Magnetic Tweezers as a Tool for Biological Physics and the Viscoelastic Characterisation of Fibrin

A thesis submitted to the University of Manchester for the degree of Master of Philosophy in the Faculty of Engineering and Physical Sciences 2013

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Abstract of thesis submitted by David Pearce for the degree of MPhil and entitled ‘Magnetic Tweezers as a Tool for Biological Physics and the Viscoelastic Characterisation of Fibrin’

6 June, 2013

Rheology is a discipline of continuum mechanics that is concerned with the mechanical properties of matter as it flows. Key to the study of rheology is the concept that materials do not behave as Newtonian liquids or as heterogeneous, homogenous materials, but as a combination of the two. This combination and blurring of the line between liquid and solid properties is known as viscosity. Furthermore, the viscosity of a material or liquid will not necessarily remain constant when it is subjected to input forces or stresses at different frequencies. This consideration brings with it the idea of viscoelasticity which can account for the variations in the characteristics of a sample medium.

Magnetic tweezers are tools that allow examination of and investigation into the viscoelastic properties of a sample on the mesoscopic scale. Magnetic matter can be inserted into and bound onto a sample. This magnetic matter can then be manipulated using an external magnetic or electromagnetic field. Calibrated magnetic tweezers apparatus can be used to investigate the mechanical and viscoelastic properties of a material with the novel application of time-variant forces and stresses. The resultant behaviour, or response, of the sample can be observed using a microscope and analysed further.

Fibrin is the highly extensible, fibre-like protein that makes up blood clots. Its particularly high levels of extensibility combined with interesting material properties such as viscoelasticity can strain-hardening make it an ideal test sample for magnetic tweezers experiments. The high elastic limit of fibrin ensures that plastic deformation does not usually occur under the range of input forces and stresses exerted by magnetic tweezers. This allows non-destructive and repeatable tests to be performed.

Magnetic tweezers have been developed and used in a series of experiments on fibrin to produce a viscoelastic characterisation of the fibrous networks. The key results in this work are the design of high-sensitivity apparatus for the experiments and associated techniques for high-frequency analysis, use of the tweezers with a high-speed CCD attached to a microscope and the analysis of the viscoelastic properties of fibrin over a several decades of frequency.
DECLARATION

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The words of the wise are like goads, their collected sayings like firmly embedded nails – given by one shepherd. Be warned, my son, of anything in addition to them.
Of making many books there is no end, and much study wearies the body.

Ecclesiastes 12:11-12, New International Version

Without the support and encouragement of my wife, I would not have finished this project. Since I have known her, she has been my rock and my life is blessed because I know her. I am continually thankful to my parents who have always supported me without condition. I hope I can live up to their example in the future.

Dr. Thomas Waigh has been a patient and understanding supervisor, advisor and a helpful guide. It is only now that I realise what a rich source of knowledge and support he is and it is painfully clear how much more I could and should have leaned on him. Along with Professor Jian Lu, I have had expert academic
mentoring in the Biological Physics Group.

The whole Biological Physics Group has been a pleasure to work alongside and I am thankful to the School of Engineering and Physical Sciences at the University of Manchester, without whose financial support I could not have continued with my studies. Professor Jian Lu also went to great lengths to secure extra funding for my work.
INTRODUCTION

1.1 Notes

1.1.1 Motivation

This project has two main motivations. The first is to develop a set of experimental techniques for investigating the use of magnetic tweezers. This is approached in two interconnected and independent ways: firstly by looking at the physical design and limitations of the apparatus and secondly by developing techniques that maximise the performance of this apparatus in the context of the established limitations.

The second motivation is the application of the techniques and methods studies in the first section in order to verify the suitability of the methods and to investi-
gate the viscoelastic properties of a suitable sample material. In this case, fibrin was chosen for the reasons discussed in the body of this thesis.

Alongside the development of techniques and apparatus, it has been necessary to apply various data analysis techniques. This has resulted in the application of novel mathematical approaches and useful sets of code that could be re-used in similar future investigations. For that reason, there is a section of this report where the code used can be found.

1.1.2 Aims

From the motivation, a specific set of aims for this project were assembled. These are four-fold: to design magnetic tweezers apparatus for researching phenomena in biological physics; to develop a set of robust experimental techniques for using this equipment; application of these techniques to a suitable test sample; and developing a number of analysis techniques to generate results from the experimental data.

Each of the aims of the project has its own set of further considerations and these are discussed in the relevant chapters of this thesis.

1.2 Structure

This document is divided into an introduction, the main chapters and the appendices. The main chapters have the following contents:
CHAPTER 1. INTRODUCTION

1.2.1 Introduction

An overview of the structure and scope of the document.

1.2.2 Literature Review

This a review of the current state of the field in the areas covered by this thesis. These areas are broadly grouped in the following way:

Rheological Techniques

A study of the major techniques used for investigating rheology visco-elastic behaviour, along with a comparative analysis of the different advantages and disadvantages of these methods.

Magnetic Tweezers

Building on the previous section, this is a review of the various considerations that are particular to magnetic tweezers, with an outline of the history of the development of the field.

Viscoelastic Behaviour and Semi-Flexible Polymers

This section is a study of the viscoelastic characteristics of various semi-flexible polymers, and their generally-expected behaviour.

Fibrin

A more detailed discussion of the items examined in the previous section, in the context of the blood-clot polymer fibrin.

Frequency Analysis

An overview of a number of aspects of control theory and a discussion of
their relevance and equivalence to the concepts discussed above.

1.2.3 Apparatus Design

The design of the magnetic tweezers apparatus used in the later chapters is presented in Chapter 3. This section is divided into four broad sections. The first two of these are notes about the existing equipment before any design work was carried out and a presentation of the results of the design process to make the new magnetic tweezers used later in this report. The latter two cover the predicted performance of the new magnetic tweezers and the results of the calibration process.

1.2.4 Analysis Techniques

Chapter 4 is concerned with the development of techniques for using the new magnetic tweezers. It contains derivations of all the analysis techniques used throughout the report. To separate the conceptual ideas required for the different types of experiment that is later used, these techniques are broken down into a number of different groups. The first group of experiments is the most straightforward, and is largely unconcerned with any time-variant inputs and responses. As the sections of the chapter develop, ideas are introduced to deal with variation of input and output in the time domain and then the focus shifts to the frequency domain and high-frequency considerations.

The chapter ends with an investigation into how the experimental techniques can be most efficiently carried out to save time, computing resources and the quality of the samples being examined.


1.2.5 Experimental Techniques

Many of the experimental techniques discussed in the later chapters of this report are variations of a common method, with changes being made to the exact implementation of the basic technique to probe different characteristics of the samples. Chapter 5 describes the experimental environment, sample preparation and basic experimental technique. Following on is an exploration of potentially difficult environmental effects and a discussion of the method in relation to the techniques introduced in the previous chapter.

1.2.6 Initial Experimentation

The chapter on initial experimentation serves three purposes. It acts as a proof-of-principle for the design of the magnetic tweezers, it validates the choice of techniques discussed in the analysis techniques and experimental techniques chapters and it also begins the process of data collection that is continues in the chapter on further experimentation.

1.2.7 Further Investigations

The chapter on further investigations furthers the results of the initial experimentation chapter in light of the appraisal in chapter 8. By investigating the effects of changing the composition of the experimental samples and other environmental factors, a more complete characterisation of the fibrin networks is developed.
1.2.8 Appendices

The Appendices in this report are used primarily for information that is relevant to the content of the body of the report but not appropriate for inclusion in the main body of text. Examples of this sort of material are code listings, data tables, and additional figures that give a complete set of data that may only be summarised in the earlier chapters.
2.1 Introduction

This chapter aims to introduce the background to the rest of the work in this thesis. It represents the majority of the non-original work in this report and the later chapters are almost entirely original and reporting on work done by the author, often based on the ideas discussed here.

The section on rheological techniques looks at the development of the research field of rheology by discussing the varied techniques that are employed today. It is coupled with the next section which contains more detail on the principles, apparatus and techniques used with magnetic tweezers. This split allows more attention to be paid to magnetic tweezers than the other methods, as they are the
focus of the apparatus design in Chapter 3.

The next two sections paired in a similar fashion. The section on viscoelastic behaviour and semi-flexible polymers explores the analysis of experimental samples in the time and frequency domains by reporting on the state of the field of research into semi-flexible polymers. The fibrin section that follows on advances the concepts of this section and applies them more specifically to the analysis of fibrin and key results.

The frequency analysis chapter introduces concepts from control systems engineering and looks at the potential for applying these well-established methods to more biological situations and experimental samples.

Each section of this chapter is designed to give insight into the current state of the field and also to present the scope for furthering the breadth of scientific understanding in these areas.

### 2.2 Rheological Techniques

#### 2.2.1 Scope

In order to understand the rationale behind the development of rheological techniques, it is first essential to understand the terms involved and the objectives in embarking on an investigation of rheology. Rheology and, for the purposes of
CHAPTER 2. LITERATURE REVIEW

this report, biorheology, is the study of the viscoelasticity of a substance - in this report that substance is typically a biological sample[32]. This means that aside from the examination of standard material properties such as shear strength or flexibility, an experimental scientist can also examine how these properties change as the applied shear stress or bending moment is applied at different frequencies.

2.2.2 Passive Particle Tracking

Passive particle tracking relies on no more specialised apparatus than a microscope and a CCD camera. Most of the applications of this technique are based on examining the difference in behaviour of particles in and parts of biological samples and comparing these characteristics with the behaviour of particles in an unconstrained environment. Because a lot of the phenomena that are investigated occur in short timescales, it is necessary to be able to record the results of an experiment and analyse it later. Figure 2.1 shows the typical set-up for this type of experiment. See the later sections and chapters of this thesis for more detailed information on the way the motion of the particles is analysed.

While the field of microrheology well established, having been approached by both Robert Brown and Albert Einstein, it was only with the advent of the video camera that it became more prevalent [22]. While it is a cheap and straightforward process to investigate a sample or material using passive particle tracking, the range of results that can be obtained is also restricted by the inability of the researcher to apply an external force to the sample medium. This is reflected in the ranges of modulus and frequency that are exposed by such an experiment.
CHAPTER 2. LITERATURE REVIEW

Figure 2.1: Apparatus for Passive Particle Tracking: This arrangement is one of the most straightforward techniques for particle tracking. The CCD will often be attached to an optical microscope to increase the accuracy of the measurements.

2.2.3 Optical Laser Trapping

Optical laser trapping is the technique that is most similar to the use of magnetic tweezers. It was first demonstrated in 1986[10]. They work by focussing a laser beam onto a very small region of a sample. When this focus is on a particle, the light bends around it and the resultant change in momentum of the light generates a force on the particle[3][27].

Figure 2.2 shows the focussing of a laser beam on a particle. The scattering of the light is equal on each side of the particle in subfigure 2.2a, but in subfigure 2.2b, it is not because the particle is not in the centre of the beam. Because of this, the change in momentum of the light has a greater component towards the left of the diagram. A simple consideration of D’Alembert’s Principle resolves a force on the bead to the right of the diagram. Similarly, if the bead moves vertically in the diagram, the ratio of scattered and refracted light components also changes, restoring the position of the particle in the vertical direction as well.
By focussing the beam in different parts of the sample or by altering the intensity of the beam, it is possible to apply a force to the particle, and thus onto the sample that is being investigated. Some research groups have applied holographic techniques to focus a laser beam on many different points in the same sample [18], and a schematic diagram of the work being carried our at New York University is shown in Figure 2.3.

One disadvantage of using optical trapping is the laser-induced heating due to the high intensity of light being focussed onto a small area of the sample[11]. For many sensitive samples this can cause damage.[4] [1]
Figure 2.3: Holographic Optical Laser Trapping: This variation of optical laser trapping uses a patterned plate (DOE) to generate multiple focus points of the light from a single source. The resultant array of foci can be used to trap multiple particles, as shown in the subfigure to the far right of the diagram. Adapted from [16]

2.2.4 Electrophoresis

Electrophoresis is a technique used for the separation of electrostatically particles in a colloid using an electric field. The electric field is generated using positive and negative electrodes that are positioned at either end of an experimental container. The electric field generates a force on the particles, which then move in response to the force and their motion can be detected with a microscope. Figure 2.4 shows the typical arrangement of a capillary electrophoresis experiment.
CHAPTER 2. LITERATURE REVIEW

Figure 2.4: Apparatus for Electrophoresis: Although not strictly a micro-rheological technique, the motion of the particles in electrophoresis provides an interesting study. The electric field gradient between the positive and negative electrodes produces a force on the particles which moves them along the capillary beneath the microscope. The other parts of the arrangement are identical to Figure 2.1. Adapted and modified from [32]

2.2.5 Atomic Force Microscopy

Atomic force microscope, of AFM is a technique that has been in common usage for around 20 years since its invention by Gerd Binnig in 1986 [12]. It works by utilising a tiny cantilever to move across the surface of a sample and the force between the tip of the cantilever and the sample can be measured. Figure 2.5 shows the experimental arrangement for an AFM experiment. Subfigure 2.5a shows the tip used in atomic force microscopy experiments with dimensions and subfigure 2.5b shows the tip moving over a surface. As the tip moves, the shape of the top of the surface causes it to lift. The deflection of the tip is often measured by examining the deflection of a laser beam that reflects off the cantilever on which the tip is mounted.
This is useful for measuring topographical changes in the surface of a sample, but it has also been applied on numerous occasions to the manipulation of molecules as well [28].

One potential downside to AFM as a technique for nano-manipulation is that its reliance on contact with a sample can also lead to physical damage of the particle.

Figure 2.5: Atomic Force Microscopy apparatus: In the left-hand subfigure, the diamond tip and gold foil cantilever of the force microscope are clearly visible. The thinness of the cantilever helps to maximize the compliance of the apparatus, minimizing the damage done as the tip moves across the sample, as shown in the right-hand subfigure. Modified from [28]

2.2.6 Summary

Figures 2.6, 2.7 and 2.8 show the ranges of modulus, force and frequency that can be investigated using the microrheological techniques discussed above.
Figure 2.6: Frequency Ranges of Rheological Techniques: In theory, a number of techniques could have very low lower-bound frequencies. However, in practice, particles could move beyond the region being studied in a long experiment, so the values reported are those found in literature. Note that electrophoresis is not included in this diagram as it is not primarily a technique where frequency can be controlled. Adapted from Waigh [31]

Figure 2.7: Force Ranges of Rheological Techniques: As passive techniques do not allow direct application of external forces, they are not included in this figure. Adapted from Waigh [31]. As passive techniques do not allow direct application of external forces, they are not included in this chart.
2.3 Magnetic Tweezers

2.3.1 Introduction

Magnetic tweezers have a similar effect on a particle to optical tweezers, but the means by which this is achieved is very different. A magnetic particle in a divergent field will experience a force due to the gradient of the magnetic field around the particle. If the particle can be attached to a biological sample, then the force can be made to act on the sample as well, allowing for manipulation of the experimental subject material.[8]

2.3.2 Principles

Magnetic tweezers work by applying a magnetic field gradient on to one or more magnetic beads. This causes a force to be applied to the bead or beads, and sub-
Figure 2.9: Magnetic Tweezers: The magnetic tweezers have a pole piece geometry designed to maximise the field gradient around the magnetic particle, thereby increasing the force on the particle.

sequently on to the substance, medium or material in which the bead has been placed. Magnetic tweezers are one of a number of technologies that can be employed to apply a force to a material, and application of such a force has a wide variety of possible applications.

