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Fully Automated Spotting Device for Thin-Layer Chromatography based on the Modification of a 3D Printer

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Abstract

Column chromatography and the subsequent analysis of the collected fractions using thin-layer chromatography (TLC) are immensely popular and frequently used methods in organic chemistry. To analyze the fractions, they have to be spotted onto the TLC plates, which can be a time-consuming task. Therefore, we have developed an automatic spotting device based on a conversion of a 3D printer kit, which could be easily built for around 500 €. All parts come directly from the 3D printer kit, are 3D printed, or are commercially available, such as syringes and tubing. In this manuscript, we describe the design and use of the spotting device. In addition, we provide a detailed step-by-step assembly instruction and all CAD files for easy replication.

Metadata Overview

Main design files: https://doi.org/10.5281/zenodo.12517787
Target group: Scientists and technicians working in chemistry, biochemistry, and associated disciplines.
Skills required: 3D printing – easy; cutting with an angle grinder – easy; mechanical assembly – easy; G-code – easy; Excel usage – easy.
Replication: So far, no replicas have been known to the authors.
See section “Build Details” for more detail.

Keywords

TLC Spotting; liquid handling; 3D printing; automation; G-code

(1) Overview

Introduction

Since thin-layer chromatography (TLC) is a quick, cheap, and sensitive chromatography technique with various post-chromatographic treatment options, it is routinely used in organic chemistry for reaction control and analysis of the fractions acquired after column chromatography.[1, 2] However, to examine the collected fractions a small sample of each fraction must be spotted onto a TLC plate. This task can be very time-consuming, especially as more and more fractions need to be analyzed.

Commercially available spotting devices are quite expensive and usually very inflexible in terms of the size of the rack and the TLC plate.[3] Particularly the usage of different rack sizes can be beneficial, as preparative column chromatography is performed with different amounts of substance and the fractions are collected in vials of different sizes. Therefore, different conditions require different rack sizes and different settings.[2, 3]

There are already several semi-automated and even fully automated devices developed for similar purposes.[4-12] However, most of these devices are designed more for liquid handling operations and do not meet our requirements for spotting TLC plates using flexible rack and TLC plate sizes.

In recent years, there have also been several projects in which low-cost 3D printers have been converted into scientific devices such as syringe pumps or HPLC fraction collectors.[13-16] This has the advantage that no programming or electronics knowledge is required, as the CNC motion control software of the 3D printer is used to control the devices.

We decided to develop a cost-effective, open hardware solution, based on an inexpensive 3D printer (Tronxy X5SA), that can be used with different rack types for TLC spotting. The system can be built for around 500 €. The Tronxy X5SA was chosen because its large print area of 330×330 mm provided the required flexibility for our application. It is also based on a core XY structure, which means that only the extruder moves in the X and Y directions, not the build plate which could have caused spilling. Using primarily the components of the Tronxy X5SA kit or 3D-printed parts, the assembly is fast and straightforward. The necessary G-code files can be easily generated with the attached Excel file, and it enables users to obtain the G-code for different rack sizes and user preferences.

The TLC robot was built to automate the spotting process but could also be used in other liquid handling tasks in the laboratory if the required sequence is programmed in G-code.

Overall Implementation and Design

Setup

The basic idea was to keep the assembly of the TLC robot as simple as possible so that anyone with basic manual skills could rebuild it. To achieve that, the design was kept as close to the 3D printer as possible, as can be seen in **Figure 1**. Originally it was considered to edit the firmware of the 3D printer to change the homing position, for example, but we decided to keep the approach as simple as possible and avoid programming tasks.

