Techniques for Single Molecule Micromanipulation

Laboratoire de Physique Statistique de l'Ecole Normale Superieure Paris France
A. Meglio, T. Lionnet, G. Lia, E. Praly, K. Neuman, F. Mosconi, J-F. Allemand, D. Bensimon and V. Croquette

I Motivations:

II Single Molecule Techniques

III Twisting DNA

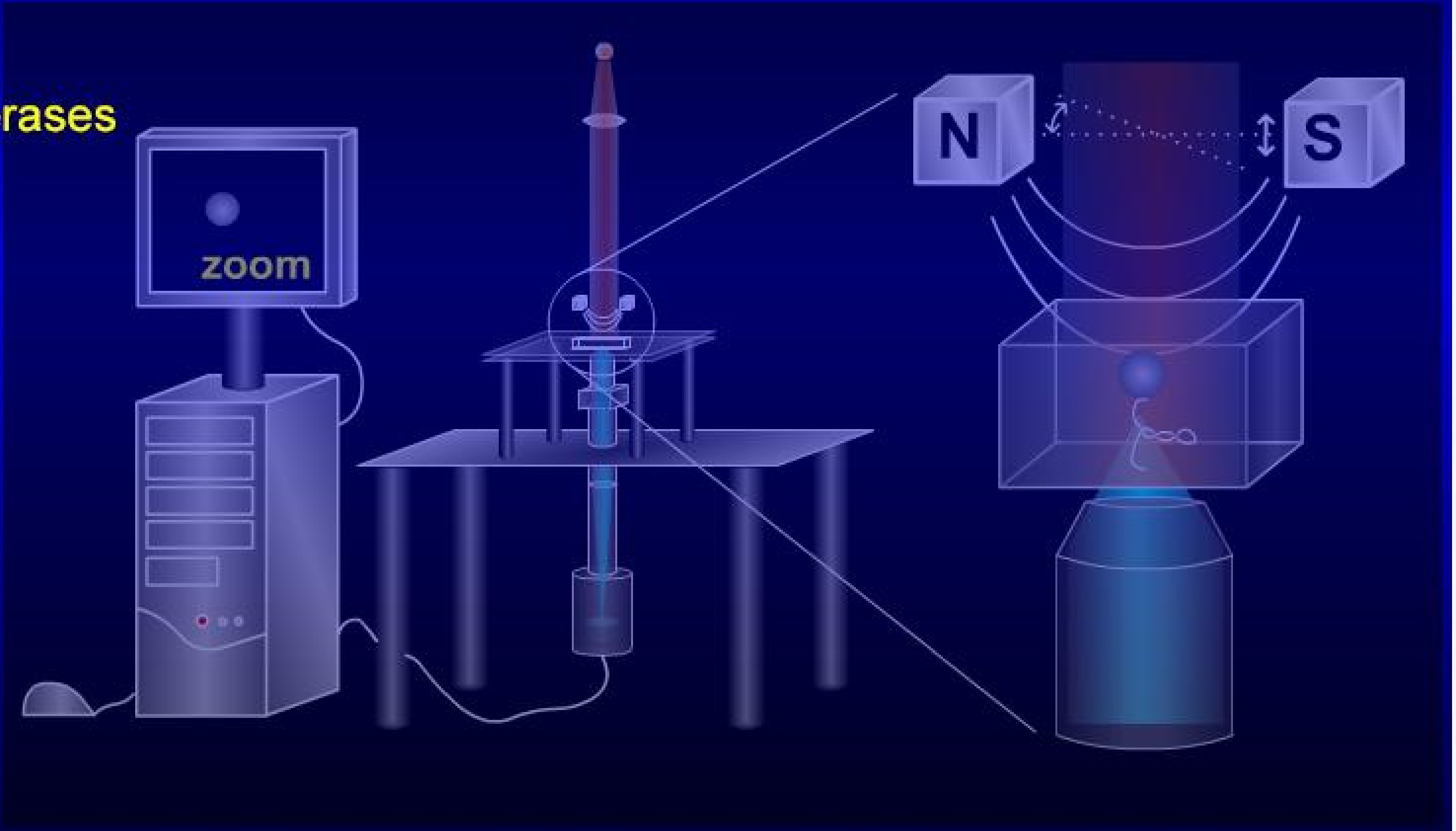
IV Topoisomerases

V Translocases

VI Helicases and polymerases

VII Genetic repressor

VI Conclusion



The group.

Croucher ASI, Hong Kong, December 4-9th, 2006.

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I Motivations:

Forces at the molecular scale,
DNA entanglement,
Myosine V steps 2 3, and rotates.
ATP synthase, Muscle.
Viral DNA packaging,
DNA sequencing.

II Single Molecule Techniques

III Twisting DNA

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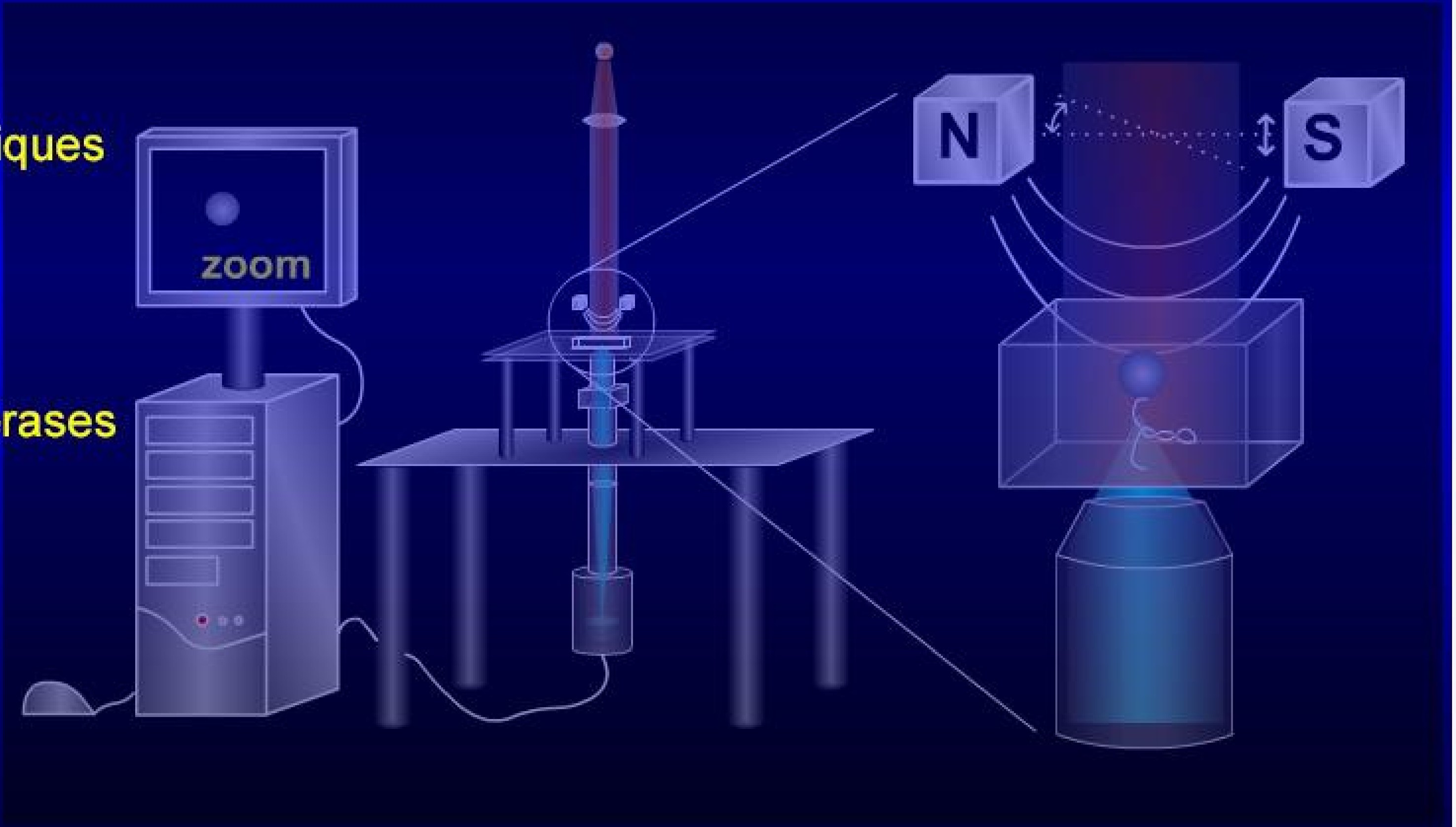

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Force order of magnitude in pN (10^{-12}N)

- Weight of a strong man → 10^{15} pN
- Covalent tensil strength : $\frac{1eV}{\text{Å}}$ → 10^3 pN
- Weak bond : $\frac{1 k_B T}{1 nm}$ → 4 pN, 1 $k_B T$ = 0.6 Kcal/mole
- Molecular motors : 1 ATP →12 $k_B T$, step size 5 to 8 nm →force stroke → 4 pN

Minimum force → sensor sensitivity

- Weight of a E-coli → 10^{-2} pN

spring k ↓ bead r ↓

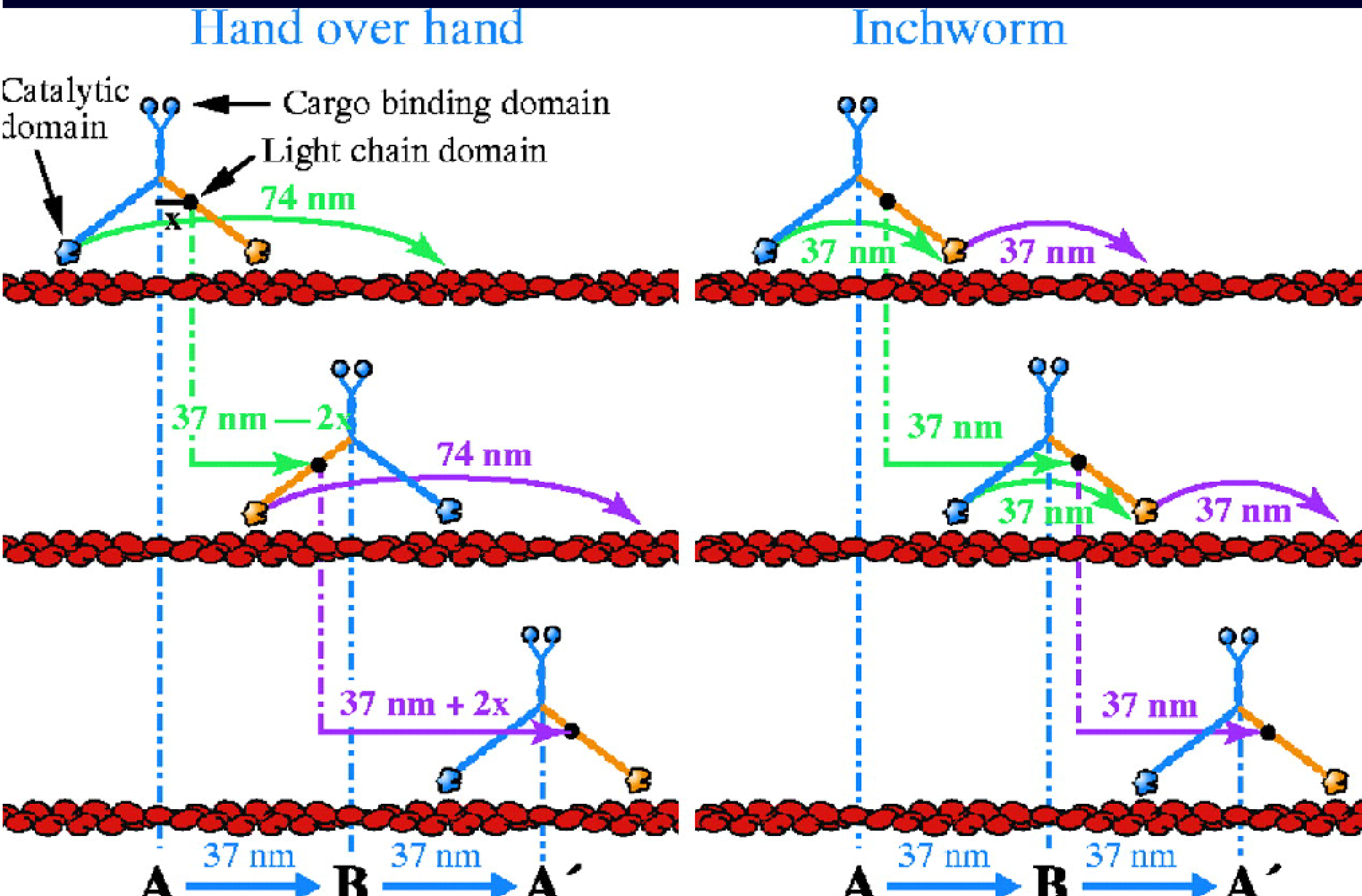
|--|/|/|/|/|/|--○

viscosity η

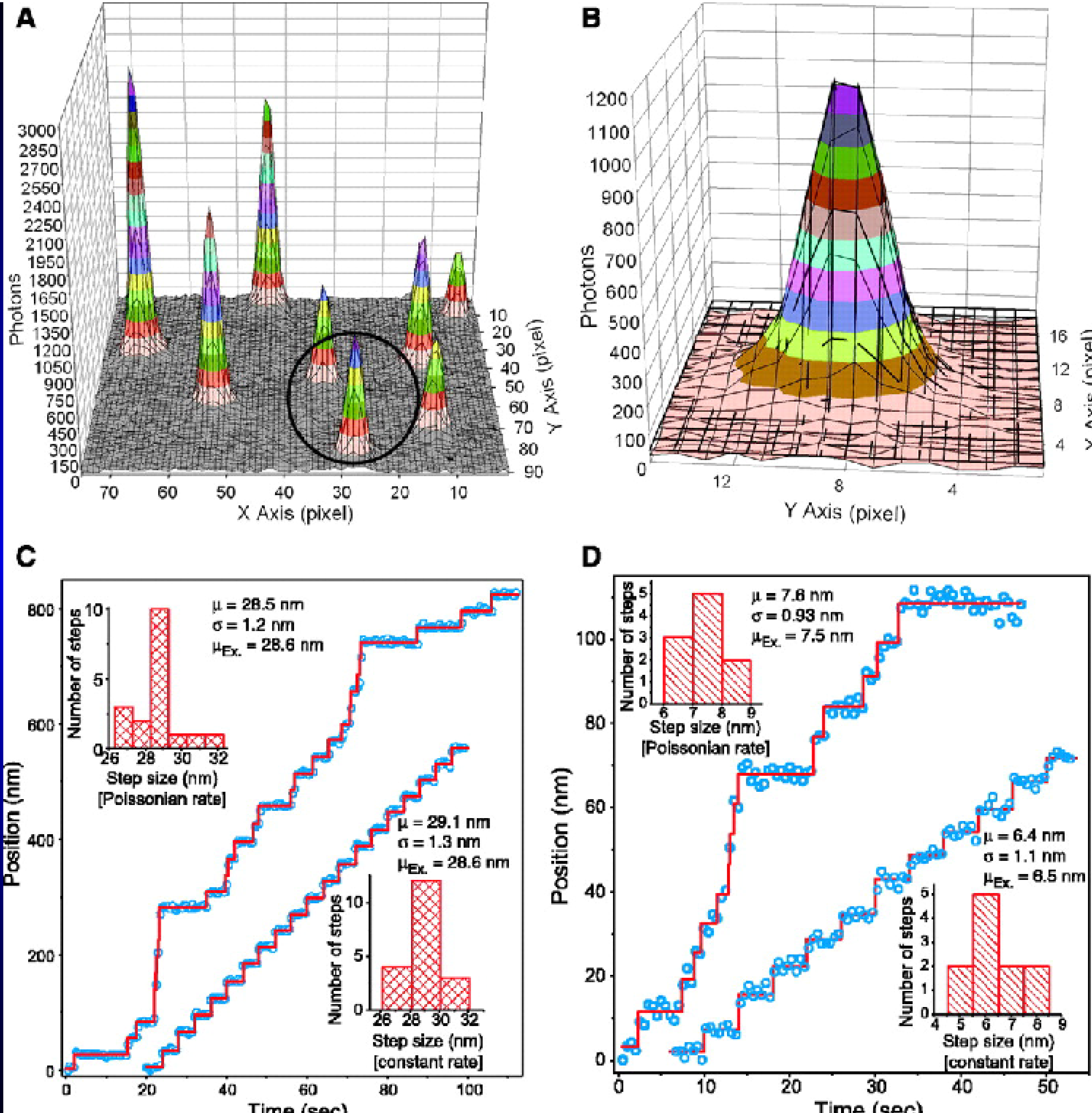
- Langevin force: $f_n = \sqrt{4 k_B T \, 6\pi\eta r \, \Delta F}$ = 0.02 pN.Hz$^{-1/2}$, r = 1 μm

