ATOMIC FORCE MICROSCOPY COMBINED WITH OPTICAL TWEEZERS (AFM/OT): FROM DESIGN TO APPLICATIONS

Filippo Pierini

Institute of Fundamental Technological Research, Polish Academy of Sciences, Warsaw, Poland

Atomic Force Microscopy (AFM)

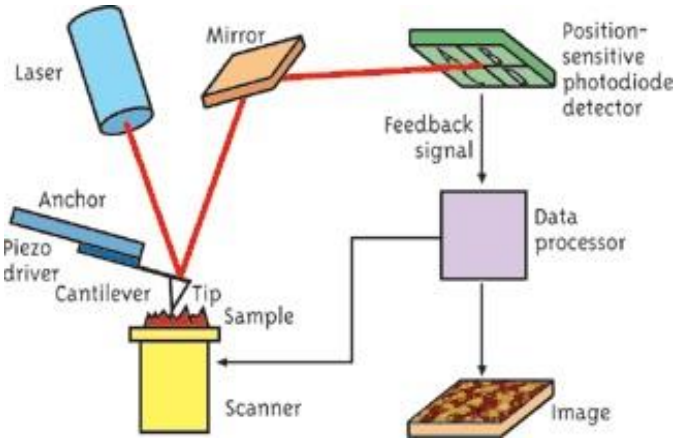
Motivation

3-D Surface Topography

History

1986 – Binnig, Quate, Gerber
1989 – the first commercially available AFM

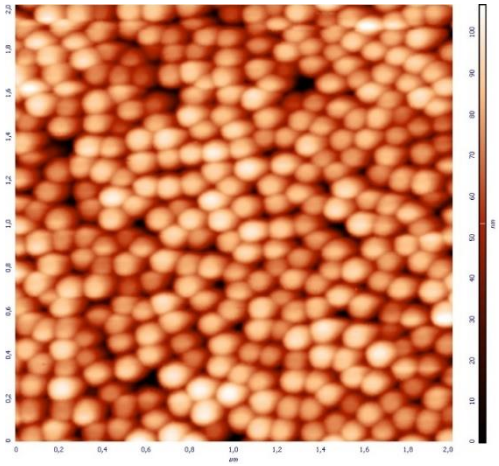
How the AFM Works



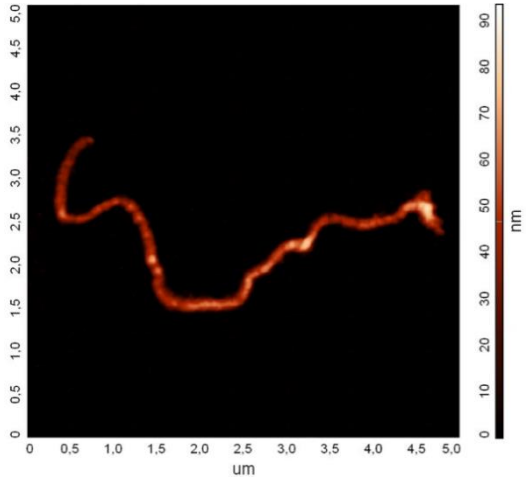
- Contact Mode
  - High resolution
  - Damage to sample
- Non-Contact Mode
  - Lower resolution
  - No damage to sample
- Tapping Mode
  - High resolution
  - Minimal damage to sample

Atomic Force Microscopy (AFM)

Results



Polystyrene nanoparticles
(diameter: 100 nm)



Hydrogel nanofilament
(contour length 7 µm, width 128 nm, height 39 nm)

Advantages

- Easy sample preparation
- Accurate height information (sub-nanometer resolution)
- Works in vacuum, air, and liquids
- Living systems can be studied

Disadvantages

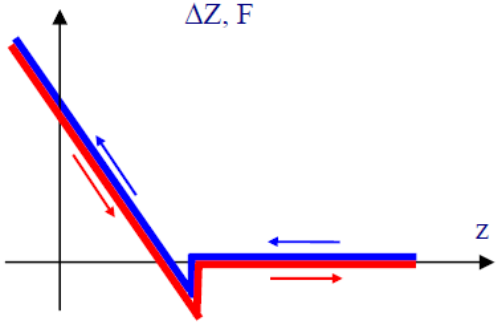
- Limited vertical range
- Limited magnification range
- Data not independent of tip
- Tip or sample can be damaged

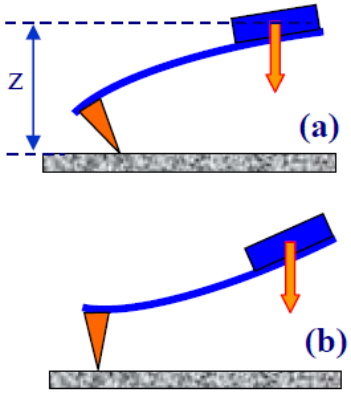
Atomic Force Microscopy (AFM)

Motivation

Forces evaluation
Sample manipulation

Force-distance curves





interaction forces between tip and sample are recorded

Applications

Study Unfolding Of Proteins
Force Measurements In Real Solvent Environments
Antibody-Antigen Binding Studies
Ligand-Receptor Binding Studies
Binding Forces Of Complimentary DNA Strands
Study Surface Frictional Forces

Disadvantages

- Force evaluation:
  inadeguate LOD (pN scale)
- Sample manipulation:
  invasive method
  lack of a feedback system

Optical Tweezers (OT)

History

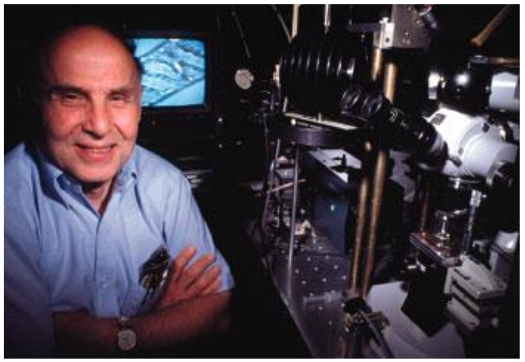
Laser: "Stimulated Optical Radiation in Ruby"
T. Maiman, Nature 187, 493 – 494 ,1960

Nanotechnology:"There's plenty of room at the bottom"
R.Feynman, 1959

- In 1970 A. Ashkin proved that light can grab and release nanometer particles by its momentum

- In 1986, A. Ashkin trap 10nm diameter particles

- In 1987, A. Ashkin showed the damage-free manipulation on cell using an infrared laser



Arthur Ashkin

- In 1997, S. Chu won the Nobel Prize in Physics for the "development of methods to cool and trap atoms with laser light"

Optical Tweezers (OT)

What are Optical Tweezers?

Optical tweezers can trap and manipulate nanometer and micrometer-sized

Optical Tweezers - one of the techniques, which use a highly focused beam to control and hold microscopic particles.

In Optical Tweezers a tightly focused laser produces a force great enough to trap particles.

Optical trap: the most versatile single-molecule manipulation technique

Used to exert forces on particles ranging in size from nanometers to micrometers

Measuring the three-dimensional displacement of the trapped particle with sub-nanometer accuracy and sub-millisecond time resolution

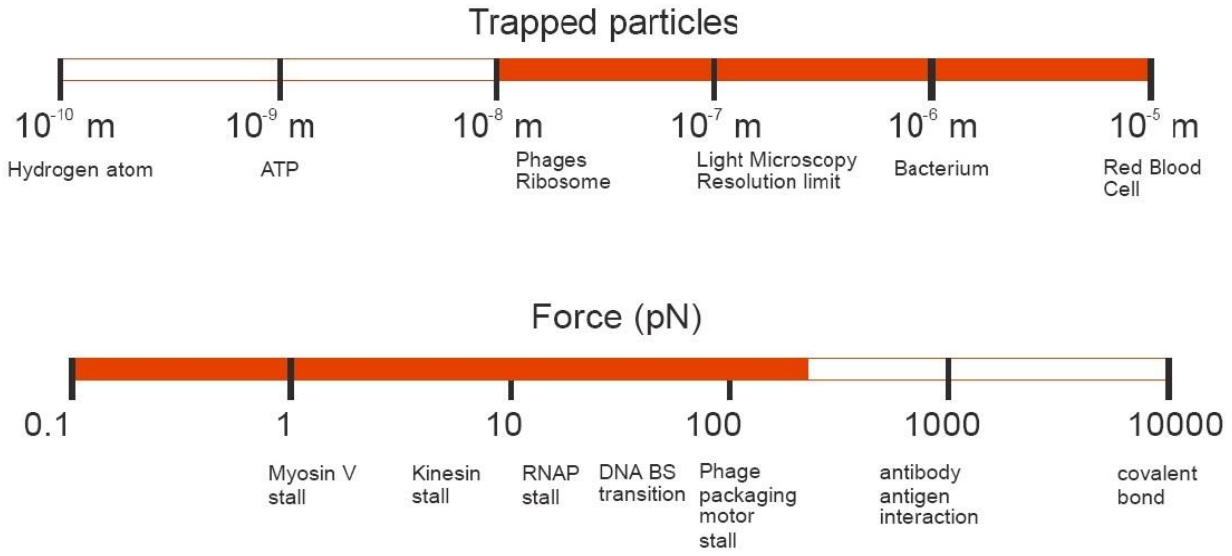
Suitable for measuring force and motion

Optical Tweezers (OT)

Describtion

A laser beam is expanded and collimated. This collimated beam is directed through a microscope objective into channel. Spheres with a higher index of refraction than the medium in will be trapped at the focus of the beam.

The scales of measurments

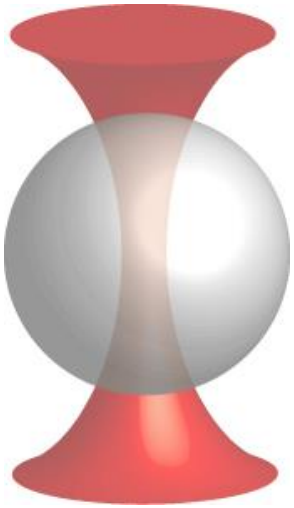


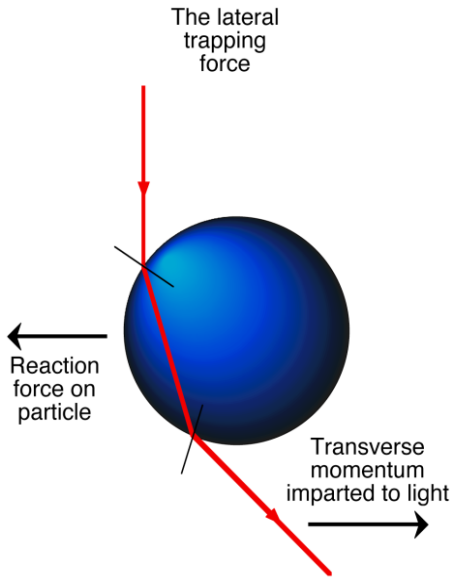
Optical Tweezers (OT)

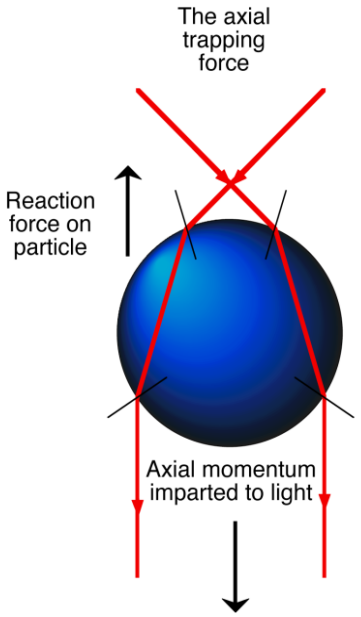
Conditions of OT – r >λ

Conditions for Mie scattering when the particle radius a is larger than the wavelength of the light λ.

