Intro to Opentrons Electronic Pipettes
Precision, Accuracy, Design, and Testing Methods
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This document and the data contained within will be updated regularly as we continue to make improvements and additions to the OT-2 and its electronic pipettes. While we are confident in the data and methods published here, we highly value dialogue with the scientific community and welcome feedback on how they can be improved. To provide transparency, we are making our raw data available in the Supplementary Data section of this document. Testing scripts and additional materials, as well as data on more pipettes, will be made available soon.
Introduction

The OT-2 from Opentrons is an affordable, easy-to-use pipetting robot that uses integrated electronic pipettes to transfer liquids and run experiments for biologists in their labs. These electronic pipettes are a novel design created by Opentrons, and provide scientists with fast, accurate, and precise liquid transfers for a fraction of the price of other lab automation systems.

This paper describes the data we've collected to ensure the accuracy and precision of our instruments, as well as the methodologies used to produce that data. We have performed gravimetric and photometric tests under real-world testing conditions similar to those our users will experience in their own labs.

Because of our commitment to open-source and reproducible science, we have also attempted to make these results easy for our users to replicate in their laboratories, as well as provided guidelines for ongoing quality analysis of their Opentrons pipettes.
OT-2 Electronic Pipettes

The OT-2 Electronic Pipettes are the first of their kind. They easily couple with the OT-2 robot's gantry, so the robot can move them precisely in XYZ space while actuating the pipette motors (more on this below).

These pipettes are based on the same principles as the manual pipettes biologists use every day. Upon reading this paper, scientists will find they are already familiar with many of the details of these new electronic pipettes (volume ranges, channels, tip use, etc), given how closely they resemble trusted manual tools they've used so often themselves.

**Figure 1**: Single-Channel Electronic Pipette  **Figure 2**: 8-Channel Electronic Pipette
Electronic Pipette Design

Opentrons electronic pipettes take the classic micropipette design and simply replace the scientists’ thumbs with electric motors. We use robust micro-stepper motors attached to a precision-molded volume chamber. The volume chamber is a cylinder with a piston that converts the linear motion of the motor into the desired volumetric output inside the attached disposable tips. The tip cones are made from a hard composite plastic designed to pick up and eject hundreds of thousands of tips over the lifetime of the pipette.

![Pipette Design Cutaway Diagram with Parts Labeled]

**Figure 3:** Pipette Design Cutaway Diagram with Parts Labeled
Volume Calculation

The accuracy of our liquid transfers is dependent on a correct conversion of millimeters traveled by the motor lead screw into volumetric microliter output within the volume chamber. The volume displaced by the plunger is calculated using the following formula:

$$\Delta V = \pi * r_{plunger}^2 * \Delta d$$

$$\Delta V = \text{change in volume (uL)}, \ r = \text{radius (mm)}, \ \Delta d = \text{distance (mm)}$$

The first conversion that needs to happen is between the motor's microsteps and the linear motion of its lead screw. Stepper motors divide a full rotation into a series of “steps” that are moved to sequentially in order to turn the lead screw. Each step of the motor’s rotation therefore translates into a corresponding precise distance of the lead screw.

Because the lead screw is connected directly to the piston in the volume cylinder, the steps per mm conversion factor defines the relationship between linear motion and volumetric output within the tip, which is the same relationship as is found in standard piston-based manual pipettes. The correct steps to mm conversion was determined empirically by collecting volume data on each pipette at different volumes with gravimetric and photometric analysis as described below in the methods sections. Once enough data was collected, we applied a curve to the data to find the appropriate function for each pipette model.

After determining the microliters per mm function for a given pipette model, the ‘constant volume’ script was executed to test both the precision (%CV) and accuracy (%d) of the pipette, which are discussed in the data analysis section below.
Gravimetric Testing Methods and Materials

Gravimetric capabilities and principles:

Gravimetry -- the measurement of weight -- is a well-accepted standard and recognized in the scientific literature as a good methodology for assessing liquid handling performance [1]. It is also relatively simple to carry out and only requires an analytical balance, an instrument found in most labs.

