PH 201 Experiment

Optical Tweezers

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Abstract

A simple optical trap is constructed, using one 10mW laser beam, and used to trap 1μm latex beads in water. Its strength is measured by studying the thermal vibrations of these beads. No corrections to the harmonic oscillator potential are found.

1 About Optical Traps

Since light caries momentum, it must exert a force on an objects which absorbs, reflects or refracts it. In most situations this force is of course negligible: walking in the Sahara, the sun bears down on you with a force of order $10^{-6}N$, one thousandth of the weight of a drop of sweat. But in the absence of other forces (as in space) or when using intense light and microscopic particles, these forces can become significant. If in addition we can control the intensity and direction of the light, then these forces can become useful: we can build traps, where the forces tend to constrain a particle to a point in space.

Geometrical Optics

Consider a sphere immersed in liquid, with the sphere having a greater refractive index than the liquid. Then a ray passing through the bead (off-centre) will be bent as it passes through, producing a force on the sphere. This refractive force is what we're interested in.

A uniform beam can't produce forces towards a point, by symmetry, but a focussed one can. Frist, for the transverse direction, consider a beam which is strongest in the centre, with a sphere placed off-centre. This is shown in the left hand part of Figure 1. The the refractive force from ray C is stronger that that from the weaker ray D, because the intensity along C is higher. So the resultant (refractive) force is towards the centre of the beam.

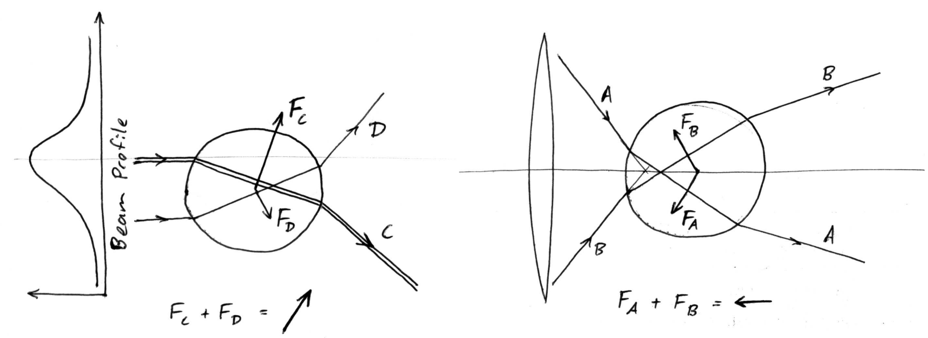

Figure 1: Ray diagrams to demonstrate trapping, both perpendicular to the beam (left) and along it (right).

Second, for the longitudinal direction (along the beam) consider a focussed beam with a sphere placed just behind the focus. Then, as is shown in the right hnd part of the figure, the net refractive force is towards the focus — the beam pulls the sphere.

Some of the light will also be reflected off the surface of the sphere, so there will be a reflective force, approximately along the beam direction. This might overcome the attraction found above, destroying the trap. One way to avoid this is to focus two beams onto the same point, from opposite directions, so that the reflective forces cancel.

A simpler way comes from noticing that the further apart rays A and B are, the more pulling they do and the less their reflections push the sphere. So the wider the cone of light converging on the focal point, the better the trap will work.

Smaller Particles

We are justified in using geometrical optics when the sphere is large compared to the photons: $R \gg \lambda$. Trapping is also possible for much smaller particles, but will need a different explanation. Our experiment used 1μm latex beads and a 633nm (Helium-Neon) laser, so $R \sim \lambda$.

A simple explanation (from [4]) runs as follows: the bead is of a dielectric material, so in an electric field (of the laser light) $\mathbf{E}$, some dipole moment $\mathbf{P} \propto \mathbf{E}$ is induced. The energy of a dipole moment in an electric field is

$$U \propto \mathbf{P} \cdot \mathbf{E} \propto -E^2 \propto -I$$

where I is the beam intensity. Thus the bead is attracted to the point of maximum intensity, the focus.

