Self-Driven Lab: Custom Liquid Handler SOP

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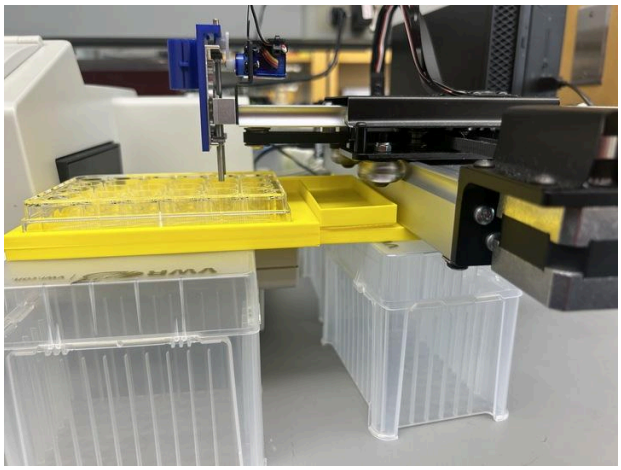
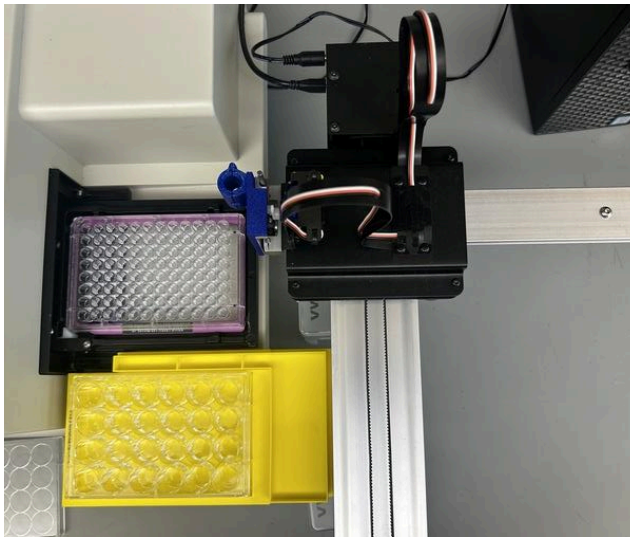
This SOP provides relevant information necessary for the overall viability and use of our device. However, it is reliant on basic understanding of other supplemental information: References to device setup will be attributed to the system build guide. References to ML/AL will be attributed to the Google Colab notebook. Some constraints of the device are in place to balance price effectiveness and system performance to provide a low cost example of a liquid handler.

Section 1. Hardware Setup

If a user creates a custom liquid handler based on the provided guide we would like to provide a small explanation of our example setup connected to a UV spectrophotometer. One of the major limitations of the device is its clearance when interacting with other devices to remedy this we used pipette tip holding boxes to elevate the device to the proper height. Any solid/stable object will work.



We align the device to the open tray of the UV spectrophotometer. The consistent measurement of this orientation is important as the positions of the measurement plate wells depend on your choice of positional values. (the positions attributed to the reagent wells and storage troughs should be always consistent as they are connected to the device).



Section 2. Software setup

To use the devices in the provided (or your own) python scripts it is important to download the relevant packages for the corresponding commands.
Axidraw - https://axidraw.com/doc/py_api/#introduction
One line install - python -m pip install https://cdn.evilmadscientist.com/dl/ad/public/AxiDraw_API.zip

Chemyx Syringe Pump -
https://chemyx.com/resources/knowledge-base/general-syringe-pump-info/computer-control-programs/python-program-installation-set-up-for-chemyx-fusion-200x-syringe-pump/

Section 3. Preparation For Experimentation

3.1. Calibration of Fluidic System

As you finish the construction of the fluidic system of the liquid handler there are additional setup steps before the device is truly experimentally ready.

