Active Transport by Biomolecular Motors: A New Tool for Nanotechnology

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S. Ramachandran, R.Tucker , D. Wu and V. Vogel

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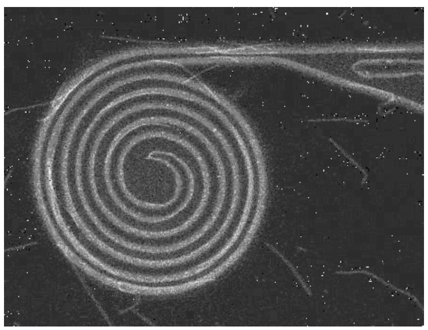
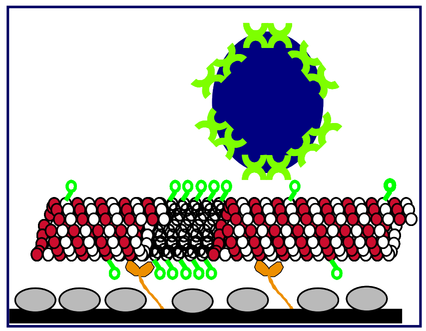
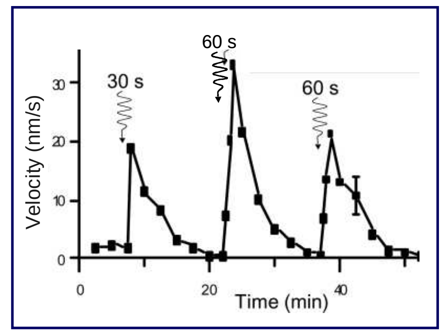
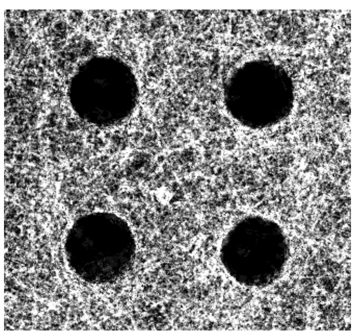
Molecular Shuttles – a Module for Nanodevices

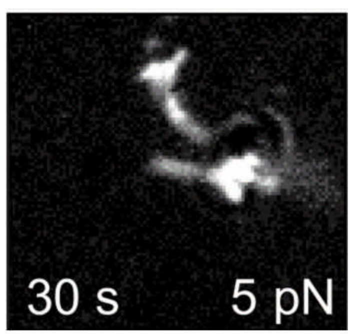




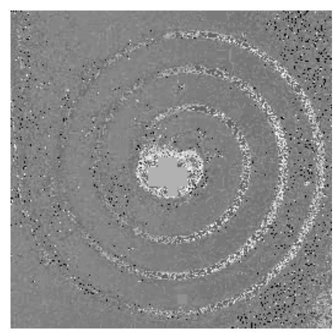




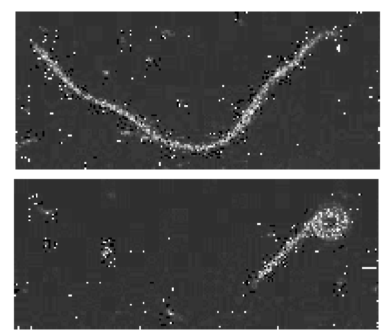
Surface Imaging



Measuring Forces



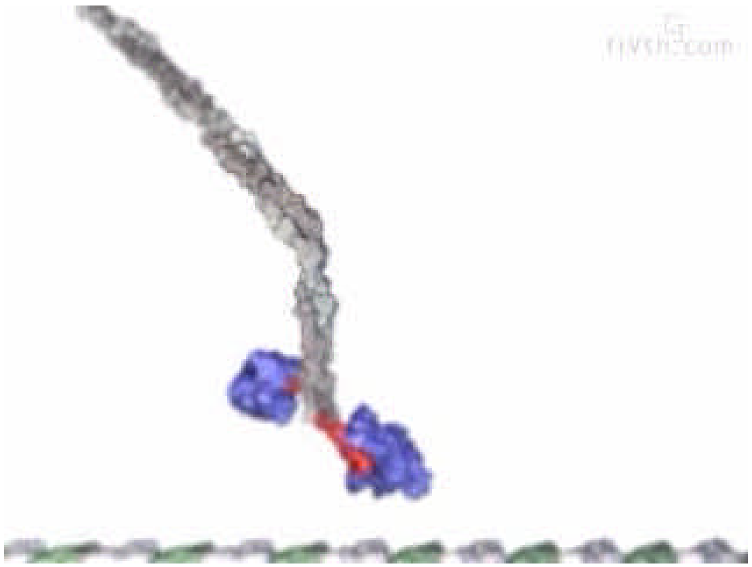
Sensors



Nanoscale Assembly

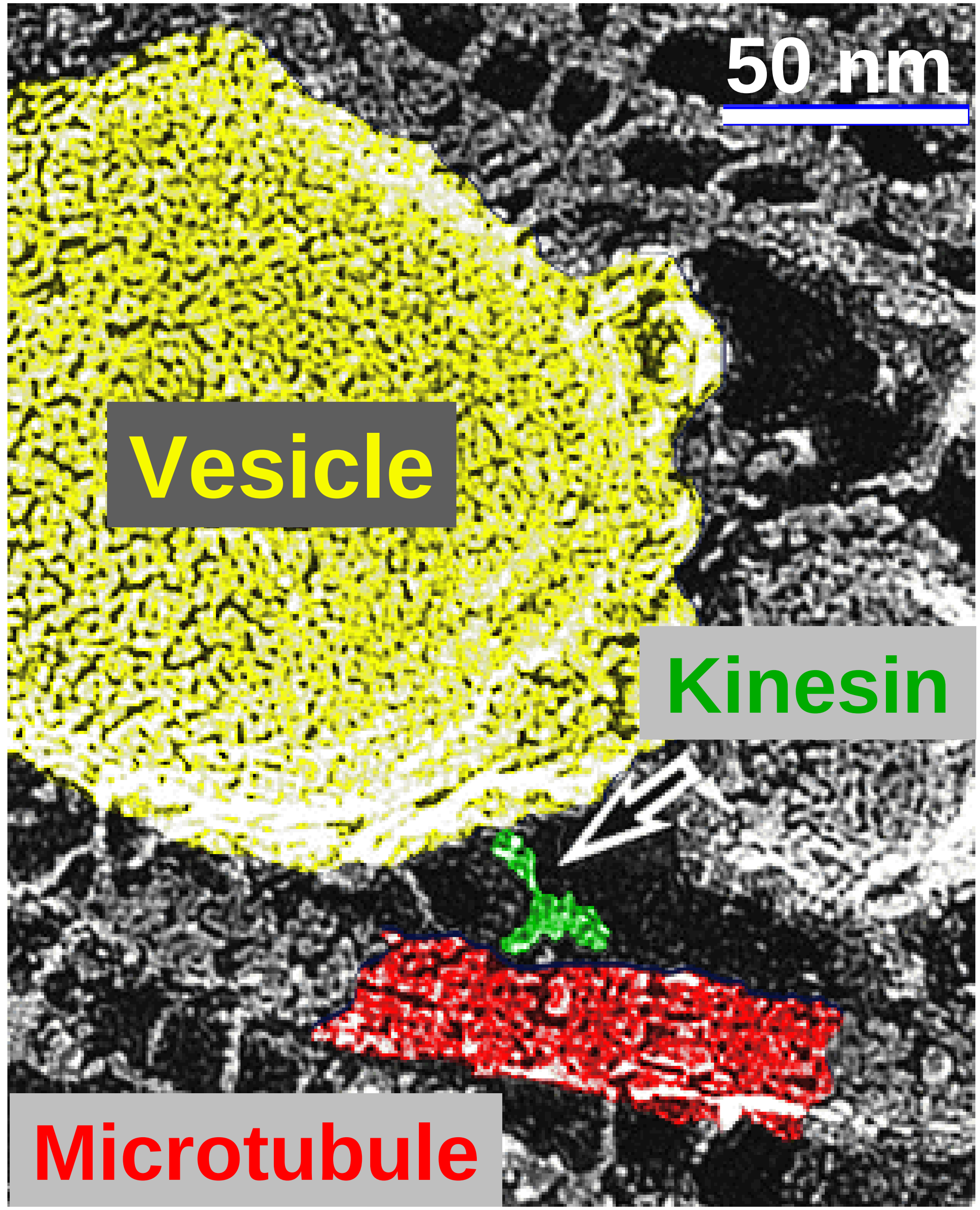
Kinesin motor

Fuel: 1 ATP per step
Force: 5 pN per motor
Speed: 100 steps/s of 8 nm



Movie from Vale lab, UCSF

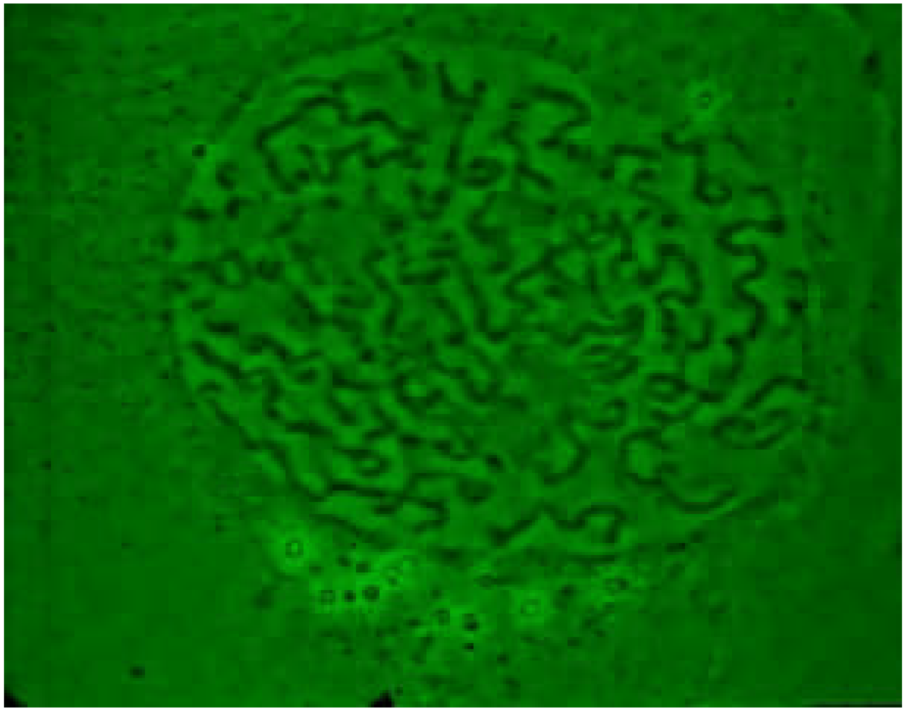
N. Hirokawa, Science **279**, 519 (1998)



