Optical Tweezers: Experimental Demonstrations of the Fluctuation Theorem

A thesis submitted for the degree of Doctor of Philosophy of The Australian National University

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October 2005
Statement of Originality

I hereby declare that this submission is my own work. To the best of my knowledge and belief this thesis contains no material previously published or written by another person, except where due acknowledgement has made in the text of the thesis. This work has not been submitted previously, in whole or in part, to qualify for any other academic award.

David Carberry

Date
Acknowledgements

First and foremost I would like to thank Dr Genmiao Wang who has taught me virtually everything I know about Optical Tweezers. Without his assistance and guidance I doubt that these experiments would have been successful. I would also like to thank Dr Edie Sevick, my principal supervisor, for providing guidance with the project when it was sought, yet allowing me to freely pursue my goals at other times. Her implicit trust in my research abilities and supervision method proved to be ideal for turning this student into an independent researcher. Prof Denis Evans' dedication and non-stop enthusiasm for his science, willingness and ability to explain difficult concepts, and overall approachability also made this project truly enjoyable.

The other members of the Sevick and Evans research groups: James Reid, Matthew Baker, Dr Stephen Williams and Dr Joanne Bright who ensured that my time spent in the lab was not all serious, and that my focus remained wider than my individual project. Additional thanks need to go to Stephen who patiently provided detailed explanations and error checking with many of the derivations in this thesis.

I would also like to thank the staff in the RSC workshop: Russell Koehne, Don Pepper, Mike Hill, Peter Devitt, Ray Filardo and Ian Clark. Their assistance building, designing and repairing devices for the Optical Tweezers has ensured that this research could proceed. Working with these people was always entertaining and gave me a chance to do some interesting engineering work.

I would also like to thank my family for listening to me complain about this PhD for 3.5 years. They ensured that life remained “relatively normal” regardless of how my research was going. The ability to visit the farm to relax and do something completely different for a few days proved vital for my sanity.

My friends, especially Christine White, Kate and Dave Osborn, Mel Ford, Monica Cooper and Richard van der Male, Belinda Haupt, Angela Ford, Marcus Uzubalis, Sara Barnes, Enesh Sejmuradova and many others have ensured that life outside of uni was truly enjoyable. I’d also like to thank the members of the RSC soccer team, who provided a much needed chance to escape the lab during lunch and ensured that I learnt how to manage people. I now understand why people say that organising academics is like herding cats. Finally, I’d also like to thank the people at the ANU gym: Charon, Angie, Nina, Emma, and the rest of the bars crowd who kept me pushing my limits while keeping things fun.
Abstract

In the late 19th and early 20th centuries famous scientists like Boltzmann, Loschmidt, Maxwell and Einstein tried, unsuccessfully, to find the link between the time-reversible equations of motion of individual molecules and irreversible thermodynamics. The solution to this puzzle was found in 1993, and the link is now known as the Fluctuation Theorem (FT). In the decade that followed theory and computer simulation tested the FT and, in 2002, an experiment indirectly demonstrated the FT.

This thesis describes original experiments that demonstrate the FT directly using Optical Tweezers. A related expression, known as the Kawasaki Identity, is also experimentally demonstrated. These experimental results provide a rigorous demonstration that irreversible dynamics can be obtained from a system with time-reversible dynamics.
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Chapter 1

Introduction

In the late 19th Century there was a public debate between several well known scientists: Loschmidt, Boltzmann, Maxwell and others. This debate, known as Loschmidt’s paradox, centered on finding the link between Newton’s equations of motion for individual molecules (part of the pioneering work by Boltzmann in statistical mechanics) and the irreversible equations in thermodynamics. Loschmidt pointed out that Newton’s equations of motion could be solved in both a time-forward and time-reverse direction from an initial set of time-reversible conditions. That is, starting at state 1 the equations of motion can be solved from time \( s = 0 \) to some arbitrary time \( t \) later in state 2, producing a certain amount of entropy. But as the equations of motion are time-reversible it is equally possible to start at state 2 at \( s = 0 \) and proceed back to state 1 over the same period of time “consuming” entropy. This is in “violation” of the second law of thermodynamics. The second law of thermodynamics states that the entropy of a closed system must increase, and these time-reversed paths clearly disobey this law. In order to overcome this paradox, and referring to the second law, Boltzmann stated “as soon as one looks at bodies of such small dimension that they contain only very few molecules, the validity of this theorem must cease” \([1]\).

In 1903 Einstein published a paper that also attempted to determine the link between Newton’s equations of motion for single molecules and thermodynamics \([2]\), however his proof was based on an incorrect assumption \([3]\). Einstein wrote “We will have to assume that more probable distributions will always follow less probable ones, that is, \( S \) always increases until the distribution becomes constant and \( S \)
has reached a maximum.\footnote{The original notation used by Einstein used \( W \) rather than \( S \). The notation has been changed so as to prevent confusion between \( W \) used by Einstein and the number of microstates available to a thermodynamic system used by Boltzmann, \( W \).} \[2\] The variable \( S \) is the thermodynamic probability, originally derived by Boltzmann to relate the entropy of the system, \( S \), to the number of microstates in the system, \( S = k_B \ln W \). Einstein’s assumption then becomes: if \( S \) is in a low probability state then a higher probability state must follow. Einstein effectively assumes second law irreversibility, and does not allow for time-reversible paths or for the surroundings to do work on the system. “It appears that Einstein was unaware of Loschmidt’s paradox.” \[3\] Einstein published a second derivation in 1904, also based on this same assumption \[4\]. Einstein’s assumption was questioned by Hertz in 1910, and Einstein did not provide any proof of this assumption.

Loschmidt’s paradox was finally overcome in 1993 when Evans, Cohen and Morriss \[5\] published the first paper on the Fluctuation Theorem (FT). This paper provided the link between the reversible equations of motion for individual molecules and thermodynamics that Einstein, Boltzmann, Loschmidt and others had attempted to find. The paper provided a heuristic proof of what is now known as the Steady State Fluctuation Theorem. In 1994 Evans and Searles \[6\] provided a formal proof for the FT. In this paper Evans and Searles describe how a thermostatted equilibrium system can be perturbed by an external field. Evans and Searles calculated the probability of observing energy being \textit{dissipated to} the surroundings and compared this to the probability of observing energy being \textit{absorbed from} the surroundings. This energy absorption is exactly what Loschmidt was trying to address in his paradox. The FT states that the probability of observing these trajectories that absorb energy from the surroundings decreases exponentially as time and/or the system size increases. In this way the FT recovers the second law of thermodynamics. Several simulations were performed following this paper to test the FT \[7, 8\].

The Kawasaki Identity, a function closely related to the FT, has also been derived recently \[9, 10, 11\]. Morriss and Evans derived the Kawasaki function in 1985 \[9\] and the Kawasaki Identity (KI) in 1990 \[10\]. However, it was not until Evans and Searles’ 1995 paper \[11\] that the KI, finite sampling and time reversibility were understood. Evans and Searles did not explore the relationship between the KI and the FT at this time, but rather explored the use of the KI as a phase space normalising term. Currently no experiments have been carried out to test the KI or to see if this can
be used when testing the FT.

In 2002 Wang et al.\cite{12} performed the first FT experiment, demonstrating an integrated form of the FT. Using Optical Tweezers Wang et al. slowly dragged a colloidal particle through an aqueous solution and measured the particle’s position as it moved with and against the flow field. In their experiment Wang et al. demonstrated that work can be done by the system on a particle for time periods out to approximately 2 seconds in an apparent “violation of the second law”. They showed that the probability of observing “second-law violating trajectories” decreases exponentially with time. In the long time limit Wang et al. only observed “second law abiding trajectories”, thus recovering the expected second law behaviour.

1.1 Thesis Goals

The experiments presented in this thesis use Optical Tweezers, a device that enables energies that are a fraction of thermal energy ($k_B T$) to be measured. In this energy regime there is a distinct probability of observing the surroundings performing work on the system and, as such, Optical Tweezers are an ideal tool to test the FT. The primary goal of this thesis is to experimentally investigate the FT and associated relations with Optical Tweezers, obtaining results that can be analysed without ambiguity. The FT needs to be experimentally demonstrated to determine whether the assumptions made in its derivation are applicable to a real system. The second goal is to explore whether the FT applies only for systems that can be modelled using deterministic or stochastic equations, or whether it can be applied to a wider range of systems. Finally, the third goal is to demonstrate the KI in an experiment and investigate its relationship with the FT.

1.2 References


Chapter 2

The Optical Tweezers

This chapter provides a brief background to the Optical Tweezers, describes some of the applications where Optical Tweezers have been used and reviews the physics required to understand the operating principles of an optical trap. It also describes the equipment and operation of our Optical Tweezers and explains the calibration techniques that are used.

2.1 Background

In 1871 Maxwell [1] theorised that the momentum of light could exert a pressure on a surface, an effect that was later called “radiation pressure”. Lebedev [2], and independently, Nichols and Hull [3] experimentally demonstrated that light could exert a pressure on an object in 1901. This pressure was very weak as there was a low photon flux. A large increase in the photon flux was achieved with the invention of the laser in 1960, and with this increase in photon flux it was realised that radiation pressure could be used to perform tasks. In 1971 Ashkin et al balanced the radiation pressure exerted by a laser and gravity to trap a 20 µm dielectric particle [4]. Ashkin, among others, continued working in the field of optically trapping particles and published several articles regarding atom and colloidal particle trapping [5][6][7][8]. This work later split into two categories: laser atom cooling and optical trapping. In 1986 Ashkin et al [9] reported the first use of a single-beam optical trap to hold particles between 25 nm and 10 µm in diameter at a fixed point in water. Shortly after this publication the apparatus that Ashkin used to trap these particles became known as Optical Tweezers and the method known as optical trapping.

Today Optical Tweezers are used in a variety of ways. In the biological and
medicinal sciences Optical Tweezers are often used to separate different cell types, manipulate sub-cellular objects without damaging the cell itself and for medicinal procedures such as invitro fertilisation. Most commercial Optical Tweezers are aimed at the biological and medical markets and, as such, the wavelength of the lasers in many commercial Optical Tweezers operate in the near infra-red to avoid damaging living cells. But Optical Tweezers are much more useful than a simple sorting and micro-manipulation tool: they can be used for force measurement. By varying the strength of the laser it is possible to exert a force on a particle ranging between 0.001 pN and 100 pN (1 pN = 10^{-12} N). This in turn can be used in single-molecule research [10, 11, 12], for example the stretching of single polymer chains, or to examine the transfer of orbital angular momentum to microscopic particles [13, 14].

New types of Optical Tweezers are also emerging. The latest variant is a set of dynamic holographic optical tweezers, where the trapping characteristics of the laser beam is modified, enabling many more systems to be studied and more applications to be found. Some examples include creating a micro-optomechanic pump [15], optical fractionation [16] and 3D manipulation of particles into crystal structures [17].

2.2 The Physics of Optical Tweezers

This section outlines the basic physics that is required to understand the operation of the Optical Tweezers. This starts by considering the effect of individual photons and concludes by analysing the potential energy landscape generated by the Optical Tweezers.

2.2.1 The Energy and Momenta of Photons

From classical and quantum mechanics it is known that individual photons have energy and momentum [18, 19, 20]. The energy of a photon is given by $E =hc/\lambda$, where $E$ is the energy, $h$ is Planck’s constant ($6.626 \times 10^{-34}$J·s), $c$ is the speed of light in a vacuum and $\lambda$ is the wavelength of the photon. The corresponding momenta of this photon is $p = h/\lambda$. For example, a photon with a wavelength of 1000 nm has $1.99 \times 10^{-19}$ J of energy and a momentum of $6.65 \times 10^{-28}$ N·s. Often
it is convenient to cast energy units in terms of force-distance: pN·nm or pN·µm. The energy of this photon is then 199 pN·nm (0.199 pN·µm). This energy can be compared with several other similar energy terms. For example the hydrolysis of ATP to ADP requires \( \approx 100 \) pN·nm, an ion moving through an ion channel takes \( \approx 30 \) pN·nm and thermal energy, \( k_B T \), at room temperature is \( \approx 4 \) pN·nm. The momentum of a single photon is extremely small and, by itself, has little effect on a nano-sized system. However by using any electromagnetic radiator (for example an LED, microwave, radio transmitter or even a simple light bulb) large quantities of photons can be generated to produce a larger effect.

2.2.2 Interactions of Photons with Objects

A beam of photons can interact with an object in a number of ways. The size of the object is critical to determine the effect of these interactions. Maxwell’s equations must be solved to determine how the object will interact with the electric field of the photons when the smallest dimension of the object is smaller than the wavelength of the photons. The review by Padgett et al [21] provides further explanation of this scenario.

When an object’s smallest dimension is larger than the wavelength of the incoming photons the concepts of reflection, refraction and absorption are used. Reflection is determined by the rule: the angle of incidence is equal to the angle of reflection (see Figure 2.1), or more simply, \( \theta_r = \theta_1 \). Where \( \theta_1 \) is the angle of incidence from the surface normal and \( \theta_r \) is the angle of reflection from the surface normal. Snell’s law can be used to determine the angle of refraction: \( n_1 \sin \theta_1 = n_2 \sin \theta_2 \), where \( n_1 \) is the refractive index of material 1, \( n_2 \) is the refractive index of material 2, \( \theta_1 \) is the incident angle measured from the surface normal and \( \theta_2 \) is the angle of the refracted photons, measured from the negative surface normal, as shown in Figure 2.1.

2.2.3 Refraction through a Mie Scattering Particle

Refraction through a Mie scattering particle (a particle whose size is similar or larger than the wavelength of light used to examine it) also imparts momentum perpendicular to the axis of beam propagation. This can be used to show how an optical trap is formed. In the following description two cases need to be considered: \( n_p > n_s \) and \( n_p < n_s \), where \( n_p \) is the refractive index of the particle and \( n_s \) is the
refractive index of the surrounding medium. I also use the terms used by Ashkin: the axial or scattering forces are forces that act parallel to the direction of the laser beam, and the transverse or dipole forces are forces perpendicular to the laser beams propagation. The terms scattering and dipole are typically used for Rayleigh particles (particles substantially smaller than the wavelength of the incident light) or atoms whereas the terms axial and transverse are typically used for Mie-sized particles.

Figure 2.2 shows the effects of multiple rays being refracted through a microscopic particle. When rays on either side of the particle have the same intensity, Figure 2.2a, the transverse components of the momentum vectors cancel. But if there is an unequal laser intensity on either side of the particle, that is, an intensity gradient is present as indicated by Figure 2.2b, then the particle will move towards (or away) from the most intense region of light. To steer a particle towards a given point, using a particle where $n_p > n_s$, intensity profiles with a strong central peak (for example Gaussian distributions, triangular profiles, etc) draw the particle into the most intense region of the laser beam. If the particle has a lower refractive index than the surrounding media, $n_s > n_p$, or is a reflective particle, a hollow profile is required. Some examples of hollow profiles include profiles with: 2D parabolic, $\cosh \theta$ functional forms and Hermite-Gaussian TEM$_{01*}$ laser modes as shown in Figure 2.3.

Using the laser intensity gradient the particle’s position in the transverse plane can be controlled. The final step in creating an optical trap is to control the particle’s
Figure 2.2: Multiple rays are shown as they refractions through a microscopic particle. $n_p > n_s$ for both the top and bottom images. The momentum vectors for each of the rays are shown to the right of the particle refraction images. The red vectors represent the incoming momentum, the purple vectors represent the refracted momentum, the green vectors represent the change in momentum, and the grey vector represents the total change in the particle’s momentum, $\Delta p_{\text{particle}}$. (a) Five rays of equal intensity are shown. The resultant vector only acts in the axial direction (in the direction of the beams propagation) after transverse components cancel. (b) Five rays of increasing intensity are refracted through the same particle. Partial cancellation in the transverse axes results, however the resulting momentum vector has components in the transverse and axial directions.
(a) Intensity profiles for when \( n_p > n_s \).

(b) Intensity profiles for when \( n_p < n_s \).

Figure 2.3: Shown are some 1D intensity profiles which can be used to hold a microscopic particle. The vertical scale, I, is the intensity while the horizontal scale is distance from a central axis of symmetry. (a) Examples of two profiles that will trap a particle when \( n_p > n_s \). (b) Examples of two “hollow profiles” that can be used to help trap a particle when \( n_p < n_s \). All of these images should be rotated about their central axis to generate 2D intensity distributions that would be required in a physical system.
position in the axial direction. By inserting a lens into the path of the laser beam, an intensity gradient is created along the laser beam’s axis. This intensity gradient will again move the particle in the same manner described above: towards the most intense region of light when $n_p > n_s$ and away from the most intense region of light when $n_s > n_p$ as illustrated in Figure 2.4. In addition to creating this axial intensity gradient, the lens effectively increases the intensity gradient in the transverse directions. It is not possible to generate a single beam optical trap for particles whose refractive index is lower than the surrounding solution, $n_s > n_p$. Instead a balance between an outside force (for example an electric field or gravity) and the change in momentum along the laser beams axis is required. This force balance will enable a particle to be trapped above or below the focal point.

A high numerical aperture lens generates a very strong gradient along the axis of beam propagation, and can effectively confine the particle within a focal plane. With such a large intensity gradient thermal fluctuations only have a minor effect on the particle’s motion perpendicular to the focal plane, and measurements can be made assuming there is little to no movement perpendicular to the focal plane.

The above concepts form a basic understanding of an optical trap, and how the forces generated by the trap move a particle towards a given point in 3D space. In addition the strength of the optical trap along each axis must be large enough to overcome the forces due to Brownian motion otherwise the particle will “escape” the trap. It should be noted that an optical trap is only formed when a particle is present within the confines of the intensity profile of the laser beam, otherwise the system is simply a focussed laser beam.

### 2.2.4 Reflection from a Mie Particle

The forces that act on a reflective particle operate in a similar manner to the forces generated when the refractive index of the solvent is larger than that of the particle, $n_s > n_p$. The momentum transferred to the particle in the transverse directions moves it away from the most intense region of the laser beam and a balance between an external force and radiation pressure must be used to create the optical trap. An example of a reflective optical trap is shown in Figure 2.5. Localised heating may also occur in these reflective solutions, which may cause other effects within the solution such as localised fluid flows. Reflective particles are generally used in Magnetic
Figure 2.4: By using a lens to focus the laser beam a point is created that the particle tries to restore itself to or move away from. The red vectors represent the incoming momentum, the purple vectors represent the refracted momentum, the green vectors represent the change in momentum, and the grey vector represents the total change in the particle’s momentum, $\Delta p_{\text{particle}}$. (a) The ray diagram for $n_p > n_s$. (b) The ray diagram for $n_s > n_p$. (c) An example of a continuous view for $n_p > n_s$. Higher light intensities are illustrated using darker red colours. (d) An example of an intensity profile with a low central intensity for $n_s > n_p$. A force balance is required to maintain the particle’s position at a given coordinate.
Figure 2.5: An optical trap that uses reflection to trap a particle. The red vectors represent the momentum of incoming photons, the purple vectors represent the momentum vectors of reflected photons, the green vectors represent the change in momentum, and the grey vector represents the total change in the particle’s momentum, $\Delta p_{\text{particle}}$ which must be balanced by an external field.

Tweezers rather than Optical Tweezers or when high localised temperatures are required.

### 2.2.5 Photon Flux

An example is given below to determine the number of photons required to manipulate a microscopic particle. The parameters and description that follows are meant only as a rough guide, several minor effects are not included.

Consider a microscopic sphere, $10 \ \mu m$ in diameter, that is moved at $1 \ \mu m\cdot s^{-1}$ through a fluid (water) with a viscosity of $\eta = 1.0 \times 10^{-3}$ using photons with a wavelength of 1000 nm. The energy of each photon is $1.99 \times 10^{-19}$ J and the momentum of each photon is $6.65 \times 10^{-28}$ N·m. The surface area of the sphere is calculated to be $3.14 \times 10^{-10} \ m^2$. Using Stokes’ drag, $F_{\text{drag}} = 6\pi \eta a V$ the force required to achieve this is $1.885 \times 10^{-13}$ N, where $a$ is the radius of the sphere. Therefore dividing $F_{\text{drag}}$ by the momentum per photon shows that $2.8345 \times 10^{14}$ photons hit the surface perpendicularly per second to maintain this velocity, assuming that every photon is absorbed by the particle. Calculating the power per unit area this corresponds to $17.95 \ \text{W} \cdot \text{cm}^{-2}$, or approximately 180 times the power flux of ordinary sunlight. The most common type of photon emitter capable of producing such an intense focussed beam is the laser.

If reflection is considered instead of absorption then the average change in mo-
momentum imparted to the sphere is \(~1.525 \) times larger\footnote{This was calculated numerically by reflecting 5000 rays off a particle, setting the laser beam width equal to the particle’s diameter and assuming a uniform intensity.} than that acquired by absorption. However in the case of refraction the average change in momentum is significantly lower. For example, if \( n_1 = 1.33 \) (water) and \( n_2 = 1.55 \) (polystyrene) then the average change in momentum imparted to the bead is only 0.0389 times that of absorption\footnote{This was calculated numerically by refracting 5000 rays through a particle, setting the laser beam width equal to the particle’s diameter and assuming a uniform intensity.}. As the relative ratio of the refractive indices increases, \( \frac{n_2}{n_1} \), there is a corresponding increase in the momentum change, although it is still only a small fraction of the momentum transfer possible with absorption. The momentum imparted to the particle moves it along the axis of beam propagation.

### 2.2.6 The Optical Tweezers as a Potential Well

Many experiments and calibrations \cite{21,22} have shown that the radiation pressure exerts a Hookian restoring force on a particle when it is located within the focal plane and its position is located near the focal point. That is \( F = -kr \) where \( F \) is an optical force that acts on the particle within the focal plane, \( k \) is the trap or spring constant and \( r \) is the displacement of the particle from the laser beam’s focal point. The potential energy, \( U \), is simply: \( U = \frac{1}{2}kr \cdot r \). Figure 2.6 shows two illustrations of potential wells with different trap constants.

Figure 2.6 illustrates how the energy of the system changes as the trap strength is increased. The yellow ring represents thermal energy, \( k_B T \) and the particle will reside below this energy if no external forces are acting on the particle. The particle in the weak trap, Figure 2.6a, is able to sample a larger region than the particle in the strong trap, Figure 2.6b. This has implications in the design of experiments that test the Fluctuation Theorem in later chapters of this thesis.

### 2.3 Instrumentation

In general, Optical Tweezers integrate several pieces of equipment to generate and control an optical trap. The Optical Tweezers used in this thesis differ significantly from most commercially available sets of Optical Tweezers as several modifications have changed the apparatus from a biological separation tool to an accurate force measurement device. These changes have enabled us to measure the forces gen-
Figure 2.6: Two Optical Tweezers potential wells. In these figures the vertical axis represents the potential energy, $U$, and the grid represents the distance from the center of the optical trap (located at the bottom of each potential well). The yellow ring in both figures represents thermal energy, or $U = k_B T$. Without any other external forces, other than the optical trap and thermal motion, the particle is expected to be located below the thermal energy ring. (a) The particle in a potential well with a weak trap constant, $k$. (b) The particle in a potential well with a strong trap constant.
erated by the optical trap and enabled better control of several Optical Tweezers components. This section describes the original, commercially available version of the Optical Tweezers and the modifications our research group has made to the device.

2.3.1 The Original, Commercial Setup

The original version of the Optical Tweezers (Cell Robotics Inc., USA) was sold as a device that could separate biological cells and other transparent particulates - it had no force measurement capabilities. This set of Optical Tweezers consisted of the following components:

- an inverted microscope,
- a stepper motor controlled microscope stage,
- a CCD camera,
- a micro-fluidic delivery device,
- an infra-red laser,
- and a computer to control the components.

