

École polytechnique de Louvain

Development of a 3D-bioprinter for the production of living tissues and organs

Design of an extrusion-based printing head mounted on a Leapfrog Creatr HS printer

Authors: **Leonardo MISSICH, Valentin TACK**

Supervisor: **Benoît HERMAN**

Readers: **Laurent FRANCIS, Greet KERCKHOFS, Jean-François REES**

Academic year 2021–2022

Master [120] in Electro-mechanical Engineering

Abstract

Researchers at UCLouvain showed an interest in the use of 3D bioprinting. This technology, much like 3D printing, allows to print three-dimensional structures but with the ability to print biological substances. The development of an affordable bioprinter has been demanded to achieve both the fabrication of 3D structures embedded with cells of marine sponges and to achieve precise deposition of nanoparticles on electronic sensors.

The identification of the needs of the clients, along with a thorough study of the state of the art, allowed to establish a list of technical requirements. These specifications set general directions for the design process.

The time constraint led to the development of a prototype of printhead mounted on the frame of a Leapfrog Creatr HS printer. The printhead relies on an extrusion-based system working with standard Luer lock syringes. It also features a heating system to ensure viability of cells and accommodate the use of temperature-sensitive biomaterials. The design suits the use of multiple printheads and its modularity makes it easy to adapt for a different frame.

The printing tests conducted with a 1% agarose solution highlighted limitations and issues of the design such as occasional clogging and poor adherence of the material on the substrate. The use of the frame of an existing 3D printer also limits the potential of the bioprinter because of the specific dimensions and electronics. Nonetheless, the prototype built proves to complete prints and maintain a stable temperature in the syringe. Many improvements can be brought in future developments, yet the current prototype will allow the clients to start proceeding with their research.

Acknowledgments

At first, we would like to thank our supervisor Benoît Herman for the topic proposed, for his support, his advises and more importantly for the trust he placed in us and in our work.

Secondly, we would like to thank Laurent Francis for supporting the project and bringing VOCSEns in the frame. We would also like to thank Jean-François Rees for his enthusiasm in sharing his ideas and his knowledge in biological sciences as well as for creating a network gathering specialists from the different fields related to bioprinting.

Then, we would like to thank Greet Kerckhofs, Professor at EPL and part of the iMMC research team, for reading our thesis with her insights on biomaterials and tissue engineering.

We would also like to thank every member of the CREDEM platform that helped us with their technical knowledge and helped us in the construction and assessment of the prototype.

Leonardo and Valentin

I would like to thank Laura for the immense support and love that she has always given me. After all these years, I never stopped feeling you close, no matter how far we might be. I love you.

I would also like to thank my parents and brothers for their constant support and understanding. I might not indulge in frequent outpouring of feelings, but remember I love you anyway.

Additionally, I would also like to thank Laura's family, for making me feel part of them. Especially to Rita, without whom I would probably not have been able to undertake this journey.

I would like to thank my friends, in particular those I have known since I was a child and still are my closest ones.

Last but not least, I would like to thank Valentin, for being an excellent thesis partner and teammate.

Leonardo

I would like to express my deep gratitude towards my friends with whom I spent countless hours in and out the academic frame, sharing stress, tears and beers. We help and uplift each other. I hope this never changes and I can't wait for our meeting in 2036.

Je voudrais aussi adresser mes plus profonds remerciements à ma sœur Laurence. Elle m'a supporté ces 24 dernières années et aidé à devenir la personne que je suis.

Je voudrais remercier mes parents bien-entendu, pour avoir su stimuler ma curiosité et me donner le goût de la découverte. Merci aussi pour leur support mais par dessus tout, pour m'avoir toujours offert l'occasion de devenir autonome.

Finally, I am thankful for all these people I encountered that took a bit of their time to share their passion, knowledge, beliefs and stories. When the memory resurfaces, it always sweetens my day.

Valentin

Contents

1	Context	1
2	State of the art	3
2.1	Overview of bioprinting technologies	3
2.1.1	Extrusion-based bioprinting	3
2.1.2	Droplet-based bioprinting	7
2.1.3	Light assisted bioprinting	12
2.2	Bioinks and biomaterial inks	15
2.3	Illustrative list of existing printers	17
2.4	Recap on the state of the art and directions	20
3	Design Objectives	22
3.1	Objective tree	22
3.2	Specifications of the bioprinter	25
4	Design of the extrusion printhead	29
4.1	Printhead mounting system on the Leapfrog printer	30
4.2	Printhead engagement system	32
4.3	Extrusion actuation system	35
4.4	Syringe heating solution	39
4.5	Bill of materials	44
5	G-code generation and printing parameters	46
5.1	Calculation of extruded volume	46
5.2	E-value computation	48
5.3	Introduction of a fill factor for extrusion tuning	51
5.4	Slicing parameters	53
6	Performance assessment and verification	57
6.1	Tests and results	57
6.1.1	Temperature tests	57

6.1.2	Printing tests	61
6.2	Verification of the design	64
Conclusion		70
References		75
Appendices		76
A:	How to start a print	76
B:	Useful G-code control commands	77

Chapter 1

Context

The first steps in the world of 3D printing were taken by researcher Hideo Kodama at the Nagoya Industrial Science Research Institute. In 1980, he filed the first patent introducing his method to solidify a photosensitive polymer with a laser and published a paper¹ reporting on experiments he conducted on three-dimensional object fabrication [1].

The growth in the field of 3D printing and the discoveries made in cell biology through the 80's and the 90's led to the creation of the first bioprinter in 2003. Nowadays, 3D bioprinting is developed and used to generate tissues such as bone, blood vessels and skin to be used as implants or to conduct tests in pharmacology. More recently, the concept of organs-on-chips was brought to light: these are microfluidic devices populated with cells for the development of drugs and disease modelling. A variety of medical and agri-food applications are under study and bioprinting could be a key element to find solutions to some of them.

Jean-François Rees, Professor in biological sciences at UCLouvain, is interested in 3D bioprinting for creating marine sponge tissue structures. His goal is to extract specific cells from sponges that are able to differentiate or transdifferentiate² and to prepare bioinks destined for 3D printing. The constructs would help analyse the viability of the cells after the process and study their behaviour depending on the structure of the deposition.

VOCSens, a spin-off from UCLouvain that develops smart sensors for gas and volatile compounds detection, is also interested in bioprinting. As they investigate new ways to detect specific gases or compounds, they are interested in automated

¹ "Automatic method for fabricating a three-dimensional plastic model with photo-hardening polymer" DOI: 10.1063/1.1136492.

² Transdifferentiation is the ability of a cell to differentiate into another differentiated type of cell without going back to the stem cell state.

solutions to perform deposition of multiple layers of micro- and nanoparticles on sensors. They are also conducting research on using biological material to detect or improve the detection of such gases or volatile compounds.

The first part of the research objective is to establish clear and comprehensive specifications for a 3D bioprinter that fulfills the common and respective needs and objectives of the clients Jean-François Rees and VOCSens. The summary of a sound analysis of the state of the art is reported in the second Chapter of this work. It allows to support the list of requirements drawn up in Chapter 3 and to motivate the choices made in the design of a prototype.

The second part of the research objective consists in designing and assembling a working prototype of a bioprinter. Considering the time available to complete this work, it was decided to focus on the development of a working printhead mounted on the frame of an existing 3D printer. The prototype is presented in Chapter 4, it is followed by a guide on G-code to select slicing parameters for the printer and a verification process of the resulting machine in Chapters 5 and 6 respectively.

Chapter 2

State of the art

In 2003, Thomas Boland creates what is known as the first bioprinter. He uses an inkjet printer to print his name with a protein previously loaded in the cartridge of the printer. The shape is not of interest but he opened the way to automated, complex, cell deposition to create structures in 3 dimensions. Nowadays, countless printers are available on the market and offer many different technologies to perform different 3D bioprinting methods. There is also a focus on the inks, some firms offer what are now standard inks with well known properties and precise guidelines on how to use them.

This chapter aims to give an overview of the main technologies used as well as of the most known bioprinters on the market and to categorise the different types of inks developed in the frame of bioprinting. This summary helps to understand the choices that are made in the following chapters regarding the selected technology for the prototype of a printhead.

2.1 Overview of bioprinting technologies

The existing printing technologies exploited in bioprinting can be separated into three categories: extrusion-based systems, droplet-based systems and light-based systems. These categories and most common related methods are described respectively in these sections.

2.1.1 Extrusion-based bioprinting

Extrusion-Based Bioprinting (EBB) is the most widely spread technology used in the field of bioprinting. Its low cost and comparatively simple design, as well as the possibility to fabricate complex structures such as porous ones for cell seeding, make it the most mature and most commercialised technology for bioprinting

[2][3]. In addition, with the spread of Fused Deposition Modelling (FDM) and the diffusion of cheap desktop 3D printers, it becomes easier to access the technology by modifying off-the-shelf printers [4].

The technology is based on a combination of a dispensing system, the extruder, in charge of the deposition of a continuous filament of biomaterial, and an automatic robotic system that allows accurate 3D displacement of the printing head thanks to Computer-Aided Design (CAD). Together they make possible the realisation of complex and solid 3D structures. In addition to the aforementioned advantages, with EBB it is possible to obtain a high cell density ($>10^6$ cells/ml [5]) over a wide range of viscosities (10^2 to 10^9 mPa·s [6]) and with great deposition and printing speed. Cell viability might raise some issues, especially in mechanical-driven approaches, but values up to 97% have been achieved [7]. On the other hand, the main drawbacks are the low resolution limited to $100\text{ }\mu\text{m}$ [6][7] and partial limitation on the biomaterial selection.

EBB mainly differentiates into four groups, based on the main cross-linking¹ strategy: Direct Ink Writing (DIW), coaxial printing, coagulation bath printing and free-form reversible embedding [9].

Direct Ink Writing DIW is the most common strategy and consists in printing a highly viscous biomaterial, with shear-thinning properties, and specific rheological² behaviour. This allows rapid recover of the mechanical properties and thus preservation of the intended shape after printing.

Coaxial printing It allows to print tube-like structures by printing a filled tube with two different materials in one go, see Figure 2.1. It can also be used to print a polymer along with a cross-linking agent thus decoupling the rheological behaviour and the printing accuracy.

¹ Cross-linking : *"In the chemical context, it is used for reactions in which molecules are linked together by introducing covalent bonds and forming three-dimensional networks."* [8]

² Rheology: Branch of physics that deals with flow of matter under specific conditions and applied forces.

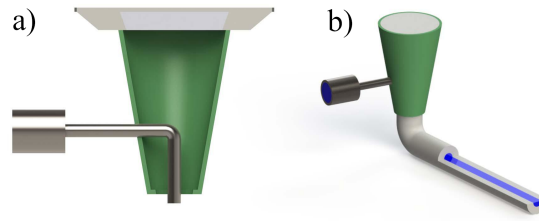


Figure 2.1: Coaxial printing: illustration of principle. a) Schematic cross section of coaxial needle, b) extrusion of a strand *adapted from* [10].

Coagulation bath printing The process takes place directly in the cross-linking agent. The gain in simplicity comes with the drawback of clogging and issues in layer adhesion.

Free-form reversible embedding The method uses a granular or colloidal bath to grant the required rheological behaviour of the printing process. An example of Free-Form Reversible Embedding of Suspended Hydrogels (FRESH) is shown in Figure 2.2.

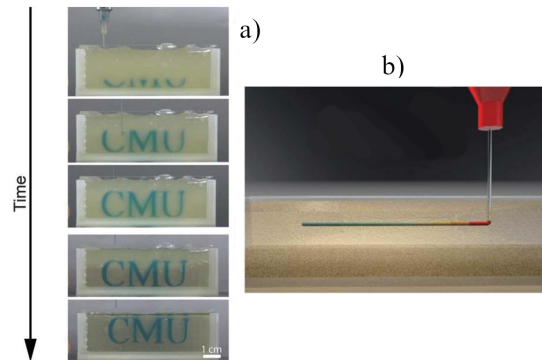


Figure 2.2: Free-form reversible embedding: illustration of principle. a) Time-lapse sequence of 3D bioprinting of the letters “CMU” using FRESH, b) close view of collagen solution extruded into the support bath *adapted from* [11].

Another major categorisation of EBB is based on the driving mechanism of the extrusion. They can be subdivided into three categories: pneumatic-driven, mechanical-driven and solenoid-driven extrusion [2][7].

Pneumatic-driven extrusion Pneumatic-based extrusion represented in Figure 2.3a, relies on compressed air coming from a compressor and pushing on either a

piston, typically the plunger of a syringe, or the free surface of the biomaterial itself. In any case, sterilisation of the air is mandatory. The flow of air can be achieved using a valve-free or valve-based design that trades a simple design for better control.

Mechanical-driven extrusion Mechanical systems are more suited for high-viscosity biomaterials and provide better control over the extrusion. Piston-driven design, reported in Figure 2.3b, is a common choice, generally implemented with a lead screw and travelling nut that convert the rotary motion of a motor into a linear displacement of the piston. Where this solution allows for good control of the extrusion flow, screw-driven design, shown in Figure 2.3c, provides better volumetric control and works best for wider ranges of viscosities. The larger pressure drop might have the drawback of damaging the cells during extrusion and therefore lowering the cell viability. An application-specific design is therefore preferable to off-the-shelf solutions.

Solenoid-driven extrusion A solenoid valve allows the flow of compressed biomaterial. This solution, along with similar valve actuating solutions such as piezoelectric-based, is not especially suited for extrusion printing and finds its main application in droplet-based printing.

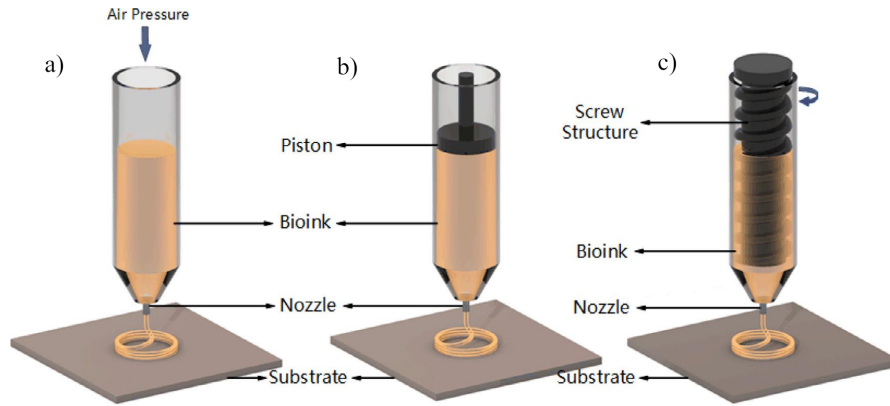


Figure 2.3: Implementations of Extrusion-Based Bioprinting (EBB) mechanisms adapted from [2]. a) Pneumatic-driven b) Piston-driven c) Screw-driven actuation.

Finally, the main parameters of EBB that influence the process and affect the final result and cell viability are mentioned hereunder [2][12].

A theoretical formulation for the printed fibre diameter d is given by Miri et al. [6]

as follows:

$$d = D \sqrt{\frac{D}{2v} \left(\frac{\eta}{K} \right)^{1/(n-1)}} \quad (2.1)$$

with D the nozzle diameter, v the biomaterial speed in the nozzle, η the viscosity and K and n material-based constants³.

Equation 2.1 shows how an increase in the nozzle diameter yields a loss in resolution due to reduced shear stress, while increasing cell viability. On the contrary, greater extrusion pressure will reduce cell viability. Additionally, for shear-thinning biomaterials ($n < 1$), higher viscosity leads to higher resolution. Temperature is also an important factor that influences cell viability in a non-monotonic trend, of which the maxima depends on the specific cells considered. An increment in normalised printing speed increases the resolution and is often related to a reduction in printing time therefore increasing cell viability as it shortens their exposition to the harsh printing conditions.

2.1.2 Droplet-based bioprinting

Second in order of spread, Droplet-Based Bioprinters (DBB) use discrete droplets to obtain the desired printed shape. Despite being the oldest technology in the field of bioprinting, the number of commercially available implementations is relatively scarce. Nonetheless, the technology offers many opportunities and advantages over competing solutions. For instance, the simpler design and versatility, as well as its high level of accuracy and control of the printing process makes the technology appealing. In addition, good results can be obtained using modified standard inkjet printers, which renders the technology easily accessible for research applications [2]. On the other hand, the high resolution comes at the cost of a lower volumetric throughput and increased printing time. Plus, the limited selection of compatible biomaterials, typically restricted to viscosity below 100 mPa·s, affects the mechanical integrity of the print and therefore reduces the reachable height of printed models.

There exist four main cross-linking strategies to build 3D structures with droplets: alternate printing, consisting in printing alternate layers of biomaterial and cross-linker; printing directly in the cross-linker solution; printing a layer of biomaterial followed by spraying of the layer with cross-linker; printing and polymerisation through UV light exposition [13]. Many variations of the technology

³ It is possible to obtain them with rheological measurements as they relate the viscosity with the shear rate such that $\eta = K\dot{\gamma}^{n-1}$ [6].

exist, including inkjet, micro-valve and acoustic.

Inkjet Inkjet printers are the most widespread and they include several sub categories based on the drop formation strategy and on the substrate interaction [13].

Continuous Inkjet CIJ printing achieves a continuous stream of drops thanks to Rayleigh-Plateau instability⁴ (Figure 2.4a). The size of the drops is quite consistent and generally around 100 μm in diameter [6]. By the use of an electric field, the unwanted drops of electrically conductive biomaterial are selectively steered away from the printing bed trajectory towards a collecting tank. The re-circulation and thus recycling of the majority of the collected drops might pose sterility issues in the procedure.

Drops-On-Demand DOD printers produce drops only in the required location, avoiding the complication of recycling and are therefore generally preferred over CIJ printers. It typically requires a viscosity greater than 30 mPa·s and generates smaller droplets, ranging from 20 to 50 μm [6]. DOD printers are in turn divided based on the droplet formation method.

Thermal Inkjet TIJ printers, shown in Figure 2.5a, use thermal resistors to generate microsecond-lasting 200-300°C heat pulses that locally vaporise the ink. The generated bubble creates a pressure pulse that propagates to the orifice and overcomes the surface tension of the biomaterial, generating a droplet. The nozzle is then supplied with fresh biomaterial via capillary action. Despite the high temperature, the heat transferred is low enough not to cause any damage to the biological material [2]. On the other hand, the lack of precise directional and size control of the of the droplets, together with frequent nozzle clogging issues, poses some challenges in the design of the printers.

Piezoelectric Inkjet PIJ printers, represented in Figure 2.5b, are similar to TIJ, with a main difference in the actuation. PIJ printers use piezoelectric actuators⁵ to achieve an abrupt change in the volume of the ink container,

⁴ Phenomenon that arises when the wavelength of the perturbed jet exceeds its initial radius by a certain limit, that is when the product of the wavenumber and the initial jet radius is less than one. The instability leads to the jet distortion that minimises its potential energy, eventually breaking into distinct drops [14].

⁵ A piezoelectric actuator responds with a deformation of its body under the application of a voltage.

which again leads to the propagation of a pressure wave responsible for the droplet formation. They are capable of reaching high frequencies (15-25 kHz) [6], which might reduce cell viability.

Electrostatic Inkjet EIJ printers are similar to PIJ printers in their functioning, with the exception that the sudden volume increment is induced by a pressure plate.

Electrohydrodynamic Jetting EHDJ printing technology, represented in Figure 2.5c, also belongs to the class of inkjet printers. Compared to other inkjet technologies, these printers rely on electric fields rather than pressure pulses, which lowers the potential damage to the cells and grants higher viability. An electric field is applied between the nozzle and the underlying substrate, this deforms the ink meniscus into a Taylor cone⁶ (Figure 2.4b). The value of the voltage has to be adjusted with respect to the distance from the substrate, and is typically between 0.5-20 kV [13]. Increasing the voltage, it transitions from discrete drops to a continuous stream. Nonetheless, single drop deposition is quite challenging and therefore renders EHDJ printers not suited for precision applications.

