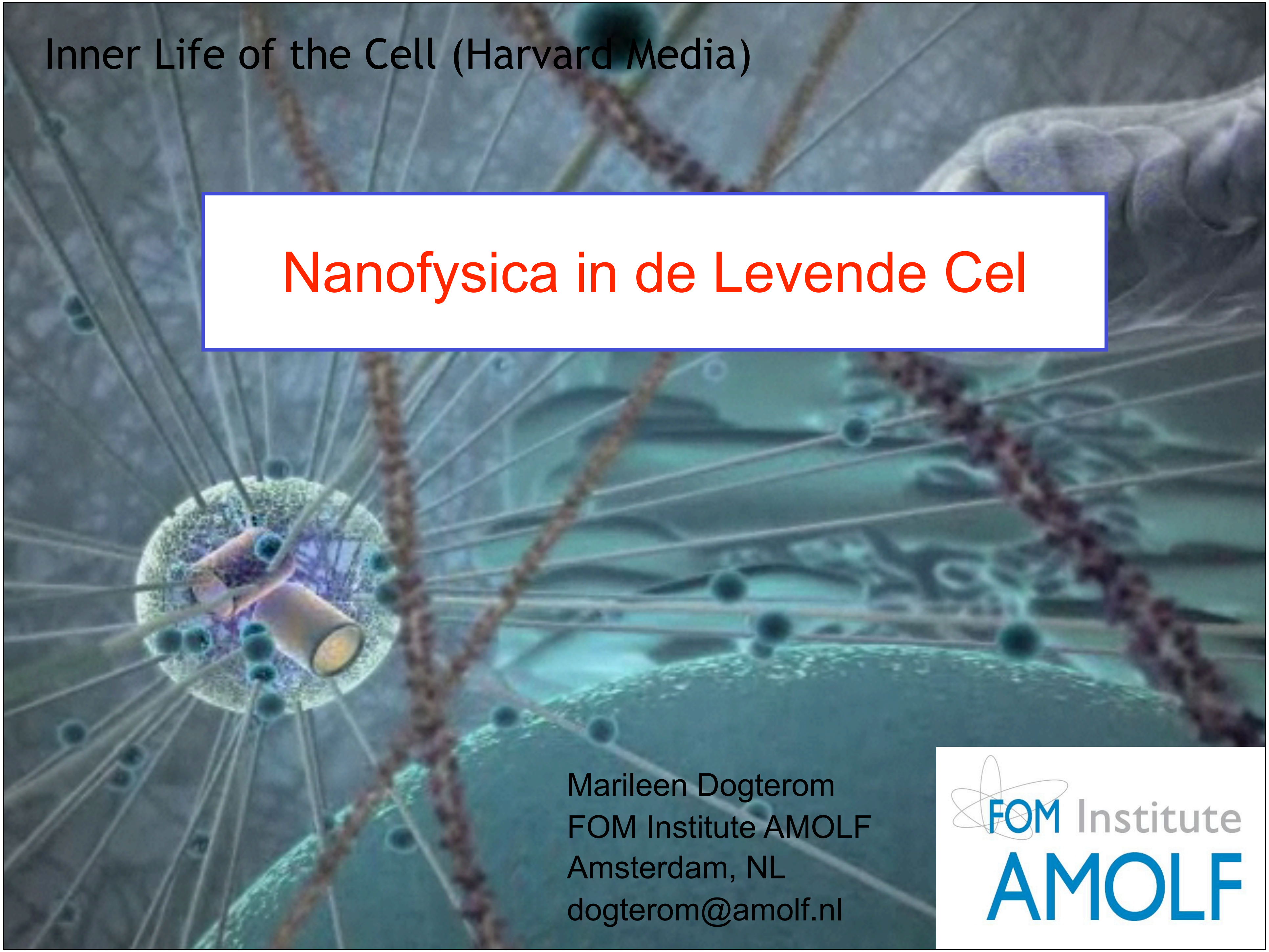
Inner Life of the Cell (Harvard Media)