A more detailed explanation for both $R \sim \lambda$ and $R \ll \lambda$ is given in [5].

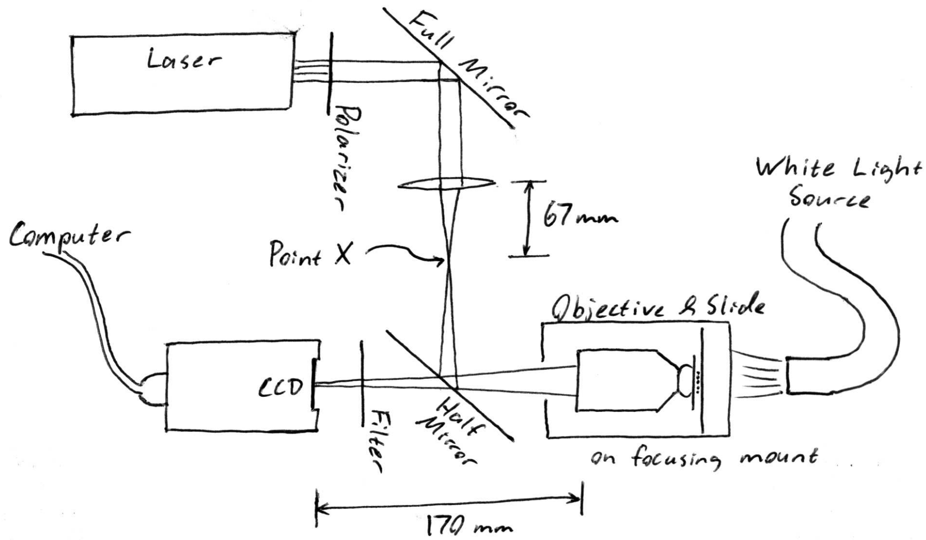

Figure 2: Layout of the experiment

Our Set-up

Figure 2 shows the layout of our experiment on the optical table. The bottom row is an open-air microscope: an incandescent light source illuminates the slide from behind, sending light through a microscope objective to project a real image onto the camera's CCD, the back focal length (170mm) away.

The same objective is also used to create the trap: a laser beam is focused by a simple lens to a point (point X in the diagram) this same 170mm from the microscope objective. The objective then re-focuses it onto the sample. The lens serves a second purpose of spreading the beam out to fill the rear aperture of the objective. This ensures we get as wide a cone of light for the trap as possible.

A semi-silvered mirror alows these two to share the objective, and a second mirror is used so as to have independed control over the position and direction of the beam entering the objective. A polariser alows us to vary the intensity of the laser beam. And a dichroic filter removes the laser's wavelength from the light reaching the camera.

The objective used is a 100x oil-immersion type. (If we used an objective designed to for an air gap between the objective and the slide, the cone angle of the trap would be limited by total internal reflection at the cover-slip–air interface.) To convert pixels on the CCD to real dimensions, the camera's specifications [3] give a pixel size of 4.65μm (square).*

*Our camera's CCD has with 1034 such pixels on the horizontal, so should be 4.8mm wide, and 6mm on the diagonal. Confusingly, this is called a $\frac{1}{3}$" type sensor, using some convention from the days of picture tubes.[2]

2 Particle Statistics

What we're going to measure is the strength of the trap, that is, the spring constant k in the potential

$$U(x) = \frac{1}{2}kx^2. \tag{1}$$

We would like to confirm that k is proportional to the beam intensity, as it must be: twice as many photons deliver twice as much momentum. To extract k from a series of snapshots of the bead's position, we need some kind of physical model.

According to the lab manual,

the time-average of $U(x)$ is just $\frac{1}{2}k_BT$, by the equipartition of energy:

$$\langle U(x)\rangle = \frac{1}{2}k\langle x^2\rangle = \frac{1}{2}k_BT$$

and hence $\langle x^2\rangle \propto k^{-1}$.

