Mass Balance Integration with the Opentrons OT-2 Robot

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ABSTRACT

Accurate automated pipetting of viscous liquids can be difficult with air displacement pipetting. By integrating a mass balance with the Opentrons OT-2 Robot, users can automate gravimetric experiments and accurately record how much liquid mass is added to a sample over time. Researchers can perform retrospective calculations to determine the precise formulation of the final sample. With the addition of a mass balance, the OT-2 can serve as a proxy viscometer by detecting dispensing errors for a fixed set of liquid handling parameters. Further, it increases the capacity of the OT-2 for novel applications. Here, we provide a step-by-step guide to integrating a mass balance inside an OT-2 robot.

INTRODUCTION

A mass balance can be incorporated with the Opentrons (see **Figure 1**) to extend its capabilities to perform gravimetric experiments. The Opentrons pipettes are calibrated using such a gravimetric analysis. However, there was no existing module nor methodology to integrate a mass balance inside the liquid handling robot. Here we show a minimally invasive method to integrate a mass balance inside the Opentrons' OT-2 robot, occupying only one of the deck's eleven slots. The method relies on rapid prototyping via 3d-printing; all design files are shared. Additionally, a code is presented to communicate with the balance thereby facilitating automated gravimetric analysis. The capacity to perform such experiments can be helpful in a range of scenarios from dealing with fluids which are difficult to pipette accurately to more creative applications such as utilising the OT-2 as a proxy viscometer.[1]

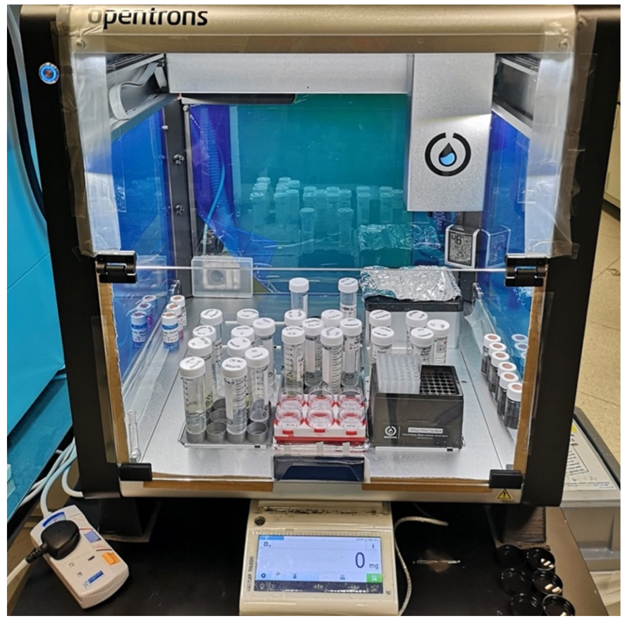

Figure 1. Retrofitted Opentrons OT-2 robot with a Mettler Toledo precision balance.

METHODOLOGY

Software: Digital Communications with the Balance

To integrate a balance with the OT-2 robot, select a balance suitable for digital communications. For example, Mettler Toledo's MS303TS/00 precision balance was selected, which can be controlled via serial communication using Python's Pyserial package. The RS232 to USB interface makes it easy to connect the balance to a local computer in the lab. Details for how to run the Opentrons balance are presented in a separate sub-section after the hardware integration.

Hardware: How to Integrate the Balance Inside the OT-2?

To physically integrate the balance within the OT-2, slot #2 on the Opentrons deck was machined away by an external vendor to the following request: machine the hole in place of slot 2 so that the edges are flush with the separation walls, following the technical drawing attached in the "design-files" on GitHub (https://github.com/Kedar-Materials-by-Design-Lab/opentrons-balance) (**Figure 2**).

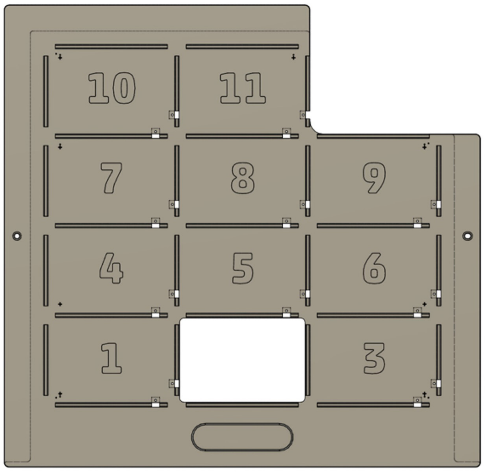

Figure 2. Cut-out slot #2 from the Opentrons deck.

