

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

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1. Opentrons Setup

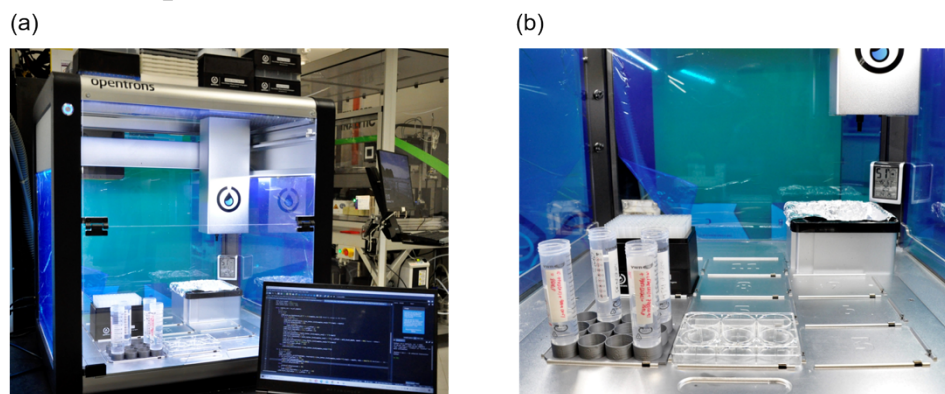


Figure S1. Experimental setup for the high-throughput viscometer. (a) Automated liquid handling robot Opentrons (OT-2). (b) Layout inside Opentrons, with the fluid reservoirs (bottom left) and dispense plate (bottom centre).

2. Opentrons Code

The protocol code and all supplementary files can be found at <https://github.com/Kedar-Materials-by-Design-Lab/Opentrons-ProxyViscometer> - 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 μ L 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: <https://docs.opentrons.com/v2/>

2.1 Setting up the Code Locally

It is recommended that the user clones the [provided repository](#) 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: <https://github.com/git-guides/install-git>.

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

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 (`opentrons_simulate`) 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: https://github.com/Kedar-Materials-by-Design-Lab/Opentrons-ProxyViscometer/blob/main/OT_Simulate-ViscometerProtocol.txt

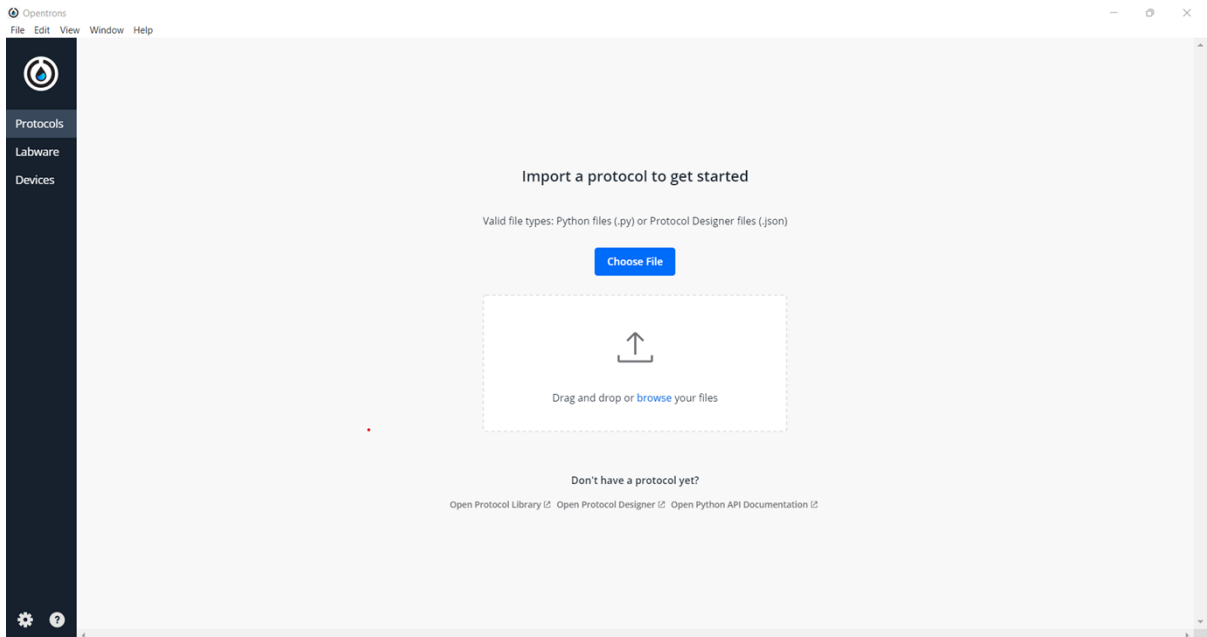
2.3 Running the Protocol on the Opentrons Robot

