

A-10 Optical and Magnetic Tweezers

From Physics 191r

Optical Tweezer Word version File:A10tweezer 10.doc

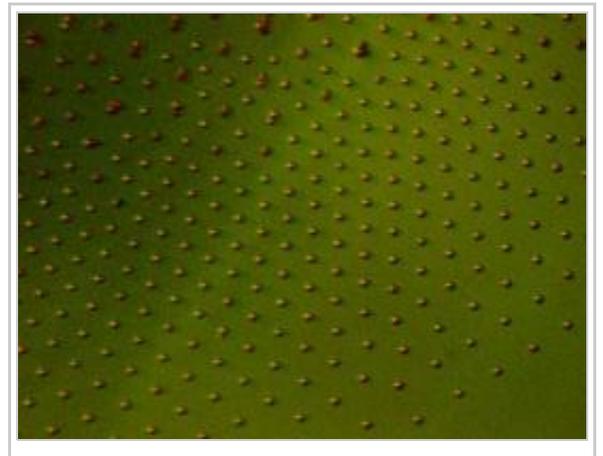
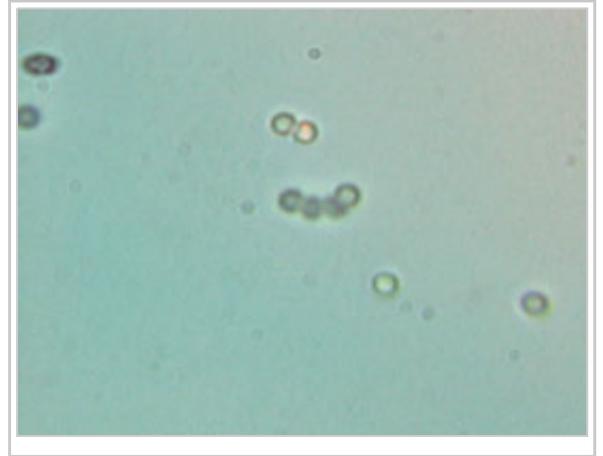
Optical Tweezer PDF version File:A10tweezer 10.pdf

ELECTROMAGNETIC TRAPPING: Optical and Magnetic Tweezers

author: Mara Prentiss, 2004

first experiment: yes

Optical tweezer video



(http://www.fas.harvard.edu/~phys191r/Bench_Notes/op_tweez.mov)

Magnetic tweezer video (http://www.fas.harvard.edu/~phys191r/Bench_Notes/mag_tweez.mov)

Contents

- 1 LEARNING GOALS

- 2 INTRODUCTION
- 3 APPARATUS
 - 3.1 Laser
 - 3.2 Laser controller
 - 3.3 Laser power monitor
 - 3.4 Laser beam profiler
 - 3.5 Sample Preparation
 - 3.5.1 Optical Tweezer
 - 3.5.2 Magnetic Tweezer
 - 3.6 Copper sample stage with temperature control
 - 3.7 Translation stage and controller
 - 3.8 Function generator/circle box/oscilloscope
 - 3.9 White light source
 - 3.10 Camera
 - 3.11 Microspheres
 - 3.12 Quadrant photodiode with I/V converter
 - 3.13 Magnetic Tweezer
- 4 EXPERIMENTAL PROCEDURE
 - 4.1 Investigations of the Optical Trap
 - 4.2 Investigations of Thermal Motion
 - 4.3 Optional Optical Tweezer Experiments
 - 4.4 Interparticle Interaction
 - 4.5 Magnetic Tweezer
- 5 NOTES
- 6 REFERENCES
 - 6.1 Optical Traps
 - 6.2 Brownian Motion
 - 6.3 Magnetic Field Gradient Traps
 - 6.4 2D Polymer Sphere Crystals
 - 6.5 2D Melting
 - 6.6 Ordered Trapped Particles
 - 6.7 Electromagnetic Traps
- 7 BENCH NOTES
- 8 APPENDIX A: Optical Tweezer Alignment Procedure
 - 8.1 Safety
 - 8.2 Beam alignment
 - 8.3 Camera alignment
 - 8.4 Trapping a particle
- 9 APPENDIX B: Particle Tracking using IDL
- 10 APPENDIX C: Particle Tracking using MATLAB
 - 10.1 Purpose
 - 10.2 Outline
 - 10.3 Details
 - 10.3.1 1) Make videos
 - 10.3.2 2) Import video and convert to grayscale
 - 10.3.3 3) Analyze the videos
 - 10.3.4 4) Analyze the data files

LEARNING GOALS

- Trap uncharged dielectric microbeads in aqueous solution with a focused laser beam.
- Calibrate the trap strength using Stokes drag.
- Analyze video with Matlab.
- Work safely with lasers.
- Observe crystalline structures of paramagnetic particles.

INTRODUCTION

The ability to manipulate small particles has revolutionized atomic physics and is beginning to have a substantial impact on biology. In recent years, three Nobel prizes have been awarded for achievements in the cooling and trapping of atoms. In addition, optical tweezers provide an important tool for investigating biological systems one molecule at a time providing information that is unavailable in investigations of bulk samples. In all of these experiments, the particles are manipulated by confining them in an electromagnetic “trap”, where the trap is formed by spatially dependent electromagnetic fields that produces a net force toward the center of the trap. Thus, if a particle in the trap is displaced from the trap center in any direction, it will be accelerated back toward the trap center.

It can be shown from Maxwell’s equations that a static electric field cannot provide a three dimensional restoring force on a charged particle toward a point in empty space. A similar argument can be made for static magnetic fields. As a consequence the earliest particle trapping experiments used AC electric fields to confine charged particles into a small volume. The exact shape of the electrodes is not important, and elementary school students have successfully constructed traps that confine dust particles in air using 60 Hz AC fields.^[1] Penning traps^[2] confine charged particles using a combination of AC electric fields and DC magnetic fields. Professor Gabrielse used a trap to confine anti-protons with which he subsequently formed anti-hydrogen.^[3]

These charged particle traps cannot trivially be extended to neutral particles, but neutral particles with permanent magnetic dipole moments can be trapped in magnetic field minima if the permanent dipole moments are aligned anti-parallel to the magnetic field. For example, if $m \sim -B$ then the energy $U \sim -\vec{m} \cdot \vec{B} \sim B^2$ is a minimum where B^2 is a minimum. The simplest magnetic field trap consists of two wire coils separated by a distance equal to their radius, where the direction of current flow in the two wires is opposite, resulting in a magnetic field zero at the center of the coils. The experiments that produced Bose Einstein condensates from collections of cold neutral atoms used magnetic trapping to contain those cold atoms with magnetic moments aligned antiparallel to B .

The AC electric fields present in light can also trap neutral particles. In these systems, the AC electric field at a frequency ω induces an AC dipole moment in the neutral particle, where the dipole moment is proportional to the electric field and also oscillates at frequency ω . The energy of an electric dipole moment, P , in an electric field, E , is given by $-P \cdot E$. The net average potential due to the AC field is the time average over one period. If P is in phase with E , the net average potential is proportional to $-|E|^2$, which is proportional to the intensity of the light. In a focused laser beam, the particle will be attracted to the focus, where the intensity is a maximum in all three directions resulting in a potential that is a minimum in all three directions. Such traps are called optical tweezers and have been used to confine atoms, molecules, sperm, individual cells, individual viruses, individual mitochondrial motors, etc.

In a purely conservative potential, particles entering the potential will leave the potential without being trapped. Stable particle confinement requires not only a trapping force, but also a damping force to dissipate energy. In the presence of a damping force, particles entering the trap lose energy as they traverse the trap, so that they do not have enough kinetic energy to escape. Thus, stable time independent particle trapping requires not only a three dimensional restoring force, but also an energy loss mechanism that permits particles entering the trap to remain confined.

If more than one particle is confined in the trap, the interactions between the particles can become important. Crystal structures have been formed in ion traps and optical tweezers when the repulsive interaction energy between the particles exceeds kT , the thermal energy of the particles. (If the repulsion is weak, the particles orient randomly.) Bose Einstein condensates result when the separation between the trapped particles is smaller than their deBroglie wavelength.

The magnetic tweezer uses a nonuniform static magnetic field to exploit the magnetic dipole/dipole interaction between paramagnetic beads. An ordered 2D crystal lattice of beads self-assembles at an air water interface. The lattice spacing in the crystal can then be controlled, allowing measurements of adhesion probability and strength of bonds between molecules such as DNA, as a function of separation distance, time, and temperature. This system can also be used to study 2D crystal formation and melting.

In this experiment, one can explore all three important components of particle traps: the trapping potential, the heating and cooling forces, and the interparticle interactions.