The problem is that $U(x)$ is a potential energy, and the equipartition theorem applies to kinetic energies (terms p^2 in the Hamiltonian.) If the interactions with water molecules were infrequent, then perhaps we could argue that the average kinetic and potential energies are the same, but here we are in the opposite limit.

Brownian Model

An appropriate equation of motion for the bead is

$$m\frac{d^2x}{dt^2} + \gamma\frac{dx}{dt} + kx = F \tag{2}$$

where γ is a viscous drag coefficient, k is the spring constant, and F is the random force due to thermal fluctuations. Because we expect the motion to be heavily damped, we will drop the kinetic term now and justify this later.

The viscous drag on a sphere of radius r moving with velocity v through a fluid of viscosity η is $F_{vis} = 6\pi\eta rv \equiv \gamma v$, giving γ in terms of known macroscopic quantities.

Since the source of the force F is uncorrelated collisions with water molecules, it is expected to contain all frequencies with equal amplitude: $|F(\omega)| = \text{const}$. The Fourier transform of (2), with $m = 0$, is

$$(i\omega + k)x(\omega) = F(\omega)$$

and then we can calculate the average of x^2 over some time τ:

$$\begin{aligned}
\langle x^2 \rangle &= \frac{1}{\tau}\int_0^\tau dt x(t) x^*(t) \\
&= \frac{1}{\tau}\int_0^\tau dt \sum_{nm} x(\omega_n) x^*(\omega_m) e^{i(\omega_n - \omega_m)t} \\
&= \tau \sum_n |x(\omega_n)|^2 \\
&= \tau \sum_n \frac{|F(\omega_n)|^2}{k^2 + \gamma^2\omega_n^2} \\
&= \frac{\tau^2}{2\pi}\int_{-\infty}^{\infty} d\omega \frac{|F(\omega)|^2}{k^2 + \gamma^2\omega^2} \\
&= \frac{\tau^2}{2}\frac{|F(\omega)|^2}{k\gamma}.
\end{aligned}$$

(I've also used τ as the length of the time-box to discretise frequency, $\omega_n = n\frac{2\pi}{\tau}$.) We have obtained $\langle x^2 \rangle \propto k^{-1}$ just from the assumtion that the force is white.

Boltzmann Distribution

Another approach is to start with the Boltzmann probablility distribution, for a classical system at finite temperature

$$p_i = \frac{e^{-\frac{E_i}{k_B T}}}{Z} \qquad \text{with } \sum_i p_i = 1.$$

Taking the states to be simply the positions of the bead (so ignoring kinetic energy, as above) this reads

$$\rho(x) = \frac{e^{\frac{-U(x)}{k_B T}}}{Z} \qquad \text{with } \int dx \rho(x) = 1. \tag{3}$$

With $U(x) = \frac{1}{2}kx^2$, we can calculate

$$\begin{aligned}
\langle x^2 \rangle &= \int dx \rho(x) x^2 \\
&= \frac{\int dx e^{\frac{-x^2}{2k_B T}} x^2}{\int dx e^{\frac{-x^2}{2k_B T}}} \\
&= \frac{k_B T}{2k}.
\end{aligned} \tag{4}$$

This fixes the constant of proportionality we were missing above.[†]

Instead of reducing everything to the variance $\langle x^2 \rangle$, we can perhaps do a little better: by measuring the position of the bead many times, we are in fact sampling the distribution $\rho(x)$. So taking the logarithm of (3)

$$U(x) = -k_B T \log \rho(x) + \text{const.} \tag{5}$$

we see that we can extract the shape of the potential directly.

Worries

How justified are we in neglecting the kinetic energy in (2) above? A rough argument is to say that the two relevant time scales in the problem are

$$\begin{aligned} \frac{\gamma}{k} &\text{ the relaxation time, and} \\ \sqrt{\frac{m}{k}} &\text{ the oscillator period.} \end{aligned}$$

With $m \approx 10^{-15}$kg, $\gamma \approx 10^{-8}$Nsm^{-1}, and $k \models 10^{-4}$Nm^{-1} (corresponding to 10mW, below) these are 10^{-4}s and 3×10^{-4}s: the motion decays in one third of a period. For lower trap powers things get better, at 1mW these become 10^{-5}s and 10^{-4}s.

