Optical Trapping at VU university physics lab

[redacted]

September 4, 2018

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1 Understanding optical trapping

Optical trapping is the technique of using lasers to manipulate small objects, such as small transparent globes (beads) or even tiny insects. It is possible to measure the mechanical properties of many small systems by manipulation of attached beads. Examples of this are the measurements of the extension of DNA, the fusing of membranes or small fluid dynamics experiments.

A basic and intuitive explanation of optical trapping can be given by a ray description. Consider a transparent bead (whose index of refraction is larger than that of surrounding medium), placed in an intensity gradient (figure 1). A ray that travels through the bead is refracted twice due to the change in index of refraction at the interfaces. The change in direction of the ray is accompanied by a corresponding change in momentum of the ray, and with an opposite change of momentum of the bead. The force the ray exerts on the bead is proportional to the number of photons in the ray, and thus to the local intensity of the beam. The resulting force of all the rays traveling through the bead is directed to highest intensity: the focus of the beam. This force is called the gradient force, due to the gradient in electric field which produces it. Light that hits the bead is not only refracted but also reflected on and scattered from the bead. This generates radiation pressure on the bead, and results in a scattering force in the direction of propagation of the beam. This can be counteracted by a gradient in this direction, which can be achieved by focusing the laser in a small area. This can be achieved by using high numerical aperture objectives, canceling the downstream radiation pressure.

Before continuing on the specifics of this setup, it is useful to set up a system of axes (figure 2). A commonly used system has its z-axis perpendicular to the laser beam, with positive z pointing away from the laser source. The x- and y-axis are perpendicular to the laser, creating a right handed system.

2 The Setup

In addition to trapping a bead we need some way of detecting the state of the sample. To do so, an imaging system based on a microscope and quadrant photodiode (QPD) are added to the setup (figure 3 and figure 4).

The imaging system is mainly used to detect the position and state of captured beads. The microscope shares part of its optical pathway with the trapping pathway, making sure the imaging and trapping are aligned. Two dichroic mirrors combine and split the trapping and imaging pathways while several filters make sure no mixing takes place.

The QPD captures the trapping laser after going through the sample. As the bead moves in the trap it will diffract the laser light, changing the QPD signal, which in turn gives us information on the position of the bead.

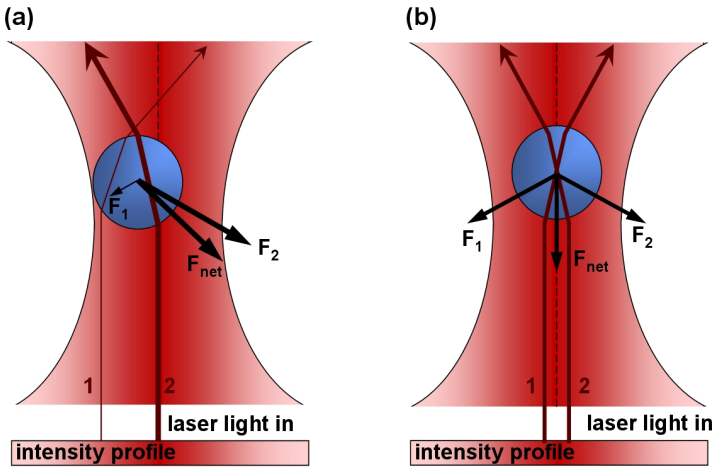

Figure 1: (a) Ray optics description of the gradient force. A bead is illuminated by a beam of light with a Gaussian intensity profile. Two representative rays of light of different intensities (represented by lines of different thickness) from the beam are shown. The refraction of the rays by the bead changes the momentum of the photons. The larger momentum change of the more intense rays results in a net force toward the focus. (b) A bead in the center of the trap, balancing all forces to create a zero net force. Image adapted from http://en.wikipedia.org/wiki/Optical_tweezers

2.1 The laser

The laser used in this setup is a single mode fibre coupled diode laser, with a wavelength of 980 nm and power of 330 mW. The high wavelength puts this laser in the infrared spectrum, making it invisible. The drawback of this is that this laser doesn't trigger a blink reflex when entering the eye. Coupled with an output of 330 mW this makes for a dangerous device. However, in normal use the laser will be contained in the setup and poses no danger. Take care when using reflecting surfaces such as microscope slides, watches and mirrors near the setup. Disable the laser when placing or removing the sample.

The laser has two controllers: one for power, the other for temperature. This set of controllers ensures that the output power of the laser will be extremely stable, which is important to maintaining a constant trapping force.

2.2 The quadrant photodiode

At the end of the laser pathway is the quadrant photodiode. This consists of 4 photodiodes, arranged in a square (fig. 5). The output of those diodes is a function of both the the power and position of the laser.

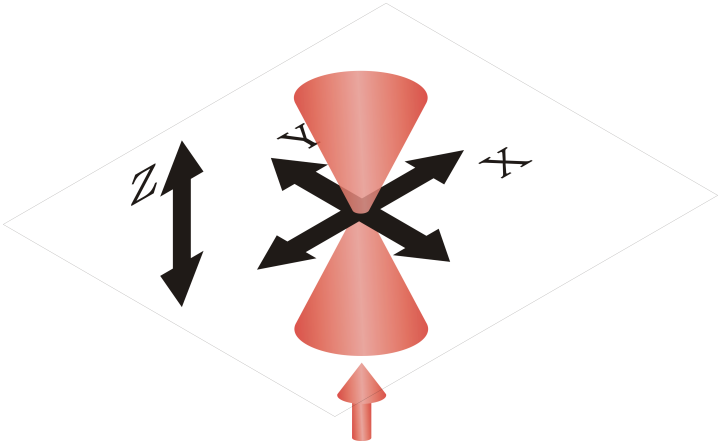

Figure 2: A system of axes commonly used in optical trapping. Parallel to the laser beam runs the z-axis, perpendicular to that is the x-y plane.

