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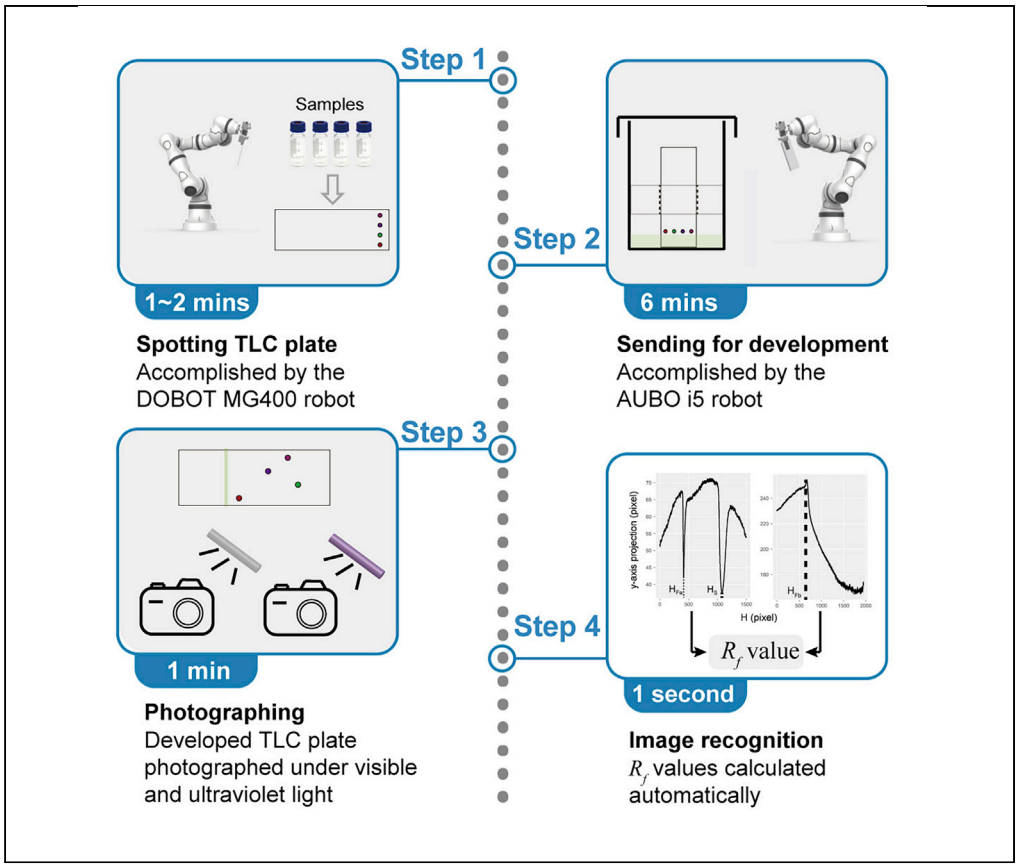
High-throughput automated platform for thin layer chromatography analysis



In this protocol, we detail steps for constructing a high-throughput automated platform for thin layer chromatography (TLC) analysis. We describe robotics and computer vision techniques that can handle 32 compounds under three different elution solvents in about 50 min. The established automated platform can obtain statistically standardized retardation factor (R_f) values and enhance reproducibility while reducing labor and time costs.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

A high-throughput automated platform for TLC analysis based on robotics

TLC plates are spotted, transferred, developed, and photographed automatically

Retardation factor (R_f) values are recognized automatically by computer vision

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High-throughput automated platform for thin layer chromatography analysis

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SUMMARY

In this protocol, we detail steps for constructing a high-throughput automated platform for thin layer chromatography (TLC) analysis. We describe robotics and computer vision techniques that can handle 32 compounds under three different elution solvents in about 50 min. The established automated platform can obtain statistically standardized retardation factor (R_f) values and enhance reproducibility while reducing labor and time costs.

For complete details on the use and execution of this protocol, please refer to Xu et al. (2022).[1]

BEFORE YOU BEGIN

Overview

Thin layer chromatography (TLC) analysis is performed extensively in the field of organic synthesis for polarity measurement and chromatographic separation. However, this process is labor-intensive and usually influenced by multiple objective factors like chamber size, loading capacity, and operation mode, resulting in irreproducibility. Moreover, determination of suitable TLC conditions is empirical, thus a large number of attempts are inevitable, which can be time consuming.

To address these limitations, we recently developed a high-throughput platform for TLC analysis, which automates all relevant experimental steps, including sample preparation, spotting samples on the plates, developing the TLC plate in elution solvents, and calculating the R_f value for each analyte. The proposed automated platform consists of two important parts: the hardware to conduct high-throughput TLC experiments and the software that analyzes the original outcomes automatically to obtain the retardation factor (R_f) value for each analyte.

The idea to use robots to assist with TLC has been around since at least 1991[2,3] and evolved into the advanced high-performance thin layer chromatography (HPTLC). As a highly integrated machine, HPTLC has higher comprehensive efficiency. However, the cost of specialization of HTPLC is the reduction of freedom. In an academic lab, scientists often need to conduct scientific exploration, which means their needs are often diversified. In this regard, the proposed automation platform has been fully expandable. After mastering the protocol, scientists can improve the automation platform according to their own requirements. At the same time, the collaborative robot can complete more actions, which means that it has great potential in automating other chemical experiment operations.

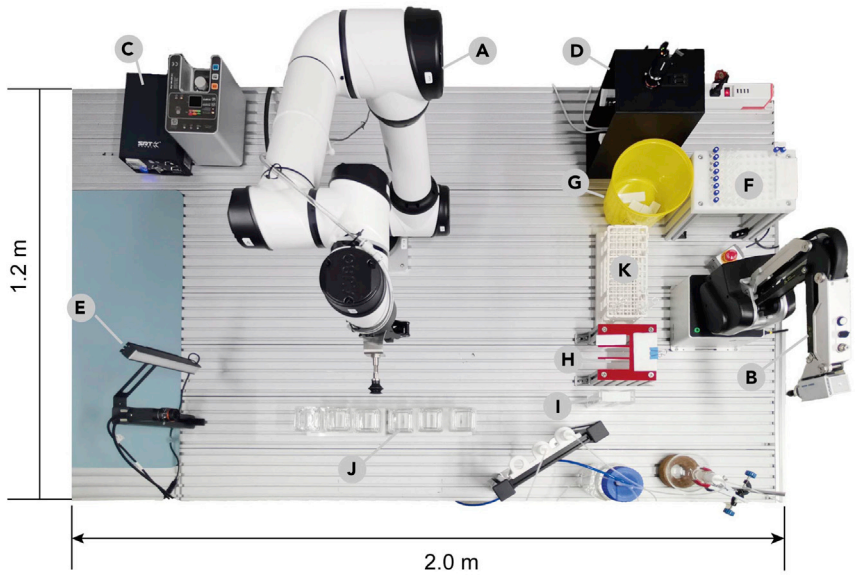

Figure 1. The layout of the designed automated platform for TLC analysis

(A) AUBO i5 robot; (B) DOBOT MG400 robot; (C) Air pumps; (D) UV light photographic device; (E) Visible light photographic device; (F) Sample tray; (G) Waste container; (H) TLC plate stand; (I) TLC plates storage case; (J) TLC development chambers; (K) Test-tube rack (optional). The platform is 2 m×1.2 m. This adapted graphic is produced with permission.[1]

In this protocol, we use this automatic platform for measuring the R_f values of 32 organic compounds under 3 different elution solvents (or a solvent system with 3 different proportions) in a working cycle of about 50 min as a representative example to illustrate step-by-step protocols for the experimental procedure and image recognition in high-throughput TLC analysis. The strategy can be readily scaled up to measure more compounds and samples consisting of multiple compounds under diversified elution solvents without extra effort.