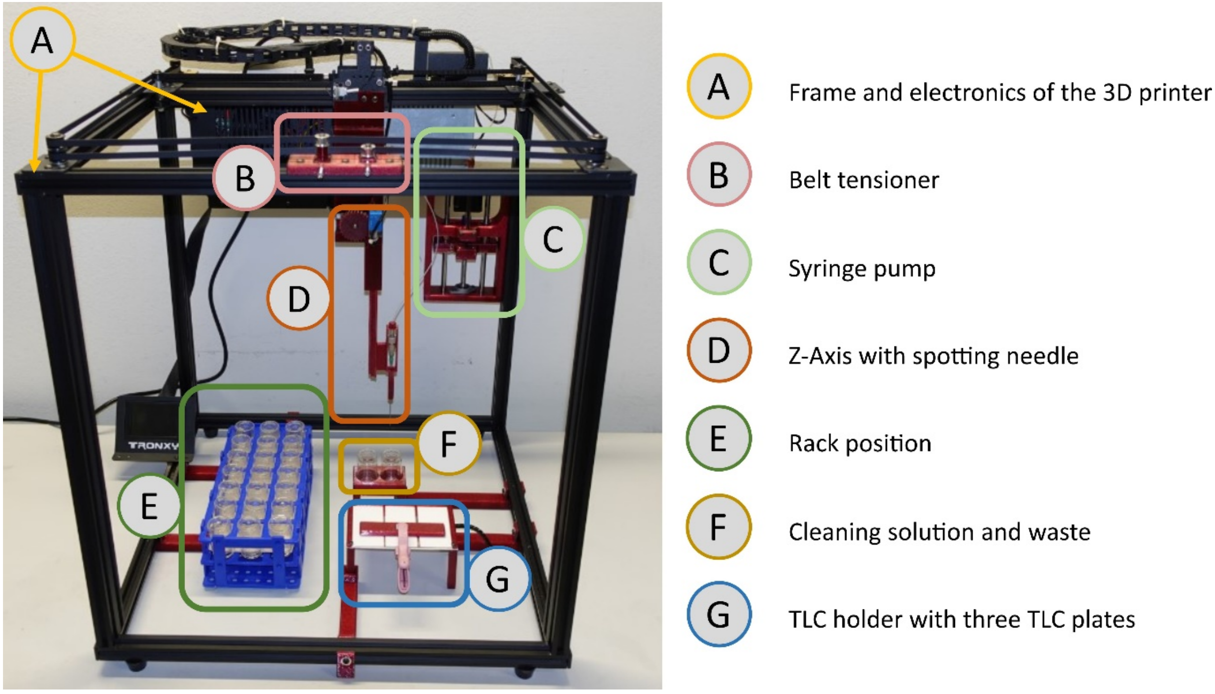

***Figure 1**: Overview of the Spotting Device.*

Movement in the X and Y directions is ensured by the 3D printer's almost unchanged CoreXY system. Only a belt tensioner (**B**) has been added so that the tension can be adjusted after assembly. The movement of the capillary in Z-direction on the other hand is facilitated by a gear wheel and gear rack to which the cannula is attached (**D**). The syringe pump at the rear of the TLC robot (**C**) enables defined quantities of liquid to be dispensed.

To be able to clean the cannula, two glasses (diameter: 3 cm, height: 5 cm) are placed inside the table at the back (**F**), one filled with cleaning solution and one empty for the waste. The required number of TLC plates (4×8 cm) can be positioned on the TLC holder (**G**) and fixed with a clamp to maintain their position throughout the spotting process. The rack with the collected fractions (**E**) can be inserted on the left side. Its position is determined by aligning it with three positioners.

The various G-code programs can be created with the Excel file and stored on the SD card of the 3D printer. If the SD card is inserted into the Host box, the different programs can be selected on the display on the left side of the base frame.

As the system is modular, the different parts can easily be replaced with alternatives, allowing further customization. For example, the two tables can easily be replaced by slightly different variants if glasses or TLC plates with different dimensions are used.

Motion in Z-Direction

In order to avoid prolonging the spotting process unreasonably, the capillary should be able to move in Z-direction at approximately the same velocity as in X- and Y-direction. Initially, a design with one lead screw and two guide rails (similar to the design of the syringe pump) was considered. However, the maximum velocity achievable with this design proved to be too low during the test. Therefore, this approach was replaced by the current design consisting of a gear wheel connected to the shaft of one NEMA motor and a gear rack inside a guiding rail to achieve the required speed (**Figure 2**). The size of the gear wheel was adjusted so that the capillary could move safely above all objects during the homing process.