The x position of the laser beam on the QPD is calculated using the difference between the left and right half of the QPD (eq. 1), the y position by taking the difference between top and bottom (eq. 2)

$$x = \frac{(A2+B2)-(A1+B1)}{A1+A2+B1+B2} \quad (1)$$

$$y = \frac{(A1+A2)-(B1+B2)}{A1+A2+B1+B2} \quad (2)$$

As the bead moves in x- or y-direction relative to the laser, it will diffract the laser. As the QPD detects changes in laser position, it gives a measure of the bead position. For small movements of the bead this relation can be assumed to be linear.

2.3 The microscope

In this setup the sample is illuminated using a very bright LED. The light emitted by the LED is made parallel using a collimator. The parallel light passes through a diaphragm and is then focussed on a plane in the sample using a condensor. The amount of light reaching the sample can be decreased by tuning a diaphragm. The condensor is at the top of the sample.

The sample scatters the light in multiple directions. Part of it is captured by a high-aperture oil immersed microscope objective, with it's focal point coinciding with the focal plane of the condensor. Together with an imaging lens further away in the setup, this effectively forms a microscope. The microscope is viewed using a CCD camera coupled to the PC. Start the program 'uc480 Viewer' (see section 3.1) to view the image. The objective is at the bottom of the sample.

To get a good view the microscope needs to be focused using the z-axis micrometer actuator. Use the edge of the sample in focusing. Try not to break the sample by

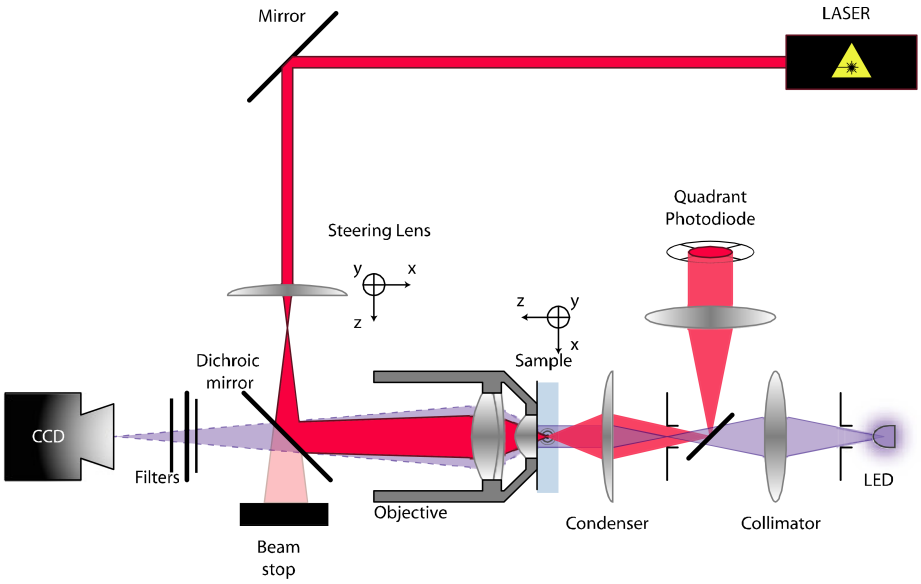

Figure 3: Schematic representation of the setup used

pushing the condensor through the sample; this obviously damages the sample, but might also damage the condensor. The focus of the microscope coincides with the focus of the laser beam, so any object trapped is automatically brought into focus.

The camera is loosely mounted to the setup. This allows removal of the camera, allowing access to an infrared blocking filter. By removing the filter the trapping laser can be visualized on camera. As the light of the laser moves away from the camera it is mostly invisible. Only when the focal plane is on an interface between glass and water the laser will be partially reflected, making it visible as a bright dot. This can be used to find the glass interfaces which is very useful focusing the microscope.

Removing the filter has a drawback: as the laser is much brighter than the background illumination used, it makes it very hard to see things at the focus of the laser. This makes it impossible to see trapped beads properly, so replace the filter to fix this.

2.4 The sample stage

The sample, often built on a microscope slide, is held in place by the sample stage. As the microscope is built upside down and views from the bottom the sample needs to be inserted with the cover slide (the thin piece of glass) at the bottom. The sample stage is used to move the sample relative to the rest of the setup, in effect moving the trap around. There are two independent mechanisms for doing this:

Micrometer actuators These devices employ precisely calibrated screws to move the

