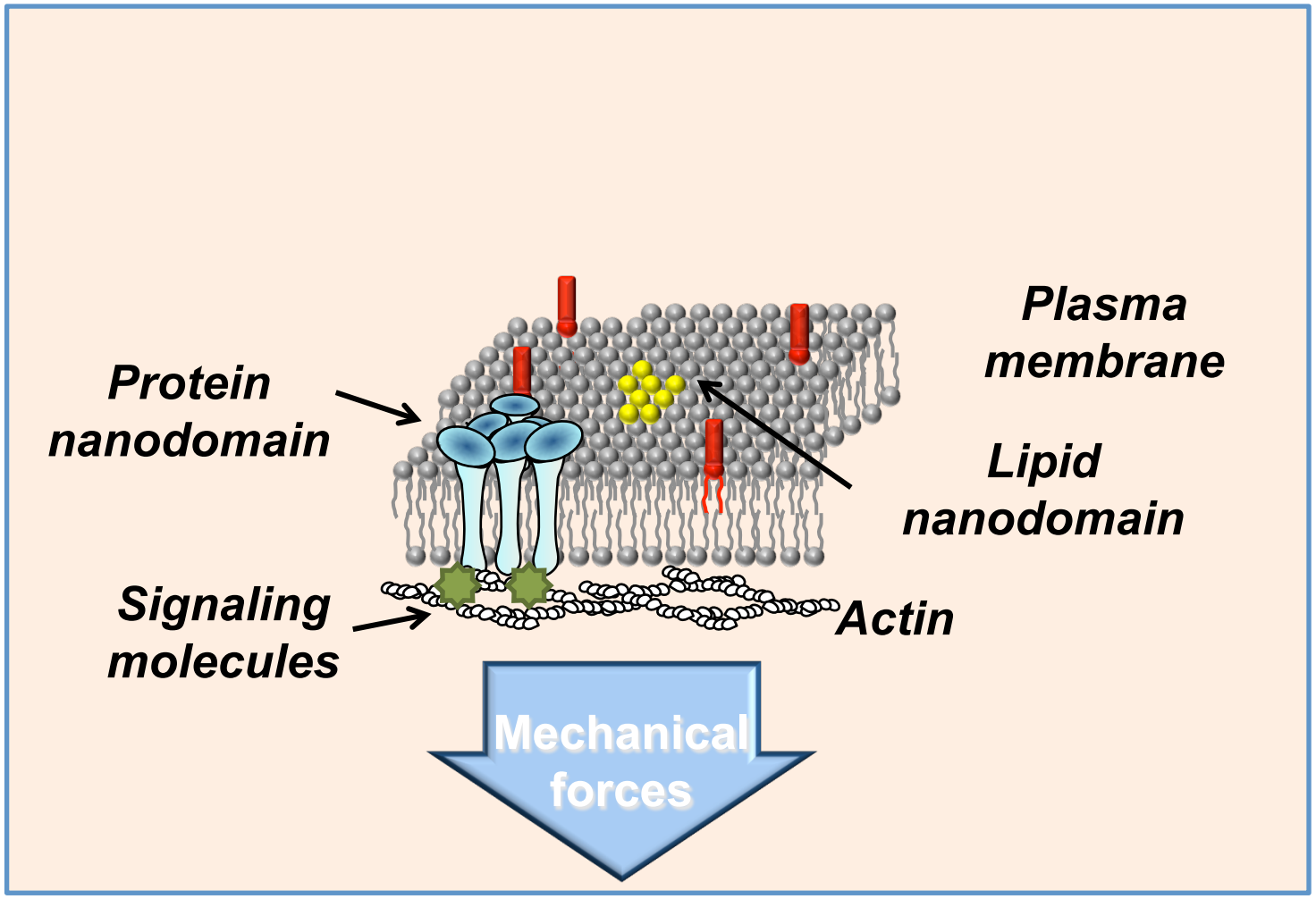
Proteins: specific signaling

A. Kusumi et al, *Trends Biochem Sci* 2011

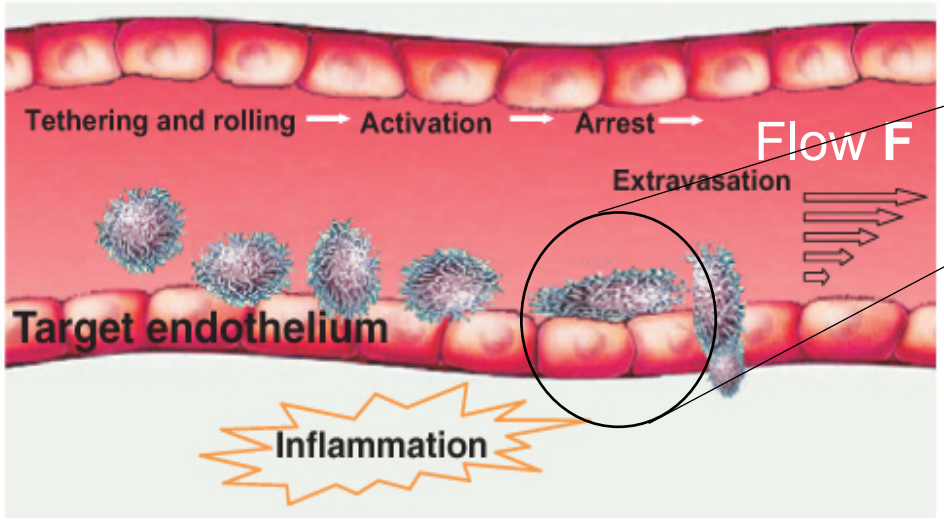
Lateral organization ⟷ **function**

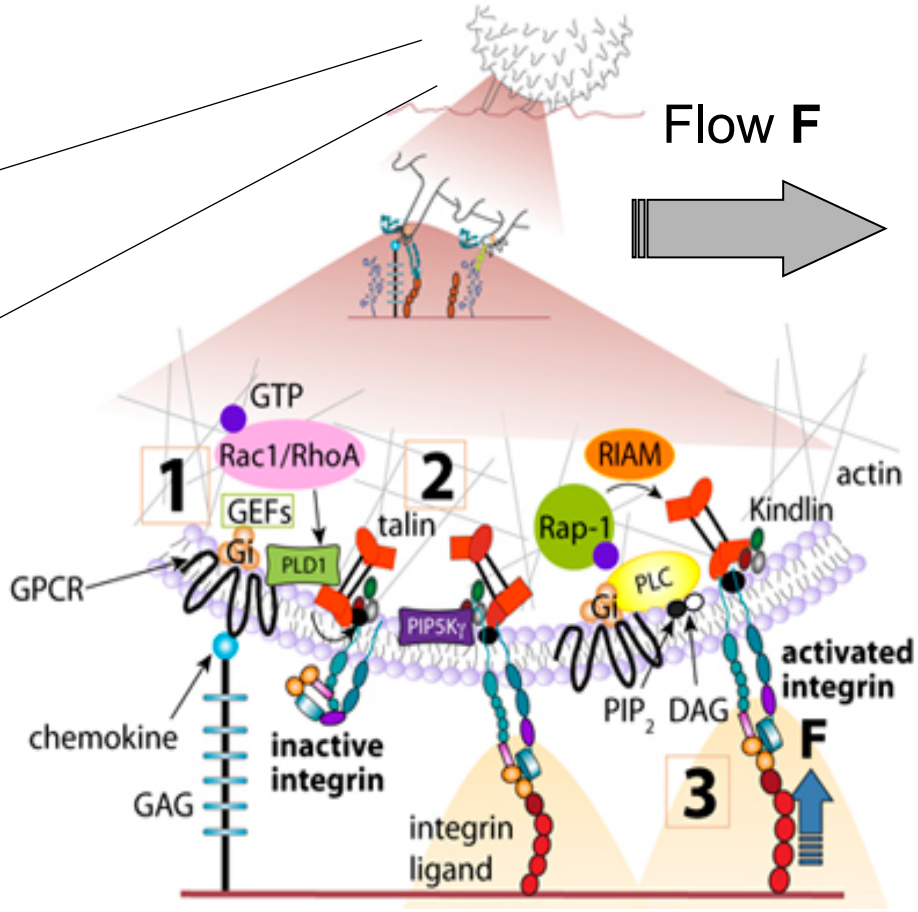
Nanometer scale organization of multiple components

Is membrane nanoscale organization influenced by mechanical stimuli ?



Example: the immune response





Adapted from R. Alon website: www.weizmann.ac.il

Complex arrangement of molecules close to the cell membrane – exposed to different type of mechanical forces

Our combined approach

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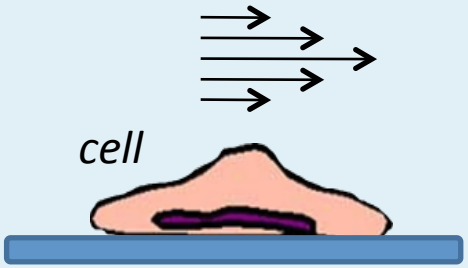
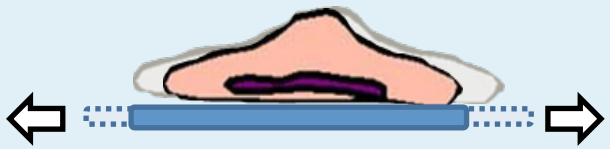
Mechanical forces

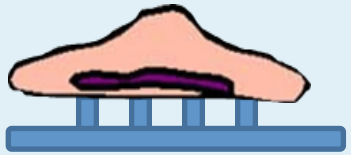
Shear flow
0.1-10 dyne/cm^2



Stretch



Substrate topology

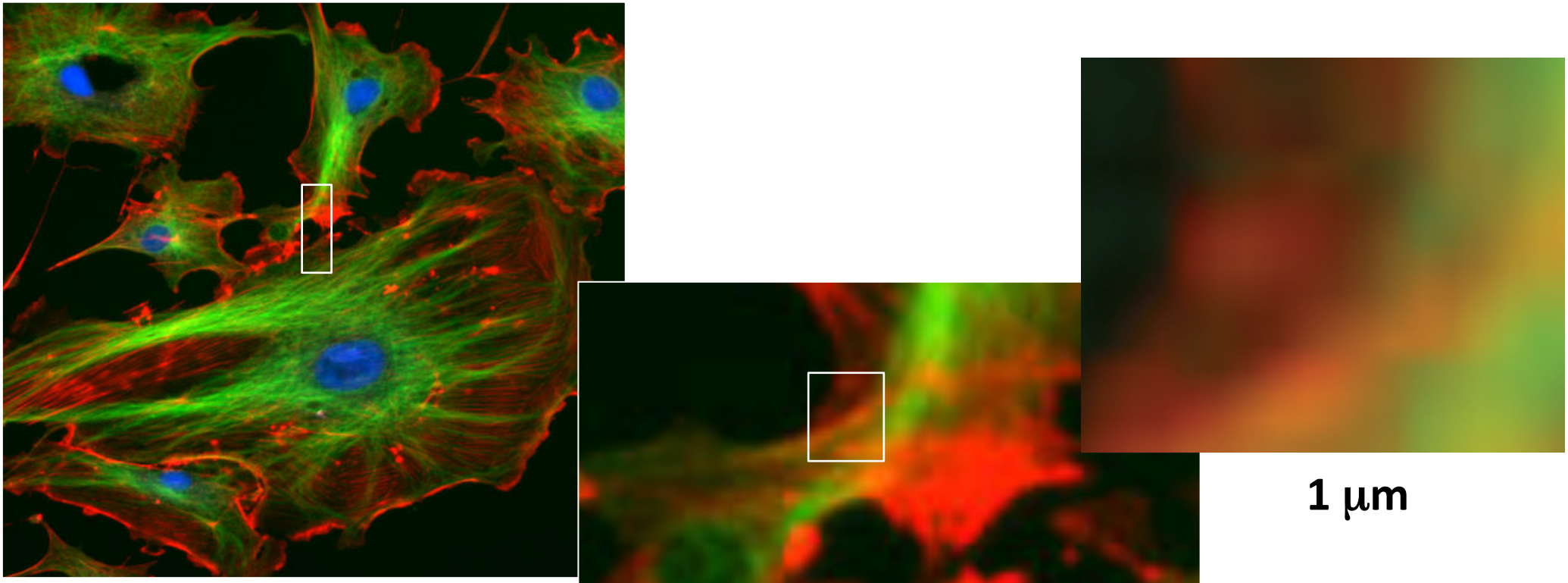


Optical toolbox

Standard optical microscopy is not good enough

Lack of resolution & sensitivity

Light for Cell Biology



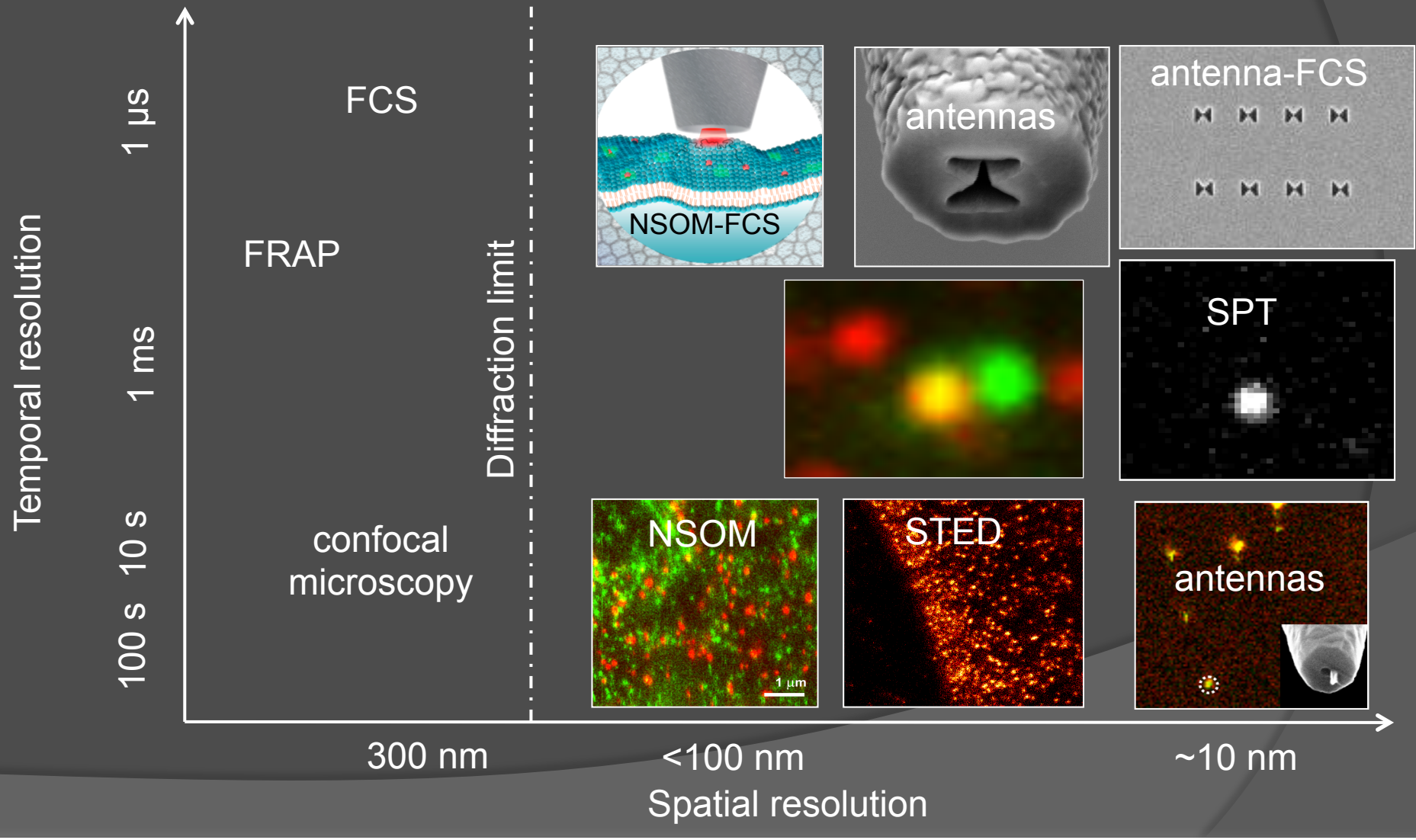
100 μm

20 μm

1 μm

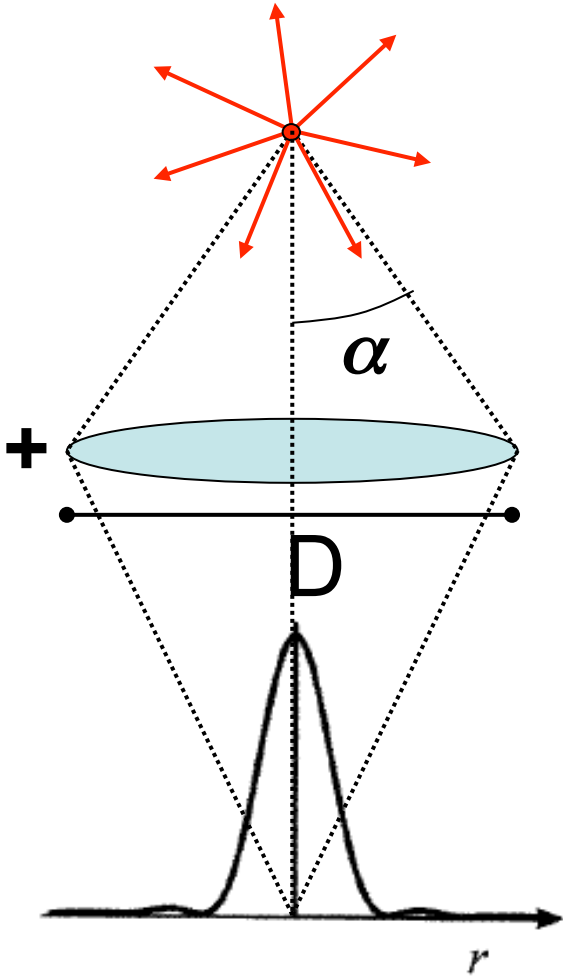
Lack of resolution: the diffraction limit of light !