A Nikon DIAPHOT 300 inverted microscope is used as a basic framework to integrate other components into the system. The microscope is fitted with 4 oil-immersion objective lenses: 40×, 60× and two 100× with numerical apertures of: 1.3, 1.4, 1.3 and 1.4 respectively and one standard 10× objective with a numerical aperture of 0.3. It should be noted that an optical trap cannot be established for the 10× lens as the numerical aperture is too low - that is, the laser beams intensity gradient along the axial direction is not large enough to trap a micron-sized particle at its focal point. In contrast the high numerical aperture lenses provide a very large gradient along the axis of beam propagation, enabling the particle to be effectively captured within the focal plane. This setup does not allow measurements of displacements along the axis of beam propagation; however this can be qualitatively judged by examining the image of the trapped particle.

The microscope stage has two servomotor-controlled micrometers acting in orthogonal directions. Each micrometer is capable of moving ±10 mm with an accu-
Figure 2.7: The photo shown was taken in June 2002, before most major modifications were made to the system. The numbered components correspond to the following: (1) Nikon Diaphot 300 inverted microscope, (2) Microscope stage, (3) MTI IFG CCD Camera, (4) Microfluidic delivery device, (5) Cell Robotics Laser, (6) Original control computer (7) light source, (8 - above) open loop piezoelectric translator, (8 below) microscope objectives, (9) quadrant photodiode position detection system, built in-house. Components (8) and (9) are additions to the original, commercial set-up.
racy of 0.1 µm, but is limited to ±8 mm in the x-direction and ±5 mm along the y-axis. The maximum controllable velocity is 200 µm·s⁻¹.

The MTI IFG 300 CCD camera is used to visualise the system. The camera is mounted on the ocular column of the microscope and projects images of the system to the computer at a rate of 25 frames per second (film speed). The resolution is 5.8 pixels/µm using a 100× lens.

An infra-red laser (Cell Robotics, USA) is fitted to the rear port of the microscope and has a wavelength of λ = 985 ± 5 nm. The laser has a variable power input, enabling the laser power output to be changed in integer steps from 1% power to 99%. By varying the output power of the laser the strength of the optical trap is modified. As the laser beams intensity profile is not a TEM₀₀ mode, the x and y trap constants are not symmetric. However, at low laser power the trap constants in the x and y directions are approximately equal, allowing us to treat the trap as symmetric. The CCD camera, laser power, objective focus and servomotors were all controlled by a dedicated computer through interfaces developed by Cell Robotics Inc. USA.

2.3.2 Trap Asymmetry

A side effect of the original commercial set-up is that the laser beam exhibits properties indicative of single slit diffraction. It is also possible that there are several different laser modes interfering with one another producing a similar result, however the sealed laser cavity prevents us from correcting the problem. Figure 2.8 shows a photo of the laser beam, taken through an IR viewer.

This non-uniform distribution of beam intensity has many advantages for a biologist wishing to sort different cell types. For example it enables cylindrical objects to align themselves with a predefined orientation within the trap. However, as there is not a uniform photon flux, difficulties arise with force measurement as the trap constant may vary depending on its orientation. When there is trap asymmetry different trap constants are obtained by rotating the detector around the axis of beam propagation. In comparison, a correctly aligned 2D-gaussian TEM₀₀ beam has equal trap constants in all directions regardless of whether it is rotated or not.

It is also common practice with Optical Tweezers to overfill the rear aperture of the trapping lens with the incoming laser beam. That is, not all of the laser
Figure 2.8: A photo of the NIR laser spot supplied by Cell Robotics viewed through an infra-red viewer. The fact the laser spot does not have a gaussian intensity distribution means that obtaining equal trap constants along both the x and y axes is very difficult.

beam is passed through the final lens, thereby enabling visual alignment of the laser beam before fine-tuning the intensity at the output. By using only the most central and intense part of the laser beam many problems due to interference can be eliminated and a pseudo-symmetric optical trap may be obtained. This is verified by performing several equipartition calibrations (Section 2.5.4) on various particles and checking to ensure that the calculated trap constants along both the x and y axes are approximately equal. Should they not be equal, the laser beam should be re-adjusted and the equipartition process repeated until the constants are approximately equal over the desired laser power range.

2.3.3 Modifications to the Optical Tweezers

Various components have been added to the Optical Tweezers to enable accurate force measurement and allow automation. The equipment acquired includes:

- a quadrant photodiode to enable force measurement,
- a second computer to monitor the image acquisition (CCD camera) independently of the control software,
- a replacement control computer with Labview software (National Instruments, LabView v7.1, USA) to automate various functions and replace the original
control software,

- a data acquisition card (National Instruments, PCI NI-6014, USA),
- two open-loop piezo translators,
- two closed-loop piezo translators (Physik Instrumente, P-814.40, Germany),
- an arbitrary function generator (Thurlby Thander Industries, TGA 1242, UK),
- a DAGE image intensifier (MTI IFG 300, USA),
- and a laser stabiliser (BEOC, LPC-NIR, USA).

The most important modification to the Optical Tweezers is the addition of the quadrant photodiode, shown in Figure 2.9. This addition enables the Optical Tweezers to measure the displacement of a particle from the focal point of the laser to within 15 nm using a 100× lens. After calibrating the quadrant photodiode system the position of the particle can be recorded and the forces acting on the particle can be calculated. The 3×3 mm quadrant photodiode (Hamamatsu, S4349, Japan) is located through the side port of the microscope and is illuminated by an image of the particle. The particle’s image produces a voltage in each quadrant of the photodiode. Using an amplifying electronic circuit, with addition and subtraction of voltages, the individual photodiode quadrant signals are converted into a voltage in both the horizontal and vertical directions. After a photodiode calibration has been performed (see Section 2.5.2) these voltages can be used to measure the displacement of the particle from the center of the optical trap.

A second computer has been purchased specifically to view and/or record the images produced by the CCD camera (P3 666MHz, 256MB RAM, 40Gb SCSI HDD). This second computer ensures that the original computer is always able to control the Optical Tweezers and record voltages using a data acquisition card (DAQ), and vice versa. In conjunction with the DAQ of the control computer, this computer allowed us to save images and the corresponding voltage signals in parallel.

A third computer (P4 2.4GHz, 512MB RAM, 80Gb HDD) was purchased with Labview software (National Instruments, LabView v7.1, USA) and a new data acquisition card (National Instruments, PCI-6014, USA). The new data acquisition card (DAQ) was required as computer architecture has changed dramatically since the original DAQ was purchased. The NI DAQ is able to record a wider range of
voltages, record data at higher sampling rates (up to 200,000 Samples/s) and has better accuracy with 16 signal bits as opposed to 12. Using Labview I have written programs to control and automate the optical tweezers and DAQ. These programs have enabled us to replace the original control computer and software, providing a more flexible system in which to operate.

Originally two open loop piezo translators were purchased, fitted to the x and y axes of the stage, enabling fine stage displacements to occur. When a voltage is applied to these open loop piezo crystals they expanded up to 60 $\mu$m. However the open-loop control system provided no feedback controls to verify that the input signal corresponds to the output translation. Testing revealed that the piezo translators operated in a non-uniform, non-linear manner and that the zero point suffers from piezoelectric drift. To overcome these deficiencies two new, closed-loop piezo translators were purchased. These piezo translators use strain gauges to provide feedback, ensuring that the piezo extends the required distance, eliminating hysteresis and zero point drift. It is claimed that these piezo crystals are accurate to within 1.2 nm, which is better than our detectors resolution. Testing has shown that the movement is indeed linear within our detection range. In addition new housing units were designed to protect the piezo translators and to enable them to be fitted to the microscope stage.

The arbitrary waveform generator (Thurlby Thander Instruments, TGA1242, UK) can output any electrical voltage ranging from -10V to 10V. It operates at frequencies ranging from 1mHz to 40MHz. The waveforms are converted from a 12 bit digital signal to an analog voltage, with an accuracy of $\pm 1$ bit (or $2.4 \times 10^{-4} \times V_{p-p}$, where $V_{p-p}$ is the input peak to peak voltage). The arbitrary waveform generator can also output all the functions available on a standard function generator. This arbitrary waveform generator is used to control several different functions, depending on the experiment being carried out. It can be used as a driving signal to move the stage along any 2-dimensional path and to supply the control signals required to automate the Optical Tweezers.

A photodiode (Honeywell, SDP86001, USA) is located above the sample cell and is used to verify when the laser intensity is increased. This photodiode is not used for quantitative data collection, but rather as a qualitative guide to the laser beam’s intensity.
Several outside factors can affect the output of the laser, such as the laser temperature and the mains power loading. The laser stabiliser is used to ensure that there is no drift in laser power over a period of time. An internal feedback mechanism, operating at 5kHz, dynamically adjusts a transmission LCD to maintain a constant laser output (to within 0.05%). However the stabiliser takes a significant time (between 2 and 70 ms) to reduce its power, especially when large changes are made to the laser power. As such the stabiliser should only be used when the decrease in the power of the optical trapping constant does not occur too rapidly.

The original laser failed in early-mid 2004 requiring a new laser to be purchased. A 4W, \( \lambda = 1064 \text{ nm} \) NIR laser with TEM\(_{00}\) output was purchased (Compass-4000M, Coherent Scientific, Australia). The laser has a feedback mechanism that checks the power of the laser to ensure that the output power corresponds to the setpoint power. A wider range of laser powers are accessible than the original Cell Robotics laser and the TEM\(_{00}\) mode (with correct alignment) overcomes the problem of producing asymmetric traps.

The fully modified Optical Tweezers can be viewed in Figure 2.9. By comparing with Figure 2.7 it is clear that many changes have been made to the system and with much of the original equipment being replaced.

### 2.3.4 The Quadrant Photodiode System in Detail

Before the exact details of the quadrant photodiode can be discussed the optical path needs to be described. Figure 2.10 illustrates the fundamental visible light path used in our system. The light source is used to illuminate our system and an image of the system is projected into the microscope objective. This image is then passed through a beam splitter and 90\% of the light is used to project an image of the system onto the quadrant photodiode, the remaining 10\% is used for the CCD camera. As the light source is very weak after passing through the 100\( \times \) magnification lens the quadrant photodiode signals are passed through a 2-stage amplifying circuit before being sampled by our DAQ card. While the 2-stage amplification reduces the signal to noise ratio (as does any additional electronics) the amplification reduces the relative amount of interference from other signal sources (such as interference from mains power) and raises the signal voltages to ranges that are suited for our DAQ. The amplifying circuits were designed within the school by
Figure 2.9: This photo was taken in June 2005, after the system was significantly modified. The components in the photo are: (1) Nikon Diaphot 300 inverted microscope, (2) Microscope objectives, (3) Microscope stage, (4) Coherent Compass 4000M Laser, (5) BEOC Laser Stabiliser, (6) MTI IFG CCD Camera, (7) Physik Instrumente Piezo Translator, (8) replacement control computer with custom written Labview software, (9) Sample Cell built in-house.
Figure 2.10: An illustration showing how the light progresses from its source (LS), passes through the sample and a microscope lens (ML) before being split 10%/90% at a beam splitter (BS). 10% of the light is delivered to the CCD with the remaining 90% being reflected off a mirror (MR) and passed to the quadrant photodiode position detector.

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The quadrant photodiode has four independent photodiodes arranged in a $2 \times 2$ array. The signals from the four quadrants are added together electronically using the following equations to give horizontal and vertical voltages, $V_H$ and $V_V$.

$$V_V = (Q_1 + Q_2) - (Q_3 + Q_4) \quad (2.1)$$

$$V_H = (Q_1 + Q_4) - (Q_2 + Q_3) \quad (2.2)$$

where $V_H$ is the output voltage for the horizontal displacement, $V_V$ is the output voltage for the vertical displacement, and $Q_x$ is the voltage produced in quadrant $x$. Figure 2.11 illustrates the location of the four quadrants of the photodiode. When calibrated these signals accurately reflect the position of a particle.

The resolution of the photodiode is directly related to the magnification of the particle’s image. In general, the higher the magnification the higher the accuracy of the photodiode’s signals. However, two factors need to be considered when determining the amount of magnification to use: the photon flux striking the quadrant photodiode and the measurement range. Firstly, a minimum photon flux is required to activate the individual elements within each quadrant of the photodiode. If this flux is not achieved then the signal obtained is dominated by the photodiode’s dark current. This scenario can occur if the image of the particle is magnified too much.
Figure 2.11: A quadrant photodiode is shown with the various quadrants labelled as marked. The quadrant photodiode is illuminated by an image of the particle as shown and the relative contributions to the voltage measured.

as the intensity, $I$, is related to the field of view, $R$, in an inverse square manner; that is $I \propto \frac{1}{R^2}$. Additionally, when amplifying the voltage signal, the bandwidth of the device is reduced preventing high frequencies from being recorded. The whole system has a maximum bandwidth of 1 kHz. Secondly, the image of the particle fills most of the active area on the photodiode as indicated in Figure 2.11. Further magnification results in the particle’s movements occurring outside the detection range of the photodiode.

The signal generated by the quadrant photodiode depends directly on the image of the particle. Figure 2.2, page 9, shows that parallel rays of light are concentrated as they refract through a particle (assuming $n_p > n_s$). If an imaging plane is inserted somewhere after the particle then it is possible to obtain the images shown in Figure 2.12. The dark regions in these images correspond to areas where the light has been refracted through the particle, and consequently there is little to no photon flux. These additional photons contribute to the bright central core before diverging. When this knowledge is applied to the photodiode one realises that the photodiode measures the following quantity:

$$Q_x = (V_{BG} - V_{Dark}) + V_{Bright}$$

(2.3)

where $Q_x$ is the voltage in one quadrant of the photodiode, $V_{BG}$ is the voltage
of the background light source alone acting over the entire quadrant, $V_{\text{Dark}}$ is the voltage that an opaque particle would project on the quadrant, and $V_{\text{Bright}}$ is the voltage of the central bright spot from the refraction through the particle. Using this model a series of calculations can be constructed to determine the voltage in the horizontal or vertical direction, $V_H$ and $V_V$ in Eqn 2.2, as a function of the particle’s position. Figure 2.13 shows that the calculated voltages are similar to those determined experimentally.

2.4 Optical Trapping and Simple Operation of the Optical Tweezers

This section briefly outlines some simple functions of the Optical Tweezers, the equipment required to perform these tasks and some standard methods to trap and move particles. Many of the functions are incorporated in the new LabView control software.

The stage of the Optical Tweezers can be moved using the control software. The user simply selects the maximum speed to move at and, while holding down a mouse button, drags the cursor to somewhere else on the display. This sends a
Figure 2.13: A comparison between the position-voltage curve of the quadrant photodiode for the model system proposed in Eqn 2.3 and an experimentally determined curve. The model’s curve is shown as (−−−) and the experimental curve is shown as (—). The data contained within the boxed section provides a unique solution when converting voltage to position and represents the photodiode’s detection range.

signal to the servomotor-controlled micrometers to move the stage in the selected x-y direction at the given speed. Coordinates can be stored so that the Optical Tweezers can investigate a different section of the sample cell and later return to the stored location. The control software also allows the laser to be switched on and off, and the laser’s output power can be modified. Furthermore the laser power stabiliser can be accessed from the software to set the desired power for constant power operation or the maximum power when modulating the laser power.

Using the control software and the 10× lens the sample cell is scanned for particles that can be optically trapped. The lens is changed when a desired particle is located (typically to the 100× N.A. 1.3 lens), the laser is switched on and the particle moves into the focal plane. This is indicated by the particle changing focus when it is not in the focal plane, or by changing its position. To ensure the particle is trapped the stage is translated slowly, which effectively applies a fluid flow relative to the trap. Provided that the particle remains near the focal point during the translation over 10’s of microns then the particle is considered to be trapped. Figure 2.12 shows the particle at various times before and after the particle has been trapped. If the particle is below the focal plane when the laser is switched on then Figures 2.12a-c result as the particle moves into the optical trap. If the particle is slightly above the optical trap then it proceeds via the route shown in going from Figure 2.12d to 2.12c.
The arbitrary function generator and the piezoelectric translators can be used to move the stage along a predefined 2D pattern. Any 2D pattern can be followed provided the hydrodynamic drag force isn’t larger than the optical trapping force, otherwise the particle will exit the optical trap. The arbitrary function generator can be used with the laser stabiliser to modulate the laser power in a predetermined manner, and hence change the optical trapping constant $k$. Microfluidic flow fields can be achieved by translating the stage at a set velocity, by using the syringe pump to inject/withdraw fluid at a specific rate or both.

2.5 Calibration of the Optical Tweezers

To ensure that experimental results obtained from the Optical Tweezers are correct each piece of equipment needs to be calibrated before it is used. Some calibrations only need to be performed once, others need to be performed before every experiment, and many of the calibrations are directly dependent upon quantities calculated from previous calibrations. For example, in order to determine the quadrant photodiode calibration it is first necessary to know the amount of magnification that occurs between the input lens and the photodiode. Similarly, to determine the strength of the optical trap (that is, the optical trapping constant $k$) it is first necessary to know the calibration curve of the quadrant photodiode. The most fundamental calibrations are presented first, followed by the dependant calibrations.

2.5.1 Optics Calibration

The most fundamental quantities in our system are the magnification factors generated by the optics within the system. All other calibrations in the system require accurate knowledge of these magnification factors. For this reason careful analysis of the optical path is required. The technique for obtaining each magnification factor is presented below.

Screen-Size Magnification

The image of the system that is projected onto the CCD camera, and consequently displayed on the computer, is used in several analysis programs. The screen magnification, $M_S$, is found by placing a standardised ruler with 10 $\mu$m increments on the
Optical Tweezers stage and bringing it to a sharp focus. The maximum number of increments shown on the screen is measured, $D_S$, and compared to the known size, $D_A$. The magnification was determined using the relation

$$M_S = \frac{D_S}{D_A}. \hspace{1cm} (2.4)$$

Using this relation, pictures recorded by the CCD camera can then be analysed to determine the actual size of image features. $M_S$ is a constant for each lens. Furthermore it is possible to determine the resolution as the CCD can only project at most $768 \times 576$ pixels. By dividing $D_S$ by the number of pixels, $N_P$, required to show the image of $D_A$ on the screen it is possible to determine the size of each pixel, $D_P$. Using $D_P = D_A/N_P$ the size of each pixel can be calculated, for example when using the 100× N.A. 1.3 lens each pixel has an area of $175 \times 175$ nm.

**Magnification at Photodiode**

To accurately measure the forces acting on a particle it is essential to know the magnification at the quadrant photodiode, $M_P$. A standardised ruler with 10 µm increments is placed on the Optical Tweezers stage and brought to a sharp focus. An imaging plane is inserted at the quadrant photodiode and the separation between several increments is measured. The separation, $D_P$, is compared to the actual size, $D_A$, and the magnification is calculated using

$$M_P = \frac{D_P}{D_A}. \hspace{1cm} (2.5)$$

$M_P$ is also a constant for each individual lens. From the previous two calibrations it is possible to determine the magnification that occurs between the CCD camera and the photodiode, $M_{SP}$. This is a constant for the entire system as the effect of differing microscope lenses cancels. It is found by

$$M_{SP} = \frac{D_S}{D_P} \hspace{1cm} (2.6)$$

The values found from these calibrations are shown in Table 2.1.

There is also a magnification that occurs between the side port of the microscope and the photodiode. By inserting the imaging plane at various distances from the side port it is possible to determine the relationship between the location of the
Table 2.1: Magnification factors for the various lenses. The values $M_S$ and $M_P$ depend upon the relative set-up and are correct at a fixed distance of the photodiode from the apparatus.

<table>
<thead>
<tr>
<th>Lens</th>
<th>$M_S$</th>
<th>$M_P$</th>
<th>$M_{SP}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>10x N.A. 0.30</td>
<td>164.3</td>
<td>44.5</td>
<td>3.692</td>
</tr>
<tr>
<td>40x N.A. 1.30</td>
<td>658</td>
<td>171.4</td>
<td>3.839</td>
</tr>
<tr>
<td>60x N.A. 1.40</td>
<td>970</td>
<td>275</td>
<td>3.527</td>
</tr>
<tr>
<td>100x N.A. 1.30</td>
<td>1600</td>
<td>400</td>
<td>4.000</td>
</tr>
</tbody>
</table>

quadrant photodiode and the imaging planes distance, although this not necessary as the quadrant photodiode is a set distance from the side port. The values in Table 2.1 are recorded at a fixed location 150 mm from the side aperture of the Optical Tweezers, at the quadrant photodiode.

### 2.5.2 Quadrant Photodiode Calibration

As has been described in Section 2.3.4, the quadrant photodiode and amplifying circuit detects the position of a particle that moves within the focal plane. A series of voltages are produced in each quadrant and, following addition and subtraction, horizontal and vertical voltages can be determined. But as the quadrant photodiode is very sensitive to the size of a particle, and the corresponding change in light intensity and pattern, this calibration needs to be performed each and every time a different particle is held in the trap if forces are to be measured. Other factors can also affect the transmitted light, including the constitution of the solution, the variability in particle size, the thickness of the lens oil and the illuminating light’s intensity.

The quadrant photodiode is calibrated using the following procedure.

i. A particle is trapped in the Optical Tweezers at high laser power and its image is projected onto the quadrant photodiode. At high laser power the particle’s Brownian motion is confined to an area smaller than the resolution of the detector, effectively immobilising the particle; that is, the particle has no fluctuations in its position.

ii. The position of the quadrant photodiode housing is precisely translated using micrometers so that a signal approximately averaging 0 V is obtained along both the horizontal and vertical axes, corresponding to the Optical Tweezers
y and x axes respectively.

iii. The photodiode is translated until the minimum signal is obtained along one axis, no change should occur to the signal on the perpendicular axis. This moves the location of the photodiode relative to the particle’s image.

iv. Using the micrometer the photodiode is moved incrementally in 0.04 mm steps and the voltage profile is recorded. This displacement corresponds to moving the photodiode 0.04 mm relative to the magnified image of a “stationary” particle, that is distances of 0.04 mm × M_p. The relative photodiode position is consequently converted into an effective particle position by dividing by the magnification factor. A different voltage is recorded for each increment in position.

v. The effective displacement of the particle is plotted against time to produce a graph with a series of “steps”, see Figure 2.14. The duration of each “step” is not important, only its voltage. The average voltage for each step is calculated and plotted against relative position, Figure 2.14 inset. A 3rd order polynomial is fitted to the points which is then used to convert the quadrant photodiode’s output voltage to the position of the particle within the trap.

vi. The quadrant photodiode is returned to its zero position and the procedure is repeated along the other axis.

The electronic noise due to the background image determines the resolution of the detector. To measure the electronic noise an image of the background (uniform illumination with no particle) is projected onto the quadrant photodiode and the resulting voltage waveforms are measured. This waveform is typical of gaussian white noise. Using an arbitrary particle’s quadrant photodiode calibration curve enables the effective positions of a hypothetical particle to be calculated. The resulting spread of values corresponds to our claimed ±15 nm resolution. An alternate method is to repeat the quadrant photodiode detectors calibration using a particle that is stuck to the bottom of the sample cell. After returning the detector to zero, a series of voltages are recorded and these are converted to displacement. Again a gaussian spread of values is generated with a distribution of approximately ±15 nm.
Figure 2.14: A graph of time vs the quadrant photodiode voltage. The duration of each step is not important provided enough points are obtained to calculate an average voltage. This average voltage (per step) is then related to the physical displacement of the quadrant photodiode, with each step corresponding to a 0.04 mm increment of the quadrant photodiode. Inset: The resulting quadrant photodiode position vs photodiode voltage graph, including a 3rd-order polynomial line of best fit.

2.5.3 Piezoelectric-stage Translations

The piezoelectric translators are calibrated so that they can be used in experiments requiring both static and dynamic positional control of the microscope stage. A standardised ruler with 10 µm increments is placed on the Optical Tweezers stage and a zero volt signal is applied to the piezoelectric translator. An image of the system is then recorded. A static calibration applies a constant DC voltage to a piezoelectric translator and allows the system to equilibrate before another image is taken. Dynamic calibrations constantly change the input voltage to the piezoelectric translator and continuously records images at 24 frames per second. The recorded images have an accuracy of ±1 pixel or \( \approx 175 \text{ nm} \) with the 100× N.A. 1.3 lens. Figure 2.15 shows the results of a dynamic calibration. The relationship between applied voltage and stage displacement can then be generated.