APPARATUS

The apparatus has two components: the optical tweezer and the magnetic tweezer. Both use optical microscopes to observe the small particles that are suspended in a liquid. In both cases, the damping is provided by Stokes drag due to the interaction with liquid. The temperature of the liquid then determines the temperature of the particles. In the case of the optical tweezer, the optical microscope objective focuses a laser beam in addition to imaging the sample. In the magnetic tweezer, a permanent magnet is used to produce a force on paramagnetic particles. The temperature of the liquid can be controlled using a thermoelectric cooler, and the motion of the microscope translation stage can be controlled electronically.

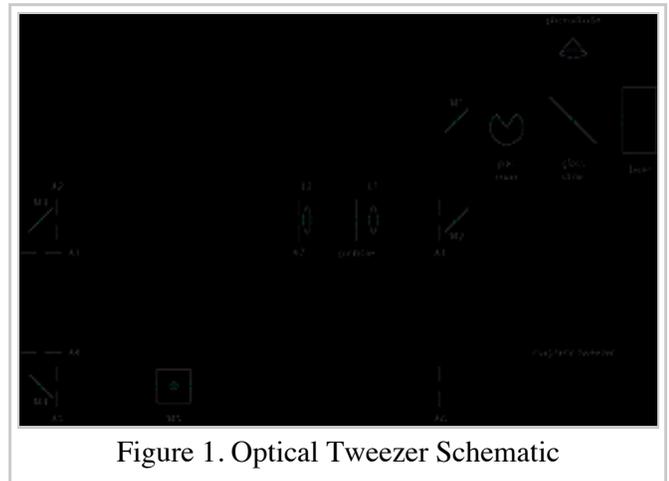


Figure 1. Optical Tweezer Schematic

This discussion assumes familiarity with the apparatus described in the AJP paper by Smith, et. al. The current apparatus is based on the one described in this paper with the difference that rather than a microscope per se, we use an inverted design in which the microscope objective faces upwards. Refer also to the schematic diagram at right. Detailed alignment procedure is described in Appendix A.

Laser

The source of laser light in this experiment is a Hitachi HL6501MG laser diode. Laser diodes work on a

principle similar to LEDs: a current flows forward through a semiconductor p-n junction where the charge carriers lose energy, emitting it in the form of a photon. The semiconductor facets are partially reflecting; they define a cavity where the lasing condition is established. The threshold for lasing is roughly 45 mA for this laser, and the light power emitted increases approximately linearly from there with diode current. A calibration curve can be found in the bench notes. The maximum current is 99 mA, where the power is approximately 35 mW. ATTEND THE RADIATION SAFETY LECTURE, AND DISCUSS SPECIFIC LASER SAFETY PROCEDURES WITH THE FACULTY OR STAFF BEFORE TURNING ON THE LASER. CAREFULLY FOLLOW THE SAFETY PRECAUTIONS DESCRIBED BELOW IN THE “ALIGNMENT HINTS” APPENDIX.

Laser controller

The laser diode is mounted in a Thorlabs TCLDM9 mount, which incorporates electrical connections for the laser diode as well as thermoelectric coolers that control the temperature of the laser. A Thorlabs ITC502 combination current/temperature controller supplies the laser diode current and controls temperature. Avoid excessive cycling of the laser power. Abrupt changes in laser current can destroy the laser. Thus it is best to leave the laser on for the duration of each lab period. A circular beam stop is in the beampath near the laser. Use it to block the beam when changing samples or inserting or removing optics from the beam path.

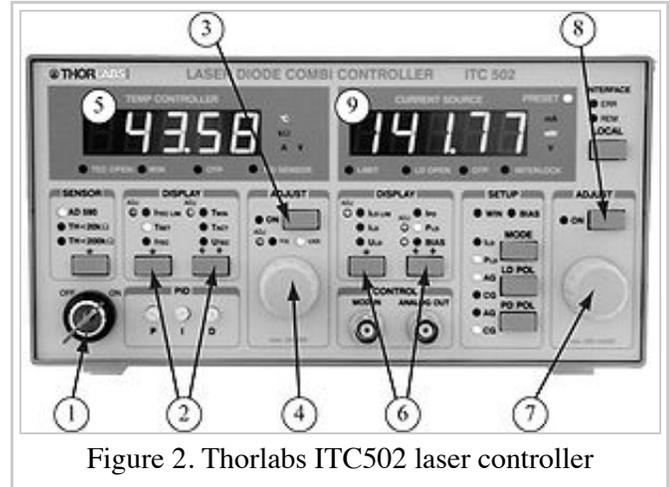


Figure 2. Thorlabs ITC502 laser controller

After discussing laser safety with the faculty or staff, when you are ready to turn on the laser, look for the laser key in the blue cabinet near the experiment. Turn on the controller with the key switch (Figure 2-1).

The left half of the ITC502 front panel is used to measure and control the laser temperature. Using navigation controls (Figure 2-2), display (Figure 2-5) T_{ACT} , the actual temperature. The units of temperature are $k\Omega$, the resistance of a thermistor mounted near the laser. Room temperature corresponds to 11.5 $k\Omega$. Display T_{SET} , and use the adjustment knob (Figure 2-4) to choose a temperature setpoint a little less than room temperature, i.e. a higher resistance value. (Why is the resistance of a thermistor inversely proportional to temperature?) Push the ON button (Figure 2-3) to initiate temperature control. Display T_{ACT} again, or I_{TEC} (current flowing to the thermoelectric coolers) using controls (Figure 2-2) and watch the controller oscillate as it tries to match the temperature setpoint. Oscillations should damp out within approximately ten minutes.

While waiting for the temperature to stabilize, prepare the laser current controls. Using navigation controls (Figure 2-6), display (Figure 2-9) I_{LD} , the laser diode current. Set the displayed value to zero, (i.e. knob (Figure 2-7) fully CCW). Turn ON the laser with power button (Figure 2-8), and gradually increase the laser current to the desired value, usually 45 – 50 mA, just above the lasing threshold. The maximum laser current is 99 mA; the controller will not permit the user to increase the current above this value.

Laser shutdown procedure is the opposite of startup: slowly reduce laser current to zero, toggle laser ON button (Figure 2-8) to OFF position. Turn off temperature controller, then switch off power using the key.

Laser power monitor

A glass slide reflects a few percent of the laser light into a Thorlabs PDA100A photodetector. A digital multimeter monitors the photodetector output for long-term laser drift.

Laser beam profiler

The beam profile of the laser diode is nonuniform. One can measure this with a photodiode/pinhole combination mounted on a 2-dimensional translation stage. In order to make a symmetrical optical trap, place a pinhole in the beam expander at the focus. Remeasure the profile with the pinhole in place to verify that it improves beam symmetry.

Sample Preparation

Optical Tweezer

To make samples for tweezing or Brownian motion studies, the microsphere stock solution is typically diluted in buffered saline solution at 1:1000 ratio. The concentrated solution ranges from 1 – 10% by volume. Several 15-ml bottles with different size microspheres are kept in the refrigerator near the tweezer apparatus. Adjustable micropipettes are available for dispensing solutions. These are shared with the Light Scattering experiment. You can either use CVS-brand saline solution formulated for use with contact lenses, or a homemade phosphate-buffered version, which also contains polyethylene glycol (for polystyrene microspheres) or bovine serum albumen (for biological samples). These additives help prevent the microspheres from sticking together. One- to three-micron diameter spheres are popular for use in the optical tweezer.

To load a sample into the tweezer, make a microsphere sandwich using a large coverslip, a parafilm spacer (with punched hole to leave space for microsphere solution), and a small cover slip on top. The 100X objective requires immersion oil, so place a drop of oil onto the bottom of the large cover slip just below the center of the sample. Slip the sandwich under the retaining springs on the PZT stage. If the particles drift toward one side of the sample, consider making sealed samples with a well slide and Canada balsam.

Magnetic Tweezer

What you need:

- 1.7 ml Eppendorf tubes
- 15 ml conical tubes
- Saline solution (sterile contact lens solution is OK; also you can use 150 mM NaCl)
- Detergent (~1:1000 of full strength) - prefer Triton X-100
- Beads - Invitrogen Dynabeads M-280 (2.8 μm) or M-450 (4.5 μm)
- MatTek glass bottom dish, 50 mm with 15 mm glass area

Procedure:

1. Dilute 500 μl 1:1000 detergent in 9.5 ml saline solution (0.005% detergent in saline).
2. Wash the glass part of the glass bottom dish 3 times with approximately 200-250 μl detergent:saline (you can use a 1000 μl pipettor).
3. Dilute 1 μl beads once in 1 ml detergent:saline, vortex 1 min, place near a magnet for 2 min, remove most of the liquid.