Set up for hardware

Timing: ~2–3 days

The automated platform requires several pieces of equipment to accomplish the procedure for TLC analysis. Therefore, the hardware should be prepared before running a sample beforehand, including the AUBO i5 robot (Figure 1A), the DOBOT MG400 robot (Figure 1B), air pumps (Figure 1C), an ultraviolet (UV) light photographic device (Figure 1D), a visible light photographic device (Figure 1E), a sample tray (Figure 1F), a waste container (Figure 1G), a TLC plate stand (Figure 1H), a TLC plates storage case (Figure 1I) and at least three TLC development chambers (Figure 1J). Most of the equipment are commercially available while others are specially designed and custom-built. The resources for all relevant hardware can be found in the key resources table, and the design diagrams of the specially designed equipment are provided in open resource data section.

Preparation for software

Timing: 2 h

In this work, the control of all equipment is realized by a controlling program written by Python on a personal computer. The communication between machines and the transmission of instructions are realized through a Wi-Fi connection access to internet by a router. Therefore, the software should be prepared before experimentation, including installing Python and relevant packages, and software

development kits (SDKs) for robots and cameras that are used to connect these devices to the computer.

1. Install Python and relevant packages.
   a. Install Anaconda: follow the instructions at https://docs.anaconda.com/anaconda/install/ to install Anaconda and Python 3.7. Some packages have been pre-installed in the Anaconda.
   b. Install Opencv and skimage: open the Anaconda prompt and use the following commands in order to install OpenCV and skimage.

```
>pip install opencv-python

>conda install scikit-image
```

   c. Install Pycharm: Pycharm is a Python integrated development environment (IDE), which can be installed from https://www.jetbrains.com/pycharm/.
2. Install software development kit (SDK) for robots and cameras.
   a. Install SDK for DOBOT MG400.
      i. Download the SDK file DOBOTSDK.py from the open resource provided by this work.
      ii. Use the following command to read this SDK in Python.

```
>from DOBOTSDK import dobotSDK
```

   b. Install SDK for AUBO i5.
      i. Download the SDK file from the url https://www.aubo-robotics.cn/download, and install the SDK following the instructions provided in the SDK file.
      ii. Use the following command to read this SDK in Python.

```
>from robotcontrol import *
```

⚠ CRITICAL: In some circumstances, using the command will report an error, which is related to the Python environment. Put the dynamic link library (DLL) folder into the external library in the pycharm may help to solve this problem.

   c. Install SDK for HIKROBOT cameras.
      i. Download the mutual visual system (MVS) client and corresponding SDK file from https://www.hikrobotics.com/cn/machinevision/service/download?module=0. Install the SDK following the instructions provided in the SDK file.
      ii. Use the following command to read this SDK in Python.

```
>from camera import *
```

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
Python	Python Software Foundation	https://www.python.org
SDK for DOBOT	DOBOT Company	https://cn.dobot.cc/
SDK for AUBO i5	AUBO Company	https://www.aubo-robotics.cn/
SDK for HIKROBOT camera	HIKROBOT Company	https://www.hikrobotics.com/cn
Pycharm	JetBrains	https://www.jetbrains.com/pycharm/

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Other		
AUBO i5 robot	AUBO Company	https://www.aubo-robotics.cn/
DOBOT MG400 robot	DOBOT Company	https://cn.dobot.cc/
Two HIKROBOT industrial cameras	HIKROBOT Company	MV-CE050-31GC
TLC plates	Yinlong	75 × 25 mm, GF254, 0.23 mm, 5–20 μm
UV lamp	Guang Xi	254 nm
Capillary	Bell Tower	0.5 mm inner diameter
Gripper	INSPIRE-ROBOTS	EG2-4C

STEP-BY-STEP METHOD DETAILS

Arrange and fix hardware

Timing: ~3–4 h

The layout of equipment is crucial for the efficiency of the automated platform as multiple steps are required during the TLC analysis. Considering that two separate robots will work collaboratively to accomplish different operations, the layout must be carefully arranged to avoid conflict. Considering that the maximum working radius of AUBO i5 and DOBOT MG400 is 88.7 cm and 44.0 cm, the two robots are suggested to keep more than 1 m apart to avoid collisions. The overall layout of equipment in this protocol is illustrated in Figure 1. In this section, we will describe the location and installation of each piece of equipment in detail to facilitate construction of the automated platform. It is worth noting that the layout is not restrictive to just this set up and can be tailored as needed. The arrangement of hardware can be done simultaneously with software manipulation, while subsequent steps require the software manipulation.

1. Fix two collaborative robots.
   a. Place the AUBO i5 robot (Figure 1B) in the middle of the workbench that is made of aluminium alloy.

      Note: The AUBO i5 is a human arm-like robot with six axes that can handle complicated tasks with six degrees of freedom. It has a large workload and is therefore arranged in the center of the workspace. We choose AUBO i5 robot here mainly because it can be controlled by Python and has six degrees of freedom, any other robots that satisfy these points can be used as a replacement.

   b. Arrange the DOBOT MG400 robot (Figure 1G) on the right of the workbench.

      Note: The DOBOT MG400 is a four-axis robot with the z-axis perpendicular to the operating plane. It has the advantage of high efficiency and easy operation. We choose DOBOT MG400 robot here mainly because it is a four-axis robot with high efficiency and can be controlled by Python. Any other robots that satisfy these points can be used as a replacement.

   c. Fix the gripper onto the AUBO i5 robot through a customized flange (Figure 2A), the design scheme of which is provided in the open source, flange_for_AUBO.dwg. Connect the gripper to the computer by the data wire.
   d. Fix the suction cup onto the AUBO i5 robot through the same flange (Figure 2A). The suction cup is driven by the air pump which is controlled by the IO interface of AUBO i5 robot. Connect the air pump to an air compressor and the pressure value is set to be 98 kpa.
   e. Fix the capillary tube onto the DOBOT MG400 robot (Figure 2B).
      i. Fix the adjustable retainer onto the DOBOT MG400 robot.

