

Optical Tweezers for Scanning Probe Microscopy

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Contents

- 0. Introduction**
- 1. Optical Tweezers**
 - 1.1 Physics of optical trapping
 - 1.2 Calibration
 - 1.3 Applications in Life Sciences
 - 1.4 Applications to Nanotechnology
- 2. Photonic Force Microscopy**
 - 2.1 Physics of PFM
 - 2.2 Applications to Life Sciences and imaging
- 3. Conclusions**

0. Introduction

- Optical tweezers are a **three-dimensional** trap for **micron** and **sub-micron** sized objects
- At its simplest an optical tweezers can be made from an inexpensive laser and a microscope
- More advanced systems can include complicated beam shaping and steering and particle tracking and detection
- This lecture aims to explain how optical tweezers work, and how they may be used for sensitive measurements of very small forces in life sciences and / or nanotechnology experiments



1. Optical Tweezers

1.1 Physics of optical trapping

1.1.1 Optical forces

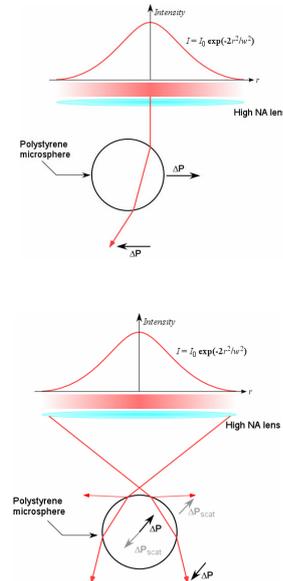
- The physics of the trapping mechanism is based on optical **gradient** and **scattering** forces arising from the interaction of strongly focussed laser light with matter
- Simple models that explain optical trapping behaviour can be applied in the **Mie scattering** ($d \gg \lambda$) and the **Rayleigh scattering** ($d \ll \lambda$) regimes depending on the size of the particle relative to the wavelength of laser light
- A real optical tweezers typically works in the intermediate ($d \approx \lambda$) regime, requiring a rigorous application of complicated approaches such as Generalised Lorentz-Mie Scattering or T-Matrix theory (beyond the scope of this lecture)
- However, insight into the trapping mechanism can be gained from studying the limiting cases

1. Optical Tweezers

1.1 Physics of optical trapping

1.1.2 'Ray Optics' model (1)

- Applied in the Mie regime $d \gg \lambda$, so that we can consider 'rays' of light being refracted at the interface between dielectric media
- For simplicity consider a **spherical** particle of refractive index n , suspended in water of refractive index n_w .
- Refraction of the 'ray' as it crosses the sphere implies a transfer of momentum from the sphere to the 'ray', and hence an equal and opposite transfer of momentum from the 'ray' to the sphere
- The gradient in intensity (number of 'rays') across the sphere produces a net transverse force towards the beam axis – an **optical gradient force**

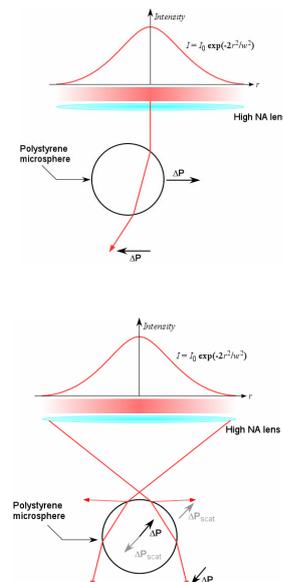


1. Optical Tweezers

1.1 Physics of optical trapping

1.1.2 'Ray Optics' model (2)

- To achieve trapping in the axial (z -) direction requires focussing of the beam where a similar argument for refraction providing an optical **gradient force** towards the focus can be made
- Axial trapping must also overcome the 'pushing' effect of the small reflection at the sphere-water interface due to the mismatch in refractive indices – the optical **scattering force**
- Stable 3D trapping requires that the gradient force exceeds the scattering force, which may be achieved with strong (high numerical aperture) focussing



