Low-cost automated tissue processor for hydration and dehydration of biological tissues

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Abstract

Imaging techniques are fundamental in biological sciences, particularly in plant science, for studying cell growth and response to environmental stimuli. Since many tissues are naturally transparent, staining is usually required to enhance visibility during imaging. However, many tissue staining protocols, such as those using propidium iodide (PI), involve laborious dehydration and rehydration steps, where tissues are placed in different concentrations of solvents, which are time-consuming and labor-intensive. To address this challenge, we have developed an affordable, automated tissue processor for the dehydration and rehydration of biological tissues. This open-source system, which costs approximately $300 to build, automates the sequential processing of tissues through solvent solutions, significantly reducing manual labor. The processor is easy to build using standardized parts, which are easily sourced from makerspaces, and can be customized for various staining protocols. Its software allows users to program and save protocols, minimizing tissue exposure to air and optimizing solvent consumption. Our design provides a low-cost, accessible alternative to expensive commercial tissue processors, offering a practical solution for biology laboratories.

Introduction

Most biological sciences, including plant sciences, heavily rely on imaging techniques that are fundamental for understanding cell growth, differentiation, and response to environmental cues (Gaion and Carvalho). Given that most tissues are transparent, a crucial step before imaging involves applying a dye for staining. Many staining methods require stepwise dehydration and rehydration to ensure full infiltration of cell layers with the desired dyes. For example, propidium iodide (PI) is a fluorescent dye commonly used in plant science; it is impermeable to live cells but penetrates cells with compromised membrane integrity, such as dead or damaged cells (ScienceDirect).

Before dye staining, tissue samples undergo meticulous preparation that includes a long sequential treatment with a series of reagents. Specifically for the PI staining, the protocol involves passing the tissue through 24 ethanol solutions every 20-30 minutes, allowing for rehydration and hydration before staining (see protocol developed by Frank Lab). Currently, this process is done manually, which can take several hours or even days, consuming significant time and productivity.

In response to this time-consuming procedure, various companies offer "tissue processors" to automate parts of the staining process. However, these machines are often prohibitively expensive and may be more sophisticated than necessary for laboratories focused on plant biology. [Expand]

To address the challenges associated with traditional tissue staining protocols, we have developed a low-cost automated tissue processor for the dehydration and rehydration of tissues. While we demonstrate its use for PI staining in this work, the processor can be programmed for any protocol requiring a dilution series. Given the high cost of existing automated processors, we provide resources and instructions to build an open-source and equally functional alternative for approximately $300. We have focused on making this build

as accessible and flexible as possible by using standardized parts and common tools found in makerspaces. This machine is designed to automatically switch the concentration of the ethanol solution within the processing chamber while minimizing the time the tissue is exposed to air. It features a self-contained software ecosystem that allows users to save the duration and concentration of exposure steps to an SD card for future use. Additionally, this project aims to reduce ethanol-related costs by utilizing the lowest effective ethanol concentrations.

Design of the automated tissue processors

Fig. 1 shows a schematic and photograph of the automated tissue processor. The processor consists of two containers: a premix container, where solvents are prepared at the desired concentration, and a tissue container, where the tissue is placed for processing. Using three peristaltic pumps, the desired concentration of solvents, or solvent mixtures, is prepared in the premix container. Once ready, the solution is transferred from the premix container into the tissue container via a top ball valve, allowing the tissue to soak in the solution for a specified time. After this step, the next solution is prepared in the premix container, and once the soaking time has passed, the solution in the tissue container is drained into a waste bottle through a bottom valve. The new solution is then introduced from the premix container. The entire process can be programmed via a keypad and display, as described in the next section. The peristaltic pumps operate with a consistent flow rate of approximately 1.1 mL/s, which is suitable for obtaining precise dosages of the solvent, as illustrated in Fig. 2.

Materials and manufacturing

Bill of materials**.** The bill of materials is shown in Table 1. Most of the listed components can be swapped out with other common materials found in makerspaces.

