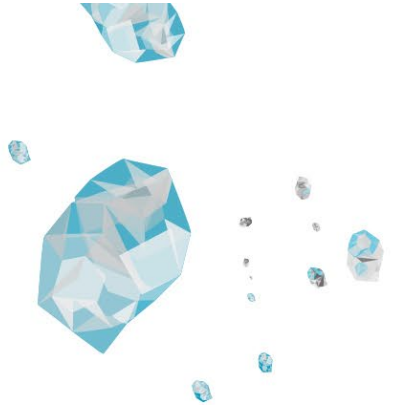




DESIGNING A PROTOTYPE CAPABLE OF LENGTHENING *IN VITRO* 3D ENGINEERED HUMAN SKELETAL MUSCLE TISSUE

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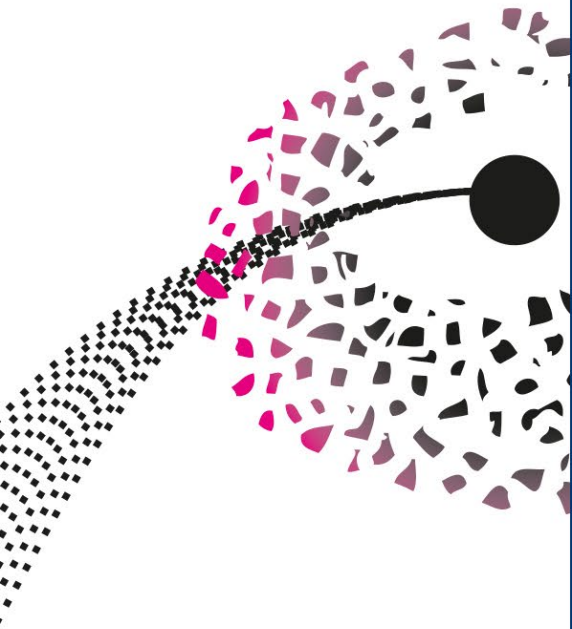
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Abstract

Researchers are able to grow small muscle tissues between two attachment points and make them contract using electrical stimulation. Currently, these attachment points are static. However, a core function of muscle tissues is their ability to change length; current devices to investigate in vitro 3D engineered human skeletal muscle tissues are not able to assess how this core function is affected during the experiment, for example, after adding a drug or applying an electrical/mechanical stimulation to the tissue. To be able to perform this function, a prototype system was designed which is able to change the distance between the attachment points. Being able to do this opens up countless possibilities for investigating tissue behaviour, from eccentric training (lengthening the tissue while electrically stimulated) to immobilisation of the tissue at a certain length. To assess how the tissue reacts to this, the force it can generate is an important metric, therefore the designed prototype is able to determine this force.

A list of functions and requirements for such a system is composed, after which it is used to design a system that should be able to fulfil them. The design is based on two parallel flexure guides; one is responsible for measuring the force, and the other for altering the tissue attachment point separation. The motion is actuated using a micro stepper linear actuator. The prototype was made, and initial tests were done to verify mechanical performance. It was concluded that the current prototype has limitations, but can be used to perform tests with and improve the design.

CONTENTS

1	Introduction	3
1.1	Context	3
1.2	Literature	4
1.2.1	Existing systems	4
1.2.2	Key takeaways	9
1.3	Goal	10
1.4	Challenges	10
1.5	Contributions	11
2	Functions and requirements	12
2.1	Functions	12
2.1.1	Possibilities	13
2.2	Requirements	13
2.2.1	Human interaction requirements	13
2.2.2	Environment requirements	13
2.2.3	Size requirements	14
2.2.4	Performance requirements	14
2.2.5	Force sensing resolution	15
3	Design	17
3.1	Main design choices	17
3.2	Material choice and specifications	19
3.3	Design overview	19
3.4	Measurement part	21
3.4.1	Measurement part displacement sensor	21
3.4.2	Parallel flexure guide measurement part	24
3.4.3	General remarks measurement part	26
3.4.4	Frequency	27
3.4.5	Gravity	28
3.4.6	Stiffness experienced by tissue	29
3.4.7	Weight	29
3.5	Movement part	30
3.5.1	General remarks movement part	30
3.5.2	Parallel flexure guide movement part	31
3.5.3	Frequency	33
3.5.4	Movement part actuator	33
3.5.5	Movement part displacement sensor	34
3.6	Prototype	35
3.6.1	Measurement part prototype	35
3.6.2	Movement part prototype	35

4	Results	37
4.1	Experiments	37
4.1.1	Experiment equipment	37
4.1.2	Determining stiffness of measurement part parallel flexure guide	37
4.1.3	Verifying force resolution and hysteresis	38
4.1.4	Verification of training motion accuracy and hysteresis	40
4.2	Results	41
4.2.1	Stiffness of measurement part parallel flexure guide	41
4.2.2	Force resolution and hysteresis results.	43
4.2.3	Verification of training motion accuracy and hysteresis	43
5	Discussion	45
6	Conclusion	47
7	Appendix	48
7.1	Formulas used for flexure calculations	48
7.2	Complete design rendering	50
7.3	Comsol simulation	52
7.4	Matlab Simulink model	52
7.5	Bogen sensor testing	53
7.6	Data mass measurement	53
	References	55

1 INTRODUCTION

In this master thesis, a prototype system was developed to change the distance between tissue attachment points to which *in vitro* 3D engineered skeletal muscle tissues are attached. To provide clarity, lengthening refers to physically increasing the distance between tissue attachment points, elongation describes the tissue's biological response through the addition of sarcomeres (contractile units) in series during myogenesis, and stretching denotes the application of tension to the tissue, which is achieved by increasing the distance between tissue attachment points. This chapter gives context about why this is done, the goal for the design, and a broad compact literature review that investigates existing systems and what can be learned from them. Furthermore, a list of design challenges and an overview of the contributions of this report are presented.

1.1 Context

Over the past decades, the field of *in vitro* skeletal muscle tissues has changed from growing 2D tissues, grown in a single plane, to 3D tissues, which grow in all directions and are superior in terms of mimicking structural and functional properties of *in vivo* muscles [1]. 3D tissues have a higher survival rate and make studying contractile functions and tissue adaptation due to mechanical loading possible [2]. All of these aspects make 3D tissues an essential tool for muscle-related investigations. Currently, all advanced 3D muscle engineered tissue systems found can only perform tests and stimulation on fixed length tissues; while being able to change length is a core function of muscle tissue, thus having a device that can also test how this core function is affected is an important step forward.

This would open up many possibilities for investigating the natural growth and adaptation of skeletal muscle tissues when exposed to length changes, both active (electrically stimulated), passive (not electrically stimulated) and over longer periods of time. Allowing research on how length changes affect tissue characteristics, such as length-force and velocity-force. At the same time: studying the effects of drugs on these properties, investigating the impact of training on damaged muscle tissue and how these characteristics recover over time are possible. The possibilities for investigating muscle response under the influence of electrical stimulations and physical training make 3D tissues very suitable for the goals set by the ERC Roboreactor grant for which this system is designed. This grant aims to investigate muscle damage recovery by electrical and mechanical stimulation [3]. However, a system that includes length changes also offers possibilities for many other tissue engineering studies.

For example, a length-force determination is essential to find how the tissue changes under the influence of an experiment. However, measuring a lower maximum force at the same tissue length after applying the test conditions does not always mean the tissue has become weaker. The tissues' relative length greatly influences the force it can exert [4], as illustrated in Figure 1.1. It could also be that the optimal length has changed when a lower force is measured,

meaning that the tissue has become stronger; however, the length at which the force peaks has changed. By finding the length-force relation, it is possible to determine if the optimal length, optimal force, or both change due to the experiment conditions; without this relation, which of the two has changed cannot be known. Furthermore, it is good to note that the optimal length is not a strict and fixed number; it will change with every measurement as the sarcomeres that make up the muscle have an optimal length range over which they are strongest [4].

One way to train tissue, is by lengthening it while being electrically stimulated, which is called eccentric training, a way of exercising muscle tissue to stimulate growth. Some variables influence the outcome of eccentric training: electrical stimulation, length range of the muscle during training, and length of the training stroke [4]. For a more detailed discussion, a reference to [4] is made, and these details will be omitted in this report; the focus is on creating a design that can perform eccentric training.

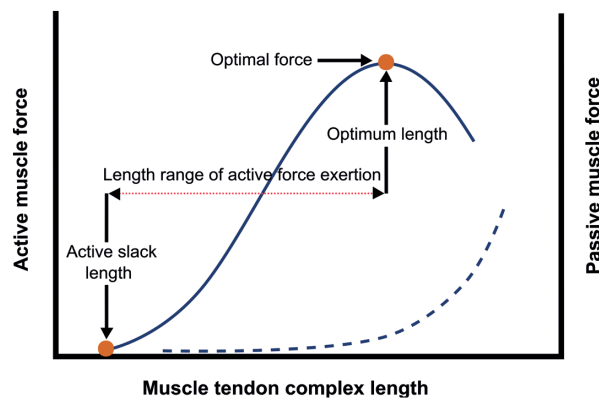


Figure 1.1: Schematic representation of length-force behaviour of skeletal muscle tissue, the relations between active (solid line), passive force (dashed line) and Muscle tendon complex length (tissue length) are shown [4]. It illustrates that there is an optimum length at which the highest muscle force is exerted during electrical stimulation, the optimal force. The tissue also has a length below which no force is generated during electrical stimulation, the slack length.

1.2 Literature

This section gives an overview of multiple existing systems selected based on their relevance to achieving this study's goal. First, all the systems are discussed, after which a list of all takeaways is given which are used for the design in this report. The main aspects that were focussed on when studying these systems are: measuring tissue force, electrical stimulation of the tissue, dimensions and conclusions drawn by the studies. Furthermore, a brief analysis is performed on the force-sensing resolution required for a system with the goal of performing eccentric training without damaging the tissue by overstretching.

1.2.1 Existing systems

PDMS pillars

System description

Initially developed for heart muscle tissue, but also suitable for skeletal muscle tissue, the system developed in [5] uses two flexible PDMS (polydimethylsiloxane) pillars; which are 0.5 mm in diameter and 3 mm long, to serve as tissue attachment points. The ends of the pillars are

coloured black using carbon black mixed with PDMS to improve visibility; onto the ends, a disk of 1 mm diameter and 0.1 mm height is moulded, which will prevent the tissue from sliding off the pillar. Six pillars are attached to one base to form three tissues per well; as a 12-well cell culture plate is used, 36 tissues are formed per plate. An overview of this system is shown in Figure 1.2.

Electrical stimulation and force measuring

A set of platinum electrodes stimulate the tissues electrically, at a maximum frequency of 1 Hz [5]. The force induced by the tissues on the pillars is measured by tracking the black pillar tips using image analysis captured by an inverted microscope; from these images, the deflection of the pillars is determined, which is converted to force using Euler-Bernoulli beam theory.

Conclusions from the paper

An advantage of this system is its modular approach [5]. One or twelve wells can be used simultaneously, depending on the needs and the experiment. Three tissues per well is advantageous, adding redundancy under the same conditions. The system uses standard commercial cell culture plates, which are readily available and optimised for the task. A significant decrease in the cross-sectional area was observed during the first five days of tissue development, related to fibrin compaction and tissue remodelling; matrix compaction happens from days 3 to 7 [5].

Conclusions about PDMS pillar design

The modular platform is a good starting point, however, creating the PDMS pillars with caps and black dots is a time-intensive manual process, this is known as it is currently being used at the university. The pillars are also not reusable, so a reusable less labour intensive design is preferred. Which still is modular and uses standard well plates, while incorporating adjustable pillar spacing.

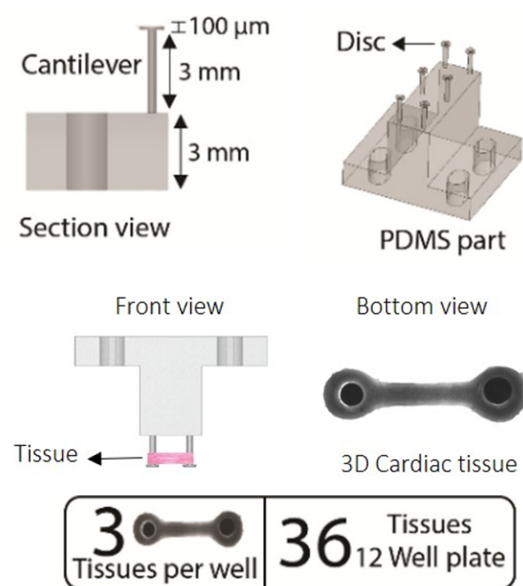


Figure 1.2: An overview of the PDMS pillar system, the tissue is grown around the cantilever(pillar), as seen in the front view. The 6 pillars are attached to one PDMS part/ In the bottom view, an image of tissue around the pillars can be seen. Figure adapted from [5].

Magnetic sensing on cardiac tissue

System description

Designed for heart tissue but also used for skeletal muscle tissues in [6], the system designed in [7] and shown here uses one flexible and one stiff pillar. The flexible pillar is made of PDMS with a 1mm^3 cubic magnet embedded in the pillar tip. The stiff pillar is also made of PDMS but reinforced with a glass capillary tube. The pillars are 12.5 mm tall, including a 0.5 mm disk on the ends, 1.5 mm in diameter and 8 mm spaced apart. One tissue per well is used with a 24-well cell culture plate. These dimensions give the flexible pillar a bending stiffness of $0.98\text{ }\mu\text{N}/\mu\text{m}$. Figure 1.3 shows an overview of the design, how the force sensing works, and photos from an experiment with tissues on the pillars.

Electrical stimulation and force measuring

To measure the forces generated by the tissue, the deflection of the flexible pillar is measured and converted to force with Euler-Bernoulli beam theory [7]. The deflection is determined by sensing the magnetic field the magnet embedded into the pillar tip creates. To make this work, each sensor had to be calibrated using 5 different pillars to account for variability. As this system is designed for heart tissue, a 7 Hz low pass filter was used as the heart tissue contracts below this frequency by itself, and electrically stimulating the tissues was done at 1.5 and 2 Hz. Finally, this system can measure forces between $26\text{ }\mu\text{N}$ and 1.96 mN. A specific force resolution was not mentioned.

Conclusions from paper

This system can be used to make real-time measurements, which is an advantage in ensuring that an experiment goes as planned [7]. Also, the measurement results can be directly converted to force without complicated image analysis, as other systems need, simplifying the experiment. Because no microscope is needed for the force measurements, the tissues can stay inside the incubator during tests, ensuring stable conditions. The pillars are disposable and not reused. Furthermore, this system cannot measure passive forces slowly developing in the tissue due to the implementation of a 0.16 Hz high-pass filter.

Conclusions about magnetic sensing design

Real-time measurements which can be directly converted to force are an important advantage which should be implemented in the design. While creating a design that can measure force inside of the incubator is the way to go for keeping stable conditions.

Magnetic sensing skeletal tissue

System description

In [6], the magnetic sensing system explained in the previous section is used for skeletal muscle tissue; it has been commercialised, but the basis is the same. It is stated that some adaptations are needed to make it work with skeletal tissue; what these adaptations are is not stated explicitly. However, instead of stimulation at 1 Hz, stimulations between 10-30 Hz are done to observe the tetanic (sustained muscle contraction) response.

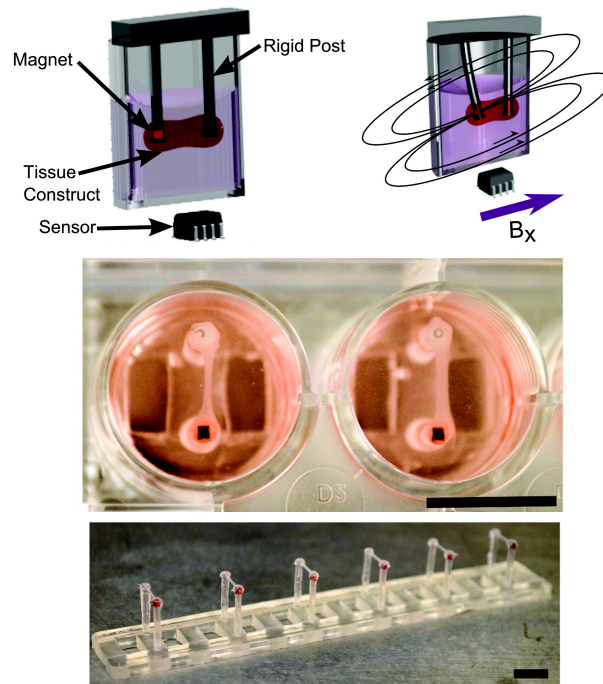


Figure 1.3: Figure showing the 2 pillars/posts with embedded magnet, how the magnetic field changes when the pillar bends, and photos of tests done with tissues attached. Figure adapted from [7]. Scale bar = 10 mm

Conslusions from paper

From day 7 onward, the tissues were strong enough to deflect the pillar, with forces improving over time [6]. Different post stiffnesses were used, where the more compliant ones already bent so much under passive force that no changes during active contraction could be observed. More compliant pillars weakened the tissues, lowering the measured forces and creating the impression that more resistance against contraction would lead to stronger muscles, as is observed *in vivo*.

Conclusions about magnetic sensing desing for skeletal tissue

Although the adaptations that need to happen to transform a system designed for hearth tissue to work with skeletal tissue are not specified, it can be concluded that there is a difference. From this paper the difference is electrical stimulation frequencies is mainly noted. Having stiffer pillars is also seen as an advantage to increase tissue strength.

Organ-on-a-chip

System overview

In [8], an Organ-on-a-chip device is made to grow skeletal muscle tissue around 2 pillars; formed between two layers of hydrogel attached to glass plates, see Figure 1.4. Cylindrical tissues were formed, of which the passive force could be calculated by measuring the strain in the hydrogel layer; by tracking fluorescent particles embedded into this layer. However, changing the length of the tissues is not possible using this approach. Therefor, it is not suitable for the goal of this research. However, important conclusions were drawn, which can be found in the next section.