3 Results & Analysis

We took data on several days, each time hoping for one good clean set, which we never quite got. Figure 7 shows all of it on one set of axes (twice). Below is some discussion about what you should see.

To begin, note that the x and y coordinates are treated as intependent data throughout. All of the analysis above is for one dimension, and nothing couples the two directions, so treating them as separately should be valid. The spacing of the two points (one a square, one a diamond) is some sort of measure of the error in the variance. (Given the quality of the data we have made no attempt at any more accurate statistical measure.)

28 September — this was our inital data, the first time we got the trap working well. Each point is from about 100 images, taken at 1s intervals.

30 September — an attempt to take one good set. The first five runs (at 3, 2, 4, 5, 6mW) were done reasonably consistently, again with about 100 images at 1s intervals. Then the camera's focus was changed, and readings taken at 7mW, 3mW (again, to check) and 1mW (as low as we could trap.) Figure 3 shows the data; the suspect points (from after this change) are circled.

[†]I guess the other derivation isn't essential, but I've typed it, and it is neat to see how white force implies this behaviour.

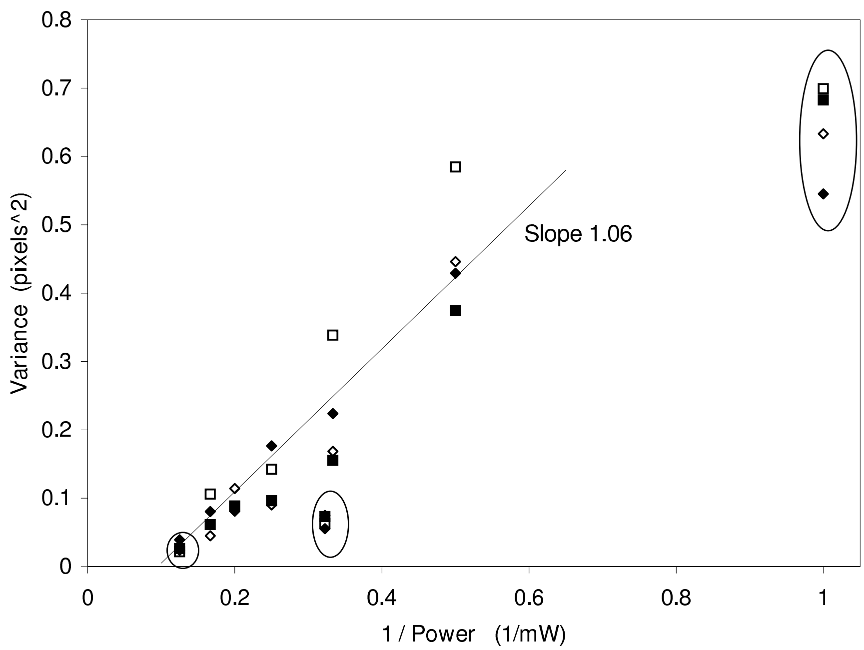

Figure 3: Data from 30 September. Diamonds and squares at the x and y directions, open symbols are from using different parameters to extract the bead's position. The circled points are suspect.

