Cell Magnetic Tweezers for the study of mechanical interactions in Cells

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1. Abstract

The intricate workings of the cell are dominated by complex chemical and mechanical processes. Therefore, a deep understanding of the mechanical processes, usually governed by proteins and the interactions between them, is crucial to understand the organism.

Cell Magnetic Tweezers have arisen in the last two decades with this very aim. They allow the study of the mechanical properties of molecules, such as proteins, *in vivo*, in other words in the Cell.

Throughout this project, a Cell Magnetic Tweezer (MT) set-up, aimed at the study of mechanical interactions in single cells, was assembled and calibrated. The set-up is able to apply forces ranging from 0.2 to 0.5 nN and was specifically designed to track the mechanics of protein-cell interactions in individual cell entities. Previous to the assembly, a series of simulations were carried out in order to understand the relation between the magnetic field generated by the setup and the force applied to the sample. In order to enhance and optimize this range of forces, a full study was done by comparing the forces obtained when changing the dimensions of the magnetic tip and the number of turns in the coil. A shorter length of the tip end and an increase of turns in the coil appeared to enhance the magnetic field and thus the force applied. In addition, the magnetic field was found to be nearly the same at an angle of $0^\circ$ and $30^\circ$ from the longitudinal axis of the rod, so any bead comprised in this range ($\pm 30^\circ$) was assumed to feel the same force and therefore, considered suitable for calibration and experiments. Once assembled, the MT was custom-programmed and made functional, using LabVIEW as the programming language. Finally, the setup was tested and consequently calibrated, tracking 3 μm paramagnetic beads in a solution of well-known viscosity (Silicone Oil, 10000 cSt). The calibration showed that, as expected, the force decayed exponentially with distance and could be fitted with a double exponential function. Furthermore, the force applied to the beads increased with the intensity of the electric current passing through the coil of the magnetic field, reaching saturation at a current of 1.5 A. The maximum forces applied at this saturation point were found to be 0.6, 0.45 and 0.3 nN at a distance of 10, 20 and 40 μm respectively. For greater distances, the force decreases and stabilizes at around 0.2 nN.

The range of forces accessible through this Cell MT (~nN) allows to apply similar pulling forces as those experienced by cells in their natural environment. Hence, the study of the mechanical interactions happening at the single cell level can be experimentally studied in detail.
2. Introduction

The unit of life as we know it is the cell. In the last centuries, huge advances have been made to comprehend its structure and function. Still, the intricate workings of the cell, which are dominated by complex chemical and mechanical processes, are not well understood. As the understanding of these complex molecular interactions has increased, more prominence has been given to the mechanical aspects of the inner working of the cells. It has been found, that most processes in the cell can be described as mechanically driven processes. These processes are usually governed by proteins and interactions between them. Therefore, understanding how the structure of these entities is modified under force, i.e., stretch, compression or twist, and how it responds to mechanical stimuli is, ultimately, understanding the organism.

Force spectroscopy techniques have arisen in the last three decades to respond to the biological questions involving mechanical processes that remain unanswered. These group of techniques comprehend AFM (Atomic Force Microscopy), OTW (Optical Tweezers) and MTW (Magnetic Tweezers) at the single-molecule and cell levels.

Focusing on Cell force spectroscopy techniques, a table is presented below showing the main differences between them and how do they comply to in vivo conditions (Table 1).

<table>
<thead>
<tr>
<th>In vivo condition or technique</th>
<th>Optical tweezers</th>
<th>Magnetic tweezers</th>
<th>Atomic force microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operate on whole cell or organism</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Physiological conditions: aqueous environment, pH ~7 body</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Temperatures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noninvasive method</td>
<td>Possible photodamage and heating</td>
<td>Yes</td>
<td>Can be used on cell surfaces only; attachment of tip might be invasive</td>
</tr>
<tr>
<td>Method works inside a cell</td>
<td>Yes</td>
<td>Yes</td>
<td>No, only on cellular surfaces</td>
</tr>
<tr>
<td>Handle inside cell</td>
<td>Can use endogenously occurring particles</td>
<td>Must be injected or endocytosed</td>
<td>Not possible without penetrating the membrane</td>
</tr>
<tr>
<td>One-to-one correspondence between molecule and handle</td>
<td>With care</td>
<td>With care</td>
<td>With care</td>
</tr>
<tr>
<td>Elimination of unspecific bindings</td>
<td>With care</td>
<td>With care</td>
<td>With care</td>
</tr>
<tr>
<td>Reliable in vivo calibration</td>
<td>Yes$^{15,25}$</td>
<td>Yes$^2$</td>
<td>Can only be calibrated outside the cell</td>
</tr>
</tbody>
</table>

Table 1. How single-molecule techniques comply to in vivo conditions. Taken from L. B. Oddershede.

The MTW technique is based on the non-invasive manipulation of magnetic particles, and thereby the biological entity they are attached to, via an externally-imposed magnetic field and/or gradient. First Magnetic Tweezers date back late 1990s and nowadays are generally still not found commercially available. For this reason,
researchers typically build their own magnets, which act as the magnetic source, around a particular microscope. Magnetic Tweezers can be distinguished by the scale at which they work, Single-Molecule and Cells Magnetic Tweezers, and by the type of magnet they use, permanent magnets and electromagnets. To date, a lot of articles employing both Single-molecule\textsuperscript{4,5} and Cell MTs\textsuperscript{6,7} can be found in the literature.

Regarding the scale at which Magnetic Tweezers work, both Single-molecule and Cell MTW work with molecules. Single-Molecule MT works with individual molecules, such as proteins, \textit{in vitro} and Cell MT allows the study of more than one molecule at a time \textit{in vivo}, in other words in the Cells.

The basic principle governing this type of setups is the following. The single molecule under study is chemically linked to a magnetic bead (~3 \textmu m). Then, a magnetic field source, constituted by a magnet, is approached to the molecule-bead system. The Magnetic bead is attached to the magnet with a force that is proportional to the distance. Controlling the relative position of the magnet, the force applied to the Magnetic bead, hence to the Single Molecule, can be controlled. By monitoring the position and movement of the Magnetic bead, the response of the Single-Molecule to a particular force and strength can be studied. The force can be either torque or translational force, depending on which type of magnetic particle is used.

Figure 1. Superparamagnetic bead attached to transmembrane proteins. In the presence of an external magnetic field, the bead creates and instant magnetic moment that aligns with the field gradient, generating a pulling force.

As for the source of magnetization, permanent magnets and electromagnets can be used. Permanent magnets are made of rare earth magnets. In contrast, electromagnets generate the field passing electrical current through a coil, which encircles a paramagnetic or ferromagnetic yoke with a sharpened tip to increase the local field gradient. This type of magnets allow to control the instant strength of the force sensed by the sample simply by modifying the current supplied to the coil\textsuperscript{8}.

The type of Cell Magnetic Tweezers implemented in this project uses an electromagnet as the magnetic source. The electromagnet is formed by a mu-metal cylindrical rod sharpened at one end and an enameled copper wire wired around it. When the current
passes through the coil, a Magnetic Field is generated and shielded through the tip towards its sharp end, where the field is tightly focused. This magnetic field decays exponentially with distance, thus, creating a magnetic field gradient in its vicinity. The gradient of magnetic field creates an attaching force that is sensed by the paramagnetic beads contained in the sample, which move according to it. The sample also contains non-magnetic beads that act as a reference. Bright-field images of beads and needle tip are taken by a camera and their relative position recorded with time. From them, the Force generated by the tip, of a few nanoNewtons, can be computed. In addition, this type of setup also allows to control the current and the position of the tip by means of a DAQ and a micromanipulator respectively. A schematic of the setup is represented in Figure 2.

Figure 2. Schematics of a Cell Magnetic Tweezers Setup with microneedle electromagnet.

The physical principles governing Cell Magnetic Tweezers will be explained below.
2.1. Physical principles of Magnetic Tweezers

When the current passes through the coil, it induces a magnetic field $H$ in the cylindrical rod made of high-permeability nickel alloy (Equation 1).

$$\vec{H} = \frac{\vec{B}}{\mu}$$  \hspace{1cm} (1)

where $\vec{B}$ is the total magnetic field and $\mu$ the magnetic permeability of the rod. This magnetic field $\vec{H}$, generated in the tip, induces a magnetic moment in the bead (Equation 2).

$$\vec{m} = \chi V \vec{H}$$  \hspace{1cm} (2)

where $V$ is the volume of the particle and $\chi$ is the magnetic susceptibility. This particle, then, can be subjected to two different forces; Torque and Translational Force (Figure 3). Torque ($\vec{\tau}$) is the force that drives the magnetic bead to align to the external field (Equation 3) and Translational Force ($\vec{F}$) is the force that makes the particle move towards regions with higher field density when the field has a gradient $\nabla \vec{H}$ (Equation 4).

$$\vec{\tau} = \vec{m} \times \vec{B}$$  \hspace{1cm} (3)

$$\vec{F} = (\vec{m} \cdot \nabla)\vec{B}$$  \hspace{1cm} (4)

Therefore, using Equations 1, 2 and 4, the Translational force can be expressed as

$$\vec{F} = V \chi \nabla (\vec{B}^2/2\mu_0)$$  \hspace{1cm} (5)

indicating that the Translational force scales with the volume and the magnetic susceptibility of the particle.