Conclusions from paper

The study observed compacting of the muscle tissue from day zero until day twelve; see Figure 1.4. This compaction creates strains which are localised around the pillars. And found a pillar diameter to inter-pillar distance ratio of 1:5 to work best.

Conclusions about Organ-on-a-chip design

The organ-on-a-chip approach seems less suitable for a system with adjustable tissue attachment point spacing. However, the compacting observed is still relevant just as the pillar diameter to inter-pillar distance ratio. Trying to prevent high strains in the tissue by accounting for compaction and moving the tissue attachment points closer to each other will be a useful function, and together with choosing the best ratio is considered for the design.

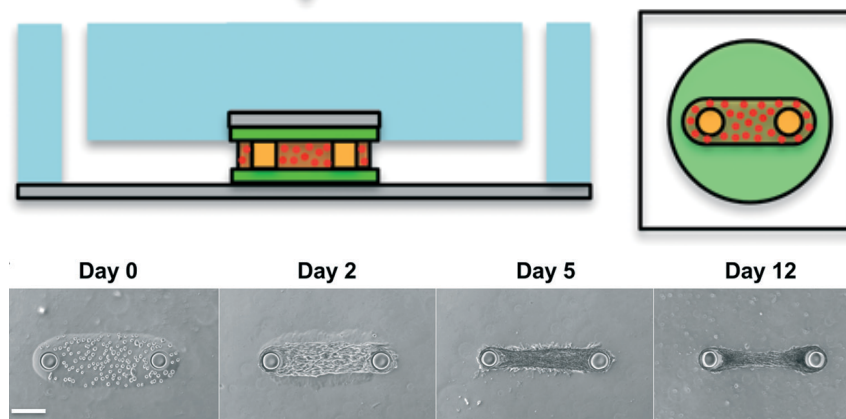


Figure 1.4: Figure showing a schematic top and side view of one device; the yellow (round) parts are the pillars, and the (red) dots represent skeletal muscle cells. Furthermore, microscope images of tissue development during 12 days are shown. The figure is composed of images found in [8]. Scalebar = 150 μm .

Interferometer measuring

System description

In [2], a system was designed specifically for skeletal muscle tissue. The system uses 1 flexible and 1 rigid stainless steel pillar. The carbon electrodes seen in Figure 1.5 are spaced 2 cm apart; no further dimensions are given for this system.

Electrical stimulation and force measuring

Measuring the deflection of the flexible pillar is done by a Fabry-Perot interferometer [2]. The deflection is determined, after which beam theory is used to calculate the force. The high interferometer sensitivity allows for nanonewton force sensitivity. Tissues were electrically stimulated up to a frequency of 75 Hz.

Conclusions from the paper

The system presented here can do nanonewton sensitive real-time measurements, with thousands of points per second of measurement frequency [2]. The system is especially suitable for drug response studies. Image based deflection measuring has limitations with respect to

resolution and cumbersome equipment. The stiffness of the flexible pillar was tested using a micromanipulator; calibration based on gravity was also used. The variance in the results of the manipulator tests was lower.

Conclusions about the interferometer system

Not much is design specifications of this system are known, however, it is an interesting concept with a very low force measurement resolution. This study goes up to 75 Hz for the electrical stimulation frequency on skeletal tissue. Which is kept in mind for the design.

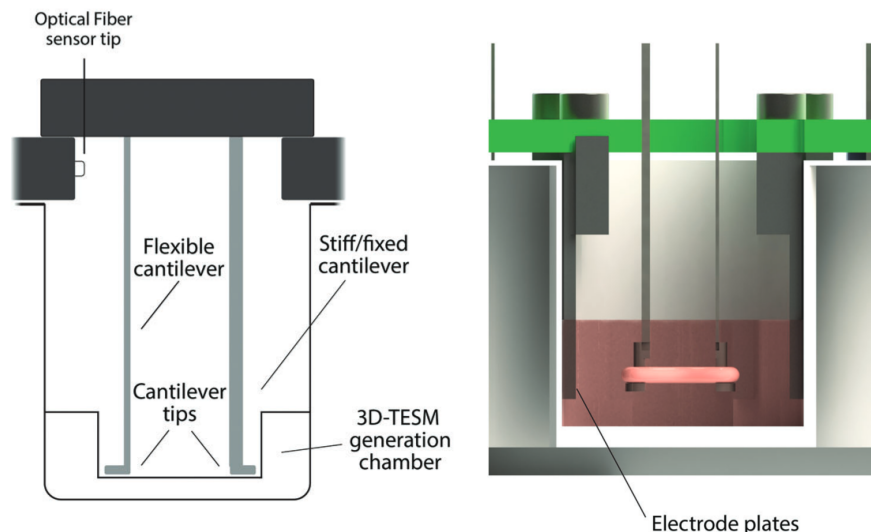


Figure 1.5: Schematic overview of the interferometer design, including optical fibre sensor tip, pillars (cantilevers), tissue and electrodes. The figure is composed of figures found in [2].

1.2.2 Key takeaways

To conclude the review of existing designs for 3D tissue engineered systems, a list summarises all the takeaways that will be used in the design.

- Multiple tissues per well creates redundant measurements under the exact same conditions, which is beneficial to confirm results [5].
- A modular approach allows for flexibility during experiments [5].
- The tissue undergoes significant compaction during the first 5-12 days [8]. To prevent strains during this phase, being able to move the attachment points closer to each other seems a good option.
- A pillar tip is needed to prevent the tissues from sliding of the pillars [5].
- Increased pillar stiffness creates muscles that generate stronger contractile forces [6].
- Force measurements are preferably done inside the incubator to have stable conditions [7].
- A pillar diameter to inter-pillar distance ratio of 1:5 works best [8].
- Real-time measurements are preferred over post-processing measurements [2, 7].
- For skeletal muscle tissues, higher stimulation frequencies (75Hz) [2] are used compared to cardiac muscle experiments (3Hz) [7].

Other remarks

Furthermore, it was noticed that most studies aim at short-term testing of muscle tissue's response to drugs, while this study aims for a design to perform long-term experiments spanning up to 4 weeks. Another difference between studies lies in how the tissues are formed; the different processes are outside the scope of this study; however, as it can affect the design, a method was chosen for this design. The choice is based on what is known by the researchers at the university and implies that liquid containing muscle cells has to be pipetted into a gelatine mould, which will form the tissue; thus, a way of inserting the pipette into the mould has to be designed. No system found can currently actively change the distance between the 2 pillars over a large range (100% lengthening), and as explained before, this opens up a range of new possible studies.

1.3 Goal

This thesis aims to design a prototype system for actively changing the length between tissue attachment points for engineered 3D human skeletal muscle tissue for experiments with a timespan of four weeks. The prototype could be used to perform the initial test, improve the design, and test the viability of the requirements. To be able to function as intended a way to measure the forces generated by the tissue while electrically stimulated is part of the prototype.

1.4 Challenges

A couple of aspects make the design particularly challenging. Starting with manufacturing limitations, to ensure an accurate, reliable, and repeatable manufacturing process, a minimum thickness of 0.3 mm was used for the electronic discharge machined parts (EDM). This limits how small parts can be made and, with that, their mass. Furthermore, the system is designed to be flexure-based, making it predictable and frictionless, but it comes with its own challenges. Concerning size, Five main design aspects add to the challenge of the design

1. System can stay in an incubator environment.
2. The eigenfrequency of the system should be maximised, as elaborated on below.
3. Design should be scalable to a full-scale experiment with at least 24 tissues.
4. The system can measure forces generated by the tissue with a resolution of 5 μN .
5. 100 % increase in tissue attachment point spacing should be possible.

For this design, the focus is on making a prototype that can be used for initial tests and verification of the requirements. However, this means that all the 5 aspects above should be met, to accomplish this. For this prototype, the system being stable for a 4 week period and being reusable is out of the scope. However, having a design which could be used for tests with tissue is.

Point 2 requires some further elaboration. A high eigenfrequency is desired as the force generated by the tissue also depends on how the tissue is electrically stimulated; the stimulation frequency determines the shape of the force-time curve and, based on that, the frequency of the movements of the pillar. The relation is shown in Figure 1.6, from which it can also be seen that the highest force is only reached once a fused tetanus is formed. The system eigenfrequency should be significantly higher than the frequencies found in the force signal, to not interfere with the measurement. However, at this point, it is not known what this highest frequency is in the

force signal. Therefore, the goal is to maximise it for this design and use the designed system to investigate which frequency is needed.

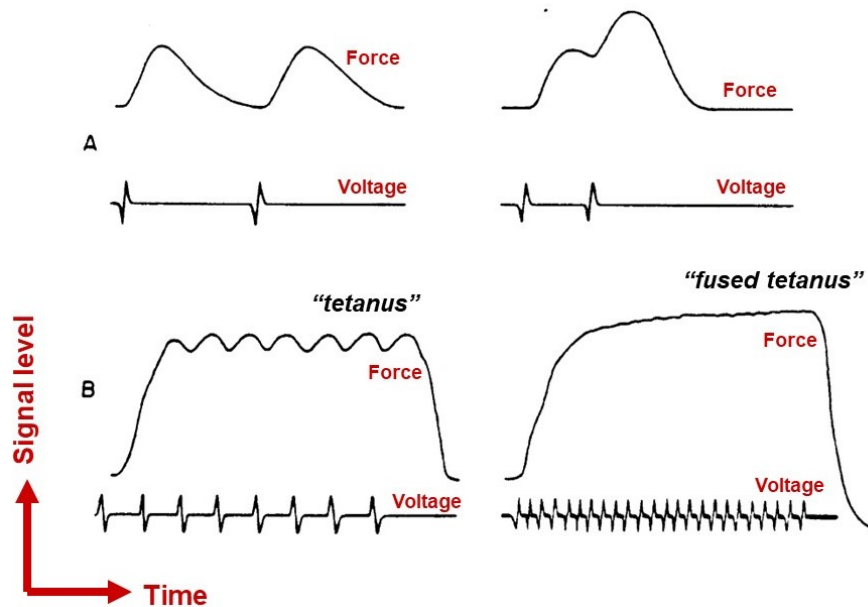


Figure 1.6: Representation of how the electrical stimulation frequency (Voltage lines in the figure), affects the force induced by the muscle tissue (Force lines in the figure) [9]. From this, it can be seen that low-frequency electrical stimulation creates low-frequency force peaks. However, the force peaks start merging together with increasing frequency; until they can not be differentiated anymore.

1.5 Contributions

The prototype design presented in this report is made to do initial testing of the idea and to validate the requirements in real lab conditions with tissues. In the next chapter, a list of requirements is set for a complete design. However, there are some assumptions which should be validated by performing tests for which a device is needed. The prototype design presented in this report incorporates an extensive range of motion of 100 % lengthening compared to the starting position, the option to compensate for tissue compaction by moving the tissue attachment points closer to each other, and a $5\ \mu\text{N}$ force sensing resolution with real-time measurements; for measuring active and passive tissue generated forces. The prototype is designed for 3D engineered skeletal muscle tissue, including the increased electrical stimulation frequencies compared to cardiac tissue systems. It should be investigated to what extent the design meets the design goals; in this report, the eigenfrequency and ability to measure $5\ \mu\text{N}$ forces will be verified. In the following chapters, first the complete overview of requirements is presented, after which a design, the reasoning behind it and the making of is given for the prototype. With this prototype tests are conducted to verify its designed mechanical properties.

2 FUNCTIONS AND REQUIREMENTS

A list of desired functions helps with knowing what to design. However, specific numbers the system should uphold are needed; making a requirement list solves this. Both are given in this section to clarify the system's demands. To start, Figure 2.1 was made to explain some terms as used below.

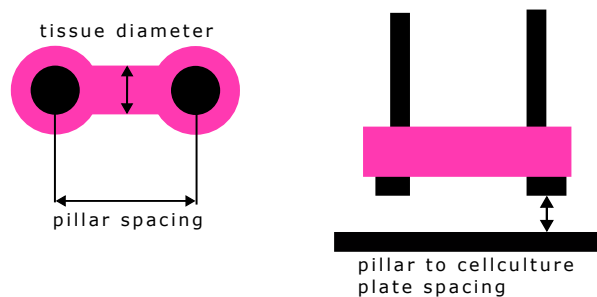


Figure 2.1: Overview of how the different distances were named.

2.1 Functions

This section lists the functions the system should fulfil, explains what they mean, and lists why they are essential. After this section, more tangible numbers, which are used for the design, will be given in the requirement section.

- **Isometrically measure force generated by the tissue in real-time** The system should be able to measure the force isometrically, which means the force is measured while the tissue length stays constant. Real-time measurements are preferred over post-processing because they allow the system to have immediate feedback during the experiment, and the tissue can be monitored in real-time.
- **The tissue can be electrically stimulated** To let the tissue contract and produce a force, it is electrically stimulated.
- **Tissue attachment point spacing can be actively increased by 100% from starting position** The distance between the two attachment points can be doubled during the experiment, leaving the tissue in an elongated situation during resting periods will stimulate length growth of the muscle [4].
- **A 3D engineered skeletal muscle tissue of 0.1 mm diameter can be formed around/ supported by the attachment points of the design** The device will be used to perform tests on 3D engineered skeletal muscle tissues, and keeping the tissues small is advantageous, as it requires fewer cells.

- **The system offers flexibility** A modular system is desired to create flexibility during the experiment. By having this, the amount of tissues can be varied; if something goes wrong, they can be removed individually.
- **The system can perform all its functions inside an incubator** This keeps the conditions as stable as possible to ensure less outside influence.
- **System has redundancy during experiments** Using two tissues per well gives redundancy under the exact same conditions so that experiment results can be verified.
- **The system is reusable** To make parts more cost-effective, reusability is a desired function of the system.

2.1.1 Possibilities

A list of possibilities for using a system with the functions mentioned above was made to show what it offers.

- **Compensate for tissue compaction** During the first days, the tissue compacts [5]. The pillar spacing should be modified to counteract the compaction, minimising strain in the tissue during this development stage and preventing the tissue from getting voids or tears. To have a safety margin, a 40% compaction in tissue length is designed for.
- **Determine slack length** As seen in Figure 1.1, the slack length is the longest length at which the tissue does not generate an active force when electrically stimulated.
- **Determine optimal length** The optimal length is the tissue length at which active force is at its maximum, as seen in Figure 1.1.
- **Perform eccentric training** Eccentric training is when the tissue is elongated while electrically stimulated. The elongation motion should be at max 1 Hz, a max stroke of 7.5% above optimal length, for 3 hours a day, for 4 weeks.

With the set of functions listed above, a set of requirements was formed that the system must meet to perform all functions. The requirements were divided into four categories: Human interaction, Environment, Size, and Performance.

2.2 Requirements

2.2.1 Human interaction requirements

- **The user can reach the tissue mould using a 20 μ L pipette tip** This is necessary for inserting the cells when forming the tissues.
- **Cell culture medium can be changed** The tissue will use substances out of the medium and excrete others. Changing the medium should be possible to keep the tissue alive.
- **Parts can be handled by the user** To make working with the system and cleaning possible, parts should withstand handling by human hands.

2.2.2 Environment requirements

- **System can work in an incubator environment for 4 weeks** The environment is CO_2 and temperature controlled with high humidity (80-95% RH) to prevent evaporation of cell medium.

- **System can withstand 121 degrees Celsius steam for 1 hour for multiple cycles** These conditions the system experiences during steam sterilisation.
- **Parts that touch tissue or medium are made of biocompatible material** Making the system biocompatible.

2.2.3 Size requirements

- **Pillar diameter is 0.5 mm** This size has proven to work [5] and is suitable for the small tissues that are desired.
- **Initial pillar spacing is 3 mm** Size that was found to work [5] and meets the 1:5 pillar diameter to inter pillar distance ratio that works best found by [8].
- **There is room for a set of electrodes** The electrodes should be placed and dimensioned to create a uniform electric field parallel and through the tissue.
- **System fits around a standard size cell culture plate** A cell culture plate has the following outside dimensions 127.7×85.48 mm. As these are readily available and proven, it is logical that the system uses them.
- **There should be room for placing a microscope camera** A clear line of sight through the bottom of the cell culture plate to see the tissues is needed. To confirm tissue generation, health and further analysis, being able to investigate the tissue by microscopy is an important requirement.
- **The bottom of the pillars is maximally 0.15 mm away from the bottom of the cell culture plate** Ensuring there is only a thin layer of liquid in between the cell culture plate bottom and the tissue, resulting in clearer microscope images while still allowing the pillars to move.

2.2.4 Performance requirements

- **Accuracy of displacements of moving pillar is $1.8 \mu\text{m}$** To accurately perform the wanted motion for eccentric training a 0.1% motion accuracy is desired. The smallest pillar spacing is 1.8 mm (if the tissue would compact 40%), 0.1% of 1.8 mm is $1.8 \mu\text{m}$, which will be the desired displacement resolution.
- **Pillar spacing can be reduced by 1.2 mm from initial position** Based on the initial pillar spacing (3 mm) and the 40 % tissue length reduction that is accounted for as maximum compaction.
- **The pillar spacing can be increased to 6 mm** From the goal of doubling the initial starting length of the tissue.
- **Force measurement range of 0-1000 μN** This comes from the goal of growing small tissues as that is desired; the starting optimal force should be around $300 \mu\text{N}$ for these tissues. The requirement leaves room for tissue growth and uncertainties.
- **Force measurement resolution of $5 \mu\text{N}$** As explained in Section 2.2.5. This resolution is desired to find the optimal length with a low enough uncertainty.
- **Force sensing bandwidth of 1000 Hz** To measure the force vs time response of the tissue, a bandwidth of 1000 Hz is desired, as the tissue will be electrically stimulated at frequencies well below this (estimated max 300 Hz), while at those frequencies the twitches have formed into a tetanus, thus the high stimulation frequency will not be seen

in the force response of the tissue. Furthermore, this bandwidth should be high enough to accomplish this experiment's goal. Taking into account the Nyquist criterium, a 2000 Hz sampling frequency for the force measurements is needed.