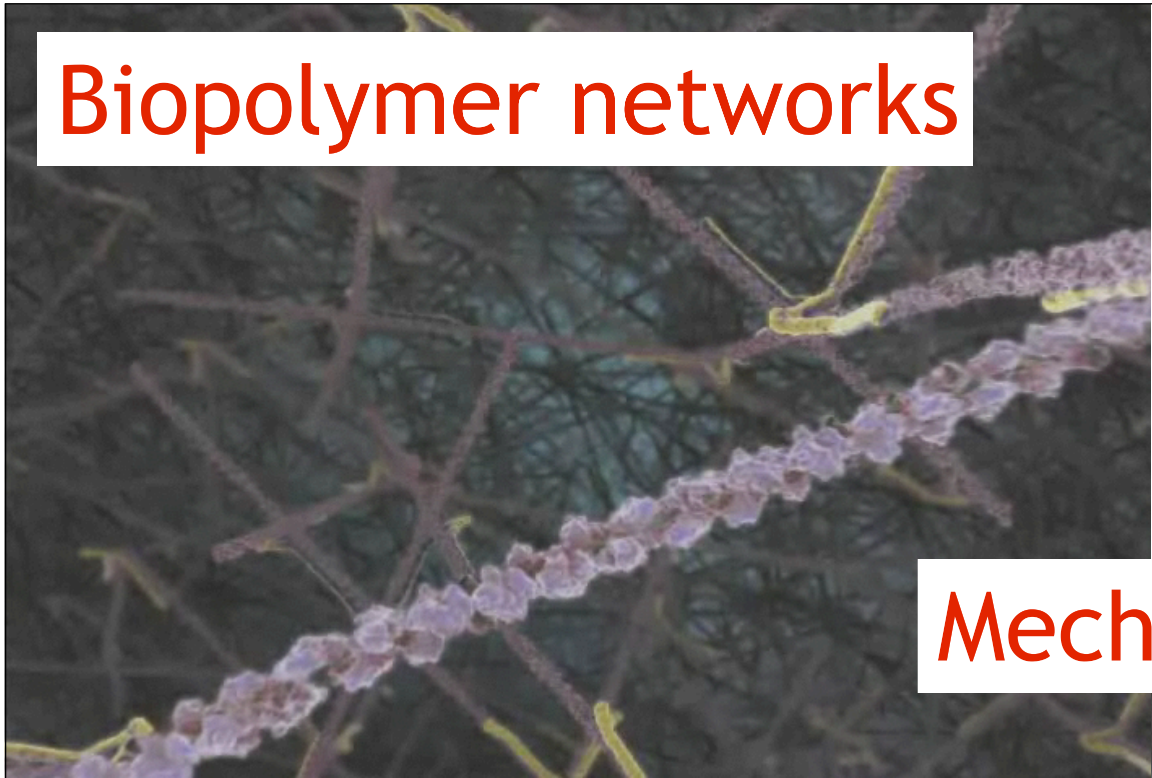


Nanofysica in de Levende Cel

Marileen Dogterom
FOM Institute AMOLF
Amsterdam, NL
dogterom@amolf.nl

FOM Institute AMOLF

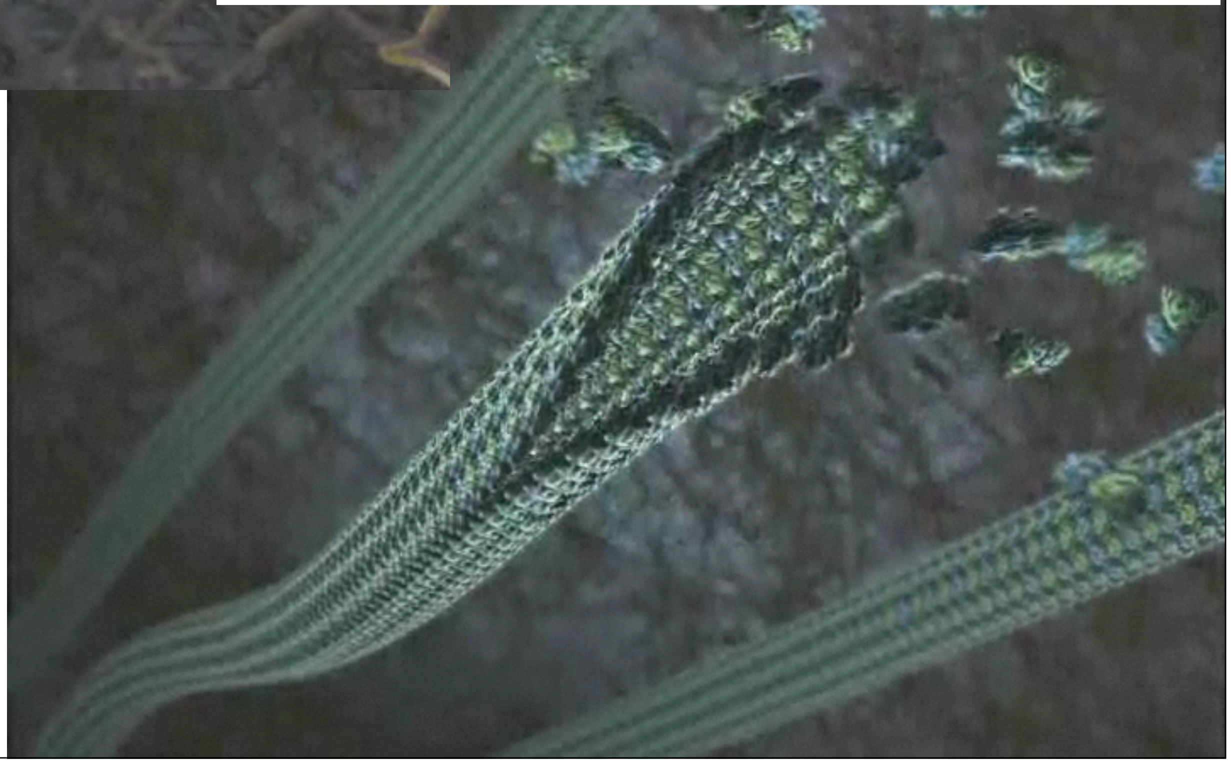
Biopolymer networks



Actin networks

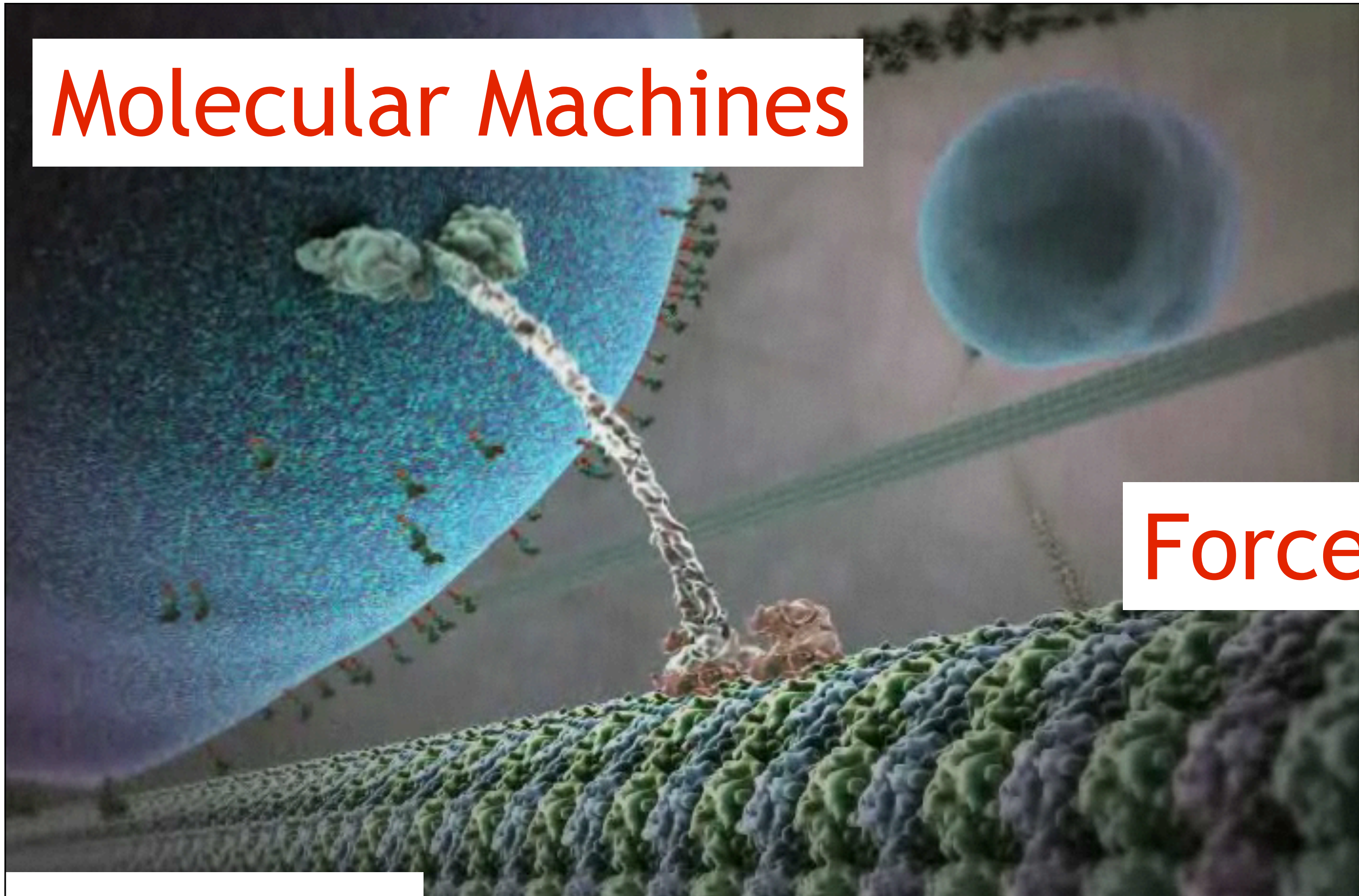
Mechanical framework

Visco-elasticity



Dynamic microtubules

Molecular Machines

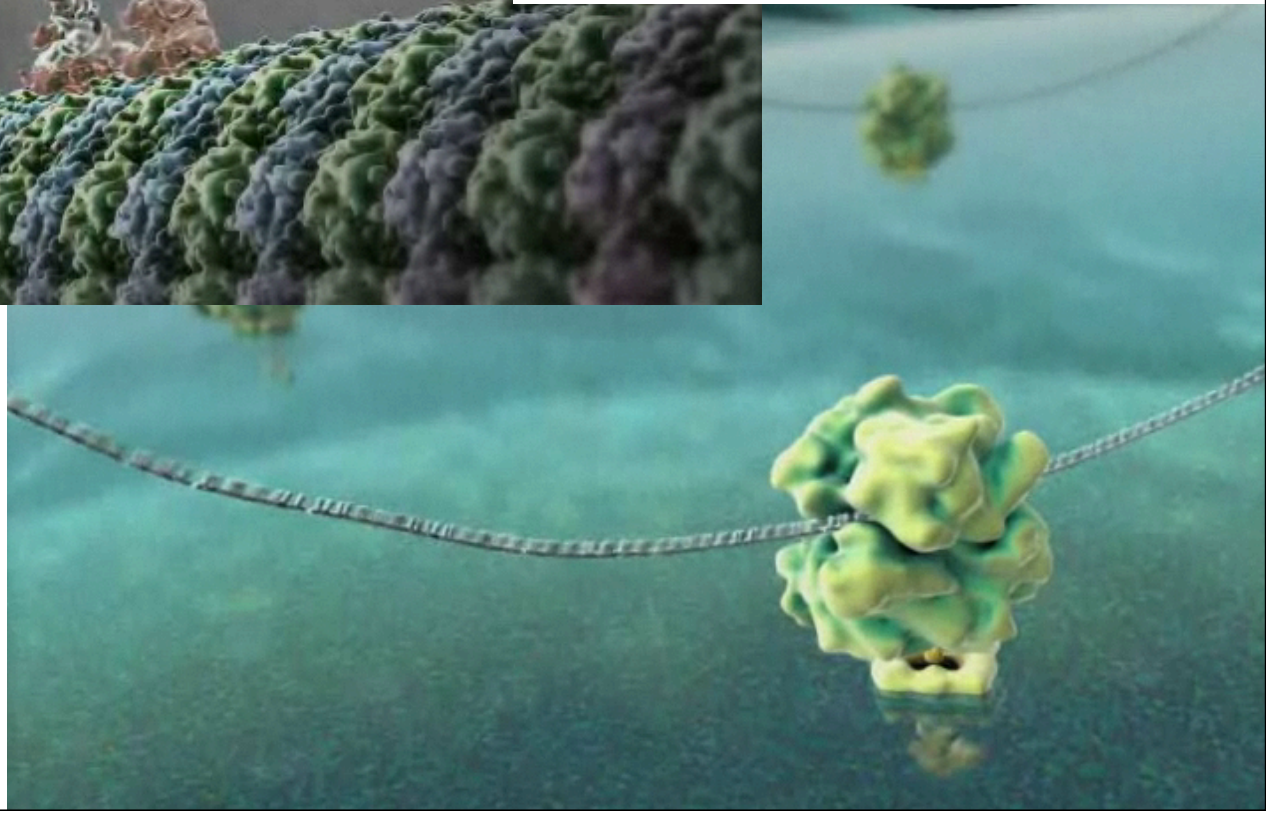


Kinesin

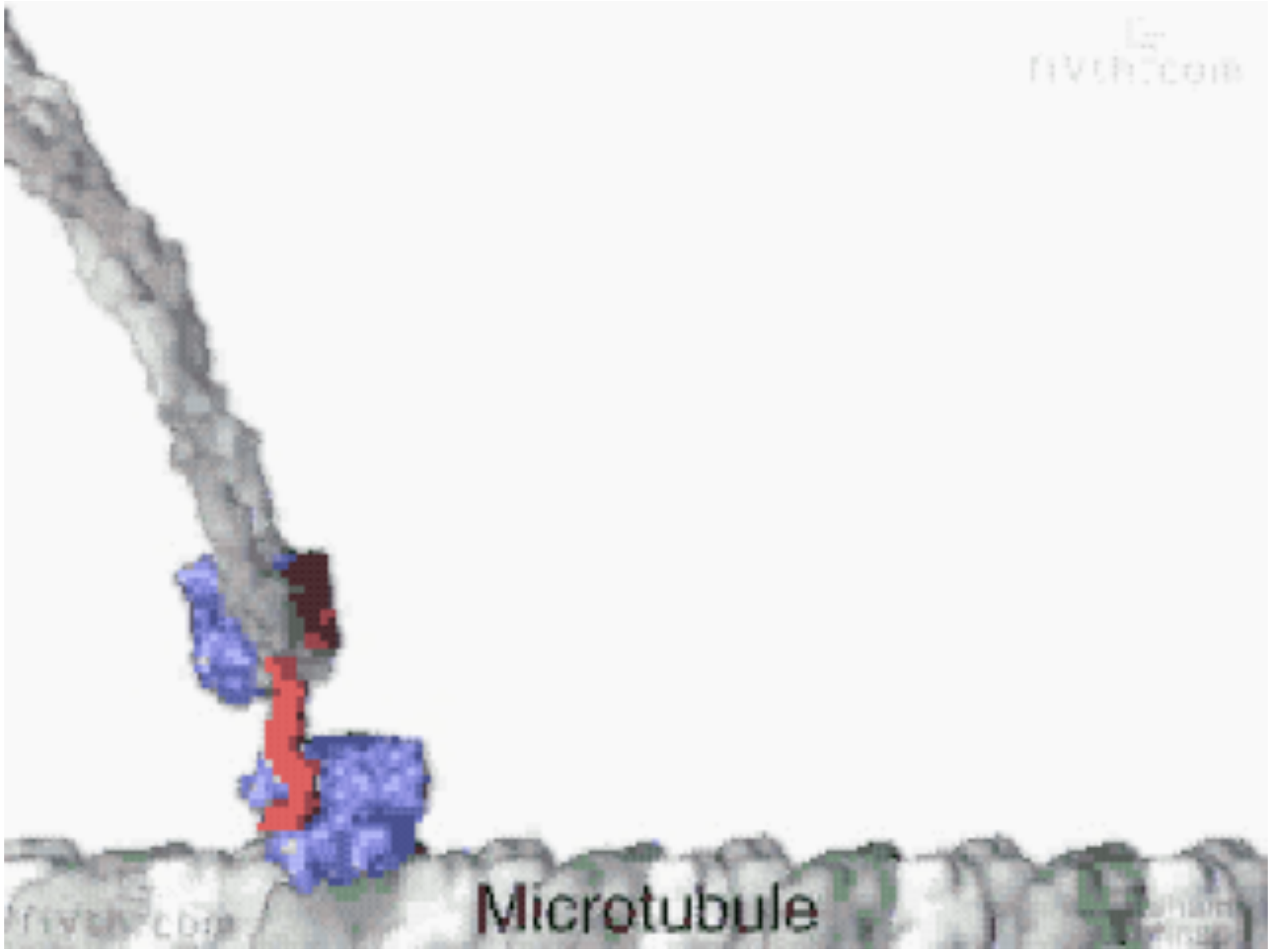
Force generation

Transport

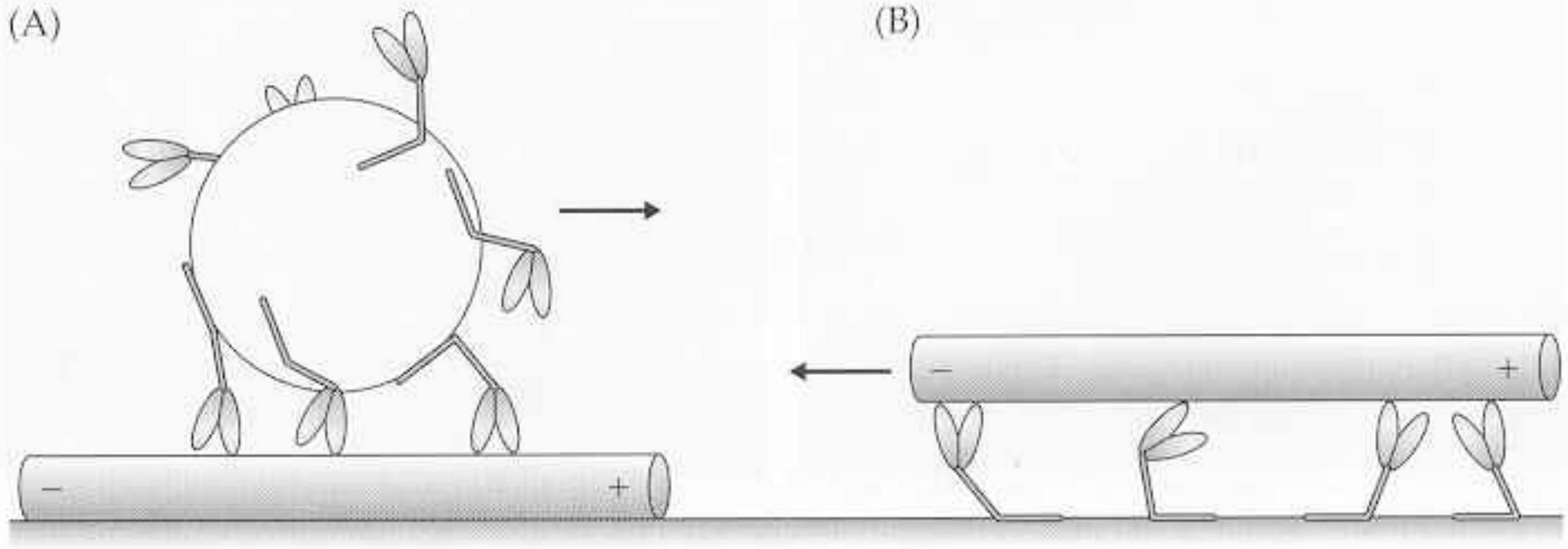
Diffusion



Ribosome


Microtubule

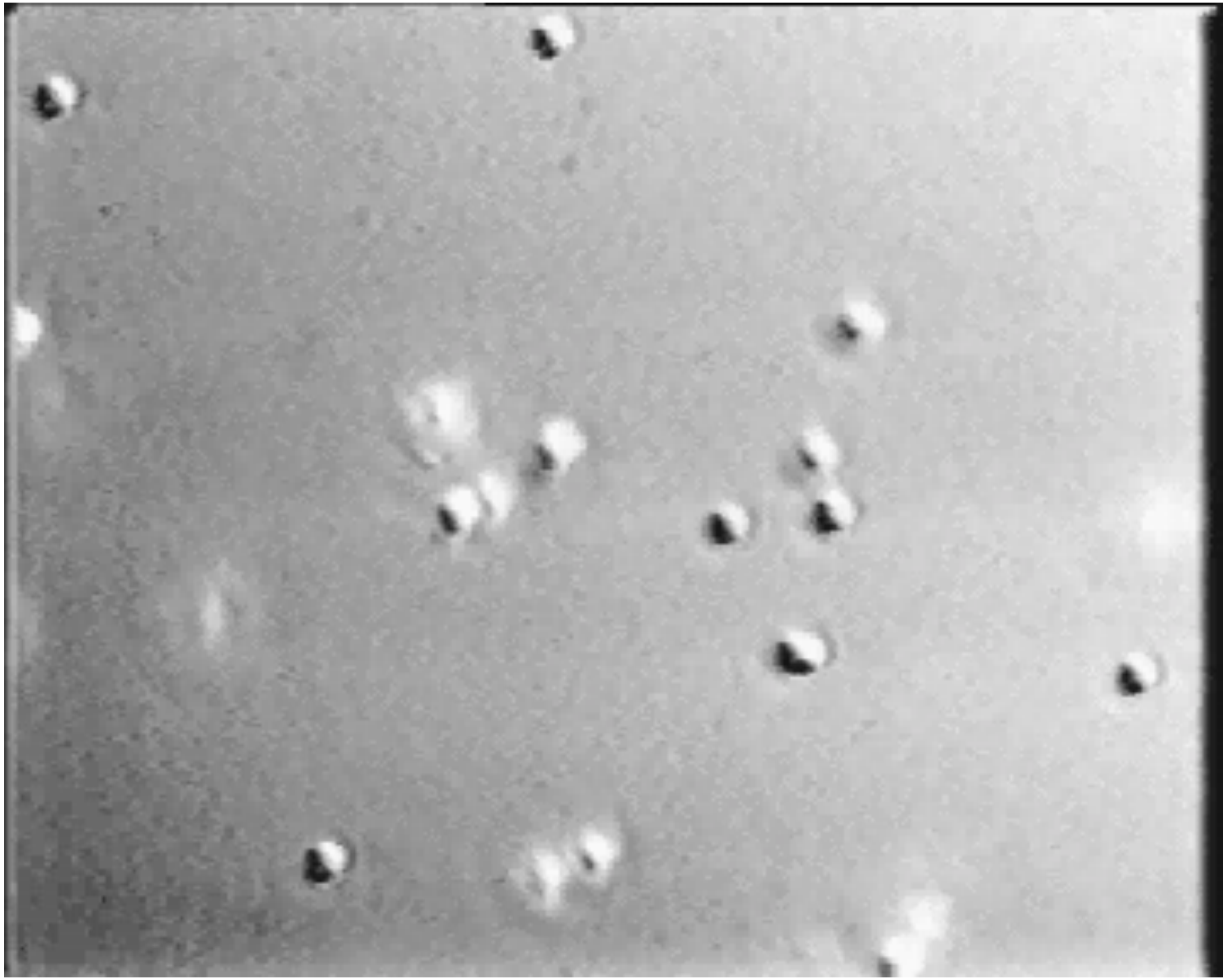
Biophysical assays



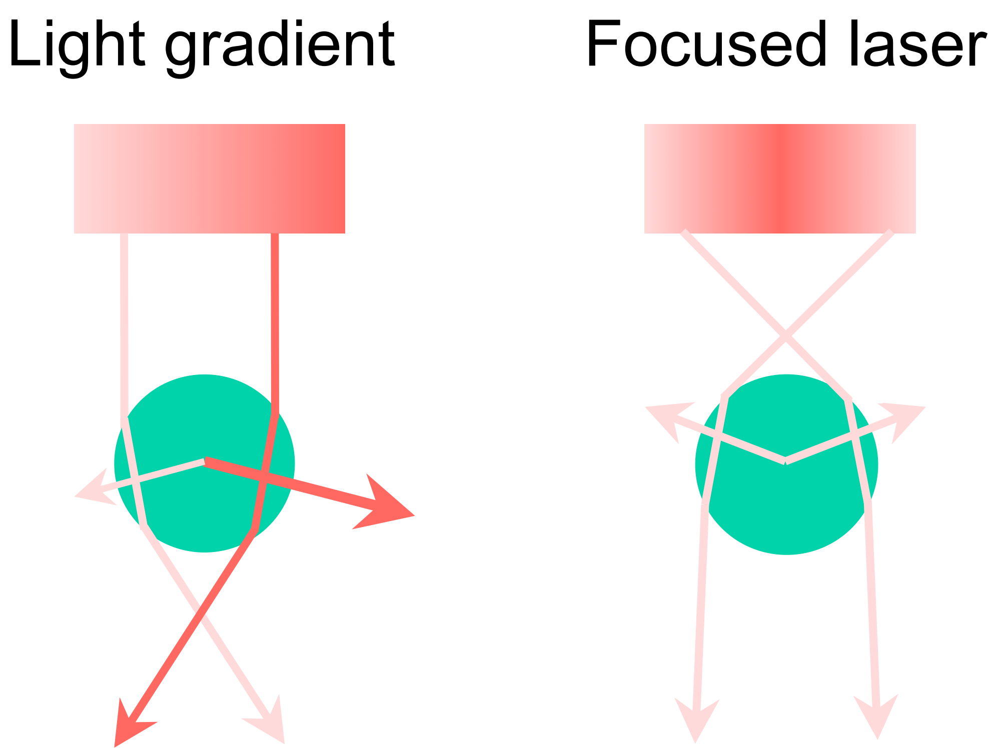
Bead assay

Gliding assay

Optical tweezers



Principles

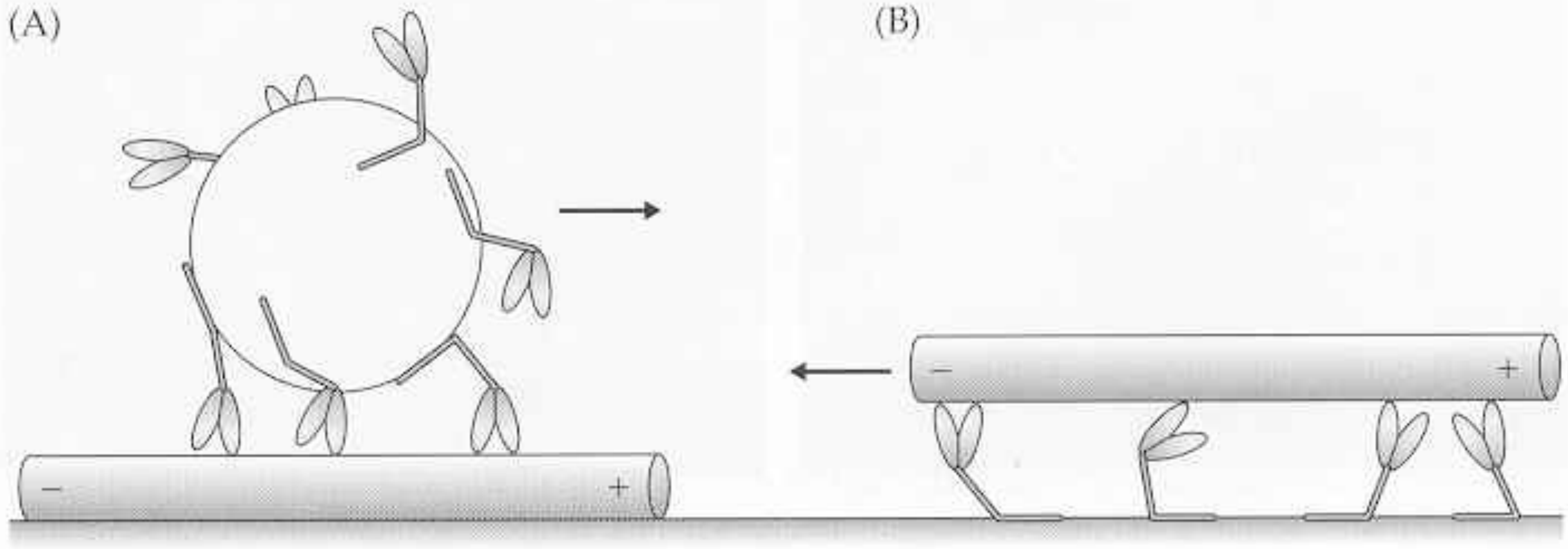


Stable trap:

Linear spring $F_x = \kappa_x \Delta x$

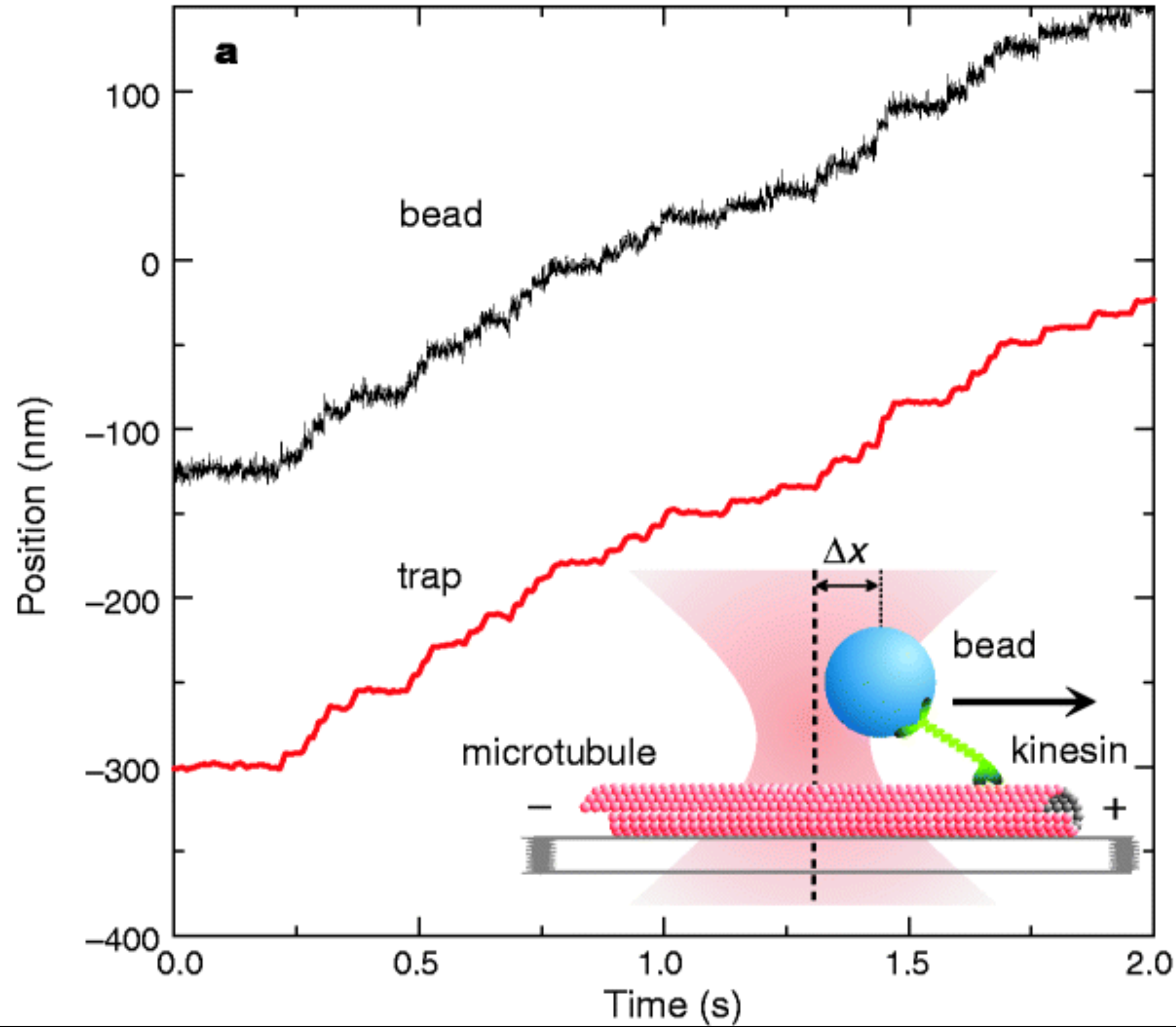
trap stiffness κ depends on *r, I, n,* …..

Biophysical assays



Bead assay

Gliding assay


a
Position (nm)
100
0
–100
–200
–300
–400
0.0
0.5
1.0