In using gravimetry we’re taking advantage of the straightforward mass-to-volume ratio of water -- that is, 1mL of water weighs approximately 1 gram [2]. Therefore, water volume can be measured reliably on an analytical balance with the appropriate sensitivity.

Limits to gravimetric analysis:

Gravimetry is limited by the precision of the analytical balance being used and the environmental conditions around it. When measuring small volumes, tiny variations in the lab environment like those caused by OT-2 robot’s movements or a person walking close to the balance will lead to skewed results in normal laboratory settings. Additionally, gravimetry is affected by a wide variety of other environmental conditions, including evaporation, static electricity, vibration, temperature, relative humidity, and more [3].

It is important to note that, as liquid volumes become smaller, both vibrational and environmental effects become more pronounced. This is why, to ensure accurate measurement, we have limited our gravimetric analysis to volumes larger than 10ul.

Gravimetric testing instrumentation and materials:

- Analytical balance with a 0.01 mg precision and USB connection (Radwag, AS82/220.R2)
- Low retention pipette tips (Eppendorf Low-Retention, 022493004)
- OT-2 Z-stage and Z motors
- Optical table by ThorLabs
- Custom testing rig made of 8/20 aluminum rail
- Windows 10 laptop computer with Microsoft Excel
- Analytical grade water (Corning, 46-000-CV, Lot 27017005)

We have developed a custom rig that allows us to collect many gravimetric data points quickly while keeping measurement conditions the same across time (pictured below in Figure 4).

The Z-Stage head design from our OT-2 robot was mounted on a standard 80/20 aluminum extrusion. The pipettes are attached in the same manner as they are to the OT-2 robot so that they could perform the same linear motion done during a typical aspirate or dispense command. A customized chamber made from acrylic was placed around the rig to limit
airflow through the fixture, which could otherwise interfere heavily with testing volumes less than 100 uL. The rig was also placed on an optical table to dampen vibrations.

In order to reduce evaporation effects, the surface area of the water was reduced to a size of a Falcon cap. Since static electricity is another factor that affects accuracy in measurement, we used specialized tips that are less hydrophobic (Eppendorf Low-Retention, 022493004) and a new tip was used for each reading.

The measurements were recorded from an analytical balance with a 0.01 mg precision (Radwag, AS82/220.R2) in a spreadsheet on a Windows 10 PC connected over USB 2.0 to the scale.

**Figure 4: Gravimetric Test Fixture**

**Gravimetric Experimental Method:**

Using the pipette being analyzed, water was aspirated and dispensed in place sequentially onto an analytical balance with a 0.01 mg precision (Radwag, AS82/220.R2). After waiting 2 seconds for the balance to level out after each aspirate and dispense, a measurement was recorded in a spreadsheet on a Windows 10 laptop computer attached to the scale over USB. For each measurement, 10 readings were taken sequentially. For a Multi-Channel Pipette, each channel was tested individually using the same methodology.

Data from the analytical balance was averaged and normalized automatically with the constant volume test script [4] developed by Opentrons test engineers to account for
random noise in the balance and fluctuations due to environmental conditions such as evaporation.

Photometric Testing Methods and Materials

**Photometric analysis capabilities and principles:**

The photometric validation method for pipetting performance uses a spectrophotometer and stable dyes that absorb light in the visible or UV range. A beam of light at a specified wavelength is passed through a solution of dye and the detector in the spectrophotometer measures the quantity of light that passes through. The amount of light absorbed by the solution is directly proportional to the concentration of dye present in the solution according to the Beer-Lambert Law. [5] This direct relationship allows us to make calculations to determine the volume of dye delivered by our pipettes.