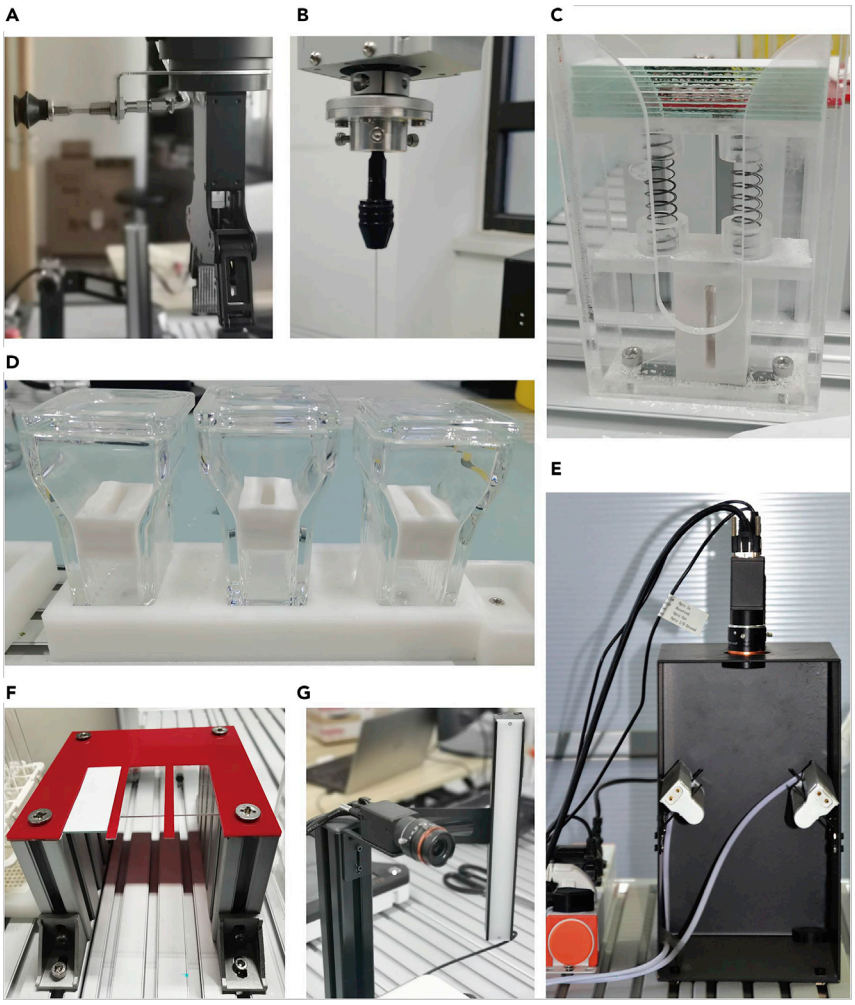

Figure 2. Photos for some pieces of equipment

(A) The flange to attach the gripper for gripping TLC plates and the suction cup for opening and closing the lids; (B) The adjustable retainer and the capillary; (C) The TLC plate storage; (D) The TLC development chambers with lids that are opened and closed by robots and the customized fixture to limit the tilt of the TLC plate; (E) The UV light photographic device; (F) The TLC plate stand; (G) The visible light photographic device. This adapted graphic is produced with permission.[1]

ii. Adjust the passable radius of the retainer to 2∼3 mm.
iii. Put the capillary tube into the retainer and rotate the retainer until the capillary is held firmly.

Note: Given that the capillary is only 0.5 mm thick and is very brittle, we use an adjustable retainer to fix the capillary, which can be adjusted to the size of the diameter by rotating on the thread. The capillary will be cleaned between samples, so fixing a new one each time is not manually needed.

2. Fix the sample tray (Figure 1E).
   a. Fix four support legs to place the sample tray.

Note: The sample tray is a customized platform where circular depressions are distributed uniformly in 10×8 grids, the design scheme of which is provided in the open source, sample_tray.dwg.

b. Place a piece of absorbent paper at the end of the sample tray to dry the capillary tube.

Note: The drying process can improve cleaning efficiency and avoid cross-contamination.

c. Put the vials (11.6 mm diameter) with samples into the circular depression sequentially.

Optional: A test tube rack (Figure 1F) can be placed in front of the robot for some special cases of a large amount of solution. For example, the test tube can be utilized to store overflow of sample that is more than the vial for backup.

3. Fix the TLC plate stand (Figure 1H).
   a. Fix the four support legs to place the plane for spotting TLC plates. The design scheme of the customized plane is provided in the open source, TLC_plate_stand.dwg.
   b. Put the TLC plates onto the notch on the plane (Figure 2F).
4. Fix the TLC plate storage (Figure 1I).
   a. Fix the plastic shell onto the workbench.
   b. Install the spring between the two diaphragms and put the diaphragms into the plastic shell.
   c. Place 18 TLC plates in the storage (Figure 2C).
5. Fix the UV light photographic device (Figures 1C and 2E).
   a. Fix the black shading shell on the workbench.

Note: The black shading shell provides a black background that facilitates the subsequent image processing.

   b. Place two UV lamps in the shell through the fixture. The UV lamps are controlled by the IO interface of the AUBO i5 robot.

Note: The two ultraviolet lamps on the left and right sides can provide balanced illumination, which facilitates subsequent image recognition.

   c. Fix an industrial camera on the top of the shading shell and adjust the focus. The camera is controlled by the computer through the router. The resolution ratio of the camera is 2592 × 1944.
6. Fix the visible light photographic device (Figures 1K and 2G).
   a. Fix the support column on the workbench and fix the visible light lamp onto the support column by a customized flange. The visible light lamp is controlled by the IO interface of the AUBO i5 robot.
   b. Fix an industrial camera onto the support column. The camera is controlled by the computer through the router. The resolution ratio of the camera is 2592 × 1944.
7. Fix the TLC chambers (Figure 1J).
   a. Fix the customized basement on the workbench.
   b. Put the TLC chambers in the basement sequentially.
   c. Put a customized fixture in each TLC chamber to limit the tilt angle of the TLC plate, which also facilitates the sending and retrieving operation of the TLC plate (Figure 2D). The fixture can be made from the design scheme provided in the open source.

Design the workflow of automated TLC analysis

Timing: ~4–5 h

After all relevant equipment is in place, the workflow of automated TLC analysis should be addressed. The core of the workflow is to plan the movement trace of two collaborative robots. In this section, we will explain how to plan the paths for both robots to accomplish operations required

