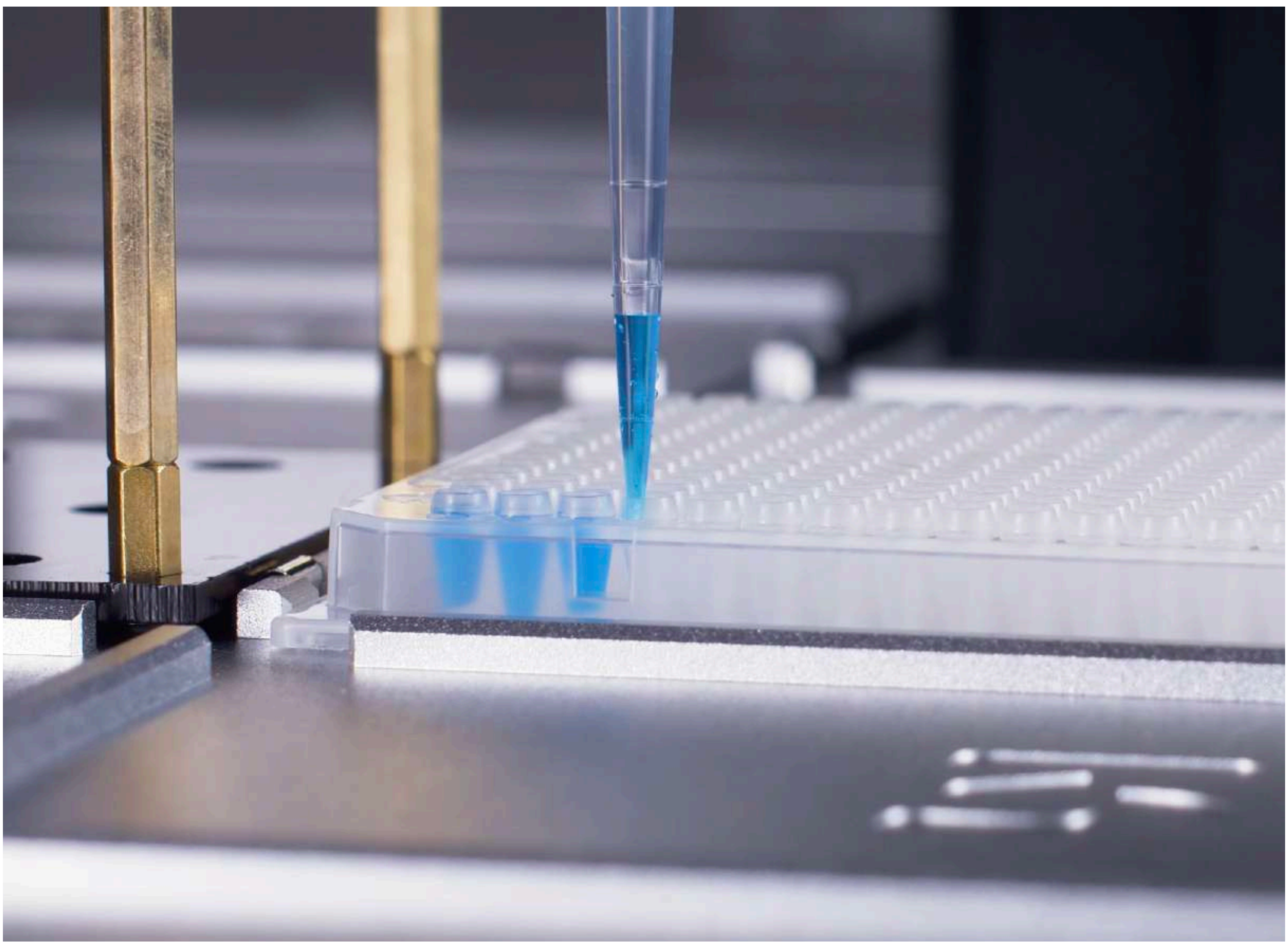
WHITE PAPER

Intro to Opentrons Electronic Pipettes **GEN1**

Precision, Accuracy, Design, and Testing Methods

Written by
Opentrons



SECTION 1

Table of Contents

NOTE: All information contained in this whitepaper directly references Opentrons GEN1 Pipettes. If you need information about Opentrons GEN2 pipettes, please refer to the GEN2 whitepaper.

SECTION 2

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 tests under real-world testing conditions similar to those our users will experience in their own labs.

SECTION 3

Product Design and Specs

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).

