

Do-It-Yourself (DIY) manufacture of a Nano-LC MALDI spotter robot using 3D printing technology

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Abstract In the era of the forth Industrial Revolution, open source code and open source hardware have gained much attention. In particular, 3D printing technology is expanding into the realms of classical science, technology and our daily lives. Relatedly, in the present study, we demonstrate the manufacture of a nano-LC MALDI spotter robot using 3D printing technology. The parts of the spotter robot were either made using a 3D printer or purchased as 3D printer parts from the 3D printer online market, so that anyone can make the robot without a deep knowledge of engineering or electronics, i.e., DIY (do-it-yourself) product. In the nano-LC MALDI spotter, the nano-LC eluent and MALDI matrix were mixed in a T-union and discharged from the capillary outlet. The eluent and matrix mixture could be spotted onto the movable MALDI plate. The MALDI plate was designed to translate in a two-dimensional space (xy plane), which was enabled by the movements of two stepper motors. In the paper, all computer-aided design (CAD) files for the parts and operation software are provided to help the reader manufacture their own spotter robot.

Key words: MALDI, Do-it-yourself, Spotter, Robot, 3D printing, liquid chromatography

1. Introduction

In recent years, 3D printing technology has emerged as a key piece of technology that can lead us into a new era, i.e., the fourth Industrial Revolution. Three-dimensional printing, also known as additive manufacturing (AM), is a technology that outputs physical materials in a layer-by-layer format by converting a digital computer-aided design (CAD) file into a 3D model. This technique was first introduced in 1984 when Charles Hull developed a 3D printing technique called stereolithography (SLA). Since then, various

3D printing technologies, such as 3D inkjet printing, selective laser sintering (SLA), fused deposition modeling (FDM), and laminated object manufacturing (LOM) have been developed and used to create prototypes in industry. In 2008, the “RepRap project” developed the first open source-based self-replicating printer. In recent years, the innovation of 3D printer manufacturing has expanded the use of 3D printing technology into small companies and individuals.^{1,2}

With the spread of the 3D technology, applications of the technology have also been diversified. Three-dimensional printing is used for biomedical engineering,

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microfluidics, education, and many other research areas.²⁻⁵ Several studies in chemistry also use 3D printers. For example, a visible spectrometer, a potential energy surface model for education,^{6,7} epifluorescence microscopes, and a template for a low-temperature plasma (LTP) probe^{8,9} have been reportedly created by 3D printers. The increase in the use of 3D printing technology is attributed to its low cost. Thus, 3D printing technology gives researchers opportunities to carry out their specific ideas with a low economic burden.

In this study, 3D printing technology was used to manufacture a nano-LC MALDI spotter robot, which is a device that combines nano-LC (liquid chromatography) and a MALDI (matrix-assisted laser desorption/ionization) mass spectrometer. Nano-LC is an effective technique to separate a mixture of different types of peptides or other complex mixtures of small molecules. MALDI is a widely used soft ionization technique and can ionize various samples with different molecular characteristics, such as peptides, proteins, and DNAs, and it provides a relatively simple spectrum while having a wide m/z analysis range.¹⁰ In addition to these advantages of nano-LC and MALDI, the combination of nano-LC and MALDI, has some advantages over LC-ESI (electrospray ionization) mass spectrometry. By archiving LC eluents on the MALDI plate, there is sufficient time for the sample analysis, regardless of the online elution time.¹¹

The general configuration of the nano-LC MALDI spotter is shown in *Fig. 1*, where the nano-LC and MALDI sample deposition setup are combined. The

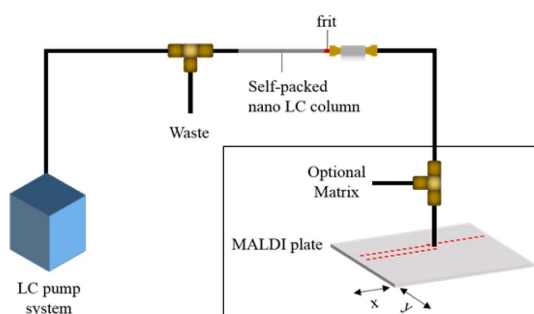


Fig. 1. Simplified schematic diagram of a nano-LC MALDI spotter robot.

nano-LC eluent and MALDI matrix can be combined and mixed using a T-union. The mixing ratio between the LC eluent and the matrix can be adjusted by controlling the LC elution flow rate and the syringe pump flow rate. Using the nano-LC MALDI spotter, a sample of sub-microliter ($0.3 \sim 1 \mu\text{L}$) separated by nano-LC can be loaded onto the spots on the MALDI plate. In this study, the schematic drawings for the manufacture of the nano-LC MALDI spotter robot and the software are provided in detail.