Motor Proteins

Mitosis –
coordinated movement,
positional control

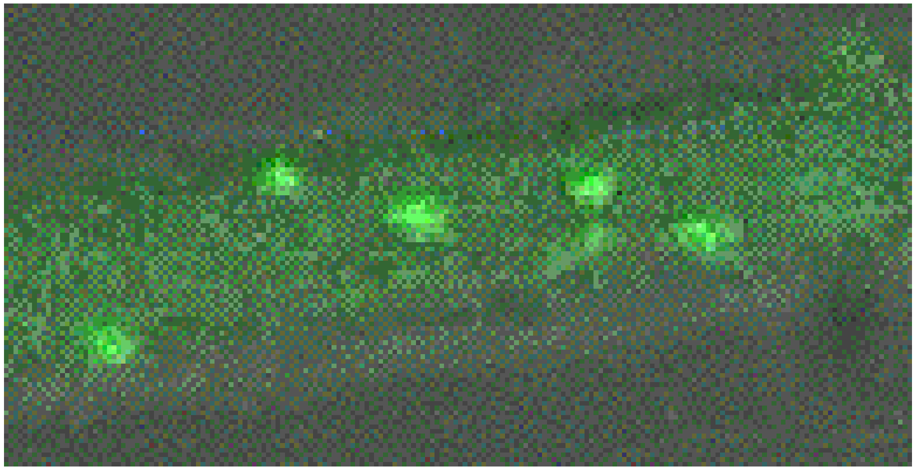
~10 μm



Smith College, Dept. of Biology website

Fast anterograde transport –
transport of vesicles (D < 100 nm)
through axons (D = 1 μm)

1 μm



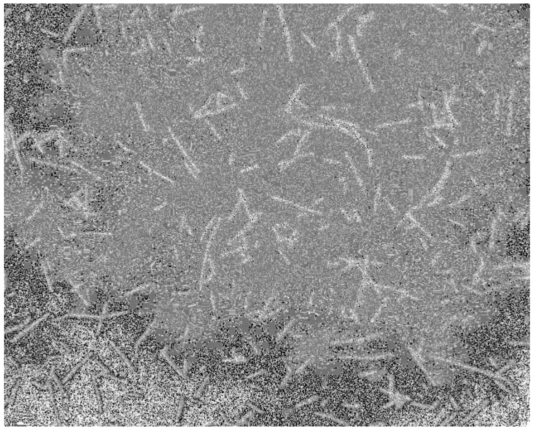
G.A. Smith et al., PNAS, 98,3466-3470

Application:

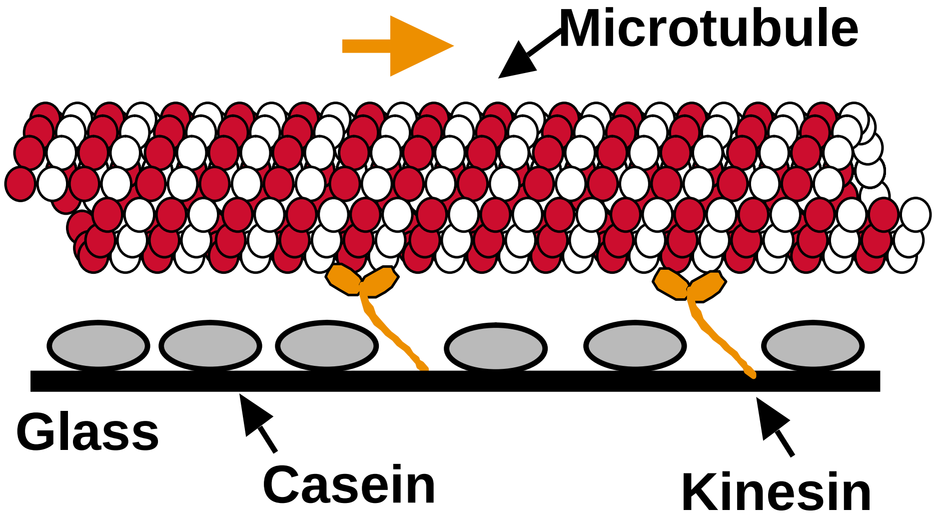
Diffusion: $\langle \Delta x^2 \rangle = 2Dt$	Active transport	Pressure-driven fluid flow: $v \sim d^2$
Intracellular transport - effective diffusion limits cell size	Mitosis Fast anterograde transport	Large scale transport – cardiovascular system
Self-assembly – upper limit for lateral dimensions	Nanofluidics Guided assembly Smart Materials	Microfluidic devices – limit for channel diameter