Since in this project particles with no permanent magnetic moment (paramagnetic beads) are used, the induced magnetic moment is already aligned with the external magnetic field when exposed to it and no Torque is applied. Therefore, Translational Force is the only force sensed by the beads and a pulling force is applied to the molecules attached to the beads.

In contrast, when ferromagnetic beads are used, their permanent magnetic moment needs to align with the external magnetic field when exposed to it, promoting a twist in the molecules attached$^{8,9,10}$. 
Throughout this report, the theoretical, chemistry and experimental methods used will be reviewed first. These include the physical principles that regulate the setup, the mathematics behind the calibration, the software used for the simulation, programming and calibration of the setup and the experimental methods employed to prepare the sample. Afterwards, the work done will be presented in the sections Setup Building and Calibration. Finally, the conclusions of the project will be commented.
3. **Methods**

3.1 **Theoretical Methods**

In this section, the mathematical fundamentals of the force calibration will be reviewed. Additionally, the reasons that motivate a simulation of the tip will be explained along with the program used.

3.1.2. Force Calibration

The force has been calibrated experimentally estimating the drag force acting on magnetic particles immersed in a viscous fluid of well-known viscosity\textsuperscript{11}. The force acting on a bead can be related to the velocity of the bead by Stokes equation of viscous drag\textsuperscript{9} (Equation 6).

\[ \vec{F} = 6\pi \eta \vec{r} \cdot \vec{v} \quad (6) \]

where \( F \) is the vector of forces, \( \vec{v} \) is the vector of absolute velocities, \( r \) is the radius of the magnetic bead and \( \eta \) is the dynamic viscosity of the fluid. The dynamic viscosity \( \eta \) can be computed from the kinematic viscosity \( \nu \) and the density of the fluid \( \rho \) (Equation 7).

\[ \eta = \nu \cdot \rho \quad (7) \]

In order to calibrate the setup, current is fixed and video frames are taken for multiple beads. Then, the relative distance between the tip and the bead can be computed for each frame of these videos. Lastly, the positions \( \vec{r} \) can be fitted with a two-term exponential (Equation 8), as the force decays exponentially from the tip end.

\[ \vec{F}(\vec{r}) = a \cdot e^{(b \cdot \vec{r})} + c \cdot e^{(d \cdot \vec{r})} \quad (8) \]

where \( \vec{r} \) is the vector of distances from the bead position to the tip and \( a, b, c, d \) are the coefficients that need to be fitted to the data. In order to calculate the real velocity of the beads, several effects have to be taken into account. Those are, the flow of the fluid and the drift of the tip. The flow of the fluid is monitored tracking the movement of non-magnetic beads and the drift of the tip is computed considering a linear non-accelerated movement (Equation 9).
\[
\vec{d}_{\text{tip}}(i) = \vec{d}_{\text{tip}}(0) + i \cdot \left( \vec{d}_{\text{tip}}(n) - \vec{d}_{\text{tip}}(0) \right)
\]  \hspace{1cm} (9)

where \(i\) is the frame number, which ranges from 0 to the total number of frames \(n\), and \(\vec{d}_{\text{tip}}\) is the position of the tip on screen, which has to components \(\vec{d}_{\text{tip}} = (x_{\text{tip}}, y_{\text{tip}})\). It is plausible to assume that the tip drift has constant velocity since the force applied in a single tracking remains constant as the current applied is constant.

The velocities of the magnetic and non-magnetic beads are deduced from their positions over time (Equation 7) and the absolute velocity is computed subtracting them (Equation 8).

\[
\vec{v}_{\text{bead}}(i) = \frac{\left( \vec{d}_{\text{bead}}(i) - \vec{d}_{\text{bead}}(i-1) \right)}{dt}
\]  \hspace{1cm} (10)

\[
\vec{v}_{\text{abs}}(i) = \vec{v}_{\text{magn bead}}(i) - \vec{v}_{\text{non-magn bead}}(i)
\]  \hspace{1cm} (11)

where \(i\) is the frame number, which ranges from 1 to the total number of frames \(n\), \(\vec{d}_{\text{bead}} = (x_{\text{bead}}, y_{\text{bead}})\) is the vector of position of the bead, \(\vec{v}_{\text{bead}} = (v_{x_{\text{bead}}}, v_{y_{\text{bead}}})\) is the vector of velocities of the bead and \(dt\) is the sampling period. Finally, equalizing Equations 6 and 8, the following relation can be made (Equation 9)

\[
6\pi \eta r \vec{v} = a \cdot e^{(b \cdot \vec{r})} + c \cdot e^{(d \cdot \vec{r})}
\]  \hspace{1cm} (12)

and the non-linear coefficients \(a, b, c, d\) can be found.

3.1.2. Simulations

Force calibration allows to compute the Force generated by the tip, but not the value of the magnetic field generated nor its dependence on the geometry of the tip. A set of simulations has been considered indispensable to have an idea of the order of magnitude of the Magnetic Field generated (in Teslas) and to visualize the shape of the lines of the Magnetic field and the gradient in the tip end. Geometry of tip and coil simulations have done using FEMM (Finite Element Method Magnetics) varying different parameters of the tip in order to study the Magnetic Field created.

3.1.2.1. FEMM

FEMM is an open source software package used in science and engineering for solving linear and nonlinear low frequency magnetic and magnetostatic problems, linear electrostatic problems and steady-state heat flow problems. The problems can be
solved on two-dimensional planar and axisymmetric domains. The computational technique used to obtain approximate solutions to the partial differential equations (Maxwell’s equations) is the finite element method (FEM), which consists in dividing the geometry into a large number of subdomains and resolve the problem for each of them. The user interface is formed by a pre-processor, where the geometry of the problem is set, and a post-processor, where the solution of the problem is displayed and measurements can be taken. In addition, the program combines geometrical adaptability with a library of materials.

3.2 Chemical methods

In this section, the chemistry processes needed to prepare the sample for the calibration will be presented. These are the preparation of the solution of beads, the cleaning of the coverslips with the plasma cleaner and the mounting of the sample in the holder.

3.2.1. Solution of beads

The solution of beads consists of Magnetic Beads (Dynabeads M-270 Streptavidin, Invitrogen) and Non-magnetic beads (Amino-polystyrene Particles, Spherotech), dissolved in a Silicone Oil (Sigma-Aldrich) solution with known viscosity (10000 cSt at 25 °C).

When purchased, both magnetic and non-magnetic beads are suspended in solution presenting too low and unknown viscosity. Therefore, it is crucial for the calibration to clean them and change the buffer to a well characterised solvent. In order to do so, both solutions of beads are suspended in high volume of Tris-HCl BSA (1 mM 1% and pH=7.4) and spin-down at 14000 rpm in a centrifuge. This process is repeated three times and, afterwards, the beads are left to dry by means of a hot plate. Finally, they are dissolved in Silicone oil.

3.2.2. Cleaning coverslips

It is important to clean the coverslips previous to any experiment, so no dirt affects the cells or stays between the field of view of the camera and the sample.

First of all, the coverslips are sonicated with MiliQ water, ethanol and acetone, one after the other and for 5 minutes each. After, they are immersed and kept in methanol until used. Before use, they are dried with N$_2$ and put inside the Plasma Cleaner (PDC-
32G, Harrick Plasma). The door is tightly closed, the vacuum pump turned on for 2 minutes, until vacuum is reached. When time has elapsed, the plasma cleaner is set to High level and the coverslips are left inside for 15 min more. The plasma cleaner and the pump are then turned off and the door is open letting the air in slowly. After the coverslips are collected, the plasma cleaner door is closed and the pump switched on until the vacuum is reached again (~ 1 min).

### 3.2.3. Sample preparation

The sample is mounted on a custom-made plastic holder. A rectangular glass coverslip (24x36 mm, Menzel) is joint to the holder with high vacuum grease (Down Cornig) and, afterwards, a plastic gasket (Sigma Aldrich) is attached to the coverslip with the same grease (Figure 4). 50 µL of Silicone Oil (10000 cSt) are put in the well formed by the gasket and. Finally, the beads solution is added to the surface of the silicone oil by using a pipette to submerge them. Consequently, the sample will be highly concentrated of beads in the surface but not in the bottom of the silicone oil.