- 4. Add 2 μl of this bead solution to 1 ml detergent:saline, vortex 1 min, sonicate 5 min, vortex for a few secs.
- 5. Add 200 μl of this working bead solution to the glass bottom dish, carefully make sure it covers the whole surface area of the glass, cover the dish.
- 6. Place the magnet at its maximum distance from the sample holder.
- 7. Place the glass bottom dish in the sample holder.
- 8. Slowly drop the magnet to about 1 mm from the top of the glass bottom dish cover.
- 9. Move the objective up until you start seeing beads no longer moving past the focus.
- 10. Move the sample holder, following the general direction of the beads' movement. Large bead clusters will get to their final location first.
- 11. The crystal's ultimate location should be obvious within about 5 minutes. The crystal gets to its equilibrium point within an hour.

Tips:

Wear gloves! You're not protecting yourself (nothing in this basic prep is particularly dangerous). You're protecting your sample from proteins that might be on your fingers.

Do not put beads in glass containers. (Use plastic instead.) They stick to the glass and to each other and they tend to attach to any gunk on the glass surface and make a mess.

Copper sample stage with temperature control

An optional sample stage with temperature control is made from two copper plates. The small upper plate is temperature-controlled. The large lower plate is attached to the PZT stage, which serves as a heat sink. Two thermoelectric coolers (TECs) can either heat or cool the upper plate. A thermistor is embedded in the upper plate with silver epoxy. It acts as the thermometer and feedback element. A rough calibration in degrees Celsius vs. resistance in $\text{k}\Omega$ is included in the bench notes. The stage can achieve temperatures between approximately 5°C and 50°C .

A temperature controller (ILX Lightwave LDT-5412), or a dc power supply provides current to the TECs.

Translation stage and controller

The sample is mounted on a Thorlabs MDT631 flexure stage. The microscope objective is mounted to a fixed platform below the sample. The relative position can be varied by $\pm 1\text{mm}$ using manual adjustment screws. If these screws go out of their adjustment range, the sample can experience unwanted vibrations. Set the X and Y micrometers (but NOT the Z micrometer) in the center of their travel and check them occasionally to make sure that they are engaging the stage. Piezoelectric transducers (PZTs) are used for fine adjustment over a total range of 15 microns. A Thorlabs MDT693 Piezo Driver controls the PZTs. Manual fine adjustment is possible using front panel knobs. External modulation inputs are used to modulate the position of the sample in conjunction with the Circle Box (see below). **IMPORTANT:** The output range is zero to +150 Volts and an input swing of ten volts generates a full-range output swing. Attempting to drive the output negative will damage the flexure stage. The input is usually a sinusoidal voltage, which swings positive and negative. This input is added to the dc level set by the front-panel knobs, therefore start with the dc levels set at midrange (75 Volts), and keep the peak input amplitude less than $\pm 5\text{V}$. The position gain is approximately 0.12 microns per output Volt. Keep in mind that the capacitance of the PZTs will limit the available bandwidth. Commercial software exists for remote control of the MDT693, but it is not recommended.

Function generator/circle box/oscilloscope

To modulate the sample position with constant velocity, drive the X and Y PZTs with sine (Figure 3-1) and cosine (Figure 3-2) waves. A home-made "Circle Box" has quadrature outputs. The amplitudes (Figure 3-3) are separately adjustable. The frequency control (Figure 3-4) on the left side of the box provides a frequency adjustment from approximately 0.2 Hz to 7 Hz. If the oscillator doesn't oscillate, or the outputs are too small, push the reset button (Figure 3-5). View the two signals in XY mode of the oscilloscope before plugging them into the PZT controller. Take care not to drive the PZT voltages out of their specified range (0 – 150 V). The oscilloscope is a Tektronix 2215.



Figure 3. Optical Tweezer Circle Box Panel

White light source

A Thorlabs OSL1 Illuminator provides the illumination for the optical microscope. The light is coupled to a fiber optic cable. From there it can be delivered to the sample area. The light bulb is powered by a dc current, but a significant 120 Hz ripple is present in the light power, especially at high intensity. Placing a simple diffuser such as a kimwipe over the end of the fiber optic cable might improve image quality. Short focal length lenses can be mounted on the fiber terminator to improve collimation.

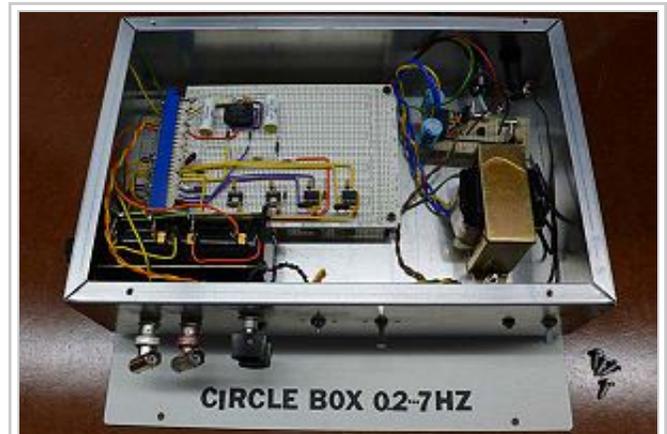


Figure 4. Optical Tweezer Circle Box Circuit

Camera

A Unibrain Fire i-400 CCD camera is used to image the microspheres. "Amcap" software displays the live image, and saves still images and video.

Microspheres

Polystyrene microspheres are available in 0.5, 1.0, 2.0, 3 and 10 micron diameters. Paramagnetic microspheres are available in three sizes: 2.8 micron, 4.5 micron, and a mixture of particles approximately less than 10 micron.

Quadrant photodiode with I/V converter

A quadrant photodiode can be used to measure the position of a beam or image, or, in conjunction with an FFT, to study fluctuations of the microspheres. (Optional section - consult the faculty or staff for availability information.)

The quadrant photodiode is one diode divided into four sections. If a light beam is centered on the diode, then the intensity falling on all four quadrants is the same. If the light is displaced up, then the top two diodes will

each receive the same intensity, which will be larger than the intensity falling on each of the two bottom detectors. Similarly, for a displacement right and left. Thus the difference between (the sum of the current for the top two quadrants) and (the sum of the bottom two quadrants), gives the up/down displacement. Similarly the left/right displacement is given by the difference between (the sum of the currents from left two quadrants) and (the sum of the right two quadrants). If one assumes the beam is Gaussian, one can convert the current differences to position differences analytically; otherwise it can be calibrated using a translation stage.

A LabVIEW program called `quaddft.vi` in conjunction with a National Instruments PCI-6023 multifunction I/O card interfaces the quadrant photodiode to the computer. If you place the quadrant detector above the sample to detect the transmitted laser light, you can monitor the frequency spectrum of the microspheres as they undergo Brownian motion. This spectrum will be different for a trapped sphere than for a free one as the active area of the photodiode is large enough to see free or trapped spheres.

Magnetic Tweezer

A vertically aligned magnet in close proximity to a droplet of liquid attracts superparamagnetic beads suspended in the liquid towards the magnet, much as iron filings are attracted towards a bar magnet. At the water-air interface, the attractive force from the applied field, and the induced repulsive dipole-dipole force between beads, balance in such a way that the beads form a 2D crystal at the interface. The lattice constant (bead-bead distance) can be controlled by varying the distance between the magnet and the liquid surface.

The configuration of the lab's magnetic tweezer is flexible and open to new development. There are currently three magnet geometries available for making bead crystals. Annular magnets in a mirror mount and a 3/8-inch cubical magnet can be placed close to the sample dish and give strong fields and field gradients at the sample location. These are good to start with because crystals form rapidly in the strong gradients, and the lattice constant of the crystals can be fairly small (several particle diameters).

The standard configuration is to orient the magnetic field perpendicular to the sample's air/water interface. This results in a triangular crystal i.e. a crystal with equal lattice constants and unit vectors at 60° .

If the magnetic field is tilted relative to the sample, other lattice geometries. Froltsov *et. al.* (2003) calculated the phase diagram for this system. The oblique and chain-like oblique phases are accessible to our apparatus. For this investigation, use one of the 2-inch by 2-inch magnets and the rotating/translating holder. This pair of magnets was donated to the lecture demonstration group by Ed Purcell (http://en.wikipedia.org/wiki/Edward_Mills_Purcell) in 1984. Rare earth magnets were a rarity at that time.

Particles of different sizes can be mixed in a single sample. This opens another level of complexity for the system, and many more crystal geometries become possible. See the paper by Chremos (2009). This is technically difficult in our apparatus because the larger particles reach the center of the crystal sooner than small ones whereas one would like the two species to mix uniformly.