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.

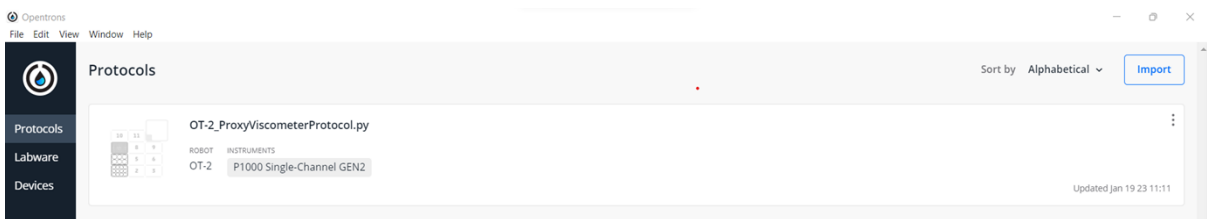
Please note the screenshots are from App Version 6.2.0 and any 6.x version of the Opentrons should look similar. (Opentrons implemented a significant UI change between the 5.x and 6.x app versions, yet the concepts remain the same.)

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.

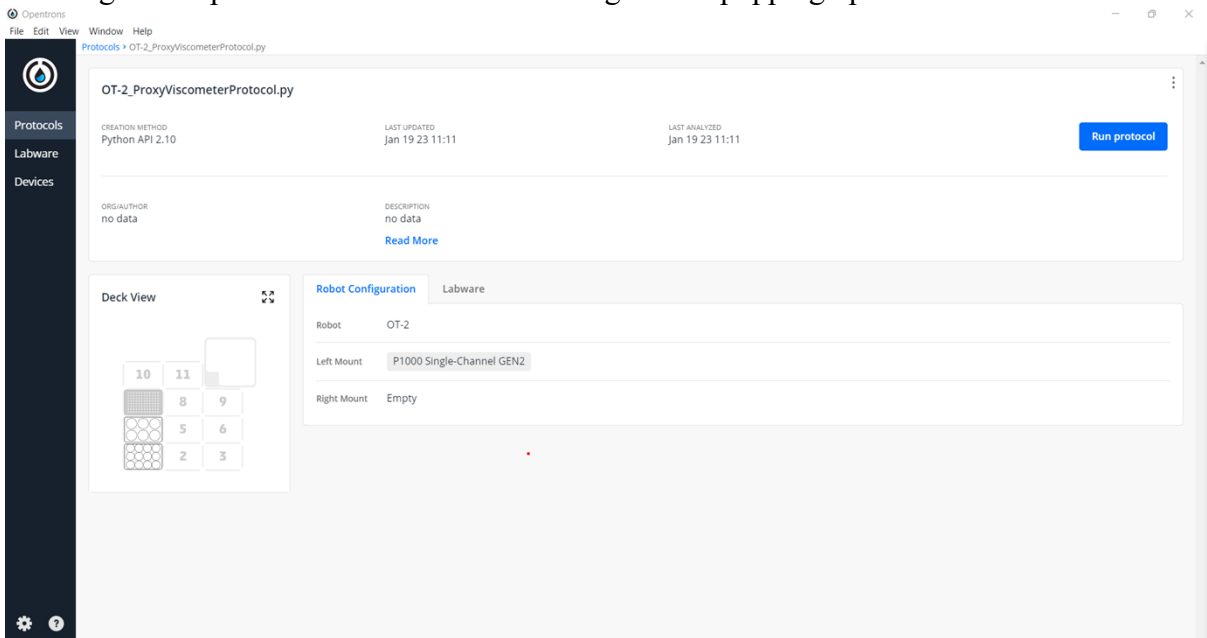
The Opentrons app can be downloaded from: <https://opentrons.com/ot-app/>. Once you open the app and navigate to the protocols tab on the right, you will see the following.