![Figure 4. Left: Gasket being attached to the coverslip by means of grease and tweezers. Right: Holder of the sample already prepared to place the solution.](image)

### 3.3 Experimental Methods

In this section, the experimental methods of the project will be presented. These include the details of the assembly of the setup, the specifications of its components and an introduction to the different softwares used to design the holder and to implement the program to control the setup; SolidWorks and LabVIEW and MATLAB respectively.
3.3.1. Setup

An overview of the fundamental parts of a Cell Magnetic Tweezers setup has been introduced in the previous chapter. The implementation of the setup in this project is presented below, detailing the specifications of its components.

As it can be seen in Figure 5, the Magnetic Tweezers components are setup on top of an Inverted Microscope (Eclipse Ti, Nikon). The main parts of this setup are:

- **Micromanipulator.** An MP-285 (Sutter Instrument) precision micromanipulator (MM) is used to move the tip. It consists of a XYZ rack mounted controller and a mobile manipulator that holds the tip with a plastic holder. The MM has a resolution down to 0.2 µm in the coarse range and 40 nm in the fine range and it can travel up to 25.4 mm on all three axes. The motion sequence can be programmed via a remote computer via a serial port or can be manually controlled using the joystick. In addition, the controller continuously displays the actuator position (in microns).

- **Data Acquisition System.** An USB-6341 X Series DAQ (National Instruments) is used to convert the digital values generated by the PC into an analogue waveform. This signal is sent to the coil. It also receives the analogue waveform that the current-voltage senses, once the signal has passed through the tip coil. It has 16-bit resolution and a ±10 V operating voltage, but will only be used between ±4 V.

- **Current-Voltage Converter.** A custom made current-voltage converter is used to transform the voltage signal set by the user using the LabVIEW PC interface and delivered by the DAQ to current signal. This current signal is sent to the tip and received back after it passes through the coil. The voltage input signal can also be controlled manually using the buttons situated in the front panel of this apparatus. For both manual and PC modes, the conversion ratio is 1.1V/1A. The output current signal coming back from the tip is converted to voltage signal and sent back to the DAQ and by extension to the PC. The Current-Voltage Converter will only be used between the ±4 V voltage range. Finally, the current-voltage converter is connected to the DAQ with two BNC wires (input and output).

- **Tip Holder.** A plastic custom holder is used to hold the tip to the micromanipulator. The holder material has been chosen in order to avoid any
interference of the electrical circuit of the actuator with the current passing through the coil, as well as to isolate the field in the tip from the environment.

- **Tip.** The tip is made of mu-metal and covered by an enameled copper wire forming a solenoid. It has two ends (in-current and out-current) which are both connected to the current-voltage converter with a 2-way locking line socket (speaker wire). The sharp end of the tip is submerged in the sample. This component will be reviewed in more detail afterwards, due its custom made character.

- **Sample Holder.** A custom designed and 3D printed holder is used to support the coverslip in the metallic vertically sliding stage of the microscope. This part will be reviewed more in detail in next chapter.

- **Coverslip.** A rectangular glass coverslip (Menzel) is used as substrate for the sample. This coverslip is attached to the plastic holder with Vacuum Grease (Dow Corning).

- **Diaphragm.** The diaphragm of the microscope is used to reduce the cone of light that is projected over the sample in order to minimize the heating of the sample. The spot of light is located in accordance with the camera field of view.

- **Camera.** The video camera used in this setup is an Infinity 2-1 R M (Lumenera). It records 1.4 MegaPixel monochrome frames at maximum resolution of 1392x1040. The frequency at which it is capable to grab with this resolution and with LabVIEW is 15 frames per second.

![Figure 5. Real Implementation of the Cell Magnetic Tweezers Setup.](image-url)
3.3.2. SolidWorks and 3D-printer

SolidWorks 2013 (Dassault Systems) has been used to design the custom-made holders of the Cell Magnetic Tweezers. It is a solid modeler software for Microsoft Windows, released in 1995 and widely used by the engineering community. It uses parametric feature-based approach to allow the user to create 3D models and assemblies, deciding their shape, dimensions and materials.

The sketches consist of geometries such as points, lines and arcs that have defined dimensions and locations in the geometry, as well as specific relations between them (tangency, parallelism, perpendicularity and concentricity). All these parameters mentioned can be modified independently. In addition, this software incorporates a tool to create drawings of the model automatically, allowing the user to choose the views displayed and the parameters included.

Once designed, the sketches were brought to the King’s College 3D printer, and printed in white plastic.

3.3.3. LabVIEW

LabVIEW (Laboratory Virtual Instrument Engineering Workbench) is a data acquisition and programming environment (from National Instruments) that allows flexible acquisition and processing of analog and digital data, as well as instrument control\(^\text{14}\). It is based in a graphical programming language named ‘G’. It was originally conceived and developed on a Macintosh computer (Apple Computer, Cupertino, CA) and was introduced in 1986, being the first software to include graphical, iconic programming techniques.

Precisely, this highly modular graphical programming language, "G", constitutes the main feature that distinguishes LabVIEW from other data acquisition programs, along
with a large library of mathematical, statistical and digital signal processing (DSP) functions. Graphical programming means that statements, variables and functions are represented by on-screen icons and "wires," rather than by lines of text. Therefore, the execution of the program is not controlled by the order in which the statements were written in the source code (line-oriented programming), but, rather, by the data that is generated (data flow oriented).

The advantage of visual programming is that the code is flexible, reusable, and self-documenting, thus the development time reduces dramatically allowing the users to develop or modify their own programs in an efficient way. This convenience has converted LabVIEW in a widely used software by engineers and scientist for more than a decade.

A LabView script, known as VI (Virtual Instrument), consists of a Front Panel and a Block Diagram (Figure 7). The Front Panel is the user interface which accepts inputs from the user by means of controls, boolean or numerical, and presents outputs by means of indicators. The Block Diagram is the panel that contains the graphical code. In this language wires represent variables that hold data and icons represent processes that act on these variables. Moreover, all basic elements of conventional programming languages are implemented, as for example case structures and for and while loops.

In this project, LabVIEW 2015 has been used to implement the control program for Cell Magnetic Tweezers, as well as the program to calibrate the Setup. In addition, some complementary software for LabView, also from National Instruments, have also been utilised. These are NI Vision Acquisition Software 2016 and NI Vision Development module 2015.

![Figure 7. Example of Front Panel (left) and Block Diagram (right). Picture from http://engineering.sdsu.edu/](http://engineering.sdsu.edu/)
3.3.4. MATLAB

MATLAB (Matrix Laboratory) is a programming language and a numerical computing environment from MathWorks. It allows matrix manipulation, plotting of functions of data, creation of user algorithms and implementation of algorithms.

In the front panel, the Command Window, the Workspace, the Editor, the Current Directory and the Command History can be found. Command Window is the interactive mathematical shell through which the user orders to execute specific commands and Command History is the window that stores these commands. Workspace displays all the variables used and generated by the code and Editor is the window where scripts are created, programmed and edited. Finally, Current Directory presents all the files contained in the current folder.

In this project, MATLAB 2014 has been used to compute the force as a function of the distance to the tip.
4. Setup Assembly

In this chapter, the components of the setup with custom-made character will be reviewed in detail, understanding and discussing the decisions taken in their process of design and building. The parts presented will be the tip, the holder for the sample and the LabVIEW programs, both to control the setup and to calibrate it.

4.1. Tip

The tip is constituted by a mu-metal rod with a very sharp end and a copper coil wired around it. Mu-metal is a mainly nickel-iron alloy which is very sensitive to low-frequency or static magnetic fields, such in our case. The coil induces a magnetic field when passing current through it and mu-metal shields the magnetic field towards the sharp end, creating a gradient that exerts a force over the particles in its vicinity.

In this section, a simulation of the tip varying its dimensions is presented in order to enhance the value and gradient of the magnetic field induced and understand the relation between them. Later on, the dimensions of the tip built according to the simulation are shown, along with the steps of building.

4.1.1. Simulations

A magnetostatic simulation of the tip, with different length of the rod, turns in the coil and sharpness of the end, has been carried out using the program FEMM (Finite Elements Method Magnetics). The aim of this study is to maximize the gradient of the generated magnetic field in order to achieve higher forces in the experiments, as well as knowing the magnitude and the lines of magnetic field and understand the relation between them.

Five different sets of simulations have been planned, varying a different parameter in each of them; length of the tip end, length of the rod in the wider end, distance between the coil and the beginning of the sharp end, number of turns in a fixed coil length and number of turns with a variable coil length. Each set of simulations is constituted by three cases and in all of them the profile of the resulting Magnetic Field has been measured along a distance of 150 µm from the tip end. The measurements have been registered at both $0^\circ$ and $30^\circ$ from the rod longitudinal axes, which constitute the angle range used in the experiments.
An open boundary condition with seven layers ($R_i=65$ mm) has been chosen in the simulation with Dirichlet boundary conditions. In addition, the materials used have been the default Air and 24 swg Copper wire included in the library of the program. A custom designed Mumetal material, according to the specifications of the provider (Magnetic Shields), is used for the rod. As mentioned, a magnetostatic frame has been considered reasonable taking into account that the current sent in the calibration is constant (1 A in the simulation). Finally, the program solves the triangular finite elements mesh with Newton method. A fixed mesh size of 1 µm is fixed in the region of interest.