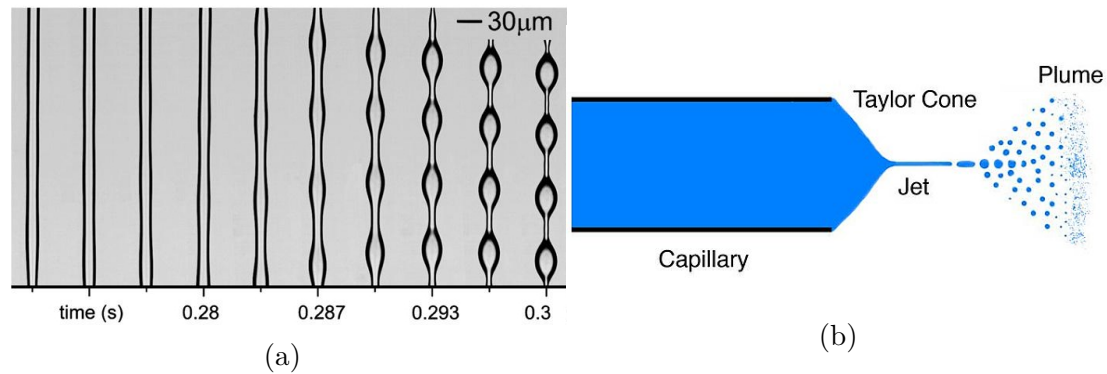


Figure 2.4: a) Time-lapse of the growth of the Rayleigh-Plateau instability in a stream of fluid *adapted from* [15] b) illustration of a Taylor cone *adapted from* [16].

Micro-valve Micro valve printers, shown in Figure 2.5d, use counter pressure and solenoid actuated micro valves. The back pressure, valve gating, nozzle diameter, ink viscosity and surface tension are key parameters for the dynamic performances.

⁶ Phenomenon occurring in electrically conductive liquids exposed to electric field, where the free surface starts to bend into a conic shape as the voltage increases. When the voltage reaches a certain threshold, a jet forms from the tip of the cone.

They generally do not form satellite drops, but the drops are larger than with inkjet printers, hence they are not suitable for high precision printing [3]. The main advantage of micro-valve printers is the synchronised deposition of biomaterial and cells through different printing heads, which allows for precise positioning of the cells and enhanced cell viability [17].

Acoustic Lastly, acoustic bioprinters, shown in Figure 2.5e, are nozzle-free printers that use acoustic waves to break the surface tension of the ink contained in small channels. They have the advantage of avoiding potential detrimental factors in the printing process, such as heat, pressure and electric fields. However, the weak acoustic wave makes it difficult to print highly viscous biomaterials and is easily disturbed by vibrations due to head positioning [3].

Overall, DBBs are capable of generating drops in the range $[20 ; 300] \mu\text{m}$, typically larger than the nozzle diameter except for EHDJ, which generates a stream of smaller droplets thanks to the formation of the Taylor cone. The final droplet projection on the substrate is larger as the droplets spread once in contact with the substrate as the spread occurs faster than biomaterial polymerisation.

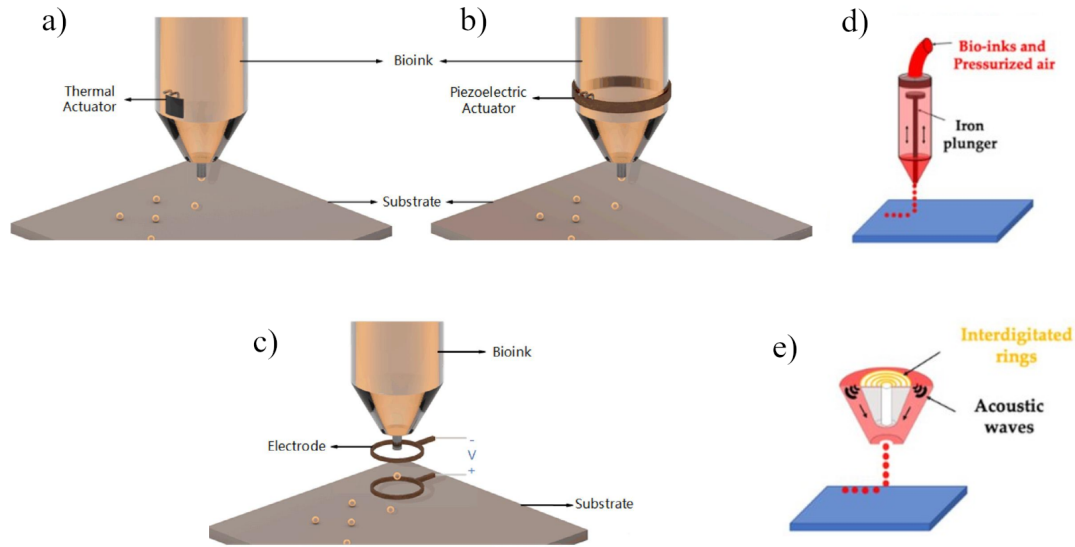


Figure 2.5: Main implementations of Droplet-Based Bioprinting (DBB) *adapted from [2][18].* a) Thermal Inkjet (TIJ) b) Piezoelectric Inkjet (PIJ) c) Electrohydrodynamic Jetting (EHDJ) d) Microvalve e) Acoustic

The printing process is normally considered possible only if the inverse of the

Ohnesorge number, Z , is greater than 1 [19]:

$$Z = Oh^{-1} = \frac{Re}{\sqrt{We}} = \frac{\sqrt{\tau\rho\iota}}{\eta} > 1 \quad (2.2)$$

where We is the Weber number, Re denotes the Reynolds number, τ is the surface tension, ρ the ink density, η represents the dynamic viscosity, and ι a characteristic length, taken as the nozzle radius for example. The main parameter is the viscosity, as the others are quite consistent among various inks.

Based on the value of Z , the properties of the droplet formation is greatly affected: for low values of Z , the droplets undergo a viscous dissipation (i.e. spreading) upon contact with the substrate, while higher figures induce splashing and satellite droplet formation [6].

Consequently the resulting resolution is greatly affected by the value of Z together with the nozzle diameter, the volume of droplets, the spacing between droplets, the printing speed and the affinity of the biomaterial with the substrate.

2.1.3 Light assisted bioprinting

Light Assisted Bioprinting (LAB) includes all the printing technologies that leverage a controlled light source to either transfer the biomaterial onto the printing substrate or to achieve selective polymerisation of the biomaterial. Various techniques exist, among which Laser Guidance Direct Writing (LGDW), Laser Induced Forward Transfer (LIFT), Stereolithography (SLA), Digital Light Processing (DLP) and Two-Photon Lithography (TPL).

Laser Guidance Direct Writing LGDW consists in using a laser beam to steer the biological materials and precisely guide them towards the printing substrate. The biomaterial is trapped and propelled by the laser beam due to optical forces that are extremely sensitive to the material refractive index [7], resulting in only a handful of compatible materials. Together with low viability of the printed cells, the reduced material selection represents the main drawback of the technique, which is progressively less used in the field of bioprinting [2].

Laser Induced Forward Transfer LIFT, shown in Figure 2.6a, offers improved viability, greater than 90%, due to indirect exposure of the biomaterial to the high energy laser source [6][20]. This technique consists in directing the laser beam towards a donor plate placed parallel above the receiving substrate. The donor plate, also referred to as ribbon structure, is usually constituted of a transparent layer of glass or quartz and an Energy Absorbing Layer (EAL) generally made of gold or titanium, and coated with biomaterial. As the laser beam hits the EAL, local evaporation of the biomaterial occurs at the interface with the metal. The pressure generated by the rapidly expanding bubble is responsible for the initiation of the jetting process.

The jetting regime and the resulting resolution of the printing are highly dependent on the laser fluence⁷. Low values might induce subthreshold regime and no printing, whereas high values end up in turbulent or plume jetting with satellite drops. This also induces high thermal diffusion in the plate with the subsequent risk of killing part of the cells [22].

Overall, LIFT offers a limited resolution compared to other LAB technologies, typically around 100 μm [6]. It allows to print single cells and the high cell viability makes it especially suitable for biological applications. The absence of the nozzle and contactless print also remove issues such as clogging and cross-contamination. On the contrary, the low printing throughput makes it difficult to print large

⁷ Fluence (or radiant exposure): *"At a given point in space, the radiant energy, Q , incident on a small sphere from all directions divided by the cross-sectional area of that sphere. SI unit is Jm^{-2} "* [21].

structures [23]. The cost is also a limiting factor, together with the difficulty in tuning the printing parameters.

Stereolithography SLA is the first known implementation of 3D printing, nonetheless its use in bioprinting is quite recent. The technology consists in scanning with a UV laser the surface of a tank filled with photosensitive biomaterial. The material then photopolymerises⁸ upon exposure to the beam of light. The scanning path is traced in 2D thanks to a movable mirror. Every time a layer is completed, the substrate moves vertically, allowing for fresh biomaterial to cover the previous layers. The vertical displacement is based on the layer thickness. With this technique, the printing speed is greatly enhanced, whereas the biocompatibility is generally lower than LIFT due to photoinitiator cytotoxicity⁹. The resolution can go down to 50 μm , but the list of compatible materials is reduced [6].

Digital Light Processing Much like SLA, DLP uses light in the UV spectrum to polymerise a photocurable hydrogel or a photosensitive resin in a layer-by-layer approach, see Figure 2.6c. The main difference is that each layer is polymerised at once by the use of dynamic masking that selectively reflects or transmits the incoming light to the substrate. The dynamic masks can be achieved using Liquid Crystal Displays (LCD) or Dynamic Micromirror Devices (DMD). While LCD offer poor resolution and slow response, in recent years the development of DMD made it possible to obtain higher resolution, down to [5 ; 50] μm , and better optical fill factor [2].

The innate advantage of DLP over SLA is the faster printing speed, independent with the layer complexity, while it still has moderate viability due to the toxicity of the photoinitiator [6].

Two-Photon Lithography TPL, represented in Figure 2.6b, is a relatively new technology. It has already proven to be very efficient in realising true 3D structures, without relying on the layer-by-layer approach of SLA and DLP. The underlying phenomenon that drives the process is called two-photon absorption. It consists in photopolymerising the material by absorbing two photons emitted by a femtosecond (10^{-15}) laser working at longer wavelengths, typically Near-Infrared (NIR) as opposed to UV light. Since most materials show low linear absorption in the NIR spectrum, the polymerisation only takes place in the focal point of the laser beam, allowing it to penetrate the material without inducing polymerisation

⁸ Photopolymerisation : *"Photochemical reaction that takes place when photon energy is provided to a matter"* [24].

⁹ Cytotoxic : *That is toxic to cells.*

in the outer region [24]. It is therefore possible to create complex 3D structures with spatial resolution below $5\ \mu\text{m}$ by moving the substrate relatively to the focal point. The drawbacks of this technology are the limited printing speed, volume and material compatibility [6].

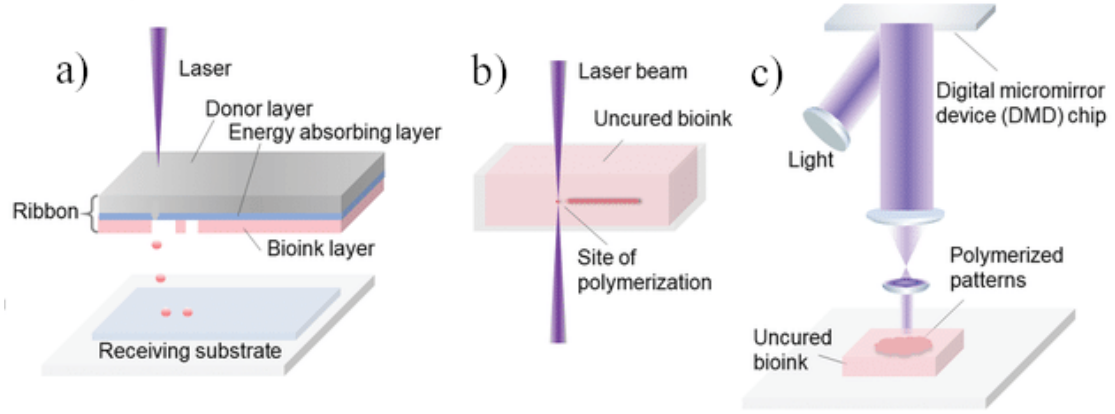


Figure 2.6: Main implementations of Light Assisted Bioprinting (LAB) *adapted from* [25]. a) Laser Induced Forward Transfer (LIFT), b) Two-Photon Lithography (TPL), c) Digital Light Processing (DLP).

2.2 Bioinks and biomaterial inks

Since the appearance of bioprinting, many research groups have tried to find suitable and efficient combinations of printing methods and printing materials thus resulting in a variety of designations for those materials.

In 2019, J. Groll et al. [26] suggested a way to differentiate the different types of materials to be used for biofabrication; they put forth that cells are a necessary component for a material to be called bioink. Although printing with simple cell-loaded solutions is achievable, it lacks structural capabilities. Therefore, polymers are often used to print scaffolds that are seeded with cells afterwards or the polymers are mixed with cells ahead of the extrusion. For the first case, these polymers are referred to as biomaterial ink. In the frame of this thesis, a third type of material called bioresin is introduced. It is a subcategory of biomaterial inks than describes inks destined to processes with selective cross-linking by light exposure, see Section 2.1.3. Bioresins are used with bioprinters such as the Lumen X+ presented in the following section. As a general term to ease the reading throughout this thesis, the word biomaterial is used.

The type of ink as well as its characteristics need to be chosen appropriately with regards to the application and the printing method. The printing characteristics of every ink need to be accounted for in order to be printed efficiently. Some inks require to be maintained at high temperature and cooled down once printed, shear-thinning inks require high shear stress to through the nozzle to flow out of the print head onto the substrate and light affected inks need to be cured layer by layer as the printing process goes.

Hydrogels Hydrogels are very popular for biological applications. They are polymers that can hold water for up to 99% [27] of their total volume so they make great reservoirs for humidity and promote hydration. In addition, their hydration level influences their biocompatibility. Hydrogels are also not hydrosoluble so it is printable within a cell culture medium while keeping structural integrity. These characteristics are very interesting when it comes to bioprinting as it allows to print cells directly within a water-rich Extracellular Matrix (ECM). Hydrogels fall into two main classes: hydrogels such as gelatin, collagen, chitosan and alginate that are naturally-derived and Pluronic®¹⁰ or Polyethylene Glycol (PEG) among others that are synthetically-derived [28].

¹⁰ 3-bloc copolymer with a hydrophobic central bloc.

Decellularized Extracellular Matrix dECM inks have also been developed by removing the cellular material of the ECM through chemical, physical and enzymatic processes. This material is gel-like, it is adequate for bioprinting and has a great biocompatibility.

2.3 Illustrative list of existing printers

Nowadays there are many bioprinters available on the market. These printers offer different printing solutions to accommodate a wide range of materials and printing conditions. The most recurrent printing method is extrusion through a syringe with a pneumatic or mechanical system. Then, depending on the manufacturer or even the models of a same brand, the printers offer other tools such as a filament extruder for polymers like PLA¹¹, a pipetting head or a photocuring head to mention the most common solutions.

To ensure adequate printing conditions, the bioprinters often feature temperature control of the bed and/or the nozzle and a clean enclosure or a streamlined profile suitable for laminar flow conditions and easy cleaning and sterilisation.

A list of commercialised bioprinters along with their specific features that are relevant for our research can be found in Table 2.1. This list is not comprehensive but it reflects the variety of bioprinters available and it allows to have a grasp of the relative cost based on their complexity and versatility.

¹¹ Polylactic Acid, most common plastic material used in fuse deposition modelling. Used in the form of filament.

Table 2.1: List of commercialised bioprinters and relevant technical characteristics.
(This list is not comprehensive)
 "# of active tools" is the number of tools that can be used in a single print.

Printer	Manufacturer	Methods	Material	# of active tools	Nozzle size	Temperature control [°C]	Printing bed [°C]	Enclosure	Others	Cost (entry)
Dr. INVIVO 4D2 Fig. 2.7 d	Rokit Healthcare	Syringe dispenser;	Collagen, Hyaluronic Acid, Gelatin, Pluronic Acid, Chitosan, etc;	2	[100 ; 600] μm ;	[-4 ; 60];	[-4 ; 60]	Yes; H14 HEPA filters; UV-C Led	Biopink warmer; Infrared light curing	\$20k
		Filament Extruder; Hot-melt pneumatic dispenser	PCL, PLA, PLGA, etc; PCL, PLA, PLGA, polyurethane, etc		[100 ; 400] μm ; [100 ; 600] μm	[RT ; 250]; [RT ; 350]				
REGENMAT3D Fig. 2.7 g	V1 BioPrinter	Extrusion syringe Metal syringe	Bioinks, biomaterials	3	[0.1 ; 1.2] mm; [0.2 ; 0.4] mm	"Refrigerated" - 50; "Refrigerated" - 50; Heated bed: [20 ; 120]	Refrigerated bed: [-20 ; 100]; Heated bed: [20 ; 120]	No	Max print speed: 20 mm/s; Manual purging system	\$25k
Allevi 3 Fig. 2.7 a	Allevi	5ml plastic/metal Luer lock syringe	Bioinks, biomaterials (optimized for their range)	3	Luer lock compatible; [233 ; 864] μm (in their range)	[4 ; 160]	[RT ; 60]	No	Photocuring {365 ; 405} nm	\$40k
BioAssemblyBot Fig. 2.7 b	Advanced Solutions	Syringes (coaxial, mix, mechanical, pneumatic), UV, pick & place, laser scan, build my own	Bioinks, biomaterials	8	<i>not mentioned</i>	3D syringe Hot: [RT ; 150]; 3D syringe Cold: [-4 ; RT]	[10 ; 60]	Yes; clean room ready; HEPA filtration	6 axis human arm	\$100k
BS3.3 Prime Fig. 2.7 e	GESIM	Syringe dispenser (mech. & pneuma.) Melt Electro spinning Nanolitre pipetting Filament extruder Plasma pen Piezo Inkjet Spotting	Bioinks, biomaterials Biopolymers (hard, soft) Filament (eg. PLA)	4	mention of 580 μm nozzle	Peltier cartridge chiller [4 ; RT]; Cartridge heater [RT ; 190]; Nozzle heater [RT ; 250]	<i>not mentioned</i>	No; Designed to be placed Biosafety Cabinet or at least in laminar flow	extrusion mixers and other tools available	(\$150k older model)
BIO X Fig. 2.7 c	CELLINK	Pneumatic printhead; Electromagnetic Droplet (EMD); Syringe (mechanical); Photocuring; Temperature -controlled printhead; Filament extruder	Universal biomaterial compatibility; Filament (eg. PLA)	3	mention of {200 ; 250 ; 410} μm	[4 ; 250]	[4 ; 65]	Yes; HEPA H14 filter; UV sterilisation		upon request
Lumen X+ Fig. 2.7 f	CELLINK	Digital light processing (crosslinking)	<i>PhotoInk™</i>	1	Irrelevant 50 μm resolution	/	/	Yes; Anti UV enclosure		upon request

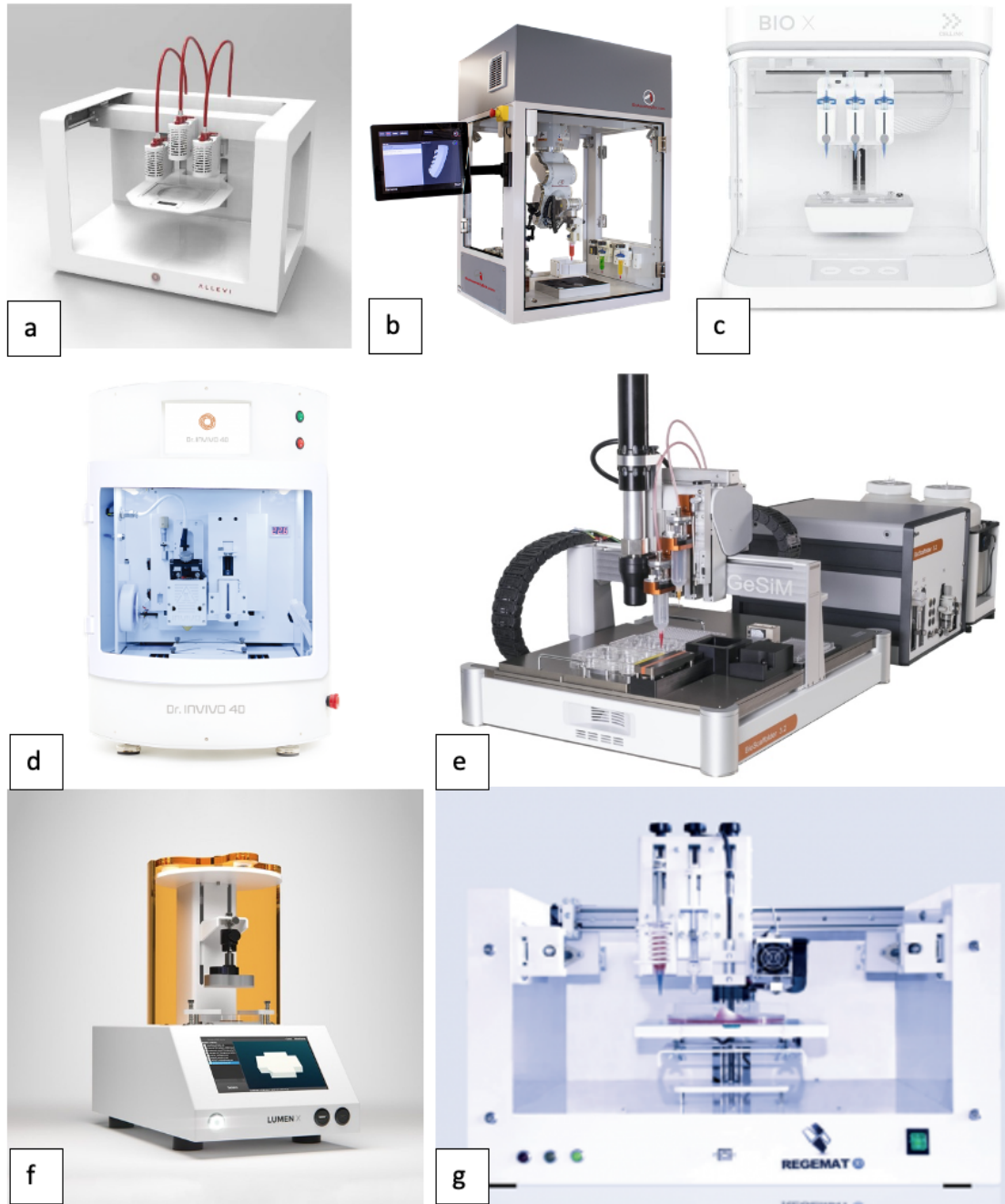


Figure 2.7: Commercialized bioprinters. a) Allevi 3 by *Allevi* adapted from [29]; b) BioAssemblyBot by *Advanced Solutions* adapted from [30]; c) BIO X by *CELLINK* adapted from [31]; d) Dr. INVIVO 4D2 by *Rokit Healthcare* adapted from [32]; e) BS3.3 Prime by *GESIM* adapted from [33]; f) Lumen X+ by *CELLINK* adapted from [34]; g) REGEMAT3D by *V1 BioPrinter* adapted from [35].