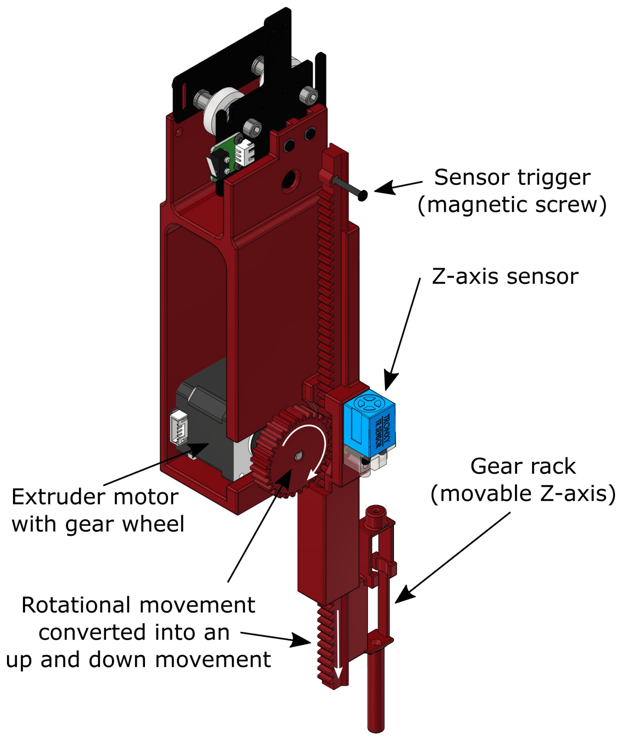

***Figure 2**: CAD drawing of the Z-axis mounting [17-20].*

Capillary Holder with Spring Mechanism

A standard medical cannula (Braun Sterican, 21G) was inserted into the rack, which represents the actual movable Z-axis (**Figure 3**). As the tip of the cannula must not be sharp, it was cut off and smoothed. A reasonably long capillary was used to ensure a long travel distance that is required to collect samples from vials with different fluid levels. To avoid the adhesion of a large fluid droplet to the outside of the cannula, a cannula with a small diameter (21G, 0.72mm) was used. As the cannula is in contact with the TLC plates during spotting, a spring mechanism was included to avoid scratching the surface of the TLC plates. This mechanism ensures that the cannula can move slightly upwards when it hits the TLC plate to provide even and gentle contact pressure and to compensate for any unevenness. The fixation was introduced to avoid rotation of the cannula. It is important to ensure that the connection with the fixation does not block the up- and downward movement of the cannula.

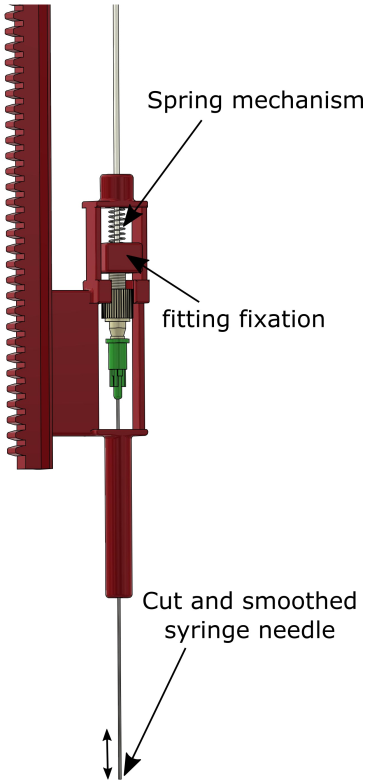

Figure 3: CAD drawing of the capillary holder with spring mechanism.

Syringe Pump

The syringe pump was designed in a way that it is possible to collect and transport defined volumes from 1 µl to 1 mL. It is based on two guide rails and a lead screw (**Figure 4**), which both originally came from the 3D printer kit, and were shortened to the appropriate length (see ESI on Zenodo/GitHub). As syringe, a 1 mL Gastight Syringe (Model 1001 TLL from Hamilton) was chosen. The pump must be placed upright so that any air inclusions are expelled during the flushing process.