4.1.2. Results

The results of the simulation show that the magnetic field at 0° and 30° are almost equal. Therefore, it will be consistent in the experiments to consider suitable for measurements any bead comprised within the boundaries of ±30°. The detailed results are presented below. In each of the cases, the varying parameter is colored with the values it adopts and the rest of the dimensions of the tip, which are fixed in all three cases, are represented in black.
4.1.2.1. Simulation with variable length of the tip end

Figure 10. Magnetic tip with variable sharpness which is achieved modifying the length of the tip end (5 mm, 10 mm and 15 mm long).

As it can be seen in Figure 11, the length of the tip that maximizes the magnetic field is the one with minimal sharpness. Clearly, the 5 mm tip length has higher magnetic field for all distances, achieving values of 0.06 T at 50 µm. The 10 mm tip shows a magnetic field of about 0.04 T at 50 µm and the 15 mm tip of about 0.03 T at the same distance. Therefore, the most suitable length for the sharp end will be 5 mm. The magnetic field is considered at 50 µm from the tip end, because this is the distance at which the cells will be placed in the experiments.

Figure 11. Plot showing the changes in the magnetic field as a function of the distance to the tip, due to alterations in the sharpness of the tip. The higher values are achieved with the 5 mm long tip (max. 0.34 T at 0 distance) and the lower values respond to the 15 mm long tip (maximum of 0.17 T at 0 µm).
4.1.2.2. Simulation with variable length of the rod in the opposite end

Figure 12. Drawing of the magnetic tip showing the variable parameter; the length of the rod in the opposite end (5 mm, 10 mm, 15 mm).

As Figure 13 shows, all tips generate a magnetic field of about $\approx 0.045$ T at 50 $\mu$m, so no substantial differences can be seen due to the change in the rod length in the opposite end. The only difference that can be observed is that the longest length (15 mm) generates a slightly higher magnetic field but not sufficiently high to be considered. This assumption can be made because the exact values are 0.0435 T for the 5 mm tail tip, 0.0470 T for 10 mm and 0.0476 T for 15 mm tip (all values taken at 0°). Consequently, it can be assumed that the length in the opposite end of the tip does not affect the overall magnetic field in the tip.

Figure 13. Graph showing the changes in the magnetic field as a function of the distance to the tip, due to alterations in the length of the not sharp end. No substantial changes can be observed since all show value of similar magnetic field ($\approx 0.046$ T at 50 $\mu$m).
4.1.2.3. Simulation with variable distance between the coil and the beginning of the sharp end

![Diagram showing the variable parameter; the length of the rod between the coil and the tip end (5 mm, 10 mm, 15 mm).]

Figure 14. Magnetic tip showing the variable parameter; the length of the rod between the coil and the tip end (5 mm, 10 mm, 15 mm).

From the data displayed in Figure 15, although a slightly greater magnetic field is achieved with the 5 mm rod length between the coil and the tip end, all tips generate similar magnetic field at 50 µm (≈0.042 T). More in detail, at 50 µm the 5 mm tip generates a magnetic field of 0.0435 T, the 10 mm tip 0.0432 T and the 15 mm tip 0.0416 T. Therefore, it can be concluded that no strong alterations take place when changing the rod length in the vicinity of the sharp end.

![Plot representing the changes in the magnetic field as a function of the distance to the tip, due to alterations in the distance between the coil and the start point of the sharp end.](chart)

Figure 15. Plot representing the changes in the magnetic field as a function of the distance to the tip, due to alterations in the distance between the coil and the start point of the sharp end. No substantial changes can be observed since all show a similar value of magnetic field (≈0.042 T at 50 µm).
4.1.2.4. Simulation with variable number of turns with fixed coil length

![Figure 16. Magnetic tip with increasing number of turns in the coil (100, 150, 200 turns) with fixed coil length (56 mm).](image)

As expected, there is a clear increase in the magnetic field when the number of turns in the coil grows. At 50 µm and at 0°, the magnetic field is 0.043 T for 100 turns, 0.056 T for 200 turns and 0.065 T for 200 turns. However, this simulation is not realistic as it has been considered that the volume occupied by the coil remains constant even if the number of turns increases. Therefore, a trade-off between the maximization of the number of turns and the available space for the coil according to the rod length will have to be considered.

![Figure 17. Plot showing the changes in the magnetic field as a function of the distance to the tip, due to variation of the number of turns in the coil. A clear tendency is observed: the magnetic field increases with the number of turns.](image)
4.1.2.5. Simulation with variable number of turns with variable coil length

The length occupied by the coil has been computed considering that only a monolayer of the coil is surrounding the rod and that the diameter of the coil is 0.56 mm. As it can be observed in Figure 19, the magnetic field increases in the case of 120 turns (67.2 mm) achieving a value of 0.050 T at 50 µm and remains nearly the same with 140 turns (78.4 mm) 0.044 T at the same distance. Both statements are made in comparison with 100 turns (56 mm) which generates a magnetic field of 0.043 T at 50 µm and 0°. Therefore, no conclusions with respect to the magnetic field can be assumed in this case since the tendency of increment or decrement is not clear.

Figure 18. Scheme of the magnetic tip showing the general geometry of the tip and the variable parameter in this set of simulations; the number of turns with associated increase in coil length around the rod. The values are 100 turns (56 mm), 120 turns (67.2 mm) and 140 turns (78.4 mm).

Figure 19. Plot showing the changes in the magnetic field as a function of the distance to the tip, due to alterations in the number of turns in the coil, considering a monolayer of coil (0.56 mm diameter). No clear tendency can be extracted.
4.1.3. Final Design

Using the information gathered from the results of the simulations, the rod is chosen to have 15 cm in length and a diameter of 6.35 mm. The length of the sharp end is 10 mm, the minimal length that allows an ease of building. The distance between the coil and the sharp end is 15 mm and the length left between the coil and the opposite end is 20 mm (Figure 20).

![Diagram](image)

Figure 20. Scheme of the fabricated mu-metal tip indicating its dimensions.

4.1.3.1. Process of building

First of all, a 15 cm long rod is cut from a mu-metal bar of 0.63 cm in diameter. A 1 cm length sharp end is polished, forming a 17.61° angle with the rod axis (Figure 21.a). Then, two plastic tube pieces are placed around the rod and stuck with contact glue with some grease (Figure 21.b). The plastic tube pieces are used as stoppers and limit the space for the copper wire. They also help to insulate the created current. In order to induce the magnetic field, a 0.56 mm enameled copper wire is coiled around the rod and between the plastic ends forming 4 layers of 625 turns in total. Each layer has as many turns as possible, ending up in an average of ≈155 turns each (Figure 21.c). To supply the current, the two ends of the copper wire are welded to a 2-way locking line socket, which will be connected to the voltage-current converter. Finally, a thermoplastic is sealed around the tip to isolate the current from the exterior (Figure 21.d).
Cell Magnetic Tweezers for the study of mechanical interactions in Cells

Aina Cabello Gómez

4.2. Holder

The holder for the sample has been designed using SolidWorks and its dimensions and shape have been chosen so it perfectly fits the metallic sliding stage of the microscope. It holds the rectangular coverslips used in the experiments (Figure 22). The hole of the holder is needed in order to allow the camera to direct observations of the sample. The holder has been 3D printed in white plastic.

Figure 21. Building of the magnetic tip. a) 15 cm mu-metal rod is cut and sharpened. b) Plastic tube pieces are stuck in both ends. c) Copper is wired around the rod. d) Tip is sealed with thermoplastic.

Figure 22. Sketch of the cell coverslips holder made with SolidWorks. All dimensions are in mm.
4.3. Programs

In this section, the programs to control the Cell Magnetic Tweezers and to analyze the videos to calibrate the setup up will be presented and detailed. They have been programmed using LabVIEW 2015 and MATLAB 2014. The control program (*MT_ControlSoftware*) is able to call the calibration program (*MT_Calibration Program*) as a SubVI and the latter opens MATLAB with a Script Node that makes the calculations of resultant forces and distance values.

4.3.1. MT Control Software

The interface that controls the setup (Figure 23) allows the user to preview the sample, record videos, take instant pictures, control the micromanipulator that holds the tip and control the voltage signal sent to the coil. From this panel it is also possible to call the subroutine that analyses the data acquired in the experiments.

![Figure 23. Front Panel caption of the custom program MT_ControlSoftware to control the Cell Magnetic Tweezers.](image)

According to what has been said, the flow of data of the main panel can be represented with the following diagram (Figure 24). Green color represents processes/inputs that affect the functioning of the whole program and blue color stands for processes/inputs that affect only a specific external instrument. Rectangles with rounded corners
represent the processes required to start or end the program, rectangles with two rounded corners symbolize manual inputs and white rectangles stand for ensemble of subprocesses that affect the same external instrument. The goal of the code implementation has been achieving a user-friendly design so the software can be easily used by non-specialist users.