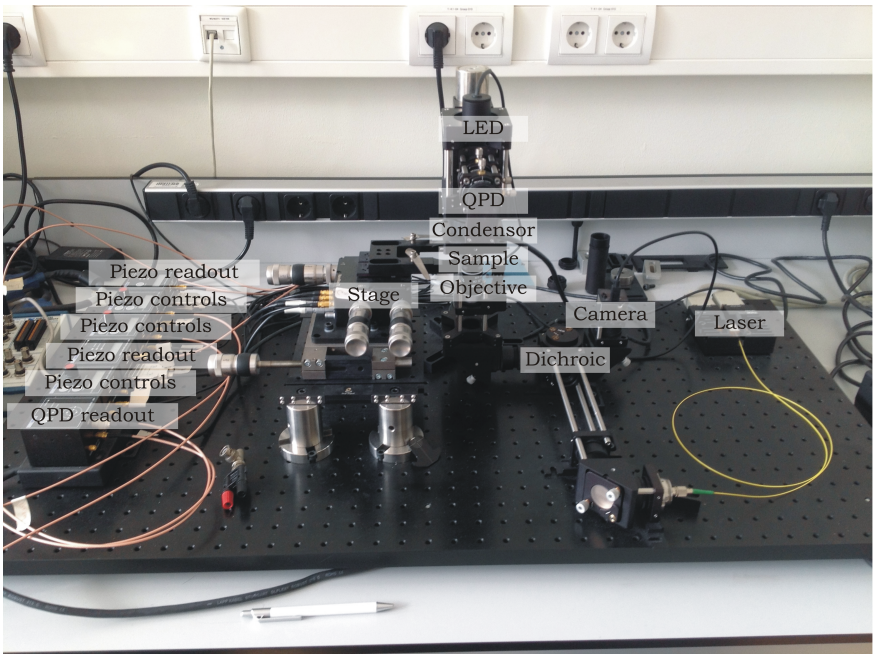

Figure 4: An actual setup with labeled parts

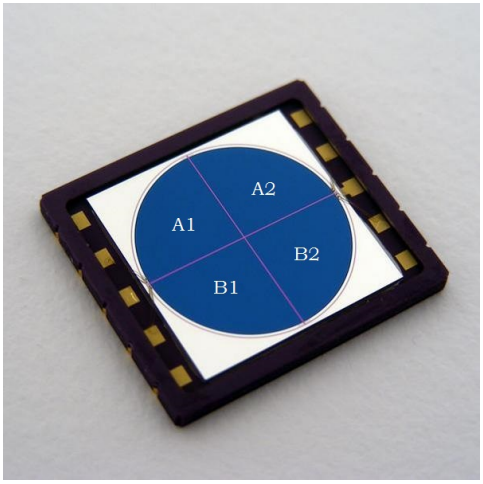

Figure 5: A quadrant photodiode with quadrants labeled. Figure adapted from Elcodis

sample around in x, y and z direction. On the actuators is a printed scale which allows direct measurements of stage position. These controls are very useful for finding/trapping beads and focusing the microscope.

Piezo elements The stage is built upon a block of piezo-crystals. Piezo crystals expand and contract when an electric potential is applied. In this setup this is used to move the stage around in x, y and z direction. The range of this movement is typically small, about 10 micrometers. By using an external input (e.g. a function generator) the stage can be moved around automatically. A built-in set of strain gauges allows acquisition of position data.

2.5 Starting the setup

1. Connect all the power plugs.
2. Switch on the laser power. Set the controls to contstant power mode (*const p*) and the display to power (*display pld*). The emission of the laser is controlled with the *laser on* button. Do not yet activate it.
3. Switch on the temperature controller at 14 °C (bottom power supply). Activate it using the *tec on* button.
4. Switch on the National Instruments (NI) data acquisition interface.
5. Switch on the Thorlabs T-cube power supply.
6. Switch on the PC and monitors, logging in using study/steady as account/ password.
7. On the PC, start the *uc480 viewer* program to visualize the sample.
8. On the PC, start the *APTuser* program to configure the piezo stage controls.
9. Put a drop of objective oil on the cover slip of the sample. Slide the sample holder to the left and place the sample in the sample holder. The sample is put in upside-down, with the cover slip and oil at the bottom. Be careful not to touch or move the objective.
10. Focus the microscope.
11. Enable the laser. Set it to about 330 mW of power, this should be enough for most experiments.
12. Trap a bead by moving the stage (using the micrometer actuators) such that a bead is in the trapping beam.
13. Move the bead into the center of the QPD for best force detection.

Powering down the setup is done in (mostly) reverse order.

3 Measurements

In an experiment two physical observables are of interest: the force exerted on the bead and the location of the bead relative to the stage. The first can be directly observed from the QPD sensor, but the latter depends on both QPD and strain gauge signal (figure 6). The position of the bead relative to the laser x_{bead} is measured by QPD, the position of the laser relative to the sample x_{laser} by strain gauge. The relative position of the bead x_{total} is then found by subtracting x_{bead} from x_{laser}.

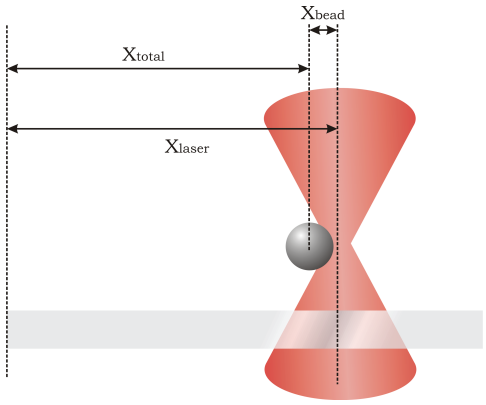

Figure 6: Finding the position of the bead relative to the stage involves multiple sensors

Almost all data acquisition will be done using the PC. Several programs are available to aid in this.

3.1 uc480 viewer (Microscope)

This program is used to view the microscope. After starting the program, press the *play* button to view the microscope.

This program also has some drawing functions; use the freehand, line and circle tools to mark features such as beads or laser position on the screen. This is especially helpful for marking the trapping laser position without filter, then replacing the filter. It can also be useful when calibrating the piezo strain gauges or checking bead size.

3.2 APTuser (Piezo elements and QPD)

This program is used both for live measurements of the Thorlabs instruments on the PC, as well as configuring them. APTuser cannot be used to collect data over time, so a LabView program is written for this.

Note that most instruments have several in- and output options. This may cause one instrument to influence the other through the inbuilt communication channels.

Because of a bug sometimes no instruments show up after starting the program. To remedy this simply restart the program until it works.

Make sure the QPD sensor is in 'monitor' mode. This setting directly links the signal of the QPD to the analog outputs. Open/closed loop are used in feedback systems. Make sure the laser input of the QPD does not exceed the maximum input. This is shown by the bar on the right of the QPD output window, which turns red when maximum input is exceeded.