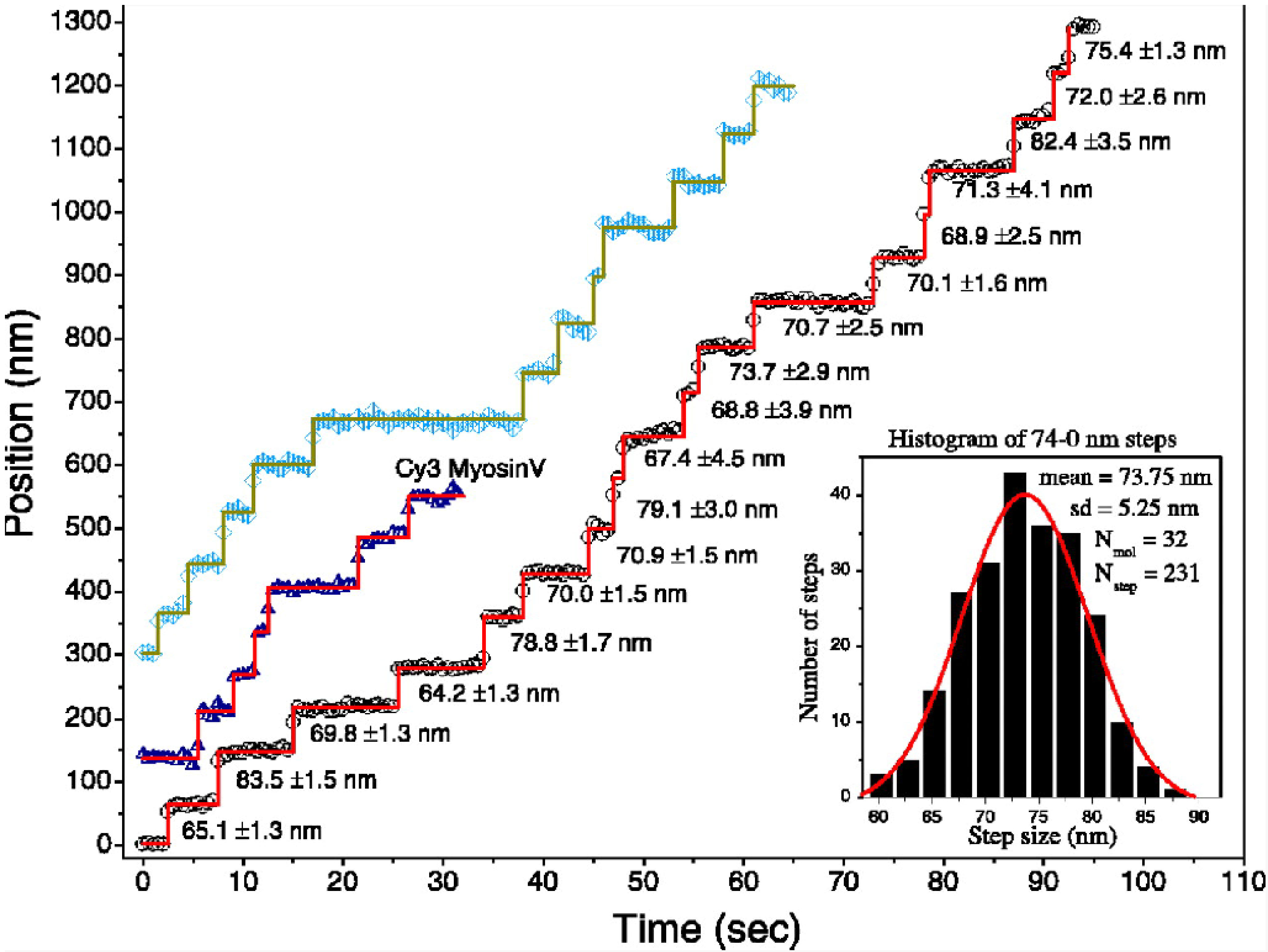
Noise comes from dissipation : η and r (not k) → small detector

Myosin V



Hand over hand
Inchworm
Catalytic domain
Cargo binding domain
Light chain domain
74 nm
x
37 nm — 2x
74 nm
37 nm + 2x
37 nm
37 nm
A
B
A´
37 nm
37 nm
37 nm
37 nm
37 nm
37 nm
37 nm
37 nm
A
B
A´


A
Photons
X Axis (pixel)
Y Axis (pixel)
B
Photons
Y Axis (pixel)
X Axis (pixel)
C
μ = 28.5 nm
σ = 1.2 nm
μEx. = 28.6 nm
Number of steps
Step size (nm)
[Poissonian rate]
μ = 29.1 nm
σ = 1.3 nm
μEx. = 28.6 nm
Step size (nm)
[constant rate]
Position (nm)
Time (sec)
D
μ = 7.6 nm
σ = 0.93 nm
μEx. = 7.5 nm
Number of steps
Step size (nm)
[Poissonian rate]
μ = 6.4 nm
σ = 1.1 nm
μEx. = 6.5 nm
Step size (nm)
[constant rate]
Position (nm)
Time (sec)



Position (nm)
Time (sec)
Cy3 MyosinV
65.1 ±1.3 nm
83.5 ±1.5 nm
69.8 ±1.3 nm
64.2 ±1.3 nm
78.8 ±1.7 nm
70.0 ±1.5 nm
70.9 ±1.5 nm
79.1 ±3.0 nm
67.4 ±4.5 nm
68.8 ±3.9 nm
73.7 ±2.9 nm
70.7 ±2.5 nm
70.1 ±1.6 nm
68.9 ±2.5 nm
71.3 ±4.1 nm
82.4 ±3.5 nm
72.0 ±2.6 nm
75.4 ±1.3 nm
Histogram of 74-0 nm steps
mean = 73.75 nm
sd = 5.25 nm
N_{mol} = 32
N_{step} = 231
Number of steps
Step size (nm)

Single-Molecule, Motion-Based DNA Sequencing Using RNA Polymerase

William J. Greenleaf[1] and Steven M. Block[1,2]*

Traditional, dideoxy-based (Sanger) sequencing of DNA is remarkably reliable and robust. However, the quest for more rapid, economical ways to sequence genomes has driven interest in alternative approaches (*1*, *2*). Methods capable of sequencing single DNA molecules represent the logical endpoint of miniaturization, leading to the maximum extraction of information from a minimum of material.

concentration, RNAP will be induced to pause at every DNA position that requires the addition of the limiting nucleotide.

To sequence DNA, we repeated the single-molecule assay four times (on four copies of the target DNA sequence) with each NTP species held rate-limiting in turn, and we inferred the template sequence directly from the ordered sequence of pauses in the set of four transcrip-

peaks, the tallest peaks were associated with the nearest unassigned windows. Last, any remaining windows were assigned to the base with the highest histogram value found at the center of the window. With this scheme, we correctly identified 30 out of 32 bases in a target region on the basis of less than 3 min of net observation time for exactly four molecules (Fig. 1). Greatly improved accuracy can be obtained by combining statistics from multiple single-molecule records and by using more a sophisticated base-calling algorithm, e.g., one based on peak deconvolution.

Read lengths of DNA sequences determined by this approach are ultimately limited, in principle, by the processivity of RNAP, which is thousands of base pairs. In practice, it has proved possible to follow RNAP at the single-molecule level with near–base pair accuracy over templates

Block's web page.

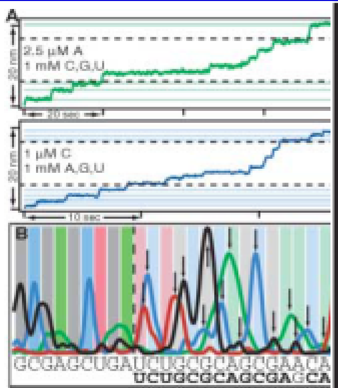
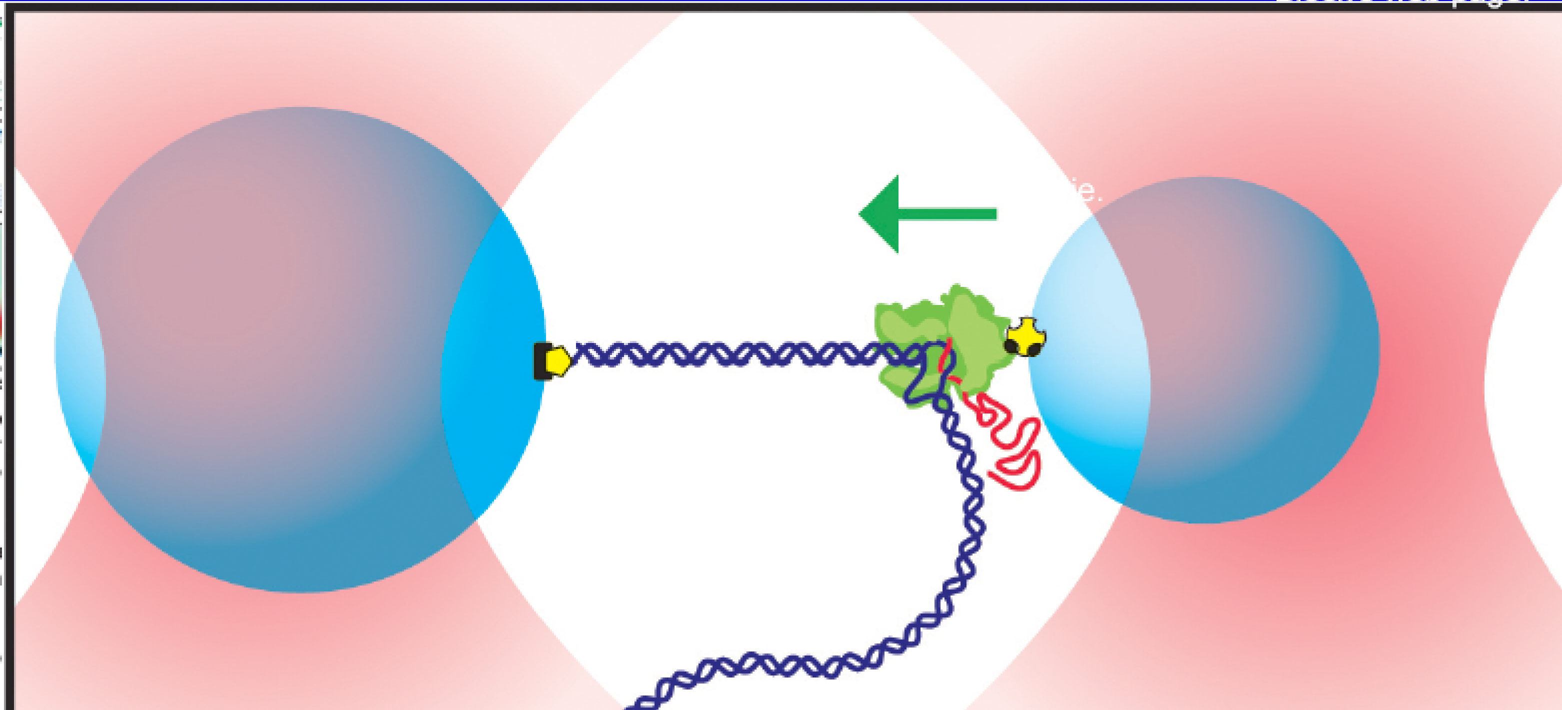
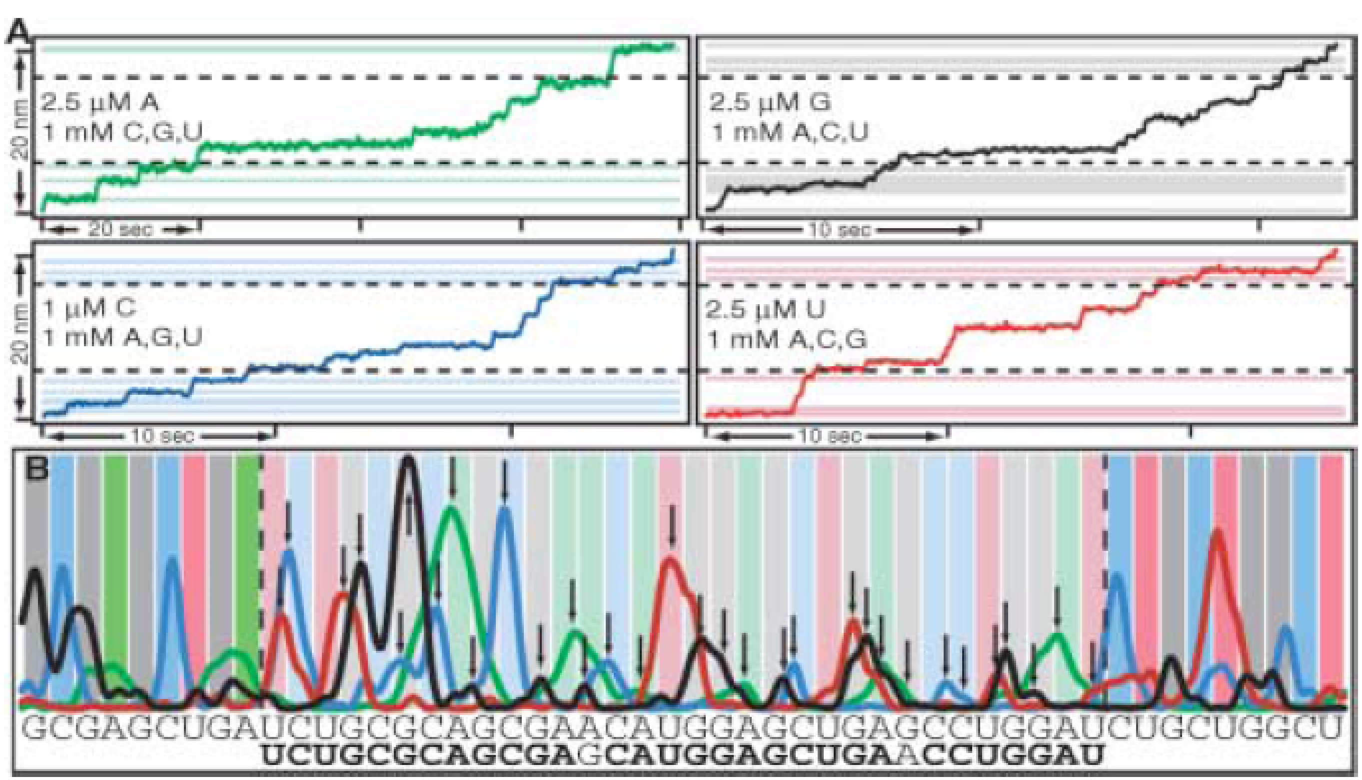


Fig. 1. Motion-based DNA sequen tional position versus time for a sir different limiting nucleotide conditi and UTP, red). Positions of expected horizontal lines) flank the region to (**B**) Position histograms for the da Flanking positions used for alignm bases to be called (light vertical ba (arrows). The true sequence of the sequence, with 30 of 32 correct bas





BREVIA

lest peaks were associated with the
igned windows. Last, any remaining
re assigned to the base with the
ram value found at the center of the
h this scheme, we correctly identi-
f 32 bases in a target region on the
than 3 min of net observation time
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acy can be obtained by combining
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more a sophisticated base-calling
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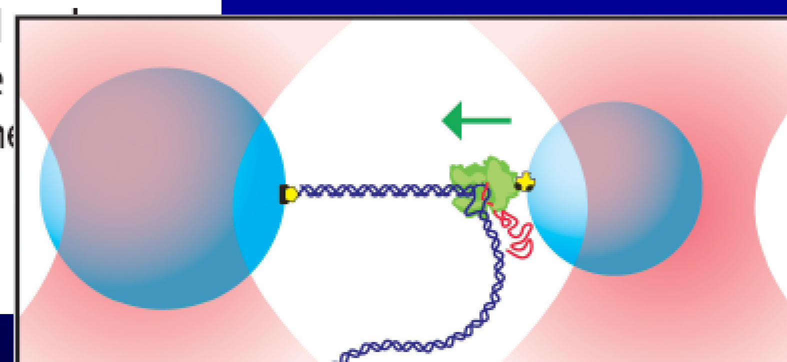
ach are ultimately limited, in princi-
processivity of RNAP, which is
base pairs. In practice, it has proved
ollow RNAP at the single-molecule
ır–base pair accuracy over templates

Block's web page.