3.1.1 Explanation of syringe choice and design

The complete design setup utilizes a two-syringe system that seeks to minimize variability of the syringe pump. Two 1ml syringes were chosen to leverage a smaller internal diameter to increase the reliability of dispense liquid at the flow rates used [Cite - Melanie A Jordan paper]. A longer syringe used within the system gives more available stroke length to leverage for better control of the stepper motor that controls the syringe pump. The discrete stopping positions of the syringe pump only allow for a specific volume of fluid to flow, and by having more discrete points it is possible to generate a greater number of specific volumes. The complete design setup utilizes a two-syringe system that seeks to minimize variability of the syringe pump. This is done by having longer syringes with small diameters. However, by optimizing for the most accuracy total volume does suffer. Larger syringes can be retrofitted into the design for an increase in possible sample volume for the cost of dispensing accuracy.

3.1.2 Storage Loop Setup

The fluidic system is based on a pressure driven syringe pump. To have the ability to draw and dispense small volumes using this system, we use a storage loop. This loop is filled with an incompressible experimentally inert fluid such as water to create consistent pressure using Pascal's principle. For this principle to be as true as possible, the minimization of air (bubbles) that is introduced into the storage solution is very important. To achieve as little air introduced into the syringes and loop the initial filling process should be done carefully.

To initially fill the syringes we fill a large vessel with the storage fluid (water) and fully submerge and evacuate the air that is trapped within. Then using the luer connectors reattach the partially filled

tubes, and evacuate any remaining air out of the tubes (this is generally done by flicking the side of the tubes to jostle the settled air bubbles.

To generally reduce the reintroduction of air bubbles in the system we were able to improve performance by the leveling of your storage loop tubing to reduce air lock formation and waterfall flow at local maximas in height.

Another possible introduction of air within your syringes itself is dependent on the syringe choice. Lower quality syringes may not have plunger tips with perfect seals when drawing or dispensing at high rates. Due to this phenomena sometimes occurring the reduction in overall flow rates may help to improve inaccuracies as a tradeoff to total experimental time.

3.1.3 Air Gap

To separate the storage loop fluid from your drawn reagents we utilize a small air gap. This idea is not perfectly ideal as it introduces pressure loss when interacting with a compressible gas. However, compared to alternative methods like using an immiscible fluid as the representative gap, issues of mixing are greatly lessened. When the' drawing protocol' is executed during experimentation this air gap is formed as the buffer and should keep as a single unit if general bubble prevention protocol is followed. If it is noticed that there is a particular breakup of the air gap it may be produced by alterations in the surface energy of the tubing. To reduce this general cleaning protocol may be necessary.

Section 4 Experimental Protocol

Section 4.1 Manual Tab

Provided with the experimental UI, we created a mechanism to independently control the spatial system for the purposes of manual setup (Due to the easy to use interface on the Chemyx syringe pump the manual UI does not have controls for the pump). The manual setup stage of the system is very important for preallocating global variables before any automation steps are tried.

4.1.1 Variable Setup

- speed_pendown, speed_penup

These values designate the added degree of speed of the needle tip moving up and down at a location (range 0-110)Values can be changed if one would like to optimize the total time of experimentation. (The notation of variables using 'pen' is based on the fact that the axidraw system usually uses its z axis to move a pen up and down for writing tasks)

- pen up/down position and limit

These values contain the desired "top" and "bottom" positions of the z axis (range 0-100). A top position at 100 signifies that we would like to have the initial position at maximum height. The bottom positions may be altered to dispense into a vessel without hitting its bottom (in our case the bottom of a single well). A known issue is droplet formation occurring and adhering at the needle tip. It may be relevant to obtain precise measurements of the dispensing/ drawing position to allow for the reduction of droplet formation interfering with the accuracy of the device. To find optimal positioning there will be some trial and error.

- Positional calibrations (X/Y positions)

Using the Manual UI you are able to alter the X/Y spatial position of the axidraw system. This capability is introduced to allow the user to identify the specific coordinates that correlate with their experimentation. This feature is used to locate the position of the first well, waste, reagent holders. However this gained knowledge must be transferred into the relevant points within the script.

1.3 Calibration Protocol

To conclude general setup we suggest using a calibration step to understand how well you have done with setting up the fluid system and identify the loss that is generated through interactions of fluid mechanics. Some additional loss factors that are intrinsic to the system such as interactions with the tubing itself can not be helped. Therefore, it is necessary to retroactively correct the volumes used for experimentation for the loss that is consistent in your system.

