Building a Bioprinter from Scratch for Bioprinting Testing

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Abstract— **Aiming to reduce the current worldwide organ shortage for transplant, tissue engineering has thrived on organ fabrication techniques. Nevertheless, building three-dimensional (3D) vascularized organs remains the main technological barrier to be overcome. New technologies like bioprinting are emerging to aid scientists to bring new solutions, since drop by drop demand allows cell deposition with high precision. In this paper it is explained a method of building a low cost 3D bioprinter, from scratch, that allows three axis printing with a reasonable resolution for this use.**

Index Terms—**3D bioprinting, step motor control, image analysis, nozzle firing control.**

I. INTRODUCTION

ORGAN FAILURE is a critical problem in the worldwide healthcare nowadays, and the demand for organ transplantation largely exceeds the supply. Conventional tissue fabrication techniques, like seeding cells onto scaffolds, are still not capable of producing thick viable tissues, because there is no control in vascularization and cell respiration at core level of the thick tissue doesn't occur [1]. Bioprinting technology brings new hopes to overcome this problem, because it allows a high rate and high precision cell deposition granting the possibility to manufacture the thick tissue with its own vascularization with the patient own cells [2]-[4].

There are many techniques being researched focused on the nozzle firing type (inkjet: thermal; piezoelectric; laser), adapting usual printers to use in this field, or building the printer from scratch. Although the overwhelming possibilities of this technology may seem, some issues like cell death caused by the firing process and cell proliferation after printed are still being researched and improved [5]-[9].

Building a bioprinter from scratch was the path that was chosen in this work, so that there are full control of the printer and the printed material. The main printing material would be human cells, among other tissue constituents. Depending on the type of cell, the size varies a bit, however in general a human cell with nucleus has 20 to 30 micrometers length. With that being said for the bioprint process to be viable the ideal printer resolution should be at around 50 micrometers [10].

II. BUILDING A BIOPRINTER FROM SCRATCH

A. Pre-build

Standard printers roll the paper at the same time as the printer head goes side to side and shoots the drops of ink, while all this parts move. Instead of trying to modulate this intricate kind of system, and since there is little information on how the circuits inside the printer work, we chose to use a simpler system that we could understand and have full control.

Our system combines this entities:

- Matlab - where the main code analyses the images to be printed and transforms the information so that it can be worked to be sent in binary to the next component.
- Micro controller – The micro controller grabs the binary information sent from the Matlab and interprets it to move the motors and firing the nozzles.
- Breadboard – Where all the driving components receive the information from the logic and power up the motors and nozzles.
- Bioprinter itself – where all the physical components are, ready to receive the commands.

As for the X and Y axis motion, bipolar step motors were chosen to allow precise start and stop motion. Other advantage on using step motor is the unnecessary use of a feedback system to know the position of the print head. However without a feedback system the only way to ensure minimal step loss is to return to position 0 at each printing line. The axis need guides so that the print head runs only on each of the axis directions, and doing guides manually wouldn't be accurate enough for the precision required, so we have chosen motors from CR-ROM drives which already has its own guide support system with good precision, and

they are easy and cheap to get. This motors provides an axis length of 3,7cm, so there is a total of $3,7 \times 3,7$ cm^2 useful print area.

As for the Z axis we opted for a servo motor for the power it needs to elevate the head. Since the Z axis doesn't need that much precision, as the X and Y axis, we chose to do the guides manually, using the same guides from the CD-ROM drives but in a different format, consenting a total length of 1 to 2cm. This length

As for the print head, little documentation about how print heads work is freely given. Large manufacturers hide this information to minimize company espionage and intellect property theft, and we didn't had the time or money to test various print heads and try figure out how they work. We chose the cartridge HP C6602 because there is information available about it from bibliography [11]. The cartridge holder HP Q2347A was considered also needed for the connection between the cartridge and the controlling board.