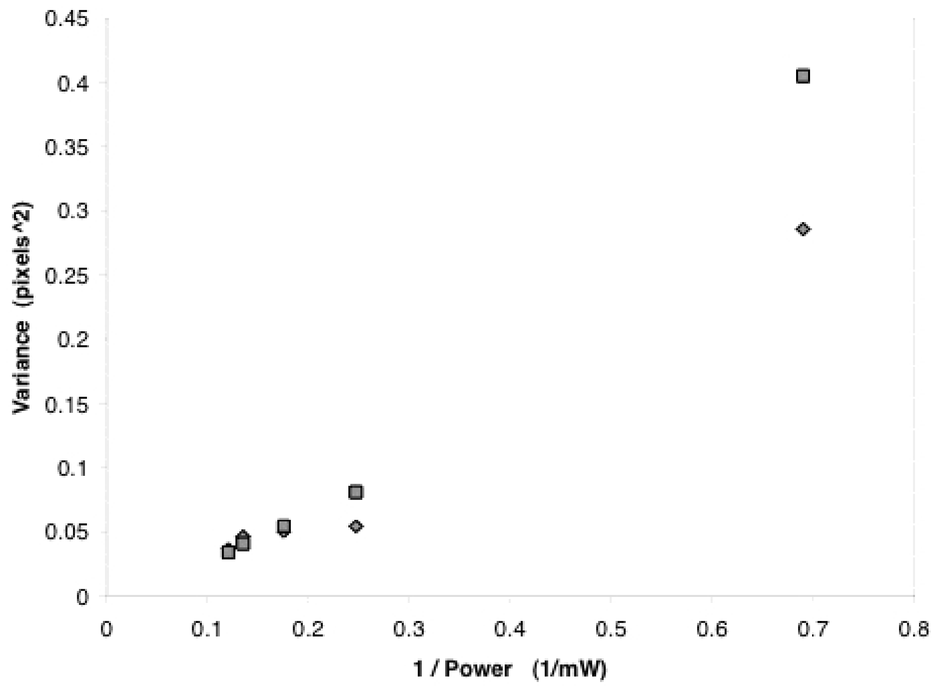

Figure 4: Data from 4 October.

Noticing that the bead's motion is a very small number of pixels, we worried about how accurately the bead-finding algorithm was working. So we asked it to find the bead both using our settings (threshold 100, minimum size 10) and its defaults (30, 20.) The results are plotted as solid and open symbols respectively.

A line has been fitted (by eye) to this data, with equation

$$\langle x^2 \rangle = (1.06 \text{ pixels}^2 \text{mW}) \frac{1}{P}.$$

4 October — deciding we needed more points, we took one more set of data, 11 runs from 1.5mW up to 10.6mW. Some of these were very long, over 500 images, most around 200 images.

Analysing this much data fully proved impossible with the time and software available. It was necessary to divide the images into smaller groups so that Scion Image could cope, and even then too hours. The results we did obtain are plotted in figure 3, and more importantly, used to study the well shape below.

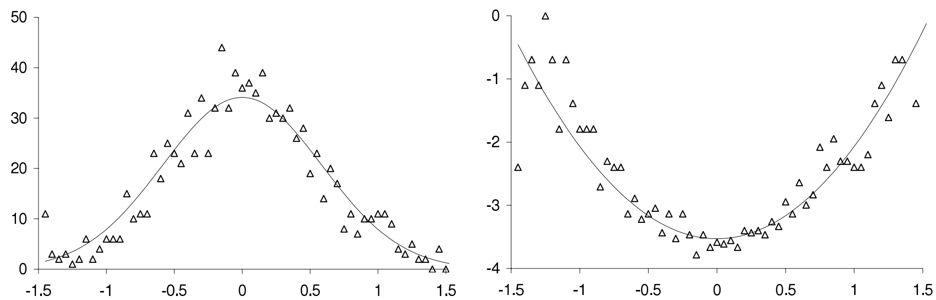

Figure 5: The distribution $\rho(x)$ and corresponding well shape $-\log \rho(x)$, at power 1.45mW, using 500x2 data points. The horizontal scale is in pixels, the vertical scale is arbitrary.

Spring Constant k

Suppose the variance $\langle x^2 \rangle$ is v pixels^2, a number of order 1. One pixel $= 4.65\mu$m on the CCD, and so $\frac{4.65\mu\text{m}}{100} = 4.65 \times 10^{-8}$m in the sample. At room temperature $k_B T = 4.14 \times 10^{-21}$Nm and so, using (4), the spring constant is

$$k = \frac{k_B T}{2\langle x^2 \rangle} = \frac{1}{v} 9.79 \times 10^{-3} Nm^{-1}.$$

Using the fit from Figure 3, $v = 1.06\text{mW}/P$, we obtain the spring constant in terms of input power as

$$\begin{aligned} k &= \frac{1}{P} 9.24 \times 10^{-6} NWm^{-1} \\ &= 92 \left(\frac{mW}{P}\right)\left(\frac{pN}{\mu m}\right). \end{aligned}$$

This matches the figure given in [5] ($92 \approx 100$).