A magnetic particle in a magnetic field will possess a property known as magnetic potential energy. Magnetic potential energy is analogous to gravitational potential for an object possessing mass in a gravitational field. In this case magnetic potential energy $U$ is represented by the formula:

$$U = -mB$$

(2.1)

Where $m$ is the magnetic dipole (analogously the mass) and $B$ is the magnetic field (c.f. gravitational field). Just as an object with mass feels a force (weight) towards a lower energy state (falling), a magnetised particle will feel a pull to-
wards a lower energy region. Therefore the magnetic force is the gradient of the magnetic potential:

\[ F_{mag} = -\nabla (mB) \]

(2.2)

Magnetic tweezers simply exploit this phenomenon by locating magnetic beads in a diverging magnetic field, causing them to move. If the particles are attached to a biomaterial or other experimental sample, then the force is transferred to the material.

2.3.3 Summary

Figures 2.10, 2.11 and 2.12 show how published results for magnetic tweezers compare with the other techniques in this chapter.
Chapter 2. Literature Review

Figure 2.10: Comparing Magnetic Tweezers - Frequency: The magnetic tweezers compare comparably with the low-frequency ranges that can be obtained with other techniques, but fall an order of magnitude short of the more responsive methods.

Figure 2.11: Comparing Magnetic Tweezers - Force: In this analysis, magnetic tweezers span the gap between the laser and AFM techniques.
Figure 2.12: Comparing Magnetic Tweezers. Modulus: Again, AFM has the higher stiffness, but the magnetic tweezers span the gap between the other methods and can even reach the levels of the AFM method.
2.4 Viscoelastic Behaviour and Semi-Flexible Polymers

2.4.1 Viscoelasticity

Viscoelasticity is the term used to describe behaviour that is neither like that of a Newtonian liquid or a solid. Figures 2.14 and 2.13 demonstrate the difference between the two types of behaviour by taking the example of a liquid, or fully dispersive sample and a solid which exhibits only elastic properties. If each of these samples is exposed to a sinusoidal force, the resultant motion for the liquid will be a quarter of a cycle out of phase with the applied force.

Materials that exhibit a combination of these two behaviours are known as viscoelastic.

2.4.2 Semi-Flexible Polymers

Macromolecules, or polymers, are chains of monomers. The behaviour of perfectly flexible polymer chains is well understood, as are the characteristics of perfectly rigid polymer chains[26]. Semi-flexible polymers is the term given to those polymers that are not rigid like Polymide or Kevlar[19], and not perfectly flexible like PEG.[24]

Semiflexible polymers typically have persistence lengths of a similar order of magnitude to their contour lengths (c.f. flexible polymers with very short persistence length, and rigid polymers with very long rigid chains[32]). This means
Figure 2.13: Solid-like Behaviour: This figure shows the relative phase angles of applied force, displacement and speed for an elastic solid. A real complex modulus, with no associated imaginary component, exhibits a stored energy value that is in phase with the applied input energy, so the force and displacement are in phase with each other, like a solid.
Figure 2.14: Liquid-like Behaviour: This figure shows the relative phase angles of applied force, displacement and speed for a Newtonian liquid. Juxtaposed to the previous figure, the liquid behavior of this ideal material is apparent. The displacement is proportional to the differential of the force, which is itself in phase with the speed of motion.
that fluctuations along the polymer can be in two different orthogonal transverse
directions and also longitudinally.

### 2.4.3 Rheological Indices

There are a number of rheological indices that can be used to analyse the be-
haviour of a viscolastic material. Many of these are defined in Waigh[31]. Wher-
ever a complex measure is given, it is often helpful to think of it in terms of a
combination of the two out-of-phase responses described above in the complex
plane. For example, the complex modulus $G^*$ is a mix of the real strength, in
phase with the application, known as the storage modulus; and a component in
the imaginary direction known as the loss modulus, so:

$$G^* = |G^*| \cos(\theta) + i |G^*| \sin(\theta) \tag{2.3}$$

The $G$ is the ratio of the shear stress and shear strain and, as such has, the units
of $Pa$.

**Complex Compliance**  Compliance is a gauge of how easily a substance is de-
formed. The complex compliance separates out the real and imaginary compli-
ances.

**Complex Modulus**  The modulus is an indication of resistance to a shear stress,
and is analysed in the frequency domain.
Creep Function and Relaxation Function  The creep and relaxation functions are found in the time domain and have similar focusses on pliability and stiffness as the previous two indices: creep indicates how much ‘give’ is in a sample over a time period after the application of a stress, and relaxation means the opposite.

Retardation and Relaxation Spectra  These two functions five the breakdown of all of the frequency components in the creep and relaxation functions that are present at the one time.

2.4.4 Modelling the Behaviour

Three widely accepted models of semi-flexible polymers exist when their frequency responses are being investigated.

The Maxwell Model  is equivalent to a spring (in-phase response) in parallel with a dashpot (out-of-phase response).

The Kelvin Model  has the same two components in a linear arrangement.

The Standard Linear Model  has the Kelvin model in parallel with a second spring, and can also be thought of as the Maxwell model in series with a different second spring.

When calculating the dynamic characteristics of these systems:

\[ F_s = ke \]  \hspace{1cm} (2.4)
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\[ F_d = d \frac{de}{dt} \]  
(2.5)

Where \( F_s \) is the spring force, \( F_d \) is the dashpot force, \( e \) is the extension of the system, \( k \) is Young’s modulus for the spring and \( d \) the damping factor in the dashpot. These models have, then components of both the real and imaginary domains and attempts can be made to fit them to known empirical data.

2.5 Fibrin

2.5.1 Introduction

Fibrin is the protein that makes up blood clots when fibrinogen and thrombin become polymerised[35]. When an injury occurs, thrombin is released into the blood that already contains fibrinogen, and clotting takes place within a few minutes. See Figure 2.15

When a blood vessel in a mammal is damaged, it is vital that it is repaired quickly. Fibrin is a protein that is formed in a damaged blood vessel when the plasma glycoprotein fibrinogen reacts with the zymogen thrombin in response to the original trauma. Once the wound has healed, the fibrin clot will disintegrate in the presence of plasmin and its components will dissolve back into the bloodstream. This process is known as fibrinolysis. The clot formation mechanism depends highly on the concentration of thrombin, and consequently thrombin production peaks soon after an injury [35]. The high activity of thrombin means that continued production of thrombin is not required to maintain a high level of clot production.
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Figure 2.15: Clot Formation due to the presence of Thrombin: Clot Formation due to the Presence of Thrombin: Note that the Thrombin is only required to catalyze the clot formation. After the thrombin levels reduce, the clot remains intact. Adapted from [35]

The structure of Fibrin is shown in Figure 2.16. It has shown to be a roughly cylindrical structure, around 46nm in length and 6.5nm in diameter.

Figure 2.16: The structure of Fibrin: Fibrin has a polymeric structure, with extended protein chains. Adapted from [20]
2.5.2 Properties

Fibrin fibres have been shown to have a very high level of elasticity, stretching up to three times their own length under an elastic regime before finally snapping at a strain of 400% [15]. This behaviour has been exhibited and demonstrated using an AFM microscope producing forces of several nN. While this is comfortably outside the usual range expected for use with magnetic tweezers, it does provide a very interesting system that can be analysed using magnetic tweezers technology.

2.5.3 Key Results

Strain Softening  At low strains, fibrin networks have been seen to strain soften.[34],[5]. It is currently thought that this effect is caused by the individual fibres in the clots aligning as the stress is increased and giving the impression of strain softening.[33],[7].

Thermal Fluctuation  MSD calculations have been demonstrated (using passive techniques) that the thermal fluctuation power-law gave the MSD as being proportional to $t^{\frac{3}{4}}$[6], and that at increasing stresses this power-law relationship should tend towards $t^{\frac{1}{2}}$.

2.5.4 Outlook

In addition to the development of new high-frequency techniques for studying biological samples, the previous work on Fibrin opens up a number of routes to
discovering and presenting new data.
3.1 Introduction

Because of the way the magnetic tweezers operate, the system can be viewed as either a number of separate modules (coils, sample container, mounting etc.) or as a set of inter-dependant components, each of which is involved in one or more of the functions of the magnetic tweezers (sample positioning, observation, calibration etc.). Because of this, a systematic approach to the development of the magnetic tweezers concept was adopted, as described by Pahl and Beitz [13]. The stages of a conceptual design using this method are listed and described below:
Component Concepts

Each component of the system being designed is separated into groups according to its function. Each function of the system is assigned a number of performance criteria that reflect how it is expected to operate. A number of concepts are produced for each of the components being considered, and the ideas are tabulated.

This allows the individual components to be designed to do their job as well as possible, before the whole system is considered in terms of which set of concepts will work together most efficiently.

Evaluation Matrix

The performance criteria for each of the functions are ranked in terms of their importance and desirability. The more important and prerequisite criteria are given a high ranking score.

Each of the component concepts is assessed with a score indicating how well it is suited to the various performance criteria set out in the previous section. Higher numbers indicate higher levels of performance.

The scores for the performance criteria are multiplied by the weighting of the performance criteria for each component.
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System Concept

Different combinations of components are grouped together in order to find the system concept that is best suited to the tasks it is required to perform. System concepts are assessed by the individual scores of their component parts.

Embodiment Design

Once a system concept has been chosen, the components in the design are developed further in an embodiment design stage. In the case of the magnetic tweezers, the embodiment design stage was carried out using Autodesk Inventor Professional, a powerful CAD package.

Iteration

Because a lot of the Pahl and Beitz design process is carried out by looking at individual components before the whole system is considered, it is often useful to revisit the designs in order to ensure compatibility, and to increase efficiency by sharing various functions between components.
3.2 Existing Apparatus

3.2.1 Introduction

At the start of the project, a magnetic tweezers set-up was available for use. Although some changes have been made since that date the principle of operation remains the same. It is therefore worth taking some time to describe the layout and properties of this equipment.

3.2.2 Apparatus

The experimental apparatus is composed of several separate parts, in an open loop configuration. It allows for the application of a magnetic field gradient to the experimental sample, and video recording of the system behaviour for post-experimental analysis.

A schematic diagram is shown in Figure 3.1.

The signal generator provides a variable voltage output signal in a number of forms, including DC, sinusoidal, square wave and saw-tooth wave. Because the current arrangement has just a single coil, the most useful output for experiments is the constant voltage (DC) signal. The magnetic field strength is related to both the number of turns on the coil and the current passing through the electromagnet, so the amplifier is used to set the coil current. Consequently, the magnitude of the generated signal is arbitrary. Preliminary tests showed that 2V was within the workable range of the amplifier.
CHAPTER 3. APPARATUS DESIGN

Figure 3.1: Original Apparatus Arrangement: The middle of this diagram matches the schematic in Figure 2.2. In addition to the imaging apparatus, an electromagnetic coil is attached to an amplifier with built-in signal generator, capable of a number of simple waveforms. The DVD recorder is limited to low-quality standard-definition television images. No separate signal generator, recording apparatus or synchronisation circuitry is present.

The amplifier has two modes of operation - voltage amplification and current amplification. For the reasons described above, it is experimentally more useful to use the current amplification feature. Built in to the amplifier hardware is a 2A current limiter. This is one of the factors that affects the maximum possible magnetic field gradient and consequently the maximum force on a magnetic particle. Without additional cooling apparatus on the coil and with the very fine copper (Φ 0.4mm) used in the winding, this 2A limit is an appropriate limit for the existing equipment.

A number of electromagnetic coils are available for use in conjunction with the other parts. The number of turns ranges from around 1700 to over 3000, and the diameter of the copper wire varies from 0.4 mm to 1 mm. The coil is mounted on
a bobbin made of either aluminium or nylon, both of which are non-magnetic in order to minimise the disruption to the magnetic field immediately surrounding the coil. The middle of each bobbin has a 5 mm round hole drilled through it along the axis of the coil, which is large enough to accommodate the soft iron pole pieces snugly.

The soft iron pole pieces are around 150 mm in length. They are designed to divert as much of the magnetic flux as possible towards the experimental sample. The end closest to the sample has been machined into either a flat, screwdriver-like ridge or a conical point. The reshaping of the end serves two purposes; it allows the magnetic flux to leave the end of the pole piece divergently, in order to maximise the field gradient in the experimental sample; it also removes some of the material that would otherwise interfere with the region that is occupied by the objective lens on the microscope.

The experimental rig allows for the mounting of a sample in a glass capillary. It is located as close to the end of the pole piece as possible, as this is the region of the greatest magnetic field divergence. The sample capillary is held in place with either rubber clips or blue-tack, and this proves adequate for ensuring that it does not move significantly during the progress of an experiment.

The Olympus BH-2 upright microscope is fitted with a 100x oil immersion objective lens. All of the apparatus is mounted on the microscope’s focussing table. No vibration suppressing devices are used on the microscope, which is placed di-
rectly onto the optical table. The microscope has two optical outputs - one which can be looked directly into and the other which has a fitting which accommodated a CCD camera and a high-speed camera, both of which are available in the laboratory.

Most of the experiments that have been carried out during this project have been using the standard speed CCD camera attached to the Olympus microscope. It provides a 25Hz output signal that can be read by the CD-DVD recorder at a resolution of 352 x 288 pixels.

The CD-DVD records on to writable and re-writable CDs in a number of formats. By recording in DVD format it is possible to then read the recorded files with freeware video software, such as VirtualDub [36]. The video files can be converted into .AVI format and viewed using a MatLab program, such as the one developed by Rogers [14].

Between the microscope platform and the experimental rig is a joystick-operated moving platform, provided by Prior Scientific Instruments. This allows the whole experiment to be moved in two dimensions around underneath the objective lens of the microscope. This changes the area of the sample that is being focussed on during the experiment, and makes it easier to locate suitable regions for analysis. The moving platform has tens of millimetres of travel in each direction, but no position feedback. It also speeds up as it moves further, making reliable repeatability and calculation of the exact position difficult.
The position of the capillary can be moved relative to the tip of the pole piece by means of two micrometer thumbscrews. This enables the sample to be lined up with the sharp edge of the pole piece, where the magnetic field divergence is greatest. The micrometer screws are graduated, but because the position of the moving platform cannot be accurately measured, these readings are not taken during experiments. Instead, the sample is lined up as accurately as possible by eye, and the screws not adjusted without a fresh calibration.

3.2.3 Operation

Power Switching

The most practical approach to switching the magnetic field on and off is to leave the signal generator on for the whole duration of the experiment and to turn the amplifier on and off when it is needed. This can potentially cause two difficulties during the experiment.

Firstly, switching a power amplifier on and off can cause fluctuations in the current, particularly a transient peak or surge in the current. This surge can be observed by looking at the current limiter light on the amplifier, which activates when the current is too high. When switching the equipment on or off, the light flashes briefly. In an experiment where the accuracy of the input current is critical, such a surge can have an impact on the results. This source of error is made worse by the lack of a current-reading feedback loop that could be used to moni-
tor the current.

Secondly, the design of the switch is such that it is not possible to time the moment of the switching with accuracy greater than $\pm 0.4$ s, which proves inadequate for time dependant tests, like observing the step response of an experimental sample.

**Calibration**

The magnetic tweezers can be calibrated by placing magnetic beads into a fluid of a known viscosity, as described by Bausch [2]. A simple mix of glycerol and water suffices for the viscous fluid. Using the viscosity data from Chapter 3 of Harrand [17] and the generalised Stoke’s law [32] for the drag force of a spherical particle in lamina flow [21]:

$$F_{\text{drag}} = 6\mu \eta v a$$

, a relationship can be built up between the coil current, the distance of the magnetic particle from the pole piece and the magnetic dipole moment of the particle. As long as the distance from the pole piece to the particle and the coil can both be measured, the force on the particle can be measured in later experiments with different samples in the capillary.
3.2.4 Appraisal

Some of the characteristics of the original apparatus proved to be less than optimal. For example, the current through the magnetic coils was controlled by a signal generator connected to an operational amplifier, with a simple analogue gauge readout. This limited the accuracy of any readings to a range of 0.1 A, or 5% of the maximum limited current. While this would suffice for a constant current experiment, where the apparatus could be accurately calibrated before the experiment, other methods may provide a better quality of result. This would be especially true if the current was changing throughout the experiment.