2.4 Recap on the state of the art and directions

An outline of the different technologies used in bioprinting is shown in Figure 2.8. It sums up Section 2.1 and gives a visual representation of the segmentation of the different printing methods available.

As a reminder, the research objective of this thesis is twofold. The aim of the first part is to produce a comprehensive list of requirements for a 3D bioprinter, the goal of the second part is to assemble a working prototype of a printhead. The prototype should allow to deposit biomaterials with and without embedded cells so both clients, Jean-François Rees and VOCSens can perform specific research.

Light assisted technologies are set aside for their complexity, limited material selection and because the viability of the cells is low on average due to recurring cytotoxicity of photoinitiators.

Extrusion- and droplet-based bioprinting stand as good candidates for this project. The wide range of biomaterials to choose from and their simple design leading to an easy setup make these technologies prone to a user-friendly experience. Moreover, their flexibility on materials allows to develop new biomaterial inks and bioinks and put them to test effortlessly.

The choice of type of materials to use as ink is left to the care of users qualified in the fields of biomaterials and biological sciences.

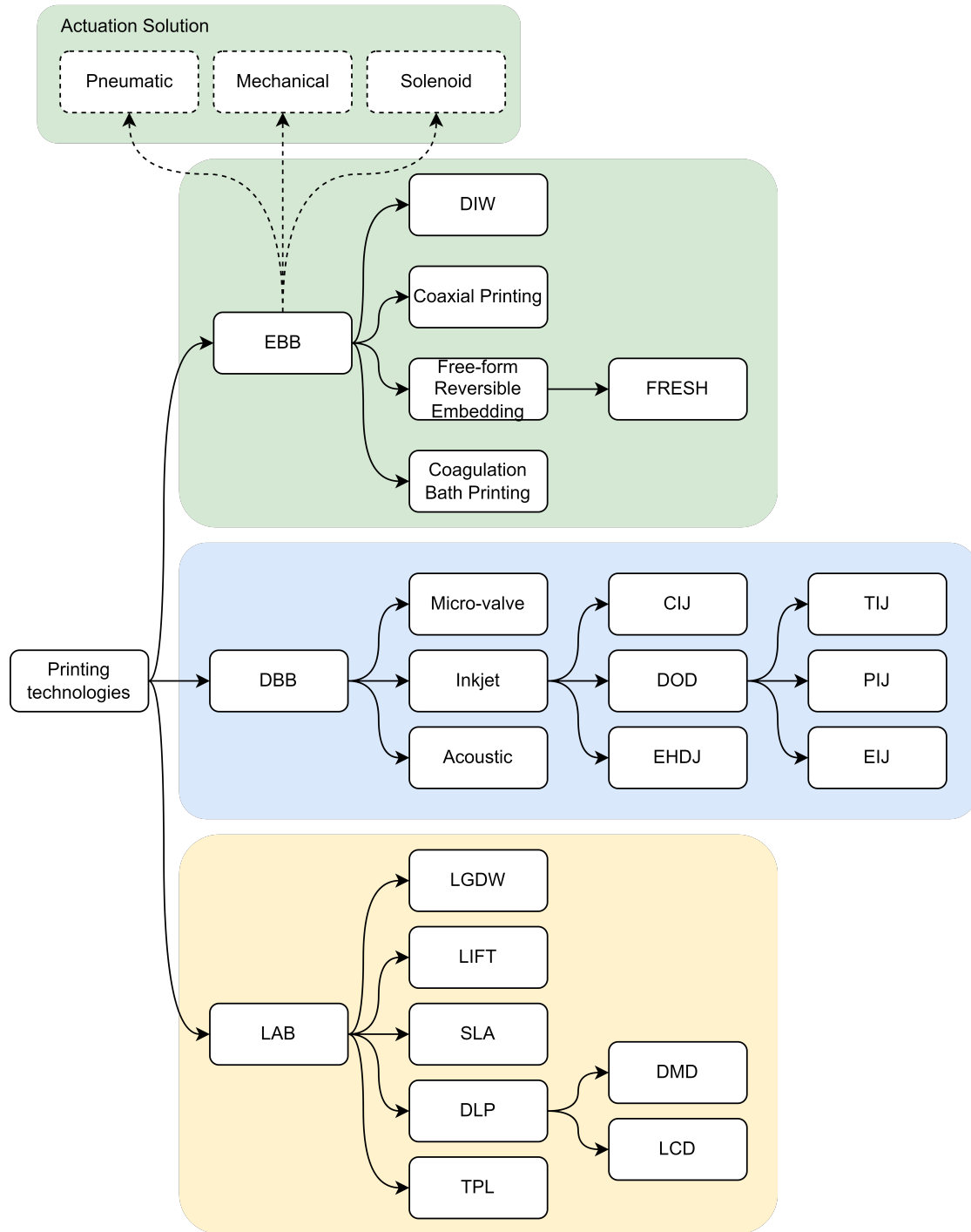


Figure 2.8: Outline of the different technologies used in bioprinting.

EBB: *Extrusion-Based Bioprinting*, DIW : *Direct Ink wWriting*, FRESH : *Free-Form Reversible Embedding of Suspended Hydrogels*, DBB : *Droplet-Based Bioprinting*, CIJ : *Continuous Inkjet*, DOD : *Drops-On-Demand*, TIJ : *Thermal Inkjet*, PIJ : *Piezoelectric Inkjet*, EIJ : *Electrostatic Inkjet*, EHDJ : *Electrohydrodynamic Jetting*, LAB : *Light Assisted Bioprinting*, LGDW: *Laser Guidance Direct Writing*, LIFT: *Laser Induced Forward Transfer*, SLA: *Stereolithography*, DLP: *Digital Light Processing*, DMD: *Dynamic Micromirror Devices*, LCD: *Liquid Crystal Displays*, TPL: *Two-Photon Lithography*.

Chapter 3

Design Objectives

Defining design objectives allow to set precise guidelines and give directions for the project. The first steps are to meet with the clients to identify their needs and to review the state of the art of the concerned scientific field. From this, objectives are derived and mapped in a structured way called an objective tree. The latter is then used to make a list of technical requirements that are sorted in functions and constraints. These specifications guide the design methodology until the end of the project when they are used to verify that the machine developed complies with the requirements of the clients.

3.1 Objective tree

The objective tree is a graphical segmentation of a project in objectives in order to have a better overview of it as a whole. These objectives are weighted by level, this helps structuring the work and sharing the time available to different tasks with respect to their importance.

The project, in full, consists in designing a complete 3D bioprinter and it is reflected in the objective tree, weighted accordingly, and in the specifications. In the frame of this thesis, the aim is to design and build a working prototype of a printing head based on an extrusion system. This head will be mounted on an existing 3D printer frame, a Leapfrog Creatr HS, already available in the iMMC¹ department.

The process of assigning weights starts from the main objective of the tree and it is completed level by level. When an objective divides into sub-objectives, a relative weight is assigned to each of them, totalling unity. The absolute weight is computed with respect to the main objective.

¹ Institute of Mechanics, Materials and Civil Engineering.

For example, Figure 3.1a shows the main objective and its direct sub-objectives. They have been weighted according to their relative relevance with respect to the others. The objective **Print material** is deemed more important than the others. In fact, if not met, the others become irrelevant. To **Meet the needs of research applications** is also fundamental as the bioprinter needs to fit the specific conditions of the research applications. **Allow different types of settings** and **Print multiple materials in the same process** are minor objectives in comparison.

The sub-level of the objective **Print material** is shown in Figure 3.1b. The main goal of the client Professor Rees is to print sponge tissue 3D constructs and the main goal of the client VOCSENS is to deposit layers of minute amount of particles in solution on a substrate. Therefore **Print ECM and/or cells** is weighted more heavily than **Deposit drops** as it can achieve both purposes whereas droplet based systems lack of 3D structure efficiency in comparison.

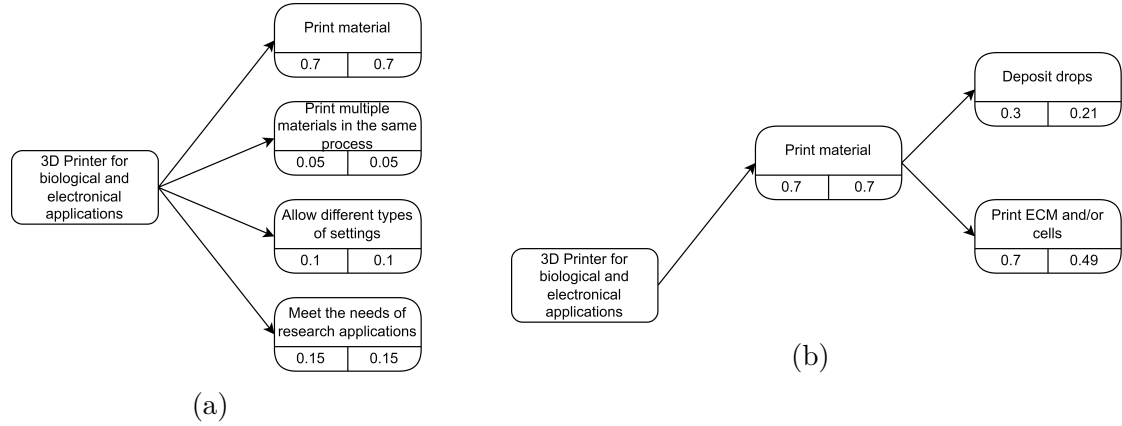


Figure 3.1: a) Representation of an objective (main objective) and its sub-objectives, b) Main objective and path to level of sub-objectives.

A similar analysis to the one reported above has been carried out for all the objectives of the tree in an iterative process. The latest version of the objective tree is shown in Figure 3.2.

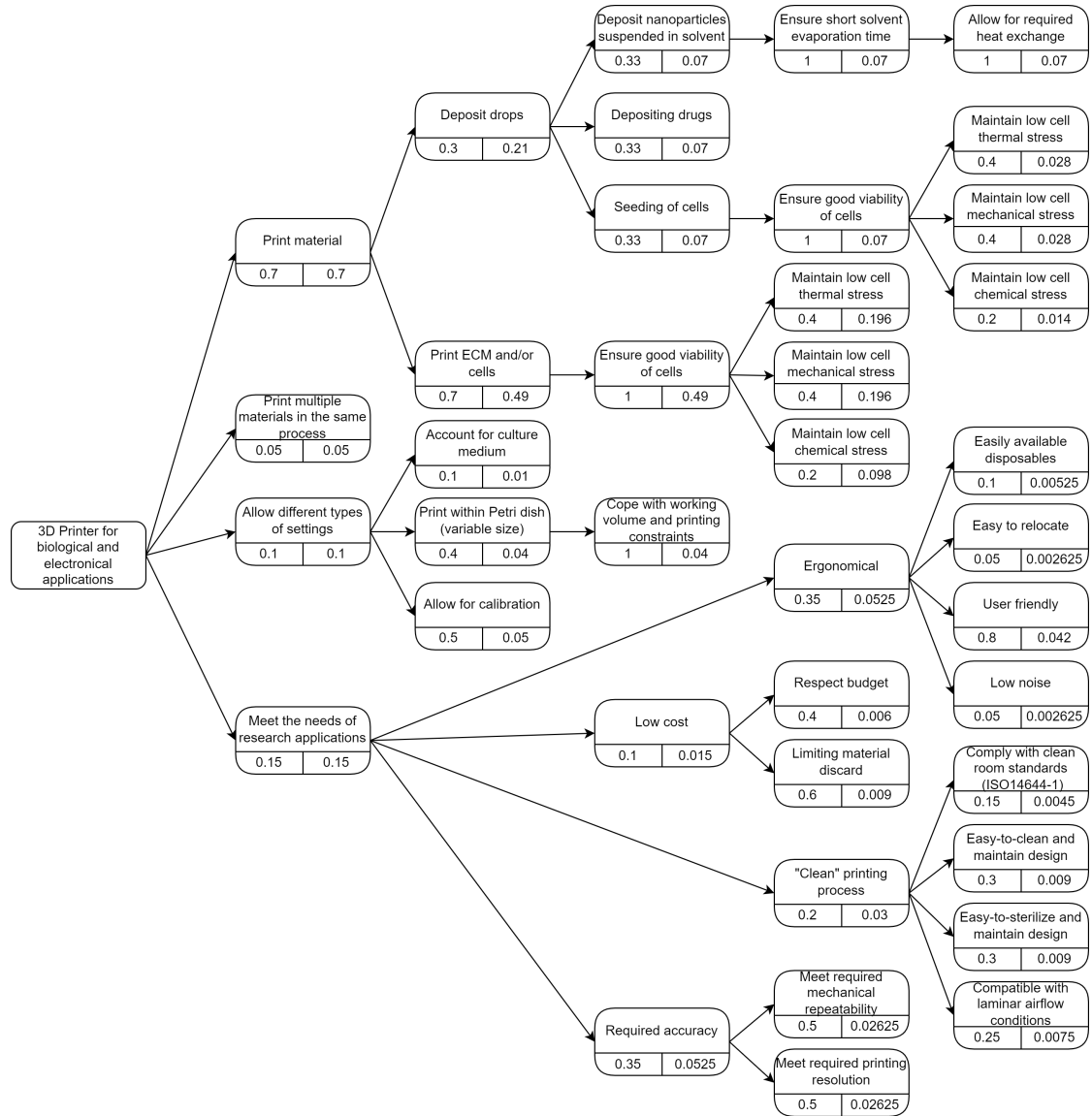


Figure 3.2: Objective tree V3. The value in the bottom left corner of each objective represents its relative weight compared to competing objectives. The value in the bottom right corner is the absolute weight computed with respect to the main objective. The sum of the relative weights of sub-objectives of a common objective amounts to unity. Similarly, the sum of the absolute weight of the extreme sub-objectives totals one.

3.2 Specifications of the bioprinter

The specifications of the bioprinter are established based on the needs of the clients and thorough research on the state of the art of existing technologies and latest results in bioprinting. The specifications are reviewed and updated through an iterative process involving the clients to set an acceptable and suitable standard for the machine.

The most recent version of the specifications, *Version: 1.4*, is available for consultation in the following pages of this Chapter. The document is separated in *Main Functions* (MF) and *Main Constraints* (MC). Each of the MFs and MCs present one criterion or several criteria denoted respectively *Criteria of Main Function* (CMF) and *Criteria of Main Constraint* (CMC).

Every MF and MC is dated based on its appearance or latest modification. A CMF (resp. CMC) added or modified after the edition of its respective MF (resp. MC) is also dated accordingly.

Leonardo Missich Valentin Tack	Specifications	Date: 18/02/2022 Version: 1.4
<i>Bioprinter</i>		

Date	Main Functions
2021-12-22	MF-1: Print material
2022-01-31	CMF-1.1: Printing of extracellular matrix (with or without cells) Cell (eukaryotic) size [10 ; 100] μm [36]
2021-12-22	MF-2: Deposit drops
2022-01-31	CMF-2.1: Deposition of nanoparticles in a solvent CMF-2.2: Deposition of drugs CMF-2.3: Seeding with cells Cell (eukaryotic) size [10 ; 100] μm [36]
2021-11-22	MF-3: Parallelise the printing process
	CMF-3.1: Multiple printing heads CMF-3.2: Different combinations of types of heads CMF-3.3: Size of the printing bed
2021-11-22	MF-4: Print multiple materials simultaneously
	CMF-4.1: Able to print different materials during a single process
2021-11-22	MF-5: Allow different types of settings (e.g.type of Petri dish, nozzle length, ...)
2022-01-31	CMF-5.1: Account for starting height (z-calibration)
	CMF-5.2: Account for starting position in build plane (x-y-calibration)
2022-01-31	CMF-5.3: Account for shape of Petri dish (substrate) Petri dishes dimensions may constraint the workspace (Impact of the bulkiness of the printhead). The height of the walls and diameter of Petri dishes. (Size $D \times h$: [60 ; 150] $\times$ [10 ; 25] mm)
2022-01-31	MF-6: Allow research on viability of cells
2021-12-22	CMF-6.1: Control of temperature of nozzle

continues on next page

2021-12-21	CMF-6.2:	Control of temperature of bed
2021-12-21	CMF-6.3:	Control of flow rate in force or position
2021-12-21	CMF-6.4:	Fast process, the impact of the printing time on cell viability should be negligible. (e.g. can move fast when not printing but cannot extrude too fast)
2021-11-22	MF-7: Ensure solvent evaporation time	
	CMF-7.1:	Control of temperature of nozzle [40 ; 60] °C
2021-11-22	MF-8: Allow the use of culture medium	
	CMF-8.1:	Allow to add culture medium during process (e.g.: add saltwater)
	CMF-8.2:	Allow to print directly within culture medium (e.g.: saltwater)

Date	Main Constraints	
2021-11-22	MC-1: Work within a clean room	
	CMC-1.1:	ISO14644-1 (WinFAB: ISO 3, class 1)
2021-11-22	MC-2: Ergonomics	
	CMC-2.1:	Low noise
	CMC-2.2:	User friendly (low technical knowledge needed)
	CMC-2.3:	Easy to relocate (weight and bulkiness)
2021-11-22	MC-3: Cost	
2022-01-31	CMC-3.1:	Research applications : [1k ; 10k] €
	CMC-3.2:	If applicable, minimise the cost of disposables (eg. Nozzles, syringes,...)
2021-11-22	MC-4: Cleanliness	
2021-12-22	CMC-4.1:	Cleaning of external agents should be easy and clean conditions should be maintained through the whole printing process
2021-12-22	MC-5: Disinfection/Sterility	
	CMC-5.1:	Sterilisation/disinfection should be easy to perform and the aseptic conditions should be held during the whole printing process [3]

continues on next page

2022-01-31	MC-6: Accuracy² and repeatability³
	CMC-6.1: Accuracy of 25 μm CMC-6.2: Repeatability of 25 μm
2022-01-31	MC-7: Printing resolution
	CMC-7.1: JF Rees: minimum resolution 500 μm (droplet and extrusion) CMC-7.2: VOCsSens: minimum resolution 10 ³ μm (droplet)
2022-01-31	MC-8: Printing volume
	CMC-8.1: XY-direction $\varnothing$ [20 ; 200] mm CMC-8.2: Z-direction 100 mm
2022-01-31	MC-9: Cost of bio inks
	CMC-9.1: Limit material discard
2022-01-31	MC-10: Laminar airflow (JF Rees application)
	CMC-10.1: Print within laminar airflow conditions Dimensions of the table : 180 $\times$ 95 cm Dimensions of the laminar airflow outlet : 180 $\times$ 73 cm

² Accuracy: maximal positioning error over a given workspace [37].