Fig. 1. Motion-based DNA sequencing. (**A**) Aligned records of transcriptional position versus time for a single molecule of RNAP under the four different limiting nucleotide conditions (ATP, green; CTP, blue; GTP, black; and UTP, red). Positions of expected pauses used for record alignment (solid horizontal lines) flank the region to be sequenced (dotted horizontal lines). (**B**) Position histograms for the data in (A), normalized and smoothed. Flanking positions used for alignment (dark vertical bars) and ... bases to be called (light vertical bars) are shown; base calls are ... (arrows). The true sequence of the template is shown above the ... sequence, with 30 of 32 correct bases (boldface type).

vie.



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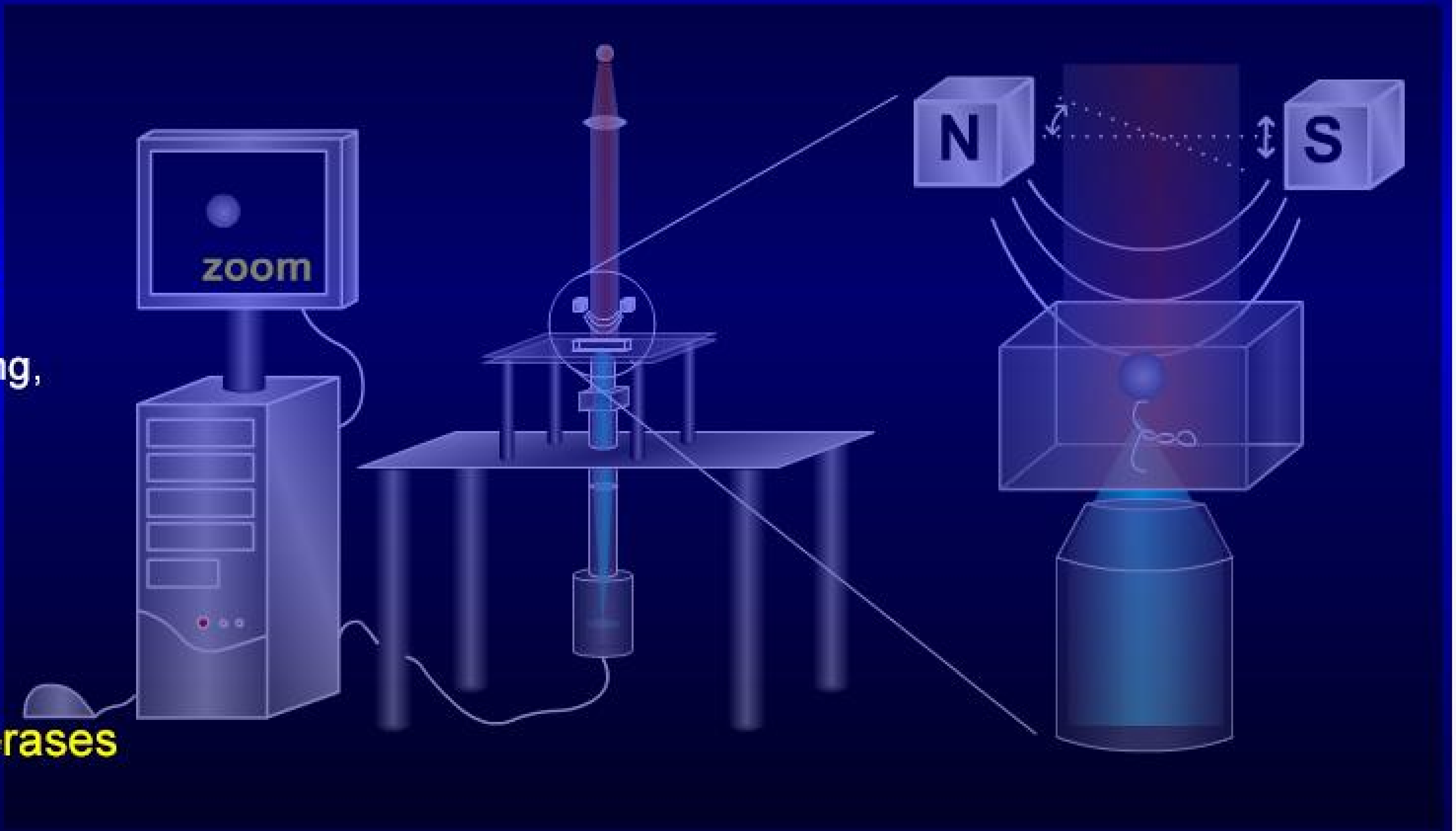
I Motivations:

II Single Molecule Techniques

Catching a single molecule.
Optical tweezers, principle.
Magnetic tweezers,
setup, beads, measure,
Force measurement
Anti-brownian trap
Polymer elasticity
tansition B-DNA to S-DNA,
DNA unzipping,and RNA,
Denaturation upon stretching,
Biotine-streptavidine,
Single strand DNA,



III Twisting DNA

IV Topoisomerases

V Translocases

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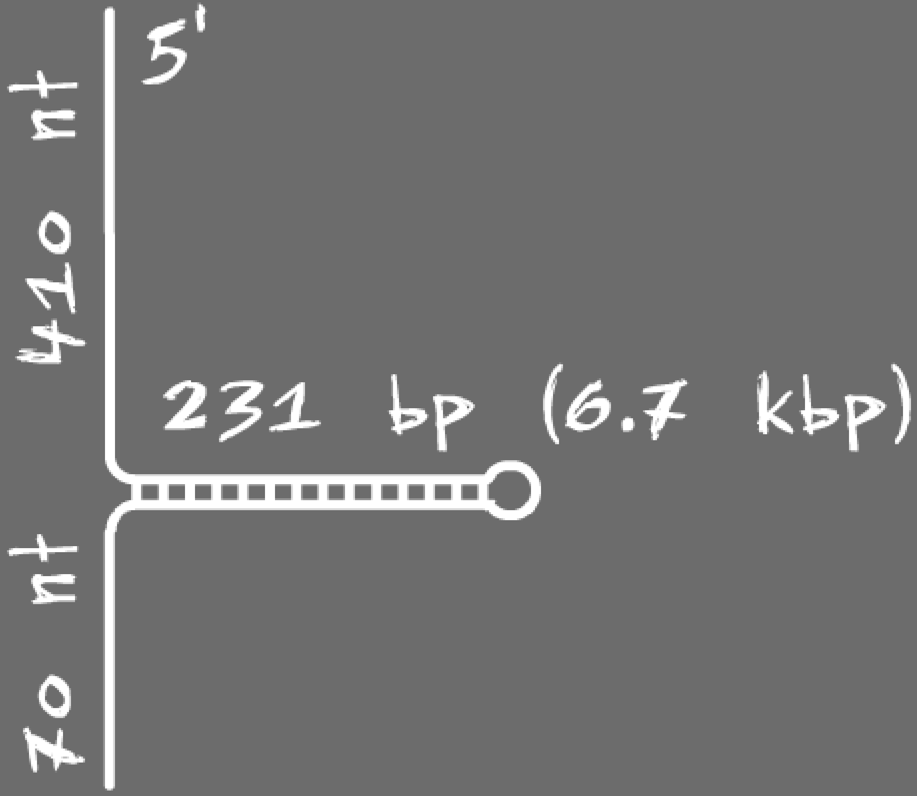
The group.