The force on a magnetic particle is critically dependent on the displacement of the bead from the tip of the pole piece. Tracking the motions of the bead during an experiment requires accurate measurement on the micron scale. Measuring the exact distance from pole piece to magnetic bead during an experiment was difficult on the old apparatus, as both could not both be seen with the 100x objective lens required during the recording of experiments. Changing the objective lens meant dropping the microscope stage, during which time the surface tension from the immersion oil could move the sample and spoil the data collection process.

Although the coil did not produce enough heat to cause a considerable problem during a short experiment, over a period of half an hour with a constant current of 2A, the temperature became unreasonably high. See Figure 3.2 for the outer wall temperature - the inner temperature would be much higher.
Figure 3.2: Coil Temperature - outer wall: Performed with a coil current of 2A. The change in gradient after 30 minutes indicates the turning off of the coil current. Note the asymmetrical heating-cooling relationships.

The calibration tests on the original apparatus gave a maximum force on a $2.7\mu m$ bead of 40pN, well below the maximum values achieved by other groups.

### 3.2.5 Scope

From the initial investigation, it was decided that a new set of tweezers should be designed that could produce a higher force, and that would be able to be used in the previously unexplored area of high-frequency magnetic microrheology.
3.3 Force Maximisation

3.3.1 Introduction and Objectives

There are four ways of maximising the force that can be produced by a set of magnetic tweezers. The magnitude of the field strength function can be increased, the field gradient can be increased, the magnetic beads can be moved closer to the tip of the pole piece or beads with higher magnetisability can be selected.

Some of these methods result in altered behaviour in the others, such as the closer bead approach meaning that the region of interest is closer to the highly divergent field lines at the tip of the pole piece where the field gradient is higher. Similarly, for a pole piece and coil combination that produces a given field function \( B(x) \), increasing the field strength by a factor of \( \alpha \) will also in turn increase the gradient of the magnetic field \( \dot{B}(x) \) by the same constant.

3.3.2 Coil Dimensions and Current

The field magnetic field generated by a coil if calculated by the relationship:

\[
B = \frac{1.6NI}{5^{1/2}a} \tag{3.2}
\]

Where \( N \) is the number of turns on the coil, \( I \) is the current passing through the coil and \( a \) is the radius of the coil.[29]. In the case where the only objective is the increasing of the maximum force available, it is therefore imperative to increase
the number of turns on the coil whilst keeping the radius as low as possible.

This concept is revisited in the section on tweezers dynamic performance, but there are some considerations that are relevant here. The number of turns on a magnetic coil of a given radius is governed by the length of the coil and the inner and outer radii of the coil. By simply using a narrower coil wire, the maximum force could be increased, but the narrower wire will be more likely to overheat if a high current is passed through it. Preliminary investigations show that a coil with 0.5mm wire can easily take an input current of 2A, so it is decided at this point to limit the variation in the coil geometry to match that of the existing equipment.

3.3.3 Pole Piece Design and Material Selection

In order to maximise the force exerted by the magnetic tweezers on the magnetic beads, there are two factors that must be optimised: the magnetic field gradient at the tip of the pole piece and the magnetic dipole moment of the magnetic beads. There are four types of magnetic material that the beads can be made out of. These are diamagnetic, ferromagnetic, paramagnetic and superparamagnetic.

Diamagnetic magnetisability is exhibited by all materials, and the magnetic force that is produced by a field passing through these materials is negligibly small. They are therefore unsuitable for use as the magnetic beads for this application. Paramagnetic beads display a magnetisation that is linearly related to the magnetic field strength at all but the highest field strengths. While the magnetic force
is a whole order of magnitude higher than the diamagnetic materials, it is still very small when compared to the forces that can be obtained using para- and superparamagnetic materials. Because paramagnetic materials are very susceptible to magnetisation, they are easily saturated. The main difference between the superparamagnetic and paramagnetic particles is that of hysteresis. Superparamagnetic materials have a much smaller hysteresis loop when they are loaded and unloaded magnetically. This is particularly beneficial for magnetic tweezers applications where it is critical that when the coils are turned off the beads do not retain their magnetisation.

Figure 3.3 shows the response of the four materials described to a magnetic field.

Figure 3.3: Magnetic Properties of Various Materials: Magnetic properties of diamagnetic (DM), paramagnetic (PM), ferromagnetic (FM) and superparamagnetic (SPM) materials. Adapted from [13]
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<table>
<thead>
<tr>
<th>Material</th>
<th>Relative Permeability $\mu_r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacuum</td>
<td>1</td>
</tr>
<tr>
<td>Ferrite U</td>
<td>8</td>
</tr>
<tr>
<td>Ferrite M33</td>
<td>750</td>
</tr>
<tr>
<td>Nickel (99%)</td>
<td>600</td>
</tr>
<tr>
<td>Ferrite N41</td>
<td>3000</td>
</tr>
<tr>
<td>Iron (99.8%)</td>
<td>5,000</td>
</tr>
<tr>
<td>Ferrite T38</td>
<td>10,000</td>
</tr>
<tr>
<td>MuMetal</td>
<td>50,000$^a$</td>
</tr>
</tbody>
</table>

$^a$after heat treatment

Table 3.1: Relative permeability of a number of common materials
Note that the MuMetal value only reaches its greatest after heat treatment.

An ideal material for use in the pole pieces is one with a high relative permeability and a low latent magnetisation when the coils are turned off. After analysing a number of commercially available materials, the alloy MuMetal was chosen as being suitable for use in the magnetic tweezers. Table 3.1 shows the benefit of using MuMetal as opposed to the existing soft iron cores, which have only around a tenth of the magnetic permeability.

Two 1500 mm round rods of MuMetal with a 6 mm radius were purchased from MuShield. One of the magnetic coils bobbin borehole widened to fit the wider pole pieces and the tips were machined and polished before being heat treated at 1950°F in a hydrogen-rich environment. The heat treatment stage was necessary to allow the metallic crystals to grow in order to maximise the magnetic permeability of the material.
3.3.4 Finite Element Modelling

In order to optimise the geometry of the tip of the pole piece, a finite element package was used to analyse the field strength around the tip of a number of different pole piece shapes. The shapes were chosen for ease of manufacture to accelerate the production process.

![Figure 3.4: Finite Element Analysis of Electromagnetic Field Strength: Calculations made at the tip of the pole piece. The plot on the right shows the relative field strength caused by each of the suggested tip shapes. The higher field gradient of the fourth pole piece shape is many times larger than the other options. All pole pieces are drawn to the same scale.](image)

Before the MuMetal pole pieces were machined, finite element analysis of the magnetic field through the pole pieces and surrounding atmosphere was carried
out. This was in order to ensure the magnetic field gradient was as high as possible once the treatment had taken place. The results for four of the end geometries are shown in Figure 3.4. A high field gradient is indicated by very close field strength contours and a steel gradient in the plots of the magnetic field strength on the right of the figure. The field gradients are scaled to indicate how each of the tip shapes would perform under the same coil input current for a generic coil.

It is clear from this figure that the pointed tip will provide the greatest field gradient in the region immediately surrounding the end of the pole piece. Note the scale on the field strength plots that indicates the optimal performance of the pole pieces will come when the sample is a fraction of 1 mm away from the tip.

The pointed tip was chosen as the final design, and one of the pole pieces was machined into this shape. The pole piece was then filed and polished for two hours. The polishing process removed all of the sharp corners from the pole piece, save for the very tip. The finite element analysis stage shows how corners cause flux leakage from discontinuities in the shape of the alloy-air boundary. It was noted that the filings that came off the pole piece became magnetised with the during the working process, and stuck to the tip, aligning themselves to the radial field gradient at this point. This helps to validate the results of the finite element analysis stage.

Once the MuMetal was heat treated above its Curie temperature, all magnetisation was gone and it was ready for use. During the heat treatment, a small
amount of oxidation left a speckled carbon deposit on part of the pole piece, but without any apparent detrimental effect on the performance of the component.

3.4 Dynamic Response

3.4.1 Introduction

The dynamic performance of a set of magnetic tweezers is governed by the dynamic performances of each of its components. By considering each of the individual components as having its own frequency-domain transfer function, the overall dynamic performance of the apparatus can be calculated by convoluting these functions in the time domain, which is equivalent to multiplying them in the frequency domain.

3.4.2 Laplace Transforms

It is necessary at this point to include a brief explanation of the Laplace transformation that is used later in this section. Consider the function in the time domain:

\[ x = x(t) \quad \text{(3.3)} \]

whose Laplace transformation is defined as:
\[ \mathcal{L} (x(t)) = \int_{t=0}^{t=\infty} e^{-st} x(t) \, dt \quad (3.4) \]

For simplification, from this point forwards, capitalised forms of functions will be used to denote those in the s-domain, so that:

\[ X(s) = \mathcal{L} (x(t)) \quad (3.5) \]

The Laplace transformation has a number of properties that are useful to the analysis techniques used later in this report, and they are described below.

**Superposition**

If we have a function that can be defined as the sum of two separate and independent functions in the time domain, it is possible to write the function as follows:

\[ x(t) = f(t) + g(t) \quad (3.6) \]

It follows that the Laplace transformation of \( x(t) \) is given by:

\[ X(S) = \int_{t=0}^{t=\infty} e^{-s t} (f(t) + g(t)) \, dt \quad (3.7) \]

Which can be rewritten as:
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\[ X(S) = \int_{t=0}^{t=\infty} e^{-st} f(t) \, dt + \int_{t=0}^{t=\infty} e^{-st} g(t) \, dt \quad (3.8) \]

this is equivalent to:

\[ X(s) = F(s) + G(s) \quad (3.9) \]

demonstrating the independence of the two functions in the time domain can be employed to calculate the Laplace transformation as the sum of two s-domain functions. This superposition allows linearity to be used in the later analyses. /

Differentiation with respect to Time

Later calculations will be dependent on the relationship between the Laplace transform of a function and the Laplace transform of the derivative of that function. The following derivation demonstrates how the first (and higher-order) time derivatives of a function in the time domain map onto the Laplace transform of the function in the s-domain.

Consider the following Laplace transform:

\[ L\left(\frac{d(x(t))}{dt}\right) = \int_{t=0}^{t=\infty} e^{-st} \frac{d(x(t))}{dt} \, dt \quad (3.10) \]

Using the notation \( \dot{f} \) to represent the first time derivative of \( f(t) \), and using the integration by parts technique, Equation 3.10 becomes:

\[ L(\dot{x}) = [e^{-st} x(t)]_{t=0}^{t=\infty} + s \int_{t=0}^{t=\infty} e^{-st} x(t) \, dt \quad (3.11) \]
which is equal to:

\[ \mathcal{L}(\dot{x}) = -x_0 + sX(s) \]  \hspace{1cm} (3.12)

where \( x_0 \) is \( x(t) \) at \( t = 0 \).

In many cases, such as the step response of a system, the analysis is primarily concerned with events that occur at the initial time, \( t = 0 \), before which no behaviour is measured, and the state of the systems being analysed is measured from this initial state. This means that in these cases we can apply causality and neglect the term \( x_0 \). Where this is done, it is explicitly stated.

**Conversion to the Frequency Domain**

It is an accepted result that the s-domain model of a system can be used to find the time response of that same system to an arbitrary time-based input by convolution of the input signal and inverse Laplace transform of the s-domain model of the system. This leads to the following relationship for a system with transfer function \( F(s) \), and input and output signal \( x(t) \) and \( y(t) \) respectively:

\[ y(t) = \mathcal{L}^{-1} \left( F(s) \right) \ast x(t) \]  \hspace{1cm} (3.13)

Which is equivalent in the s-domain to:

\[ Y(s) = F(s) \cdot X(s) \]  \hspace{1cm} (3.14)
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What is more if we have an easily-transformed input signal, such as a step response, Equation 3.14 allows us to easily de-convolute the input and output signals like so:

\[ F(s) = \frac{Y(s)}{X(s)} \] (3.15)

For a step response, \( x \) is the Heaviside step function, equal to 1 for \( t > 0 \) and 0 otherwise, so that:

\[ X(s) = \int_{t=0}^{t=\infty} e^{-st} dt \] (3.16)

\[ X(s) = \frac{1}{s} \] (3.17)

Giving:

\[ F(s) = sY(s) \] (3.18)

To calculate the behaviour of the same system in frequency domain, we wish to understand the relationship between a frequency-dependent input signal \( X(\omega) \) and the resultant frequency-dependent output signal \( Y(\omega) \). This requires the frequency response of the system to be known, which is simply the step response of the system at \( s = i\omega \).
3.4.3 Coil Induction

The inductance for a coil is given by the function [29]:

\[ L = k\mu_0 N^2 a \]  \hspace{1cm} (3.19)

Where \( L \) is the inductance, \( \mu_0 \) the magnetic permeability, \( N \) the number of turns in the coil and \( a \) the radius of the coil.

Consider an electrical circuit consisting of an inductance \( L \) in series with a resistance \( R \). The current \( i \) flowing through the inductive part of the circuit is related to the inductance and voltage drop across the inductance, \( V_L \):

\[ V_L = L \frac{di}{dt} \]  \hspace{1cm} (3.20)

The current flowing through the resistance can be calculated from the voltage drop \( V_R \) across the resistive part of the circuit:

\[ i = \frac{V_R}{R} \]  \hspace{1cm} (3.21)

Now this model of an inductance and resistance in series can be used to model a coil. If the total voltage drop across the coil is \( v_c \), it follows that:

\[ v_c = V_R + V_I \]  \hspace{1cm} (3.22)
Combining this relationship with Equations 3.20 and 3.21 leads to the differential equation:

\[ L \frac{di}{dt} + Ri - v_c = 0 \]  
\[ (3.23) \]

Taking a Laplace transformation of this function and applying the superposition and differentiation rules derived above yields the following (where \( I \) is the Laplace transformation of \( i \), \( V_c \) the Laplace transformation of \( v_c \) and the initial current is \( i_0 \)):

\[ sLI - i_0 + RI - \frac{V_c}{s} = 0 \]  
\[ (3.24) \]

\[ I = \frac{V_0 + si_0}{sL + R} \]  
\[ (3.25) \]

In more common notation, assuming the initial coil current is 0A, the transfer function of the coil, \( G_c(s) \) can be described in this way:

\[ I(s) = G_c(s).V_c(s) \]  
\[ (3.26) \]

So that:

\[ G_c(s) = \frac{1}{sL + R} \]  
\[ (3.27) \]

This can be converted to a complex frequency dependent function be setting
$s = i\omega$. Furthermore, this frequency-dependent current can be translated to the field strength at a given location using the measured field profile so that for a given location, the field gradient is proportional to the current:

$$\frac{dB}{dx} = f(x).I$$  \hspace{1cm} (3.28)

### 3.4.4 Amplifier Performance

The amplifier characteristics will be considerably more complicated than the simple coil equations as the internal electronics and control systems are more complicated. In the simplest case, a high-powered amplifier has the ability to track the input signal exactly, meaning that its transfer function will simply be a constant $K$, that represents the gain on the amplifier.