1. Optical Tweezers

1.1 Physics of optical trapping

1.1.3 'Electric dipole' model

- Applied in the Rayleigh regime $d \ll \lambda$, so that we can consider an electric dipole that is polarised by the application of an electric field
- A separation of charge (electric dipole) is **induced** in the dielectric by the applied field:

$$\underline{p} = \alpha \underline{E}$$

- The interaction energy of the dipole is

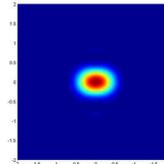
$$U_{\text{dip}} = -\underline{p} \cdot \underline{E} = -\alpha \underline{E} \cdot \underline{E} \propto -I(r)$$

- Remembering that the intensity distribution is gaussian in the transverse plane we see that for small displacements from the axis we have

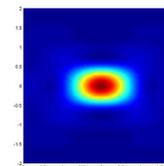
$$\underline{F}_{\text{grad}} = -\nabla U_{\text{dip}} \propto \nabla I(\underline{r}) = -\kappa_i x_i$$

- i.e. a force proportional to the gradient in intensity
- Strong confinement is therefore achieved by strong focussing

x-y plane



x-z plane



1. Optical Tweezers

1.1 Physics of optical trapping

1.1.4 Optical tweezers characteristics (1)

- Both models for limiting cases give similar behaviour for the forces in optical tweezers
- A particle is trapped close to the focus of the laser beam (in fact the equilibrium position is just beyond the focus due to the scattering force)
- For small displacements from equilibrium the restoring force on the particle is proportional to the displacement and directed towards the equilibrium position, i.e. it behaves as a mass-spring oscillator with spring constant κ .
- The spring constant is proportional to the trap intensity
- The spring constant in the axial (z-) direction is different from (and weaker than) the transverse (x- and y-) directions (in fact for nanoparticles the spring constants in x- and y- are also different from each other due to polarization induced symmetry breaking)

1. Optical Tweezers

1.1 Physics of optical trapping

1.1.4 Optical tweezers characteristics (2)

- Typical parameters for an optical tweezers are
 - Particle size, $d \sim 0.1 - 10 \mu\text{m}$
 - Maximum trapping force, $F_{\text{max}} \sim 10 - 100 \text{ pN}$
 - Potential well depth, $U \sim 10 - 100 \times 10^{-21} \text{ J}$ i.e. $\mathcal{O}(k_{\text{B}}T)$ at biological temperature
 - Spring constant, $\kappa \sim 1 - 10 \text{ pN } \mu\text{m}^{-1}$
- However, all these parameters depend on laser wavelength, objective numerical aperture, particle size and refractive index, suspending liquid refractive index, lens aberrations...so $\kappa = \kappa(d, \lambda, \text{NA}, n, n_w, \dots)$ etc

1. Optical Tweezers

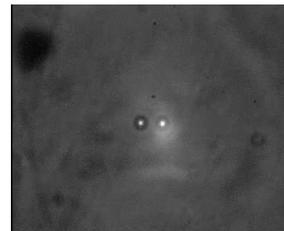
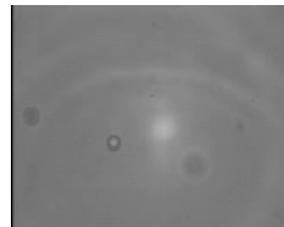
1.1 Physics of optical trapping

1.1.5 Optical trapping and manipulation

- Once a particle is trapped it may be dynamically manipulated by steering the trap position: in the movie this is achieved using scanning mirrors
- The particle will remain trapped provided that the viscous drag force of the suspending liquid does not exceed the maximum trapping force, i.e.

$$F_{\text{drag}} = -\gamma v < F_{\text{max}}$$

- Multiple particles can be trapped by ‘jumping’ the trap position quickly between a number of locations
- Alternative methods include holographic optical tweezers for multiple traps, individual particle steering and optical rotations



1. Optical Tweezers

1.2 Calibration

1.2.1 Experimental method (1)

- An optical tweezers becomes a useful tool for quantitative measurements when the spring constant can be calibrated
- Due to the difficulties of a theoretical description of optical trapping in a realistic size regime this must typically be done experimentally
- A segmented or **quadrant photodiode** is placed in a plane conjugate with the back aperture of the microscope condenser lens
- The laser light transmitted through the microscope carries an interference pattern between unscattered and forward scattered light from the trapped particle