1.5
2.0
Time (s)
bead
trap
Δx
bead
microtubule
kinesin
–
+

Steps: First direct observation Nature **365**, 721 (1993)

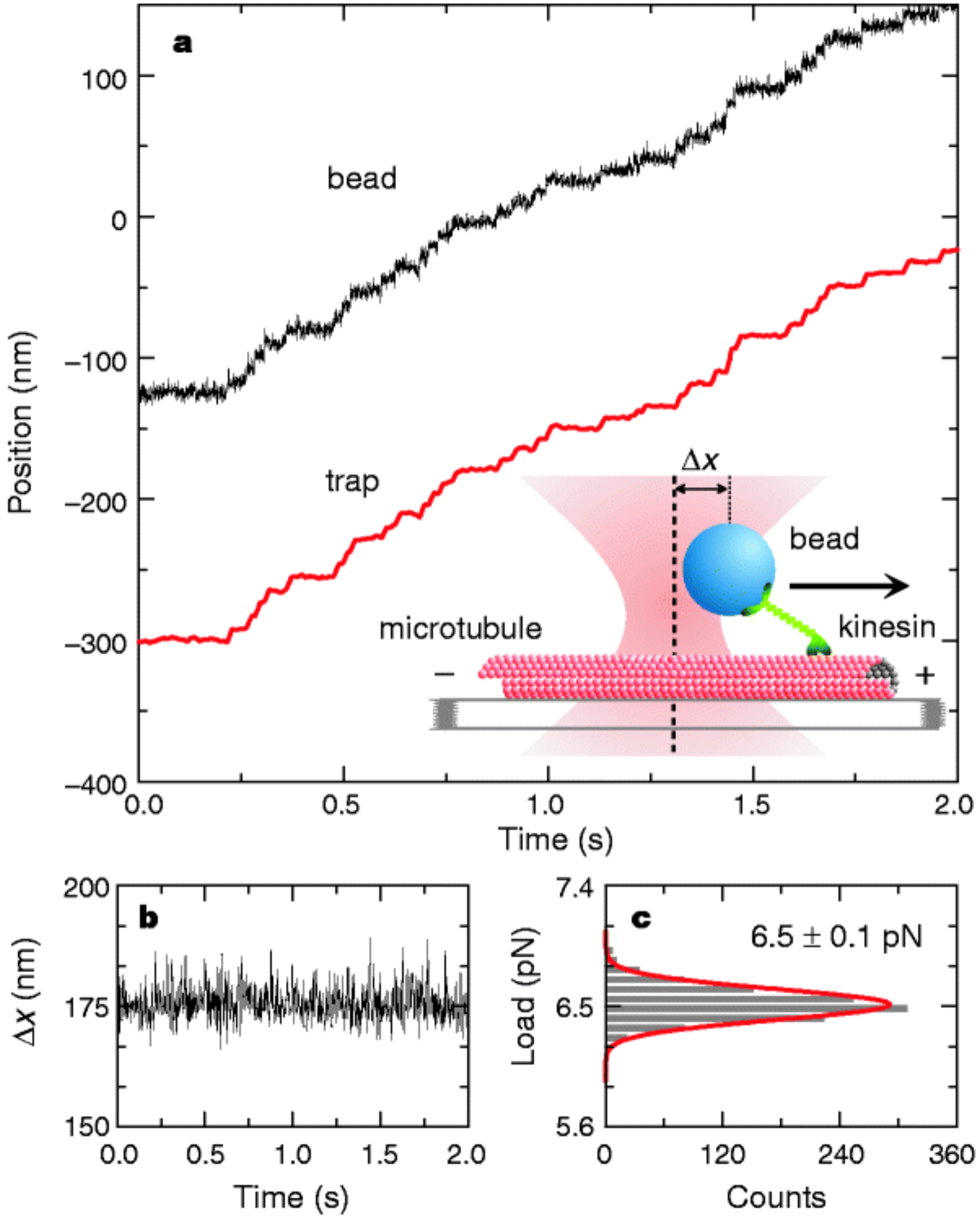
Direct observation of kinesin stepping by optical trapping interferometry

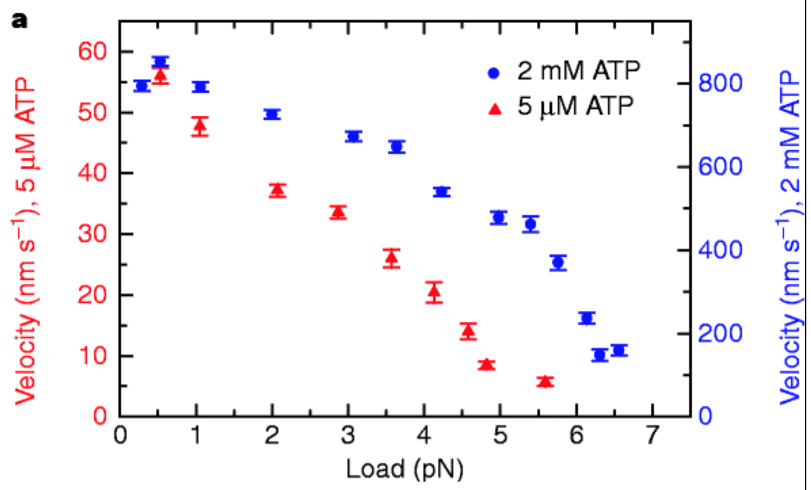
Karel Svoboda*†, Christoph F. Schmidt*‡, Bruce J. Schnapp§ & Steven M. Block*||

* Rowland Institute for Science, 100 Edwin Land Boulevard, Cambridge, Massachusetts 02142, USA
† Committee on Biophysics, Harvard University, Cambridge, Massachusetts 02138, USA
§ Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115, USA

Do biological motors move with regular steps? To address this question, we constructed instrumentation with the spatial and temporal sensitivity to resolve movement on a molecular scale. We deposited silica beads carrying single molecules of the motor protein kinesin on microtubules using optical tweezers and analysed their motion under controlled loads by interferometry. We find that kinesin moves with 8-nm steps.

