

Watching Single Motor Molecules at Work: From Cellular Function to Bionanotechnology

Stefan Diez

Bionanotechnology and Optical Technology Development
<http://www.mpi-cbg.de/~diez>



Andor EMCCD Workshop, Water's Edge (CT), April 14-16, 2005



Max-Planck-Institute of Molecular Cell Biology and Genetics, Dresden

BioNano-Group



Max-Planck-Institute of Molecular Cell Biology and Genetics

opened in 2001

300 scientists
from 28 countries

Biopolis Dresden

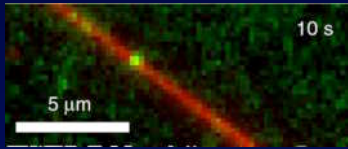


Max-Planck-Institute of Molecular Cell Biology and Genetics, Dresden

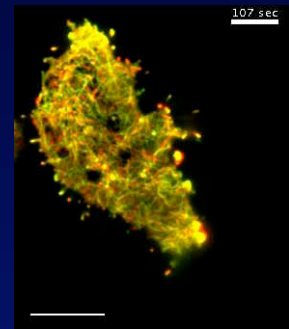
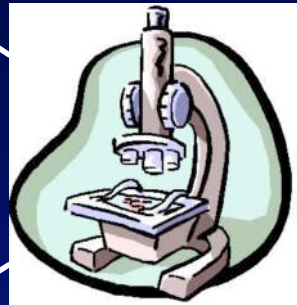
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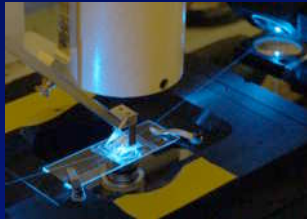
Outline



single molecule imaging

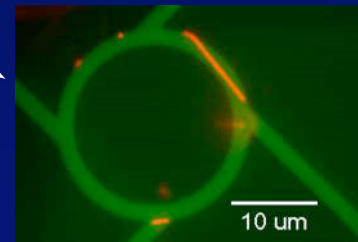


cell motility



optical technology development

speed + sensitivity matters !!!



bionanotechnology



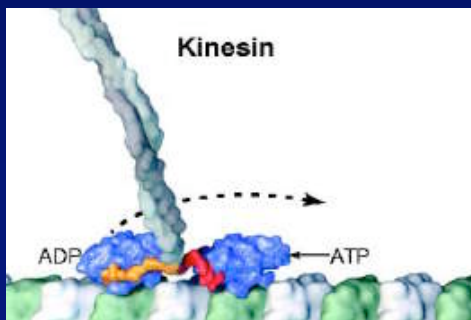
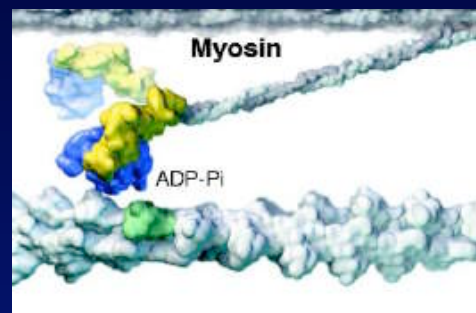
Molecular Motors Operating on the Cytoskeleton

... are the active workhorses in cells

... are complexes of 2+ proteins

... convert chemical energy (ATP) into mechanical work (and v.v.)

Directly!!! Important because confinement of heat is impossible on the nanoscale.

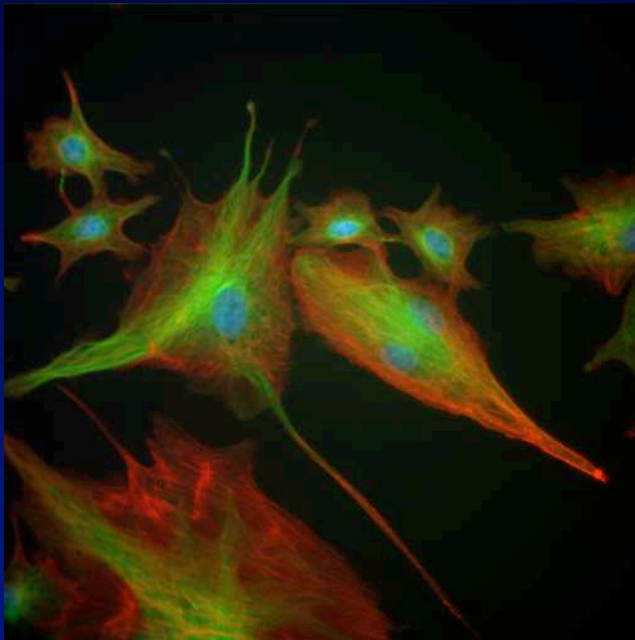


... are needed to generate force and to overcome diffusion by directed motion

e.g. passive diffusion of a small protein to the end of a 1m long neuron would take about 1000 years - with kinesin it takes a week

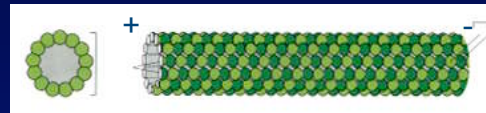


Microtubules: Structure and Cellular Function



≈ 100 μm

Microtubules



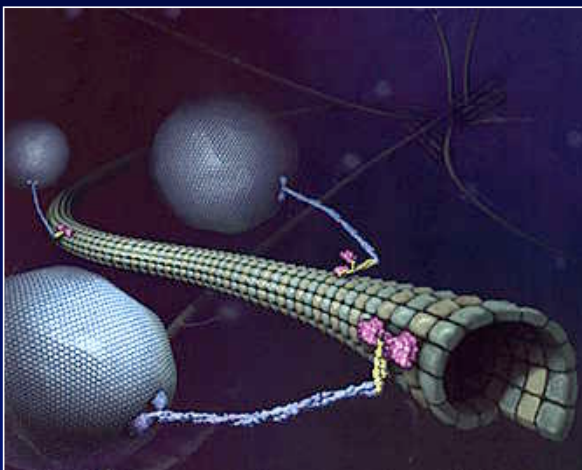
25 nm

Tubulin Dimer (α and β)

- organisation of the cytoplasm
- cell division
- road system

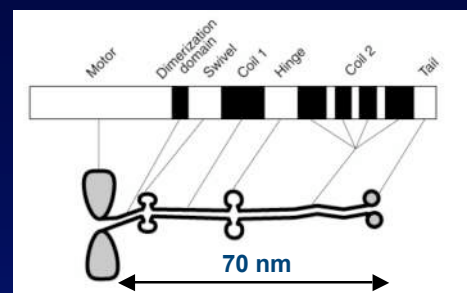


Kinesin walking on Microtubules



- step size: 8 nm
- speed: 0.8 μm/s (100 steps/s !!!)
- energy source: ATP hydrolysis