A second method is employed to check the calibration when small voltages are applied to the system. This method uses a microscopic particle stuck on the bottom of the sample cell. A quadrant photodiode calibration is performed as in Section 2.5.2 and then a voltage is applied to the piezoelectric translator. The quadrant photodiode output is recorded and the displacement of the particle calculated. This
Figure 2.15: Shown are two graphs used in the piezoelectric calibration. (a) The initial result showing the relationship between time and the signals collected. The triangular voltage used as an input (—) had an amplitude of 95 V with a frequency of 0.1 Hz. The corresponding stage displacement (—) has an amplitude of 57 µm and frequency of 0.1 Hz. (b) The resulting piezo displacement vs applied voltage curve. The line of best fit has a slope of 5.9359 µm/V.

calibration method is more accurate, but can only be applied to stage displacements of ±3 µm. Translations larger than this move the image of the particle outside detection range of the quadrant photodiode.

2.5.4 Trap Strength Calibrations

These calibrations are carried out to determine the trapping force exerted by the laser upon the particle. There are several ways to calculate the optical trapping constant, $k$. The 3 most common methods to determine $k$ are: equipartition, drag, and power spectral density (PSD) methods. Equipartition uses Brownian motion to relate the average position of a particle from the center of the optical trap to the thermal energy of the system. The equipartition method can only be used for low laser powers where particle fluctuations can be clearly resolved. At higher laser powers electronic noise can be comparable to position fluctuations greatly reducing the accuracy of the calibration. The stage is translated at a known velocity, imposing a known fluid flow on a trapped particle, in the drag calibration. The displacement of the particle from the center of the trap is measured and the Stokes’ drag force calculated. A Lorentzian is fitted to the power spectral density of the recorded particle displacements in the PSD calibration. To use either the drag or PSD method the viscosity of the solution must be known. Depending on how well the viscosity
is known, errors of $\approx 10\%$ can result. The details of each calibration method are described below.

**The Equipartition Method**

A particle is trapped in the Optical Tweezers at a low laser power. Using the DAQ and the quadrant photodiode the particle’s position relative to the centre of the optical trap, $r$, is recorded as it fluctuates with Brownian motion. A sampling rate greater than 200 Hz is used and the position is monitored over several minutes. The average potential energy of the particle, $U = \frac{1}{2}k\langle r \cdot r \rangle$, is equal to the thermal energy, $\frac{1}{2}k_BT$ per degree of freedom. To ensure that the effects of an asymmetric trap are considered, the data is analysed along each axis separately to provide independent trap constants. The calibration performed solely along one axis therefore becomes

$$\frac{1}{2}k_x\langle r_x^2 \rangle = \frac{1}{2}k_BT,$$

$$k_x = \frac{k_BT}{\langle r_x^2 \rangle}. \quad (2.7)$$

The data set is split into $N$ subsets, each of which yields a separate value for $r_x^2$. The standard deviation of these averages, $\sigma(r_x^2)$, is then calculated. Knowing that the error in $k_BT$ is negligible in this experiment, then

$$\frac{\delta k_x}{k_x} = \frac{\delta(r_x^2)}{r_x^2},$$

where $\delta k_x$ is the error in $k_x$, $\delta(r_x^2)$ is the error in $r_x^2$, and $\delta k_x = \sigma(r_x^2)/\sqrt{(N)}$. In all of the experimental results given, the error bars are calculated using $N = 100$.

**The Drag Method**

This calibration technique needs to be performed twice, along the $x$ and $y$ axis in the focal plane. The technique presented here assumes that all quantities act along this one axis. A particle is trapped in the Optical Tweezers at the desired laser power. The photodiode is positioned so the voltage signal is approximately centered around zero. A piezoelectric translator is supplied with a triangular signal causing the stage to translate at a constant velocity. The frequency of the triangular signal and its voltage range are used to determine the velocity of the stage using $V = 2f(V_H - V_L)\Delta$; where $f$ is the triangular waves frequency, $V_H$ is the maximum
Figure 2.16: An example of photodiode voltage vs time that results from the particle moving away from the trap center during a drag calibration. The input triangular signal used to move the stage has a frequency of 0.08 Hz. Data was recorded at 200 Hz. The dashed lines represent the average voltage produced by the quadrant photodiode once the particle has reached a steady state. The scatter around this average value indicates there is a large error associated with this method.

triangular wave voltage, $V_L$ is the minimum triangular wave voltage and $\Delta$ is the stage offset per volt (60$\mu$m/100V). Figure 2.16 shows the particle’s position as the stage is translated, $V_L$ and $V_H$ correspond to the dotted lines. The drag force acting on the particle at a particular frequency is determined using Stoke’s Law, $F = 6\pi\eta a V$, and is equal to the restoring force felt by the particle due to the optical trap in the steady state. Here $\eta$ is the viscosity of the solvent and $a$ is the hydrodynamic radius of the particle in the trap.

This procedure is repeated at several different frequencies. At each frequency, the average maximum and average minimum photodiode voltage is measured. Using the line of best fit calculated in Section 2.5.2 the maximum and minimum displacement, $D_{Max}$ and $D_{Min}$, of the particle are calculated. The maximum displacement is caused by the drag force acting on the particle as it moves in one direction, the minimum drag force when it is moved in the opposite direction. The amount of data scattered above and below $D_{Max}$ and $D_{Min}$, shown in Figure 2.16, indicates that there are substantial error margins using this technique.

By realising that the optical force and the Stokes drag force must be equal $F_{opt} = F_{drag}$, or $k \cdot r = F_{drag}$. Using this relationship it is possible to directly calculate the trapping constant $k$ by plotting the values of “half-wave displacement”
Figure 2.17: An example graph showing the drag force vs the displacement of the particle. The line of best fit is also plotted. The gradient of this best fit line is the optical trapping constant, \( k \). Error bars of approximately 10% should be added to each data point.

as \( r = \frac{1}{2}(D_{\text{Max}} - D_{\text{Min}}) \), against \( F_{\text{drag}} \) as shown in Figure 2.17. The gradient of the line of best fit yields \( k \).

The Power Spectral Density Method

This method can be used independently or in conjunction with the equipartition method. An excellent review on this method is provided by Sorensen et al [22]. A particle is trapped in the Optical Tweezers at a low laser power and using the DAQ and quadrant photodiode the particle’s position is recorded. The PSD is calculated using the discrete fourier transform,

\[
X(n) = \delta t \sum_{j=1}^{N} \exp(i2\pi f_j t_j) x(t_j),
\]  

where \( x(t_j) \) is the particle’s position recorded as discrete data, \( \delta t \) is the time interval between samples, \( f_j = j/(N\delta t) \), \( t_j = j\delta t \) and \( X(n) \) is the Fourier transform of \( x(t_j) \). The Power Spectral Density (PSD), \( P_n \), is then calculated using

\[
P_n = \frac{|X(n)^2|}{N\delta t},
\]
Figure 2.18: An example plot of the particle’s displacement frequency vs its power spectral density. The fitting Lorentzian and $f_c$ are also shown.

Following this the Lorentzian,

$$P_n = \frac{D}{2\pi^2(f_c^2 + f_n^2)}, \quad (2.10)$$

is fitted to the data. Here $D = k_BT/\xi$ is the diffusion constant, $\xi = 6\pi\eta a$ is the friction coefficient, $\eta$ is the viscosity of the solution, $a$ is the hydrodynamic radius of the particle in the optical trap, $f_n$ is the frequency of sample $n$ and $f_c$ is a fitting parameter known as the corner frequency. By fitting $f_c$ to the data, $k$ is calculated using $k = 2\pi f_c \times 6\pi\eta a$. An example is shown in Figure 2.18.

**Comparison of Calibration Methods**

Each of the different calibration methods has its strengths and weaknesses. Large amounts of data can be recorded quickly and easily for equipartition, and a fast algorithm can yield an accurate trap constant almost immediately. However equipartition can only be used with weak optical traps. The PSD method can be limited by the fluid properties of the solution and the bandwidth of the position detection system (quadrant photodiode and circuit). Again large numbers of data can be obtained, although calculating the Fourier transform of large sets of data can be computationally expensive and take between a few seconds and several hours depending on the quantity of data collected. The accuracy of the Stokes’ drag method depends upon the number of frequencies investigated, the fluid properties, and the
Figure 2.19: A graph showing trap constants determined using equipartition (●), drag (●), and PSD (●) constants as a function of laser power. At low laser powers the methods provide equivalent results. However at high laser powers equipartition is no longer able measure the particle’s displacement with enough accuracy to provide the correct result.

hydrodynamic radius of the particle. The statistics are much poorer for Stokes’ drag, that is only 10-20 data points are collected compared to 10,000 - 20,000 generated using equipartition or PSD. The choice of method to calibrate the optical trap clearly depends on the system to be investigated.

Figure 2.19 compares these three different calibration methods at various laser powers using the same particle. It can easily be seen that for low laser powers the equipartition method is equivalent to the Stokes drag and PSD methods. However this starts to fail at higher laser powers. Stokes drag and PSD both provide good results, although PSD has a much better set of statistics to sample from.

2.6 References


Chapter 3

The Fluctuation Theorem

3.1 Background

The Second Law of Thermodynamics states that the entropy production must be greater than or equal to zero. However the Second Law was formulated for macroscopic systems observed over relatively long periods of time (over several milliseconds, seconds or more). Despite this strict limitation, the Second Law’s application is often treated as being universal because the size of most systems can be considered large when compared to atomic length and time scales. However several systems of current scientific interest, such as nanomachines and protein motors, operate at length and time scales where the system cannot be considered large. At the nano- and micro-scales the thermal energy available per degree of freedom can be comparable to the work performed by the system, enabling the system to operate in reverse for short periods of time. Clearly classical thermodynamics does not apply to these small systems.

The puzzle of how the time-reversible microscopic equations of classical mechanics lead to the time-irreversible macroscopic equations of thermodynamics has been a paradox since the days of Boltzmann. Boltzmann simply side-stepped this 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.” [1] [2] Einstein also attempted to solve this paradox and published two proofs [3] [4] [5]. However these proofs both made an incorrect assumption: “We will have to assume that more probable distributions will always follow less probable ones, that is, $W$ always increases until the distribution
becomes constant and $W$ has reached a maximum.” [3] No proof or justification for this assumption was provided. In 1993 the first quantitative description of entropy production in finite systems was given by the Fluctuation Theorem (FT) of Evans et al [6]. The FT describes the transition from time-reversible, microscopic equations of motion to irreversible macroscopic behaviour. In its most general form, the theorem provides an analytic expression for the probability that a dissipative flux flows in the direction opposite to that required by the Second Law of Thermodynamics.

The original 1993 FT paper by Evans et al provided a heuristic proof of the Steady State FT. In the following year, 1994, Evans and Searles [7] provided a formal proof for the FT by perturbing an equilibrium state. This form of the FT became known as the Transient FT, although its application extends beyond the transient response of the system. In 1995 Gallavotti and Cohen derived another equation, which they called the Gallavotti-Cohen Fluctuation Theorem (GCFT) [8, 9]. In 2004 another form of the GCFT, known as the extended heat theorem, was produced by von Zon and Cohen [10]. The GCFT and the extended heat theorem are different from the FT described throughout this thesis. Throughout this thesis the term FT shall apply solely to the FT derived by Evans et al [6, 7].

The FT has been verified in a number of Molecular Dynamics simulations [6, 7] and it is possible to demonstrate the predictions of the theorem in an experiment involving a colloidal particle and an optical trap. Wang et al [11] recorded the trajectories of a colloidal particle localised in a translating optical trap and evaluated the entropy production for a large number of trajectories. Wang et al were unable to demonstrate the FT directly, but showed that the frequency of trajectories with “entropy production” as opposed to “entropy consumption” agreed quantitatively with the integrated form of the FT. The terms “entropy production” and “entropy consumption” were used loosely: entropy is a macroscopic thermodynamic quantity and not a quantity that can be used to describe energy or work performed along a single path. Wang et al used these terms to refer to positive and negative values of the dissipation function, which shall be discussed later in this chapter. Further experimental evidence of the FT is presented in Chapters 4 and 5.
3.2 Derivation of the Fluctuation Theorem

Below are two derivations of the FT. The first is a deterministic derivation of the FT following the principles outlined by Evans and Searles in their review article [12]. The second derivation uses the stochastic approach presented in Reid et al [13] and in Reid [14]. Furthermore, the FT can be derived using other methods, such as using Lyapunov exponents as shown in [12].

3.2.1 Derivation of the FT using Deterministic Dynamics

Consider a system containing $N$ atomistic or molecular particles, with each particle having 3 momentum coordinates $p_i \equiv (p_{xi}, p_{yi}, p_{zi})$ and 3 position coordinates $q_i \equiv (q_{xi}, q_{yi}, q_{zi})$, where $i$ is the particle number. This corresponds to $6N$ variables within the system. If each variable is used to represent a spacial dimension then the system can be fully described in $6N$-dimensional space, or “phase space”. If the distribution of all $p_i$'s and $q_i$'s at a particular time $s$ in phase space is known then the phase space vector at time $s$, $\Gamma(s)$, can be found by solving the equations of motion, Eqn 3.1. Additionally, one can introduce an external field $F_e$ to the system and solve the equations of motion to determine how the system responds to the external field. The equations of motion are

\[ \dot{q}_i = \frac{p_i}{m_i} + C_i(\Gamma) \cdot F_e, \]

\[ \dot{p}_i = F_i(q) + D_i(\Gamma) \cdot F_e - S_i \alpha(\Gamma)p_i, \] (3.1)

where $C_i(\Gamma)$ and $D_i(\Gamma)$ are second-rank tensors that describe how $F_e$ interacts with each particle, $F_i$ is the interatomic force on particle $i$, $\alpha$ is a thermostat multiplier and $S_i$ is a switch that determines whether the particle interacts with the thermostat. The thermostat is introduced so that the system remains at a constant temperature despite the effect of external fields. Without the presence of the thermostat simulated particles in an external field keep increasing their temperature. The thermostat introduced in Eqn 3.1 is a synthetic device that does not occur in nature, but is still a useful model. The thermostating particles can be placed a large distance away from the region of interest, ensuring that the system is not “aware” of the thermostat. Furthermore, Williams et al [15] showed that the specific details of the thermostat had no effect on the derivation of the FT. Williams et al also found
that “when the number of degrees of freedom in the thermostat is large compared
to the number of degrees of freedom in the nonequilibrium system, the fluctuation
theorem is insensitive to the details of the thermostating mechanism”. In addition
Morris & Evans have shown that thermostats do not affect the linear response of
the system \[16\].

In many systems of interest an initial equilibrium probability density \(f(\Gamma, 0)\)
can be constructed. This probability density propagates according to Liouville’s
continuity equation
\[
\frac{\partial f(\Gamma, t)}{\partial t} = -\frac{\partial}{\partial \Gamma} \cdot [\dot{\Gamma} f(\Gamma, t)]
\tag{3.2}
\]
or in Lagrangian form
\[
\frac{df(\Gamma, t)}{dt} = -f(\Gamma, t) \frac{d}{d\Gamma} \cdot \dot{\Gamma}
= -\Lambda(\Gamma)f(\Gamma, t),
\tag{3.3}
\]
where \(\Lambda(\Gamma)\) is the phase space compression. The Liouville equation is used to
determine how the probability density changes as a function of time.

The probability that a phase space coordinate, \(\Gamma(s)\), will be observed within an
infinitesimal phase space volume of size
\[
\delta V_\Gamma = \lim_{\delta q_1, \delta p \to 0} \delta q_1 \delta q_{1,1} \delta q_{1,2} \ldots \delta q_{1,N} \delta p_{x_1} \ldots \delta p_{z_N}
\]
about the phase point \(\Gamma(t)\) at time \(t\) is given by
\[
P(\delta V_\Gamma(\Gamma(t), t)) = f(\Gamma(t), t)\delta V_\Gamma(\Gamma(t)),
\tag{3.4}
\]
where \(f(\Gamma(s), t)\) is the probability density of observing the phase space configuration
\(\Gamma(s)\) at time \(t\). By integrating the Liouville equation, Eqn 3.3 the distribution at
any time \(t\) can be found if the initial configuration \(f(\Gamma(0), 0)\) is known,
\[
f(\Gamma(t), t) = \exp \left[ -\int_0^t \Lambda(\Gamma(s)) ds \right] f(\Gamma(0), 0).
\tag{3.5}
\]
By definition the number of trajectories contained within the volume \(V_\Gamma(\Gamma(t))\) re-
mains constant as time evolves, so it is required that

\[ V_\Gamma(\Gamma(t)) = \exp \left[ \int_0^t \Lambda(\Gamma(s)) ds \right] V_\Gamma(\Gamma(0)). \tag{3.6} \]

A system’s trajectory, \( \Gamma(t) \), from an initial state \((q_0, p_0)\) at time 0 to a state \((q_t, p_t)\) some time \( t \) later is a solution of Newton’s equations of motion. As Loschmidt [17] pointed out in 1876, since these equations are time-reversible, for every trajectory \( \Gamma(t) \) that satisfies the equations of motion there is a time-reversed trajectory or anti-trajectory which is also a solution to the equations. This anti-trajectory, represented by \( \Gamma^*(t) \), evolves from an initial state \((q_t, -p_t)\) to a final state \((q_0, -p_0)\). Thus, \( \Gamma(t) \) and \( \Gamma^*(t) \) represent a pair of conjugate trajectories that are related by time-reversal symmetry according to \( \Gamma(\tau) = \Gamma^*(t - \tau) \).

Consider a set of trajectories that start at time \( s = 0 \) inside an infinitesimal volume \( \delta V_\Gamma(\Gamma(0)) \equiv \delta q_0 \delta p_0 \) about an initial state \((q_0, p_0)\), and the set of conjugate trajectories that start inside the volume element \( \delta V_\Gamma(\Gamma^*(0)) \equiv \delta q_t \delta p_t \) about \((q_t, -p_t)\). Figure 3.1 illustrates these sets of trajectories in the \((q, p)\)-space. Every trajectory initiated within \( \delta V_\Gamma(\Gamma(0)) \) has a conjugate trajectory initiated in \( \delta V_\Gamma(\Gamma^*(0)) \). The dissipation function is defined as the probability of observing trajectories initiated within \( \delta V_\Gamma(\Gamma(0)) \), \( P(\delta V_\Gamma(\Gamma(0)), 0) \), divided by the probability of observing trajectories initiated within \( \delta V_\Gamma(\Gamma^*(0)) \),

\[
\frac{P(\delta V_\Gamma(\Gamma(0)), 0)}{P(\delta V_\Gamma(\Gamma^*(0)), 0)} = \exp(\Omega_t). \tag{3.7}\]

It has also been assumed that the initial distribution of velocities is symmetric under time reversal; that is, the anti-trajectory’s momentum distribution at time \( t = 0 \) is equal to the forward-trajectory’s distribution at time \( t \), or \( f(\Gamma^*(0), 0) = f(\Gamma(t), 0) \). The forward trajectory is related to the conjugate trajectory through a time reversal mapping. Knowing this the phase-space volume of the forward-trajectory bundle at time \( t \) must be equal to the phase-space volume of the anti-trajectory bundle at time \( t = 0 \), \( \delta V_\Gamma(\Gamma(t)) = \delta V_\Gamma(\Gamma^*(0)) \). By equating the probabilities of observing the
volume elements of the trajectory and anti-trajectory bundles at time zero one gets

\[
\frac{P(\delta V_T(\Gamma(0)), 0)}{P(\delta V_T(\Gamma^*(0)), 0)} = \frac{f(\Gamma(0), 0)\delta V_T(\Gamma(0))}{f(\Gamma^*(0), 0)\delta V_T(\Gamma^*(0))} = \frac{f(\Gamma(0), 0)\delta V_T(\Gamma(0))}{f(\Gamma(t), 0)\delta V_T(\Gamma(t))}
\]

\[
\exp(\Omega_t) = \frac{f(\Gamma(0), 0)}{f(\Gamma(t), 0)} \exp\left[ -\int_0^t \Lambda(\Gamma(s))ds \right]
\]

(3.8)

after substituting in Eqn 3.6.

Figure 3.1: An illustration of a set of neighboring trajectories initiated in a volume element \(\delta V_T(\Gamma(0))\) (top tube) and the corresponding set of anti-trajectories initiated in \(\delta V_T(\Gamma^*(0))\) (lower tube) in coordinate-momentum \((q, p)\) and time, \(t\), space. The ratio of the probability of observing trajectories initiated within \(\delta V_T(\Gamma(0))\) to those in \(\delta V_T(\Gamma^*(0))\) is a measure of the system’s irreversibility. Every trajectory in the \((q, p)\) space corresponds to a solution of Newton’s time-reversible equations of motion. Thus, for every trajectory \(\Gamma(t)\) that starts at \((q_0, p_0)\) and ends at \((q_t, p_t)\) some time \(t\) later, there exists its time-reversed or anti-trajectory \(\Gamma^*(t)\). This anti-trajectory starts at \((q_t, -p_t)\) and ends at \((q_0, -p_0)\) at time \(t\). For thermostatted Newtonian systems the probability of observing trajectories initiated in the volume element \(\delta V_T(\Gamma(0))\) is proportional to the size of the volume element and the probability density of trajectories at \((q_0, p_0)\).

The Fluctuation Theorem is defined according to the probability of observing the dissipation function equal to a particular value \(\Omega_t = A\) as opposed to the probability
of observing the reverse process with $\Omega_t = -A$

$$\frac{P(\Omega_t = A)}{P(\Omega_t = -A)} = \frac{\sum_{i|\Omega_{t,i} = A} P(\delta V_{\Gamma}(\Gamma_i(0)), 0)}{\sum_{i|\Omega_{t,i} = -A} P(\delta V_{\Gamma}(\Gamma_i(0)), 0)}. \tag{3.9}$$

The sum $\sum_{i|\Omega_{t,i} = A}$ ensures that all trajectories with $\Omega_t = A$ are used to compute the probability of observing $\Omega_t = A$ and the sum $\sum_{i|\Omega_{t,i} = -A}$ ensures that all trajectories with $\Omega_t = -A$ are used to compute $P(\Omega_t = -A)$. Due to the time-reversal mapping each trajectory with $\Omega_t = -A$ is simply an anti-trajecory when $\Omega_t = A$. Therefore it is possible to change the sum to include the anti-trajectories in the following manner

$$\frac{P(\Omega_t = A)}{P(\Omega_t = -A)} = \frac{\sum_{i|\Omega_{t,i} = A} P(\delta V_{\Gamma}(\Gamma_i(0)), 0)\exp(-\Omega_t = -A)P(\delta V_{\Gamma}(\Gamma_i(0)), 0)}{\sum_{i|\Omega_{t,i} = -A} P(\delta V_{\Gamma}(\Gamma_i^*(0)), 0)}. \tag{3.10}$$

By re-arranging Eqn 3.7 and substituting into the above equation the following is obtained

$$\frac{P(\Omega_t = A)}{P(\Omega_t = -A)} = \frac{\sum_{i|\Omega_{t,i} = A} P(\delta V_{\Gamma}(\Gamma_i(0)), 0)}{\sum_{i|\Omega_{t,i} = -A} \exp(-\Omega_t = -A)P(\delta V_{\Gamma}(\Gamma_i(0)), 0)}. \tag{3.11}$$

As $\exp(-\Omega_t = -A)$ is a constant in the summation, it can be moved in front of the sum. After cancelling the following equation remains

$$\frac{P(\Omega_t = A)}{P(\Omega_t = -A)} = \exp(A), \tag{3.12}$$

which is the definition of the Fluctuation Theorem found in the literature [11,12,18].