3D printing. Table 2 presents a comprehensive list of all the 3D parts required for the assembly, along with the materials needed for printing each part. All the CAD files for 3D printing are available in the Supplementary Information (S.I.). These files were created using SOLIDWORKS and are provided in both STEP and SLDPRT formats.

Assembly procedure

The mechanical design can be separated into four main mechanisms.

Glassware Tower: When securing the prints to the bottom MDF plate, use short wood screws and ensure that the MDF does not separate during this process. Drill 13/32” holes into the PVC pipe to fit the assembly pins. To achieve proper placement, mark the center of these holes with a sharpie by assembling the mechanism and holding the pipe in a vice while drilling. Exercise caution with the ball valve and funnel mechanism, as extra tolerance in the prints may cause flex. When transporting the device, support one end under the MDF base plate and use one hand to stabilize the ball valve and funnel mechanism.

If you find insufficient clearance on the pipe side of the ball valve holder for M2.5 screws and nuts, utilize the clamping mechanism as a replacement. Should you opt for a different

separatory funnel, ensure to adjust the funnel holder CAD so that the valve aligns with the servo, as off-axis strain may break the glassware. Cut the metal rod to a length that allows the separatory funnel to comfortably fit inside the bottom glassware when lowered, while enabling easy removal of the lid when the funnel is raised.

Keypad Box: When securing the display and keypad with M2 screws, be aware of potential self-tapping issues. To create threads, screw the screws in without the components first. If using a common Arduino USB-B cable (the translucent blue type), you might need to strip the blue rubber off the Arduino side and bend it for better clearance within the box. Avoid directly soldering wires to the display and keypad; instead, use headers and M-F jumper cables. Since the wiring can become messy, utilize rainbow ribbon jumper cables for organization. For wires connecting to the relay box, consider using 2-prong Wago connectors to convert breadboard jumper cables to regular wires.

Relay Box: This box is the most cramped, so opt for space-efficient relays to connect to the ball valve and pumps. Use 5-prong Wago connectors and zip ties to maximize space. The switch controlling the flow of the 9V input should be pre-soldered to the female XT60 connector and a loose wire. This wire will connect to a Wago, linking it to the buck converters and the ball valve. Installing this component may require inserting the XT60 connector through the switch hole.

Pump Box: The Adafruit pumps come with both ends of silicone tubing, but one side must be swapped with the extra tubing to reach the top of the glassware. Use blue tubing to clearly indicate the pump outputs. It is advisable to label the ends of the tubing with tape to confirm input and output. Use a sharpie on the box to label each pump according to its respective liquid, and ensure that the leftmost pump has extra-long wires to reach the relay box.

Electrical wiring

Fig. 3 shows a schematic of the electrical wiring for the system, illustrating how each component is connected. In addition, Table 3 lists all the pinouts, providing detailed information on the connections required for proper functionality.

Software design and operation

The Arduino code is provided in the Supplementary Information (S.I.). The code requires the following libraries in the Arduino IDE: Adafruit_GFX.h, Adafruit_ST7735.h, Adafruit_Keypad.h, and ArduinoJson.h. Fig. 4 presents a flowchart of all the options available in the software. Below, we provide an explanation of each menu option to guide users through the functionalities and features of the software.

Run Program: Runs the program after selecting a Routine ID. The display shows the current step, concentration, and ETA for routine being run.
Modify Programs: Reads and writes programs to the JSON file on the SD card under the display. All functionality in this block can be replaced by directly plugging the SD card into a PC, and modifying the file with a text editor.
Flush System: Opens all valves and runs all pumps to ensure that machine is clear of all liquid before storage.

Settings | Sanity Check: Checks that all hardware elements work as expected, and should be run after first assembly. Ensures that all pinout variables are correctly assigned.
Settings | Calibrate: Volume / time calibration variables are stored on the SD card for easy modification, in which they are set by this process.
Settings| View Calibration Variables: Displays the variables set on the SD Card.