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

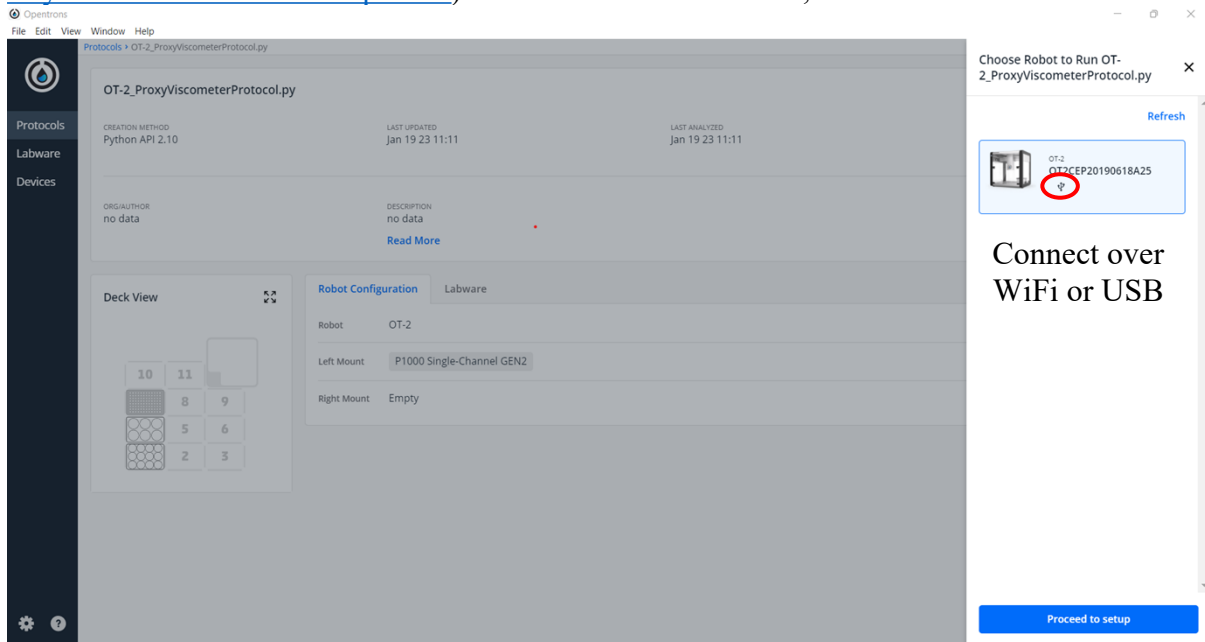


Clicking on the protocol results in the following details popping up.