The OT-2 balance was then raised on 90 mm high 3d-printed polycarbonate feet, printed with 6 perimeters at 0.6mm nozzle using Prusament PC blend filament, with 10% gyroid infill (Opentrons_feet.3mf file with pre-set configurations provided on the GitHub repository). The design for these feet is shown in **Figure 3**. An M8 hex bolt needs to be inserted into the printed feet and used to replace the original feet.

Next, the draft shield around the balance must be taken down. Please follow the instructions in the balance manual. Additionally, the weighing pan must be removed, thereby exposing the balance's load cell. A mass balance adapter comprising four parts, as shown in **Figure 4**, was designed to replace the weighing pan and make the balance compatible with the geometry of the cut-out Opentrons slot (i.e., an MTP well-plate).

- The top & bottom halves were printed on Prusa SL1S, using the Elegoo water washable 4k grey resin, at 50-micron layer height and 2.5 seconds per layer exposure time. Parts were oriented in such as to not require supports. After being washed and dried, the parts were cured in the Prusa CW1S for 6 minutes.

- The top plate & clip were printed on Prusa Mk3S+, using Prusament ASA. Both parts were printed using a 0.4 mm nozzle, 0.2 mm layer height, 4 perimeters, 5 bottom layers, 6 top layers, and 15% cubic infill. All parts were oriented in such a way as to not require support structures.

After all the parts were printed, additional pieces of approximately 5cm long Prusament ASA filament were inserted into the top plate to form a tight loop, as shown in **Figure 5b**, with the ends melted in place to lock the position. These loops acted as a spring to firmly hold the well-plate during the experiments.

The mass balance adapter must weigh the same mass, to the mg, as the weighing pan being replaced. The top half, as seen in **Figure 4a**, was designed hollow so that it could be filled with heavy materials till it matched the required weight. Here loose nuts & bolts were used to achieve

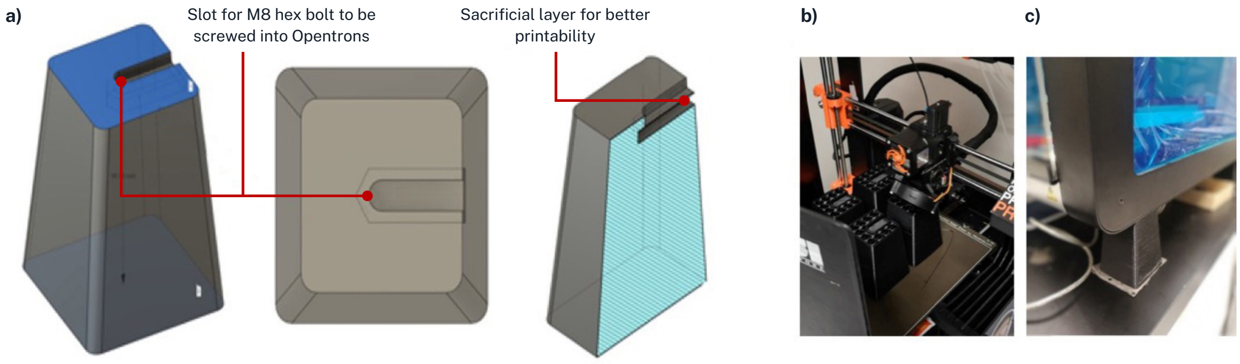

Figure 3. a) Design for the new Opentrons feet which are, b) 3d-printed, c) and after they have been installed the height of the Opentrons is raised such that a balance can be slotted underneath.

this. Such an approach was selected for cheap (less of the relatively expensive 3d-printing filament is used) and rapid prototyping, however, to make a more polished product, one could design a solid print, not a hollow one, to match the mass of the weighing pan. With the presented design the top plate can simply fit onto the top half, but if aiming for a solid print these two parts could be designed together too. The design should however remain purposefully split into a bottom and top half due to the part annotated in **Figure 4a**. The balance's load cell has a groove into which the balance adapter must fit, however, this groove is offset at an angle. The angle was neither made available by the manufacturer nor is it the same across different balances even of the same model (MS303TS/00). The presented design in **Figure 4** allows the bottom half to be rotated independently such that it slots in and then the clip can be used to fix the top half on such that the piece fits correctly through the cut-out slot of the Opentrons deck. With this, the balance adapter is complete and should look as shown in **Figure 5**.