Two-dimensional bead crystals can be annealed and melted. Annealing is accomplished with an ac magnetic field generated by a coil placed next to the sample dish. Overnight anneals may reduce the number of dislocations. Increasing the distance between magnet and sample decreases the "binding energy" of the crystal, decreasing its melting point. You may need to adjust the amount of surfactant (Triton-X) used in the water to tune the surface tension. If the surface tension is too small, the microspheres sink before the crystal melts.

The technical details of how pros do 2D bead crystals are available in the archived article by Ebert (2009).

EXPERIMENTAL PROCEDURE

Investigations of the Optical Trap

First demonstrate stable three-dimensional trapping of a 1-micron or 3-micron polystyrene sphere. Then use the PZTs on the translation stage to move the sample in a circle at a velocity v . Increase the frequency of the signal generator until the bead falls out of the trap. The velocity v is approximately the maximum velocity at which a particle will remain trapped. It is the velocity where the Stokes drag is equal to the maximum restoring force.

Optimize the trap depth using the following procedure. Change the spacing between the coverslip and the focused spot by moving the translation stage. This can be done either with the manual adjustment, or the "Z" PZT. Measure whether capture velocity increases or decreases. If it increases, go in the same direction, if it decreases, go in the opposite direction. This optimizes the trap depth for a given position of the 160 mm focusing lens (upstream of the objective). Plot capture velocity as a function of lens separation. Note: Stokes drag does not describe the drag very near a surface and this discrepancy should be measurable. Change the spacing between the 160 mm lens and the microscope objective and iterate to fully optimize trap depth. If time runs out, this entire step can be skipped.

First mandatory step: Measure the trap depth as a function of the intensity of the light. Explain the dependence. To do this, plot the escape velocity vs. intensity. This will be linear at high intensities. At low intensities random thermal motion will knock the sphere out of the trap. The escape velocity can then be converted into maximum force using the Stokes drag experiment. The trap depth is the product of the maximum force times the displacement. The displacement from the center of the trap at which the sphere escapes can be measured using the video. Alternately, assume that the size of the trap is approximately the diffraction limited spot size of the microscope objective, which can be calculated from the numerical aperture. The trap depth is then approximately the product of the force times this distance, which should be effectively constant for all intensities.

Additional parameters affecting trap depth: index difference between the sphere and the liquid, the depth of focus of the trap in the liquid, the size of the sphere. Adding salt to water, for example, can control index difference. Investigate one or more of these parameters if time allows.

Investigations of Thermal Motion

Particles in a fluid are buffeted by collisions with molecules in the liquid. The Brownian motion of a particle can be measured by measuring the displacement of the particle as a function of time. This diffusion is fundamentally related to the damping properties of the liquid.

Second Mandatory Step: Measure random walk diffusion of a spherical particle in water with NO trap and compare with Brownian theory. Deduce Boltzmann's constant from this measurement.

Optional: Repeat with various particle sizes and various solute viscosities.

Optional Optical Tweezer Experiments

Even "trapped" particles experience some random motion due to collisions. One can measure the trap depth by measuring the RMS deviation of the particles in the trap. This deviation can be measured using the camera in conjunction with IDL or Matlab Software, or by using the quadrant photodiode.

Optional: Measure RMS deviation of trapped particles as a function of intensity. The product of the measured force vs. intensity from the previous section times the deviation in this section gives the depth of the potential well as a function of intensity.

Trapped particles can be knocked out of the trap by these collisions. The average time required for a particle to leave the trap gives a measure of the trap depth at a given temperature.

Optional: Measure the time required for a particle to spontaneously leave the trap as a function of the trapping intensity. Note that this is only practical for weak traps, as the particle stays trapped indefinitely at high laser powers. As a starting point, use the weakest trap that you were able to measure in the Stokes' drag experiment. Since the probability of escaping spontaneously is proportional to the Boltzmann factor, $e^{-U/kT}$ where U is the trap depth, the trap lifetime (1/probability) will be highly non-linear. Produce a graph of average trap lifetime as a function of U .

Note that in practice fluctuations that can be described in terms of kT really represents many small momentum collisions. As a result, the above discussion is not an accurate measure of the effectiveness of a trap when a single fluid molecule can knock a particle out of the trap. Thus the approximation breaks down when U becomes small and for very small particles. Evaporative cooling of trapped atoms in the approach to BEC exploits this random process – the hottest atoms leave the trap first.

Optional: Experiments that use different liquids with different viscosities will provide information on the relationship between diffusion and damping. The viscosity of glycerol has very strong temperature dependence near room temperature. So controlling the temperature of the glycerol using a TE cooler provides an opportunity to make a smooth continuous investigation of the effects of viscosity.

Interparticle Interaction

If more than one particle is trapped in the optical tweezer, the two particles interact with each other. If the particle interaction is weak, the two particles move randomly with respect to each other, but if it is sufficiently strong the particles will order into a crystal. The transition from order to disorder can be obtained by changing the light intensity. The dependence of the transition depends on the trap depth, intensity, and size and number of the trapped particles can be measured and analyzed.

Magnetic Tweezer

The crystal ordering is more easily observed using magnetic tweezers. In this experiment, a permanent magnet is used to attract particles in the fluid toward the magnet. The highly paramagnetic, 2.8-micron and 4.5-micron particles develop an induced magnetic moment parallel to the external field of the permanent magnet. If the surface tension of the liquid is sufficient, the particles will be trapped at the air/water interface. These samples should be prepared without a cover slip, so the air/water interface presents a curved two-dimensional surface where the bead crystal can form. Determine the particle ordering for the case where the magnetic field is perpendicular to the surface. Compare to the case where the field is parallel. What is the interparticle spacing as a function of the distance of the magnet from the air water interface? What is the approximate interparticle force? These systems have been used to study crystallization and to mimic the motion of defects through superconductors.

The optical tweezer can be combined with the magnetic tweezer, where the optical tweezer is used to displace a single bead in the magnetic tweezer lattice. The motion of the “defect” through the lattice can be examined, as

well as return of the system to equilibrium after the optical tweezer perturbation is removed.

NOTES

1. ↑ H. Winter and H.W. Ortjohann, “Simple demonstration of storing macroscopic particles in a “Paul trap,” Am. J. Phys., 59, 807 (1991).
2. ↑ http://wswww.physik.uni-mainz.de/werth/g_fak/penning.htm
3. ↑ G. Gabrielse, et. al., “First Capture of Antiprotons in a Penning Trap: A Kilolectronvolt Source,” Phys. Rev. Lett., 57, 2504 (1986). and D.S. Hall and G. Gabrielse, “Electron Cooling of Protons in a Nested Penning Trap,” Phys. Rev. Lett., 77, 1962 (1996).

REFERENCES

Optical Traps

Stephen P. Smith, Sameer R. Bhalotra, Anne L. Brody, Benjamin L. Brown, Edward K. Boyda, and Mara Prentiss, “Inexpensive optical tweezers for undergraduate laboratories (<http://www.fas.harvard.edu/~phys191r/References/a10/Smith1999.pdf>) ,” Am. J. Phys. 67 (1) 26-35 (1999). The current apparatus is based on this article.

A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, and Steven Chu, “Observation of a single-beam gradient force optical trap for dielectric particles (<http://www.fas.harvard.edu/~phys191r/References/a10/Ashkin1986.pdf>) ,” Opt. Lett. 11 (5), 288–291 (1986).

A. Ashkin, “Forces of a single-beam gradient laser trap on a dielectric sphere in the ray optics regime (<http://www.fas.harvard.edu/~phys191r/References/a10/Ashkin1992.pdf>) ,” Biophys. J. 61, 569-582 (1992). Referring to Smith, et. al. (1999), the constant Q in eq. (1), is written out explicitly in this paper.

Keir C. Neuman and Steven M. Block, “Optical trapping (<http://www.fas.harvard.edu/~phys191r/References/a10/Neuman2004.pdf>) ,” Rev. Sci. Instrum. 75, 2787 (2004).

M.S. Rocha, “Optical tweezers for undergraduates: Theoretical analysis and experiments (<http://www.fas.harvard.edu/~phys191r/References/a10/Rocha2009.pdf>) ,” Am. J. Phys., 77, 704 (2009). Calculation of trap stiffness.

K. Svoboda and S.M. Block, "Biological Applications of Optical Forces (<http://www.fas.harvard.edu/~phys191r/References/a10/Svoboda1994.pdf>) ,” Annual Review of Biophysics and Biomolecular Structure, 23, 247 (1994).