- **System can survive 4 million training motion cycles** 1 Hz movement for 3 hours per day for 4 weeks yields 302400 cycles per experiment. Accounting for 10 experiments and including a safety margin, a fatigue life of 4 million cycles is chosen.
- **Maximum displacement speed of 3 mm/s can be reached** Based on the eccentric training function description, the maximum speed of that motion was determined to be 2.83 m/s; hence, 3 mm/s was chosen.
- **Maximum acceleration of 18 mm/s²** From the eccentric training motion function, the maximum acceleration was determined to be 17.77 mm/s²; hence, 18 mm/s² was chosen.

2.2.5 Force sensing resolution

A force sensing resolution must be set for the requirement list. To determine this resolution, more literature research needed to be done to make an estimate, as no exact number could be found; this research is presented here and is required for performing eccentric training by knowing its limitations.

Eccentric training limitations

There are limitations to the eccentric training of muscle tissue. Eccentric training is much more prone to causing damage to tissue as passive stretch. 15% eccentric stretch training has proven to cause damage in *in vivo* tissue [10]. This setup is not mentioned in Section 1.2.1 as it is not performed on 3D engineered muscle tissues but on real humans. Therefore, as the engineered skeletal muscle during the experiment is expected to be more fragile, an upper limit of 10 % stretch above the optimal length is taken to ensure the tissues are not damaged. However, the optimal length will likely differ depending on the specific type of tissue used, the number of contracting units (sarcomeres) in series and the measurement setup used. Thus, this is more of a guideline than a fixed number, and a more precise number should be determined using the design made in this study.

Force sensing resolution needed

To ensure the upper limit of 10% lengthening is not exceeded, the optimum length should be determined within 5% of the true optimal length, ensuring that no damage will be done at 5% lengthening during eccentric training. As said before, optimal length is not a true fixed number, and it will shift around, but how much is not exactly known at this point and should be tested; however, for this reasoning, the assumption is made that the shifting is neglectable. The precision with which the optimal length can be found relies on how accurately the optimal force can be measured.

A look was taken at real length-force measurement results to see the effect of force sensing resolution on optimal length determination uncertainty. As no measurement results for 3D tissue engineered human skeletal tissue are available, a plot from the soleus muscle of a mouse is used, which should be comparable; the plot is shown in Figure 2.2. The forces are of a different order; however, this does not influence the shape of the curve. The force is around 1000 times bigger than the expected force of the 3D tissue engineered skeletal muscle tissues.

From Figure 2.2, it can be determined that a force sensing resolution of 7.5 μ N or better is

needed to determine the optimal length within a certainty of 5 %. To account for some uncertainties and possible differences, the requirement was to 5 μN .

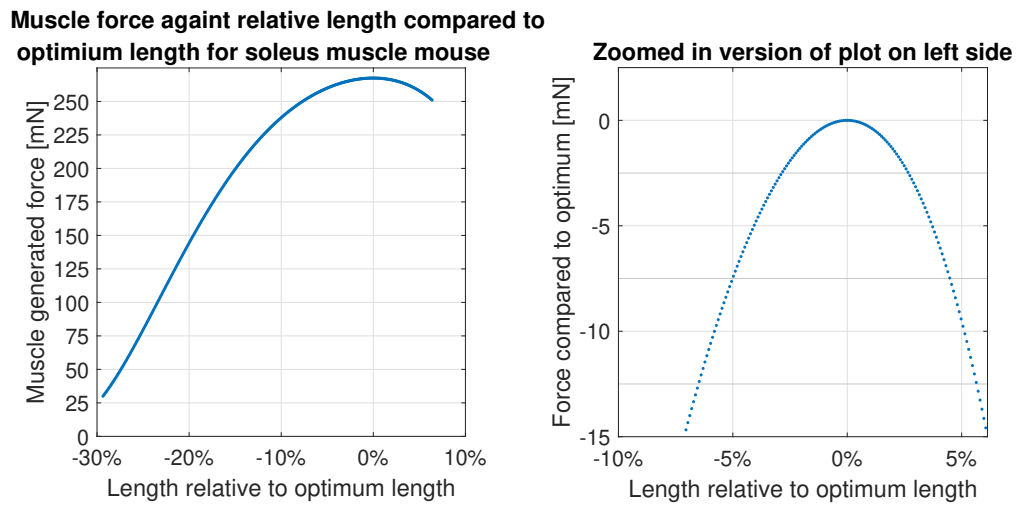


Figure 2.2: Trend line of the length-force curve for the soleus muscle of a mouse. The force generated by the tissue is shown against the muscle length relative to the optimum length. On the right an enlarged version of the same line. Based on data from [11].

3 DESIGN

The design chapter needs some explanation. First, solutions for the design's main functions are formulated in the main design choices section. After that, the material choice and equations for determining the guide's characteristics are presented. This is followed by an overview of the complete design and a more detailed discussion of the main two parts. The measurement part and the movement part, what these parts are will become clear in the design overview section. This chapter concludes with the presentation of the prototype.

3.1 Main design choices

To start the design process, first decisions need to be made on how to accomplish the main functions of the design, as these choices will have a major influence on the outcome. For this design, the following functions were identified as the most important. The functions do not fully overlap with the functions of the previous section. The changing tissue attachment point spacing function is split up into 2 separate ones:

1. Tissue attachment points
2. Changing tissue attachment point separation
3. Measuring force generated by tissue
4. Support and guidance of tissue attachment point movement

After identifying these main design choices, a morphological chart was made, listed in this Table 3.1. After this a motivation is given as to why a certain choice is made.

Tissue attachment points

Using two stiff pillars is chosen to serve as the attachment point for the tissue. The decision is made to measure the force not down in the well of the cell culture plate, but next to it to have more space available for the sensor and moving of the pillar. To transfer the force generated by the tissue to this location, without having too much deflection in this part, a relatively stiff pillar will be used.

Changing pillar separation

For changing pillar separation, the choice was made for an electromagnetic actuator that physically pushes the part to be moved. Transducing actuators were deemed a lesser solution as they would not keep their position without power, while hydraulics would require pumps and hoses. Electromechanical actuators are readily available, easy to control, and only need an electrical connection, making them the most suitable choice.

System function	Solution 1	Solution 2	Solution 3	Solution 4
Tissue attachment points	2 stiff pillars	2 flexible pillars	1 stiff and 1 flexible	Other types of tissue support (for example between wires)
Changing pillar separation	Electromagnetic actuator	Transducing actuator	Hydraulic actuator	
Measuring force generated by tissue	Strain gauge (load cell)	Pressure	Displacement	Force measuring using actuator
Support and guidance of pillar movement	Linear slide	Flexures	Air/magnetic bearings	Pivot joints

Table 3.1: Morphological chart with different solutions to make the main design functions possible.

Measuring force induced by tissue

Different options were considered, but to reach the requirements and functions of 5 μN , real-time results and a 1000 Hz bandwidth, the choice was made to measure displacement and convert this to force. It turned out that it is easier to find very accurate displacement sensors compared to off the shelf force sensors, no load cell was found that met the requirements or could be implemented into the system. Besides this, force measurements using the actuator proved no benefit, as the displacement sensor that would be used in the actuator could also be used stand-alone to do the force measurement. The selection of which type of sensor is used will be explained further in Section 3.4.1.

Support and guidance of pillar movement

To support the moving pillar and guide its motion, a decision needs to be made on the type of guidance system to use. Different options were considered, however, preventing hysteresis (position history dependence on the system) and contamination were important factors in making the decision. Hysteresis will create uncertainty in the displacement measurement of the measurement part, causing force measurements to be less accurate, while friction could create small particles that could end up contaminating the tissues. In order to prevent both these problems, a frictionless solution is preferred. Leaving flexures and contactless bearings (air and magnetic) as options, when price and simplicity are also considered, flexures are the most suitable choice. Flexures have no friction, do not need lubrication, minimising the contamination risk, while being very predictable, making the force measurements more accurate and stable. Different kinds of flexure-based guidings could be used; however, for this design, the parallel flexure guide was selected as the preferred choice for supporting and guiding the moving parts in this design.

Parallel flexure guides ensure an approximately straight movement while also being compact in the width direction; compared to using six folded leave springs linear guide, this compactness is a benefit for placing more units around a well plate. Parallel flexure guides do suffer from some parasitic motion; there is some up and down movement in Figure 3.1, but this is managed by using a small stroke and choosing a suitable flexure length as longer flexures have less parasitic motion at the same stroke.

3.2 Material choice and specifications

The functions, reusability, and biocompatibility were considered for choosing a material. In the end, the choice was made to go with metal due to its stability over time and ductility. This is essential for reuse and longer-running experiments, ensuring the results are not altered by changing material properties. Ceramics are also very stable over time; however, they are less suitable for the repeated bending the material would encounter. To also comply with the biocompatibility function, grade 5 titanium is chosen for the part that comes in contact with the tissues, as titanium is the most bio-compatible metal [12] and grade 5 the strongest titanium alloy [13]. For the components that are not close to the tissue where biocompatibility is far less of a concern, aluminium was selected, as it will not corrode due to its protective oxide layer and is less expensive compared to titanium. However, aluminium is less suitable for making bending parts out of, as it has a lower fatigue strength [14], compared to other metals (Table 3.2). Therefore, another more suitable metal was selected for those parts: stainless steel 316, which is used for medical devices [15]. The relevant mechanical properties of these materials are listed in Table 3.2 as they are needed for the design calculations.

	Titanium (grade 5)	Aluminium(6061)	Stainless steel (316)
Yield strength (tensile)	880 MPa [16]	276 MPa [17]	332 MPa [18]
Fatigue strength (10,000,000 cycles)	510 MPa [16]	50 MPa [14]	270 MPa [19]
Modulus of Elasticity	113.8 GPa [16]	68.9 GPa [17]	165 GPa [18]
Density	4430 kg/m ³ [16]	2700 kg/m ³ [17]	8000 kg/m ³ [20]

Table 3.2: Material properties of the materials used in the design.

3.3 Design overview

A design is made that incorporates the main design choices of Section 3.1, and is capable of realising the goal. In this section, an overview of the design, followed by a more in-depth analysis of the individual parts, is presented.

To meet the modular design function, each pillar with its corresponding sensor and actuator was mounted to an individual movement and measurement unit (MMU). Each MMU can be fastened to the base frame by itself such that an experiment can be done with one or sixteen tissues for each base frame. An overview of how this could look, including electrodes and the stationary pillars, can be seen in the appendix in Figure 7.1.

The remainder of the report will focus on the design of the individual MMU. Although some choices are influenced by the full design, this will be mentioned when this is the case. Figure 3.1 shows a complete overview of one MMU.

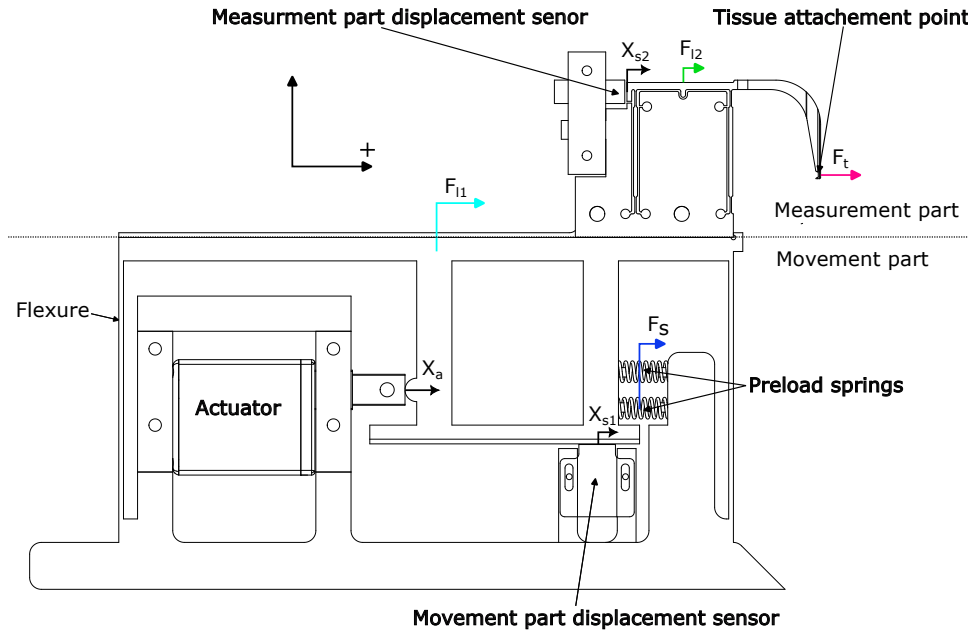


Figure 3.1: Overview of one complete movement and measurement unit as was made during prototyping, with the names of important parts. The most important forces acting on the design are shown, F_{l1} (Leaf springs 1), F_{l2} (Leaf springs 2), F_t (Muscle tissue force) and F_s (Preload spring force). Besides the forces the actuator and sensor positions are shown, X_{s1} (Sensor bottom part), X_{s2} (Sensor top part) and X_a (Actuator position). Bolts holding the parts together are excluded to prevent cluttering.

To illustrate how the system would move, the following images were made to show the movement (Figure 3.2).

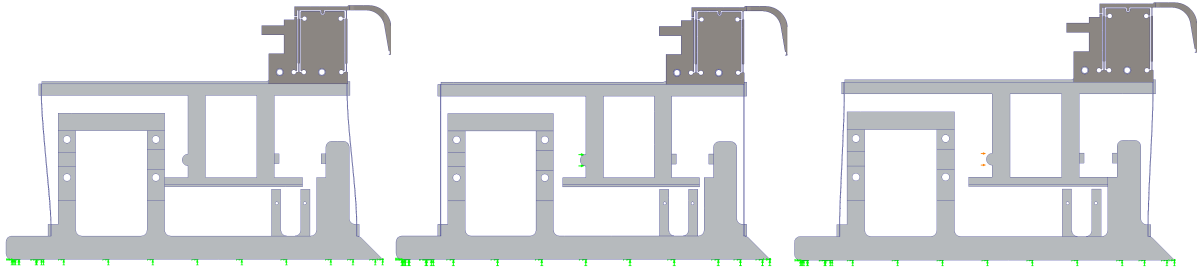


Figure 3.2: Picture series of the movement range of the MMU.

From here onward, a distinction is made between the measurement part and the movement part. First, a detailed design of the measurement part will be given, followed by the movement part.

3.4 Measurement part

In this section, the dimensions and other important nuances of the measurement part are discussed. As the name suggests, this part is responsible for measuring the forces generated by the tissue. This is done by measuring the deflection of the parallel flexure guide under the load of the tissue force and converting this to force using the stiffness against displacement of the guide.

The stiffness against displacement is the most important specification of the measurement part. It determines the size of the flexures in the parallel flexure guide, and the eigenfrequency of the system. However, the sensor resolution is the limitation in choosing a stiffness. The desired stiffness against displacement is based on the sensor resolution and the force resolution requirement; with these numbers, the highest allowable stiffness against displacement is calculated to meet the requirements and maximise stiffness. A high stiffness against displacement is desired concerning the vibration modes of the system. An example of the calculation for the maximum stiffness against displacement is given here:

$$k_{x,wanted} = \frac{\text{Force sensing resolution}}{\text{Sensor resolution}} = \frac{5\mu\text{N}}{2\text{nm}} = 2500 \text{ N/m} \quad \text{max stiffness against displacement} \quad (3.1)$$

So to find out what the stiffness of the system can be first a sensor needs to be selected.

3.4.1 Measurement part displacement sensor

Choosing a sensor for this application is an elaborate consideration that significantly affects how well the system will meet its functions. A robust, precise, fast sensor is desired, which is also not too expensive, as 24 are needed for a full-scale experiment; while functioning in challenging humidity conditions. The sensor specifications also significantly affect the design, in addition to maximum stiffness against displacement. For example, the type of sensing affects whether a measurement scale is needed and, with that, an increase in moving weight and a decrease in eigenfrequency. Therefore, the sensor properties are strongly connected to the system properties, dimensions, and price tag.

In the following sections, in-depth reasoning is given about how the choice of sensor was made. First, the implications of sensor choice are explored in detail, after which possible sensor options are presented and concluded by informing about which sensor was chosen.

Connection between sensor choice and design

Three main parameters come into play for this choice, the sensor resolution, stiffness against displacement of the parallel flexure guide, and the moving mass of this guide. They together determine the performance metrics: force sensing resolution and eigenfrequency. To get a feeling of how the parameters affect the performance metrics, the mathematical functions that connect them are examined. The relation between stiffness, force sensing resolution, and displacement sensing resolution is linear (Equation 3.1). Making it easy to imagine how one variable will react to changing another. However, the relation of stiffness and mass to eigenfrequency is a square root relation (Equation 3.2). Figure 3.3 was made to show this square root relation. In this figure, the force sensing resolution is used to convert stiffness into the displacement sensing resolution (Equation 3.1), which is needed to have a 5 μN force sensing resolution; this is done as stiffness will be based on the sensor resolution and not chosen by itself. Furthermore, the effect of mass on the frequency is shown in this graph, as this can be altered to a certain extent by adjusting dimensions.