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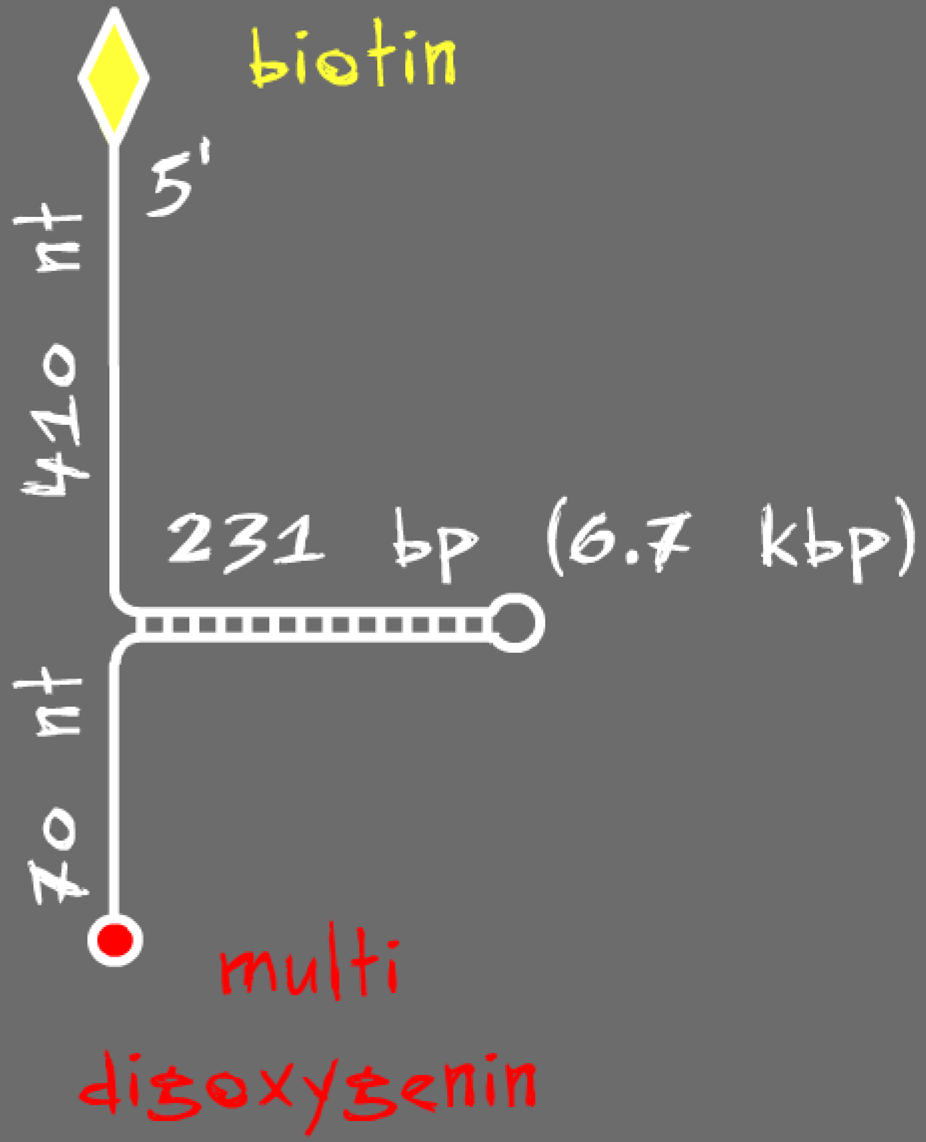
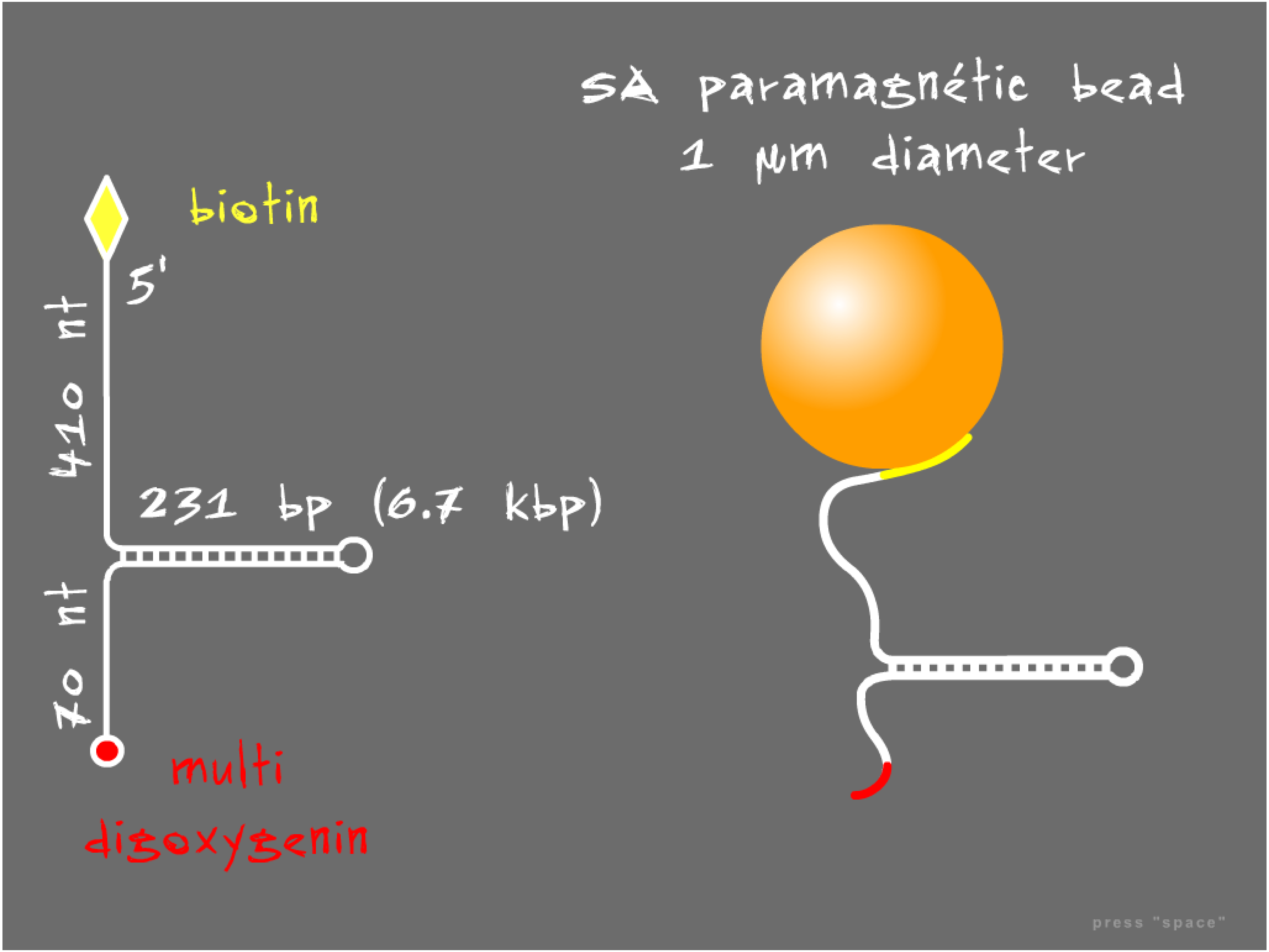
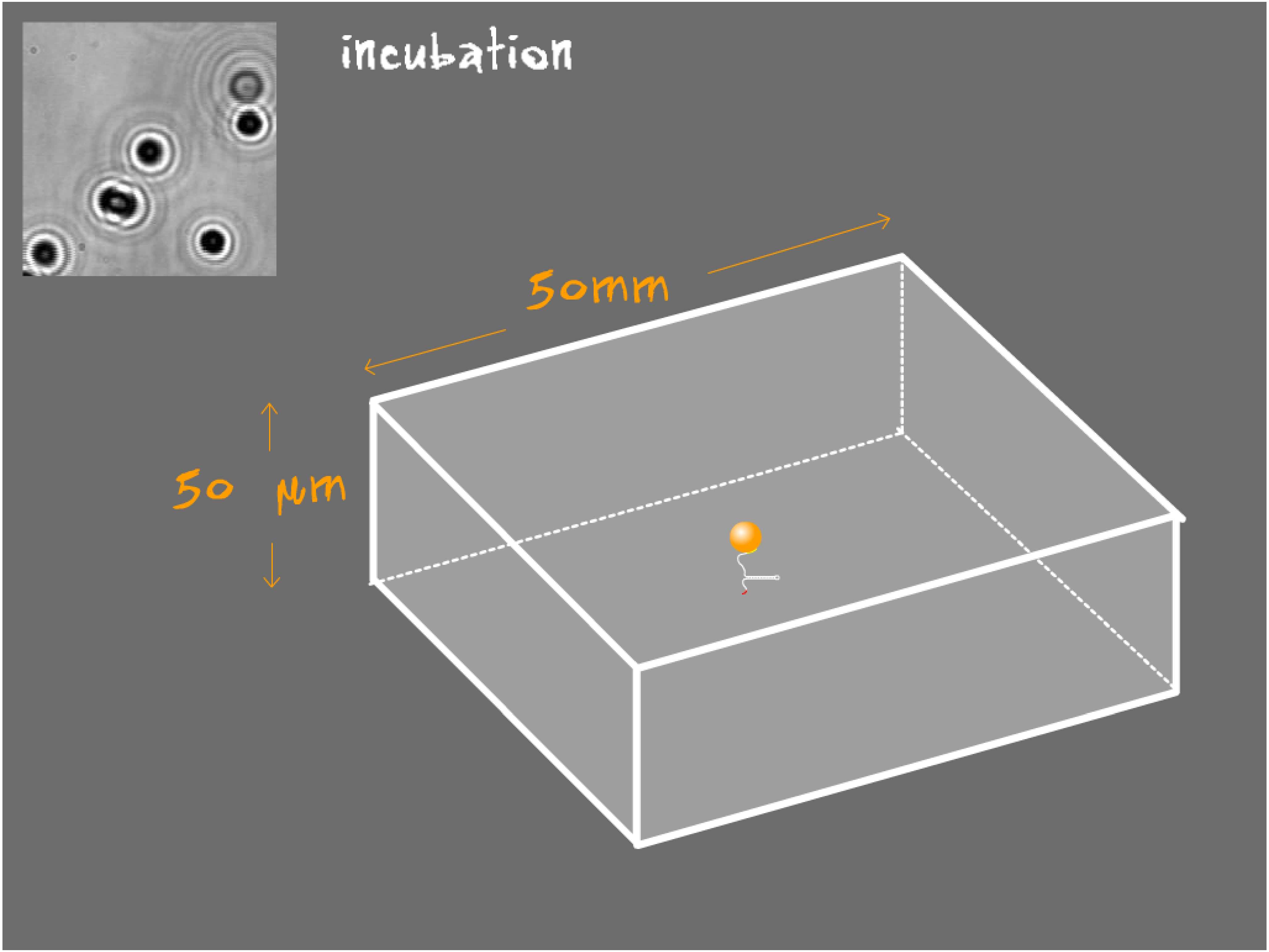
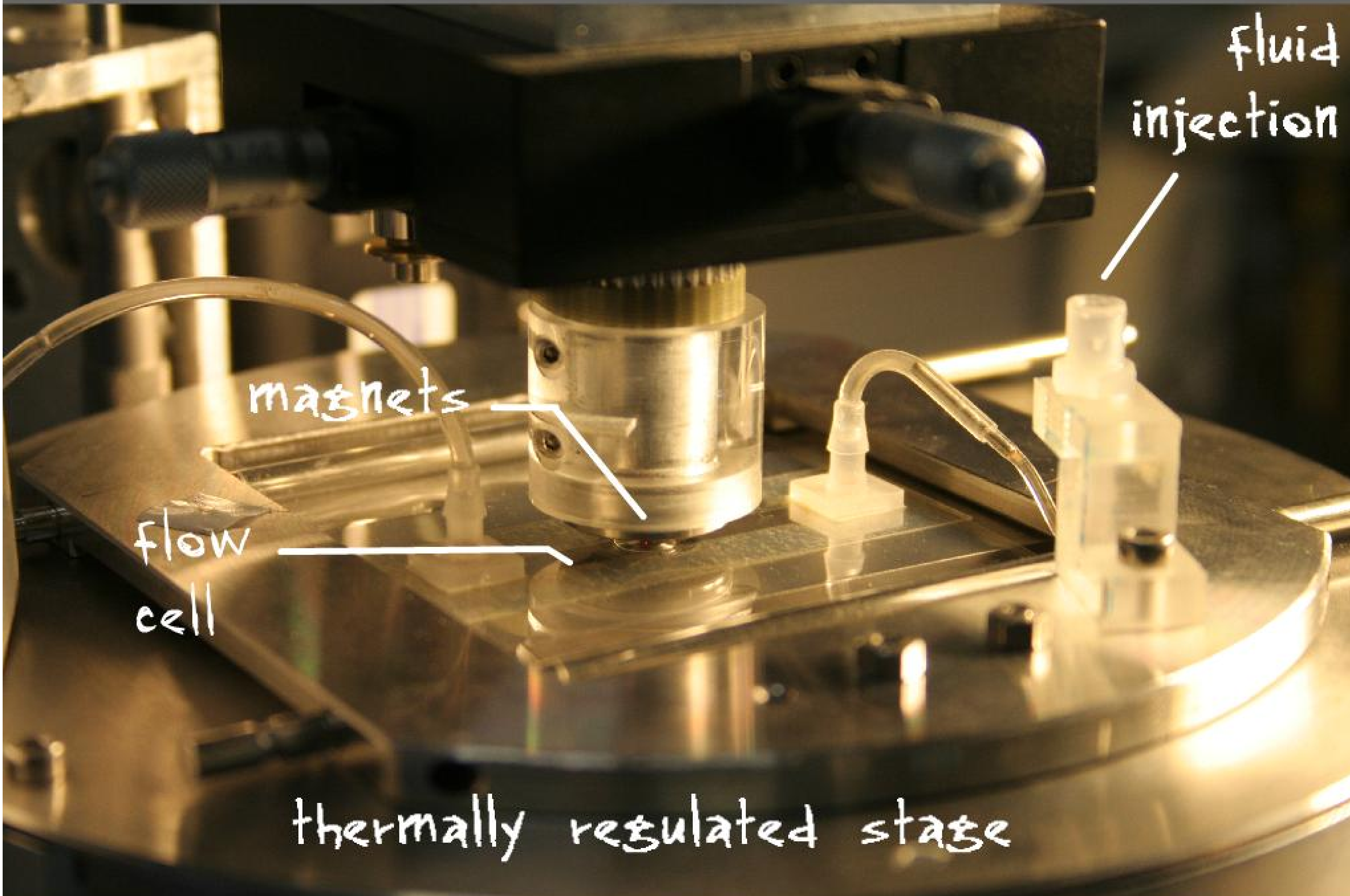
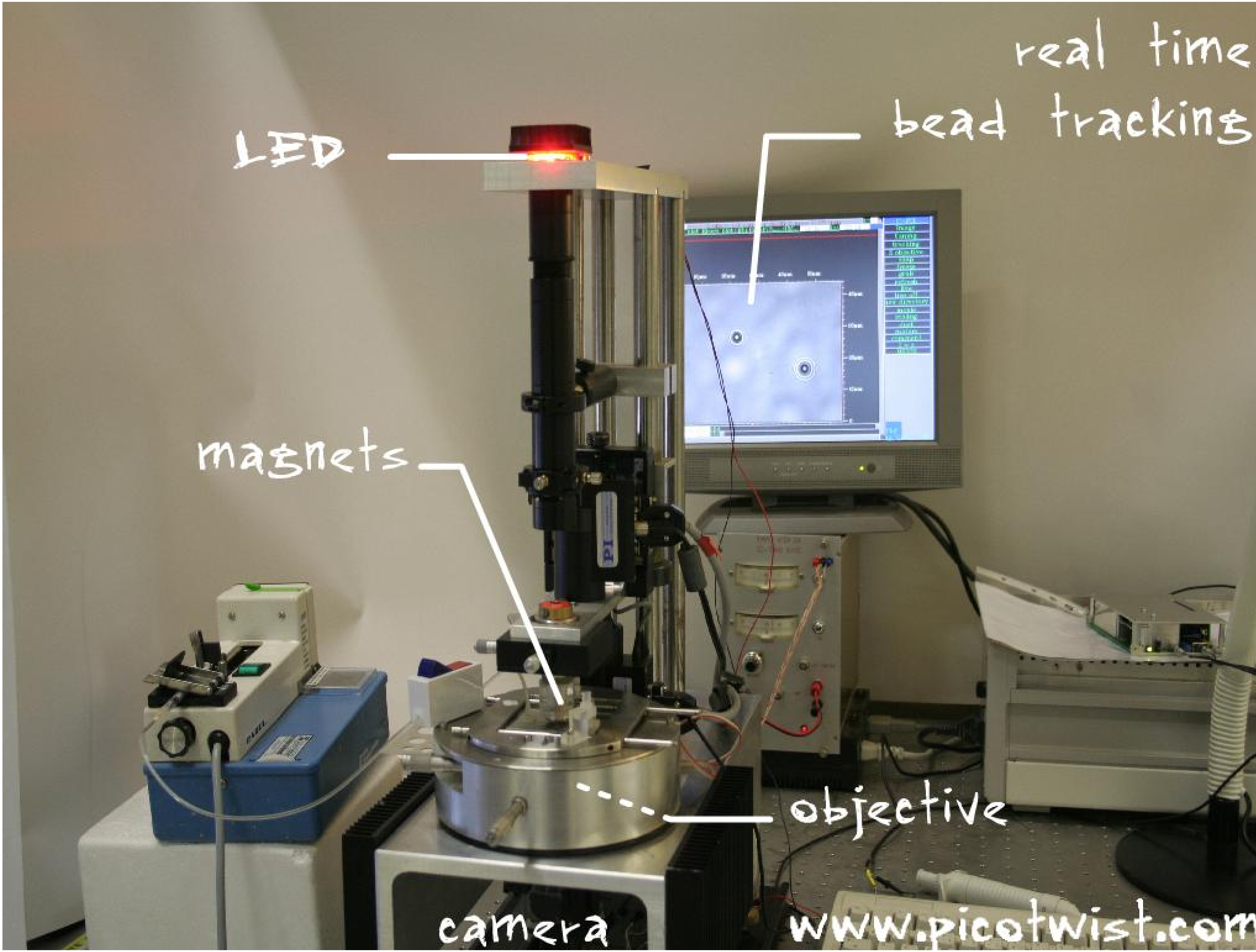
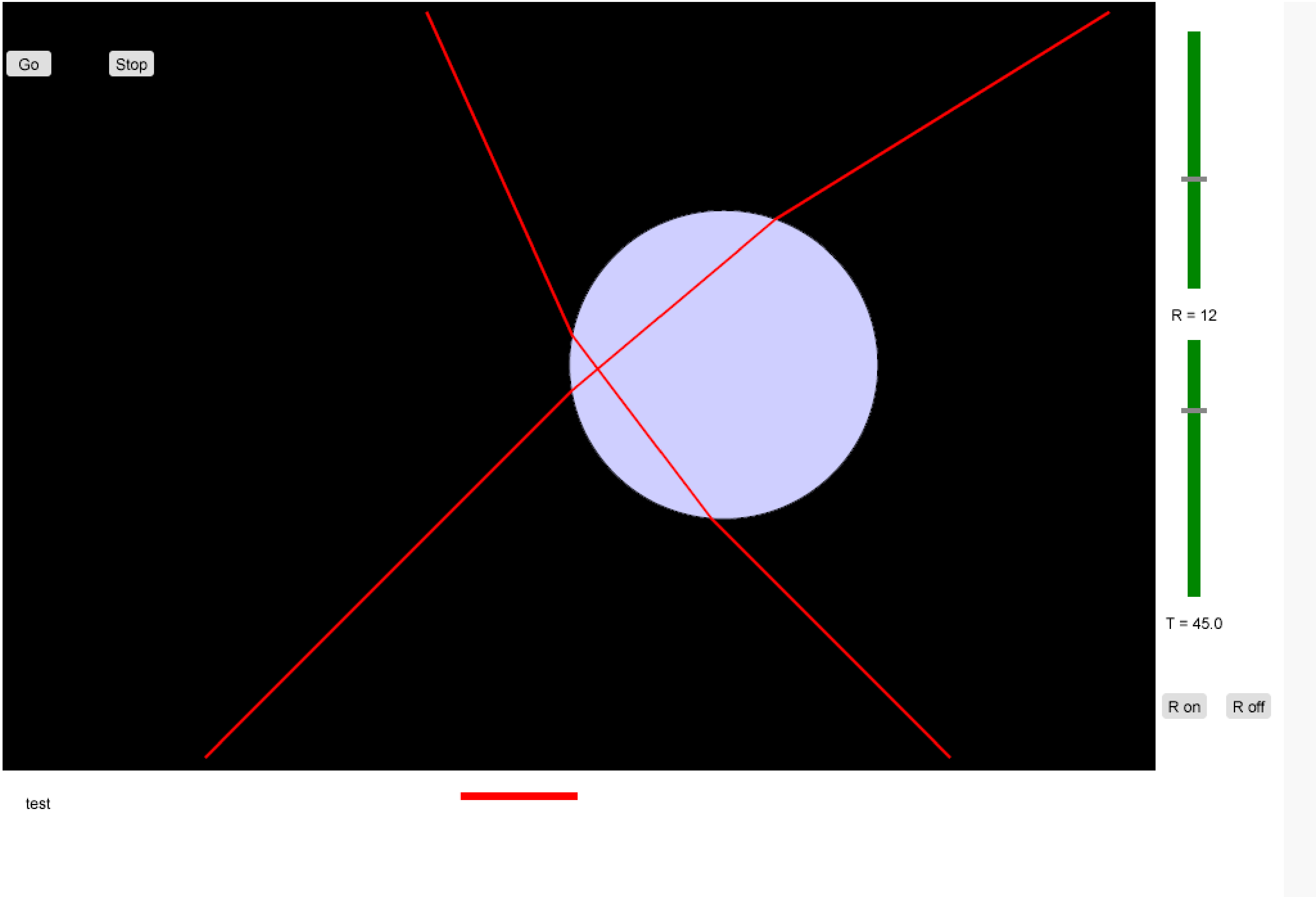

biotin
5'
410 nt
231 bp (6.7 kbp)
70 nt
multi
digoxygenin

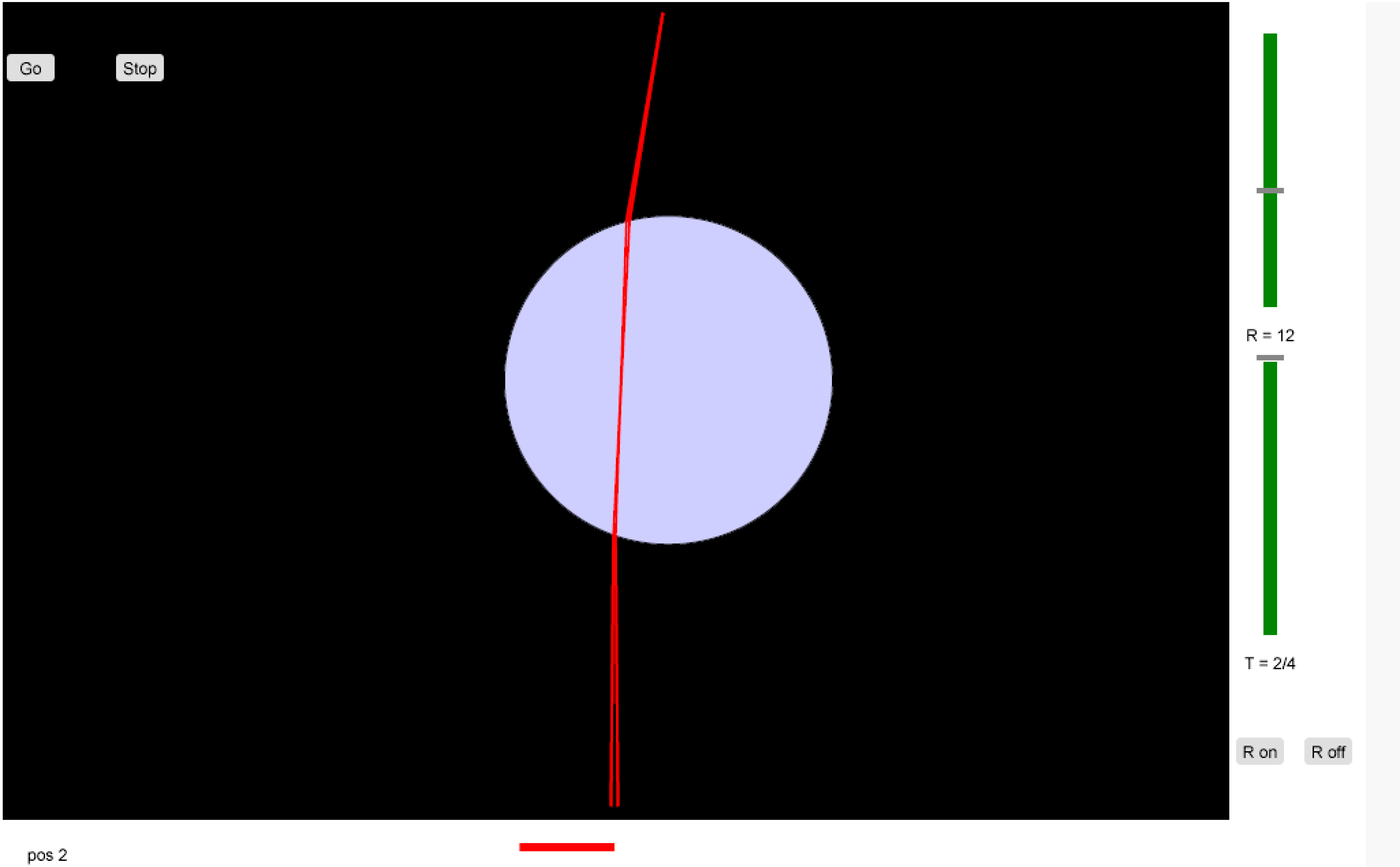

SA paramagnétic bead
1 µm diameter
biotin
5'
410 nt
231 bp (6.7 kbp)
70 nt
multi
digoxygenin
press "space"