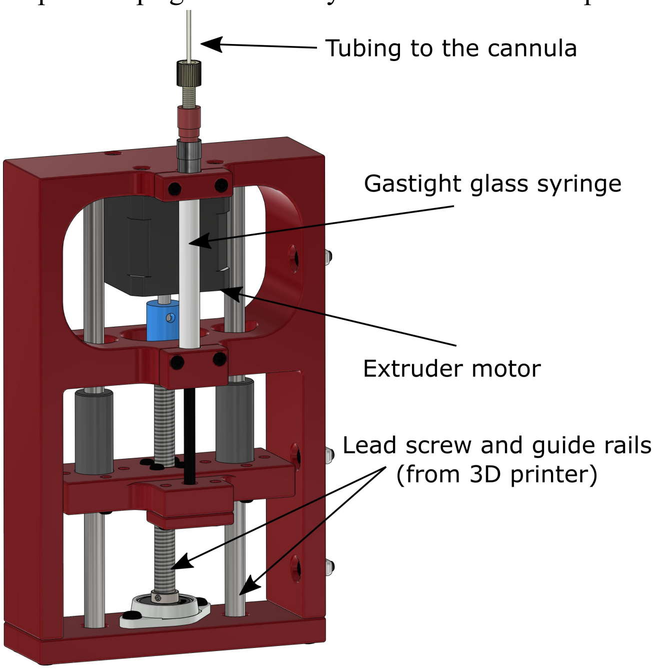

Figure 4: CAD drawing of the syringe pump.

TLC Holder

To keep the TLC plates in place, a table based on an aluminium plate and a 3D-printed frame was used (**Figure 5**). As the size of our TLC plates (Machery Nagel POLYGRAM® SIL G/UV254, 40×80 mm) varied by 3 mm, a frame with small separators ensures that the plates remain in the same position during the spotting process. If larger TLC plates need to be used, the separators must either be cut off or removed in the CAD file before printing.

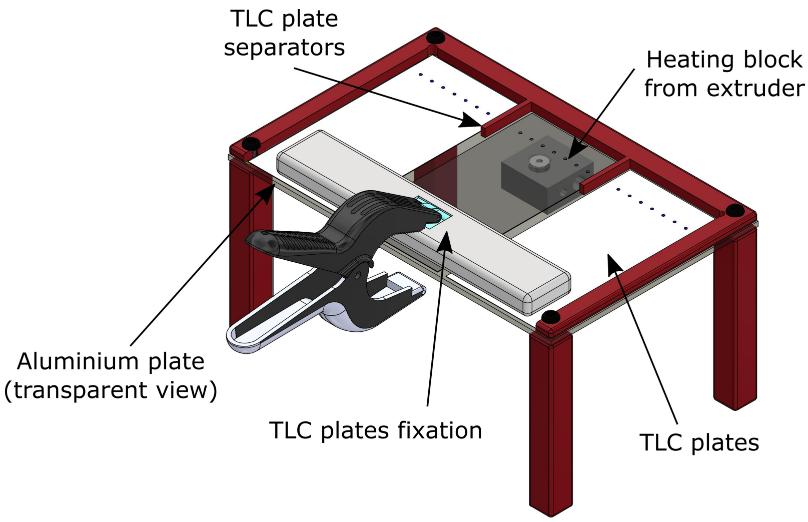

***Figure 5**: CAD drawing of the TLC holder [21].*

To speed up the spotting process, the heating block from the 3D printer was connected to the bottom of the aluminum plate. The TLC plates are thus heated during the spotting process, which leads to faster evaporation of the solvent. This is particularly useful when high-boiling solvents such as water, toluene, or dimethyl sulfoxide (DMSO) are used. A spring clamp in combination with a 3D-printed extended end was used to hold the TLC plates in place.

Belt Tensioner

As the tension of the tension belts decreased over time and the movement of the TLC robot became less and less precise, a belt tensioner was introduced. The belt tensioner was taken from an existing design without any modifications.[22]

(2) Quality Control

Safety

Entanglement or entrapment in moving parts: Since the cannula moves abruptly and fast in all three directions during the process, tasks inside the base frame should be avoided while the TLC robot is running. If a problem occurs during a run of the TLC robot, the run should always be stopped first before any adjustments inside the base frame are made. The host box at the back left corner allows the operator to stop each program instantly by turning off the power while staying away from moving parts.

Risks of burning fingers while inserting or removing the TLC plates: Since the aluminum plate of the TLC holder is heated up during the spotting process, the operator can slightly burn their fingers while changing the TLC plates. To minimize the risk of burning, the maximum temperature selectable for the heating block was limited in the Excel file to 70 °C and the default value was set to 50 °C. Furthermore, the heating is stopped after the last spot is made. Therefore, the heated elements can already cool down while the spotting program is finished.