Brownian Motion

R. Newburgh, et. al., “Einstein, Perrin, and the reality of atoms: 1905 revisited (<http://www.fas.harvard.edu/~phys191r/References/a10/Newburgh2006.pdf>) ,” Am. J. Phys., 74, 478 (2006).

T. Greczylo and E. Debowska, “Brownian Motion with Data Video (<http://www.fas.harvard.edu/~phys191r/References/a10/Greczylo.pdf>) .”

Magnetic Field Gradient Traps

L. Assoud, F. Ebert, P. Keim, R. Messina, G. Maret and H. Löwen, "Crystal nuclei and structural correlations in two-dimensional colloidal mixtures: experiment versus simulation (<http://www.fas.harvard.edu/~phys191r/References/a10/Assoud2009.pdf>) ," Journal of Physics: Condensed Matter **21** (2009) 464114.

L. Assoud, R. Messina and H. Löwen, "Stable crystalline lattices in two-dimensional binary mixtures of dipolar particles (<http://www.fas.harvard.edu/~phys191r/References/a10/Assoud2007.pdf>) ," Europhysics Letters **80** (2007) 48001.

A. Chremos and C. Likos, "Crystal structures of two-dimensional binary mixtures of dipolar colloids in tilted external magnetic fields (<http://www.fas.harvard.edu/~phys191r/References/a10/Chremos2009.pdf>) ," Journal of Physical Chemistry B **113** (2009) 12316.

F. Ebert, P. Dillmann, G. Maret and P. Keim, "The experimental realization of a two-dimensional colloidal model system (<http://www.fas.harvard.edu/~phys191r/References/a10/Ebert2009.pdf>) ," arXiv:0903.2808v4 [cond-mat.soft] 20 Apr 2009.

F. Ebert, P. Keim and G. Maret, "Local crystalline order in a 2D colloidal glass former (<http://www.fas.harvard.edu/~phys191r/References/a10/Ebert2008.pdf>) ," European Physical Journal E **26** (2008) 161.

V.A. Froltsov, R. Blaak, C. N. Likos and H. Löwen, "Crystal structures of two-dimensional magnetic colloids in tilted external magnetic fields (<http://www.fas.harvard.edu/~phys191r/References/a10/Froltsov2003.pdf>) ," Physical Review E **68** (2003) 061406.

V. A. Froltsov, C.N. Likos and H. Löwen, "Anisotropic mean-square displacements in two-dimensional colloidal crystals of tilted dipoles (<http://www.fas.harvard.edu/~phys191r/References/a10/Froltsov2005.pdf>) ," Physical Review E **71** (2005) 031404.

P. Keim, G. Maret and H.H. von Grünberg, "Frank's constant in the hexatic phase (<http://www.fas.harvard.edu/~phys191r/References/a10/Keim2007.pdf>) ," Physical Review E **75** (2007) 031402.

I. Varga, H. Yamada, F. Kun, H. G. Matuttis and N. Ito, "Structure formation in a binary monolayer of dipolar particles (<http://www.fas.harvard.edu/~phys191r/References/a10/Varga2005.pdf>) ," Physical Review E **71** (2005) 051405.

K. Zahn, J.M. Mendez-Alcaraz and G. Maret, "Hydrodynamic Interactions May Enhance the Self-Diffusion of Colloidal Particles (<http://www.fas.harvard.edu/~phys191r/References/a10/Zahn1997.pdf>) ," Physical Review Letters **79** (1997) 175.

2D Polymer Sphere Crystals

P. Pieranski, "Two-dimensional interfacial colloidal crystals (<http://www.fas.harvard.edu/~phys191r/References/a10/Pieranski1980.pdf>) ," Phys. Rev. Lett. **45**, 569 (1980).

N.D. Denkov, O.D. Velev, P.A. Kralchevsky, I.B. Ivanov, H. Yoshimura, and K. Nagayama, "Two-

Dimensional Crystallization (<http://www.fas.harvard.edu/~phys191r/References/a10/Denkov1993.pdf>) ,” Nature (London) 361, 26 (1993).

H.H. Wickman and J.N. Korley, “Colloid crystal self-organization and dynamics at the air/water interface (<http://www.fas.harvard.edu/~phys191r/References/a10/Wickman1998.pdf>) ,” Nature (London) 393 (6684), 445 (1998).

2D Melting

C. Eisenmann, P. Keim, U. Gasser and G. Maret, "Melting of anisotropic colloidal crystals in two dimensions (<http://www.fas.harvard.edu/~phys191r/References/a10/Eisenmann2004.pdf>) ,” J. Phys.: Condensed Matter, 16, S4095 (2004).

K. Zahn, R. Lenke, and G. Maret, "Two-Stage Melting of Paramagnetic Colloidal Crystals in Two Dimensions (<http://www.fas.harvard.edu/~phys191r/References/a10/Zahn1999.pdf>) ,” Phys. Rev. Letters, 82, 2721 (1999).

K. Zahn and G. Maret, “Dynamic Criteria for Melting in Two Dimensions (<http://www.fas.harvard.edu/~phys191r/References/a10/Zahn2000.pdf>) ,” Phys. Rev. Letters, 85, 3656 (2000).

K. Zahn, et. al., “Elastic Properties of 2D Colloidal Crystals from Video Microscopy (<http://www.fas.harvard.edu/~phys191r/References/a10/Zahn2003.pdf>) ,” Phys. Rev. Letters, 90, 155506-1 (2003).

Ordered Trapped Particles

F. Diedrich, E. Peik, J. M. Chen, W. Quint, and H. Walther, “Observation of a phase transition of stored laser-cooled ions (<http://www.fas.harvard.edu/~phys191r/References/a10/Diedrich1987.pdf>) ,” Phys. Rev. Lett. 59, 2931 (1987).

D. J. Wineland, J. C. Bergquist, W. M. Itano, J. J. Bollinger, and C. H. Manney, “Atomic-ion coulomb clusters in an ion trap (<http://www.fas.harvard.edu/~phys191r/References/a10/Wineland1987.pdf>) ,” Phys. Rev. Lett. 59, 2935 (1987).

J. Hoffnagle, R. G. DeVoe, L. Reyna, and R. G. Brewer, “Order-chaos transition of two trapped ions (<http://www.fas.harvard.edu/~phys191r/References/a10/Hoffnagle1988.pdf>) ,” Phys. Rev. Lett. 61, 255 (1988).

R. G. DeVoe, R. G. Brewer , “Observation of superradiant and subradiant spontaneous emission of two trapped ions (<http://www.fas.harvard.edu/~phys191r/References/a10/Devoe1996.pdf>) ,” Phys. Rev. Lett. 76, 2049 (1996).

S. A. Tatarikova, A. E. Carruthers, and K. Dholakia, “One-Dimensional Optically Bound Arrays of Microscopic Particles (<http://www.fas.harvard.edu/~phys191r/References/a10/Tatarikova2002.pdf>) ,” Phys. Rev. Lett. 89, 283901 (2002).

W. Singer, M. Frick, S. Bernet, and M. Ritsch-Marte, “Self-organized array of regularly spaced microbeads in a fiber-optical trap (<http://www.fas.harvard.edu/~phys191r/References/a10/Singer2003.pdf>) ,” J. Opt. Soc. Am. B 20, 1568, (2003).

Electromagnetic Traps

H. Winter and H.W. Ortjohann, "Simple demonstration of storing macroscopic particles in a Paul trap (<http://www.fas.harvard.edu/~phys191r/References/a10/Winter1991.pdf>) ," Am. J. Phys., 59, 807 (1991).

M. H. Anderson, J. R. Ensher, M. R. Matthews, C. E. Wieman, and E. A. Cornell, "Observation of Bose-Einstein condensation in a dilute atomic vapor (<http://www.fas.harvard.edu/~phys191r/References/a10/Anderson1995.pdf>) ," Science 269, 198 (14 July 1995).

M. Amoretti et al., "Production and detection of cold antihydrogen atoms (<http://www.fas.harvard.edu/~phys191r/References/a10/Amoretti2002.pdf>) ," Nature (London) 419, 456-459 (2002).

G. Gabrielse, et. al., "First Capture of Antiprotons in a Penning Trap: A Kiloelectronvolt Source (<http://www.fas.harvard.edu/~phys191r/References/a10/Gabrielse1986.pdf>) ," Phys. Rev. Lett., 57, 2504 (1986).

D.S. Hall and G. Gabrielse, "Electron Cooling of Protons in a Nested Penning Trap (<http://www.fas.harvard.edu/~phys191r/References/a10/Hall1996.pdf>) ," Phys. Rev. Lett., 77, 1962 (1996).

