ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

TRAVAUX PRATIQUES AVANCES DE PHYSIQUE
3ème ANNEE

CONFINEMENT OPTIQUE

Salle PH B1 395

Assistant 2008: Elena Bertseva,
Office: PH K1 494, tel. 33998
elena.bertseva@epfl.ch

Motivation

An optical trap is essentially highly focused laser beam that can capture and then move objects of diameters from 5 nm to 10 μm with forces up to 100 pN and resolution down to 100 aN [1,2]. Various particles, like plastic or glass spheres, cells, carbon nanotubes, oil droplets, cell organelles can all be trapped.

A free particle in water will move erratically due to constant bombardment from surrounding water molecules – motion called Brownian.

A particle trapped in the optical tweezer performs the Brownian motion in a volume restricted by the optical trap. The analysis of such confined motion allows to measure the force of the optical trap which is crucial for the interpretation of the trapping experiments.

Objective

During these four days, you will learn to:

1. construct the optical microscope and the optical trap
2. develop procedure for analysis of particle's motion using various software packages
3. analyze and interpret free and confined Brownian motion

Safety

In general, you must wear laser safety goggles designed for red HeNe laser light at all times unless the beam intensity is set below 1 mW.

General remarks

It is important to plan your four days with the end objective in mind, and revise these plans if necessary. The final report carries 50% of the final mark; the other 50% will come from your performance in the laboratory and your motivation. The report has to be written as a journal article – providing all the information for any outside reader to understand what you have done. A nice example of how the report should look you can take from the article [3].

Since this is an experimental technique, one can always run into problems and experience delays. I provide an outline that can serve as an orientation to track your progress. If you're behind, don't despair, if you're ahead, you could do more. ;)

Sessions 1-2

Read references 4-8 and be ready to answer the questions:

1. What is the random walker? What is the origin of Brownian motion?
2. What forces are involved in Brownian motion of a microparticle in water? Write Langevin equation.
3. Solution of Langevin equation. Diffusion coefficient, D, and Einstein's relation.

List of tasks:
1. Construct an optical microscope
2. Record free Brownian motion of isolated particles with three different radii (R = 0.5, 1.25 and 2.25 μm). *Software*: *Logitech ImageStudio*
3. Extract particle position from recorded videos. *Software: VirtualDub, ImageJ, Particle Tracker*[13]
4. Calculate mean-square displacement for all particles and obtain the diffusion coefficient. *Software: programming language of your choice.*

For the report:
1. Experimental setup (drawing)
2. Figure: Mean-square displacement versus time for different particle sizes
3. Table: Diffusion coefficient – theoretical and experimental values

Sessions 3-4

Read references [8-11] and be ready to answer the questions:

1. What forces create optical trap? How do we describe the motion in the trap?
2. What is the equipartition theorem? Use it to obtain trap stiffness.
3. What is the Boltzmann law? Use it to obtain the energy potential of the trapped particle.

List of tasks:

1. Add an optical trap to the optical microscope you constructed before (some explanations in reference [8])
2. Record motion of a trapped particle.
3. Extract particle position from recorded video. *Software: VirtualDub, ImageJ.*
4. Analyze confined Brownian motion

For the report (Figures):
1. Experimental setup (drawing)
2. Figures: Position histograms and energy landscape for confined particle
3. Table: Trapping coefficient – experimental values

Key optical notions

Numerical Aperture (NA)

The concept of *numerical aperture* is relevant to all microscope objectives. The NA is a dimensionless number that characterizes the range of angles over which the system can accept or emit light. NA indicates the *resolving power* of a lens. The size of the finest detail that can be resolved is proportional to λ/NA, where λ is the wavelength of the light. Low magnification objectives have very low NA's, around 0.1, while high powered objectives can have NA's as high as 1.6. The NA is given by the simple formula:

$$NA = n \sin \theta$$

where θ is the angular aperture of the objective, a value fixed by its internal optics, and n is a refractive index of the medium outside the objective.

Immersion objective

State of the art objectives can have NA of up to 0.95. Because $\sin\theta$ is always less than or equal to unity, the numerical aperture can never be greater than unity for an objective lens in air. If the space between the objective lens and the specimen is filled with oil, however, the numerical aperture can obtain values greater than unity. This is because oil has a refractive index greater than 1. The biggest NA are obtained with the *oil immersion* objectives.