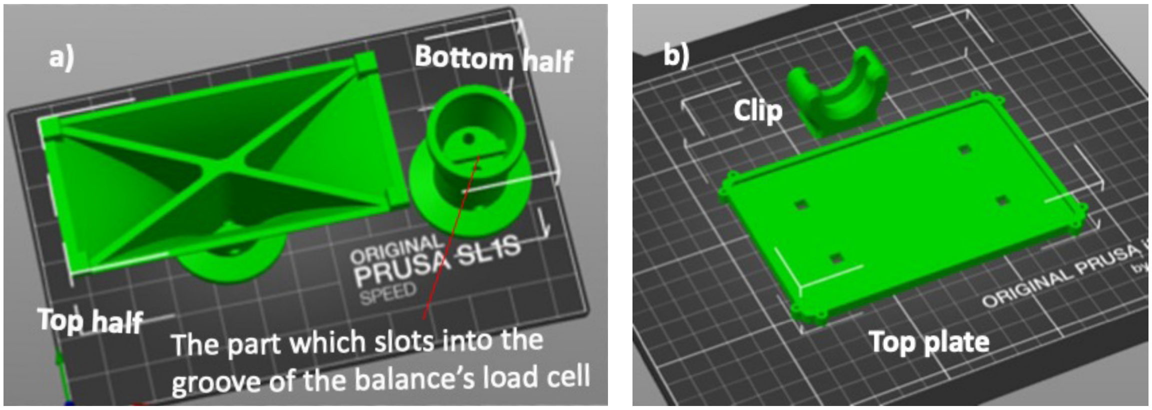

Figure 4. Parts to 3d-print the mass balance adapter: a) from "Top_half+Bottom_half.3mf", b) from "Clip+top_plate.3mf" with both files being found under "design-files" on the provided GitHub repository.

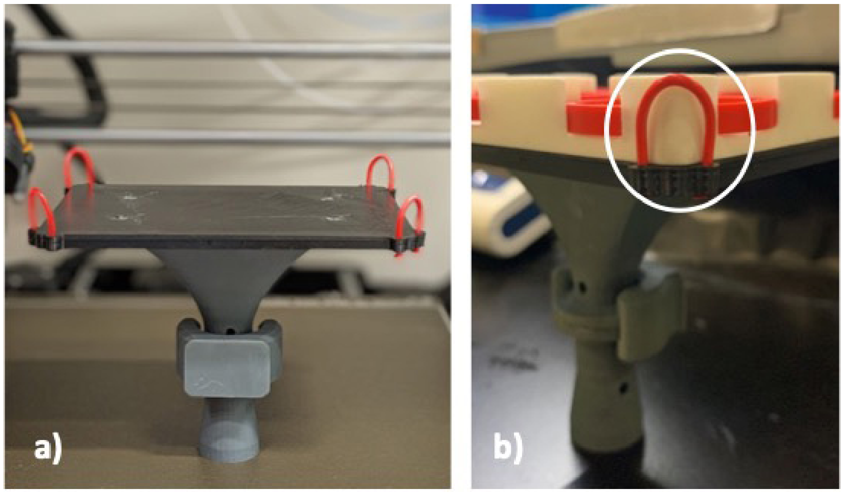

Figure 5. a) Assembled mass balance adapter (front-on view). The bottom part sits over the balance's load cell and any MTP-compatible labware can be loaded on the top plate; b) Side-angle view of the adapter highlighting the loop holder which secures loaded labware in place.

The balance adapter can hold any standard MTP labware. Frequently, the labware definition for these pieces is already made available in Opentrons' labware library, however, here, as the labware will be raised on the balance, a custom labware definition will need to be created. This can be easily done using: https://labware.opentrons.com/create/. Shown in **Figure 6** is the balance adapter holding a couple of different 6-well plate options.

Software: Running the Integrated OT-2 Balance

In addition to hosting the design files on the GitHub repository, its primary purpose is to share the code for running the Opentrons balance. Firstly, it is recommended to set up a virtual environment and initialise a Git repository on your computer locally. Then, please "`git clone` [`https://github.com/Kedar-Materials-by-Design-Lab/opentrons-balance`](https://github.com/Kedar-Materials-by-Design-Lab/opentrons-balance)" (+ "`pip install -r requirements.txt`") and open the "OT-2_BalanceAutomation" Jupyter Notebook. Note that the balance is not directly connected to the OT-2, as the Raspberry Pi computer onboard the Opentrons is not capable of supporting non-native Python packages, *e.g.*, matplotlib, which is used for live visualisation of the logged mass data. Instead, the logging of the mass data must be run concurrently *via* the Jupyter Notebook along with the standard execution of whichever Opentrons protocol you wish to run using the Opentrons App. To be clear, you are running your Opentrons protocol as usual and then simply logging the balance data in parallel, which can then be processed further, for the intended use case.

