Supplementary Information for Automated Pipetting Robot for Proxy High-Throughput Viscometry of Newtonian Fluids

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1. Opentrons Setup

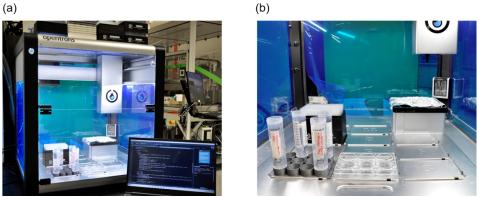


Figure S1. Experimental setup for the high-throughput viscometer. (a) Automated liquid handling robot Opentrons (OT-2). (b) Layout inside Opentrons, with the fluid reservoirs (bottom left) and dispense plate (bottom centre).

2. Opentrons Code

The protocol code and all supplementary files can be found at <u>https://github.com/Kedar-Materials-by-Design-Lab/Opentrons-ProxyViscometer</u> - the protocol could be run on sample fluids of unknown viscosity and the calibration curve presented in Fig. 3 used to characterize the viscosity of these fluids, subject to them being Newtonian.

The following labware is required to re-produce the experiment:

- Pipette tips 1000 µL wide bore tips (Thermo Scientific[™] 3591) were the primary choice in this study, with which the calibration curve in Fig. 3 was developed.
- Dispense well Corning 6 Well Plate 16.8 mL Flat

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• Reservoir for sample fluid - 50 mL Falcon® centrifuge tubes (VWR) supported in a custom-designed and 3D-printed holder

The pipette tips, well plate and centrifuge tubes can be purchased commercially, whilst details to make the centrifuge tubes holder can be found under the "Manufacturing Custom Labware" section below.

The Opentrons robot needs to know the geometries of all labware; the pipette tips and well plate are already pre-defined as labware options in the Opentrons labware library and the code is self-contained such that a custom labware definition for the sample fluid reservoir is directly included in the `OT-2_ProxyViscometerProtocol.py` file.

The following is a guide to get started with running the code on the Opentrons. Familiarity with the Opentrons is advantageous and the reader is directed to comprehensive documentation found at: <u>https://docs.opentrons.com/v2/</u>

2.1 Setting up the Code Locally

It is recommended that the user clones the <u>provided repository</u> from GitHub directly. In order to do this, one needs Git installed on their machine. If using Mac OS (or Linux), Git usually comes pre-installed by default. For instructions on how to install Git on Windows, please see the following: <u>https://github.com/git-guides/install-git</u>.

Next, create a new directory, e.g., named `OT-2_Viscometer` at a chosen location on your computer locally. Using either Git Bash on Windows, or the Terminal on Mac, cd into this directory and enter git init – this initialises the directory as a Git repository.

Now, type the following to clone the remote repository:

```
git clone https://github.com/Kedar-Materials-by-Design-Lab/Opentrons-
ProxyViscometer.git
```

*Note, if you are using Windows, you will want to close Git Bash and move into using either Command Prompt or PowerShell. Change directory into the `OT-2_Viscometer` folder or equivalent that you previously created.

Next, it is recommended to create a virtual environment in this directory to avoid any package dependency conflicts with other projects on your machine. (This is general programming advice, and more information can be found online.)

- Guide for Windows: <u>https://mothergeo-py.readthedocs.io/en/latest/development/how-to/venv-win.html</u>
- Guide for Mac OS: <u>https://sourabhbajaj.com/mac-setup/Python/virtualenv.html</u>

Ensuring that you have pip installed, enter pip install -r Opentrons-ProxyViscometer/requirements.txt - all the package dependencies for this project have been bundled into the `requirements.txt` file.

Now, type cd Opentrons-ProxyViscometer where all the cloned files, including the Python protocol to be uploaded to the Opentrons robot, can be found.

2.2 Opentrons Simulate (Optional)

N.b. This sub-section is entirely optional and does not need to be followed to execute the protocol on the OT-2 robot correctly, it may however be of interest to some readers.