Basic experimental setup:
Inverted motility assay

J. Howard et al, *Methods in Cell Biology* 39 (1993) 137

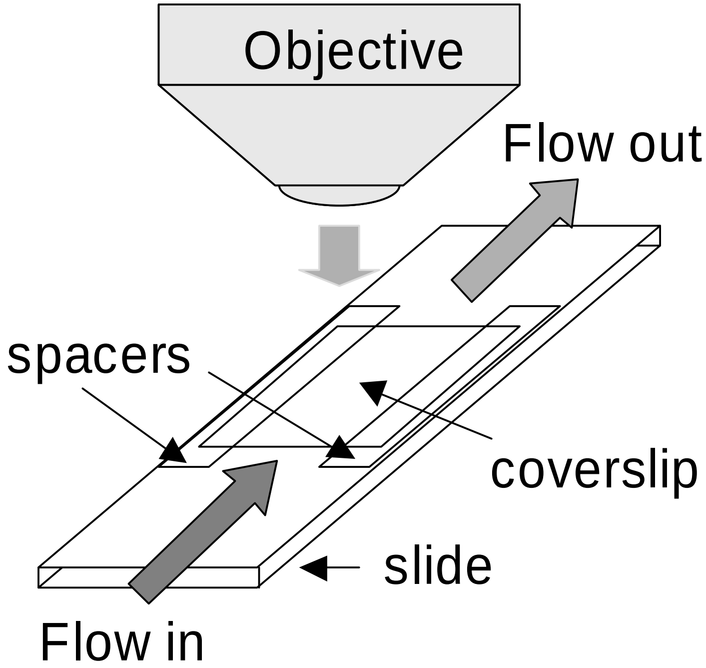


Force: 5 pN per Kinesin

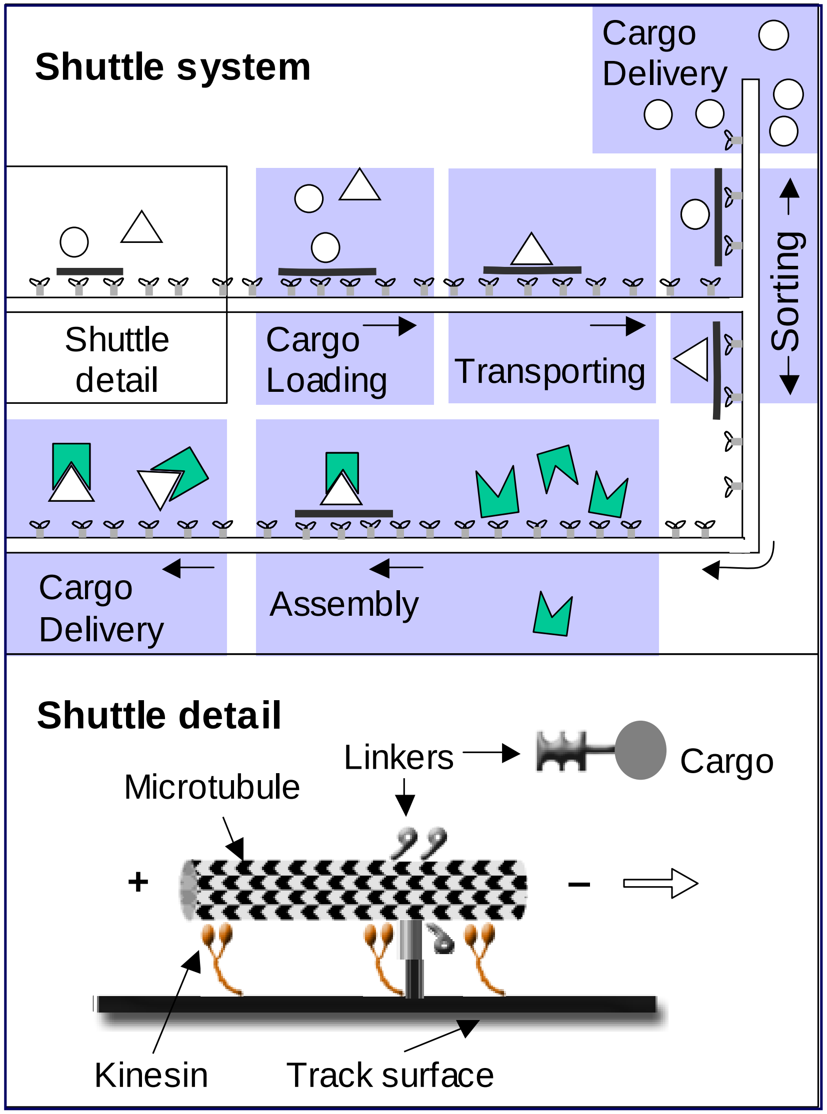


Speed: 800 nm/s at 1 mM ATP

Fluorescence microscope

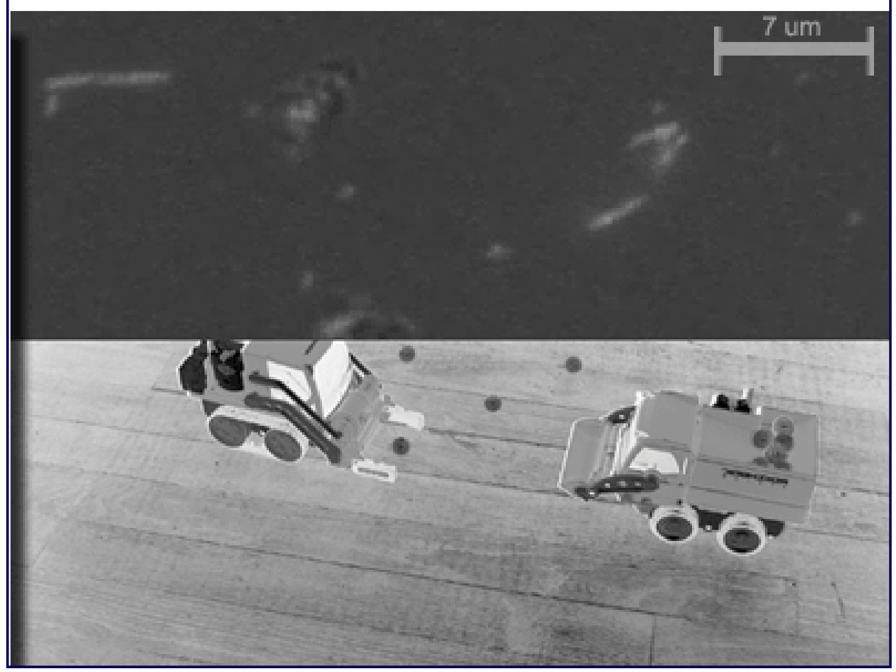


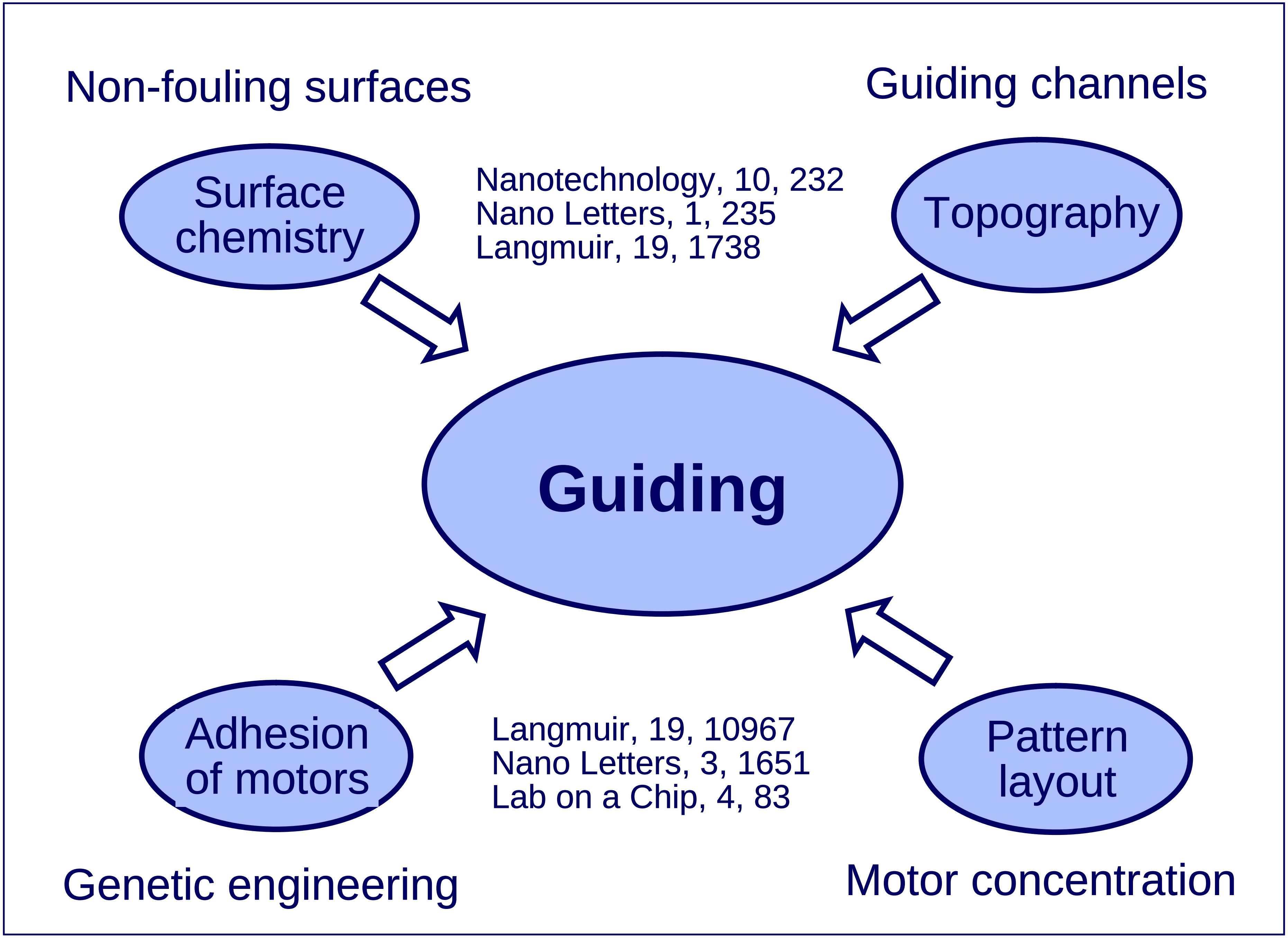
Molecular Shuttles – a Nanoscale Transport System



Engineering challenges:

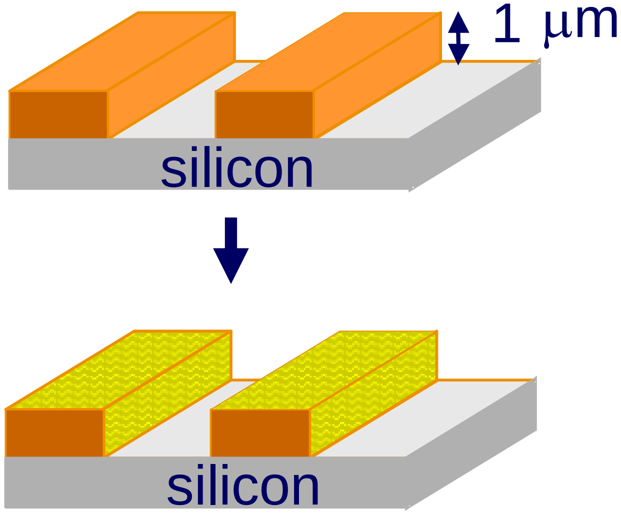
- Guiding along tracks
- Control of movement
- Loading and unloading




Non-fouling surfaces
Guiding channels
Surface chemistry
Nanotechnology, 10, 232
Nano Letters, 1, 235
Langmuir, 19, 1738
Topography
Guiding
Adhesion of motors
Langmuir, 19, 10967
Nano Letters, 3, 1651
Lab on a Chip, 4, 83
Pattern layout
Genetic engineering
Motor concentration

Guiding:

Photolithography
+ Detergent to prevent
Protein adsorption

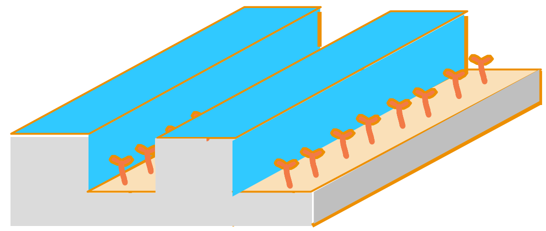


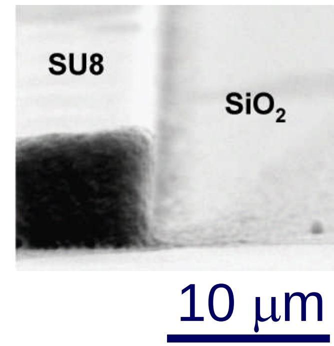
Photoresist SU8-2

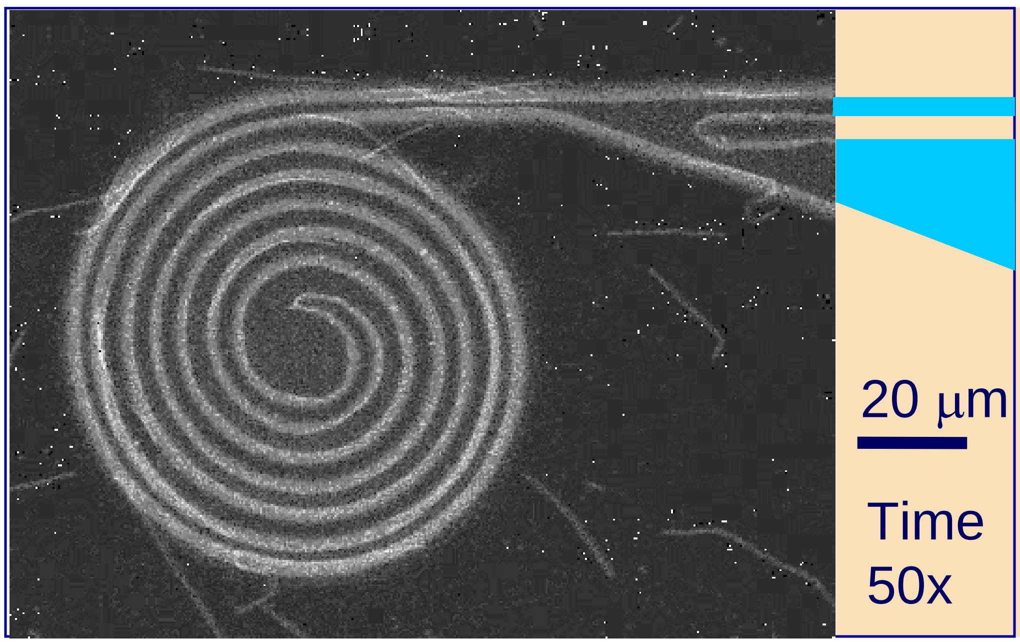
Adhesive: Bare silicon

Non-adhesive: F108 pluronic [PEO_{129}-PPO_{56}-PEO_{129}]