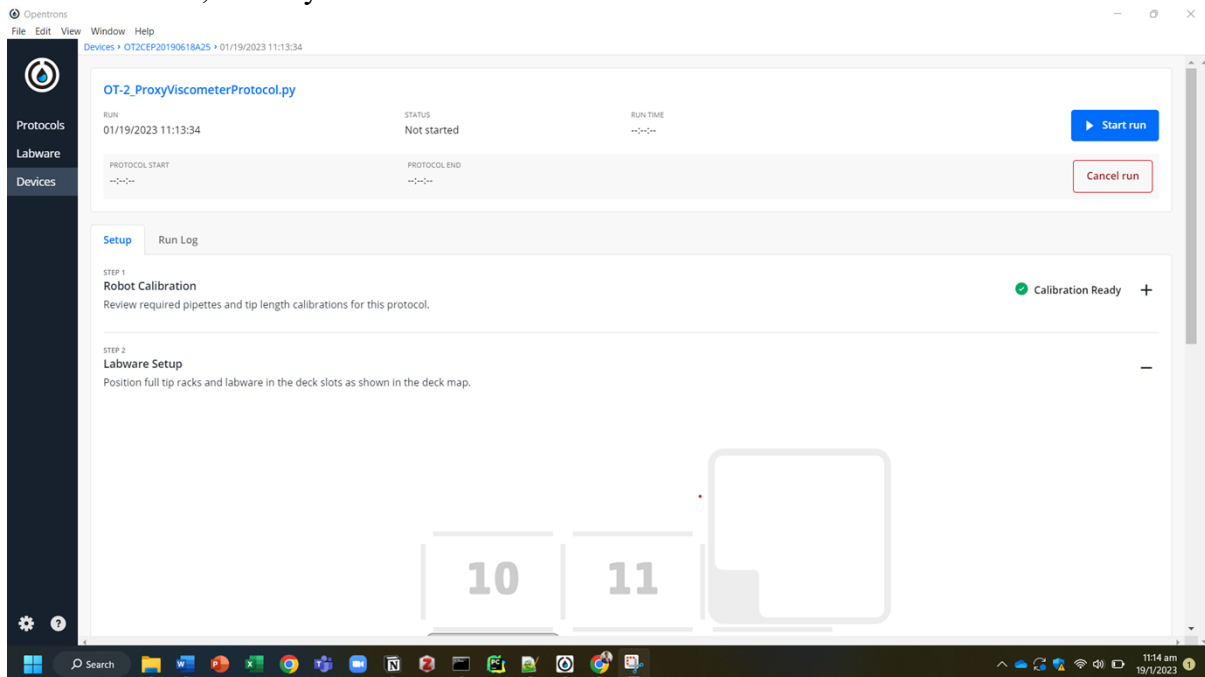


The “Deck View” on the left shows you where your labware should be placed. Ensure to set-up the labware appropriately, then click on “Run protocol”.

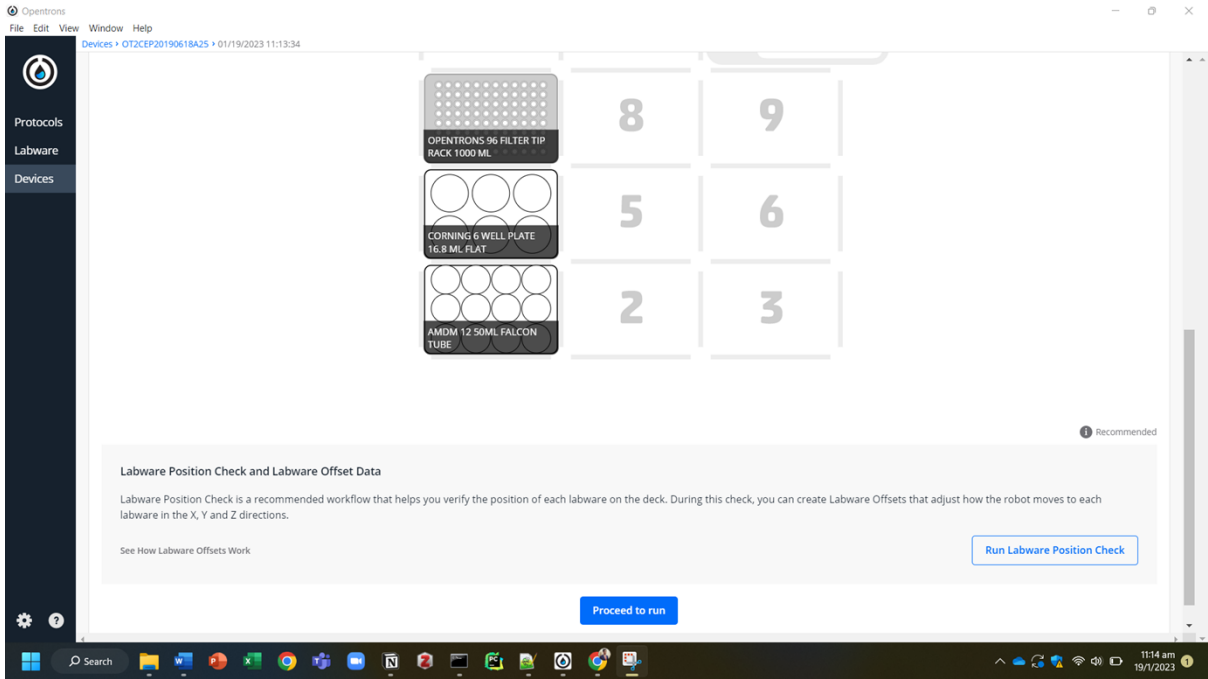
This gives you the option to select the robot to run the protocol on. You can set-up the OT-2 robot, either over WiFi (see how: <https://support.opentrons.com/s/article/Get-started-Connect-to-your-OT-2-over-Wi-Fi-optional>) or over a USB connection, as shown here.