Opentrons has developed a simulation function (<code>opentrons_simulate</code>) which can be used as a debugging tool and to verify that the instructions programmed in a particular Python protocol do indeed result in the robot actions that the user was expecting. More details are available at: https://support.opentrons.com/s/article/Simulating-OT-2-protocols-on-your-computer

If you are within the `Opentrons-ProxyViscometer` directory, where you should be from following the instructions in SI Section 2.1, then the following can be used to simulate on your machine:

- Windows (Command Prompt/Powershell): python -m opentrons.simulate OT-2_ProxyViscometerProtocol.py
- Mac (Terminal): opentrons_simulate OT-2_ProxyViscometerProtocol.py

The expected output is the step-by-step instructions which the OT-2 robot follows. The following shows the result of running the simulate function on the viscometer protocol: <u>https://github.com/Kedar-Materials-by-Design-Lab/Opentrons-</u> <u>ProxyViscometer/blob/main/OT Simulate-ViscometerProtocol.txt</u>

2.3 Running the Protocol on the Opentrons Robot

After following the steps in SI Section 2.1 the proxy viscometer protocol is available on your machine locally, ready to be uploaded to the OT-2. The following screenshots serve to guide you through the process of starting a run.

Please note the screenshots are from App Version 6.2.0 and any 6.x version of the Opentrons should look similar. (Opentrons implemented a significant UI change between the 5.x and 6.x app versions, yet the concepts remain the same.)

The protocol API version is 2.10.0 and the robot software and firmware versions are v6.2.0 and

v1.1.0-25e5cea, respectively, which support protocol API versions between versions 2.0 - 2.13, so there is both backward and forward compatibility.

The Opentrons app can be downloaded from: <u>https://opentrons.com/ot-app/</u>. Once you open the app and navigate to the protocols tab on the right, you will see the following.

Opentrons File Edit View Window Help		-	Ö	×
\odot				*
Protocols				
Labware				
Devices	Import a protocol to get started			
	Valid file types: Python files (.py) or Protocol Designer files (.json)			
	Choose File			
	<u>↑</u> .			
	Drag and drop or browse your files			
· · · ·				
	Don't have a protocol yet? Open Protocol Library (2: Open Protocol Designer (2: Open Python API Documentation (2:			
	open Protoco Lunary & open Protoco Designer & open Python APLOCOMENTATION &			
* 0				~

Upload the `OT-2_ProxyViscometerProtocol.py` file here and you will see it appear in the window as below.

 Opentrons File Edit View 	Window Help		- 0 X	
٨	Protocols		Sort by Alphabetical ~ Import	•
Protocols Labware Devices	10 11 10 1 10 1	OT-2_ProxyViscometerProtocol.py ROBOT ROBOT ROBOT POTOD Single-Channel GEN2	EUpdated Jan 19 23 11:11	

Clicking on the protocol results in the following details popping up.

OT-2_ProxyViscometerP		LAST UPD	ATED	LAST ANALYZED	_
Python API 2.10		Jan 19	23 11:11	Jan 19 23 11:11	Run
ORG/AUTHOR		DESCRIPT no data			
		Read M	fore		
Deck View	53	Robot Configuration	Labware		
		Robot OT-2			
10 11		Left Mount P100	0 Single-Channel GEN2		
8 9		Right Mount Empty			
5 6 8888 2 3					
löööö					

The "Deck View" on the left shows you where your labware should be placed. Ensure to setup the labware appropriately, then click on "Run protocol". This gives you the option to select the robot to run the protocol on. You can set-up the OT-2 robot, either over WiFi (see how: <u>https://support.opentrons.com/s/article/Get-started-Connect-to-your-OT-2-over-Wi-Fi-optional</u>) or over a USB connection, as shown here.

