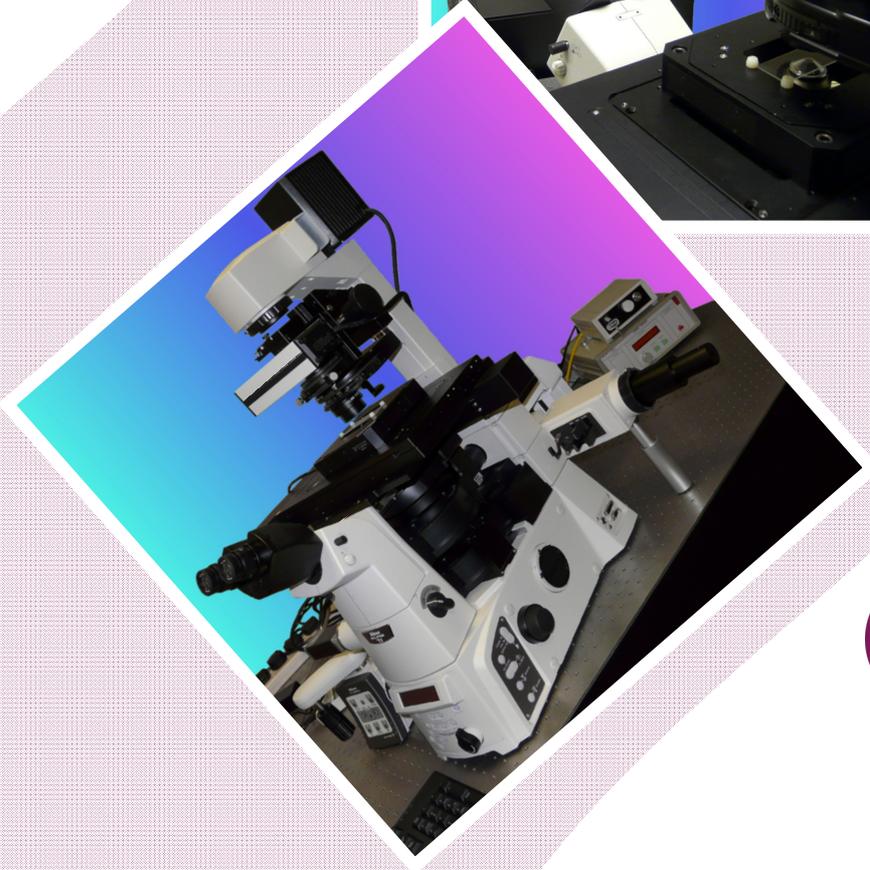


Optical Tweezer Application Notes

1. Trap Stiffness



ELLIOT SCIENTIFIC OPTICAL TWEEZERS APPLICATION NOTES

1. TRAP STIFFNESS

Optical micromanipulation has engendered some major studies across all of the natural sciences at the mesoscopic scale. Though over forty years old, the field is finding new applications and has lost none of its dynamic or innovative character: a trapped object presents a system that enables a calibrated minuscule force (piconewtons or less) to be exerted at will, enabling precision displacements right down to the ångström level to be observed. The study of the motion of single biological molecular motors has been revolutionised and new studies in the physical sciences have been realised.

Optical tweezers are based on a standard microscope that uses a high numerical aperture objective lens that enables the tightly focused light beam required for operation. The optical forces exerted on the particle cause it to act rather like a microscopic version of a Hookean spring: that is, force is proportional to displacement. A number of methods exist for recording the trap properties, including hydrodynamic drag, use of the equipartition method, and recording the power spectrum of the motion of the trapped particle. This latter technique is the one we concentrate upon here.

The power spectrum (PSD) of the thermal Brownian motions of a trapped particle of known radius may readily be used to calculate the trap stiffness. Figure 1 is an example of the PSD recorded in each axis, for an 800 nm diameter polystyrene micro-particle trapped 5 µm above the lower surface of the trapping chamber. The Lorentzian profile of the PSD is fitted (bold lines, figure 1) to find the 'roll-off' frequency (or 'corner frequency' as it is also commonly known). From this, the trap stiffness is readily calculated using the equations and methods outlined in [1,2]. Briefly:

$$f_0 = \alpha(2\pi\beta)^{-1}$$

where f_0 is the roll-off frequency, α is the trap stiffness and β is the drag coefficient.

For a particle trapped near a surface, additional drag effects due to the boundary must be considered. The full expression for the drag coefficient is given by the following equation, where h is the height of the particle above the surface, a is the radius of the particle, β_0 is the Stokes drag and η is the viscosity of the medium:

$$\beta = \beta_0 \frac{4}{3} \sinh \alpha \sum_{n=1}^{\infty} \left(\frac{n(n+1)}{(2n-1)(2n+3)} \right) \times \left[\frac{2 \sinh(2n+1)\alpha + (2n+1)\sinh 2\alpha}{4 \sinh^2 \left(n + \frac{1}{2} \right) \alpha - (2n+1)^2 \sinh^2 \alpha} - 1 \right], \text{ where } \alpha = \cosh^{-1} \left(\frac{h}{a} \right) \text{ and } \beta_0 = 6\pi\eta a$$

For typical biological applications of optical trapping, we find that the roll-off frequency is well below 1 kHz. This is much lower than the resonant frequency, confirming the very over-damped nature of the oscillations. We are in the low Reynolds number regime: viscosity dominates and inertial and gravitational forces can largely be ignored. In addition to providing the damping force, the surrounding fluid can act as a thermal bath that assists in minimising the heating effect of the laser light.