Electric shock: As with any other electrical device, there is a risk of electric shock. However, only the power supply of the host box is operated with main voltage (230 V). The other cables, which only have a voltage of 48 V, are covered and permanently installed. Nevertheless, all cables should be checked for damage before operating the robot and replaced if necessary.

Chemical exposure: When working with volatile solvents or other hazardous substances, appropriate safety precautions must be taken, e.g. placing the device in a fume hood.

Calibration

Before using the TLC robot for the first time, a calibration is required. These procedures are described in detail in the ESI on Zenodo/GitHub and summarized below. Once the calibration is complete, recalibration is only required if the syringe in the syringe pump is replaced, the positioners that ensure that the objects are always in the same position are moved in X or Y direction, or if a new rack is inserted.

Calibration of the Syringe

To calculate the correct number of E units required to draw and dab a given volume, three parameters must be determined: the nominal volume of the syringe in the syringe pump, the residual volume in the syringe in its zero position and the E value required to fill the syringe to its nominal value.

Determination of all Coordinates

To generate the correct G-code, the coordinates of all components must be determined and entered in the Excel file. In case of the TLC holder, the waste bin, and the cleaning solution the X-, Y- and Z-values of one specific position have to be determined, as well as the corresponding height for movement between these parts. For each rack size on the other hand, it is necessary to determine the x- and y-coordinates of two vials in diagonally opposite corners, which enables the positions of the other vials to be calculated. Furthermore, the corresponding Z-values for the fluid collection and movement above the rack have to be determined.

Generation of the G-Code Programs

The G-code can be generated with the provided Excel file. At least one G-code program per rack is required to use each rack with the TLC robot. However, is possible to create different programs for each rack to fulfill different user preferences.

To do so, first, all parameters obtained during the calibration process must be entered. Subsequently, the user can implement their preferences to the program. The G-code file must be exported and saved on an SD card that can be inserted into the host box. The new program can be selected on the display of the TLC robot.

General Testing

The spotting robot has been in use for about a year, on average at least 10–20 times a week. During this time, there have been a few minor issues that have been resolved easily.

- The belt tension decreased, resulting in imprecise movement in X and Y directions. As a result, a belt tensioner was introduced, as mentioned above.
- The cannula was able to rotate freely during the spotting process, which led to imprecise spotting points with a slightly bent cannula. A fixation was therefore introduced, as can be seen in **Figure 3**. This prevents rotation and precise spotting points are achieved even with a slightly bent cannula.

The TLC spotting robot has been extensively tested with two different rack types, one 3×7 rack (size 26.5×12.5 cm) and one 4×7 rack (size 23×16 cm) with snap lid jars or tubes with a diameter of 3 cm. These are our standard collection vials for column chromatography. During the tests, the racks have been filled with vials with a height of either 5 cm (small), 10 cm (middle), or 20 cm (large). Smaller test tubes with a diameter of 15 mm were also successfully tested.
With the current settings, it is possible to use the TLC robot for racks with a size of up to 32×19 cm. It may also be possible to use a larger rack. This would require changing the homing positions in the firmware and rearranging all other components.
The maximum possible height of a rack with the vials inside is 21 cm. If higher racks are required, the length of the Z-axis mounting has to be shortened in the CAD software. The height of the two tables (TLC and washing table) has to be changed accordingly.
With the current settings, the capillary can only be lowered down to approximately 5.5 cm above the bottom. Therefore, if the height of the rack is smaller than 7 cm, it should be elevated for example, by a lifting platform until a height of approximately 10 cm.

In order to check whether the spotting process leads to carry-over into subsequent spots, an experiment with two different concentrations of a Nile red solution was carried out. After each vial with Nile red solution, several vials filled with pure solvent (acetone) were spotted and visually examined to see whether carryover occurred (**Figure 6**).