### 3.2.2 Derivation of the FT using Stochastic Dynamics

A stochastic approach is often used to characterise the trajectories of a colloidal particle. When the force of inertia is negligible the inertia-less Langevin equation [19] can be used,

$$\xi \frac{dr(t)}{dt} = f_{ext}(r(t)) + g(t). \tag{3.13}$$

The state of the colloidal system is no longer described by the set of coordinates and momenta of all constituent molecules, but is reduced to only the coordinates of the colloidal particle, $r(t) = r_t$. The external force exerted on the particle is given
by $f_{\text{ext}}(r_t)$. The many degrees of freedom associated with the solvent molecules are recast into the macroscopic material property of viscosity, $\eta$, and the buffeting action of the solvent molecules on the particle is represented by a fluctuating random force, $g(t)$. The random force is assumed to be uncorrelated Gaussian noise with zero mean, $\langle g(t)g(t') \rangle = 2\xi k_B T \delta(t - t')$, and the drag coefficient for a particle of radius $a$ is $\xi = 6\pi \eta a$. Unlike Newtonian dynamics, this stochastic equation cannot be used to construct conjugate pairs of trajectories through time-reversal as the random force is Markovian. As the particle’s position is not unique to any given trajectory, there exists an infinite number of trajectories that originate at $r_0$ and a small subset of these arrive at a given destination $r_t$ at time $t$. Let $\{r_0, r_t\}$ represent those stochastic trajectories that evolve from $r_0$ to $r_t$, and let $\{r_t, r_0\}$ represent a conjugate set of trajectories evolving from $r_t$ to $r_0$. This is illustrated in Figure 3.2. Let $\delta V_{r,r}({\{r_0, r_t\}})$ represent an infinitesimal volume of sets of trajectories that initiate at $r_0$ and terminate at $r_t$, then

$$\delta V_{r,r}({\{r_0, r_t\}}) \equiv \lim_{\delta r_0, \delta r_t \to 0} \delta r_0 \delta r_t. \quad (3.14)$$

The probability of observing a stochastic trajectory with a colloidal particle located at an initial position between $r_0$ and $r_0 + \delta r_0$ and a final position between $r_t$ and $r_t + \delta r_t$ is then

$$P(\delta V_{r,r}({\{r_0, r_t\}})) = P(r_0, r_t) \delta r_0 \delta r_t, \quad (3.15)$$

where the normalised distribution $P(r_0, r_t)$ can be expressed using well-known probability distributions and where the volume elements are the same size, $|\delta r_0| = |\delta r_t|$.

The reversibility of a system subjected to a change at $t = 0$ does not depend on whether Newtonian mechanics or Langevin stochastics is used to model it. Therefore the dissipation function for stochastic dynamics can be expressed, by analogy to Eqn 3.7 as

$$\Omega_t(r_0, r_t) = \ln \left[ \frac{P(\delta V_{r,r}({\{r_0, r_t\}}))}{P(\delta V_{r,r}({\{r_t, r_0\}}))} \right]. \quad (3.16)$$

Substituting in Eqn 3.15 this simplifies to

$$\Omega_t(r_0, r_t) = \ln \left[ \frac{P(r_0, r_t)}{P(r_t, r_0)} \right]. \quad (3.17)$$

Eqn 3.17 applies not only to single, colloidal particles, but also to a many-particle
colloidal system. In that case \( \mathbf{r} \) is a \( ND \) vector where \( N \) is the number of colloidal particles embedded in \( D \)-dimensional space. However the probability distributions of Eqn 3.17 cannot be described analytically for many-particle systems.

Figure 3.2: An illustration of a subset of the stochastic trajectories that initiate at \( \mathbf{r}_0 \) and terminate at \( \mathbf{r}_t \), denoted by \( \{\mathbf{r}_0, \mathbf{r}_t\} \), and a corresponding subset of conjugate trajectories, \( \{\mathbf{r}_t, \mathbf{r}_0\} \). Such stochastic trajectories are represented by the position of the Brownian particle(s) at time \( s \), \( \mathbf{r}_s \), from \( 0 < s < t \). The degrees of freedom of the solvent molecules are reduced to viscosity and a random fluctuating force. The stochastic Langevin equation is not time-reversible and consequently, it is not possible to construct an anti-trajectory that is conjugate to any particular trajectory as is possible using Newtonian dynamics. Also, as position is not unique to any given stochastic trajectory, a bundle of trajectories is determined by both initial and terminating positions, \( \mathbf{r}_0 \) and \( \mathbf{r}_t \). This is different to deterministic trajectories where each trajectory is fully-defined by only one point, \( \Gamma(s) \), at any time \( s \) along its trajectory.

The probability density associated with observing a set of trajectories of duration \( t \) for which \( \Omega_t = A \) is

\[
P(\Omega_t = A) = \int dr_0 dr_t \delta(\Omega_t(\mathbf{r}_0, \mathbf{r}_t) - A) P(\mathbf{r}_0, \mathbf{r}_t). \tag{3.18}
\]

Likewise, the density associated with a trajectory with \( \Omega_t = -A \) is

\[
P(\Omega_t = -A) = \int dr_0 dr_t \delta(\Omega_t(\mathbf{r}_0, \mathbf{r}_t) + A) P(\mathbf{r}_0, \mathbf{r}_t) \tag{3.19}
\]

\[
P(\Omega_t = -A) = \int dr_0 dr_t \delta(\Omega_t(\mathbf{r}_t, \mathbf{r}_0) - A) P(\mathbf{r}_0, \mathbf{r}_t) \tag{3.20}
\]
where $\Omega_t(r_0, r_t) = -\Omega_t(r_t, r_0)$ has been used. Using the definition of $\Omega_t$ from Eqn [3.17] $P(\Omega_t = -A)$ can be rewritten as

$$P(\Omega_t = -A) = \int d\mathbf{r}_0 d\mathbf{r}_t \, \delta(\Omega_t(\mathbf{r}_t, \mathbf{r}_0) - A) \exp(-\Omega_t(\mathbf{r}_t, \mathbf{r}_0)) \, P(\mathbf{r}_t, \mathbf{r}_0) \quad (3.21)$$

Thus

$$\frac{P(\Omega_t = A)}{P(\Omega_t = -A)} = \frac{\int d\mathbf{r}_0 d\mathbf{r}_t \, \delta(\Omega_t(\mathbf{r}_0, \mathbf{r}_t) - A) P(\mathbf{r}_0, \mathbf{r}_t)}{\int d\mathbf{r}_0 d\mathbf{r}_t \, \delta(\Omega_t(\mathbf{r}_t, \mathbf{r}_0) - A) \exp(-\Omega_t(\mathbf{r}_t, \mathbf{r}_0)) \, P(\mathbf{r}_t, \mathbf{r}_0)} \quad (3.22)$$

Recognising that the integration variables, $\mathbf{r}_0$ and $\mathbf{r}_t$, can be written arbitrarily, the FT is obtained.

$$\frac{P(\Omega_t = A)}{P(\Omega_t = -A)} = \exp(A). \quad (3.23)$$

### 3.3 The Second-Law Inequality

The FT is often described as a Second Law-like theorem. In some experimental systems $\Omega_t$ can be related to the accumulated entropy production, $\Sigma_t$, from time $s = 0$ to $s = t$ in the system. In these particular cases the FT reduces exactly to the Second Law for large systems observed over long times. This can be shown directly from the FT, which has been re-written as

$$\frac{P(\Omega_t = -A)}{P(\Omega_t = A)} = \exp(-\Omega_t) \quad (3.24)$$

The FT tells us that the probability of observing a negative dissipation function decreases exponentially when compared to the probability of observing a positive dissipation function. As the values of $\Omega_t$ get larger the probability of observing positive dissipation becomes overwhelmingly likely. In the limiting case of $\Omega_t \to \infty$ it is easy to show from Eqn [3.24] that $P(\Omega_t = -A) = 0$. This simply states that the dissipation function will not have negative values in large systems, thus reducing to Second Law-like behaviour.

It is also possible to prove that the ensemble average of the dissipation function is always positive using the FT [18]. Starting with the definition

$$\langle \Omega_t \rangle = \int_{-\infty}^{\infty} A \, P(\Omega_t = A) \, dA \quad (3.25)$$
and splitting the integral to obtain

$$\langle \Omega_t \rangle = \int_0^\infty A \, P(\Omega_t = A) \, dA + \int_{-\infty}^0 A \, P(\Omega_t = A) \, dA$$

$$= \int_0^\infty A \, P(\Omega_t = A) \, dA + \int_0^\infty -A \, P(\Omega_t = -A) \, dA$$

$$= \int_0^\infty A \left( P(\Omega_t = A) - P(\Omega_t = -A) \right) dA$$

By substituting in the FT for $P(\Omega_t = -A)$ one gets

$$\langle \Omega_t \rangle = \int_0^\infty A \left( P(\Omega_t = A) - \exp(-A) \, P(\Omega_t = A) \right) dA$$

which simplifies to

$$\langle \Omega_t \rangle = \int_0^\infty A \, P(\Omega_t = A) \left( 1 - \exp(-A) \right) dA$$

and then to

$$\langle \Omega_t \rangle = \langle \Omega_t (1 - \exp(-\Omega_t)) \rangle_{\Omega_t \geq 0} \geq 0 \quad \forall t. \quad \text{(3.26)}$$

This equation shows that the average dissipation function is always positive. The exponential term inside the brackets, $\exp(-\Omega_t)$, can only take on values between 0 and 1 as $\Omega_t \geq 0$. As such the term $(1 - \exp(-\Omega_t))$ can only be $\geq 0$. This ensures that the value of the entire function is $\geq 0$.

By knowing that the ensemble average of the dissipation function equates to entropy in some experimental systems, $\langle \Omega_t \rangle = \Sigma_t$, it is clear that the FT predicts a positive entropy, and hence Second Law behaviour. In other systems where $\langle \Omega_t \rangle \neq \Sigma_t$ there is still a positive quantity that mimics entropy. It is for these reasons that the FT is referred to as a Second Law-like theorem.

### 3.4 The Integrated Fluctuation Theorem

An integrated form of the Fluctuation Theorem (IFT) can easily be derived and is experimentally very useful. It considers the probability ratios of the trajectories
with positive and negative $\Omega_t$ values rather than ratios of individual values. This theorem is particularly useful when the data is limited and statistical sampling is insufficient to show the FT directly. The probability of observing $\Omega_t < 0$ as opposed to $\Omega_t > 0$ is given by

$$
\frac{P(\Omega_t < 0)}{P(\Omega_t > 0)} = \frac{\int_0^\infty dA \, P(\Omega_t = -A)}{\int_0^\infty dA \, P(\Omega_t = A)}.
$$

(3.27)

Substituting in the FT for $P(\Omega_t = -A)$ yields

$$
\frac{P(\Omega_t < 0)}{P(\Omega_t > 0)} = \frac{\int_0^\infty dA \, \exp(-A) P(\Omega_t = A)}{\int_0^\infty dA \, P(\Omega_t = A)},
$$

(3.28)

which is the definition of an ensemble average taken over all trajectories where $\Omega_t > 0$. Therefore the IFT can be written as

$$
\frac{P(\Omega_t < 0)}{P(\Omega_t > 0)} = \langle \exp(-\Omega_t) \rangle_{\Omega_t > 0}.
$$

(3.29)

Alternately a second form of the IFT can be derived. Starting from Eqn [3.27] and realising that you can re-write it is as

$$
\frac{P(\Omega_t < 0)}{P(\Omega_t > 0)} = \frac{\int_{-\infty}^0 dA \, P(\Omega_t = A)}{\int_{-\infty}^0 dA \, P(\Omega_t = -A)}.
$$

(3.30)

Then substitute the FT for $P(\Omega_t = -A)$ to obtain

$$
\frac{P(\Omega_t < 0)}{P(\Omega_t > 0)} = \frac{\int_{-\infty}^0 dA \, P(\Omega_t = A)}{\int_{-\infty}^0 dA \, \exp(-A) \, P(\Omega_t = A)}.
$$

(3.31)

This equation simplifies to

$$
\frac{P(\Omega_t < 0)}{P(\Omega_t > 0)} = \langle \exp(-\Omega_t) \rangle_{\Omega_t < 0}^{-1}.
$$

(3.32)
This form of the equation is not as valuable as Eqn 3.29 due to the lower frequency of observing anti-trajectories. Consequently many more trajectories (experimental or simulated) need to be collected to ensure that this method works with acceptable error margins.

3.5 The Dissipation Function

As has been shown in the previous section there are two alternate definitions for the dissipation function depending on whether one uses stochastic or deterministic derivations. For the remainder of this thesis \( \Omega_t(\Gamma) \) will be used to represent the dissipation function derived deterministically, \( \Omega_t(r_0, r_t) \) has been shortened to \( \Omega_t(r) \) and will used to represent the dissipation function derived stochastically, and \( \Omega_t \) will be used when the description applies to all derivations of the dissipation function.

The dissipation function, \( \Omega_t \), is a measure of the thermodynamic irreversibility of a system. Specifically it quantifies the amount of energy dissipated to the surroundings. It is an extensive parameter that increases with system size and generally scales with observation time. Furthermore it’s ensemble average \( \langle \Omega_t \rangle \) is always positive. When applied to large systems observed over long periods of time the probability of observing trajectories with \( \Omega_t < 0 \) decreases exponentially and the irreversible behaviour of the Second Law is recovered.

If the probability of observing a trajectory bundle is equal to the probability of observing the anti-trajectory bundle, that is \( P(\delta V_{r_t}^{\ast}(\Gamma(0)), 0) = P(\delta V_{r_t}(\Gamma(0)), 0) \) or \( P(\delta V_{r,r}(\{r_0, r_t\})) = P(\delta V_{r,r}(\{r_t, r_0\})) \), then the system is thermodynamically reversible and \( \Omega_t = 0 \). This measure of a system’s irreversibility is consistent with our qualitative observations on how events proceed in time. To illustrate this point, consider a colloidal particle residing in an optical trap whose strength increases instantaneously from \( k_0 \) to \( k_1 \) at \( t = 0 \). The properties of the particle are such that it undergoes Brownian motion, but is confined within the optical trap. Furthermore, consider a set of trajectories characterised by the particle spiraling into the harmonic trap to become more tightly confined to its center. A corresponding set of anti-trajectories is characterised by the particle spiraling out and escaping away from the trap center, despite the increase in trapping strength. These anti-trajectories are less likely to be observed than their time-forward counterparts, and this probability imbalance is a measure of the system’s irreversibility. If these same trajectories are
explored in the limit of \( t \to 0 \), there is little difference in the frequency of observation of these two sets of trajectories and it is possible to say that such short trajectories are nearly reversible. However, as the time of the particle’s trajectory increases, the probabilities of observing forward-spiralling trajectories and their conjugate anti-trajectories become more unbalanced. On average, the anti-trajectories become increasingly infrequent with trajectory duration, and \( \langle \Omega_t \rangle \) becomes larger, reflecting the growing irreversibility of the system.

### 3.6 Steady State FT

In the literature there are two different labels for the FT, depending upon how the theorem is applied. The Transient Fluctuation Theorem or TFT is simply Eqn 3.12 applied to transient systems that evolve from a known initial equilibrium state towards a final (equilibrium or non-equilibrium) steady-state. The Steady-State Fluctuation Theorem or SSFT refers to the steady-state application of the theorem, where the dissipation function is evaluated over trajectory segments of duration \( t \), sampled wholly under nonequilibrium steady-state conditions. When \( \Omega_t \) is evaluated for steady-state trajectories, the theorem is said to hold only in the long time limit,

\[
\lim_{t \to \infty} \frac{P(\Omega_{ss}^t = -A)}{P(\Omega_{ss}^t = A)} = \exp (-A),
\]

(3.33)

where the term \( \Omega_{ss}^t \) is the steady state approximation of \( \Omega_t \) (as shown below). The asymptotic limit in the SSFT is a result of approximations made in the dissipation function, \( \Omega_t \). When it is possible to express \( \Omega_t \) exactly, the asymptotic limit is no longer needed and the operative theorem under steady-state conditions is the FT. This is explored more fully in Chapter 5, page 75. Thus, while the literature and its nomenclature might indicate that there are two different theorems, the FT is general and applicable to both transient and steady-state conditions. The labels SSFT is used in this thesis to indicate the application of the asymptotic limit in Eqn 3.33.

As \( \Omega_t(\Gamma) \) requires that the relative probabilities of trajectories be made under initial, equilibrium conditions it is not possible to construct exact expressions for \( \Omega_t(\Gamma) \) for trajectory segments of duration \( t \) that are wholly at a nonequilibrium steady-state. However, when the dissipation function is extensive with time, an approximate steady-state dissipation function can be constructed in the following
way. \( \Omega_t(\Gamma) \) can be cast in terms of its instantaneous rate of change, \( \Omega(s) \) at time \( s \), accumulated from an initial equilibrium state at time \( s = 0 \) to some arbitrary time \( t \)

\[
\Omega_t(\Gamma) = \int_0^t ds \, \Omega(s) + \int_\tau^t ds \, \Omega(s).
\]  

(3.34)

Here \( \tau \) has been introduced as an arbitrary “cut-off” time that is sufficiently large so that the system can be regarded as being in steady-state for \( s > \tau \). This means that \( \Omega_t \) is cast as a sum of transient and steady-state contributions. The steady-state contribution is identified with the steady-state dissipation function, \( \Omega_t^{ss} \), which can be used to approximate \( \Omega_t \) with an error of order \( \tau \).

\[
\Omega_t(\Gamma) \approx O(\tau) + \Omega_t^{ss}
\]  

(3.35)

It is instructive to express these dissipation functions as time-averages, \( \bar{\Omega}_t = \Omega_t/t \), such that

\[
\bar{\Omega}_t \approx \bar{\Omega}_t^{ss} + O\left(\frac{\tau}{t}\right).
\]  

(3.37)

This shows that the error invoked by approximating \( \bar{\Omega}_t \) with \( \bar{\Omega}_t^{ss} \) vanishes in the long time limit as \( \tau/t \). However, the fluctuations in \( \bar{\Omega}_t^{ss} \) also vanish in the long time limit and, in order that the SSFT be of any utility, it is necessary that these fluctuations vanish more slowly than \( O(\tau/t) \). The measure \( \bar{\Omega}_t^{ss} \) along the steady-state portion of a trajectory is

\[
\bar{\Omega}_t^{ss} \equiv \frac{1}{t} \int_0^t ds \, \Omega(s),
\]  

(3.38)

which can be re-expressed as a sum of measures taken along contiguous trajectory segments of duration \( \Delta t \)

\[
\bar{\Omega}_t^{ss} \equiv \frac{1}{t} \sum_i \int_{(i-1)\Delta t}^{i\Delta t} ds \, \Omega(s)
\]  

(3.39)

\[
\equiv \frac{1}{t} \sum_i \Omega_i \Delta t.
\]  

(3.40)

If \( \Delta t \) is larger than the longest correlation time in the system then the sum \( \sum \Omega_i \Delta t \) contains independent measures and, consequently, the variance in the sum is proportional to the number of measures or \( t/\Delta t \). The factor \( 1/t \) in front of the sum
decreases the variance of the sum by a factor $t^2$. Thus the standard deviation of the
measure $\bar{\Omega}^{ss}_{t}$ along a steady-state portion of a trajectory diminishes as $1/\sqrt{t}$, slower
than $O(\frac{t}{t^3})$. Therefore $\Omega_t$ can be approximated by the steady-state dissipation func-
tion $\Omega_t^{ss}$ in the FT (see Eqn 3.12), leading to the SSFT

$$\lim_{t \to \infty} \frac{P(\Omega_t^{ss} = A)}{P(\Omega_t^{ss} = -A)} = \exp(A). \quad (3.41)$$

In this way the SSFT is an approximation to the FT that is accurate in the long
time limit. That is, the transient contribution to the dissipation function becomes
negligible before the fluctuations in $\Omega_t^{ss}$ vanish. For some systems described using
stochastic dynamics it is possible to construct distributions of trajectories that are
wholly in a non-equilibrium steady-state. In such cases it is not necessary to apply
the SSFT as the FT can be used directly. However if approximations are made to
$\Omega_t(r)$ then the SSFT will be required.

An integrated form of the SSFT also exists and is derived in the same manner
as the IFT:

$$\lim_{t \to \infty} \frac{P(\Omega_t^{ss} < 0)}{P(\Omega_t^{ss} > 0)} = \langle \exp(-\Omega_t^{ss})\rangle_{\Omega_t^{ss} > 0}. \quad (3.42)$$

Again the average on the RHS is an ensemble average taken over all trajectories
where $\Omega_t^{ss} > 0$.

### 3.7 References

[1] L. Boltzman, Rejoinder to the heat theoretical considerations of Mr. E. Zermelo
(1896).

1983).


[5] A. Pais, *‘Subtle is the Lord’… the Science and the Life of Albert Einstein*
(Oxford University Press, 1983).


Chapter 4

The Capture Experiment

In this chapter the Fluctuation Theorem (FT), Eqn 3.12, is directly demonstrated by observing the time-dependent relaxation of a colloidal particle. In this experiment a particle is localised in a stationary optical trap of strength $k_0$ long enough for its position to be described by an equilibrium distribution. At $t = 0$ the optical trap strength is increased discontinuously from $k_0$ to $k_1$ so that the particle is more tightly confined or “captured”. The particle’s position is recorded as it relaxes to its new equilibrium distribution and the dissipation function, $\Omega_t$, is evaluated over an ensemble of trajectories. It is shown that $\Omega_t$ obeys the FT and the Integrated Fluctuation Theorem (IFT), Eqn 3.29. In addition, the results show that the experiment and FT predictions are in quantitative agreement with Langevin dynamics.

4.1 The Optical Trap Experiment

An 8.0 ml transparent sample cell is filled with an aqueous solvent containing 4.0 ml of 10 mM Tris-HCl with 1 mM EDTA maintained at a pH of 7.5 (TE), and 1.0 ml of glycerol. A stock solution of latex particles (6.3 µm diameter, Interfacial Dynamics Co., USA), containing approximately $3 \times 10^8$ particles is diluted with TE and a 5 µl aliquot, containing approximately 3000 particles, is injected into one end of the sample cell. The glycerol is added to maximise the viscosity of the solution and increase the time it takes for the latex particles to relax to an equilibrium distribution. Further addition of glycerol disrupts the neutral buoyancy of the latex particles and prevents optical trapping in our system. A glass coverslide or mineral oil is added to the top of the sample cell to prevent evaporation of the solvent. Using high laser power, one particle is optically trapped, dragged at least 2 mm
from the injection site to ensure its isolation from other particles, and used to calibrate the quadrant photodiode position detector as described in Section 2.5.2, page 30. The equipartition theorem (Section 2.5.4, page 33) is used to determine the value of the trapping constant, $k$. The particle’s position was measured at each laser power at 200 Hz for 120 seconds and the equipartition theorem applied: $k = k_B T / \langle \mathbf{r} \cdot \mathbf{r} \rangle$. Deviations in the symmetry of the trap require that the trap constants be evaluated independently in each of the orthogonal directions, and also require $\Omega_t$ to be calculated along each axis independently before being summed to produce a total effect. The strength of the optical trap is then cycled discontinuously and evenly between the initial trap strength, $(k_{0,x}, k_{0,y}) = (1.24 \pm 0.05, 1.17 \pm 0.06) \text{ pN/\mu m}$, and a final trap strength, $(k_{1,x}, k_{1,y}) = (2.96 \pm 0.07, 2.70 \pm 0.13) \text{ pN/\mu m}$ with a period of 20 seconds. A “trajectory” corresponds to the particle’s position over 10 seconds in the weak trap ($-10 < t < 0$ seconds) and a further 10 seconds in the strong trap ($0 \geq t > 10$ seconds), as indicated in Figure 4.1. It is important to recognise that the $-10 \leq t \leq 10$ time intervals are recorded in the continuous laboratory time-frame. Each trajectory is then separated and the relative time from the $k_0$ to $k_1$ transition is used to calculate the dissipation function.