Nanoscopy in space and time



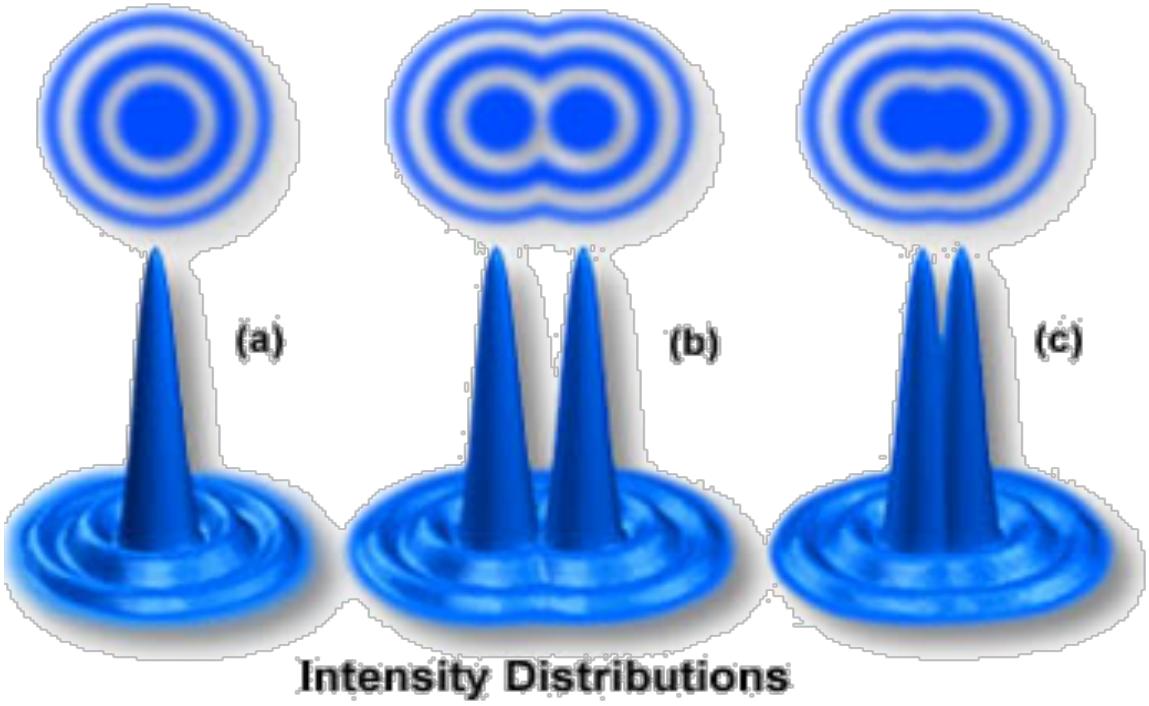
The diffraction limit: Point Spread Function

Image of a point source in a microscope:
One only collects part of the angular spectrum of the source !



Airy pattern: PSF
Point Spread Function

Rayleigh criterion



$$d = \frac{\lambda}{2NA}$$

~ 300-400 nm

Superresolution optical nanoimaging

- Using conventional optics

In the far field: relies on fluorescence emitters

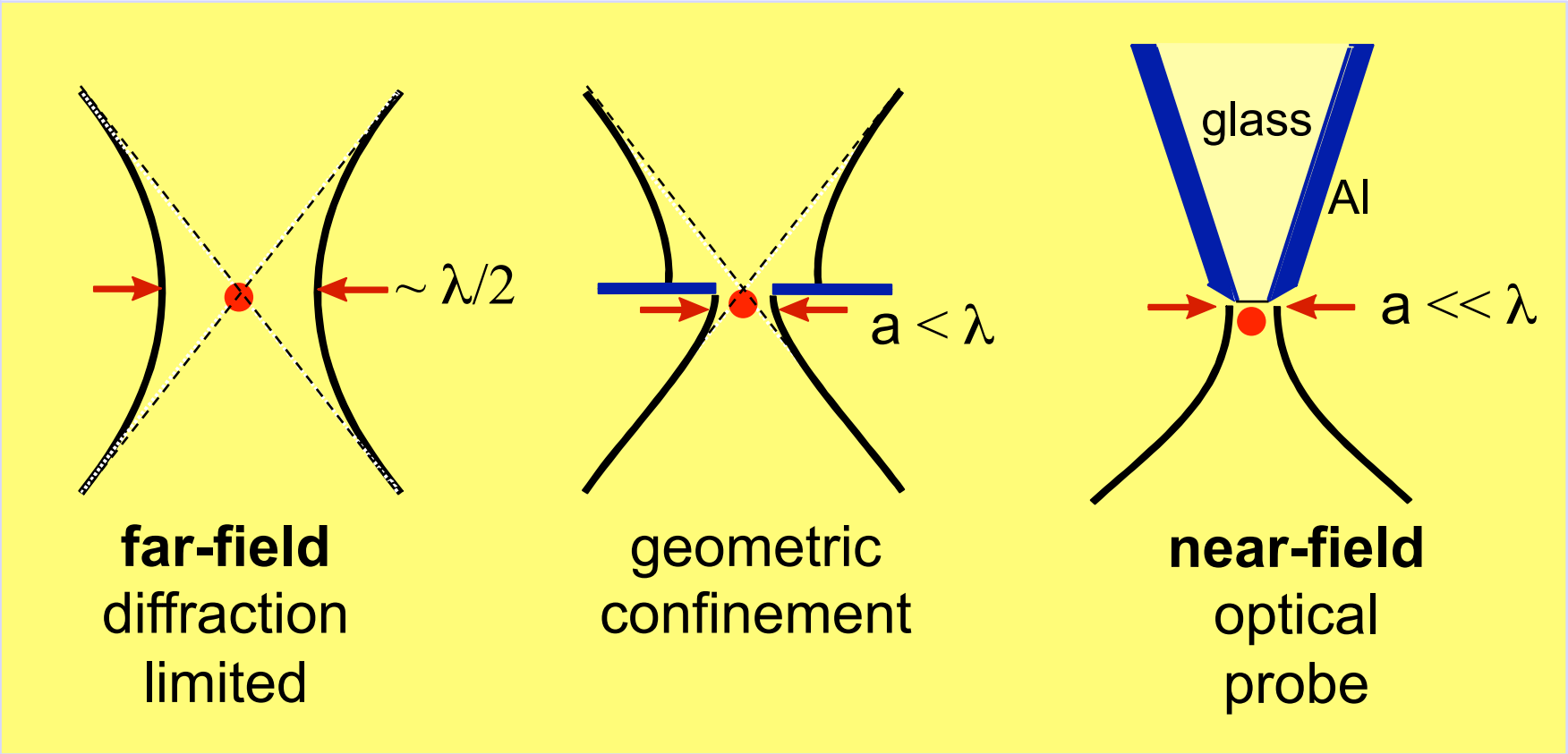
-Getting rid of lenses

In the near-field: making used of near-field optics

Truly breaks the diffraction limit of light

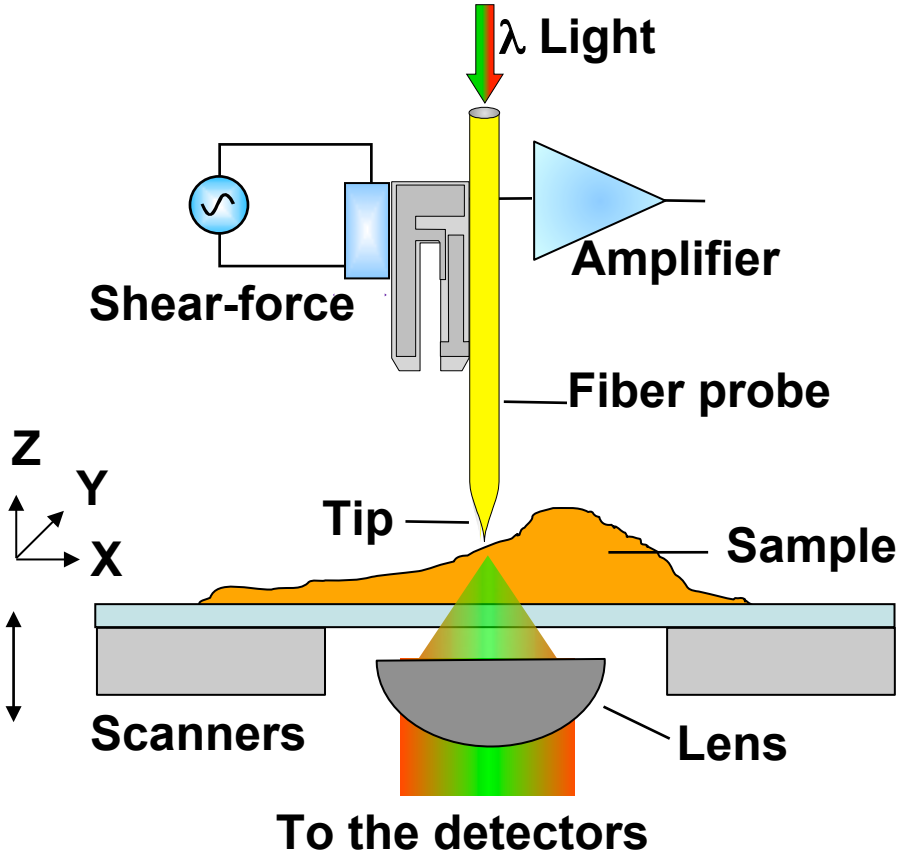
Ideal to address the cell membrane

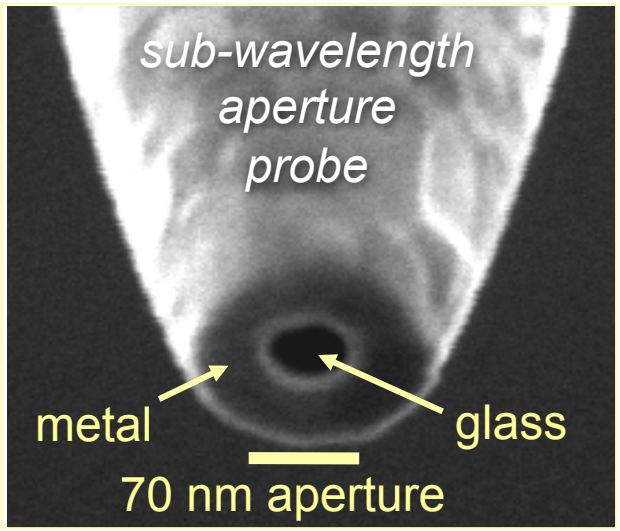
Beyond the diffraction limit in the near-field



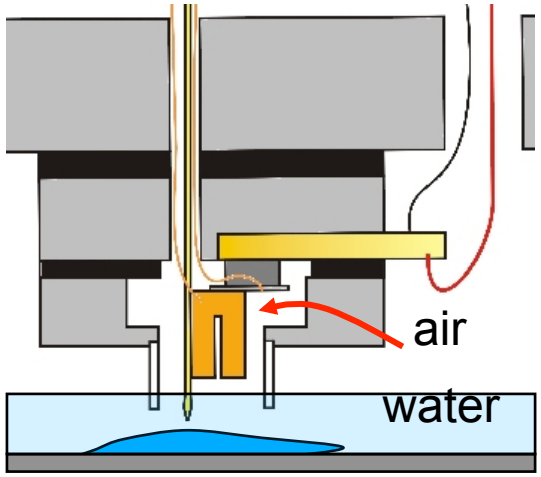
Already suggested in 1928 by Synge !

Near-field scanning optical microscopy - NSOM





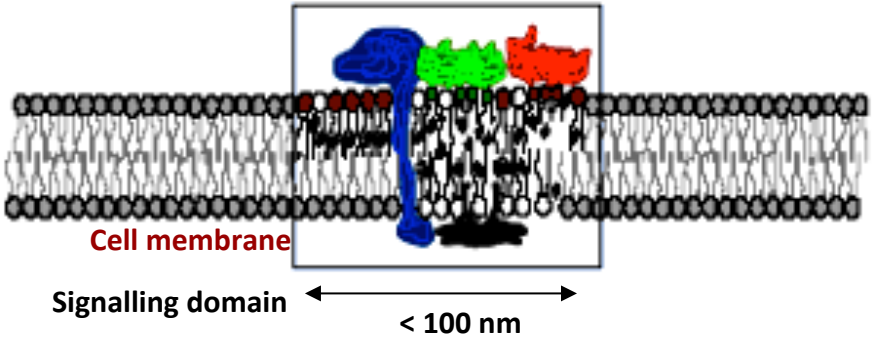
Liquid operation

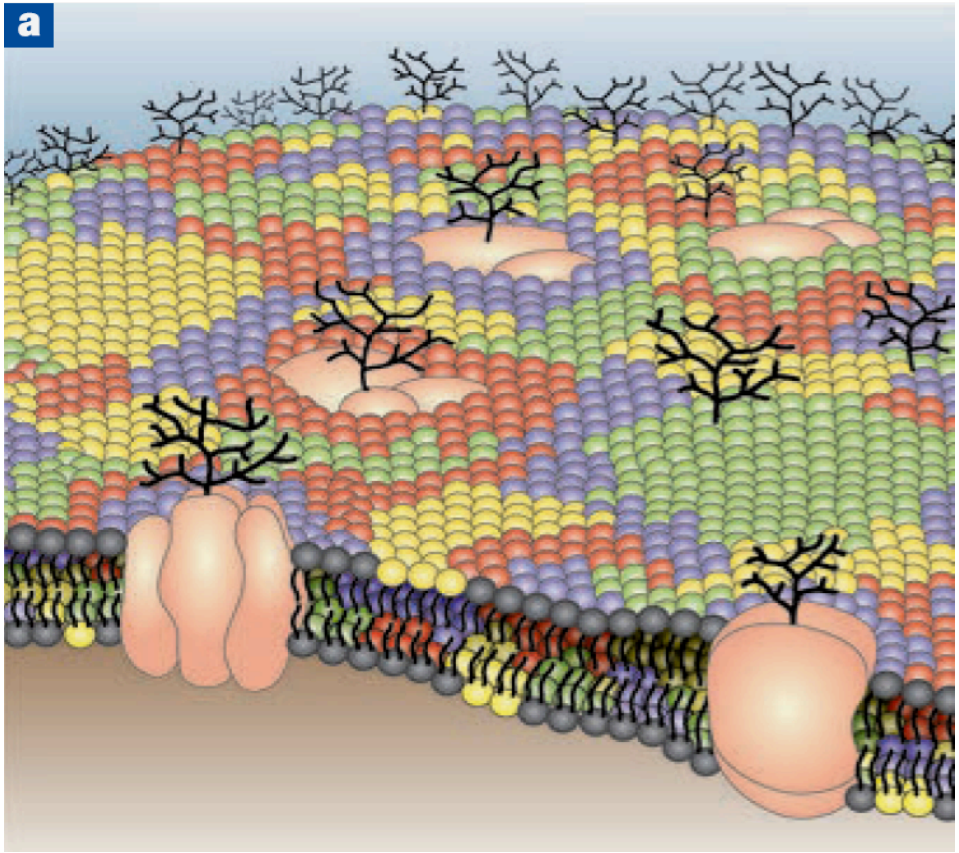


- Near-field interactions– does not depend on emitter's properties
- Simultaneous topography & fluorescence imaging, resolution ~ 70nm
- Extreme sensitivity in z-direction: ~ 10nm
- Multi-color excitation, no aberrations
- Low background, ultra low illumination volumes
- Slow – based on scanning