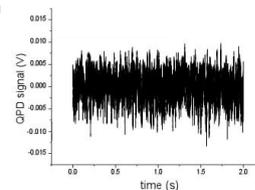
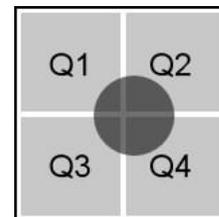


1. Optical Tweezers

1.2 Calibration

1.2.1 Experimental method (2)

- The spatial distribution of the interference pattern depends on the position of the particle relative to the waist of the focussed laser beam
- Combining the signals from the four quadrants of the QPD gives signals that measure the particle's displacement from equilibrium in along of the Cartesian axes e.g. the signal $S_x = (Q1 + Q3) - (Q2 + Q4)$ is proportional to the displacement in the x -direction
- Fluctuations in position due to the particle's Brownian motion can be tracked by the QPD and appear as a randomly fluctuating signal voltage, or 'noise'
- A number of techniques exist for analysing the 'noise' that enable us to deduce the characteristics of the optical tweezers trapping potential



1. Optical Tweezers

1.2 Calibration

1.2.2 The Langevin equation

- Equation of motion of a damped harmonic oscillator subject to a randomly fluctuating force:

$$m \frac{d^2x}{dt^2} + \gamma \frac{dx}{dt} + \kappa x = \xi(t)$$

- The term $\xi(t)$ describes random (uncorrelated) fluctuations in force with zero mean, i.e.

$$\langle \xi(t) \rangle = 0 \quad \langle \xi(t + \tau) \xi(t) \rangle = 2\gamma k_B T \delta(\tau)$$

- Where the angled brackets indicate a *time-averaged* quantity
- 3D particle tracking and analysis of the Brownian position fluctuations reveals the trap parameters
- Start by assuming the oscillator is **heavily overdamped**, i.e. that the inertial term is negligible compared to viscous and trap forces

1. Optical Tweezers

1.2 Calibration

1.2.2 The Langevin equation: autocorrelation function analysis (1)

- Equation of motion in the overdamped regime:

$$\gamma \partial_t x(t) = -\kappa x(t) + \xi(t)$$

- Calculate the **autocorrelation** of position fluctuations:

$$C_{xx}(\tau) = \langle x(t)x(t + \tau) \rangle$$

- And differentiate with respect to the **lag time**, τ :

$$\partial_\tau C_{xx}(\tau) = \langle x(t) \partial_\tau x(t + \tau) \rangle$$

- Substitute for the derivative of x , and note that the time average of the second term is zero

$$x(t) \partial_\tau x(t + \tau) = -\frac{\kappa}{\gamma} x(t)x(t + \tau) + \frac{1}{\gamma} x(t)\xi(t + \tau)$$

1. Optical Tweezers

1.2 Calibration

1.2.2 The Langevin equation: autocorrelation function analysis (2)

- Giving a differential equation for the autocorrelation function:

$$\partial_{\tau} C_{xx}(\tau) = -\frac{\kappa}{\gamma} \langle x(t)x(t+\tau) \rangle = -\frac{\kappa}{\gamma} C_{xx}(\tau)$$

- The solution to which is straightforward:

$$C_{xx}(\tau) = C_{xx}(\tau = 0) \exp(-\omega\tau)$$

- A exponential decay with lag time, τ , with the decay constant given by

$$\omega = \frac{\kappa}{\gamma}$$

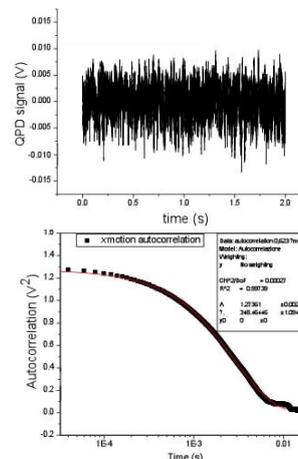
- Provided the viscous drag coefficient, γ , is known the spring constant, κ , can be calculated from a fit