Photometry is a well-accepted method for assessing pipetting performance, and is particularly suited to assess volumes less than or equal to 10uL [6]. It’s much less sensitive to environmental fluctuations than gravimetry [7] and can provide good information about each channel in a multichannel device in a streamlined manner. We chose to use a straightforward peer-reviewed single-dye method described in the Journal of Laboratory Automation (now SLAS Technology) to assess the performance of our pipettes at volumes below 10uL [8].

**Limits to photometric analysis:**

While photometric analysis provides better data at low volumes than gravimetric analysis in a typical laboratory environment, it is a more complicated and lengthy process that requires special dye and more expensive equipment (specifically, a 96 well plate format spectrophotometer). In addition, when comparing results to manually-pipetted standard curves as described below, the operator must make sure to correct for possible variance in the dye preparation and manual procedure by using trusted, well-calibrated pipettes and adding additional verification steps to the method. Since gravimetry is more straightforward for higher volumes, we have used photometric analysis primarily for volumes less than or equal to 10ul.

**Photometric instrumentation and materials:**

- Analytical grade water (Corning, 46-000-CV, Lot 27017005)
- Optical grade 96-well flat bottom microplate (Corning, Special Optics 3720)
- Orange G dye (Fisher Chemical, O267-25, Lot 133297)
- Automation-friendly plate seals (Biochromato, R80.120.00)
- Tecan Infinite F200 Pro spectrometer with Magellan analysis software
- 10ul Opentrons pipette tips (GEB, PT0010-9-NS)
- Windows 10 laptop computer with Microsoft Excel
**Photometric experimental method:**

**Preparation of dye solutions**

We prepared 250 mL of a 10mg/mL stock solution of Orange G dye (Fisher Chemical, O267-25, Lot 133297) in a foil-wrapped 500mL glass bottle using molecular biology grade water (Corning, 46-000-CV, Lot 27017005). While Orange G is described as relatively photostable in solution [9], the foil provided extra assurance that the dye would not photodegrade over time. The solution was then mixed vigorously on a magnetic stirrer overnight (>16h). We used the same stock solution for each test to reduce variables and ensure consistency in results.

We then prepared 1.33 mg/mL working solution as described in the Stangegaard et al paper referenced above, hereafter referred to as WS-A. This solution was prepared as needed in a 50 mL Falcon tube using our Orange G stock solution and molecular biology grade water.

**Preparation of standards**

A six-step standard series was prepared in 15 mL Falcon tubes using WS-A according to Table 1 below. All standards were prepared using manual DragonLabs pipettes.

<table>
<thead>
<tr>
<th>Standard</th>
<th>WS-A Volume (uL)</th>
<th>Water Volume (mL)</th>
<th>Final Concentration (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STD1</td>
<td>750 uL</td>
<td>9.25 mL</td>
<td>0.09975</td>
</tr>
<tr>
<td>STD2</td>
<td>500 uL</td>
<td>9.5 mL</td>
<td>0.0665</td>
</tr>
<tr>
<td>STD3</td>
<td>250 uL</td>
<td>9.75 mL</td>
<td>0.03325</td>
</tr>
<tr>
<td>STD4</td>
<td>100 uL</td>
<td>9.9 mL</td>
<td>0.0133</td>
</tr>
<tr>
<td>STD5</td>
<td>50 uL</td>
<td>9.95 mL</td>
<td>0.00665</td>
</tr>
<tr>
<td>STD6</td>
<td>25 uL</td>
<td>9.975 mL</td>
<td>0.003325</td>
</tr>
</tbody>
</table>

**Table 1**: Preparation of Standards with Working Solution A.
Plate preparation

Each test was run on a 64 wells of an optical grade 96-well flat bottom microplate (Corning, Special Optics 3720), with the other 24 wells reserved for standards and blanks. Standards were plated in quadruplicate using a DragonLabs manual pipette in columns 1 - 4 of test plate as seen in Figure 5 below. Then, 200 uL of standard was added to each well. Water was plated using a DragonLabs manual pipette in wells G1 - G4 to establish a series of blanks. Wells H1 - H4 were left empty.