Kinesin



<http://valelab.ucsf.edu>

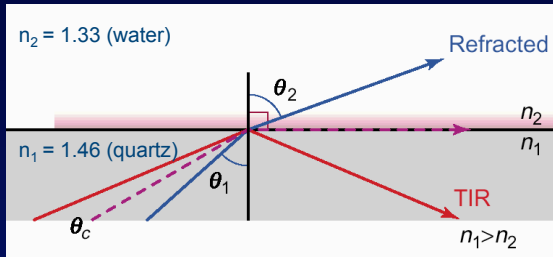
→ Single Molecule Imaging



Total Internal Reflection (TIR)

$$\theta_{\text{critical}} = 65.7 \text{ deg}$$

$$d_p \sim 100 \dots 200 \text{ nm}$$



Snell's law:

$$n_1 \sin \theta_1 = n_2 \sin \theta_2$$

θ = angle of incidence, n = refractive index

Critical angle:

At the critical angle, θ_c , $\theta_2 = 90^\circ$; $\sin 90^\circ = 1$;

$$n_1 \sin \theta_c = n_2$$

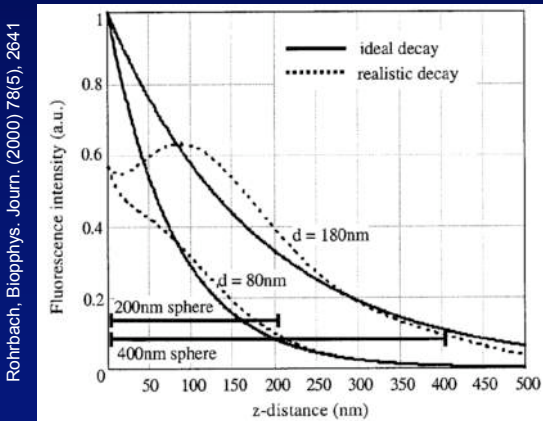
$$\theta_c = \sin^{-1}(n_2/n_1)$$

Evanescent field:

$$I_z = I_0 \exp^{-z/d_p}$$

$$d_p = \frac{\lambda}{4\pi \sqrt{n_1^2 \sin^2 \theta_1 - n_2^2}}$$

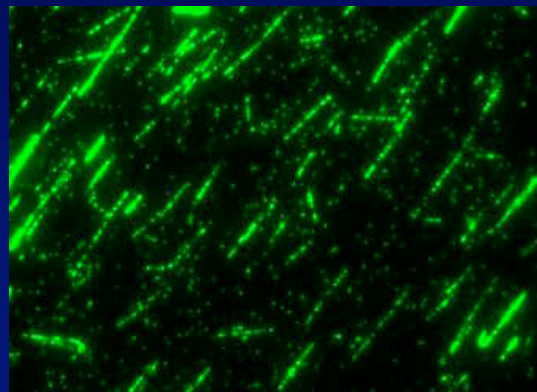
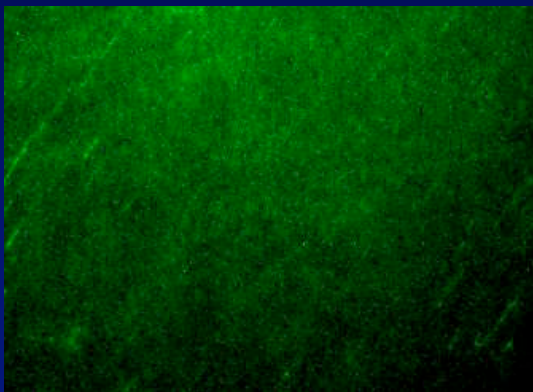
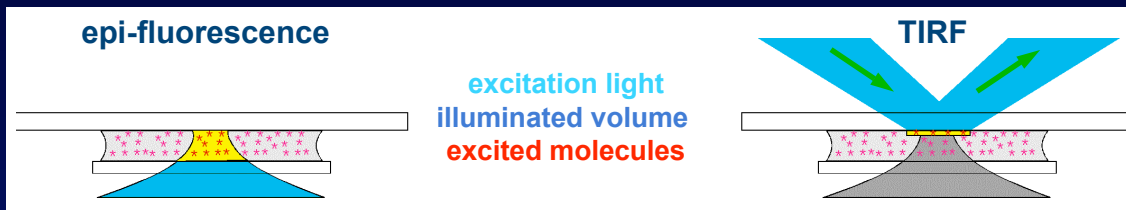
I = intensity, z = distance, λ = wavelength,
 d_p = penetration depth



from D. Toomre and D. J. Manstein, Trends in Cell Biology 11 (2001) 298



Total Internal Reflection Fluorescence (TIRF) Microscopy

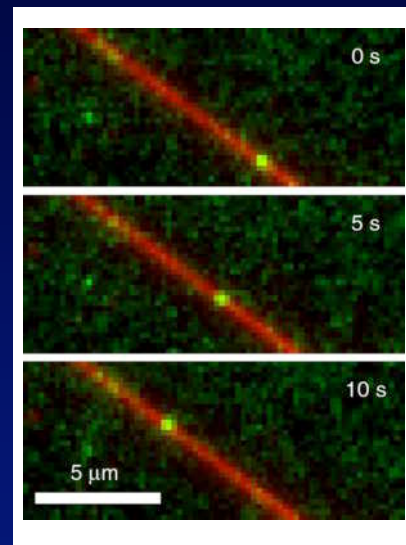
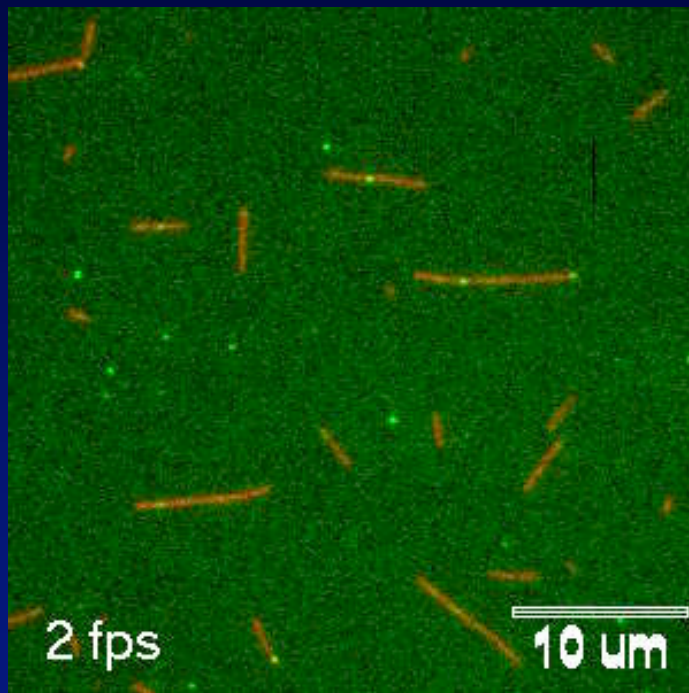