Programming the processor. The processor can be used to make a new protocol for each step of a procedure. In order to create a new protocol, the machine is turned on, and then the interface is used along with the keypad below it. As programmed, it is best to first go to "Modify Routine", and then "New Routine". Then, for each step a duration is listed and a concentration. The pound key (#) is used to move forward in the coding, the star key (*) is used to move backwards, and "any key" except the pound key is used to add a new step when needed. It is recommended that a user returns to the "Modify Routine" to "View Routine" and confirm that the desired steps and concentrations are as written. There will also be an opportunity before a routine is run to check the steps again.

PI Staining protocol

The following protocol has been used in the tissue processor for PI staining but can be modified as needed for any other dye protocol that requires stepwise dehydration and/or rehydration. Tissues from tomatoes stem were collected and directly put into FAA (50% EtOH, 3.7% Formaldehyde, 5% acetic acid) with enough volume to completely submerge all tissues. The same day, the tissues were vacuum infiltrated in the FAA for 1 hour. The samples were kept at 4℃ overnight. A program was then written onto the machine with 30-minute intervals for the following nine concentrations: 50%, 70%, 85%, 95%, 100%, 100%, 95%, 85%, 70%. The samples were kept in the chamber at 70% overnight. The following day, a new protocol was created with 30 minute intervals for the following five concentrations: 50%, 30%, 15%, 0% (deionized water), 0%. The samples were shaken at approximately 150 rpm in 100 mL of 2% propidium iodide solution for 1 hour while covered in foil. The samples were then placed into the chamber, and a new protocol was created with 30-minute intervals for the following six concentrations: 0%, 0%, 15%, 30%, 50%, 70%. The samples were held in the chamber overnight at 70%. The following day, a new protocol was created with 30-minute intervals for the following four concentrations: 85%, 95%, 100%, 100%. Following the completion of the final dehydration sequence, the samples are cleared in 1:1 ethanol: methyl salicylate on a shaker for 1 hour, followed by storage in 100% methyl salicylate at 4℃ for a minimum of 48 hours prior to imaging.

The samples were then mounted in 100% methyl salicylate and imaged using a Zeiss 880 confocal microscope (Germany) using an argon laser 514 nm beam. Fig. 5 shows confocal images of the stained tomato stem.