Kinesin stepping in force clamp: Δx kept constant through feed-back

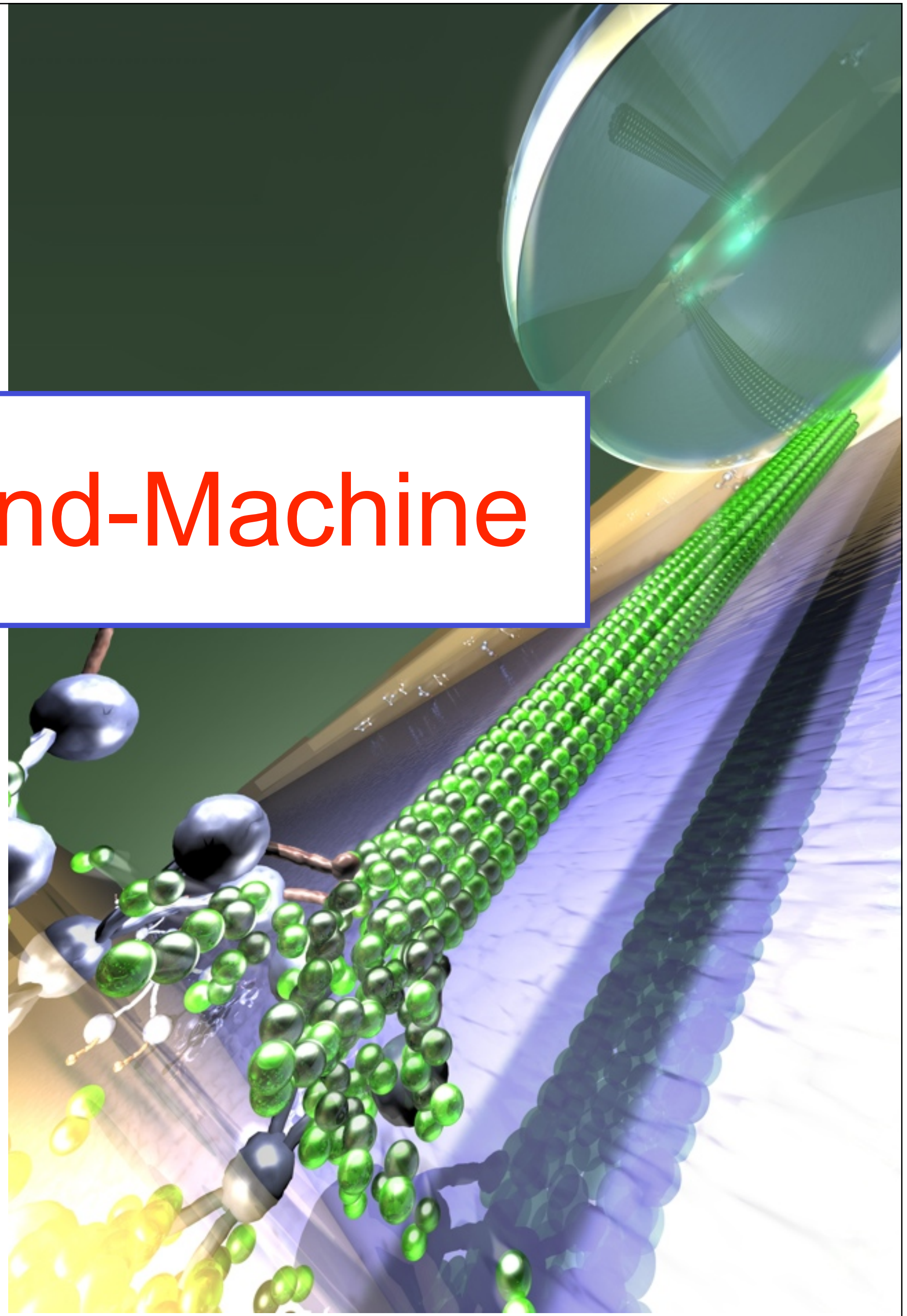




8 nm x 6 pN = 48 pNnm
= 60 % ATP

Visscher et al, Nature, '99

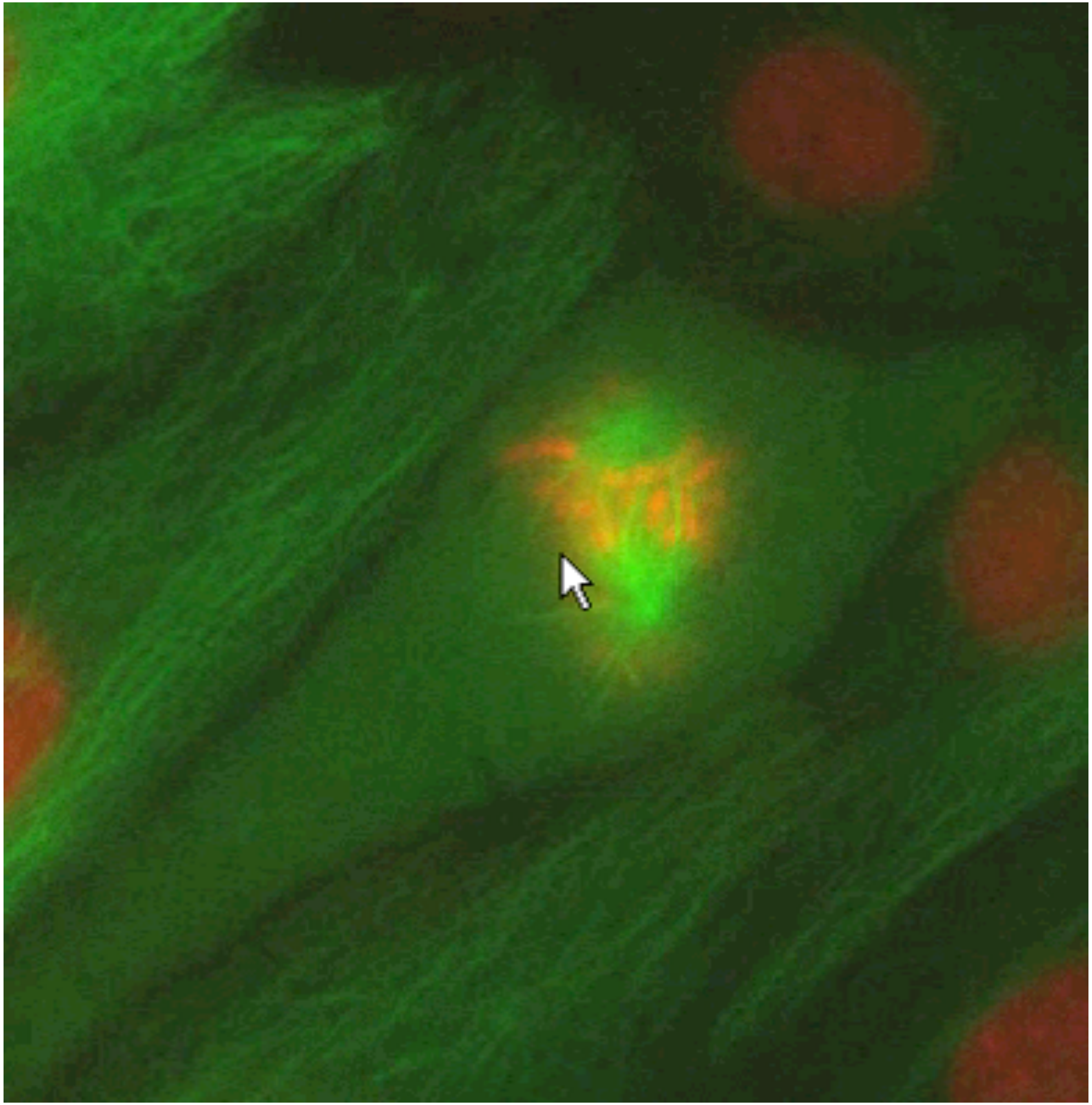
FOM Institute AMOLF



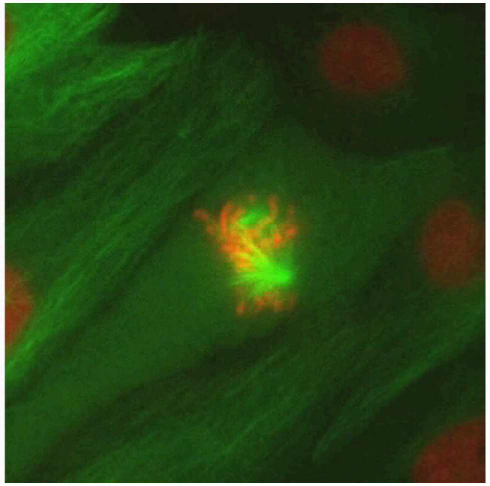
The Microtubule End-Machine

Svenja-Marei Kalisch
Olga Sytina
Magdalena P. Lopez
Pierre Recouvreux
Sophie Roth
Nuria Taberner

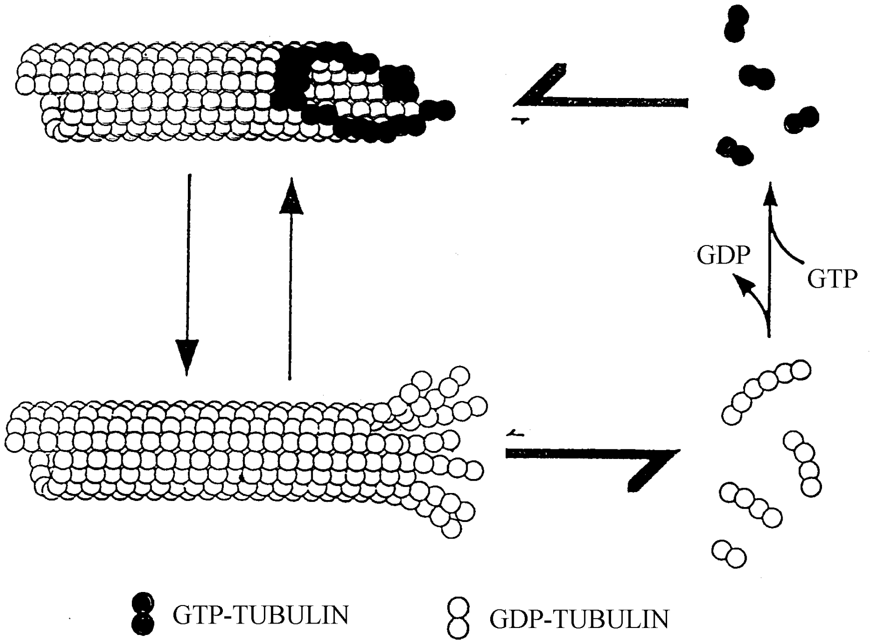
Former:
Liedewij Laan,
Julien Husson, Laura Munteanu,
Jacob Kerssemakers, Christian Tischer, Marcel Janson, Martijn van Duijn