BENCH NOTES

- Thorlabs ITC 502 Laser Diode Controller (http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/thor_its502.pdf)
- Hitachi HL6501MG Laser Diode (http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/ld.pdf)
- Laser Power Calibration (pdf) (http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/laser_calib.pdf)

LASER POWER CALIBRATION

laser current (mA) -- power at laser (mW) -- power at objective (mW)

40	0.0854	0.048
45	0.218	.146
50	2.89	1.87
55	6.64	3.97
60	10.4	6.16
65	14.4	8.39
70	16.0	10.5
75	22.3	13.0
80	26.0	15.2
85	29.2	17.5
90	31.8	19.8
95	33.8	22.2

- Thorlabs TCLDM9 Laser Diode Mount

- (http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/ldmount.pdf)
- Thorlabs LG4 Laser Safety Glasses
(http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/goggles.pdf)
- Thorlabs PDA100A Photodetector
(http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/PDA100A.pdf)
- Thorlabs MDT 693 Piezo Driver
(http://www.fas.harvard.edu/~phys191r/Bench_Notes/D4/Thorlabs_mdt693a.pdf)
- Microscope Primer (http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/micprimer.pdf)
- Shortpass Filter (http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/filter.pdf)
- 4423 Quadrature Oscillator (Circle Box)
(http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/4423.pdf)
- ILX Lightwave LDT-5412 Temperature Controller
(http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/ldt5412.pdf)
- Thermistor Calibration (http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/thermistor.gif)
- Fiber Optic Illuminator (http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/lamp.pdf)
- DIN Micro-Plan (Semi-Plan) Objective
(http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/objective.pdf)
- Microspheres MSDS (http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/2_0micron.pdf)
- Bovine Serum Albumin (http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/bsa.pdf)
- Quadrant Photodiode (http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/quadrant.pdf)
- Quadrant Photodiode Pinout
(http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/quadrant_pinout.pdf)
- Quadrant Photodiode Current to Voltage Converter
(http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/qpiv.pdf)
- Quadrant Photodiode PCI-6023 Interface
(http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/ni6023e.pdf)
- Quadrant Photodiode PCI-6023 Interface Pinout
(http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/qpiv.pdf)
- Vortex Mixer (http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/vortexer.pdf)
- Ultrasonic Cleaner (http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/ultrasonic.pdf)
- Triton-X Detergent MSDS (http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/triton-x.pdf)
- Protocol for preparing a magnetic tweezer sample (Efrain)
(http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/efrain_protocol.txt)
- MATLAB files (Efrain) (http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/Matlab.zip)
- MATLAB files (Efrain) -- June 2011
(http://www.fas.harvard.edu/~phys191r/Bench_Notes/A10/tweezer_june11.zip)

Links

- Penning Trap (http://www.physik.uni-mainz.de/werth/g_fak/penning.htm)
- Evaporative Cooling (http://www.colorado.edu/physics/2000/bec/evap_cool.html)
- Free image analysis software: ImageJ (<http://rsb.info.nih.gov/ij/>)

Options for Particle Tracking software:

- IDL (Crocker and Weeks) (<http://www.physics.emory.edu/weeks/idl/tracking.html>) LINK BROKEN
- Matlab - based on IDL algorithm (<http://physics.georgetown.edu/matlab/>)
- UW particle tracking resource page (Graham Milne) - includes LabVIEW
(<http://faculty.washington.edu/gmilne/tracker.htm>)

APPENDIX A: Optical Tweezer Alignment Procedure

Optical alignment is best done systematically. The general procedure is to align the laser beam with rows of holes on the optical table, then insert lenses and other components, preserving the existing beam path.

Safety

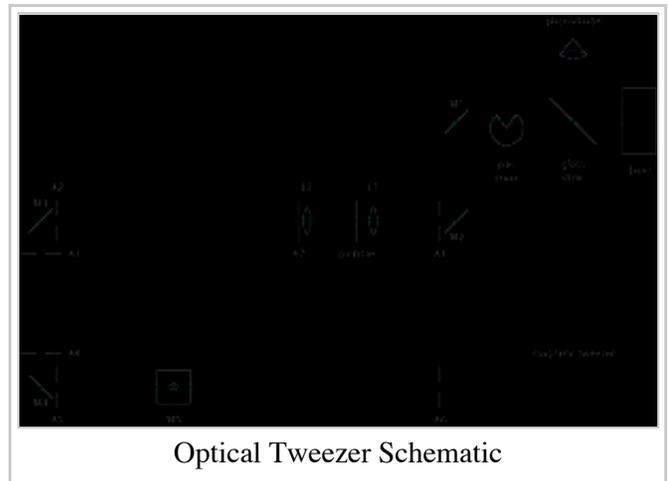
Laser safety is important because a laser accident can have a lasting impact on your vision. The most likely time to have a laser accident is during alignment. Placing optical components into the beam, taking them out of the beam, or manipulating them may create stray beams in unpredictable places. Wear laser safety glasses. When inserting or extracting components from the beam path, block the beam with the rotating shutter near the laser. Never place your eye at the same height as the laser beam.

Take care not only to avoid eye contact with the direct beam, but also with the specular reflection from any surface. Never allow stray reflections to leave the footprint of the optics table. Use black foamcore to block them.

For alignment, tune the laser current just above the lasing threshold to produce a beam of one milliwatt or less power. See the bench notes for a calibration of power vs. current.

Beam alignment

Refer to the schematic diagram at right for names and numbers of the components referred to in this section.



1. The first component to align is the laser itself. This has probably been done by previous users. If you choose to change the configuration, start by choosing a beam height, then set the angle of the laser housing so that the beam follows a row of holes on the optical table. The beam may not be parallel to the tabletop; correct this with the first pair of mirrors, M1 and M2.
2. Check the laser collimation. If needed, tweak the small collimating lens in the laser housing by using a spanner wrench to rotate the black disk in which the lens is mounted.
3. Place mirrors M1 through M4 on the table as indicated in the schematic. The ultimate target is the rear aperture of the objective. Use a wooden ruler to measure the beam height at various places along the beam path. Adjust the vertical tilt of the mirrors so that the beam height is uniform within one millimeter or so.
4. Insert apertures A1 and A2 and adjust their heights to match the beam height. Install collars on the posts so that they can be moved while keeping the same height. The post holders should automatically center the lateral position of the apertures above a row of holes on the optical table.
5. Stop down the apertures A1 and A2 and adjust the position and lateral tilt of M2 so that the beam goes through both apertures and is centered on M2.
6. Move the apertures to locations A3 and A4 and repeat the previous step for M3.
7. Move the apertures to locations A5 and A6 and repeat the previous step for M4.
8. Insert L1 and L2 keeping the beam path unchanged. L1 and L2 form a beam expander that enlarges the beam to fill the rear aperture of the objective. A 50 mm and a 300 mm lens are suggested; other options are available.
9. Place A7 directly after L2 and leave about 20 cm for the beam profile device. This is a good opportunity

- to measure the profile of the expanded beam.
10. Place a pinhole, P1 at the focus of the 50 mm lens. Use the mini translation stage and post holder with height adjustment. Fine-tune the pinhole position for a symmetrical diffraction pattern centered on the optical axis. Remeasure the beam profile.
 11. Remove the profiler and mount the microscope objective on the flexure stage. Position M5 (actually a dichroic filter that reflects red and transmits blue) directly below the objective. A plumb bob might help with this. Also verify that the objective is above a hole in the optical table. *Note on laser safety:* The laser light that passes through the objective diverges strongly enough that it is safe to view with the naked eye. It is annoying however so block it with a piece of paper. The paper also functions like a screen. The diffuse light transmitted by the paper is an indicator of alignment and can also be used to identify trapping.
 12. Adjust the beam angle, position and size to match the microscope objective.
 1. Angle: In order to adjust the angle of the beam, hold a glass slide against the rear aperture of the objective. When the incident beam is perpendicular to the slide, the reflected beam will travel back along the optical axis. Using an index card with a hole punched in it, check the position of the reflected beam, and align it with the incident beam by adjusting the angle of M5. Holding the card farther upstream gives better angular resolution.
 2. Position and size: Tune the location of M5 so that the beam is centered on the rear aperture of the objective. The beam should slightly overfill the objective aperture. Stop down A7 to make the beam smaller. To make the beam larger, reconfigure the beam expander. Place a spare mirror on the tabletop to help you see the objective aperture without putting your eye near the beam.
 3. Iterate these adjustments until no further changes are needed. Very small position adjustments can be made with M3.
 13. L3 is a 160 mm lens that sets the curvature of the beam at the objective. It is actually two lenses (200 mm & 750 mm) in the same holder. Place it on the 2-inch translation stage about 32 cm from the objective aperture, maintaining the existing beam path. This lens is needed because of the optical design of the Objective. Microscope objectives are optimized to produce an image at 16 cm. In this application, if the laser light seems to originate from a point source 16 cm away, the objective gives a tighter focus than it would for a collimated beam.