![Flowchart Diagram](image)

**Figure 24.** Scheme representing the flowchart diagram of the software control.

Once the *Run* button is pressed, a window pops-up and asks to create or open a folder to save the experiments of the session. If the folder does already exist, it is not overwritten but opened and extended. The corresponding path is displayed in *Saving Folder* and can be modified using the folder icon. After that, the camera is configured and the sample is previewed on screen. Then, the program waits in the Listen state until the user interacts with the interface introducing any input with the controls.

The program, as said before, is also able to call the SubVI *MT_Calibration Program* by means of the *Analyse* button and is stopped when the *Close Program* button is pressed.

A detailed discussion of the implementation of the code will be presented below. The explanation will be split in sections, according to the external instrument that the program controls in each case. In addition, captures of the graphical code can be found in Annex I.

**4.3.1.1 Camera**

As said previously, once the program is open, the camera is automatically initialized and configured. Then, the computer waits for the user to interact with the interface in the *Listen* state. The sample is previewed from the very first moment, even though the user does not execute any action. Relative to the camera, the user has only three
buttons that can press; Start Acquisition, Take a Shot and Stop Acquisition. At the beginning, the user can only actuate the two first buttons mentioned, as Stop Acquisition is disabled (Figure 25). It is worthy to mention that this part of the program is the only one that generates outputs to be saved.

Figure 25. Snapshot of the Front Panel representing the controls and indicators relative to the camera control. In the picture the Saving Folder indicator, the AVI Name indicator and the Append to File Name control are shown. Below them, the Start Acquisition, Stop Acquisition and Take a Shot buttons along with the screen can be seen.

On the one hand, if the user presses Start Acquisition, the images that are being displayed on screen start to be saved as frames of an AVI file (.avi). Moreover, the Stop Acquisition button is abled and in turn the Start Acquisition is disabled. The AVI file created is named video_ plus an appendix chosen by the user by means of Append to File Name plus the hour in which the video has started to be saved. The Append to File Name appendix has been added so the conditions in which the experiment takes place can be indicated in the name of the video. This very name is displayed in AVI Name indicator and the recording is stored in the folder created when the program is started. Each AVI has only 450 frames (≈10 s), so the buffer does not run out of memory. Once an AVI has reached 450 frames, a new video is created and stored automatically following the steps already explained. The videos are created one after the other until the user activates the button Stop Acquisition. When this event
takes place, the latter is disabled and *Start Acquisition* is abled again. Therefore, the length of a video is always ≤450 frames, depending on when the *Stop Acquisition* was pressed.

On the other hand, if the user activates *Take a Shot*, the frame that is displayed in the very moment is saved as a TIFF image (.tif). The picture is named `image_` followed by the hour in which it is taken. This process can be activated as many times as wanted and stops with the rest of the program.

![Flowchart Diagram](image)

**Figure 26.** Scheme representing the flowchart diagram relative to the control of the camera.

This part of the code is implemented in two while loops, one for video recording and the other for picture taking. Each of the loops contains a Case Structure regulated by the value of *Start Acquisition* in the first case and *Take a Shot* in the second. In addition, the latter loop has an event structure so it is only activated when the user presses *Take a Shot*. For this implementation, the functions included in the Vision Acquisition Software (2016) have been used. Both loops stop simultaneously, by means of a local variable, when the *Close Program* button is pressed. The Flow data diagram of the routine of the camera is represented in Figure 26.
4.3.1.2. Micromanipulator

The micromanipulator is the instrument that controls the tip movement and, at the same time, acts as a support for it. As mentioned in 3.2.2. Setup, the move of the tip is of the order of microns (µm).

As it can be seen in Figure 27, the control interface of the micromanipulator is a control console that allows the user to move the tip along one axis each time. It also permits to decide the velocity at which the tip travels with the control Velocity (µm/s) and to monitor the current position with current X, Y and Z indicators. However, this current position only corresponds to the position of the tip once it stops after a commanded movement. The movements can be of a 10 µm fixed length, using the inner green arrows without top-line, or of custom length, using the outer green arrows with top-line. In the latter case, the distance travelled will be the one indicated in X position, Y position and Z position in each case. It is worthy to note that the sign of the position put in X position, Y position and Z position will not affect the direction of the movement, as the arrows are the ones that set the direction. Finally, the horizontal arrows direct the movement along the X-axis, the vertical light arrows along the Y-axis and the dark arrows on the left hand side of the panel along the Z-axis.

In addition, two other buttons are set with very different aims. The function of the house button is to communicate the micromanipulator that the actual position will be set as the new origin. The red octagonal button acts as a break and interrupts the movement in case the travel distance set by the user is too large and puts the sample at risk. As all other parts of the program, this routine will stop when the Close Program button is pressed.

![Figure 27. Section of the front panel that controls the Micromanipulator. On the left, the arrows that move on the Z-axis can be seen. On the central part, the X-axis arrows (horizontal) and Y-axis arrows (vertical) can be found, along with the Break (red octagon with white cross button, left) and Set Origin (house button, right). Finally, the controls that set the distance travelled and the velocity plus the indicators of the current position are observed on the right.](image-url)
The micromanipulator control has been implemented as an event-driven state machine. This programming architecture is conceived as an abstract machine that can be in one of a finite number of states and can only be in one state at a time. The machine waits until an event occurs and this event triggers the execution of the appropriate case to handle that event.

An event-driven state machine in LabVIEW consists of a While Loop, a Case structure, an Event Structure and a shift register. Each state of the state machine is a separate case in the Case structure and each case contains the code that the state should execute within the appropriate event. A shift register stores the state that should execute upon the next iteration of the loop and the Event Structure makes possible to recognize the events triggered by the user (i.e. press a button).

In this case, the possible states are Start, Listen, Set Origin, Move 10 X, Move 10 Y, Move 10 Z, Move X, Move Y, Move Z and Close. As said in the previous paragraph, the states of the Shift Register are tightly related with the cases in the Case Structure, as the shift register sets which case is going to be executed in every iteration. Below is a summary listing the main actions that take place in each case of the Case Structure.

- **Start.** This is the state that initializes the state-machine. It waits for 100 ms and sets the Shift register to Listen.
- **Listen.** This state contains an Event Structure that waits until an event occurs and then executes the appropriate case to handle that event, setting the shift register according to it. The expected events are changes of value in any of the buttons associated with the micromanipulator control. In addition, a Timeout is added in case none of the events takes place since the last iteration.
- **Set Origin.** In this case the order ‘set the current position as Origin’ is sent to the micromanipulator. Therefore, the position displayed in the micromanipulator screen becomes $x = 0 \, \mu \text{m}; y = 0 \, \mu \text{m}; z = 0 \, \mu \text{m}$. The command sent is ‘o’CR, where the Return Constant is ASCII CR (13 decimal, 0D hexadecimal). Once finished, the shift register is set to Listen.
- **Move X, Move Y and Move Z.** These states are triggered when one of the green arrows with a top-line is pressed. In each of them the program sends to the micromanipulator the velocity at which it has to travel, 10 \, \mu \text{m/s} by default, and sets the sense of the movement with a Boolean indicator (left/right or up/down). After that, it reads the respective position where it has to travel ($X Position$, $Y Position$ or $Z Position$) and proceeds to send the order to the
micromanipulator. The command is ‘move to specified position’ and is sent as ‘m’xxxxyyyyyyyyyyyyyyzCR, where each xxxx consists of a signed long (32-bit) integer value in microsteps. When the code is executed, the shift register is set to Listen for the next iteration.

- **Move 10 X, Move 10 Y, Move 10 Z.** When pressing one of the green arrows without line, one of these cases is activated. In all of them, the velocity is read and the tip travels 10 µm in the direction or sense that agrees with the button pressed by the user. After that, the shift register is set back to Listen.

- **Close.** This state is triggered when the Close Program button is set to true with a local variable. It stops the state-machine, interrupting the while loop.

In order to have a general overview of the functioning of the state-machine, a flowchart is presented in Figure 28.

![Flowchart Diagram](image)

**Figure 28.** Scheme representing the flowchart diagram relative to the control of the micromanipulator.

### 4.3.1.3. DAQ. Voltage in the tip

The DAQ is the instrument that sends and senses the voltage to and from the tip. It transforms the digital signal sent by the PC into an analogue signal and, after the current has passed through the coil, it carries out the inverse process. From now on, Output and Input will be seen from the point of view of the DAQ. Therefore, Output
will refer to the part of the code relative to the signal from the PC to the tip and Input will be relative to the waveform from the coil of the tip to the PC.

As it can be observed in Figure 29, the front panel associated with the control of the DAQ has four buttons (Send Once, Repeat Send, Stop Sending and Demagnetize), Manual Signal panel that configures the signal sent to the coil of the tip and a screen that displays simultaneously both the waveform sent to the tip in blue (In-PC) and the voltage sensed in return in red (Sensor).