In the present study, the nano-LC MALDI spotter was designed to maximize the use of the 3D printer and the 3D printer parts available in the online market, which enables DIY (do-it-yourself) manufacturing. Careful attention was paid to the 3D CAD design to minimize the printing error; a printed object tends to have manufacturing errors due to swelling, misalignment, etc. When designing the parts, some error margins are provided to minimize the manufacturing error. Furthermore, to facilitate self-maintenance, the parts were designed to minimize the use of adhesive. Based on the spirit of the so-called “open source hardware and code”, the information necessary for making a nano-LC MALDI spotter robot is available to the public, so researchers can make this robot or use it for their project. The source CAD file for the parts and the source code to operate the nano-LC MALDI spotter robot can be obtained at <https://github.com/tlatnlagho/nano-LC-MALDI-spotter>.

2. Materials and Method

2.1. 3D printer and designing

Many of structural parts necessary for manufacturing the Nano-LC MALDI spotter robot were printed using a commercial FDM-type 3D printer (Moai clone, Sandimall, Seoul, Korea). This 3D printer is equipped with a heating bed and an auto-leveling function. A polylactic acid filament (PLA, Aplus, China) was used as a printing material. The parts were designed using AutoCAD 2017 (the computer aided design (CAD) software program). The designed figure was converted into the STL (standard tessellation language or STEREO-lithography) file format. The

printing options were set as follows: fill-density, 20 %; nozzle temperature, 220 °C; bed temperature, 65 °C; support-type, everywhere; and platform adhesion type, brim. The CAD files and STL files for the parts used to manufacture the nano-LC MALDI

spotter robot can be obtained at the website <https://github.com/tnlatnlagho/nano-LC-MALDI-spotter>.

2.2. Parts

The schematics of the parts used for the spotter robot are shown in *Fig. 2*, and the parts are listed in

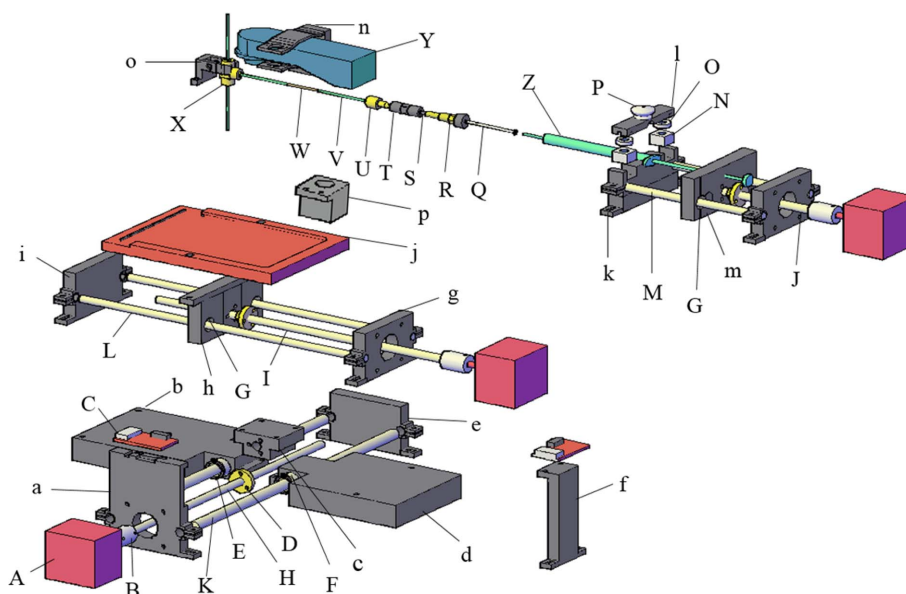


Fig. 2. Parts of the nano-LC MALDI spotter robot. The parts denoted by upper-case letters were purchased as 3D printer parts in the online market and the parts denoted by lower-case letters were 3D printed.