The remaining wells are used for the analysis samples. The plates were prepared with molecular biology grade water in each of the sample wells so that the volume of water plus the testing volume equals 200 uL. So, for example, if we are testing the pipette at 10 ul, the sample wells would be prepared with 190 uL of water. The final configuration of the test plates can be seen in Figure 5 below.

After preparation, each plate was covered with an automation-friendly plate seal (Biochromato, R80.120.00) prior to its placement on the deck.

Figure 5: Photometric test plate layout
Volume testing procedure

We instructed the OT-2 to use the pipette being tested to transfer the target volumes of dye from a reservoir to the sample wells in the test plate using a new disposable tip (GEB, PT0010-9-NS) for each well.

We attempted to use best practice pipetting techniques for each test, including tip pre-wet, precise plunger speeds for both aspirate and dispense, exact tip placement within the well, and more. These best practices were established through iterative testing, and are now implemented as consistent behaviors for each OT-2 electronic pipette. These behaviors are captured in our python test scripts[10] written with the Opentrons API [11].

Additionally, an automation friendly plate seal (Biochromato, R80.120.00) was put onto each test plate to ensure accurate liquid transfers. The plate seal was used to reduce evaporation effects and to prevent carry-over liquid from the outside of the tip from going into the well, while ensuring that all liquid was transferring from the inside the tip into the well.

Photometric volume reading

After the OT-2 completed the test protocol and filled the testing wells with dye at the target volume, the plates were put into a microplate spectrophotometer for analysis (Tecan Infinite F200 Pro equipped with 450 nm absorbance filter).

The Tecan plate reader is equipped with a movable XY stage with shaking capabilities, so we added a process to our plate reader method that shook the plates before they were read. This ensured that the dye was evenly incorporated into the solution before reading. For users with plate readers that do not have this capability, we suggest placing the prepared test plate(s) onto an orbital shaker at 150 rpm for at least 10 minutes prior to reading.

Absorbance was read at 450 nm using our Tecan spectrometer with accompanying Magellan analysis software.
Data Analysis

Gravimetric data processing:

For our gravimetric tests, the test script extracted the measured weights from the analytical balance and output the values in a CSV file format that was then imported into an Excel spreadsheet. The weights were then converted directly into volumes according to the direct relationship between the density and volume of water, and averaged to find the test mean.

Photometric data processing:

For our photometric tests, we constructed a method in the Magellan software that takes factors such as standard curve and blank reduction into account, then exports the raw data into Excel. We verified each standard curve in Excel by averaging the absorbance readings from the standards and calculating the curve using linear regression. Plates with a curve carrying an $R^2$ value of >0.99 were accepted for final data analysis. Each plate was read twice in the spectrophotometer to ensure consistent results.

Using the standard curve, the Magellan software calculated the concentrations of each sample in the series and exported those concentrations to Excel.

The following formula was used to convert these concentrations into volumes:

$$V_f = \frac{C_{\text{well}} * V_W}{C_{\text{sol}}} \times 1000 \ \text{uL/mL}$$

- $V_f$ is the volume of the working solution in a given well (in uL).
- $C_{\text{well}}$ is the concentration in a given well found using Beer’s Law (in mg/mL).
- $V_W$ is the total volume found in a given well (0.2 mL).
- $C_{\text{sol}}$ is the concentration of the working solution used (in mg/mL).

Precision and accuracy calculations

The calculated volumes from both gravimetric and photometric testing were then averaged and compared with the intended volumes. We assessed random error, or precision, using the following equation:

$$CV = \frac{\sigma}{\bar{X}} \times 100$$

- $CV$ is the coefficient of variance (expressed as a percentage).
- $\sigma$ is the standard deviation of the sample set.
- $\bar{X}$ is the mean of all of the sample volumes.
We assessed systematic error, or accuracy, using the following equation:

\[ d = \left[ \frac{(\bar{X} - V_{\text{test}})}{V_{\text{test}}} \right] \times 100 \]