incubation
50mm
50 µm


fluid
injection
magnets
flow
cell
thermally regulated stage


real time
bead tracking
LED
magnets
objective
camera
www.picotwist.com


Go
Stop
R = 12
T = 45.0
R on
R off
test


Go
Stop
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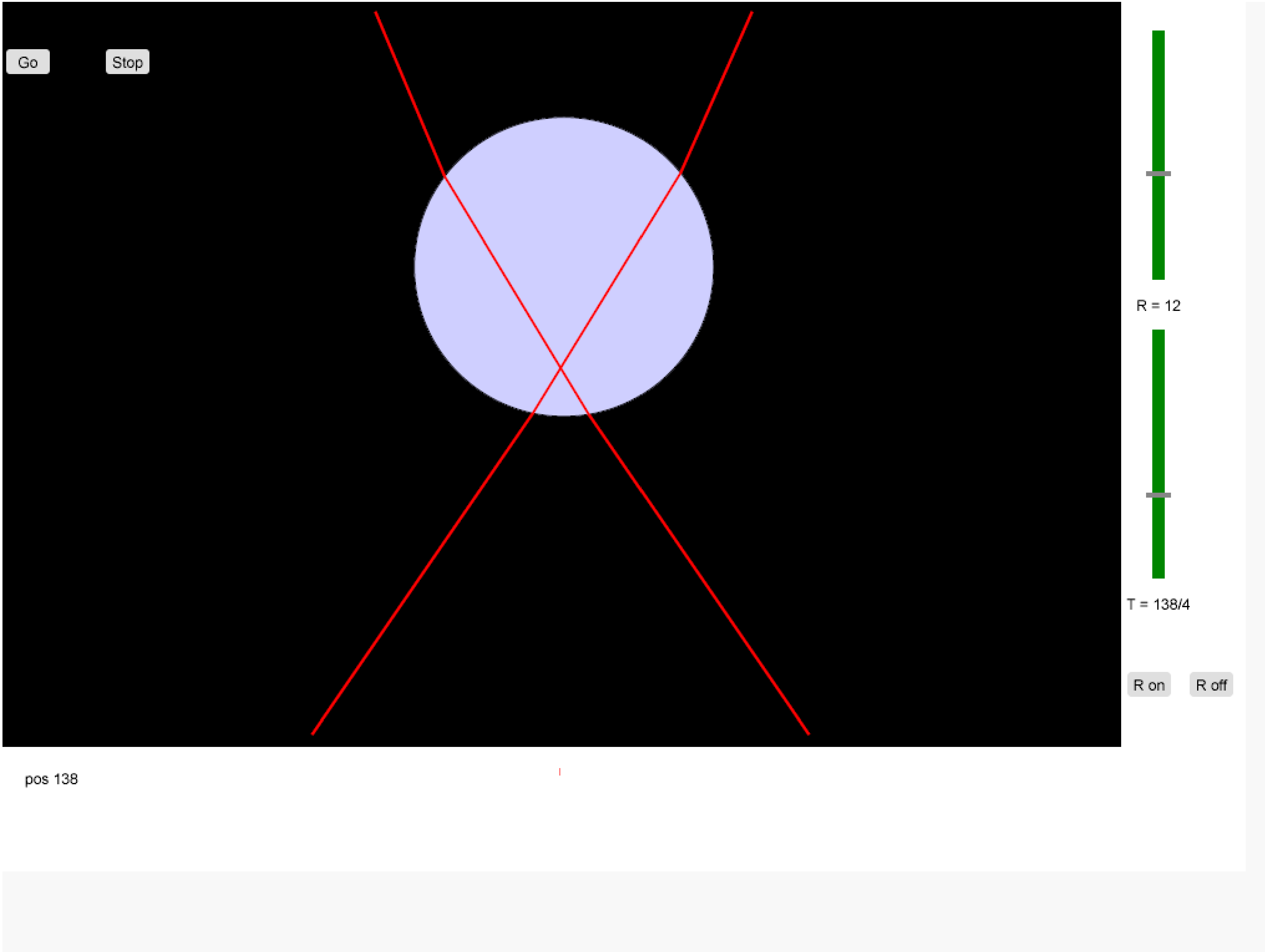
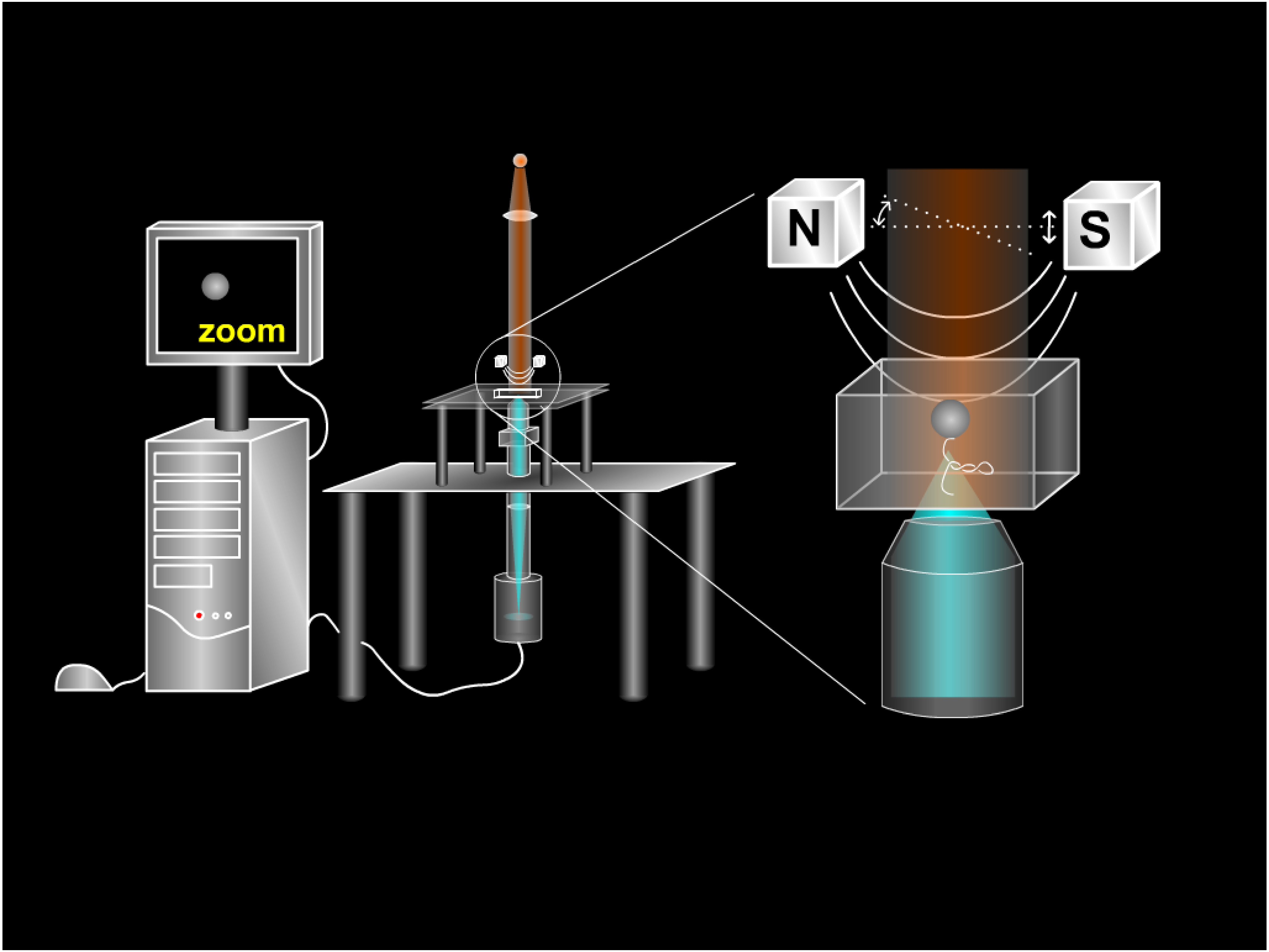
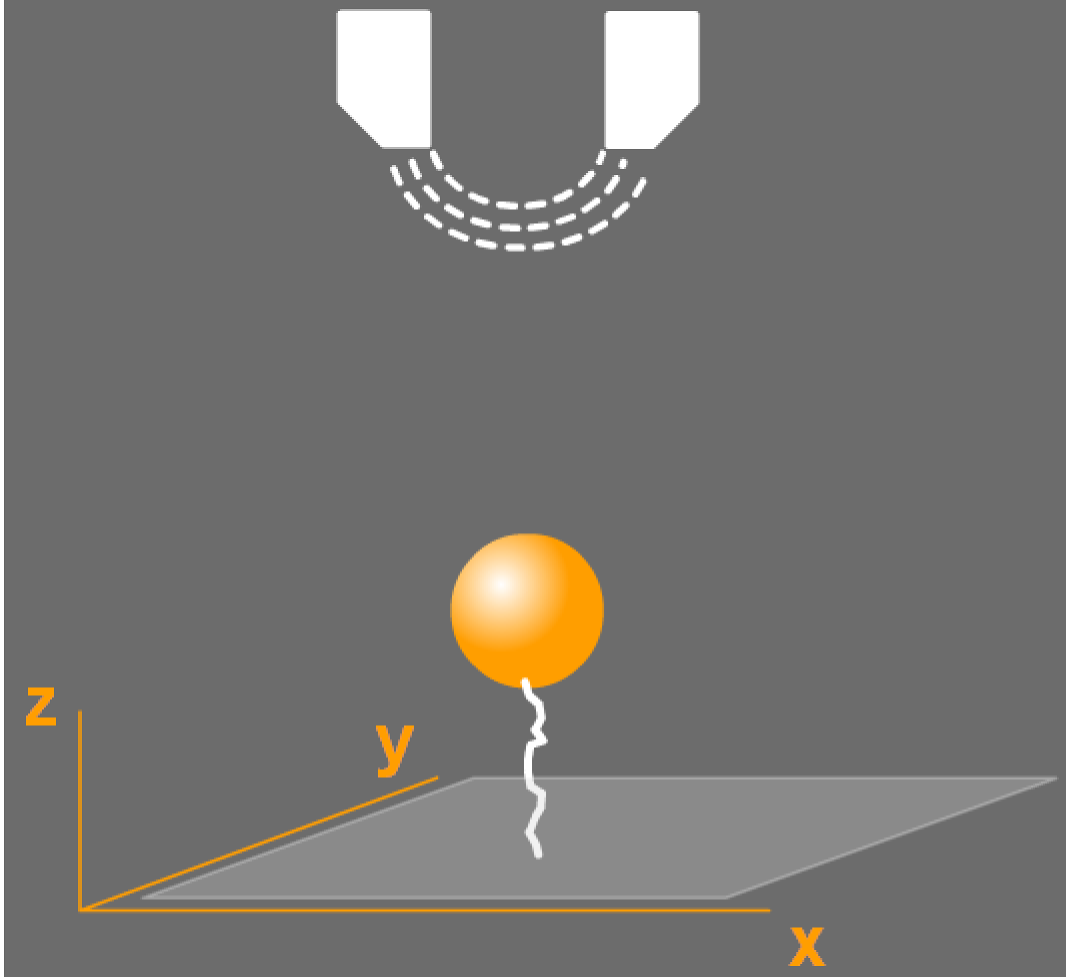
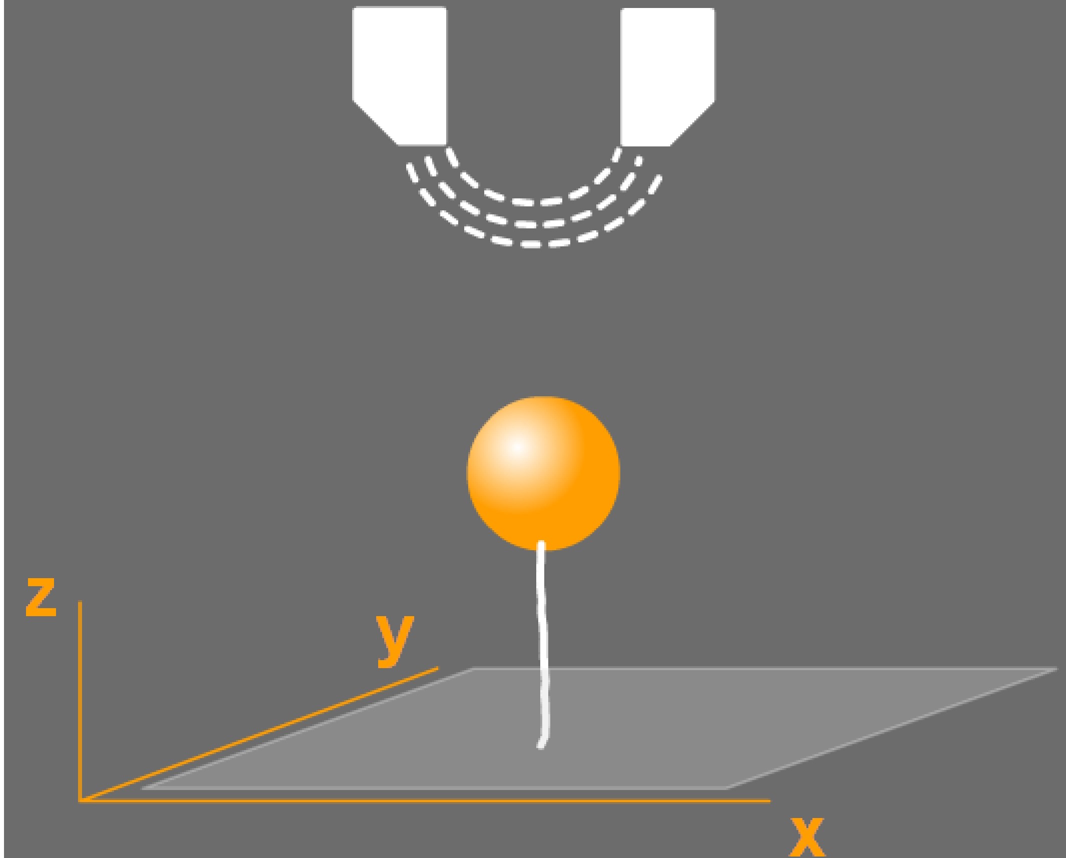
T = 2/4
R on
R off
pos 2


Go
Stop
R = 12
T = 138/4
R on
R off
pos 138

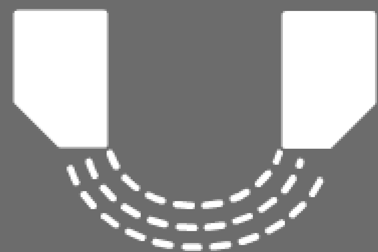

zoom
N
S

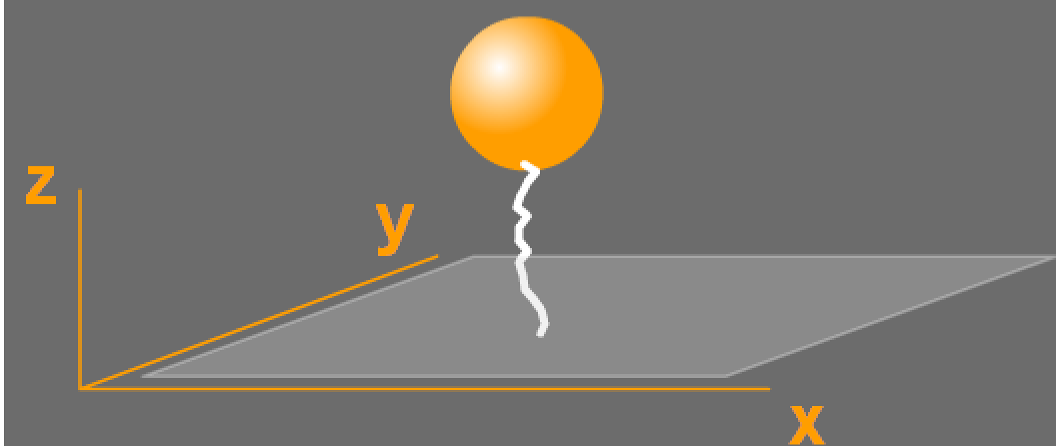


press "space"