1. Optical Tweezers

1.2 Calibration

1.2.2 The Langevin equation: autocorrelation function analysis (3)

- Calculate the autocorrelation of the randomly fluctuating position signal
- Fit to an exponential decay
- Two fitting parameters: time constant of decay, ω^{-1} , gives the trap spring constant; zero-time intercept (amplitude) gives the detector sensitivity in V / nm.
- Together they enable measurement of displacement with sub-micron precision, and force with sub-piconewton precision



1. Optical Tweezers

1.2 Calibration

1.2.2 The Langevin equation: power spectrum analysis (1)

- An alternative method of extracting the trap spring constant is to consider the **power spectrum** of position fluctuations. Starting again with the **Langevin equation** in the overdamped regime:

$$\gamma \partial_t x(t) = -\kappa x(t) + \xi(t)$$

- Now consider the power spectrum of the random force fluctuations:

$$|\tilde{\xi}(f)|^2 = 4\gamma k_B T$$

- Where \sim indicates a Fourier transform. The power spectrum is independent of frequency – it is an ideal **white noise** source.

1. Optical Tweezers

1.2 Calibration

1.2.2 The Langevin equation: power spectrum analysis (2)

- If we define the Fourier transform of the position fluctuations with:

$$x(t) = \int_{-\infty}^{\infty} \tilde{x}(f) e^{-2\pi i f t} df$$

- Then we can write for the particle velocity:

$$\partial_t x(t) = -2\pi i f \int_{-\infty}^{\infty} \tilde{x}(f) e^{-2\pi i f t} df = -2\pi i f x(t)$$

- And we can take the Fourier transform of both sides of the Langevin equation:

$$-2\pi i f \gamma \tilde{x}(f) + \kappa \tilde{x}(f) = \tilde{\xi}(f)$$

- Or (re-writing in terms of angular frequency, $\omega = 2\pi f$):

$$\tilde{x}(f)(\omega_c - i\omega) = \tilde{\xi}(f)/\gamma$$

1. Optical Tweezers

1.2 Calibration

1.2.2 The Langevin equation: power spectrum analysis (3)

- Where we have defined the **corner frequency**:

$$\omega_c = \frac{\kappa}{\gamma}$$

- The **power spectrum** $S_x(\omega)$ of the position fluctuations is the squared modulus of the frequency spectrum (Fourier transform), and hence:

$$S_x(\omega) = \frac{4k_B T}{\gamma} \frac{1}{\omega^2 + \omega_c^2}$$

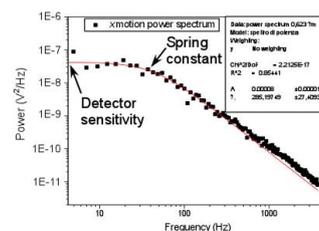
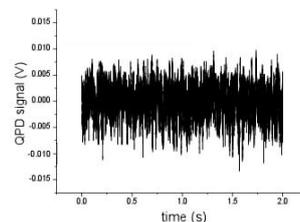
- Which is a **Lorentzian** of characteristic (half-) width ω_c , from which the spring constant can be determined.
- The zero-frequency intercept can be used to make a calibration of the position detection system in V / nm.

1. Optical Tweezers

1.2 Calibration

1.2.2 The Langevin equation: power spectrum analysis (4)

- Fourier transform randomly fluctuating QPD signal and plot modulus squared against frequency
- Fit to a Lorentzian
- Two fitting parameters: corner frequency, ω_c , gives the trap spring constant; zero-frequency intercept (amplitude) gives the detector sensitivity in V / nm.
- Together they enable measurement of displacement with sub-micron precision, and force with sub-piconewton precision