Camera alignment

The laser alignment above should produce a trap. Additionally however, we want to be able to see the trap on the camera. To align the camera, use a mirror to reflect the laser light back down through the objective and take advantage of the small but finite red transmission of M5 to view the laser spot with the camera.

1. Insert the viewing mirror below M5 and align it by eye. Mount the camera on the camera rail. The optimum position for the CCD plane is 16 cm from the objective. Note that this is a bare CCD. Adjust the height of the viewing mirror to match the height of the camera.
2. Move the z-adjustment of the flexure stage all the way up, fully CW.
3. Load a 22 mm x 50 mm cover slip in the sample holder. By hand, very carefully move the objective upwards until it slightly deforms the cover slip. Fix the objective in this position with the two shoulder washers.
4. Move the z-adjuster back down 1 - 2 turns CCW.
5. Take the cover slip back out, place a small drop of immersion oil in the center, and reload the cover slip so that the oil contacts the objective.
6. Place a small front surface mirror on top of the cover slip.
7. Connect the camera to the computer firewire card. Run AMCap.exe to view the image on the CCD.
8. Find the optimum z-position by watching the reflected light on the downstream side of A4 as you move the objective in the z-direction. Look for the place where the circle of reflected light is smallest.

9. By hand, scan the angle and height of the viewing mirror until a reddish spot is visible on the CCD image. Lock the viewing mirror in place and center the spot on the CCD with the fine controls of the viewing mirror. This will be the location of the trap.

Trapping a particle

To check for trapping, prepare and load a sample of 1-micron spheres according to the sample prep guidelines above. Increase the laser power to 5 mW. Viewing transmitted laser light, circular diffraction patterns of microspheres should drift through the field of view. Move the X and Y controls of the flexure stage to bring a particle close to the center of the field of view. Adjust Z (focus) to make this sphere larger. The signature of trapping is unambiguous: a sphere will be pulled into the trapping region and appear very large. If the trap is weak, it may only trap in two dimensions. The signature of this is a trapped sphere that gets pushed out of the trap along the direction of light propagation after a few seconds. To improve the strength of the trap, optimize the curvature of the beam.

In order to see the trap on the camera, install the white light source above the objective. Increase intensity until you see an image on the camera. If the camera alignment was done carefully, you will see the trap immediately. If not, establish that you have a trapped particle by viewing the transmitted laser light and scan the position and angle of the viewing mirror.

APPENDIX B: Particle Tracking using IDL

IDL is not licensed for use on lab computers in Physics 191. These notes are provided for students with prior IDL experience and access to a current license.

Based on <http://www.physics.emory.edu/~weeks/idl/tracking.html> by John C. Crocker and Eric R. Weeks. Adapted for Physics 191/247 Lab by Richard Conroy, Pamela Gay and Joe Peidle. November 2004.

Purpose: Find the (x,y) coordinates of a trapped particle in each frame of a video clip.

- Outline:
- 1) Trap a particle and make a video clip of it using "Firei" software (movie.avi).
- 2) Uncompress the file using "VirtualDub."
- 3) Convert uncompressed video to stack of TIFF images using "ImageJ" software (stack.tif)
- 4) Read stack.tif into IDL.
- 5) Find particles in a sample image. Determine parameters needed by software.
- 6) Repeat step 5 for all frames.
- 7) Link particles frame-to-frame.
- 8) Save (x,y) coordinates to text file.
- 9) Calculate rms deviation of the particle.

Details:

- 1) "Firei" software controls the CCD camera. You can save individual frames in bitmap format by pressing <ctrl><space> while in Preview mode. There is also a "Frame Capture" mode which saves a frame at pre-determined time intervals. Establish a trap and compose your image in Preview mode with color (YUV 4:1:1). The background will appear bluish because of the shortpass filter. When a particle is in the trap, some laser light will be scattered into the camera. This is useful for alignment, but can create

problems during IDL analysis of the images. You can reduce it by placing a second shortpass filter in the imaging beam path.

For video capture, change to grayscale: Pixel Format: Y_MONO Resolution: 640x480 Frame Rate: 30.0

Arrange the image for best contrast. IDL expects bright particles on a dark background. When ready to capture video, click on the "Video Capture" link to set the destination filename. To actually begin capture, check the "Video Capture" box, and uncheck it to stop. A few seconds of video are enough; the file sizes will be tens of Mbytes.

- 2) The files created by Firei are in compressed .avi mode, but uncompressed images are needed below. Uncompress the .avi file using "VirtualDub:" Open VirtualDub, then open your video file. Select Video:Compression:(Uncompressed RGB). Save as AVI. Close VirtualDub.
- 3) Open the ImageJ application. Make sure that adequate memory is allocated (Edit:Options:Memory). The memory setting should be greater than your largest file size. From the Plugins menu, choose Shortcuts:avi_read. This will open your .avi file as a stack of images. Check that the Image:Type is set to 8-bit (which converts to grayscale if you kept color info in your video). Save the image stack with File:SaveAs:Tiff. (Type in the .tif extension explicitly.) Close ImageJ.

Note that ImageJ has many useful features such as cropping the image stack, and adjusting brightness and contrast. In particular, cropping at this point saves much labor later. Cropping the images makes IDL run much faster, and since you only process the trapped particle locations, you don't have to edit out untrapped particles later.

- 4) Copy the IDL project "247tracking.prj" in c:\students into your data directory. The filename might be "191tracking.prj" depending on who used it last – the content should be the same. Open "247tracking.prj." You can enter commands at the prompt at the bottom of the window. Change directories into your data directory:

```
IDL>cd,'c:\students\your_name'
```

Read your tiff stack into a variable called a:

```
IDL>a=readtiffstack('stack.tif')
```

```
IDL>help,a
```

This should return information about the 3D array, a such as

```
A BYTE Array(640,480,n)
```

where n is the number of frames in your stack. To view the mth image, first transfer it to a variable b:

```
IDL>b=a(*,*,m)
```

```
IDL>tv,b
```

The image should appear in a window called IDL0. Note that in ImageJ, pixel numbers are referenced to the upper left corner of the image, whereas in IDL, they are referenced to the lower left corner, so the image appears upside-down.

- 5) Next we have to identify the particles of interest with software. We will do this for our mth image now called b :

Filter the image to make a black background:

```
IDL>c=bpass(b,5,15)
```

The parameters 5,15 here are given as an example. Adjust them to suit your image.

View the filtered image:

```
IDL>tv,c
```

The two numbers five and fifteen are typical values for the “low cutoff” and “high cutoff” of the filter. Play around with the parameters to make the nicest filtered image. A useful shortcut is the up-arrow key, which recalls previous commands.

Next, find coordinates of features that are potentially interesting:

```
IDL>f=feature(c,15)
```

This parameter should be close to the size of the particles; at most it should equal the high cutoff. If too many features are found, increase the feature size. To see the result, view the array f:

```
IDL>print,f
```

f is a 2D array containing five columns: x-centroid, y-centroid, brightness, radius, and eccentricity (which is zero for circles and one for lines). The feature.pro subroutine will probably find many features that are actually just random variations in the background. Compare the features with the original image:

```
IDL>fo=fover2d(b,f) {shortcut key = <alt>v}
```

White circles should appear over your original image in the IDL0 window. (You can also choose to “fover2d(c,f)” to view the filtered image.) If your original image had good contrast, the particles will be much brighter than the random features. They will also have uniform size. We can see this in a plot of brightness as a function of radius:

```
IDL>plot,f(3,*),f(2,*),psym=6 {shortcut key = <alt>b}
```

psym=6 makes square symbols in the plot. Hopefully, the real particles are much brighter than the random features; they should appear near the top of the plot. We can discriminate against dim features using the parameter masscut:

```
IDL>f=feature(c,15,masscut=20000)
```

```
IDL>fo=fover2d(b,f)
```

20000 is a brightness level chosen greater than the random features and less than the particles of interest. The second parameter, here 15, is the feature size used above. If you can't discriminate against random features, return to bpass and attempt to choose better parameters. The worst-case scenario is to take more data after adjusting the imaging system for better contrast.