The value of NA determines the gradient of light intensity near the focal point, and therefore the gradient force in the parallel direction. The bigger NA, the higher is the trapping efficiency. But for the optical tweezers experiment the choice of NA is a tradeoff between the efficiency and the depth of the trap with respect to the sample surface. A high NA will lead to a high trapping efficiency, but a shallow trapping depth. A low NA will lead to a deep trapping depth, but a low trapping efficiency.

Tube length

Contrary to simple lenses, common microscope objectives are not designed to work properly with parallel light; instead they are designed to receive light with a particular divergence. This divergence defines the *tube length* of the microscope; the distance between the back of the microscope objective and the *intermediate image*. This distance is generally a number etched into the objective and is usually a value of either 160 or 170 mm. As a result of this feature, an object at the front focus of the objective will generate an image about 160 mm behind the objective.

Experimental details

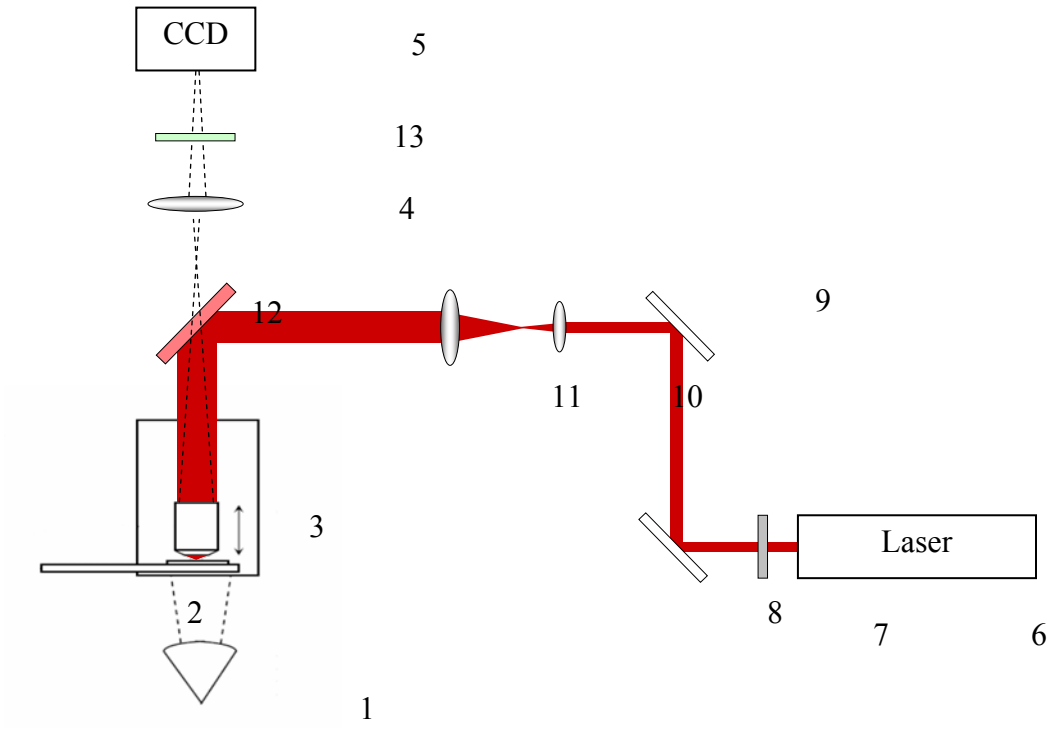

Figure 1. Optical scheme.

Microscope: 1 - illumination source, 2 – sample, 3 – objective, 4 – eyepiece lens, 5 – camera. Optical tweezer: 6 – laser, 7 – neutral filter, 8,9 – mirrors, 10,11 – lenses, 12 – dichroic mirror, 13 – shortpass filter.

The experimental setup is illustrated in Figure 1. The scheme contains two light paths – for microscopy and for optical trapping. They are coupled through a dichroic mirror which reflects the laser light and transmits the light from the illumination source.

The equipment needed in the experiment includes:

- 100x Oil Immersion Objective, NA=1.25, TL=160 mm
- Laser Power Technology Inc. PMP (LD1240), λ=658nm P_{max} =35 mW
- LDCU5 power supply, and PM–ACS–HS heat sink
- Dichroic Mirror LDM-670-1037-45
- Pulnix Analog Camera, 1/2”, 768x493 pixels, with PCI frame-grabber.
- White lamp
- Lenses, mirrors, optics mounts
- Slide, slide covers, double sided scotch
- PC Computer

Optical microscope

The microscope is composed of the illumination lamp, 100x Oil Immersion Objective, and the CCD camera. The classical principle of the microscope operation is illustrated on Fig. 2. The object (**O**) is a distance (**a**) from the objective (L**ob**) and projects an intermediate image (**O**') at the finite tube length (**b**), which is further magnified by the eyepiece (**Ley**) and then projected onto the retina at **O**''[12].