B. Motors

The motors used in this work run at 5V, 500mA and have a step resolution of 18 degrees. They are bipolar step motors in the category of two-phase step motors, and have a single winding per phase. There is no common wire so the current in a winding needs to be reversed in order to reverse a magnetic pole, typically this motors are driven with an H-bridge [12]. This H-bridge allows the current to flow in each winding on both directions at different times as Fig. 1 shows. The motor drives chosen from Texas Instruments, allows step motors to do microstepping which would increase greatly the resolution of the axis, however after testing all together both motors didn't respond to microstepping.
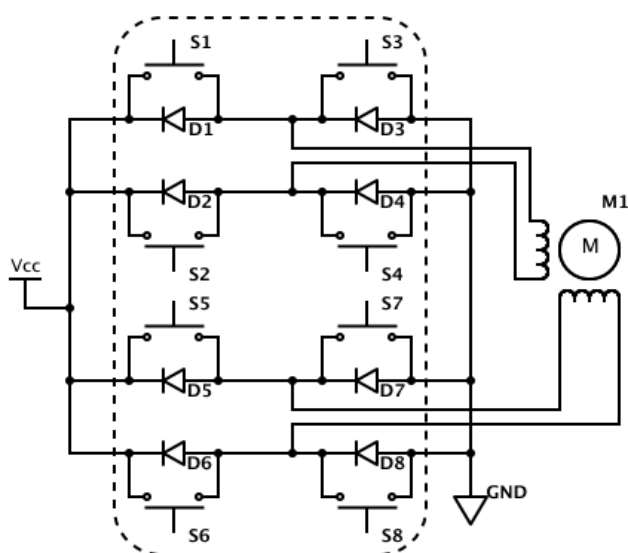

Fig. 1. Step motor simple drive schematic showing the two H-Bridges, each one providing the current and its direction for each motor winding.

When testing, the X axis motor performed the total axis length with 252 full steps, and the Y axis motor with 166 full steps, this difference is because the screw rotors in both motor differ, making the carriage move differently. Since the total length of the axis is 3,7cm this means that for the X axis we get a 146μm step and for the Y axis we get 223μm.

This numbers are a bit far from the ideal 50μm, but considering the low cost and low time to build this prototype

they were considered as valid numbers.

As for the Z axis, a servo was chosen because of the torque that this motor performs. It is rotary actuators that will allow the print head to move along the Z axis with gearing through the guides provided [13].

C. Print head

The print head is the combination of the HP C6602 cartridge and its carriage HP Q2347A. This carriage facilitates the signal transmission between the control board and the cartridge besides holding and protecting the cartridge. The HP C6602 cartridge was chosen not just because of the available information to run it but also because most recent cartridges have nozzle dimensions that doesn't allow them to be used to print biologic material. There is a dimensional limits for the living cells to be fired with high survivability and old cartridges with their larger nozzles are a better choice in this field.

It has 12 nozzles disposed in 3mm, 250μm from nozzle to nozzle, it has an average resolution of 96 dpi which will be increased with our system build, and it has an average drop volume of 160pL.

The nozzles fires drops of ink through heating, and it requires pulses, of 20 to 21V, of 6μs for each nozzle with a resting time of 800μs. Also the nozzles can't be fired all at the same time there must be a delay of 0,5μs between two different nozzle fire.

D. Coding

The matlab communicates with the microcontroller through RS232. And to ensure the data acquisition, the coding includes feedback mechanisms which makes the micro controller to resend back the data receives so that the main code at matlab continues with the operation, Fig. 2.
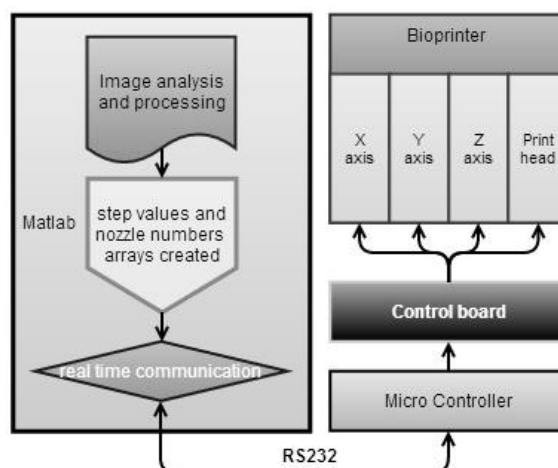

Fig. 2. Matlab communicates through RS232 with the micro controller which feeds back the data received to ensure no data loss. The micro and the control board connect to the printer devices through unifilar wires.

As said before the total resolution of the printer is 252 x 166, so only images with this resolution or less can be analyzed and printed. The whole process starts with a prompt asking for the image to be printed, after that another prompt asks for the confirmation of the printing process, since it is in real time and the process takes some time to finish, at the end a final prompt asks the user if another layer

of tissue is going to be printed.