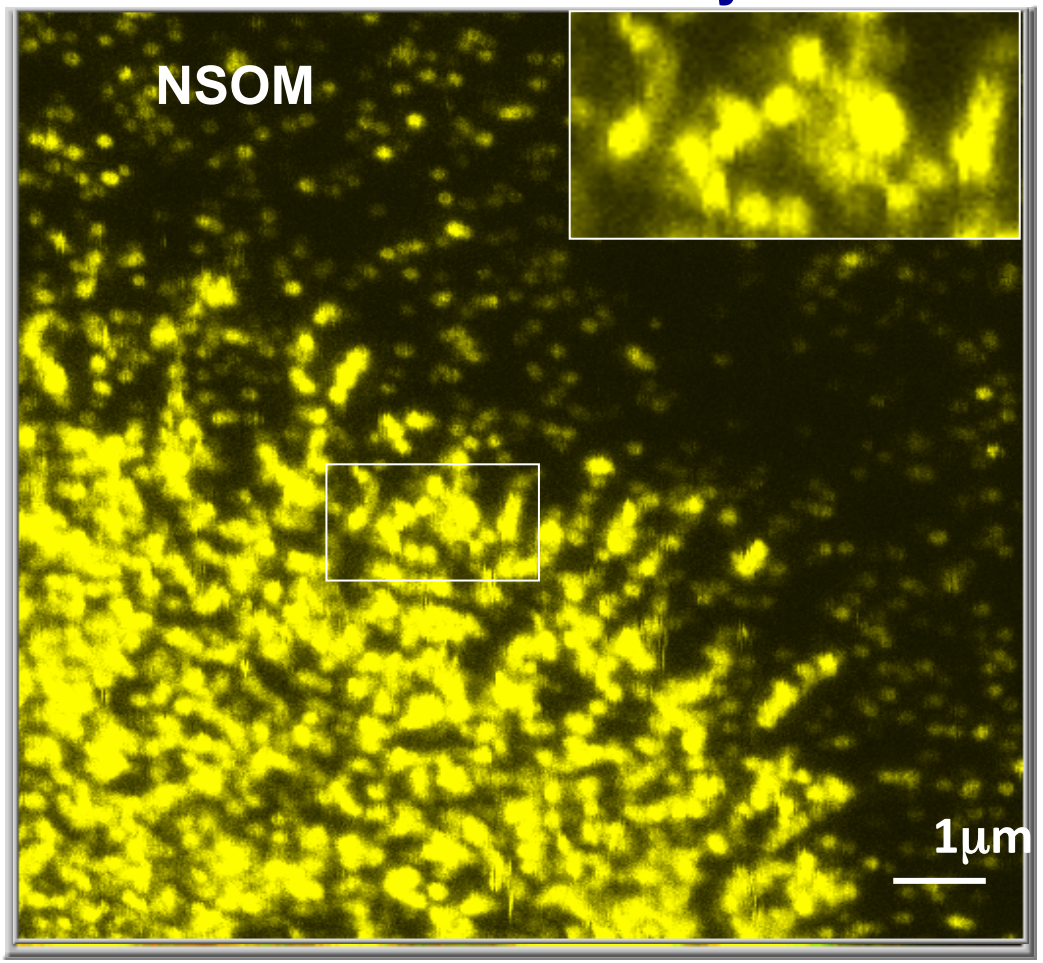
Hinderdorfer, Garcia-Parajo, Dufrene, Acc. Chem. Res. 2011

Nano-signaling platforms using NSOM



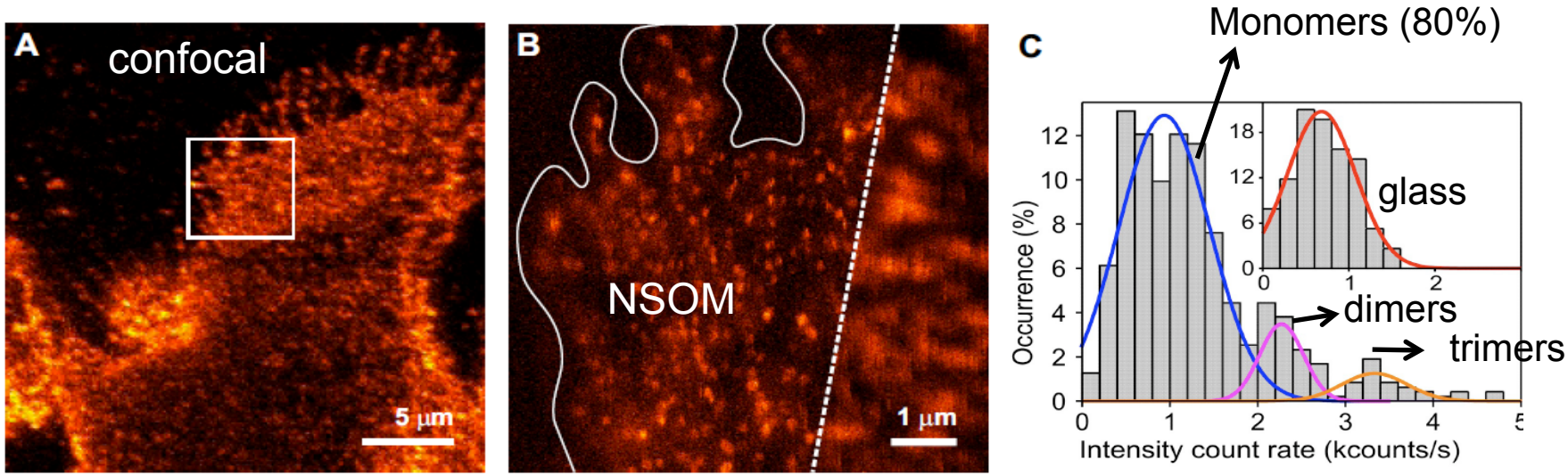


GM1-CTxB on monocytes



T.S. van Zanten, J. Gomez, C. Manzo, R. Reigada, M.F. Garcia-Parajo, *PNAS 2010*

GPI-anchored proteins involved in signalling



van Zanten et al, PNAS 2009

Lipid-protein interactions at the nanoscale

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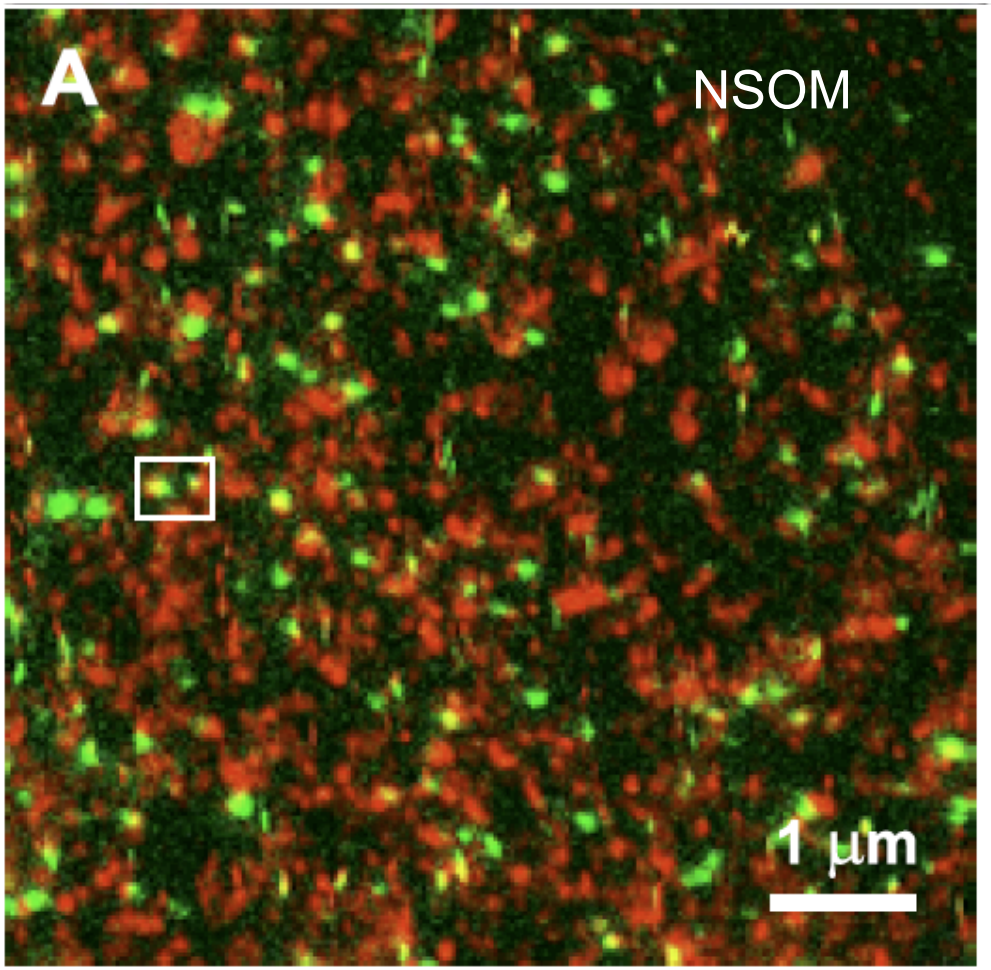
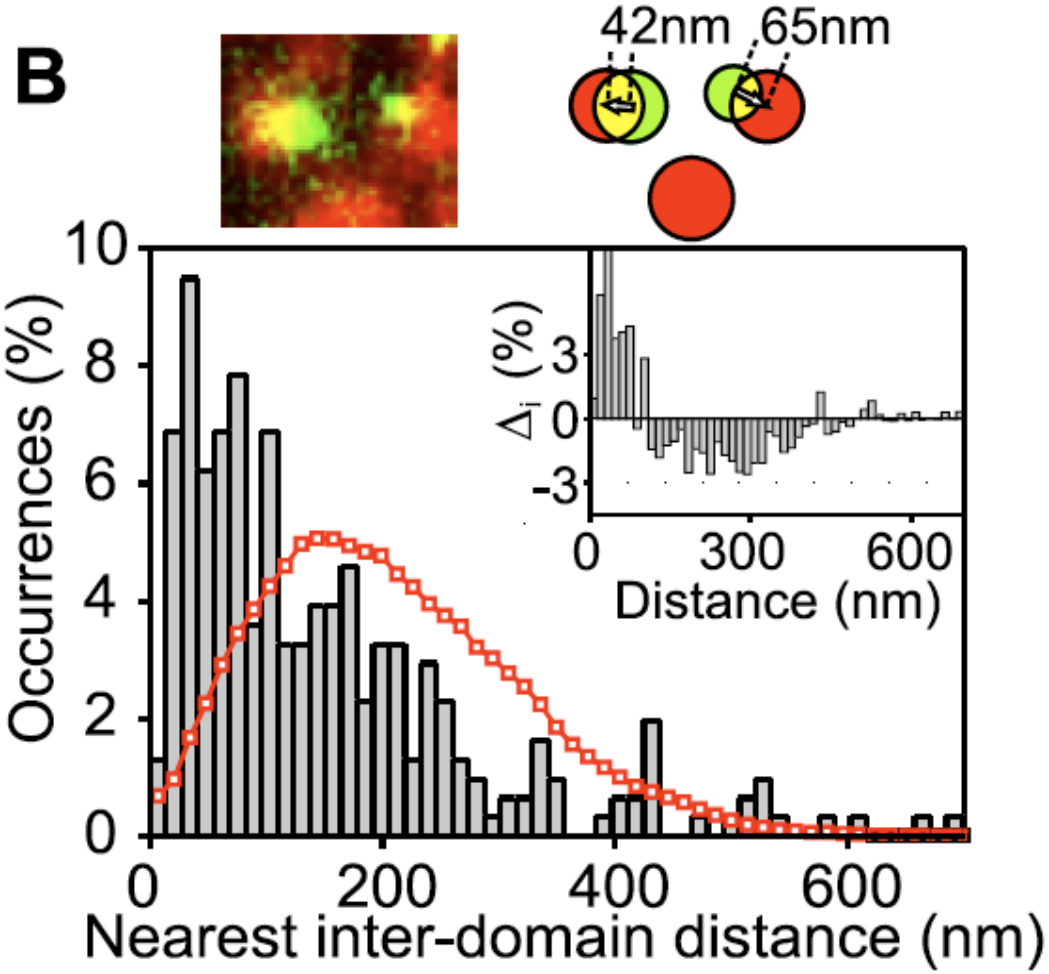
LFA-1 --- GPI-AP





T.S. van Zanten, A. Cambi, M. Koopman, B. Joosten, C.G. Figdor, M.F. Garcia-Parajo, *PNAS 2009*.

Membrane order & function

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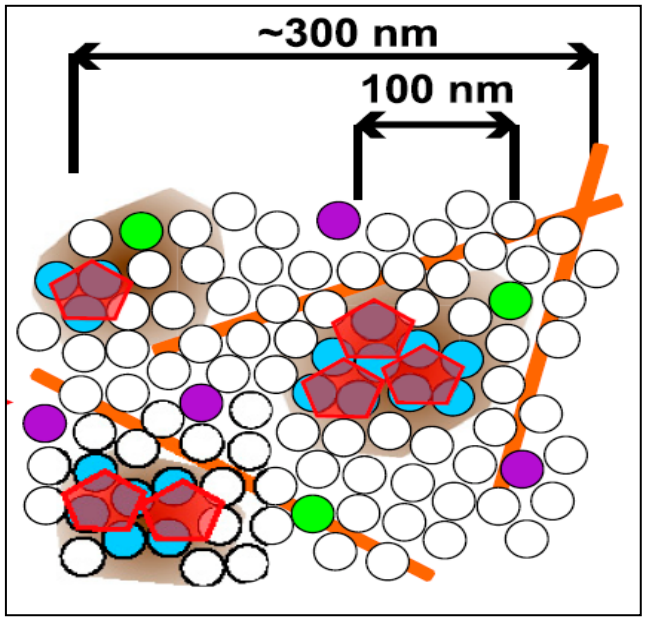
SPACE