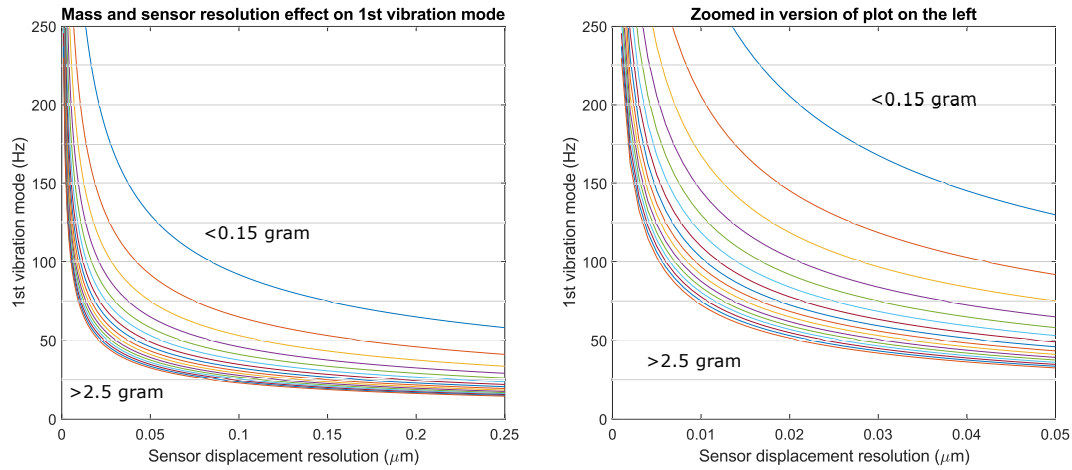


Figure 3.3: Plot showing how stiffness (here converted to needed sensor resolution based on the force resolution requirement) is related to the first vibration mode of the system, for different moving weights, ranging from 0.15 to 2.5 grams, at 0.15 gram intervals. On the right a zoomed-in version of the same plot.

From these relations and the plot, it can be concluded that to increase system performance, either the mass should be lowered or the displacement sensor resolution increased (and with that stiffness against displacement can be increased). However, the effectiveness of changing a variable is not always the same.

While also taking the other requirements and functions into account an optimum needs to be found; however, for example, making the system sturdy enough to handle by hand is hard to express in numbers, making this a difficult optimisation task.

In finding this balance it is good to look at what is needed and which boundaries are set by this. To give an idea, for achieving an eigenfrequency above 100 Hz, a sensor resolution of 75 nm or lower is minimally desired at a 0.15 gram moving weight, and 5 nm at 2.5 gram moving weight, as was read from Figure 3.3.

Finally, the conclusion can be drawn that a sensor with a maximum resolution of 150 nm would be needed (to have an eigenfrequency above 75 Hz), as reducing the weight to 0.15 grams or beyond would be very challenging with the current design aspects and manufacturing limitations, as a much lower stiffness out of the leaf spring would be needed (33.33 N/m at $5\mu\text{N}$ force resolution and 150 nm displacement resolution), it may even be impossible. As lowering the stiffness requires the flexures to become longer, thereby increasing the weight. The 75 Hz is based on the minimal wanted eigenfrequency in order to check if the electrical stimulation frequency causes tetanus contraction in the muscle.

In addition to which resolution is wanted in theory, which resolution is achievable in real life is also considered. As measurements on the nanometer level are susceptible to outside influences, vibrations and temperature changes that can affect the result. A low resolution is wanted to increase force sensing resolution. However, it comes at the cost of more susceptibility to outside influences, therefore careful considerations need to be made.

Possible displacement sensor options

By having identified that the maximum sensor resolution is desired to be 150 nm and considering other factors like added weight by the sensor (moving weight), dimensions, cost, measurement range, working environment, sensing area required, noise, and alignment, different sensors

RMS resolution	0.78 nm
Bandwidth	14000 Hz
Measurement range	50 μm
Dimensions(diameter)	5 mm

Table 3.4: Lion precision 6-D capacitive sensor specifications [21]

were selected and rated on how well they would suit the needs for this system. An overview of various kinds of sensors with their corresponding measurement range, added weight, resolution, and cost are given in Table 3.3. How well the sensors handle the humidity mostly depends on whether the conditions are condensing or not, as water droplets will disturb the measurements. However, most(possibly all) sensors in this precision regime are not tested in these conditions by the manufacturers. This makes it difficult to conclude without testing the sensor under these conditions if it can survive the humidity or not, especially for elongated periods, so testing should be done.

After balancing the weight, the size of the part, manufacturability, eigenfrequency, and stiffness, the choice was made for a sensor with a resolution of 2 nm or better, which at the corresponding design weight and stiffness would result in an eigenfrequency above 200 Hz.

Ultimately, the best sensor for this case was determined to be an optical encoder, mainly because it costs significantly less than other options that could work. The added weight is also a downside of the optical encoder; however, this is very manageable on the design moving weight. How well it handles humidity will need to be tested.

Sensor type	Measurement range	Added weight (per cm range)	Resolution	Cost (€)
Interferometer	0-4 mm	-	<1nm	8000-10000
Capacitive	0-10 mm resolution dependent	-	<1nm	6000-8000
Optical encoder	Limited by scale length	0.05 gram	$\simeq$ 1nm	800
Magnetic encoder	Limited by scale length	0.72+0.36/cm	$\simeq$ 250nm	100

Table 3.3: Different sensor options, numbers are based on requested quotes and online information of available sensors. References are not included as not all information is publically available.

Prototype displacement sensor

Due to the timeframe of this thesis, and the aim of first testing the design using the prototype before significant amounts of money are spend. The choice was made to use an available capacitive sensor. The capacitive sensor is a Lion Precision C6-D, combined with a CPL290 driver. In Table 3.4 the critical specifications from the calibration report of this specific sensor can be found. According to these specifications, the sensor will suffice to verify the functioning of the prototype.

A clamp is designed to keep the displacement sensor fixed to the measurement part. The clamp works by adjustable friction fits for keeping the sensor secure to the clamp and the clamp to the measurement part. The clamp in its location can be seen in Figure 3.4.

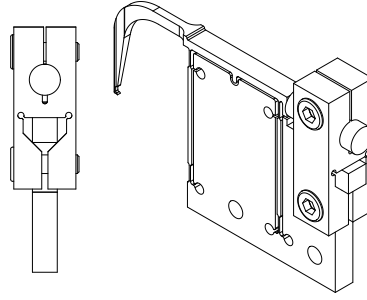


Figure 3.4: Rendering of measurement part including the clamp and capacitive sensor.

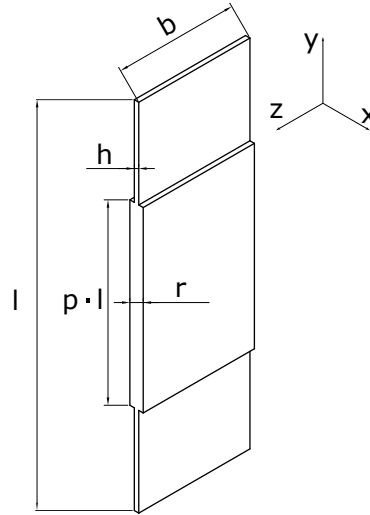


Figure 3.5: Drawing of how the different dimensions of a flexure are defined. l =length, h =height, b =width, r =reinforced height, p =reinforcement percentage.

3.4.2 Parallel flexure guide measurement part

The design of the parallel flexure guide for the measurement part is crucial, as it affects all system functions related to force measurement. With the sensor resolution and coupled desired stiffness against displacement known, the guide can be designed. First, the manufacturing limitation is repeated; the minimum material thickness should be 0.3 mm to make reliable manufacturing possible. To eliminate hysteresis, the measurement part is designed to be made of one solid disk of titanium; the downside is that the minimum flexure height is equal to the manufacturing limitation, which would not be the case if the guide was assembled. However, the assembly would add hysteresis. The height, width, and length dimensions of the flexures are discussed in individual sections.

Height The flexures were designed to have a height of 0.3 mm; lower would be preferred, but it is impossible due to the manufacturing limitation. Thicker would require longer or narrower flexures to keep the same stiffness against deformation, which is unwanted to keep the design compact and stable; therefore, the minimum height is chosen.

Width Different factors influence the choice for the flexure width, to start with the sensor; depending on the type of sensor and how it measures, different widths are required to be able to perform measurements on or to attach the measurement scale to. Besides the sensor, the out-of-plane stiffness and how fragile the part becomes are important factors in choosing a width. In the end, 4 mm was selected as a suitable number for this design; after a prototype is made, this number should be revised based on the findings. Currently, the part's width is constant except where it tapers down to the pillar dimension. More width changes based on where they

are needed could be considered, but for now and for manufacturing simplicity, the width is kept at 4 mm.

Length With the height and width of the flexure selected, the length is the only variable remaining to adjust the stiffness against displacement; thus, the length is used to tune this stiffness.

Using the desired stiffness against displacement and considering manufacturing uncertainties, a design stiffness against displacement can be selected with which flexure length is determined. This design was aimed at around 2000 N/m stiffness against displacement using analytical formulas that did not account for fillets. However, they do account for the reinforcements incorporated into the flexure design. Reinforcing the flexures significantly improved manufacturability by reducing the lengths over which a strict dimensional tolerance must be achieved from 23 mm to 7.685 mm (in two sections) per flexure. The consequence of reinforcing is an increased mass, thereby lowering the eigenfrequency of the parallel flexure guide and lowering the flexure's internal frequencies; however, simplifying manufacturing was preferred for this design. 71 per cent of the flexure length is reinforced, as this was found to be a good compromise [22].

The full design, including fillets and reinforcements, was analysed using Comsol (Appendix 7.3) to calculate the estimated stiffness against displacement accurately. At 26.5 mm long flexures with 71% of the length reinforced, an estimated stiffness of 2380 N/m was found, which is a good number to aim at for the design. A stiffness lower than the maximum will result in a less optimal eigenfrequency, while a higher stiffness will cause the stiffness not to meet the force sensing requirement.

Measurement part parallel flexure guide specifications

The specifications of the parallel flexure guide with the dimensions from the previous section can be found here. Using the formulas listed in Table 7.1, the results in Table 3.5 were calculated and supplemented with simulations performed in Comsol. Multiple conclusions are drawn from Table 3.5. Starting with the max stress staying well below the 10 million cycle fatigue limit, implying the 4 million cycle requirement is met. Also, the pillar tip to cell culture plate spacing requirement is no problem as the shortening effect over the 0.4mm stroke is only 0.2µm. The stiffness against displacement, computed using Comsol to take the fillets into account, was 2380N/m. In-plane guiding stiffness, buckling force, internal vibration frequency, and out-of-plane stiffness do not require a specific number to meet the requirements. However, the stiffnesses are wanted to be 3 times higher than the displacement stiffness to limit movement in those directions. At the same time, the buckling force says more about how sturdy the part is for this design, and the internal vibration frequency of the flexures should be double the eigenfrequency of the guide to ensure it does not interfere with measurements. Last but not least, the reinforcement ratio is calculated; this number should be kept at least above 20 to ensure the reinforced parts can be considered rigid compared to the thin parts of the flexure, which it is for this design [22].

Movement tracking

A Simulink simulation is performed to ensure the stiffness of the flexures is high enough to follow the movements created by the movement part. The part is modelled as two masses attached together and to the ground with springs; one mass can be actuated, and the position of the other is measured. An overview of this model can be seen in Appendix 7.4. The masses and spring constants are according to the design, and a damping constant of 0.05 N·s/m was assumed. As an input, the cyclic training motion is given, and as output, the motion of the pillar tip. Figure 3.6 shows how the pillar follows the movement part. There is some difference between the two lines. However, the lines are never 1µm or more apart, which is acceptable.

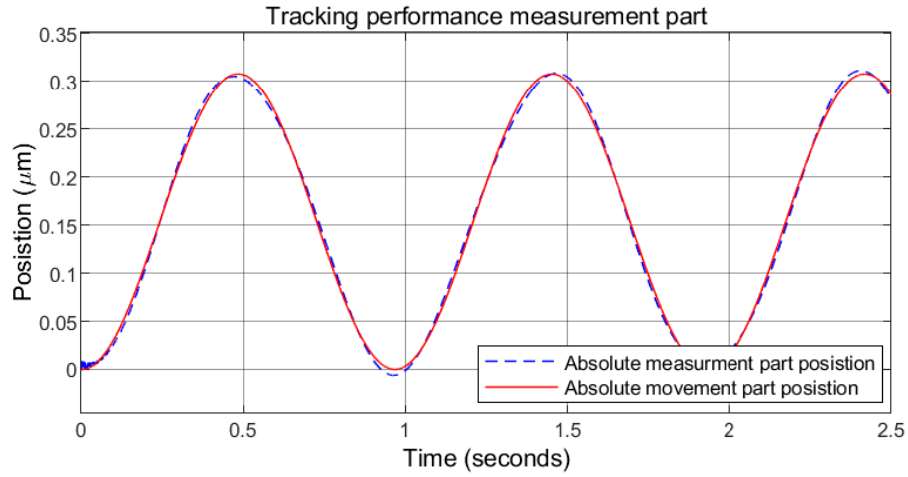


Figure 3.6: Showing the tracking behaviour of the measurement part compared to the movement part.

Dimensions($l \times b \times h$)(mm)	$26.5 \times 4 \times 0.3$ 71% reinforced
Max bending stress due to translation	$90.8 MPa$
Shortening	$-0.2 \mu m$
Stiffness against displacement	$2380 N/m$ computed using Comsol
In-plane guiding stiffness	$1.7 \cdot 10^7 N/m$ at $\Delta x = 0.4 mm$
Correction for unconstrained situation made using graph found in [22] (per flexure)	(while constrained in x) $0.85 \cdot 10^7 N/m$ at $\Delta x = 0.4 mm$ (while unconstrained in x)
Buckling force (per flexure)	$684 N$
Internal vibration frequency	$1.82 \cdot 10^7 Hz$
Out of plane stiffness	$30199 N/m$ at $\Delta x = 0 mm$ and $y = 26.5$
Correction for deflected position made using graph found in [22] (Formula for unreinforced flexure is used, values will be higher)	$5210 N/m$ at $\Delta x = 0.3 mm$ and $y = 26.5$
Reinforcement ratio	22.74

Table 3.5: Overview of important specifications measurement part parallel flexure guide.

3.4.3 General remarks measurement part

Dimensions of measurement part

The measurement part dimensions, as seen in Figure 3.7, are mainly based on the dimensions for the flexures of the parallel flexure guide (Section 3.4.2), available room (considering the function of working with a standard well plate and place for electrodes) and manufacturing (lower limit of 0.3 mm on thickness, manufacturing time/cost and needs for example start holes) and usability considerations (making the part too fragile could result in deformation during handling and manufacturing). Now four important features of the design shown in Figure 3.7 are discussed.

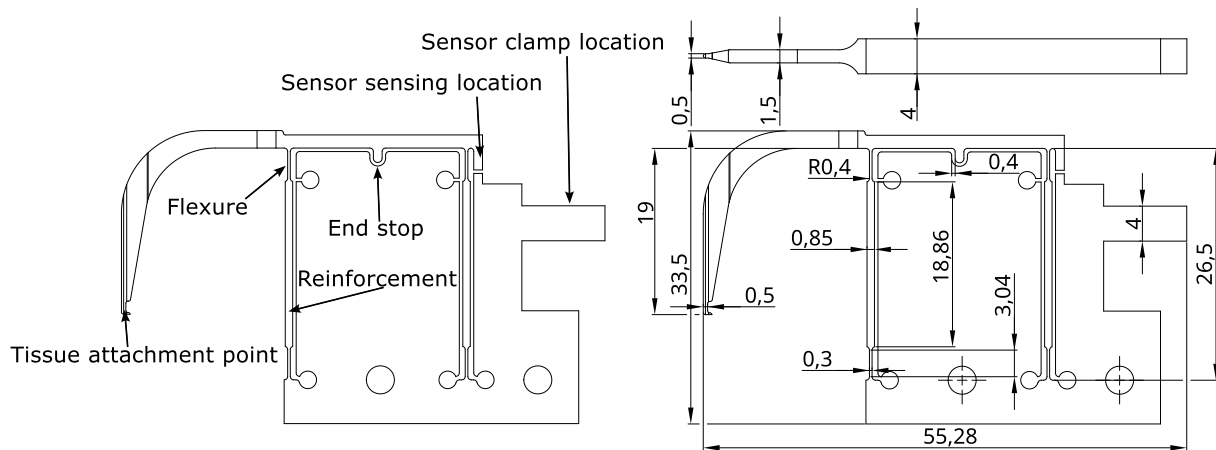


Figure 3.7: On the left, the measurement part, with names of parts indicated. On the right, a technical drawing of the measurement part, with the most important dimensions in mm. 71% of the total leaf length has the reinforced height ($p=0.71$).

Sensor clamp location

In Figure 3.7, a square part of 4×4 mm can be seen; this part is designed to act as a clamping location for attaching the sensor, which measures the displacement. More on this part and how the sensor is attached to it in the sensor section (Section 3.4.1).

Sensor sensing location

To give the capacitive sensor an area to sense, a flat area of 4×4 mm was included for this purpose.

Tissue attachment point

The end of the pillar seen in Figure 3.7, serves as an attachment point for the tissue. It is 0.5 by 0.5 mm, with one side rounded off and the other side having sharp corners. However, this cannot be avoided due to limitations in access during the manufacturing process. The end is L shaped to make sure the tissue can not slide off. Tests should be done to verify this design will work, or adaptations need to be made. For example, removing sharp edges by etching material away.