![Figure 29. Section of the Front Panel that corresponds to the DAQ control. On the left, the panel to configure the waveform sent to the tip can be seen and on the centre, the buttons that control the sending. On the right the screen that monitors the signal sent to (blue)/received from (red) the tip.](image)

According to the possibilities that this panel offers, the user can configure a signal and send it once pressing Send Once or send the same signal repeatedly with Repeat Send. In the second case, the waveform will be sent continuously until the button Stop Sending is pressed. On top of this, the Demagnetize button sends a pre-configured signal to the tip that is employed to remove its residual magnetism. This waveform is an alternating voltage degauss signal of about ±2V and 100 Hz frequency that reduces the voltage to 0 V in less than 3 seconds. It is important to note that while Repeat Send is active, Send Once and Demagnetize buttons are disabled. The sent voltage (output) and the sensed voltage (input) are displayed on the screen at all times. The code is stopped when the Close Program button is pressed.

This code has been implemented in two different loops, one for the Input, which is a While Loop, and the other for the Output, which is an event-driven State Machine again.
Figure 30. This Scheme represents the flowchart diagram relative to the control of the camera. The output event-driven state-machine will be discussed below in more detail, due its complexity. The states available in this Case structure are *Start*, *Listen*, *Once*, *Repeat*, *Demagnetize*, *Stop* and *Close*. A diagram showing the flow of data is represented in Figure 30.

- **Start.** Initializes the state-machine configuring a 0 V DC signal in the DAQ. When finished, it sets the Shift register to *Listen*.

- **Listen.** This case contains an Event Structure that waits until an event occurs and then executes the appropriate case to handle that event, setting the shift register according to it. The expected events are changes of value in any of the following buttons: *Send Once*, *Repeat Sending*, *Stop Sending* or *Demagnetize*. In addition, a Timeout is added in case none of the events has taken place since the last iteration. Timeout sets the Shift Register to *Listen* state again and configures a 0 V DC signal that is sent to the DAQ.

- **Once.** If the *Send Once* button is set to true, this state is executed. It consists of configuring the signal set in *Manual Input* and send it to the DAQ just once.

- **Repeat.** This state is triggered when the *Repeat Send* button is pressed. It configures the signal set in *Manual Input* panel and sends it to the DAQ repetitively until the *Stop Sending* boolean is true.

- **Stop.** When *Stop Sending* is pressed, the previous signal is stopped and a 0 V DC waveform order is sent.

- **Demagnetize.** As explained before, this state is executed when the *Demagnetize* button is pressed and removes the residual magnetic field in the tip sending a preconfigured AC signal with decreasing amplitude.
- **Close.** This is the last state which is activated when the *Close Program* button is set to true. It stops the while loop, stopping at the same time the state-machine.

In conclusion, the main program is capable of controlling the camera, the micromanipulator and the DAQ, as well as recording videos and saving images of the sample. As said before, it is also capable of opening the Analyse SubVI, with the *Analyze* button, and display the results worked out by the latter in the main Front Panel. This is accomplished by means of a global variable. The *MT_Calibration Program* will be further explained below.

### 4.3.2. MT Calibration Program

The aim of this program is to analyze the videos to calibrate the Cell Magnetic Tweezer Setup. This program allows the user to track the beads, magnetic and non-magnetic, and from the positions obtained, compute the associated force as a function of the distance to the tip. This calculation is made thanks to a MATLAB subroutine that can be called from LabVIEW. Lastly, it fits the points into a double exponential (see 3.1.2. Force Calibration), and displays the results in the *F vs. d Graph*. The VI can be run stand alone or can be called from the main program *MT_ControlSoftware*.

![Figure 31. Front Panel of the MT_Calibration Program VI.](image)

On the left, the screen can be found. On the centre, the buttons that control the frame displayed and the *Define first and last frames* buttons are seen. Finally, the *Locate Magnetic and Reference Beads* and the *Analyze* buttons are observed, along with the X-Y graph that shows the results of the Force vs. distance calculation and its curve fitting in a double term exponential.
First of all, once the program starts to run, a window appears asking if the video that has to be analyzed has to be acquired or loaded from a folder. If the first option is chosen, a file dialog opens to select the folder where the video will be saved and the Start Recording button is abled. When pressed, the Stop Acquisition control is also abled and the video is recorded until this very button pressed. If the option chosen is to load a video, a file dialog pop-ups and the user is asked to select the video to be opened. It is worthy to mention, that all the output files generated afterwards are automatically saved in the same folder as the video, so it is convenient to create a folder exclusively for the video in order to have all the results together.

Afterwards, the first frame of the video is opened on the screen and the buttons Previous Frame, Next Frame and Go to ____ make possible to change the displayed frame. The aim of the frame navigation is to be able to identify the First and the Last frames for the tracking. The total number of frames are represented in Total Number of Frames and the frame that is on screen is indicated in Current Frame. Clearly, the Current Frame has to be between zero and the total number of frames. The first frame is defined with the green button Define First Frame and the last frame is set with the analogue button Define Last Frame. A TIFF image (.tif) of each of these frames is saved.

Once the first and last frames have been selected, the dark green button Locate the Magnetic and Reference Beads has to be set to true. After, the first frame of the tracking opens and the user is asked to select the Magnetic Bead with the green rectangle provided. This step is known as selecting the Region of Interest for the tracking. When the Ok button is pressed, confirming that the user is pleased with the ROI, the program automatically tracks the magnetic bead and shows this process on screen (see subsection 4.3.2.1. LabVIEW Tracking). The same procedure is repeated for the non-magnetic Bead. When both tracking processes are finished, a message will pop-up displaying that the position measurements have

![Figure 32. Magnetic Bead being tracked on screen. The tip is on the left and the bead is on the central lower part of the image.](image-url)
been successfully completed. Each tracking generates as an output a .csv file containing the position of the bead, magnetic and non-magnetic, in each frame. This files show three columns; frame, x position (pixels) and y position (pixels) and will be used by the MATLAB routine afterwards.

When the tracking is completed, the user can press the Analyse button, which triggers the calling of MATLAB and runs the code contained in Matlab Script Node externally. Through MATLAB, the script asks the user to select the end and final position of the tip, showing both frames, and computes the Force as a function of the distance to the tip end. After the computation is completed, two output files are generated, one for the force and one for the distance, and both are saved in .csv format.

Finally, the (distance,Force) points are plotted on the $F$ vs. $d$ Graph in blue along with a double term exponential curve fitting made with LabView in red. The non-linear coefficients of the fitting are also saved in a .csv file. In addition, the points and the fitting are saved in a global variable and displayed in the Front Panel of the main program, $MT\_ControlSoftware$, if open.

A diagram is presented in Figure 34 in order to ease the understanding of the functioning of the program. As it has been exposed, the purpose of this code is concrete, the calibration, but it will be used as a base for specific programs developed in the future to respond to the experiments carried out in the laboratory. Some of its parts can remain unaltered, as the loading of the video and the tracking of the beads, and some will have to be changed according to the needs of the experiment. The function of the program can be transformed easily just changing the code in the MATLAB Script Node. Finally, the Block Diagram of the VI can be found in Annex II, showing how the program has been implemented.
4.3.2.1. LabVIEW Tracking

The tracking in LabVIEW is carried out thanks to the Vision Module Software (2015). This software of National Instrument provides an object tracking tool that allows the user to track the beads using Mean Shift Algorithm or Shape Adapted Mean Shift Algorithm. The option employed for this project is the second, Shape Adapted Mean Shift Algorithm, as it not only localizes the position of the bead in each frame, but also considers changes in size and shape from frame to frame. This percentages of variation have to be fixed in advance, as well as the maximum number of iterations allowed to find the bead per frame. Object tracking for an image frame is performed by a combination of histogram extraction, weight computation, derivation of new location and

Figure 33. This Scheme represents the flowchart diagram relative to the *MT_Calibration Program*.

Figure 34. Example of tracking using Adapted Mean Shift Algorithm. Image taken from www.zone.ni.com
update of covariance matrix. The algorithm has three stages; Target Model, Mean Shift Convergence and Update of Location and model.

4.3.2.2. Force-distance Code

As mentioned before, this code has been programmed in MATLAB language and inserted in the MT_Calibration Program by means of a Matlab Script Node. Its purpose is to compute the force as a function of the distance to the tip. The whole code can be found in Annex III.

First of all, the constants that refer to the conditions at which the experiments are carried out are read. These are the magnification of the objective, the real size of the pixel (ratio µm/pixel), the kinematic viscosity of the solution (Centistokes), the density of the solution (kg/m³), diameter of the magnetic bead (µm) and the sampling period (s). After that, the program loads the first and last frames of the tracking and requests the user to mark the initial position of the tip, showing the first frame, and the last position of the tip, presenting the last frame. This positions are used to compute the drift of the tip. Consequently, a vector of positions of the tip is generated considering a uniform linear movement. It is important to mention that the position of the tip has to be selected in the same very point where the bead tracked arrives in order to have an accurate calibration.