It is important to explain how we might calibrate and use a trap to exploit this knowledge. Visualising and tracking the motion of a trapped microsphere naturally leads to an in situ measurement of the trap stiffness and shape and depth of the optical potential: this determination of the exact centre of the trapped microsphere can lead to resolutions well in excess of the wavelength of light and allows biologists to perform studies at nanometric and even ångström resolution.

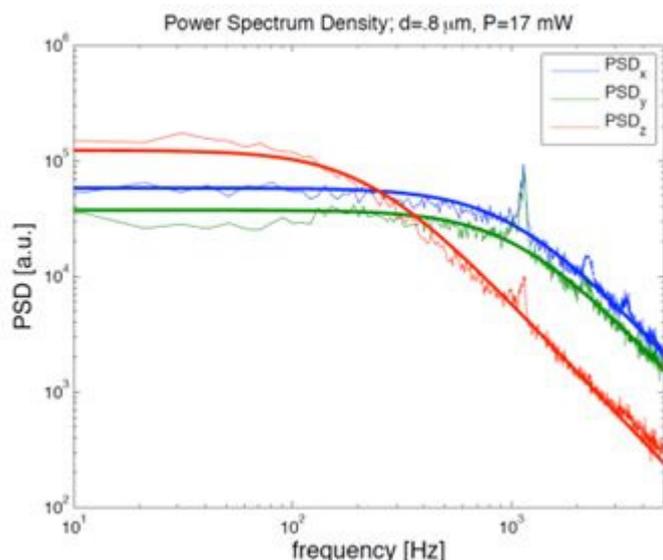


Figure 1: Power Spectral Density (PSD) of the thermal Brownian motion of an 800nm polystyrene micro-sphere trapped 5µm above the bottom of the trapping chamber. Laser power (17mW in this example) was measured at the back aperture of the objective. Lorentzian fits to the PSD (bold solid lines) in each axis provide the 'roll-off' frequency from which the trap stiffness in that axis is derived (see Figure 2).

Here we see measured data for the trap stiffness in the Elliot AOD system as a function of power for an 800 nm diameter polystyrene microsphere in water.

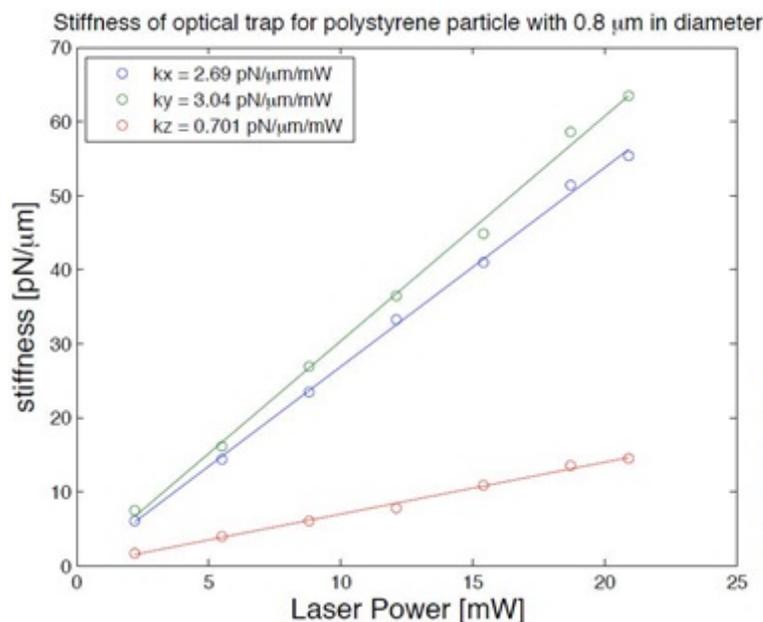


Figure 2: trap stiffness in 3D as a function of the laser power entering the trapping objective for the Elliot AOD system, using an 800nm polystyrene micro-sphere trapped 5µm above the bottom of the trapping chamber.

[1] Kirstine Berg-Sørensen and Henrik Flyvbjerg: "Power spectrum analysis for optical tweezers", Rev. Sci. Instrum. 75, 584-612 (2004).

[2] Keir C Neuman and Steven M Block: "Optical Trapping", Rev. Sci. Instrum. 75, 2787-2809 (2004).

This case study was performed at the University of St. Andrews using a standard Elliot Scientific E3500 AOD Optical Tweezers system.

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