We can use a ray optics treatment and look at the transfer of momentum

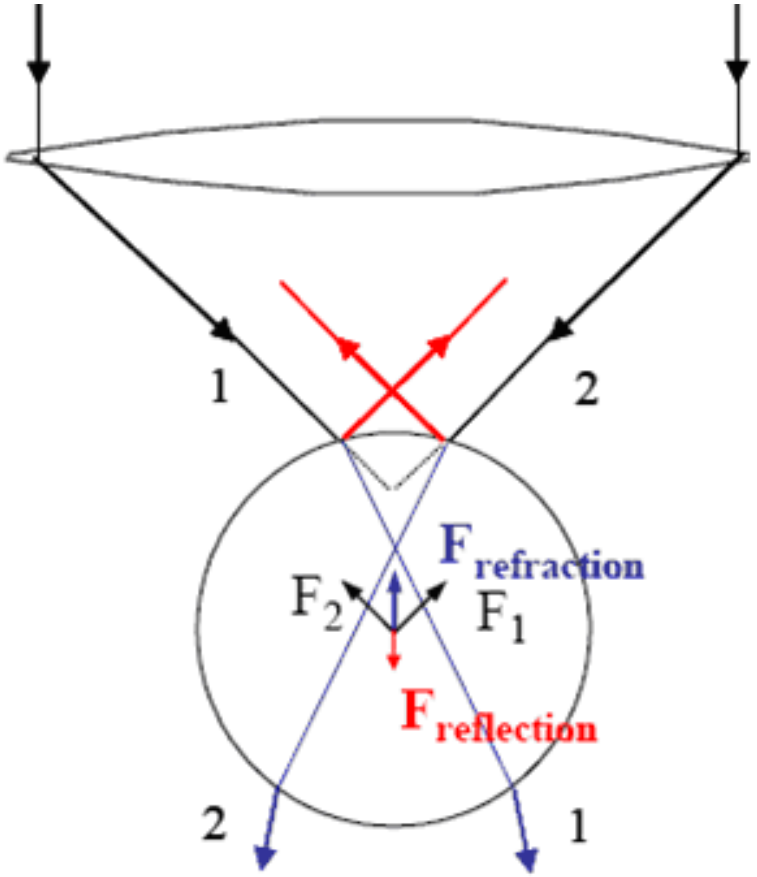






Optical Tweezers (OT)

The Ray Optics Approach



A) The reflected photons create a scattering force.

B) The refracted photons create a restoring force towards the focus of the beam.

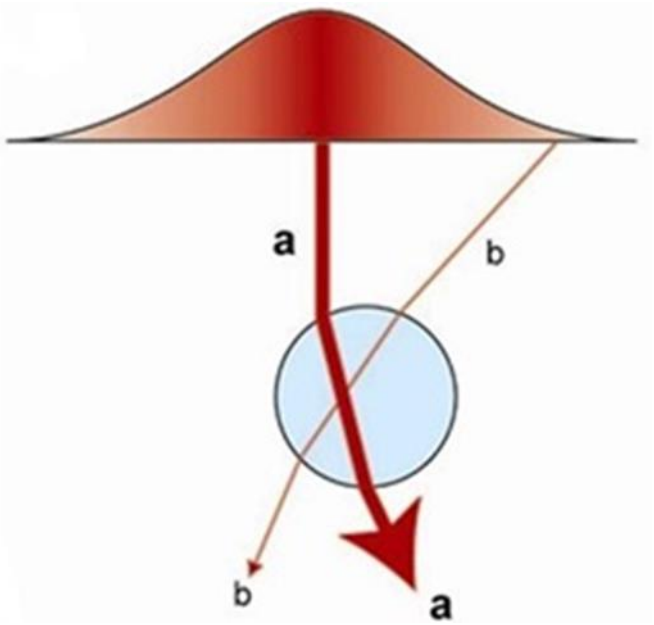
$p = h/\lambda$

$F = dp/dt$

Optical Tweezers (OT)

Bead moves to left or right

Newton's third law – for every action there is an equal and opposite react

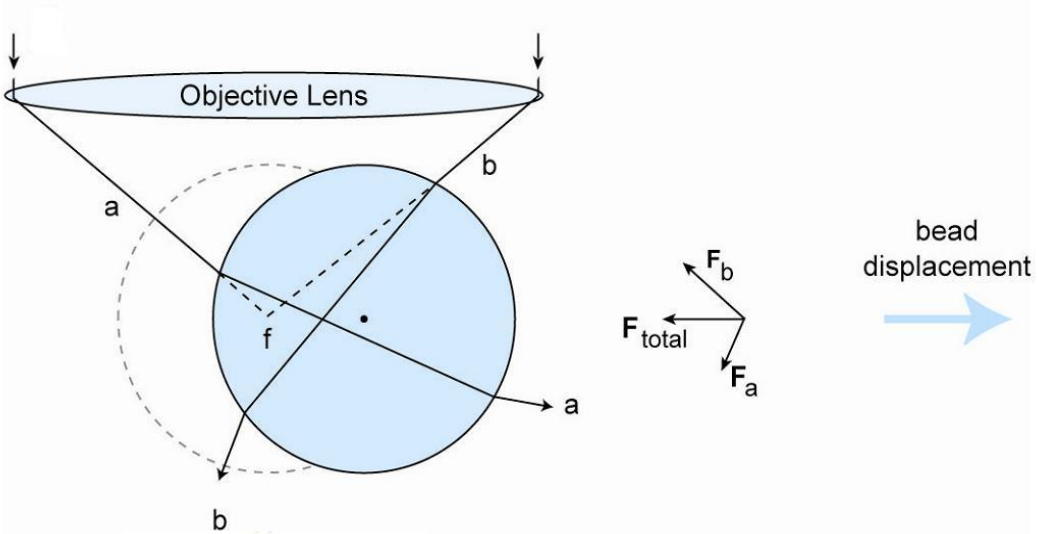


The force from a single beam gradient optical trap with Gaussian intensity profile.
The central ray, a, is of higher intensity than ray b

Optical Tweezers (OT)

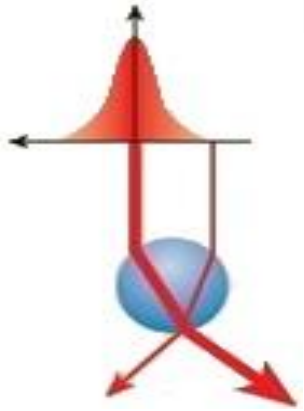
Bead moves to left or right

Newton's third law – for every action there is an equal and opposite



F_a and F_b represent the forces imparted to the bead by rays a and b

F_{total} is the sum of these two vectors and points to the left.

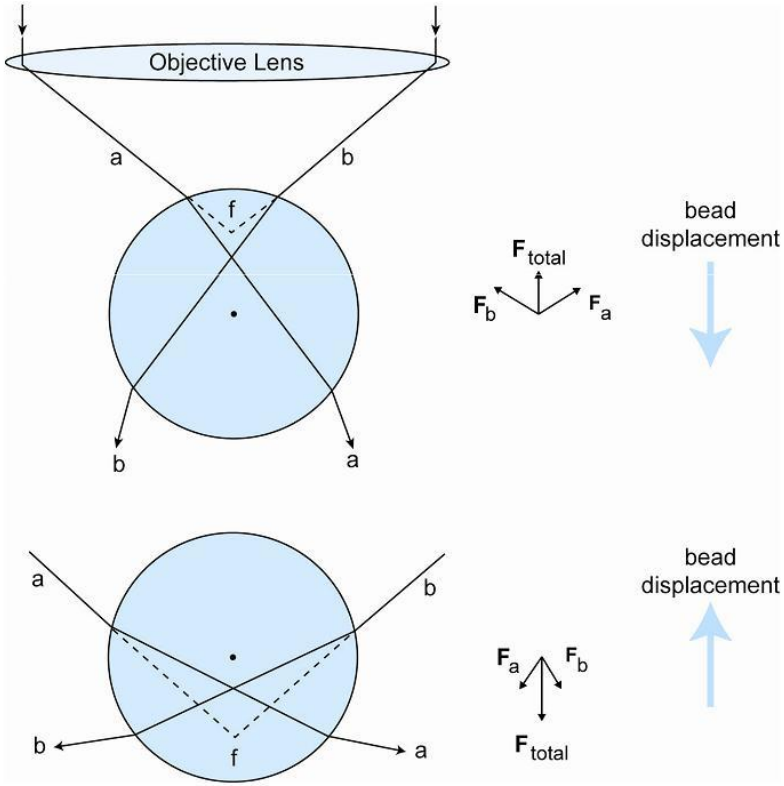


Object feels a force toward brighter light

Optical Tweezers (OT)

Bead moves forward or backward

Newton's third law – for every action there is an equal and opposite reaction



When the bead is displaced below the laser focus, the deflected rays a and b are more convergent, and resulting force points upward

When the bead is displaced above the laser focus, the deflected rays a and b are more divergent, and the resulting force points downward

Object feels a force toward focus
Force ~ gradient intensity

Optical Tweezers (OT)

Technical requirements

Trapping lasers: Gaussian output intensity profile to achieve the smallest focal spot producing the largest optical gradient

A trapping laser with superior pointing and power stability: fluctuations in beam pointing increase noise.

Trapping lasers: Near infrared wavelengths (800 – 1100 nm) minimize optically induced damage in biological specimens. Diode-pumped neodymium-doped yttrium aluminum garnet (Nd:YAG) with a wavelength of 1064 nm

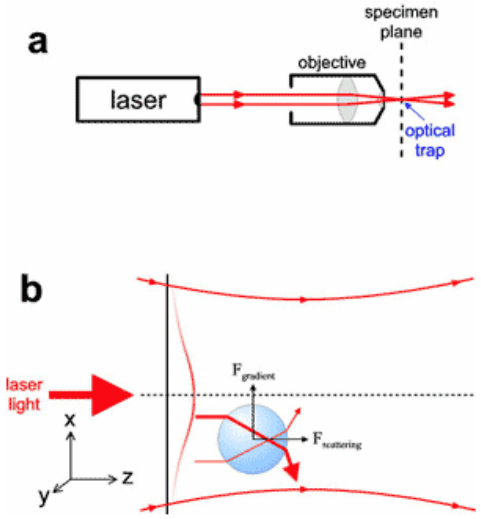
Focused laser beam to a diffraction-limited spot with a high numerical aperture (NA) microscope objective: light-gathering ability and resolution

The NA of the trapping objective: at least 1.2 to achieve the steep focus needed to create a stable optical trap.