Afterwards, the code loads the files containing the positions of both the magnetic and reference non-magnetic beads. These files have three columns, the first enlist the frame of the video and the second and third columns show the x and y position of the bead in pixels. From this data, the velocities in x and y directions can be computed.

Once the velocities have been computed for both the magnetic and reference beads, the distance between the bead and the tip is calculated in each iteration.

Finally, the force is computed considering the absolute velocity, so the flow of the solution is removed from the analysis. The output force and distance results are stored in a vector and passed to LabVIEW.

4.4. Discussion

In this section, the simulations of the tip, its final design and procedure of building and the LabVIEW programs to control and calibrate the setup are discussed.
As far as the simulations are concerned, it has been proved that the magnetic field at 0° and ±30° is nearly the same. Therefore, any bead in this range of angles will be considered suitable to be taken into account in the calibration and in further experiments. Additionally, the Magnetic Field generated by the tip in its vicinity can be fitted with a double exponential, which agrees with the Force Calibration assumption. Moreover, the value at a distance of 50 µm is around 0.045 T and at 0 µm is always below 0.4 T.

As for the geometry of the tip, as shorter is the length of the sharp end, the greater is the magnetic field. The distance between the coil and the sharp end and the distance in the opposite end do not affect much the magnetic field in the tip. Lastly, an increase of turns in the coil favor an augment in the magnetic field.

In order to improve the design, more tip dimensions could be simulated and results could be compared with another simulating software.

Related to the assembling, as it has been learnt, the magnitude of the magnetic field gradient, and hence the force magnitude, depends crucially on the shape of the needle of the tip. From literature, it is known that highest forces and steepest gradient are obtained using the smallest possible radius. However, a limitation of the sharpness is fixed with the stiffness of the mu-metal material and the radius of the rod is subjected to the products available in the market. Therefore, the best possible material fit to date has been chosen, but other options may appear in the future.

In addition to the holder presented, another simple holder for a rectangular coverslip has been designed and 3D printed, but has not been included because it will not be used for this setup.

Finally, with relation to the codes for the control and calibration programs, they have been really useful to learn and get confident with a widely used language; LabVIEW. In addition, the control program (MT_ControlSoftware) will be fully functional for future experiments carried out in the laboratory. In contrast and according to what has been previously said, the function of the calibration program (MT_Calibration Program) is very specific, but the code has been implemented so it can be easily adapted to the needs of future trials just changing the code in the Matlab Script Node.
5. Calibration

In this chapter, the method to take a video to conduct an accurate calibration and the procedure to obtain the parameters to describe the experiment environment in the calibration code will be described.

5.1. Calibration procedure

The process of calibration consists in recording a video and analyzing it, tracking the beads and computing the resultant force and distance associated. Five beads have been tracked for each intensity. The intensities considered are 0.5 A, 1 A, 1.5 A, 2 A and 2.5 A.

5.1.1. Record a video

To record a video suitable for calibration a spot with well-defined beads has to be found and focused, having the tip in the field of view.

In the beginning, the objective is as far as possible from the coverslip and it slowly approaches the latter until the top of the glass is in focus (when the edge of the drop is focused). Then, it is raised until beads are focused, which means reaching the superior part of the drop. The tip is lowered until its sharp end enters the liquid and then it is moved manually with the joystick until its shadow can be seen in the screen. The tip is carefully lowered until the sharp end is in focus and it is in the same X-Y plane than the beads. Afterwards, the microscope stage is moved horizontally until a spot with equally magnetic and no-magnetic beads is in the vision zone of the camera. Additionally, by means of the computer, the position of the tip can be adjusted. Finally, the button *Start Acquisition* is pressed and the voltage applied to the coil in order to induce a magnetic field.

Additionally, in order to acquire a good video and to have a correct functioning of the setup, the tip has to be connected and disconnected of the voltage-current converter previous and after every experiment session. This precaution is taken because strange behaviour of the beads was observed when leaving the tip connected overnight, due to residual currents.
5.1.2. Enter parameters in Matlab Code

In order to calibrate accurately the force of the Cell Magnetic Tweezers, it is essential to introduce in the Matlab Force distance Code, the parameters that describe the conditions in which the experiment is done. These are the magnification of the objective, the size of the pixel (ratio µm/pixel), the kinematic viscosity of the solution (Centistokes), the density of the solution (kg/m$^3$), diameter of the magnetic bead (µm) and the sampling period (s).

The magnification of the objective, the kinematic viscosity of the solution, the density of the solution and the diameter of the magnetic bead are obtained from the specifications sheet provided by their respective fabricators. The solution is 378402 Silicone Oil from Sigma-Aldrich and the magnetic beads are Dynabeads M-270 Streptavidin from Invitrogen. The values used are displayed in Table 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinematic Viscosity</td>
<td>10000</td>
<td>cSt</td>
</tr>
<tr>
<td>Density</td>
<td>0.971 · 10$^3$</td>
<td>kg/m$^3$</td>
</tr>
<tr>
<td>Diameter of the bead</td>
<td>2.8</td>
<td>µm</td>
</tr>
<tr>
<td>Magnification</td>
<td>40</td>
<td>x</td>
</tr>
</tbody>
</table>

Table 2. Several parameters used in the calibration, according to the sample characteristics.

It is worthy to mention that the kinematic viscosity and the density of the solution are the values provided for 25 °C, the temperature at which the experiments are carried out.

In contrast, the real size of the pixel and the sampling period are not as straightforward to obtain. The procedures by which they have been figured out will be following explained.

5.1.2.1. Size of the pixel

To relate the pixels with its dimension in the real world, we use a microscope stage micrometre 3”x1” (Thorlabs). The stage micrometer is a coverslip of glass with marks of known size and distance between them (Figure 35). In this case, the distance between the thin lines is 10 µm and between the wide lines is 100 µm. To calibrate, first of all, a picture of the stage in focus is taken and, afterwards, ImageJ is used to correlate the pixels length with its real dimension. This step is accomplished measuring
the length of a straight line with known length in real world using the tool Analyze > Measure, which gives the result in pixels. Then, relating the length in pixels with its real dimension in µm is trivial. The process is repeated several times and an average of all cases is computed in order to have a more accurate value. For each picture, a line of real size 10, 20, 50 and 100 µm is measured.

Figure 35. Image with an ImageJ ruler. The relation here is 528.607 pixels = 0.6 mm.

The results are shown in Table 3.

<table>
<thead>
<tr>
<th>Image</th>
<th>Dimension in µm</th>
<th>Dimension in pixels</th>
<th>Magnification of the lens</th>
<th>µm/pixel</th>
<th>Pixel size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10</td>
<td>89.373</td>
<td>40</td>
<td>0.111891</td>
<td>4.475625</td>
</tr>
<tr>
<td>A</td>
<td>20</td>
<td>176.126</td>
<td>40</td>
<td>0.113555</td>
<td>4.542203</td>
</tr>
<tr>
<td>A</td>
<td>50</td>
<td>436.458</td>
<td>40</td>
<td>0.114559</td>
<td>4.582342</td>
</tr>
<tr>
<td>A</td>
<td>100</td>
<td>877.104</td>
<td>40</td>
<td>0.114012</td>
<td>4.560463</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>88.091</td>
<td>40</td>
<td>0.113519</td>
<td>4.540759</td>
</tr>
<tr>
<td>B</td>
<td>20</td>
<td>177.459</td>
<td>40</td>
<td>0.112702</td>
<td>4.508084</td>
</tr>
<tr>
<td>B</td>
<td>50</td>
<td>436.735</td>
<td>40</td>
<td>0.114486</td>
<td>4.579436</td>
</tr>
<tr>
<td>B</td>
<td>100</td>
<td>878.504</td>
<td>40</td>
<td>0.11383</td>
<td>4.553195</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>88.04</td>
<td>40</td>
<td>0.113585</td>
<td>4.543389</td>
</tr>
<tr>
<td>C</td>
<td>20</td>
<td>172.129</td>
<td>40</td>
<td>0.116192</td>
<td>4.647677</td>
</tr>
<tr>
<td>C</td>
<td>50</td>
<td>439.064</td>
<td>40</td>
<td>0.113879</td>
<td>4.555145</td>
</tr>
<tr>
<td>C</td>
<td>100</td>
<td>878.306</td>
<td>40</td>
<td>0.113856</td>
<td>4.554221</td>
</tr>
</tbody>
</table>

Table 3. Values of measurements in pixels of dimensions of known size in µm and associated pixel size.
Therefore, the relation pixel size considered for the calibration of the force as a function of the distance is 4.55 µm/pixel.

\[
\text{average ratio} = 0.114 \pm 0.001 \text{µm/pixel} \\
\text{pixel size} = 4.55 \pm 0.04 \text{µm/pixel}
\]

5.1.2.2. Sampling Period

The theoretical sampling frequency (1/sampling period) of Lumenera Infinity 2-1 R camera at maximum resolution (1392x1040) when using LabVIEW is 15 fps. However, this value does not correspond to the reality because, when recording videos with the custom-made LabVIEW programs, the time required per frame is not only the acquisition time, but the time to display the frame on screen and to store it in an AVI file. Therefore, the experimental sampling frequency is slower than the theoretical sampling frequency.