More complex than this is the idea that the amplifier will have a lag in its operation. The Laplace transformation of a time delay of $\tau$ is given below:

$$\frac{G_A(\omega)}{K}S = \int_{0-}^{\infty} u(t - \tau)e^{-st}dt = e^{-s\tau}$$  \hspace{1cm} (3.29)

### 3.4.5 Bead Response

There are two regimes in which magnetic beads can behave magnetically. They can be either saturated or unsaturated. Consider the equation determining the magnetic force acting on a bead:
For paramagnetic and superparamagnetic beads, the magnetisation \( m \) is proportional to the applied magnetic field \( H \) for small field intensities, after which the beads saturate. This discontinuity in the behaviour of the beads could lead to problems in the analysis, especially as the varying value of \( m \) is present at the short time scales associated with high-frequency data.

One solution to this is to prepare step response tests to start at a non-zero input voltage. The added advantage of this is that any nematic or fibre alignment behaviour is removed from the list of possible sources of error in the test.

Once the bead is saturated magnetically, the force is directly proportional to the field gradient. Where the magnetic field around the bead is treated as a linear system, the field gradient at a given displacement from the tip of the pole piece will also be proportional to the field strength, so there is no frequency-dependent element to the transfer function of the beads. More formally, we can define the transfer function that takes the field gradient to the applied force as being:

\[
G_b(s) = -m_{\text{sat}}
\]  

(3.31)

Where \( m_{\text{sat}} \) is the saturated magnetisation of the bead.
3.4.6 System Transfer Function

By combining the elements of the various components, the relationship between the input signal and the bead force \( G_{\text{tweezers}}(s) \) can be calculated as being:

\[
G_{\text{tweezers}}(s) = G_c(s)G_a(s)f(x) \tag{3.32}
\]

For a calibration experiment where the bead is free to move in a Newtonian fluid, the Reynolds’ number of the motion is sufficiently low that inertial effects can be ignored, and the following relationship holds:

\[
F_{\text{drag}} = 6\pi \eta a v = 6\pi \eta ax \tag{3.33}
\]

Taking the Laplace transform of this function gives the following relationship:

\[
F_{\text{drag}}(s) = 6\pi \eta ax(s) \tag{3.34}
\]

Which defines the complex modulus of the Newtonian fluid as being \((6\pi \eta as)^{-1}\). Setting \( s = i\omega \) should produce a transfer frequency response for a bead in a calibration experiment that is proportional to \( s^{-1} \)

3.4.7 Step Response

Measuring the step response of the whole chain of components is the easiest was to get the full dynamic characterisation of the apparatus. This removes the
necessity for calculating the individual component responses individually and combining them later.

The unit step response is the response of a step increase in the input to a system. The derivative of a unit step \((\gamma(t))\) is the dirac delta function. By applying a Laplace transformation to the step input, the frequency components of a step input \((\Gamma(s))\) are given as\([9]\) \(\frac{1}{s}\) when \(s = i\omega\).

### 3.5 Design

#### 3.5.1 Introduction

In addition to the theoretical considerations outlined in the preceding few chapters, the final magnetic tweezers design is highly dependent on each of the components working in unison to achieve a useable set of apparatus.

#### 3.5.2 Concepts for Evaluation using the Systematic Design Process

In order to control the magnetic field strength, the following three concepts have been considered:

1. Varying the current with the amplifier
2. Varying the amplifier input voltage by using the signal generator controls
3. Moving the electromagnetic coil towards and away from the sample while keeping the current constant
Two performance criteria have been identified for this function:

1. Accurate, easily quantifiable field strength readings
2. Minimisation of the eddy currents that come with switching the coil current

For the measurement of the distance from the pole piece tip to the region of interest, there are three options that have been considered:

1. Keeping the sample still while moving the coil relative to it using micrometer using a stepper motor
2. Keeping the coil still while moving the sample relative to the coil using micrometer screws
3. Moving the coil and sample together like the original apparatus using the moving platform

The following performance criteria have been identified for this function:

1. Accurate measurement of the tip-sample distance
2. Reliable repeatability

### 3.5.3 Evaluation Matrices and Concept Selection

From this method, the ideas of a) moving the electromagnetic coil towards and away from the sample while keeping the current constant and b) Keeping the sample still while moving the coil relative to it using micrometer using a stepper
motor have the highest scores. Fortunately, both of these are compatible with each other and so an embodiment design process was begun with these two high level concepts in mind.

### 3.5.4 Embodiment Design

The coil it moved towards and away from the sample using a Physik Instrumente M-126 microcontroller, which can move 25 mm at 15 mm/s, with 0.1µm repeatability. With a full characterisation of the magnetic field around the pole piece, the displacement of the coil and the current will be all the information required to know the field gradient in the microscope view. The coil is mounted next to the stepper motor, rather than on top of it, so that it leaves the greatest amount of vertical space in the rather cramped gap under the objective lens.

Not shown in Figure 3.5 is the micrometer screw adjustment for setting the position of the sample under the microscope. Because the base of the apparatus will
be rigidly attached to the microscope frame, moving the sample independently of the coil will not affect the force distribution in the region under the objective lens. Therefore there is no requirement for a high level of accuracy in the setting of the sample position as long as its fixed height remains level with the tip of the pole piece.
3.6 Magnetic Field Analysis

3.6.1 Objectives

These experiments are designed to determine the three-dimensional magnetic field around the tip of the pole piece to aid the calibration process.

3.6.2 Introduction

The magnetic force on a bead in a magnetic tweezers experiment can be calculated with the equation:

\[ F_m = -m \nabla B \]  \hspace{1cm} (3.35)

Where \( F_m \) is the magnetic force, \( m \) the magnetisation of the bead and \( B \) is the magnetic field around the bead. The design of magnetic tweezers is deliberately such that \( \nabla B_x \) is much larger than \( \nabla B_y \) or \( \nabla B_z \), so that the component of the force in the \( x \) direction will dominate the behaviour of the particle.

Superparamagnetic beads will typically saturate magnetically in low field levels. Close to the magnetic pole piece, the magnitude of the magnetic field will be great enough to saturate the beads, making \( m \) a constant.

Because of this, the force on a bead in the \( x \) direction can be determined simply
by examining the field gradient in the same direction.

\[ E_m = -m \frac{\delta B_x}{\delta x} \]  \hspace{1cm} (3.36)

This magnetic field gradient can be calculated from measuring the magnetic field with a gauss meter.

### 3.6.3 Vector Field B

While the force in the \( x \) direction can be calculated by measuring the \( x \) component of the magnetic field, there will still be a component of the magnetic field in the \( y \) and \( z \) directions. Each of these will vary in magnitude as well, resulting in lateral and vertical forces on the beads. These forces exist when one or both of these conditions are met:

- The bead is not placed along the \( x \) axis of the pole piece, where the \( y \) and \( z \) axes act as lines of symmetry.

- The sample container in which the bead is placed is not aligned to the \( x \) axis of the pole piece, so that the measured motion of the bead is not in the direction of the force on the bead.

This can cause a problem for a measurement of the shear strain of a sample, because the exact shear force is required to derive the shear strength. However, if the direction of motion is known, then by assuming the bead moves in the direction of the greatest magnetic field gradient the exact force can be derived.
CHAPTER 3. APPARATUS DESIGN

For a well-modelled set of magnetic tweezers, the $x$-component of the force due to the magnetic field is often easily determined. In an experiment where the most significant force on a bead is due to the magnetic field, the magnetic force on a magnetic bead dominates its movement. Therefore, the direction of motion of the bead will be in the direction of the applied magnetic force. From the starting point that:

$$F_m = |F_m| \hat{r} \quad (3.37)$$

Defining the ratio of the magnitudes of the total magnetic force $F_m$ and the $x$-component of the magnetic force $F_x$ as $R$:

$$R = \frac{|F_m|}{|F_x|} \quad (3.38)$$

Equation 3.37 becomes:

$$F_m = R|F_x|\hat{r} \quad (3.39)$$

It follows that $R$ can be calculated using the component of $F_x$ in the $\hat{r}$ direction:

$$R = \frac{|F_x|}{F_x \hat{r}} \quad (3.40)$$

So that:
\[ F_m = \frac{|F_x|^2}{F_x \hat{r}} \]  

(3.41)

3.6.4 Magnetic Field Strength

Method

The magnetic field in the \( x \) direction was measured using a Hall Effect gauss meter. Because experimental use of the magnetic tweezers often results in a gap between the tip of the pole piece and the sample that can be in any direction, the results were measured along 20 different \( y \) positions either side of the pole piece, as well as 9 different vertical displacements. The total range in the \( y \) direction was 10mm and in the \( z \) direction it was 4mm.

The magnetic field strength was recorded using a digital storage oscilloscope attached to a pc. The datasets were taken by moving the pole piece towards the tip of the pole piece at 5mm/s, over the whole nano-positioning stage range of 25mm. Results for \( B_x \) were plotted for each value of \( z \).

Results

The field strength in Figure 3.6 matches well the finite element predicted valued for an angled tip profile in the Apparatus Design chapter. Further understanding of the governing equations can be found in the next part of this section, before the field gradient is calculated.
3.6.5 Field Equations

Introduction

Three-dimensional data is easy to visualise but more difficult to understand when it is only in its graphical form. If an equation can be found to match the full behaviour of this function throughout the near-tip region of interest, then the later experimental analyses will become more straightforward.
CHAPTER 3. APPARATUS DESIGN

Method

The magnetic field was then analysed to fit an equation to its magnitude. Starting from the assumption that the field strength was a function of the distance from the pole piece, an equation of the form $B_x = c_1 x^{c_2}$, where the decaying field strength would be provided by a negative value for $c_2$. However, the geometry of the magnetic coil and pole piece does not provide an intuitive reason why the tip of the pole piece should be the point of peak magnetic field. By incorporating this consideration and an allowance for the background magnetic field in the laboratory, the equation for $B_x$ was refined to the following:

$$B_x = c_1 (x + c_2)^{c_3} + c_4$$ (3.42)

The four coefficients were found in the following ways. The ambient magnetic field was measured with the gaussmeter while the coil was switched off. $c_2$ was found by considering the logarithms of $B_x$ and $c_1 (x + c_2)^{c_3}$ across a range of values for $c_2$. An examination of a plot of $\log B_x$ with respect to $\log (x + c_2)$ shows a change in shape of the magnetic field strength line. For low values of $c_2$, the gradient of the line is decreasing and for higher values it is increasing while staying negative. If a value of $c_2$ can be found that gives a straight line on the logarithmic plot, this would indicate a pure power law relationship.

The value of $c_2$ which gave the greatest negative correlation coefficient between $\log B_x$ and $\log (x + c_2)$ was the value of $c_2$ that was used. The corresponding linear gradient of the plot with this value of $c_2$ was deemed to be $c_3$. The final coefficient $c_4$ was found by simply examining the magnitude of the magnetic field at a known value of $x$. 
CHAPTER 3. APPARATUS DESIGN

The Matlab function for producing the coefficients if given in the Appendices in Section B.3.

Results

Figure 3.7: 3d Field Model: The coefficients from Equation 3.42 are determined using the top two plots in this figure. Firstly, the virtual centre, \( c_2 \) of the magnetic field is calculated on the top plot. The value for \( c_2 \) is optimal when the gradient of the plots is constant. The second coefficient to be calculated if \( c_3 \), which is optimal when the field strength power law is most correlated. The third plot shows the accuracy of the calculation, with very little deviation between the modelled and actual field strengths.

Figure 3.7 shows the results from the one of the data points for the linearisation of the field strength data. The full set can be found in the Appendices in Section
A.1

The top plot shows how the shape of the logarithmic $B_x$ plot changes from a convex to a concave shape, straightening up at an optimum value of $c_2$.

The middle plot shows the correlation of $\log B_x$ and $\log x + c_2$ as $c_2$ is varied in green. The blue line is the gradient of the plot as $c_2$ changes. The minimum value of the correlation, where the line on the upper plot is straightest is highlighted in red. The point where the red and blue lines intercept provides a value for $c_3$, the power law coefficient.

The lowest plot in Figure 3.7 is an overlay of the magnetic field strength from the experiments and the fitted results with the coefficients that have been calculated. The curve fitting technique has proved successful. Over the whole range of measurements taken, 95% of the fitted values fall within 2% of the measured values.

3.6.6 **Gradient $B_x/x$**

**Method**

Because of the accuracy of the curve fitting technique, it is possible to provide an analytical solution for the magnetic field gradient along each of the measurement lines.
This equation proves useful for calculating the force exerted on the magnetic beads because it is a direct integration of the accurate fitted equation. The gauss meter provides data that is subject to noise that fluctuates with a larger magnitude than the local magnetic field gradient as the coil is moved towards the probe. A direct piecewise differentiation of the gauss meter signal without and smoothing would reveal large localised magnetic field gradients that are artefacts from the measurement technique. While it is possible to eliminate these local anomalies by averaging the readings over a longer time, the number of data sets taken in this investigation (over $4 \times 10^5$ measurement points) would make this idea impractical.

**Results**

The three-dimensional sliced image of the magnetic field gradient is shown Figure 3.8.

This plot clearly shows that variation on the position of the bead from side to side (in the $y$ direction) moves the experiment away from the higher force central region.

**3.6.7 Conclusions**

This technique for matching the field gradient to an analytical set of equations makes the later analysis of bead forces much more simple, as the calculated value
Figure 3.8: 3D Field Gradient: Magnetic field gradient $\frac{dB}{dx}$ as a function of $x$, $y$ and $z$ in the space around the tip of the pole piece. These measurements are taken from Equation 33.43, with the coefficients calculated from the method described in Figure 3.7.

can be used to determine the input profile.
3.7 Force Generation

By applying the step input signal to the amplifier shown in Figure 3.9, it was possible to determine the generated force on a bead of 1 µm by observing its motion.

![Signal-Time.png](Signal-Time.png)

Figure 3.9: Calibration Input Profile: y supplying a step input to the amplifier via the signal generator, all positive frequencies are activated in the apparatus, and the frequency response to all of these will be calculated.

The movement of the bead was tracked using the particle tracking software and the force derived using the formulae described in Chapter 2, giving the a resultant force-time relationship. By repeating this experiment at a range of displacements (set with the travelling stage), a full dependency of the force on time and distance could be generated. This is given in Figure 3.10.
Figure 3.10: Dependency of Force on Distance and Time: The force is taken along the centre line of the pole piece, using the model for field gradient calculated in previous sections and the input profile from Figure 3.9 to develop a full model of the step response of the apparatus.

3.8 Frequency Response

The details of methods used for calculating the frequency response of the apparatus are given in the next chapter, but the results of the apparatus calibration are given in the Bode plot in Figure stepBodeInput. The ratio between the magnitude of the generated shear stress at 0 Hz and the magnitude of the generated shear stress at 10 kHz is several orders of magnitude. This is a key indicator of how accurate the higher-frequency results will be.
3.9 Discussion and Conclusions

The high-frequency transfer function described in the previous section shows that there is a strong enough component of the input function to measure data up to 10 kHz, exceeding the previously-reported value of 1 kHz in Figure 2.10 by a factor of 10.

The generated forces are in a range up to 40 pN, which is close to the top of the range of values that can be measured by the apparatus reported in Figure 2.11.

The moduli range that will be generated by these magnetic tweezers will depend on the sample being used, but the performance of the apparatus on the calibration experiments is promising.
Figure 3.11: Re-designed apparatus Frequency Response: Note that the magnitude gradient and phase angle hold constant up to around 50Hz. Above this frequency, the frequency response of the apparatus begins to dominate. See Chapter 4 for a full analysis of the techniques required to take this into account.
CHAPTER

FOUR

ANALYSIS TECHNIQUES

4.1 Introduction

In order to optimise the use of the magnetic tweezers, it is essential to give extended consideration to the techniques being employed. This chapter covers the interpretation of data from the experiments described in later chapters. Particular attention is paid to the suitability of the methods to an arbitrary medium so that these methods can be applied to a wide range of samples.

