

In the past decade, Atomic Force Microscopy (AFM) and Optical Tweezers (OT) have revolutionised molecular science by measuring picoNewton forces over lengthscales from 1 to  $10^4$  Angstroms. In our laboratory, we have an Optical Tweezer Apparatus which has been extensively modified for our force measurement experiments in polymer and colloids science. The apparatus consists of an optical trap that weakly “holds” a micron-sized bead. The trap is formed by a focused laser beam that is refracted through the transparent bead. The refracted rays differ in intensity over the volume of the colloidal bead and exert a force on the bead, drawing it towards the region of highest light intensity. The optical trap is harmonic near the focal point: the optical force acting on a colloidal particle positioned at  $x$  from the trap centre is  $F_{\text{opt}} = -kx$ , where  $k$  is the trapping constant which can be tuned by adjusting the laser power. In this way, the optical trap generated by the OT serves to both localise a colloidal particle and to measure the small, sub-picoNewton scale forces acting on the particle. With substantial modifications, our OT apparatus probes forces at small lengthscales and over small timescales that are necessary in studies of nonequilibrium statistical mechanics and polymer/biopolymer science.



### Probing Thermodynamics of Small Systems over Small Timescales using Optical Tweezers

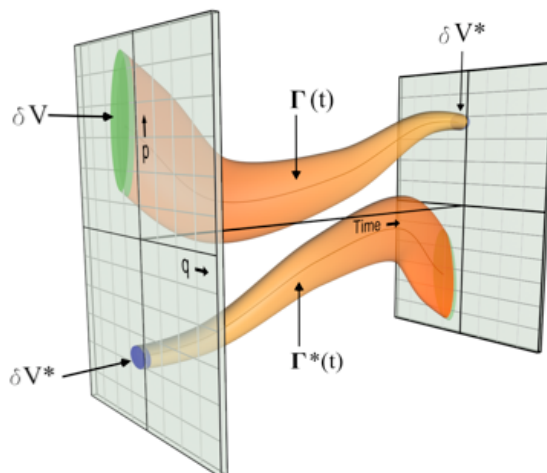
The puzzle of how time-irreversible microscopic equations of mechanics lead to the time-irreversible macroscopic equations of thermodynamics has been a paradox since the days of Boltzmann. Boltzmann simply side-stepped this enigma by stating “as soon as one looks at bodies of such small dimension that they contain only very few molecules, the validity of this theorem [the Second Law of Thermodynamics and its description of irreversibility] must cease.” Today we can state that the Fluctuation Theorem, first proposed by Denis Evans and colleagues in 1993, is a generalized, Second-Law like theorem that bridges the microscopic and macroscopic domains and links the time-reversible and irreversible descriptions. The predictions of the FT should be relevant to many nanotechnological applications. Our work in 2003 had three major themes:

### Experimental Demonstrations of the FT

The first experimental demonstration of the FT was achieved only in 2002, in a joint collaboration between the Evans and Sevick groups. We continue to demonstrate experimentally the FT predictions using a modified OT to measure picoNewton forces acting on a colloidal bead over nanometre to micron distances. Last year, we demonstrated an integrated form of the Transient Fluctuation Theorem (ITFT) through analysis of trajectories of an optically-trapped colloidal particle subject to solvent flow field. This was the first experimental demonstration in the FT field and it received considerable international press. This year, after further modifications to the OT, we demonstrated *directly* the Transient Fluctuation Theorem (TFT), for the first time, using the “capture” experiment. The experiment follows the transient trajectories of a colloidal particle in a stationary optical trap whose strength changes discontinuously. Unlike our previous experiment, the measured averages of the dissipation function also agreed with analytic and numerical predictions of the Langevin equation. (with D.M. Carberry, J.C. Reid, G.M. Wang, D.J. Searles [(Griffith U.), and D.J. Evans)

## Stochastic Descriptions of the FT

The argument of the FT is the dissipation function, which is a measure of the system's irreversibility. It was originally defined for deterministic, Newtonian equations of motion or "detailed balance", but has been applied inconsistently to systems where the relevant time/length scales are too large to be simulated using Newtonian dynamics. In 2003 we presented a definition for a stochastically-derived dissipation function for systems described by stochastic, Langevin dynamics. We demonstrated this stochastically-derived dissipation function in experimental analyses and showed that, for a given system, there can be "different" forms of the dissipation function that obey the FT. (with J.C. Reid, D.M. Carberry, G.M. Wang, D.J. Searles [(Griffith U.)], and D.J. Evans)



An illustration of a set of neighbouring Newtonian trajectories initiated in a volume element  $\delta V$  (top tube) and corresponding set of anti-trajectories initiated in  $\delta V^*$  (lower tube) in coordinate-momentum ( $q, p$ ) and time,  $t$ , space. For every trajectory  $\Gamma(t)$  initiated in  $\delta$ , there exists a time-reversed or anti-trajectory  $\Gamma(t)^*$  that is initiated in  $\delta V^*$ . The ratio of the probability of observing trajectories initiated within  $\delta V$  to those in  $\delta V^*$  is a measure of the system's irreversibility.

## Kawasaki Identity and the FT

We have shown that the FT implies that the Kawasaki function is equal to unity. More importantly, we demonstrated that deviation of the Kawasaki function from unity can be used as a diagnostic tool in simulation and experiment, indicating the quality of the sampling of data. (with D.M. Carberry, S.R. Williams, G.M. Wang, and D.J. Evans)

## Optical Tweezers for Biopolymer Studies

By attaching specially-coated latex beads to the ends of modified DNA, we are able to use an optical trap and micropipette to stretch a single bead-DNA-bead assembly and to construct a force versus extension profiles of several DNA systems. These force profiles are used to study the effect of different salts and the interactions of specific binding proteins on DNA. In 2003 and in conjunction with the Protein Synthesis and Evolution Group headed by Nick Dixon, we focused upon different end-tethering techniques so that we can efficiently stretch *ds*-DNA where one strand is linked to the beads, and where both strands are linked to the beads. (with G.M. Wang, D.M. Carberry, P. Schaeffer, and N.E. Dixon)

<http://rsc.anu.edu.au/research/sevick.php>