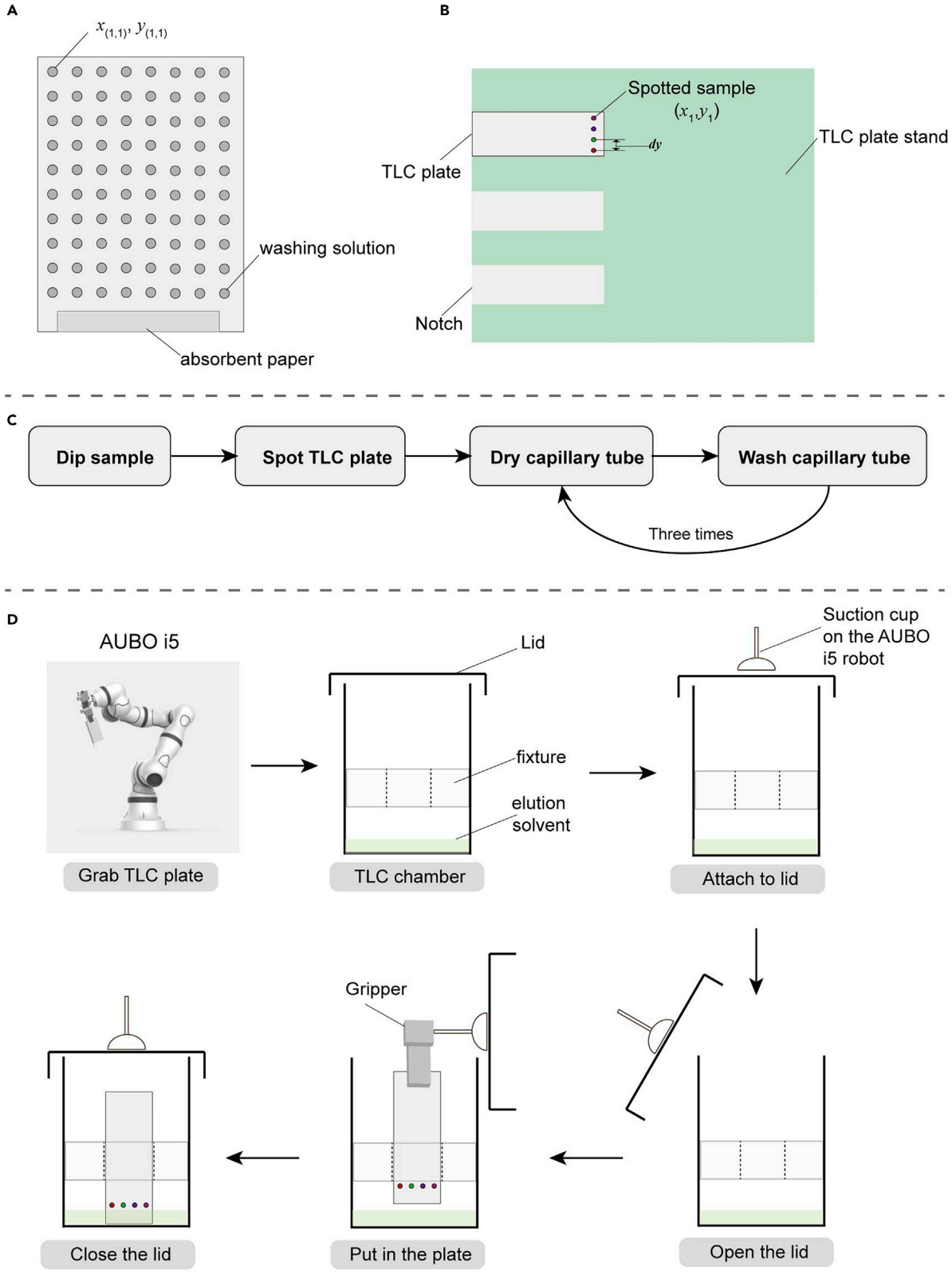
A
$x_{(1,1)}$, $y_{(1,1)}$
washing solution
absorbent paper
B
Spotted sample
(x_1,y_1)
dy
TLC plate
TLC plate stand
Notch
C
Dip sample
Spot TLC plate
Dry capillary tube
Wash capillary tube
Three times
D
AUBO i5
Grab TLC plate
Lid
fixture
elution solvent
TLC chamber
Suction cup on the AUBO i5 robot
Attach to lid
Open the lid
Gripper
Put in the plate
Close the lid

Figure 3. The sketch map of spotting samples on the TLC plates and sending TLC plates for development

(A) The sketch map of sample tray; (B) The sketch map of TLC plate stand; (C) The workflow of spotting samples on the TLC plates; (D) The sketch map of transferring TLC plates for development.

in different stages. For the AUBO i5 robot, we employ it to transfer the TLC plates between different devices, including placing, transferring, and retrieving the TLC plates. For the DOBOT MG400 robot, it is utilized to complete the task of dipping samples and spotting them on the TLC plates, which requires fast and accurate vertical movement.

8. Spot samples on the TLC plates. The workflow is illustrated in Figure 3C.
   a. Hand-eye calibration for dipping samples (Figure 3A).
      i. Move the robot arm of DOBOT MG400 physically or with the software to the directly above the bottle with samples in the first row and the first column of the sample tray, and record the *x*-*y* coordinates of the robot arm as $(x_{(1,1)}, y_{(1,1)})$ in the robot reference coordinate system.
      ii. Record the coordinates of 9 points at different positions as $(x_{(i,j)}, y_{(i,j)})$, where i and j refers to the index of row and column.
      iii. Solve the least squares solution of overdetermined equations to obtain the calibration coefficients a_1, b_1, a_2, b_2:

$$\begin{aligned} a_1 \cdot i + b_1 \cdot j &= x_{(i,j)} \\ a_2 \cdot i + b_2 \cdot j &= y_{(i,j)} \end{aligned}, \text{ for each recorded location } (i, j) \qquad \text{(Equation 1)}$$

      iv. Obtain the coordinate of each bottom in row i and column j of the sample tray by Equation 1 with the calculated calibration coefficients.
   b. Design the movement trace of dipping samples onto the TLC plate. Troubleshooting 1.
      i. Move the robot arm of DOBOT MG400 to the position higher than the bottle and record the *z* coordinate z_{Hdip}.
      ii. Reduce z_{Hdip} until the capillary reach near the bottom of the bottle, and then record the height of the robot z_{Ldip}.
      iii. The controlling code for dipping the sample placed in the bottle in i^{th} row and j^{th} column is written in the Python program as:

```
> Dobot_dip_high=[x(i,j), y(i,j), zHdip, 0, 0, 0]

> Dobot_dip_low=[x(i,j), y(i,j), zLdip, 0, 0, 0]

>dobotSDK.MovJ(api, Dobot_dip_high, isBlock=True)

>dobotSDK.MovL(api, Dobot_dip_low, isBlock=True)

>dobotSDK.MovL(api, Dobot_dip_high, isBlock=True)
```

Note: The coordinate of DOBOT MG400 has six dimensions, including the information of location (the first three dimensions) and rotation (the last three dimensions). In this protocol, we only consider the location coordinates. In the robot system, MovJ means joint movement while the MovL means linear movement.

   c. Determine the coordinates of spotting location (Figure 3B).
      i. In this protocol, the robot spots 4 samples on each TLC plate, and the TLC plate stand holds 3 plates simultaneously. The *x*-*y* coordinates of the first spotting locations (x_1, y_1) on each TLC plate are recorded. The distance between the spots is set beforehand as *dy*.

Note: The TLC plate utilized in this work is 7.5 cm long and 2.5 cm wide and *dy* is set to be 0.5 cm.

ii. Adjust the robot arm by the software to a proper initial height so that it will not touch other objects during movement and record the z coordinate as z_{Hspot}.
iii. Reduce the z_{Hspot} until the capillary is close to the TLC plate but has not touched it (<5 mm). Record the *z* coordinate as z_{Mspot}.
iv. Reduce the z_{Mspot} until the capillary touches the silica gel layer of TLC plate and record the corresponding *z* coordinate as z_{Lspot}. Troubleshooting 2.
v. The controlling code for spotting i^{th} dot on a TLC plate is written as:

```
Dobot_spot_high=[x1, y1+i*dy, zHspot,0,0,0]

Dobot_spot_middle=[x1, y1+i*dy, zMspot,0,0,0]

Dobot_spot_low=[ x1, y1+i*dy, zLspot,0,0,0]

dobotSDK.MovJ(api, Dobot_spot_high, isBlock=True)

dobotSDK.MovL(api, Dobot_spot_middle, isBlock=True)

dobotSDK.MovL(api, Dobot_spot_low, isBlock=True)

time.sleep(0.1) #spot sample

dobotSDK.MovL(api, Dobot_spot_middle, isBlock=True)
```

Note: Please refer to troubleshooting 3 for accelerating the speed of spotting TLC plates.

d. Wash and dry the capillary.
i. Put a bottle in the last row of the sample tray and fill it with washing solution (Figure 3A). Record the *x*-*y* coordinates of the bottle with washings solution as (x_{wash}, y_{wash}).
ii. Move the DOBOT MG400 robot arm to the center of the absorbent paper and record the *x*-*y* coordinates as (x_{dry}, y_{dry}).
iii. Move the robot arm to (x_{dry}, y_{dry}, z_{Hdip}) and reduce the height until the capillary touches the absorbent paper. Record the z coordinate as z_{Ldry}.