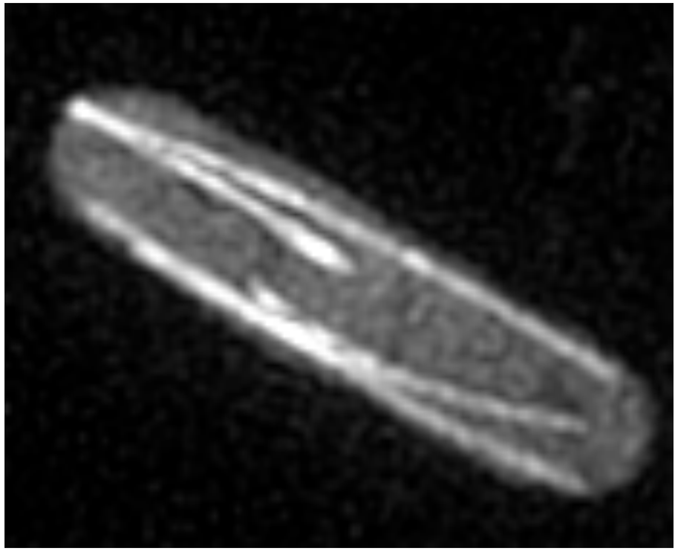


dividing
kidney
cell



DYNAMIC MICROTUBULES GENERATE PUSHING AND PULLING FORCES IN CELLS

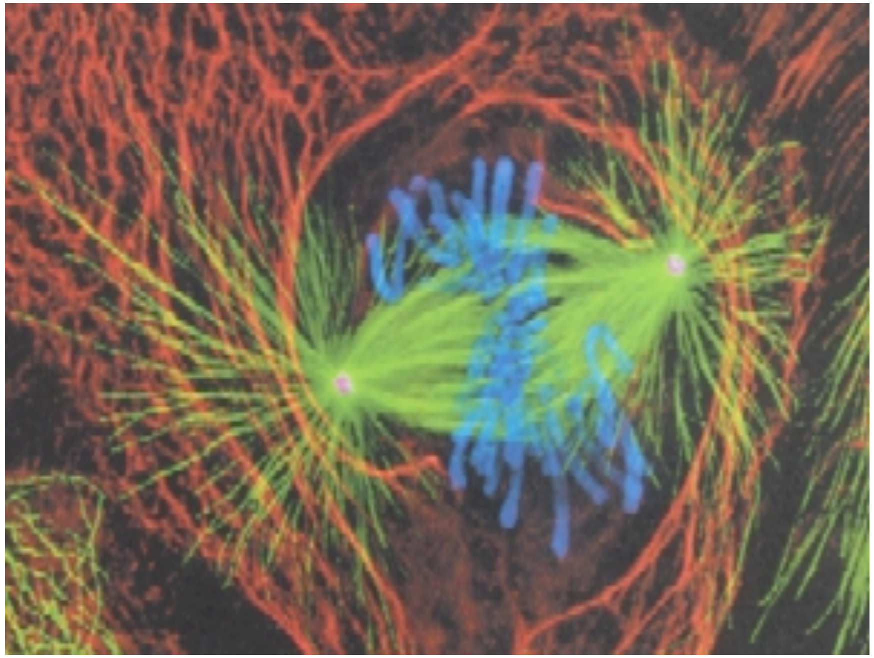




MICROTUBULE FORCES IN CELLS

Pushing and pulling at microtubule – kinetochore interface

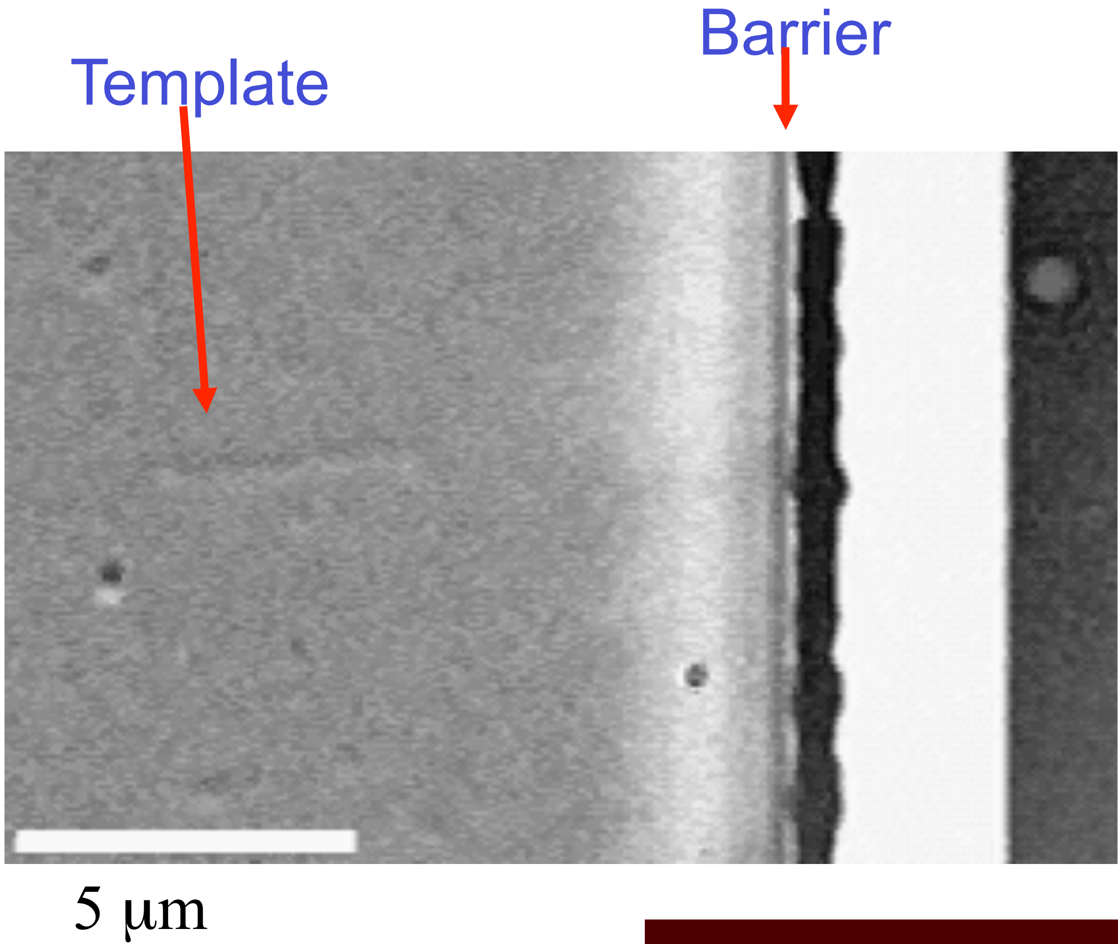
Inoue & Salmon, Mol. Biol. Cell, 1995

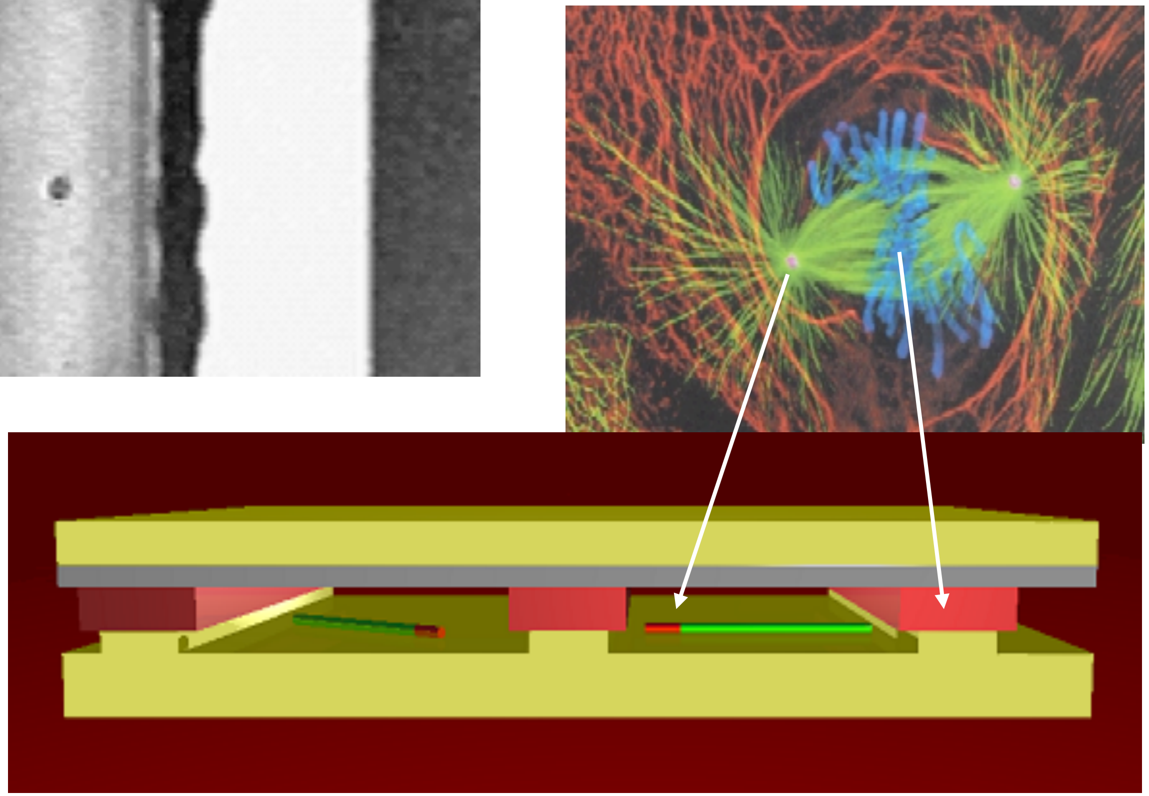


Schibler et al.

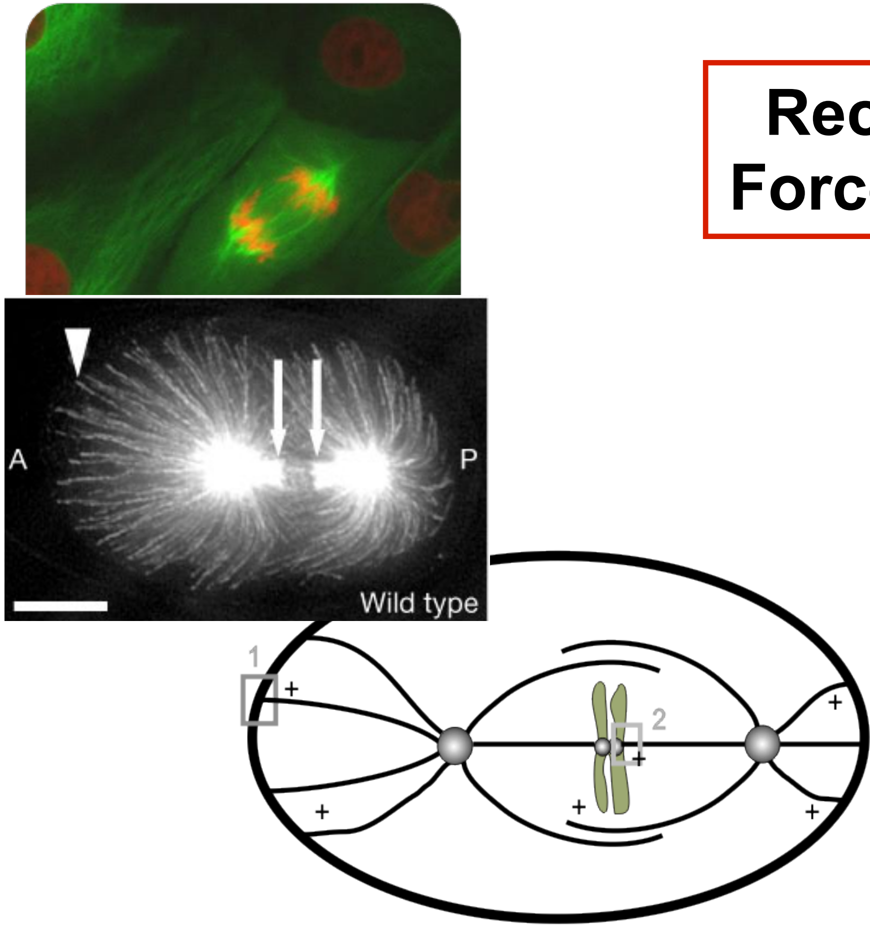
Rieder et al.

Microfabricated model environment

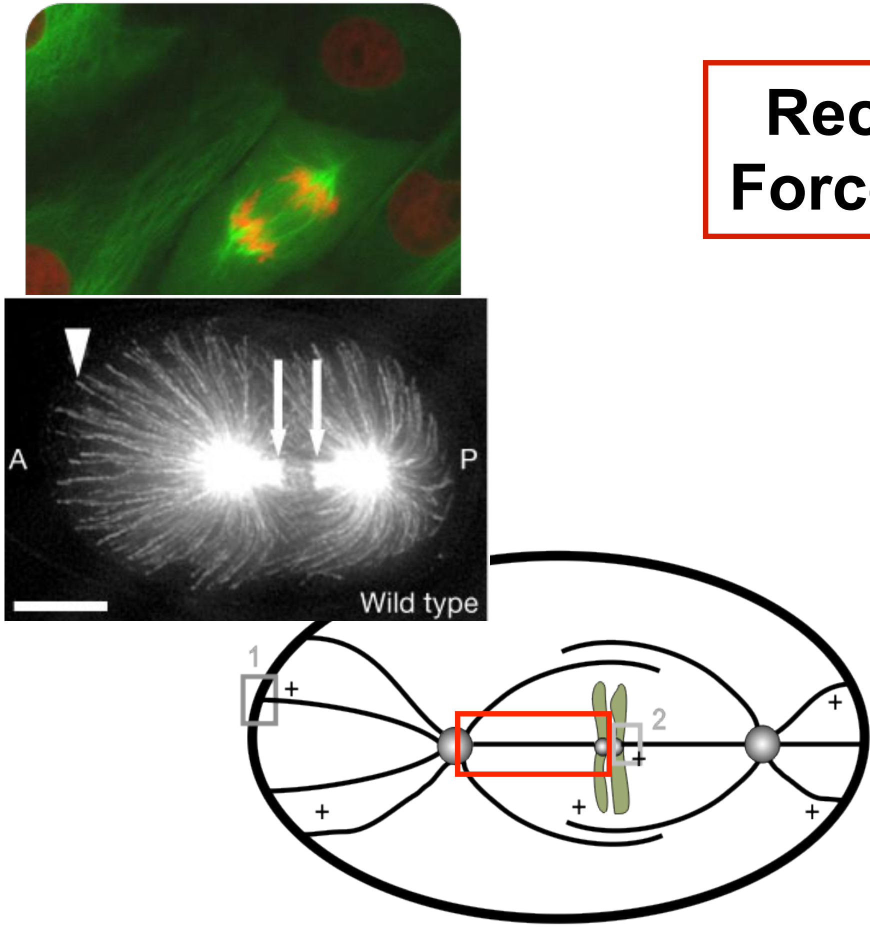


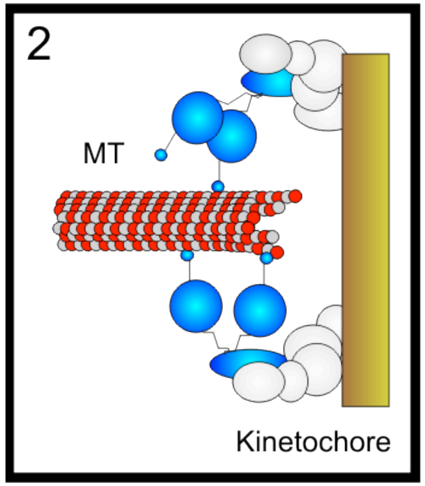


Reconstituting the Pulling Force Generating Machinery

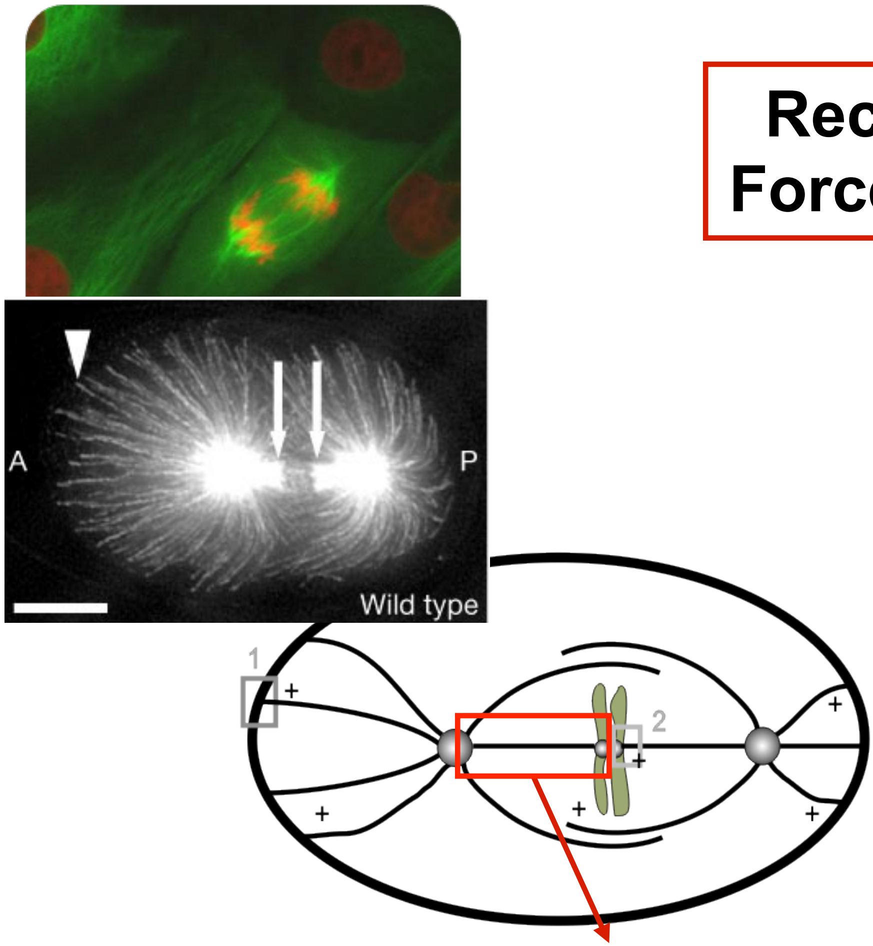


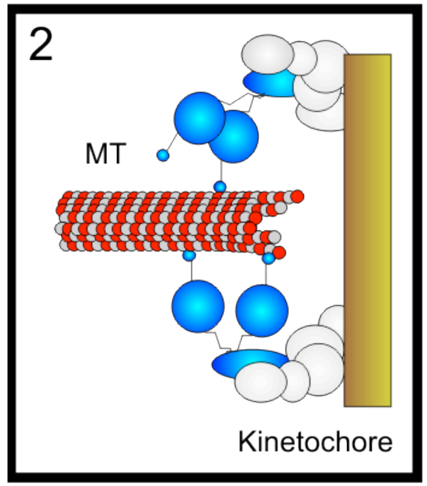
Reconstituting the Pulling Force Generating Machinery

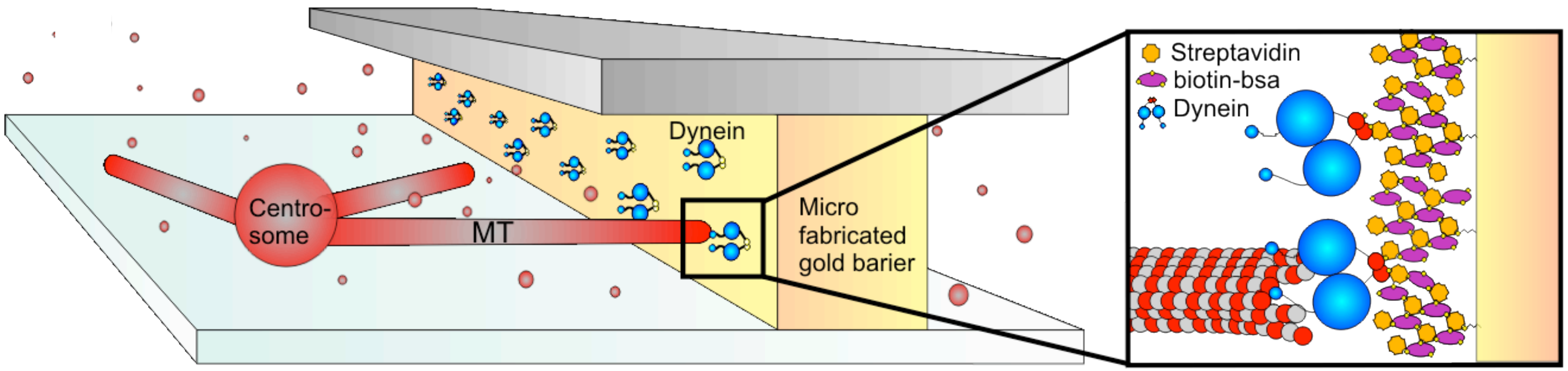




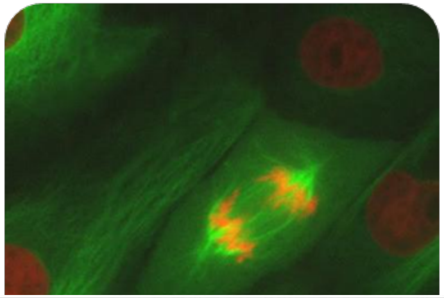
Reconstituting the Pulling Force Generating Machinery