Conclusion

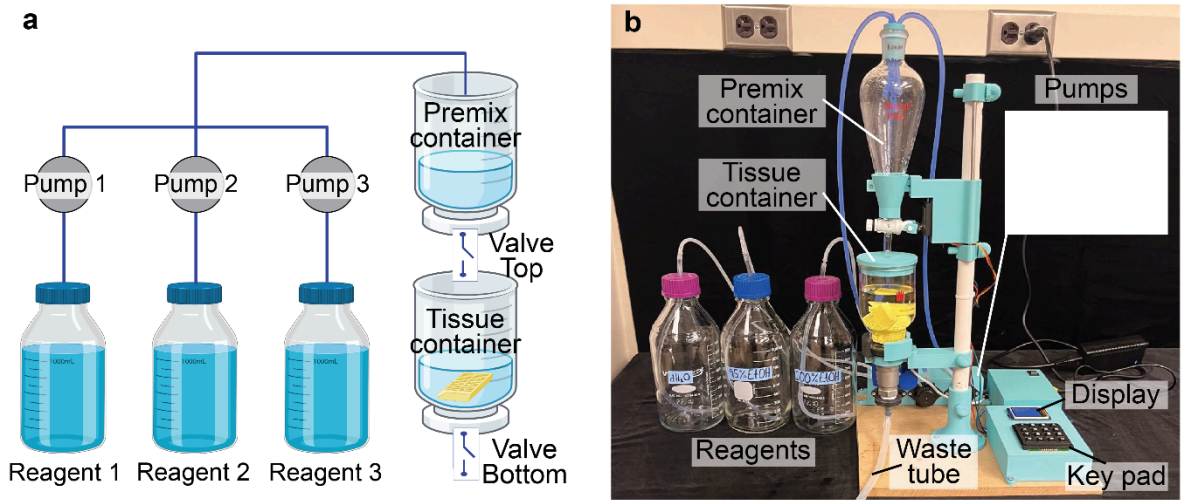

Fig. 1. **a) Schematic and b) photograph of the automated tissue processor.** The system consists of two main containers: a premix container for preparing solvents and a tissue container for processing. Using three peristaltic pumps, solvents are mixed to the desired concentration in the premix container and transferred to the tissue container via a top ball valve. After the tissue soaks for a programmed time, the solution is drained into a waste bottle through a bottom valve, and the next solution is introduced. The process is fully programmable via a keypad and display.

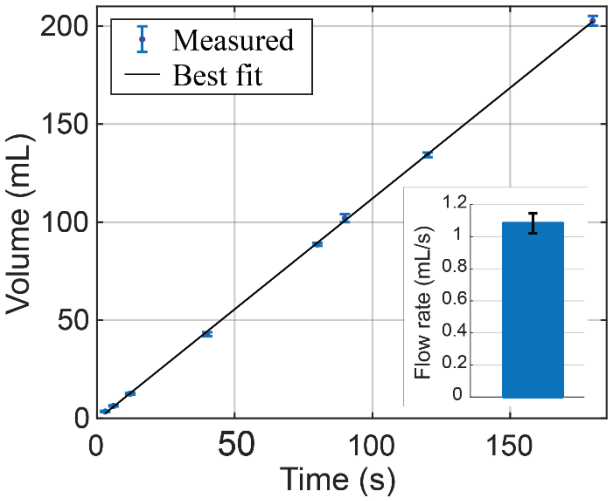

Fig. 2. Experimental measurement of the flow rate of the peristaltic pump. To obtain this plot, we operated the pump for a given time, displayed on the x-axis, and measured the volume of liquid dispensed on the y-axis. We define the flow rate as the ratio between the pump's volume and the time. The inset shows the average flow rate based on the measured values, indicating a flow rate of approximately 1.1 mL/s.

Table 1. List of materials including electrical and mechanical components.

Components	Qty	Price	Link
Electrical Components			
Keypad	1	5.95	Adafruit
Display	1	24.95	Adafruit
Arduino Mega	1	48.9	Amazon
MG995 Positional Servo	1	19.6	Amazon
Peristaltic Pump	3	24.95	Adafruit
Relay Board	1	9.99	Amazon
9V 5A Power Adapter	1	7.49	Amazon
Buck Converter	1	7.99	Amazon
Motorized Ball Valve	1	35.9	Amazon
Wago Connectors	1	19.99	Amazon
Barrel Jack Connector	1	0.95	Adafruit
22 AWG Electrical Wire	1	12.99	Amazon
Mechanical Components			
Graduated Filtration Funnel	1	104.5	Amazon
250 ml Addition Funnel	1	21.97	Amazon
Steel Rod	1	6.99	Amazon

0.75" ID, 0.85" OD PVC Pipe	1	10.96	HomeDepot
12"x12"x1/2" MDF Board	1	6.97	McMaster
3.5mm ID Silicone tubing	1	7.99	Amazon
7.5 mm ID Silicone Tubing	1	11.99	Amazon
Teflon Tape	1	2.99	Amazon
225 Oring	1	5.91	Amazon
Wood Screws	1	6.99	Amazon
Metric Screws	1	15.59	Amazon

Table 2. List of 3D printed parts and type of resin used for the printing. The CAD files of all the parts are provided in the S.I.

Part:	Note	Material
Rail Link	Quantity = 2	
Assembly Pin	Quantity = 5 Print Orientation: On round side with standard supports; Otherwise, pin shears easily.	
Funnel Angle Locker	Quantity = 3 Print Orientation: On large round side with standard supports; Otherwise, clamping lips shear easily.	
Funnel Ball Valve Adapter Separatory Funnel Holder / Ball Valve Holder	Material: Needs to be resin printed to be waterproof. Print Setting: Need to split part into two objects to have them both print on flat sides.	
Keypad Box / Pump Box / Relay Box	Print Orientation: Needs to be printed on one of the two flat edges such that the screw tabs are not along layer lines; Use tree supports for faster printing times + cleaner support removal.	