Note: In this protocol, the capillary tube is washed and dried three times after dipping and spotting each sample. For better drying effect, a random deviation is added to (x_{dry}, y_{dry}) to dry the capillary tube at different places on the absorbent paper.

9. Developing spotted TLC plates in the elution solvent (Figure 3D).
a. Grab the spotted TLC plates.
i. Adjust the posture of the AUBO i5 robot to make the gripper face the TLC plate stand and record the angles of six joints as AUBO_grab_initial=(deg1, deg2, deg3, deg4, deg5, deg6).
ii. Move the robot arm until the gripper is aligned with the center of the target TLC plate, and record the corresponding posture as AUBO_grab_loc.
iii. Retract the robot arm horizontally until the front end of the gripper leaves the TLC stand and record the corresponding posture as AUBO_grab_prepare. This posture is utilized to prevent crash onto the plate stand when grabbing the spotted plate.
iv. Move the robot arm to AUBO_grab_loc and grab the TLC plate by the gripper. Raise the robot arm to a sufficient height so that other objects will not be affected during subsequent operations. Record the posture as AUBO_grab_high.
b. Transfer the spotted TLC plates into the TLC development chamber.
i. Move the AUBO i5 robot arm to the location right above the first TLC development chamber and align the suction cup with the center of the chamber lid. Record the posture as AUBO_suck_high.

ii. Decrease the height of the robot arm until the suction cup touches the chamber lid. Record the posture as AUBO_suck_low.
iii. Open the air pump to suck up the lid. Change the posture of the robot arm so that the TLC plate is facing the gap of the customized fixture in the TLC development chamber. Record the posture as AUBO_put_high.
iv. Decrease the height of the robot arm until the TLC plate is close to the fixture. Carefully adjust the location so that the TLC plate can pass through the gap in the middle of the fixture. Record the posture as AUBO_put_middle.
v. Continue to decrease the height of the robot arm until the TLC plate touches the bottom of the TLC chamber and open the gripper to release the plate. Record the posture as AUBO_put_low.
vi. Move the robot arm to the right until the gripper touches the released TLC plate and makes it tilt to the same direction. Record the posture as AUBO_put_right.

c. Close the TLC chamber for development.
i. Move the AUBO i5 robot arm to AUBO_put_high and then move to AUBO_suck_high.
ii. Move the robot arm to AUBO_suck_low and then stop the air pump through IO interface to put down the lid and close the TLC chamber.

d. Code for developing spotted TLC plates in the elution solvent as shown below.

```
#Grab the TLC plate from TLC plate stand
>robot.move_joint(AUBO_grab_initial)
>robot.move_joint(AUBO_grab_prepare)
>robot.move_line(AUBO_grab_loc)
>movetgt(0) #close the gripper
>time.sleep(2)
>robot.move_line(AUBO_grab_high)
#Put the TLC plate into the TLC chamber
>robot.move_joint(AUBO_suck_high)
>robot.move_line(AUBO_suck_low)
>robot.set_board_io_status(5, 'U_DO_01', 1) #open air pump
>robot.move_line(AUBO_suck_high)
>robot.move_joint(AUBO_put_high)
>robot.move_line(AUBO_put_middle)
>robot.move_line(AUBO_put_low)
> movetgt(100) #open the gripper
>robot.move_line(AUBO_put_right)
# Close the TLC chamber
>robot.move_line (AUBO_put_high)
>robot.move_joint(AUBO_suck_high)
>robot.move_line(AUBO_suck_low)
>robot.set_board_io_status(5, 'U_DO_01', 0) #close air pump
>robot.move_line(AUBO_suck_high)
```

10. Take out the plate and send to photograph under visible light and UV light.
    a. Take out the plate.
        i. Open the chamber lid in the same manner as mentioned above (step 9b).
        ii. Move the AUBO i5 robot arm to AUBO_put_low and close the gripper, then move the robot arm to AUBO_put_high.
        iii. Close the chamber lid in the same manner as mentioned above (step 9c).
    b. Photograph under visible light.
        i. Move the robot arm to the location that the camera can catch the TLC plates clearly. Record the posture as AUBO_photo_visible.
        ii. Open the visible light through the IO interface and control camera to take a photograph. The photograph is automatically saved in the save dir.
        iii. Close the visible light through the IO interface.

⚠ CRITICAL: A standard lab TLC plate has two sides where the front side is silica and the back side is the glass. when photographing under the visible light, the TLC plate is photographed from the back side. It is critical because that the solvent front is easy to be identified from the photo taken from the back side since the glass surface is more reflective.

    c. Photograph under UV light.
        i. Move the robot arm to the front of the UV light photographic device. Record the posture as AUBO_photo_UV_prepare.
        ii. Send the TLC plate into the black shading shell and hold the TLC plate under the UV light. Adjust the posture until the TLC plate is completely visible by the camera.
        iii. Open the UV light through the IO interface and control camera to take a photograph. The photograph is automatically saved in the save dir.
        iv. Close the UV light through the IO interface.
        v. Move the robot arm to AUBO_photo_UV_prepare.
    d. Drop waste.
        i. Send the photographed TLC plate to the upper part of the waste container and open the gripper to make the TLC plate fall into the waste container. Treat the waste centrally after the experiment.
11. Retrieve new TLC plates.
    a. Move the AUBO i5 robot arm above the TLC plate storage and adjust the posture to make the suction cup faced the TLC plate storage. Record the posture as AUBO_retrieve_prepare.
    b. Decrease the height of the robot arm until the suction cup presses the blank TLC plate. Record the posture as AUBO_retrieve_low.
    c. Open the air pump through the IO interface and raise the robot arm to AUBO_retrieve_prepare.
    d. Move the robot arm to the top of the notch on the TLC stand where the plate is to be placed, and record the posture as AUBO_place_high.
    e. Decrease the height of the robot arm until the TLC plate is put onto the notch precisely and then close the air pump. Record the posture as AUBO_place_low. Troubleshooting 4.

Note: Every time a TLC plate is retrieved from the customized TLC storage device, the spring will push up the next TLC plate in order to be retrieved for the next experiment. In this protocol, the TLC plates storage device can hold 18 TLC plates at most.

Conduct high-throughput TLC experiments

Timing: 50 min per cycle

After the workflow of each dedicated step for TLC analysis has been determined, the high-throughput TLC experiments can be conducted by combining these workflows in series through a

Python program. In this section, we will describe the preparation of sample analyte and solvents before the high-throughput experiments and the workflow of the automated TLC experiments.

12. Preparation before the high-throughput experiments.
    a. Prepare samples. Each compound is dissolved in dichloromethane (CH_2Cl_2), and the concentration is between 1–5 mg/mL. Next, the samples are placed into the vials on the sample tray through a dropper at 1.5 mL.
    b. Prepare elution solvents. By calculating the solvent ratio, the total volume of the elution solvent prepared each time is 100 mL. Put the prepared elution solvents (5 mL) into different TLC chambers.

    Note: The elution solvents are prepared by using a 100 mL graduated cylinder (division value: 1 mL) and a disposable plastic tip dropper. The TLC chambers are labeled in the software and the elution solvent is replaced after each running cycle.

    c. Prepare TLC plates. Fill the TLC plate stand and storage device with blank TLC plates.
    d. Prepare washing solvent and absorbent paper. Fill the bottle for washing capillary tubes with washing solvent (CH_2Cl_2, 1.5 mL) and replace the absorbent paper with a pristine one.

    Note: The washing solvent and absorbent paper are replaced after each running cycle.