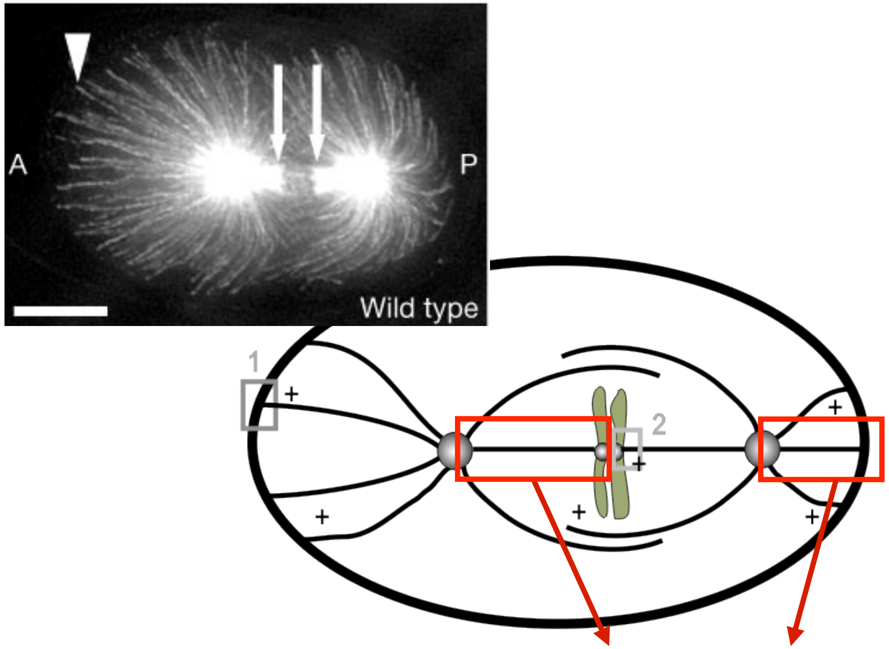


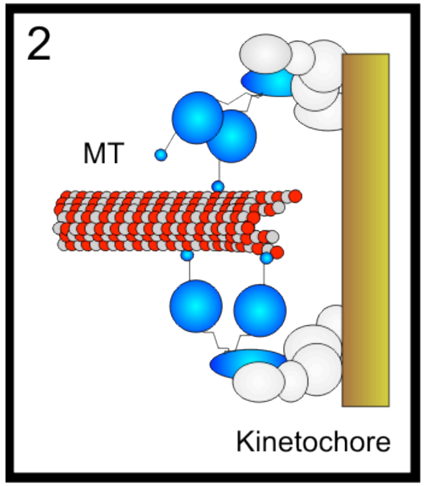


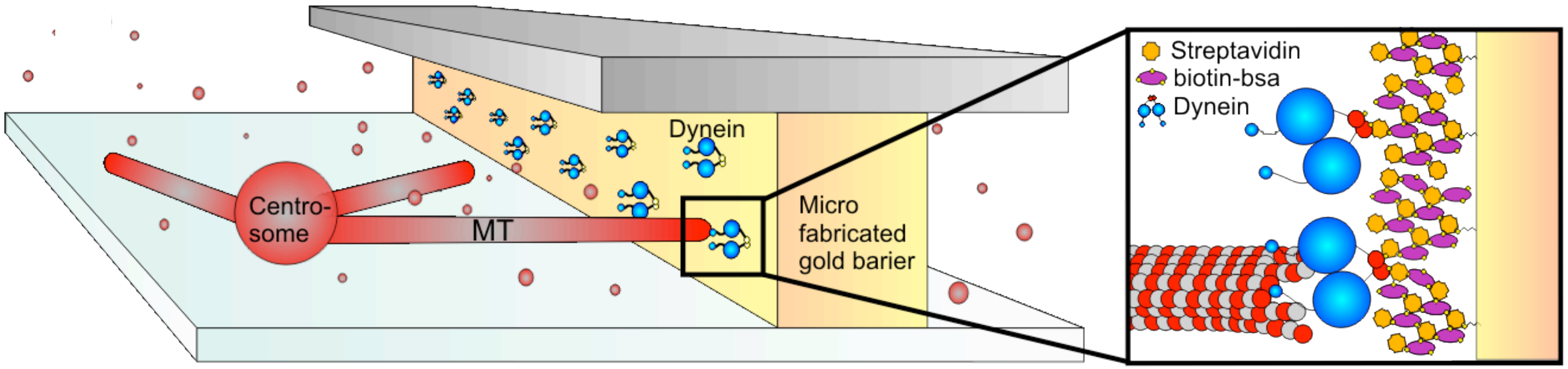


Reconstituting the Pulling Force Generating Machinery

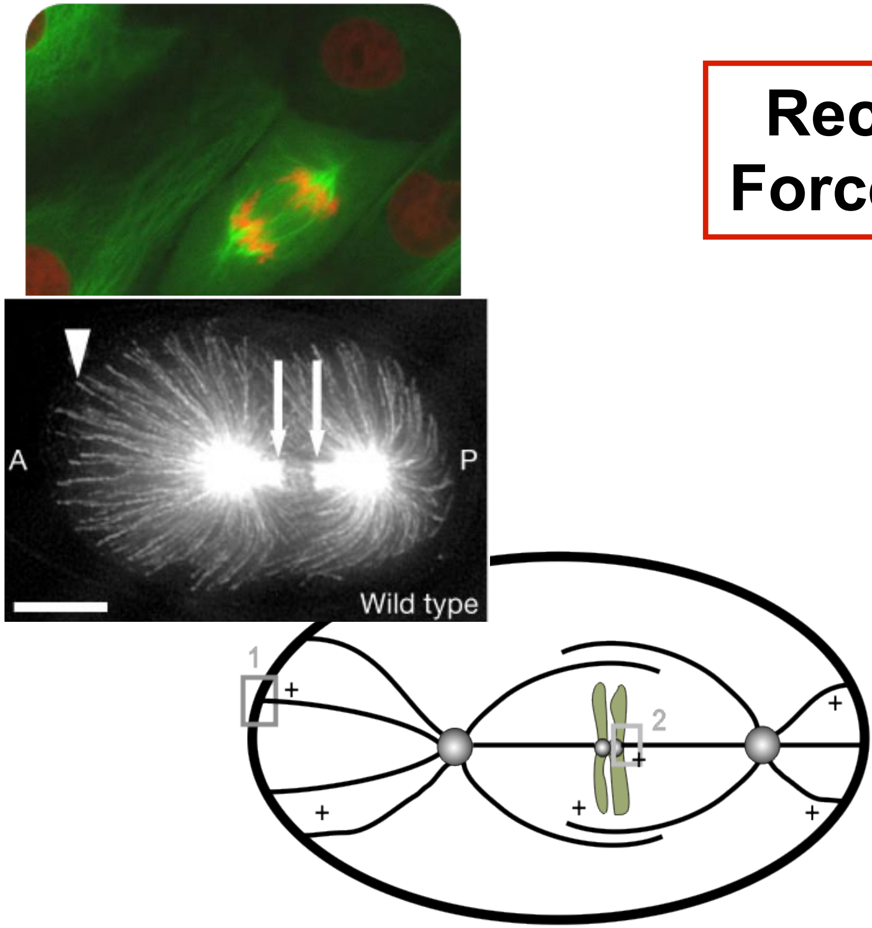


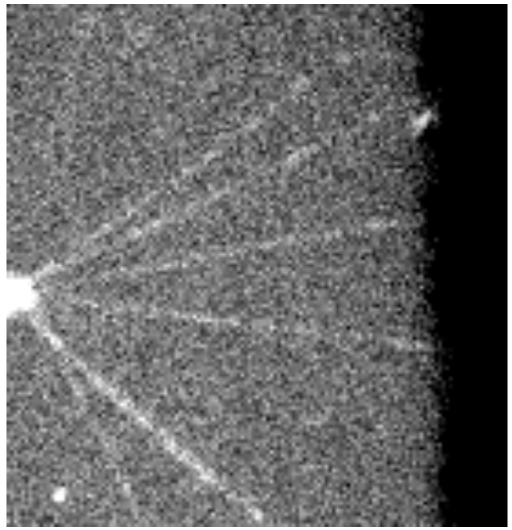


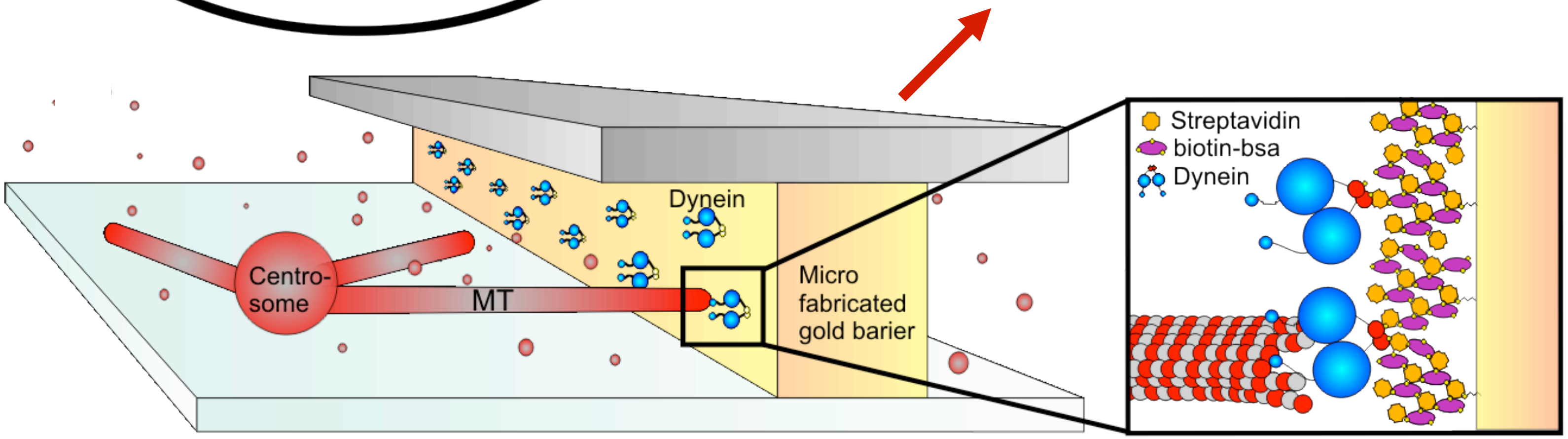




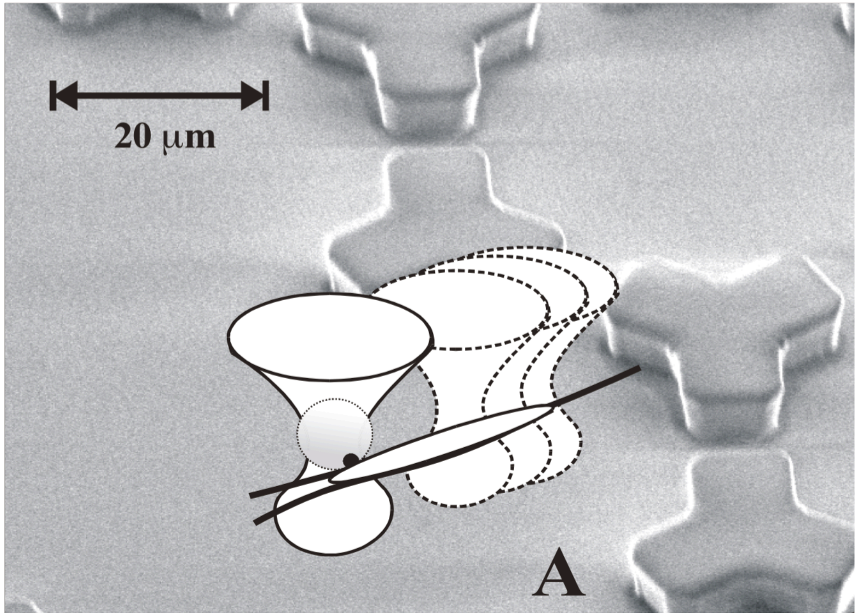
Reconstituting the Pulling Force Generating Machinery