The program starts by transforming the image in gray scale since RGB color isn't needed in this process, also in gray scale, the dark spots are more easily identified than in RGB. The next step is to transform the image in a matrix composed of 0 at the brighter areas and 1 at the darker areas, as said before histologic images usually have colored (darker) nucleus which we can roughly define as the positions of the cells. The Fig. 3 shows a schematic of the proposed code.

As said before the print head is composed with 12 nozzles so the print line is composed of 12 positions running the length of the whole image. The next step is to cut the image into slices of 12 lines, and matlab allows us to save all the slices in the same matrix, in different layers of the matrix third dimension. Having this slice matrix we just need to search for the 1s, for them to be saved. With this information two 1 column matrixes are created, one for the position of the firing column, where the X motor have to dislocate the print head, and other for the number of the nozzles that fires at each firing column.
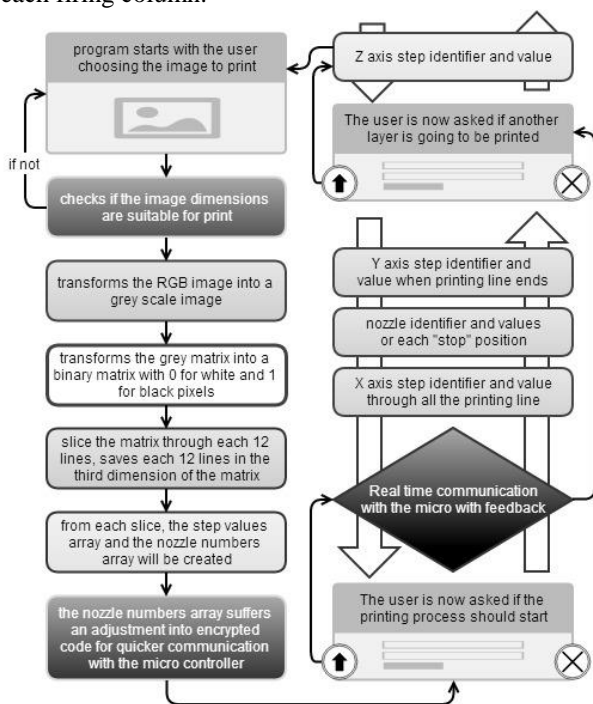

Fig. 3. Schematic of how the code works, from the process of choosing the image to print to the end of the printing process.

Although we have the X positions for each firing column, we need to send to the micro controller the values of the steps needed to reach those positions, not the number itself. Another 1 column matrix is created with the step values for each firing column, i.e. the number of steps needed for the motors to perform to reach the positions where there is a firing column.

Before the print process begins we opted to encrypt the nozzle numbers into 2 bytes so the communication is faster. Nozzles from number 1 to 8 are sent in the first byte, as 1 to 8 are respectively 1, 2, 4, 8, 16, 32, 64, and 128, and nozzles from 9 to 12 are sent in the second byte, as 9 to 12 are respectively (1, 2, 4, 8) + 240. So for example if a firing column has the nozzles 2, 4, 7, 8, 9, and 11 matlab sends 2

bytes being the first 2+8+32+64=106 and the second 249.

For this a 2 column matrix has to be created to save the numbers of the nozzles, in bytes, at each firing point so that they can be sent at the same time to the micro and received from it to ensure no loss of communication.

Now the main code has enough information to start the real time data send process, the script runs the 1 column matrix for the step values and sends the values to the micro controller, at the same time it is running the 2 column matrix for the nozzle numbers and after the step value is sent, the 2 bytes with the numbers of the nozzles are sent. This communication is a bit slow because it has to count with the motor motion timing and the feedback checkup of the data received from the micro. Also this is a stop and print method not a print while running used in most all commercial printers.

Each time the code finishes running each layer of the third dimension of the step column matrix (which represent the different 12 line slices) Matlab sends the number of steps that the Y motor needs to perform for the next printing line. Since the print line is 3mm wide, and the Y motor performs 223µm per step, the motor needs to move 14 full steps, 14×223=3122mm, so that number is sent to the micro controller at the end of each printing line.

Every time the print line ends the X motor returns to its default position (home position) to minimize step loss, likewise when the full image is printed both X motor and Y motor returns to home position.