³ Repeatability: unpredictability of the behaviour of a manipulator along periodic tasks to be repeated over and over [38].

Chapter 4

Design of the extrusion printhead

The aim of this thesis is to design and build a working prototype of a printing head based on an extrusion system. This head will be mounted on an existing 3D printer frame, a Leapfrog Creatr HS already available in the labs. The printing frame is a 3-axis cartesian robot. The printing head moves in the XY plane and is fixed in the Z direction (vertical), the vertical displacement is achieved by the movement of the bed, see Figure 4.1.

The printer runs with Marlin, a very common G-code firmware. It can be controlled via its own control panel or by connecting a computer running Simplify3D¹.

The design solution of the printhead is versatile, as it is composed of separate interchangeable parts: a base, an engagement system and an extrusion system. They are all independent and can be changed based on the requirements and the working frame.

Unless stated otherwise, the 3D renderings and the technical drawings have been generated using SOLIDWORKS.

¹ <https://www.simplify3d.com/>

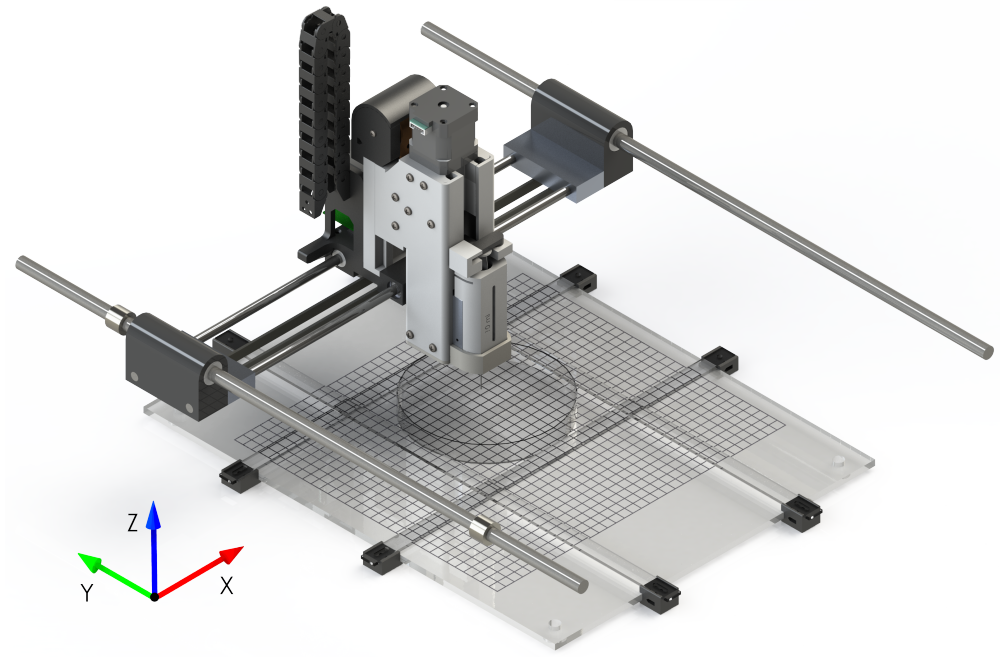


Figure 4.1: Printing frame set up with designed head.

4.1 Printhead mounting system on the Leapfrog printer

The printhead is mounted on the X-axis of the Leapfrog Creatr HS printer. It consists in a linear guidance system made of four linear bushings travelling on two $\varnothing 8$ parallel guiding rods spaced by 60 mm. This confers a minimum encumbrance in the Y direction of the moving part. In the design process, the lateral (X) dimension is minimised to allow for multiple printheads mounted side by side while maximising the printing area.

The base of the printhead provides support and guiding. It can be modified based on the frame configuration of the printer.

Regarding the support, the design of the base makes it easy to correctly place the extrusion assembly thanks to an alignment slot and two M6×35 screws, as shown in Figure 4.2a. This also simplifies the assembly, so the two parts can be easily replaced.

The design ensures the rigidity of the structure, needed for the accuracy of the printing. In addition, the base supports a fixing frame to attach the cable chain,

as well as the PCB connecting the motors and the heating system.

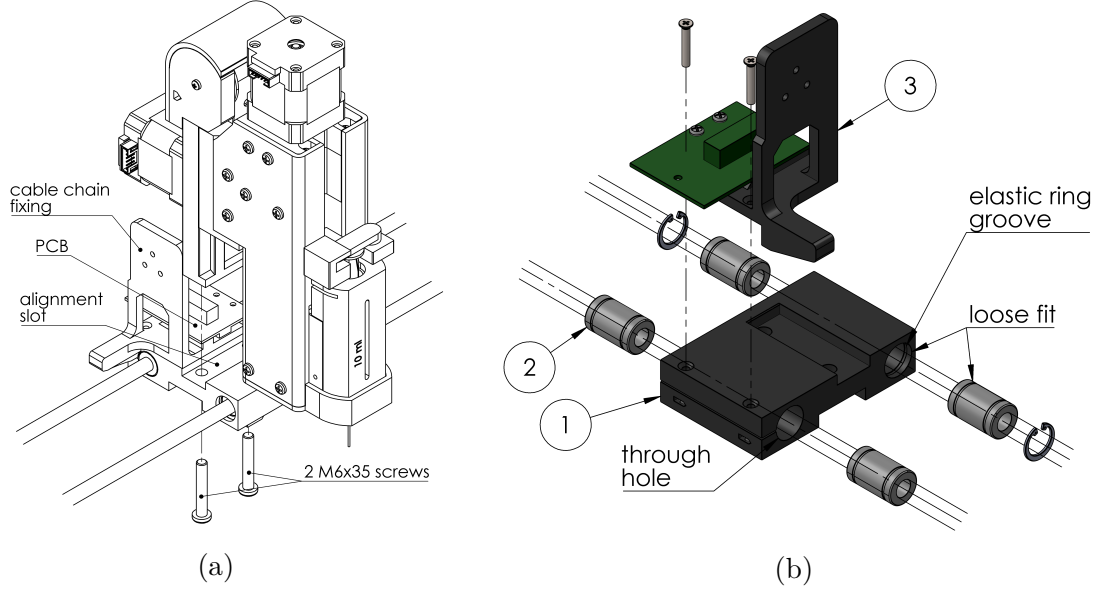


Figure 4.2: a) Exploded view showing mounting of the extrusion assembly on the base b) exploded view of the design solution for the mounting of the linear guides. The indexing of the ballooning refer to Table 4.3.

Misalignment of the linear guides should be accounted for so the base travels smoothly along the X-axis, thus limiting noise and wear. Our design solution is shown in Figure 4.2b, where an exploded view of the base and linear guides can be seen. One pair of bushings is fixed to the base with a vise. This is obtained directly with the compliance of the printed part and can be tightened with a pair of screws when the bushings are in place, fixed both axially and radially. The other pair of bushings is loosely inserted in the part so the radial play can cope with slightly skewed guides. The axial locking is provided by a circlip on each side.

The pre-existing cable chain is used for the routing of the electrical connections from the controller board to the printing head. Its minimum bending radius prevents the printhead from reaching the limit of the X-axis and from making contact with the calibration microswitch. To avoid breaking the cable chain and allow for correct calibration, an extension is added to press the microswitch, as shown in Figure 4.3. Since the design is modular, it is again possible to substitute the part in order to easily cope with different frame configurations.

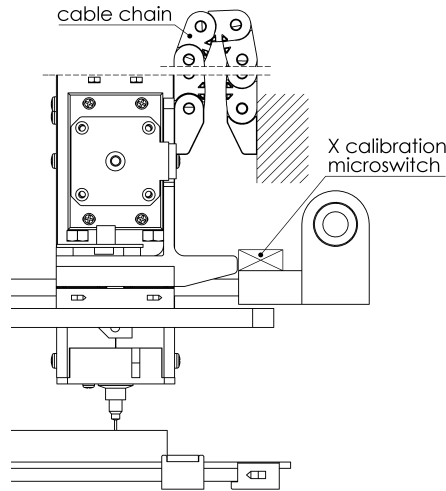


Figure 4.3: Extension of the moving base to cope with cable chain encumbrance and allow for triggering of the calibration microswitch.

4.2 Printhead engagement system

A major requirement for the bioprinter is to allow for multiple printing heads to work during a single print. An intrinsic problem arises with the idle heads that are also in the workspace and might hit the substrate, the construct or the borders of the substrate (i.e. the walls of a Petri dish). This is solved by implementing a solution consisting of a rail with a wedge profile, fixed to the base, on which moves a sled carrying the extrusion assembly.

This is in line with the idea of modularity and flexibility of the design, as the design of the different parts is independent and a modification on a given subassembly will not affect the others. A representation of the multi head setup is shown in Figure 4.4.

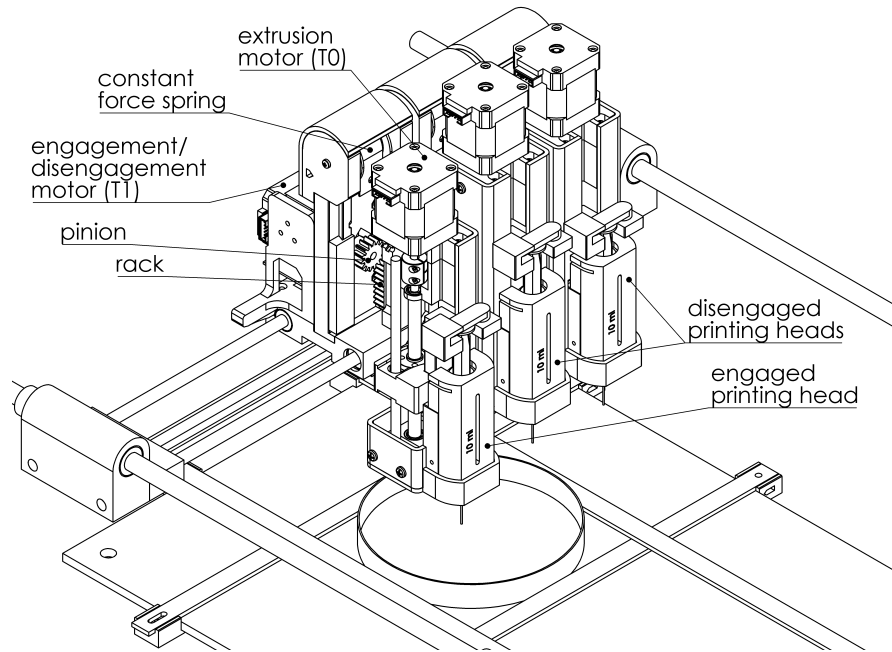


Figure 4.4: Multi head configuration with partial cut view of the rack and pinion mechanism.

The engagement and disengagement of the printing head is achieved through its vertical displacement. This motion is provided by a rack and pinion mechanism. An E3D stepper motor [39], mounted perpendicularly to the extrusion axis, is used to perform the task. A flange is used to adjust the horizontal position and grant good meshing of the pinion with the rack, as shown in Figure 4.5.

To reduce the encumbrance of the design, the rack is incorporated to the sled as one single piece. This can be mounted on the rail from the top as the wedge profile of the rail prevents it from separating. The design partially encloses the gears, keeping the printhead cleaner. To prevent the sled from getting off of the rail, a stop plate is mounted on top.

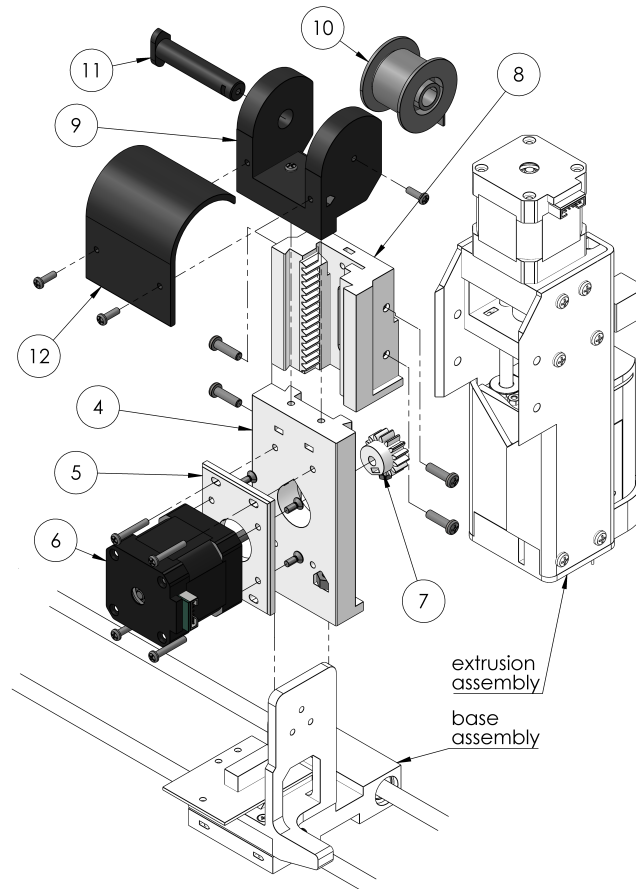


Figure 4.5: Exploded view of the head engagement mechanism. The indexing of the ballooning refers to Table 4.3.

The idle printheads resting on the top position of the rail requires to constantly apply a voltage to the motor in order to overcome the torque applied by the weight of the extrusion assembly through the pinion. To avoid this unwanted power consumption, a constant force spring is used to pull the sled upwards and balance the weight of the extrusion assembly. The rating of the spring has been chosen based on the weight calculation in Table 4.1. In a preliminary estimation, a weight of 650 g was calculated so a 1 kg spring was selected. A measurement of the extrusion system was performed once completely assembled, with a total weight of 865 g. This confirms the selection of the spring as the weight of the assembly is less than 1 kg.

The friction between the sled and the rails compensates for the 135 g difference, so the sled stays still after it is moved as every position is stable. This further reduces the required power consumption as the motor does not need to hold the printhead

down. It also adds a safety feature as the printhead does not move unexpectedly when the printer is powered off. The spring is mounted inside an enclosed frame that prevents dust accumulation.

Table 4.1: Estimation of the weight of the extrusion system for spring selection and total mass weighted with full assembly for validation.

Weight estimation for spring selection		
Parts	qty	Total estimated weight [g]
3D printed parts	N/A	112.81
Extrusion motor	1	360
Lead screw	1	50
Lead screw nut	1	15
Linear guide	2	70
Bearings	2	20
Bushings	2	12.58
Fastners	N/A	10
Total mass (estimated)	-	650.39
Total mass (weighted)	-	865

4.3 Extrusion actuation system

An exploded view of the assembly of the extrusion system is shown in Figure 4.6. The extrusion is achieved through the use of a lead screw and a travelling nut that is fixed to a moving frame. This in turn pushes on the plunger of the syringe, placed parallel to the lead screw. The guiding of the moving part is achieved through the use of two rods and bushings for smooth functioning.

The actuation is provided by a stepper motor, placed in line with the screw, on top of the assembly. This reduces the encumbrance as no additional transmission is needed and it keeps the lower part of the printhead clear.

The design also features two removable side panels that serve for structural integrity as they link the parts of the assembly together and they connect the assembly to the head engagement system. They also enclose the inner mechanism to avoid contamination of the printing environment. The syringe is held in place by a removable cover that is attached to the heating jacket with a pair of magnets. This makes it easy to place the syringe for printing and remove it afterwards.

The main constraint faced when designing the mechanism was determined by

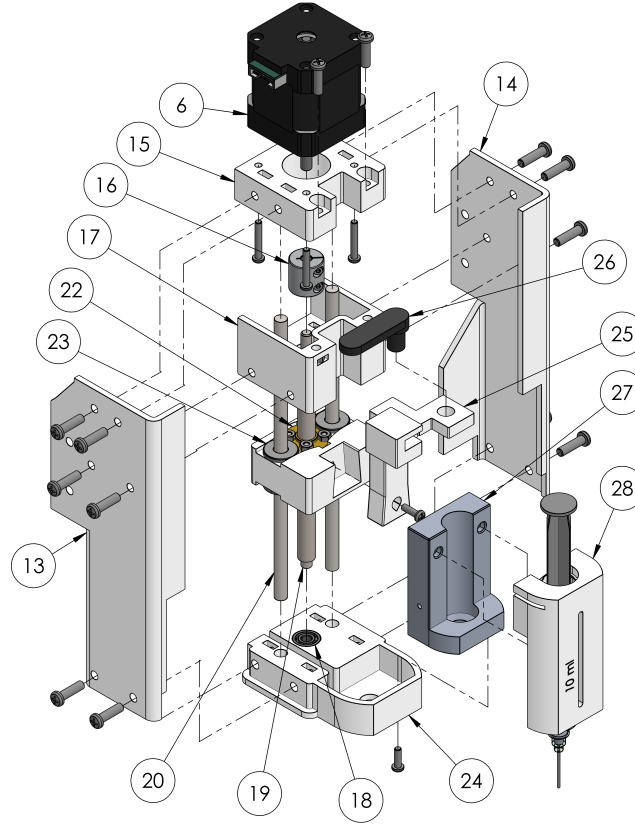


Figure 4.6: Exploded view of the extrusion system. The indexing of the ballooning refers to Table 4.3.

the size of the compatible syringes. It was chosen to allow for the use of syringes of 3, 5 and 10 ml with a Luer lock system. These syringes are very common and can be found in different materials, reusable or disposable. The Luer lock system is a feature that allows to screw the needle to the syringe, hence ensuring physical locking of both together and avoiding the loss of the needle during a print. The main constraining dimensions are reported in Table 4.2.

Table 4.2: Syringe dimensions (see Figure 4.7a).

		Syringe (vol in ml)		
		3	5	10
Dimensions [mm]	a	71.78	67.38	84.34
	b	22.66	26.68	31.42
	c	01.50	12.10	16.48
	stroke ²	52	48	65

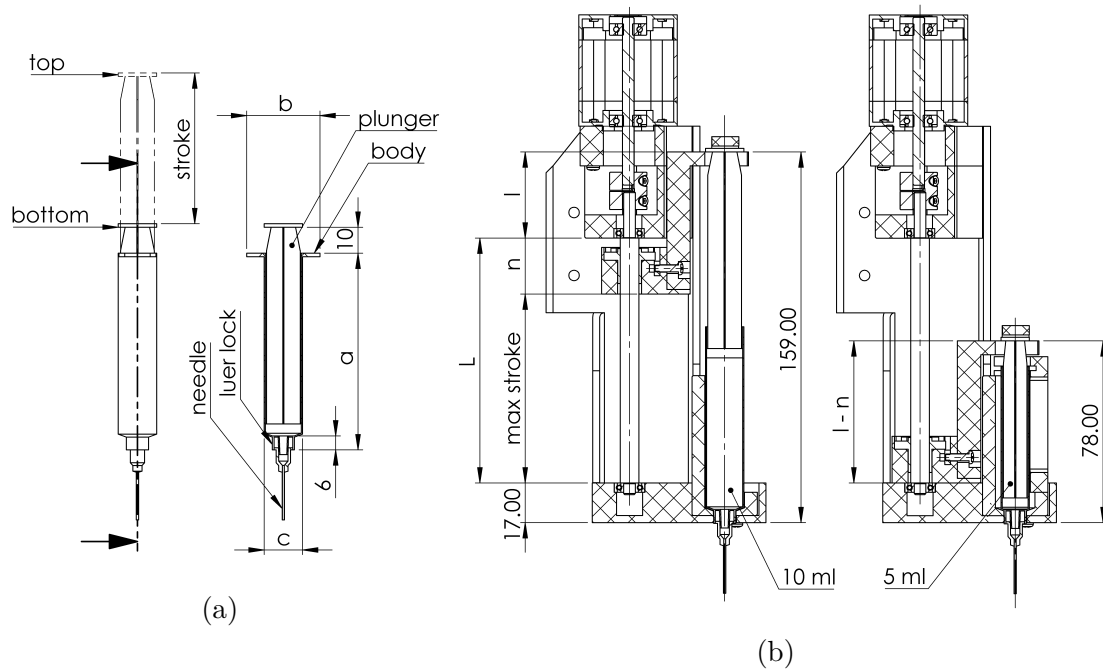


Figure 4.7: a) Schematic representing main dimensions of disposable syringes b) section view of extrusion system mounting a fully extended 10 ml syringe (left) and a fully pressed 5 ml one (right).

Two section views of the extrusion mechanism with a focus on the key design dimensions are reported in Figure 4.7b. It supports the selection of the threaded length of the screw, denoted with L , and of the plunger holder, denoted with l .