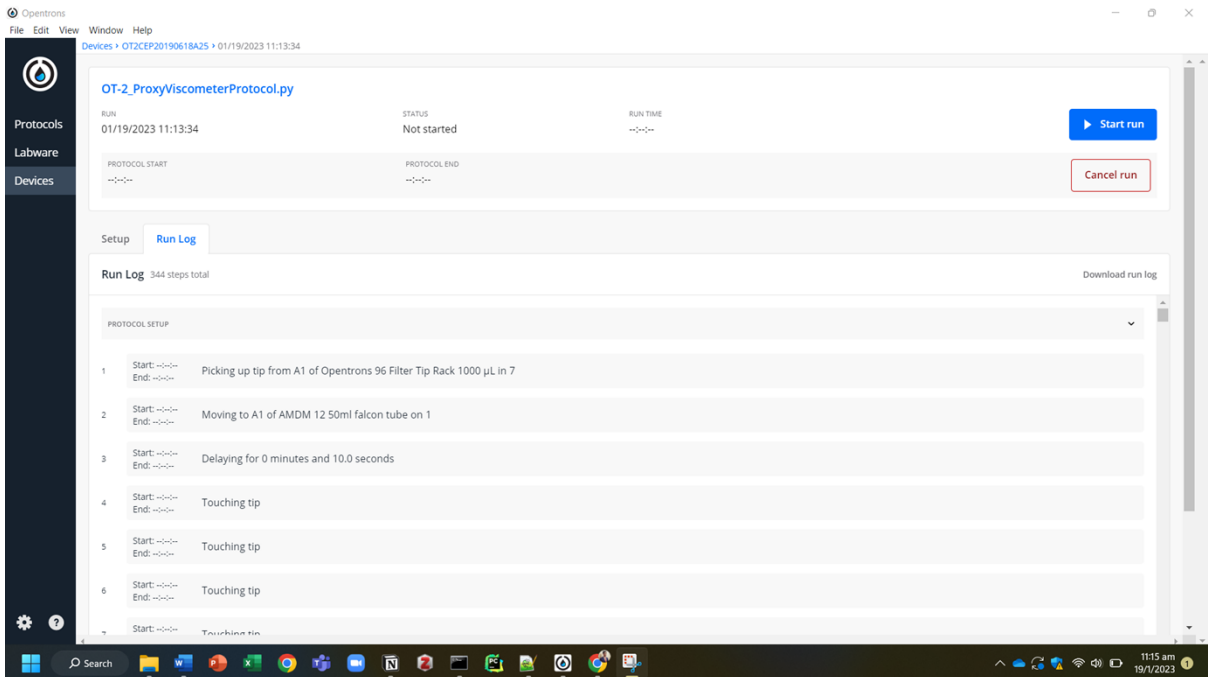
This takes you to the following screen. Here the 1st set-up step checks that the robot itself has been calibrated, which you can see here that it has been.



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.



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



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: <https://github.com/Kedar-Materials-by-Design-Lab/Openetrans-ProxyViscometer/blob/main/Data%20SI.xlsx>