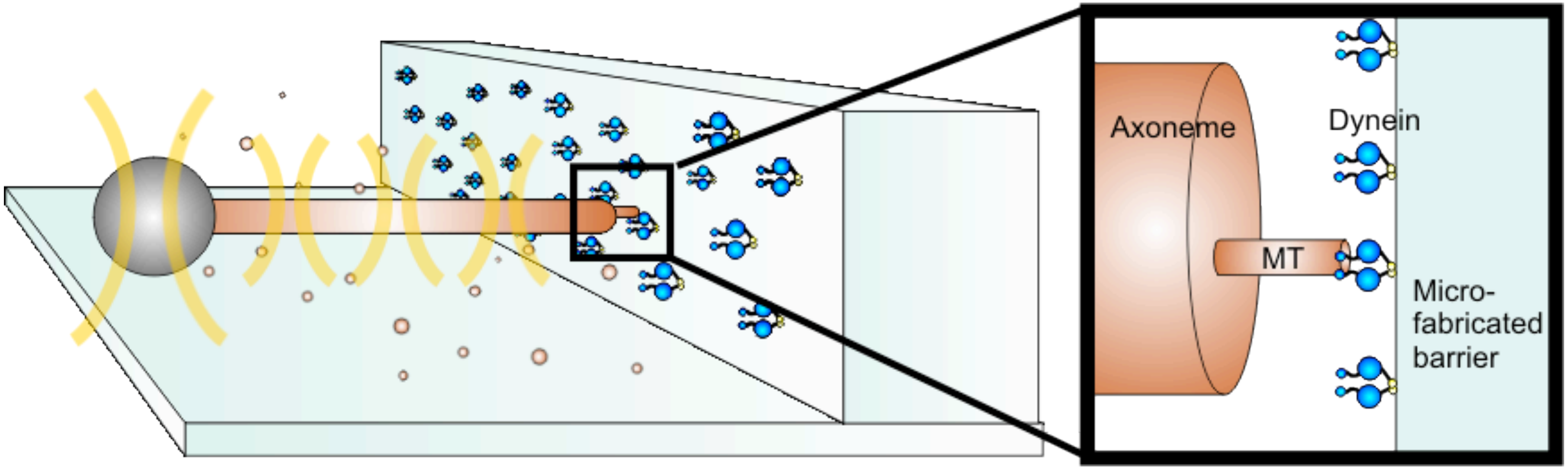


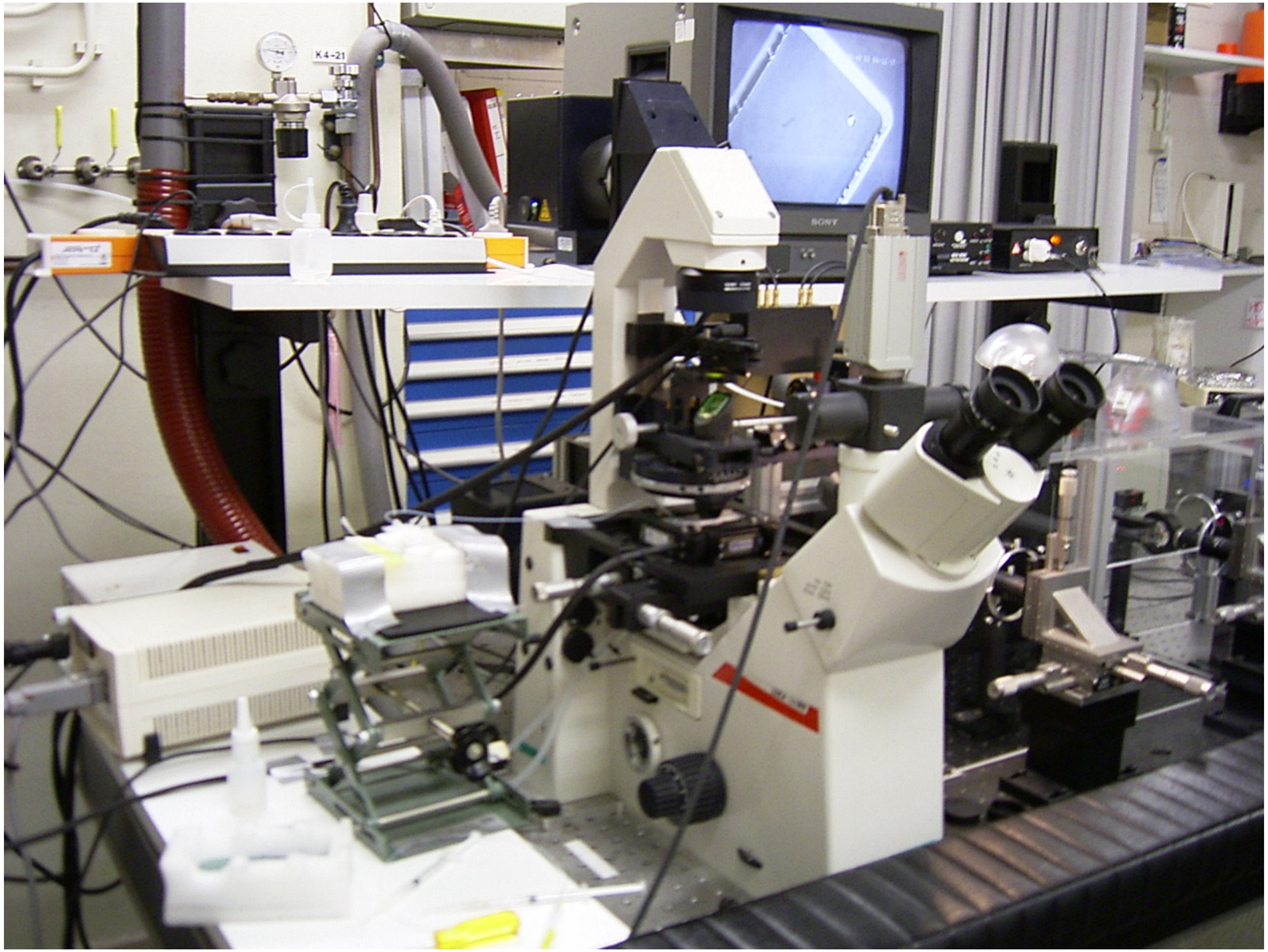


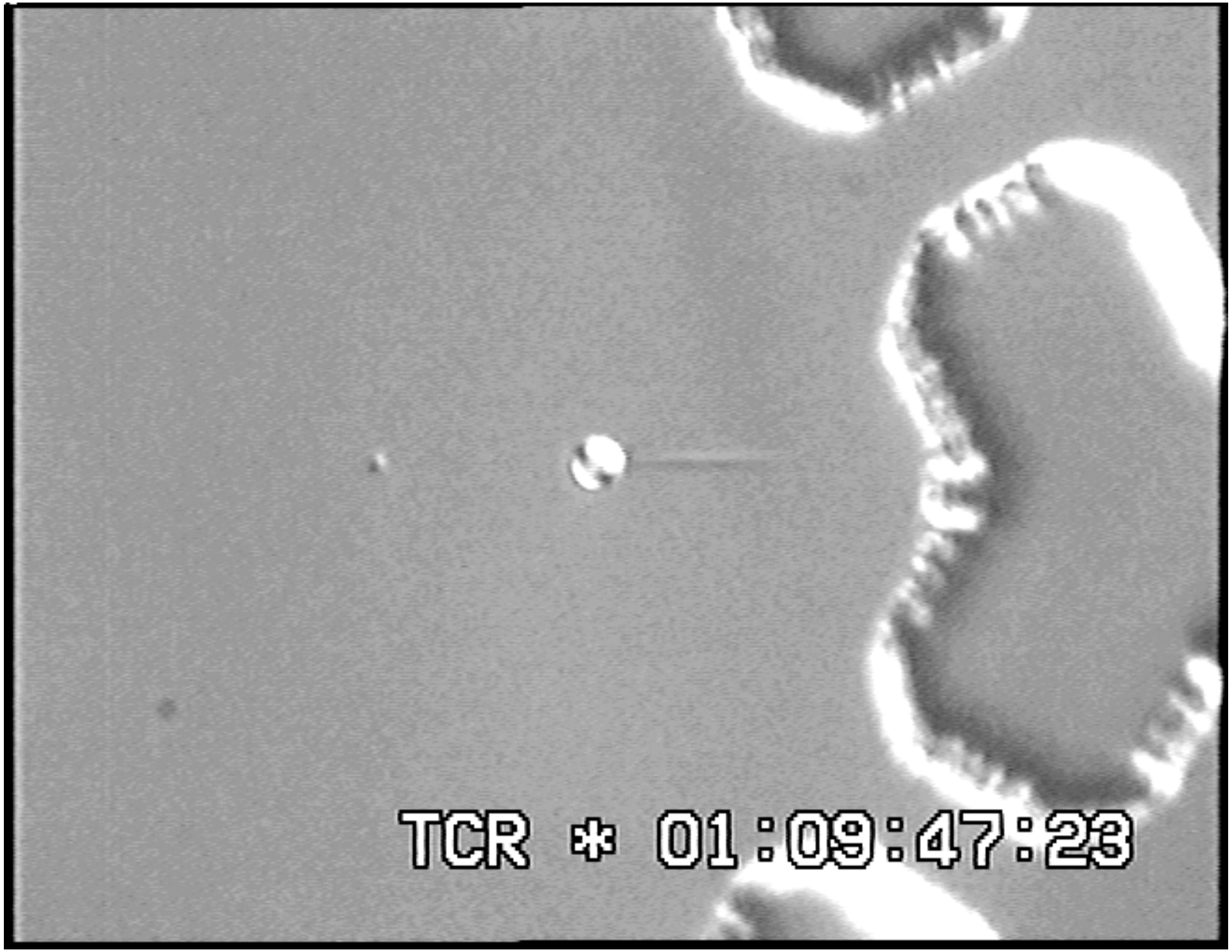


Keyhole optical trap

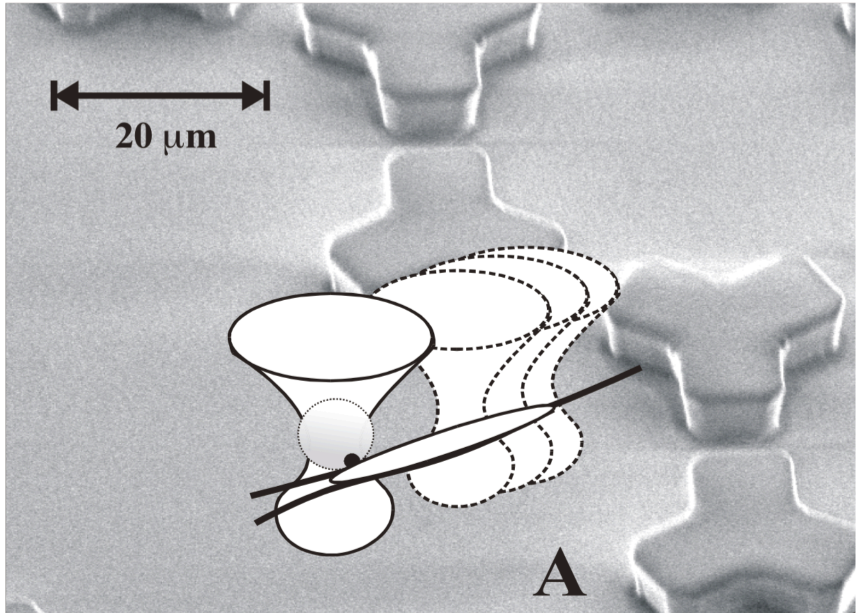


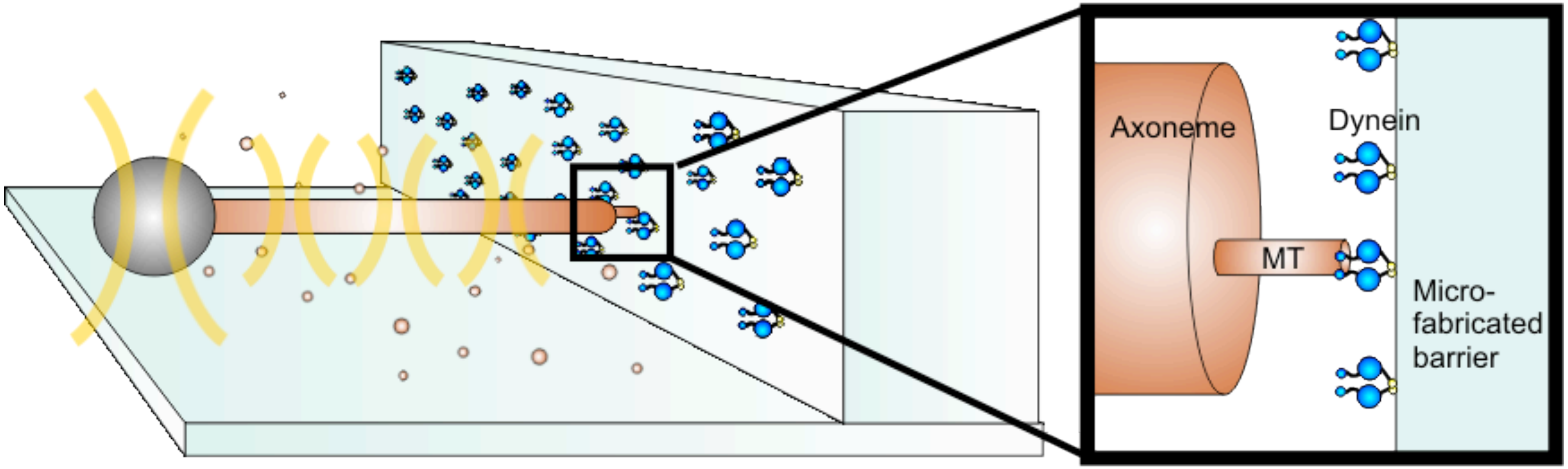



K4-21
SONY

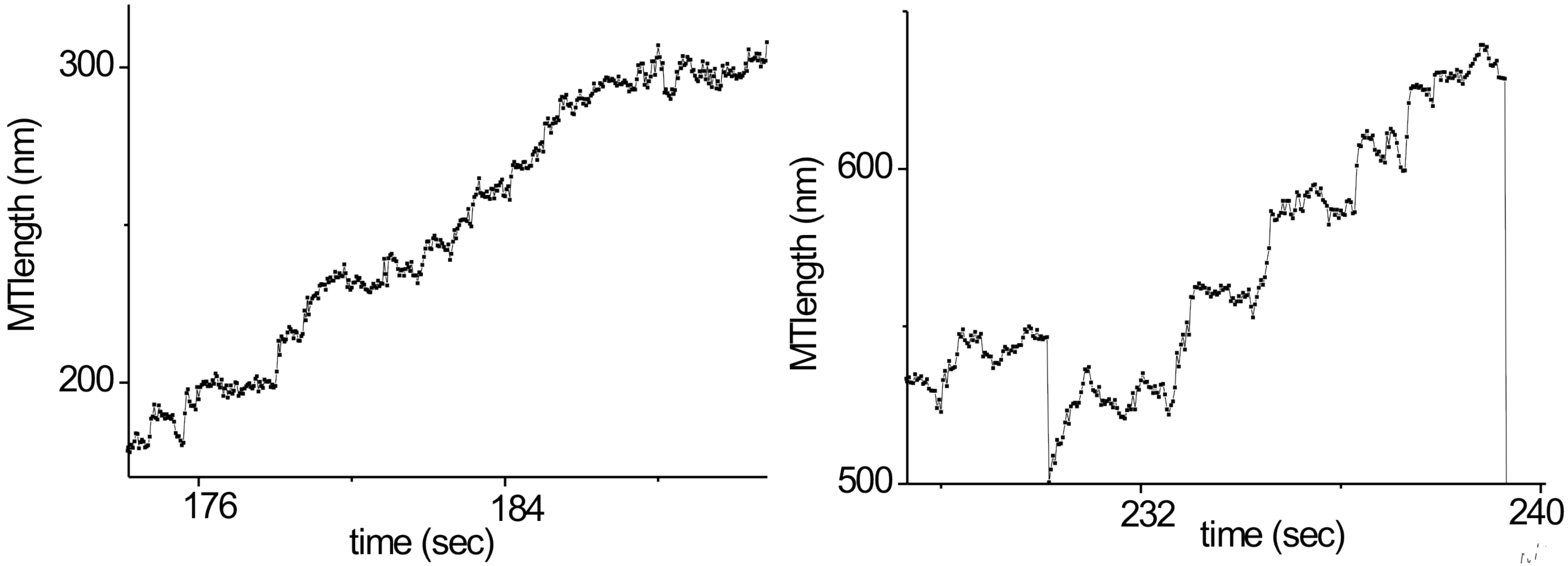

TCR * 01:09:47:23

Keyhole optical trap





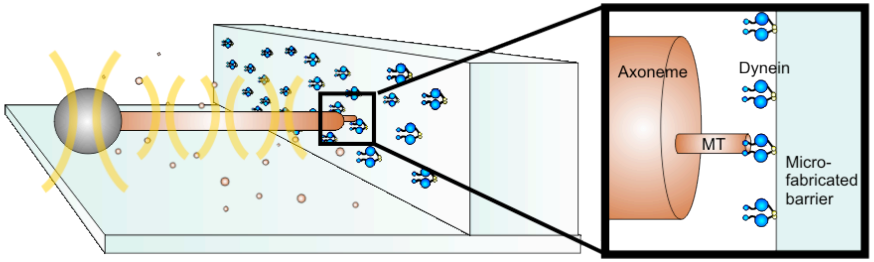
Microtubule Growth Details under Force

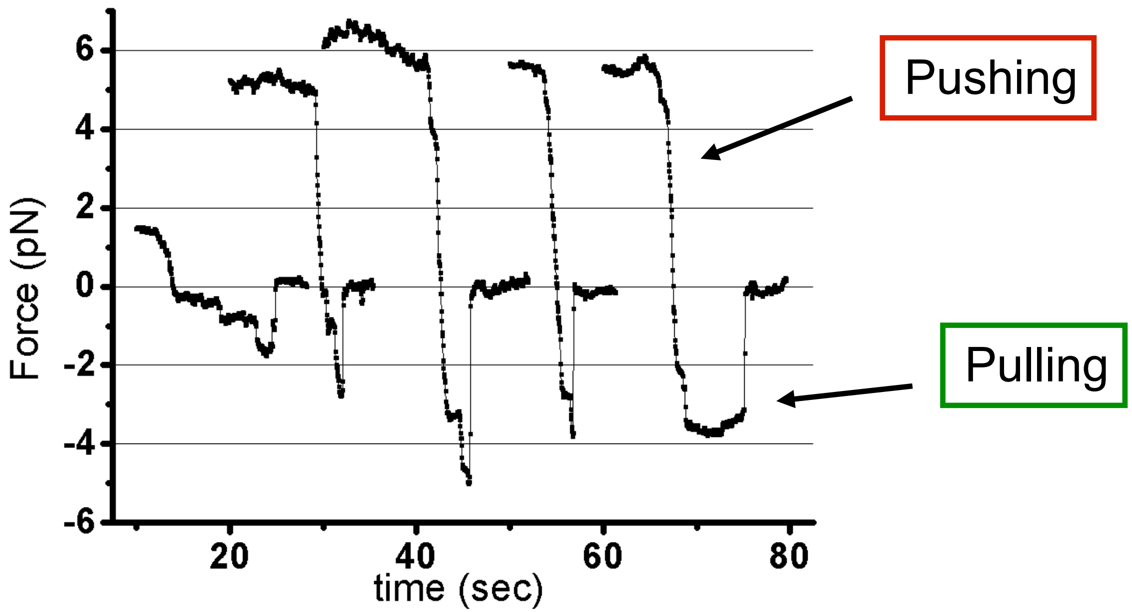


Recent results: few nm noise (effectively)

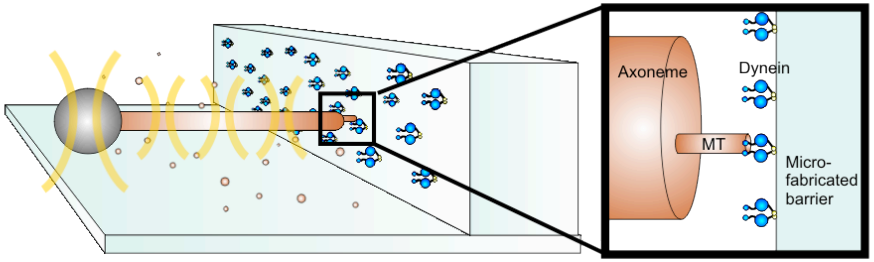
Older results: Kerssemakers et al, Nature 2006

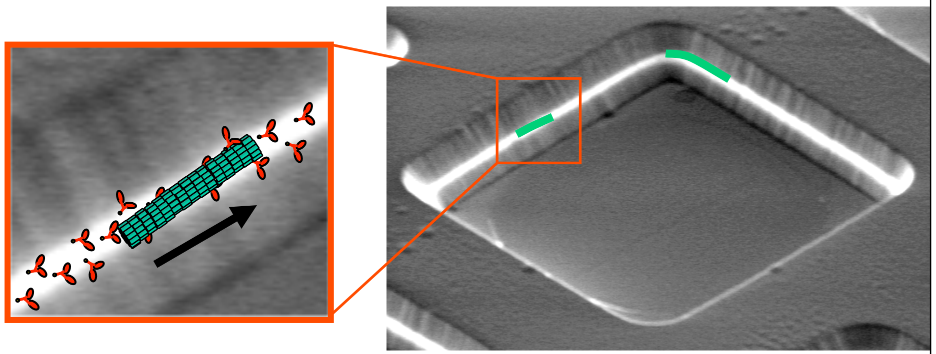
Laura Munteanu, Julien Husson

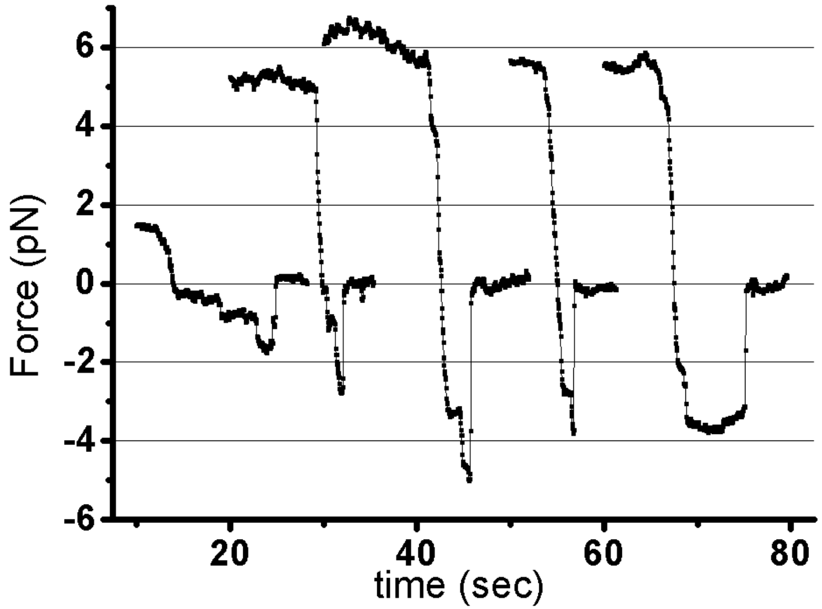




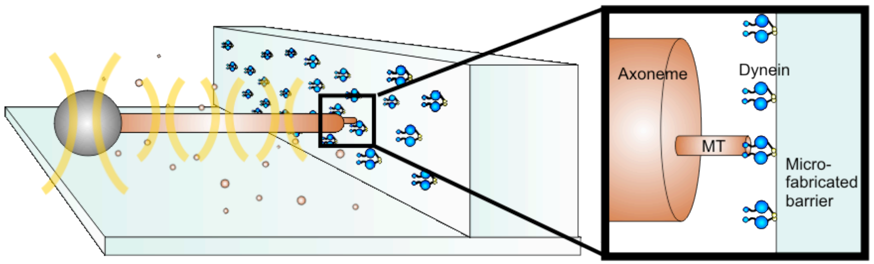
The MT-dynein connection generates pulling forces up to 5 pN