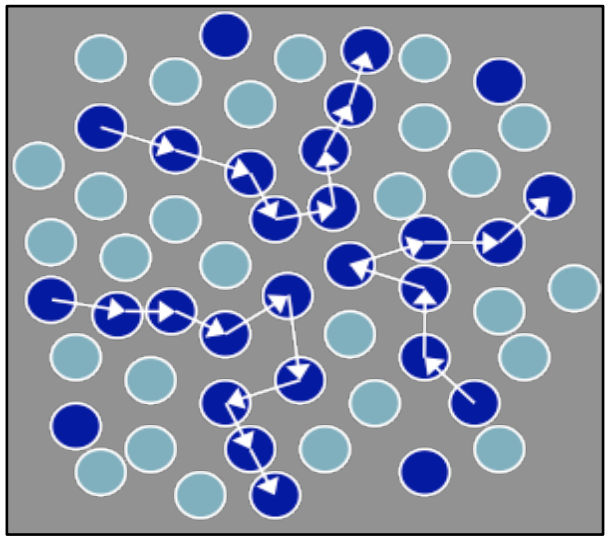
- Clustering
- Compartmentalization



Length scale:
~10 nm → ~1 µm

TIME

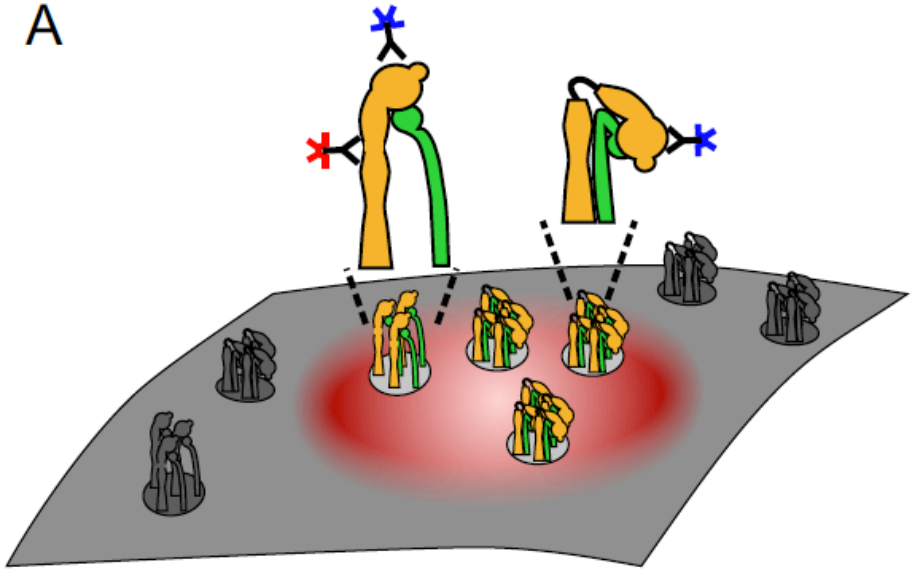
- Fluid environment
- Diffusion

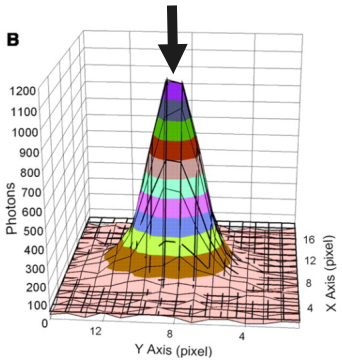


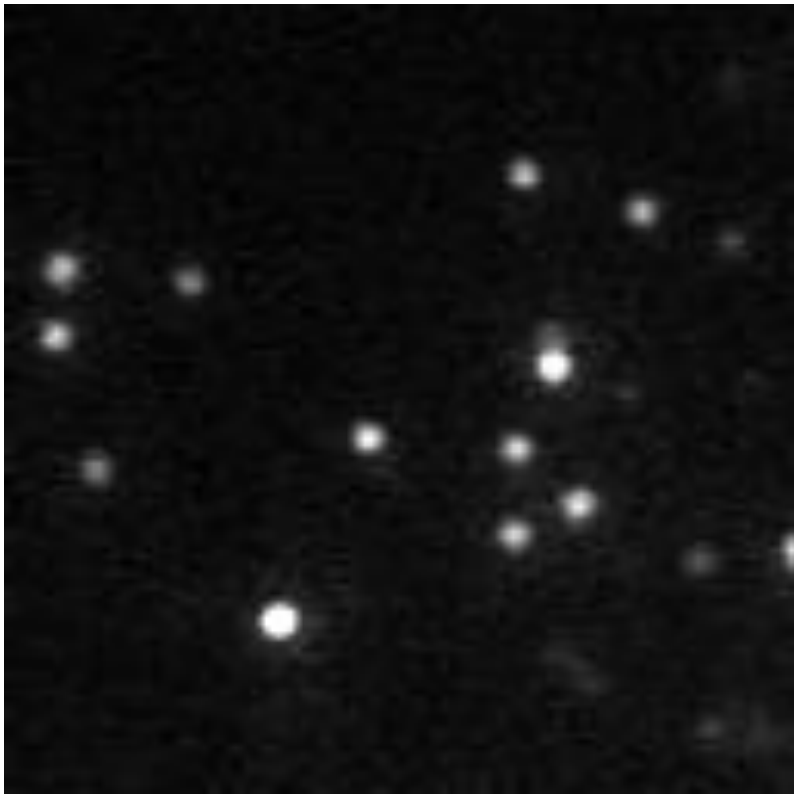
D ~10^{-3} → ~1 µm^2/s

Time scale: ~µs

Approach 1 to measure diffusion: Single particle tracking





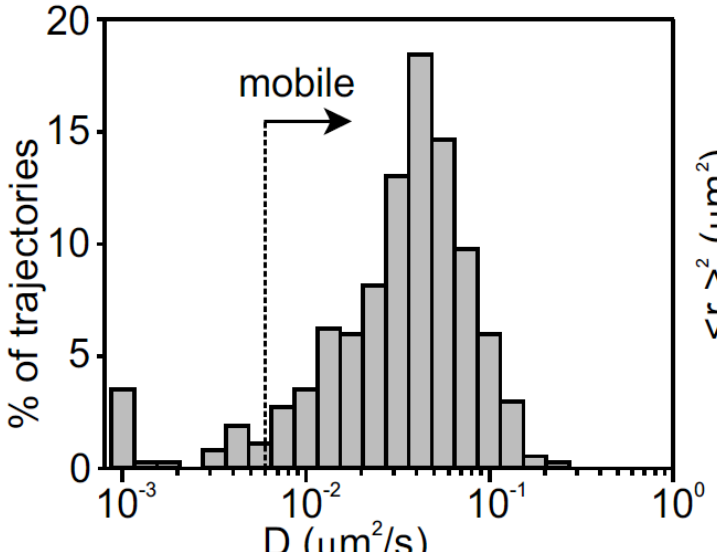


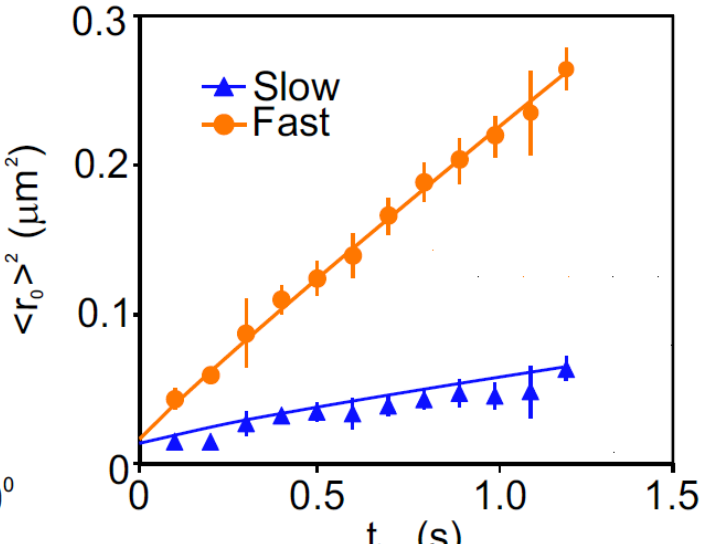
σ ~ 10nm for Qdots

σ ~ 30nm for dyes

σ ~ 40nm for XFP

Diffusion of the integrin LFA-1 on monocytes

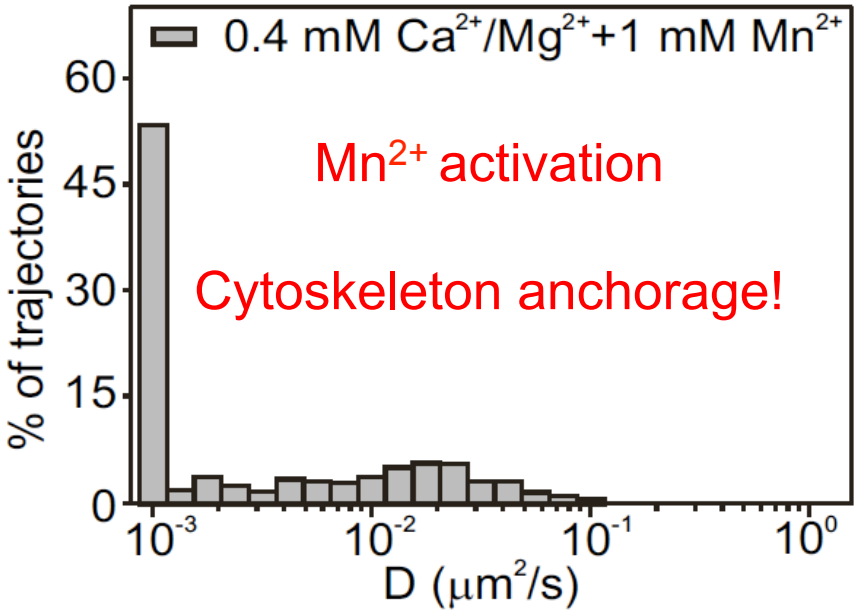


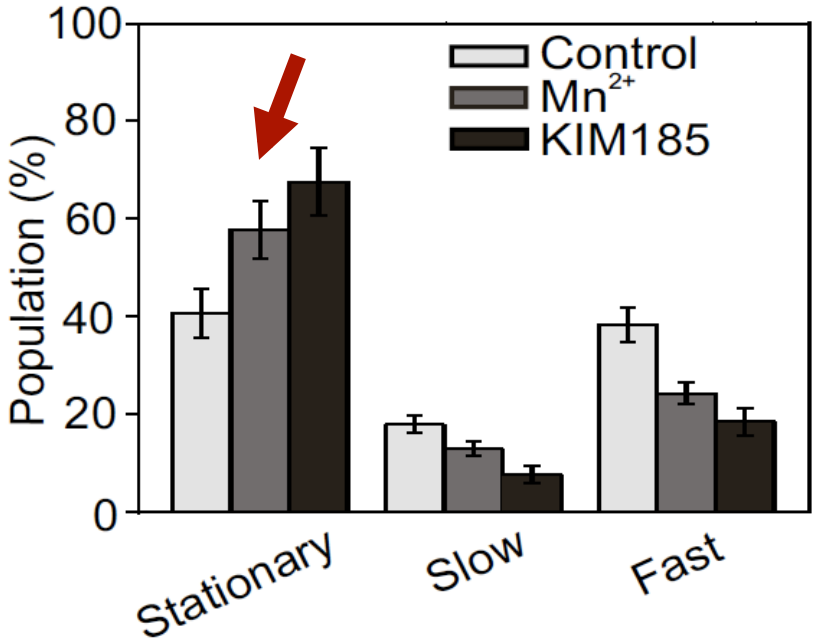


FREE Diffusion

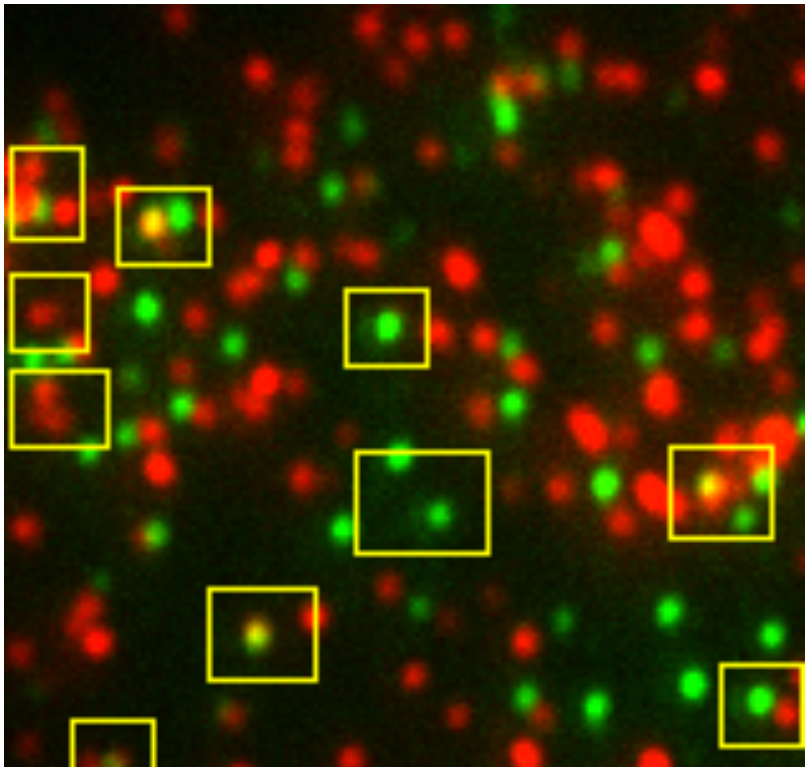
- **~ 95% mobile**
- **Brownian diffusion**
- **No cytoskeleton interaction**

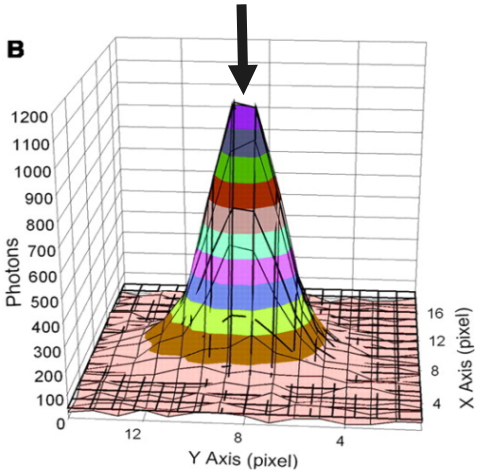
Bakker et al, PNAS 2012





Dynamics by dual color single particle tracking

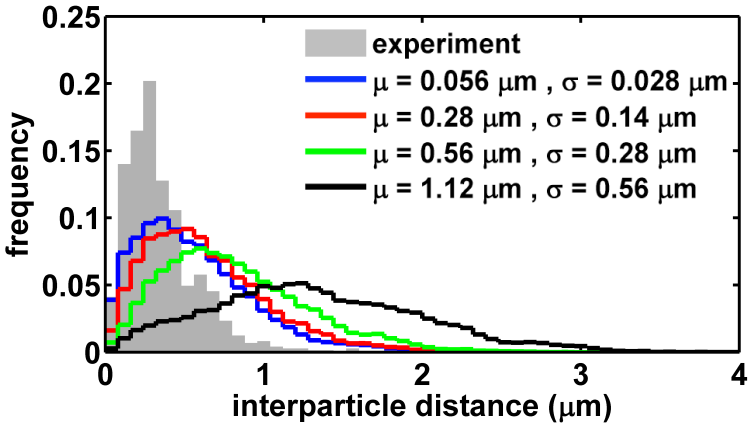


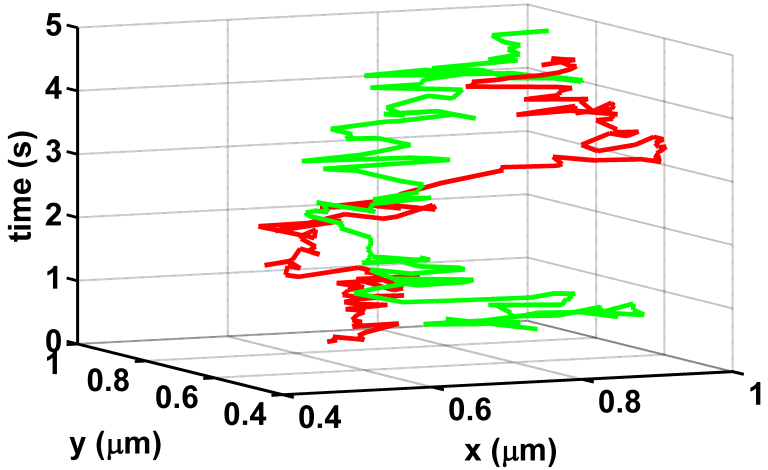


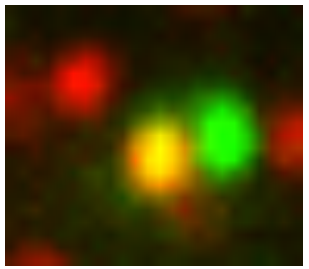
σ ~ 10nm for Qdots

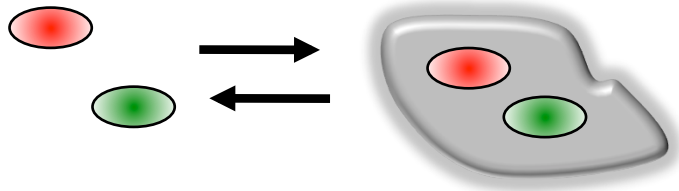
σ ~ 30nm for dyes

σ ~ 40nm for XFP





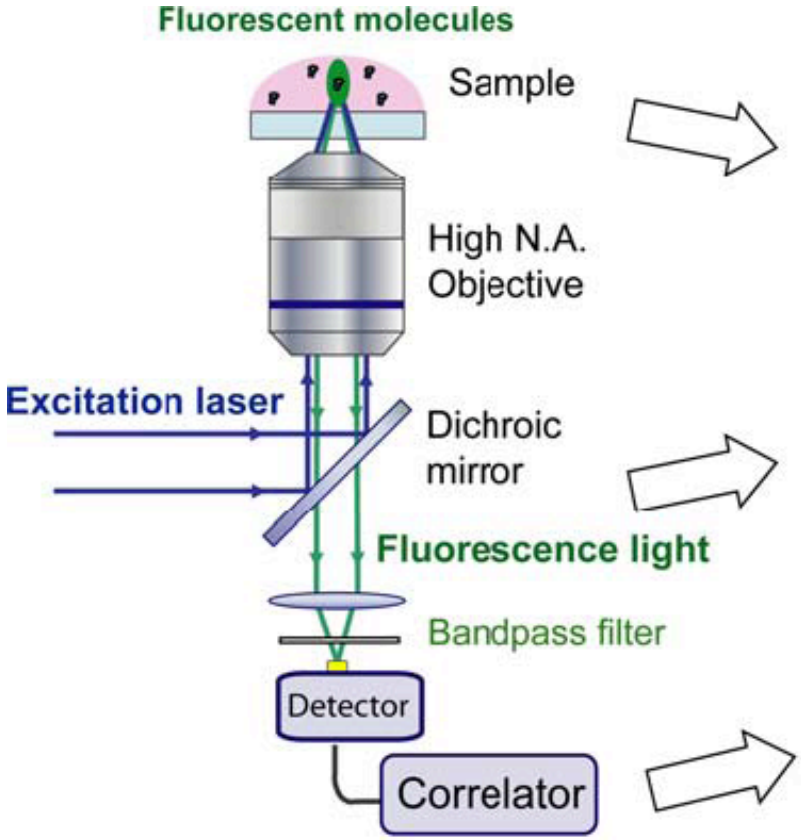


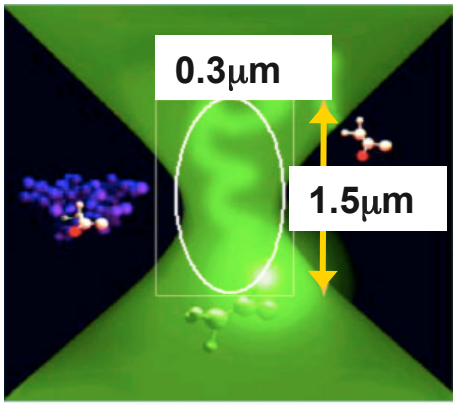


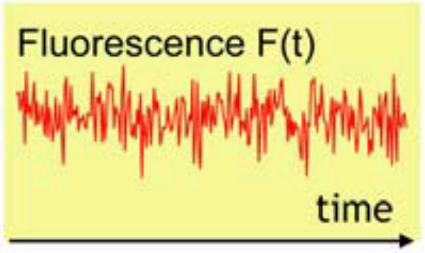
State 1 - Free

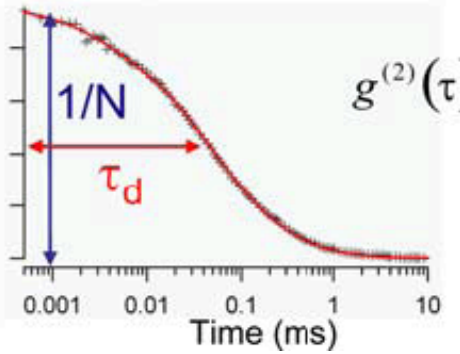
State 2 - Domain

Approach 2 to measure diffusion: FCS









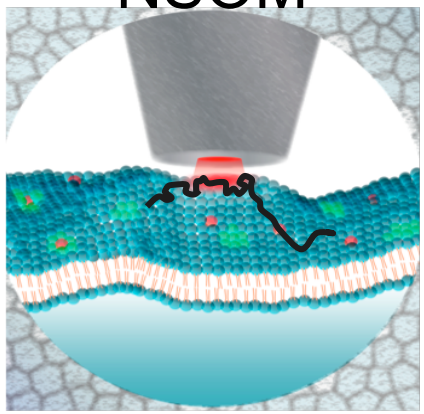
$$g^{(2)}(\tau)=\frac{\langle F(t)\cdot F(t+\tau)\rangle}{\langle F(t)\rangle^2}$$