GFP-MCAK bound to the lattice of microtubules

10 μm



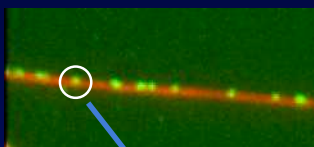
GFP-Kinesin Molecules Walking on Microtubules



→ speed, run length, directionality, inhibition



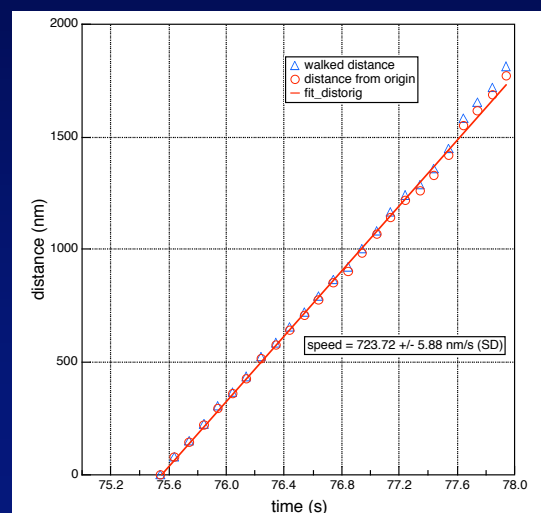
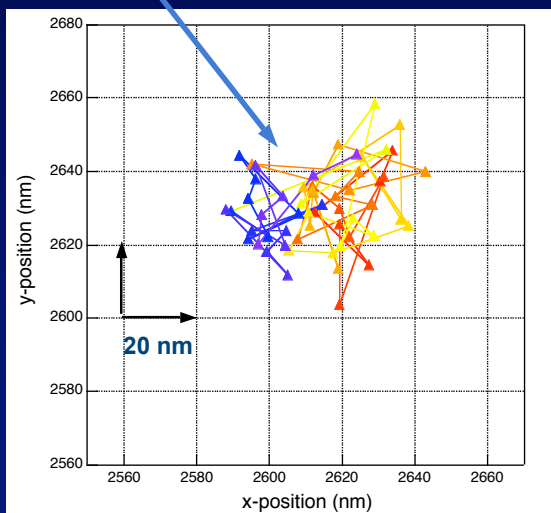
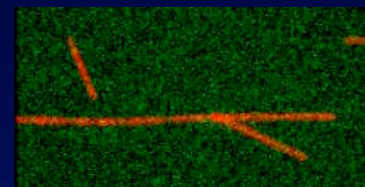
Nanometer Tracking of Kinesin Motors



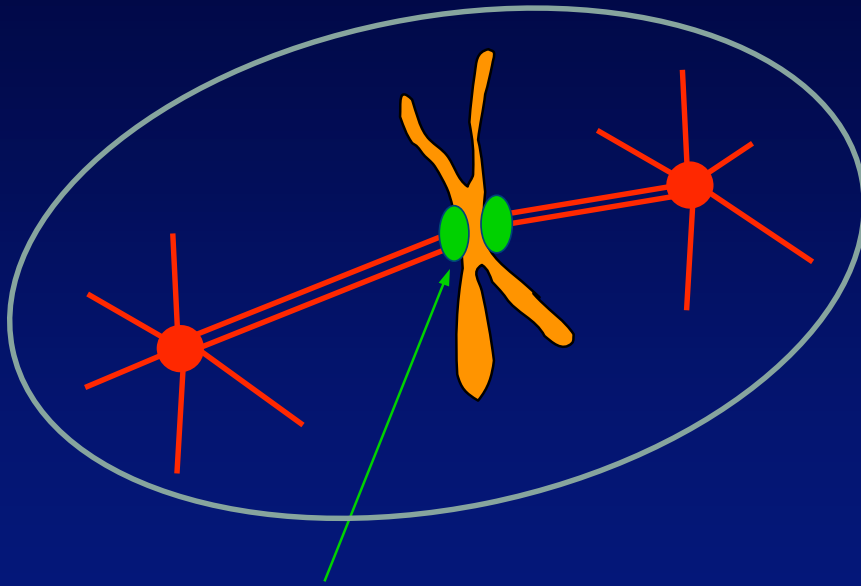
w/o ATP

micro / nano-fluidics

with ATP



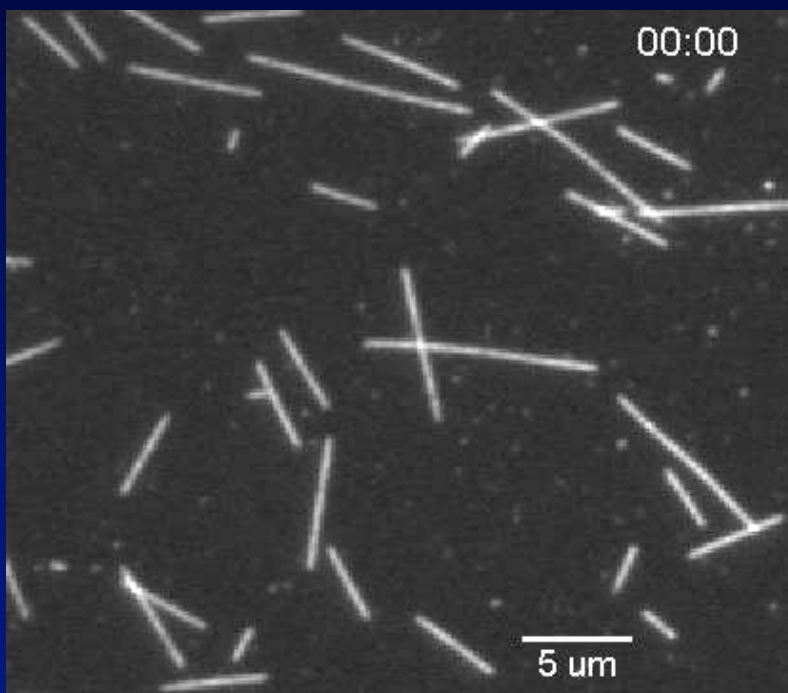
Other Kinesin-Related Motor Proteins: MCAK