The other dimensions are considered fixed at this stage. In order to use the full stroke of the syringes, the following relations on the dimensions need to hold true:

² The stroke is approximated by subtracting the length of the Luer lock and the plunger from the total length and leaving 1 cm clearance on top.

$$\begin{cases} L + l + 17 = h_{max} \geq a_{10} + stroke_{10} + 10 \approx 159 \\ l + n + 17 = h_{min} \leq a_5 + 10 \approx 78 \\ L - n = stroke_{max} \geq stroke_{10} \approx 65 \end{cases} \quad (4.1)$$

where n is the length of the travelling nut, a_5 and a_{10} are the length of the barrel of the 5 ml and 10 ml syringes respectively and $stroke_{10}$ the stroke of the 10 ml syringe. The lead screw is provided with a 20 mm nut. Using the values of Table 4.2, the relations become:

$$\begin{cases} L \geq 142 - l \quad \wedge \quad L \geq 85 \\ l \leq 41 \end{cases} \quad (4.2)$$

In order to have the shortest lead screw, and therefore the smallest encumbrance of the mechanism, the minimum acceptable length of the plunger holder is selected. That results in $l = 41$ mm and $L \geq 101$ mm. Considering a comfortable margin, $L = 105$ mm is chosen.

One end of the lead screw is inserted in the coupling towards the shaft of the motor, the other is inserted in a ball bearing. Both ends are machined to have a 5 mm diameter and the length set to fit within the assembly, see Figure 4.8.

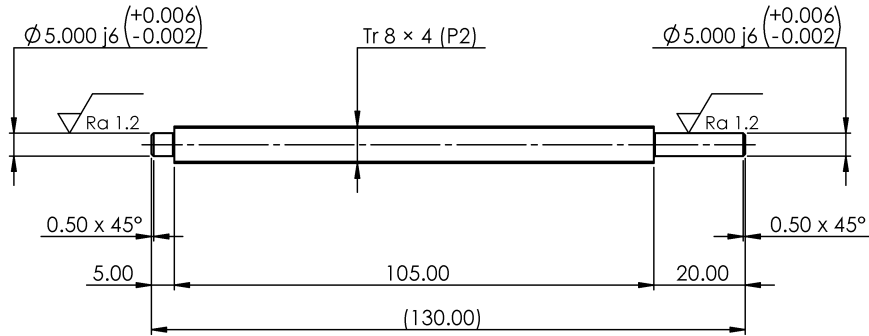


Figure 4.8: Technical drawing of the lead screw with both ends stepped.

4.4 Syringe heating solution

The specifications require to allow for printing at temperatures ranging from room temperature up to 60°C. A heating element is needed to maintain the temperature of the biomaterial during the printing process.

Due to the complexity of the final design, the part would be manufactured in CREDEM³. Aluminium is a well-suited candidate for its high thermal conductivity⁴, low cost, machinability and availability on site.

A first study was conducted with a simple shape, with relevant dimensions, in which the heating resistance of the former printer can be inserted. The results of the simulation confirmed that aluminium is a sound choice for heat transfer as the heat spreads well across the whole part. Moreover, the temperature in the region of interest that is the hollow cylinder wrapping the syringe, is rather constant with a temperature difference of 0.5°C between the local maximum and minimum, and 0.8°C across the part, see Figure 4.9a and 4.9b respectively.

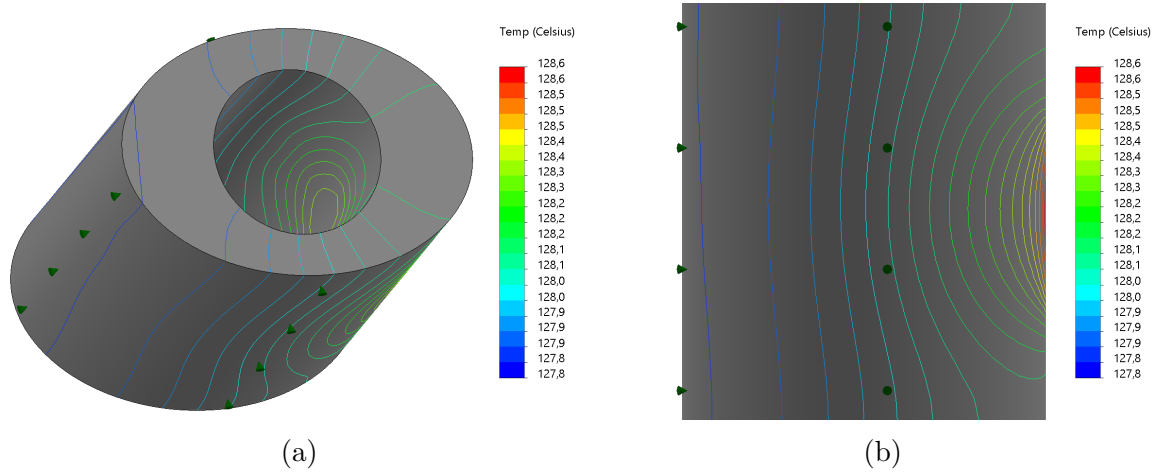


Figure 4.9: Study of the heat conduction of aluminium in a part with a general geometry adapted to fit a syringe. a) View of the hollow cylinder for syringe insertion, b) side view of the part.

A raw version of the part, closer to the final design, is manufactured to perform practical tests. These aim to verify that the heat spreads homogeneously in the part and to measure the temperature reached for a given setpoint. It would also show if the printer sensor works better in a parallel or perpendicular position with

³ Plateforme de Conception, Réalisation et Essais de Dispositifs Electro-Mécaniques.

⁴ Steel : $\approx 40 \text{ W m}^{-1} \text{ K}^{-1}$ < Aluminium: $\approx 237 \text{ W m}^{-1} \text{ K}^{-1}$ < Copper: $\approx 390 \text{ W m}^{-1} \text{ K}^{-1}$

respect to the heater and reveal a potential offset of the sensor of the former printer as it is not used in its dedicated range of temperature.

The experimental setup can be seen in Figure 4.10a. Commands with different temperature setpoints to the heating resistor connected to the printer. There are four different configurations possible for the pair sensor-heater, see Figures 4.11a to d. Temperature measurement were performed continuously during the experiments with the sensor of the printer and with four thermocouples⁵, independent from the printer, spread over the part and fixed with aluminium tape, see Figure 4.10b.

The configurations with the sensor close to the heater were performed first (Figures 4.11a and 4.11c). Positioning the sensor close to the heating element intuitively leads to think that the response of the sensor is faster, hence the overshoot due to the nature of the control loop is limited.

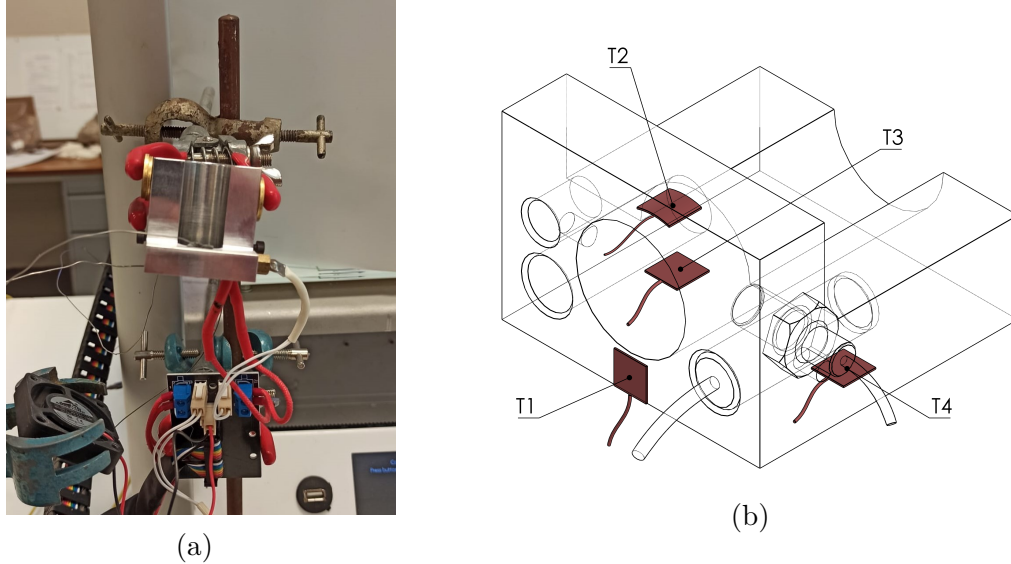


Figure 4.10: a) Experimental setup for the heating tests with the raw part, b) Locations of the four thermocouples T1, T2, T3 and T4.

⁵ Passive sensor, made by junction of at least two dissimilar conductors, that generates a voltage response to temperature[40].

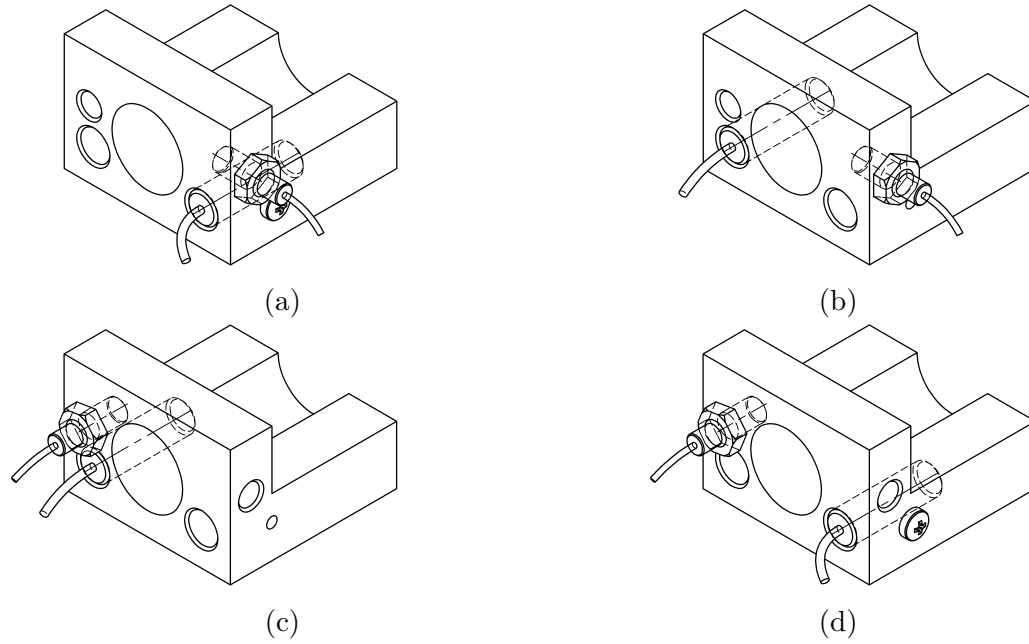


Figure 4.11: Four configurations of the sensor-heater pair⁶. a) sensor $\perp$ heater - sensor close to heater , b) sensor $\perp$ heater - sensor away from heater , c) sensor $\parallel$ heater - sensor close to heater , d) sensor $\parallel$ heater - sensor away from heater.

The results are plotted in Figure 4.12. These graphs show that the reading of the printer sensor, T_{sens} is lower than the mean of the actual temperature of the aluminium part, $\bar{T}_{Al}$, measured with the external thermocouples. There is an offset in steady state, it will be characterised accurately in Section 6.1.

The light coloured shade spread along the similarly coloured line shows the standard deviation σ between the four thermocouples, it is scaled by 20% for reading purposes. When reaching steady state, after 1.4 min, σ becomes almost null meaning that the temperature is constant across the aluminium part. For this reason, and the minimisation of the overshoot mentioned earlier, configurations with the sensor away from the heater (Figures 4.11b and 4.11d) were not tested. These results also comfort the choice of aluminium and the geometry of the part.

⁶ $\perp$ means *perpendicular to*, $\parallel$ means *parallel to*.

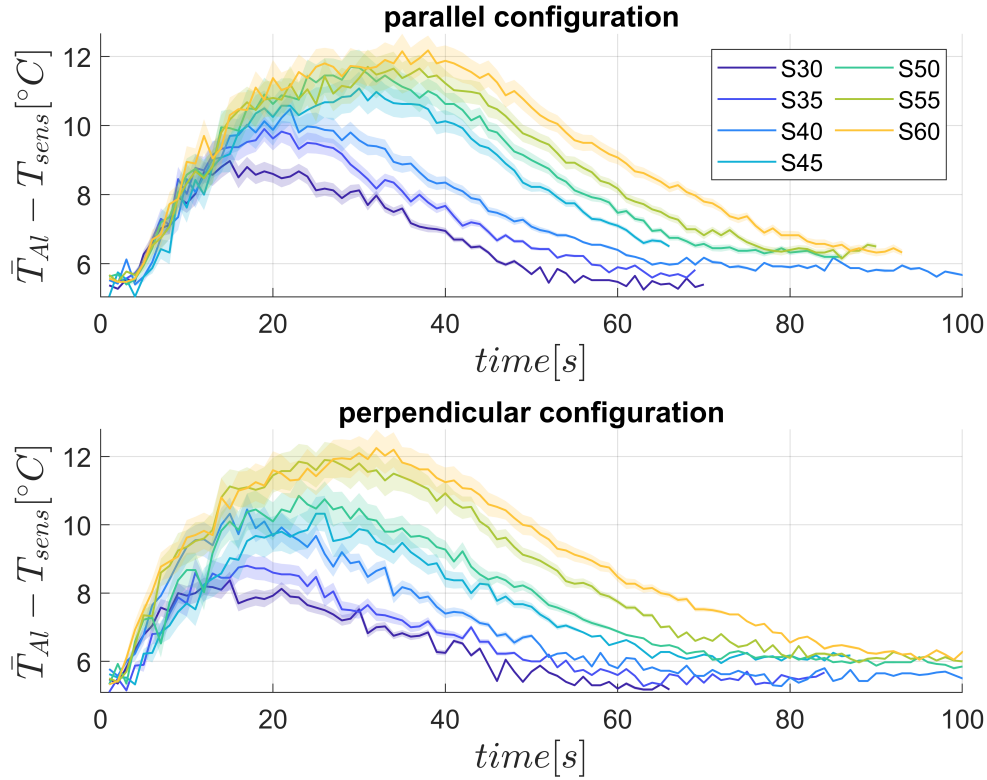


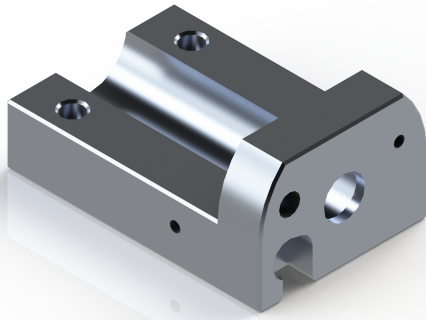
Figure 4.12: Plot of the difference of the temperature readings of the four thermocouples and the sensor of the printer over the transient for given commands with setpoint $\{30, 35, 40, 45, 50, 55, 60\}^{\circ}\text{C}$. The coloured shade along a similarly coloured line shows the standard deviation σ between the four thermocouples (scaled down to 20% for clarity).

The final version of the heating part made of aluminium is represented in Figure 4.13a. The validation tests performed and underlying results are presented in Chapter 6.

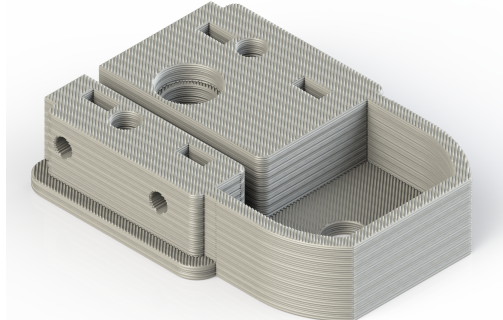
The part shows features to insert magnets used to hold the plastic cover that maintains the syringe in place. It also contains a cut dedicated for routing of the heating resistance cable.

The required working temperature while printing can reach up to 60°C , and the temperature of the aluminium part can reach 75°C while heating because of the overshoot resulting from the control loop. Therefore, the bottom piece that fits the aluminium part cannot be made of PLA as it softens at temperatures above

60°C. In these conditions, ABS⁷ is preferred as it has a higher melting point, hence it withstands better temperatures around 70°C. The part also has features that allow for the routing of the heating resistance sensor towards the back.



(a)



(b)

Figure 4.13: a) Final design of the aluminium part dedicated to the heating of the syringe. b) Bottom part made of ABS.

⁷ Acrylonitrile Butadiene Styrene, a common polymer in the family of thermoplastics.

4.5 Bill of materials

A comprehensive list of all the parts used to build and mount the printhead on the printer frame is drawn up in Table 4.3. It is numbered consistently with the ballooning in the Figures shown in Sections 4.1 to 4.3, it specifies the quantity of each part and whether the part is bought, 3D printed, machined or was already available from the Leapfrog printer.

Table 4.3: List of the parts of the assembly of the printhead. Numbers correspond to numbering in the Figures showing the assembly in Chapter 4. Parts printed in PLA or ABS, machined parts, parts bought and material already available are indicated in the columns dedicated.

#	Part	qty	Source
1	y_frame_guide_V5_bottom	1	P-PLA
2	Linear bushing (LMU8)	4	bought
3	PCB_cable_frame	1	P-PLA
4	y_frame_guide_V4_top	1	P-PLA
5	motor adjustment	1	P-PLA
6	E3D Stepper Motor	2	already available
7	custom_pinion (1.25)	1	P-PLA
8	custom_sledV4	1	P-PLA
9	stop_plateV7	1	P-PLA
10	Spring 1kg (CSF1_0)	1	bought
11	spring_shaft	1	P-PLA
12	spring_coverV6	1	P-PLA
13	side_cover_L_V1	1	P-PLA
14	side_cover_R_V1	1	P-PLA
15	extrusion_motor_frameV2	1	P-PLA
16	shaft coupling (D5xD5)	1	bought
17	bearing_fixing_topV3	1	P-PLA
18	ball bearing, double seal (695-H-ZZU76U-01)	2	bought
19	lead screw adapted	1	bought + machining
20	round_bar (D6x155)	2	bought
21	nut_frameV2	1	P-PLA
22	travelling_nut	1	bought
23	Oil free bushing (SHFZ6_10)	4	bought
24	bottom_plateV3_ABS	1	P-ABS

table 4.3 continued from previous page

#	Part	qty	Source
25	plunger_fixerV2	1	P-PLA
26	plunger_fixer_tab	1	P-PLA
27	heating_jacketV3	1	Machining aluminium
28	Syringe10ml_cover	1	P-PLA
29	M3 nut	18	bought
30	M3x8, flat head	4	bought
31	M3x10, pan head	7	bought
32	M3x20, pan head	10	bought
33	Magnet (D5x3)	6	bought
34	M3x20, flat head	6	bought
35	M4 nut	18	bought
36	M4x15, pan head	18	bought
37	M6 nut	2	bought
38	M6x35, pan head	2	bought
39	Internal circlip D15	2	bought
40	PCB	1	already available
41	Heating resistance	1	already available
42	Temperature sensor	1	already available

Chapter 5

G-code generation and printing parameters

The aim of this chapter is to provide general guidelines to choose the slicing parameters when generating the G-code with Cura¹. A thorough study of the printing system has also been performed in order to cope with the changes made to the Leapfrog Creatr HS printer, especially concerning the modifications on the extrusion system. That mainly involved the assessment of the relation between the E-value², generated automatically by a slicing software, and the effective printed volume.

Regarding the slicer, we opted for Cura by Ultimaker, a free and open source software. The software is easily accessible to the inexperienced user, it is yet powerful and provides plenty of customisation and control of the printing parameters. The software also provides the basic settings to use a Leapfrog Creatr HS printer.

5.1 Calculation of extruded volume

First the relation between the extruded volume and the E-value is evaluated. As discussed in Section 4, the pre-existing filament extrusion mechanism involving a hot-end extruder with pulley gear (Figure 5.1a) is swapped for a lead screw driving the plunger of a syringe (Figure 5.1b).

¹ Download here: <https://ultimaker.com/en/software/ultimaker-cura>.

² E-value: "An absolute or relative coordinate on the E (extruder) axis (in current units). The E axis describes the position of the filament in terms of input to the extruder feeder" [41].