There are four different groups of experiments described in the following sections, with the following definitions:

Constant Data

This is data that is either constant over time or stochastic but able to be averaged out over a suitably long time scale.
Slowly-Varying Data

This is data that is not constant but is varying so slowly that over a given short time interval it can be treated as being so.

Periodic Responses

This refers to the dynamic responses of an experimental sample to a pre-determined or quantifiable periodic input, such as a sine wave.

High-Frequency Data

This is data that has been sampled at very short time intervals and requires special considerations because of this.

After the development and definition of the various techniques, there are two further sections on methods for modelling the various types of responses described above and the practical considerations of efficiently carrying out experiments on to extract these data types.

4.2 Apparatus

For all of the techniques in this section, assume the following definitions and theoretical experimental set-up.

The methods and techniques described in this section are designed to be apparatus-agnostic. All that is required is a sample medium to which a shear stress can be applied and the corresponding response, or shear strain, of the sample can be measured.
It is worth noting that analogous experimental systems can also be defined with paired inputs and responses. For example:

- Force and extension of a spring
- Input voltage and output sound in an electronic speaker
- Thermostat and temperature in a heated building

### 4.3 Constant and Slowly-Varying Data

#### 4.3.1 Scope

Constant data is the term assigned to data that is time-invariant, such as a constant applied force or the resultant displacement of a part of a sample in force equilibrium due to this applied force. In reality much of the force and position data produced by the magnetic tweezers in an experimental situation is subject to variations and fluctuations. In the case of positional variations, these may be due to stochastic thermal fluctuations in the experimental medium, external vibrations, methodical errors in the interpretation of experimental videos or some other cause. Similarly, the force variations could have a range of causes such as non-constant amplification of the input signal, stray magnetic field lines or fluctuations of other provenances. As every set of data will be due to some sort or time-dependent variation (whether significant enough to be measured or not), it is important to be able to separate the fluctuation from the important experimental data. This is done by averaging the data over a period of time that is at least one order of magnitude greater than the reciprocal of the lowest-frequency
fluctuation that can be measured. In short, constant data that is referred to in this (and following) chapters is often the result of averaging over a sufficiently long time period.

In this chapter, the concept of slowly-varying data is used for values that do not significantly vary over the time it takes to measure them. For example, the section on quasi-static experiments relies on the fact that a slowly ramped force increasing over the duration of an experiment ($t_{\text{exp}}$) is approximately constant over a sampling time ($t_{\text{sample}}$) provided that the sample time is much shorter than the experimental time so that $t_{\text{exp}} \ll t_{\text{sample}}$.

When this approximation is used, it also takes into account the fact that some phenomena, such as thermal fluctuations in a sample, occur over a third time scale ($t_{\text{fluct}}$). The ratio between the sample time and the fluctuation time must be sufficiently high that any random vibrations in order to allow the average value of the reading to be taken. This gives the full relationship between the three timescales as:

$$t_{\text{fluct}} \ll t_{\text{sample}} \ll t_{\text{exp}} \quad (4.1)$$

### 4.3.2 Introduction

Two similar types of experiments are described in this section. Passive techniques involve the observation of the thermal motion of a particle in a biological sample and the material properties that can be inferred from analysis of these motions. Quasi-static techniques are similar, but feature the addition of a fixed force to the sample (in this case by subjecting the magnetic particle to a magnetic field).
CHAPTER 4. ANALYSIS TECHNIQUES

Variation of the force should provide a variation in the behaviour of the samples, as well as a variation in the equilibrium position of the bead. A third, more traditional type of experiment is the establishing of a relationship between applied force and displacement, or shear stress and shear strain.

This section first explores these passive techniques and then discusses how the analysis techniques should vary with the application of the force. Further discussion of stitching together the data from passive experiments filmed at different frame rates and directional analysis of the particle motion are found in sections 4.3.5 and 4.3.6. There then follows a description of the techniques for acquiring stress-strain relationships and the effect of a slowly increasing force on the sample behaviour.

Each part of this section includes a discussion of the theory, analysis techniques, suitability and methodical approach to each of the methods, which are tested in the next chapter of this report.

4.3.3 Unstressed Thermal Fluctuations

Consider the motion of a bead in a visco-elastic fluid with an external force acting on it.

The mean-squared displacement for the position of the bead \( \langle r^2 \rangle \) will vary as \( t^n \), where \( n = 1 \) represents diffusive motion, \( n < 1 \) represents sub-diffusive motion and \( n > 1 \) represents super-diffusive motion. However, where the bead is trapped
in a network of semiflexible fibres, the power law relationship has been shown to tend towards [30]:

\[ \langle \Delta r^2(t) \rangle = 0.47 \left( \frac{k_B T}{\eta} \right)^{0.75} \left( \frac{1}{T \rho} \right)^{0.25} t^{0.25} \]  

(4.2)

The timescales available for analysis in this type of experiment vary from the interval between the frames, equal to the inverse of the frame rate, up to the total time of the experiment. Beyond the accuracy of particle tracking, which is determined by the image resolution, brightness, bead contrast and focus, the main source of systematic errors in this test is that of repetition of measurements. For a dataset with \( N \) measurements, a time interval of \( t_0 \) and a total experimental time of \( T = N t_0 \), every time interval in the set

\[ t = n t_0, n = 1, 2, 3...N \]  

(4.3)
will exist at least once. However, there will be only one occurrence of the time interval \( t = T \), but \( N \) occurrences of the interval \( t = t_0 \). the subsequent error will be much lower on the longer time intervals.

### 4.3.4 Pre-Stressed Thermal Fluctuations

One variation on the passive method described in Section 4.3.3 is to apply a constant force to the sample at the same time as tracking the particle. The beads will once again be constrained to one location, with freedom to move short distances around this equilibrium point. Where semi-flexible polymer theory suggests there will be a reduction in the power law relationship between the mean-squared displacement and time, this power will naturally decrease as the fibres or gel network become tighter and the bead is effectively pinned.

A number of principles can be demonstrated by such an experiment, the first being nematic fibre behaviour. In an unstressed network of fibres in a clot such as fibrin, the stress in each fibre is determined by the biological and chemical processes during the clotting process. Some may be stressed and others unstressed. For a bead attached to a fibre that is unstressed, the restoring force due to that fibre will not become evident until the fibre is taut.

Up to this point, the sub-diffusive behaviour evident from the fibre force will not become evident. Beyond this point, the gradient of the mean-squared displacement plot will decrease. For a bead in a pre-stressed network, there will be no fibre ‘looseness’, and the lower power-law behaviour will be evident at an earlier
Figure 4.2: Fibre tension: Note that the resultant force on this bead due to the tension of the fibre shall be to the left.

This has been quantified as [30]:

\[
\langle \Delta r^2(t) \rangle = 0.47 \left( \frac{k_B T}{\sigma} \right)^{0.75} L^{0.25} \eta^{0.5} t^{0.5}
\]  

(4.4)

### 4.3.5 Multi-Rate Thermal Fluctuation Analysis

Maximisation of the data range in the passive techniques described above can be achieved by increasing the frame rate and extending the time of the experiment. However, in order to increase the data range by a factor of \( F_{upper} \) at the top of the scale in reduce the minimum time interval by a factor of \( F_{lower} \) at the bottom of the scale would mean an overall increase, \( F \), in the number of frames recorded of:

\[
F = F_{upper} F_{lower}
\]  

(4.5)
CHAPTER 4. ANALYSIS TECHNIQUES

To add a decade at each end of the data range would increase the total recorded file size by 100 times. As discussed above, the lowest time intervals would then have 100 times as many readings over which to be averaged and the highest time intervals would still only appear once in each dataset.

Consider a rating applied to a dataset for each of its intervals that indicates the ‘efficiency’ of the data for that interval. Clearly, increasing the number of repetitions of that interval increases the accuracy of the result, making the data quality better. At the same time, increasing the number of readings for each interval increases the file size, reducing the benefits in data size and increasing analysis time. Across the range of a dataset, the ‘efficiency’ of the data will then be measured using the composite function of these two parameters.

If a number of datasets can be ‘stitched’ together, each of roughly equal file sizes, the amount of total recorded data and processing time can be minimised.

4.3.6 Fibre Alignment and Fluctuation

Consider the movement of a bead on a fibre in a gel and the schematic representation of the fibre extension in Figure 4.3.

Assuming the centre of fibre of length $l$ with fixed ends is moved trasversely by a distance $s$, the extension of the fibre, $e$ can be determined by describing a right-angled triangle with vertices at one end of the fibre, at the displaced centre-point of the fibre and at the original centre-point of the fibre. Pythagoras’ theorem gives:

\[
(l + e)^2 = l^2 + s^2 \tag{4.6}
\]
\[ e^2 + 2el - x^2 = 0 \] (4.7)

\[ e = \frac{-2l + 2\sqrt{l^2 - x^2}}{2} \] (4.8)

\[ e = \sqrt{\left(1 - \frac{x^2}{l^2}\right)} - l \] (4.9)

In Figure 4.3, the relative extensions due to longitudinal and transverse displacements is shown. As the transverse displacement always causes a smaller extension, the resultant restoring force from the fibre will be lower than it would be in the longitudinal direction. From this information it should be possible to determine the fibre alignment as being perpendicular to the principal direction of movement.

One other method that can be applied is the separation of the two orthogonal mean-squared displacement functions. The squared displacement \( r^2 \) is defined as follows:

\[ r^2 = x^2 + y^2 \] (4.10)

And this addition of squares helps to keep the two orthogonal components distinct in the calculation of the full mean-squared displacement:

\[ \langle r^2 \rangle = \langle x^2 \rangle + \langle y^2 \rangle \] (4.11)
Figure 4.3: Extensions: For transverse motion, it is expected that the fibre will be taut both before and after the bead position. For longitudinal motion, there may be an unstressed part of the fibre.

Any difference in these two behaviours would give clues to the structure of the fibrous clot being investigated.

4.3.7 Stress-Strain Relationships

The stress-strain relationship is a key method of determining the material properties of a sample in many disciplines of science and engineering.

A typical stress-strain relationship has an initial hookean linear extension, before the sample begins to yield and then plastic deformation continues towards failure. Many materials become more extensible at the yielding point as demonstrated in the example. However some materials, such as polythene become stronger as the strain increases, as discontinuities on the molecular makeup of the material begin to align and prevent stress flow. As discussed in the literature, this is a phenomenon that has been seen in Fibrin [33] [7].
CHAPTER 4. ANALYSIS TECHNIQUES

Application of a shear stress to a biological sample will provide a resultant displacement. If the input stress is held constant, the sample will approach its equilibrium position, where the displacement can be measured. This in turn can be manipulated to give the modulus $E$ of the sample, using the relationship:

$$E = \frac{\sigma}{\gamma}$$  \hspace{1cm} (4.12)

Where $\sigma$ is the applied stress and $\gamma$ is the resultant strain.

4.3.8 Ramped Stress Response

Shear Stress Profile

Later in this chapter the concept of a periodic shear stress input to a viscoelastic sample is discussed, the magnitude and phase relationships between this stress and the resultant shear strain are calculated. Consider an input stress profile that has a high magnitude but also a long wavelength. The input stress can be written as:

$$\sigma(t) = A \sin(\omega t)$$  \hspace{1cm} (4.13)

where $A$ is the magnitude and $\omega$ the frequency in Hz. The first differential of this function is:

$$\dot{\sigma}(t) = A\omega \cos(\omega t)$$  \hspace{1cm} (4.14)

For small values of $\omega t$, along a fraction of this wave function, small-angle theory
applies, so

$$\cos(\omega t) \approx 1 \quad (4.15)$$

$$\dot{\sigma}(t) \approx A\omega \quad (4.16)$$

$$\sigma(t) \approx A\omega t \quad (4.17)$$

Figure 4.4: Slowly-increasing stress as a segment of a sine-wave: A ramped stress can be modelled as the start of a sinusoidal input profile, assuming the frequency of the sine wave is suitably low, or that the sampling time is suitable short.

This gives a constant gradient of stress. This represents a slowly increasing stress. Calculation of the input stress profile in this manner allows any higher-frequency input data to be ignored, and the stress-strain relationship can be reconstructed from points along the shear stress curve which will correspond to the shear stress at that time.
Advantages of the Method

This method has two advantages over the traditional method of building a stress-strain relationship as given in section 4.3.7. Firstly, if an experiment is being filmed at a high enough frame rate, a great many data readings can be taken in much less time. Secondly, using the signal generator attached to the apparatus to produce a linear output stress greatly simplifies the experimental process and reduces the chance for human error to enter into the results.

Creep

While section 4.3.7’s method does not have the same fidelity as this technique, it is important to understand the creep function for a sample. In this regime, where the applied shear stress is sufficiently high, the low-frequency creep characteristic dominates the behaviour of the sample. To understand this phenomenon, consider a slowly increasing force that is held at a constant force some way into the experiment. If the constant force is low enough or the rate of force increase before this point has been low enough, the bead position should match only be changing while the force is changing.

If, however the rate of force increase has been too high, then the time constant of the input stress function has been of a similar order of magnitude to that of the sample. In either of these two cases some level of creep would be seen in the bead position after the point where the input stress had become level, and the assumptions of the method would no longer be valid.
Figure 4.5: Response to a sudden flattening of the input stress profile: It is expected that the response of the sample medium will be found between Outputs 1 and 2. Output 1 represents a material with a very low phase angle that will not be subject to any inertial effects or time lags between the flattening of the input profile and the response of the material. Output 2 suggests that a sample may have some complex component to its characteristics, and that this allows for a time delay in the response of the system.

4.4 Periodic Responses

4.4.1 Definitions

Periodic data is information that varies over time and has a distinctive shape that repeats over a given length of time, known as its frequency.

Two properties of sinusoidal periodic data that are important to the experiments described in this chapter are its magnitude and its frequency. A signal $F$ of the form

$$F = A \sin \omega t$$

(4.18)
Where $F$ has a magnitude $A$ and a frequency (in $\text{rads}^{-1}$) of $\omega$.

When comparing two sinusoidal signals where one is the response to the other, the ratio of the magnitudes is critical, but the frequencies should be the same. There is a third property that becomes useful, and this is the phase lag or the time lag. For an input signal $F_1$ with a response signal $F_2$, the following equations can be calculated:

$$F_1 = A_1 \sin \omega t$$ \hspace{1cm} (4.19)

$$F_2 = A_2 \sin (\omega t - t_{\text{lag}})$$ \hspace{1cm} (4.20)

From these equations, the ratio of the magnitudes is $A_1/A_2$ and the time lag is $t_{\text{lag}}$. This is equivalent to a phase lag of $2\pi t_{\text{lag}}/\omega$ radians.

When a periodic function is not purely sinusoidal, it can be reconstructed out of a number of constituent sinusoidal functions using the Fourier summation:

$$F(\theta) = \sum_{-\pi}^{\pi} (a_n \cos n\theta + b_n \sin n\theta)$$ \hspace{1cm} (4.21)

Where

$$a_n = \frac{1}{\pi} \int_{-\pi}^{\pi} F(\theta) \cos n\theta d\theta$$ \hspace{1cm} (4.22)

$$b_n = \frac{1}{\pi} \int_{-\pi}^{\pi} F(\theta) \sin n\theta d\theta$$ \hspace{1cm} (4.23)
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This method provides a number of separate sinusoidal functions. Because each has its own frequency, which is always a one of the harmonics of the base frequency, they can be discussed and analysed as a number of independent inputs and responses superimposed on top of each other. These can then be analysed in using the ratios discussed after Equation 4.20.