Table 1. List of the parts used to manufacture the nano-LC MALDI spotter robot. The components, which are denoted by either upper-case or lower-case letters, correspond to the parts shown in *Fig. 2*. The parts that are denoted by upper-case letters were purchased from the online market, and parts denoted by lower-case letters were 3D printed

Component	Description	Component	Description	Component	Description
A	stepper motor	O	iron-embedded acrylic	c	lead screw nut holder
B	stepper motor	P	acrylic knob	d	floor 2
C	coupler	Q	connection tube	e	round rod holder 2
D	endstop limit switch	R	rhexflex nut	f	limit switch holder
E	lead screw nut	S	ferrule	g	round rod holder 3
F	linear ball bush 1	T	microtight union	h	lead screw nut holder 2
G	linear ball bush 2	U	microtight fittings with ferrules	i	round rod holder 4
H	lead screw 1	V	sleeve	j	MALDI plate holder
I	lead screw 2	W	silica capillary	k	syringe holder
J	lead screw 3	X	T-union	l	acrylic knob holder
K	round rod 1	Y	microsyringe	m	lead screw nut holder 3
L	round rod 2	Z	camera	n	camera clamp
M	round rod 3	a	round rod holder 1	o	T-union clamp
N	magnet-embedded acrylic cube	b	floor 1	p	waste tube holder

Table 1. In *Fig. 2*, the 3D-printed parts are denoted by lower-case letters, whereas the purchased parts are denoted by upper-case letters. The stepper motors (A), driver A4988, and end-stop mechanical limit switches (C) were purchased from Aplus (China). A 12 V 30 W DC power supply was available at Eltion SMPS (Geeltech, Gyeongsangbuk-do, Korea). A USB microscope (Y) was purchased from UM-CAM (China). The diameters and lengths of the round rods (K, L, and M), which were used as the MALDI-plate moving-stage axes (K and L) and the syringe pump movement axis (M), were 8/26 mm, 6/24 mm, and 6/14 mm, respectively. The linear ball bushes (bearing, Aplus, China) were used to guide the round rod, and their inner diameter, outer diameter, and length were 8 mm, 15 mm, and 45 mm for F, respectively, and 6 mm, 12 mm, and 19 mm for G, respectively. The lead screws H and I were used to move the MALDI-plate, and their diameters and lengths were 5 mm and 20 mm, respectively. The lead screw J for the syringe pump had a diameter and length of 5 mm and 10 mm, respectively. Three stepper motor couplers (B) (Aplus, China) were used and their diameter, length, and hole diameter were 15 mm, 20 mm, and 5 mm, respectively. A snap ring (E) was used to incorporate the bearing (linear ball bush) into the 3D printed structure blocks. The schematics for the container case and the cover acrylic plates can be obtained at the website <https://github.com/tlatnlagho/nano-LC-MALDI-spotter>. A micro syringe (Z, 1705RNR, volume 50 μ L, needle size 22s, Hamilton, Reno, NV, USA) was used as a matrix loader. A needle port (Q, R, S) (P/N 9013, Rheodyne, Rohnert Park, CA, USA) was used to connect the micro syringe to the silica capillary (W).

2.3. Software

Arduino mega 2560 (Aplus, Ningbo, China) was used as a digital I/O board to operate the instrument. An operation software, which was coded using Labview™ 2014 (National Instruments, Austin, Texas, USA), was used to control the Arduino mega 2560, the stepper motor drivers A4988, and the end-stop mechanical limit switches. LINX was used to interface

the Arduino's digital I/O board with Labview™.

3. Results and Discussion

3.1. Hardware design

3.1.1. Design concept

The components necessary for making the spotter robot are shown in *Fig. 2*. The spotter robot consists of two main parts; the MALDI plate moving stage (shown in the lower part of *Fig. 2*); and the syringe pump operation, T-union (X), and USB camera monitoring (in the upper part of *Fig. 2*). In the T-union, the LC eluent and MALDI matrix (from the matrix-loaded syringe) can be mixed for the sample loading onto the MALDI plate. The MALDI plate moving stage components, shown in *Fig. 2*, are located within the container frame, whereas the syringe pump and USB camera are located on top of the container case (outside the container). Additionally, a T-union, an Arduino microprocessor, a circuit board, and a power-supply were located on top of the container but enclosed in another small box for protection from dust. The container case is designed with two front doors that allowed access to the moving stage. The exterior of the container case is made of an acrylic plate, whose dimensions can be obtained at the website <https://github.com/tlatnlagho/nano-LC-MALDI-spotter>. The moving stage was designed so that the MALDI can move in two-dimension: x- and y-directions on the plane. This stage is fixed to the bottom plate of the container acrylic plate.