The first half of the trajectory cycle provides ample time for the particle to equilibrate in the weak trap. The second half of the cycle provides the data necessary for analysis of the FT, that is $\mathbf{r}_0$ and a range of $\mathbf{r}_t$ for $0 < t < 10$ seconds. The relaxation time of the particle in the second half of the cycle, $\tau = 2\xi / |(k_{1,x} + k_{1,y})|$, is $37.5 \pm 4.5 \text{ ms}$, where the friction coefficient was determined by Stokes drag as $\xi = 1.05 \times 10^{-7} \pm 0.05 \times 10^{-7} \text{ Ns/m}$. The trap strength is cycled continuously, providing several hundred particle trajectories in one experiment. It is important that each experiment proceeds with a particular particle as the calibration of the quadrant photodiode position detector is sensitive to slight differences in size and light transmission, as discussed in Section 2.3.4, page 22.

4.2 Experimental Expressions for the Dissipation Function

To test the FT, one must first determine the functional form of $\Omega_t$. This can be done using Newton’s equations of motion or by using Langevin dynamics, as demonstrated
Figure 4.1: This schematic shows how the optical trap strength, $k$, varies with respect to laboratory time. The trap cycles between a low trap strength, $k_0$, and high trap strength $k_1$ discontinuously with a period of 20 seconds. A single “trajectory” (grey box) corresponds to the particle positions recorded over a single cycle, starting at a trajectory time of $t = 0$ when the trap strength is increased.

by Reid et al [1] and in Reid [2]. Below, both methods are used to derive the functional form of $\Omega_t$. Considerable care has been taken to properly derive $\Omega_t$ and show the steps. In some of the current literature the dissipation function has been hypothesised and, as such, fails to satisfy the FT as it does not have the correct functional form.

4.2.1 Deterministic Derivation of $\Omega_t$ for Capture

In Newtonian dynamics this experiment is represented as a system of $N$ molecular particles, with each particle being identified with an index $i$. The system’s dynamics are tracked in terms of the position, $q_i$, and momentum, $p_i$, of each particle. Each particle’s motion is influenced by the inter-particle force $F_i$. Particle $i = 1$ is used as our optically trapped particle and is subjected to a harmonic trap with potential energy

$$\Phi_{\text{trap}}(q_1(t), t) = \frac{1}{2} k(t) q_1(t) \cdot q_1(t).$$

The trap constant is defined such that $k(t < 0) = k_0$ and $k(t \geq 0) = k_1$, as in Figure 4.1. $q_1$ is defined relative to the center of the optical trap.

As the experiment is in thermal contact with its surroundings, a thermostat
is introduced\textsuperscript{1}. The Nose-Hoover thermostat controls the temperature of reservoir particles using a switch, $S_i$; where $S_i = 1$ for each of the $N_W$ thermostated particles, and $S_i = 0$ for non-thermostated particles. The thermostat also introduces the thermostat multiplier $\alpha$, and the equations of motion are written as

\[
\begin{align*}
\dot{q}_i(t) &= \frac{p_i(t)}{m_i} \\
\dot{p}_i(t) &= F_i - \delta_{i1}k(t)q_i(t) - S_i\alpha(t)p_i(t) \\
\dot{\alpha}(t) &= \frac{1}{Q} \left[ \sum_{i=1}^{N} \left( \frac{S_i p_i(t) \cdot p_i(t)}{m_i} \right) - DN_w k_B T \right]
\end{align*}
\]

for particle $i$. Here $\delta_{i1}$ is the Kronecker delta function, $D$ is the number of Cartesian dimensions for the system, $k_B$ is the Boltzmann constant, $T$ the thermostat temperature, $m_i$ is the mass of particle $i$, $F_i$ is the interparticle force and $Q$ the effective mass of the thermal reservoir. The addition of the thermostat only affects those particles that have been designated as reservoir particles. In addition the thermostat multiplier applies the feedback only to the reservoir particles.

The Hamiltonian for the system is defined as

\[
H_0 = K(p) + \Phi(q) + \Phi_{\text{trap}}(q_1(t), t) + \frac{1}{2} Q\alpha^2
\]

where the kinetic energy is $K(p) = \sum_i \frac{p_i \cdot p_i}{2m_i}$, the interparticle potential energy $\Phi(q)$ is a known function, the optical trap potential is $\Phi_{\text{trap}}(q_1(t), t)$ and the effect of the thermostats is given by $\frac{1}{2} Q\alpha^2$. This additional thermostatting term is required so that the phase-space compression be related to $\dot{H}_0$.

The distribution function at $t = 0$ for the capture experiment is therefore defined as

\[
f(\Gamma, 0^-) = \exp(-\beta H_0) = \exp(-\beta[K(p) + \Phi(q) + \Phi_{\text{trap}}(q_1(t = 0^-), t = 0^-) + \frac{1}{2} Q\alpha^2]).
\]

An expression for the dissipation can then be obtained by using Eqn 3.8,

\[
\Omega_t(\Gamma_0) = \ln \left( \frac{f(\Gamma(0), 0^-)}{f(\Gamma(t), 0)} \right) - \int_0^t ds \Lambda(\Gamma(s)). \quad (4.1)
\]

\textsuperscript{1}See Section 3.2.1, page 43, regarding the use of artificial thermostats for the derivation of the FT and associated quantities.
The variable $\Lambda(\Gamma(s))$ corresponds to the phase space contraction of a trajectory bundle in time (see Figure 3.1, page 46). This contraction or expansion is due to the action of the thermostat upon the system. Expanding the first term on the RHS of Eqn 4.1 gives

$$\ln \frac{f(\Gamma(0), 0^-)}{f(\Gamma(t), 0)} = \ln \frac{\exp \left( -\beta \left[ K(p(0)) + \Phi(q(0)) + \Phi_{\text{trap}}(q_1(0), 0^-) + \frac{1}{2} Q\alpha^2(0) \right] \right)}{\exp \left( -\beta \left[ K(p(t)) + \Phi(q(t)) + \Phi_{\text{trap}}(q_1(t), t) + \frac{1}{2} Q\alpha^2(t) \right] \right)}$$

$$= \beta \int_0^t ds \left[ \dot{K}(p(s)) + \dot{\Phi}(q(s)) + \dot{\Phi}_{\text{trap}}(q_1(s), s) + Q\alpha \dot{\alpha} \right]$$

$$= \beta \int_0^t ds \left[ \dot{K}(p(s)) - \sum_i F_i \cdot \dot{q}_i(s) + \dot{\Phi}_{\text{trap}}(q_1(s), s) + Q\alpha \dot{\alpha} \right]$$

Substituting in exact terms for $K(p(s))$, $\Phi_{\text{trap}}(q_1(s), s)$, and $\alpha(s)$ results in the following equation

$$\ln \frac{f(\Gamma(0), 0^-)}{f(\Gamma(t), 0)} = \beta \int_0^t ds \left[ \sum_i \frac{\dot{p}_i \cdot p_i}{m_i} + \sum_i F_i \cdot \dot{q}_i + q_1 \cdot \dot{q}_1 k + \alpha \left( \sum_i \frac{S_i p_i \cdot p_i}{m_i} - D N W k_B T \right) \right].$$

Where the functional dependence on the integration times has been omitted. Substituting $\dot{p}_i$ into the equation produces

$$\ln \frac{f(\Gamma(0), 0^-)}{f(\Gamma(t), 0)} = \beta \int_0^t ds \left[ \sum_i \frac{\dot{p}_i \cdot p_i}{m_i} \cdot (F_i - \delta_{ii} k q_i - S_i \alpha p_i) - \sum_i F_i \cdot \dot{q}_i + q_1 \cdot \dot{q}_1 k + \alpha \sum_i \frac{S_i p_i \cdot p_i}{m_i} - D N W k_B T \right].$$

Expanding the terms yields

$$\ln \frac{f(\Gamma(0), 0^-)}{f(\Gamma(t), 0)} = \beta \int_0^t ds \left[ \sum_i F_i \cdot \dot{q}_i - k q_1 \dot{q}_1 - \sum_i S_i \alpha \frac{p_i \cdot p_i}{m_i} - \sum_i F_i \cdot \dot{q}_i + q_1 \cdot \dot{q}_1 k + \alpha \sum_i \frac{S_i p_i \cdot p_i}{m_i} - D N W k_B T \right].$$

After cancellation the above equation simplifies to

$$\ln \frac{f(\Gamma(0), 0^-)}{f(\Gamma(t), 0)} = \beta \int_0^t ds \left[ - q_1 \cdot \frac{d q_1}{ds} k - \alpha D N W k_B T \right].$$
Further simplification yields
\[
\ln \frac{f(\Gamma(0), 0^-)}{f(\Gamma(t), 0)} = \frac{1}{2} \beta [k(s)] \left[ \mathbf{q}_1(s) \cdot \mathbf{q}_1(s) \right]_0^t - \int_0^t ds D N W \alpha(s).
\]
After evaluation this produces
\[
\ln \frac{f(\Gamma(0), 0^-)}{f(\Gamma(t), 0)} = \frac{1}{2} \beta (k_0 - k_1)(\mathbf{q}_1^2(t) - \mathbf{q}_1^2(0)) - \int_0^t ds D N W \alpha(s). \tag{4.2}
\]
As \( \int_0^t ds D N W \alpha(s) \) is equal and opposite to the phase space compression integral, \( \int_0^t ds \Lambda(\Gamma(s)) \), Eqns [4.1] and [4.2] can be combined to produce the dissipation function,
\[
\Omega_t = \frac{1}{2} \beta (k_0 - k_1)(\mathbf{q}_1^2(t) - \mathbf{q}_1^2(0)) \tag{4.3}
\]

### 4.2.2 Stochastic Derivation of \( \Omega_t \) for Capture

The dissipation function can also be formulated using the Langevin equation,
\[
\xi \frac{dr}{dt} = -kr + g(t), \tag{4.4}
\]
where \( r \) represents the position of the particle and \( g(t) \) is a fluctuating random force, or Markovian. When the particle is equilibrated within a harmonic potential its position is distributed according to the Boltzmann distribution,
\[
P_B(r, k) = \frac{Dk}{2\pi k_B T} \left[ \frac{D}{2k_B T} \right]^{D/2} \exp \left( -\frac{Dk \cdot r}{2k_B T} \right). \tag{4.5}
\]
in D dimensional space. The Green’s function provides the probability of observing a particle at \( r_t \) in a harmonic potential of strength \( k \), given its position \( r_0 \) at time \( t \) earlier,
\[
G(r_0, r_t, k, t) = \frac{Dk}{2\pi k_B T(1 - \exp(-2t/\tau))} \left[ \frac{D}{2k_B T} \right]^{D/2} \exp \left( -\frac{Dk(r_t - r_0 \exp(-t/\tau))^2}{2k_B T(1 - \exp(-2t/\tau))} \right), \tag{4.6}
\]
where \( \tau = \xi/k \) is the characteristic relaxation time of the particle residing in a harmonic potential of strength \( k \). In the limit \( t \to \infty \) the Green’s function reduces to the time-independent Boltzmann distribution. Using Eqns [4.5] and [4.6] it is possible to generate the probability distributions of the particle at various time intervals.
The probability that an equilibrated particle resides at \( r_0 \pm dr_0 \) at \( t = 0 \) in a trap of strength \( k_0 \) is given by \( P_B(r_0, k_0)dr_0 \). The probability of observing the particle at \( r_t \) some time \( t \) after the trap strength was changed to \( k_1 \), given the initial position \( r_0 \), is \( G(r_0, r_t, k_1, t) \). The probability density of forward trajectories is then \( p(r_0, r_t) = P_B(r_0, k_0)G(r_0, r_t, k_1, t) \). Likewise, the probability density of the corresponding anti-trajectories is \( p(r_t, r_0) = P_B(r_t, k_0)G(r_t, r_0, k_1, t) \). Substituting these into Eqn 4.15 yields

\[
\exp(\Omega t) = \frac{P_B(r_0, k_0)G(r_0, r_t, k_1, t)}{P_B(r_t, k_0)G(r_t, r_0, k_1, t)}.
\]

(4.7)

After some algebra this simplifies to

\[
\Omega t = \frac{1}{2k_BT}(k_0 - k_1)(r_t \cdot r_t - r_0 \cdot r_0)
\]

(4.8)

### 4.2.3 Details about \( \Omega_t \) for Capture

By comparing Eqns 4.3 and 4.8 noting that the definition of \( q_1 \) is identical to that of \( r \), it can be seen that the dissipation function in this experiment has the same functional form when derived using deterministic and stochastic mechanics. This may not be the case in other experiments (such as the SSFT Drag experiment detailed in Chapter 5). Due to the possibility of asymmetry in the optical trap the trap constants \( k_0 \) and \( k_1 \) were evaluated in the \( x \) and \( y \) coordinates separately. Similarly, the \( x \) and \( y \) contributions to \( \Omega_t \) are evaluated separately and summed to produce the complete dissipation function.

For a physical interpretation of Eqn 4.8, consider the dimensionless work done during one period as a result of the change in the trap strength, which can be written as

\[
\frac{1}{k_BT} \int_0^t ds \Delta f \cdot v \equiv \frac{1}{k_BT} \int_0^t ds [(f \cdot v) - (f_0 \cdot v)]
\]

(4.9)

where \( f \) is the optical force acting on the particle in the strong trap, \( f_0 \) is the force in the weak trap, \( \Delta f \) is the difference in the forces and \( v \equiv dr/ds \) is the velocity of the particle. The first term on the RHS is the work accumulated along the particle’s path of duration \( t \). The second term on the RHS is a hypothetical work: it is the work accumulated over the same path had the trap strength not been increased.

An analogy can be made with the work done by a hiker trekking up a snow-covered
mountain. The first term is analgous to the work done by the hiker during his ascent up the snow-covered mountain, the second is the amount of work that he would have done along the same path without snow. The difference is the extra work that the hiker performed due to the snow. For this experiment the extra work, attributed to the increase in the trap strength, reduces to Eqn 4.3.

The dissipation function in this experiment differs considerably from the dissipation function used by Wang et al [3] in their 2002 “drag” experiment. In the capture experiment $\langle \Omega_t \rangle$ is not a measure of the entropy of the system. Instead the experiment measures the amount of energy dissipated when one equilibrium distribution relaxes to another. It also illustrates why the term ”dissipation function” has been used in the definition of the FT rather than a more specific term related to an entropy production.

4.3 Results and Discussion for the Capture Experiment

Figure 4.2 shows histograms of the dissipation function, as defined in Eqn 4.3, constructed using 3300 experimental trajectories at various times after the trap strength was increased from $k_0$ to $k_1$. At $t = 0$, the instant the trap strength is increased, $\Omega_t = 0$ for every trajectory and the FT is meaningless. As the trajectory time increases the distribution of $\Omega_t$ spreads out spanning a larger range of positive and negative values until the maximum breadth of the distribution is obtained. Also, the distribution of $\Omega_t$ becomes skewed towards positive values as time increases, indicating that the system is increasingly characterised as irreversible. The average value of the dissipation function is always greater than zero and at long times reaches a maximum plateau value. Figure 4.2a, recorded at $t = 2$ ms, shows that the distribution of $\Omega_t$ is roughly symmetric about $\Omega_t = 0.1$, with similar numbers of trajectories in histogram bins on either side of the average. By comparing the number of trajectories with a specific value of $\Omega_t$ to the number of trajectories with the same specific value but with the opposite sign, it can be seen that the FT should be obeyed. At $t = 20$ ms, Figure 4.2b, the distribution of $\Omega_t$ has increased its range, its average value and shows a distinct skew in the distribution. Figure 4.2c shows the results after the system has relaxed to a new equilibrium state, the histogram indi-
cates that the distribution of $\Omega_t$ has increased its range and average to a maximum value. Again there is a distinct skew in the distribution.

It should be emphasised that after the trap strength is increased the probability of observing negative-$\Omega_t$ distributions increases with increasing time. This is immediately apparent if one examines the simulated Langevin data in Figure 4.2. This increase in observing $P(\Omega_t > 0)$ arises due to the nature of the dissipation function. At $t = 0$ there is no dissipation and the FT is trivially satisfied. Time is required for thermal fluctuations to influence the system and allow negative-$\Omega_t$ trajectories to occur. As time increases the proportion of trajectories with minor particle excursions from the $t = 0$ particle position also increases. This increases the range of the $\Omega_t$-distribution, and consequently increases the probability of observing negative-dissipative trajectories. In the long-time limit the distribution doesn’t change any further and the probability of observing negative-$\Omega_t$ trajectories remains constant. In this system $P(\Omega_t < 0)$ remains non-zero; in other experiments, such as the SSFT experiments detailed in Chapter 5, this may not be the case.

The histogram data can be recast to directly demonstrate the FT, Eqn 3.12. Let $N_i$ be the number of experimental trajectories with a dissipation function evaluated to be between $\Omega_{t,i} - \Delta/2$ and $\Omega_{t,i} + \Delta/2$, where $\Delta$ is the size of the histogram bin in dimensionless units of $\Omega_t$ and $\Omega_{t,i} = i\Delta$. The experimental analog of the LHS of the FT then becomes $\ln \left( \frac{N_i}{N_{-i}} \right)$. Figure 4.3 shows $\ln \left( \frac{N_i}{N_{-i}} \right)$ versus $\Omega_{t,i}$ evaluated for trajectories at $t = 26$ ms. Also shown in the figure is a line of slope unity, representing the FT prediction. The slope of the least squares fit, $0.95 \pm 0.26$, shows that the FT is obeyed within experimental uncertainties. This plot is representative of the experimental data at nearly all sampled times; however at early times, more scatter is observed as the distribution of $\Omega_t$ is highly peaked at early times and the wings of the distribution are more infrequently sampled.

For each data point located at $(x, y)$ in Figure 4.3 there is a corresponding point at $(-x, -y)$. This is directly due to the FT, Eqn 3.12. When plotting $\Omega_{t,i} = i\Delta$ vs $\ln \left( \frac{N_i}{N_{-i}} \right)$ from $\Omega_t = -k$ to $k$ ($-k$ is the most-negative, occupied histogram bin) the experimental data is effectively plotted twice. Mathematically, the correct analysis uses both positive and negative values of $\Omega_t$. In practice, this full analysis which includes the experimental points twice ensures that the $y$-offset is located at the origin when performing a least squares fit.

A plot of the slope of the least squares fit vs “trajectory time” is shown in
Figure 4.2: A histogram showing the distribution of the dissipation function, $\Omega_t$, evaluated over 3300 experimental trajectories at times (a) $t = 0.002$ s, (b) $t = 0.02$ s and (c) $t = 0.2$ s after the trap strength is increased from $(k_{0,x}, k_{0,y}) = (1.24 \pm 0.05, 1.17 \pm 0.06)$ pN/µm to $(k_{1,x}, k_{1,y}) = (2.96 \pm 0.07, 2.70 \pm 0.13)$ pN/µm. The width of each bin is $\Delta = 0.2$, which corresponds to $\frac{1}{5}$ of the standard deviation of the $\Omega_t$ distribution sampled at $t = 1$ ms. (d) The Langevin-predicted distributions, $P(\Omega_t)$, for similar $t/\tau$ and trap strengths.
Figure 4.3: Logarithm of the ratio of number of trajectories in histogram bins, \( \ln \left( \frac{N_i}{N_{-i}} \right) \), versus the value of the dissipation function associated with the \( i \)th bin, \( \Omega_{t,i} \), evaluated for trajectories of time duration \( t = 26 \text{ ms} \). The data used is from the histogram shown in Figure 4.2. The line of best fit has a slope of 0.95 ± 0.26 which agrees with the FT predicted slope of 1 (shown as a red line). Similar plots can be constructed for all times.

Figure 4.4. The plot shows that the FT is obeyed within fitting error for all times. The data range can be extended out to 10 s, when the optical trap is reduced in power and equilibration for the next cycle occurs. The plot is displayed on a shorter timescale so that greater detail can be seen. The errors in Figure 4.3 and Figure 4.4 were obtained by realising that there is an error of \( \pm N_i^{-\frac{1}{2}} \) in each histogram bin. Using error propagation the error of data point \( i \), \( \ln \left( \frac{N_i}{N_{-i}} \right) \), is \( \pm N_i^{-\frac{1}{2}} \pm N_{-i}^{-\frac{1}{2}} \). It should be noted that least squares fitting is not ideal to test the FT. The most important data for a least squares fit lies on the outer edges of the distribution, but this data on the edges of the distribution contains the most erroneous FT data. The slope of the line of best fit should be used more as a qualitative guide, and an inspection of representative FT plots should be performed to check for compliance with the FT. Furthermore, the size and nature of the bins has an effect on the overall result. Histogram bins that are too large smooth the results, and histogram bins that are too small contain large error bars. In addition, bin sizes based on a particular fraction of a standard deviation provide results that are substantially different from bin sizes with a constant value, especially at short times. The error bars significantly differ depending on which method and value are used to determine bin sizes. For this reason the error bars are called fitting errors rather than experimental errors.
The IFT, Eqn 3.29, is also demonstrated from the experiments and the result is shown in Figure 4.5. The ratio of experimental trajectories having a negative dissipation function to those with a positive dissipation function, \( N_{i<0}/N_{i>0} \), is evaluated over time \( t \) and is represented as black points (×) in the figure. This number ratio corresponds to the experimental measure of the LHS of the IFT. The exponential of \(-\Omega t\), averaged over all trajectories having a positive dissipation function, \( \langle \exp (-\Omega t) \rangle_{\Omega t>0} \), is represented in the figure as the red line (—). This average, constructed at millisecond time intervals, corresponds to the experimental measure of the RHS of the IFT. Over the complete 10 second duration of the trajectories, the experimental values of the LHS and RHS of the IFT are nearly equivalent, thereby demonstrating the IFT. Both show that the relaxation of \( \langle \Omega t \rangle \) is roughly on the timescale of the motion of the colloidal particle, that is \( \tau = 37.5 \pm 4.5 \text{ ms} \).

It should also be noted that our experimental apparatus is incapable of producing an instantaneous switch in the trapping constants; the switching time between weak and strong traps is 2-3 ms. This switch time is fast in comparison to the dynamic timescale of the particle’s relaxation, but not on the time scale of our measurements. The scatter in the early-time FT plots, that is plots similar to Figure 4.3 compiled at early times, and the mismatch in the IFT plot, Figure 4.5, over the first few milliseconds are likely to be a consequence of this switch time. Using Langevin
Figure 4.5: The LHS and RHS of the IFT, $N_{i<0}/N_{i>0} \ (\times)$ and $\langle \exp (-\Omega_t) \rangle_{\Omega_t>0} \ (\longrightarrow)$ versus time, $t$. Both sets of data were evaluated from 3300 experimental trajectories of a colloidal particle, sampled at millisecond time intervals. This confirms the earlier experiments by Wang et al [3] that use a translating optical trap to demonstrate the integrated form of the theorem.

Independent, numerical predictions of the dissipation function can be constructed using inertia-less Langevin simulation and compared with our experimental results. A Langevin trajectory, representing the motion of a colloidal particle in an optical trap, is generated using the difference form of the Langevin equation:

$$r(t + \Delta t) = r(t) + \frac{k_1 r(t)}{\xi} \Delta t - \sqrt{\frac{2k_B T \Delta t}{\xi}} g(t)$$ \hspace{1cm} (4.10)

where $\Delta t$ is an arbitrarily small time interval, $g(t)$ is a number chosen randomly from a Gaussian distribution with zero mean and unit variance, and the initial location of the particle, $r(t = 0)$ is chosen randomly from an equilibrium distribution of particle positions in a trap of strength $k_0$, and $\xi$ is the friction coefficient. The particle position is recorded over several million trajectories of duration $t = \tau$ with $\Omega_t$ evaluated over each trajectory. The value of $\Omega_t$ is calculated for each trajectory at various time steps and are placed in histogram bins, providing a Langevin-predicted probability distribution, $P(\Omega_t)$. Figure 4.2d shows $P(\Omega_t)$ constructed from $3 \times 10^6$ Langevin
Figure 4.6: The average of the dissipation function, $\langle \Omega_t \rangle$, versus time, $t$, is compared with the predictions of Langevin dynamics (red line). The Langevin model uses the experimentally determined values $k_0 = 1.22$ pN/µm and $k_1 = 2.80$ pN/µm with $\tau = 37.5$ ms. The experimental averages are evaluated over 3300 experimental trajectories of a colloidal particle.