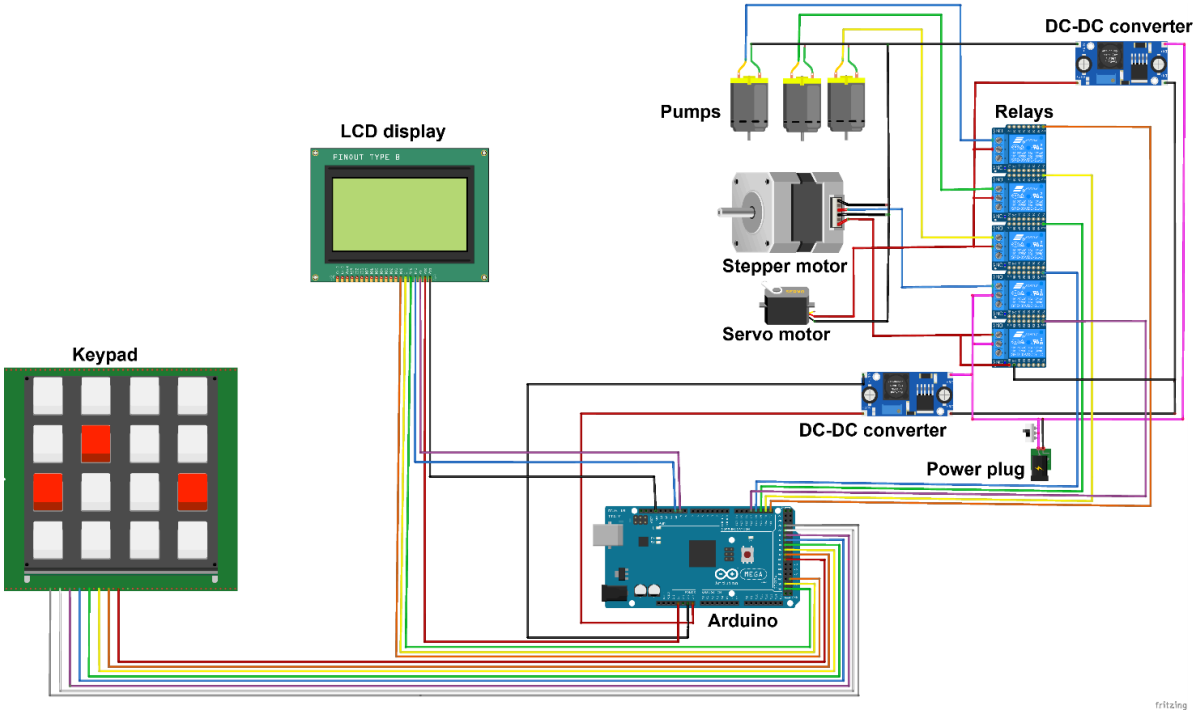

Fig. 3. Schematic of the electrical wiring for the system. The pin numbering is shown in Table 3.

Table 3. Numbering of pins for the electrical wiring.

Item	Pin
Display TFT_CS	10
Display TFT_DC	8
Display SD_CS	11
Display MOSI	51
Display MISO	50
Display SCK	52
Keypad	[26-40], even
Servo	41
Pumps (Water, EtOH96, EtOH100)	37,35,33