End stop

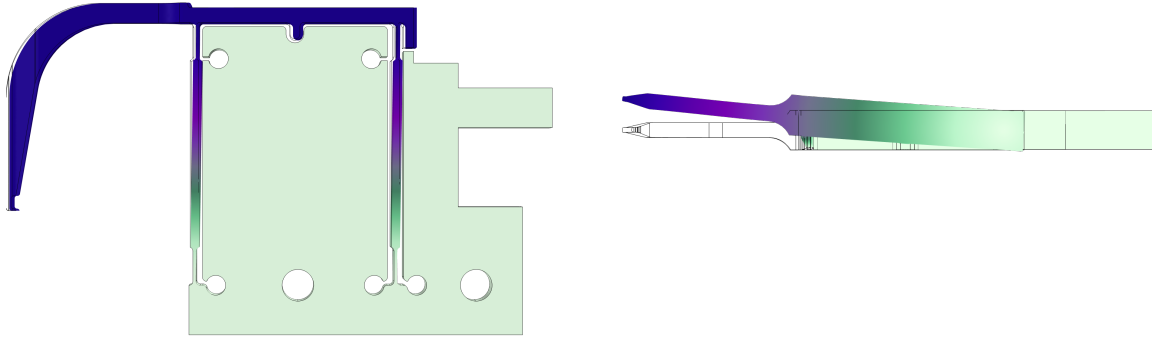
To prevent altering the properties of the flexures by overly deflecting them, an end stop was incorporated, limiting the stroke to 0.4 mm in both directions (Figure 3.7); which also prevents the flexures from coming in contact with other sections of the measurement part and causing damage. The end stop was incorporated in the centre of the parallel flexure guide to provide symmetry.

3.4.4 Frequency

Vibration modes

To ensure the measurement part can actually measure changes in the force generated by the tissue at the desired frequency, the system's vibration modes are evaluated. When the force

signal's frequency becomes close to the first vibration frequency, it will vibrate at that frequency and disturb the force measurements. Therefore, this is an important number for the system's performance. The first and second vibration modes of the top part were analysed using Comsol; the results can be seen in Figure 3.8. The first mode is the most important and is found at 212.63 Hz; this will limit the frequency of the force measurements, if this frequency is sufficient has to be tested using the prototype.



(a) First vibration mode of the system (212.63 Hz), (b) Second vibration mode of the system (1025.8 Hz), torsional mode of the part.

Figure 3.8: First and second vibration mode of the measurement part as analysed using Comsol. A fixed constraint was applied to the bottom face.

Predicted moving weight

With the stiffness and the first vibration mode frequency calculated using the model, the expected amount of moving weight can also be calculated. Using the following formula for the first vibration mode in Hz:

$$f = \frac{1}{2\pi} * \sqrt{\frac{k}{m}} \quad (3.2)$$

Based on the parallel flexure guide's modelled stiffness and frequency and the equation, the expected moving weight is 1.333 grams.

3.4.5 Gravity

If the measurement part is not exactly level in the direction of motion, gravity will deflect the parallel flexure guide. As the forces that need to be measured are in the order of μN , gravity does pose a problem when moving the device after the force has been zeroed. A minute change in the tilt already affects the results; the following calculation was made to see how big this effect is. To know how much the guide deflects under the influence of gravity, the component of gravity in the movement direction of the guide needs to be determined. Figure 3.9 was made to show how this is done. Using the sin equation, the $F_{g,x}$ force component can be determined, which caused the guide to deflect (ΔX), based on the stiffness in the displacement direction of the guide

$$F_{g,x} = \sin(\theta) \cdot m \cdot g \quad (3.3)$$

$$\Delta X = F_{g,x}/k \quad (3.4)$$

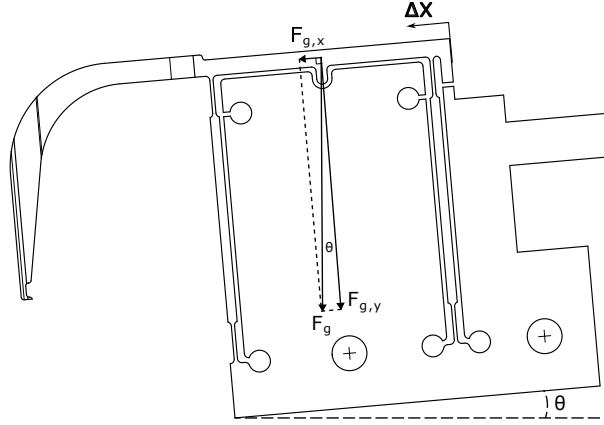


Figure 3.9: Figure showing how the gravity force acting on the measurement part can be dissected into 2 components.

$$\frac{d\Delta X}{d\theta} = (\cos(\theta) \cdot m \cdot g)/k \approx (m \cdot g)/k \quad (3.5)$$

Using the expected moving weight of 1.333 grams and a 1 degree tilt angle, $F_{g,x}$ is calculated to be 0.000228 N, which is 228 μN . $\frac{d\Delta X}{d\theta} = 5.49 \mu\text{m/rad} = 95.8 \text{nm/degree}$ Therefore, the system is susceptible to tilting; a small change in tilt angle will cause the results to be off.

3.4.6 Stiffness experienced by tissue

The stiffness the tissue experiences during a contraction differs from the parallel flexure guide stiffness against displacement, as the bar connecting the flexures can not be assumed rigid. So when a force is applied at the tissue attachment point, the displacement at the sensing location differs from that at the tissue attachment point (Figure 3.7). As stiffness is the amount of force applied divided by the corresponding displacement of the system at that location. The stiffness differs depending on the location. The Comsol model (Appendix 7.3) was used to determine the stiffness in movement direction at the tissue attachment point to be $\approx 2030 \text{ N/m}$, deviating slightly depending on the exact location, which is seen as the tissue attachment point. When the tissue contracts, this stiffness determines how much the tissue shortens depending on the force generated by the tissue. Thus for a generated maximum force of 1000 μN , a displacement of $\frac{1000 \mu\text{N}}{2025 \text{ N/m}} = 493 \text{ nm}$ is to be expected for the tissue attachment point. On a tissue of 3 mm long, a shortening of 493 nm is $\frac{493 \cdot 10^{-9}}{3 \cdot 10^{-3}} \cdot 100 \% = 0.016 \%$. Hence the tissue length stays almost constant during the entire contraction force measurement, with almost no concentric part (the muscle length decreases while being electrically stimulated), before the isometric measurement.

3.4.7 Weight

The total weight of the measurement part is around 25 grams, accounting for the titanium part, the sensor and the sensor clamp. When the movement part is moved, this weight is also moved, thus it should be considered in the calculations for the movement part.

3.5 Movement part

Following the discussion of the measurement part, the report now shifts focus to the movement part. As the name suggests, the movement part is responsible for changing the pillar separation. To make the movement possible, a parallel flexure guide is used for the support, while a micro stepper actuator is used for creating the movement. The whole design will be elaborated on in the upcoming sections.

3.5.1 General remarks movement part

The design shown in this section differs slightly from the design shown in Figure 3.1; this is due to the prototype being made using 3D printing, while the part is designed out of aluminium and stainless steel. Due to the different manufacturing processes, some differences come to exist, but the adaptations are limited to the leaf springs, preload spring(s) and their attachment points.

The dimensions of the measurement part are presented in this section, along with explanations concerning these dimensions and other important nuances of the design. To start, the most important dimensions of the movement part can be seen in Figure 3.10

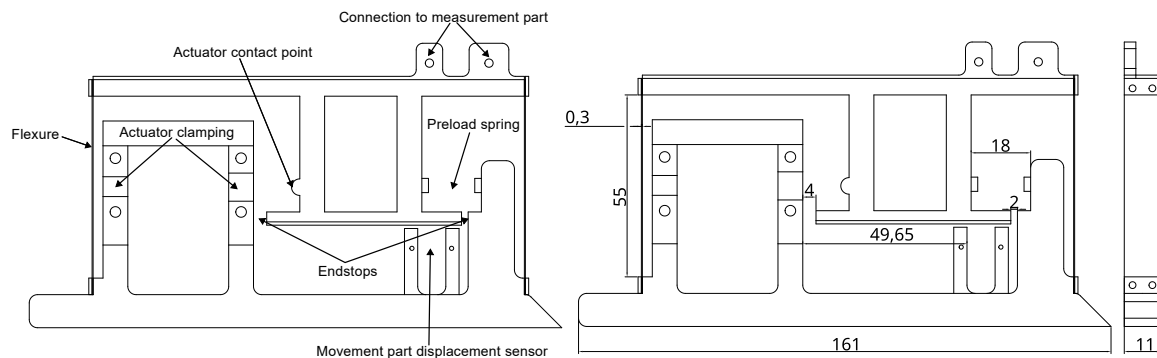


Figure 3.10: On the left, the movement part, with names of parts and locations. On the right, a technical drawing of the movement part with the most important dimensions in mm. Fasteners keeping the assembly together, the sensor and actuator are not included in this figure.

The dimensions of the flexures of the parallel flexure guide will be discussed in a separate section, but the length of the flexures mainly determines the height of the movement part. The 11 mm width is based on incorporation in the full design (Appendix 3.1); when equally spaced around the cell culture plate (standard 12-well cell culture plate) with 2 MMUs per well; each one has 13 mm of space available. 11 mm was chosen such that the MMUs would not touch, there would be more room to remove one if needed, and to run cables. The sensor and actuator chosen mainly influence the length of the part, as the sensor would pick up the magnetic field generated by the coils in the actuator if they are too close together, thus adequate spacing was designed. The amount of spacing needed was found by testing at what distance the influence seemed to stop, plus an added safety margin.

Endstops

To not allow the parallel flexure guide to deflect past its normal stroke, to prevent damage, endstops are incorporated. One side is at 2 mm, and the other is at 4 mm to allow for compaction compensation and lengthening of the tissue as set by the requirements.

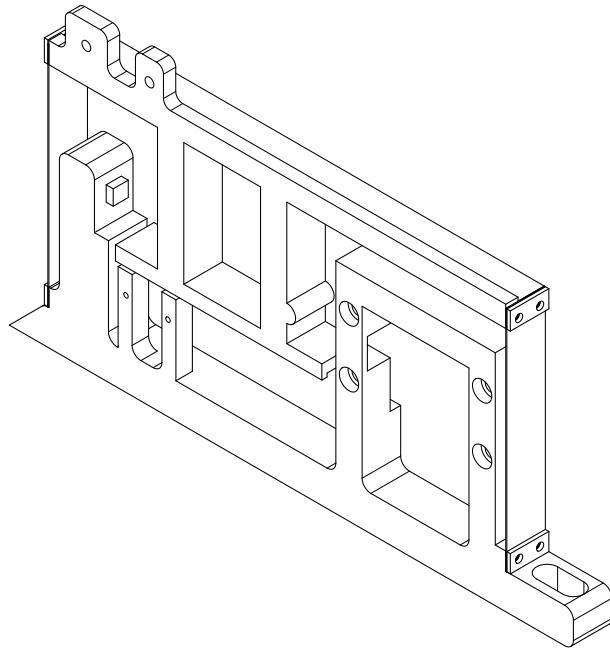


Figure 3.11: Backside of movement part, where the countersunk holes for the bolts holding the actuator can be seen.

Actuator contact point

To give the actuator a smaller area of contact to push onto, lowering the effect of surface irregularities, a small semicircular protrusion was designed for the actuator to push against.

Actuator clamping

Two separate plates are designed to keep the actuator fixed in; the plates feature threaded holes for the bolt to thread into, while the bolt fits into countersunk holes to prevent them from sticking out (Figure 3.11).

Connection to measurement part

The measurement part is fastened to the movement part using two bolts to make them move together, as seen in Figure 3.12. To ensure that the position of the pillar is as expected, this connection has to be secure with limited hysteresis. In the line of the tissue force, a positive stop is created to help prevent movement. The force on this joint is small due to the light weight of the top part and small accelerations, limiting the force caused by inertia. The weight is 25 grams, with a 10 mm/s acceleration, yields a force of $F=m \cdot a=0.025 \cdot 0.01 = 0.25 \text{ mN}$. A bolt in the direction of the forces was preferred. However, this would decrease the manufacturability, Hence this solution was chosen to simplify the part.

3.5.2 Parallel flexure guide movement part

Contrary to the measurement part, hysteresis is less of a problem in the movement part, which allows this part to be an assembly. Individual parts can be made and bolted together to form the complete part; both flexures will be made separately from the top and bottom. Making it also possible to use two different materials, the top and bottom will be aluminum for easy manufacturing and light mass; while the flexures are made out of stainless steel, for its strength

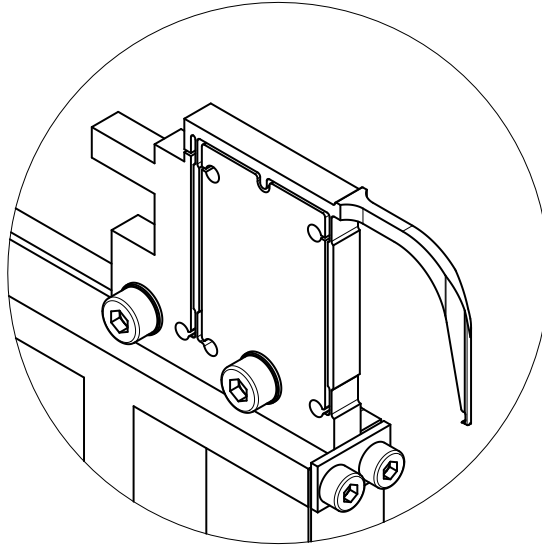


Figure 3.12: Connection between movement and measurement part is shown, including the M3 bolts that hold them together.

and fatigue limit. The dimensions of the flexures were determined to keep the movement stable and within the requirements; height, width, and length will now be discussed separately.

Height The height of the flexures was chosen based on the stiffness against displacement, the out-of-plane stiffness, the buckling force, and the max bending stress to translation. 0.3 mm height was found to be a good spot to be, based on the specifications for the corresponding parallel flexure guide. Which will be explained below.

Width As explained above, the width of the measurement part is chosen to be 11 mm based on incorporation into a full design with multiple MMUs next to each other; the width of the flexures is selected the same to optimise the out of plane stiffness of the system.

Length The distance between the end of the pillar and the bottom of the cell culture plate should be maximally 0.15 mm. The shortening effect of the leaf springs should be 0.1 mm or less to have clearance for moving the pillar; flexures of 55 mm are needed at the maximum deflection of 4 mm to achieve this. The shortening of the measurement guide is neglected, as it is minimal.

Movement part parallel flexure guide specifications

Using the formulas listed in Section 7.1, the relevant specifications of this parallel flexure guide were calculated. The results can be found in Table 3.6.

The max stress stays well below the fatigue limit and is calculated conservatively. The shortening is just below 0.1 mm at 3 mm deflection, which is within the requirements; however, it falls outside at the end stop at 4 mm deflection. This was done deliberately, as reaching a shortening below 0.1 mm at 4 mm deflection would mean increasing flexure length to 100 mm, which is undesired, and in practice, the guide does not reach its end stop during the experiments. The stiffness against displacement of the flexures is 589 N/m; however, the specific number is not important as long as it is not too high, the actuator can move the guide, and it does not affect the preload too much. The in-plane guiding stiffness, buckling force and out-of-plane stiffness are all several times higher than the stiffness against displacement without the preload added, which is desired as only the x direction should be a motion direction. The out-of-plane stiffness was calculated keeping the measurement part on top in mind by setting the force application point for the calculation at the top of the measurement part.

Dimensions($l \times b \times h$)	55×11×0.3 mm 0% reinforced
Max bending stress due to translation	196.36 MPa at $\Delta x = 4$ mm deflection
Shortening	-0.098 mm at $\Delta x = 3$ mm deflection
Stiffness against displacement	589 N/m
In-plane guiding stiffness	2.44 · 10 ⁶ N/m at $\Delta x = 4$ mm (while constrained in x)
Correction for unconstrained situation made using graph found in [22] (per flexure)	7337.7 N/m at $\Delta x = 4$ mm (while unconstrained in x)
Buckling force (per flexure)	53.29 N
Internal vibration frequency	- Hz
Out of plane stiffness	13351 N/m at $\Delta x = 0$ mm at $y = 85$ mm
Correction for deflected position made using graph found in [22]	3060.7 N/m at $\Delta x = 4$ mm at $y = 85$ mm
Reinforcement ratio	-

Table 3.6: Overview of important specifications movement part parallel flexure guide.

3.5.3 Frequency

To check the eigenfrequency of the bottom part, a simple calculation was done using Formula 3.2. The stiffness of the movement guide, without taking into account preload, is 589 N/m, and the weight of the moving parts is estimated to be 75 grams; based on the 25 gram measurement part, 44 grams for the aluminium, and 6.5 grams for the flexures, bolts and plates. Using these numbers, an eigenfrequency of 14.1 Hz was found, this does not consider the preload in the system. The preload causes the stiffness of the guide to be the actuator's stiffness, which will increase it significantly. To determine how much the actuator's stiffness would need to be known. However, the 14 Hz is already higher than the 1 Hz motion frequency; this part will see.

3.5.4 Movement part actuator

One of the system's most important functions is changing pillar separation; to do this, an actuator is needed. In the morphological overview, the decision is made to use a mechanical actuator. An abundance of options are available, and after some investigation, an Actuonix P8 ST actuator is chosen. This linear stepper actuator is selected for a few key points: precision, ease of control, strength, size, duty cycle, no power back drive force and cost. The specifications of the actuator can be seen in Table 3.7. This actuator stands out compared to other options mainly because of its small size, 100% duty cycle, small movement resolution, and reasonable price. One downside is the relatively large mechanical backlash. However, this is solved by adding a preload to the system.

Step size	1.8 μ m
Max force (at 10 mm/s)	35 N
Backdrive force(no power)	25 N
Duty cycle	100 %
Dimensions($l \times b \times h$ fully retracted)	45×11×25 mm
Cost(actuator+driver)	€150
Mechanical backlash	<0.5 mm

Table 3.7: Specifications of the Actuonix P8 ST actuator [23].