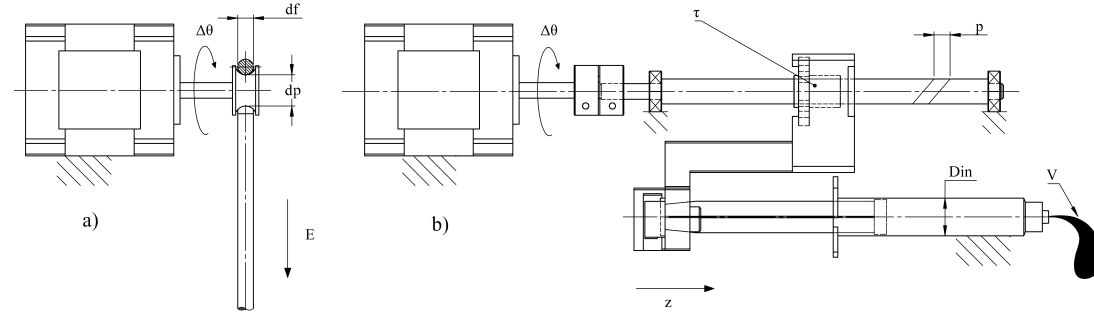


Figure 5.1: Schematics of the extrusion system configurations:
a) original, b) new.

The extruded volume is first a function of the specific syringe used, as different syringes have different cross sectional areas. It is possible to relate the linear travel of the plunger z with the theoretical extruded volume V_{th} as follows:

$$V_{th} = \frac{D_{in}^2 \pi}{4} z(E) \cdot 10^{-3} \quad (5.1)$$

where D_{in} is the inner diameter of the syringe. Notice that the linear dimensions are expressed in mm, while the volume is expressed in ml.

Then the theoretical relation between the E-value and the linear travel of the plunger is given by the following equation:

$$z = \frac{2\tau}{d_p + d_f} E \quad (5.2)$$

where τ is the transmission coefficient of the lead screw, equal to $p/2\pi$, with p the pitch of the screw, d_p the diameter of the pulley and d_f the diameter of the filament. Together, Equations 5.1 and 5.2 yield:

$$V_{th} = m_{V,th} E = \frac{p D_{in}^2}{4(d_p + d_f)} \cdot 10^{-3} E \quad (5.3)$$

For example, in the case of a 10 ml syringe setup, the known dimensions can be substituted to find the linear coefficient $m_{V,th}$ that links the theoretical printed volume with the E-value. Assuming $d_p = 3.5$ mm, $d_f = 1.75$ mm, $p = 4$ mm and $D_{in} = 15.1$ mm and found $m_{V,th} = 4.343 \cdot 10^{-2}$ ml/mm.

To evaluate this result, practical tests are performed on the printer to measure $m_{V,exp}$. The tests consisted in sending a command with a set E-value to the printer via the control terminal and measuring the linear displacement of the plunger with a caliper and reading the extruded volume on the measurement lines on the syringe.

From the collected data, the linear least squares method is used to find the experimental value of the coefficient, obtaining:

$$m_{V,exp} = \frac{\sum_{i=1}^n E_i V_{exp,i}}{\sum_{i=1}^n E_i^2} = 4.37 \cdot 10^{-2} \text{ ml/mm} \quad (5.4)$$

which is consistent with the results from the theoretical analysis.

5.2 E-value computation

Once the relation between the E-value and the printed volume is known, it is worth knowing how the slicer computes the E-value based on the geometry of the part to print, as well as the influence of the control parameters.

Despite being open source, it is difficult to derive the precise computation procedure for the E-value from the source code. Instead a new set of tests is performed and the process is reverse engineered.

A notation is introduced with Figure 5.2 showing the dimensions of the part to print and a close cross section view of the extrusion head. A simplified geometry consisting in a fully infilled rectangular parallelepiped printed only with concentric contours is used.

For the printed part, L is the length, T the width and H the height of the part. Note that L is always considered to be greater than T . The printing head traces the path drawn with the dashed line, it travels a distance Δx for each layer and is divided in N parallel passes, whereas M denotes the number of layers. The height of each layer is h and is equal to the ratio between the height of the piece and the number of layers. Similarly, the thickness t is the ratio between T and N .

The section view shows the nozzle with a diameter denoted as d_n , and the extruded filament with a diameter before extrusion d_f^3 .

The volume continuity equation is found by equating the volume extruded by the nozzle and the printed volume:

$$\frac{d_f^2 \pi}{4} E = A_p \Delta x \quad (5.5)$$

where the cross sectional area of the printed filament A_p is assumed to be a rectangle

³ It is important to note that in the previous section the filament diameter was fixed and defined according to the firmware of the printer, while here it is a parameter defined in the slicing software.

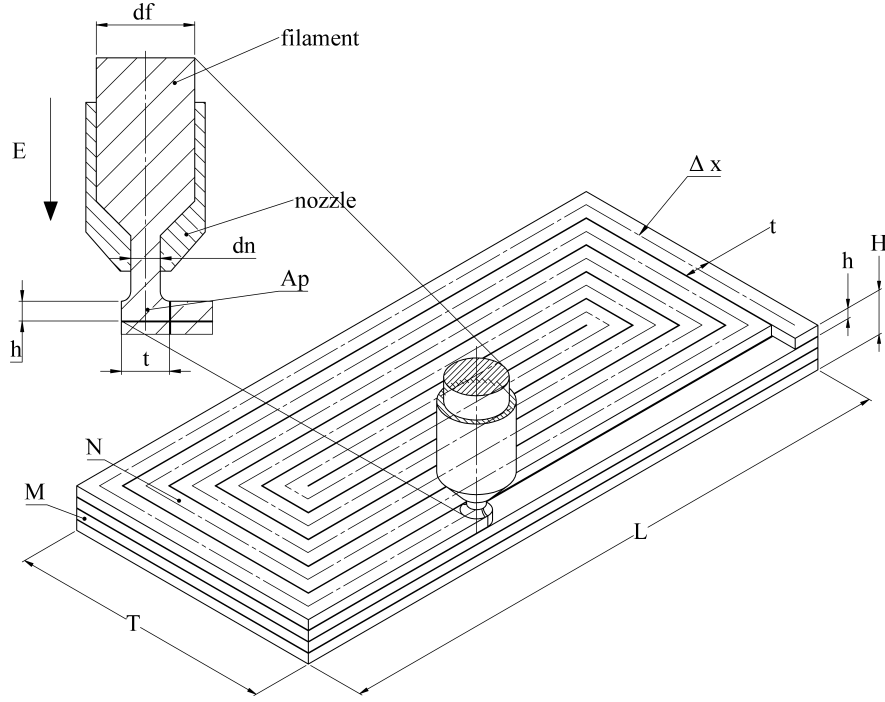


Figure 5.2: Section of the nozzle and diagram of the printing path with dimensions and annotations.

with base equal to the layer width and height equal to the layer height⁴. With this assumption, Equation 5.5 becomes:

$$E = \frac{4hMt\Delta x}{d_f^2\pi} \quad (5.6)$$

To verify the equation, the results are compared with the G-code-generated E-values. A rectangular parallelepiped is sliced with T and H so the part is a single line with a single layer (i.e. $N = M = 1$). In this case, Δx is equal to the length L , subtracted by the width $T = t$ in this case.

Therefore Equation 5.6 becomes:

$$E_{single\ line} = \frac{4hT(L - T)}{d_f^2\pi} \quad (5.7)$$

The results are reported in Figure 5.3a showing the E-values generated by the slicer together with the theoretical values computed with Equation 5.7 while varying T .

⁴ This is how Cura seems to compute the volume according to [42] and it was verified mathematically. Other slicers use different assumptions. For example, the Open source 3D printing toolbox Slic3r uses a rectangle with semicircular ends instead [43].

The width (T) is normalised by the nozzle diameter, and the E-value is normalised by the product of the nozzle diameter (d_n), the layer height (h) and travel of the printhead (Δx).

Note that the values computed with Equation 5.7 coincide with the values generated with Cura, down to $T/d_n < 0.85$, at which point the normalised E-value is fixed to a minimal extrusion of 0.35 by the software⁵.

By extending the reasoning to a fully infilled rectangular parallelepiped such as in Figure 5.2, E is computed with Equation 5.6, where Δx can be written as:

$$\Delta x = \begin{cases} \sum_{i=1}^{\lceil N/2 \rceil} 2(L + T) - 4(2(i-1) + 1)t, & N \text{ even} \\ \sum_{i=1}^{\lceil N/2 \rceil - 1} 2(L + T) - 4(2(i-1) + 1)t + L - (2(\lceil N/2 \rceil - 1) + 1)t, & N \text{ odd} \end{cases} \quad (5.8)$$

The number of contours N is a function of the width of the part and of the nozzle diameter. The latter determines the distance between two lines. N can be approximated such that $N \approx r = T/d_n$. However a more precise computation based on the G-code results is proposed here below and plotted in Figure 5.3b:

$$N = \begin{cases} 1 & \text{if } r < 1.71 \\ 2 & \text{if } 1.71 \leq r < 2.85 \\ 2 \left(\left\lfloor \frac{r-2.85}{2} \right\rfloor + 1 \right) + 1 & \text{if } \text{frac}^6 \left(\frac{r-2.85}{2} \right) < 0.425 \text{ and } r \geq 2.85 \\ 2 \left(\left\lfloor \frac{r-2.85}{2} \right\rfloor + 1 \right) + 2 & \text{if } \text{frac} \left(\frac{r-2.85}{2} \right) \geq 0.425 \text{ and } r \geq 2.85 \end{cases} \quad (5.9)$$

⁵ This limitation seems to be set based on physical constraints of the extrusion.

⁶ Reminder: $\text{frac}(x) \triangleq x - \lfloor x \rfloor$, $x > 0$.

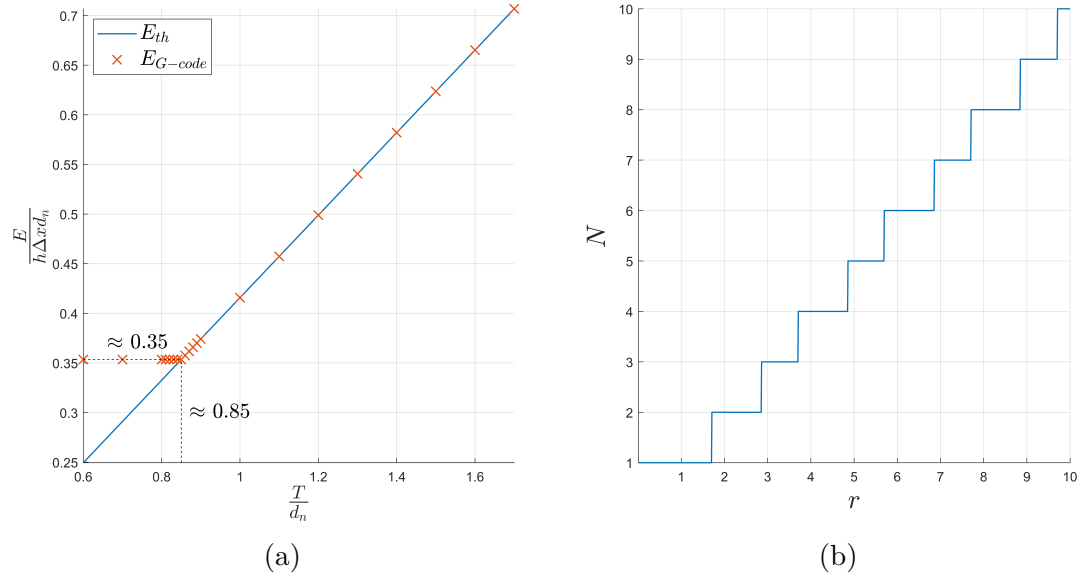


Figure 5.3: a) Comparison of the theoretical E-value and G-code generated results with varying parameter T , b) number of contours VS ratio T/d_n .

5.3 Introduction of a fill factor for extrusion tuning

The design of the extrusion system allows for syringes of various volumes. Syringes of 3, 5 and 10 ml can already be used. The inner diameter D_{in} of these 3 syringes is different, so the volume extruded for a given E-value is different. Thus, the slicing parameters need to be adjusted.

For simplicity, a new parameter is introduced: the fill factor, ρ , that is the ratio between the extruded volume and the volume occupied by the printed part (volumes expressed in mm^3):

$$\rho = \frac{V_{extruded}}{V_{part}} = \frac{m_V E}{LTH} \cdot 10^3 \quad (5.10)$$

By fixing the geometry of the part and varying d_n and d_f over a range of values, the fill factor is computed and the plot in Figure 5.4 is retrieved.

It can be seen that the number of contours is inversely related to d_n , as shown previously in Equation 5.9. Yet, the effect of the nozzle diameter is marginal in

the computation of ρ as the value of the latter changes slightly in the y direction. As the nozzle diameter increases, Δx decreases while t increases, thus the product between Δx and t remains almost constant. In practice:

$$t\Delta x \approx TL \quad (5.11)$$

Rewriting Equation 5.10 using 5.6 and 5.11 the following equation is obtained:

$$\rho \approx \frac{4m_V LTH}{d_f^2 \pi LTH} = \frac{4m_V}{d_f^2 \pi} \quad (5.12)$$

As a result, the fill factor is a function of the chosen syringe and the filament diameter set in the slicer parameters.

Numerically it becomes:

$$\rho \approx 0.2425 \cdot \left(\frac{D_{in}}{d_f} \right)^2 \quad (5.13)$$

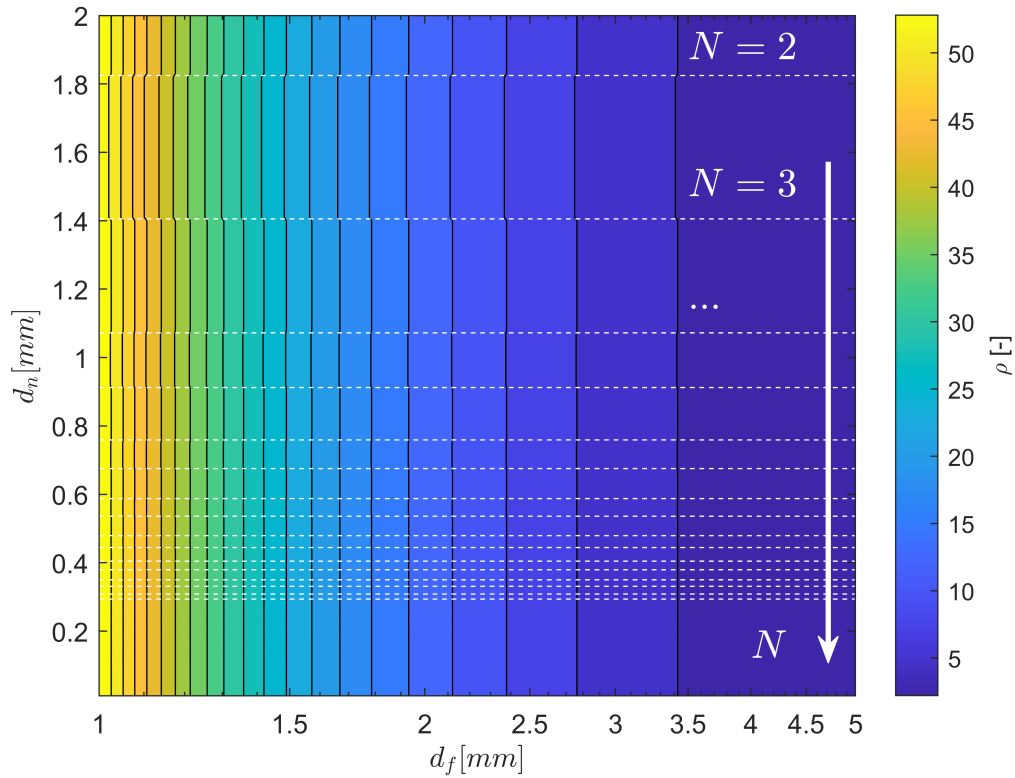


Figure 5.4: Volumetric fill factor over a range of value of d_n and d_f , for a part with $L = 60$ mm, $T = 5.2$ mm, $H = 0.4$ mm, $h = 0.2$ mm

The graph in Figure 5.4 shows values of ρ ranging from 5 to 50, meaning that the printer would extrude up to 50 times the volume of the part to print it. Except from specific trials, ρ should be equal to 1 to extrude a volume equal to the volume of the part to print. Cura offers a parameter called *flow* that allows to scale the extrusion of material. Here it will be referred to as $f_{compensation}$. Equation 5.13 becomes:

$$\rho \approx 0.2425 \cdot \left(\frac{D_{in}}{d_f} \right)^2 \cdot f_{compensation} \quad (5.14)$$

Thus, for $\rho = 1$:

$$f_{compensation} = 4.1233 \cdot \left(\frac{d_f}{D_{in}} \right)^2 \quad (5.15)$$

To conclude, scaling of the extrusion with the flow parameter coherently with the choice of syringe and calibration of the extrusion must be performed to avoid overextrusion ($\rho > 1$) or underextrusion (ρ too low). The main extrusion rate parameter identified are the filament diameter, or equivalently the flow compensation that is easier to set in the slicer. The calibration of d_n and h is required to ensure the quality of the print. The nozzle diameter is chosen to have the required spacing between printed strands and h to enforce the layer height. The optimal values are highly material dependant. A good first guess for d_n will be the value obtained from Equation 2.1 as, if d_n is equal to the diameter of the strand, these should not overlap.

5.4 Slicing parameters

The configuration used in Cura for the tests performed in Chapter 6 is reported in Table 5.1. Some of the values have been left as default⁷ while the others have been modified. The main parameters that need to be modified based on a specific material are:

- *Layer Height*: it determines the height of the layers h . Thus a reduction positively affects the quality of the print, while it increases the printing time;
- *Line Width*: in Cura, by default it is equal to d_n . This has to be selected based on the required quality of the print. Thinner lines will allow for a better resolution and sharper details;
- *Wall Thickness* and *Top/Bottom Thickness*: have to be chosen based on the required number of outer contours and top/bottom full layers;

⁷ Default values of the existing profile for a Leapfrog Creatr HS printer in Cura.

- *Infill Density*: filling percentage of the bulk of the part (shell excluded). It will affect ρ ;
- *Printing Temperature*: will be chosen based on the specifics of the used biomaterial. The value has to be computed from the required temperature considering the offset and gain error reported in Chapter 6;
- *Build Plate Temperature*: has to be chosen based on specific material and substrate pair;
- *Flow*: has to be chosen based on the required volume to be extruded, as discussed previously;
- *Printing Speed*: affects the speed of the extruder relatively with the bed during extrusion phases. Common values in bioprinting are below 20 mm/s [35].

The G-code header and footer developed for this machine are reported in Table 5.2. It is important to override the cold extrusion to be able to print at any temperature. Alternatively `M302 [S<temp>]` could be used to set a new minimum temperature for cold extrusion. Also note that T0 is selected as the working tool as it is wired for the extrusion, while T1 is responsible for the engagement of the printhead.

The printer and extruder dimensions are reported in Table 5.3 (see Figure 6.6).

Quality		<i>Travel Speed</i>	10 mm/s
<i>Layer Height</i>	0.2 mm	<i>Initial Layer Speed</i>	10 mm/s
<i>Initial Layer Height</i>	0.2 mm	<i>Enable Acceleration Control</i>	×
<i>Line Width</i>	0.1 mm	<i>Enable Jerk Control</i>	×
<i>Initial Layer Line Width</i>	100 %	Travel	
Shell		<i>Enable Retraction</i>	✓
<i>Wall Extruder</i>	Right extruder	<i>Retraction at Layer Change</i>	×
<i>Wall Thickness</i>	1 mm	<i>Retraction Distance</i>	2 mm
<i>Top/Bottom Extruder</i>	Right extruder	<i>Retraction Speed</i>	25 mm/s
<i>Top/Bottom Thickness</i>	0.8 mm	<i>Combing Mode</i>	All
<i>Top/Bottom Pattern</i>	Lines	<i>Avoid Printed Parts When Traveling</i>	✓
<i>Optimise Wall Printing Order</i>	×	<i>Avoid Supports When Traveling</i>	×
<i>Outer Before Inner Walls</i>	✓	<i>Travel Avoid Distance</i>	1 mm
<i>Fill Gaps Between Walls</i>	Everywhere	<i>Z Hop When Retracted</i>	×
<i>Horizontal Expansion</i>	0 mm	Cooling	
<i>Enable Ironing</i>	×	<i>Enable Print Cooling</i>	✓
Infill		<i>Fans Speed</i>	100 %
<i>Infill Extruder</i>	Right extruder	<i>Regular/Maximal Fan Speed Threshold</i>	10 s
<i>Infill Density</i>	20 %	<i>Initial Fans Speed</i>	0 %
<i>Infill Pattern</i>	Grid -	<i>Regular Fan Speed at Height</i>	0.2 mm
<i>Infill Line Multiplier</i>	1	<i>Minimum Layer Time</i>	5 s
<i>Infill Overlap Percentage</i>	10 %	<i>Minimum Speed</i>	10 mm/s
<i>Infill Layer Thickness</i>	0.2 mm	<i>Lift Head</i>	×
<i>Gradual Infill Steps</i>	0	Support	
Material		<i>Generate Support</i>	×
<i>Printing Temperature</i>	40 °C	Buildplate Adhesion	
<i>Printing Temperature Initial Layer</i>	40 °C	<i>Buildplate Adhesion Type</i>	None
<i>Initial Printing Temperature</i>	40 °C	Dual Extrusion	
<i>Final Printing Temperature</i>	40 °C	<i>Enable Prime Tower</i>	×
<i>Build Plate Temperature</i>	0 °C	Special Modes	
<i>Build Plater Initial Layer</i>	0 °C	<i>Surface Mode</i>	Normal
<i>Flow</i>	100 %	<i>Spiralize Outer Contour</i>	×
<i>Standby Temperature</i>	35 °C	Experimental	
Speed		<i>Make Overhang Printable</i>	×
<i>Print Speed</i>	10 mm/s	<i>Use Adaptive Layers</i>	×

Table 5.1: Used printing parameters (✓: enabled; ×: disabled).

