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

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INTRODUCTION

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

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

Forces distance curves
Interaction forces between tip and sample are recorded
Optical Tweezers (OT)

History

**Laser: “Stimulated Optical Radiation in Ruby”**

**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

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

Arthur Ashkin
Optical Tweezers (OT)

What are Optical Tweezers?

Optical tweezers can trap and manipulate nanometer and micrometer-sized particles.

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)

Description

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.
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 \( \lambda \).

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.

\[ p = \frac{h}{\lambda} \]

\[ F = \frac{dp}{dt} \]
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 $b$

$F_{\text{total}}$ 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 the 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 $\sim$ 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.
INTRODUCTION

Optical Tweezers (OT)

Types of OT

Single Beam Optical Tweezers

Dual beam Optical Tweezers

Holographic Optical Tweezers

INTRODUCTION
- Atomic Force Microscopy (AFM)
- Optical Tweezers (OT)

SCOPE

AFM/OT SETUP

CALIBRATIONS
- Beams alignment
- QPD detector calibration
- Force calibration

EXPERIMENTS
- Nanomanipulation and high resolution imaging
- Colloidal particles interaction forces
- DNA stretching

OUTLOOKS

CONCLUSIONS

IPPT, April 04 2016, Warsaw
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.
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Polyacrylamide filament (length: 2 μm - diameter: 0.6 μm) (10 fps)
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

Calibration using a 1.0 μm polystyrene bead immobilized in hydrogel

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 $(\alpha_x, \alpha_y)$ for $S_x$ and $S_y$ are respectively 0.0026 and 0.0031 in arbitrary units.
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 $\frac{1}{2} k_B T$ where $k_B$ is the Boltzmann constant and $T$ is the absolute temperature. According to this theorem it is possible to evaluate the trap stiffness ($k$) by solving the equation:

$$k = \frac{k_B T}{\langle \Delta x^2 \rangle}$$

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

![Graphs showing the relationship between laser power and trap stiffness for Equipartition X and Y.](image-url)
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 $\eta$, and velocity $U$ of the water

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

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

- 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

External Force X

External Force Y

Graph showing the relationship between laser power (mW) and trap stiffness (pN/nm) for X and Y directions.

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     - Force calibration

3. EXPERIMENTS
   - Nanomanipulation and high resolution imaging
   - Colloidal particles interaction forces
   - DNA stretching

4. OUTLOOKS

5. CONCLUSIONS
**Force calibration**

- **Equi-partition Calibration**
  - Non-linear correlation over the studied range of trapping laser power due to:
    - temperature increasing (4°C/W)
    - viscosity decreasing
    - convective flow generation
  - Low standard deviation for the calibration measurements 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 measurements 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

<table>
<thead>
<tr>
<th>Trapping laser power (mW)</th>
<th>Axes direction</th>
<th>Escape force (pN)</th>
<th>Escape force standard deviation (pN)</th>
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<tr>
<td>1.74</td>
<td>X</td>
<td>4.91</td>
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<td>2.62</td>
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<td>7.26</td>
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<td>4.24</td>
<td>Y</td>
<td>10.89</td>
<td>0.18</td>
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</table>
Equipartition Calibration

Microfluidic device
Nanomanipulation and high resolution imaging

Glass slide functionalization: 3-aminopropyltriethoxysilane (APTES)

AFM measurement:
- tapping mode
- in water
- scan frequency of 0.2 Hz.
- $5.0 \mu m \times 5.0 \mu m$

Optical Microscope

AFM
Colloidal particles interaction forces

DLVO Theory

potential

potential barrier

distance from the particle surface

electrostatic repulsion

addition of acting potentials

van-der-Waals attraction

addition of acting potentials including Born repulsion

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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
- \( \kappa^{-1} \) = double-layer thickness, Å
- \( 10^{10} \) = length conversion, Å/m
- \( 1000 \) = volume conversion, L/m³
- \( e = \) electron charge, \( 1.60219 \times 10^{-19} \) C
- \( N_A = \) Avagadro’s number, \( 6.02205 \times 10^{23}/\text{mol} \)

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

\[ \varepsilon = \text{permittivity relative to a vacuum (\( \varepsilon \) for water is 78.54)} \]

\[ \varepsilon_0 = \text{permittivity in a vacuum, 8.854188 \times 10^{-12} C^2/J \cdot m} \]

\[ k = \text{Boltzmann constant, 1.38066 \times 10^{-23} J/K} \]

\[ T = \text{absolute temperature, K (273 + °C)} \]

<table>
<thead>
<tr>
<th>C in mol/L³</th>
<th>Debye length ( \delta_\kappa ) of different types of electrolytes in nm</th>
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<tr>
<td></td>
<td>(1,1)</td>
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<tr>
<td>10⁻¹</td>
<td>0.96</td>
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<td>10⁻²</td>
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<tr>
<td>10⁻⁴</td>
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Colloidal particles interaction forces (AFM)

**Substrate preparation**

**Method**

Colloidal probe

AFM scanner

Hydrophilic surface

Results

Poor sensitivity

Double layer perturbation by particle confinement

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**EXPERIMENTS**

Colloidal particles interaction forces (AFM/OT)

**Particle probe preparation**

**RESULTS**

- **High sensitivity**
- **Long range interaction**
- **Optimized experimental conditions**

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DNA stretching

- Persistence Length

AFM topography
Sample: DNA in Tris buffer and NiCl₂ solution
Substrate: freshly cleaved mica
Technique: tapping mode in air

Preliminary studies

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

Tangent – tangent correlations: 118 nm

- Motion

Persistence Length = 139 ± 0 nm (2D fit)
Bending Rigidity = 5.7e-028 ± 0 N.m²
Determination Coeff = 0.961

Persistence Length = 118 ± 0 nm (2D fit)
Bending Rigidity = 4.9e-028 ± 0 N.m²
Determination Coeff = 0.779

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DNA stretching

Experimental method

DNA modification

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DNA modification

JOJO-1 IODIDE

BIOTIN-STREPTAVIDIN

THIOL-GOLD

(excitation/emission: 529/545 nm)
**DNA stretching**

**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 \(\beta\)-mercaptoethanol (1%).
glucose oxidase 10 \(\mu\)g/ml
catalase 120 \(\mu\)g/ml

1 \(\mu\)m streptavidin-coated microspheres interaction with fluorescent functionalized 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
EXPERIMENTS

DNA stretching

AFM/OT experiment

Buffer: NaCl 1M; 20 mM Tris (pH 7.5); 1 mM EDTA Triton X-100 (0.0005%)
Ballistic Brownian Motion

\[ \tau_p = \frac{M}{6\pi\eta R} \quad t < \tau_p \]

\( \tau_p \) = momentum relaxation time \((\tau_p \text{ of } 1 \mu m \text{ silica particle in water } \sim 10\mu s)\)

M = particle mass
\( \eta \) = viscosity of the fluid
R = particle radius
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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

Project is funded by NCN grant no. 2011/03/B/ST8/05481