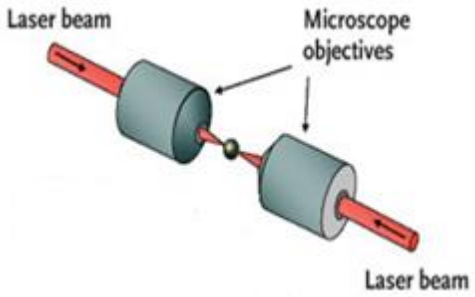
Optical Tweezers (OT)

Types of OT

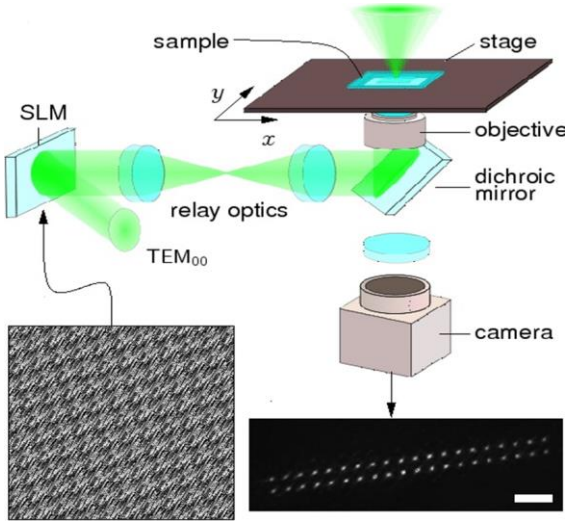
Single Beam Optical Tweezers



Dual beam Optical Tweezers



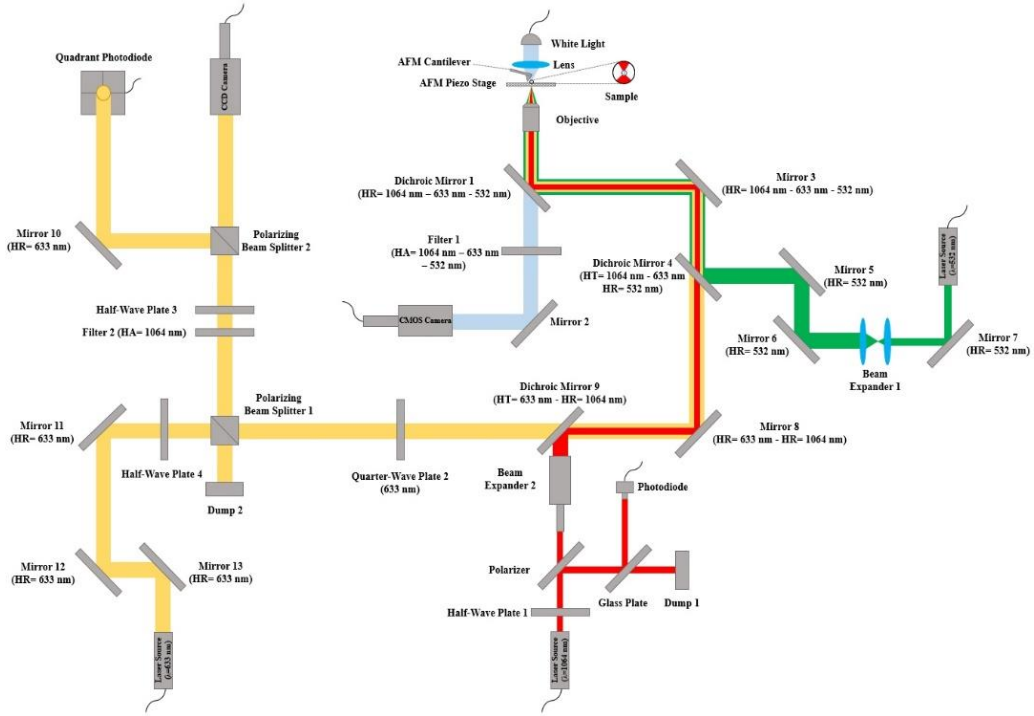
Holographic Optical Tweezers

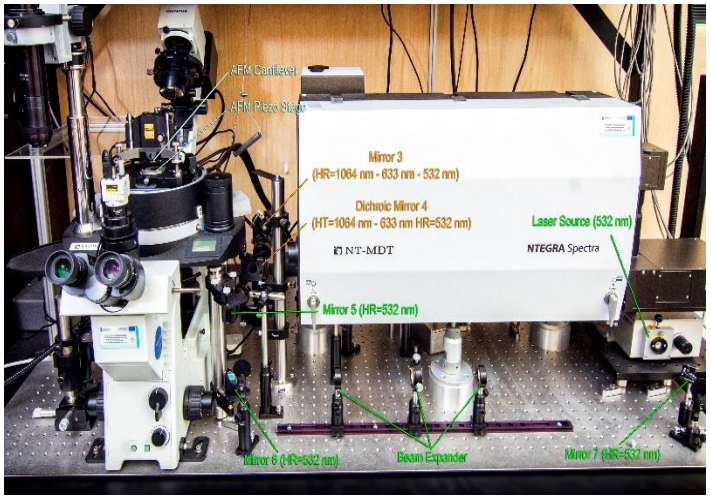


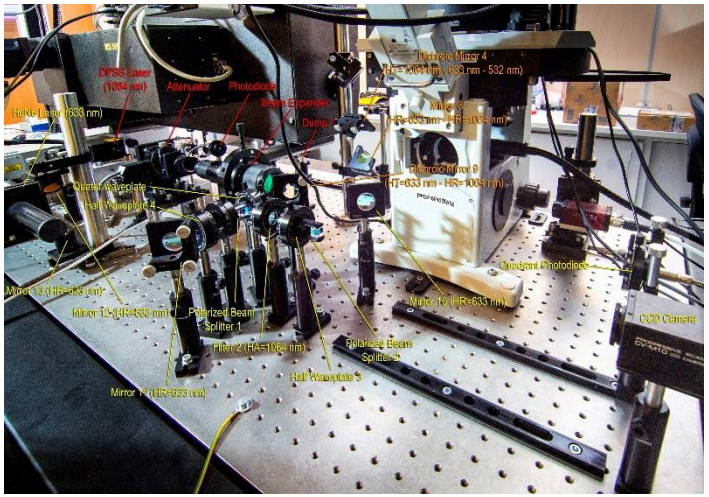
SCOPE

The aim of the present research is to demonstrate the possibility of extending the capability of a commercial AFM system by combining it with optical tweezers.
It permits to obtain a high-quality imaging instrument able to trap and modify nanometric materials and to measure force in the subpiconewton scale.

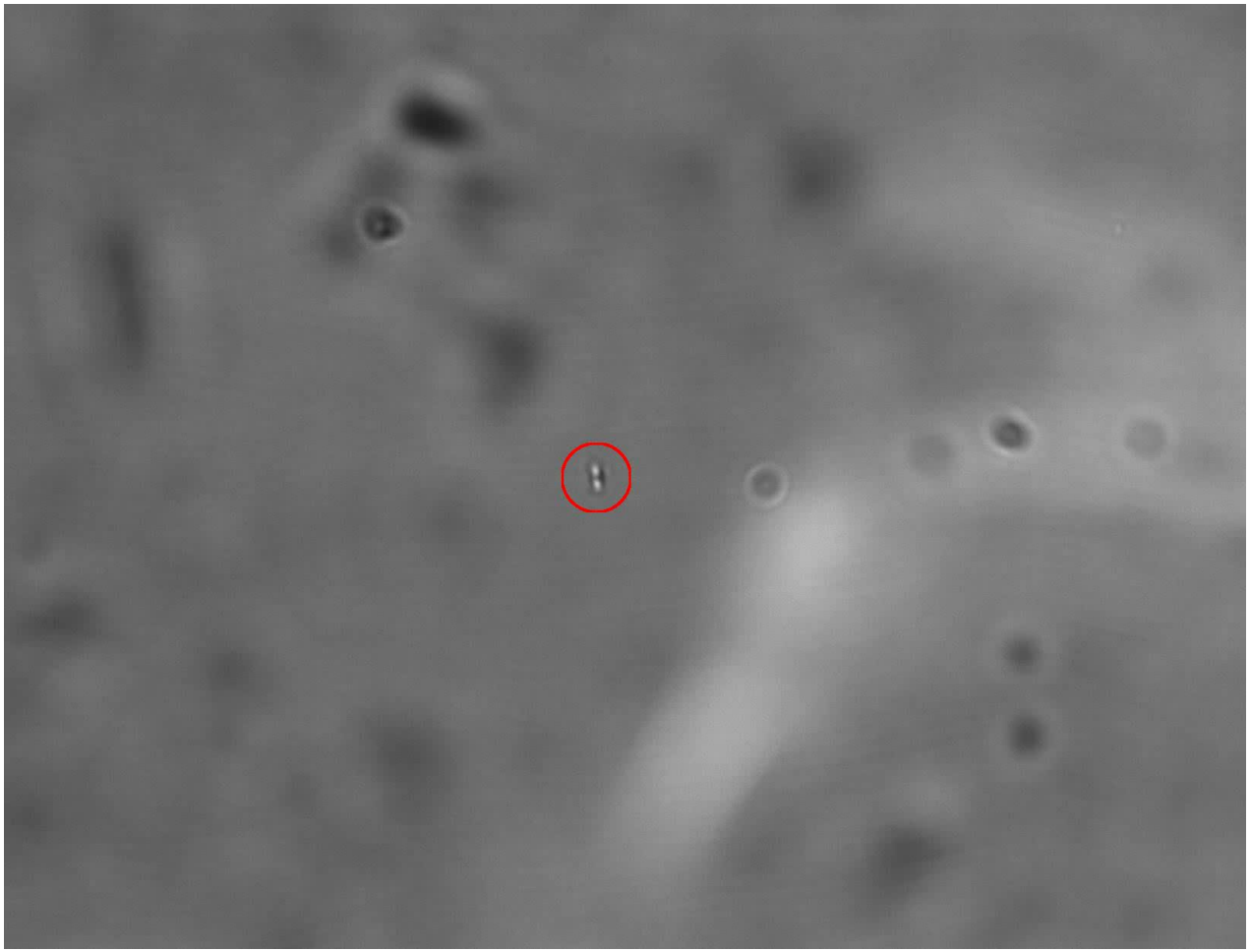
AFM/OT SETUP





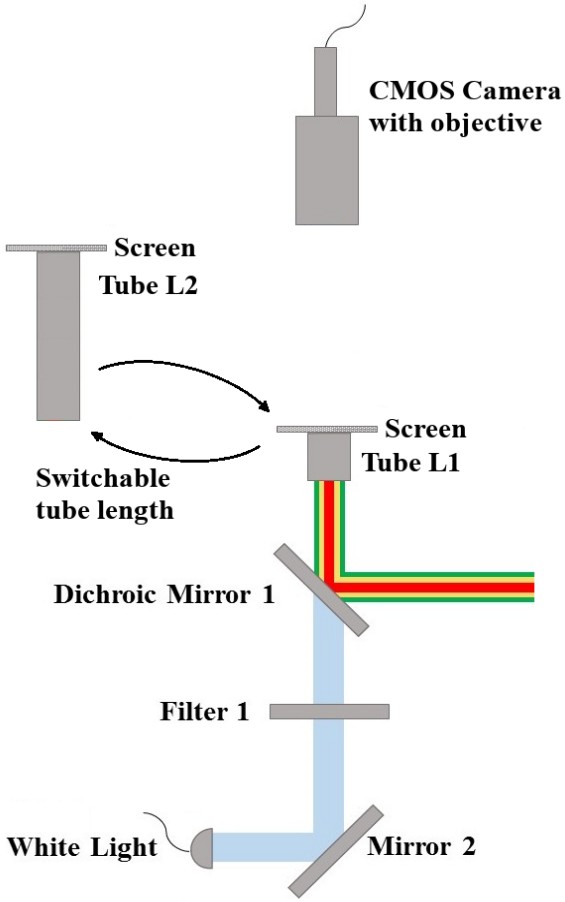


AFM/OT SETUP



Polyacrylamide filament (length: 2 μm - diameter: 0.6 μm) (10 fps)

Beams alignment

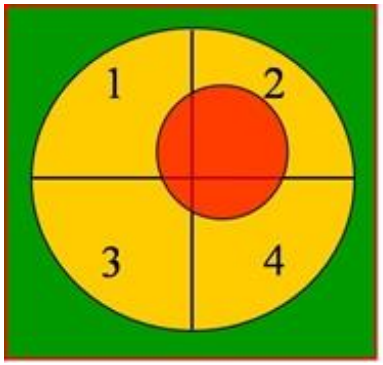


The CMOS camera and the White Light source are swapped.
The microscope objective is replaced with a short (L1) or a long tube (L2).
Semi-transparent screen on their top side of the tubes.

Alignment procedure :

1. Finding the center of the Tube L1 and L2.
2. Alignment of the trapping laser.
3. Alignment of the detection laser.
4. Alignment of the fluorescent laser. Each part is described in detail below.