3.1.2. Hardware component manufacturing

To load the LC eluents onto the MALDI plate, either the tip of the capillary, from which the eluent discharges, or the MALDI plate must move. In this study, we chose to mobilize the MALDI plate instead of the tip of capillary. The MALDI plate moving stage is designed to move in the two-dimensional plane. The moving stage was translated using the rotation of a lead screw connected to the stepper motor (see *Fig. 3*). To achieve the two-dimensional motions, two lead screws were orthogonally used for

the movements of the MALDI plate stage. Most of the mechanical parts were those used to manufacture the 3D printer, and therefore are readily available from 3D printer online shops. The syringe pump was also made using the lead screw connected to the stepper motor.

Many of the parts that were used to manufacture the spotter robot were made using the 3D printer, for example, a MALDI plate holder (j in Fig. 2) that was designed to contain the MALDI plate (in this study, MTP 384 ground steel BC targets, BRUKER Daltonics, Bremen, Germany) and a waste tube holder (p in Fig. 2) for a 1 mL Eppendorf tube. The CAD file for

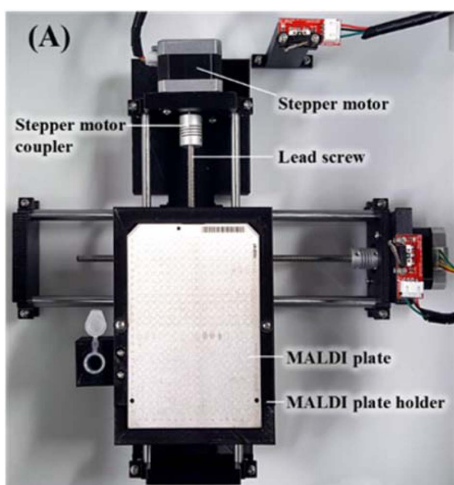


Fig. 3. Pictures of (A) the MALDI plate moving stage.

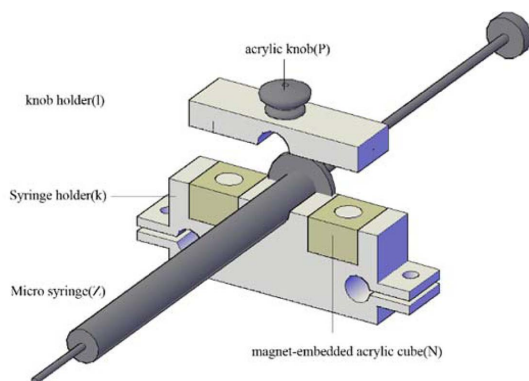


Fig. 4. Schematic drawing of the syringe holder assembly to mix the nano-LC eluent and MALDI matrix. The CAD file can be obtained at the website <https://github.com/tnlatnlagho/nano-LC-MALDI-spotter>.

the MALDI plate holder can be obtained at the website <https://github.com/tnlatnlagho/nano-LC-MALDI-spotter>. this file can be modified to accommodate MALDI plates of different dimensions. The syringe holder (k) was also made using the 3D printer (see Fig. 4); a commercial magnet-embedded acrylic cube (N), an acrylic knob (P), and a 3D printed knob holder (i) were assembled to hold the syringe.

4. Software Design

4.1. Design concept

The operation software, which was coded using Labview™ consists of four sub-programs (Fig. 5): (1) interface set-up, (2) motor control, (3) autohome, and (4) spotting.

(1) “Interface set-up” sub-program

This program initiated the serial communication between the Arduino micro-processor and the Labview™ software.

(2) “Motor control” sub-program

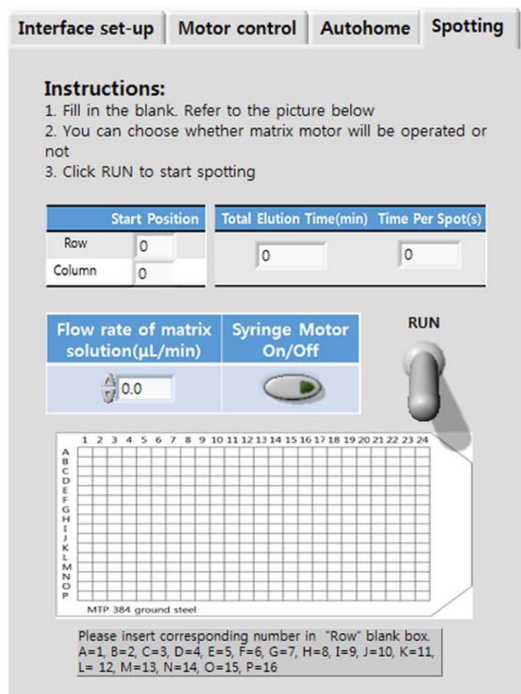


Fig. 5. Graphic user interface of the operation software. This software was coded using Labview™. This screen shot shows the front panel of the spotting sub-program.