    ⚠ CRITICAL: Ensure that all devices are powered up and connected to the PC. Check the condition of each equipment to avoid problems during the experiment. If possible, additional sensors can be equipped to double-check the position and guarantee safety.

13. Construct the work cycle of automated TLC experiments.

    Note: In this protocol, a work cycle is composed of 8 sub-cycles. In each sub-cycle, the R_f values of 4 compounds under 3 different elution solvents are measured.

    a. Spot four compounds in sequence by DOBOT MG400 robot (Figure 4A). Wash and dry the capillary tube three times after spotting each sample.
    b. The AUBO i5 robot sends these spotted TLC plates to three different TLC chambers with different solvents for development, respectively.

    Note: The developing time is 300 s in this protocol (Figure 4B). It is worth noting that the developing time can be freely and easily changed in the program to handle different conditions.

    c. While developing, the AUBO i5 robot retrieves three new blank TLC plates from the TLC plate storage and puts them on the TLC plate stand. Afterwards, the DOBOT MG400 robot spot the following 4 compounds on the pristine TLC plates.
    d. When the developing time reaches the set value, retrieve the developed TLC plate by the AUBO i5 robot from the chamber and send it to the visible light and UV light photographic devices to take photographs (Figure 4C). Then, drop TLC plates into the waste container. Troubleshooting 5.
    e. Transfer the TLC plates spotted with following 4 compounds by the AUBO i5 robot for development.
    f. This cycle will continue until all 32 compounds have been tested.

    Note: Refer to troubleshooting 6 for how to make one cycle run more time and measure more compounds in a cycle.

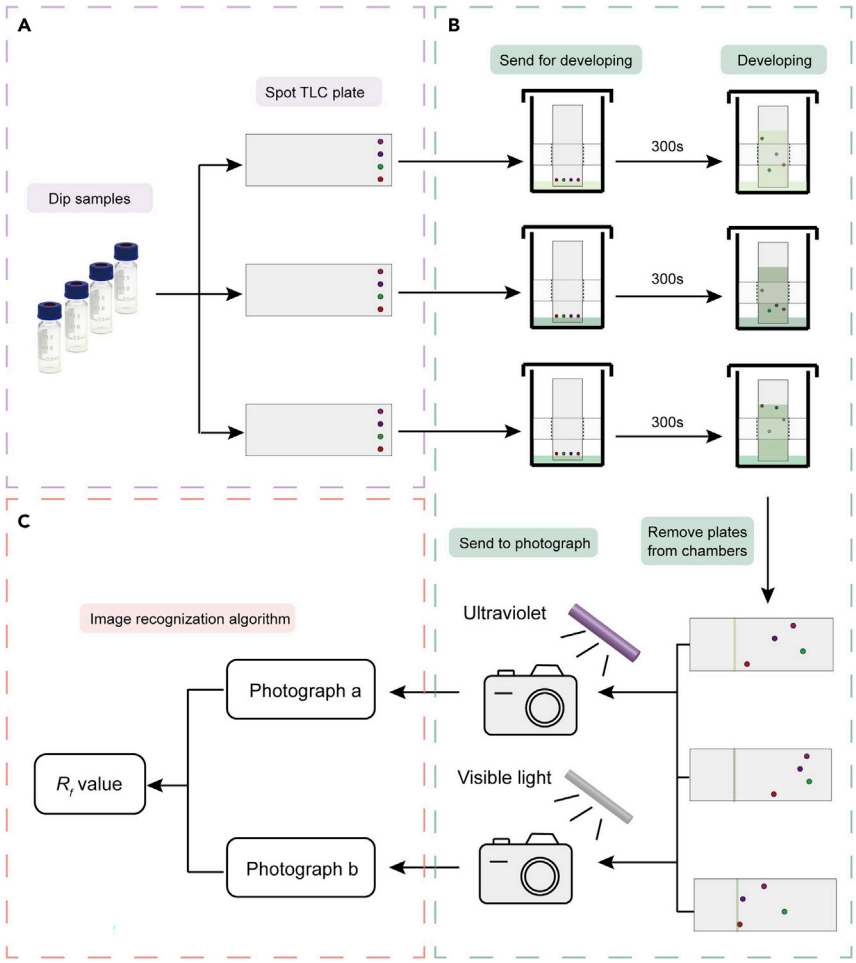

Figure 4. The workflow of the sub-cycle of the automated TLC experiments

(A) Dipping and spotting 4 samples on 3 TLC plates.

(B) Sending the TLC plates for development in the elution solvents and sending them to photograph under the visible light and UV light.

(C) Obtaining the R_f values by the image recognition algorithm.

Image recognition algorithm for calculating the R_f values

Timing: 1 s for photographs of each TLC plate

For high-throughput experiments, automated processing of obtained original data is crucial. In the TLC analysis, the R_f value is the ultimate outcome. In the automated platform, the original data is photos taken under the visible light and UV light. Therefore, an image recognition algorithm is provided in this protocol to calculate the R_f values automatically from photographs. The R_f value is defined to be the ratio of distance traveled by the solute to distance traveled by the solvent. Here, the solvent front is recognized from the photographs under visible light and UV light, while the heights of developed samples are identified from the photograph under UV light. Therefore, the two photographs work together to calculate the R_f values. Refer to troubleshooting 7 for handling UV-inactive compounds.