The Jupyter Notebook is fully commented out for ease of use; however, a summary of steps is provided here. The first cell to be run will import any Python packages required. And then, as shown in **Figure 7**, the port for connecting the balance to the computer needs to be selected.

Then you have a couple of options in Section 2 of the notebook for data collection: (i) live visualisation of the mass vs. time data, or (ii) simply logging the data without plotting. **Figure 8** shows screenshots of using either option, including which button (circled in red) to terminate data logging once the Opentrons protocol is complete.

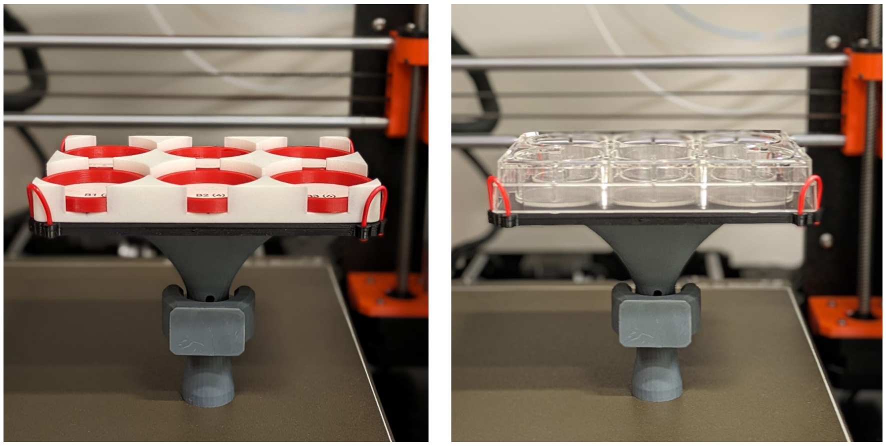

Figure 6. The mass balance adapter is compatible and interchangeable with different labware.

1. Port for Mass Balance

Find the port number on Windows through Device Manager or on Mac by running this command (`ls /dev/tty.*`) on the Terminal

```
port = '/dev/tty.usbserial-14130' # Mac
#port = 'COM5' # Windows
```

Figure 7. Set the port to connect the balance to the computer.

And finally, the last section of the code saves the data into (i) a Pandas dataframe, which is a convenient format to analyse your data in, and (ii) a .CSV file to be exported.

RESULTS AND DISCUSSION

Successfully following the presented methodology should result in the Opentrons now appearing as shown in **Figure 1**. One may wish to consider that as we have removed the balance's draft shield, for it to continue functioning with high precision and accuracy, blocking the airflows and minimising the vibrations around the instrument is important. The first step is to seal any air gaps around the Opentrons, particularly around the newly installed feet of the Opentrons. (Simply using tape suffices.) Otherwise, airflows from below will disturb the balance readings. Additionally, there is an exhaust at the back of the OT-2 robot, this should be covered too. If possible, place the retrofitted Opentrons setup on an anti-vibration table. This is relatively common practice to put high-precision balances on such a "balance table" as it helps mechanically decouple the setup from other lab equipment, helping achieve a reliable baseline performance. However, this is not a necessity. For example, as seen from the tared balance in **Figure 8a** (used without a balance table), a stable zero reading is recorded. And with this, the integration of the mass balance with the OT-2 is complete.

The integrated mass balance can be used in a variety of applications and is most useful when dealing with difficult-to-pipette liquids, *e.g.*, viscous fluids. **Figure 9** shows an example application in a liquid formulation project where a wide range of ingredients are being used to prepare shampoo formulations. The ingredients ranged from moderately to highly viscous (≈ 100 – 10,000+ cP) so it was very challenging to accurately dispense the ingredients using the air displacement pipetting technology of the Opentrons robot, even after following the guidance from the viscous liquids handling application note.[2] However, in said project, if one could accurately know the composition of the prepared samples that would suffice. Therefore, by recording the mass vs. time data as shown for a sample run in **Figure 9**, we can back-calculate and analyse the amount of ingredient added for each formulation and thereby its composition. In the case of viscous liquids handling, one needs to customise the liquid handling parameters for each fluid. This can be a very laborious task, however, with the integration of the mass balance one could perform automated gravimetric experiments and even envisage the self-optimisation of the liquid handling parameters. Furthermore, Soh et al. have shown that the Opentrons could also be used as a proxy viscometer by recording the error in set dispenses for a fixed set of liquid handling parameters; utilising the knowledge that the Opentrons will under-dispense viscous fluids with its default settings.[1] These are just some examples, and it is hoped through open-sourcing this tool with the Opentrons community, the interested parties would find novel applications for their use cases.