To be able to compute the real sampling period, a simple LabVIEW program has been implemented. The VI consists in a loop that records a video of 450 frames and a timer (Figure 36). Both activate when Start Acquisition button is pressed and the timer stops when the last frame is acquired, displayed and stored. In this way, the time showed in the timer when the VI stops is the time required to record and store a video of 450 frames, the same length used in the experiments. From this value, which is 39.82 s, it is trivial to compute the experimental sampling frequency (fps) and, inverting the latter, the sampling period (s) (Equations 12 and 13).

\[
\text{Sampling frequency} = \frac{450 \text{ frames}}{39.82 \text{ s}} = 11.3 \text{ fps} \tag{6}
\]

\[
\text{Sampling period} = \frac{1}{\text{Sampling frequency}} = \frac{1}{11.3 \text{ fps}} = 0.0885 \text{ s} \tag{7}
\]

Therefore, the sampling period, which is the time elapsed between frames and is referred in the code as \(dt\), is 0.0885 s.
5.2. Results and Discussion

The measurements have been repeated for five different intensities and 25 beads in total, as already mentioned. The results of calibration are represented with two graphs, each of them enlightening different characteristics of the setup. In addition, detailed results for each intensity are found in Annex IV.

On the one hand, the first plot shows the force as a function of the distance for different intensities (Figure 37). On the other hand, the second plot represents force as a function of the intensity at different distances from the tip (Figure 38).
Figure 37. Force as a function of distance at different intensities.

Figure 38. Force as a function of the electrical current intensity at different distances from the tip.
As it can be observed in the figures above, the force applied over the beads increases as the intensity of the electric current applied to the coil increases and decays exponentially with distance. The forces have been fitted with a two-term exponential using the cftool of Matlab and considering a 95% confidence boundary.

From the results, it can be concluded that the tip saturates at 1.5 A, understanding by saturation that the force does not increase even though higher currents supplied. The reason for this is that all the microscopic magnetic domains of the mu-metal rod are fully aligned to the external field with 1.5 A, so the magnetization does not further increase with increasing external magnetic field. However, in the vicinity of the tip (1 µm), an increment of ~0.7 nN is observed from 1.5 A to 2.5 A. Unfortunately, this value is not relevant as no experiments will be planned at such near distances.

In addition, at a distance of 20 µm, which would be the first distance realistic for experiments, currents higher than 1.5 A are not worthy to apply, as the increment is very low. At such distance from the tip, the resultant force is 0.44 nN at 1.5 A and 0.29 nN at 0.5 A. This two values are enough differentiable to consider that an increase of current drives an increase in force.

For middle distances (40 µm), the force ranges from 0.23 nN at 0.5 A to 0.31 nN at 1.5 A. According to what said previously, at this distance no difference is made for currents of 1.5 A and higher.

Finally, at distances as far as 60 µm, very little differences between intensities are observed. The forces generated are of about 0.18 nN at 0.5 A and 0.25 nN at 2.5 A. Therefore, it will be nearly equivalent to apply any intensity.

As for the decrement of force with distance, it is made clear from figure 38 that the force decays exponentially in the first 10 µm from the tip and decreases describing a nearly straight line with very little slope at greater distances.

Additionally, literature states that the forces needed for experiments range from 0.1 nN to 7 nN. Therefore, the tip is functional only for experiments requiring little forces, as distances as short as 1 µm are not practical. In case higher forces are required, a new tip will have to be built.

Lastly, the saturation current could be lowered decreasing the number of turns in the tip and the forces could be enhanced with a steeper gradient. This gradient could be achieved having a sharper tip, not rounded in the end, or smaller rod radius.
5.3. Experimental Problems

The calibration of the setup has been the part of the project that has taken more time due to the observation of an unexpected phenomenon related to the behavior of the beads. This event was seen when recording the videos of the calibration and consisted in the attraction and consequent repulsion of the beads when no intensity was applied. In order to solve it, different measures were taken. The first was changing the holder of the tip and replacing it to a fully plastic 3D printed holder, so the metallic screws of the old holder could no longer interfere with the current passing through the coil, hence could not affect the magnetic field. After this, the incident was still observed, so the decision of rebuilding the tip was made. Unfortunately, the new tip showed the same behavior. Then, the suspicion was centered on the voltage-current converter. According to this, the electrical circuit was revised and an EU-UK voltage converter was plugged to the voltage-current converter, but no changes were observed. Afterwards, the tip and the converter were sent to Barcelona and proved in a similar setup (IBEC). In the trials carried out there, the phenomenon was no longer observed. Therefore, it was guessed that the problem lied on the procedure to prepare the experiments and not in the setup itself. Once back in London, the solution was found and consisted in not leaving the tip connected overnight, as was also done in Barcelona. Finally, the phenomenon observed was explained as a residual field created by the residual current of the converter that after many hours could be enough powerful to induce a magnetic field in the tip.
6. Conclusions and Future Perspectives

In conclusion, the assembly, programming and calibration of a Cell Magnetic Tweezer with an electromagnet as a magnetic source has been presented in this project. First, the setup has been assembled. Second, a simulation of the tip has been done to understand the magnetic field generated and to enhance its value. These simulations have been carried out as accurate as possible and the tip has been designed according to the guidelines deduced from it. These are that a shorter length of the tip end and an increase of turns in the coil enhance the magnetic field, while the distance between the coil and the sharp end and between the coil and the opposite end have no perceptible effect on the field. In addition, any bead comprised in a range of ±30° can be considered for calibration and experiments, as the magnetic field is nearly the same at 0° and 30°. Third, a program to control all the individual parts of the setup has been implemented in LabVIEW and an additional software has been programmed to calibrate the setup, joining LabVIEW and Matlab. Lastly, a tip has been built and calibrated. The calibration has shown that, as expected, the force decays exponentially with distance and can be fitted with a two-term exponential. In addition, the force applied over the beads increases with the intensity of the electric current passing through the coil until reaching saturation at 1.5 A. The maximum forces are of about 0.6 nN at 10 μm, 0.45 nN at 20 μm and 0.3 nN at 40 μm. For greater distances, the force decreases and stabilizes around 0.2 nN. Therefore, the tip is only functional for experiments requiring low forces from 0.2 nN to 0.5 nN.

In order to add functionalities to the setup, a fluorescent camera will be added. With this modification, the visualization of fluorescent proteins and other entities inside the cell will be enabled while applying forces and recording the movement of the beads. Therefore, it will possible to see how the cells reacts to certain mechanical stimuli in real time. In addition, new tips will be built and calibrated learning from the process of building described in this project. Consequently, different ranges of force will be available for experiments in the laboratory.

As for the direction of research, the first step will be to reproduce experiments found in literature. This part was supposed to be included in this project but could not be done due to experimental problems with the calibration. After, an experiment will be carried out to test the hypothesis that an external force exerted on a cell (pulling force)
can induce an activation or a deactivation of the transcription factors. These results will be compared with the AFM (pushing force) results, which have been already acquired in the laboratory.

7. Acknowledgements and Personal thoughts

First of all, I would like to thank Dr Sergi Garcia Manyes for giving me the opportunity to do an internship in his laboratory and all the laboratory members for making me feel at home. Special thanks are due to Dr Marta Castro for supervising my project and helping me with any questions throughout these months and Dr Elvira Infante and Marc Mora for teaching me about biology and biophysics, while accompanying them in their experiments. Then, Dr Jose Francisco Trull for accepting to codirect my project at the distance. Dr Jose Antonio Lazaro for making possible the experience of doing the TFG abroad in an institution such as King’s College London. UPC (Universitat Politecnica de Catalunya) and King’s College London for facilitating an Academic agreement and the Erasmus Scholarship for the economic support. Finally, I would like to express my gratitude to my family for their unconditional love and support.

From a personal point of view, the project has been really enriching as I have been able to spend 5 months in an interdisciplinary laboratory, learning from all their members, and being able to get in touch with the high technology and resources available. Additionally, the project has allowed me to learn LabVIEW and SolidWorks, which will be really useful for my future career, and has helped me to become more autonomous and critic with the results obtained. Unfortunately, due to problems during the project, it has not been possible to include the biology experiments in the project. However, being in the biology laboratory, and learning how to grow cells, has given me another interesting perspective of science. In conclusion, it has been an experience from which I have learnt a lot and it has definitely helped me to grow as a scientist and as a person.
8. References