14. Post-processing the photographs.

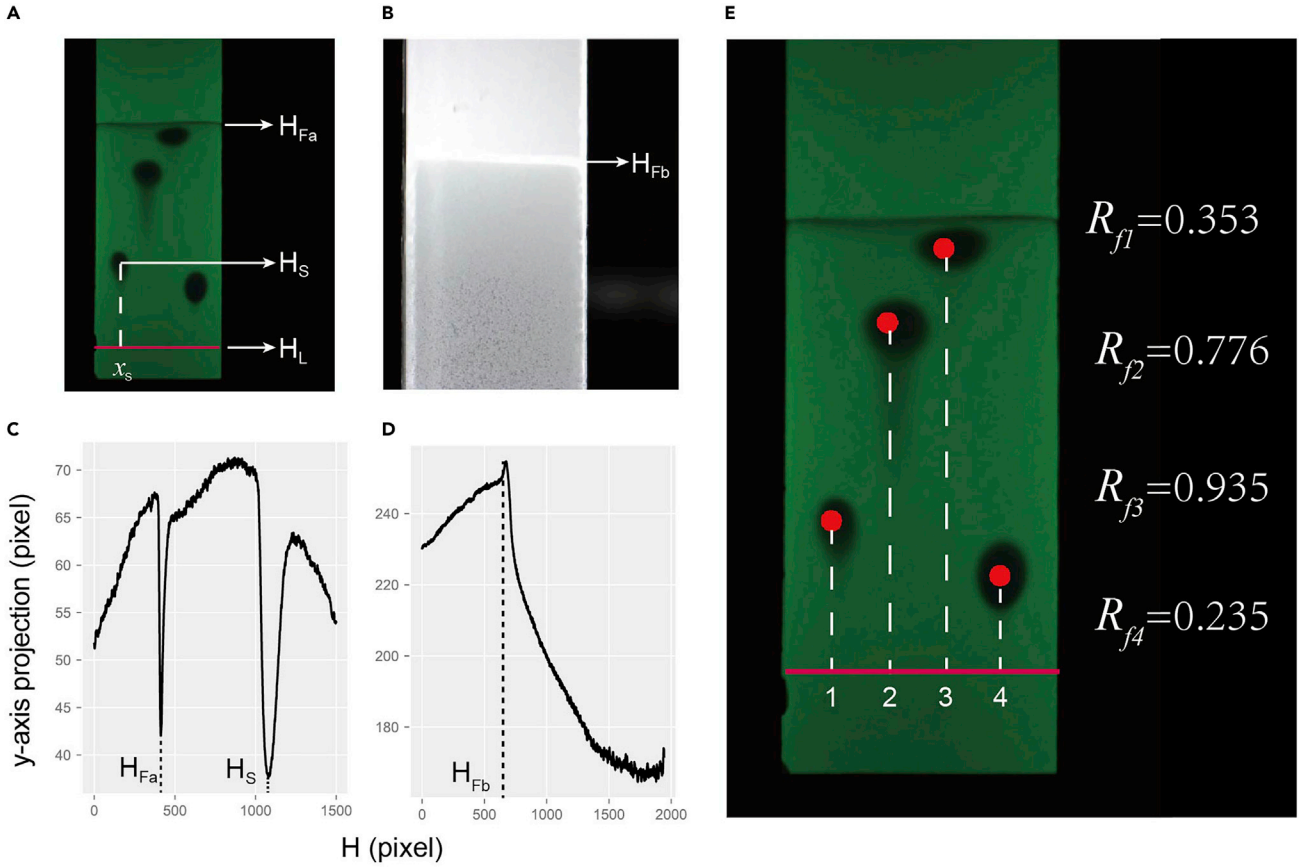

Figure 5. The automatic recognition and calculation of R_f values

(A) The photograph taken under the UV light.

(B) The photograph taken under the visible light.

(C) The average pixel value after the *y*-axis projection is performed on the identification neighborhood of the first sample spot.

(D) The average pixel value after the *y*-axis projection is performed on the TLC plate photographed under visible light.

(E) The output of the recognized spot and calculated R_f. In this figure, H_{Fa} and H_{Fb} refers to the height of the solvent front in both photos, respectively, H_L refers to the initial height of the sample spot, H_s refers to the height of the TLC spot, and x_s represents the horizontal position of the TLC spot.

a. For the photographs taken under visible light and UV light, crop to extract the approximate range of the TLC plate.

b. Convert photographs into grayscale by the Python package Opencv.

```
> cv2.cvtColor(image, cv2.COLOR_BGR2GRAY)
```

c. Identify the precise edge of the plate by the grayscale value since the main body of the plate is significantly different from the surroundings, which will lead to differences of the grayscale value.

15. Identify the heights of developed samples from the photograph under UV light. The initial positions of sample spots (x_s, H_L) (s=1, 2, 3, 4) are fixed beforehand (Figure 5A).

a. Determine identification neighborhood. For each point of sample, the neighborhood space [$x_s-\Delta x$, $x_s+\Delta x$] is established, and the recognition algorithm is implemented for each neighborhood. The size of the neighborhood Δx is selected according to the distance between the points which is fixed in advance.

b. *y*-axis projection in the identification neighborhood. In each neighborhood space, *y*-axis projection is performed to calculate the average value of pixels at each height (Figure 5C).

c. Peaks identification. In general, there will be two obvious peaks (i.e., minimum values) in the *y*-axis projection curve, which represent the height of the developed sample H_S and the elution solvent front H_{Fa}. By identifying the peaks, the H_S and H_{Fa} can be obtained (Figure 5C).

Note: In this protocol, single analyte recognition is provided as an example. For mixed analytes, each separated component will have a peak. Therefore, the height of each component can be obtained also by identifying the peaks for calculating corresponding R_f values.

d. Obtain the map f_a between the height of any coordinate on the TLC plate and the location in the photo.

Note: The focal length of the camera and the spatial location of the TLC plate are predetermined, the mapping relationship between the pixel distance on the photo and the actual distance can be determined in advance.

Note: Before recognition, the y-axis projection can be cleaned and smoothed by digital filters to eliminate noise and avoid recognition errors.

16. Identify the solvent front from the visible light photograph (Figure 5B).

Note: Considering the solvent front is the brightest under visible light illumination, the y-axis projection technique is utilized here.

a. Conduct y-axis projection. Specifically, the pixel values in each row in the photo are averaged to form a *y*-axis projection curve.
b. Find the minimum value and its location in the *y*-axis projection curve (Figure 5D). The location is referred as H_{Fb}.
c. In the debugging stage, we mark two points on the TLC plate. According to the position in the photo and the actual position, we can get the map f_b between the height of any point on the TLC plate and the location in the photo.

Note: In some cases, the solvent front is not apparent and cannot be accurately identified under ultraviolet light, which means that there will be one peak to identify H_S. In this circumstance, the H_{Fb} identified from the photo taken under visible light plays an important complementary role.

17. Calculate the R_f value.
a. The actual height of the solvent front H_F is expressed as:

$$H_F = \begin{cases} \frac{1}{2}(f_a(H_{Fa}) + f_b(H_{Fb})), & \text{if } \frac{|f_a(H_{Fa}) - f_b(H_{Fb})|}{f_b(H_{Fb})} < 5\% \\ f_b(H_{Fb}), & \text{other condition} \end{cases}. \quad \text{(Equation 2)}$$

Note: Here, other conditions include the difference between H_{Fa} and H_{Fb} is large, or H_{Fa} is not identified successfully.

b. The R_f value can be calculated by:

$$Rf_s = \frac{f_a(H_s) - f_a(H_L)}{H_F - f_a(H_L)}. \quad \text{(Equation 3)}$$

c. The calculated R_f value and corresponding experiment information about samples and elution solvents are saved automatically.

Note: Refer to troubleshooting 8 for common problems and complex situations that may occur in the image recognition algorithm.

EXPECTED OUTCOMES

In this protocol, the automated platform is expected to measure 32 compounds under 3 different elution solvents (e.g., hexane/ethyl acetate system, dichloromethane/methanol system, and hexane/diethyl ether system) in 50 min. For each TLC plate, original data is obtained with two photographs that are taken under the visible light and ultraviolet light. The R_f values are calculated quickly and automatically via an image recognition algorithm which is saved in the personal computer along with corresponding information about samples and elution solvents. We expect this high-throughput automated platform for TLC analysis can be combined with the prediction model proposed in Xu et al.[1] to free researchers from tedious and time-consuming experiments and accelerate scientific progress.

LIMITATIONS

The high-throughput automated platform for TLC analysis described in this protocol should readily apply to any chemical compounds that can show color under the ultraviolet. However, some limitations still remain. Firstly, additional designs should be considered for UV-inactive compounds. For example, phosphomolybdic acid or other methods to facilitate showing color need to be added to the system. Secondly, after each cycle, it is necessary to manually replenish TLC plates in the TLC plate storage device and replenish (or replace) the elution solvents, which may limit the automation ability of the platform. Meanwhile, an additional safety system (e.g., block the robot automatically when detecting external elements in the work area) needs to be adopted to guarantee safety. Thirdly, this protocol is designed for glass-based TLC plates, and needs to be further improved when faced with non-glass TLC plates (e.g., aluminium-based TLC plates). Lastly, when calculating R_f values by the image recognition algorithm, some difficult situations may emerge that are challenging for the algorithm to judge, such as sample tailing, paler color, and fusion of two sample spots (see troubleshooting 8). Therefore, manual verification by expert experience is required to deal with these extreme situations to guarantee the accuracy and reliability of the obtained results. In this protocol, the frequency of extreme situations is about 5%. To deal with this problem and improve efficiency, the automated part can be limited to photography and analysis of signals to give a consultation, while the ultimate recognition can be carried out by a specialized operator. Meanwhile, the automated platform requires knowledge of robotics and Python programming, which will take some time to build this system.