MCAK - Mitotic Centromere-Associated Kinesin



Depolymerization of Stabilized Microtubules by MCAK



min

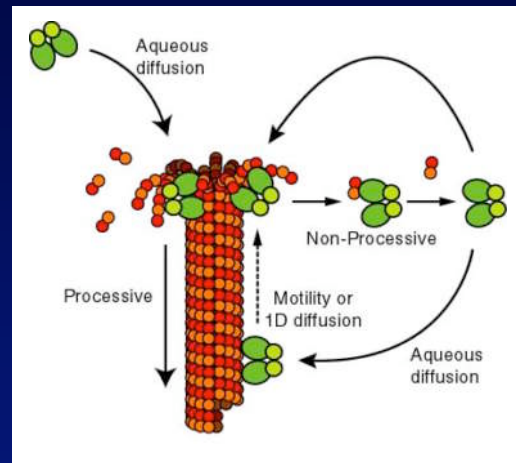
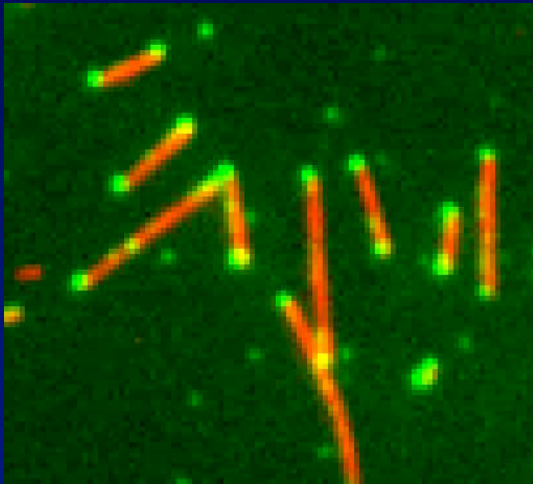
depol. rate
up to 1 $\mu\text{m}/\text{min}$

depolymerizes
from both ends:
40% speed (+ end)
60% speed (- end)



Depolymerization of Microtubules by MCAK

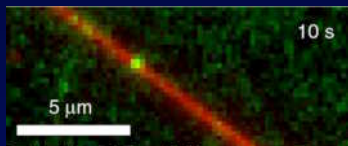
- targets the end of microtubules
- depolymerizes from both ends
- energy source: ATP hydrolysis



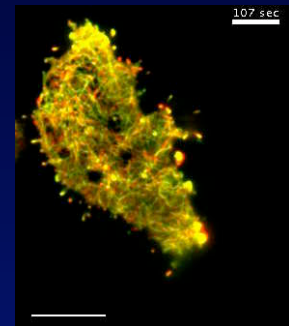
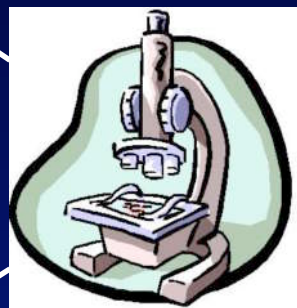
- How does MCAK get to the MT end?
- Processive / Nonprocessive?



Outline



single molecule imaging

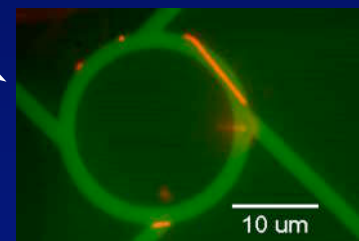


cell motility



optical technology development

**speed + sensitivity
matters !!!**

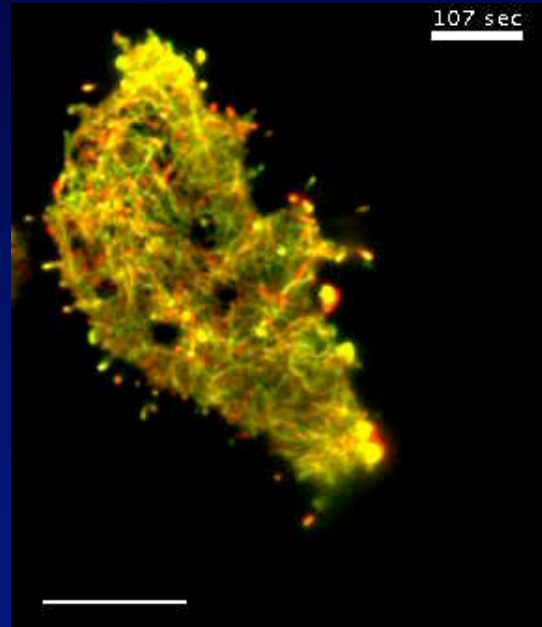
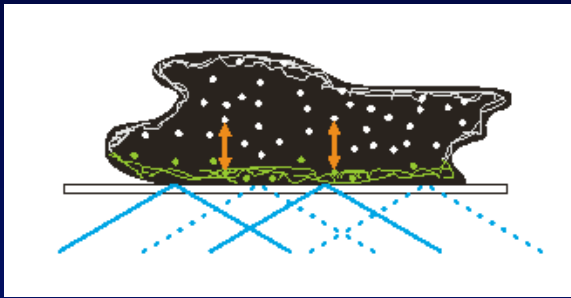


bionanotechnology



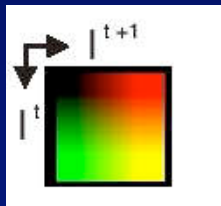
Cortical Actin Dynamics of Motile Cells

Current Biology (14) 1-10, 2004.