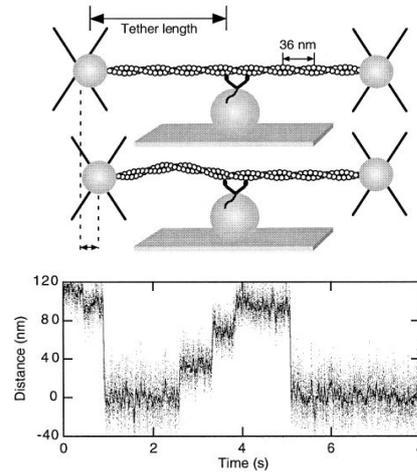


1. Optical Tweezers

1.3 Applications to Life Sciences

1.3.1 Motor protein step size

- A 'classic' optical tweezers experiment which uses a 'dual beam' trap
- Two polystyrene beads are held in optical tweezers with an actin filament stretched between them
- The filament is lowered towards a third sphere which has a low density coating of the motor protein Myosin V
- The Myosin 'steps' along the actin filament in a progressive manner driven by hydrolysis of ATP producing small displacements of the optically trapped microbead
- The regular step size of 36 nm is evident in the particle tracking signal



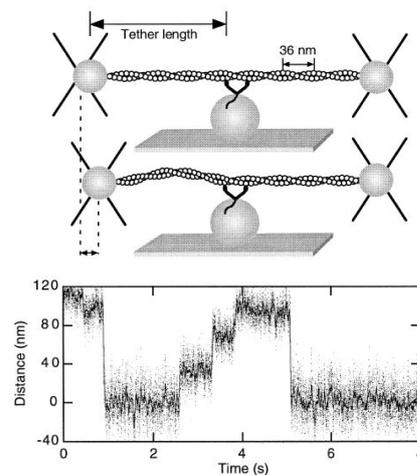
Data from: A. Mehta et al, *Nature* 400 590 (1999)

1. Optical Tweezers

1.3 Applications to Life Sciences

1.3.2 Motor protein forces

- The spring constant of the optical trap is also calibrated
- Typically the protein was found to perform 3 – 5 steps before 'stalling' when the bead returns to the equilibrium position in the trap
- The force required to 'stall' the motor was therefore measured to be 3.0 ± 0.3 pN



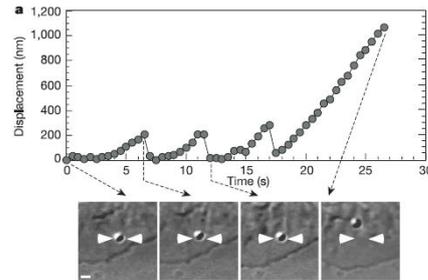
Data from: A. Mehta et al, *Nature* 400 590 (1999)

1. Optical Tweezers

1.3 Applications to Life Sciences

1.3.3 Single molecule bond strength

- An optically trapped microbead is allowed to bond to an integrin on the surface of a migrating cell
- Talin binds the cytoplasmic tails of the integrins to the actin cytoskeleton
- The migrating cell pulls the bead out of the calibrated trap until the trap restoring force exceeds the strength of the talin 'slip bond'
- The microbead 'handle' returns to the trap centre, and from the maximum displacement the force required to break the bond is deduced to be 2 pN



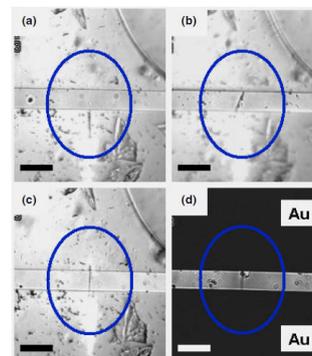
Data from: G. Jiang et al, *Nature* **424** 334 (2003)

1. Optical Tweezers

1.4 Applications to Nanotechnology

1.4.1 Nano-assembly

- Optical tweezers can be used to trap and manipulate nano-materials for the assembly of complex nanostructures
- In this example metal oxide (CuO) nanorods are manipulated with optical tweezers and used to bridge a gap between two gold electrodes deposited on a glass slide



Data from: T. Yu et al, *Nanotechnology* **15** 1732 (2004)