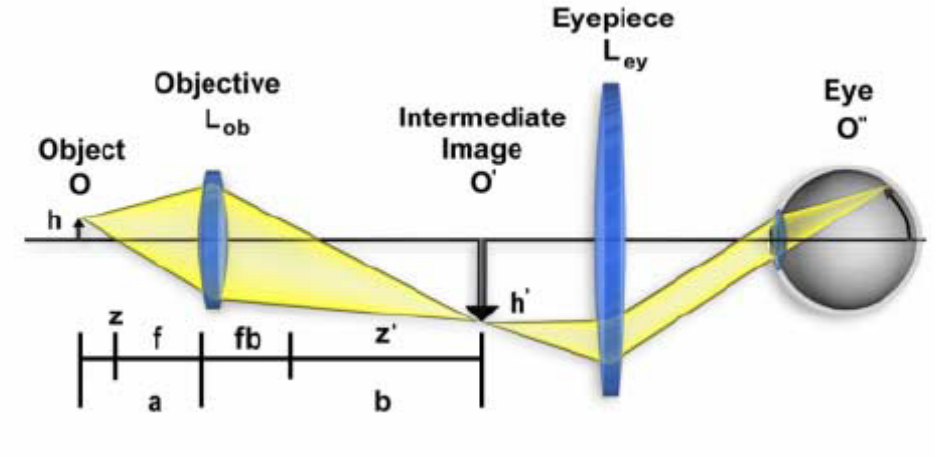

Figure 2. Finite tube length microscope ray path

In our case, we observe the image not by eye, but by a camera. Therefore, we adapt the eyepiece lens to produce the focused image of the intermediate image on the surface of the camera. To calculate the distance from the back surface of the Objective to the camera we should add the tube length parameter of the Objective to the solution of the lens equation of the eyepiece lens.

The answer and the final magnification will depend on the choice of the focal length of the eyepiece lens.

The distance between the objective and the camera shall be big enough to hold the dichroic mirror which we use to introduce the laser beam for optical trapping.

A illumination lamp is used to illuminate the slide. As no condenser is used, we are not able to get the commercial microscope quality image. The contrast can be improved with a hand-made diaphragm.

Optical Tweezer

To align the trapping optics put the neutral density filter in front of the laser to minimize the intensity to the safety Class 1. Aligning the optics begin with the mirrors. Once the mirrors have been aligned, add the lenses. Use the apertures and the principle of back reflection to align the beam along the proper optical paths.

The laser beam first passes through a focusing lens serving two purposes, to expand the beam filling the back lens of the microscope objective and to focus the beam to a point at the tube length of the objective.

From the lens, the beam reflects off a dichroic mirror placed above the objective. The dichroic mirror reflects only at specific wavelengths. In our case it is chosen to reflect at the wavelength of the laser. This dichroic mirror is used to reflect the beam into the objective and, due to its transparency, it allows us to use the objective not only to create our trap, but also view it with our camera.

Once the beam passes through the objective, it reaches the sample, where it focuses the beam to a point. By moving the sample slide with help of translation stage the position of the focal point can be placed anywhere within the sample solution.

Making a sample

1. Place two stripes of double scotch on the sides of the sample glass
2. Place the cover slip on top of the glass with double scotch and press along the sides.
3. Use the pipet to fill the place between the surfaces with the solution of beads in water
4. Seal the open sides with the nail polish and wait until it dries.
5. Place a drop of immersion oil over the sample on the cover slip.
6. Place the slide on the translation stage and bring the objective up to the slide so the immersion oil adheres to both the cover slip and the objective.

Software (recommended)

Use CVA LEO/ARIES to view the video and “VB AVIRec OCX" to record. The later is constantly storing video to disk, so the disk overfill can happen. The former allows to zoom the image if needed.

To start recording in VB AVIRec OCX press “Load Image” and load the file C://Program Filesn/Common Vision Blox/Drivers/cvleo.vin

Verify the frame rate (the movie player say that it is 25 frames per second, but they don’t know at which speed it has been taken).