Dictyostelium
(LimEDcoil-GFP)

TIRF Imaging
100ms exposure
1 fps



collaboration with Günther Gerisch and Till Bretschneider (MPI Martinsried)



Max-Planck-Institute of Molecular Cell Biology and Genetics, Dresden

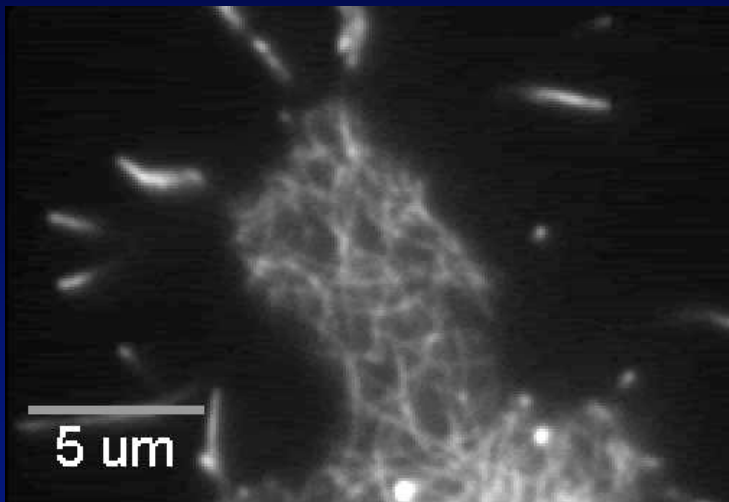
BioNano-Group



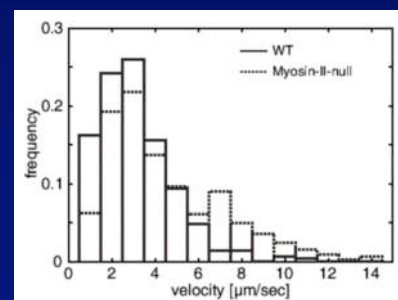
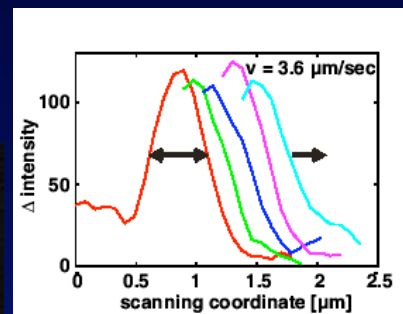
Fast Actin Reorganization in Cell Motility and Chemotaxis

Dictyostelium (LimEDcoil-GFP)

TIRF Imaging, 100ms exposure, 10 fps



PNAS, 2005 (in press)



collaboration with Günther Gerisch and Till Bretschneider (MPI Martinsried)

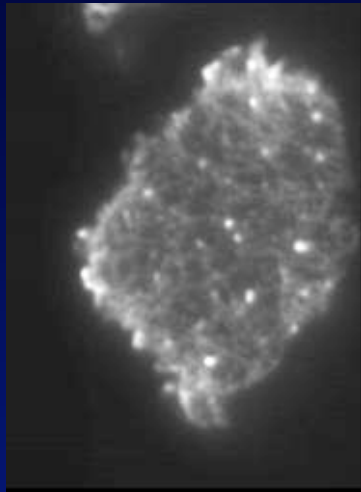


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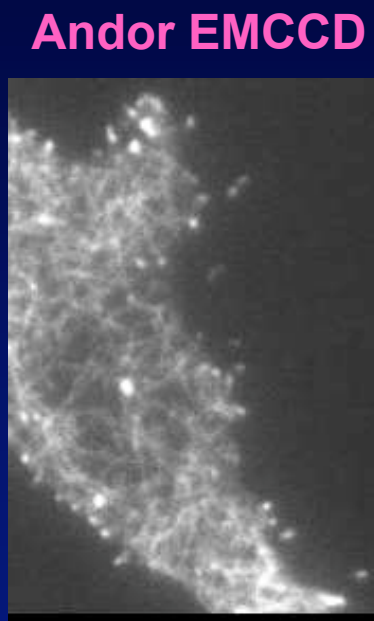


Fast Actin Reorganization in Cell Motility and Chemotaxis



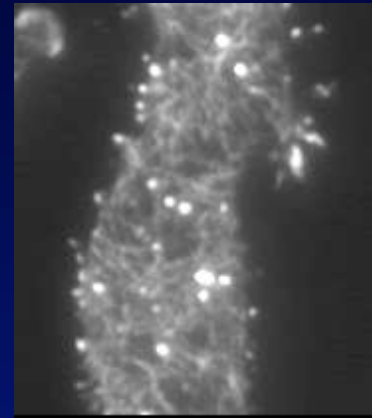
10 fps

light-induced
rounding up
of cells



Andor EMCCD

42 fps



10 fps

focussing
while
streaming

collaboration with Günther Gerisch and Till Bretschneider (MPI Martinsried)

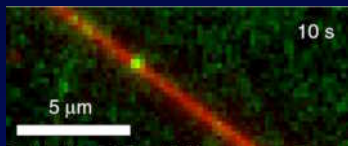


Max-Planck-Institute of Molecular Cell Biology and Genetics, Dresden

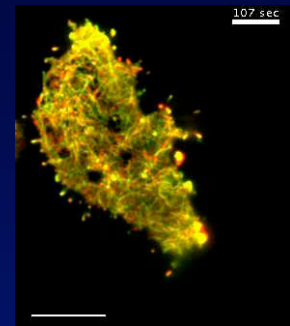
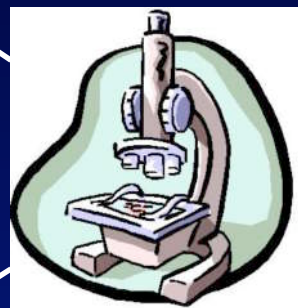
BioNano-Group



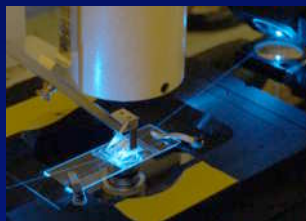
Outline



single molecule imaging

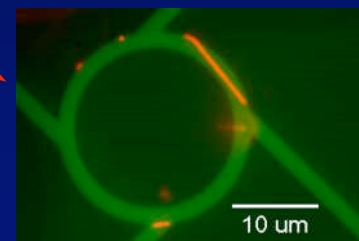


cell motility



optical technology development

**speed + sensitivity
matters !!!**



bionanotechnology



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Generation and Manipulation of Nanostructures ...