Trajectories, generated with values $k_0 = 1.22$ and $k_1 = 2.80$, similar to those of the experiment. These Langevin-predicted distributions are comparable with the experimental frequency of $\Omega_t$-trajectories, shown in Figures 4.2a-c. Qualitatively, it appears that Langevin dynamics captures the time-evolution of the $\Omega_t$-distribution, and further evidence of this is provided in Figure 4.6 where the average value of the dissipation function $\langle \Omega_t \rangle$ is shown for both the capture experiment and Langevin predictions. The experimentally-determined $\langle \Omega_t \rangle$ follows the Langevin prediction, $\langle \Omega_t \rangle \equiv (k_0 - k_1)(\frac{1}{k_1} - \frac{1}{k_0})(1 - \exp(-2t/\tau)) [1]$, with a timescale close to $\tau = 37.5$ ms.

4.4 Capture Conclusions

This experiment has shown that the FT can be shown directly in an experiment, Figure 4.3. The previous experiment by Wang et al [3] demonstrated the IFT rather than the FT directly, and this experiment provides further evidence that the FT describes a real phenomenon that can be measured experimentally. The experiment also demonstrates how the IFT varies with time, Figure 4.5. In addition the results are in quantitative agreement with Langevin dynamics, indicating that the FT applies to colloidal length and time scales.
4.5 References


Chapter 5

Steady State Fluctuation Theorem

Experiments

In this Chapter the FT is demonstrated for steady-state conditions using a system where the dissipation function can be expressed exactly for stochastic dynamics, but requires approximations for deterministic dynamics. This system is based upon the 2002 drag experiment used by Wang et al \cite{1}. In Wang’s experiment a colloidal particle was weakly held in a stationary optical trap. At a known point in time the stage was uniformly translated with velocity $v_{opt}$.

Two drag experiments, similar to that performed by Wang et al, are reported in this chapter. These drag experiments use linear or circular translation of a particle-filled optical trap to evaluate the dissipation function under steady-state conditions. The experimental results show that the FT holds asymptotically in the long time limit, consistent with the previous literature on the SSFT, but only when $\Omega_t$ is derived approximately. When $\Omega_t$ is derived exactly the FT holds for all time, and not just in the asymptotic limit.

The SSFT was also tested by Garnier & Ciliberto \cite{2} at approximately the same time that our experiments were being performed. Garnier & Ciliberto’s experiment used an electronic circuit, consisting of a parallel resistor and capacitor, that was driven out of equilibrium by a small current. They analysed the results with Langevin dynamics and found that the SSFT was obeyed; that is, the FT was obeyed in the long-time limit.
5.1 Experimental setup

Approximately 50 particles (6.3 $\mu$m in diameter) were added locally into a stage-mounted, glass-bottomed cell, containing 3.0 ml aqueous solution of 10mM Tris-HCl+1mM EDTA, maintained at a pH of 7.5. One particle was optically trapped, moved at least 2 mm from the local particle reservoir to isolate it from the other particles, and was used to calibrate the quadrant photodiode position detector and optical trap strength. The optical trapping constant, $k$, was determined by sampling the particle’s position in a stationary trap for 120 seconds at 200 Hz and applying the equipartition theorem: $k = k_B T / \langle r \cdot r \rangle$. The particle’s trajectories were recorded as the stage was translated in one of two ways: (i) in a series of linear translations or (ii) using a continuous circular translation. Further details about these two experiments are given below.

5.1.1 Linear Drag

An ensemble of particle trajectories was generated by linearly translating the microscope stage in an alternating-sign square-wave velocity profile: the stage was stationary for 5 seconds, translated at $v_{opt}$ for 20 seconds, stationary for another 5 seconds, and then translated at $-v_{opt}$ for an additional 20 seconds. This is shown in Figure 5.1a. Figure 5.1b shows the position of the microscope stage which corresponds to the input signal. The stationary time is sufficiently long to ensure that the system is in equilibrium before further translations occur. This sequence was repeated, enabling the collection of hundreds of cycles, with the particle’s position being recorded at millisecond intervals. When the trap translates the particle is displaced from its equilibrium position until the average velocity of the particle is equal to the trap velocity. When this occurs the average position of the particle is determined by a balance between the optical force and hydrodynamic drag. From this point on the system is in a non-equilibrium steady-state. During the first 10 s of translation the particle’s position, measured relative to the trap center, follows neither an equilibrium nor steady-state distribution. In this transient period the particle’s position was used to analyse the dissipation function under transient conditions. For the remaining translation, $10 < t < 20$ s, the distribution of particle positions follows a time-independent, steady-state distribution. This portion of the particle’s trajectories were used to construct the steady-state dissipation functions.
The dissipation function is calculated along each axis individually before being combined to form the total dissipation. The translation velocity is equal along each axis.

### 5.1.2 Circular Drag

A single long trajectory is generated by continuously translating the microscope stage in a circular path. This is achieved by feeding synchronised sine and cosine voltage signals, shown in Figure 5.2, to the two perpendicularly mounted piezo crystals attached to the microscope stage. This single, long trajectory is advantageous for studying steady-state trajectories as it maximises the amount of steady-state data; only the first few seconds of the initial, transient trajectory are discarded from the steady-state analysis. The long trajectory is evenly divided into 75 s, non-overlapping intervals. Each interval is then treated as an independent steady-state trajectory from which the steady-state dissipation functions are evaluated. The dissipation function is calculated along the two axes separately, with the total dissipation being the sum of the two results. This circular drag experiment allows the steady-state dissipation function to be analysed over longer times than the linear drag experiment. As only one trajectory is recorded the dissipation function cannot be determined under transient conditions.
In the following sections an expression for the dissipation function is obtained using deterministic and stochastic dynamics. The separate expressions are used to analyse the results using different dynamics.

5.2.1 Deterministic Derivation of \( \Omega_t \)

In deterministic dynamics the system is represented as a particle in an optical trap surrounded by a sea of identical particles unaffected by the trap. The position and momentum of the particles at time \( s \) are given by \( q_i(s) \) and \( p_i(s) \), with \( q_1(s) \) and \( p_1(s) \) representing the position and momenta of the optically trapped particle. At time \( s = 0 \) the position of the optical trap is moved at a constant velocity relative to the particles. This is the molecular analog of Wang’s original colloidal experiment \[1\]. The optical trap potential at time \( s \geq 0 \) is given by

\[
\Phi_{opt}(q_1(s)) = \frac{1}{2} k(q_1(s) - v_{opt}s)^2,
\]

where \( v_{opt}s \) is the position of the trap centre, initially located at the origin.
The equations of motion for this system are given as

\[ \dot{q}_i(s) = \frac{p_i(s)}{m_i} \]  
(5.2)

\[ \dot{p}_i(s) = F_i - \delta_1 k_1 q_i(s) + \delta_1 k_1 v_{opt} s - S_i \alpha(s) p_i(s) \]  
(5.3)

\[ \dot{\alpha}(s) = \frac{1}{Q} \sum_{i=1}^{N} \left( S_i \frac{p_i^2(s) \cdot p_i^2(s)}{m_i} \right) - DN_w k_B T \].  
(5.4)

The \( \delta_1 k_1 v_{opt} s \) term in Eqn \( 5.3 \) is required to ensure the correct time-reversal occurs. That is, the optical trap center needs to be located at \( v_{opt} t \) at the start of an anti-trajectory.

To derive an expression for the dissipation function Eqn \( 3.8 \)

\[ \Omega_t(\Gamma_0) = \ln \left( \frac{f(\Gamma(0), 0^-)}{f(\Gamma(t), 0)} \right) - \int_0^t ds \Lambda(\Gamma(s)) \]  
(5.5)

is used. The distribution function at time \( s \) is given by

\[ f(\Gamma(s), 0) = \exp \left( -\beta H_0(s) \right), \]  
(5.6)

where the Hamiltonian of a thermostatted equilibrium system is given by

\[ H_0(s) = K(p(s)) + \Phi(q(s)) + \Phi_{opt}(q_1(s)) + \frac{1}{2} Q \alpha^2. \]  
(5.7)

In the above equation the kinetic energy is \( K(p) = \sum_i \frac{p_i \cdot p_i}{2 m_i} \), the interparticle potential energy \( \Phi(q) \) is a known function, the optical trap potential is \( \Phi_{opt}(q_1) \) and the effect of the thermostats is given by \( \frac{1}{2} Q \alpha^2 \).

Taking the derivative of the Hamiltonian with respect to \( s \) gives

\[ \dot{H}_0(s) = \dot{K}(p) + \dot{\Phi}(q) + \dot{\Phi}_{opt}(q) + Q \alpha \dot{\alpha}, \]

\[ \dot{H}_0(s) = \sum_i \frac{\dot{p}_i \cdot p_i}{m_i} - \sum_i F_i \cdot \dot{q}_i + k_1 (\dot{q}_1 - v_{opt})(q_1 - v_{opt}s) \]

\[ + \alpha \left[ \sum_i \left( S_i \frac{p_i^2 \cdot p_i^2}{m_i} \right) - DN_w k_B T \right]. \]  
(5.8)
Substituting in Eqn 5.3 gives

$$\dot{H}_0(s) = \sum_i \frac{F_i \cdot p_i}{m_i} - \sum_i \frac{\delta_{ij} k_i q_i \cdot p_i}{m_i} + \sum_i \frac{\delta_{ij} k_i v_{opt} s \cdot p_i}{m_i} - \sum_i \frac{\alpha S_i p_i \cdot p_i}{m_i} - \sum_i F_i \cdot \dot{q}_i$$

$$+ k_1 (q_1 - v_{opt})(q_1 - v_{opt}s) + \alpha \left[ \sum_i \left( \frac{S_i p_i \cdot p_i}{m_i} - DN_w k_B T \right) \right].$$

(5.9)

This simplifies to

$$\dot{H}_0(s) = \sum_i F_i \cdot \dot{q}_i - \frac{k_1 q_1 \cdot p_1}{m_i} + k_1 v_{opt} s \cdot \dot{q}_1 + \sum_i \frac{\alpha S_i p_i \cdot p_i}{m_i} - \sum_i F_i \cdot \dot{q}_i$$

$$+ k_1 (q_1 - v_{opt})(q_1 - v_{opt}s) + \sum_i \frac{\alpha S_i p_i \cdot p_i}{m_i} - \alpha DN_w k_B T,$$

(5.10)

and further cancels to

$$\dot{H}_0(s) = -\frac{k_1 q_1 \cdot p_1}{m_i} + k_1 v_{opt} s \cdot \dot{q}_1 + k_1 (q_1 - v_{opt})(q_1 - v_{opt}s) - \alpha DN_w k_B T.$$ 

(5.11)

Defining the term $f_{opt} \equiv -\frac{d\Phi_{opt}}{dq_1} = -k_1(q_1 - v_{opt}s)$ and substituting the product $f_{opt}v_{opt}$ into the above equation yields

$$\dot{H}_0(s) = -k_1 q_1 \cdot \dot{q}_1 + f_{opt} v_{opt} + k_1 q_1 (q_1 - v_{opt}s) + k_1 q_1 v_{opt}s - \alpha DN_w k_B T.$$ 

(5.12)

This simplifies to

$$\dot{H}_0(s) = f_{opt} v_{opt} - \alpha DN_w k_B T.$$ 

(5.13)

As the phase space compression factor is given by $\Lambda(\Gamma) = -DN\alpha$, this further simplifies to

$$\dot{H}_0(s) = k_B T \Lambda(\Gamma) + f_{opt} v_{opt}$$

(5.14)

Referring back to Eqn 5.5, the dissipation function can be equated.

$$\Omega_t = \ln \left( \frac{\exp \left( -\beta H(\Gamma_0) \right)}{\exp \left( -\beta H(\Gamma_0^*) \right)} \right) - \int_0^t ds \Lambda(\Gamma(s))$$

$$= \frac{1}{k_B T} \left( H(\Gamma_0^*) - H(\Gamma_0) \right) - \int_0^t ds \Lambda(\Gamma(s))$$

$$= \frac{1}{k_B T} \left( H(\Gamma_0^*) - H(\Gamma_0) \right) - \int_0^t ds \Lambda(\Gamma(s))$$

$$= \frac{1}{k_B T} \left( H(\Gamma_0^*) - H(\Gamma_0) \right) - \int_0^t ds \Lambda(\Gamma(s))$$
\[ \Omega_t = \frac{1}{k_B T} \left( H(\Gamma_t) - H(\Gamma_0) \right) - \int_0^t ds \Lambda(\Gamma(s)) \]
\[ = \frac{1}{k_B T} \int_0^t ds \left( \frac{dH(\Gamma_s)}{ds} \right) - \int_0^t ds \Lambda(\Gamma(s)) \]

Substituting the evaluated Hamiltonian, Eqn 5.14, into the dissipation function yields

\[ \Omega_t = \int_0^t ds \Lambda(s) + \frac{1}{k_B T} \int_0^t ds f_{opt} v_{opt} - \int_0^t ds \Lambda(s) \]

Simplifying we obtain the dissipation function

\[ \Omega_t(\Gamma) = \frac{1}{k_B T} \int_0^t ds (f_{opt} \cdot v_{opt}). \quad (5.15) \]

It is important to emphasize that the system is constrained to be at equilibrium at the lower time integration limit, \( t = 0 \). For this experiment, the dissipation function physically corresponds to the work required to translate the trap with constant velocity \( v_{opt} \). If the trap contained no particle, no work would be done translating it.

As the deterministic definition of \( \Omega_t \) requires that the relative probabilities of trajectories be made under initial, equilibrium conditions, it is not possible to construct exact expressions for \( \Omega_t(\Gamma) \) for trajectory segments of duration \( t \) that are wholly at a nonequilibrium steady-state. As the dissipation function is extensive in this experiment an approximate steady-state dissipation function can be constructed as described in Section 3.6. This results in a steady-state dissipation function of

\[ \Omega_{tss}(\Gamma) = \frac{1}{k_B T} \int_\tau^t ds (f_{opt} \cdot v_{opt}) \quad (5.16) \]

where the time integration starts under steady-state conditions.

### 5.2.2 Stochastic Derivation of \( \Omega_t \)

By applying an arbitrary reference frame, \( a \), to Eqn 3.15,

\[ \Omega_t(a_0, a_t) = \ln \left( \frac{P(a_0, a_t)}{P(a_t, a_0)} \right), \quad (5.17) \]

it is possible to equate the dissipation function using stochastic dynamics. In the above equation \( P(a_0, a_t) \) is the probability that the colloidal bead will start at
coordinate \( \mathbf{a}_0 \) at time zero and finish at \( \mathbf{a}_t \) at time \( t \), and \( P(\mathbf{a}_t, \mathbf{a}_0) \) is the probability that the colloidal bead will start at coordinate \( \mathbf{a}_t \) at time zero and finish at \( \mathbf{a}_0 \) at time \( t \). It is also assumed that the system is in a steady state. Two reference frames are defined for the system: a laboratory reference frame, \( \mathbf{r} \), and a translating reference frame, \( \mathbf{x} \). The fixed laboratory frame, \( \mathbf{r} \), is defined so that its origin is located at the position of the optical trap at time \( s = 0 \). After translation starts the optical trap moves along \( \mathbf{r} \) at velocity \( v_{\text{opt}} \). The Langevin equation of motion for this system is

\[
\xi \frac{d\mathbf{r}}{dt} = -k(\mathbf{r} - v_{\text{opt}}t) + \mathbf{g}(t),
\]

where \( \mathbf{r} \) is measured in the sample cell’s reference frame, \( \xi \) is the friction co-efficient, and \( \mathbf{g}(t) \) is uncorrelated Gaussian noise with zero mean and \( \langle \mathbf{g}(t)\mathbf{g}(t') \rangle = 2\xi k_B T \delta(t - t') \). The second frame, \( \mathbf{x} \), is related to \( \mathbf{r} \) through the transform \( \mathbf{x} = \mathbf{r} - v_{\text{opt}}t + \xi v_{\text{opt}}/k \), and the equation of motion for the system is simply

\[
\xi \frac{d\mathbf{x}}{dt} = -k\mathbf{x} + \mathbf{g}(t).
\]

Using the \( \mathbf{x} \) frame the initial distribution of particle positions in the steady state is simply the Boltzmann distribution,

\[
P_B(\mathbf{x}) = \left[ \frac{Dk}{2\pi k_B T} \right]^\frac{D}{2} \exp \left( -\frac{Dk\mathbf{x} \cdot \mathbf{x}}{k_B T} \right),
\]

and the propagator function is simply the Greens function,

\[
G(\mathbf{x}, \mathbf{x}_t, t) = \left[ \frac{Dk}{2\pi k_B T(1 - \exp(-2t/\tau))} \right]^\frac{D}{2} \exp \left( -\frac{Dk(\mathbf{x}_t - \mathbf{x} \exp(-t/\tau))^2}{2k_B T(1 - \exp(-2t/\tau))} \right),
\]

where \( \tau = \xi/k \). Realising that \( P(\mathbf{x}_0, \mathbf{x}_t) = P_B(\mathbf{x}_0) \times G(\mathbf{x}_0, \mathbf{x}_t, t) \), the dissipation function can be found after substitution into Eqn [5.17]. This then reduces to the trivial result of

\[
\Omega_t(\mathbf{x}) = 0
\]

as, in this moving frame, the particle remains in equilibrium.

The dissipation function can also be derived in the laboratory frame, \( \mathbf{r} \). The
Boltzmann function for \( r_0 \) and \( r_t \) are therefore

\[
P_B(x = r_0 + \frac{\xi v_{opt}}{k}) = \left[ \frac{Dk}{2\pi k_B T} \right]^{\frac{D}{2}} \exp \left( -\frac{Dk(\frac{r_0 + \xi v_{opt}}{k})^2}{k_B T} \right)
\]

\[
P_B(x = r_t - v_{opt}t + \frac{\xi v_{opt}}{k}) = \left[ \frac{Dk}{2\pi k_B T} \right]^{\frac{D}{2}} \exp \left( -\frac{Dk(\frac{r_t - v_{opt}t + \xi v_{opt}}{k})^2}{k_B T} \right)
\]

Substituting \( P(r_0, r_t) = P_B(x = r_0 + \frac{\xi v_{opt}}{k}) \times G(r_0, r_t, t) \) and \( P(r_t, r_0) = P_B(x = r_t - v_{opt}t + \frac{\xi v_{opt}}{k}) \times G(r_t, r_0, t) \) into Eqn 5.17 gives the following

\[
\Omega_t(r) = \frac{Dk v_{opt}t}{k_B T} \frac{(r_t - r_0)}{(1 - \exp(-t/\tau))}.
\]

(5.23)

### 5.3 Results

It is important to recognise that two different sets of experimental data were collected: trajectories where the stage is linearly translated, and trajectories where the stage is circularly translated. For each set of trajectories, dissipation functions are calculated using both deterministic and stochastic dynamics.

#### 5.3.1 Transient FT using Linear Drag

The linear drag experiment was performed using an optical trapping constant of \((k_x, k_y) = (0.49 \pm 0.03, 0.48 \pm 0.03) \text{ pN/\mu m} \) with a stage velocity of \(|v_{opt}| = 0.29 \text{ \mu m/s} \).

Only 400 cycles, corresponding to 800 trajectories, could be collected - after this time the thickness of the lens oil varied substantially, changing the optical trapping constant, \( k \), and introducing errors in later results. The dissipation function is accumulated for each trajectory, starting from \( t = 0 \) when the particle is equilibrated in the stationary optical trap, to some time \( t \) after the stage begins to translate. Histograms were constructed at each time step, two of which are shown in Figure 5.3.

The histogram at 50 ms is nearly symmetrical and roughly centered around zero. As time progresses the distribution’s range increases and the mean dissipation shifts to positive values. The histogram bins were set at 25% of the distribution’s standard deviation to provide an adequate number of histogram bins, while simultaneously ensuring enough trajectories are available for analysis.

As shown in Chapter 4.3, the FT can be demonstrated directly using the histograms. This is achieved by dividing the number of trajectories in histogram bin
Figure 5.3: Histograms of $\Omega_t(\Gamma)$, Eqn 5.16, are shown at (a) 50 ms and at (b) 3000 ms.

$i, N_i$, by the number of trajectories in histogram bin $-i$, $N_{-i}$. The natural log of this division can then be plotted against $\Omega_t = i \pm di$, and a comparison with the FT is possible. Figure 5.4 shows the FT results using the two histograms shown in Figure 5.3. As can be seen there is a good correlation between the FT and the experimental results, especially considering the relatively low number of experimental trajectories (800 as opposed to 2500 in Chapter 4’s capture experiment). It can also be seen that there is more scatter in the early plots, which arises due to infrequent sampling of the wings of the distribution. As time increases the variability improves. Least squares fitting of the data shows that the data at 50 ms has a slope of $1.19 \pm 0.22$ and the data at 3000 ms has a slope of $0.94 \pm 0.31$. While these results deviate from the expected value of 1.00 they are within the fitting error margins. The error in each histogram bin is estimated to be $N_i^{-1/2}$, therefore the total error for the FT point $\ln \frac{N_i}{N_{-i}}$ is $\pm N_i^{-1/2} \pm N_{-i}^{-1/2}$. Due to the low number of trajectories, many histogram bins have few data points and the error becomes quite significant, especially on the edges of the distributions. Furthermore, as discussed in Section 4.3, least squares fitting is not ideal for FT data as outlying data points have a disproportionately large effect. The slope of the least squares fit should rather be used as a general guide, and a visual examination of the central FT data points should be made to determine correspondence with the FT.

The process is repeated using $\Omega_t(r)$ instead of $\Omega_t(\Gamma)$, and Figure 5.5 shows the
Figure 5.4: FT results for linear drag using $\Omega_t(\Gamma)$ at (a) 50 ms. The least squares fit has a slope of $1.19 \pm 4.22$. The data in (b) was recorded at 3000 ms, the slope of the least squares fit is $0.94 \pm 0.31$. In each figure $\ln[P(\Omega_t = A)/P(\Omega_t = -A)]$ is plotted against $A$. The red line represents the FT prediction and the points are from the experimental data.

The least squares fit for each FT graph is calculated at each time step, and the slope of this fit is plotted against time. Figure 5.6 shows the slope of the least-squares fit plotted against time for $\Omega_t(\Gamma)$ and $\Omega_t(r)$. The error bars are shown above and below the line. The graphs show that there is significant variation in the early data points and that a fit close to 1.00 is achieved. In the case of $\Omega_t(r)$ the graph has been truncated from 10 s to 0.8 s. Data in the range 0.8 s to 1.2 s is extremely noisy and difficulty arises separating the data from the error bars. This is caused by very few trajectories having a negative dissipation function. Data after 1.2 s is meaningless as no trajectories have negative dissipation functions: the ratio $\frac{N_r}{N_{-r}}$ is either 0 or $\infty$. The decrease in the number of trajectories with a negative dissipation function is best seen using the IFT.

In Figure 5.7a the IFT is analysed using the deterministically-derived dissipation results of the stochastic FT. The results also indicate that the stochastic FT holds, but more statistics would remove much of the scatter seen at short times. The slope of the lines of best fit are $0.65 \pm 0.66$ for 50 ms and $1.18 \pm 0.42$ for 500 ms. The 50 ms result does deviate from 1.00, but the central data indicates that the FT is obeyed. The data at 500 ms fits the FT well, both sets of data fit the stochastic FT within fitting error. This result shows that Langevin dynamics correctly describe the system.
Figure 5.5: FT results using the stochastically derived dissipation function, $\Omega_t(r)$, at (a) 50 ms. The slope of the least squares fit is $0.65 \pm 0.66$. (b) The FT is more obvious at 500 ms, and the slope of the least squares fit is $1.18 \pm 0.42$.