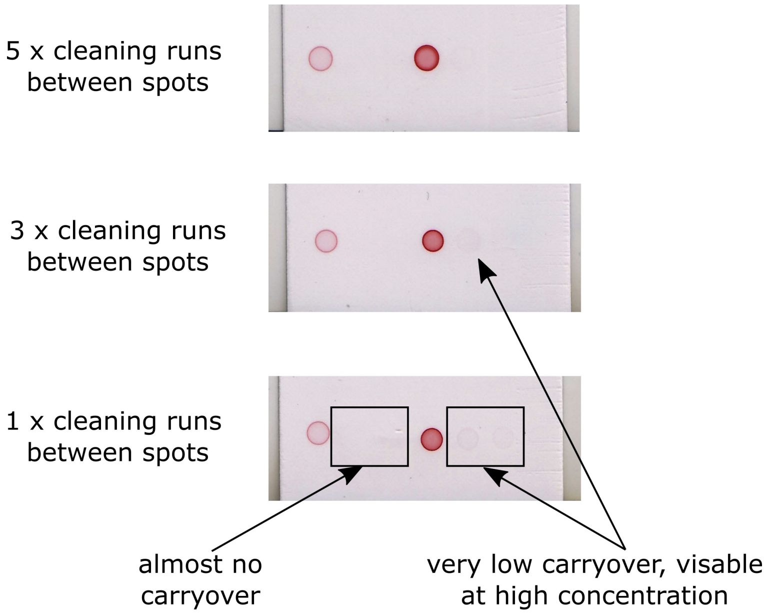

Figure 6: Overview of the complete process.

During the experiment, the cannula was automatically cleaned before entering a new vial. The number of times the cleaning process was run each time was gradually increased from 1 to 5. With 5 cleaning runs between spotting, almost no carryover was seen even at high concentrations. With a single cleaning run, very low carryover, which was only visible at very high concentrations (saturated solution), was observed. For the application as a spotting device for TLC, a single run cycle between the spots is sufficient. This can significantly reduce the duration of the whole spotting process. A whole spotting process of our standard racks (3×7 jars) during column chromatography takes approximately 7 minutes.

(3) Application

Use Case

We only used the TLC spotting device for the application of spotting TLCs. Figure 7 gives an overview of standard column chromatography followed by automatic spotting on the TLC plates and the development of those plates. A video showing the TLC robot in action can be found here.

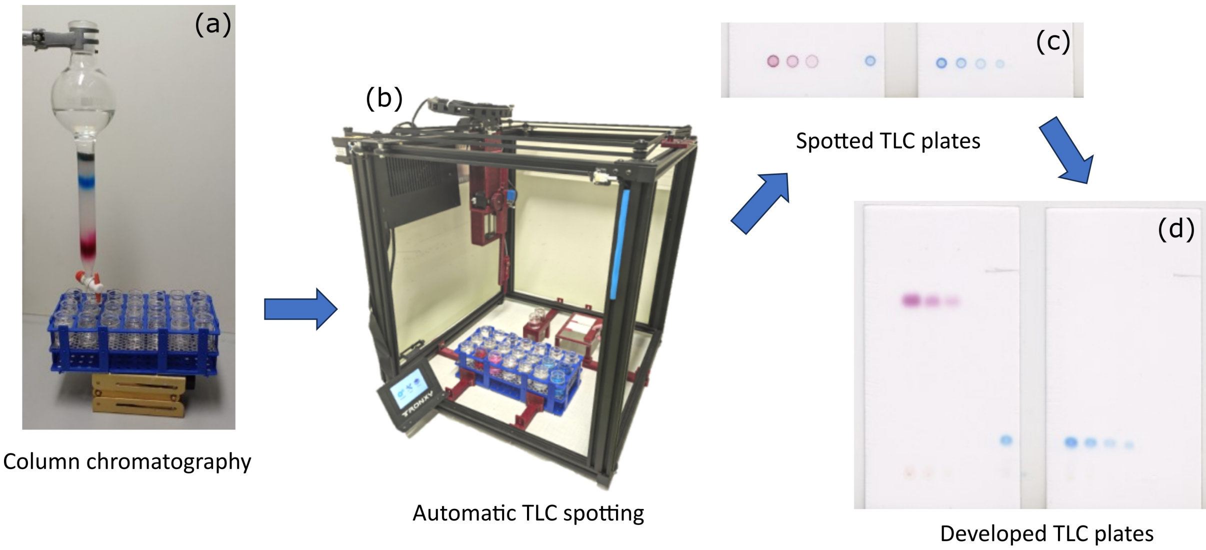

Figure 7: Overview of the complete process.