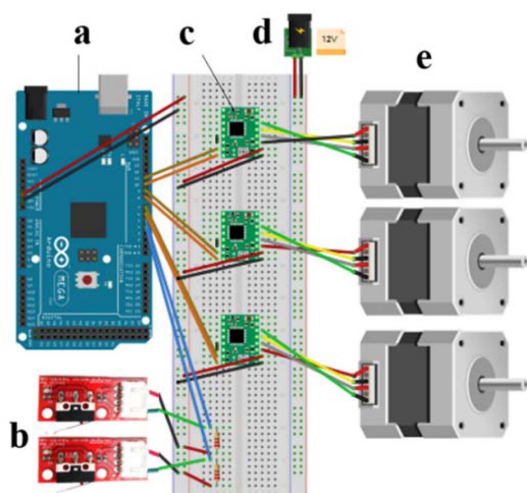


Fig. 6. Wiring circuit diagram to control the stepper motors, including: (a) an Arduino board, (b) end-stop mechanical limit switches, (c) stepper motor drivers A4988, (d) a 12 V power supply, and (e) stepper motors. This circuit diagram was drawn using the open source, free software Fritzing (Friends-of-Fritzing foundation).

This program enabled the movements of the MALDI plate and the syringe plunger by operating three stepper motors. Each motor was independently controlled using its own controller panel. The motor rotation was coordinated through the stepper motor driver A4988 and Fig. 6 shows the wiring schematic among the Arduino board, driver A4988, and the motors.¹² The motor speed and rotational direction were controlled by adjusting two parameters. The motor speed was controlled using the pulse width modulation (PWM) method. The motor rotational direction was controlled using the LED boolean constant: LED ON, clockwise; LED OFF, counter-clockwise. The default motor speed for the MALDI plate movement was set to 450 Hz and was readily adjustable. The syringe motor was set to 50 Hz as the default value, which enabled a fine syringe infusion.

(3) “Autohome”

The function of the “autohome” sub-program was to set the MALDI plate to the initial position. This sub-program is essential for reliably spotting the mixture of the analyte LC eluent and matrix onto the MALDI plate as specified by the analyst. In the

spotter, the initial position was set to the center of the waste tube holder.

(4) “Spotting”

The spotting of the analyte LC eluent (plus matrix) was mainly coordinated in the “spotting” sub-program. In this sub-program, there are three input parameters for spotting: the start position, the total elution time, and the time per spot. When the program begins, the MALDI plate moves to position (1, A) where the capillary tip is positioned directly above this position (see Fig. 5). After a moment, the MALDI plate moved to the start position of the spotting which was designated by the analyst. It was designed to allow each experiment to avoid the un-preferred spots on the MALDI plate. From the start position, the MALDI plate moved with the one-spot step size. The residence time (designated as the time per spot (seconds) on the graphic user interface) at one spot could be controlled using the adjustable parameter. This residence time could be determined in correlation with the LC flow rate.

In this study, the MALDI plate was translated along the numbered row, i.e., 1-24; when the plate reached the end of the row, it was shifted down to the next row ($n^{\text{th}} \rightarrow (n+1)^{\text{th}}$). In the next row, the translational direction was reversed. For example, the translational direction is from left to right in the n^{th} row and from right to left in the $(n+1)^{\text{th}}$ row.

In this sub-program, the flow rate of the matrix solution from the syringe could also be controlled. By changing the rotational speed of the stepper motor, the flow rate could be adjusted. The optimal rotational speed of the stepper motor varied based on the diameter of the syringe.

5. Conclusions

In the present study, a nano-LC MALDI spotter robot was fabricated to automate the spotting of a mixture of nano-LC eluent and MALDI matrix onto the MALDI plate. In fabricating the spotter robot, the skeletons and components were either made using the 3D printer or purchased as 3D printer parts from the online shop. The CAD files for the 3D

printed parts are available in the Supplementary information and the operation software can be downloaded from <https://github.com/tnlatnlagho/nano-LC-MALDI-spotter>. This paper is aimed to enable readers to make the nano-LC MALDI spotter robot on their own, i.e., DIY production.

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