- 6) Knowing the parameters determined in step 5), we can use the pretrack subroutine (version t) to identify the particles of interest in each frame of stack.tif and plot their coordinates.

tpretrack.pro reads data directly from stack.tif and produces a file called xys.stack.tif :

```
IDL>tpretrack,'stack.tif',[5,50,50,30000]
```

Note the square brackets. The parameters are [low cutoff, high cutoff, particle size, masscut brightness]. If tpretrack.pro finishes without errors, xys.stack.tif should appear in your directory. Read it into IDL:

```
IDL>pt=read_gdf('xys.stack.tif')
```

```
IDL>print,pt
```

pt is an array like f, but with a sixth column containing the frame number in which the feature was detected. Again we can plot brightness as a function of radius:

```
IDL>plot,pt(3,*),pt(2,*),psym=3
```

psym=3 sets the symbol type to a dot. If any spurious particles passed the filters, you can see them in this plot. The real particles will be in a distinct cloud or clouds. To see where the particles were found, plot x-centroid vs y-centroid:

```
IDL>plot,pt(0,*),pt(1,*),psym=3 {shortcut key = <alt>c}
```

You can adjust the plot range with qualifiers such as /yzero or ,xrange=[m,n],yrange=[o,p]

If you have only one particle in each frame, you're done – skip to step 8). Otherwise,...

- 7) The last step is to link the particles found in each frame with all the other frames. For this we need a parameter to define a search radius – how far a particle is likely to be displaced between adjacent frames.

track.pro reads information from array pt, and takes three additional parameters:

```
IDL>t=track(pt,20,goodenough=10,memory=5)
```

The first parameter is the search radius; goodenough=10 discards particles which are tracked for less than ten frames, such as particles which drift out of focus; memory=5 keeps particles which disappear for less than five frames. t is an array like pt, but with a seventh column containing a unique particle identifier. If you are tracking more than one particle, it can be useful to isolate a single one (particle number 5 in this example) and plot its coordinates:

```
IDL>u=where(t(6,*) eq 5)
```

```
IDL>plot,t(0,u),t(1,u)
```

- 8) The 2D array, t (or pt, if you skipped step 7)) contains the desired information. Save it to a text file:

```
IDL>openw,1,'result1.txt'
```

```
IDL>printf,1,t
```

IDL>close,1

1 (one) is the unit identifier for the data file.

APPENDIX C: Particle Tracking using MATLAB

- Based on software developed by Efraim Feinstein (Prentiss group) for magnetic tweezer analysis
- The tweezer computer can be accessed by Windows Remote Desktop. It's IP address is 140.247.29.216. Turn off sleep/hibernate if you want to use this computer remotely after hours.

Purpose

To analyze videos from the optical tweezer, Brownian motion and magnetic tweezer. The software finds the (x,y) coordinates of particles in all frames in a series of video clips, and calculates the mean square displacement and correlation coefficients.

Outline

- 1) Make a series of videos.
- 2) Convert the video files to grayscale.
- 3) Analyze the videos, i.e., find the (x,y) coordinates of each particle in each frame and save them in data files.
- 4) Analyze the data files.

Details

1) Make videos

Prepare a sample and configure it according to the experimental goals. Use "AMCap.exe" software to capture video files.

2) Import video and convert to grayscale

At the MATLAB prompt, change to your data directory:

```
>> cd c:\students\your_directory
```

```
>> movieObj=VideoReader('filename.avi')
```

Reads in the .avi file. (Commands in MATLAB are case sensitive.) VideoReader assumes that movies are RGB24, so even if you re-read a grayscale movie you have to convert it to grayscale as described below. First however, convert the Object to a Struct array:

```
>>mov=MakeMovStruct(movieObj)
```

Converts the VideoReader Object into a struct array. For a 10-second video taken at 15 frames per second MATLAB returns:

mov=1x151 struct array with fields:

cdata

colormap

```
>> bwmov=rgbmovie2gray(mov)
```

Converts the movie to grayscale. The struct array bwmov is a $1 \times nFrames$ array where nFrames is the number of frames in filename.avi. Each element consists of two arrays, cdata and colormap. cdata is an array of intensity values with an element for each pixel of the CCD camera, 480x640 in this case. The elements are unsigned 8-bit integers corresponding to 8-bit grayscale. Colormap is a 256x3 array with row elements equal to each other, corresponding to RGB and columns increasing linearly from zero to one.

Optionally, save the grayscale movie as filename2.avi:

```
>> movie2avi(bwmov,'filename2.avi','compression','none')
```

3) Analyze the videos

The analysis algorithm works best if particles appear as black dots against a uniform light background. In the magnetic tweezer this is usually the case. For the optical tweezer one should invert the grayscale images. The following example code writes the first frame to a file, inverts all frames, and writes the inverted first frame to another file:

```
>> imwrite(bwmov(1,1).cdata,'test.jpg','jpg')
```

```
>> nFrames = 151
```

```
>> for i = 1:nFrames;InvBwMov(1,i).cdata=255 -  
bwmov(1,i).cdata;InvBwMov(1,i).colormap=bwmov(1,i).colormap;end
```

```
>> imwrite(InvBwMov(1,1).cdata,'inverttest.jpg','jpg')
```

If this looks good, optionally save the inverted movie:

```
>> movie2avi(InvBwMov,'filename2inverted.avi','compression','none')
```

Then try the analysis program:

```
>> AnalyzeAVIVideos7S(InvBwMov,0.5,293,0.3,15,'filename3',0)
```

The parameters are: (movie struct array name, particle radius (microns), temperature (K), camera calibration (microns/pixel), frame rate (frames/second), output data filename base, detection algorithm number [zero if particles appear as black dots, or one otherwise]).

Be patient. This step takes time. MATLAB returns:

M of N done. Tracking P particles Q tracks total

(Repeats with different M's, P's and Q's.)

M is the current frame.

N is the total number of frames.

P is the number of particles.

Q is the number of tracks.

The results are saved in a file beginning with filename3.mat.

The previous version, AnalyzeAVIVideos6S, has the capability of reading avi files from disk and analyzing a series of them automatically instead of operating on a single array in the workspace. This can be useful for magnetic tweezer studies of crystal formation.

4) Analyze the data files

```
>> load('filename3.mat')
```

The format of data in filename3.mat is:

```
% tracks = raw track data in the form:
```

```
% tracks(:,1) = x coordinates (pixels)
```

```
% tracks(:,2) = y coordinates (pixels)
```

```
% tracks(:,3) = time (frames)
```

```
% tracks(:,4) = particle number
```

```
% tracks(:,5) = particle brightness on a scale of zero to one
```

```
% tracks(:,6) = radius of gyration
```

```
% complete_tracks is like tracks, except that: incomplete particle tracks are ignored (if the particle disappeared, for example), and the x,y coordinates are corrected to the center of mass of particles that remain in the image (to remove any drift in the field of view). Note that this is not useful for optical tweezer especially if there is a single particle in the field of view. Take data directly from tracks(:,1:2).
```

```
% complete_track_ns = the particle numbers of tracks that are considered complete
```

```
% cmx = array of x coordinates of center of mass as a function of time
```

```
% cmy = array of y coordinates of center of mass as a function of time
```

- If the tracking software found lots of things that are obviously not particles, run AnalyzeAVIVideos7S again with a larger (at least double) particle radius.
- One can calculate mean square deviation directly from tracks for the particles identified in complete_track_ns (suitable for optical tweezer and Brownian motion).

- Alternately, use AnalyzeCorrelations3S (suitable for magnetic tweezer):

```
>> AnalyzeCorrelations3S('filename3', 'filename4', 1, 'self')
```

The parameters are: (input data filename base, output filename base, file sequence number to analyze, correlation type: self, RR, RQ, QR or QQ).

MATLAB returns:

Calculating 001 of L...

Loading...

Calculating...

self

nParticles=

P

nCombinations=

R

```
>> load filename4-001-ii.mat
```

Reads in the analysis file for video number 1.

```
>> who
```

Displays the variables loaded into memory

```
>> size(dR2)
```

Displays the size of the array dR2. Each element of dR2 is an array of msd vs. time for the corresponding particle. The column length thus equals the number of particles.

```
>> plot(dR2{1})
```

Plots the msd vs. frame number for particle number 1.

```
>> tim=1/15:1/15:length(dR2{1})/15
```

Builds an array of time points with the units of seconds (assuming that the video was taken at 15 frames/second). The length of this array has to be the same as the length of dR2{1}.

```
>> plot (tim,dR2{1})
```

Plots msd vs. time in seconds.

```
>> msd=zeros(length(dR2{1}),1);for i=1:length(dR2), msd=msd+dR2{i};end;msd=msd/length(dR2{1});
```

Averages the msd over all particles.

```
>> plot (tim,msd)
```

Plots average msd vs. time in seconds.

Retrieved from "https://coursewikis.fas.harvard.edu/phys191r/A-10_Optical_and_Magnetic_Tweezers"

- This page was last modified on 26 February 2013, at 13:52.