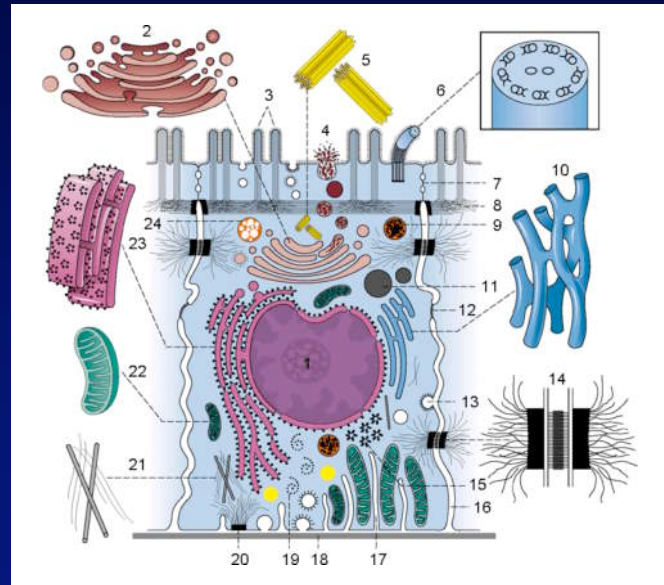
Advantages:

- highly optimized processes
- small dimensions
- parallel processing possible
- molecular-specific interaction
- energy efficient
- potentially cheap

Challenges:

- synthetic environment
- controlability
- reproducibility

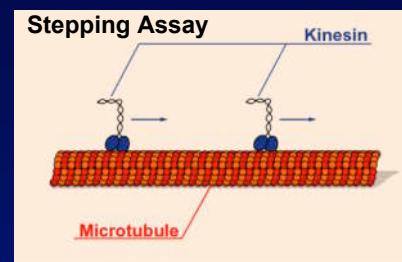
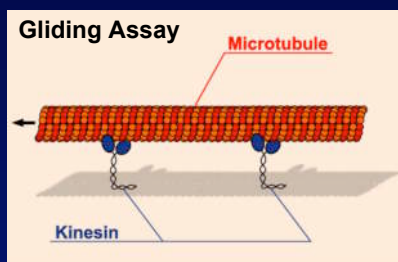
... using Cellular Machines!



→ Kinesin + Microtubule



Nanotechnology: Kinesin-Microtubuli Assays

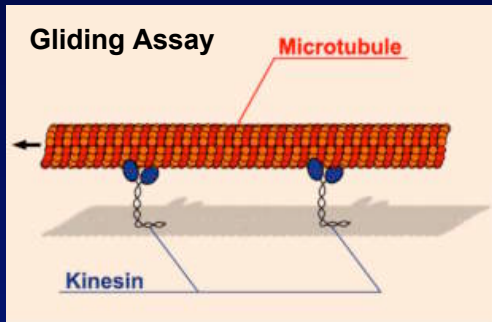


- microtubules act as nanohandles
- large forces possible (6-100pN)
- flexible carrier surface

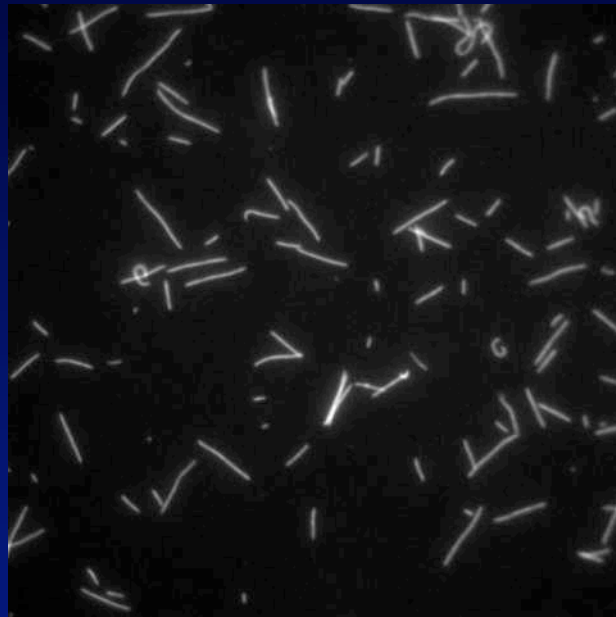
- kinesin molecules act as nanohandles
- 8 nm discrete steps
- single molecule gripper



Gliding Motility Assay to study Kinesin



→ control over motility



10 μm



Surface Topography

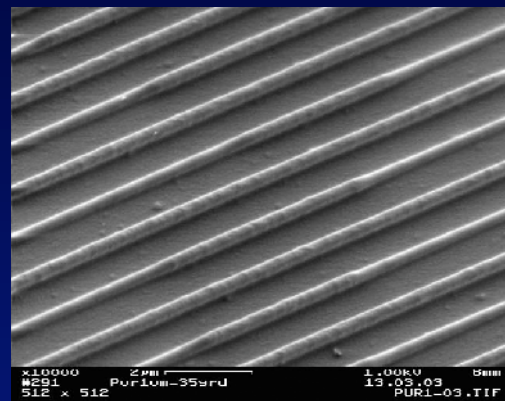
Replica-Molding:
Si Master → PDMS → Polyurethan (PU)



channel width: 500 nm
channel depth: 300 nm



500 nm PU-structure on glass



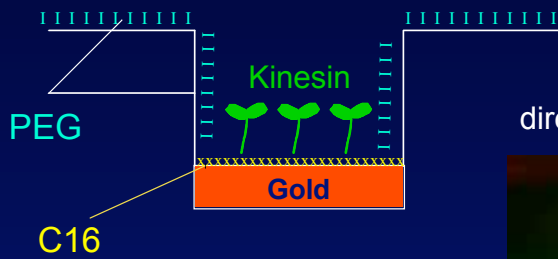
directed movement

changing between channels due
to kinesin on the walls and rims

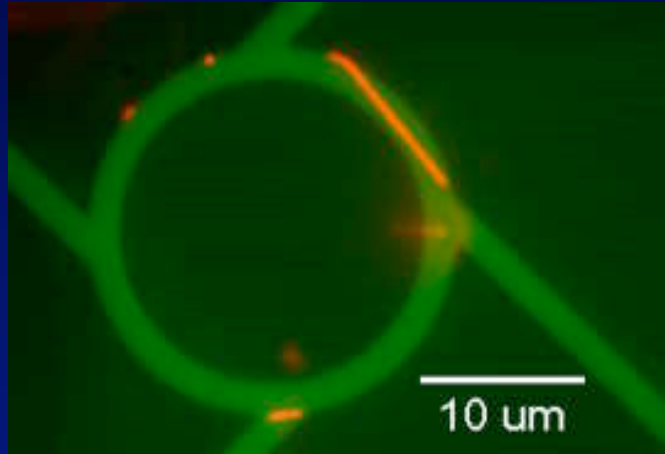
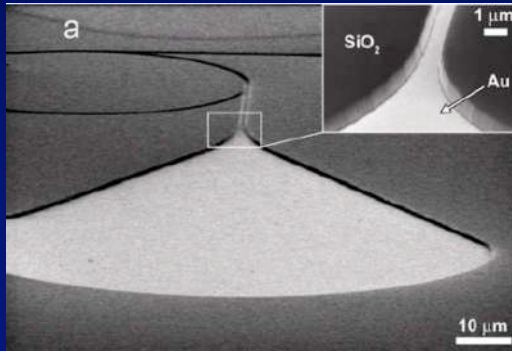
Si-master by Tilo Pompe (IPF Dresden)