\( d \) is the systematic error (expressed as a percentage).
\( \bar{X} \) is the mean of all of the sample volumes.
\( V_{\text{test}} \) is the specified test volume.
Precision and Accuracy Data

<table>
<thead>
<tr>
<th>Pipette</th>
<th>Target Volume (uL)</th>
<th>Random Error (CV)</th>
<th>Systematic Error (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>uL</td>
</tr>
<tr>
<td>p10 single channel</td>
<td>10</td>
<td>±0.5</td>
<td>±0.05</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>±3</td>
<td>±0.15</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>±5</td>
<td>±0.05</td>
</tr>
<tr>
<td>p10 8-channel</td>
<td>10</td>
<td>±2</td>
<td>±0.2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>±5</td>
<td>±0.25</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>±5</td>
<td>±0.05</td>
</tr>
<tr>
<td>p300 single channel</td>
<td>300</td>
<td>±0.3</td>
<td>±0.9</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>±0.4</td>
<td>±0.6</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>±1.5</td>
<td>±0.45</td>
</tr>
<tr>
<td>p300 8-channel</td>
<td>300</td>
<td>±0.5</td>
<td>±1.5</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>±0.8</td>
<td>±1.2</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>±2.5</td>
<td>±0.75</td>
</tr>
</tbody>
</table>

Table 2: Precision and accuracy of OT-2 Electronic Pipettes at Target Volumes

Data Discussion

When compared with other liquid handling robot performance data freely available online [12], the data above shows that the OT-2 performs as well as robots 10x more expensive. Our novel electronic pipette designs have proven capable of the precision and accuracy required in executing delicate biological experiments in common laboratory environments.

We are also making our raw data and analysis scripts open-source to allow our users to see the full picture (including the breakdown on individual pipettes). It is our hope that this makes it easy for others to then reproduce this data in their own labs, and even share more data with us to be incorporated into later versions of this paper. We will be releasing more pipettes in the future, and will include regular updates and test results for those here as well.
Supplementary Data

Below you can find the raw data from the accuracy and precision tests run in the Opentrons lab. These links are continually updated with additional data as it becomes available.

**p10 Single Channel**
- [https://drive.google.com/drive/folders/1OaT0K09PrIamUTeKFXzIOhCZCv4CnDXz?usp=sharing](https://drive.google.com/drive/folders/1OaT0K09PrIamUTeKFXzIOhCZCv4CnDXz?usp=sharing)

**p10 8-Channel**
- [https://drive.google.com/drive/folders/1SbOUQ3K-En3yL7PetLBQhZ2pVW9kXGJ4?usp=sharing](https://drive.google.com/drive/folders/1SbOUQ3K-En3yL7PetLBQhZ2pVW9kXGJ4?usp=sharing)

**P300 Single Channel**
- [https://drive.google.com/drive/folders/1JcXUVpRXuOsjqVqWbctUTRN_lGbo71R6?usp=sharing](https://drive.google.com/drive/folders/1JcXUVpRXuOsjqVqWbctUTRN_lGbo71R6?usp=sharing)

**P300 8-Channel**
- [https://drive.google.com/open?id=1T_Subqgi59_Hu0W4kHMtu_0ljWlRmuaA](https://drive.google.com/open?id=1T_Subqgi59_Hu0W4kHMtu_0ljWlRmuaA)
Citations


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We want to thank the entire Opentrons team, from Shenzhen to Brooklyn. Without the collective efforts of our amazing design, marketing, support, engineering, production, and scientific teams, this product would not have become a reality.

We are also grateful to our scientific advisors, notably Dr. Stephen Ekker, who has provided us with excellent feedback on the test methods and analysis techniques presented in this document.

Finally, we’d like to thank the Opentrons community. All of the scientists who have used OT-One, beta tested OT-2, given us valuable feedback, and contributed to our open-source platform have helped us to build better, more user-friendly, and more collaborative tools for scientists everywhere.