Start G-code	End G-code
M107 ; start with the fan off	M104 S0 T0 ; turn off right extruder
G92 X0 Y0 Z0 E0 ; reset software positions	M104 S0 T1 ; turn off left extruder
T0	M140 S0 T0 ; turn off bed
M302 ; override cold extrusion	G1 Y-5 F1200 ; move for next line
G1 E2.0000 F500	G28 X0 ; home X axis
G1 X5	M84 ; disable motors
G92 X0 Y0 Z0 E0 ; reset software positions	

Table 5.2: Used header and footer G-codes.

Printer and Right extruder configurations	
X (Width)	260 mm
Y (Depth)	180 mm
Z (Height)	-
X min	-58 mm
X max	29 mm
Y min	-18 mm
Y max	161 mm
Gantry Height	-
Number of Extruders	1
Nozzle size	d_n
Compatible material diameter	d_f
Nozzle offset X	57.78 mm
Nozzle offset Y	57.60 mm

Table 5.3: Printer and extruder configurations.

Chapter 6

Performance assessment and verification

6.1 Tests and results

Various tests have been carried out to verify the prototype in accordance with the specifications presented in Chapter 3. To do so, the temperature control and the printing process have been assessed to characterise the performances of the printer.

6.1.1 Temperature tests

As specified in Section 4.4, the function of the heating jacket is to keep the temperature in the syringe constant in the range from room temperature to 60°C. To achieve this control, the original heating system of the Leapfrog Creatr printer, consisting in a heating resistance and a temperature sensor, is mounted on the new design of the heating jacket, as it was shown in Figure 4.13a. This can easily and reliably drive the temperature of the aluminium part to the setpoint temperature. Despite that, this setup does not allow to measure the temperature of the material within the syringe. Therefore, tests have been performed to study the evolution of the temperature of the biomaterial with regards to the setpoint.

The system under study can be simplified with the block diagram in Figure 6.1. The signal represented are r , the reference (or setpoint), e , the feedback error, u , the actuation command, y_1 , the temperature of the aluminium measured with the sensor and y_2 , the temperature of the material inside the syringe measured with an external thermocouple. Only y_1 is controlled with a feedback loop. The block labelled with C represents the controller, while G_1 and G_2 are the

Transfer Functions (TFs) that encompass the dynamics of the heat transfer from the heating element to the embedded sensor and to the syringe content, respectively.

The controller defined in the Marlin firmware consists in a discrete time PID¹ controller, defined as follows:

Algorithm 1 PID Controller

Input: the setpoint r and the measured temperature y

Output: the actuation command u

```

1:  $y_{prev} \leftarrow 0$ 
2:  $I_{sum} \leftarrow 0$ 
3:  $D_{prev} \leftarrow 0$ 
4: while running do
5:    $e \leftarrow r - y$ 
6:    $P \leftarrow K_p \cdot e$ 
7:    $I_{sum} \leftarrow I_{sum} + e$ 
8:    $I \leftarrow K_i \cdot I$ 
9:    $D \leftarrow (1 - K_1) \cdot K_d \cdot (y - y_{prev}) + K_1 \cdot D_{prev}$ 
10:   $u \leftarrow P + I + D$ 
11:  if  $u > Max$  then
12:    if  $e > 0$  then
13:       $I_{sum} \leftarrow I_{sum} - e$ 
14:    end if
15:     $U \leftarrow Max$ 
16:    if  $e < 0$  then
17:       $I_{sum} \leftarrow I_{sum} - e$ 
18:    end if
19:     $u \leftarrow 0$ 
20:  end if
21:   $y_{prev} \leftarrow y$ 
22:   $D_{prev} \leftarrow D$ 
23:  return  $u$ 
24: end while

```

After initialisation, the algorithm runs in a continuous loop that computes the feedback error and generates a proportional term P , an integral term I and a derivative term D . They are then added together to generate the actuation control u , which is constrained between 0 and Max . In case of saturation, the cumulative sum of the error I_{sum} is clamped to prevent windup of the integral error. The

¹ Proportional Integral Derivative, a very common controller architecture.

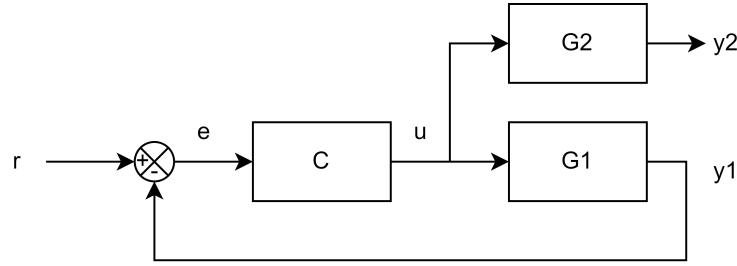


Figure 6.1: Block diagram of the heating system.

variables y_{prev} and D_{prev} are updated every cycle with the previous values of y and D . The proportional, integral and derivative coefficients are K_p , K_i and K_d respectively. The coefficient K_1 is a smoothing factor.

The default values for the parameters are $K_p = 11.07$, $K_i = 0.44$, $K_d = 49.82$, $K_1 = 0.95$ but they can be modified and tuned in G-code if required. The output is saturated to stay in the range $[0-127]^2$ corresponding to the maximum and minimum value of the command. The TFs G_1 and G_2 are driven by the design choices such as material used, volume of the parts, relative distance between heat source and sensor, as well as the syringe content that can have different thermal characteristics.

In order to measure y_2 during the tests, a thermocouple is placed in the syringe barrel filled with water to model the biomaterial. Water is a good candidate to create a generic model as it is a very common component of biomaterials used in bioprinting and it makes the tests easy to replicate. The tests consist in a series of setpoint steps applied to the printer while logging the evolution of the measurements. A 40°C step response is reported in Figure 6.2. The dynamics of y_1 is much faster than the dynamics of y_2 . Since y_1 rapidly reaches the steady state, the actuation command is reduced and only the integral term remains, which maintains the temperature and compensates for the heat losses with the outside.

Figure 6.2 shows that the value of y_2 in steady state is offset compared to the setpoint. This introduces a steady-state error in the temperature of the syringe that must be accounted for. This error is composed of an offset error e_{offset} and a proportional error e_{prop} , so several tests at various temperature have been carried out. A time evolution of the setpoint and measured temperature in the syringe during a test is shown in Figure 6.3a. The steady-state error is better highlighted in Figure 6.3b, where the measured temperature is plotted with respect to the setpoint temperature. The interpolation of the values shows that the offset error

² The output is a signed 8 bit integer.

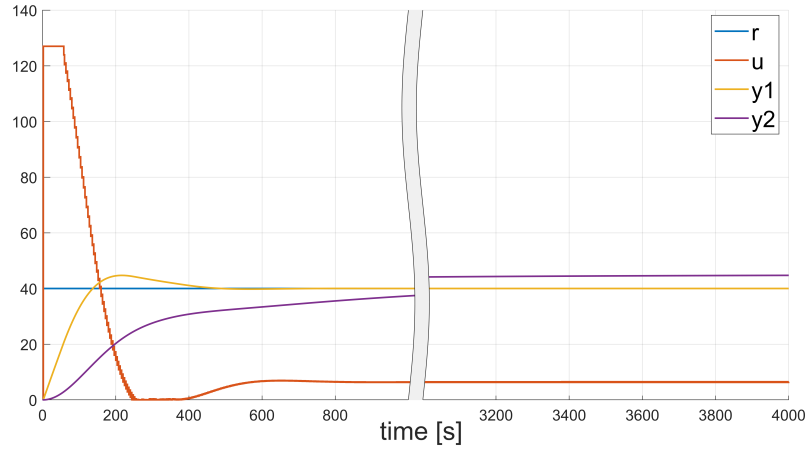
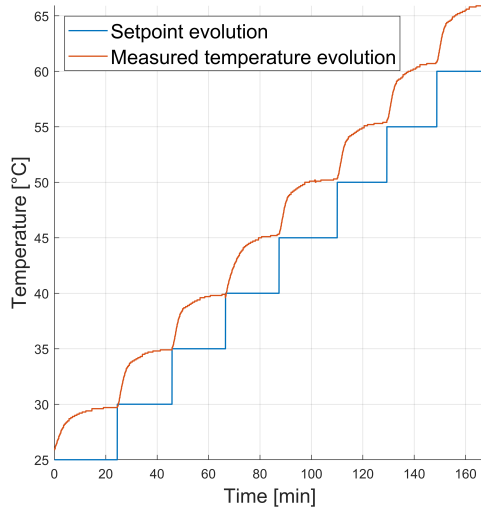


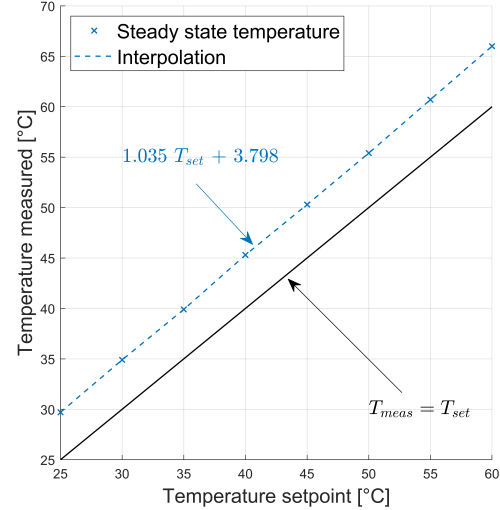
Figure 6.2: Response of the system to a 40°C step input.

amounts to 3.798°C, while the proportional error is equal to 0.035. In order to cope for the steady-state error, one can simply use the offset and proportional errors and compute the setpoint based on the required temperature, T , as follows:

$$r = \frac{T - e_{offset}}{1 + e_{prop}} \quad (6.1)$$



(a)



(b)

Figure 6.3: a) Time evolution of the measured temperature with respect to the applied setpoint, b) steady-state error for various setpoint temperatures and linear interpolation of the results.

6.1.2 Printing tests

Printing tests were performed with a 1% solution of agarose [44]. The solution is kept at 60°C in a shaking water bath [45] to maintain it liquid³ and ready to use with the bioprinter. The solution remains fluid above 37°C thus the printing temperature is set at 40°C so the solution solidifies rapidly once extruded.

The first objective is to find a suitable extrusion flow at a printing speed of 10 mm/s. Printing speed are very rarely mentioned in the literature on bioprinting. Moreover, the study of the speed is more relevant when working with living cells. A suitable speed will depend on both the application and the bioink characteristics. As a reference, the maximum printing speed found in commercial bioprinter datasheets reaches 20 mm/s, see Table 2.1.

The extrusion rate is adjusted by tuning the line width of the print. Successive lines are printed with a line width ranging from 0.10 to 0.30 mm with a 0.05 mm step. The results are shown in Figure 6.4. A line width of 0.10 mm seems to produce suitable results at the speed aforementioned. As the line width increases, over-extrusion is observed so the lines are less even and more asymmetrically spread along their length.

It was noticed later after this experiment that the extrusion can be adjusted more efficiently by following a more systematic procedure. The details of this procedure are reported in Chapter 5.

³ A solution of agarose is liquid at temperatures above $\approx 40^\circ\text{C}$, depending of the quality of the agarose. Under said temperature, solution gellates, which means it solidifies in the form of a gel.

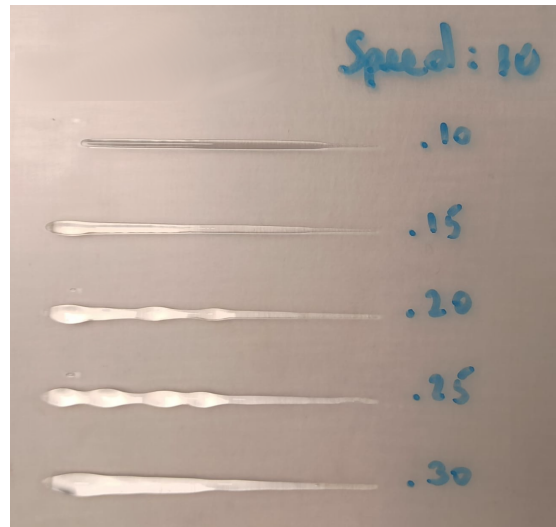


Figure 6.4: Printing tests performed at 10 mm/s speed with different line thickness to adjust extrusion rate.

The results of the first printing trials showed important adhesion problems between the material and the substrate. Three ideas were investigated: changing the surface of the substrate, changing the temperature of the substrate and lowering the printing temperature so the agarose solution is closer to its gelation point. Working at lower printing temperature bore no positive results but it raised an issue on the design. The temperature of the needle is not controlled, so clogging issues arise when working with materials with temperature-sensitive cross-linking. In order to test the effect of the temperature of the substrate, a Petri dish was embedded in ice. The low temperature induces a very rapid gelation of the solution extruded. However, water condensation quickly covers and contaminates the substrate and the adherence is dramatically reduced, see Figure 6.5c. To test the remaining lead, the surface of the Petri dish is finely sanded. The adherence between the material and the substrate improved but the result remains unsatisfying, see Figures 6.5a and 6.5b.

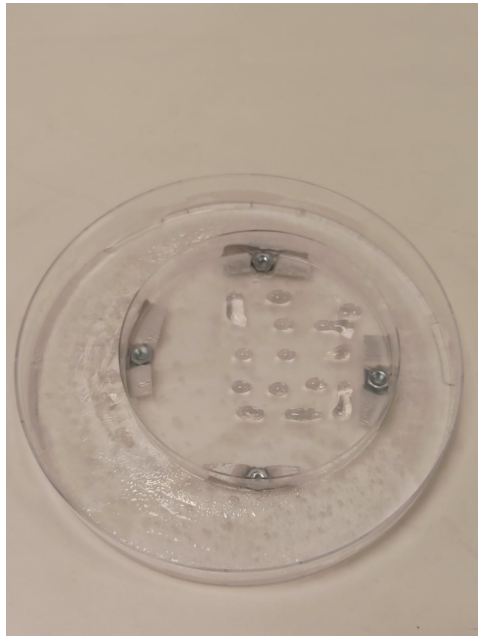
The effect of the printed shape is also studied to identify types of geometry prone to printing defaults. Common test shapes identified in the literature were printed [46][47]. The result of one print is shown in Figure 6.5d. Straight and curved lines seem to print well whereas 90° angles show unsatisfying results with the material retracting in the corners. There can be multiple factor to this result, including poor adherence, printing speed or extrusion not adapted, printing temperature. Many parameters can be tuned in accordance with the biomaterial used, further studies and trials are needed to obtain better printing performances.



(a)



(b)



(c)



(d)

Figure 6.5: Trials to improve printing performances. Pattern printed on a) smooth and b) rough substrate to compare adherence between substrate and biomaterial. c) Printing on cold substrate inducing rapid gelation of material. Results in poor adhesion due to condensation contamination of substrate. d) Printing of different shapes to identify problematic geometry. Retraction of the material in the corners of 90° angle, reducing printing performances.

6.2 Verification of the design

In this section the specifications are reviewed with a classification of each MF, MC, CMF and CMC and a comment to validate or provide an explanation for the non completion. The MF/MC/CMF/CMC can be either

- **Completed:** the requirement is met;
- **In progress:** part of the requirement is met or, in the case of a MF/MC, some criteria are not completed;
- **Not addressed:** the requirement has not been addressed;

Remember that the specifications were established with the objective to build a completely new 3D bioprinter whereas, in the frame of this thesis, only a printhead was developed to be mounted on the frame of a former FDM printer. Hence some of the requirements are not completed or have not even been addressed.

State	Main Functions
Completed	MF-1: Print material
	CMF-1.1: The printhead allows to extrude material from a pre-filled syringe. Syringes of 3, 5, and 10 ml with a Luer lock system can be used along with fitting needles of various lengths and diameters.
Not addressed	MF-2: Deposit drops
In progress	MF-3: Parallelise the printing process
Completed	CMF-3.1: The engagement system allows to use multiple print-heads in a single print. The frame of the Leapfrog printer can be mounted with 2 printheads, however its current motherboard does not offer necessary connection ports to plug additional motors.
In progress	CMF-3.2: This was not addressed because only an extrusion system has been developed. Yet, the modular design allows for easy changes to add a new type of printhead.

continues on next page

Not addressed	CMF-3.3: The effective working area of the printing bed is directly constrained by the original dimensions of the frame. The bulkiness of the new printhead is such that two of them could be used while preserving a reasonable printing area. The motherboard issue (CMF-3.1) prevails.
Completed	MF-4: Print multiple materials simultaneously
	CMF-4.1: The use of multiple printheads allows to use syringes filled with different materials. See CMF-3.1.
Completed	MF-5: Allow different types of settings (eg.type of Petri dish, nozzle length, ...)
	CMF-5.1: The z-calibration relies on the pre-existing calibration system of the Leapfrog printer. It can be tuned by hand to account for the height of the substrate. CMF-5.2: The x and y calibration relies on the pre-existing calibration system of the Leapfrog printer. It should be performed before starting a print. Then the user moves the printhead to the desired starting position over the substrate (see Section 5.4 and appendix A "How to start a print"). CMF-5.3: The design of the extrusion head and the flexibility in the selection of the needle allow to use Petri dishes with the following dimensions: $D \times h$: $[60 ; 150] \times [10 ; 25]$ mm.
In progress	MF-6: Allow research on viability of cells
In progress	CMF-6.1: The temperature of the syringe can be controlled but there is no direct control on the temperature of the needle.
Completed	CMF-6.2: The Leapfrog printer has an embedded bed temperature control [Room temp ; 90]°C.
Completed	CMF-6.3: The extrusion system is mechanically driven by a lead screw actuated by a stepper motor with a 1.8° step angle. Information on the relation between the vertical movement of the plunger in the syringe and the extruded volume can be found in Chapter 5.
Not addressed	CMF-6.4: No tests were conducted with cells.

continues on next page

Completed	MF-7: Ensure solvent evaporation time
	CMF-7.1: The syringe can be heated up to the specified range [40 ; 60]°C.
In progress	MF-8: Allow the use of culture medium
Completed	CMF-8.1: The workspace is accessible during the printing phase, culture medium can be poured in during a process.
In progress	CMF-8.2: Printing directly within a culture medium is possible as the length of the needle can be long enough to be dipped in the medium. Nonetheless, it has not been tested. Issues like clogging may arise if the printed material is temperature sensitive and the temperature of the medium is not set accordingly.