N: number of molecules

τ_d: residence time

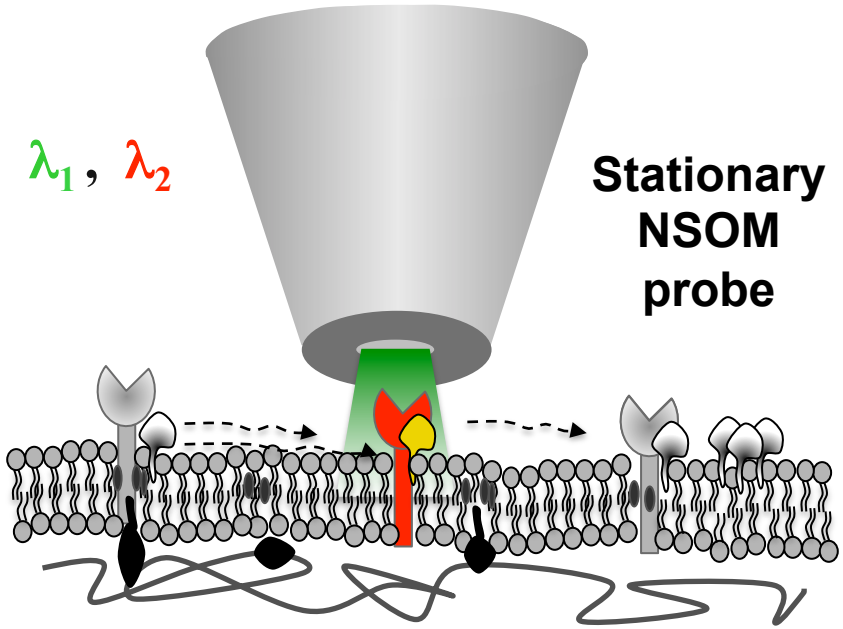
Diffraction limited lateral & axial

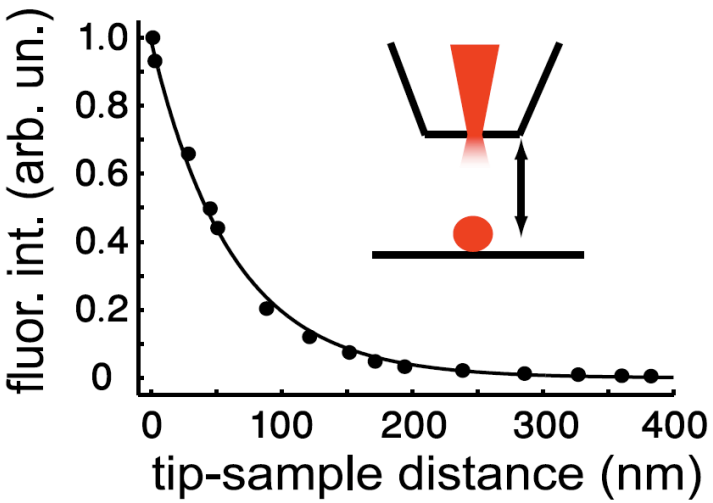
NSOM



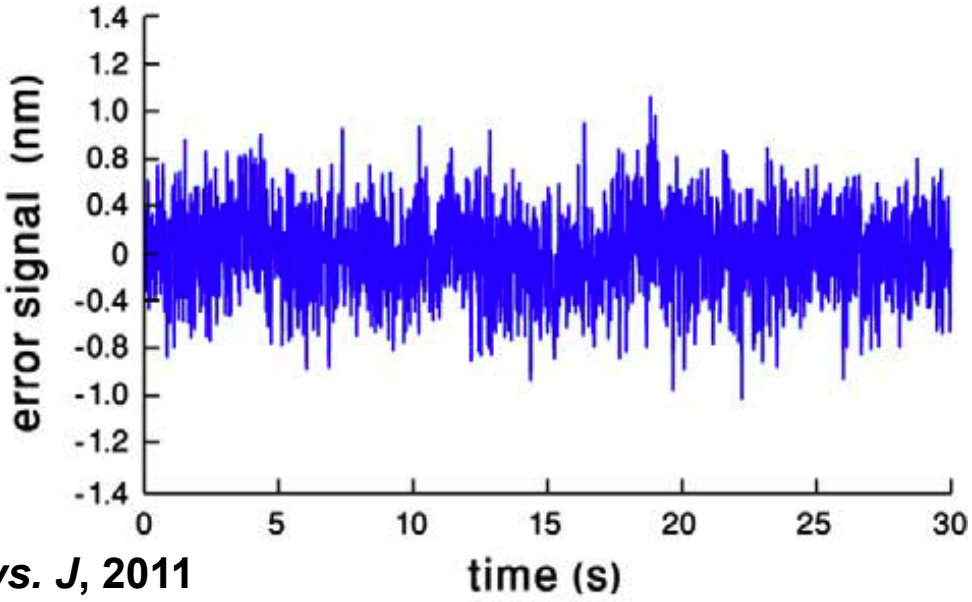
Smaller illumination volumes are needed to resolve diffusion at the nanoscale

Implementation of FCS-NSOM





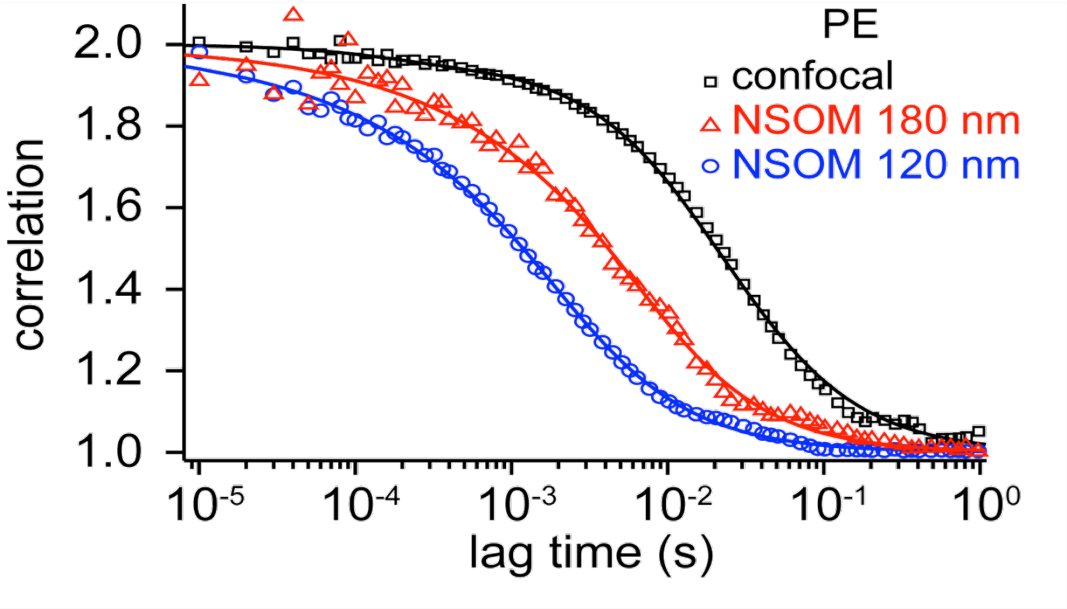
- Diffusion on the membrane & proximal cytosolic regions
  (< 70nm)
- Extreme sensitivity in *z*
- Not sensitive to vertical fluctuations of the cell membrane
- Dual color cross correlation

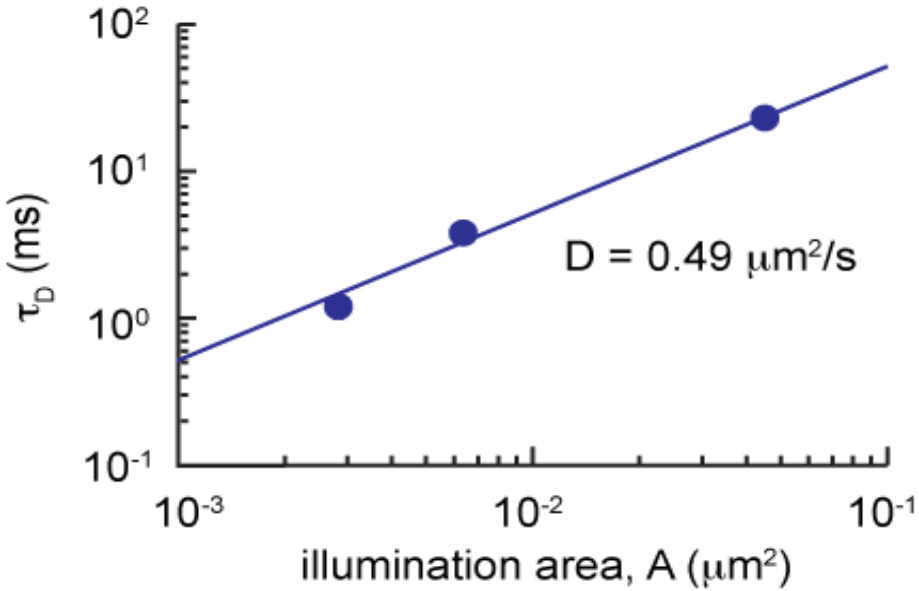


C. Manzo, T.S. van Zanten, M.F. Garcia-Parajo, *Biophys. J*, 2011

Dynamics at the nanometer scale (FCS-NSOM)

Phospholipid PE-ATTO647 on living CHO cells





$$G(\tau) = \left[erf\left(2\frac{\tau_D}{\tau}\right)^{\alpha/2} - \frac{1}{2\sqrt{\pi}}\left(\frac{\tau}{\tau_D}\right)^{\alpha/2}\left(1 - e^{-4\left(\tau_D/\tau\right)^{\alpha}}\right)\right]^2$$

α=1 → random diffusion
α<1 → anomalous diffusion

Brownian diffusion, α=1
τ_D is linear with illumination area

C. Manzo, T.S. van Zanten, M.F. Garcia-Parajo, *Biophys. J*, 2011

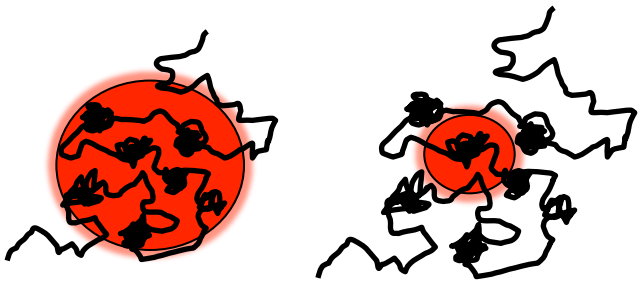
Diffusion of Sphingolipid SM

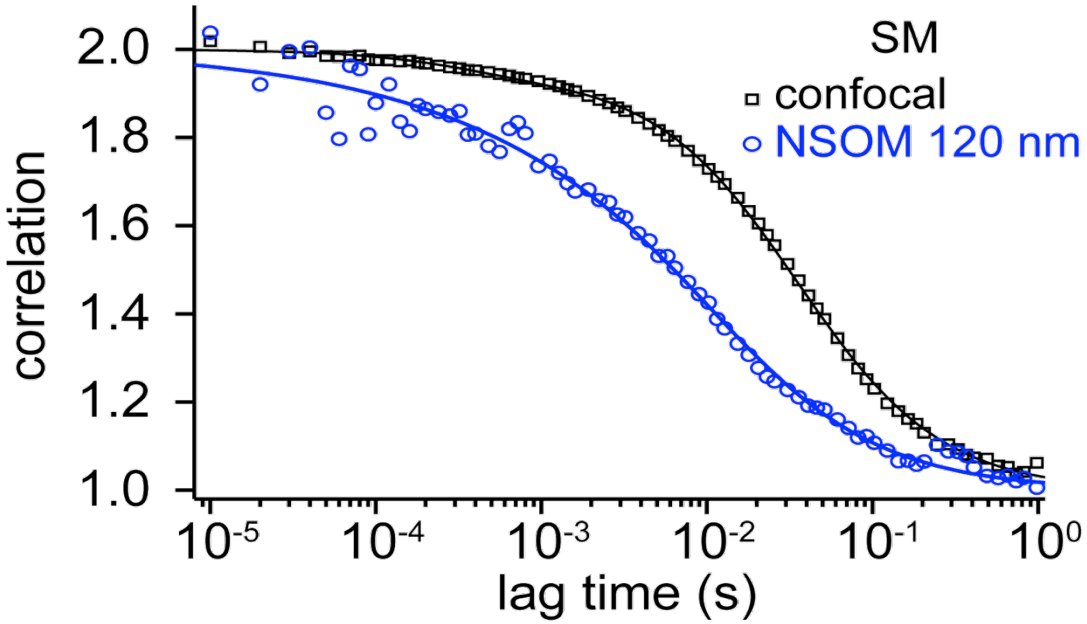
SM-Atto647N
Living CHO cell

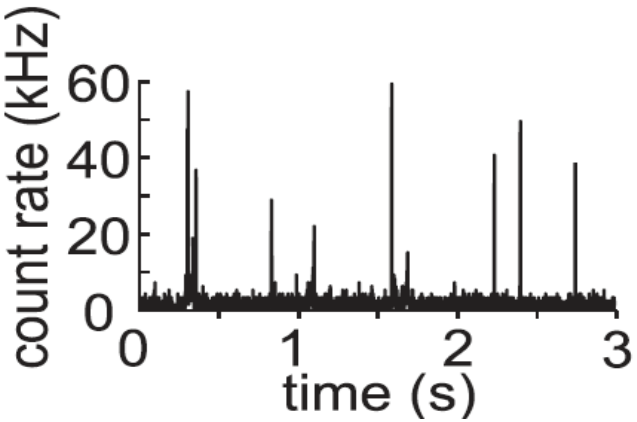
Anomalous diffusion

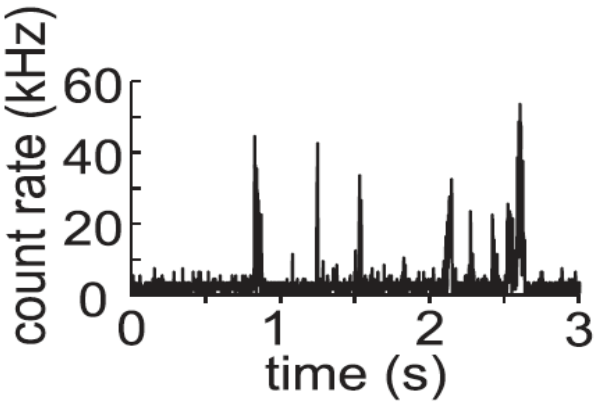
α = 0.79 ± 0.07

Consistent with cholesterol-induced confinement of sphingolipids





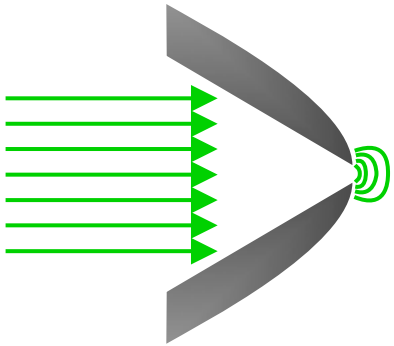




NSOM-FCS reveals nanoscale membrane compartmentalization

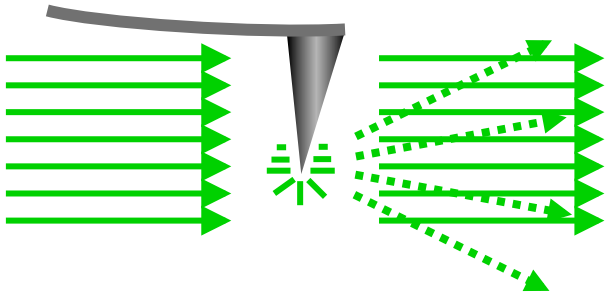
Challenging to further reduce the illumination volume …

How to reduce the illumination volume further



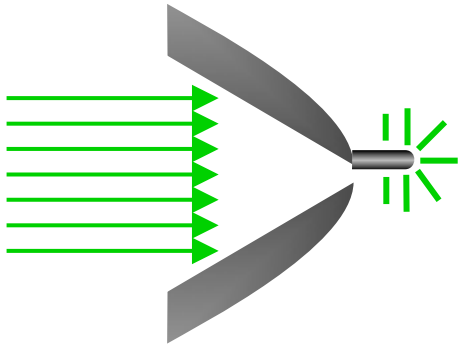
Classical aperture-NSOM

- Low background
- Optical resolution > 50nm
- Low throughput - slow…



Apertureless-NSOM

- Optical resolution > 10nm
- Very large background
- Difficult to decouple signal from background