Preload

Preload springs are incorporated into the design to deal with the actuator's mechanical backlash, make the operation more reliable, and ensure the actuator stays in contact with the contact point. This is needed because the actuator is only used for pushing and not pulling to avoid inducing hysteresis in the connection between the actuator and the parallel flexure guide.

The manufacturer recommends a preload of 50-75% of the maximum force the actuator can deliver for a certain given speed [23]. For the required maximum speed of 3 mm/s, the actuator can provide 48 N of force. Seventy-five per cent of this would be $0.75 \cdot 48 = 36$ N, and fifty per cent of this would be $0.5 \cdot 48 = 24$ N. Therefore, with a 12 N difference over a 6 mm stroke, a maximum spring constant of 2 N/m is selected; furthermore, the maximum diameter is 11 mm, and the minimum maximum force is 36 N while being made out of stainless steel. With these requirements a selection from the available springs was made, of which the one with the smallest dynamic length was chosen to keep the design compact. For the specifications of this spring, see Table 3.8.

To achieve the preload, the spring is compressed to $\text{Initial length} - (\text{max preload} / \text{spring constant}) = 36 - (36 / 1.799) = 15.988$ mm at the end of the stroke, which means the spring is compressed to 18 mm in the guide's initial position. The effect of the stiffness of the flexures is neglected in this calculation, as the force created by this is less than 10% of the preload force.

Material	Stainless steel
Dimensions ($l \times b \times h$)	$36 \times 8 \times 1 \varnothing$ mm
Spring constant	1.799 N/mm
Minimum dynamic length	14.29 mm
Maximum force dynamic use	39.055 N

Table 3.8: Stainless steel compression springs specifications[24].

3.5.5 Movement part displacement sensor

Although the actuator used can take steps of less than $1.8 \mu\text{m}$, which satisfies the requirement, to ensure the stability of the position over longer periods of time, a position sensor was added to provide feedback and ensure the actuator does not miss a step. For this, a magnetic linear encoder was selected based on its small size, availability (there was one available at the university), resolution (± 300 nm), and cost (80 euros). The sensor specifications are in Table 3.9. The resolution in the table and stated above do not match, as testing using this sensor proved 300 nm to be a reliable resolution; based on tests conducted, results can be found in Appendix 7.5. This sensor can be used for closed-loop control on the actuator to ensure the motion is as intended. By itself, this sensor is not suitable for the incubator environment, it should be shielded from the humidity by applying a protective layer around the whole sensor, only leaving the cable sticking out.

Resolution	20 nm
Max movement speed	0.18 m/s
Operating temperature	-40 to +125 °C
Dimensions ($l \times b \times h$)	$15.4 \times 15.8 \times 4.76$ mm

Table 3.9: Linear magnetic encoder specifications [25]

3.6 Prototype

As the last step of the design process, a prototype is made (Figure 3.15), which is used to perform tests to check whether the design meets the requirements and functions it was designed for. This section discusses the making of the prototype, while the results of the testing done using it are in the next chapter.

3.6.1 Measurement part prototype

The measurement part (Figure 3.13) was made according to the design described above; wire electronic discharge machining (EDM) was combined with grade 5 titanium staff material. Staff material was chosen as it has less internal stress than rolled material, which helps with deformation during manufacturing. However, some deformation was observed in the cut-offs; thus, heat treating the staff material would be recommended.

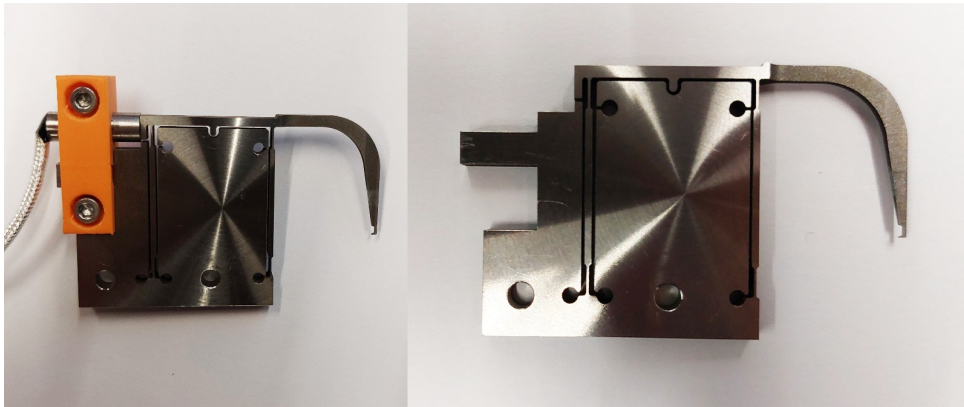


Figure 3.13: Foto showing the manufactured measurement part of the design, with and without the sensor connected.

3.6.2 Movement part prototype

Due to time constraints (manufacturing times) and cost, as well as long-term stability not being a concern for the planned tests and purpose of the prototype, the bottom part was made by 3-D printing out of polylactic acid (PLA). Compared to the design presented above, some changes were made to make it more suitable for 3D printing. The part was adapted to remove the assembly, making it one solid part. It has thicker leaf springs, matching the stiffness of the metallic version. Furthermore, two smaller preload springs are used as the PLA part is weaker. All other dimensions are kept the same to test the dimensions. The result can be seen in Figure 3.14, including the actuator, sensor, and preload springs.

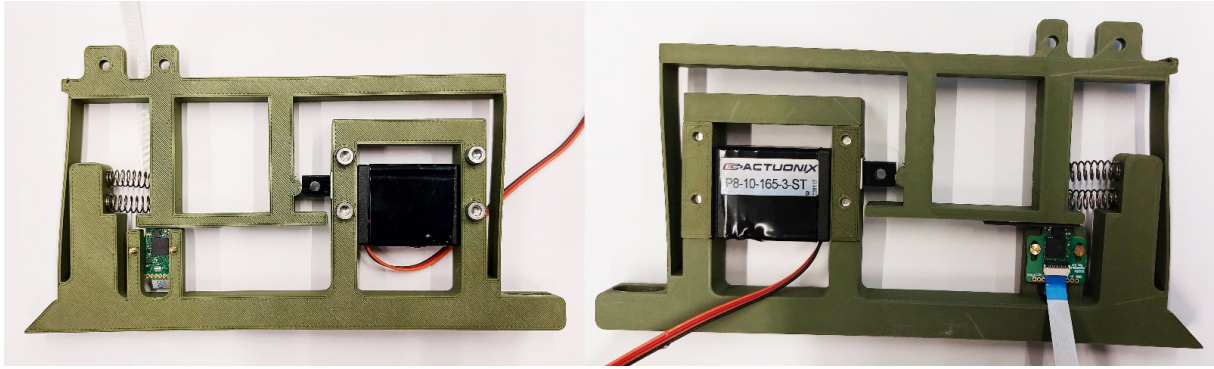


Figure 3.14: Figure showing the movement part manufactured by 3D printing.

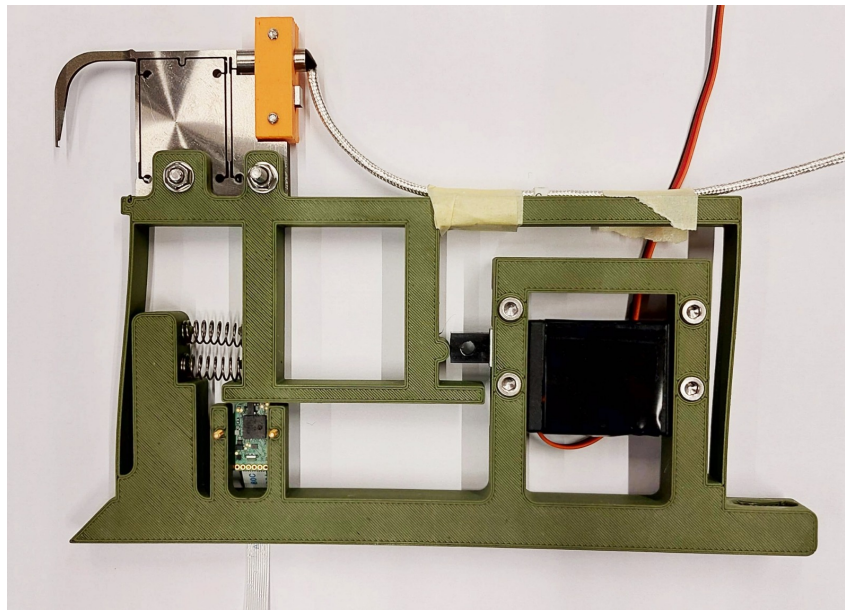


Figure 3.15: Picture of prototype part, with the sensors, springs, and actuator.

4 RESULTS

Experiments are performed using the prototype to assess whether the design meets the requirements and to determine the prototype's specifications. In this chapter, these experiments are introduced and explained, where after the results are shown.

4.1 Experiments

The experiments focus on testing the design and performing the procedures to make it functional. As manufacturing tolerances will cause the prototype to differ from the exact design dimensions, testing determines the prototype's specifications. The main specification that needs to be determined is the stiffness against displacement of the measurement part parallel flexure guide, which is required to convert the sensor's deflection measurement to force. Furthermore, the moving weight and eigenfrequency are determined as they are used for calculating stiffness. Finally, the actuator movement and hysteresis of the system are verified.

4.1.1 Experiment equipment

The following equipment is used to conduct experiments on the prototype. The Lion Precision 6-D capacitive sensor is set to a measurement bandwidth of 1000 Hz for the nm displacement sensing. To digitise the analogue signal from the Lion Precision CPL290 amplifier, a National Instruments 6221 DAQ was used in collaboration with Matlab Simulink Realtime 2015b to collect the data at 2000 Hz. The measurements were conducted on a passively damped optical table with pneumatic isolation from Newport Corporation to prevent outside vibrations from influencing the measurement. Furthermore, the magnetic sensor (Section 3.5.5) and the actuator (Section 3.5.4) were used, the actuator driver being controlled by an Arduino Mega 2560. The output signal of the magnetic sensor (RS422) was converted to TTL by differential line receivers. This TTL signal was converted to steps by the 6221 DAQ set to read out a quadrature encoder signal.

4.1.2 Determining stiffness of measurement part parallel flexure guide

Determining the stiffness of the measurement part was done by measuring the eigenfrequency and moving mass; with these numbers and Equation 3.2, the stiffness of the flexures could be determined.

Determining eigenfrequency

The eigenfrequency of the measurement part parallel flexure guide is determined by giving it an impulse and measuring its displacement. From this signal, the frequency components were

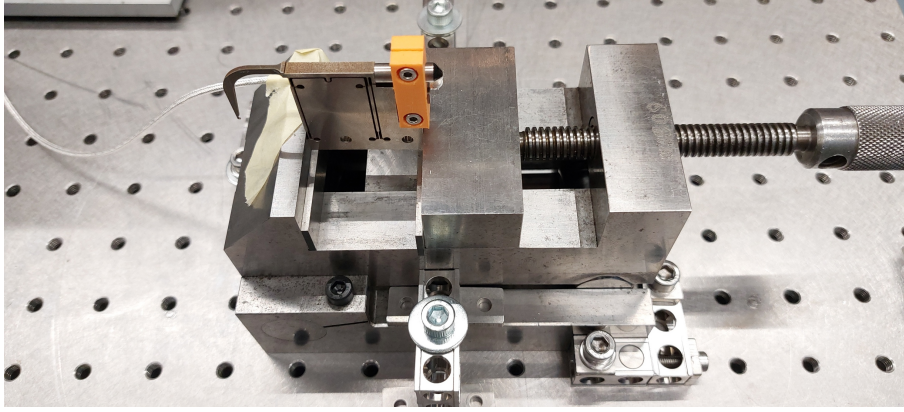


Figure 4.1: Figure showing the setup used for measuring the eigenfrequency, the measurement part, with sensor clamp and sensor can be seen, held in place by the sineclamp. The parallel flexure guide was excited mechanically after which it vibrates at its eigenfrequency.

determined using a Fourier transformation, which shows a spike for the eigenfrequency of the system. The setup used for measuring is shown in Figure 4.1.

Determining moving mass

The mass is determined by repeating the eigenfrequency measurement with a known amount of mass added to the parallel flexure guide; the added mass lowers the eigenfrequency, and these 2 numbers, together with the system's eigenfrequency, can be used to determine the moving mass (Equation 4.1). In which a is the known amount of mass added, and ω^* is the eigenfrequency with the mass added. Equation 4.2 is derived from Equation 4.1, as the stiffness against displacement of the system should stay equal (neglecting the effect caused by the weight preloading the guide) they can be equated and solved for m .

$$\omega = \sqrt{\frac{k}{m}} \qquad \omega^* = \sqrt{\frac{k}{m+a}} \qquad (4.1)$$

$$m = \frac{\omega^{*2} a}{\omega^2 - \omega^{*2}} \qquad (4.2)$$

4.1.3 Verifying force resolution and hysteresis

To test if the designed force resolution is met and to verify the design of the measurement part is hysteresis free, tests were performed. For these tests, gravity is used as a calibration force; tilting the part a precise amount induces a small force in the measurement direction, as discussed in Section 3.4.5. To verify how accurate this method is, as the force caused by gravity does not go through the centre of compliance of the parallel flexure guide (Figure 4.3), the Comsol model (Appendix 7.3) was used. The model was adapted by removing the tissue force and applying gravity at a specified angle. The theoretical applied force based on angle and gravity is plotted compared to the force based on stiffness and deflection, which is calculated by the model. (Figure 4.2). For a one degree tilt angle, a $25 \mu\text{N}$ difference in force is seen. Increasing linearly with larger angles.

A flexure based setup was made to measure the tilt of the part (Figure 4.4); for this design, a cross flexure is used, which acts as a pivot. A magnetic linear sensor (Section 3.5.5) measures

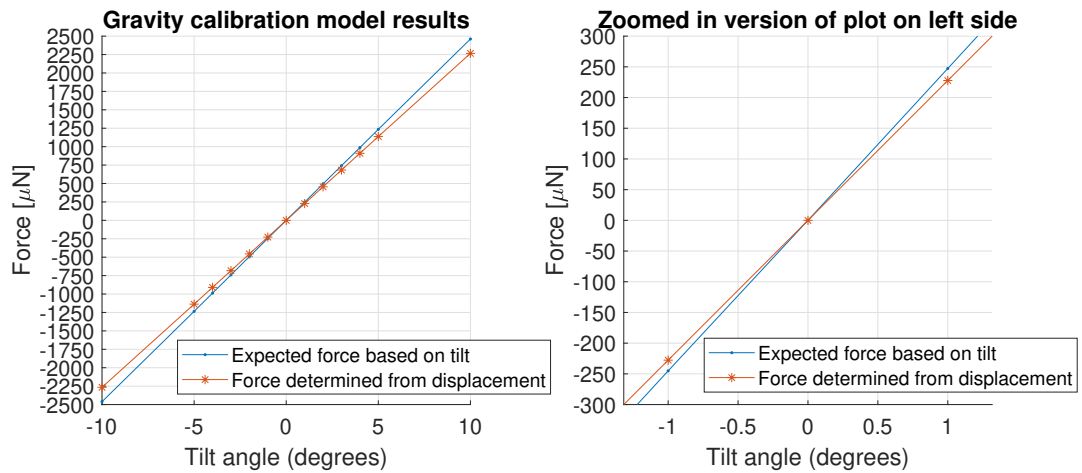


Figure 4.2: Plots showing the discrepancy between the expected and the measured force based on the Comsol model when the top part is tilted between -10 and 10 degrees.

the amount of deflection at 75 mm from the pivot, which can be converted to an angle. While the capacitive sensor (Section 3.4.1) is mounted to measure the deflection of the top part parallel flexure guide. A small cup of water is added to dampen the system and shorten its stabilisation time. The adjustment screw allows for small angle changes, and the springs keep a preload on the screw. Using the adjustment screw has friction at its contact point; thus, in the end, blocks of material were used to adjust the tilt by placing and removing them between the screw and the tilting part.

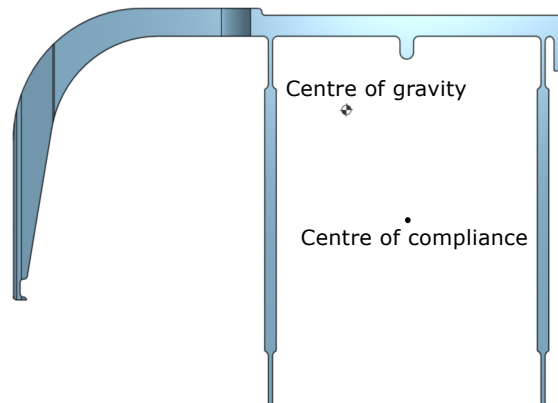


Figure 4.3: Figure showing the centre of compliance and the centre of gravity locations of the measurement part.