3.3 Simple version Aquire data_NI_MX (Piezo elements and QPD)

This program is used to acquire data over time. Together with a function generator this can be used to automatically generate datasets of many types. After starting the program, select your datachannels using the 'browse data' function. Choose the maximum number of samples to take, along with the type of output required (.csv or .calib). Then press the run button to set the program to stand by.

Press the 'write to disk' button to start taking samples. When the maximum number of samples is taken or the 'stop' button is clicked the data will be saved to disk.

4 Calibration

All collected data will have units of volt. Typically, we want the position of the stage in some unit of length and force in some unit of force. To get there the strain gauges and QPD need to be calibrated.

4.1 Calibration of strain gauges

The stage can be moved both using the micrometer actuators and piezo elements. Attached to the piezo elements are strain gauges, which create a voltage linearly related to the position of the stage. Calibrating these is done as follows:

1. Attach a multimeter to the output of the strain gauge to be calibrated.
2. Find an object stuck to the glass, for example a bead or some dirt.
3. Use the camera software to mark the location of the object.
4. Apply a voltage to the piezo element, moving the object away from the mark.
5. Use the micrometer actuator to move the object back to the mark.
6. Record output voltage of strain gauge.
7. Record micrometer readout.

Repeat steps 4-7 several times, covering the whole voltage range (0-75 V) a few times. Note that some hysteresis occurs, so take measurements moving in both the positive and negative direction.

After obtaining the data do a linear fit to find the relation between position and voltage readout.

4.2 Calibration of QPD

The quadrant photodiode is used both for tracking the position of the bead relative to the laser and force detection, in both x-and y-directions. This gives us four observables while the QPD gives only two signals. Therefore there need to be two calibrations: one of force in N/V and one of position in m/V. There are several methods of calibration, all of which are described more detail in the article by Appleyard et al.:

Scanning By simply scanning the laser over a bead stuck to the surface of the sample the relation between position of the bead and QPD output can be determined. This can be done seperately for x- and y-direction. This only gives a m/V calibration.

The power spectrum The force applied to the bead can be modelled as an harmonic potential. Without friction this makes the bead a harmonic oscillator, which can be used for calibration. This method is simplified by a LabVIEW program simply called 'powerspectrum'. It gives both a calibration in N/V and m/V.

Fluid dynamics A small sphere moving in a fluid is influenced by drag. It is possible to calculate this drag using Stoke's law and compare it to QPD readouts, thus calibrating the device in N/V.

Equipartition The movement of the sphere is caused by thermal fluctuations. The equipartition theorem gives a measure of the energy of these fluctuations on each independent axis. This can be used to calculate the trap stiffness from noise in the bead position. This gives a calibration in N/V, thus requiring an additional a calibration in m/V.

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Assistant version

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1 Understanding optical trapping

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In addition to trapping a bead we need some way of detecting the state of the sample. To do so, an imaging system based on a microscope and quadrant photodiode (QPD) are added to the setup (figure 3 and figure 4).

The imaging system is mainly used to detect the position and state of captured beads. The microscope shares part of its optical pathway with the trapping pathway, making sure the imaging and trapping are aligned. Two dichroic mirrors combine and split the trapping and imaging pathways while several filters make sure no mixing takes place.

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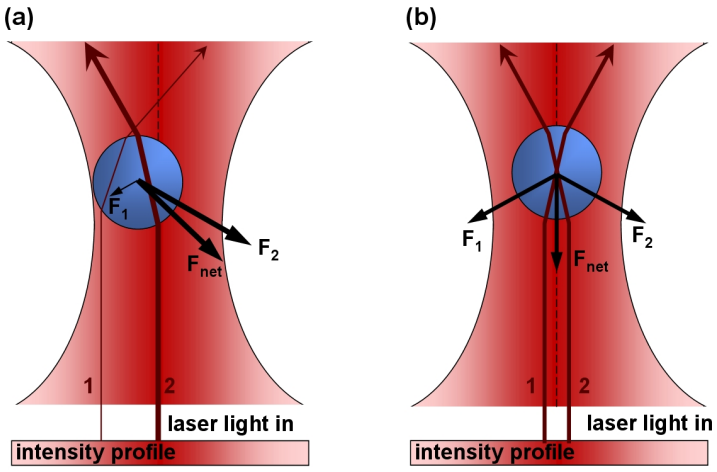

Figure 1: (a) Ray optics description of the gradient force. A bead is illuminated by a beam of light with a Gaussian intensity profile. Two representative rays of light of different intensities (represented by lines of different thickness) from the beam are shown. The refraction of the rays by the bead changes the momentum of the photons. The larger momentum change of the more intense rays results in a net force toward the focus. (b) A bead in the center of the trap, balancing all forces to create a zero net force. Image adapted from http://en.wikipedia.org/wiki/Optical_tweezers

2.1 The laser

The laser used in this setup is a single mode fibre coupled diode laser, with a wavelength of 980 nm and power of 330 mW. The high wavelength puts this laser in the infrared spectrum, making it invisible. The drawback of this is that this laser doesn't trigger a blink reflex when entering the eye. Coupled with an output of 330 mW this makes for a dangerous device. However, in normal use the laser will be contained in the setup and poses no danger. Take care when using reflecting surfaces such as microscope slides, watches and mirrors near the setup. Disable the laser when placing or removing the sample.

The laser has two controllers: one for power, the other for temperature. This set of controllers ensures that the output power of the laser will be extremely stable, which is important to maintaining a constant trapping force.