Combination of surface chemistry and topography

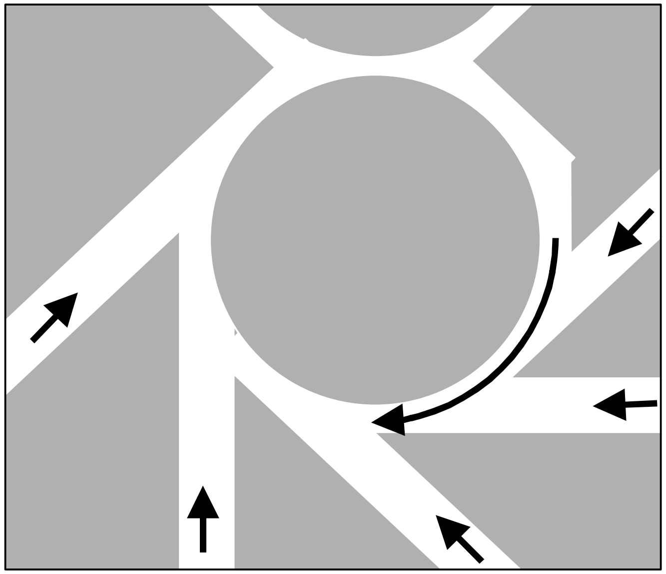


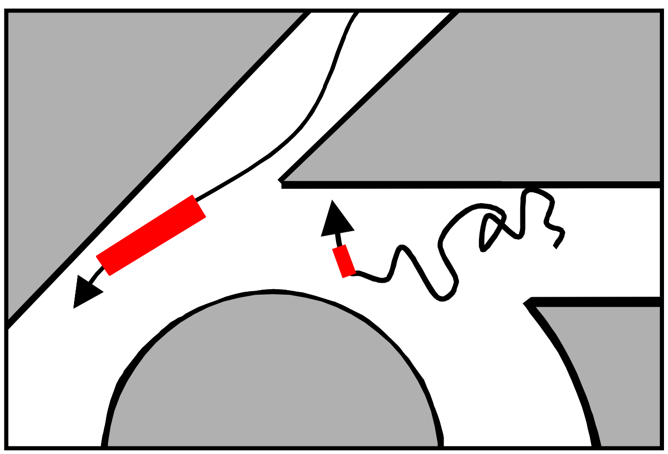


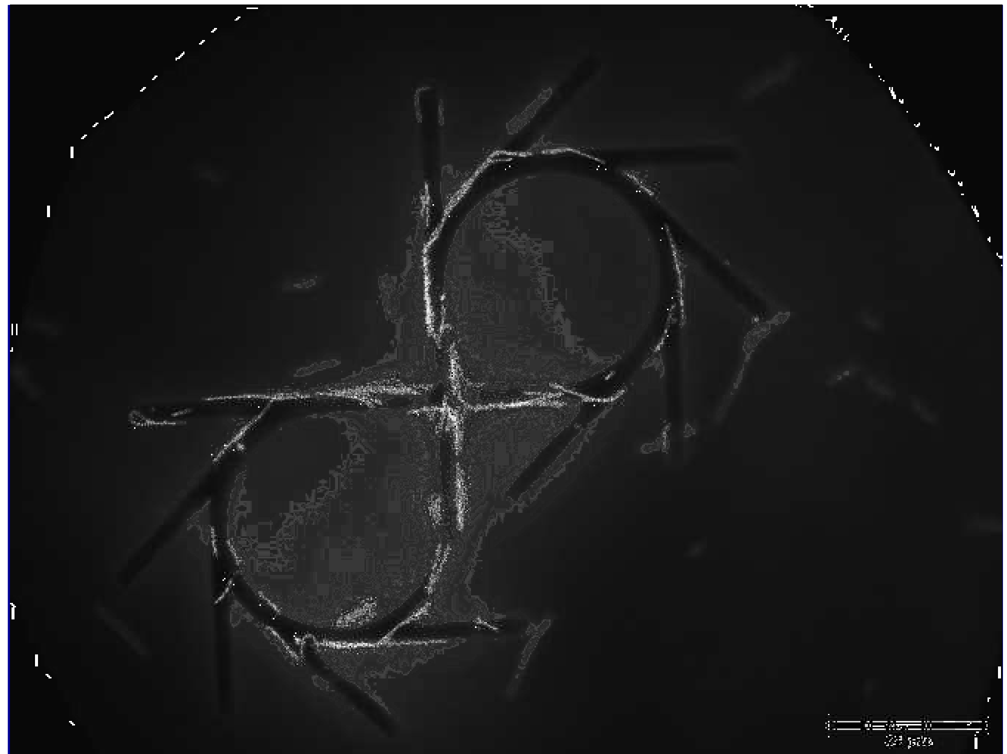


Guiding:

Directional sorting is accomplished by track geometry





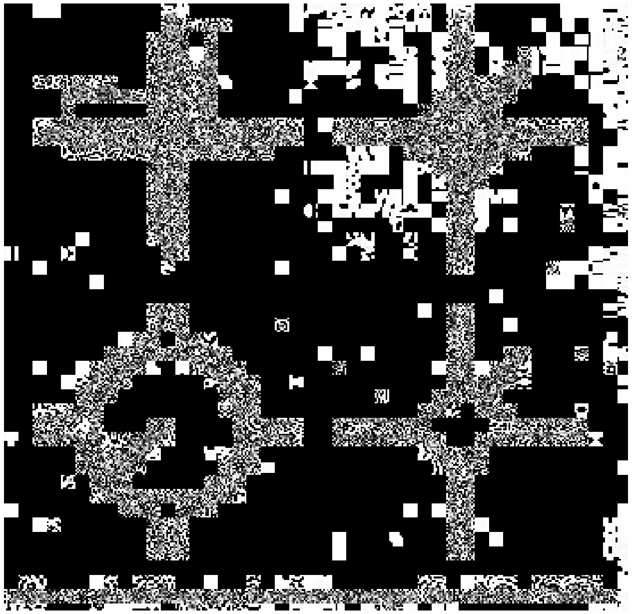


20 μm

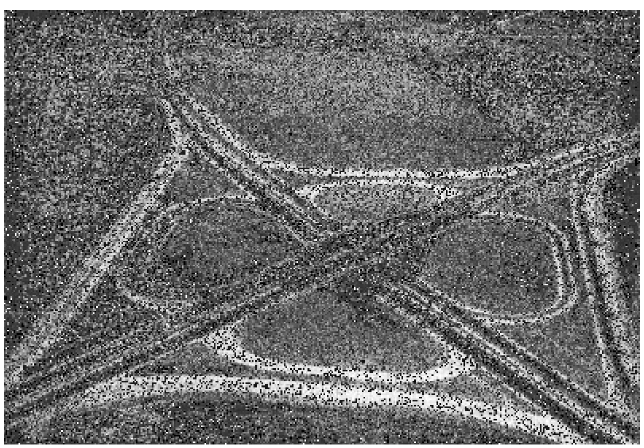
Time 50x

H. Hess et al.: **“**Ratchet patterns sort molecular shuttles”, Appl. Phys. A **75**, 309 (2002)

“Der Straßenbau“ - 1929

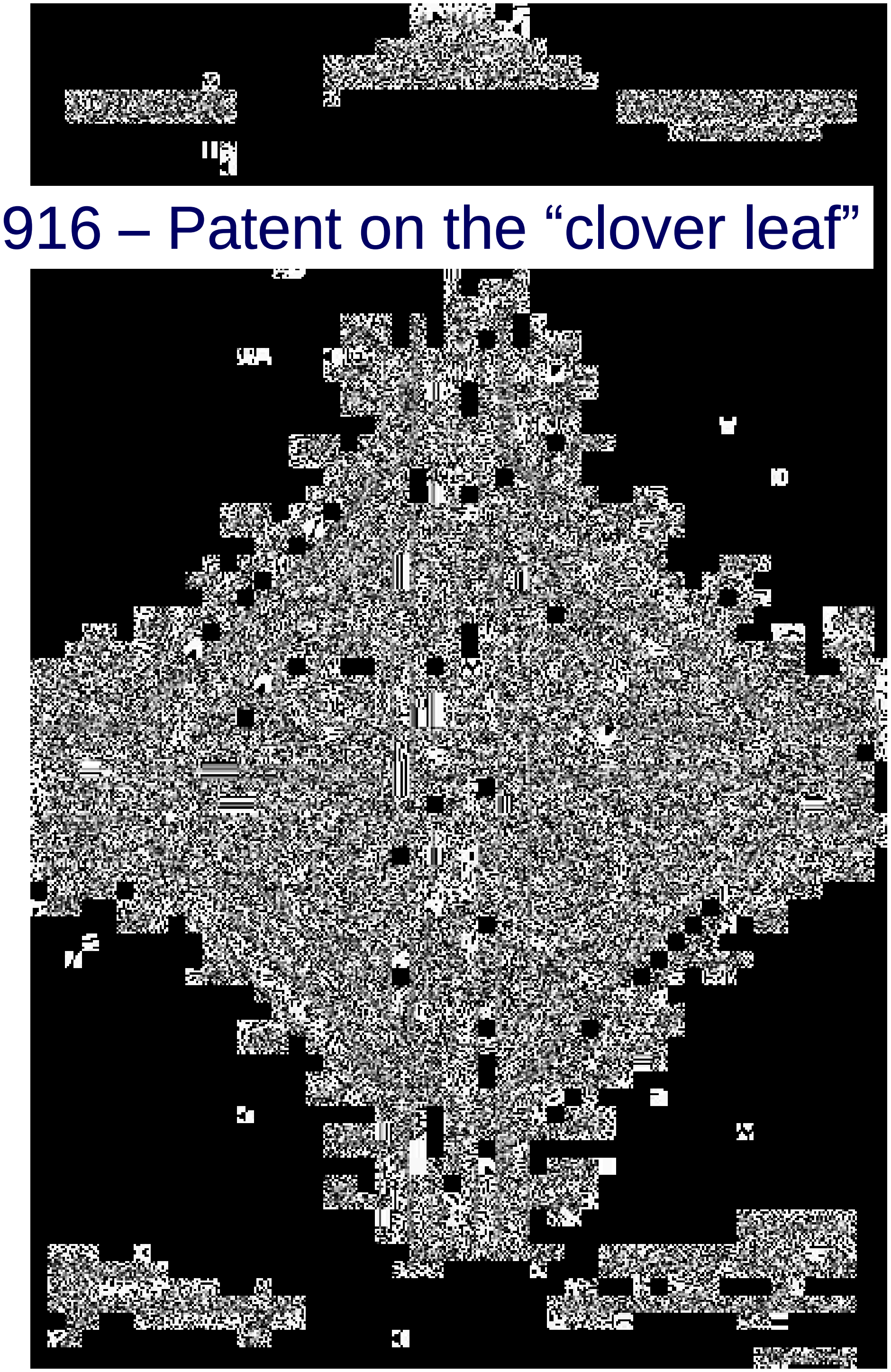


Woodbridge, NJ - 1928

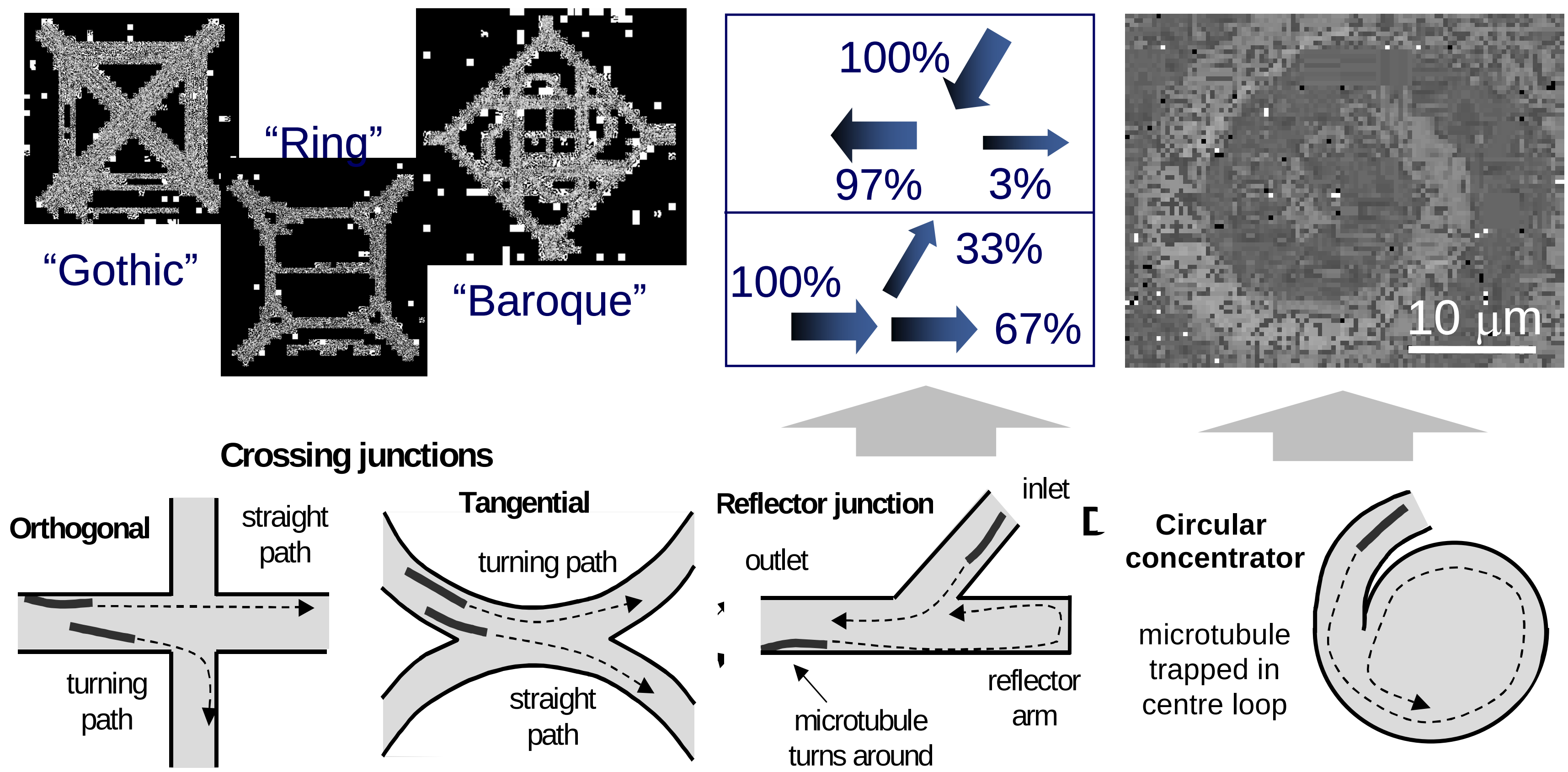


http://members.a1.net/wabweb/frames/kreuzf.htm

1916 – Patent on the “clover leaf”

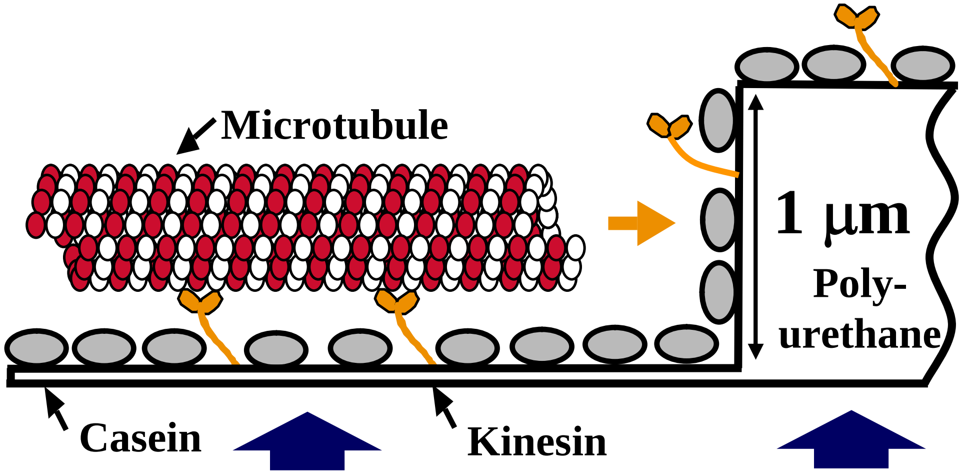


Guiding: Complex track networks

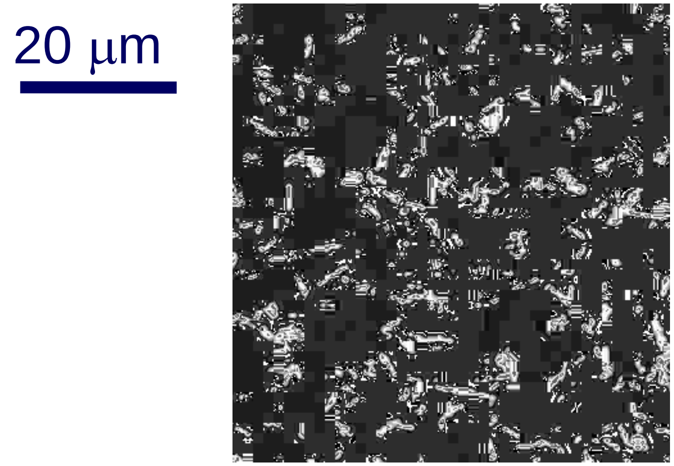


J. Clemmens et al.: **"**Motor-protein "roundabouts": Microtubules moving on kinesin-coated tracks through engineered networks, Lab-on-a-Chip **4**, 83 (2004)

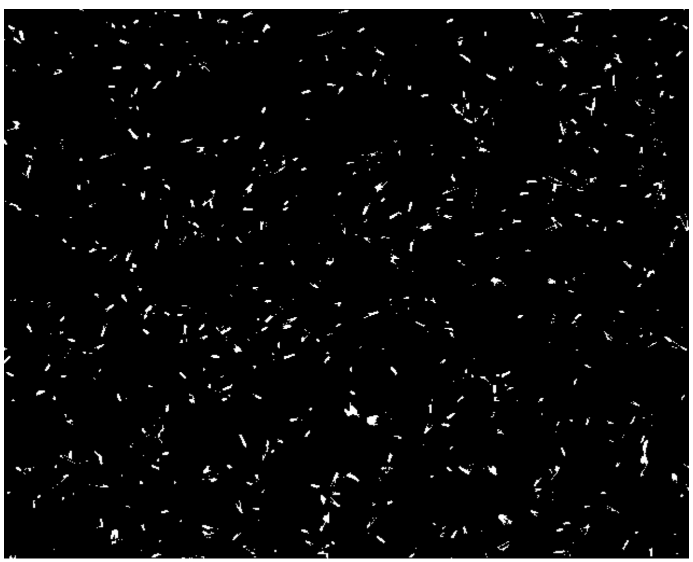
Molecular shuttles image surfaces



accessible **inaccessible**

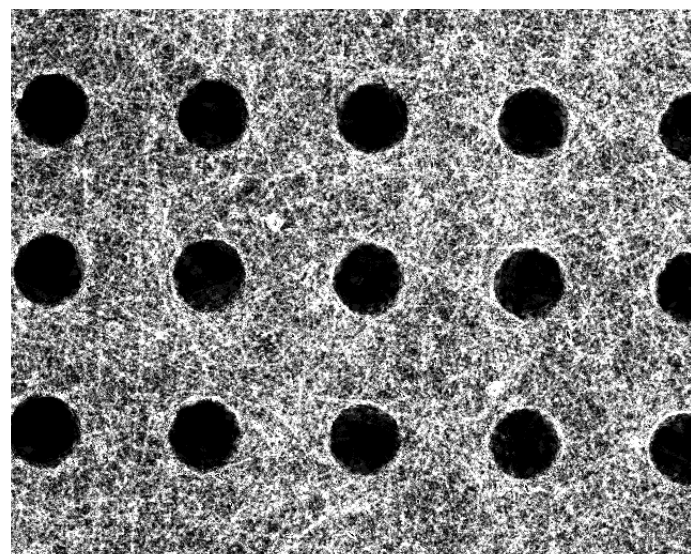


Nano Letters **2**, 113 (2002)