2. Photonic Force Microscopy

2.1 Physics of PFM

2.1.1 Optical Tweezers as a scanning probe microscope

- Optical tweezers as a tool for scanning probe microscopy was first suggested by L. P. Ghislaine & W. W. Webb, *Opt. Lett.* **18** 1678 (1993)
- The mechanical cantilever and tip of the atomic force microscope (AFM) is replaced with an optically trapped micro- or nanoparticle to make a **photonic** force microscope (PFM)
- PFM has some advantages over AFM for particular applications, especially for imaging of soft structures
- The first commercial OT based PFM system has recently become available from JPK Instruments, developed in conjunction with EMBL, Heidelberg



2. Photonic Force Microscopy

2.1 Physics of PFM

2.1.2 PFM vs AFM

	Photonic Force Microscope (PFM)	Atomic Force Microscope (AFM)
Spring constant (stiffness), κ / Nm^{-1}	$10^{-4} - 10^{-5}$	0.1 - 1
Force resolution, $\delta F / \text{N}$	10^{-13}	10^{-10}
Resonant frequency, f_0 / Hz	~ 1000	~ 2000

- PFM can achieve high force resolution as the OT is a very 'soft' spring, but keeps a high resonant frequency well above mechanical vibrations

2. Photonic Force Microscopy

2.1 Physics of PFM

2.1.3 Brownian motion in a potential well (1)

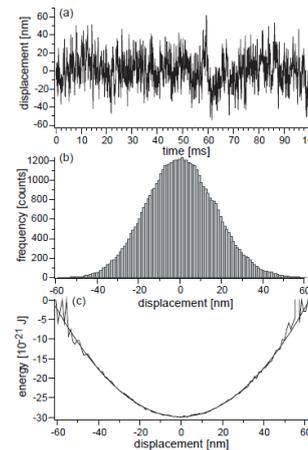
- The Brownian position fluctuations of the optically trapped probe particle can be tracked with nanometre resolution by a calibrated position detection system
- A histogram of the position fluctuations can be built up. For a harmonic potential, by equipartition of energy:

$$\frac{1}{2}\kappa\langle x^2 \rangle = \frac{1}{2}k_B T$$

- The optical potential well can be reconstructed by assuming Boltzmann statistics:

$$p(x)dx \propto \exp(-E(x)/k_B T)$$

- A quadratic fit shows the harmonic approximation is reasonable



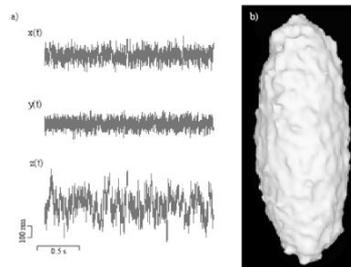
Data from: E.-L. Florin et al, *Appl. Phys. A* **66** 75 (1998)

2. Photonic Force Microscopy

2.1 Physics of PFM

2.1.3 Brownian motion in a potential well (2)

- Particle motion can be tracked in three dimensions as shown opposite. Note that the fluctuations in the axial (z) direction are slower than the transverse directions
- The three-dimensional potential well can be represented by the **3D energy isosurface** plotted at $E = 5k_B T$ above the potential minimum
- Slower fluctuation in the axial direction are a consequence of the lower potential curvature (smaller spring constant) in this direction



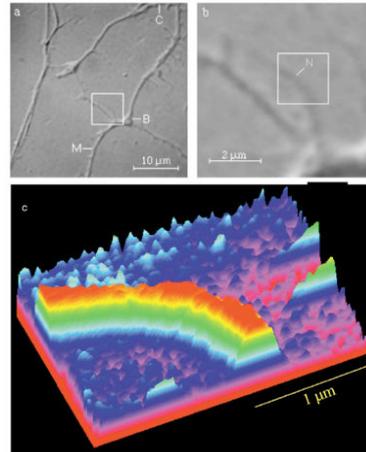
Data from: A. Pralle et al, *Single Mol.* **1** 12 (2000)

2. Photonic Force Microscopy

2.2 Applications of PFM

2.2.1 PFM as a scanning probe

- Scanning the trapped probe over a structured surface reveals information about surface morphology with a resolution determined by the size of the probe, which may be a fraction of the optical wavelength.
- Figure shows optical DIC images of neural dendrites compared with a PFM image acquired using a 200 nm diameter latex bead as the probe.
- The PFM was operated in 'constant height' mode using a fluorescent nanosphere, the changing intensity of fluorescence emission providing a sensitive measure of axial displacement as the probe is scanned over the surface