Well Shape

In addition to plotting the variance $\langle x^2 \rangle$, we should be able to tell something about the shape of the well using (5).

We took a very long run, 500 images, at low power, 1.45mW, in the hope of seeing some deviation from Gaussin spread, corresponding to a non-parabolic potential. Figure 5 is the result (treating x and y as independent data.) Unfortunately I don't think we can claim to have seen this.

Figure 6 is the corresponding graph at very high power, 8.3mW.

4 Conclusion

We were able to construct the trap, and it certainly works. It would be nice to get better data showin the linear relation (4), but it is at least clear that

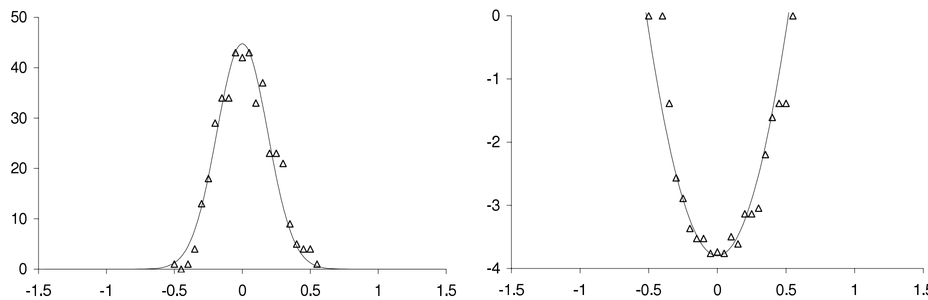

Figure 6: Distribution and well shape at 8.3mW, using 200x2 data points.

the trap strength varies with insensity. The strengths found are in agreement with published values.

It would be even nicer to be able to accurately characterise the shape of the potential well, but like [5] , we were unable to do so.

Noise

One worry not adequately addressed here is that of 'shot noise': since the movements we are detecting in the images are small, often of order 1 pixel, we should worry about how much of the variance is due to noise.

I hoped we might see this in the graphs of variance against 1/power, where it should add a constant to what should otherwise be a straight line through zero. [5] Unfortunately our straight line seems, if anything, in intercept the y-axis below zero.

It might also be possible to measure this directly, by 'taking data' of one of the beads stuck to the cover slip's surface. I thought of this too late to try it.

Thanks

are due to Marc-Andre for persuading me not to even try to analyse all the data, and to Steve for giving up a night of his holiday to help out in the lab.

Articles consulted but not cited above include an overview of all forms of optical trapping by pioneer Arthur Ashkin [1], and an article by Mark Williams [6] in which Brownian motion is used to derive a different method of determining k, by measuring a critical frequency.

References

[1] Arthur Ashkin. Optical trapping and manipulation of neutral particles using lasers. *Proc. Natl. Acad. Sci. USA*, 94:4853–4860, 1997.