In this example, a manual preparative column chromatography was first carried out with a dye mixture of Sudan Red 7B and Oil Blue 35. The fractions were collected in a 3×7 rack **(Figure 7a)**. For this rack, a G-code program with 3 applications per spot was created in Excel and executed with the TLC robot **(Figure 7b)**. The TLC plates spotted in this way **(Figure 7c)** were developed using toluene as the mobile phase **(Figure 7d)**.

Reuse Potential and Adaptability

Since we designed the TLC robot to be as flexible as possible, it is possible to create a G-code for almost any type of rack and TLC plates for spotting. The TLC robot could also be used for other liquid handling tasks, e.g., transferring reagents from different containers to one or more reaction vessels. This, for example, could be useful for reaction screening in parallel synthesis.[23, 24] Therefore, some changes are required: Some new positioners would need to be printed calibrated. The Excel file would have to be adapted to generate a matching G-code.

The device could also be used for biological tests where 96-well plates are used, such as ELISA assays.[25] We tested the precision of the TLC robot with a 96-well plate as a rack and found no problems with precision during the run.
Another application of the TLC robot could be the usage for quantitative TLC, as a precise and adjustable amount of solution is always spotted onto the TLC.[26-28] Dilution series of different substances would have to be examined and calibration curves have to be created to see whether the accuracy of the extruding syringe is sufficient for this application.

(4) Build Details

Availability of Materials and Methods

Most of the components were either part of the Tronxy X5SA kit or were produced using 3D Printing, the STL- and STEP-files can be found on Zenodo/GitHub. The other materials are readily available from online suppliers. A complete material list, including URLs of suppliers, is included in the ESI file on Zenodo/GitHub. The TLC robot can be assembled using normal tools. An angle grinder is recommended to cut the slide rods to the correct length and a bench drill with a vice to drill accurate holes in the aluminum plate. A 3D printer with a print area of at least 300×250 mm is also required to print the rather large components. As printing material PETG was used due its prior temperature, chemical and impact resistance. If PLA is used as printing material it is necessary to ensure that the maximum temperature of the heating block is limited to 50 °C. A detailed step-by-step instruction with sample photos and CAD drawings of the components can be found in the ESI file on Zenodo/GitHub.

Ease of Build / Design Decision

The general idea was to keep building the TLC robot as easy as possible. Therefore, it was opted to not shorten the vertical aluminum profiles but to print a longer casing of the Z-axis mounting instead. Changes in the electronic set-up were kept to a minimum and only require plugging and unplugging of cables and pins. Changes in the firmware were avoided. The TLC robot can be easily built and calibrated within around 2 days.

Operating Software and Peripherals

The original CNC motion control software of the 3D printer is used for controlling the processes of the TLC robot. The necessary G-code can be generated with the provided Excel file. However, Excel 2010 or a newer version is required for using our file. For the calibration process, any slicer software with a machine control panel, e.g., Cura, Simplify3D, can be used.

Hardware Documentation / Build Instructions / Files Location:

Name: Zenodo/GitHub: TLC spotting device
Persistent identifier: https://doi.org/10.5281/zenodo.12517787
Publisher: Jochen M. Neumaier
Date published: 2024-06-24

(5) Discussion

Conclusions

In this paper, we have described a programmable fully automated spotting device that is capable of performing a spotting process for thin-layer chromatography. Primarily, this robot was developed to automate and simplify the TLC spotting process after column chromatography. Due to the flexible design, almost all types of racks and TLC plates can be used. This device was built using a 3D Printer Kit and mainly 3D-printed parts for a total cost of around 500 €.

Due to the many setting options, e.g., number and velocity of spotting operations, pause between spotting, or heating of the TLC plates, it is possible to apply relatively large volumes on the TLC plates and still obtain spots with relatively small diameters and good accuracy.

Furthermore, under the same settings, the TLC robot should be able to reproduce more reproducible results than a human operator.

Future Work

The current version of the TLC robot is fully functional and fulfills its tasks reliably. Nevertheless, some further improvements could be considered in the future:

For ease of use, an Excel file was used to generate the G-code. However, it would be conceivable to adapt the program to an open-source version such as OpenOffice or LibreOffice, so that no Excel license would be required, and the replication of the TLC robot would be easier. If necessary, the settings in the firmware could be changed so, that, for example, the homing position could be altered. This allows the various components to be placed closer together or larger racks to be inserted into the frame.

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Competing Interests

The authors declare that they have no competing interests.

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