QPD detector calibration



$$S_x=(V_2+V_4)-(V_1+V_3)$$

$$S_y=(V_1+V_2)-(V_3+V_4)$$

Calibration using a 1.0 μm polystyrene bead immobilized in hydrogel

Instrumental noise

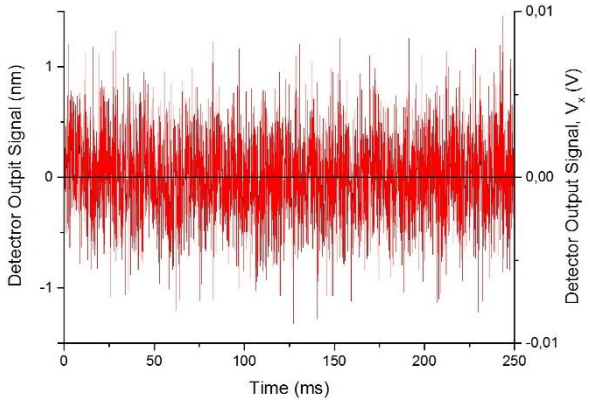
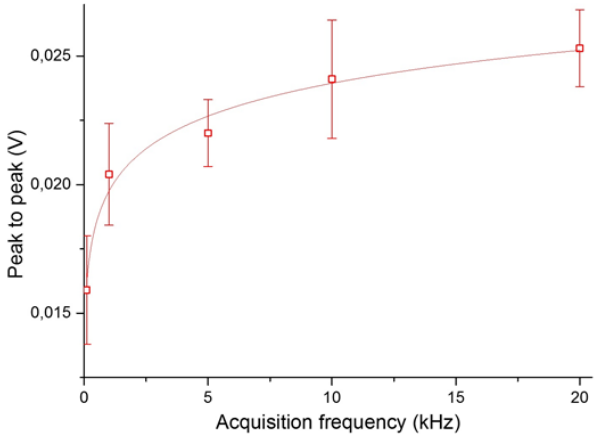
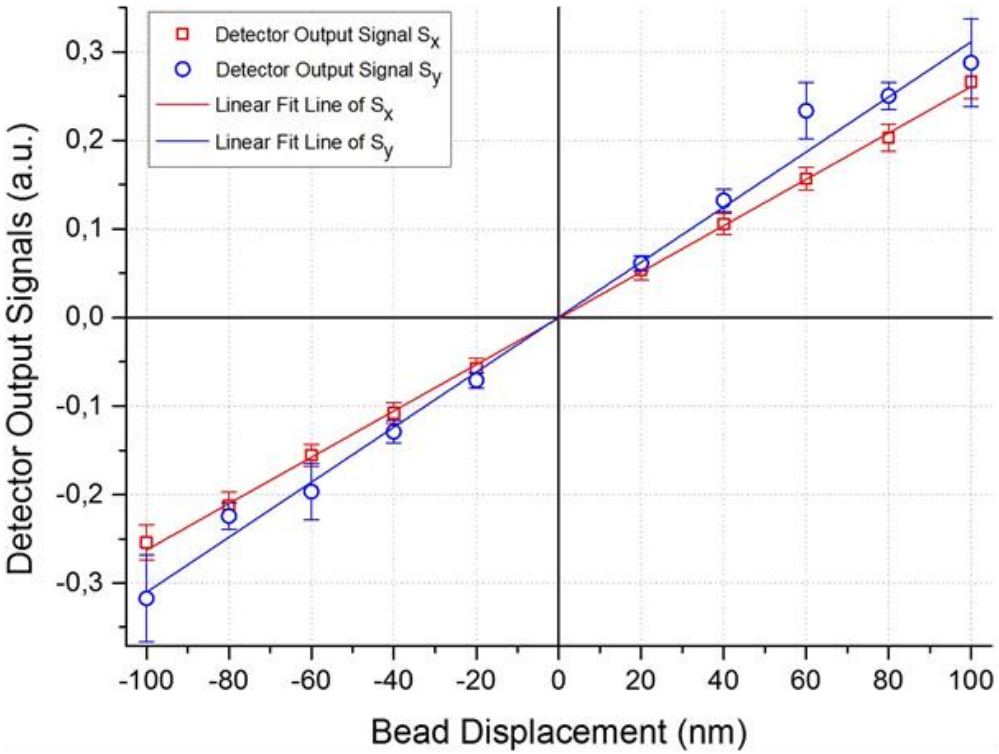


Diagram of photodiode output signal in X direction as a function of time (5000 samples per second)



Instrumental noise highlighted as the peak to peak value

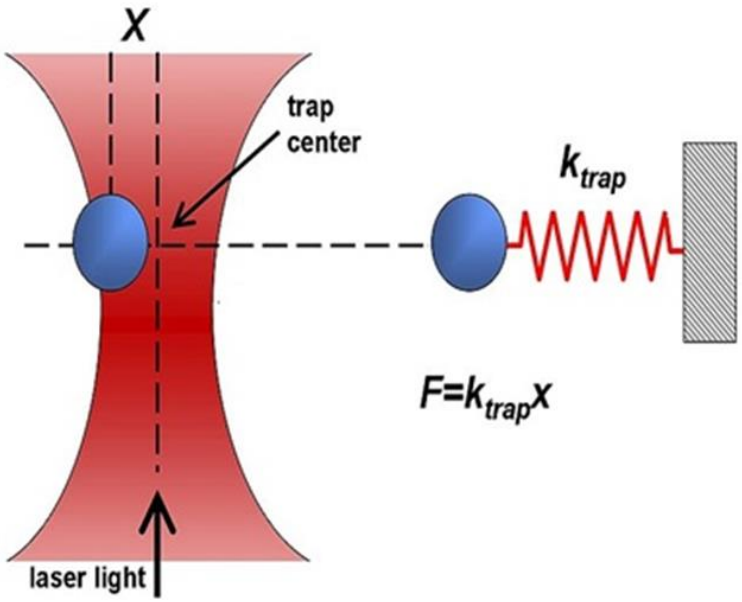
QPD detector calibration



Quadrant photodiode output signals (S_x and S_y) versus particle displacement curves recorded by moving the bead through the optical trap.

The slope of the fit lines (α_x ,α_y) for S_x and S_y are respectively 0.0026 and 0.0031 in arbitrary units.

Force Calibration



Object is attracted to the center of the beam

The force applied on the object depends linearly on its displacement from the trap center just as with a simple spring system

The spring constant, or stiffness: optical gradient, laser power, properties of the trapped object and solvent

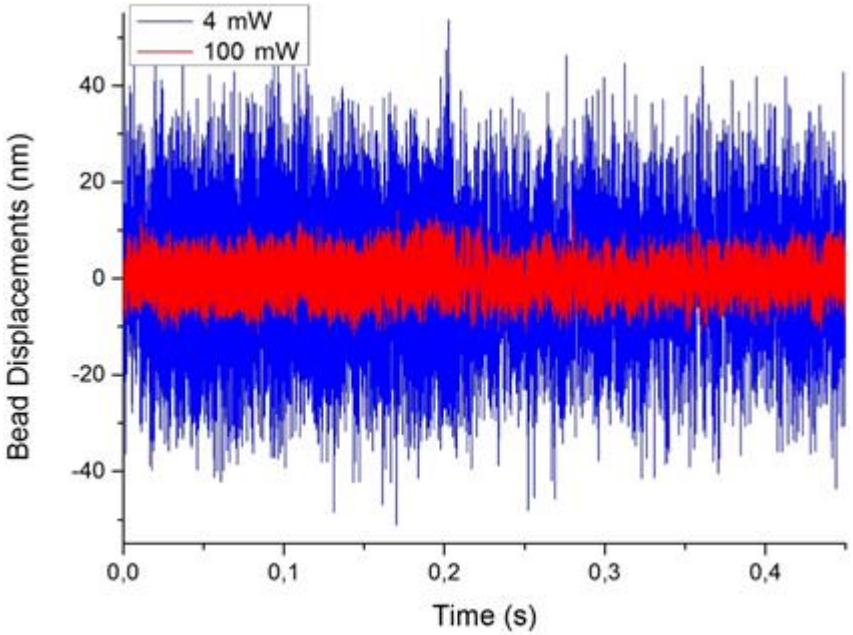
Equipartition Calibration

- The trapped bead oscillates randomly near the focal point of the laser beam when it is in thermal equilibrium.
- The particle flocculation is due to the Brownian motion which tend to displace the bead stochastically.
- The bead spatial position is well-described by a Gaussian function centred in the focal laser point

The equipartition theorem defines the average translational kinetic energy of a particle for each translational degree of freedom as ½ $k_B T$ where k_B is the Boltzmann constant and T is the absolute temperature.
According to the this theorem it is possible to evaluate the trap stiffness (k) by solving the equation

$$k = k_B T / \langle \Delta x^2 \rangle$$

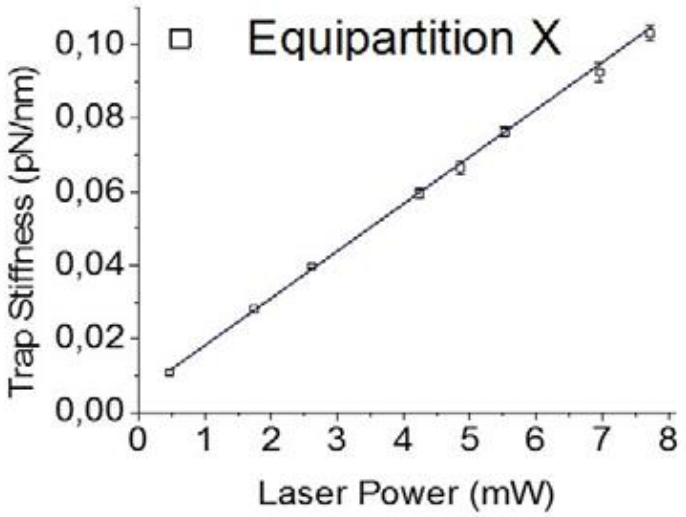
where $\langle \Delta x^2 \rangle$ is the statistical variance in the particle position

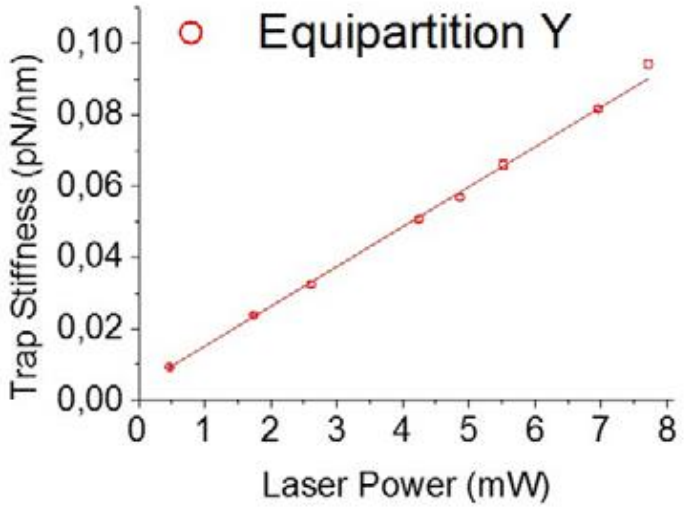


Particle: 1.0 µm polystyrene bead
Trapping laser: 4 mW and 100 mW
Sampling: 10 kHz

Equipartition Calibration

Results





External Force Calibration

We apply a force to the trapped sphere by flowing water through the cell. This force is dependent on radius r, viscosity η, and velocity U of the water

$$F_{drag} = 6\pi\eta r U$$

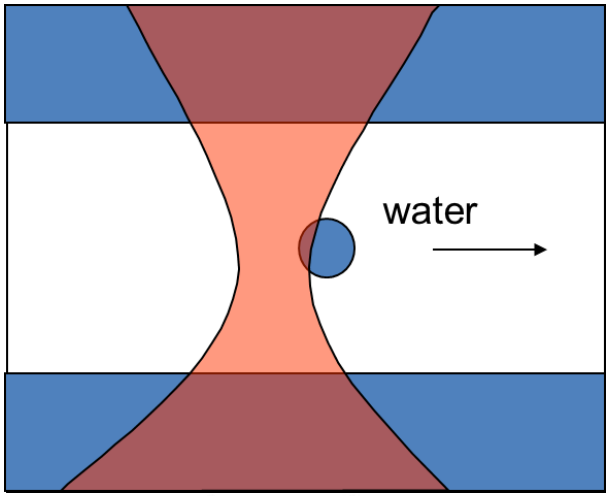
Within the limits of the strength of the trap, the sphere remains trapped, but undergoes a displacement under the influence of this external force just like a mass on a spring