Dynamic data is time-variant but in the examples discussed in this chapter, it is tackled separately from the more easily defined periodic data of the previous subsection. Where the experimental system has an input force that contains a range of frequency-dependent information, the resultant output (whether displacement, generated field or force) will also contain a range of frequency-dependent information. The chapter on analysis techniques contains a detailed exposition on extracting the correlation function between a given input and a given output, and this can be used to construct graphs of the complex defining functions being investigated. Once the mathematical analysis has been carried out on the experimental data, the functions for magnitude and phase can be plotted (as described above), and the key system properties extracted.

The concept of dynamic data is applied to input and output signals that are not purely sinusoidal and therefore more complicated than those described in the periodic data section. Again, care must be taken to consider the sources of error in these functions: where the component of a signal at a given frequency is particularly low, then the relative error becomes large, and the ratio between two values with large relative errors is resultantly much larger. This is often defined as the signal to noise ratio (SNR).
4.4.2 Introduction

A useful transition from constant force calculations to full dynamic analysis is the use of periodic force inputs.

When a pure sine wave input is applied to a linear system, the natural frequency of that sine wave is the only frequency of input entering the system, and so the response of the system should consist of a pure sine wave output. Although a sample that demonstrates characteristics such as strain hardening or strain softening cannot be strictly defined as a linear system, small perturbations around an equilibrium position will produce approximately linear behaviour.

Slightly more advanced is the consideration of an input that consists of a number of component frequencies, each with its own magnitude. Separation of the input and response into these component frequencies will produce a set of relationships that determine the material properties of the sample at each of the contained frequencies.

Where the relationship between an input signal and an output response can be described in the following way:

\[
\frac{A_{\text{out}}}{A_{\text{in}}} = R = \sin(\omega t - \phi) \tag{4.24}
\]

Where \( A_{\text{in}} \) and \( A_{\text{out}} \) are the magnitudes of the input and output signal whose ratio is \( R \), and the \( \phi \) is the phase lag.
In the two-dimensional complex plane, the phase angle $\phi$ represents a rotation of the sinusoidal function into complex space so that the complex ratio $R^*$ is given by

$$R^* = R(\cos \phi + i \sin \phi) \quad (4.25)$$

In the case of an investigation of the relationship between the applied shear stress and resultant shear strain at a given frequency, this ratio $R^*$ will be proportional to the complex modulus $G^*$. The real and imaginary components of the complex modulus are known as the storage and loss moduli respectively.

This section of the report discusses the considerations that must be taken into account when conducting investigations into the viscoelastic properties of a sample, where a periodic input profile is used and the tweezers have one coil.

### 4.4.3 Half-Sinusoidal Response

Providing a sinusoidal input signal to the amplifier controlling a single magnetic coil on a set of magnetic tweezers does not produce a sinusoidal input stress to the sample. One coil can only produce a positive force on the magnetic colloids and a full sine wave has both positive and negative components. What will in fact be produced is a rectified sine wave input to the experimental system, as shown in Figure 4.6.
Figure 4.6: Rectified Sine Wave: Use of a rectified sine wave allows the mathematically difficult negative forces associated with a full sine wave to be ignored.

This shape matches exactly the shape of a sine wave over the first half-wave, but is then equal to the negated shape of the wave for the other half of the time period. For an experiment to determine the dynamic behaviour of a bead in low Reynolds’ number conditions, this would be an acceptable method.

This is because the low Reynolds’ number means that the drag forces on the bead dominate and the inertial effect of the motion becomes negligible. Under these conditions the speed of motion of the bead is proportional to the drag force in the
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experimental medium, and there is a very low d’Alembert (inertial) reaction to any acceleration of the bead. These two factors combine to mean that the speed of motion of the bead is proportional to the applied force. In short, the bead has no ‘memory’ of its previous motion.

Full Fourier analysis of a rectified cosine wave is given by [9]:

\[ g(t) = \frac{2}{\pi} \sin \frac{\pi}{2} \left[ 1 + 2 \sum_{n=1}^{\infty} \frac{(-1)^{n+1} \cos(2n\omega_0 t)}{4n^2 - 1} \right] \]  \hspace{1cm} (4.26)

This function indicates the presence of an infinite number of harmonics in the input data signal which will all have a corresponding response in the output signal, but the calculation of the individual parts is more complicated than a simple step function.

In addition to this, if the apparatus used to generate the magnetic field is subject to an inductance-led time lag or has its own specific dynamic response, the purity of the signal will further degenerate and cause a further calculation level to measure the exact input signal before the response is analysed.

The concept of introducing a single frequency input signal to the system breaks down with a single coil because of its inability to produce bi-directional force profiles.
4.4.4 Full (Pre-Stressed) Sinusoidal Response

Consider a magnetic tweezers experiment where an offset sinusoidal signal is provided to the amplifier. The input voltage \( V \) will have the following form:

\[
V(t) = V_0 + V_1 \sin(\omega_0 t)
\]  

(4.27)

Where \( V_0 \) and \( V_1 \) are the d.c. component of the signal and the sinusoidal amplitude, and \( \omega_0 \) is the frequency of the input signal. Provided that \( V_1 > V_0 \), this signal will always be positive.

Assuming that the combination of the amplifier, coil and pole piece have a combined frequency dependent transfer function, \( g \), for each specific value of \( \omega \) there will be a given amplitude \( A(\omega) \) of response at which the magnetic tweezers can produce a fully sinusoidal stress function \( \sigma \) to the bead, so that:

\[
\sigma(\omega) = g(\omega)V(\omega)
\]  

(4.28)

\[
|g(\omega_0)| = A(\omega_0)
\]  

(4.29)

\[
|\sigma(\omega_0)| = A(\omega_0) |V(\omega_0)|
\]  

(4.30)

A phase lag will also exist between the stress and voltage functions but once the apparatus has been calibrated, this can be calculated. From the known magnitude
and phase characteristics of the transfer function from the signal to the bead, the exact magnitude and phase of the input signal is known. The measured bead response can be compared to the input signal and the complex modulus at $\omega_0$ can be calculated.

Repetition of the method can yield results in a two ways. Firstly, by repeating the same reading for a number of cycles, the response can be averaged. This will help to eliminate the stochastic thermal vibrations from the measured bead position. Secondly, the experiment can be repeated at a range of different frequencies to help to build up a fuller viscoelastic characteristic of the sample.

This technique does suffer from a number of limitations that dictate how it should be used and what useful information can be found through its use. The most limiting factor is the dynamic response of the field generating apparatus. A coil with a high number of turns has a high inductance. This means that a high voltage is required to speed the switching to higher frequencies. The performance of the amplifier is key in driving a heavy load through quick voltage changes. This is discussed in more detail in the design chapter.

The second practical difficulty is the synchronisation of the input stress and output strain. The geometry of the experimental apparatus is such that either the magnetic field can be measured using a gauss meter only when there is no sample on the apparatus. A relationship between the input and the output from the tweezers can be established but the field cannot be measured at the same time.
as the experiment to assess any changes in the behaviour of the apparatus at this point.

Thirdly, the maximum non dynamic performance of the amplifier limits the magnitude of the signals that can be generated for input into the system. If the input signal from the signal generator is too high, the output signal will be subject to clipping. This affects the purity of the input signal in a manner that is similar to the half-sine wave approach described in Section 4.4.3. Once again, the basis of a single frequency test would break down at this point. Any sinusoidal experiment must have a method for checking the shape of the input stress function.

4.4.5 Full (Arbitrary) Waveform Analysis

The Fourier transformation of a wave shape as laid out in Equations 4.21, 4.22 and 4.23 is useful for producing a more complete dynamic analysis of the sample than a pure sine-wave. In this respect, some distortion of the input signal can be useful in extending the utility of an experiment.

Where the previous methods were reliant on the waveform being standard, breaking the input signal into its component parts is easily calculated, especially with a computer. A periodic input is also good because the time period is constant so it is straightforward to average the bead position and applied stress over a number of cycles without the risk of the synchronisation becoming misaligned.
4.5 High-Frequency Data

High-frequency data is one of the types of data that these investigations aim to produce. Although no fixed frequency has been defined in the report to define the transition from dynamic data to high-frequency data, higher frequencies bring extra considerations for the experimental researcher. In the case of magnetic tweezers, high frequency data can be produced in one of three ways:

- High-speed recording of passive experimental methods
- High-frequency periodic input force signals to the sample
- High-speed switching of the input signal

Each of these brings is associated with different types of experiment but all three are subject to the same considerations. High-speed recording of passive experimental methods is designed to produce mean-squared-displacement plots of the stochastic fluctuations in the position of a bead within a sample, from which various material properties can be gleaned. High-frequency periodic input forces use an input signal at the upper limits of the dynamic performance of the tweezers’s force generating capacity, to investigate the magnitude and phase of the displacement response. High speed switching is a technique designed to apply as many different frequency inputs to the sample as possible at the same time in order to determine the frequency response over the applied spectrum. The factors that must be considered in detail in these types of experiments exist in the lower-frequency experiments as sources of error but tend to dominate when the frequency is increased.

Small deviations in the interpretation of bead position from the recorded video
could be interpreted as a faster motion when the time-step between frames is reduced. This is coupled with lower positional interpretation as the shutter time (and resultant light to the CCD) is reduced in order to record a higher-frequency experiment.

A misalignment or poor synchronisation between the input and output signals greatly affects the interpretation of frequency information for periodic or high-speed switching experiments. In the case of a periodic input, poor synchronisation of just 100 µs can represent a whole input cycle at 10 kHz. By not correctly aligning the input and output signals in a high-speed switching test, a factor of $-i\omega$ is introduced to all data values, becoming increasingly significant at higher frequencies and leading to the characteristic second plateau demonstrated in the validation of techniques chapter.

High frequency data extends the range of useful information, with the caveat that it brings with it more difficulties in analysis of data that must be properly understood, accounted for and assessed.

### 4.5.1 Introduction

Division of the experimental techniques into frequency-independent and frequency-dependent methods helps to differentiate between the different material properties that are being investigated. Further subdivision of the frequency-dependent data into lower and higher frequencies demonstrates the novel types of data that are being produced by using a camera with a very high frame rate setting. Higher frame rates allow for higher frequencies of data recording. This in turn can lead to results at very high frequencies but only if the implications of this increase in speed are fully understood.
A number of dynamic experiments such as those investigating the linear creep of a sample make certain assumptions about the profile of the applied force. While these do not always stand up to rigorous mathematical investigation, the effects of phenomena such as non-square step inputs do not dominate the response of the sample. When the short time-scale data in a fast-switching becomes important.

This section starts by examining existing traditional and more recent techniques in obtaining high-frequency data, and then suggests modifications to existing analyses that take account of short timescale discrepancies in earlier assumptions.

### 4.5.2 Step Response and Shear Creep

The step response of a linear system is important because it can be used to determine the dynamic response to any given input.

While the stress-strain relationship can be used to calculate the modulus of a sample material, if specifically concerned with the final value of strain to an applied stress. Once the system has reached this strain, it is in equilibrium and will not change any further and this ratio is the dynamic response at a frequency of 0Hz. The position of the beads does not always reach the equilibrium position immediately but often take some time to approach this position, known as the relaxation time.
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A traditional shear creep experiment determines the creep compliance \( J(t) \) of the sample when it is subjected to a step increase in the shear stress \( \sigma_0 \) exerted on it by measuring the subsequent shear strain experienced by the sample. These three properties are linked by the relationship:

\[
\gamma(t) = J(t)\sigma_0
\]  

(4.31)

From this, it is possible to use the following relationship between the creep compliance and the complex shear modulus \( G^*(\omega) \) to evaluate the frequency-dependent complex shear modulus, and separate it into its component storage and loss moduli, \( G'(\omega) \) and \( G''(\omega) \):

\[
G^*(\omega) = \frac{1}{i\omega J(\omega)} = G'(\omega) + iG''(\omega)
\]  

(4.32)

This is useful since it separates the liquid-like component from the elastic component. The method is reliant on the assumption that the shear stress generated by the apparatus follows a square step, switching instantaneously from 0 to \( \sigma_0 \) at the start of the experiment. In reality, however, the dynamics of the magnetic tweezers mean that the generated force takes some time to increase from 0 to the peak force. While this delay may only be a few hundredths of a second, it becomes of vital importance as the frequency at which the experiments are recorded is increased to hundreds or thousands of Hertz.

By applying superposition theory to the relationship between input and response of the system, it is possible to derive a more accurate value for the complex
modulus that takes the delays into account. Consider a sample with a given creep compliance function $J(t)$. If the sample is subjected to a step increase $\sigma_s$ in the shear stress applied to it at a time $t_s$, the resultant strain $\gamma_t$ caused by that particular stress will be:

$$\gamma(t) = J(t - t_s)\sigma_s$$  \hspace{1cm} (4.33)

Superposition of a series of step stress increases $\sigma_1, \sigma_2, ..., \sigma_N$ at times $t_1, t_2, ..., t_N$ gives the following relationship for the resultant strain:

$$\gamma(t) = \sum_{n=1}^{N} J(t - t_n)\sigma_n$$  \hspace{1cm} (4.34)

Consider a continuous stress function that varies with time. If the stress and its derivative at a given time $t$ are $\sigma$ and $\dot{\sigma}$, and the stress at a time $(t + \delta t)$ is $(\sigma + \dot{\sigma}\delta t)$, then there has effectively been a step increase in the stress in that time-step of $\dot{\sigma}\delta t$.

Combining this fact with the expression for $\gamma_t$, we get:

$$\gamma(t) = \sum_{n=1}^{N} J(t - t_n)\dot{\sigma}(t_n)\delta t$$  \hspace{1cm} (4.35)

As $\delta t \to 0$, this becomes the convolution integral:

$$\gamma(t) = \int_{0}^{t} J(t - t_n)\dot{\sigma}(t_n) dt = J(t) * \dot{\sigma}(t)$$  \hspace{1cm} (4.36)
In the frequency domain, this convolution becomes the simple multiplication of two frequency dependent functions, and can be related back to the complex shear modulus using the relationship between $G^*(\omega)$ and $J(\omega)$.

\[
\gamma(\omega) = J(\omega)\dot{\sigma}(\omega) = i\omega J(\omega)\sigma(\omega)
\]  
(4.37)

\[
G^*(\omega) = \frac{1}{i\omega J(\omega)} = \frac{\sigma(\omega)}{\gamma(\omega)}
\]  
(4.38)

This relationship is consistent with Equation 4.31 in which the input stress is assumed to be a step input:

\[
\sigma = \sigma_0 u(t)
\]  
(4.39)

Which is equal to $\sigma_0$ for all $t > 0$, but 0 otherwise. The Laplace transformation of this value is calculated to be:

\[
\int_{0}^{\infty} u(t)e^{-st}dt = \frac{1}{s}
\]  
(4.40)

Differentiation with respect to time can be represented in the Laplace domain:

\[
\int_{0}^{\infty} \frac{dg}{dt} = sG(s) - g(0)
\]  
(4.41)

and $g(0)$ in this case is equal to zero. This gives Equation 4.37 as:
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\[ \gamma(\omega) = i\omega \int (\omega) \frac{s}{s} \sigma(\omega) \]  

(4.42)

Now that this relationship has been established, the remaining challenge is to convert the time-dependent shear stress and strain functions into the frequency domain. Two main approaches are available for this process: the values for \( \sigma(\omega) \) and \( \gamma(\omega) \) can be directly calculated using the method described by Tassieri et al.[25], or analytical expressions can be deduced to describe the functions \( \sigma(t) \) and \( \gamma(t) \), which can then be modified using the Laplace transform.