Gile Edit Mar				
File Edit View	w Window Help Protocols > OT-2_ProxyViscometerProtocol.py			
٢	OT-2_ProxyViscometerProtocol.py	· · · · · · · · · · · · · · · · · · ·		Choose Robot to Run OT- 2_ProxyViscometerProtocol.py
	01-2_rroxyviscometerrrotocol.py			
Protocols Labware	CREATION METHOD Python API 2.10	LAST UPDATED Jan 19 23 11:11	LAST ANALYZIO Jan 19 23 11:11	01.2 0122CEP20190618A25
Devices	ORGIAUTHOR no data	DESCRIPTION no data		
		Read More		Connect over
	Deck View	Robot Configuration Labware		WiFi or USB
		Robot OT-2		
	10 11	Left Mount P1000 Single-Channel GEN2		
	8 9	Right Mount Empty		
	888 z 3			
* 0				Proceed to setup

This takes you to the following screen. Here the 1st set-up step checks that the robot itself has been calibrated, which you can see here that it has been.

RUN 01/19/2023 11:13:34	STATUS RUN TIME Not started	8	► Start ru
PROTOCOL START	PROTOCOL END		Cancel run
Setup Run Log			
STEP 1 Robot Calibration Review required pipettes and tip length calibr	itions for this protocol.		 Calibration Ready
STEP 2 Labware Setup Position full tip racks and labware in the deck	slots as shown in the deck map.		

If you scroll down, there's the option to perform a "Run Labware Position Check" (highly recommended). The instructions are easy to follow on screen. This calibrates any required offsets to the nearest 0.1 mm, so that the robot moves to the positions accurately when picking up the pipette tips, etc.

Devic	dow Help es > OT2CEP20190618A25 > 01/19/2023 11:13:34						
ocols ware		OPENTRONS 96 FILTER TIP RACK 1000 ML	8	9			
ices		CORNING 6 WELL PLATE CORNING 6 WELL PLATE	5	6			
		AMDM 12 50ML FALCON TUBE	2	3			
						Recomme	ended
	Labware Position Check and Labware Offset	Data					
	Labware Position Check is a recommended work labware in the X, Y and Z directions.	flow that helps you verify the position of each lab	ware on the deck. During t	his check, you can create La	bware Offsets that adj	ust how the robot moves to each	
	See How Labware Offsets Work					Run Labware Position Check]
0			Proceed to run				

When the labware position check is complete, click "Proceed to run".

01	r-2_ProxyVisc	ometerProtocol.py				
RUN 01/	/19/2023 11:13:	34	STATUS Not started	RUN TIME		Start ru
	ROTOCOL START		PROTOCOL END			Cancel run
Set	tup Run Lo	8				
Ru	In Log 344 steps	total			t	ownload ru
PS	ROTOCOL SETUP					~
1	Start:() End:()	Picking up tip from A1 of O	pentrons 96 Filter Tip Rack 1000 µL in 7			
2	Start:::- End:::-	Moving to A1 of AMDM 12 5	i0ml falcon tube on 1			
3	Start:::- End:::	Delaying for 0 minutes and	10.0 seconds			
4	Start:() End:()	Touching tip				
5	Start:() End:()	Touching tip				
6	Start: End:	Touching tip				
	Start:	Touching tip				

Finally, one can start the run with the button at the top right.

3. Manufacturing Custom Labware

To run the experiment, as shown, one needs to use the `falcon_tube_holder.stl` file (in the `Labware_Files` directory of the provided GitHub repo) to 3d-print the MTP-sized labware holder for the 50 mL Falcon® centrifuge tubes (VWR).

The holder was printed on the Prusa SL1S, using the Elegoo water washable 4k grey resin, at 50-micron layer height and 2.5 seconds per layer exposure time. The part was oriented in such to not require supports. Then the part was washed and dried, after which it was cured in the Prusa CW1S for 6 minutes.

Additionally, the Autodesk Fusion (Computer-Aided Design) file for the customised 1 mL pipette tip shown in Figure 4b has been provided as `CustomDesignedTip_1000uL.f3d` as an initial example of the many possibilities via which the design of the pipette tips could be modified to alter the range of viscosities measurable with the presented methodology.

4. Raw Data

The raw data used to plot the calibration curve in Fig. 3 is available at: <u>https://github.com/Kedar-Materials-by-Design-Lab/Opentrons-</u> ProxyViscometer/blob/main/Data%20SI.xlsx