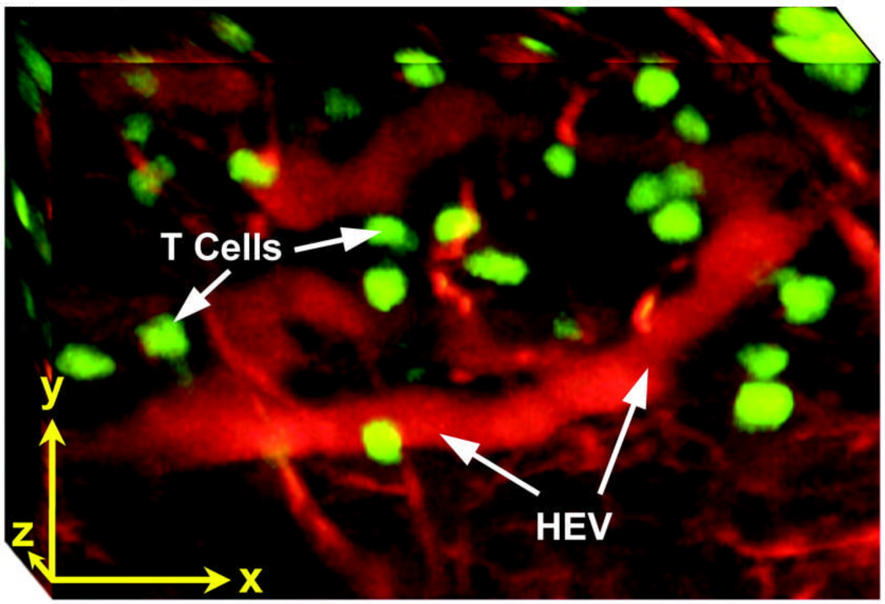
500 frames observed in 2500 s

20 µm

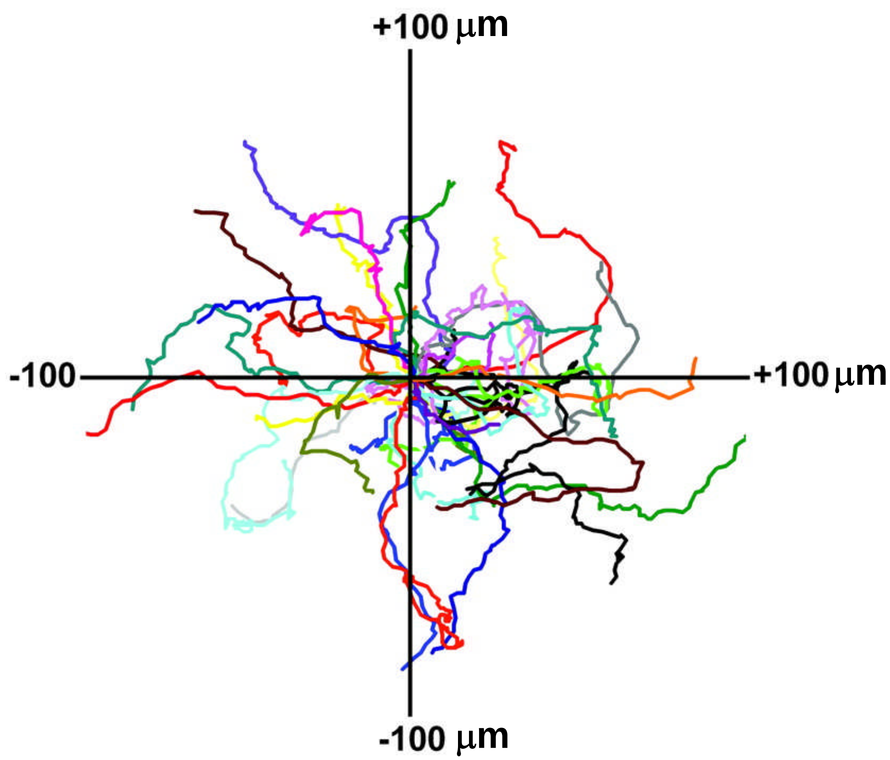


Biological Analogue: T cell trafficking

M. J. Miller, et al.: **“**Autonomous T cell trafficking examined *in vivo* …”, PNAS **100**, 2604 (2003)



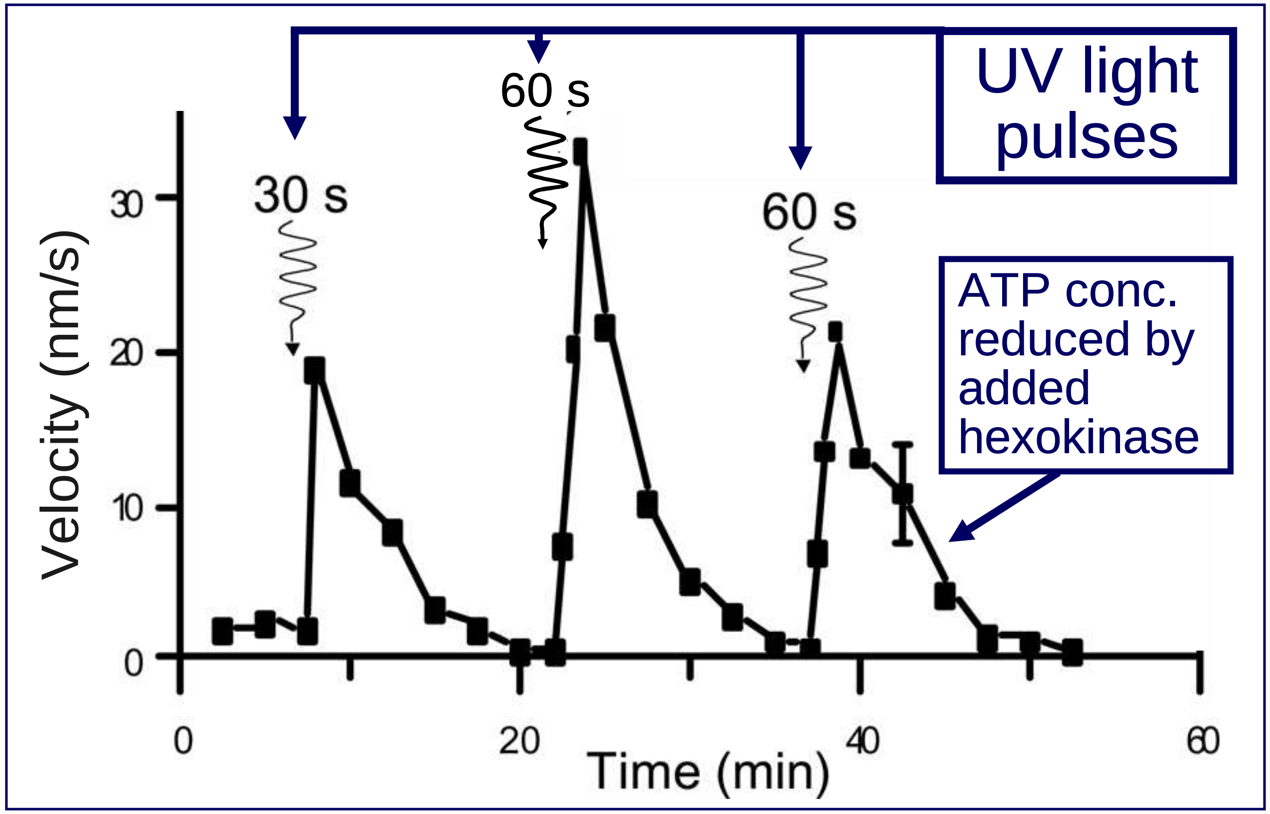
25 μm

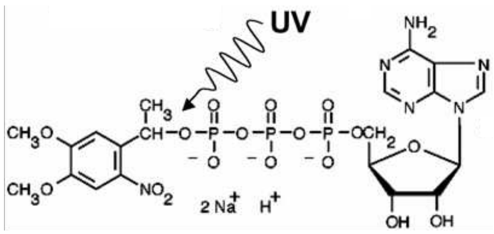


Intravital imaging of vessels (red) and cells (green) in a living lymph node

T cell perform Random Walk to sample local landscape of antigens

Control of movement:

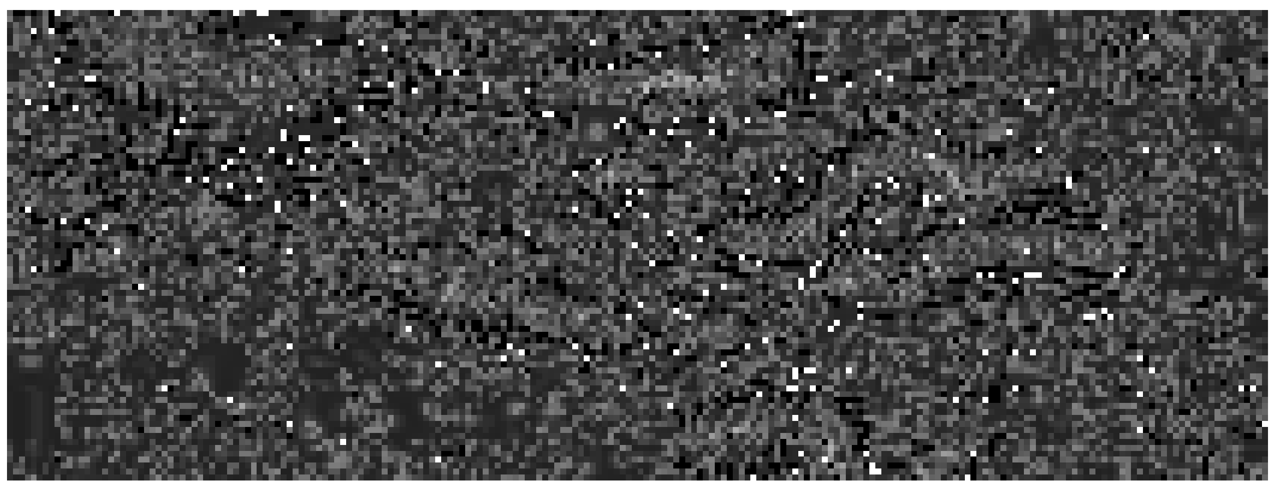




Caged ATP is released by UV light

10 μm

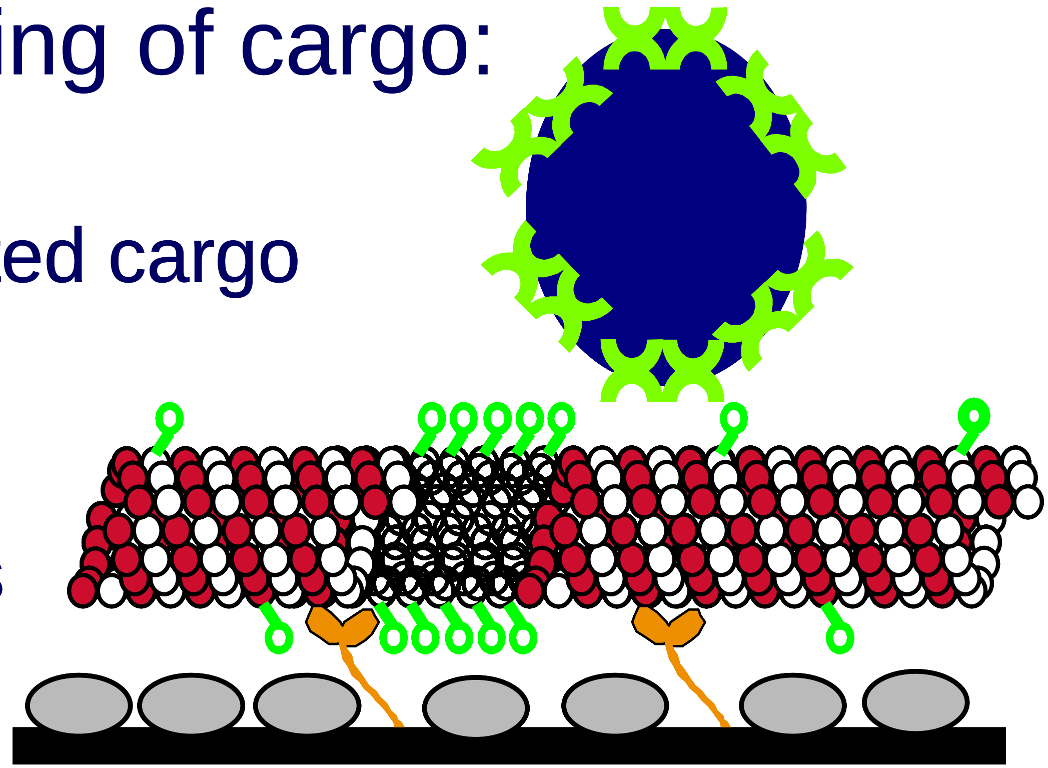
1 s movie = 300 s real time



Loading / unloading:

H. Hess, et al., Nano Letters, 1, 235 (2001)

Selective binding of cargo:



Streptavidin-coated cargo

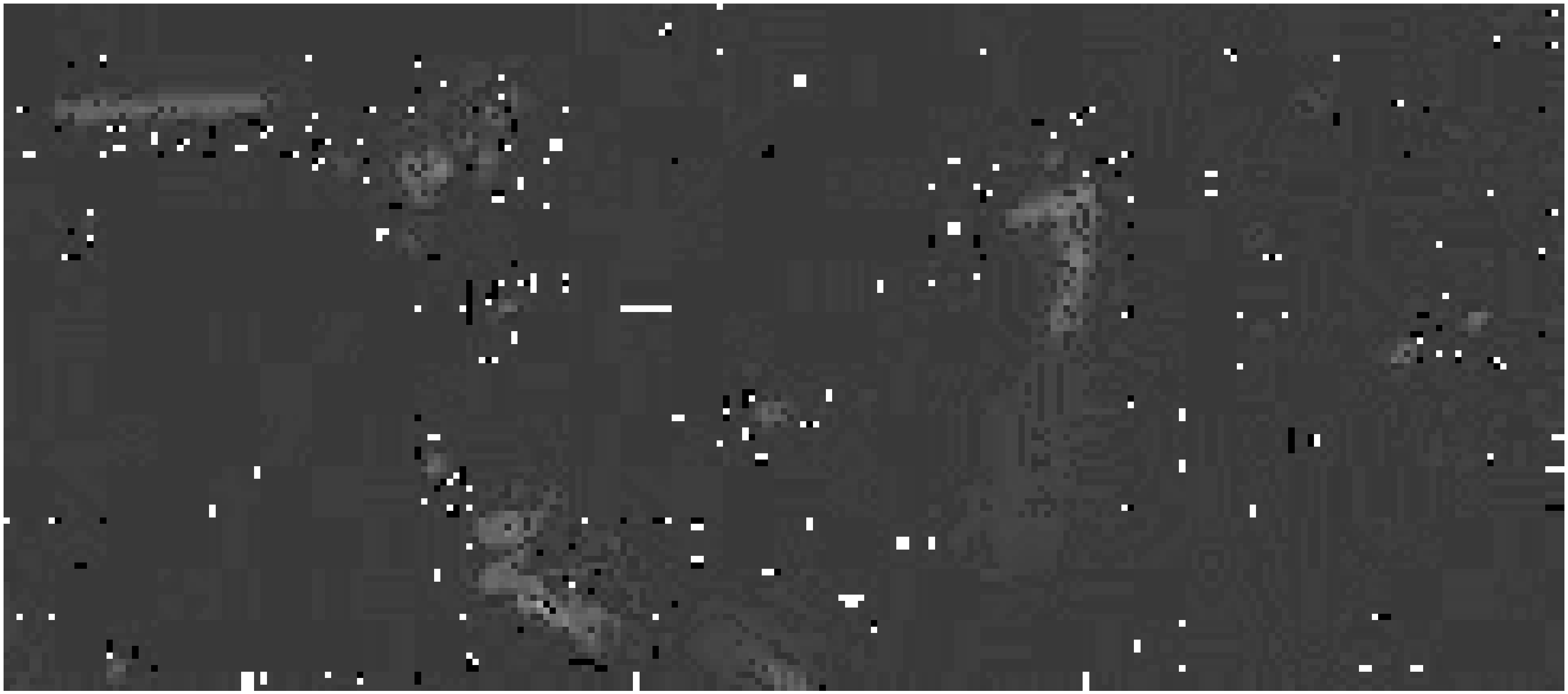
Microtubules with biotin-linkers

Kinesin-coated surface

H. Hess, J. Howard, and V. Vogel:

A piconewton forcemeter assembled from kinesins and microtubules,

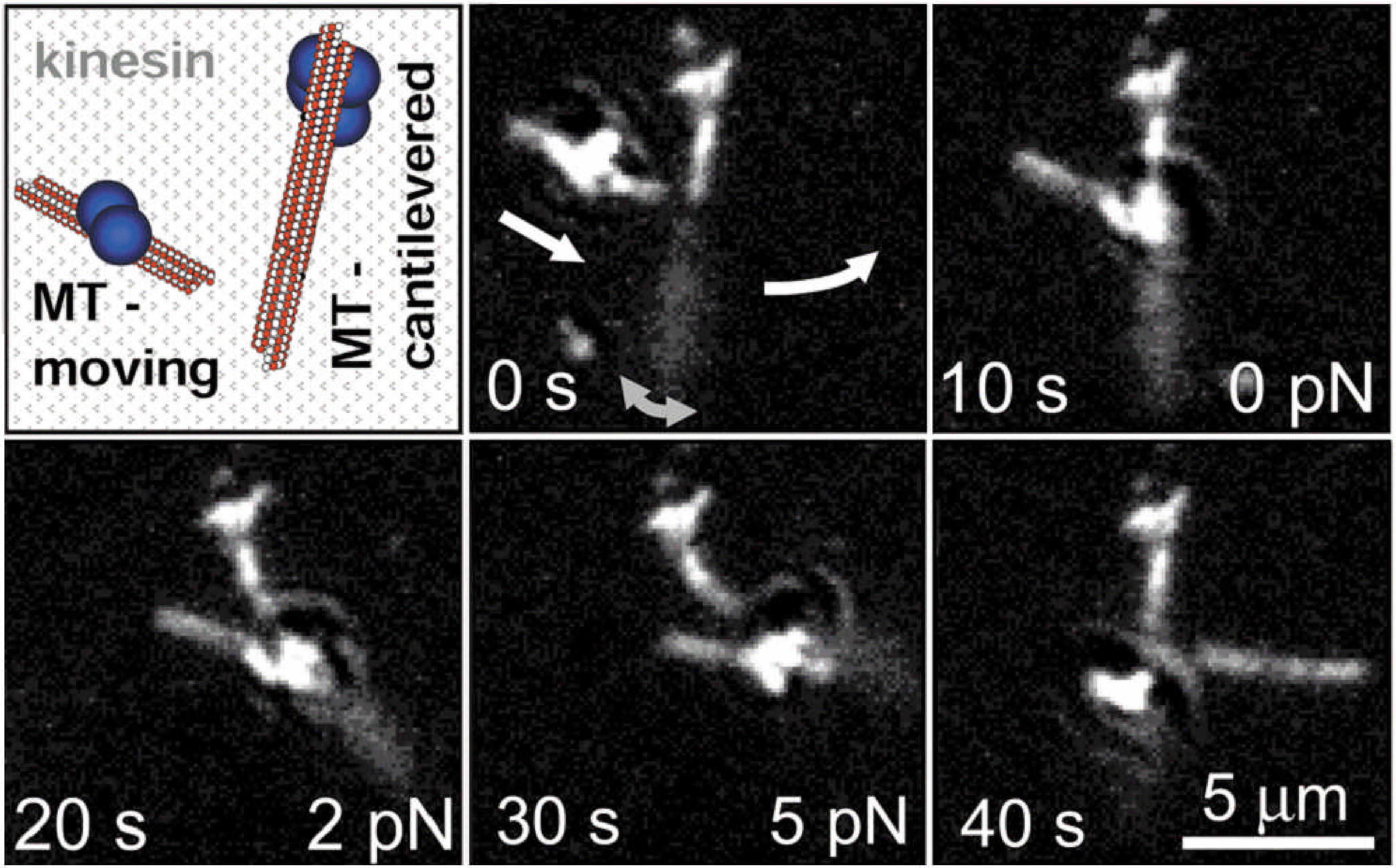
Nano Letters, 2(10), 1113 (2002)



5 μm

1 s movie = 50 s real time

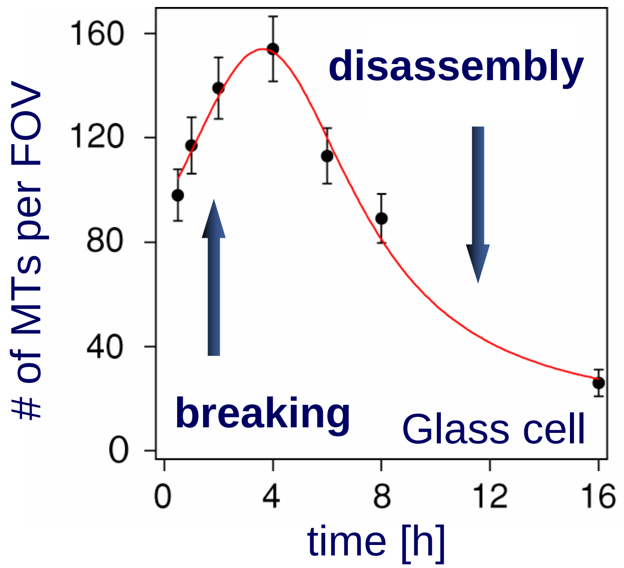
A piconewton forcemeter:



Lifetime of bionanodevices: A benchmark

(1) Kinesin motors are dimeric proteins undergoing large conformational changes

(2) Microtubules are supramolecular assemblies of the protein tubulin, their natural equilibrium between assembly and disassembly is affected by the stabilizing anti-cancer drug taxol (used in our devices)



(1) Kinesin motors remain active for at least 1-2 days at room temperature.