It is important to note that causality dictates that the strain due to a given stress is only apparent after the strain has been applied, and that the stress before the creep test is started is taken to be zero. No information therefore exists about the system for \( t < 0 \), and so the Laplace and Fourier integrals will both have limits from 0 to \( \infty \). This allows for the two integrals to be interchanged within the two methods, with the substitution \( s = i\omega \).

The summation suggested [25] for a stress-step shear creep test is the following:

\[ G^*(\omega) = i\omega [A + B + C]^{-1} \]  

(4.43)

Where the initial compliance \( A \) at time \( t = 0 \) is given by:

\[ A = J_0 \]  

(4.44)
B refers to the final viscosity, the gradient of the curve at time $t_N$ and is given by:

$$B = \frac{e^{-i\omega t_N}}{\eta_0}$$  \hspace{1cm} (4.45)

Finally $C$ is the summation of all the stepwise gradient changes in the continuous function $J(t)$, calculated with the sum:

$$C = \sum_{k=1}^{N} \left( \frac{J_k - J_{k-1}}{t_k - t_{k-1}} \right) \left( e^{-i\omega t_{k-1}} - e^{-i\omega t_k} \right)$$  \hspace{1cm} (4.46)

### 4.5.3 Calculation of the Complex Shear Modulus with a Varying Applied Shear Stress

Two methods are proposed to calculate the complex shear stress. The first uses an adaptation of the previous theory while the second uses curve fitting and optimisation to provide an analytical data fit.

**Piecewise Calculation**

An adaptation of this technique can be used to deal with the actual shear stress from the experiment by summing the step changes in gradient at each time interval. At every time interval, the change in gradient of both the input and outputs to the system can be treated as a step at that point in time, with its own step response at all times after that point. This, combined with the principles of linearity, causality and superposition, means that it is possible to build up the response of the experimental sample over a long time period to an arbitrary input by treating
that input as a series of discrete step inputs, each with its own delay.

\[
\frac{\sigma(\omega)}{\gamma(\omega)} = G^*(\omega) = \frac{i\omega\sigma(\omega)}{i\omega\gamma(\omega)} \tag{4.47}
\]

Because each individual step input only causes an effect in the times after it acts, it can be isolated to have no resultant response at time \( t = 0 \), the conversion from s-domain back to the frequency domain can be reversed, setting \( s \) to \( i\omega \):

\[
G^*(s) = \frac{s\sigma(s)}{s\gamma(s)} = \frac{L\dot{\sigma}(t)}{L\dot{\gamma}(t)} \tag{4.48}
\]

This means that performing the direct transformation on the gradients of the stress and strain will produce a value for \( G^*(\omega) \). This leaves the following expression:

\[
G^*(\omega) = \frac{D}{E + F} \tag{4.49}
\]

With the values \( D, E \) and \( F \) being:

\[
D = \sum_{k=1}^{N} \left( \frac{\sigma_n - \sigma_{n-1}}{t_n - t_{n-1}} \right) (e^{-i\omega t_{n-1}} - e^{-i\omega t_n}) \tag{4.50}
\]

\[
E = \dot{\gamma}_M e^{-i\omega t_M} \tag{4.51}
\]

\[
F = \sum_{k=1}^{N} \left( \frac{\gamma_m - \gamma_{m-1}}{t_m - t_{m-1}} \right) (e^{-i\omega t_{m-1}} - e^{-i\omega t_m}) \tag{4.52}
\]
Where $\sigma_n$ is the shear stress at time $t_n$, $\gamma_m$ is the shear strain at time $t_m$ and $\dot{\gamma}_M$ is the linear gradient of the shear strain at the final data point (time $t_M$). There is no term as the shear stress reaches its limit and is therefore constant at the end of the test, leaving a zero gradient.

**Curve Fitting**

The second technique for determining the complex shear modulus of the sample is to fit functions to the time-dependent stress and strain. This is advantageous as it provides a continuous function across which to perform further analyses, rather than having to interpolate between discrete data points. Two features are expected in the experimental curves: an initial value of 0 and a long-time linear element that dominates once any initial transient effects have decayed. A suitable prototype function would look like this:

$$y = At + B(f(t))$$

Where the constant $A$ provides the linear factor. It is necessary that $f(t)$ is 0 at short times and tends towards 1 at long times. This behaviour can be modelled using this relationship:

$$f(t) = 1 - e^{-at}$$

So that:
\[ y = At + B(1 - e^{-at}) \] (4.55)

While this would allow for a simple curve fitting, the initial gradient of the curve would be constrained to \( A + \alpha \) at the same time as having a decaying element with a time coefficient of \( \alpha \). To increase the accuracy of the data fitting, it is desirable to be able to include more than one transitory function:

\[ y = at + B \left( C_1(1 - e^{\alpha_1 t}) + C_2(1 - e^{\alpha_2 t}) \right) \] (4.56)

Where the coefficients \( C_1 + C_2 = 1 \). In practice, the accuracy of this model can be improved even further with more transient elements. The following expressions define the curves that can be fitted to the shear stress and strain in the experiments:

\[ \sigma = At + B \left( \sum_{p=1}^{P} C_p \left( 1 - e^{-D_p t} \right) \right) \] (4.57)

\[ \gamma = Et + F \left( \sum_{q=1}^{Q} G_q \left( 1 - e^{-H_q t} \right) \right) \] (4.58)

Where integers \( P \) and \( Q \) are increased until an accurate fit of the data is achieved, and \( \sum C_p = \sum G_q = 1 \). Again, the linear gradient of the shear stress will be zero at \( t \) approaches \( \infty \), so the term \( At \) can be neglected. The same is not necessarily true for the shear strain. Once appropriate values are found for the variables, a Laplace transformation of the function gives the following expressions:
\[ \sigma(s) = \sum_{p=1}^{P} \left( C_p \left( \frac{B}{s} - \frac{B}{D_p + s} \right) \right) \]  

(4.59)

\[ \gamma(s) = \frac{E}{s^2} + \sum_{q=1}^{Q} \left( G_q \left( \frac{F}{s} - \frac{F}{H_p + s} \right) \right) \]  

(4.60)

Substitution of \( s = i\omega \) gives frequency-dependent expressions for the shear stress and shear strain.

A computer program has been written to fit values of \( A, B, C..H \) to the experimental data by generating valid sets of parameters that minimises the error functions \( e_\sigma \) and \( e_\gamma \):

\[ e_\sigma = \sum_{n=1}^{N} (\Sigma_n - \sigma_n)^2 \]  

(4.61)

\[ e_\gamma = \sum_{n=1}^{N} (\Gamma_n - \gamma_n)^2 \]  

(4.62)

Where \( \Sigma(t) \) and \( \Gamma(t) \) are the fitted values of the shear stress and shear strain.

For these high frequency properties to be presented accurately, the frequency range of analysis should be maximised. To do this, it is preferable to increase both the frame rate and the length of the experiment. The highest valid frequency for this test is derived from sampling theory, where the upper bound on the experimental data is \( f_{max} = \frac{f}{2} \) where \( f \) is the frame frequency, or \( 2\pi \) multi-
plied by the frame rate. The lower bound on the measured frequency will become 

\[ f_{\text{min}} = \frac{1}{f} \]

Synchronisation Errors

The bulk of the usable data in a step response test appears in the first fractions of a second after the apparatus is triggered. This is the region of the experiment where the gradient of the shear stress curve is closest to the 'perfect discontinuity' of a sharp step. Because of this, collecting high-speed data relies on an accurately prepared triggering mechanism.

Consider a step function where the measurements of the input stress and the output strain are misaligned be a value \( \tau \). The Laplace transform of a time delay is given below:

\[
\int_{0^-}^{\infty} x(t - \tau)H(t - \tau)e^{-st}dt = e^{-s\tau} \bar{x}(s) \quad (4.63)
\]

This pre-factor \( e^{-s\tau} \) can conceivably affect either the stress measurement or the strain measurement depending on which is being measured first. The relative error in a test with a frame frequency of \( \omega_0 \) Hz where the synchronisation error is \( N \) frames is given by:

\[
\text{error} = \exp^{-\omega N/\omega_0} \quad (4.64)
\]
For this to represent a 5% error, the upper bound on the usable frequencies becomes:

\[
\log 1.05 = 0.488 = \frac{\omega N}{\omega_0} \quad (4.65)
\]

Giving a value for \( \frac{\omega}{\omega_0} \) of 0.0488/\( N \), losing over a decade of usable information for a synchronisation error of just one frame in the data.
EXPERIMENTAL TECHNIQUES

5.1 Introduction

5.2 Apparatus and Environment

The experimental equipment used is the magnetic tweezers apparatus developed in the design chapter of this report and shown in Figure 5.1. The shear stress applied to the beads was changed using either controlled current supply to the apparatus or by moving the pole piece towards the sample with the travelling stage attached to the coil.
5.3 Sample Preparation

5.3.1 Technique

Fibrin clots were prepared using materials from Calbiochem. Lyophilised human thrombin was reconstituted in pure Tris-EDTA buffer, and lyophilised, plasminogen depleted human fibrinogen was dissolved at 37°C, again in pure Tris-EDTA buffer. The fibrinogen was then mixed with carboxylic acid coated superparamagnetic beads of 1 and 2.7 μm radius before the diluted thrombin was added. The final concentrations of fibrinogen, thrombin and beads were 3 mg/ml, 1.5 U/ml and 4.2% by mass respectively.

Once the ingredients had been quickly mixed, they were immediately transferred into the 0.5 mm square glass capillaries. This was achieved by inserting a capillary into the end of a plastic pipette tip, and using the pipette to draw the mixture into the capillary. The samples were then sealed in place with the glue gun, and left for around twenty minutes to allow the clot to form and stabilise.

5.3.2 Repeatability and Reliability

The sample preparation process is difficult and great care must be taken at all stages to make sure no difficulties occur. During the preparation of the samples, around 30% of the sample material was damaged and not suitable for continued experimentation. Because of the delays in clot stabilisation and analysing the
CHAPTER 5. EXPERIMENTAL TECHNIQUES

experimental data, it was often not possible to identify spoilt samples until the end of an experiment. Some of the causes of damage are detailed below.

**Reconstitution and Over-Vigorous Mixing**  When mixing the lyophilised human thrombin or the plasminogen depleted human fibrinogen in the Tris-EDTA buffer, it was necessary to gently stir the mixtures with a stainless steel spatula or magnetic spinner. If this is done without proper care, or the undissolved thrombin or fibrinogen clumped together, the mixing process began to introduce air into the mixture. This led to frothing in the glass beaker. In some cases, the mixture could still be dissolved successfully but where that was not the case, it was not possible to quantify the amount of wasted raw material, and therefore the final concentrations in the experimental sample.

**Fibrin Network Rupture**  When transferring the sample to the glass capillary, the pipette was placed in the end of the capillary and the sample sucked into the makeshift syringe. If this process was carried out too quickly, it led to high concentrations of shear stress within the capillary, and if the fibrin clot had already begun to form, this often caused it to begin to break up. Any broken sections of the clot in the sample would then move freely within the capillary and not provide any resistance to the applied stresses through the network.
5.4 Methods

5.4.1 Introduction

Figure 5.1 shows the arrangement of the apparatus, as described in the Apparatus Design chapter. This arrangement remained unchanged for the initial set of experiments.

Figure 5.1: Experimental Apparatus Arrangement: Note the gradually increasing complexity of the apparatus throughout this report. In addition to previous instances of this diagram, more measuring apparatus has been added in the form of the digital storage oscilloscope, and the computer is now being used to control all the functionality of the apparatus.

For all of these experiments, the amplifier was set to be controlled by the current the coil, rather than voltage across it. Precise adjustment of the applied shear stress was controlled by a combination of coil current and pole piece displacement.

Unless otherwise stated, the fully calibrated 1µm beads were used in the follow-
5.4.2 Constant Data

The two test cases for the constant data were an unstressed MSD plot and the establishment of a stress-strain relationship. The unstressed MSD plot required no input to be applied to the apparatus, and data was obtained by filming the positions of the beads at a range of frequencies. The input profile for the stress-strain relationship is shown in Figure 5.2, and consisted of the application of a constant current through the coil in 0.2\(A\) intervals from 0\(A\) to 2\(A\), and held at each setting for 10s. As stated in earlier chapters, the accuracy of the data would increase if the steps in current were held for longer, but this would have introduced heat dissipation problems. In order to make sure that no plastic deformation had taken place, the applied current was returned to 0\(A\) at the end of the test and the original bead position was re-measured.

5.4.3 Quasi-Static Methods

The slowly-changing applied shear stress required for this set of experiments was a triangular ramped input function as shown in Figure 5.3.

5.4.4 Periodic Responses

A number of different waveforms were used to excite fibrin samples at various frequencies to develop a frequency-based characterisation of the behaviour of the
5.4.5 High-Frequency Data

High-Speed Periodic Responses Each of the waveforms used in the periodic responses experiments were re-applied at a range of frequencies and with the CCD frame rate set at 10,000 fps.

Step Response A periodic square wave force was applied to the fibrin/magnetic bead network using the electromagnet. This was achieved by programming the signal generator to provide a square wave of frequency 1Hz and magnitude 10V. This signal was used as the current-controlling input to the amplifier, which generated a peak current of 2A. A resultant periodic force was exerted on the magnetic beads attached to the fibrin clot, with a peak to peak range of 25pN. The periodic movement of the beads and fibrin was then filmed at 10,000 fps using the high-speed camera. The particle tracking program was then used to calculate the displacement of the fibrin under shear force over several cycles. The force and position information was averaged over a number of cycles and then analysed.
5.5 Linearity and Experimental Efficiency

5.5.1 Introduction

There are some common elements to the methods described in this chapter, especially for the frequency-based data investigations. The main three are linearity, frequency and time ranges for valid data and concurrent analyses that can speed up the experimental process. Linearity is the idea that some systems have constant properties, such as the step response, that are not dependent on the size of the input. The frequency limits and time ranges for valid data discussion is about the methods that are employed to make sure unwanted artefacts do not enter into the experimental results. The idea of concurrent analysis is one of increasing the experimental efficiency by carrying out a number of different tests at the same time. This section tries to formalise some of these concepts to maintain a sense of coherence across the various experimental techniques. Its subsections are divided into the three groups introduced above.

5.5.2 Linearity and Partial Characterisation

There are two criteria that should be fulfilled in order to describe a system as being linear.

- Superposition (Homogeneity and Additivity)
- Shift Invariance and Causality
Consider a system that has a transfer function $G(s)$. Homogeneity is the concept that a system will behave in a similar manner regardless of the size of its input. So for a system with a transfer function $G$ subject to an input $x$, the output $H(s)$ is defined by:

$$H(x) = G(x)$$  \hspace{1cm} (5.1)

Homogeneity dictates that scaling the input function should scale the output function:

$$G(\alpha x) = \alpha H(x)$$  \hspace{1cm} (5.2)

Additivity takes this idea further and removes the limitation of the input being a scaled version of one function, but it could be two separate functions. Let the second input be $y$:

$$G(x + y) = H(x) + H(y)$$  \hspace{1cm} (5.3)

By combining these two rules, superposition is developed, which gives:

$$G(\alpha_1 x + \alpha_2 y) = \alpha_1 H(x) + \alpha_2 H(y)$$  \hspace{1cm} (5.4)

Obviously when a sample demonstrates properties such as strain hardening, it becomes more difficult to determine the linearity of the system. In this report, the consistency of the data provides the justification for applying methods from control system engineering to a novel subject.
5.5.3 Frequency and Time Limits

When the experiments described in this chapter are used practically, it is important to remember the concept of frequency and time windows. Sampling theory defines the maximum frequency where data is valid as being one-half of the sampling frequency. Above this value, it is impossibly to reconstruct the waveforms that are being measured.