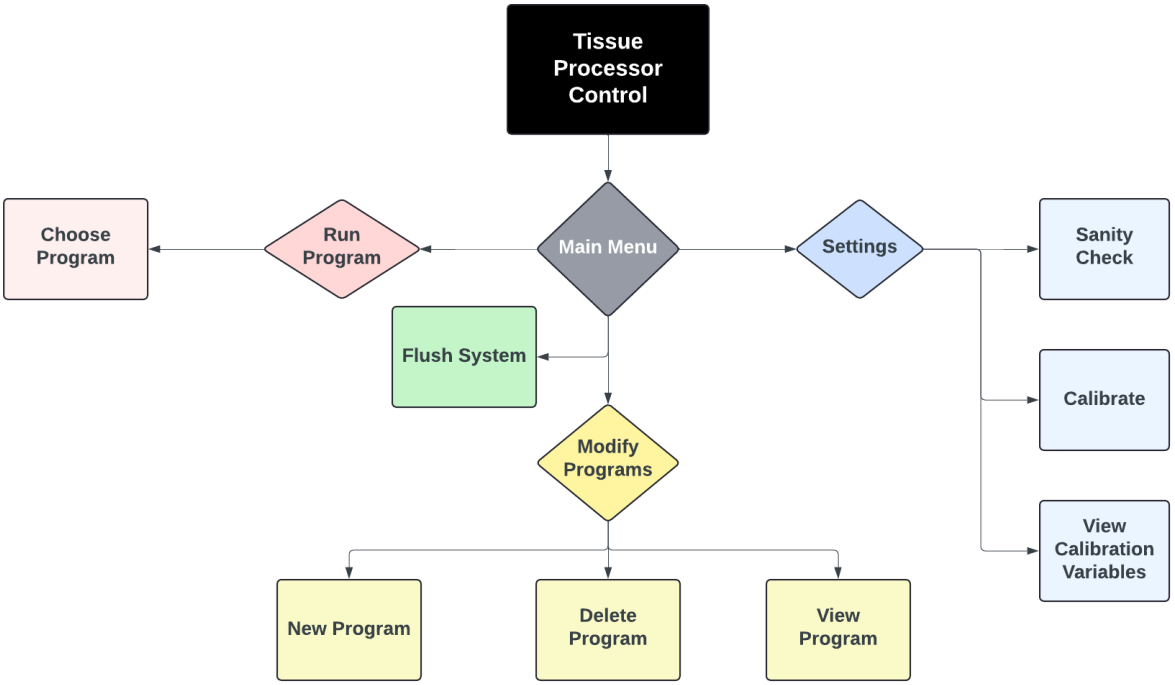

Fig. 4. Flowchart of all the options available in the software.

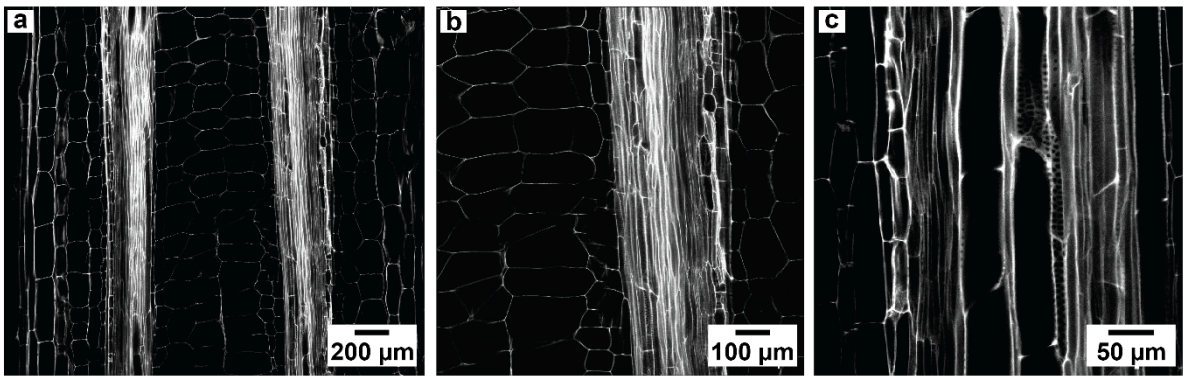

Fig. 5 a-c) Confocal images of PI stained tomato stems.