State	Main Constraints
Not addressed	MC-1: Work within a clean room
In progress	MC-2: Ergonomics
In progress	CMC-2.1: The extrusion system produces very a low noise when actuated, the play between the lead screw and the traveling nut as well as the play between the oil free bushing and the guiding bars are very small (H7-g6 for the bushing-bar pair). The noise of the motors and the xyz movements remain as they were before the Leapfrog printer was transformed although the original, worn bushings have been replaced by new bushings so the noise has been slightly lowered.
Completed	CMC-2.2: The design has been studied to ease the operations that need to be performed by hand such as inserting a syringe. All the necessary information to select adequate slicing parameters were given in Chapter 5 and a step by step guide is given in appendix A "How to start a print". The user still needs to handle a slicing software and the control terminal of the printer in the Simplify3D software, this is accessible by anyone with little to no 3D printing experience.

continues on next page

Not addressed	CMC-2.3: The frame of the Leapfrog printer has not been modified so its weight and bulkiness have not been considered.
In progress	MC-3: Cost
In progress	CMC-3.1: The cost of the parts bought for the printhead reached 117.47€. The cost of the PLA and ABS is not evaluated, <1 kg of PLA filament is needed and <50 g of ABS is needed. For information, the Leapfrog Creatr HS printer is not sold anymore.
Completed	CMC-3.2: Luer lock syringes and compatible needles are very common, they are easy to find and low cost, especially in large quantities. These are the only potential consumables.
Not addressed	MC-4: Cleanliness
	CMC-4.1 The cleaning was not addressed, although the design of the printhead is thought to minimise contamination of and from the mechanisms that are purposely enclosed.
Not addressed	MC-5: Disinfection/Sterility
	CMC-5.1: The disinfection and sterilisation have not been addressed, yet a watchful utilisation of the printer with disinfected/sterilised syringes, needles and substrates will help provide appropriate printing conditions.
In progress	MC-6: Accuracy and repeatability
Completed	CMC-6.1: The Leapfrog Creatr HS has an accuracy of 16.9 μm in x and y directions and 20 μm in the z direction.
Not addressed	CMC-6.2: The mechanical repeatability of the printer is not given in its datasheet. It was not assessed in this project. Yet, it is calibrated before every print so the repeatability should be positively impacted.
In progress	MC-7: Printing resolution
Completed	CMC-7.1: 500 μm resolution can be achieved with an adequate needle. Higher resolution can be reached as the diameter of the needles can reach below 300 μm .

continues on next page

Not addressed	CMC-7.2: The drop deposition system has not been addressed (MF-2).
Completed	MC-8: Printing volume
	CMC-8.1: The printing area extends to 180 mm in diameter (Fig. 6.6). CMC-8.2: Printed structure can reach 100 mm in height, substrate height included.
Completed	MC-9: Cost of bio inks
	CMC-9.1: The syringe can be selected between 3, 5 and 10 ml in volume to come as close as possible to the volume needed for the print. They can also be filled with only as much material as needed.
Not addressed	MC-10: Laminar airflow (JF Rees application)
	CMC-10.1: Laminar flow conditions have not been tested. Yet, the side panels of the printer can be removed to allow for the airflow to go through the printer, more precisely through the working space of the printhead.

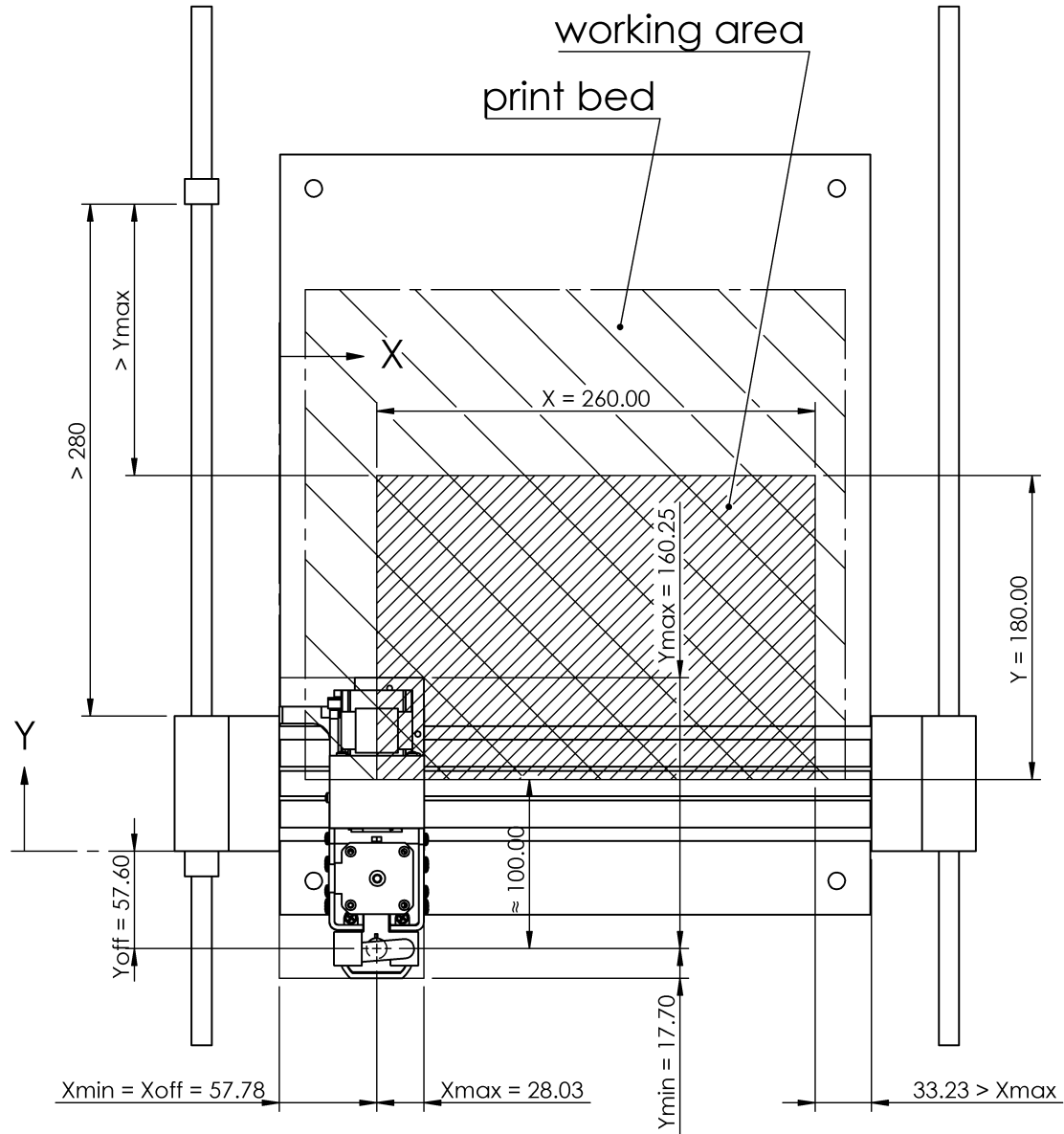


Figure 6.6: Top view of the printer actuation system. Printing area resulting from the dimensions of the printing frame, the encumbrance of the head in the XY plane and relative position of the needle with respect to the origin.

Conclusion

Since the apparition of the first bioprinter in 2003, the field of bioprinting has experienced a rapid growth and gained the interest of researchers worldwide. In the past few years, researchers at UCLouvain started investigating the benefits of using 3D bioprinters for specific applications in their respective fields. Jean-François Rees, Professor in biological sciences, expressed his interest in the technology to create 3D structures of marine sponge. In that context, the development of a 3D bioprinter was suggested as a Master's thesis. The local spin-off VOCSENS also rapidly showed its common interest in the topic. The research objective is twofold: the first part consists in identifying the needs of the clients and generating a list of technical requirements for design purposes while the second part involves the realisation and assessment of a prototype.

The specifications were established in an iterative process encompassing meetings with the clients, the identification of the objectives and a review of the state of the art. These specifications give general directions for the design process. Considering the limited time frame available, the conception is limited to the design and assembly of an extrusion-based printhead mounted on the frame of an existing 3D printer.

The prototype developed features a solution to extrude material through a syringe. The design is modular and flexible, it fits different sizes of syringes and includes an engagement mechanism of the head so multiple heads can be used in a single print. It can also be easily modified to be mounted on a different printer frame. The printhead presents a heating system to control the temperature of the material in the syringe. It allows to use temperature-sensitive biomaterials and to increase cell viability.

The new design of the extrusion system features a different transmission chain than the original extrusion system of the printer. Therefore the relation between the slicing parameters and the output of the extrusion has changed and led to inconsistent results in the printing. Tests and calculations have been performed to

create a procedure to tune the specific parameters and cope with the modifications.

The assessment of the prototype includes tests and measurements relevant to verify the specifications established at the beginning of the project. Some specifications are not fulfilled as only the printhead design is considered in the frame of this thesis.

Tests on the heating system showed an error between the required and the actual temperature of the material inside the syringe. In addition, the long transient time of the heating requires the syringe to be preheated. The printing tests proved that the printer is functional and it is able to produce prints. However, the tests also highlighted issues with the extrusion and the adherence of the material on the substrate. These are partially due to starting height calibration, clogging of the needle and extrusion adjustment. Overall, most of the specifications within reach have been completed.

The current prototype enables the clients to start proceeding with their respective research. Yet many improvements could be brought to the design of the printhead and the bioprinter in general. The clogging issue aforementioned was due to the lack of control of the needle temperature. Either an extension of the current heating system or the addition of a dedicated one could solve the problem, although likely at the cost of additional encumbrance.

The transient time of the heating system could be improved by performing an identification of the system to carry out a shaping of the input command. This adapted input would increase the dynamic performances of the control loop. Including new heating and sensing elements could also greatly improve the response of the system.

The current motherboard lacks of external ports to connect additional print-heads and sensors. These extensions would allow to use multiple heads and to develop a calibration routine for the engagement system. This could be achieved by redesigning the electronics and including additional ports.

Another limiting factor of working with an existing printer is the size of the frame. An increase in the length of the X and Y axis would allow to add printing heads and even to increase the working area on the printing bed. The design of a new specific frame should be considered to improve the potential of the bioprinter.

In future involvements in the project, we suggest to focus both on the design of a custom frame with appropriate electronics and on the design of a droplet-based system to access a wider range of applications.

References

- [1] Mathilde Berchon. *Le grand livre de l'impression 3D*, page 26. 2020.
- [2] Zeming Gu, Jianzhong Fu, Hui Lin, and Yong He. Development of 3d bioprinting: From printing methods to biomedical applications. *Asian Journal of Pharmaceutical Sciences*, 15:529–557, 9 2020.
- [3] Ibrahim T. Ozbolat, Kazim K. Moncal, and Hemanth Gudapati. Evaluation of bioprinter technologies. *Additive Manufacturing*, 13:179–200, 1 2017.
- [4] Melanie Kahl, Markus Gertig, Phillipp Hoyer, Oliver Friedrich, and Daniel F. Gilbert. Ultra-low-cost 3d bioprinting: Modification and application of an off-the-shelf desktop 3d-printer for biofabrication. *Frontiers in Bioengineering and Biotechnology*, 7, 2019.
- [5] Jesse K. Placone and Adam J. Engler. Recent advances in extrusion-based 3d printing for biomedical applications. *Advanced Healthcare Materials*, 7, 4 2018.
- [6] Amir K. Miri, Iman Mirzaee, Shabir Hassan, Shirin Mesbah Oskui, Daniel Nieto, Ali Khademhosseini, and Yu Shrike Zhang. Effective bioprinting resolution in tissue model fabrication. *Lab on a Chip*, 19:2019–2037, 6 2019.
- [7] Ibrahim T. Ozbolat and Monika Hospodiuk. Current advances and future perspectives in extrusion-based bioprinting. *Biomaterials*, 76:321–343, 2016.
- [8] NETZSCH *Proven Excellence*. Curing (crosslinking reactions). <https://analyzing-testing.netzsch.com/en/training-know-how/glossary/curing-crosslinking-reactions>, consulted on July 11 2022.
- [9] Elham Davoodi, Einollah Sarikhani, Hossein Montazerian, Samad Ahadian, Marco Costantini, Wojciech Swieszkowski, Stephanie Michelle Willerth, Konrad Walus, Mohammad Mofidfar, Ehsan Toyserkani, Ali Khademhosseini, and Nureddin Ashammakhi. Extrusion and microfluidic-based bioprinting to fabricate biomimetic tissues and organs. *Advanced Materials Technologies*, 5, 2020.

-
- [10] Ashwini Rahul Akkineni, Tilman Ahlfeld, Anja Lode, and Michael Gelinsky. A versatile method for combining different biopolymers in a core/shell fashion by 3d plotting to achieve mechanically robust constructs. *Biofabrication*, 8, 10 2016.
- [11] A. Lee, A. R. Hudson, D. J. Shiwerski, J. W. Tashman, T. J. Hinton, S. Yerneni, J. M. Bliley, P. G. Campbell, and A. W. Feinberg. 3d bioprinting of collagen to rebuild components of the human heart. *Science*, 365(6452):482–487, 2019.
- [12] Amit Panwar and Lay Poh Tan. Current status of bioinks for micro-extrusion-based 3d bioprinting. *Molecules*, 21, 6 2016.
- [13] Hemanth Gudapati, Madhuri Dey, and Ibrahim Ozbolat. A comprehensive review on droplet-based bioprinting: Past, present and future. *Biomaterials*, 102:20–42, 2016.
- [14] Lord Rayleigh. On the instability of jets. *Proceedings of The London Mathematical Society*, 1:4–13.
- [15] Anke Lindner and Christian Wagner. Viscoelastic surface instabilities, 11 2009.
- [16] Wikipedia. Cone de taylor. https://fr.wikipedia.org/wiki/Cône_de_Taylor, consulted on August 1 2022.
- [17] Wei Long Ng, Jia Min Lee, Wai Yee Yeong, and May Win Naing. Microvalve-based bioprinting-process, bio-inks and applications. *Biomaterials Science*, 5:632–647, 2017.
- [18] Junjie Yu, Su A. Park, Wan Doo Kim, Taeho Ha, Yuan Zhu Xin, Junhee Lee, and Donghyun Lee. Current advances in 3d bioprinting technology and its applications for tissue engineering, 12 2020.
- [19] Brian Derby. Bioprinting: Inkjet printing proteins and hybrid cell-containing materials and structures. *Journal of Materials Chemistry*, 18:5717–5721, 2008.
- [20] Amer B. Dababneh and Ibrahim T. Ozbolat. Bioprinting technology: A current state-of-the-art review. *Journal of Manufacturing Science and Engineering, Transactions of the ASME*, 136, 12 2014.
- [21] S. E. Braslavsky. Glossary of terms used in photochemistry 3rd edition: (iupac recommendations 2006), 3 2007.

-
- [22] A. A. Antoshin, S. N. Churbanov, N. V. Minaev, Deying Zhang, Yuanyuan Zhang, A. I. Shpichka, and P. S. Timashev. Lift-bioprinting, is it worth it?, 9 2019.
- [23] Ali Bakhshinejad and Roshan M D’Souza. A brief comparison between available bio-printing methods. 2 2015.
- [24] V. Harinarayana and Y. C. Shin. Two-photon lithography for three-dimensional fabrication in micro/nanoscale regime: A comprehensive review, 10 2021.
- [25] Min Tang, Jeremy N. Rich, and Shaochen Chen. Biomaterials and 3d bioprinting strategies to model glioblastoma and the blood–brain barrier, 2 2021.
- [26] J. Groll, J. A. Burdick, D. W. Cho, B. Derby, M. Gelinsky, S. C. Heilshorn, T. Jüngst, J. Malda, V. A. Mironov, K. Nakayama, A. Ovsianikov, W. Sun, S. Takeuchi, J. J. Yoo, and T. B.F. Woodfield. A definition of bioinks and their distinction from biomaterial inks. *Biofabrication*, 11(1), 2019.
- [27] Futura Sciences. Hydrogel : qu’est-ce que c’est ? <https://www.futura-sciences.com/sciences/definitions/chimie-hydrogel-4917/>, consulted on July 13 2022.
- [28] Monika Hospodiuk, Madhuri Dey, Donna Sosnoski, and Ibrahim T. Ozbolat. The bioink: A comprehensive review on bioprintable materials. *Biotechnology Advances*, 35(2):217–239, 2017.
- [29] aniwaa. Allevi 3. <https://www.aniwaa.com/product/3d-printers/allevi-allevi-3/>, consulted on July 11 2022.
- [30] Advanced Solutions. Bioassemblybot 400. <https://www.advancedsolutions.com/bioassemblybot-400>, consulted on July 11 2022.
- [31] CELLINK. Bio x. <https://www.cellink.com/bioprinting/bio-x-3d-bioprinter/>, consulted on July 11 2022.
- [32] Rokit Healthcare. Dr. invivo 4d2. <https://rokithealthcare.com/dr-invivo-4d2/?ckattempt=1>, consulted on July 11 2022.
- [33] Analytik. Bioscaffolder 3d bioprinter series. <https://analytik.co.uk/product/3d-bioprinter-bioscaffolder/>, consulted on July 11 2022.
- [34] CELLINK. Lumen x+. <https://www.cellink.com/bioprinting/lumen-x/>, consulted on July 11 2022.

-
- [35] aniwaa. Regemat3d. <https://www.aniwaa.com/product/3d-printers/regemat3d-v1-bioprinter/>, consulted on July 11 2022.
- [36] Anon. Cell size. <https://bio.libretexts.org/@go/page/12711>, Accessed December 22, 2021.
- [37] Bruno Siciliano and Oussama Khatib. *Springer handbook of robotics*, page 237. 2016.
- [38] Bruno Siciliano and Oussama Khatib. *Springer handbook of robotics*, pages 447–448. 2016.
- [39] Schneider Electric. *NEMA size 17 1.8° 2-phase stepper motor*. http://www.autoflexible.com/file_upload/product/attach/NEMA%2017.pdf.
- [40] Jacob Fraden. *Handbook of Modern Sensors*, page 626. 2016.
- [41] Marlin. G0-G1 - Linear Move. <https://marlinfw.org/docs/gcode/G000-G001.html>, consulted on August 1 2022.
- [42] User "burtoogle" on Ultimaker Community of 3D Printing Experts. Cura flow calculations. <https://community.ultimaker.com/topic/28492-flow-calculations/>, consulted on August 3 2022.
- [43] Slic3r. Manual - flow math. <https://manual.slic3r.org/advanced/flow-math>, consulted on August 1 2022.
- [44] InvitrogenTM. *UltraPureTM Low Melting Point Agarose*. <https://www.thermofisher.com/order/catalog/product/16520100?SID=srch-srp-16520100>.
- [45] THERMOLAB®. *Shaking Water Baths GFL®1083*. https://www.gfl.de/m129_upload/GFL-SWB_e.pdf.
- [46] Guo Liang Ying, Nan Jiang, Sushila Maharjan, Yi Xia Yin, Rong Rong Chai, Xia Cao, Jing Zhou Yang, Amir K. Miri, Shabir Hassan, and Yu Shrike Zhang. Aqueous Two-Phase Emulsion Bioink-Enabled 3D Bioprinting of Porous Hydrogels. *Advanced Materials*, 30(50), 2018.
- [47] Nan Chen, Kai Zhu, Yu Shrike Zhang, Shiqiang Yan, Tianyi Pan, Mieradili-jiang Abudupataer, Guodong Yu, Md Fazle Alam, Li Wang, Xiaoning Sun, Yanlei Yu, Chunsheng Wang, and Weijia Zhang. Hydrogel Bioink with Multi-layered Interfaces Improves Dispersibility of Encapsulated Cells in Extrusion Bioprinting. *ACS Applied Materials and Interfaces*, 11(34):30585–30595, 2019.

Appendices

A: How to start a print

Prerequisites

- Printer is plugged in and powered on;
- A computer with Simplify3D installed is connected to the printer and the printer is in computer control mode;
- The the G-code file with header and footer as specified in Section 5.4 for the print is uploaded in the list in the Simplify3D control panel;
- The material for extrusion is preheated at the target temperature;

Procedure to start the print

1. Calibrate XYZ axis **without** syringe and substrate
2. Heat up the nozzle and bed to application target temperature
3. Move Z 40 mm positive (build plate moving downwards)
4. Place sacrificial Petri dish under printhead (it will collect potential dripping from the syringe during following steps)
5. Place syringe in the heating jacket with adapted syringe cover
6. Type and send "M302" in the communication tab to override safety for cold extrusion
7. Adjust extrusion height to lock plunger
8. Remove sacrificial Petri dish
9. Place substrate on printing bed and lock with the plexiglass slats
10. Adjust XY position with respect to the substrate (origin in front left corner)
11. Adjust Z axis: move bed so the substrate is as close to needle as needed for application
 - if the same print must be performed multiple times with similar substrates in a row, adjust Z calibration screw (if substrate height ≤ 10 mm)
12. start G-code
13. wait for end of the print

B: Useful G-code control commands

This appendix provides a set of useful G-code control commands but it is not comprehensive. Those commands are grouped in Table 3.

All the existing commands as well as further information on G-code can be found on this website: <https://marlinfw.org/meta/gcode/>.

Useful G-code control commands	
G0	Linear move (non-extrusion movement)
G1	Linear move (extrusion movement)
G28	Auto home of X Y Z
G90	Absolute positioning
G91	Relative positioning
G92 X0 Y0 Z0 E0	Specify nozzle current X Y Z E position is 0, 0, 0, 0
M302	Override cold extrusion
X<val>	Move in x direction by <val> mm
Y<val>	Move in y direction by <val> mm
Z<val>	move in z direction by <val> mm
E<val>	Extrude <val> mm
F<val>	Set movement speed
T0	Activate tool 0
T1	Activate tool 1

Table 3: Useful G-code control commands.

UNIVERSITÉ CATHOLIQUE DE LOUVAIN

École polytechnique de Louvain

Rue Archimède, 1 bte L6.11.01, 1348 Louvain-la-Neuve, Belgique | www.uclouvain.be/epl