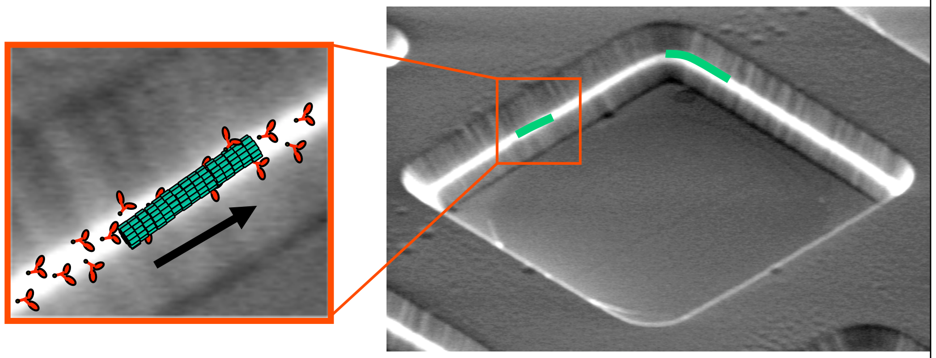


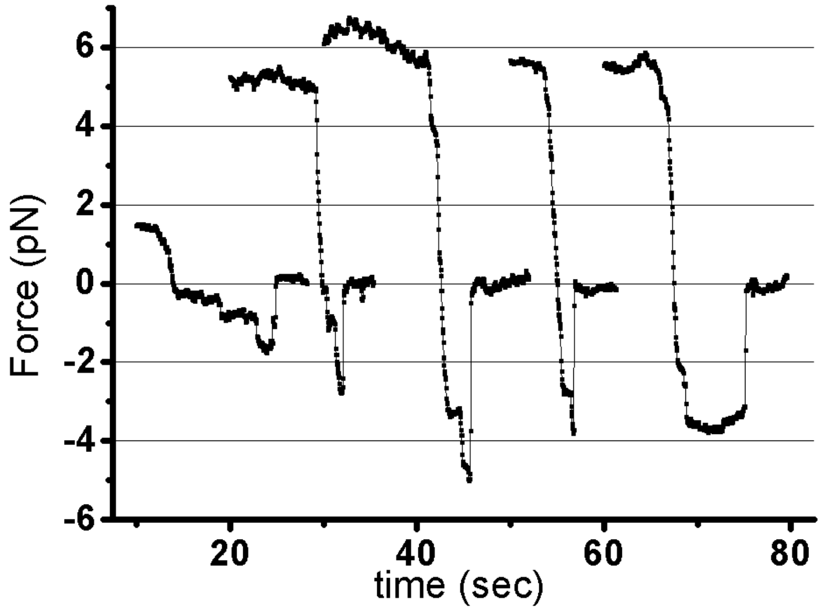


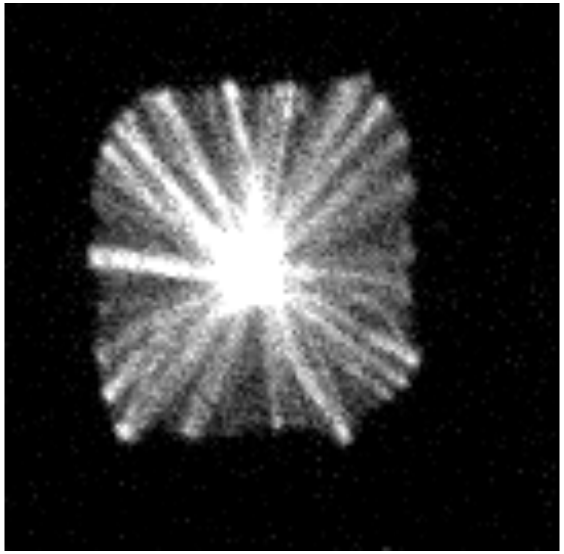


The MT-dynein connection generates pulling forces up to 5 pN,

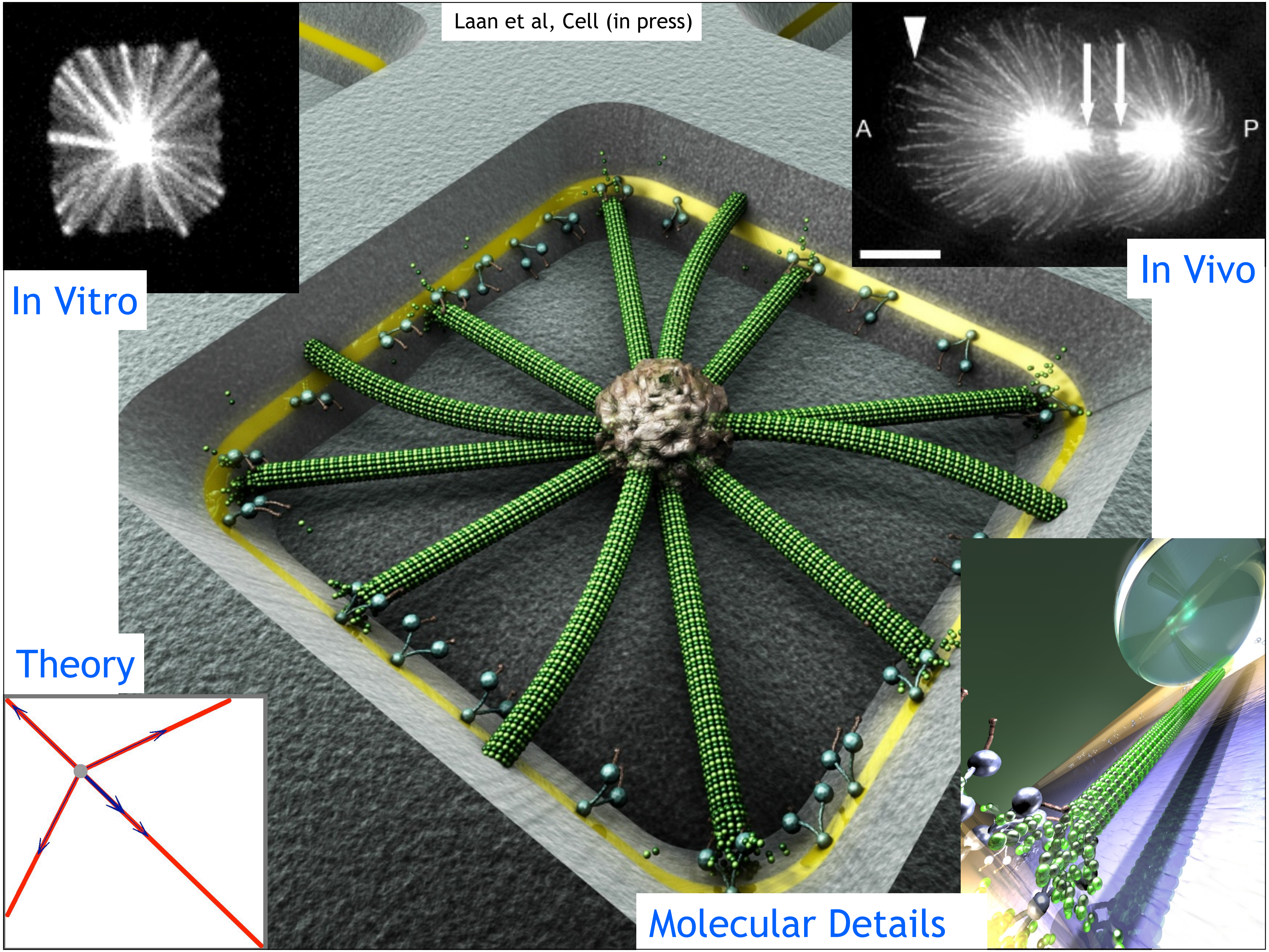








The MT-dynein connection generates pulling forces up to 5 pN, which position microtubule asters in confined spaces!


Laan et al, Cell (in press)
In Vitro
A
P
In Vivo
Theory
Molecular Details

Acknowledgements

Axonemes:

Matt Footer (Stanford)

Julie Theriot (Stanford)

Dynein:

Sam Reck-Peterson (Harvard)

Ron Vale (UCSF)

Positioning by pulling, theory:

Nenad Pavin (MPI-PKS Dresden)

Frank Juelicher (MPI-PKS Dresden)

Centrosomes:

Claude Celati (Curie Paris)

Michel Bornens (Curie Paris)

Funding:

FOM

NWO-ALW

HFSP

EU