Surface Topography and Chemistry on Silicon



directed movement of microtubules on Si-chip



Nano Letters, in press, 2005.

collaboration with group of Cees Dekker (TU Delft)



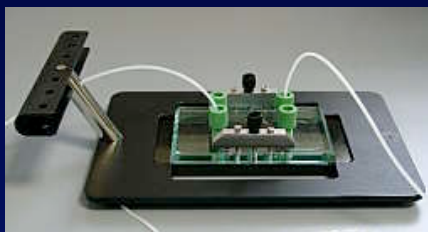
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External Control over Microtubule Motility

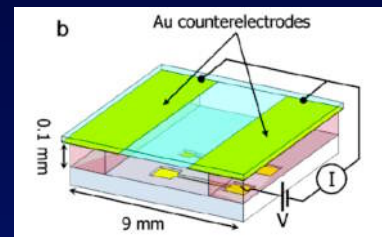
hydrodynamic fields



Microfluidics and Nanofluidics, in press

flowcell development together with GeSim (S. Howitz)

electrical fields



collaboration with TU Delft (C. Dekker)

Nano Letters (5), 235, 2005.



directional control



temporal control



electrical docking

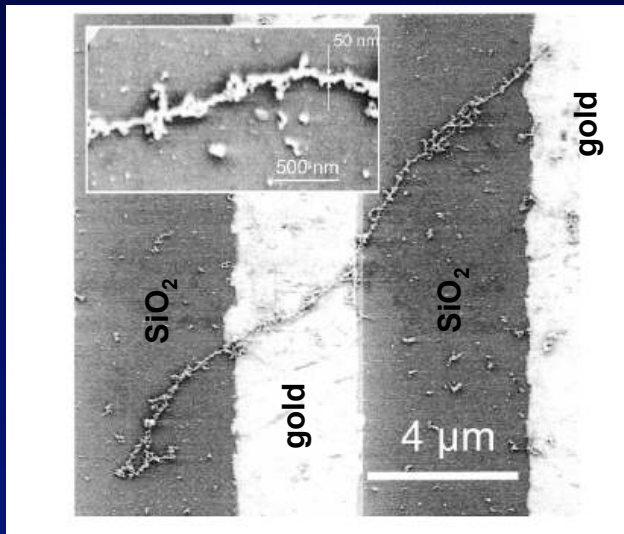


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Building Nano Wires and Networks with DNA



DNA strand metallized with palladium bridging two gold electrodes (group of M. Mertig and W. Pompe, TU Dresden)

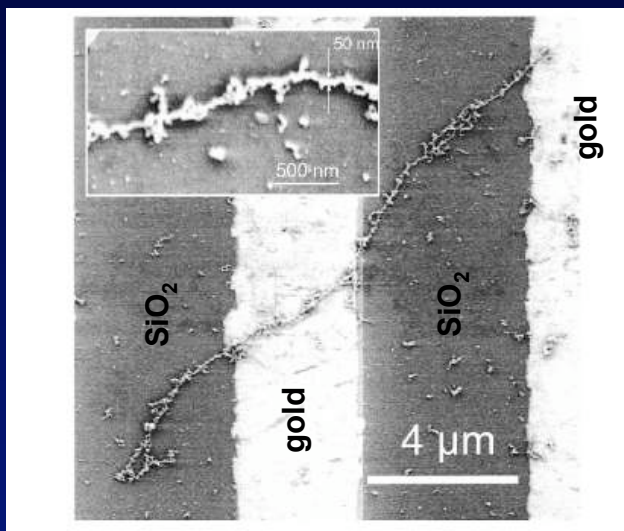
Stretching DNA in a hydrodynamic flow



Flow: 0.5 μl/s



Building Nano Wires and Networks with DNA



DNA strand metallized with palladium bridging two gold electrodes (group of M. Mertig and W. Pompe, TU Dresden)

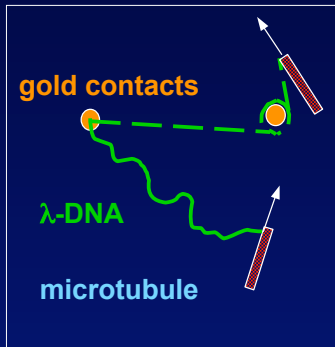
Towards Nanoelectronics:

Use
biological machinery
and
evolutionary mechanisms of cells
to build
networks
on the nanoscale.



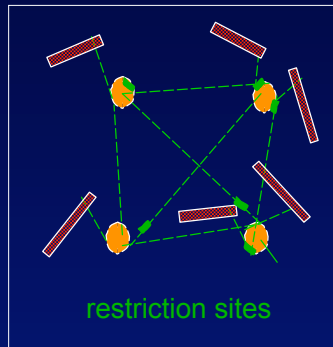
Generation of DNA-based Nanocircuits

Network Assembly



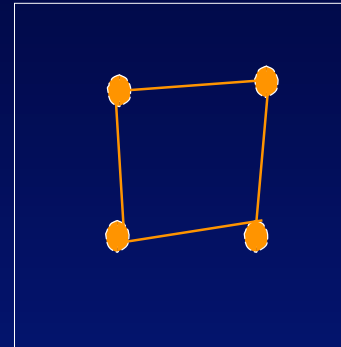
DNA binding to gold-contacts and microtubules
stretching + transport of DNA by microtubules
deposition of DNA on second gold contact

Optimization



creation periodic DNA-network by parallel processing
structural selection by sequence-specific restriction enzymes