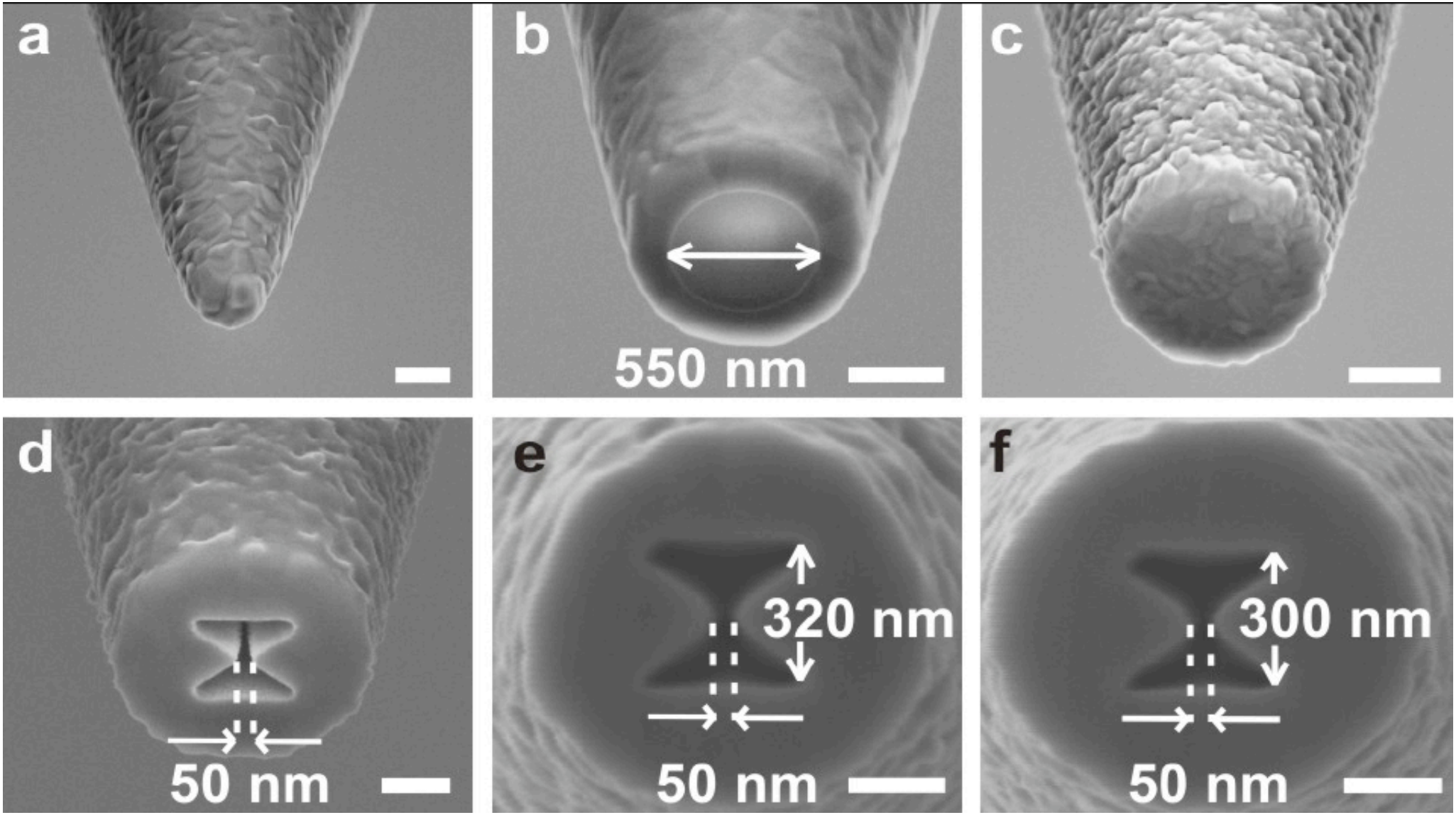
Tip-on-aperture NSOM (optical antenna)

- Low background
- Optical resolution > 10nm
- High throughput
- Ultra-small illumination volumes

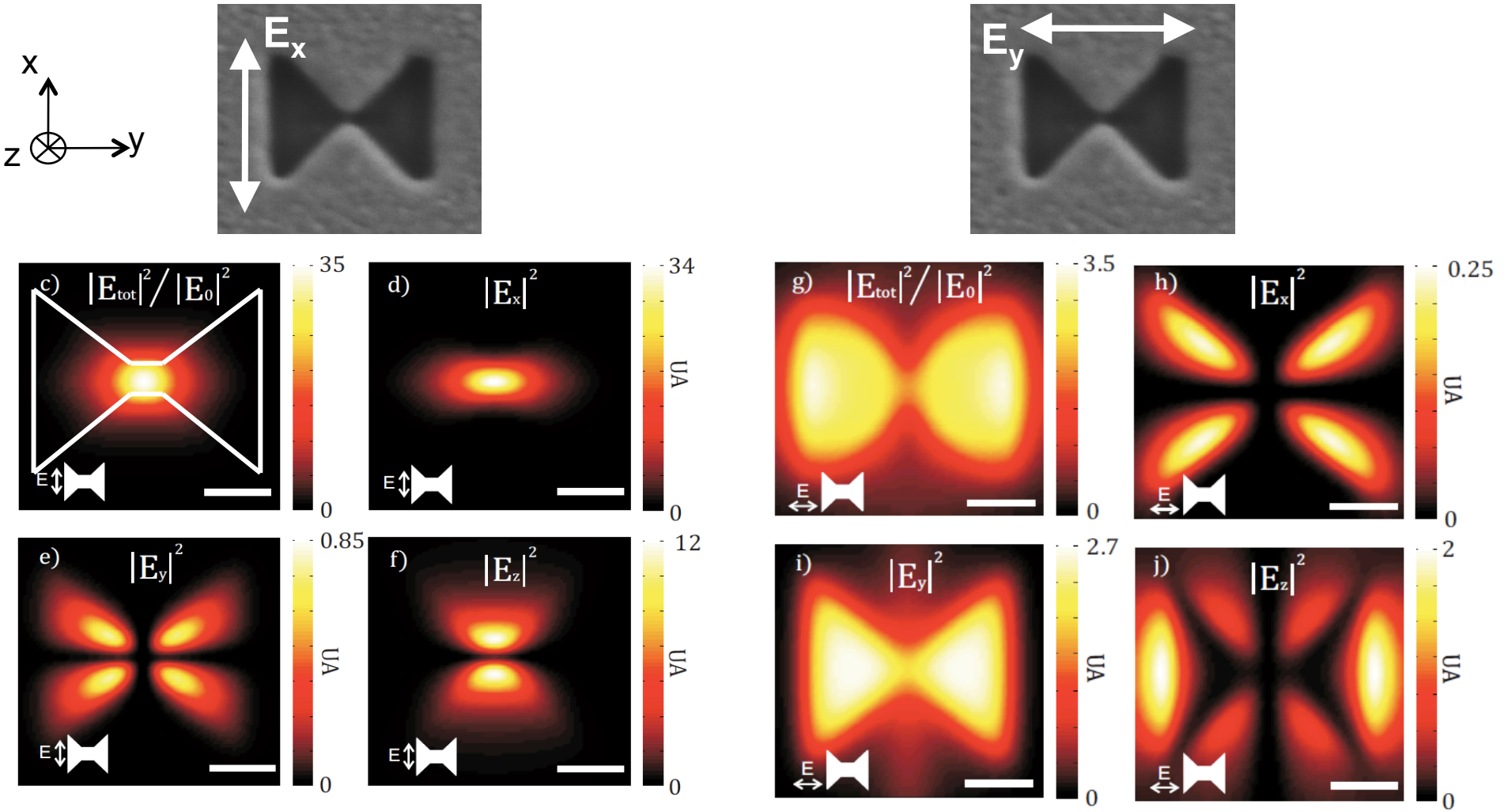
Towards FCS using antenna probes

ICFO

Bowtie nano-aperture antenna (BNA)

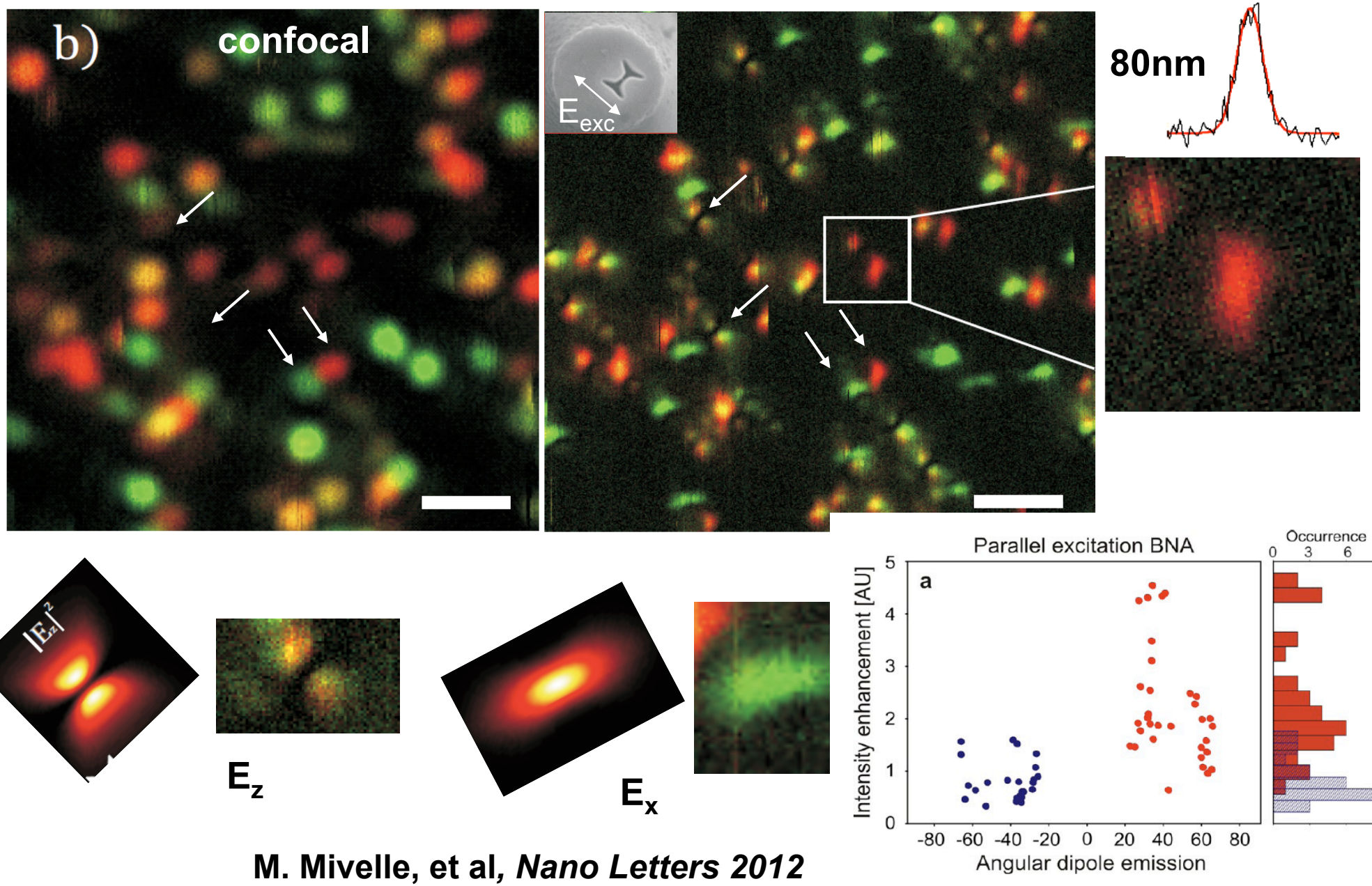


BNA excitation: confinement & enhancement



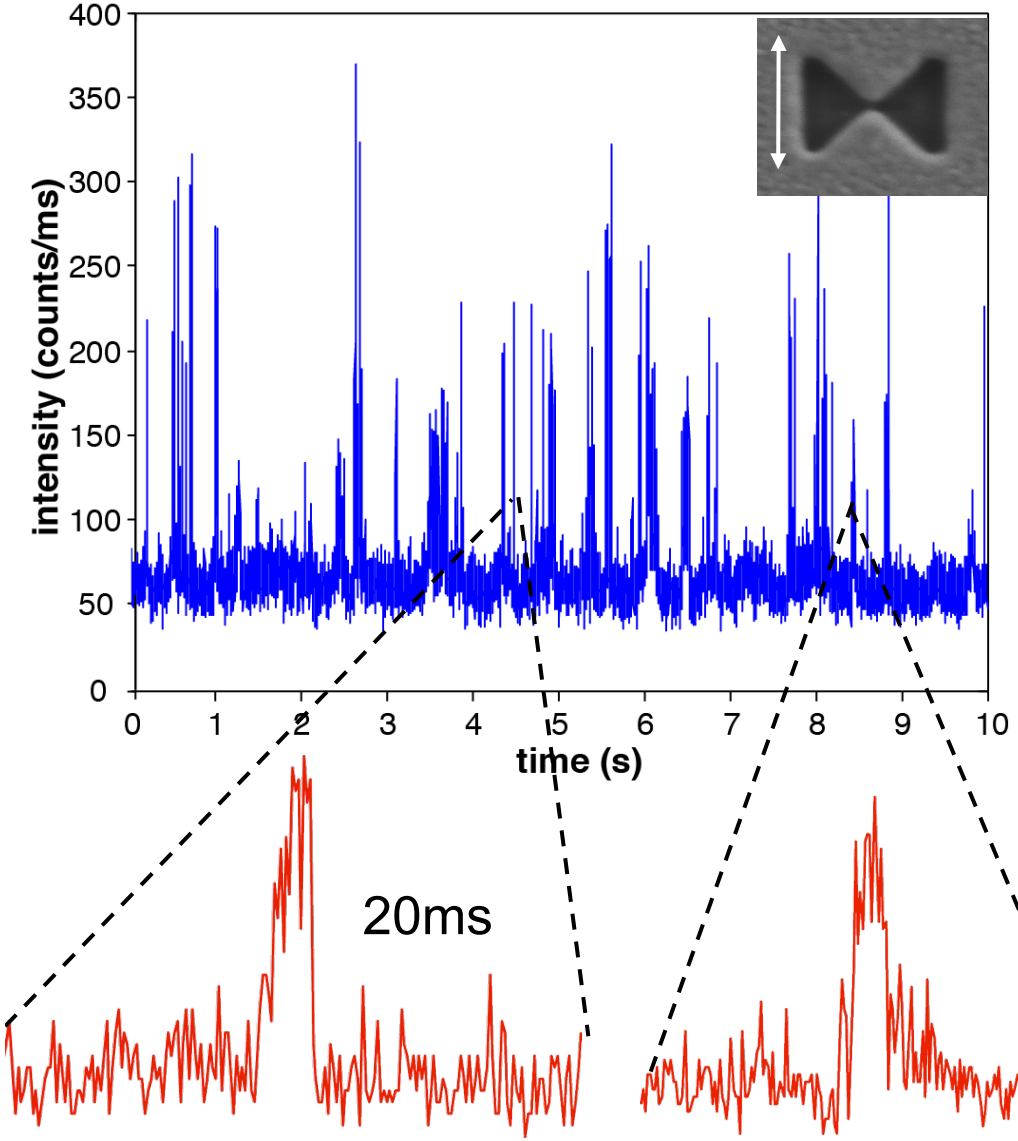
- 3D-components of the E near-field
- Tuning of the effective confinement region according to the excitation polarization
- 35x enhancement and ~ 10^3 larger compared with subwavelength apertures, same size

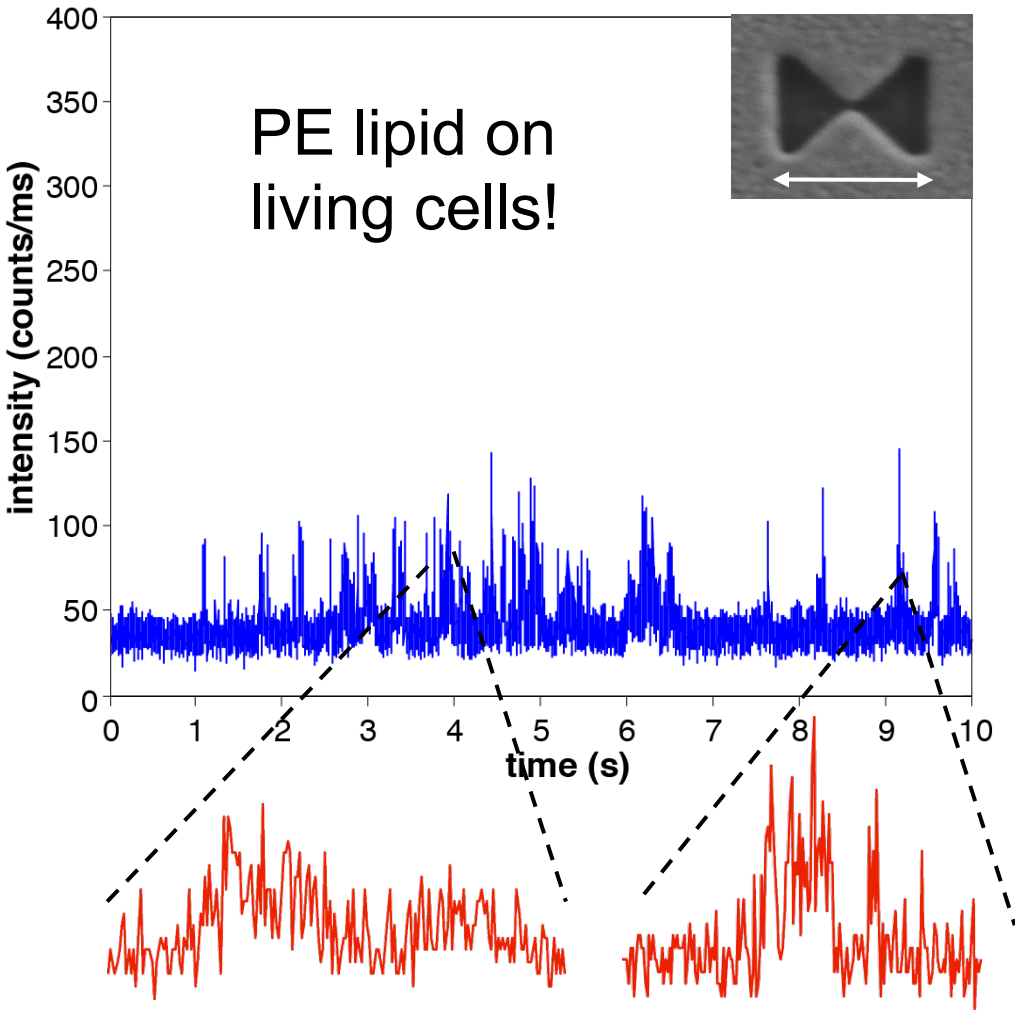
Single molecule mapping of near-field of BNA