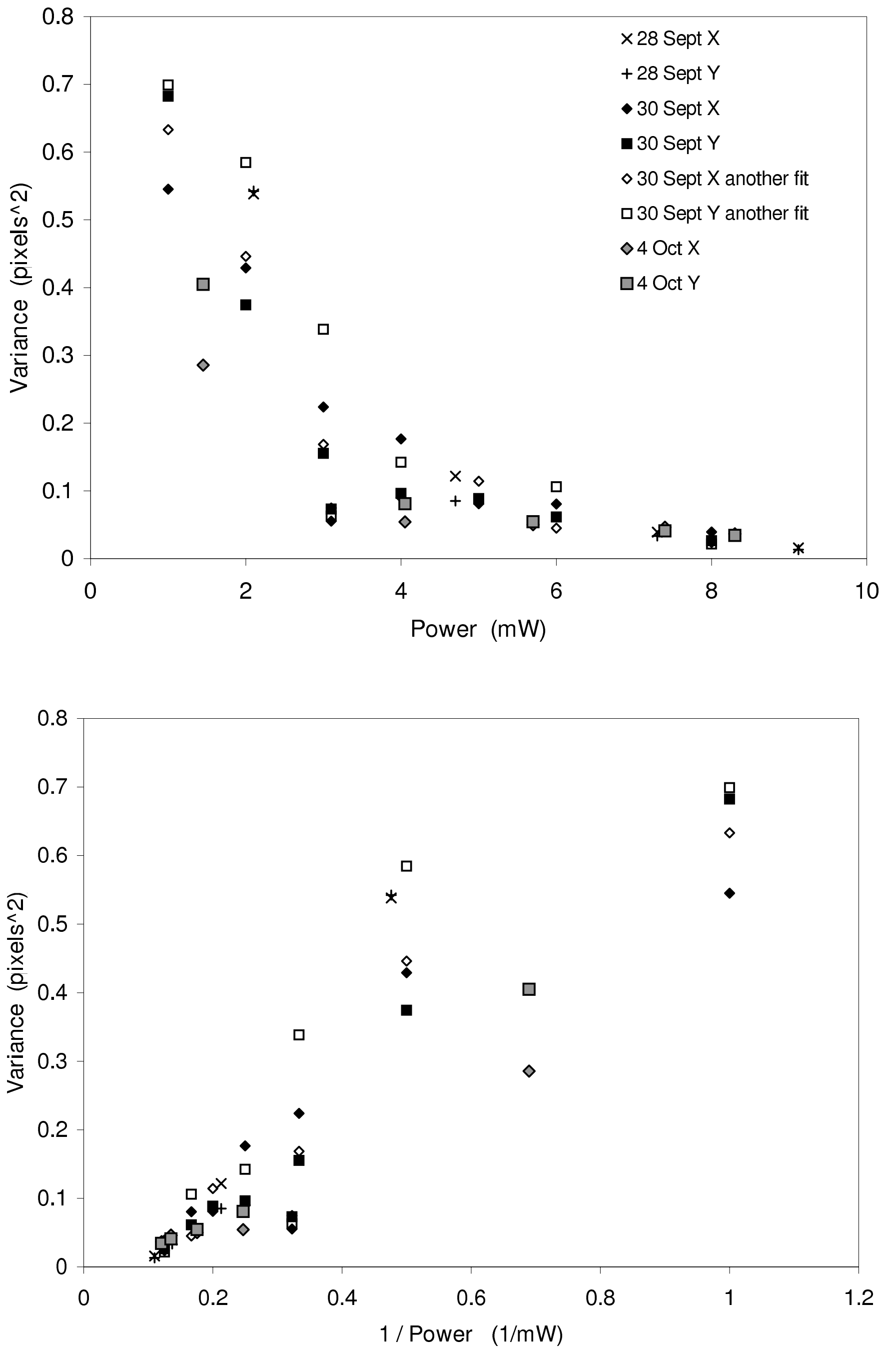

Figure 7: All the data! According to (4) the second graph here should be linear. See text for detailed discussion.

[2] Vincent Bockaert. Sensor sizes article for dpreview.com.

[3] Sony Corporation. Specifications for xcd-x710 camera. 2003.

[4] Stephen P. Smith et. al. Finexpensive optical tweezers for undergraduate laboratories. *Am. J. Phys.*, 67:26–35, 1999.

[5] Scott Wilson John Bechhoefer. Faster, cheaper, safer optical tweezers for the undergraduate laboratory. *Am. J. Phys.*, 70:393–400, 2002.

[6] Mark C. Williams. Optical tweezers: Measuring piconewton forces. 2002. http://www.biophysics.org/education/williams.pdf.