Assistant info

The setup as-is is quite safe: the laser is contained in the setup. The only places where the laser can be accessed are where it's divergent, thus posing a reduced risk. The unsafest thing that can be done is removing the QPD which will allow a parallel beam

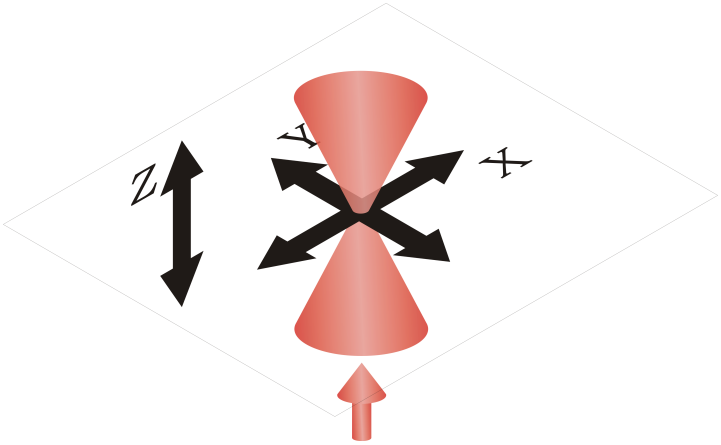

Figure 2: A system of axes commonly used in optical trapping. Parallel to the laser beam runs the z-axis, perpendicular to that is the x-y plane.

to exit the setup at about eye's height.

2.2 The quadrant photodiode

At the end of the laser pathway is the quadrant photodiode. This consists of 4 photodiodes, arranged in a square (fig. 5). The output of those diodes is a function of both the the power and position of the laser.

The x position of the laser beam on the QPD is calculated using the difference between the left and right half of the QPD (eq. 1), the y position by taking the difference between top and bottom (eq. 2)

$$x = \frac{(A2+B2)-(A1+B1)}{A1+A2+B1+B2} \quad (1)$$

$$y = \frac{(A1+A2)-(B1+B2)}{A1+A2+B1+B2} \quad (2)$$

As the bead moves in x- or y-direction relative to the laser, it will diffract the laser. As the QPD detects changes in laser position, it gives a measure of the bead position. For small movements of the bead this relation can be assumed to be linear.

Assistant info

When the signal from the QPD is bad, the laser needs to be realigned. To do this:

- Switch the laser OFF.
- Insert a sample into the setup.
- Loosen the four screws on the QPD mount, then remove the mount together with the QPD and diafragm.

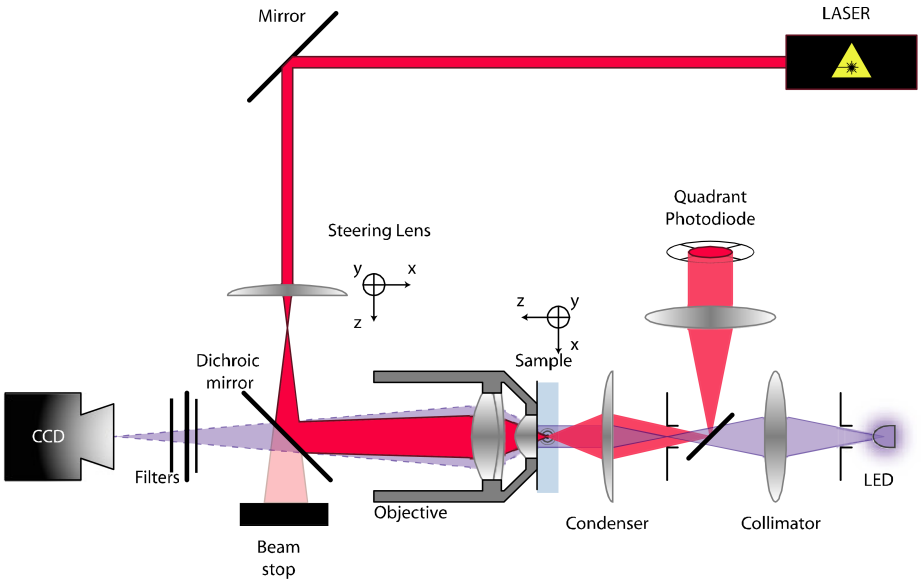

Figure 3: Schematic representation of the setup used

- Loosen the four screws on the lens mount, then remove it.
- Get someone to hold the IR card where the QPD used to be, standing in such a way that the laser is blocked by his body if it misses the card.
- Switch the laser ON.
- Move the IR card away until the focus of the beam is found.
- Rotate the knob next to the objective to make the beam more parallel, moving the focus away from the setup
- Use the three screws on the upper dichroic mirror housing to align the beam to the 4 steel posts.
- Switch the laser OFF.
- Replace the lens and mount.
- Replace and fasten the QPD, diafragm and mount.
- Slide the lens and mount assembly to maximise the QPD signal.
- Fasten the lens mount.
- Align the QPD and lens.