At this point the user is asked if another layer of the tissue is going to be printed, since the printing process is done layer by layer of material to produce the 3D thick tissue. If the user chooses to print another layer an increment value is sent to the micro for the Z axis motor, and the process starts all over again.

The secondary code, or receiving code, at the micro controller, sends back the data received to ensure data integrity. The step values received are analyzed and the correct signals are sent to the drives to move the motors. As for the bytes comprehending the numbers of the nozzle the code needs to analyze it and decrypt it. Although they are not fired at the same instant, the time between each nozzle fire at each firing column is very low, yet respecting the minimum of 0,5µs imposition for each different nozzle, and 800µs from each fire at same nozzle.

E. Control and wiring

The bread board used to control the devices connects the micro controller to the motor drives and to the demultiplexer used to fire the nozzles, as shown in Fig. 1 and Fig. 4.

There are 2 different voltages running in the board, the 3,3V logic from the micro controller, and the 8V to feed the motor drives. Both drives require the 8V, and since the motors work at 5V, two 27Ω resistors were added in series to each winding, to reduce the voltage given to the motors, and excess of current. The nozzle firing requires 21V which is connected directly to the demultiplexer. This 16-channel demultiplexer allows to reduce significantly the number of wires required from the micro to fire the all 12 nozzles. Four inputs that permit 16 different combinations, one for each output from the demultiplexer. For this project the micro

was instructed to send the code as 0 0 0 1 for the first nozzle, 0 0 1 0 for the second and so on until the 1 1 0 0 that represent the twelfth nozzle.

In total, 15 wires connects the micro controller to the control breadboard, 3 wires per motor, 4 wires for the print head and 2 for the 3,3V and ground.
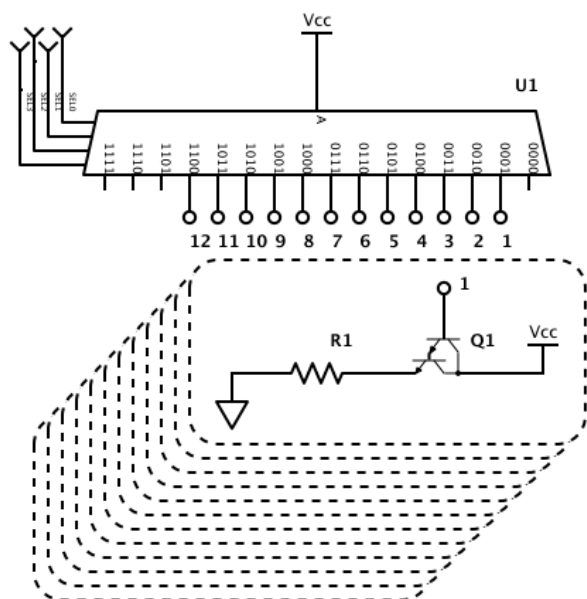

Fig. 4. Circuit schematic of the: demultiplexer, with 4 inputs and only 12 outputs used; Darlington transistor for each output from the demultiplexer, providing the current needed to the heating resistor at the firing chamber.

F. Structure

The material chosen to support the motors and axis was Poly (methyl methacrylate) (PMMA). Also called acrylic glass, or the most common name Plexiglas, its handling and cutting and low cost makes it a top choice for many projects, including our own bioprinter. The two Plexiglas sheets were bolted with four 15,5cm screws with a 10cm gap, for the print head to fit between them and move freely, Fig. 5.
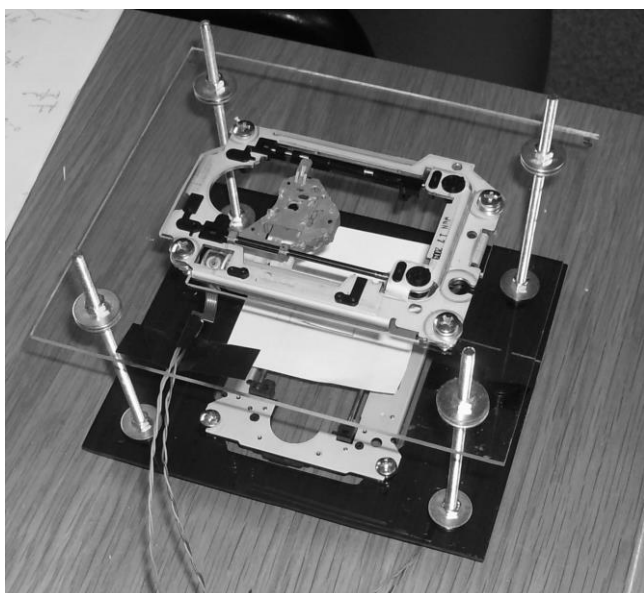

Fig. 5. Fotograph of the bioprinter X and Y axis; bottom axis is the X axis and moving the plate; upper axis the Y holding the heald and the Z axis.