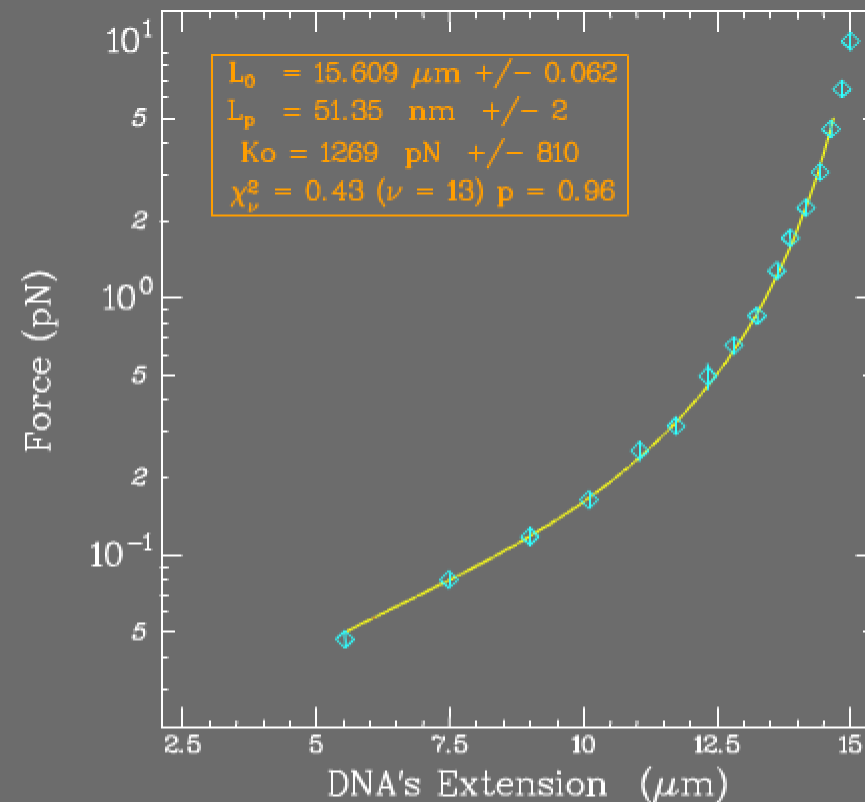


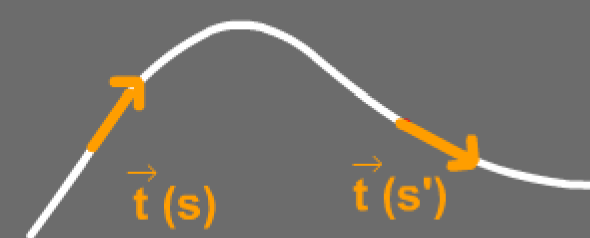
press "space"



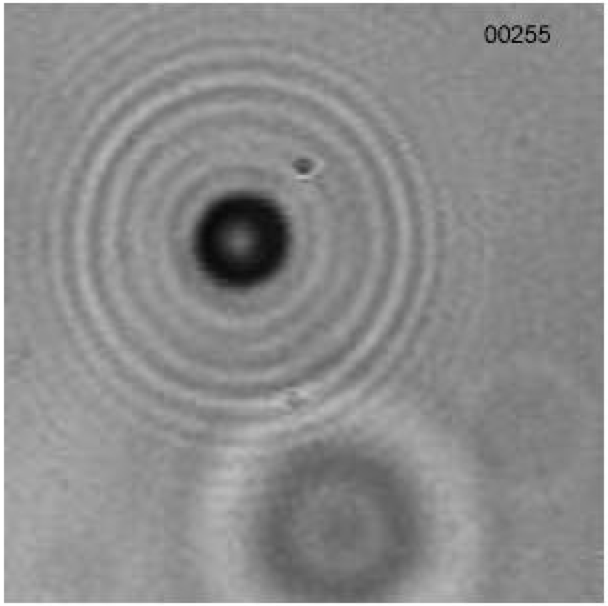


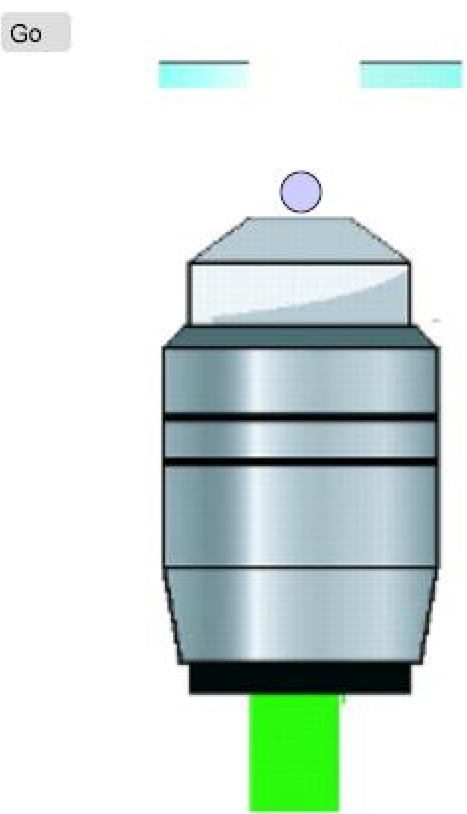
$$\frac{1}{2}\left(\frac{F}{\langle z \rangle}\right)\langle \delta x^2 \rangle = \frac{1}{2} k_b T$$

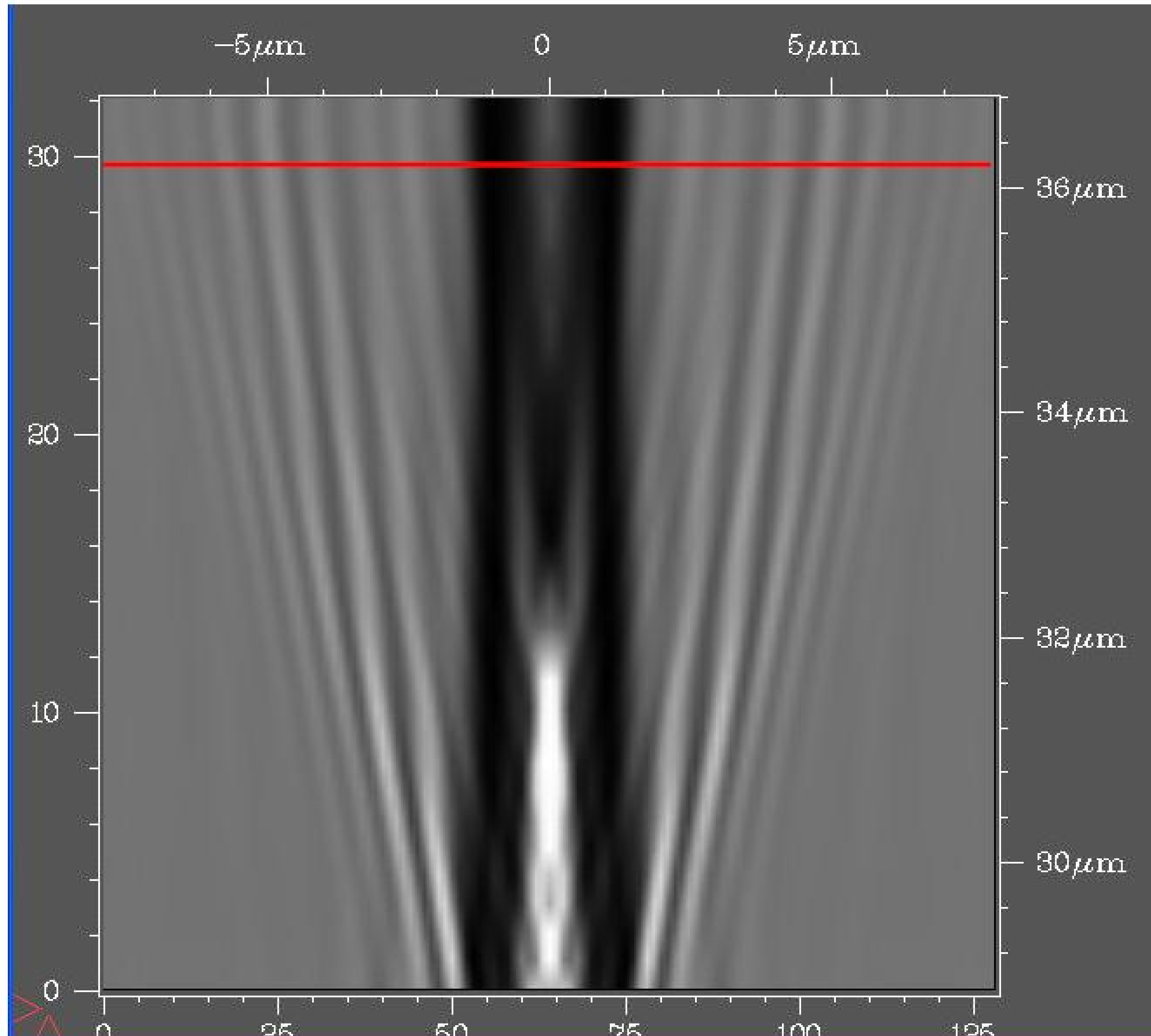


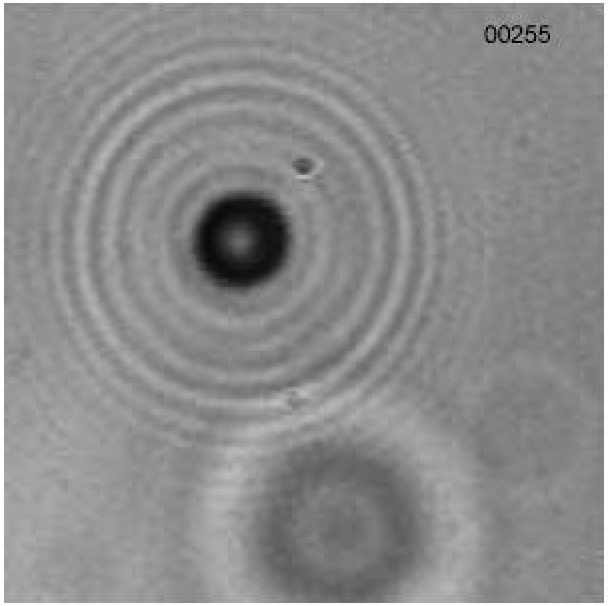


$$\langle \vec{t}(s) \cdot \vec{t}(s') \rangle = e^{-|s-s'|/L_p}$$


00255


Go


−5μm
0
5μm
30
20
10
0
36μm
34μm
32μm
30μm
0
25
50
75
100
125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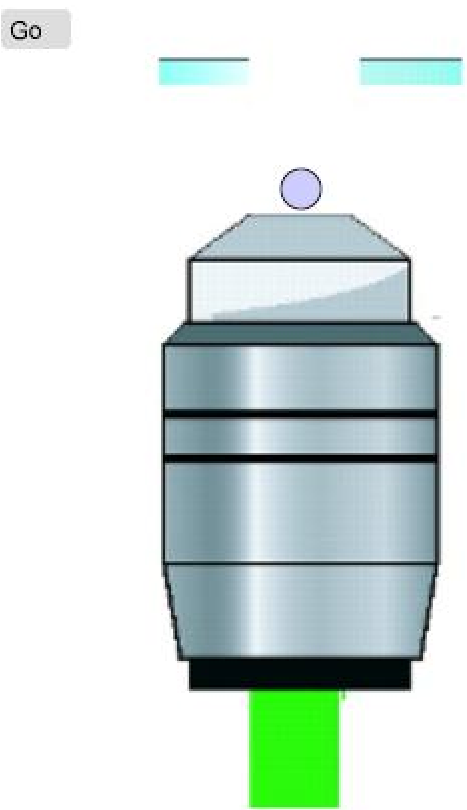
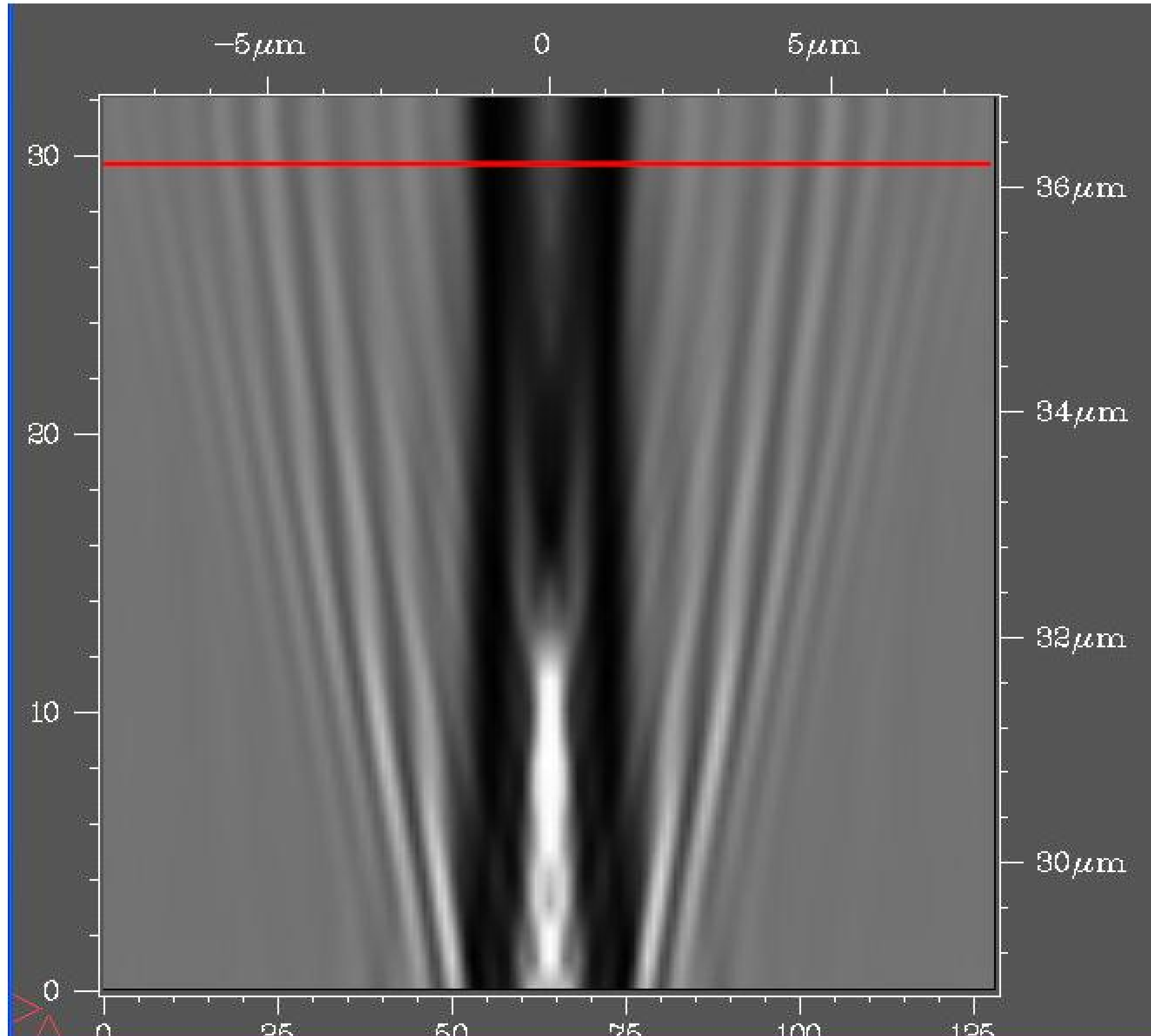
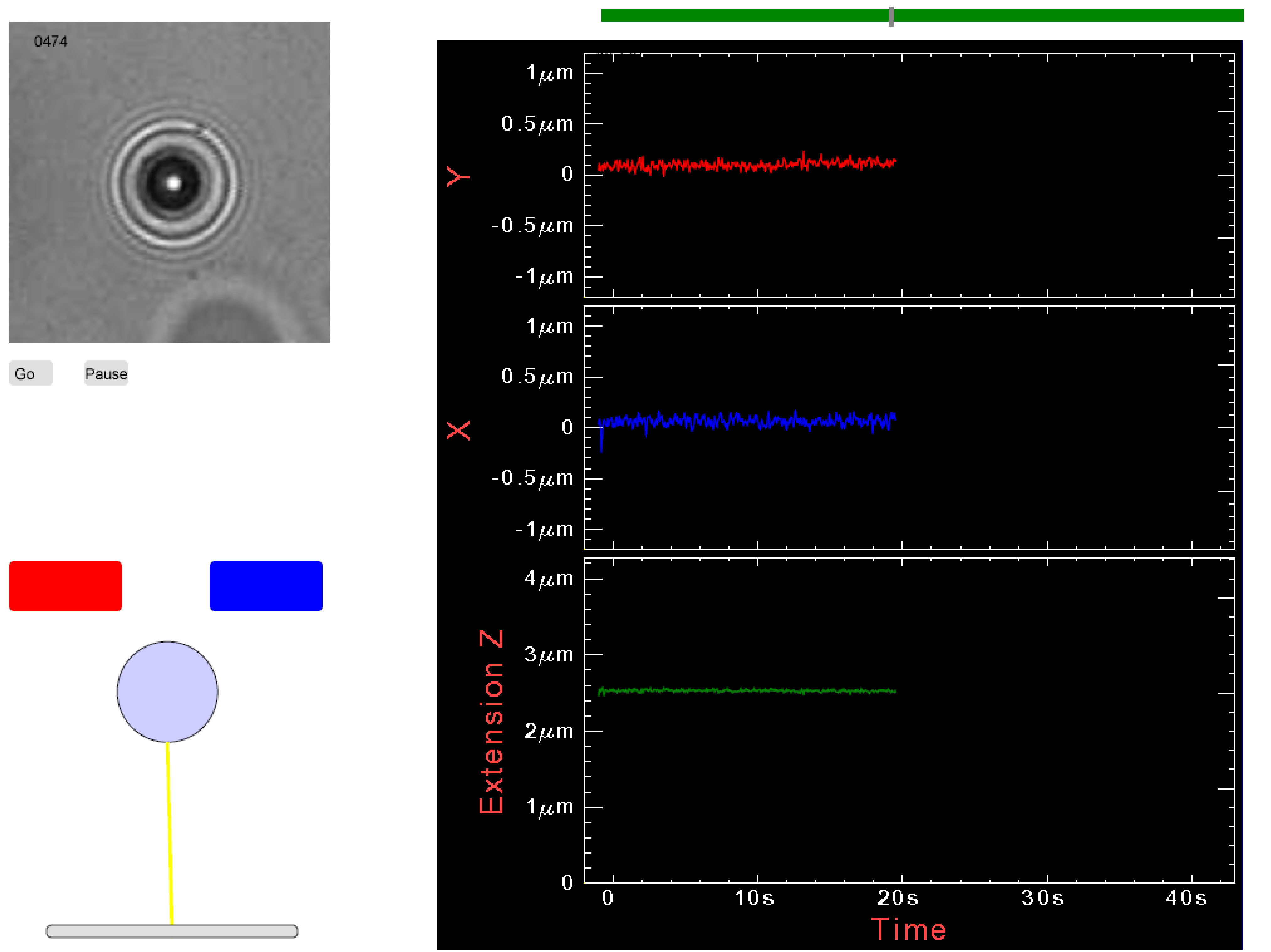
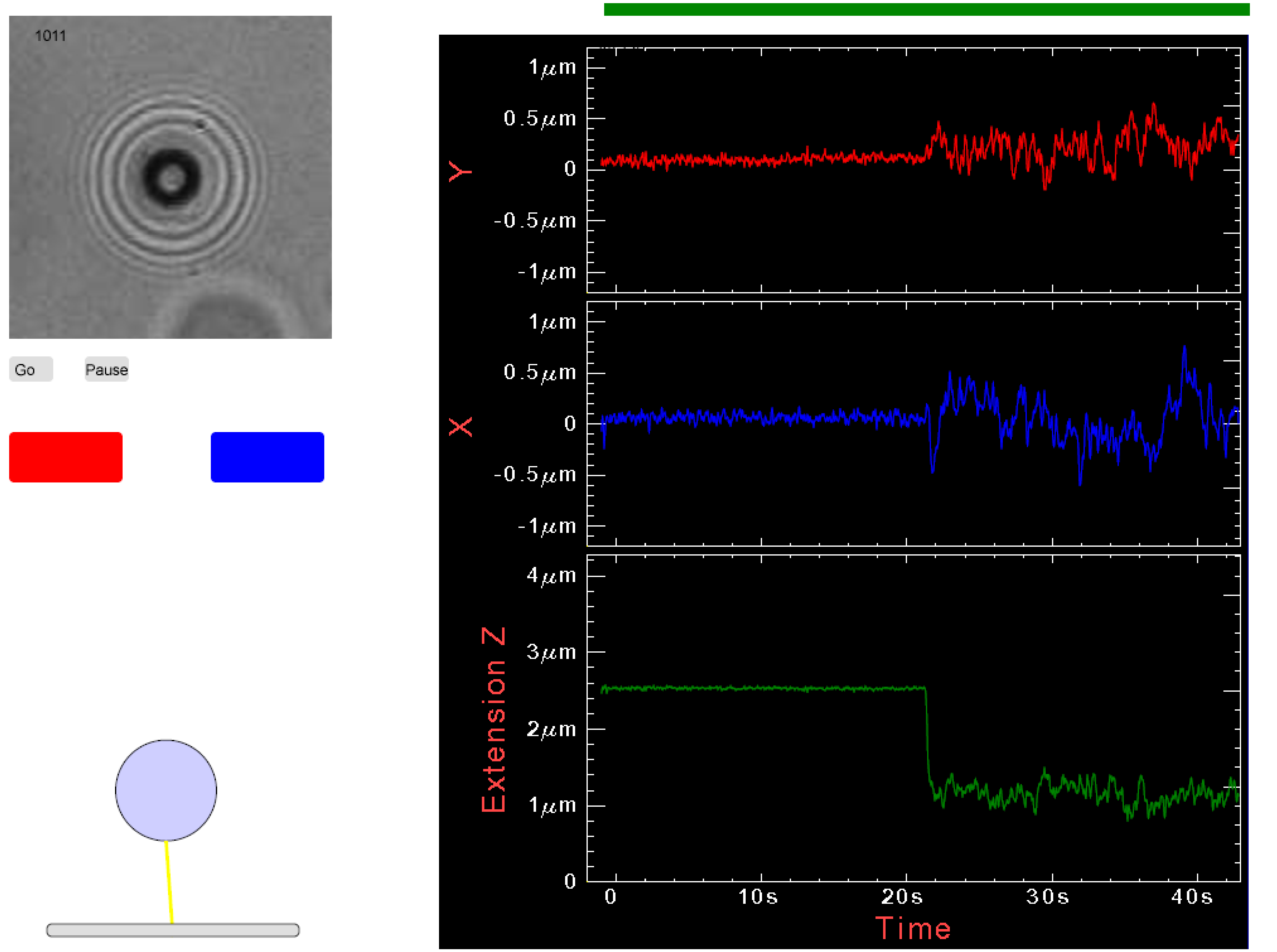