M. Mivelle, et al, *Nano Letters 2012*

Implementation of FCS-antenna probes

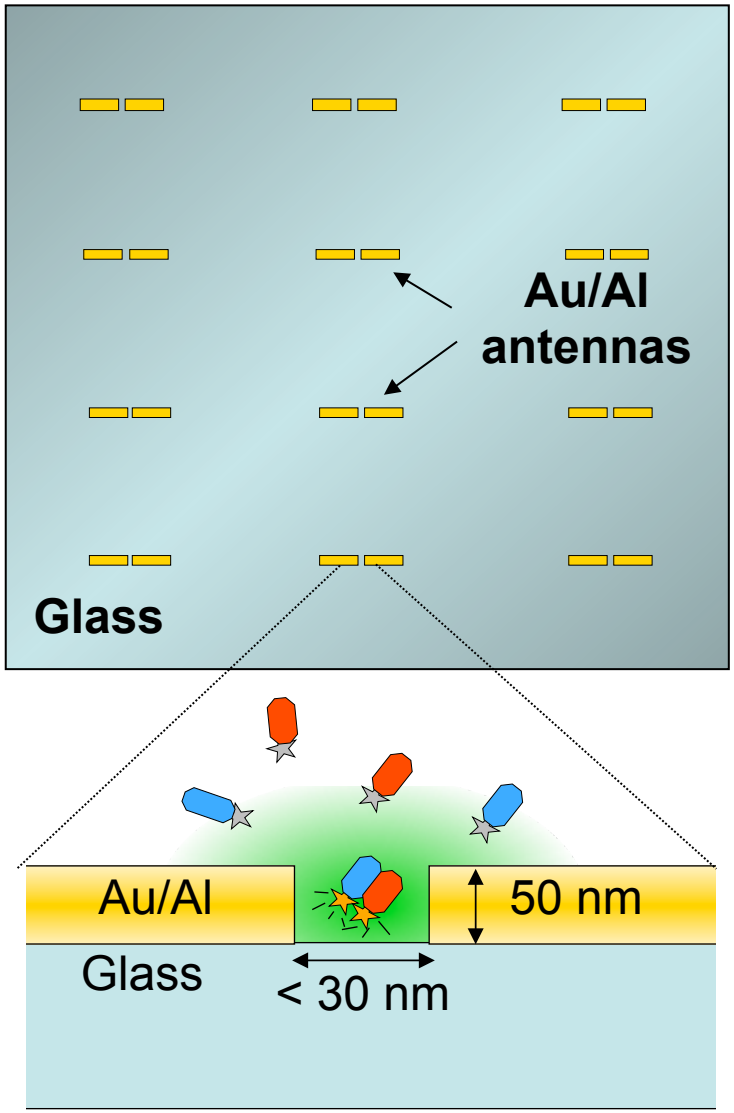




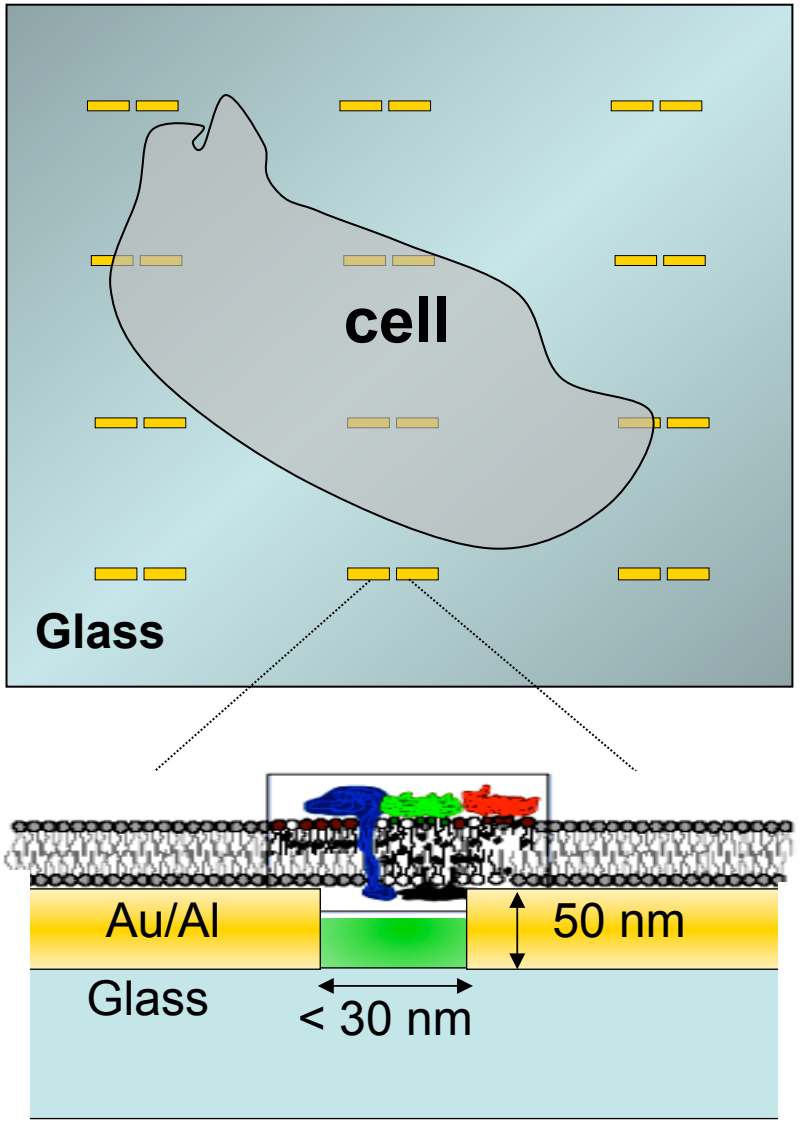
See talk Thomas van Zanten

τ_D and burst intensity consistent with antenna excitation

2D-antenna geometries



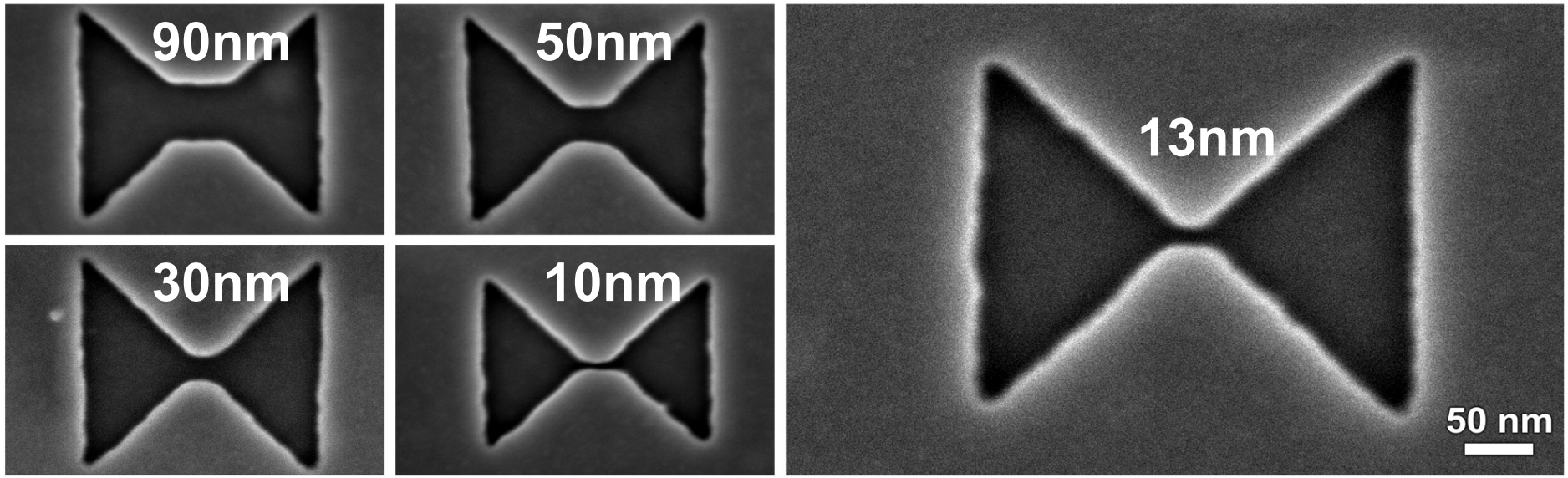
Ultra-sensitive detection & high sample concentrations



Ultra-small illumination volumes for nano-FCS in living cells

Fabrication of 2D antenna geometries

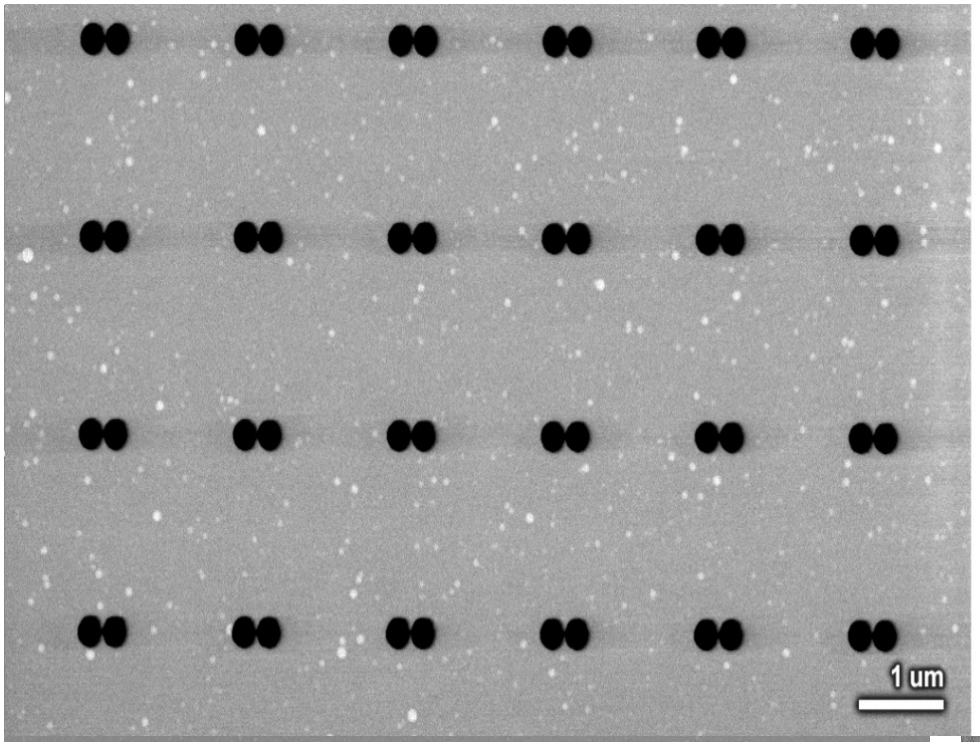
– EBL and post processing optimized for 50nm SiN
– Gaps down to 10nm wide transfered in SiN

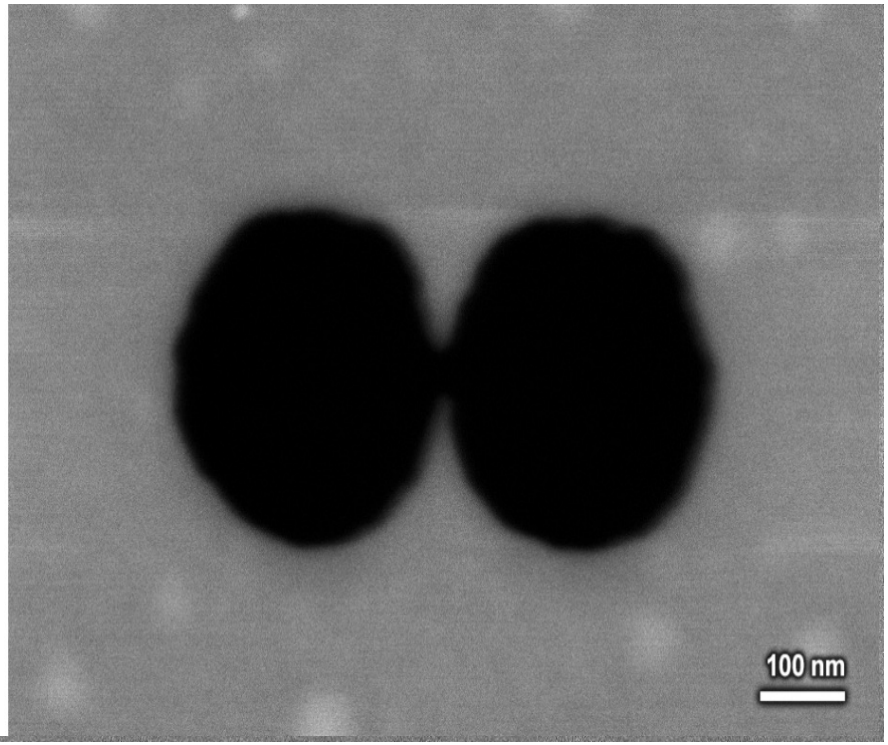


V. Flauraud – EPFL unpublished data

Fabrication of 2D antenna geometries

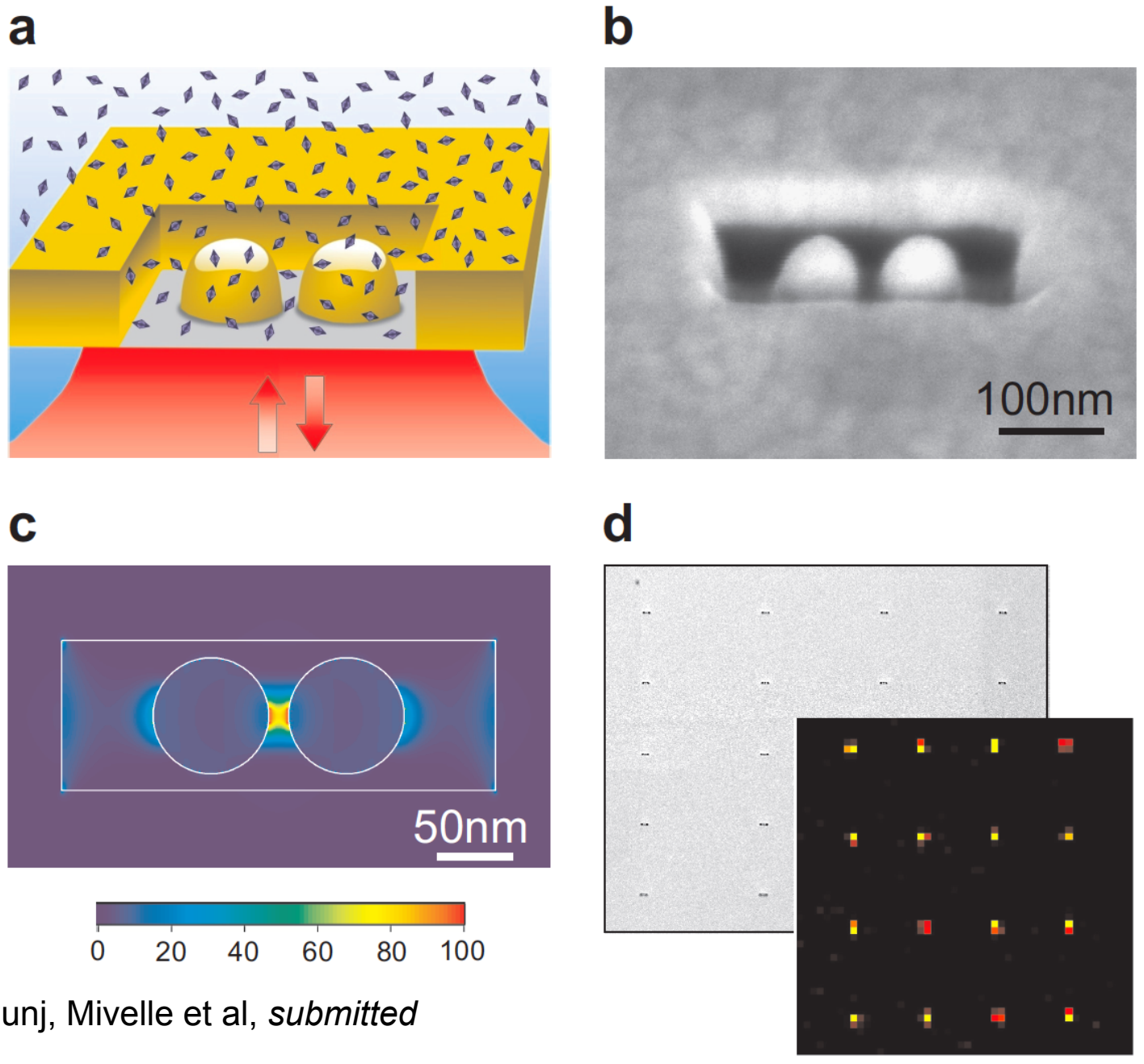
- EBL process carried out for BTA arrays
- Final free standing SiN stencils released