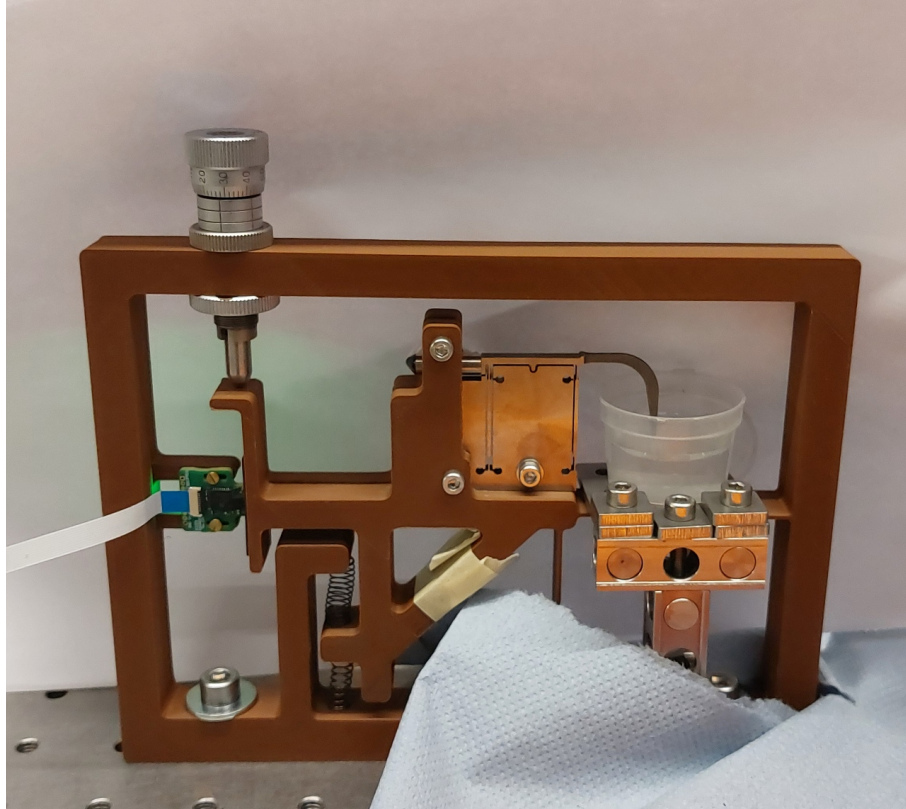


Figure 4.4: Picture of tilting setup as used for experiments. The bottom spring is chosen stronger to ensure a permanent upward force.

4.1.4 Verification of training motion accuracy and hysteresis

To simulate the tissue applying force at the tissue attachment point, a spring with a spring constant of $2\mu\text{N}/\mu\text{m}$ was used (RZ-006KI [26]). One end was hooked on the pillar, and the other was fixated. When the actuator of the movement part displaces the top part, thereby moving the pillar tip, a force is applied to the pillar tip by the spring (Figure 4.5). As the spring constant is known, the force the spring exerts depends on actuator movement; by moving the pillar, a prescribed amount of micrometre, micro newtons of force can be exerted onto the tissue attachment point. The movement is measured using the magnetic sensor too not rely on the accuracy of the actuator steps while also being able to verify the accuracy of the actuator.

To verify the hysteresis in the system, the spring is stretched in five discrete steps, after which the tension is partially released following the same steps, stretched again, and finally fully released in steps. Hysteresis can be determined by comparing the force measurement at corresponding amounts of stretch.

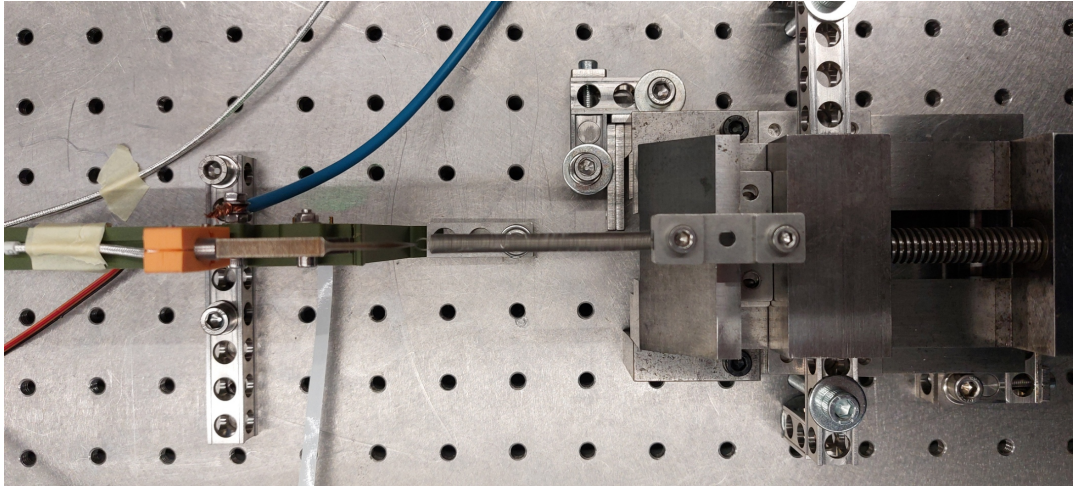


Figure 4.5: Picture of setup used for experiments using spring. With the spring in the centre, the fixed end on the right, and at the left side, the MMU with the spring hooked onto the tissue attachment point. The spring is stretched to overcome its initial tension, after which the force measurement is set to zero.

4.2 Results

In this section, the results of the experiments introduced above are presented.

4.2.1 Stiffness of measurement part parallel flexure guide

First the results of the eigenfrequency and moving mass test are shown, which are used to calculate the stiffness of the part.

Eigenfrequency results

The eigenfrequency measurement results can be seen in Figure 4.6. In this plot, a clear peak can be seen at the eigenfrequency (highest peak), while some peaks at higher frequencies can be seen; these are of no concern and are most likely harmonics of the eigenfrequency. The eigenfrequency is determined to be 195.602 Hz $\pm$ 0.06 Hz by finding the highest peak of the plot.

Mass results

Adding 4 different masses onto the flexure guide lowers its eigenfrequency; the results are in Table 4.1 and Figure 4.7. Using the lowered eigenfrequency and Formula 4.2, the moving mass is determined to be 1.2539 grams.

Determining stiffness

The stiffness of the measurement parallel flexure guide was determined to be 1894.05 N/m $\pm$ 1.16, from the measured mass, eigenfrequency and Formula 3.2. The uncertainty on the stiffness calculation comes from the frequency resolution of the Fourier transformation, which in this case was $\pm$ 0.06 Hz. To check if this is accurate enough, the effect of the uncertainty on determining the force is calculated. The effect will become stronger with increasing force. Therefore, the impact around the maximum force requirement of 1000 μ N is observed. To do

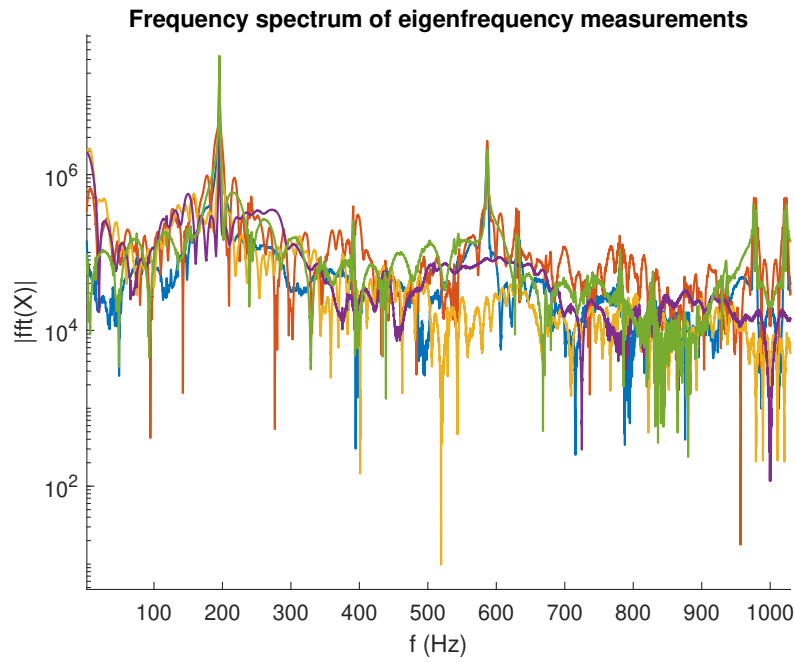


Figure 4.6: Frequency spectrum of eigenfrequency measurements of the measurement part. The results of 5 different measurements are shown in this plot, which all have the highest peak at 195.602 Hz.

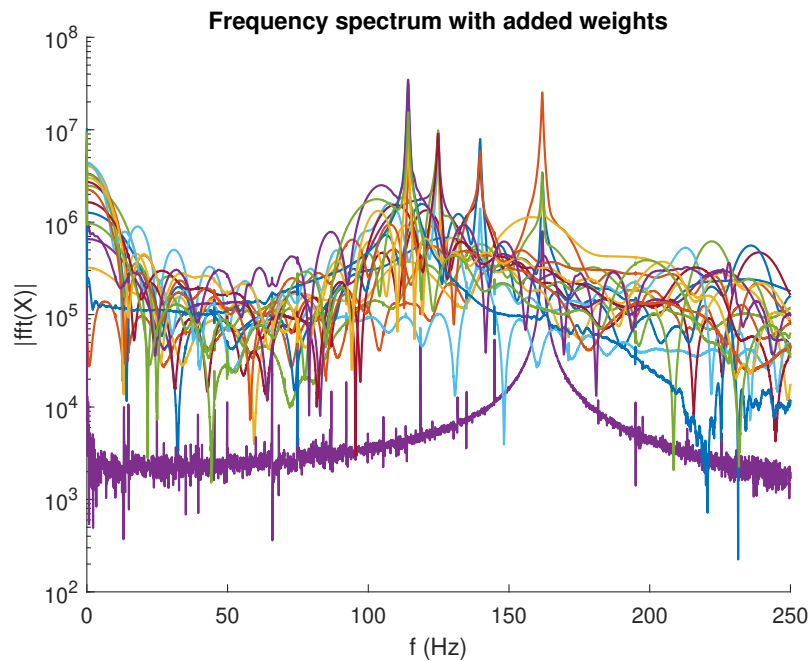


Figure 4.7: Frequency spectrum of eigenfrequency measurements with added weight to parallel flexure guide, the 4 different peaks correspond to the four amounts of mass added. The experiment was done multiple times for each weight, explaining the nineteen lines. To show the accuracy of the measurement, the exact peak number of each measurement can be found in Appendix 7.6.

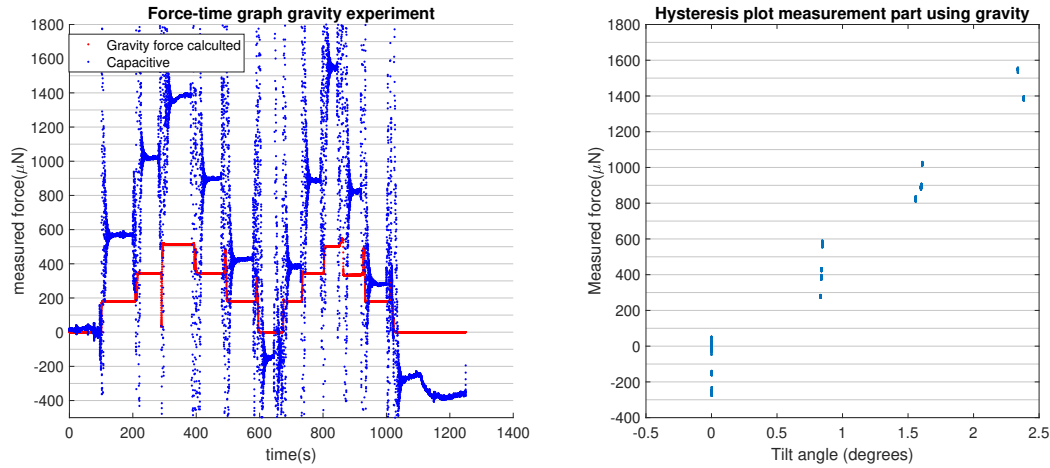


Figure 4.8: Left: the applied force based on gravity and tilt angle, and the force measured based on capacitive sensor reading and stiffness against displacement plotted over time. Capacitive was lowpass filtered to 5 Hz (measurement frequency 200 Hz). Right: Force based on capacitive reading and stiffness against displacement plotted against tilt angle to see hysteresis; for this plot, the sections of the capacitive measurement were used where it has stabilised.

this, a deflection of 530 nm is assumed, which results in a force difference of $0.6\mu\text{N}$. Which is found to be sufficiently accurate.

Added weight (gram)	0.580004	1.20156	1.82084	2.42745
Average frequency (Hz)	1.61822	139.741	124.813	114.181
Calculated moving weight of guide (gram)	1.2580	1.2525	1.2505	1.2547
Average calculated weight (gram)	1.2539			

Table 4.1: Moving weight measurement and calculation results.

4.2.2 Force resolution and hysteresis results.

Figure 4.8 shows the results of one test using the setup that uses gravity to apply a force. It can be seen that the applied and the measured force do follow each other; however, there seems to be about a 3x difference in the force measurement results. From the hysteresis plot, it can be seen that there is 200-300 μN of hysteresis in this setup. Also, the noise in the capacitive sensor measurement of about 25-35 μN can be seen. Corresponding to 13-18 nm of noise in the displacement measurement.

4.2.3 Verification of training motion accuracy and hysteresis

The result of the testing using the spring can be found in Figure 4.9. It can be seen that the force based on capacitive reading is lower than expected based on the spring deflection. Also, the underdamping of the system causes an overshoot in the capacitive measurement. The hysteresis plot shows a 50-75 μN difference between applying the same force with the spring. From this plot, it can also be seen that the actuator is repeatable and can go to the same position multiple times within 5 μm ; adding feedback control using the sensor will improve this. However, it is good to verify the step size of the actuator.

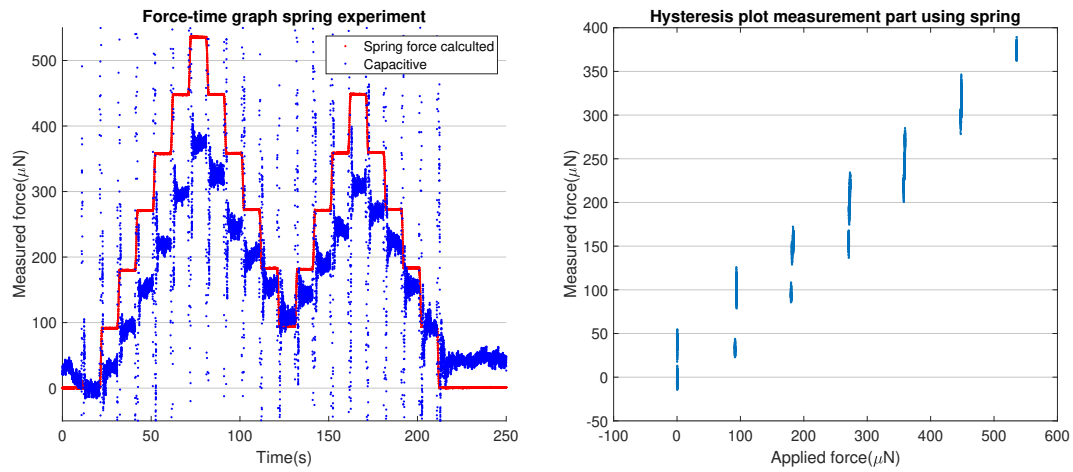


Figure 4.9: Left: the force based on the displacement of the movement part and the spring constant, and the force based on the capacitive reading and the stiffness against displacement are shown over time. Capacitive was lowpass filtered to 5 Hz (measurement frequency 100 Hz). Right: Force based on capacitive reading and stiffness against displacement plotted against applied force based on spring constant and deflection to see hysteresis; for this plot, the sections of the capacitive measurement were used where it has stabilised.

5 DISCUSSION

In this report, a system design was made to investigate the contractile properties of 3D tissue engineered human skeletal tissue under the influence of mechanical and electrical stimulation. To achieve this, a list of functions and requirements was formed, which was used to design a prototype for testing. This prototype is needed to verify the validity of the requirements, as some are educated guesses based on similar research on other types of muscle tissue. However, this adds uncertainty; having a system to validate the requirements and adjust them where necessary will ensure the system functions as intended. One number that needs to be verified is the percentage of how much stretch the tissue can handle above its optimal length during eccentric training needs to be verified (section 2.2.5).

During the design process, it was found that some requirements contradict one another. Therefore, the goal was to find a balance to make a working design. Things that needed to be balanced include: -Eigenfrequency/force measurement resolution -Eigenfrequency and force resolution/sensor resolution -Sensor resolution/vibration, thermal stability -Sensor resolution/sensor cost Eventually, a balance was found, using which a prototype was made. To verify whether the right balance was found, experiments using real tissue need to be conducted using the prototype.

Besides finding this balance, aspects like manufacturing set boundaries on what is possible. The 0.3mm material thickness limitation set for the wire EDM process means that it becomes impossible to make the parallel flexure guides very small without increasing the stiffness against displacement. This limits the design's lower end weight, thereby strongly influencing the eigenfrequency that can be achieved. However, the current design is not optimised for moving weight, and there is room for improvement, but the trade-off between saving weight and complicating manufacturing needs to be made. Other design choices, like the measurement part flexure width, were partly based on making it rigid enough to handle. A 4 mm width was chosen, and it is certainly rigid enough after handling the prototype during testing, so by reducing some dimensions also, weight could be saved.

Furthermore, tests must be done using this design to verify its usability in real test conditions, inside an incubator, being sterilised, influenced by lab vibrations, and with actual muscle tissue. Also, experiments with electrical stimulation of the tissue need to be done to verify if the eigenfrequency is high enough. This will show the system's limitations and where improvements need to be made. Also, the system's long-term stability must be assured before it can be used for 4-week testing.

The lower stiffness found during the measurements on the measurement part (1894 measured, 2380 modelled) can be explained by manufacturing tolerances. Reducing flexure height to 0.29 mm lowers the stiffness against displacement to 2165 N/m, while flexure length also has a strong (¹³) influence on stiffness. Thus, slight deviations of both can explain the difference. The stiffness difference explains the differences in eigenfrequencies and mass of the prototype compared to the design as they are linked.