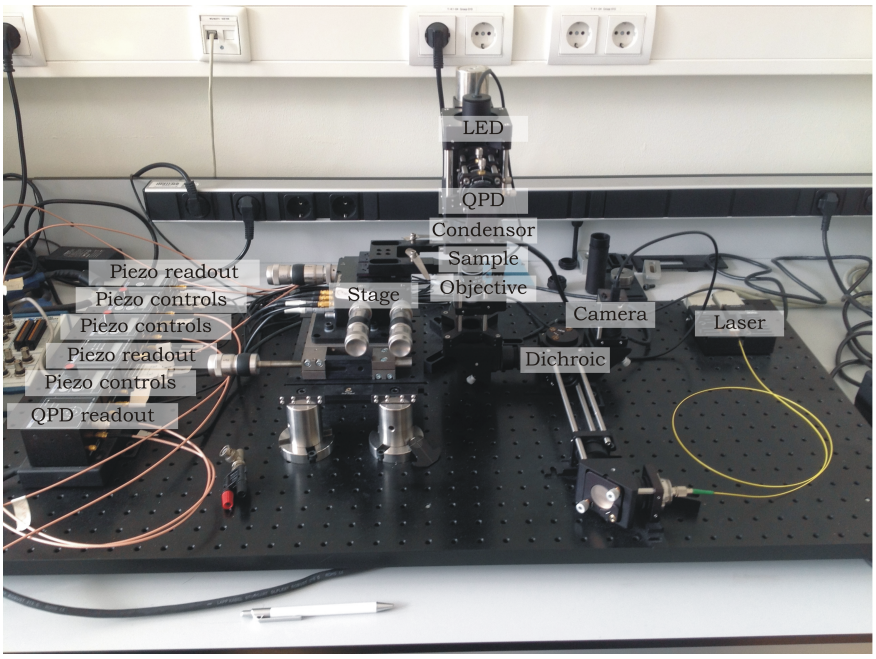

Figure 4: An actual setup with labeled parts

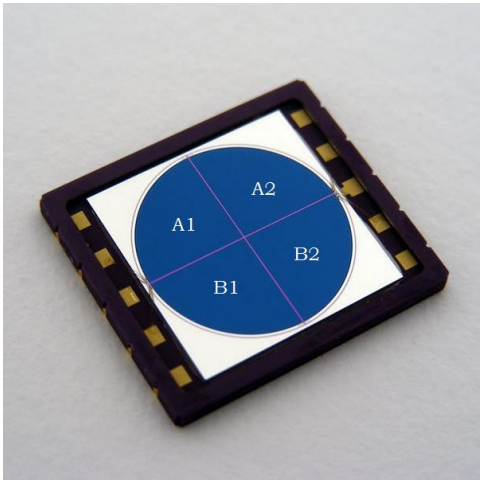

Figure 5: A quadrant photodiode with quadrants labeled. Figure adapted from Elcodis

Choosing the right laser intensity also increases signal quality: saturation will induce clipping of the sensor while a low intensity will decrease the signal to noise ratio significantly.

2.3 The microscope

In this setup the sample is illuminated using a very bright LED. The light emitted by the LED is made parallel using a collimator. The parallel light passes through a diaphragm and is then focussed on a plane in the sample using a condensor. The amount of light reaching the sample can be decreased by tuning a diaphragm. The condensor is at the top of the sample.

The sample scatters the light in multiple directions. Part of it is captured by a high-aperture oil immersed microscope objective, with it's focal point coinciding with the focal plane of the condensor. Together with an imaging lens further away in the setup, this effectively forms a microscope. The microscope is viewed using a CCD camera coupled to the PC. Start the program 'uc480 Viewer' (see section 3.1) to view the image. The objective is at the bottom of the sample.

To get a good view the microscope needs to be focused using the z-axis micrometer actuator. Use the edge of the sample in focusing. Try not to break the sample by pushing the condensor through the sample; this obviously damages the sample, but might also damage the condensor. The focus of the microscope coincides with the focus of the laser beam, so any object trapped is automatically brought into focus.

The camera is loosely mounted to the setup. This allows removal of the camera, allowing access to an infrared blocking filter. By removing the filter the trapping laser can be visualized on camera. As the light of the laser moves away from the camera it is mostly invisible. Only when the focal plane is on an interface between glass and water the laser will be partially reflected, making it visible as a bright dot. This can be used to find the glass interfaces which is very useful focusing the microscope.

Removing the filter has a drawback: as the laser is much brighter than the background illumination used, it makes it very hard to see things at the focus of the laser. This makes it impossible to see trapped beads properly, so replace the filter to fix this.

Assistant info

Most of the beads will be found at the glass surfaces on the top and bottom of the channel. Focusing the microscope can best be done at the edge of the channel; move the sample in y-direction until the parafilm/tape becomes visible, then use the z-controls to focus on the film. Continue until some beads become visible too. The laser can also be used to find the glass interfaces: when the glass is in focus, the laser will be reflected as a small dot instead of the usual large interference rings.

Removing and replacing the camera to remove the filter will usually de-align the camera somewhat: the apparent position of the laser shift. Nudging the camera can remedy this, but it usually isn't a problem.

2.4 The sample stage

The sample, often built on a microscope slide, is held in place by the sample stage. As the microscope is built upside down and views from the bottom the sample needs to be inserted with the cover slide (the thin piece of glass) at the bottom. The sample stage is used to move the sample relative to the rest of the setup, in effect moving the trap around. There are two independent mechanisms for doing this:

Micrometer actuators These devices employ precisely calibrated screws to move the sample around in x, y and z direction. On the actuators is a printed scale which allows direct measurements of stage position. These controls are very useful for finding/trapping beads and focusing the microscope.

Piezo elements The stage is built upon a block of piezo-crystals. Piezo crystals expand and contract when an electric potential is applied. In this setup this is used to move the stage around in x, y and z direction. The range of this movement is typically small, about 10 micrometers. By using an external input (e.g. a function generator) the stage can be moved around automatically. A built-in set of strain gauges allows acquisition of position data.

Assistant info

The micrometer actuators are the most versatile movement system. Being totally analog, they react instantly to input and don't lag at all. They have a wide range of movement (several cm) in the coarse controls and a few mm in fine. If the fine control knob turns the coarse ring, the fine control is at its end. Rotate it back and compensate using the coarse controls. The piezo elements are only useful for automated measurements: by using a function generator repeatable motions can be made while the DAQ card collects data.

2.5 Starting the setup

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4. Switch on the National Instruments (NI) data acquisition interface.
5. Switch on the Thorlabs T-cube power supply.
6. Switch on the PC and monitors, logging in using study/steady as account/ password.
7. On the PC, start the *uc480 viewer* program to visualize the sample.

8. On the PC, start the *APTuser* program to configure the piezo stage controls.
9. Put a drop of objective oil on the cover slip of the sample. Slide the sample holder to the left and place the sample in the sample holder. The sample is put in upside-down, with the cover slip and oil at the bottom. Be careful not to touch or move the objective.
10. Focus the microscope.
11. Enable the laser. Set it to about 330 mW of power, this should be enough for most experiments.
12. Trap a bead by moving the stage (using the micrometer actuators) such that a bead is in the trapping beam.
13. Move the bead into the center of the QPD for best force detection.