Figure 5.6: The slope of the least squares fit to the FT results are plotted vs Time. The (—) represents data with no errors applied. (—) represents the upper and lower error bars on this data. In (a) $\Omega_t(\Gamma)$ is used as the dissipation function to calculate the FT, and (b) uses $\Omega_t(r)$.
Figure 5.7: IFT results with (a) $\Omega_t(\Gamma)$ and (b) $\Omega_t(r)$ for $0 \leq t \leq 10$. (—) represents the LHS of the IFT while (●) represent the RHS of the FT. It can be seen that the IFT holds for all time.

function, $\Omega_t(\Gamma) = (k_B T)^{-1} \int_0^t ds (f_{opt} \cdot v_{opt})$. It can be seen that the LHS and RHS of the IFT, $P(\Omega_t(\Gamma) < 0)$ and $\langle \exp(-\Omega_t(\Gamma)) \rangle_{\Omega_t(\Gamma) > 0}$ respectively, are approximately equal. Negative values of the dissipation function can be seen out to approximately 10 s after the stage starts translating. This is approximately $5 \times$ longer than observed by Wang et al. [1] in their original experiment. Figure 5.7b analyses the IFT using the stochastically derived dissipation function, $\Omega_t(r)$. It can be seen that there is a difference in the time it takes for the dissipation function to return to zero. This is due to the difference in the functional forms of the two dissipation functions. Also seen is that the two sides of the IFT decay to zero, unlike the capture experiment.

If the drag FT is sampled for longer and longer times the experimental FT and IFT fails. This is caused by finite sampling preventing the system from observing increasingly-rare negative dissipations.

By comparing Figures 5.7a and 5.7b it is clear that the decay time of the deterministic dissipation function varies substantially to the decay time of the stochastic dissipation function. This is caused by the difference between the functional forms of the dissipation function. Importantly, despite the difference in the functional forms of the $\Omega_t(\Gamma)$ and $\Omega_t(r)$, the FT and IFT results work correctly for both deterministic and stochastic systems.

Data similar to that shown in Figure 5.7a was first published in 2002 by Wang et al. [1] as an experimental demonstration of the transient application of the Fluctua-
tion Theorem using a nearly identical experiment. By repeating Wang’s experiment it is now possible to obtain more information than was available at the time of the original experiment - that is, Figures 5.4, 5.5, 5.6 and 5.7b can be obtained.

5.3.2 Steady State FT using Linear Drag

The steady-state portions of the same 800 trajectories can be used to test the FT in the steady state. The first 10 seconds of data for each trajectory, corresponding to the transient response of the system to the step change in the stage’s velocity, is skipped and the remaining data points along the trajectory are used to construct the dissipation functions, $\Omega_{ss}^t(\Gamma)$ and $\Omega_t(r)$. These truncated trajectory segments, from $10 \leq 10 + t \leq 20$ s, are judged to be in the steady-state as the initial 10 seconds of transient data far exceeds the relaxation time of the stationary system, $\tau \equiv \xi/k = 120 \pm 15$ ms.

Figure 5.8a and Figure 5.8b plot the FT directly at $t = 0.05$ s and $t = 3.0$ s respectively. The number of recorded trajectories with $\Omega_{ss}^t(\Gamma) = A \pm dA$, $N_i$, is divided by the number of trajectories with $\Omega_{ss}^t(\Gamma) = -A \pm dA$, $N_{-i}$, and $\ln \left( \frac{N_i}{N_{-i}} \right)$ is plotted against $A$. Referring specifically to Figure 5.8a, it is clear that the FT is not obeyed - the observed data points do not coincide with the FT prediction. The slope of the least squares fit is $7.65 \pm 3.86$, larger than the values produced by data expected to fit the FT. Furthermore, an inspection of Figure 5.8a shows that the central points fail to follow the FT indicating that the deviation in the least squares fit is not due to outlying points. In contrast, the experimental results agree very well with the FT prediction at $t = 3.0$ s indicating that at long times the FT is obeyed. The slope of the best fit line at $t = 3.0$ s is $0.77 \pm 0.25$, indicating that the FT is obeyed at longer times. These figures indicate that the SSFT, Eqn 3.33 page 54, is obeyed: the data only matches the FT in the long time limit.

Figure 5.9a and Figure 5.9b also plot the FT but use the stochastically derived dissipation function $\Omega_t(r)$ instead of the deterministic dissipation function. The times over which the dissipation function is accumulated are $t = 0.05$ s and $0.5$ s, with the least squares fits having slopes of $0.62 \pm 0.70$ and $0.92 \pm 0.30$ respectively. From Figures 5.9a and 5.9b it is clear that the FT is obeyed at all times using the stochastically derived dissipation function. Plots at times less than $t = 0.05$ s (not shown) also indicate that the exact, stochastically derived dissipation function
Figure 5.8: FT results with $\Omega_t^{SS}(\Gamma)$ for $10 \leq 10 + t \leq 20$. In each figure $\ln \left[ P(\Omega_t^{SS} = A)/P(\Omega_t^{SS} = -A) \right]$ is plotted against $\Omega_t$. The theoretical FT prediction is shown as a continuous line. (a) The FT using $\Omega_t^{SS}(\Gamma)$ is plotted at $t = 0.05$ s. Its line of best fit as a slope of $7.65 \pm 3.86$. (b) The FT results at $t = 3.0$ s. The line of best fit has a slope of $0.77 \pm 0.25$.

obeys the FT at early times, although they exhibit more scatter on the edges of the distribution.

The least squares fit for each FT graph is also calculated for each time step, and the slope is plotted against time in Figure 5.10. An examination of Figure 5.10a shows that this figure is substantially different from Figures 5.6a, 5.6b and 5.10b. The lower error bar at small times converges towards unity from a positive value rather than from a negative value. In comparison, Figure 5.10b shows that the stochastically derived dissipation function obeys the FT even at very short times. As the FT can be shown directly using $\Omega_t(r)$, the long time limit is not required and the operative theorem is the FT. This shows that the long time limit required for the SSFT arises from the approximations made when deriving the deterministic dissipation function, $\Omega_t^{ss}(\Gamma)$.

Figure 5.11 shows the IFT results for this data. In Figure 5.11a the IFT is plotted using the deterministic results. The LHS and RHS of the IFT, $\frac{P(\Omega_t^{ss}(\Gamma) < 0)}{P(\Omega_t^{ss}(\Gamma) > 0)}$ and $\left\langle \exp \left( -\Omega_t^{ss}(\Gamma) \right) \right\rangle_{\Omega_t^{ss}(\Gamma) > 0}$, only agree at larger time segments, $t > 2$ s. This differs significantly from the plot shown in Figure 5.7a where approximations have not been made. Figure 5.11b plots the IFT using the stochastic results. The LHS and RHS of the IFT, $\frac{P(\Omega_t(r) < 0)}{P(\Omega_t(r) > 0)}$ and $\left\langle \exp \left( -\Omega_t(r) \right) \right\rangle_{\Omega_t(r) > 0}$, agree for all times as they do in Figure 5.7b. This also indicates that the long time limit in the SSFT arises
Figure 5.9: FT results with $\Omega_t(r)$ for $10 \leq 10 + t \leq 20$. In each figure $\ln \left[ \frac{P(\Omega_t = A)}{P(\Omega_t = -A)} \right]$ is plotted against $\Omega_t$. The theoretical FT prediction is shown as a continuous line. (a) The FT using $\Omega_t(r)$ is plotted at $t = 0.05$ s. The least squares fit has a slope of $0.6184 \pm 0.6956$. (b) The FT using $\Omega_t(r)$ is plotted at $t = 0.5$ s. The least squares fit to the data has a slope of $0.9174 \pm 0.2991$.

Figure 5.10: The slope of the least squares fit to the FT results are plotted vs Time. Again, (—) represents data with no errors applied and (---) represents the upper and lower error bars on this data. In (a) the $\Omega_t^{**}(\Gamma)$ is used as the dissipation function to calculate the FT, and (b) uses $\Omega_t(r)$ for $10 \leq 10 + t \leq 20$ seconds. The plot scales have been truncated so that times with no recorded negative dissipation functions are not displayed.
Figure 5.11: IFT results with (a) $\Omega_t(\Gamma)$ and (b) $\Omega_t(r)$ for $10 \leq t \leq 20$ s. (—) represents the LHS of the IFT while (•) represent the RHS of the FT. It can be seen that the IFT holds only after $\approx 1.2$ s for (a) but holds for all time in (b).

from approximations made in deriving $\Omega_t^{ss}(\Gamma)$. It should also be noted that the asymptotic limit of the SSFT is met while $\Omega_t^{ss}(\Gamma)$ is appreciably larger than zero, ensuring that the function is not satisfied in a trivial limit.

5.3.3 Steady State FT using Circular Drag

This section reports the results of the circular drag experiment. The experiment was run with an optical trap constant of $(k_x, k_y) = (0.14 \pm 0.01, 0.11 \pm 0.01) \text{ pN/\mu m}$, traversing a circle with a diameter of 14.6 \text{ \mu m} at 4 \text{ mHz} - corresponding to a tangential velocity of 0.18 \text{ \mu m/s} (assuming $k_x = k_y = 0.12 \text{ pN/\mu m}$). Only one trajectory was recorded, so the transient version of the FT could not be analysed. This single trajectory was then cut into 75 s intervals, with each interval being treated as an independent steady-state trajectory. A total of 665 intervals were collected in this manner. As the optical trap constants and drag velocity are substantially smaller than those used in the linear drag experiments negative dissipation functions can be observed over a longer period of time: 75 s for circular drag compared to 10 s for linear drag.

The FT is obtained in the same manner as in Section 5.3.2 Figures 5.12 and 5.13 show data similar to that in the steady-state linear drag experiment. Figure 5.12a shows an example where an inspection of the FT plot indicates that the FT is not obeyed, but where the least squares fitting error, $2.34 \pm 3.81$, is large enough to
Figure 5.12: FT results for circular drag are shown. In (a) $\Omega_{tss}(\Gamma)$ is used to plot the FT at $t = 0.25$ s. The least squares fit provides a result of $2.34 \pm 3.81$. In (b) $\Omega_{tss}(\Gamma)$ is used to plot the FT at $t = 2.5$ s. The least squares fit is $1.32 \pm 0.76$.

indicate that the FT “could” hold. Relying on the visual inspection, this data does not fit the FT. Figures 5.12b, 5.13a and 5.13b are all observed to fit the FT within visual and fitting accuracy. Due to the scatter for large values of $|\Omega_{t}|$, that is at the edges of the distributions, the fitting errors will have a major effect. All these figures support the premise that the long time limit in the SSFT is required when the approximated deterministic dissipation function, $\Omega_{tss}(\Gamma)$, is used and not when the dissipation function has been determined exactly.

The slope of the least squares fitting lines are shown in Figure 5.14. The time axis has been shortened to 4 s for both $\Omega_{tss}(\Gamma)$ and $\Omega_{t}(r)$ so that greater detail can be observed. Figure 5.14a indicates that the FT does not hold at early times, although the lower fitting error does allow for this possibility. Inspection of the raw FT plots rather than this least squares fit further demonstrates this. Figure 5.14b shows that the FT is likely to be obeyed for all times.

Figure 5.15 shows the IFT using steady-state segments to calculate the steady-state dissipation functions, $\Omega_{tss}(\Gamma)$ and $\Omega_{t}(r)$. Figure 5.15a shows the first 10 seconds of data plotted using $\Omega_{tss}(\Gamma)$. Like the linear drag results there is a lack of equivalence of the LHS and RHS of the IFT over short segment times, as expected from using the approximated dissipation function $\Omega_{tss}(\Gamma)$. Figure 5.15b shows the first 5 seconds of data plotted using $\Omega_{t}(r)$. Just like the linear drag case, the IFT works for all times using the non-approximated stochastic dissipation function.
Figure 5.13: The FT for circular drag using the stochastically derived dissipation function, $\Omega_t(r)$, is shown. In (a) the data is plotted at $t = 0.25$ s, and the least squares fit is $0.56 \pm 0.81$. Further inspection indicates that the poor value for the FT is due to the edge points. A fit using only the data points where $|\Omega_t(r)| \leq 1$ produces a slope of $1.00 \pm 0.64$. In (b) the data is plotted at $t = 2.5$ s with the least squares fit being $0.83 \pm 0.24$.

Figure 5.14: The slope of the least squares fit to the circular drag FT results are plotted vs time. (—) represents data with no errors applied and (—) represents the upper and lower fitting error bars. In (a) the $\Omega^{ss}(\Gamma)$ is used as the dissipation function to calculate the FT, and (b) uses $\Omega_t(r)$. Both plot scales have been truncated to 4 s so that greater detail can be displayed. The $\Omega^{ss}(\Gamma)$ data can be extended out to approximately 70 s before the numbers of trajectories with a negative dissipation functions approaches zero, and the $\Omega_t(r)$ data can be extended to 5 s.
The experiments in this chapter have demonstrated the application of the FT under nonequilibrium steady-states for all times. Under steady-state conditions it is not possible to construct an exact expression for the dissipation function using deterministic dynamics and it is necessary to approximate the dissipation function with $\Omega_{\text{ss}}^t(\Gamma)$. Consequently, when $\Omega_{\text{ss}}^t(\Gamma)$ is used as an argument in the FT, the FT holds only in the long time limit. In contrast, when a closed-form expression of the steady-state dissipation function is derived exactly using stochastic dynamics the FT holds over all time. This shows that the asymptotic limit in the SSFT is due to approximations in the argument of the theorem, and that when the argument of the theorem is derived exactly, the FT is operative over all time. It is important to recognise that it may not always be possible to construct exact, closed-form expressions for steady-state dissipation functions using stochastic dynamics. In such cases approximate dissipation functions are necessary and the FT will only hold in the long time limit.
5.5 References


Chapter 6

The Kawasaki Identity and the Fluctuation Theorem

This chapter describes the Kawasaki function, \( \langle \exp(-\Omega_t) \rangle \), and shows that the Kawasaki function follows an identity when the Fluctuation Theorem is obeyed. Using data from the capture experiment described in Chapter 4, the Kawasaki Identity (KI) is confirmed. Furthermore, the Kawasaki Identity’s use as a diagnostic tool is illustrated.

While much research has focused on the FT \([1, 2, 3, 4, 5, 6, 7, 8]\), the Kawasaki function \([9]\), which is intimately related to the FT, has rarely been discussed. The Kawasaki function was originally defined by Morriss & Evans \([10]\) in 1985, and was named after Kawasaki for his original work on non-linear response theory \([9]\). Morriss & Evans \([11]\), and later Evans & Searles \([10]\), showed that the Kawasaki function obeys the identity,

\[
\langle \exp(-\Omega_t) \rangle \equiv 1. \tag{6.1}
\]

But these publications, \([10, 11]\), do not investigate the link between the KI and the FT. In the following section the KI is derived directly from the FT showing how the two are intimately linked.
6.1 Derivation of the Kawasaki Identity using the Fluctuation Theorem

The KI follows directly from the FT, Eqn 3.12, and the definition of an ensemble average. The ensemble average of the Kawasaki function can be written as

$$\langle \exp (-\Omega_t) \rangle = \int_{-\infty}^{\infty} P(A) \exp (-A) dA, \quad (6.2)$$

where $P(A)$ is the normalised probability density of observing a collection of trajectories of duration $t$ with dissipation production $\Omega_t = A$. Substituting the FT for $P(A) \exp (-A)$ gives

$$\langle \exp (-\Omega_t) \rangle = \int_{-\infty}^{\infty} P(-A) dA. \quad (6.3)$$

A change of variable plus the normalisation condition of $P(A)$ shows that

$$\langle \exp (-\Omega_t) \rangle = \int_{-\infty}^{\infty} P(-A) dA = \int_{-\infty}^{\infty} P(B) dB = 1. \quad (6.4)$$

This proves that if the FT holds, then the KI is satisfied. However, the KI is not a sufficient condition for the FT, as illustrated by S.R. Williams [12] in the following counter-example.

Consider a distribution function, $P(A)$, that is the sum of two Gaussian distributions, $G_1(A)$ and $G_2(A)$, which have the same mean but different variances. The normalised distribution function is

$$P(A) = \frac{1}{2} G_1(A) + \frac{1}{2} G_2(A), \text{ or}$$

$$P(A) = \frac{1}{\sqrt{2\pi}} \left[ \frac{1}{2} \exp \left( -\frac{(A - \mu)^2}{2} \right) + \frac{1}{4} \exp \left( -\frac{(A - \mu)^2}{8} \right) \right] \quad (6.5)$$

where $\mu$ has been chosen to ensure that $\langle \exp (-A) \rangle = 1$. This occurs for $\mu = 1.508266$. However the distribution function, $P(A)$, fails to satisfy the FT. That is, $P(A) \neq P(-A) \exp (A)$, as shown in Figure 6.1. As the KI is not a sufficient condition for the FT, the KI may be used as a screening aid when analysing trajectories that test the FT, but may not be used as a definitive test for the FT.

As the derivation above shows rare negative-$\Omega_t$ trajectories, that are contrary to expectations of the Second Law, are necessary for the KI to hold. Positive-$\Omega_t$
Figure 6.1: From Eqn. 6.5 the distribution functions $P(A)$ and $P(-A) \exp(A)$, $\Delta$ and $\times$ respectively, are plotted versus $A$. The mean of $P(A)$ was chosen to be $\mu = 1.508266$ so that the distribution is normalised and the Kawasaki Identity is satisfied. Notice that the distribution $P(-A) \exp(A)$ is different from $P(A)$. In order for the FT to be satisfied, these distributions must be equivalent, or $P(A) = P(-A) \exp(A)$.

trajectories frequently contribute to the Kawasaki function, but each contribution is small in magnitude due to the negative sign in the exponential. As the dissipation produced along a trajectory increases (that is becomes more positive), its contribution to the Kawasaki function rapidly decreases. On the other hand, the infrequent negative-$\Omega_t$ trajectories contribute rarely to the average, but each contribution is exponentially significant. Without the occurrence of these rare negative-$\Omega_t$ trajectories, it is impossible for the KI to hold.

In the limiting case where there is only a weak change in the trap strength and the trajectory is observed over infinite time, $t \to \infty$, the KI and the FT are equivalent. That is distributions, $P(\Omega_t)$, that obey the KI must also obey the FT and there is a direct mathematical equivalence between the KI and FT. This is a result of the central limit theorem dictating that $\lim_{t,\Delta t \to \infty} P(\Omega_t)$ is a Gaussian distribution (see [13] for a discussion about this double limit). It should also be noted that $P(\Omega_t)$ will always obey the KI by virtue of the Liouville equation in this infinite time, weak change limit. In the more general case explored here, where the trajectory time is finite and the change in trap strength is appreciable, the central limit theorem no longer applies and $P(\Omega_t)$ is not necessarily Gaussian. Consequently, $P(\Omega_t)$ may satisfy the KI but need not satisfy the FT, as shown by the example. However, if $P(\Omega_t)$ satisfies the FT, then the KI must hold. [12]
6.2 KI Experimental Procedure

To demonstrate the KI in an experiment the time-dependent relaxation of a colloidal particle in an optical trap is investigated. With the exception of different trap constants, this experiment is identical to the capture experiment performed in Chapter 4, page 59 and only a brief summary is repeated here.

A colloidal particle is initially localised in a trap of strength $k_0$ over a sufficiently long time so that its position is described by an equilibrium distribution. At $t = 0$ the optical trap strength is increased discontinuously from $k_0$ to $k_1$ so that the particle is more tightly confined. The particle’s position is recorded as it relaxes to its new equilibrium distribution and the dissipation function of each trajectory is evaluated at various time steps using

$$\Omega_t = \frac{1}{2k_B T} (k_0 - k_1)(r_t \cdot r_t - r_0 \cdot r_0).$$

(6.6)

In this equation $r_0$ is the initial position of the particle along a trajectory and $r_t$ is the position of the particle along the same trajectory at time $t$ after the trap strength has been changed. More detailed experimental details can be found in Section 4.1, page 59 and [8]. The derivation of Eqn 6.6 from stochastic and deterministic dynamics is presented in Section 4.2.

6.3 Results of the KI Experiment

Two sets of data demonstrate how the Kawasaki function can distinguish phase space sampling errors. Figure 6.2 illustrates the Kawasaki function (line), the LHS of the IFT ($\times$) and the RHS of the IFT ($+$) evaluated over 2500 particle trajectories. In general, there is moderate agreement between the LHS and RHS of the IFT as well as between the Kawasaki function and unity. However, strong intermittent peaks appear in the background of the Kawasaki function and there is a decrease in the Kawasaki function from approximately 2 at $t \approx 0$ s to unity at $t \approx 0.05$ s. Careful inspection of the IFT over this period of decay shows that the LHS and RHS of the IFT do not agree. This deviation could be attributed to a number of possible errors; including systematic errors such as trap misalignment and miscalibration, or random errors such as electronic noise and laser power fluctuations. The large spikes in the Kawasaki function are the major concern, especially since the IFT does not
Figure 6.2: An example of the IFT and Kawasaki function, constructed from a data set containing a sampling error. The Kawasaki function is denoted as the (line), the LHS of the IFT as (×), and the RHS of the IFT as (+). It can be seen that the Kawasaki function is much larger than 1 at early times, indicating that the Fluctuation Theorem is not be satisfied by this collection of trajectories. Additionally, large spikes in the Kawasaki function indicate that an error has occurred in the data collection process. The optical trap constants were \( (k_{0,x}, k_{0,y}) = (0.39 \pm 0.02, 0.46 \pm 0.03) \) pN/\( \mu \)m and \( (k_{1,x}, k_{1,y}) = (0.74 \pm 0.02, 0.77 \pm 0.03) \) pN/\( \mu \)m. 2500 trajectories were collected and used in this figure.
show any errors when the peaks occur. For each peak in Figure 6.2 the individual \( \Omega_t \) components at the relevant time interval were examined and histograms showing the \( \Omega_t \) distributions were calculated, as shown in Figure 6.3. Careful examination of each histogram bin in the negative regime showed a trajectory (which could differ between histograms) with an anomalously-large, negative value of \( \Omega_t \). Following their identification the suspect trajectories were manually examined. An example of a normal positional signature is shown in Figure 6.4a, while a suspect trajectory is shown in Figure 6.4b.

When analysing the FT, and in particular when scrutinising individual trajectories, one must be extremely careful about discarding data or assigning errors. While an event may initially appear abnormal, it may be a rare event that is required for the FT and KI to hold. In this light data should only be discarded when an obvious problem can be identified.

This need to be cautious was applied when investigating the cause of the peaks observed in Figure 6.2. In four of the six investigated peaks an electronic signature, characteristic of a second particle entering the optical trap and replacing the original particle, was observed. The positional trace of a particle exchange event is quite recognisable, with an example being shown in Figure 6.4b. As can be seen there is a large difference between the normal fluctuations shown in Figure 6.4a and the \( \approx 0.4 \) second region highlighted in Figure 6.4b as particle exchange. Details related to how this signal can be generated computationally are found in Section 2.3.4, page 22.
Figure 6.4: (a) A typical segment of a normal trajectory. The average position is centered around zero and fluctuations are not considered “excessive”. (b) A positional trace of a suspected particle exchange event. The exchange occurs during the time interval contained within the box. (c) The plot of \( \exp(-\Omega_t) \) for this individual trajectory.

By analysing the suspect trajectory’s signal it is seen that there is an initial increase in the particle’s position, which rapidly increases to a maximum value. Eqn 6.6 shows that a large \( \mathbf{r}_t \cdot \mathbf{r}_t \) value produces a very negative \( \Omega_t \), which in turn produces a very large Kawasaki value due to the exponential in \( \langle \exp(-\Omega_t) \rangle \). In the remaining trajectories an unknown event has caused the particle to move a long way from the centre of the optical trap, causing the peaks.

Table 6.1 contains a summary of when the major peaks were observed, the value of \( \Omega_t \), the suspect trajectory identified and the likely cause of the error. As can be seen the Kawasaki function has also identified two trajectories with different anomalies, trajectories 0152 and 0823. The positional trace of these trajectories shows a very rapid spike. These spikes occurred over a time period much shorter than that required for particle exchange and have a different functional form. Further oscillations in the particle’s position were observed after the spike occurred. Still the large spike in the positional trace, which has an abnormally large value of \( \mathbf{r}_t \cdot \mathbf{r}_t \), can be attributed to the spike in the Kawasaki function.