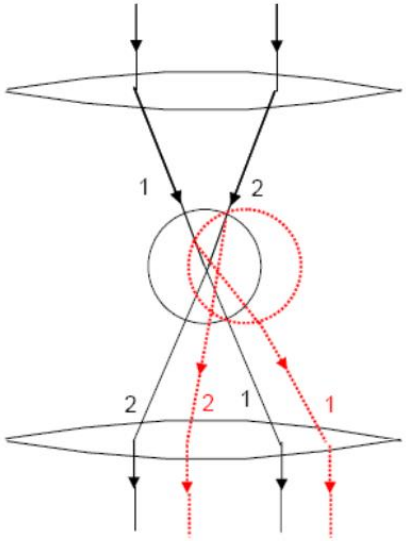
$$F = kx$$

External Force Calibration

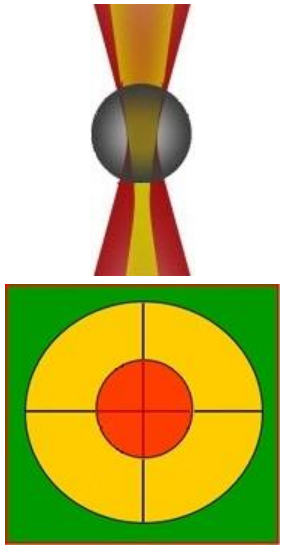
If a known force is applied, and the displacement is measured, the 'stiffness' of the optical trap may be determined

$$k = \frac{6\pi\eta r U}{x}$$

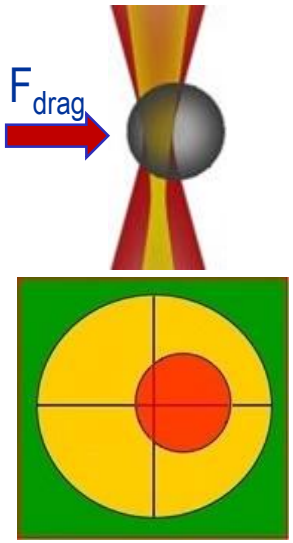




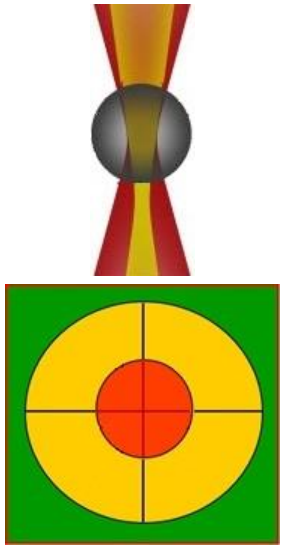
External Force Calibration



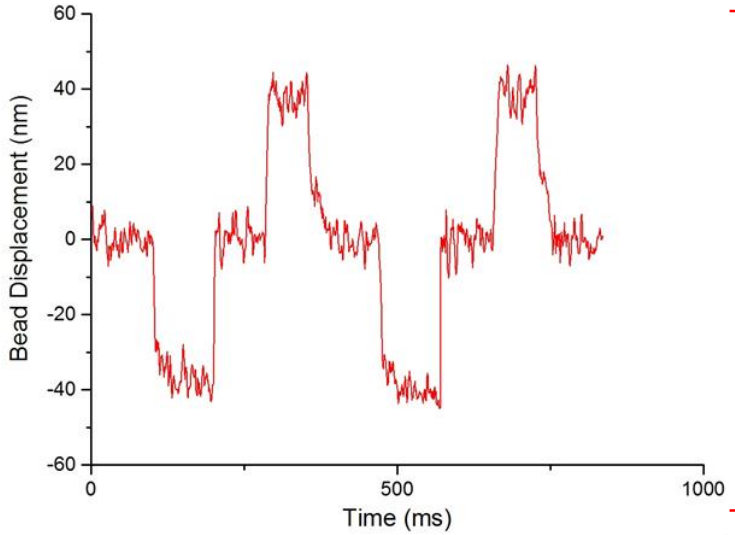
Initial position



Displaced position



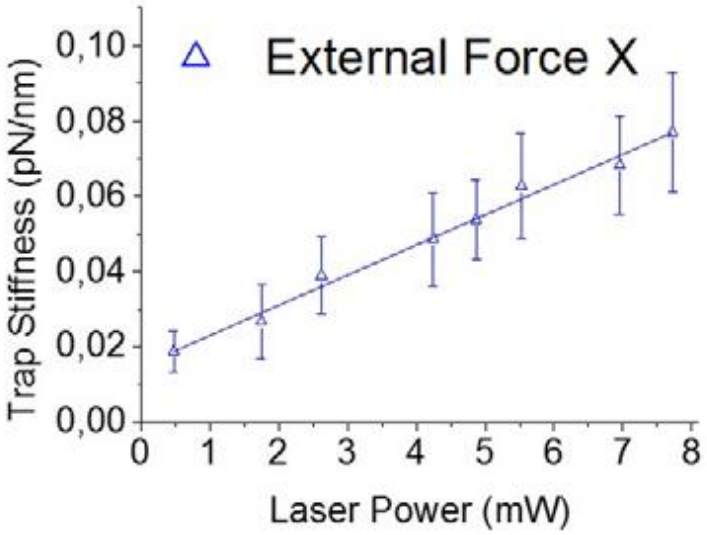
Final position

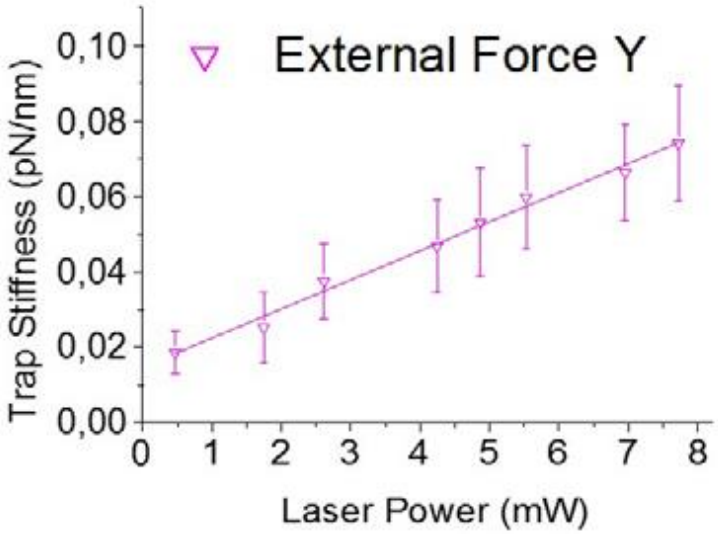


- Polystyrene bead diameter: 1.0 μm
- Trapping laser: 50 mW
- Piezo stage movement: 100 μm
- Piezo stage movement speed: 1400 μm/s
- Piezo stage movement delay: 100 ms
- Sampling: 1.0 kHz

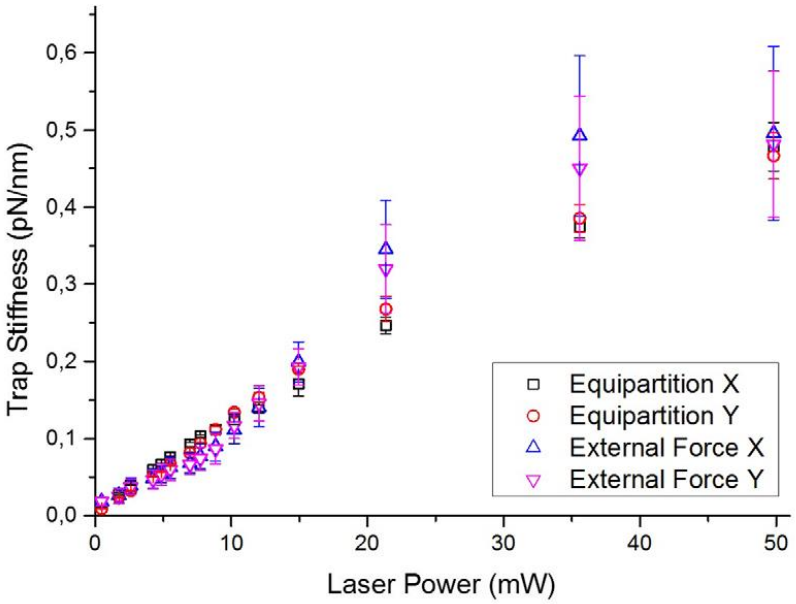
External Force Calibration

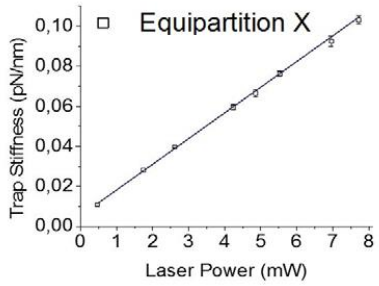
Results

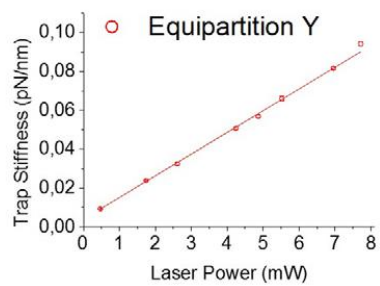


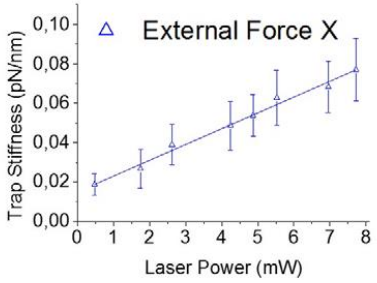


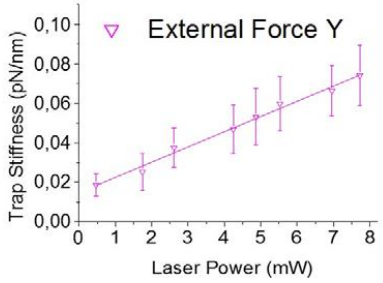
Force calibration











Equipartition Calibration

Non-linear correlation over the studied range of trapping laser power due to:
- temperature increaseing (4°C/W)
- viscosity decreaseing
- convective flow generation

Low standard deviation for the calibration measurments obtained using low of trapping laser

External Force Calibration

Linear correlation over the studied range of trapping laser power

Large standard deviation for the calibration measurments obtained using low of trapping laser