Powering down the setup is done in (mostly) reverse order.

Assistant info

The blinking of the strain gauge readouts can be suppressed by pressing the *mode* button for a few seconds. This will cause the gauges to re-zero and stop blinking. When powering down it is important to shut down the APTuser *before* disabling the T-cube power supply. If this is done in reverse order, APTuser will generate a never ending string of errors which can only be ended by killing the APTuser process. The rest doesn't matter too much.

3 Measurements

In an experiment two physical observables are of interest: the force exerted on the bead and the location of the bead relative to the stage. The first can be directly observed from the QPD sensor, but the latter depends on both QPD and strain gauge signal (figure 6). The position of the bead relative to the laser x_{bead} is measured by QPD, the position of the laser relative to the sample x_{laser} by strain gauge. The relative position of the bead x_{total} is then found by subtracting x_{bead} from x_{laser}.

Almost all data acquisition will be done using the PC. Several programs are available to aid in this.

Assistant info

All data acquisiton and instrument configuration runs over USB. USB has a limited amount of bandwidth. This is especially noticable when using both the uc480 viewer and aquire data program at the same time. The view on the webcam will lag and the data aquisition might drop frames. Shut down any programs not in used to free up bandwidth on the USB.

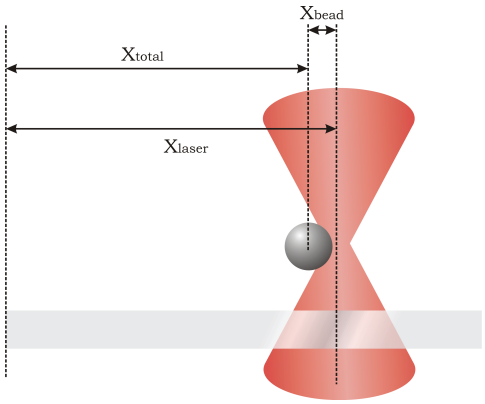

Figure 6: Finding the position of the bead relative to the stage involves multiple sensors

3.1 uc480 viewer (Microscope)

This program is used to view the microscope. After starting the program, press the *play* button to view the microscope.

This program also has some drawing functions; use the freehand, line and circle tools to mark features such as beads or laser position on the screen. This is especially helpful for marking the trapping laser position without filter, then replacing the filter. It can also be useful when calibrating the piezo strain gauges or checking bead size.

3.2 APTuser (Piezo elements and QPD)

This program is used both for live measurements of the Thorlabs instruments on the PC, as well as configuring them. APTuser cannot be used to collect data over time, so a LabView program is written for this.

Note that most instruments have several in- and output options. This may cause one instrument to influence the other through the inbuilt communication channels.

Because of a bug sometimes no instruments show up after starting the program. To remedy this simply restart the program until it works.

Make sure the QPD sensor is in 'monitor' mode. This setting directly links the signal of the QPD to the analog outputs. Open/closed loop are used in feedback systems. Make sure the laser input of the QPD does not exceed the maximum input. This is shown by the bar on the right of the QPD output window, which turns red when maximum input is exceeded.

Assistant info

Make sure the Thorlabs PSU is actually on, otherwise the students will keep rebooting the software.

This program will be mostly used to change the settings of the setup. When using the QPD, make sure it's set to *monitor*, otherwise no data will be generated. The input of the piezo controllers needs to be set to accept the analog output if a function generator is to be used.

3.3 Simple version Aquire data_NI_MX (Piezo elements and QPD)

This program is used to acquire data over time. Together with a function generator this can be used to automatically generate datasets of many types. After starting the program, select your datachannels using the 'browse data' function. Choose the maximum number of samples to take, along with the type of output required (.csv or .calib). Then press the run button to set the program to stand by.

Press the 'write to disk' button to start taking samples. When the maximum number of samples is taken or the 'stop' button is clicked the data will be saved to disk.

Assistant info

The .csv option sometimes loses data, so it's safer to save data as a .calib file. I couldn't get .calib to load in Origin as it is an unknown binary format, so it needs to be converted later. With each measurement it is important to write down relevant data, such as what kind of bead/dna/sample is used and what each data channel represents. Also the number of channels is important as .calib files don't save this: conversion from .calib to .csv using a wrong number of channels will mix all data.

4 Calibration

All collected data will have units of volt. Typically, we want the position of the stage in some unit of length and force in some unit of force. To get there the strain gauges and QPD need to be calibrated.

Calibration

Because all data is in units of volt a great deal of confusion might arise. Drawing some sort of flowchart wich shows all data conversion often helps. A common mixup is

1. Bead position (from QPD)
2. Stage position (from strain gauge)

because both deal with position. Drawing a schematic with positions of laser, bead and stage usually helps. Another common mixup is

1. Bead position (from QPD)
2. Bead force (from QPD)

because both signals are derived from the QPD signal. That these are related by the spring constant is usually a good enough explanation.

4.1 Calibration of strain gauges

The stage can be moved both using the micrometer actuators and piezo elements. Attached to the piezo elements are strain gauges, which create a voltage linearly related to the position of the stage. Calibrating these is done as follows:

1. Attach a multimeter to the output of the strain gauge to be calibrated.
2. Find an object stuck to the glass, for example a bead or some dirt.
3. Use the camera software to mark the location of the object.
4. Apply a voltage to the piezo element, moving the object away from the mark.
5. Use the micrometer actuator to move the object back to the mark.
6. Record output voltage of strain gauge.
7. Record micrometer readout.