TROUBLESHOOTING

Problem 1

During the experiment, the solvent in the bottle may evaporate (step design the workflow of automated TLC analysis, 8).

Potential solution

Cover the bottle with a film and puncture the film when dipping samples.

Problem 2

Some edges and corners are not smooth when manually cutting the TLC plates, which may cause the plates being unevenly placed on the stand, further leading to breaking the capillaries. Moreover, some plates with abnormally larger thicknesses of silica gel will cause the glass capillary to break

when spotting the sample onto the plate, and it will require stopping the cycle and replace the capillary (step design the workflow of automated TLC analysis, 8).

Potential solution

- Cut TLC plates by machine.
- Remove inappropriate plates in advance.
- Replace the capillary with a steel needle that is not easy to break.

Problem 3

Low efficiency for spotting TLC plates (step design the workflow of automated TLC analysis, 8).

Potential solution

The same compound can be spotted at the same position on three TLC plates with only one sampling operation, which enhances the efficiency.

Problem 4

When retrieving plates, the blank plates sometimes cannot be placed on the notch of the TLC stand that is designed to adjust a TLC plate perfectly, which may cause the TLC plates to be angled and subsequently affect the sample spotting process (step design the workflow of automated TLC analysis, 11).

Potential solution

When planning the path, be very precise to ensure that the TLC plate is placed in the notch. Meanwhile, remove inappropriate plates in advance, especially oversized or disproportionate.

Problem 5

How to control each TLC plate to develop the same time (step conduct high-throughput TLC experiments, 13).

Potential solution

Considering the time required to send the plate to develop and the time required to take photos after development, it is necessary to plan the time when each TLC plate starts to develop in advance to ensure that the robot arm is free when the development time reaches the set value to take out the plate immediately. In this protocol, we pause for a certain time to control the time after sending each TLC plates for photographing.

Problem 6

The automated time in a cycle without manual operation needs to be improved (step conduct high-throughput TLC experiments, 13).

Potential solution

The time that the automated platform can run automatically in a cycle is restricted by two main factors: replenishment of the elution solvents and the maximum number of TLC plates in the TLC plates storage container. To handle these issues, redesigning the TLC plates storage container with a larger capacity and inventing a device to refill elution solvents automatically may enable the automated platform to run continuously for longer periods of time.

Problem 7

Some compounds are UV-inactive (step image recognition algorithm for calculating the Rf values).

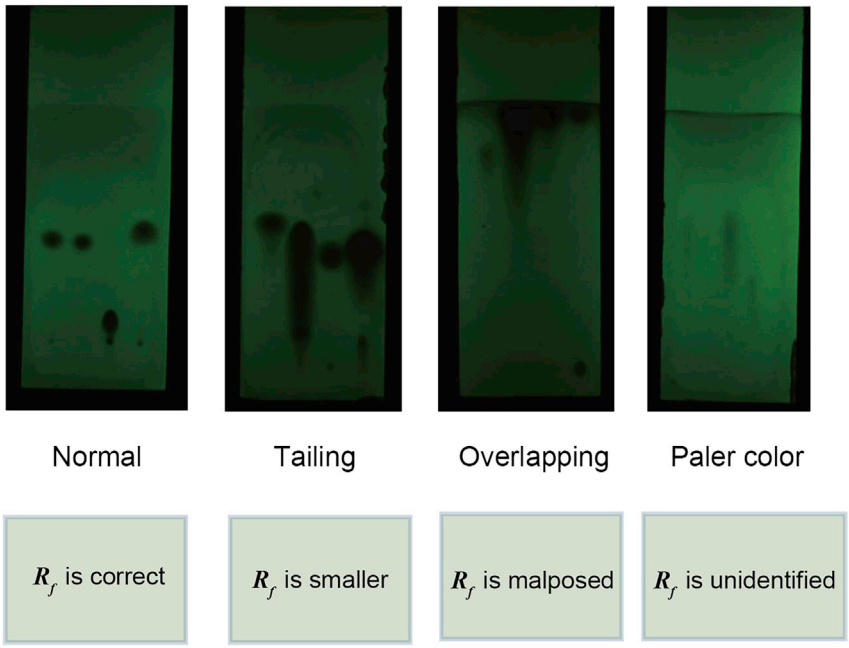

Figure 6. Common problems and complex situations that may occur in the image recognition algorithm

Potential solution

For UV-inactive compounds, phosphomolybdic acid is often used for plate visualization. This process can be adapted to the automated workflow with some adjustment. Specifically, a vessel filled with 80 mL phosphomolybdic acid (0.1 g/mL) and a plate heater are added to the automated platform. The heater is set to be 180°C in order to quickly dry the plate. After the TLC plate has been developed, the AUBO i5 robot grabs the plate and soaks it in the vessel with phosphomolybdic acid. Then, the plate is sent onto the plate heater and heated for a while. The heating time is set beforehand to guarantee the staining spots are clear. Then, the heated plate is sent to the visible light photographic device to take photos. It is worth noting that the front side is taken for UV-inactive compounds. Nevertheless, there still remain some challenges since although the plate is stained and the spots are visible to human eyes, it is still a little blurry for image recognition in some circumstances, so it requires manual verification in some cases through the verification program provided in this protocol.

Problem 8

Common problems and complex situations (Figure 6) that may occur in the image recognition algorithm (step image recognition algorithm for calculating the Rf values, 17).

Potential solution

- Changing the concentration of samples. For example, tailing and overlapping require reducing the concentration, while the concentration needs to be increased in the case of paler color.
- Add an alarm mechanism into the image recognition program to find the TLC plates with problems that need manual verification.
- Adopt more advanced methods, such as deep learning techniques, to improve recognition accuracy.
- Improve the efficiency of manual verification. A program of visual interface is developed that makes manual verification easy by simply clicking on the correct sample spot, starting point,

and solvent front on the image and the image recognition algorithm will correct the mistakes automatically.

- Faced with certain kinds of compounds (e.g., carboxylic acid and amine compounds), the corresponding treatment measures (e.g., adding tailing-suppressing reagents) can be flexibly added to this automatic system to facilitate the TLC process.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Fanyang Mo (fmo@pku.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

The design schemes for customized objects generated during this study are available at the website https://github.com/woshixuhao/Protocol_automated_TLC/tree/main/design_scheme.

The code generated during this study are available at the website https://github.com/woshixuhao/Protocol_automated_TLC/tree/main/Controlling_code.

The version of record of the GitHub repo is https://doi.org/10.5281/zenodo.7275985.

A video associated with this project can be found via the following links:

English version: https://www.bilibili.com/video/BV1am4y1o7yE/.

Chinese version: https://www.bilibili.com/video/BV17R4y1j7jz/.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

H.X. and F.M. built the robotics platform for high-throughput experimentation. H.X. and F.M. wrote the manuscript. F.M. conceived the idea and designed the overall research. F.M. and D.Z. supervised the whole project.

DECLARATION OF INTERESTS

F.M., H.X., and D.Z. are inventors on two patent applications (CN 202111638511.2 and 202122346010.9) submitted by Peking University that cover an organic chemistry laboratory automation system and a machine learning method for TLC conditions prediction, respectively.

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