III. PRINTER ASSEMBLY

At this point the bioprinter still doesn't have the print head mounted and working due to technical issues related to the Z axis, the axis guides are showing some building complications. Although both axis X and Y works fine with no step loss nor overheating. We expect to have the Z axis assembled to the bioprinter and ready to be tested soon.

IV. PRINTING RESULTS

There are no real printed results yet since the Z axis is being built, however as shown in Fig. 6 the main code is running as predicted.

Fig. 6. Virtual print of an image from matlab main code on the bottom, with its original image at the top.

V. CONCLUSION

Biopriting has been showing high potential in the field of tissue engineering, allowing researchers to manipulate the biologic materials. With this advantage scientists in a near future might be creating artificial organs, with the patient own cells, to surpass the worldwide situation on organ shortage.

It was with this intent that we started this work, and we show a low cost way to produce and control a bioprinter from scratch, that provide high precision with reasonable resolution and full control over the printed material.

REFERENCES

[1] Ozbolat, I. T. and Y. Yu. "Bioprinting towards Organ Fabrication: Challenges and Future Trends" Ieee T on Biomedical Engineering, 2013, vol. 60, no. 3, pp. 691-99.

[2] Cui, X., D. Dean, Z. M. Ruggeri, T. Boland. "Cell damage evaluation of thermal inkjet printed Chinese hamster ovary cells", *Biotechnology and bioengineering* ", 2010, vol. 106, no. 6, pp. 963-72.

[3] Xu, T. *et al.* "Viability of Neural Cell Structures Generated by the Inkjet Printing Method." *Biomaterials*, 2006, vol. 27, no. 19, pp. 3580-88.

[4] Catros, S., *et al.* "Layer-by-Layer Tissue Microfabrication Supports Cell Proliferation in Vitro and in Vivo." *Tissue Eng Part C Methods*, 2012, vol.18, no. 1, pp. 62-70.

[5] Durmus, N. G., S. Tasoglu and U. Demirci. "Bioprinting Functional Droplet Networks." *Nature Materials*, 2013, vol. 12, no. 6, pp. 478-1.

[6] Hong, S. *et al.* "Cellular Behavior in Micropatterned Hydrogels by Bioprinting System Depended on the Cell Types and Cellular Interaction." *J Biosci Bioeng*, 2013, vol. 116, no. 2, pp. 224-30.

[7] Koch, L., A. Deiwick, S. Schlie, S. Michael, M. Gruene, V. Coger, D. Zychlinski, *et al.* "Skin Tissue Generation by Laser Cell Printing." *Biotechnol Bioeng*, 2012, vol. 109, no. 7, pp.1855-63.

[8] Arai, K., *et al.* "Three-Dimensional Inkjet Biofabrication Based on Designed Images." *Biofabrication*, 2011, vol. 3, no. 3, pp. 034113.

[9] Duan, B., L. A. Hockaday, K. H. Kang, and J. T. Butcher. "3d Bioprinting of Heterogeneous Aortic Valve Conduits with Alginate/Gelatin Hydrogels." *J Biomed Mater Res A*, 2013, vol. 101, no. 5, pp. 1255-64.

[10] Xu, T., J. Jin, C. Gregory, J. J. Hickman and T. Boland. "Inkjet Printing of Viable Mammalian Cells." *Biomaterials*, 2005, vol. 26, no. 1, pp. 93-101.

[11] Gilliland, Matt. "*Inkjet Applications.*" Woodglen Press, 2005.

[12] Hayner, David A., and Michael E. Mitchell. "Motor controller, integrated circuit, and method of controlling a motor." U.S. Patent No. 6,081,091. 27 Jun. 2000.

[13] Ferrigno, Joseph T., Timothy J. Liska, and Kevin Moriarty. "Servo motor control." U.S. Patent No. 5,798,626. 25 Aug. 1998.