Repeat steps 4-7 several times, covering the whole voltage range (0-75 V) a few times. Note that some hysteresis occurs, so take measurements moving in both the positive and negative direction.

After obtaining the data do a linear fit to find the relation between position and voltage readout.

Assistant info

This is the easiest of calibrations and probably the best way to start with the setup. It will often yield a very nice straight line with a low error. It teaches the students almost all functions of the setup in an easy and forgiving way. After first starting the setup and focussing the microscope, they need to use both ways of stage movement: micrometer actuators to find a nice bead and piezo elements for the calibration. Strain gauge readouts can be taken in APTuser, the data acquisition program or using a simple multimeter. The QPD doesn't need to be used yet.

In this calibration the students will probably encounter both hysteresis and creep, as the piezo elements take a long time to reach their equilibrium position. They will have to decide on quick measurements vs slow measurements. Keep in mind that the final experiment, taking a DNA stretching curve, is probably done using a function generator operating at about 1 Hz.

4.2 Calibration of QPD

The quadrant photodiode is used both for tracking the position of the bead relative to the laser and force detection, in both x-and y-directions. This gives us four observables while the QPD gives only two signals. Therefore there need to be two calibrations: one of force in N/V and one of position in m/V. There are several methods of calibration, all of which are described more detail in the article by Appleyard et al.:

Scanning By simply scanning the laser over a bead stuck to the surface of the sample the relation between position of the bead and QPD output can be determined. This can be done seperately for x- and y-direction. This only gives a m/V calibration.

The power spectrum The force applied to the bead can be modelled as an harmonic potential. Without friction this makes the bead a harmonic oscillator, which can be used for calibration. This method is simplified by a LabVIEW program simply called 'powerspectrum'. It gives both a calibration in N/V and m/V.

Fluid dynamics A small sphere moving in a fluid is influenced by drag. It is possible to calculate this drag using Stoke's law and compare it to QPD readouts, thus calibrating the device in N/V.

Equipartition The movement of the sphere is caused by thermal fluctuations. The equipartition theorem gives a measure of the energy of these fluctuations on each independent axis. This can be used to calculate the trap stiffness from noise in the bead position. This gives a calibration in N/V, thus requiring an additional a calibration in m/V.

Assistant info

It's a good idea to do the scanning calibration first as it gives a good idea of the possibilities and limits of the QPD. It's also a nice introduction into the workings of the QPD, data aquisition program and function generator. Additionally it gives a lot of information on the QPD signal: if the signal is really nonlinear, it's probably improperly aligned or focussed. The methods using equipartition and power spectrum are really fast, and can be done from the same dataset. There's no reason not to try them. Just take a measurement of a loose bead and analyse the data. If there isn't enough time to do a DNA stretching experiment, or it somehow fails due to sample problems, the fluid dynamics method of calibrating can be turned into a nice backup experiment. It has some additional theoretics and has enough errors for a proper statistical analysis.

5 Samples

The students probably aren't allowed in the labs, so the assistant has to make the samples. The Physics of Living Systems group has all the required materials and knowledge on how to make those.

The loose beads sample takes about an hour to make and is very useful for all calibration steps. It will work for a few days, so making one at the start of the experiment is a good first step. Small air pockets may form but this shouldn't impact the working of the sample much.

The DNA tethering sample takes longer to make, best results are obtained when the antiDIG has incubated overnight and the sample is finished in the morning. This has

the added benefit of giving the students all day to do measurements. After use store the sample in a wet cell in cold storage, this *may* extend the life of the sample by one day. If it doesn't work anymore it can sometimes be used for calibration measurements.

5.1 DNA tethering sample

Building a flow cell

1. Take 1 microscope slide (76×26 mm) and 1 large cover slip (60×24 mm).
2. Clean with acetone.
3. Clean with ethanol.
4. Clean with mQ.
5. Dry the glass using a paper tissue.
6. Cut 2 strips of parafilm with a width of about 5 mm, put them on the sides of the cover slip.
7. Place the microscope slide orthogonally on the parafilm.
8. Melt the parafilm on a heatblock at 80 °C and push the two sides firmly together.

Casein buffer

- 1000 µl 10× RE-buffer
- 50 µl 20 mg ml^{-1} Casein
- 3.95 ml mQ

Beads

1. Dilute 5 µl of biotin coated beads in 50 µl of Casein buffer.
2. Spin the beads down at 13.2 kRPM for 2 minutes.
3. Remove the supernatant.
4. Resuspend the pallet in 50 µl Casein buffer.
5. Sonicate the beads for at least 15 min.

DNA tethers

1. Fill a flowcell with antiDIG ($20\,\mu g\,ml^{-1}$) and incubate overnight in a cold wet cell.
2. Flow in the DNA (Sfil 2 sites) diluted in 400 µl Casein buffer and incubate for 60 min in a cold wet cell.
3. Flush the flow chamber 4 times with Casein buffer.
4. Flush in the beads.
5. Gravity flush the beads with Casein buffer. Remove excess fluid.
6. Close the sample using molten parafine.

5.2 Bead sample

Building the cell

1. Take 1 microscope slide (76 mm×26 mm) and 1 cover slip (24 mm×24 mm).
2. Put two 50 mm strips of double sided tape on a smooth surface.
3. Cut two 3 mm and two 5 mm strips out of the tape using a razorblade.
4. Place the strips of tape on the microscope slide, wider ones at the outside, creating three channels.
5. Place the cover slip on top and press the two sides firmly together, removing air pockets from the tape.

Bead solutions

1. Dilute 5 µl of beads in 250 µl of mQ.
2. Dilute 5 µl of this dilution in 250 µl of mQ.
3. Now dilute 5 µl of the previous dilution in 250 µl of mQ.

Creating the sample

1. Fill the three channels with the three dilutions of beads. Remove excess fluid.
2. Close the sample usin nail polish.