Use ImageJ (version 1.4.1 or higher) to edit the recorded videos. Add « memory » to make it run faster (but not more than ¾ of all available memory). Use the plugins "particle tracking” [13] and "background subtractor” [14], as well as menus “Crop”, “Invert” and “Adjust - Brightness/Contrast”

Data analysis

The Brownian motion has to be long enough to obtain the information about the error of measurement (50-100 sec). The quality of the particle tracking will highly depend on the contrast of the recorded video.

Use the Invert, Contrast and Crop functions in the video analysis software.

The diffusion coefficient is can be approximated theoretically as

$$D = \frac{k_B T}{\gamma_0}, \tag{1}$$

where $\gamma_0 = 6\pi r\eta$ is the viscosious drag, or derived from the fit of the mean square displacement (MSD) of the freely diffusion particle plotted against the time:

$$MSD(t) = 2dDt \tag{2}$$

where d – is the number of dimensions of space used for the calculation of MSD, and

$$MSD(t) = \langle (x(t'+t) - x(t'))^2 \rangle = \frac{1}{N - t/\delta t} \sum_{i=1}^{N-t/\delta t} (x(i\delta t + t) - x(i\delta t))^2 \tag{3}$$

where $1/\delta t$ is the sampling frequency and N is the total number of position measurements.

The trap stiffness can be approximated using the Equipartition Theorem:

$$\left\langle r\frac{\partial H}{\partial r}\right\rangle = k_B T \qquad V = \frac{1}{2}k(x^2 + y^2) = \frac{1}{2}kr^2 \qquad \Rightarrow \qquad k\left\langle r^2\right\rangle = k_B T \tag{4}$$

where k is the "spring constant" or our trap, k_B is Boltzmann's constant, T is temperature in Kelvin and $<r^2>$ is the average of the squared deviation from the mean particle position. Calculate the force per unit displacement in the x and y directions on a particle using sensible force and length units. Measure this restoring force for a number of different beam intensities and plot the force as a function of intensity.

In using the Equipartition Theorem, the assumption of a parabolic potential was made. To check the validity of this assumption a probability density for particle position can be created. The probability $p(x)dx$ of finding a molecule or a small particle in a potential V*(x)* in a volume dx in thermal equilibrium is described by Bolzmann statistics:

$$p(x)dx = C\exp\left(-\frac{V(x)}{k_B T}\right) \tag{5}$$

Calculating the distributions $p(x)$ for different displacements allows to calculate the potential of the trap as:

$$V(x) = -k_B T \ln(p(x)) + k_B T \ln C \tag{6}$$

Fit this potential with a Gaussian from Eq.4 and find the trap stiffness, k.
This method requires the absolute calibration of the displacements, which can be obtained translating an immobile object in the sample over a given distance measured with the calibrated translational stage.

Literature:

1. D. G. Grier, "A revolution in optical manipulation", *Nature* **424** (2003) 810
2. Visualizing Single DNA-bound proteins using DNA as a scanning probe http://www.nat.vu.nl/en/sec/compl/dualdna/index-en.php
3. P. Nakroshis, M. Amoroso, J. Legere, and C. Smith, "Measuring Boltzmann's constant using video microscopy of Brownian motion," Am. J. Phys. **71**, 568–573 (2003).
4. F. Reif, *Simple Discussion of Brownian Motion* (pp 560-567), in *Fundamentals of Statistical and Thermal Physics* (McGraw-Hill, Singapore, 1985)
5. Feynman, Leighton, Sands, *The Brownian Movement* (pp 41-1/41-4 and pp 41-12/41-15), in *The Feynman Lectures on Physics*
6. Abramowitz M, Davidson MW (2007). Optical Microscopy, Review Article.
7. Basic Concepts in Optical Microscopy
8. J. Bechhoefer and S. Wilson, "Faster, cheaper, safer optical tweezers for the undergraduate laboratory, *American Journal of Physics* **393** (2002) 393.
9. P. Galajda, "Principles of optical tweezers"
10. http://en.wikipedia.org/wiki/Optical_tweezers
11. http://www2.bioch.ox.ac.uk/~oubsu/ebjknight/toc.html
12. Oil immersion
13. ETH - CBL - Particle Tracking Plug-In for ImageJ
14. ETH - CBL - Background subtractor for ImageJ
15. Viewing and Projection Eyepieces