Go


−5μm
0
5μm
30
20
10
0
36μm
34μm
32μm
30μm
0
25
50
75
100
125

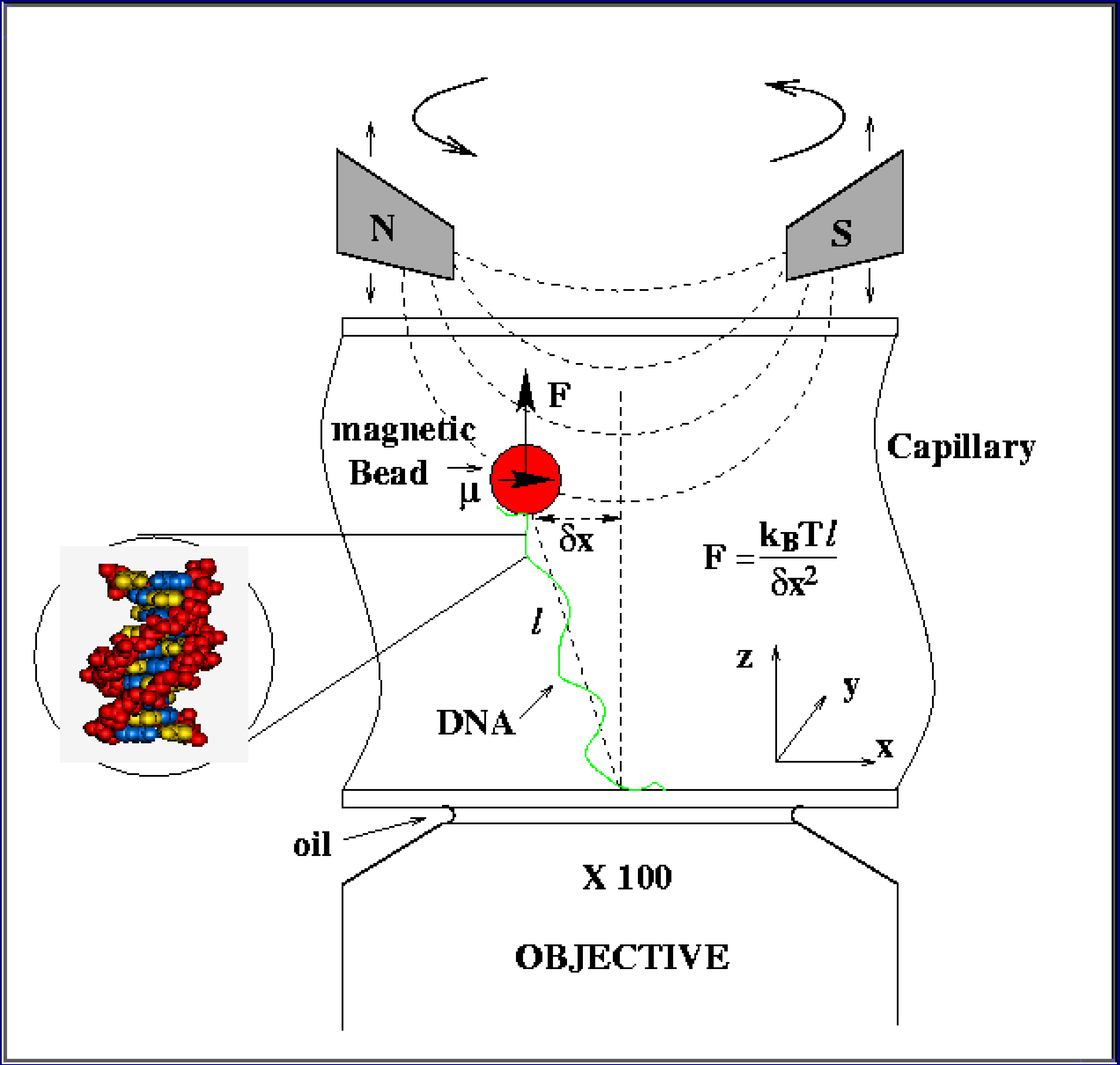


0474
Go
Pause
1μm
0.5μm
0
-0.5μm
-1μm
Y
1μm
0.5μm
0
-0.5μm
-1μm
X
4μm
3μm
2μm
1μm
0
Extension Z
0
10s
20s
30s
40s
Time



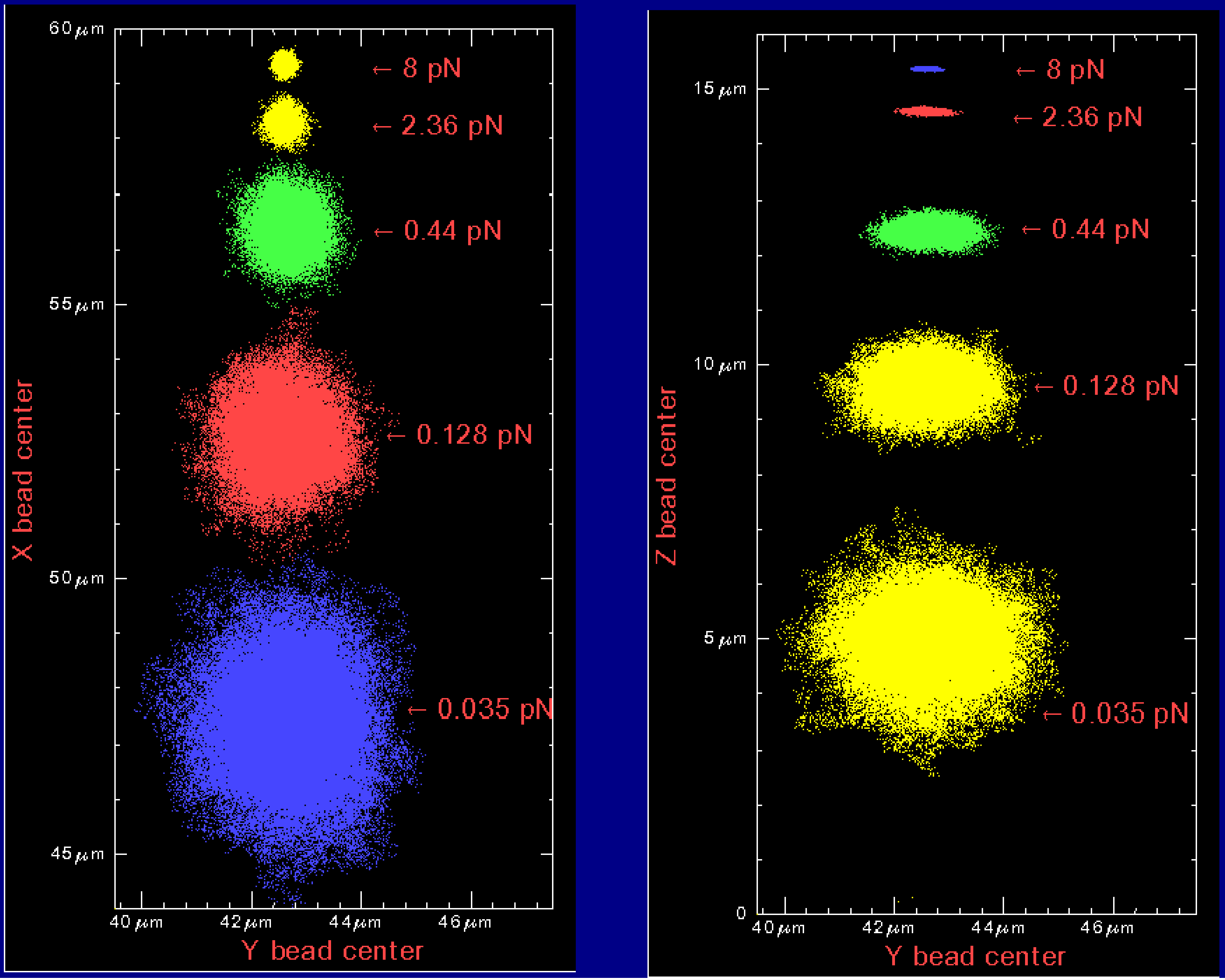
1011
Go
Pause
1μm
0.5μm
0
-0.5μm
-1μm
Y
X
Extension Z
4μm
3μm
2μm
1μm
0
10s
20s
30s
40s
Time

Force Measurement using Brownian motion

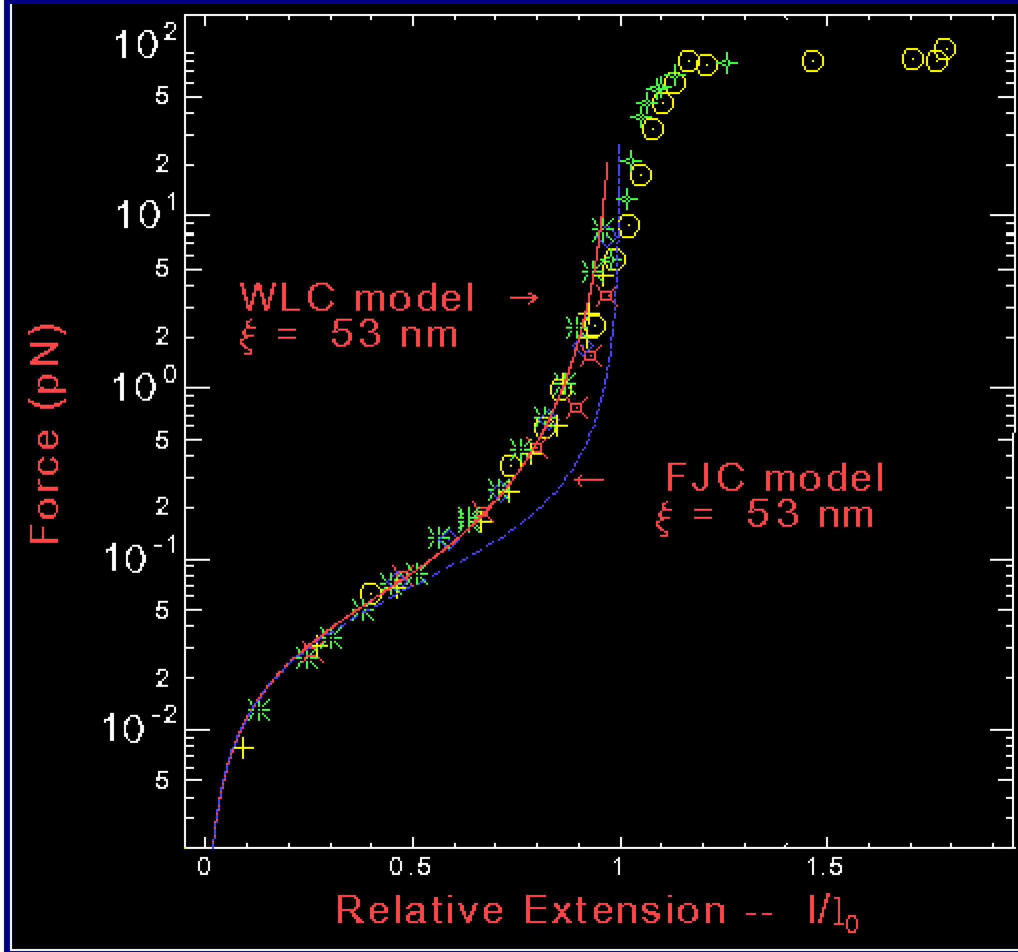


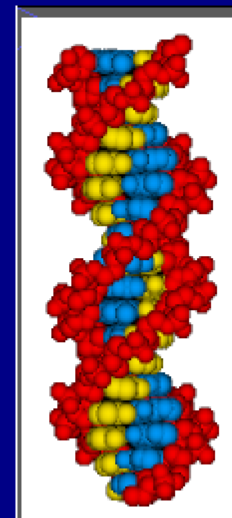
Strick T., Allemand J.F., Bensimon D., Bensimon A., et Croquette V., (1996). Science, 271; 1835 --1837. Strick T., Allemand J.-F., Bensimon D. et Croquette V,(1998). Biophys. J., 74; 2016--2028.