Metallization

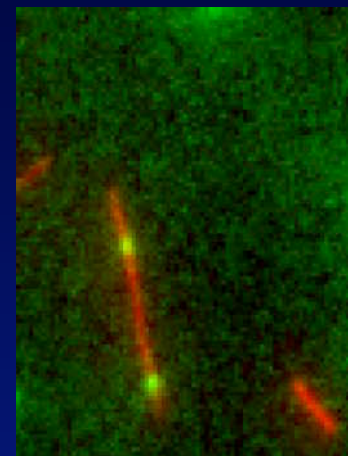
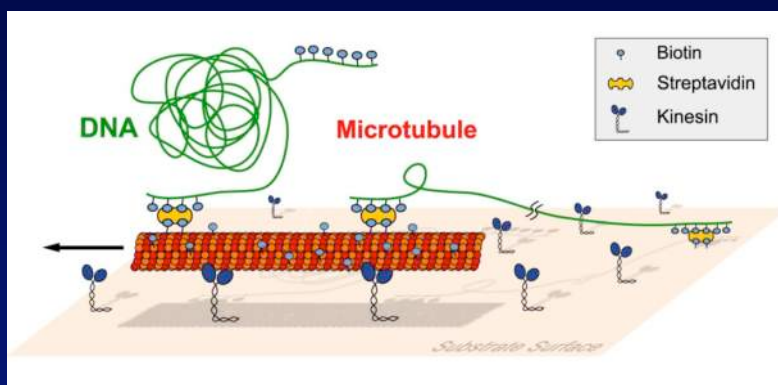


deposition of metal complexes (Pt, Pd, Au, Ag)
...or also carbon-nanotubes, quantum dots



Binding and Transport of DNA on motile Microtubules

Nano Letters (3),1251, 2003.



Biotinylated Microtubules :

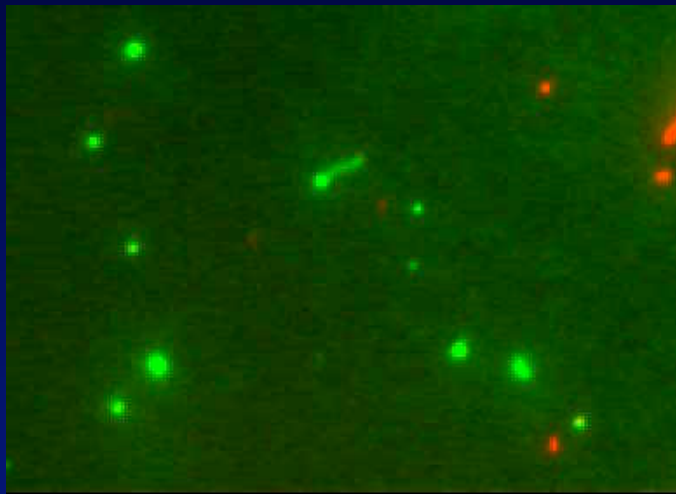
- Biotin randomly incorporated
- (9 tub / 3 Rhod-tub / 4 Biotin-tub)
- labeled with Rhodamine

λ-phage DNA ds :

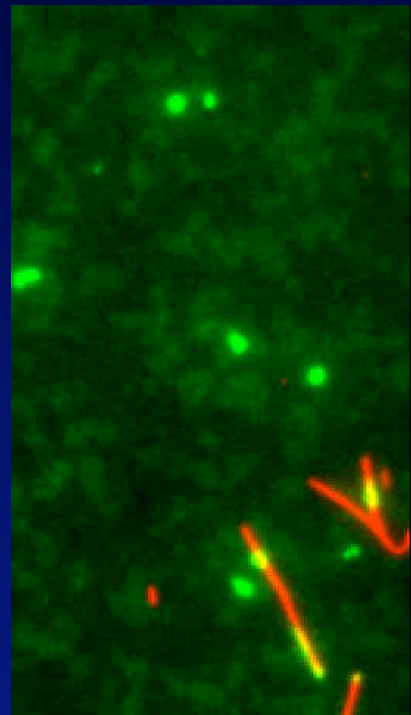
- 17μm long, 2nm in diameter
- coiled molecules
- biotinylated at both ends
- labeled with YOYO



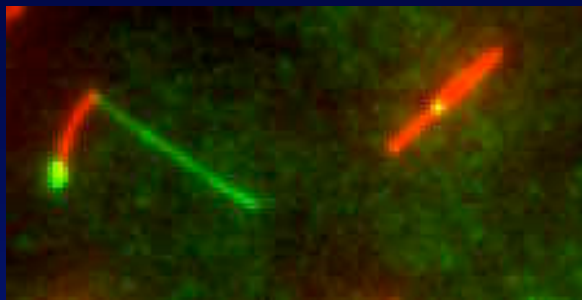
Pickup and stretching of DNA by motile MT



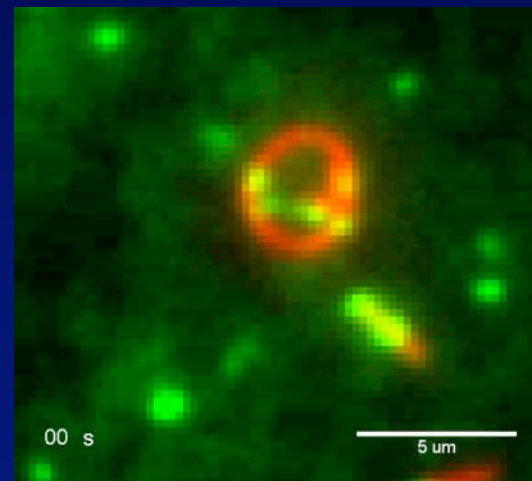
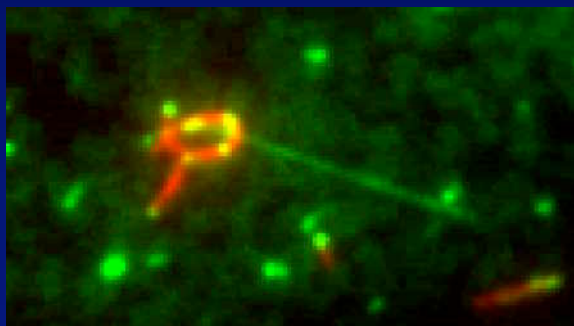
5 μm



Restriction of MT movement by stretched DNA



... guiding of microtubules



00 s

5 μm



Acknowledgements

<http://www.mpi-cbg.de/~diez>



Jacob Kerssemakers,
Bert Nitzsche, Felix Ruhnow
(**Single Molecule Techniques**)

Cordula Reuther, Cerasela Dinu,
Leonid Ionov, Dimitar Stamov
(**Nanotec**)

Corina Bräuer
(**Technician**)



funded by the BMBF
Nanotechnology

Collaborations:

MCAK:

J. Helenius, G. Brouhard, **Joe Howard**
(MPI-CBG)

Actin Dynamics:

T. Bretschneider, G. Gerisch
(MPI Martinsried)
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