The decay of the Kawasaki function from \( \approx 2 \) to 1 that occurs from \( 0 < t < 0.05 \) can also be attributed to the invading particles. All trajectories were analysed using the original particle’s quadrant photodiode calibration curve, but trajectories containing rogue particles require different calibration curves, as slight differences in the particle diameters and light transmission significantly affect the recording of the particle’s position (and hence all other measurements and analyses, such as the FT). Consequently, a significant fraction of the trajectories provide inaccurate
Table 6.1: This table illustrates times when there is a peak in the Kawasaki function, as shown in Figure 6.2. It also shows the corresponding $\Omega_t$ values, the trajectory that the unusual $\Omega_t$ value occurred in and what the error has been attributed to. The separated value comes from Figure 6.8 at early times, and is reduced towards zero when many samples have been collected.

<table>
<thead>
<tr>
<th>Peak Time (s)</th>
<th>$\Omega_t$</th>
<th>Suspect Trajectory</th>
<th>Likely Cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.017</td>
<td>-8.28</td>
<td>1637</td>
<td>Particle Exchange</td>
</tr>
<tr>
<td>0.044</td>
<td>-8.40</td>
<td>1826</td>
<td>Particle Exchange</td>
</tr>
<tr>
<td>0.195</td>
<td>-10.99</td>
<td>0823</td>
<td>Unknown Event</td>
</tr>
<tr>
<td>0.342</td>
<td>-9.35</td>
<td>0921</td>
<td>Particle Exchange</td>
</tr>
<tr>
<td>0.347</td>
<td>-9.12</td>
<td>0921</td>
<td>Particle Exchange</td>
</tr>
<tr>
<td>0.065</td>
<td>-7.24</td>
<td>0152</td>
<td>Unknown Event</td>
</tr>
</tbody>
</table>

The separated value comes from Figure 6.8 at early times, and is reduced towards zero when many samples have been collected.

The quadrant photodiodes calibration curve, which relates the photodiode signal to the particle’s position, is a cubic function approximately passing through the origin, as shown in Figure 2.14, page 32. The calibration error is greatest when the rogue particle is located furthest from the trap centre, but this error decreases the closer the particle gets to the centre of the optical trap. The particle is more likely to be far from the trap centre in the weak trap and closer to the trap centre in the strong trap. Immediately after the increase in the strength of the optical trap the particle is likely to be far from the trap centre where the calibration error is greatest. As time progresses this error shall reduce as the particle relaxes to the centre of the strong trap. Thus a miscalibrated particle contributes a large error to the Kawasaki function at small times. As time progresses, and the particle’s position becomes confined closer to the trap centre, the error decreases.

The second set of data is illustrative of a set of properly sampled trajectories in a properly calibrated optical trap. Figure 6.6 shows the Kawasaki function (line), the LHS of the IFT ($\times$), and the RHS of the IFT ($\pm$). By comparing the two sides of the IFT it can be seen that the IFT is obeyed within statistical uncertainties. But it is easier to assess the sampling of the data by comparing the Kawasaki function to unity. The Kawasaki function indicates that there have been no erroneous events, such as particle exchange, and that the data is well sampled. This is corroborated by the agreement between the two sides of the IFT. By examining the data shown in the inset of Figure 6.6, where the Kawasaki function is not quite unity, one can see that there is a lower correspondence with the IFT. Consider the points around $t = 0.35$ s where the Kawasaki function has an average value of 0.97. In this case
Figure 6.5: A schematic of the optical trap strength $k$, versus laboratory time, showing the series of trajectories containing the original particle (solid line), a particle exchange event (arrow), and a subsequent series of trajectories containing the rogue particle (dotted line). Trajectories containing the rogue particle contain errors as the rogue particle has a different calibration curve, and consequently trajectories recorded after an exchange event should not be analysed.

when the Kawasaki function does not equal one the LHS and RHS of the IFT do not match exactly. While the results are still good, and within experimental errors, the difference between the Kawasaki function and unity is attributed to “finite-sampling”. Finite-sampling effects are expected due to the relatively low number of trajectories sampled, especially when compared to the number of trajectories that can be generated in computer simulations.

There was a minor difference between the experimental protocols used to collect the two sets of data shown in Figure 6.2 and Figure 6.6. In the original experiment, which yielded the poor Kawasaki results shown in Figure 6.2, a dilute evenly-mixed solution of particles was used. This solution was searched until a free particle was found, its coordinates were stored and any other nearby particles were removed from the general area using the optical trap. The particle was then calibrated and the experiment performed. In later experiments a high local concentration of particles was injected at one end of the sample cell, creating a local “reservoir” of particles, before confining one in the optical trap. The trap was then moved a large distance (2 mm or more) from the particle reservoir. This ensured that the new particle position had a very low local concentration of particles at the start of data collection. This second protocol yielded a local concentration of particles that was
Figure 6.6: An example of the IFT and Kawasaki function, constructed from a well sampled data set using the same scale as Figure 6.2. The Kawasaki function is denoted as the (line), the LHS of the IFT as (×), and the RHS of the IFT as (+). The Kawasaki function is approximately 1, showing the KI holds. The IFT results are also in good agreement. The optical trap constants were \((k_{0,x}, k_{0,y}) = (1.66 \pm 0.04, 1.82 \pm 0.05) \text{ pN}/\mu\text{m}\) and \((k_{1,x}, k_{1,y}) = (2.82 \pm 0.07, 3.30 \pm 0.07) \text{ pN}/\mu\text{m}\). 2100 trajectories were collected.

substantially lower than that of the original protocol, and reduced the frequency of rogue particles interfering with the experiment.

Finite sampling of statistically rare events has a major effect on the value of the Kawasaki function. This is particularly evident for the 2002 Wang experiment [7]. As the probability of observing rare, negative-\(\Omega_t\) events decreases, more trajectories need to be sampled in order to ensure the Kawasaki Identity remains valid. In the long time limit, where the probability of observing these rare, negative-\(\Omega_t\) trajectories approaches 0, the experimental estimates of the Kawasaki function deviate from 1. Finite-sampling can also be shown using the capture experiment. In Figure 6.7 the Kawasaki function is shown to improve with increasing numbers of sampled trajectories. As more trajectories are analysed the Kawasaki function approaches unity.

In Figure 6.8 the Kawasaki function is calculated using the poor Kawasaki results, with varying numbers of sampled trajectories. The expected pattern of the Kawasaki function becoming smoother with more samples is only observed if one excludes the large peaks associated with particular events. Originally in traces (a) to (c) the
Figure 6.7: The Kawasaki function is plotted with various numbers of experimental trajectories, as indicated. The horizontal axes are in time (s).
Kawasaki function becomes more stable, and slowly evolves towards unity. Then in (d) a new set of peaks is observed, due to the anomaly recorded in trajectory 0152. The data again smooths itself in (e) and (f). Two events occurred between (f) and (g) providing new sets of peaks to be investigated. If one ignores the peaks and focuses on the time segment \(0 \leq t \leq 0.05\) it can be seen that after the first anomaly there appears to be a minor decay from about 1.5 towards unity. Although this decay can also be seen before the anomaly there are too few sampling points to conclude that a miscalibration has occurred or whether it is a finite sampling effect. However, after several particle exchange events the decay becomes much more pronounced and the number of trajectories becomes significant. With more trajectories sampled it is possible to conclude that the decay in the Kawasaki function is most likely due to processing the data using miscalibrated signals.

6.4 Concluding Remarks

It has been shown that the Kawasaki Identity is a necessary, but not sufficient, condition for the FT to hold. Additionally, it has been shown that the Kawasaki function is an extremely useful diagnostic tool when analysing experimental data. It provides an excellent indicator to the quality of the results, shows times where phase space sampling has been insufficient and indicates where errors may have occurred. The Kawasaki function also provides a basis to explore finite sampling effects.
Figure 6.8: The Kawasaki function, plotted with the same number of time steps as shown in Figure 6.7, shows how the Kawasaki function indicates anomalous results. The horizontal scales are in time (s).
6.5 References


Chapter 7

The Fluctuation Theorem in Complex Systems

So far the FT has only been investigated in systems that can be modelled using stochastic or deterministic dynamics. The next logical step is to test the FT in a more complex system. One of the easiest methods to increase the complexity of the system is to change the solution properties from purely viscous to viscoelastic. While numerous viscoelastic models are available, for example the Maxwell-Wiechart or the Voight-Kelvin models, experiments are required to obtain fitting parameters in these models.

7.1 The Viscoelastic Capture Experiment

The experiment presented here is almost identical to that performed in the capture experiment, Chapter 4. A brief overview of the viscoelastic capture experiment is presented here, further details on the general capture experiment method are presented in Section 4.1, page 59.

An 8.0 ml transparent sample cell is filled with a visco-elastic solvent containing 0.25 wt% of $8 \times 10^6$ MW polyethyleneoxide (PEO), also known as polyethylene-glycol, dissolved in water. Approximately 3000 particles are injected locally into the sample cell, one particle is optically trapped and dragged at least 2 mm from the injection site to ensure its isolation from the other particles. The position of the particle is recorded at 500 Hz for at least 600 s and equipartition is used to determine the optical trapping constant $k$ at low and high laser powers. The
optical trapping constants are \((k_{0,x}, k_{0,y}) = (2.32 \pm 0.07, 2.24 \pm 0.08)\) pN/µm and \((k_{1,x}, k_{1,y}) = (4.62 \pm 0.09, 4.19 \pm 0.11)\) pN/µm. The optical trap strength was cycled with a period of 8 s. That is, the optical trap strength is \(k_0\) for \(0 \leq t < 4\) s and \(k_1\) for \(4 \leq t < 8\) s. Approximately 3500 trajectories are collected in this experiment at a sampling rate of 500 Hz.

### 7.2 The Dissipation Function for Viscoelastic Capture

In Chapter 4 detailed expressions of the dissipation function are obtained using both deterministic and stochastic dynamics. In the experiment presented here it is not possible to model the system using stochastic dynamics, empirical data is required to model the elastic effects. The deterministic derivation is still valid, the effects of the polymer are incorporated at an atomic level into the interparticle (or interatomic) force, \(F_i\). As such the dissipation function has the exact same form given in Eqn 4.9

\[
\Omega_t = \frac{1}{k_B T} \int_0^t ds [ (\mathbf{f} \cdot \mathbf{v}) - (\mathbf{f}_0 \cdot \mathbf{v}) ].
\]  

(7.1)

where \(\mathbf{f} = -kr\) is the optical force acting on the particle, \(\mathbf{v} = \frac{dr}{ds}\), and \(\mathbf{r}\) is the position of the particle relative to the center of the optical trap.

Eqn 7.1 shows that \(\Omega_t\) is the difference between the accumulated work performed along a path after some change in the initial conditions, \(\frac{1}{k_B T} \int_0^t ds (\mathbf{f} \cdot \mathbf{v})\), minus the work accumulated along the path had the system not changed, \(\frac{1}{k_B T} \int_0^t ds (\mathbf{f}_0 \cdot \mathbf{v})\). Using the analogy presented in Section 4.2.3, this change in work is equivalent to calculating the work due to snow when hiking up a snow-covered mountain. That is, the amount of work that a hiker performs walking up a snow covered mountain subtract the work required for the hiker to walk up the same path without snow gives the amount of work due to the snow. In the viscoelastic experiment instead of climbing a snow-covered mountain the hiker is walking through a snow covered forest. If the hiker subtracts the work it takes to walk along a path with no snow from the work it takes to walk along the same path covered in snow, then the difference is still the work performed due to the snow. That is, the effect of the viscoelastic or viscous solvent (the terrain in the analogy) is not important for the dynamics observed in the deterministic system: the dissipation function arises solely...
Figure 7.1: The calculated power spectral density for data collected in the viscoelastic solvent. The Lorenzian fitting lines are shown as the solid lines underlying the data. As the gradient of the data in the PEO solution is more positive than $-2$ there is an elastic response in this system and the PEO solution is judged to be viscoelastic.

from the increase in the optical trapping strength.

## 7.3 Results

Before the FT can be analysed it is essential to ensure that the solution has viscoelastic properties. Schmidt et al. [1, 2] showed that the power spectral density (PSD) of the particle’s movement could be used to illustrate viscoelastic properties. The PSD should have 3 distinct regions: a plateau at low frequencies, a curve near the “corner frequency”, and a linear response at higher frequencies. The slope of the linear response should equal -2 if the solution is purely viscous, and becomes more positive as the elastic contributions become dominant. Figure 7.1 shows the PSD result of the PEO solution and water. The slope of the linear response for the PEO solution is -1.85. This is more positive than that observed in a purely viscous liquid and, as such, the solution is judged to be viscoelastic. Due to limitations in the amplification of our quadrant photodiode’s voltage signals it is not possible to determine the storage and loss moduli over a wide enough range of frequencies to fully characterise the PEO solution. This limitation could be overcome in the future by using a quadrant photodiode/photomultiplier circuit capable of detecting the position of a trapped particle at much higher sampling rates.
Assuming that equipartition generates the correct optical trapping constants, the friction coefficient, $\xi$, can be obtained by working backwards through the PSD calibration. This yields a value of $\xi = 0.475 \pm 0.02$ pN·s·µm$^{-1}$ for the PEO solution. Assuming a purely viscous solution an approximate relaxation time, $\tau = \frac{\xi}{|k|}$, can be generated for the solution. Using the calibrated trap constants it is seen that $\tau_1 = \frac{\xi}{|k_1|} = 0.104 \pm 0.007$ s and $\tau_2 = \frac{\xi}{|k_0|} = 0.212 \pm 0.011$ s. It should be noted that these values are indicative only, the elastic response of the solution is not considered.

After performing the viscoelastic capture experiment histograms of the dissipation function, Eqn [7.1], are constructed. Both the $k_0$ to $k_1$ and the $k_1$ to $k_0$ change in optical trap strength are used to construct the histograms shown in Figure [7.2]. In Figures [7.2a] and [7.2d], corresponding to data recorded 2 ms after the change in the optical trap constant, the data is approximately symmetric about zero with a slightly larger number of trajectories having a dissipation function with positive values. The range of values present in Figure [7.2d] is less than that shown in Figure [7.2a] due to the differences in the times relative to the relaxational time constants, $\frac{t}{\tau_1} = 0.0192$ and $\frac{t}{\tau_2} = 0.0095$. Using these relative times the data in Figure [7.2d] should resemble a spike at zero much more than Figure [7.2a]. That is, the data should have a smaller range and larger weights close to zero. This is exactly what is observed in Figure [7.2d]. The number of trajectories with a positive dissipation function increases as time increases for both $k_0$ to $k_1$ and $k_1$ to $k_0$. Furthermore, the range of the data increases as time increases. Figures [7.2b] and [7.2e] show the histograms after 20 ms have elapsed, corresponding to $\frac{t}{\tau_1} = 0.192$ and $\frac{t}{\tau_2} = 0.095$, the total number of trajectories having close to zero dissipation decreases. The positive and negative limits approach the steady state values, but the populations in the outer bins are still low. When $\frac{t}{\tau_1} = 1.92$ and $\frac{t}{\tau_2} = 0.95$, corresponding to Figures [7.2c] and [7.2f], the system has approximately relaxed to its new equilibrium distribution. The range and shape of the histograms remains constant for further increases in time despite the fact that the dissipation function of individual trajectories still varies.

The FT plots in Figure [7.3] are calculated from the histograms in Figure [7.2]. $\ln \left( \frac{N_i}{N_{-i}} \right)$ is plotted vs $\Omega_t = A$, where $N_i$ is the number of trajectories where $\Omega_t = A \pm dA$ and $N_{-i}$ is the number of trajectories where $\Omega_t = -A \mp dA$. Figures [7.3a] and [7.3d] show the FT results at 2 ms. The data shown in Figure [7.3a] does not clearly match the FT, and the least squares fit indicates the FT fails. However, an inspection of the most central points indicates that the data could satisfy the FT.
Figure 7.2: Histograms of $\Omega_t$ for the viscoelastic capture experiment are shown. In (a) - (c) the optical trapping constant is varied from $k_0$ to $k_1$. The histograms are recorded at (a) 2 ms, (b) 20 ms and (c) 200 ms. In (d) - (f) the optical trapping constant is varied from $k_1$ to $k_0$. The histograms are also shown at (d) 2 ms, (e) 20 ms and (f) 200 ms.

The discrepancy may be due to the 2-3 ms it takes for the laser power stabiliser to increase the strength of the optical trap constant, may be due to viscoelastic effects in the solution, or may be due to some other undetermined source. Figure 7.3 indicates that the FT is obeyed at short times, although it’s data range is smaller than that shown in Figure 7.3a. The least squares fit provides a value of 1.17 ± 0.65, further indicating that the FT is obeyed. The FT results are shown at 20 ms in Figures 7.3b and 7.3e. Inspection of the plots and the least squares fits indicate that the data obeys the FT. Figures 7.3c and 7.3f show the FT after the system has relaxed to its new equilibrium state and show that the FT is obeyed. Plots between 200 ms and 4 s resemble Figures 7.3c and 7.3f with similar least squares fits and errors.

Figure 7.4 plots the slope of the least squares fit vs time. With the exception of the first point in Figure 7.4a, that is the data point at 2 ms, the error bars indicate that the FT holds in the viscoelastic solution. After approximately 20 ms, while the system is still in a transient state, the fit approximately equals 1.00. Further increases in time show the fit averages around unity. In Figure 7.4b the data initially
Figure 7.3: The FT is plotted from the histograms shown in Figure 7.2. The points represent the experimental data and the (—) represents the FT prediction. In (a) - (c) the optical trapping constant is varied from $k_0$ to $k_1$. The FT and least squares fit to the data at various times are (a) 2 ms, slope = 0.42 ± 0.47, (b) 20 ms, slope = 0.84 ± 0.37 and (c) 200 ms, slope = 1.08 ± 0.36. In (d) - (f) the optical trapping constant is varied from $k_1$ to $k_0$. The FT plots are shown at (d) 2 ms, slope = 1.17 ± 0.65, (e) 20 ms, slope = 1.00 ± 0.36, and (f) 200 ms, slope = 1.21 ± 0.34.
fits the FT prediction, but its average lies at 1.20 rather than unity. Referring back to Figure 7.3b, it is likely that this increase from 1.00 to 1.20 is due to the outer points in the FT graphs. The inner points are all extremely close to the FT prediction. Both plots in Figure 7.3 can be extended out to 4 s when the next change in optical trap strength occurs, it has been shortened to 0.5 s so that greater detail can be seen. Comparing Figures 7.4a and 7.4b to Figure 4.4 page 70, it can be seen that the error bars for viscoelastic capture are approximately 50% larger than for the original capture experiment. As the numbers of trajectories are similar between the two experiments the difference is most likely due to the change of solvent.

The IFT and KI are also plotted for this experiment. Figure 7.5a shows the IFT calculated using the $k_0$ to $k_1$ transition. The data shows that the IFT holds for the displayed time. Data from 0.5 s to 4.0 s remains constant at approximately 0.6. The KI is also shown to approximately hold. As described in Chapter 6, minor variations can be expected in the data, erroneous variations have extremely large values and are easily identified. The trajectories that led to these minor peaks in the KI were identified and examined: no clear errors are seen. An increase in the positional trace occurs over the short time period in only one channel, although the positional trace is substantially smaller than that seen for a secondary object entering the optical trap. The peaks resemble an elastic response rather than an error. Figure 7.5b shows the IFT and KI results of the $k_1$ to $k_0$ transition. These are also in good
agreement and indicate that both the IFT and KI hold in a viscoelastic solvent. Furthermore, the accuracy of the KI indicates that the fitting errors applied to the FT by least squares fitting is significantly overstated. The accuracy of the KI is a better indicator of the sampling and final results as errors are now a result of all the data points rather than those in a particular histogram bin.

7.4 Concluding Remarks

The experiment in this chapter has shown that the FT, IFT and KI are all obeyed in this viscoelastic solution. This indicates that, provided an expression can be obtained for $\Omega_t$, the FT may be applied to more complex systems.

7.5 References


Chapter 8

Conclusions and Future Work

With many new concepts and experiments presented in this thesis it is important to re-emphasise the main points. In Chapter 1 the scene is set: Loschmidt’s paradox - a major problem in classical physics that Boltzmann, Loschmidt, Maxwell, Einstein and numerous other scientists were unable to solve. Then in Chapters 2 the physics, operation and design of Optical Tweezers are described. The most important of the points presented is that the Optical Tweezers can be used as a tool to measure forces and energies that are a fraction of $k_B T$. Chapter 3 shows the derivation of the FT using both deterministic and stochastic equations of motion. Furthermore, a proof is presented that shows the ensemble average of the dissipation function is always positive, and therefore has entropy-like properties.

The capture experiment is shown in Chapter 4. In this experiment the strength of the optical trap was varied in a stepwise manner from a weak optical trap to a strong optical trap. An expression for the dissipation function is obtained using both deterministic and stochastic dynamics, with both having the exact same functional form. The FT is demonstrated by collecting 3300 trajectories, constructing histograms of the dissipation function at various time intervals, and calculating the relative probabilities of observing positive and negative values for the dissipation function. Furthermore, this experiment shows that the FT applies to systems that are not extensive with time.

Chapter 5 determines the source of the long-time limit in the SSFT and revisits the original experiment by Wang et al, the translation of a colloidal particle through a viscous solvent. The dissipation function is expressed using both deterministic and stochastic dynamics, but in this experiment the two expressions have different
functional forms. The transient response of the FT and IFT are plotted using both
dissipation functions and it is found that both obey the FT despite the differences in
functional forms. The data is also analysed in the steady state, however the deter-
ministic dissipation function had to be approximated in order obtain an expression.
It is found that this approximated dissipation function does not match the FT at
early times, rather it obeys the FT only in the long-time limit. The stochastic dissi-
pation function can be expressed without any approximations, and it is shown that
it obeys the FT for all times. Combined, these experiments show that the long time
limit in the SSFT is a result of approximations made when obtaining expressions
for the dissipation function.

The Kawasaki Identity is derived using the FT in Chapter 6. Consequently, if
the FT is obeyed then the KI must hold. Using data from two separate capture
experiments the Kawasaki function is shown to be a valuable diagnostic tool. The
Kawasaki function equals unity when data is correctly sampled. However when an
anomalous event occurs, such as a second particle entering the optical trap, large
spikes in the positional trace give rise to extremely large values for the Kawasaki
function. These spikes in the Kawasaki function provide a marker to investigate
the time and trajectory where such an error has occurred. In addition, it has been
shown that the Kawasaki function can detect erroneous events that the FT and IFT
cannot.

A more complex system is analysed in Chapter 7. Instead of using a purely vis-
cous solution that can be well modelled using deterministic and stochastic equations
a viscoelastic solution is used where only the deterministic dissipation function can
be expressed. It is shown that the FT, IFT and KI are all obeyed in this viscoelastic
solution. Furthermore, the results are analysed using both the increase and decrease
in the strength of the optical trap, and both sets of results demonstrate the FT.

The majority of the experiments presented in this thesis are carried out in rela-
tively “clean” systems, but it is believed that the FT can be applied to more complex
systems. The viscoelastic capture experiment is just the first step in applying the
FT towards ever more complex systems. The complexity in the experiments need to
be increased so that they can be applied to nano- and bio-technology. As researchers
and engineers build ever smaller nanotechnological devices, the amount of work that
these devices perform will approach \( k_B T \). When the work per duty cycle is of the
same order as \( k_B T \) the effects of the FT will need to be considered, else sufficient
redundancies may not be implemented in their design. Failure to consider the FT means that the probability of observing nano-machines operating in reverse are unlikely to be quantified or have redundancies built in. This would greatly reduce the time-efficiency of nano-machines as the system is likely to behave in an N steps forward, M steps back manner. Finally, the experiments presented in this thesis show that Loschmidt’s paradox has been resolved. The original FT derived by Evans et al, and these experiments (amongst some other recent experiments), demonstrate that irreversible dynamics can be obtained from a system with time-reversible dynamics.