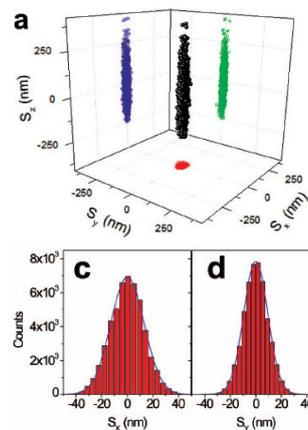
Data from: A. Pralle et al, *Single Mol.* 1 12 (2000)

2. Photonic Force Microscopy

2.2 Applications of PFM

2.2.2 PFM with a nanoprobe

- Nanoscale material such as carbon nanotubes can be used as the optically trapped probe in PFM
- 3D particle tracking of a trapped CNT bundle shows a large asymmetry in the trap aspect ratio
- The nanometre-scale diameter of the CNT bundle enables tight transverse confinement and high spatial resolution, whereas the extended structure makes the longitudinal spring constant small, and therefore a very sensitive probe of forces in the axial direction with resolution < 10 fN



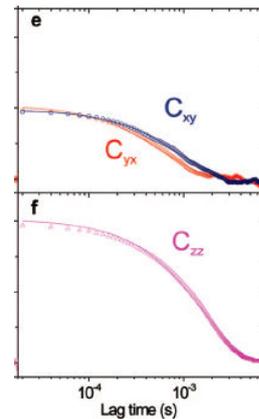
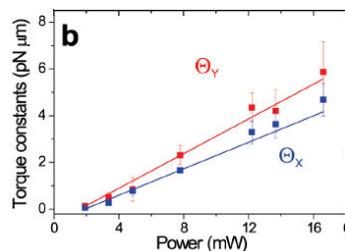
Data from: O Marago et al, *Nano Lett.* 8 3211 (2008)

2. Photonic Force Microscopy

2.2 Applications of PFM

2.2.3 Photonic Torque Microscopy

- Unlike a trapped sphere a rod-like particle such as a CNT bundle can perform angular fluctuations also
- **Cross-correlations** of the particle tracking reveal angular motion, allowing us to define an optical **torque** constant, and measure a torque of $\sim 1\text{pN} \cdot \mu\text{m}$



Data from: O Maragò et al, *Nano Lett.* **8** 3211 (2008)

3. Conclusions

3.1 Suggested further reading

- 'Optical trapping', K C Neuman & S M. Block. *Rev. Sci. Instrum.* **75**(9) 2787-2809 (2004)
- 'Lights, action: optical tweezers', J E Molloy & M J Padgett. *Contemp. Phys.* **43**(4) 241-258 (2002)
- 'Signals and noise in micromechanical measurements', F Gittes & C F Schmidt. *Methods in Cell Biology* **55** 129-156 (1998)
- 'Recent advances in optical tweezers', J R Moffat, Y R Chemla, S B Smith & C Bustamente. *Annu. Rev. Biochem.* **77** 205-208 (2008)
- 'Light at work: The use of optical forces for particle manipulation, sorting, and analysis', A Jonáš & P Zemánek. *Electrophoresis* **29** 4813-4851 (2008)
- 'Photonic force microscopy: from femtonewton force sensing to ultra-sensitive spectroscopy', O M Maragò, P G Gucciardi & P H Jones in *Scanning Probe Microscopy in Nanoscience and Nanotechnology 1* (Springer) B Bushan (Ed.) (2010)
- UCL Optical Tweezers website: www.ucl.ac.uk/~ucapphj
- The 'Holoassembler': www.holoassembler.com. State-of-the art micro and nanomanipulation with fingertip control!

Projects

- CoMPLEX MRes and Nanotechnology MSc projects in optical trapping & nano-optics may be available this year.
- See website www.ucl.ac.uk/~ucapphj or contact philip.jones@ucl.ac.uk