The 5 μ N force resolution which is designed for is currently not met; more noise is coming from the capacitive sensor 13-18 nm than expected based on its specifications. Tests conducted with the sensor mounted rigidly confirmed this. Lowpass filtering with a cut-off frequency of 200 Hz improves the signal but leaves at least 5nm of noise. Furthermore, the hysteresis observed during the experiments undermines the 5 μ N force resolution. What causes this hysteresis is not exactly known. Multiple causes are assumed depending on the measurement setup. For the gravity setup, creep in the 3D-printed beams due to the preload of the springs is expected. In addition, movement between the measurement part and the capacitive sensor is expected, as every nm of movement here will affect the results. The next steps would be to make a version of the setup that is less susceptible to creep and verify the readings of the capacitive sensor to explain the 3X force difference. For the spring test setup, hysteresis in the spring itself, at the point where the spring contacts the tissue attachment point, the sensor clamp, and the connection between movement and measurement part are suspected. To check where it comes from, they should be ruled out individually. This was not done due to time constraints, but glueing the spring to the tissue attachment point and verifying there is no movement in the connection between the parts by directly measuring the displacement of the measurement part using another sensor, would be a logical next step. Hysteresis in the spring and the possible nm movement in the sensor clamp are difficult to measure and rule out.

Furthermore, it is recommended that different non-magnetic sensing options for the movement part be considered. This would remove the interference between the sensor and actuator and allow for making the movement part smaller. Implementing an inclination sensor to monitor tilt would make moving the complete device possible by allowing for a changed tilt, which causes a changed influence of gravity.

6 CONCLUSION

In this report, a prototype system is designed to induce length changes in engineered 3D skeletal muscle tissue. A list of possibilities using the system is given here:

- Eccentric training
- (isometric)Force measurements
 - Active
 - Passive
- Length-Force measurements
- Force-velocity measurements
- Compaction compensation
- 100 % lengthening of tissue

The system sets itself apart by being designed for long-term testing (4 weeks) of muscle contractile properties, while most existing systems are aimed at drug studies. By adding the pillar spacing adjustment, other new functions also become available, which can help in understanding muscle contractile behaviour. The system developed is designed to have a precise force measurement resolution of $5\text{ }\mu\text{N}$ and an eigenfrequency above 200 Hz. The current prototype has a 195.5HZ eigenfrequency. The $5\mu\text{N}$ requirement is currently not met by test results. However, it is not ruled out, and further testing should be done. Finally, this system should, most of all, be used to verify if the requirements are sufficient or should be improved. This design enables these tests to be done and the hidden problems with the design to be found. This is a significant step toward a final design that can be used for experiments.

7 APPENDIX

During the preparation of this work, I used Grammarly, Overleaf, DeepL, Microsoft Word, and Google to correct my writing. After using this tools/services, I thoroughly reviewed and edited the content as needed, taking full responsibility for the final outcome.

7.1 Formulas used for flexure calculations

In this section the formulas that are used to perform analytical computations on the parallel flexure guides are given.

Maximum bending stress due to translation	$\sigma_{b,max} = \frac{3 \cdot E \cdot h \cdot \Delta x}{l^2} \cdot \frac{1}{1 - p^3} \quad (7.1)$
Shortening effect	$\Delta y = -0.6 \cdot \frac{(\Delta x)^2}{l} \quad (7.2)$
Stiffness against displacement (for one flexure)	$c_x = 12 \cdot \frac{E \cdot I}{l^3} \cdot \frac{1}{1 - p^3} \quad (7.3)$
Second moment of area	$I = \frac{b \cdot h^3}{12} \quad (7.4)$
In-plane guiding stiffness (constrained in x direction) corrections can be made for the free case (for one flexure)	$c_{y,0} = \frac{E \cdot b \cdot h}{l \cdot (1 - p)} * \frac{1}{1 + \frac{1}{1-p} \cdot \frac{12}{B} \cdot (\frac{\Delta x}{h})^2} \quad (7.5)$ $B = 700 \cdot \frac{(1 + q) \cdot (1 + 3q + 3q^2)^3}{1 + 10q + 45q^2 + 105q^3 + 105q^4} \quad (7.6)$ $q = \frac{p}{1 - p} \quad (7.7)$
Buckling force (for one flexure)	$F_{buckle} = \frac{4\pi^2 E \cdot I}{l^2} \cdot \left(\frac{1}{1 - p} \right)^2 \quad (7.8)$
Internal vibration frequency of reinforced flexure	$f_e = \frac{1}{2\pi} \cdot \sqrt{\frac{24E \cdot I}{(\rho \cdot l \cdot b \cdot h) l^3}} \quad (7.9)$
Out of plane stiffness(formula for unreinforced) y being the position of force application relative to halfway the flexure, corrections are made for stiffness at deflected position	$(c_{z,tot})^{-1} = (c_z)^{-1} + (k_\varphi/y^2)^{-1} \quad (7.10)$ $c_{z,0} = \frac{12 \cdot E \cdot (\frac{1}{12} \cdot b^3 \cdot h)}{l^3} \quad (7.11)$ $k_{\varphi,0} = \frac{E \cdot (\frac{1}{12} \cdot b^3 \cdot h)}{l} \quad (7.12)$
Reinforcement ratio	$RR = \frac{(E \cdot I)_{reinforced}}{(E \cdot I)_{uniform}} \quad (7.13)$

Table 7.1: Equations used to calculate flexure properties [22]. In these equations, different dimensions relating to the flexures are used; their definition can be found in Figure 3.5.

7.2 Complete design rendering

To show how a complete system could look, the following model was made (Figure 7.1). The model was used to make sure a full design is feasible and no space conflicts between parts exist. Room for electrodes, pipettes and lengthening of the pillar spacing was assured.

Each stationary pillar is mounted separately, such that it can be removed to make room for pipetting in the cell liquid, after which the pillar can be placed back, and the tissue will form around it.

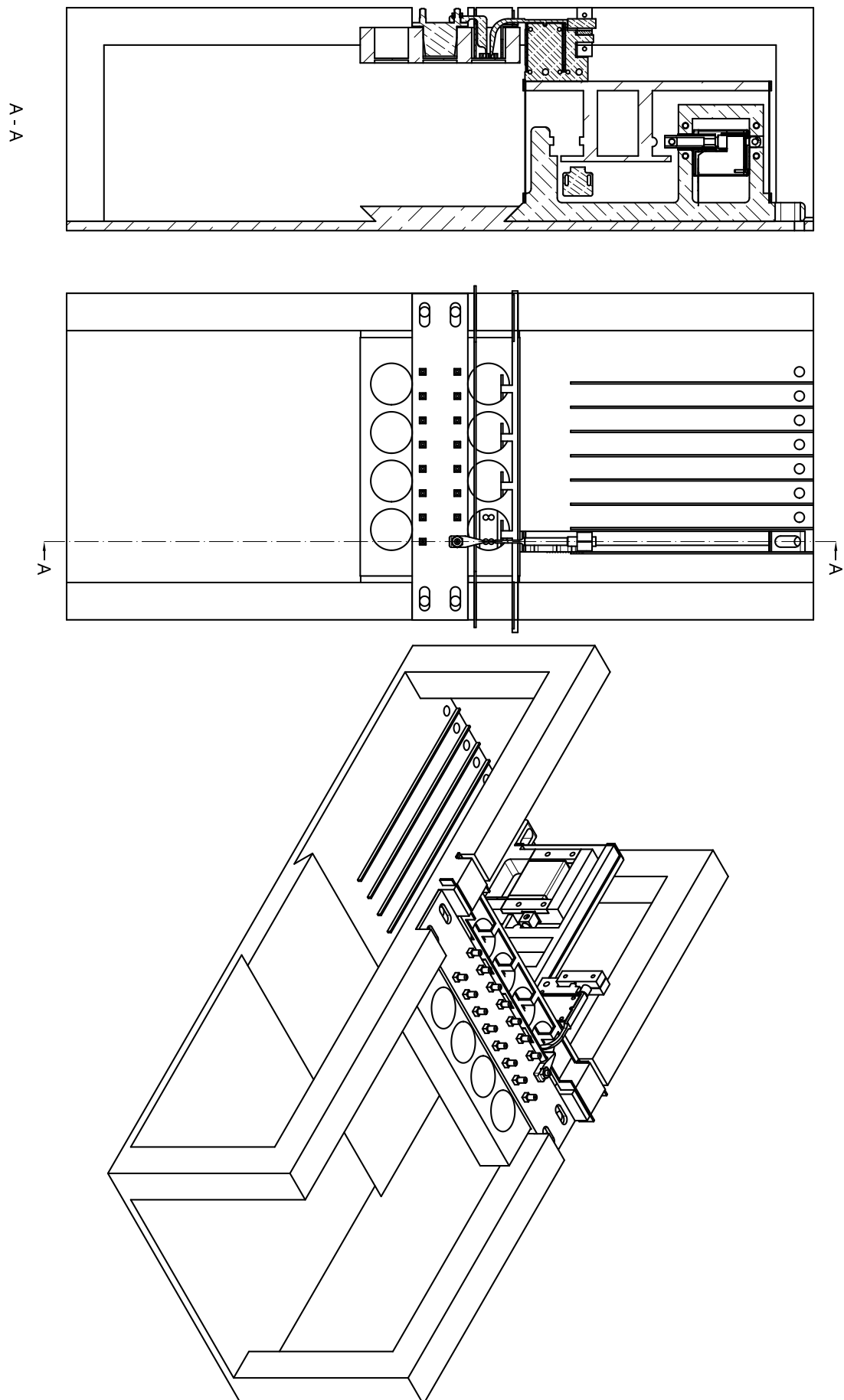


Figure 7.1: Drawing of possible full working experiment design. 16 MMUs could be placed around one 12-well cell culture plate. Only one MMU and fixed pillar are shown for clarity.

7.3 Comsol simulation

The stiffness against displacement and the vibration modes of the measurement part were analysed using COMSOL Multiphysics. The exact geometry was loaded into the program, after which the material, boundary conditions and load were selected. The choice was made to use an extremely fine physics-based mesh; convergence was checked by selecting more coarse meshes, while no significant change in results was observed. The locations where the boundary conditions and load are applied can be seen in Figure 7.2

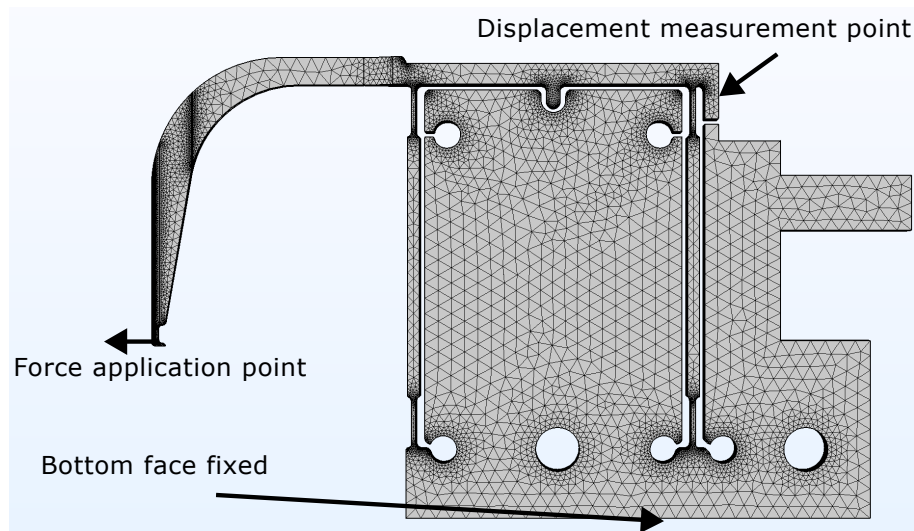


Figure 7.2: Render of the mesh used to perform simulations on the measurement part, with the boundary condition and load application locations specified.

7.4 Matlab Simulink model

To predict how the system would behave, a Simscape model was made in Simulink (Figure 7.3). For the model, the weights and stiffnesses as designed were used. The speed of the actuator was set, as would be the case during eccentric training.

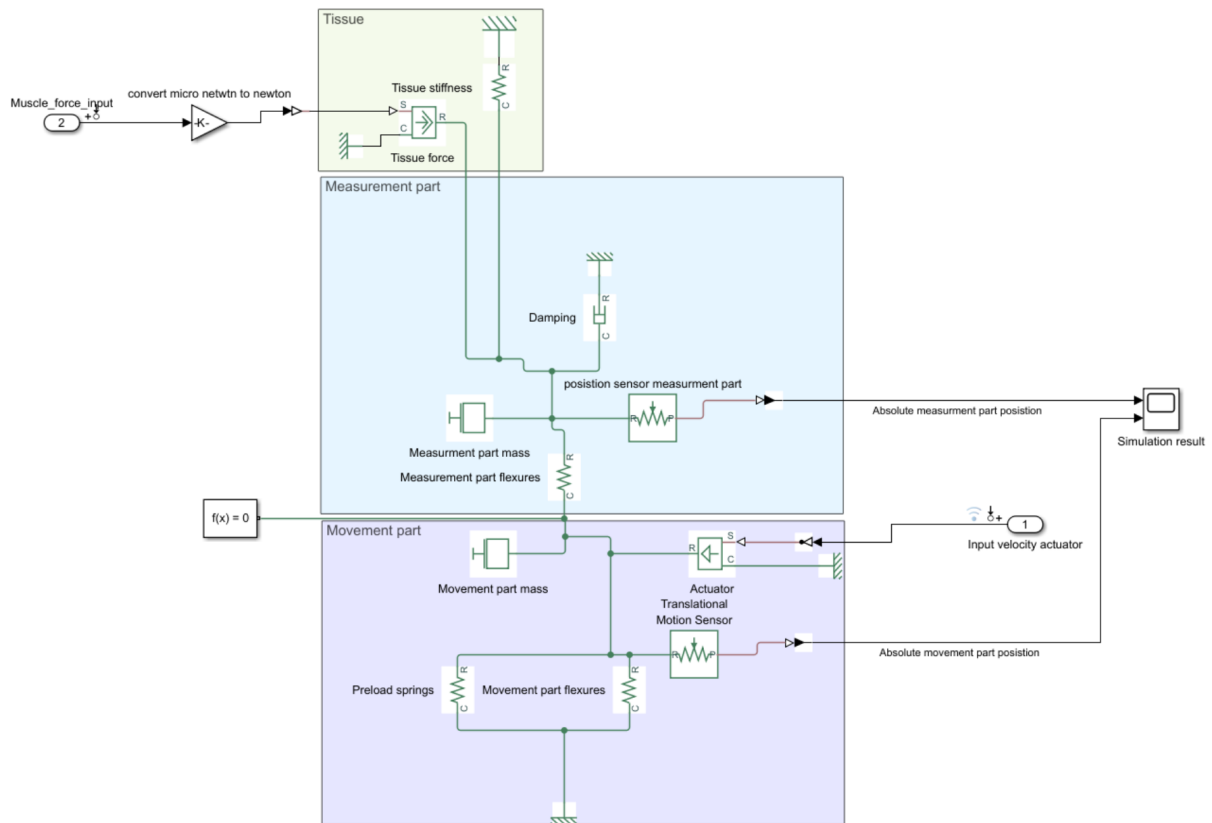


Figure 7.3: Overview of Simscape model, of 2 parallel flexure guides on top of each other.

7.5 Bogen sensor testing

With the Bogen magnetic sensor mounted fixed above its measurement scale, the following data was observed (Figure 7.4). From this, it can be seen that the 20 nm resolution is not met. It was concluded that this sensor can be assumed to have a workable resolution of +/- 300 nm.

7.6 Data mass measurement

In this section, the measurement data from the moving mass experiment is shown.

Added weight (grams)	Weight 1 added (0.58004 gram)	Weight 2 added (1.20156 gram)	Weight 3 added (1.82084 gram)	Weight 4 added (2.42745 gram)
Measurement 1	161,822	139,741	124,741	114,121
Measurement 2	161,822	139,741	124,861	114,181
Measurement 3	161,822	139,741	124,861	114,181
Measurement 4	161,822	139,741	124,801	114,181
Measurement 5	161,822	-	124,801	114,241
Average	161,822	139,741	124,813	114,181

Table 7.2: Added weight frequency measurement results, all frequency measurements are +/- 0.06 Hz, One measurement with weight 2 went wrong, thus for this weight, there are only 4 measurements.

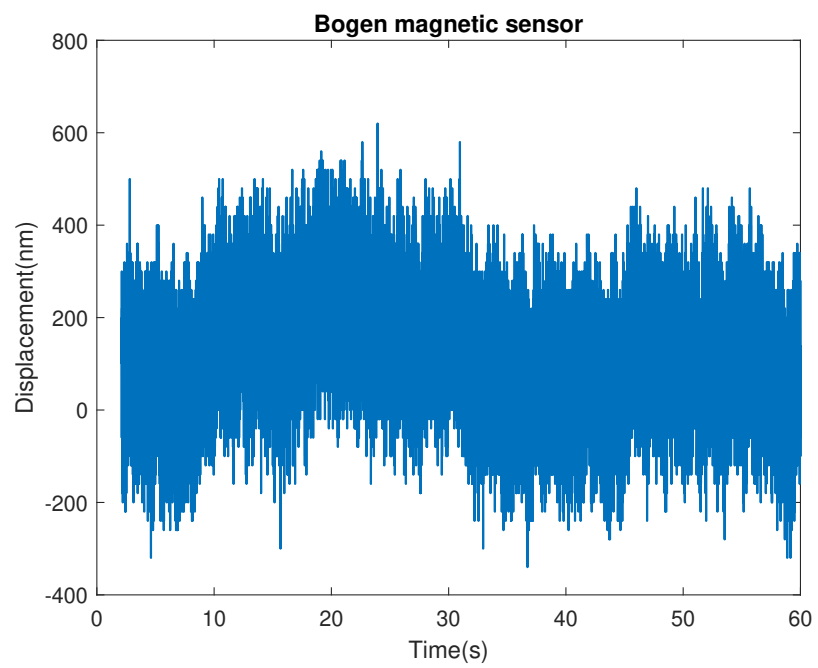


Figure 7.4: Displacement measurement captured using Bogen IKP11 sensor, while kept stationary.

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