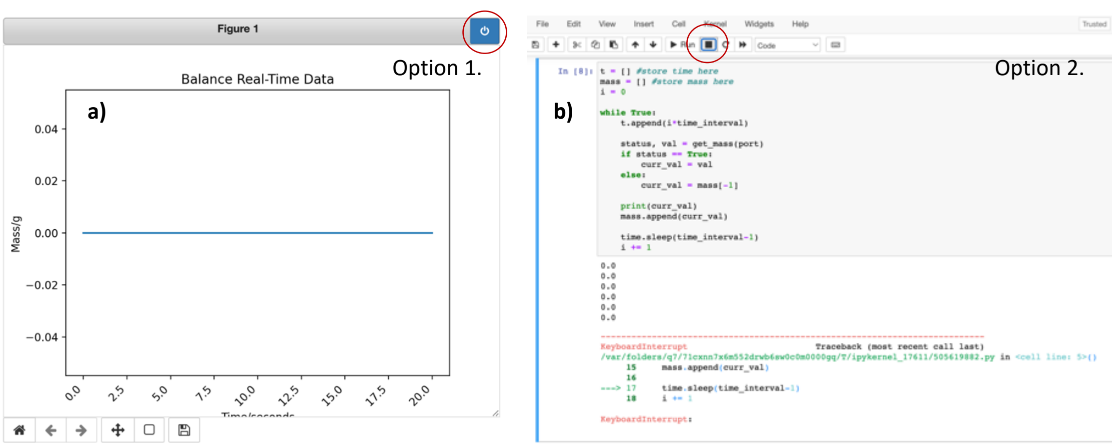

Figure 8. a) Live plotted mass data (option 1) from a tared balance; b) Logging data into a list (option 2).

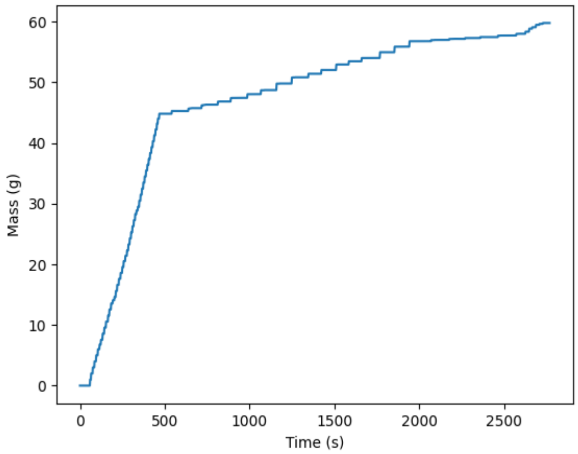

Figure 9. Illustrative mass vs. time profile using the mass balance integrated with the Opentrons OT-2 robot.

CONCLUSION

This application note presents how to integrate a mass balance with the Opentrons OT-2 robot. A similar method could also be followed for the newer Opentrons Flex. Access to machining tools and 3d-printing is required to replicate the hardware modifications. All the design files to reproduce the complete modifications are provided as supplementary information under the linked GitHub repository: https://github.com/Kedar-Materials-by-Design-Lab/opentrons-balance. This additionally contains a fully-commented Jupyter Notebook to communicate with the balance. With this retrofitted setup, the user can run automated gravimetric experiments, which are particularly useful for handling difficult-to-pipette fluids.

REFERENCES

1. Soh, B. W.; Chitre, A.; Lee, W. Y.; Bash, D.; Kumar, J. N.; Hippalgaonkar, K. Automated Pipetting Robot for Proxy High-Throughput Viscometry of Newtonian Fluids. *Digital Discovery* **2023**. https://doi.org/10.1039/D2DD00126H.
2. Kanase, A.; Watson, K. Viscous Liquid Handling Automation Using Opentrons OT-2, **2021**.