Conversely, in a mean-squared displacement measurement, the maximum time measurement available is the maximum length of the experiment and the minimum is the inverse of the sampling rate. However, this fact must be taken with the caveat that some of these measurements will not have sufficient repetitions in a dataset to be accurate.

While it is disappointing to be bound by the upper frequency limit of one-half of the sampling frequency, the energy being dissipated at these higher frequencies is not lost from the experimental method. An input to a system at a given frequency can only cause a measurable output at the same frequency in the analyses discussed here. Therefore, just because some frequency information is lost in the output, the fact that it is not measured in the input means that all the inputs and outputs are accounted for.

When a frequency analysis is taking place over a range of frequencies, there will be some frequencies that do not have a large magnitude in either the input or the output signals. When these are encountered it is important to remember that
$A/B$ becomes indeterminate as $A, B \to 0$, and this problem is compounded when
the size of the error is on a similar order of magnitude to either $A$ or $B$

### 5.5.4 Multiple Concurrent Analyses

Some of the experiments in this chapter must be carried out singly, whereas
others can be followed at the same time. The key recordable data in these experi-
ments is the position of the bead that is attached to the sample, and every single
experiment described is a variation on this measurement. It therefore makes sense
to collect data for as many tests as possible at the same time. Some examples of
experiments that can be carried out simultaneously are given below.

Either of the two methods for calculating the stress-strain relationships (simple
stress-strain or slowly-ramped input) can be carried out at the same time as cal-
culations of the mean-squared displacement. The more straightforward method
lends itself more readily to this application as the bead stress is held constant
for the duration of a reading, which can be up to several seconds. At a high
enough frame rate, this can produce a range of frequency information for the
mean-squared displacement plot.

Calculation of the mean-squared displacement from a slowly ramped stress is
more difficult, especially as the measurements can take place across the transi-
tion in the power law relationship in the sample. However, judicious separation
of the slowly changing equilibrium position from the bead’s thermal fluctuations
can yield high-frequency results. The lower-frequency data may be more affected
by the slow position change, and these readings should be treated with caution. Where the ramped input is used for mean-squared displacement plots, it is perfectly possible to use a moving average in the calculations to show the gradual transition of the curve shape.

5.6 Discussion

In this chapter, a number of experimental techniques have been discussed and developed. The key result is the derivation of the formula for the analysis of non-square step response experiments, which effectively sidesteps the inaccuracies in the data that would be caused by inductive (and other) lags in the magnetic field generating apparatus. This means the user can fully calibrate the apparatus, and discover its dynamic response and then use only the output from the signal generator to know with certainty what the force on, and subsequent shear stress generated by, the bead will be.
Figure 5.2: Constant Data Input Cases: The unstressed MSD plot required a coil input current of 0 A for 30 s, and the stress-strain relationship was determined using the steps shown above. Note that the step size could be increased to allow multiple MSD plots to be calculated.
Figure 5.3: Slow-Ramped Input Function
6.1 Introduction and Scope

This chapter contains the details of the initial experimentation that proves the principle of operation of the tweezers and allows the analysis techniques derived earlier. The breakdown of experimental types is roughly similar to those described in the previous chapter.
CHAPTER 6. INITIAL EXPERIMENTATION

6.2 Constant Input Responses

6.2.1 Force and Displacement

Figure 6.1 shows the displacement of the bead attached to the fibrin network described in the previous chapter as the applied force is applied. Note that as the force increases, the gradient of the curve drops as the nematic behaviour of the fibres becomes significant when they align themselves with the applied force field. Over the range of forces applied, it was not possible to discern any strain-hardening behaviour.

Figure 6.1: Force-Displacement: Note the jump in the curve as the force increases, indicating the sudden onset of softening behavior. This is discussed above.
6.2.2 MSD

Figure 6.2 shows the mean-squared displacement of the fibrin network observed over 200 seconds. The low-stress curve shows the expected power law of $t^{0.75}$. The second curve is discussed in the next chapter on further investigations.

![Figure 6.2: MSD of the Fibrin Network](image)

Figure 6.2: MSD of the Fibrin Network: Under differing stresses, the gradient of the plot is shown to be different. As the gradient decreases under increases stress, it is apparent that the modulus increases here, across a range of frequencies.

6.3 High-Frequency Response

The high-frequency response characteristics of the fibrin network are discussed in this section

6.3.1 Inputs

Figure 6.3 shows the input signal voltage, to the experimental system. This gave an input force to the system, as derived from the calibration measurements and a gaussmeter held at the experimental distance as shown in Figure 6.4. By running
multiple repeats of the input signal through the apparatus, averaging the data and curve fitting, the fitted force function in Figure 6.5 was generated. This was the data that was used in the later analysis of the frequency response of the system.

Figure 6.3: Input Voltage:
Figure 6.4: Step Force Input: Raw Data. Note the limited resolution afforded by the storage oscilloscope.
Figure 6.5: Step Force Input: Averaged force against time, taken from 7 readings and then fitted to an exponential function.
6.3.2 Responses

Figure 6.6 shows the initial measurements of the bead position over time from the input described above. This was averaged and fitted in a similar manner to the input signals to produce the data shown in Figure 6.7, and the overall strain relationship of Figure 6.8.

Figure 6.6: Bead Position With Time for a Step Input: Note that the position profile varies considerably between runs. This is due to larger, slower thermal fluctuations.
Figure 6.7: Bead Position With Time for a Step Input: By taking averages of all the readings and fitting a linear + exponential function to the data, an easily manipulated function if generated.
Figure 6.8: Fibrin Strain with Time: An alternative view of the displacement of the bead, taking into account the shear strain that the movement indicates in the sample.
6.3.3 Frequency Analysis

The data for each of the input and output curves was then analysed using the piecewise method described in the Analysis Techniques chapter, giving the input Bode plot shown in Figure 6.9, and the output Bode plot shown in Figure 6.10. If the input force profile was assumed to be square, then the complex shear modulus of the Fibrin network could be taken from Figure 6.10, but as shown previously, a full frequency analysis of the Fibrin is required. By taking the ratio of the shear stress and shear strain across the whole curve, the resolved complex shear moduli have been calculated, and are shown in the Bode plot in Figure 6.11.
Traditionally, the complex shear moduli of biological samples are shown in terms of the complex and imaginary parts of the $G^*$, rather than as the magnitude and phase angles of Figure 6.11. This representation of the same data is given in Figure 6.12, with the range of acceptable data windowed in accordance with the previous analyses.

### 6.4 Discussion and Conclusions

Note that in the complex shear modulus, the phase angle makes a transition from 90 degrees at very low frequencies to 0 degrees at high frequencies. This is also demonstrated by the two lines on the loss and storage moduli plot, as $G'$ dominates at low frequencies (indicating a liquid-like behaviour), before the storage modulus $G''$ becomes larger than the imaginary component at higher frequencies. This indicates that the fibrin network becomes ‘more solid’ at higher frequencies, and more able to absorb shocks. This is behaviour that is ideal for a blood clot making up part of a scab.

The complex moduli plot also shows a ‘double-plateau’, where the storage modulus levels off at around 10 Hz and then again at 1 kHz. The reason for this is unclear, but further examination of the Bode plot of the same data shows that the phase angle reaches a localised peak at the inter-plateau region of increased gradient. This shows a deviation away from the solid-like behaviour of the fibrin, as a phase difference between the input and output functions develops. This indicates there may be some damping effects at around 300 Hz in the fibrin, much like mechanical dampers in industrial machines.
Figure 6.9: Step Stress Input: The stress power-law gradient and phase angle hold steady up to around 50Hz. Above that frequency, they are less applicable, but all recorded data is used to generate the final curves in the next section.
Figure 6.10: Step Strain Output: Bode plot. There is a definite shift in the phase angle at around 7Hz, where the liquid behaviour of the sample dominates.
Figure 6.11: Frequency Response of Fibrin Network: Bode Plot. After unifying the input and output values, the effect of the phase shift is mitigated. As the phase angle drops from 90° to 0°, the fibrin moves from liquid-like behaviour at lower frequencies and to solid behaviour at higher frequencies.
Figure 6.12: Resolved Complex Shear Moduli: Shear Moduli. Comparing these curves to Figure 6.11, it is apparent that the crossover point is where the two moduli are equal, and the phase angle is $45^\circ$. As the storage modulus begins to dominate at higher frequencies, the phase angle settled to zero.
7.1 Introduction

Following on from the previous section, further work was carried out on examining how the behaviour of the fibrin network would change under different input conditions. These are described in this chapter.

7.2 MSD

In the previous chapter Figure 6.2 presented the MSD behaviour of the Fibrin Network. If is reprinted as Figure 7.1 below in order to discuss the second curve on the chart.
CHAPTER 7. FURTHER INVESTIGATIONS

Figure 7.1: MSD of the Fibrin Network: The reduction in gradient as the stress increases indicates strain hardening as well as a question about the linearity of the fibrin.

During the initial experimentation, only the lightly-stressed experiment was carried out. Initially, this was intended to be an unstressed MSD plot, but as the experiment was started, it became apparent that the high shear stresses of loading the samples into the capillary had damaged them, and parts of the fibrin networks were able to move around unconstrained. As the investigations in this thesis are about the behaviour of individual fibres within networks, it was decided that it was possible to ‘pin’ the fibrin network with a light strain, and that this would prevent the larger-scale movement of the clot, but not present too much inhibition to the thermal fluctuations of the bead in the localised area of the sample as it was being investigated. This theory was confirmed with the use of polystyrene spheres as well as the magnetic ones, and the MSD relationships proved identical.

As part of the additional experimentation, the pinning stress was increased and the behaviour of the beads was examined. Note in Figure 7.1 that the power-law ratio reduces in line with that expected in the Literature Review.
7.3 Modulus

Figure 7.2 shows the storage moduli of a fibrin network as the pinning force was increased. This was calculated by direct modulus derivation from MSD plots, and indicates the reduction on the modulus as the pinning force is increased from 10 pN to 20 pN. This indicates a softening of the clot as the strain is increased. This is again in line with the predictions of strain softening. At the higher pinning force of 30 pN, the modulus again begins to increase, indicating the onset of strain hardening, but it does not rise above the initial 10 pN result.

Figure 7.2: The Qualitative effect of Increasing Pinning Force: Logarithmic vertical axes. Again, the variation in not only magnitude, but also shape of these plots indicates a loss of linearity.
7.4 Discussion

Both of the additional experiments carried out in this section back up the results of the first data section, showing the evidence of strain softening at mid-range pinning forces and then strain hardening as the fibrin becomes more strained.
A.1 Magnetic Field Analysis

Section 3.6 features one of the analysis sets for the calibration of the magnetic field strength. For completeness, the full set are included here. Each curve is defined by the displacement in the $y$ and $z$ direction from the central axis of the pole piece.
APPENDIX A. ADDITIONAL DATA
APPENDIX A. ADDITIONAL DATA
APPENDIX A. ADDITIONAL DATA
B.1 Introduction

This chapter contains some of the functions written to analyse the data from magnetic tweezer experiments.

B.2 Mean-Squared Displacement

This function is designed to return the MSD data from a given particletracker dataset.

function msdout = msd(datain)
% Convert pixel position to actual position
sf = 1/375;
l1 = size(datain,1);
dt = diff(datain(1:2,3));
msdout = zeros(l1-1,2);
datain = datain(:,1:2)*sf;

% For each time interval
for n = 1:(l1-1)
    msdout(n,1) = dt*n;
    msdout(n,2) = mean( (diff(datain(1:n:end,1))).^2 + ...
                        (diff(datain(1:n:end,2))).^2 );
end

B.3 Magnetic Field Modelling Code

This function is used in section 3.6 to calculate the coefficients required to mathematically describe a magnetic field around the tip of a pole piece.

function [c1 c2 c3 c4] = fieldfunction(B, X, c2range, y, z)

% Assuming the magnetic field is of the form
% c1 + (x+c2)^c3 + c4
% This function will find the best fit for the coefficients
% Inputs should be Bx in Tesla and X in metres
% c2range = [c2min c2max] should be the range of x
% offsets to examine

% Initial parameters
Bx = B(:,y,z);
attempts = 1001;
Bmin = 1.23e-3;
curvecount = 5;

% Take the empirical background field for c4
c4 = Bmin;
myB = Bx - c4;

% c2 and c3 can be found by looking at the linear gradient
% and straightness of loglog(B,X)
% Use cvals to note offset, multiplier, gradient and goodness of fit
cvals = [linspace(c2range(1),c2range(2),attempts)
            zeros(2,attempts)];
goodness = zeros(1,attempts);

% Set up plots and colourmap
mycols = jet(curvecount+1);
figure('units','normalized','outerposition', [0 0 1 1]);
subplot(3,1,1);
ccount = 1;
mylabels = [];

% Calculate coefficients
for i = 1:attempts
    myX = log10(X' + cvals(1,i));
    myY = log10(myB);
    coeffs = polyfit(myX,myY,1);
    if mod(i,fix(attempts/curvecount)) == 0 || i == 1
        loglog(X'+cvals(1,i),myB,'color',mycols(ccount,:));
        ccount = ccount+1;
        hold on;
        mylabels = strvcat(mylabels,_
        sprintf('c2 = %1.1fmm',cvals(1,i)*1000));
    end

% Goodness is a least squared fit
gcorr = corrcoef(myX,myY);
goodness(i) = gcorr(2,1);
% [offset;gradient;multiplier;goodness]
cvals(2:3,i) = coeffs';
end

% Format the top graph
legend(mylables,'location','west');
title(sprintf('B_x against x+c_2 for various _
values of c_2 for y = %1.1fmm and z = %1.1fmm',
(y-10)/2,(z-5)/2));
xlabel('x (mm)');
ylabel('B_x (T)');

% Find minimum value of least squared error
myC = mean(cvals(:,goodness==min(goodness)),2);

% Extract the coeffieients
c1 = 10^myC(3,:);
c2 = myC(1,:);
c3 = myC(2,:);

% Build the fitted curve
Bx2 = c1 * (X + c2).^c3 + c4;

% Plot the optimisation curves to show the coefficient derivation
subplot(3,1,2);
[a h1 h2] = plotyy(cvals(1,:)*1000,cvals(2,:),cvals(1,:)*1000,goodness);
title('Optimisation of values for c_1, c_2 and c_3');
xlabel('c_2 (mm)');
ylabel(a(1),'c_3');
ylabel(a(2),'Correlation between log(B_x) and log(c_2)');
hold on
plot(cvals(1,goodness==min(goodness))*[1 1]*1000,ylim,'r');
text(c2*1000,c3,sprintf('\n c_2 = %1.1f\text{mm} \n c_3 = %1.1f',c2*1000,c3),...
   'HorizontalAlignment','left','VerticalAlignment','top');

% Plot the original and overlayed data to show the quality of fit
h3 = subplot(3,1,3);
plot(X*1000,Bx);
hold on
plot(X*1000,Bx2,'-r');
title('Fitted and Experimental values of B_x');
xlabel('x (mm)');
ylabel('B_x (T)');
legend('Experimental field strength'...
   sprintf('Fitted field strength \nc_1 = %1.1E, c_2 = %1.1E \nc_3_ = %1.1E, c_4 = %1.1E',
   c1, c2, c3, c4));