Escape Force

Particle: 1.0 μm diameter polystyrene bead

Trapping laser: from 1.74 mW to 2.24 mW

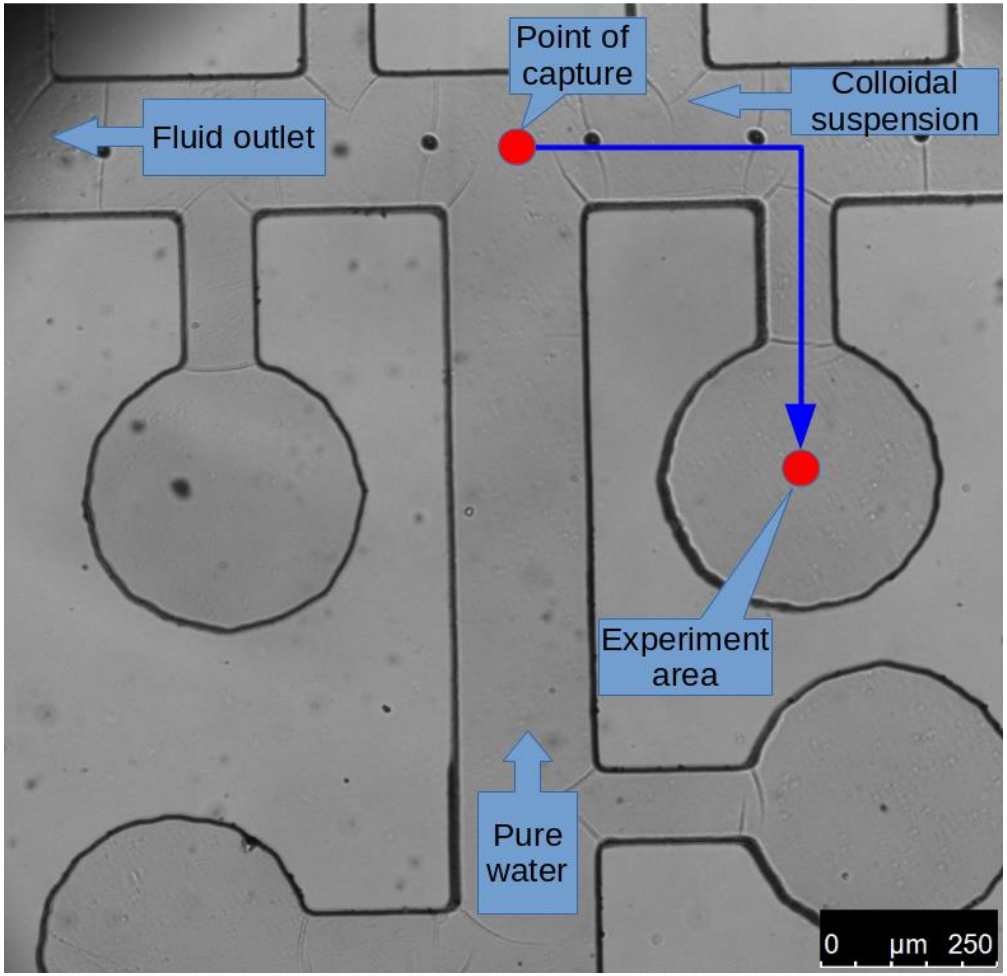
Stage speed: up to 1400 μm/s

Sampling: 1.0 kHz

Trapping laser power (mW)	Axes direction	Escape force (pN)	Escape force standard deviation (pN)
1.74	*X*	4.91	0.35
2.62	*X*	7.22	0.22
4.24	*X*	11.17	0.74
1.74	*Y*	4.89	0.11
2.62	*Y*	7.26	0.13
4.24	*Y*	10.89	0.18

Equipartition Calibration

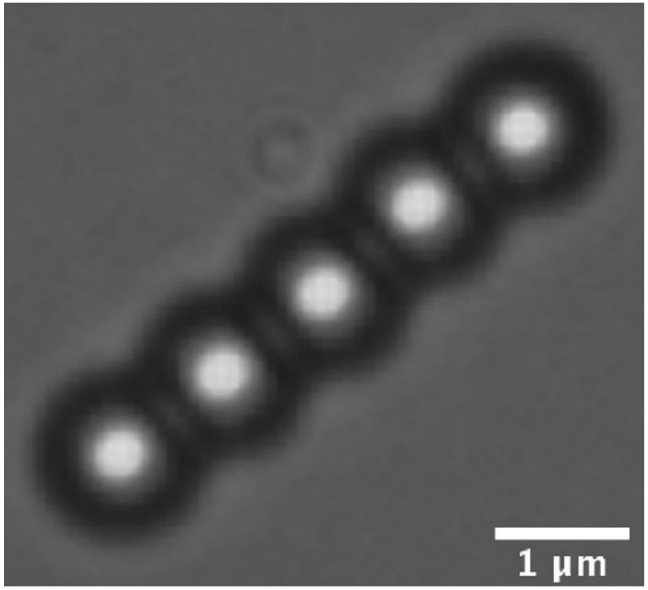
Microfluidic device



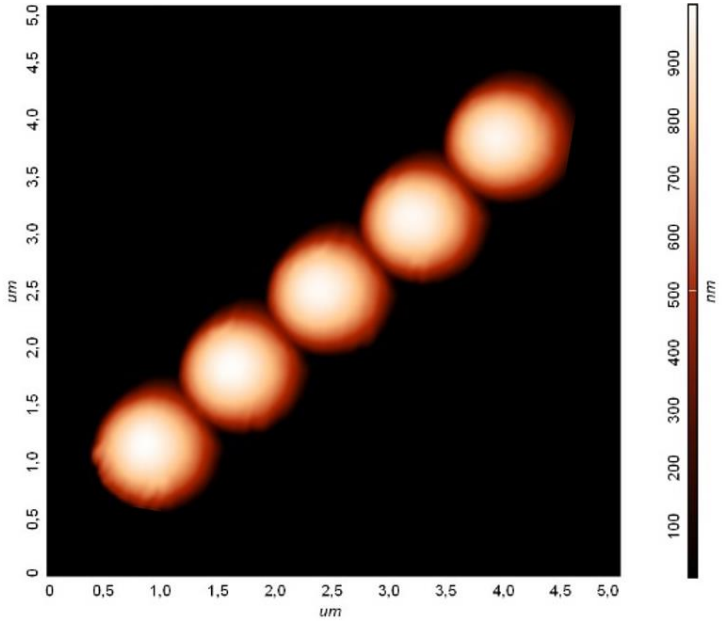
Nanomanipulation and high resolution imaging

Glass slide functionaliztion: 3-aminopropyltriethoxysilane (APTES)

AFM measurment:
- tapping mode
- in water
- scan frequency of 0.2 Hz.
- 5.0 μm × 5.0 μm



Optical Microscope



AFM

Colloidal particles interaction forces

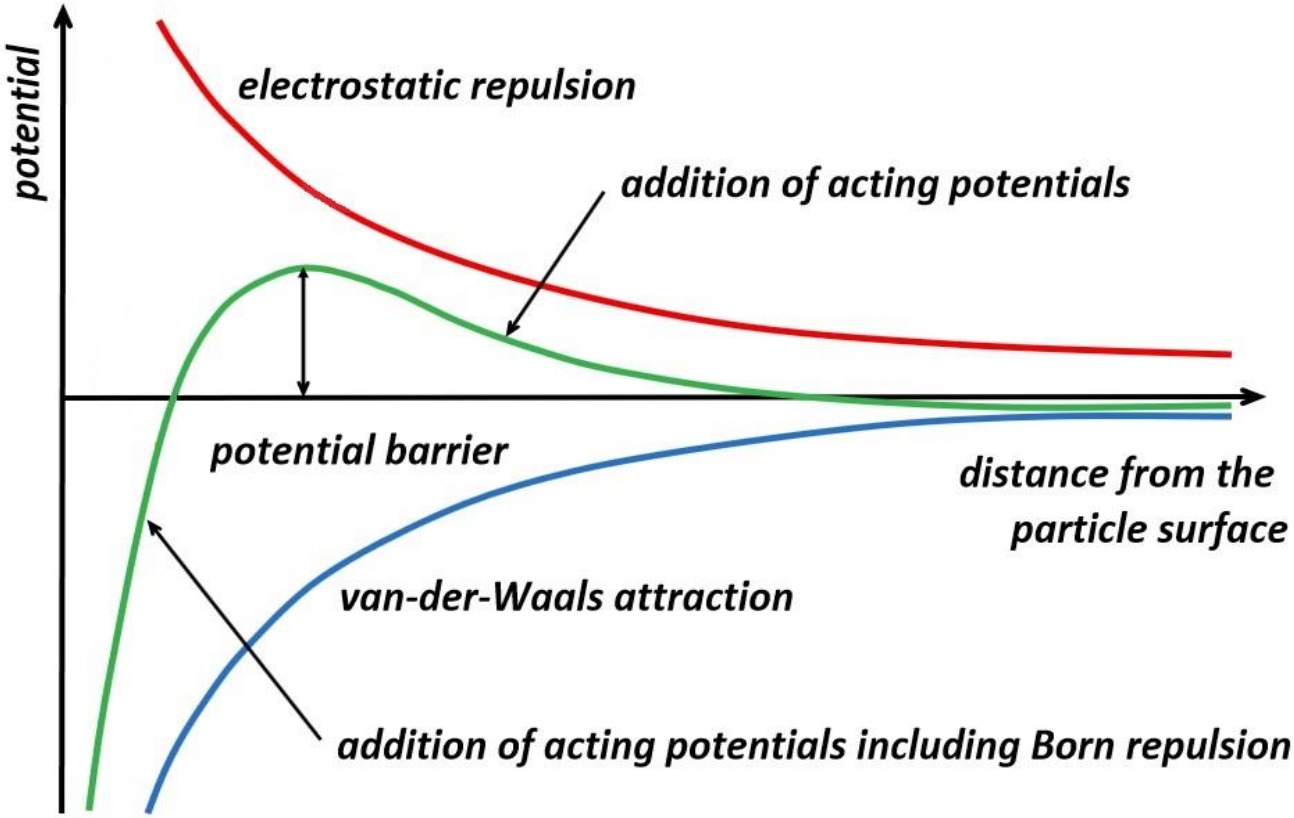
DLVO Theory


Boris Derjaguin


Lew Landau


Evert J.W. Verwey


J.T.G. (Theo) Overbeek



Colloidal particles interaction forces

Solute concentration vs Stability and motion

$$\kappa^{-1} = 10^{10}\left[\frac{(2)\,(1000)\,e^2 N_A I}{\varepsilon\varepsilon_0\,kT}\right]^{-1/2}$$

where

κ^{-1} = double-layer thickness, Å

10^{10} = length conversion, Å /m

1000 = volume conversion, L/m^3

e = electron charge, 1.60219×10^{-19} C

N_A = Avagadro's number, 6.02205×10^{23}/mol

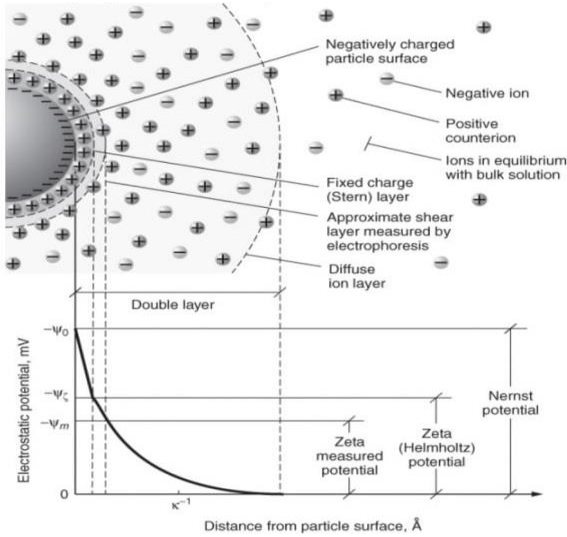
I = ionic strength, $\frac{1}{2}\sum z^2 M$, mol/L

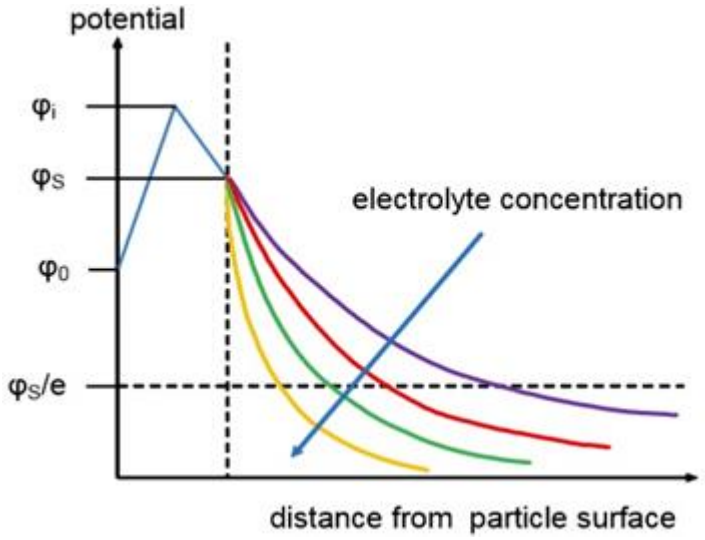
ε = permittivity relative to a vacuum (ε for water is 78.54)

ε_0 = permittivity in a vacuum, 8.854188×10^{-12} C^2/J • m

k = Boltzmann constant, 1.38066×10^{-23} J/K

T = absolute temperature, K (273 + ° C)