To do this we use the relationship between mass and volume with a fluid of known density. We execute a sweep of volumes across the probable dispensing range (5-200ul) (Manually controlled through the Chemyx UI). In between each volume dispensed we measure the change in mass using a zeroed scale. This is recorded in triplicates to obtain readings that compare the desired dispensing volume and the measured volume. Using this data we can identify any loss trends and calibrate the system to correct for the error. Finally using the new calibration factor we identify the new accuracy using the same method. Alternative methods that can be used to identify the accuracy at specific volumes may include slowly drawing the dispensed volumes using a pipette. However, this method may be slower and more susceptible to human error.

Section 4.2 Experimental tab

4.2.1 How to configure an experiment

You will need to design your parameter space, so you will need:

1. Reagent list with concentrations (and pHs if necessary)
2. Mixing Order
3. Reagent Concentration bounds (Final concentration in the well)
4. Desired activity (vMax)

5. The initial number of samples (Calculate the total volume of dispensing for each reagent and have it be under 1.6ml, including “filler”)

Capabilities of the program:
Can group reagents together, where each group gets a specific molarity based on the bounds of the reagents in the group. Then one of the reagents in the group will be selected. You can control what percentage of the seed library will contain reagents from a group. # Half of the seed library will contain reagents from a group.

Furthermore, you can add buffers, with different pHs. You can also have different buffer types, and then the program will select a buffer molarity, buffer type, and then a pH for each composition.

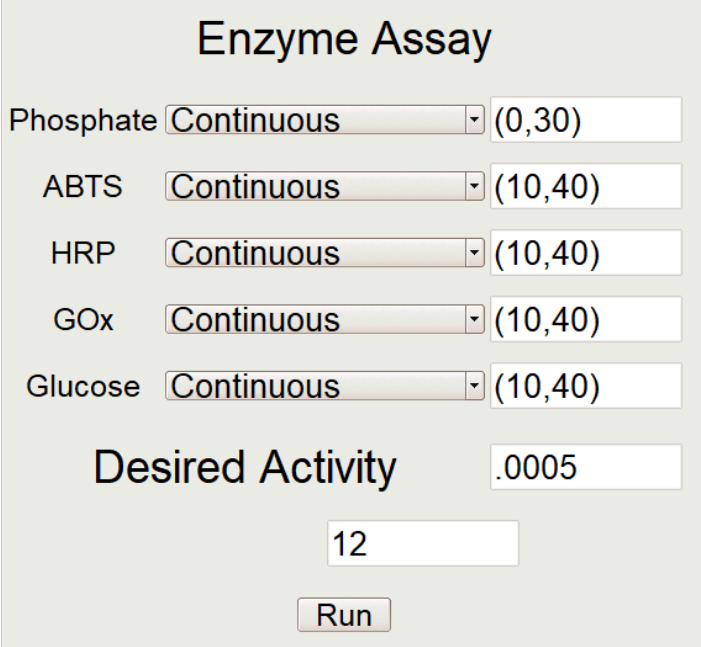
4.2.2 User Interface (UI) Info Guide

Once a wanted experimental pipeline has been thought up, one must use the UI to translate it into an automated loop.

1) You should first name the reagents in order of which they show up in your reagent reservoirs. The filler is not included in this bar, it is implied that the first reservoir is your filler material.
2) You must then identify if any reagent identifies. [i.e if you are using 3 different pH of the same buffer solution they would be in the same buffer 1 group]
3) Then you must input all reagent concentrations, each reagent concentration is non dimensionalized with respect to volume. [i.e. ABTS 200 is a 1X solution of 0.00173 mg/uL, while ABTS 100 represents a 0.5X solution] The order of concentration values determine the placement of your reagent reservoirs.
4) If a pH value is required [buffer group] you mark this reagent and leave all other reagents blank.