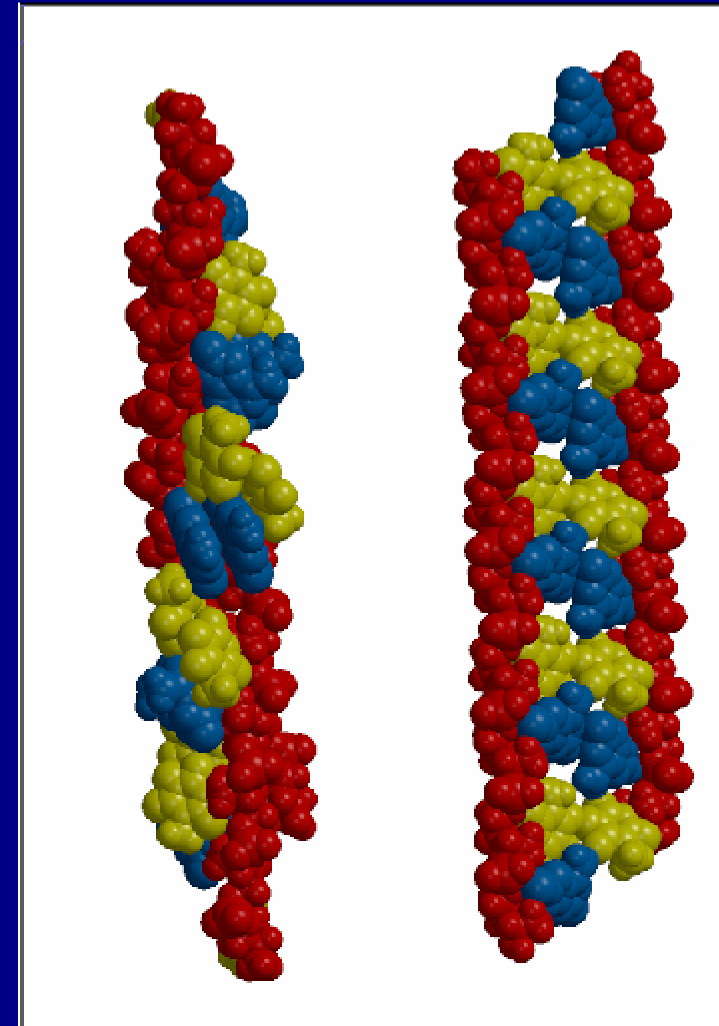
Brownian motion of a bead in x,y,z

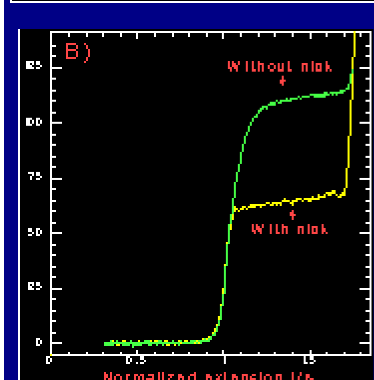


Structure du S-DNA



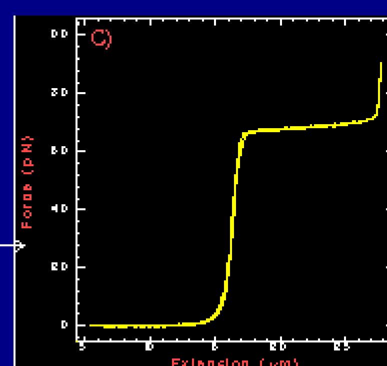




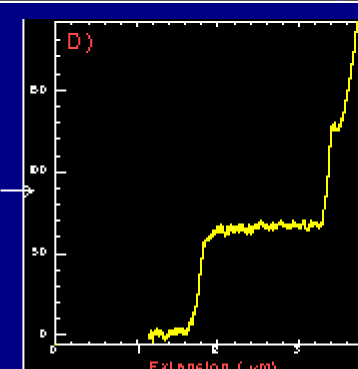


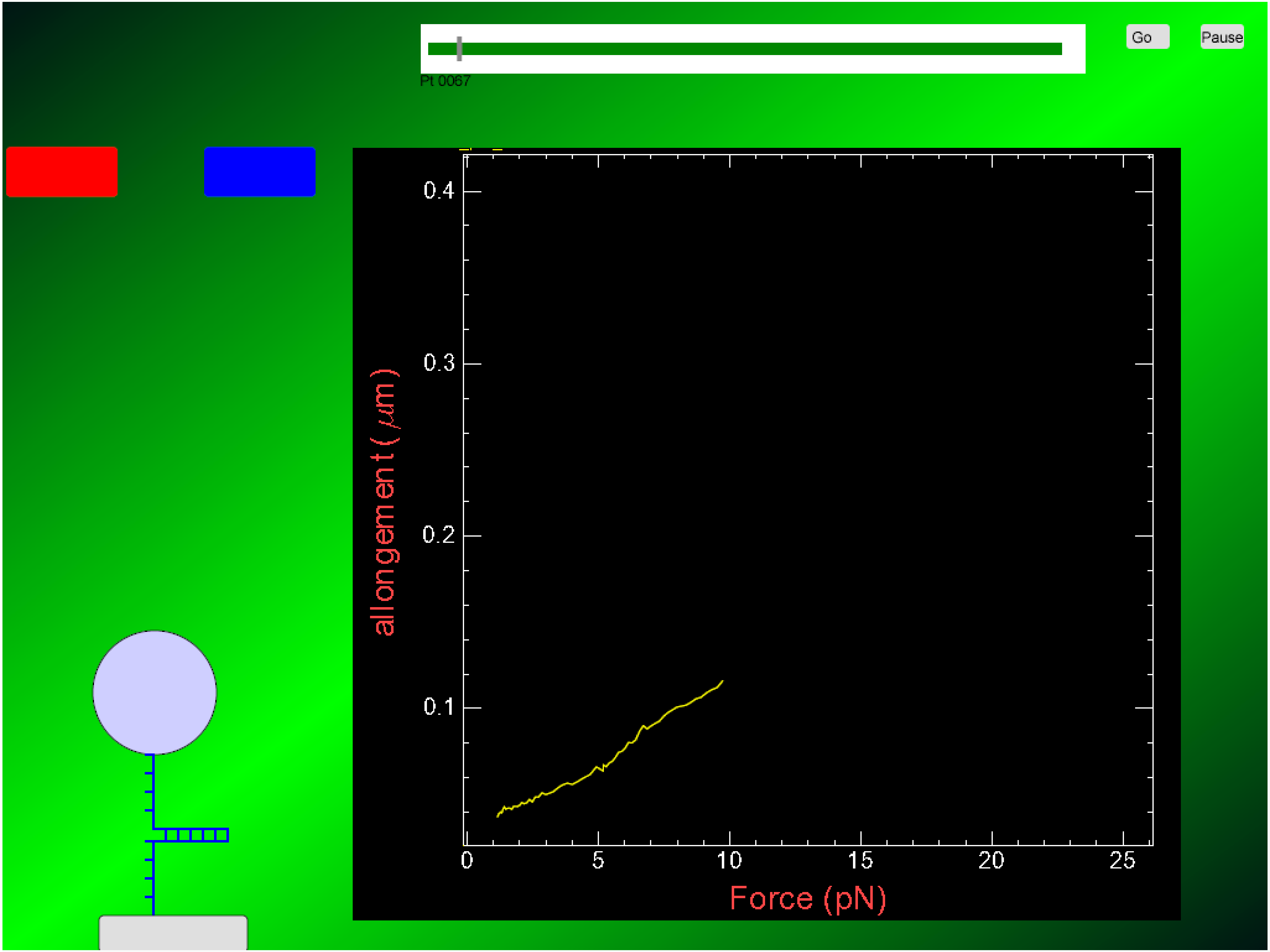
← D. Chatenay
J-F. Léger

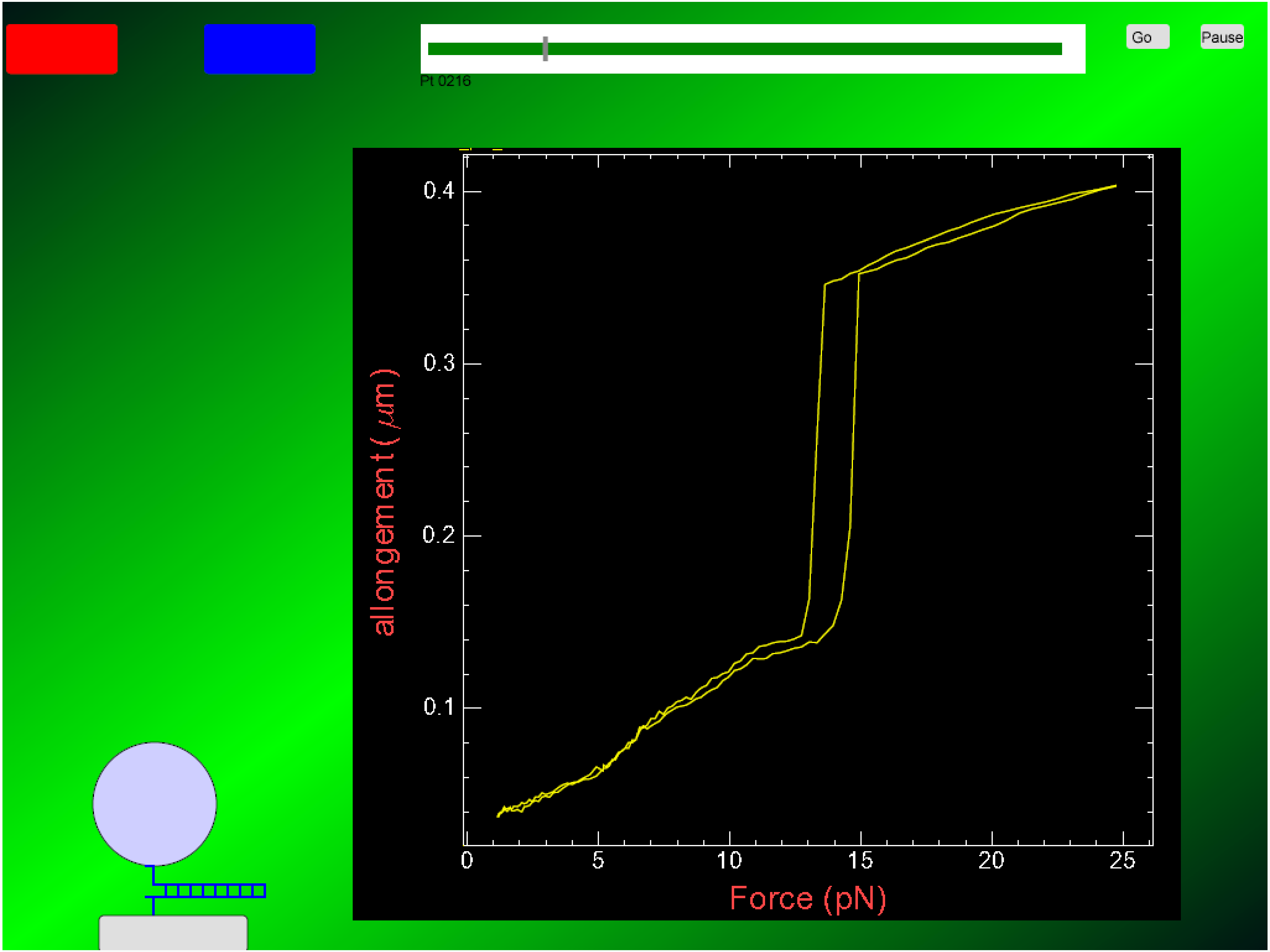
C. Bustamante
S. Smith
et al →



H. Gaub
et al →

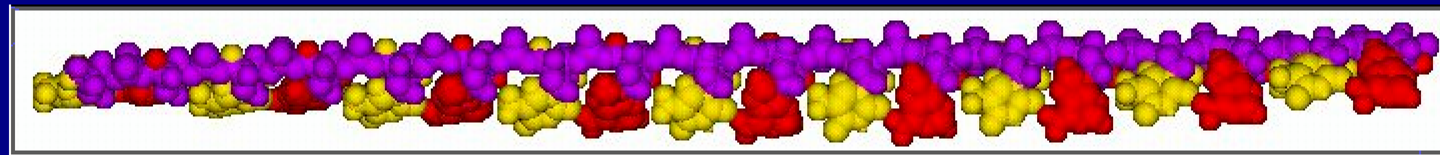



Go
Pause
Pt 0067
0.4
0.3
0.2
0.1
allongement (μm)
0
5
10
15
20
25
Force (pN)

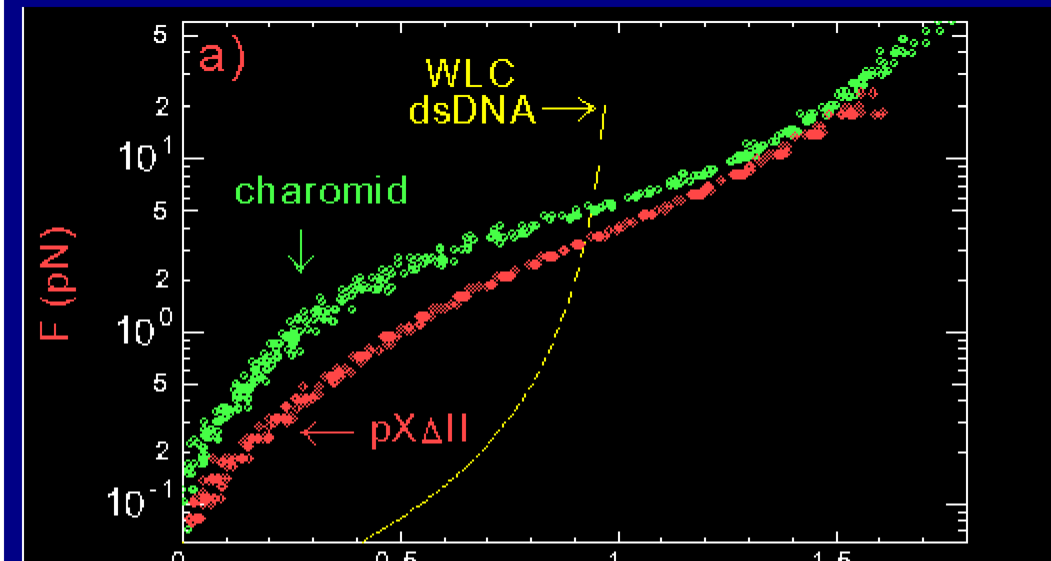


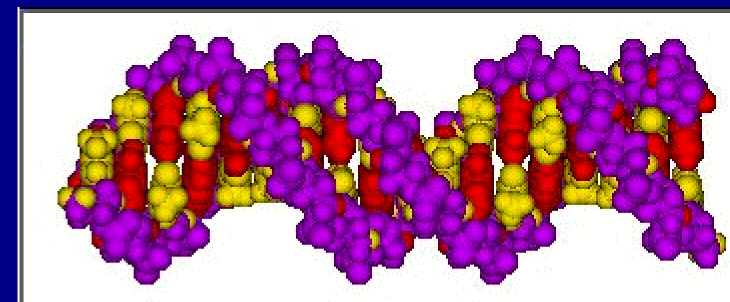
Go
Pause
Pt 0216
allongement (μm)
Force (pN)
0.4
0.3
0.2
0.1
0
5
10
15
20
25

Elasticité d'une molécule d'ADN simple brin



ADN simple brin (18b)





ADN-B (18bp) R. Lavery