(2) Microtubules have a lifetime of ~12 hours.

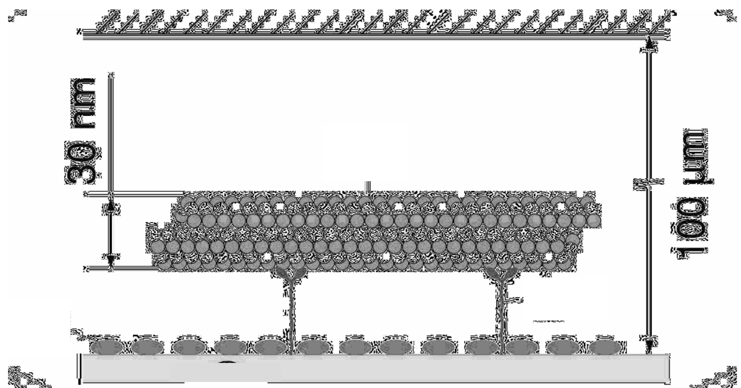
(3) The ATP fuel lasts for ~10 days.

Microtubules are the weak link!
More aggressive stabilization by chemical cross-linking required.

C. Brunner, K.H. Ernst, H. Hess, V.Vogel: "Lifetime of biomolecules in hybrid nanodevices", Nanotechnology **15**, S540 (2004)

Lifetime of bionanodevices: Packaging materials

Cover: Microfabricated, transparent, inert



Patterned surface: Well-characterized materials (photoresist, glass) in contact with motors

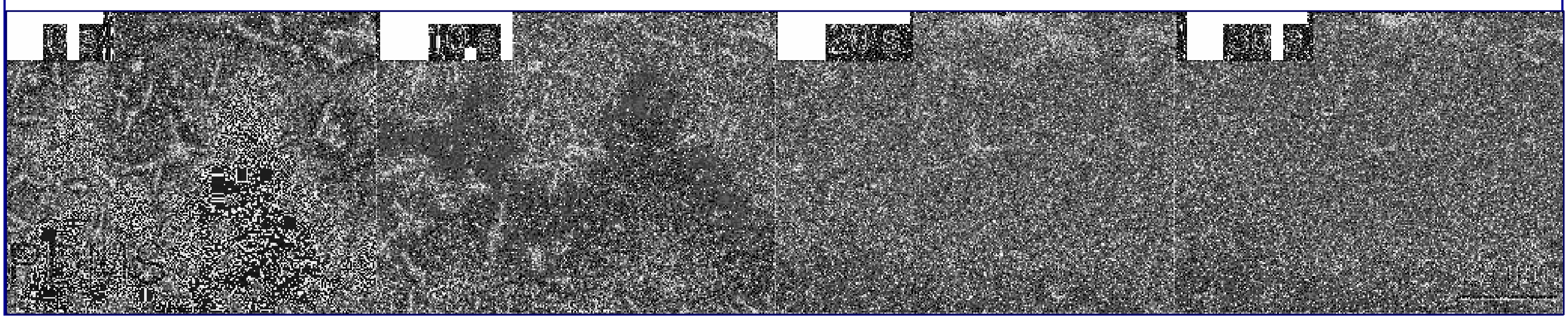
Materials scan:

Glass, PDMS, PMMA, PU, EVOH

In the absence of intense illumination:
Microtubule lifetimes of several hours

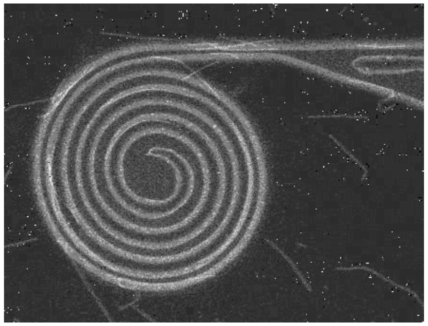
Under illumination in the fluorescence microscope:
Rapid decay for PDMS,
glass cell without oxygen scavenger

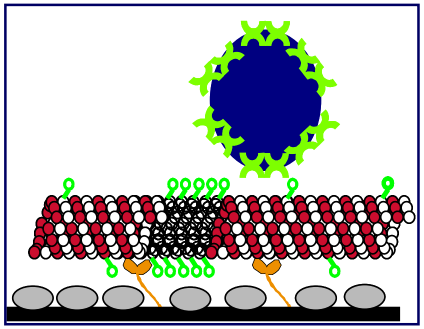
O_2 permeability affects compatibility

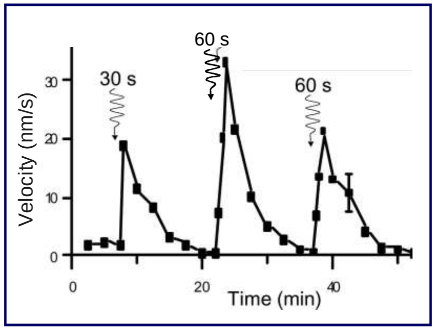


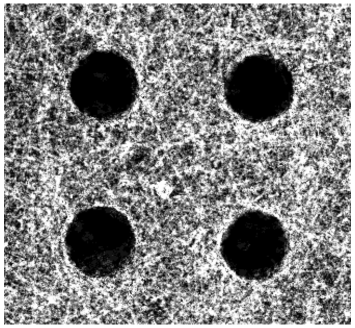
C. Brunner, K.H. Ernst, H. Hess, V.Vogel: "Lifetime of biomolecules in hybrid nanodevices", Nanotechnology **15**, S540 (2004)

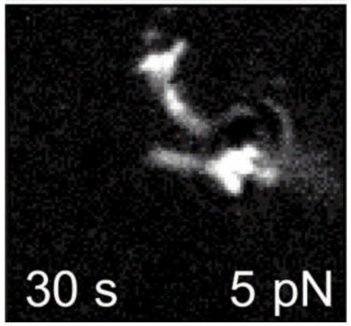
Molecular Shuttles –
a Module for Nanodevices

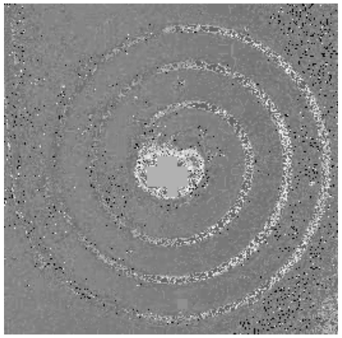












Surface Imaging

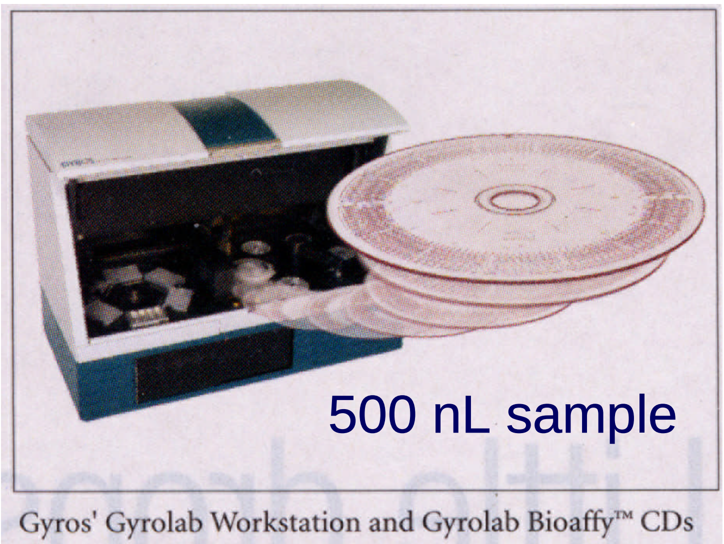
Nanoscale Forcemeter

Biosensor

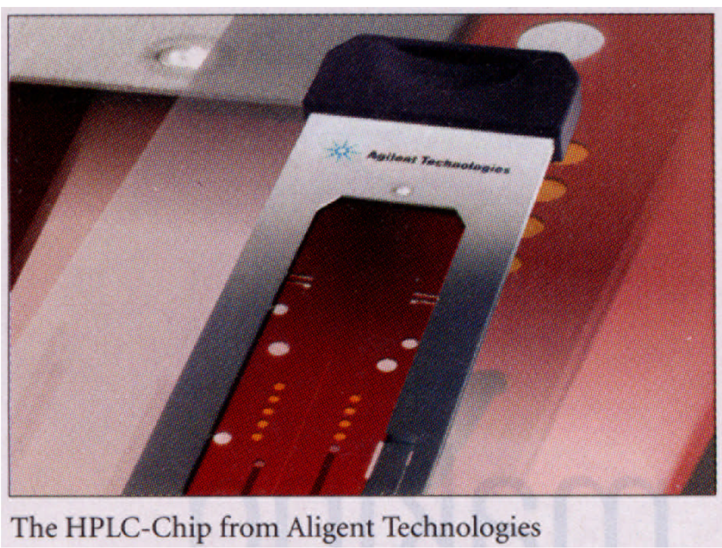
Acknowledgements:

The UW molecular shuttle team:	Viola Vogel Jonathon Howard (until 2002) John Clemmens, Robert Doot, Christian Brunner, Karl-Heinz Ernst Robert Tucker, Sheila Luna, Di Wu, Sujatha Ramachandran Scott Phillips
The SNL motor team:	Bruce C. Bunker George D. Bachand Carolyn M. Matzke, Susan B. Rivera Andrew K. Boal, Joseph M. Bauer
Kinesin expression:	Mike Wagenbach & Linda Wordeman
Non-fouling surfaces by plasma deposition:	R. Lipscomb, Y. Hanein, B. Ratner, K. Böhringer
Funding:	NASA, DOE-BES DARPA Biomolecular Motors Program

Microfluidics versus Nanofluidics



Gyros' Gyrolab Workstation and Gyrolab Bioaffy™ CDs



The HPLC-Chip from Aligent Technologies

Channel diameter:
50 µm
vs.
500 nm

Sample volume:
500 nL
vs.
10 fL

Flow velocity:
1 mm/s
vs.
1 µm/s