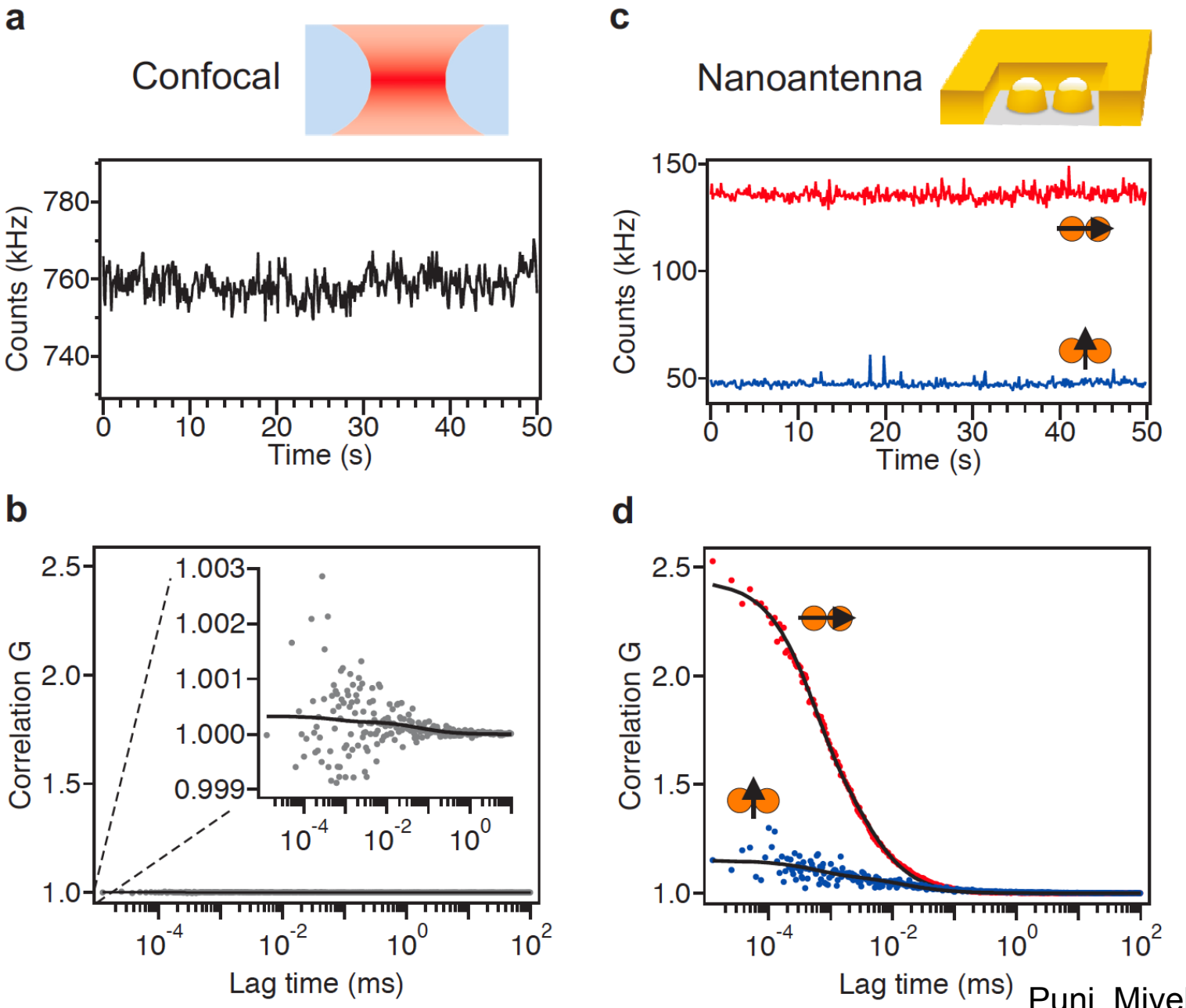
V. Flauraud – EPFL unpublished data

Antenna-in-a-box design for SMD @ high concentrations



Punj, Mivelle et al, *submitted*

Antenna-in-a-box design for SMD @ high concentrations

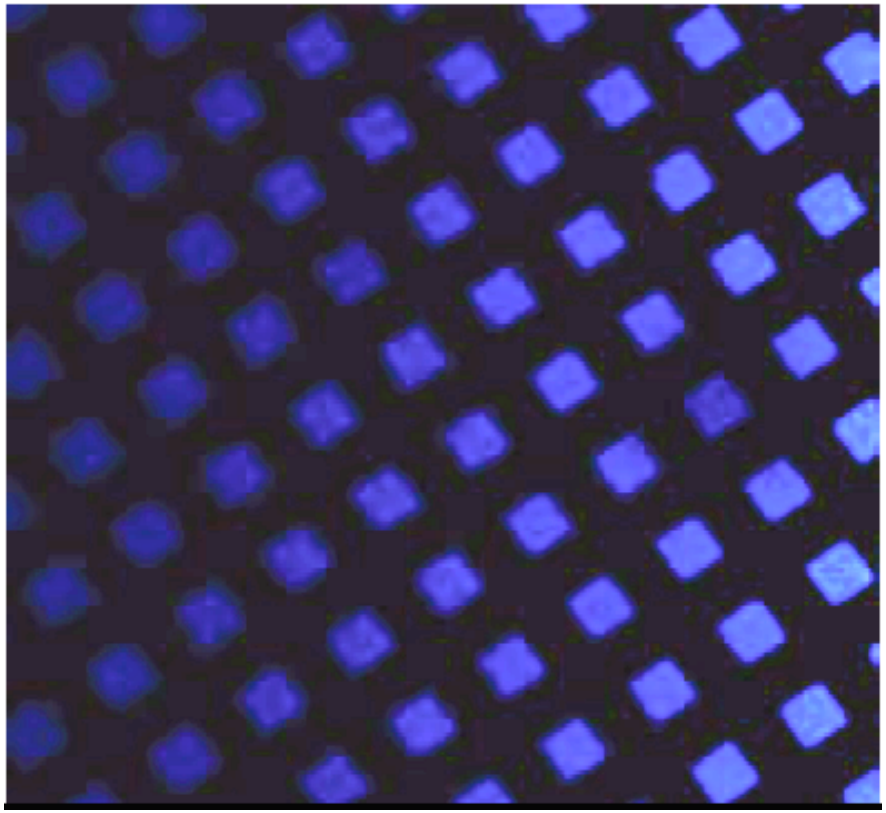


Punj, Mivelle et al, *submitted*

Mechanical tools compatible with optics

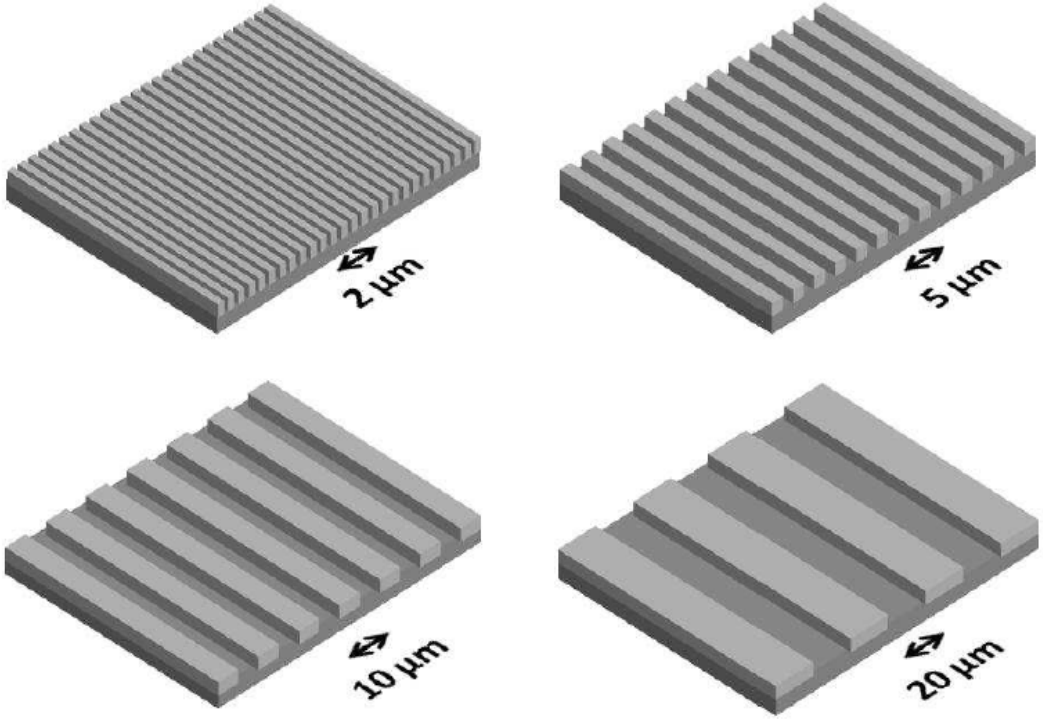
Micro-patterned substrates

Chemical contrast



μ-contract printing, different ligands, geometries, spacing

Topographic contrast

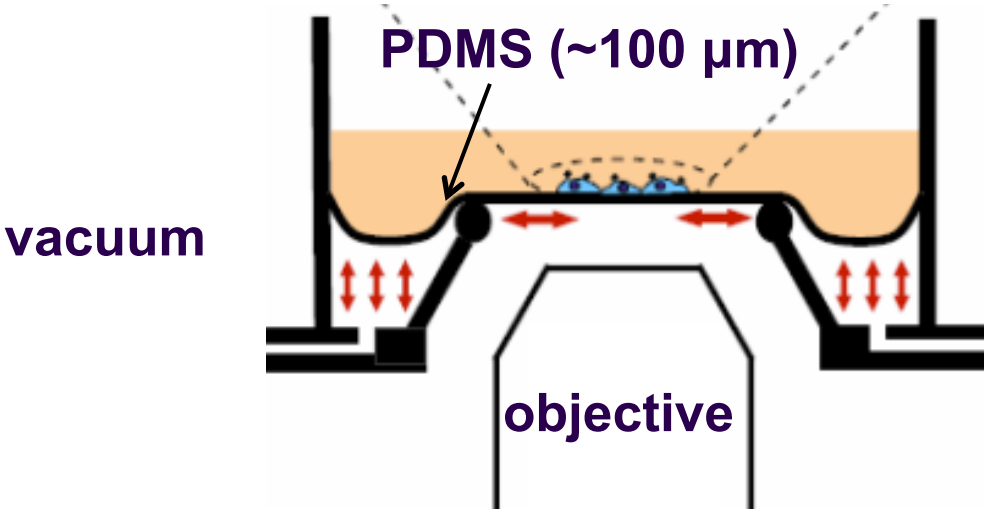


Hot embossing on polymers (PS, PMMA), different size, geometries, height…

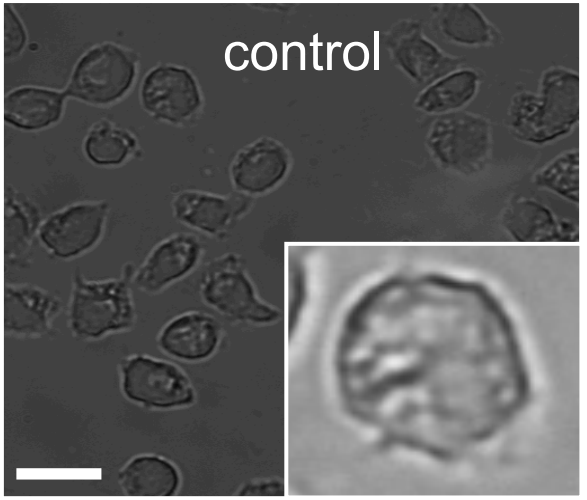
Mechanical tools compatible with optics

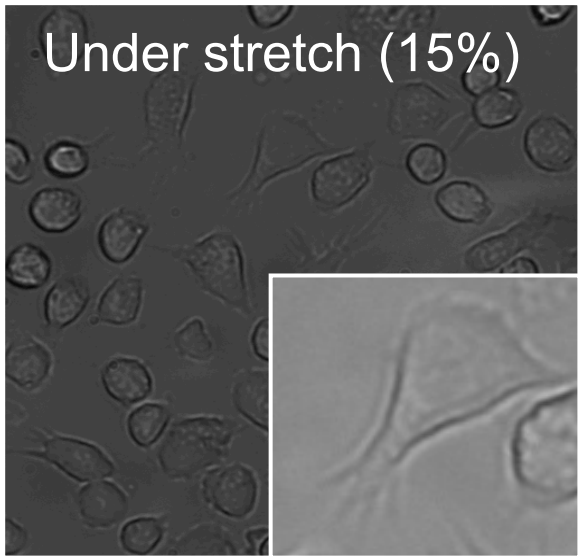
Stretching device compatible with SPT & superresolution

Cell stretching apparatus



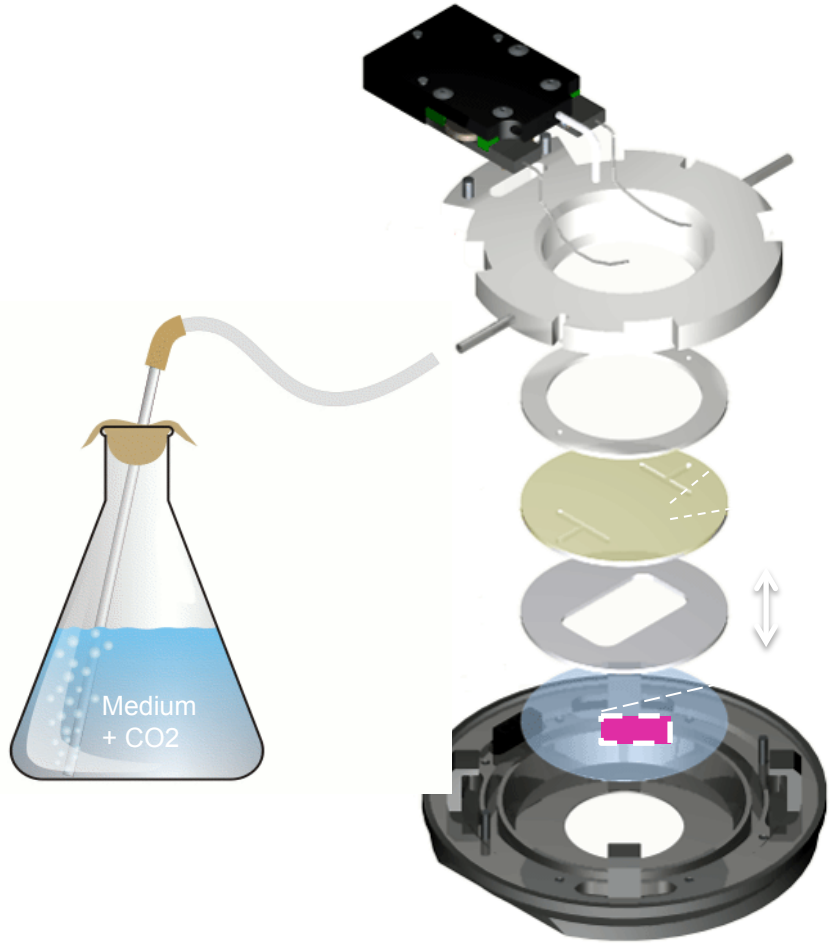
Trepat et al, *Nature 2007*

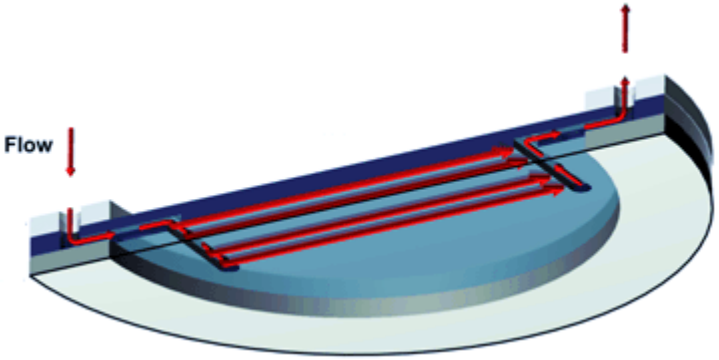


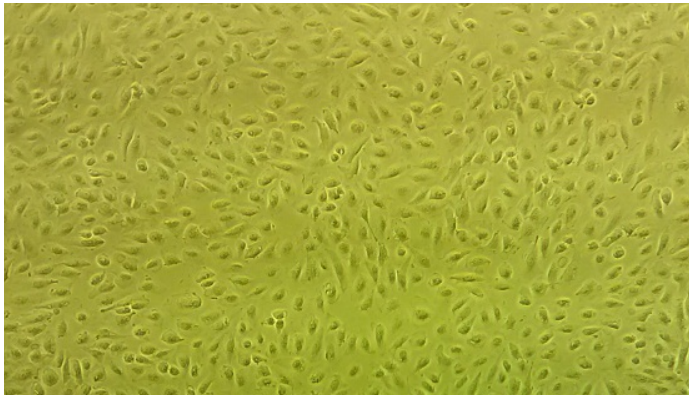


Mechanical tools compatible with optics

Shear flow compatible with SPT



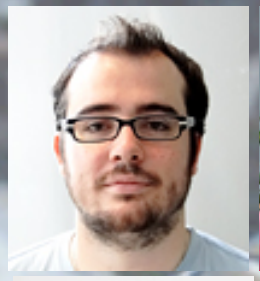




See talk Izabela Piechocka

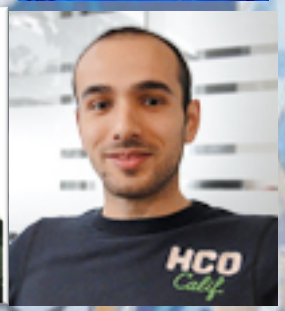
The players

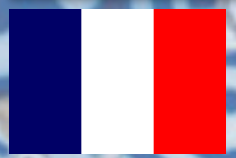








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- ✓ Herve Rigneault, IF, Marseille
  - ○ Jerome Wenger
- ✓ Juergen Brugger, EPFL
- ✓ Niek van Hulst, ICFO, BCN
- ✓ Jitu Mayor, NCBS, Bangalore

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