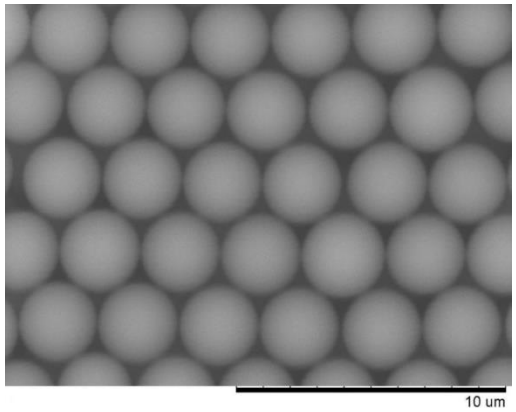


C in mol / L^3	Debye length δ_κ of different types of electrolytes in nm			
	(1,1)	(1,2)	(2,2)	(1,3)
10^{-1}	0.96	0.55	0.48	0.39
10^{-2}	3.04	1.76	1.52	1.24
10^{-3}	9.60	5.55	4.81	3.93
10^{-4}	30.40	17.60	15.20	12.40

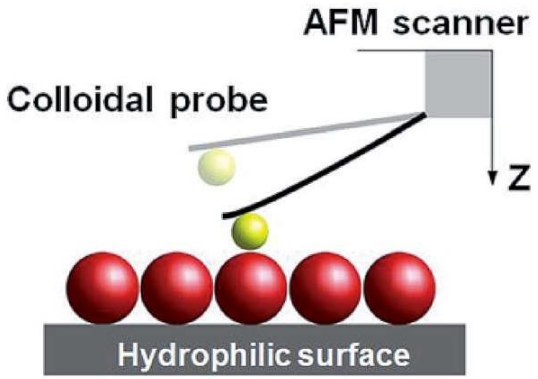
layer radii in nm for different salt types in water at 298 K

Colloidal particles interaction forces (AFM)

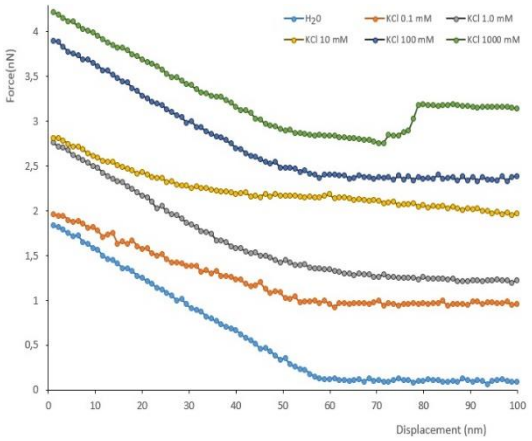
Substrate preparation



Method



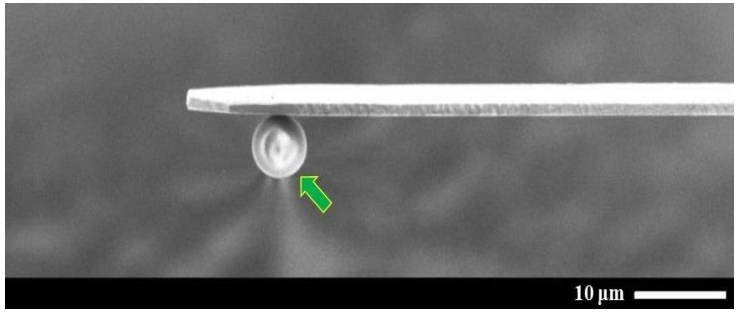
Results



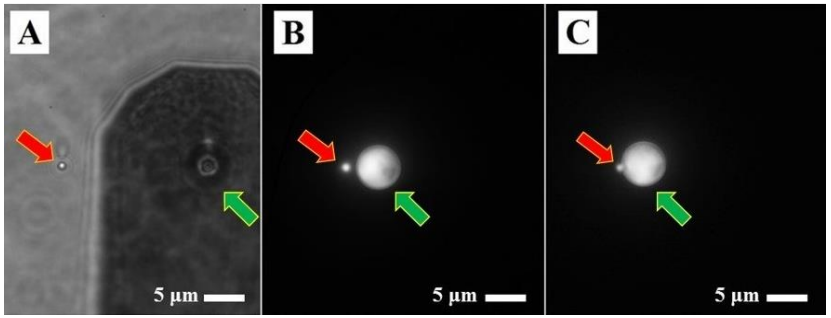
- Poor sensitivity
- Double layer perturbation by particle confinement

Colloidal particles interaction forces (AFM/OT)

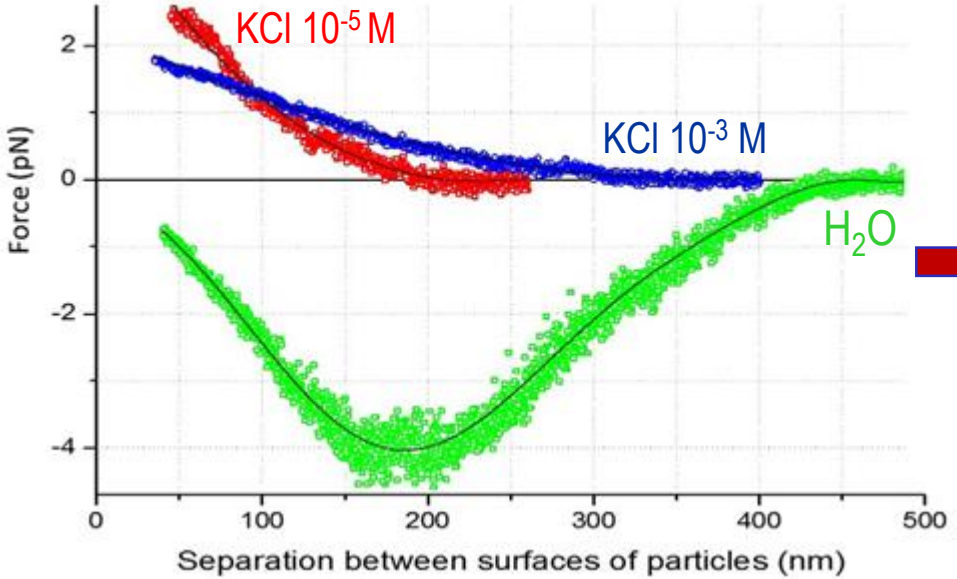
Particle probe preparation



Method



Results

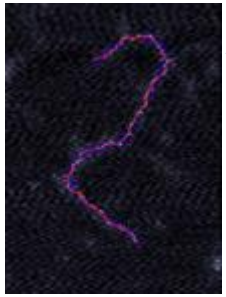


- High sensitivity
- Long range interaction
- Optimized experimental conditions

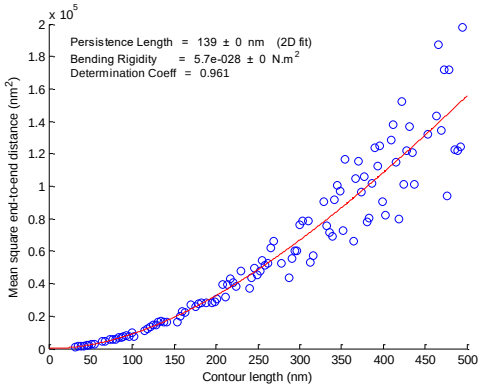
DNA streatching

Preliminary studies

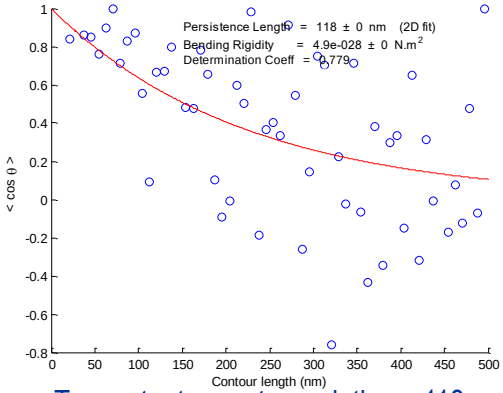
- Persistence Length



AFM topography
Sample: DNA in Tris buffer and $NiCl_2$ solution
Substrate: freshly cleaved mica
Technique: tapping mode in air

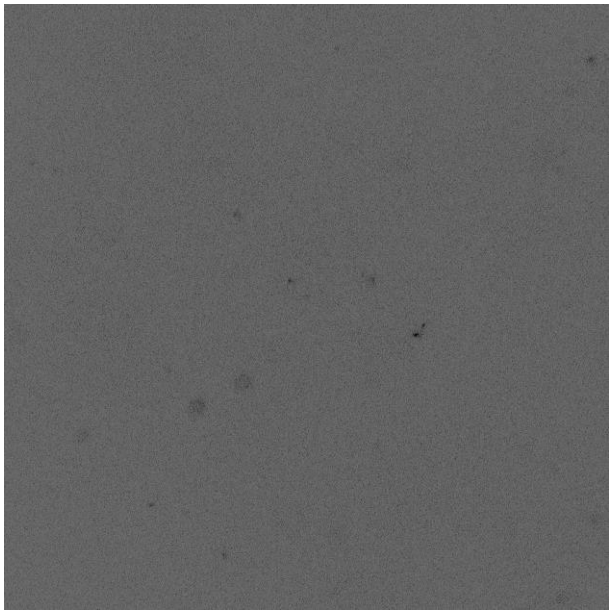


Contour length versus end-to-end distance: 139 nm



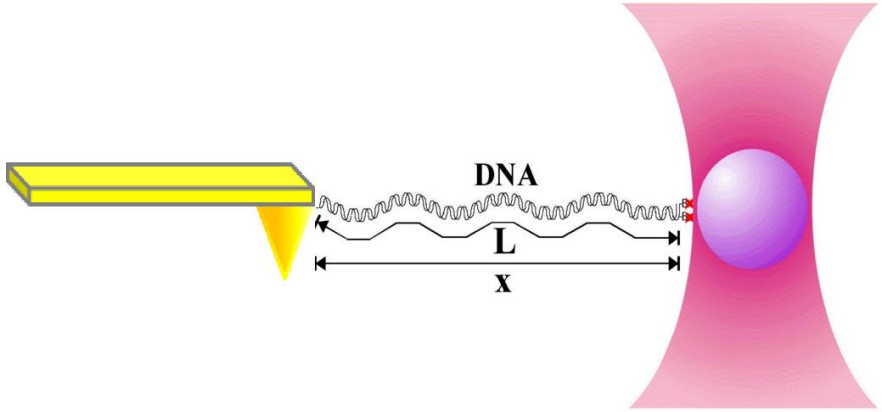
Tangent – tangent correlations: 118 nm

- Motion



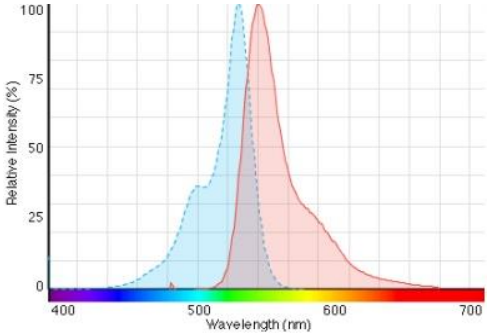
DNA streatching

Experimental method



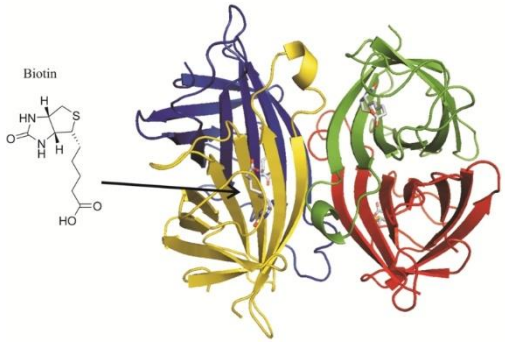
DNA modification

JOJO-1 IODIDE

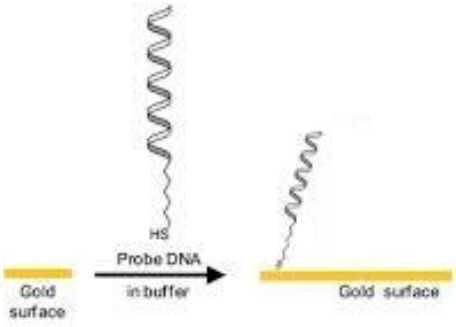


(excitation/emission: 529/545 nm)