	Reagent 1	Reagent 2	Reagent 3	Reagent 4	Reagent 5	Reagent 6
Name	Phosphate	ABTS	HRP	GOx	Glucose	
Concentration	200	200,100,25,6.2	200	200	200	
Ph	7.4					
	Buffer 1	No Group	No Group	No Group	No Group	No Group

Non dimensionalized to volume : equivalent to the concentration value.



4.3. Notes on Experimentation

This section will identify helpful information for the development of your experimental procedures.

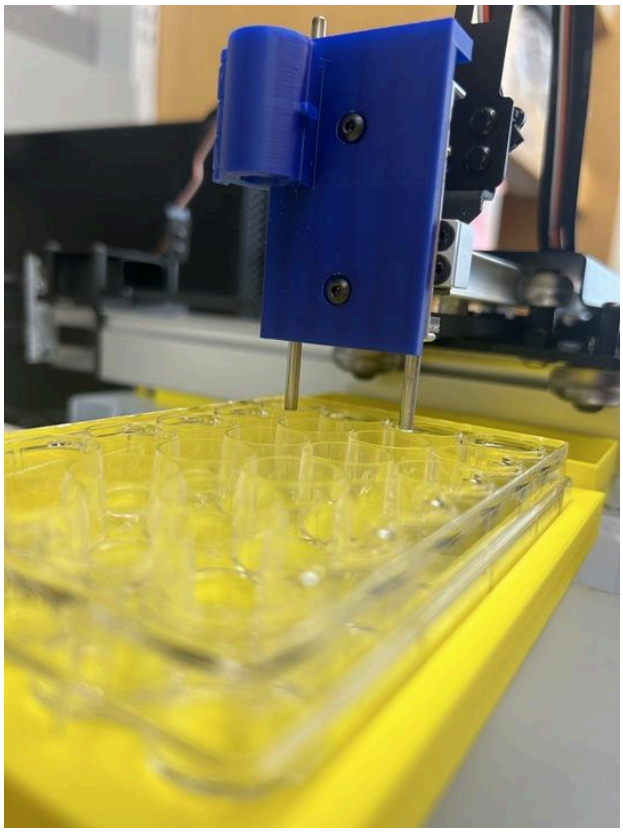
4.3.1 Choosing Reagent Order

A small note when setting up your design space and it is translated into the data sheet that runs the experiments. It is important to understand the ordering that the device will draw and dispense a specific reagent to formulate your mixtures. In our case, an enzymatic process does not begin its reaction until a specific substrate has been introduced. This required the ordering of the dispensed reagents to be tailored so that the reaction does not begin before we are ready to analyze the mixtures. The order may also need taking into account when thinking about possible mixing that could occur using the low cost system. Within the storage loop residue is reduced as much as it can with a cleaning stage, but the tubing may still have interactions with subsequent reagents. It may be necessary to order the reagents in order for reduction of fouling your experiment.

4.3.2 Reagent, Waste, Storage Solution, and Cleaning Troughs

For each component of your experiment the specific locations must be identified and logged into the script for use within the dispensing/drawing procedure. An issue that is apparent with the limited z axis depth is that to have additional objects within the range of the axidraw's capabilities it must not interfere with X/Y movement. This produces a strict height requirement. To work within this constraint we utilized a 24-well well plate to hold our reagents, this is to hold a larger amount of volume of individual reagents that can be used uninterrupted over each iteration.. Our waste, storage, and cleaning troughs are all held in in-house creations of 3D printed troughs to be within these parameters. As an added

complication to integrate with an analysis device it may be necessary to raise the entire device using stable structures to be able to correctly interface.



4.3.3 Compatibility (biologics, solvents, highly viscous materials)

Compatibility with solvents will be designated by tubing and luer products reference sheets. Compatibility with biological and viscous media may interfere with the fluid mechanics that drive our pressure driven system, however alterations in design or calibration may remedy these issues.

4.3.4 Cleaning Protocol

Standard care procedures should be taken into consideration. Flushing the system with a relevant cleaning solution after repeated use may be necessary. Replacement of disposable components (needle) may also be in order after repeated use. After cleaning protocol, it may be necessary to check the calibration.

Thank you for trying to use our device as a platform for a SDL. Please reach out if you have any further questions.