BIOTIN-STREPTAVIDIN



THIOL-GOLD



DNA streatching

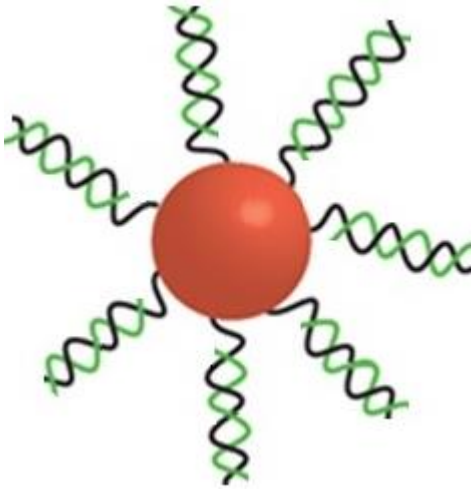
Preparation of functionalized particles

JOJO-1 interaction with biotinylated and thiole modified bacteriophage λ DNA

NaCl 10 mM; Glucose (0.1%); Tris 10 mM (pH 7.5); EDTA 1 mM β-mercaptoethanol (1%). glucose oxidase 10 μg/ml catalase 120 μg/ml

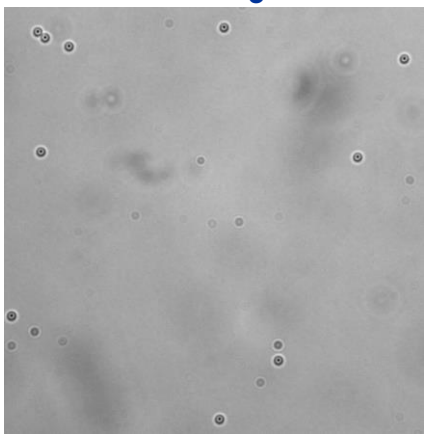
1 μm streptavidin-coated microspheres interaction with fluirescent funtionalized λ DNA

NaCl 1M; 20 mM Tris (pH 7.5); 1 mM EDTA; Triton X-100 (0.0005%)

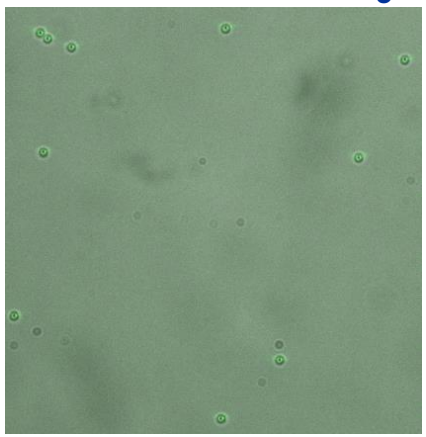


Results

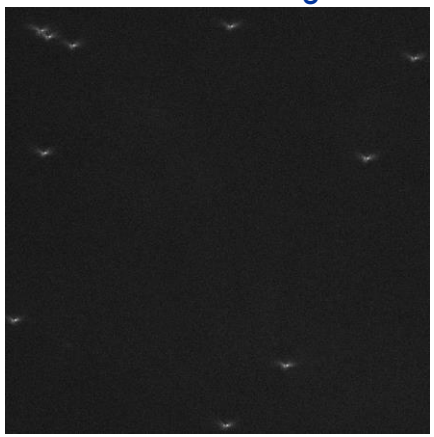
White light



Fluorescent and white light

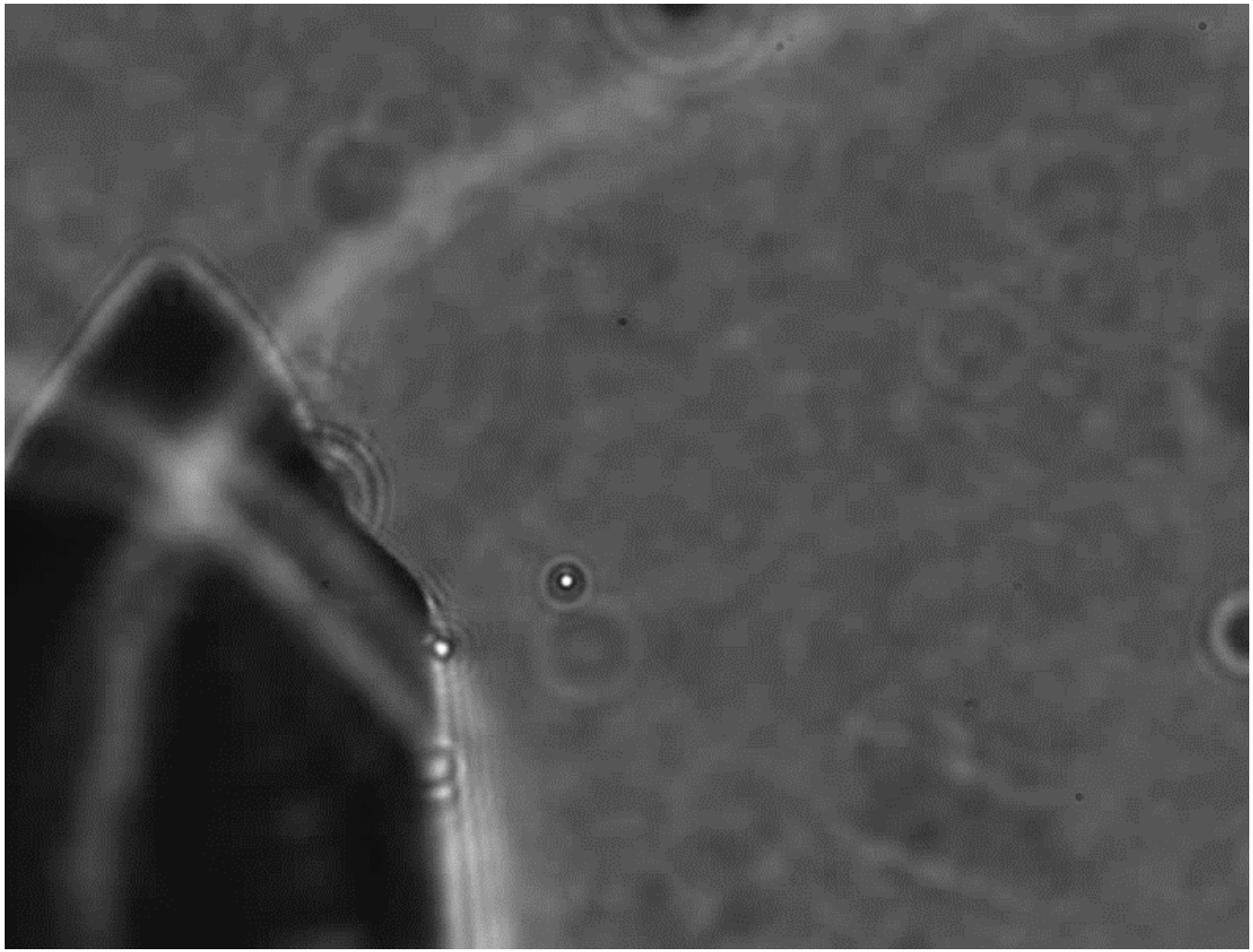


Fluorescent light



DNA streatching

AFM/OT experiment



Buffer: NaCl 1M; 20 mM Tris (pH 7,5); 1 mM EDTA Triton X-100 (0,0005%)

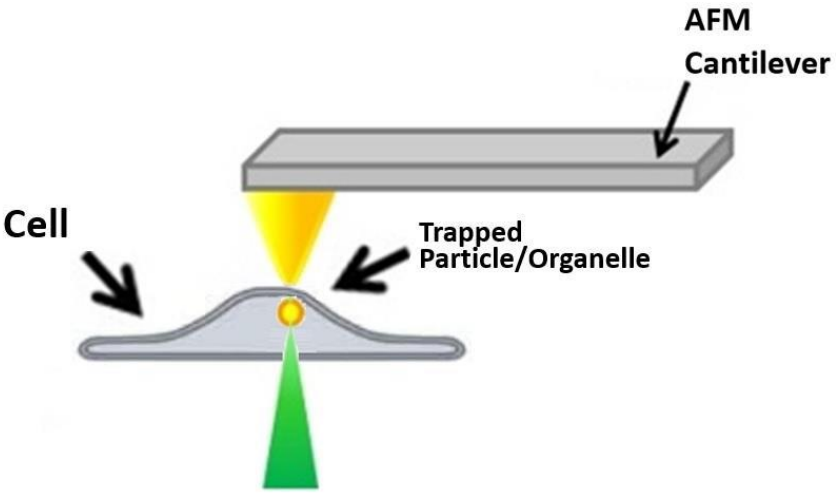
Ballistic Brownian Motion

$\tau_p = M/6\pi\eta R \Longrightarrow t < \tau_p$

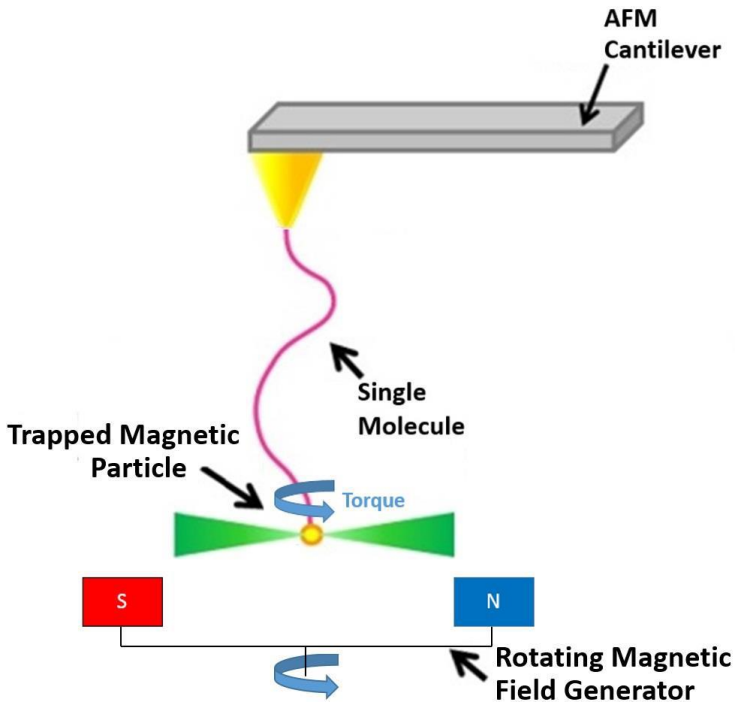
τ_p = momentum relaxation time (τ_p of 1 μm silica particle in water ~ 10μs)
M = particle mass
η = viscosity of the fluid
R = particle radius

OUTLOOKS

Cell double probing by AFM/OT



Stretching and/or twisting of single molecules or nano-objects



Concluding Remarks

- We have designed and developed a combined AFM/OT equipment.
- We have calibrated and used the instrument in three different experiment proving its high potential in nanomechanics, molecules manipulation and biological studies.
- We have demonstrated the possibility to the possibility of extending the capabilities (force sensing, nanomanipulation and simultaneous double probing) of a commercial AFM equipment by combining it with optical tweezers.

Acknowledgements

K. Zembrzycki S. Pawłowska P. Nakielski Prof. T. A. Kowalewski

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[F. Pierini, K. Zembrzycki, P. Nakielski, S. Pawłowska, and T.A. Kowalewski, *"Atomic force microscopy combined with optical tweezers (AFM/OT)"*, Measurement Science and Technology, 27 (2016) 025904]