FIGURE 1

Single-Channel Electronic Pipette

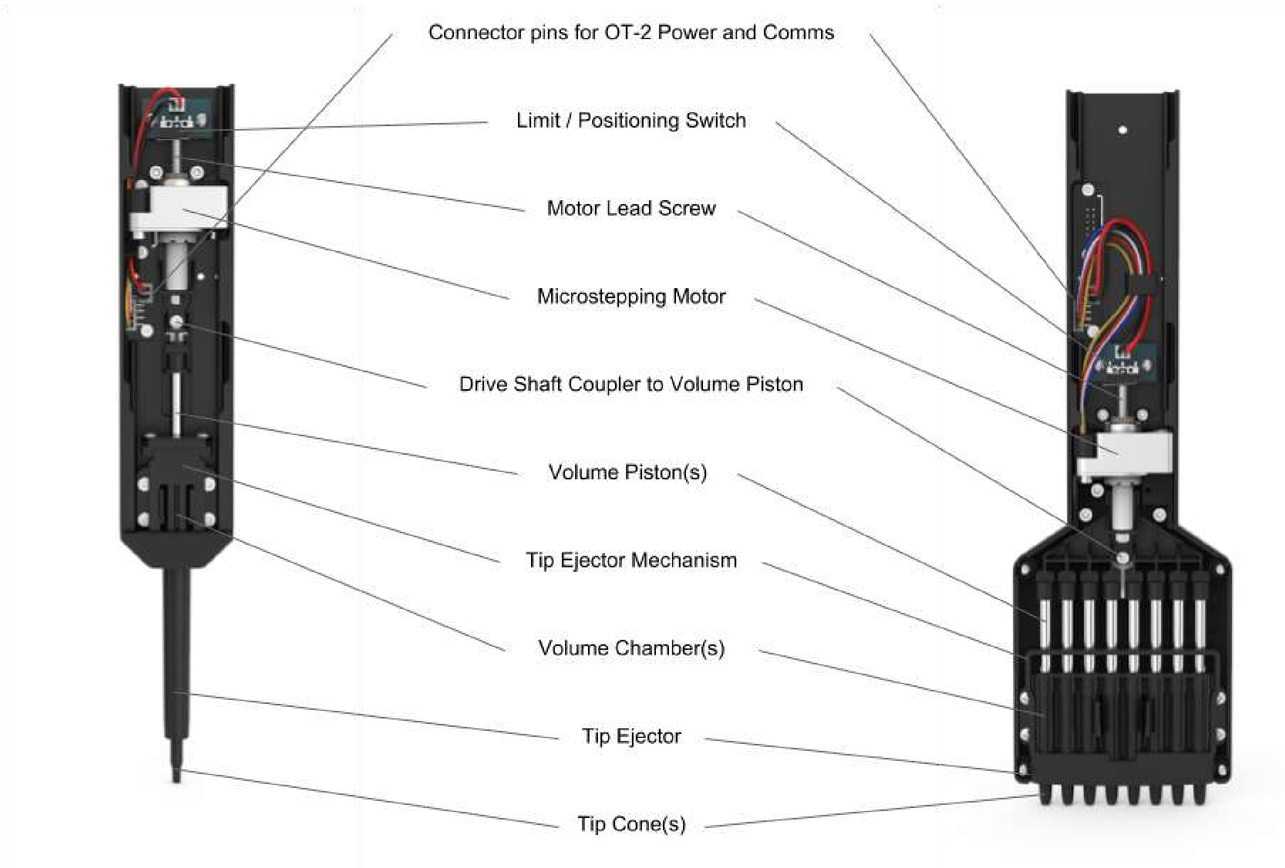
FIGURE 2

8-Channel Electronic Pipette

Opentrons electronic pipettes take the classic micropipette design and simply replace the scientists' thumbs with electric motors. We use robust a stepper motor attached to a precision-ground linear shaft. The shaft creates a seal with an o-ring to create a piston (or volume chamber) which accurately controls the volume of liquid aspirated and dispensed inside the disposable tips. The tip cones are made from a hard polypropylene plastic designed to pick up and eject 100,000 tips over the lifetime of the pipette.

FIGURE 3

Pipette Design Cutaway Diagram with Parts Labeled



VOLUME CALCULATION

The accuracy of our liquid transfers is dependent on the conversion of millimeters traveled by the pipette lead screw into a corresponding change in fluid within the pipette tip.

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 travel distance of the lead screw.

Because the lead screw is connected directly to the piston in the volume cylinder, we can translate the mm traveled to the volumetric change at the tip. This is the same relationship as is found in standard piston-based manual pipettes. The correct mm to µl conversion was determined empirically by collecting volume data on each pipette at different volumes with gravimetric 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, advertised in the Testing Data section of this whitepaper

SECTION 4

Testing Methods

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 from 1 µL to 10 µL.

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 with modified deck**
- **Optical table by ThorLabs**
- **Windows 10 laptop computer with Microsoft Excel**
- **Analytical grade water (Corning, 46-000-CV, Lot 27017005)**
- **Draft protection (to ensure the scale does not provide false readings from the air moving in the testing room)**

We have developed a custom rig that allows us to collect many gravimetric data points quickly while keeping measurement conditions the same across time.

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 µl. 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.

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.

SECTION 5

Testing Data

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.

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:

$$CV = \frac{\sigma}{\overline{X}} * 100$$

CV is the coefficient of variance (expressed as a percentage).

σ is the standard deviation of the sample set.

X is the mean of all of the sample volumes.

We assessed systematic error, or accuracy, using the following equation:

$$d = \left[\frac{(\overline{X} - V\,test)}{V\,test} \right] * 100$$

d is the systematic error (expressed as a percentage).

X is the mean of all of the sample volumes.

V_{test} is the specified test volume.

TABLE 1

Precision and Accuracy of OT-2 Electronic Pipettes at Target Volumes

PIPETTE	TARGET VOLUME (µL)	RANDOM (%CV)		SYSTEMATIC (%d)	
		%	µL	%	µL
P10 Single Channel	10	+/- 1	+/- 0.1	+/- 2	+/- 0.2
	5	+/- 3	+/- 0.15	+/- 5	+/- 0.25
	1	+/- 5	+/- 0.05	+/- 15	+/- 0.15
P50 Single Channel	50	+/- .4	+/- 0.2	+/- 1	+/- 0.5
	25	+/- 0.6	+/- 0.15	+/- 1.5	+/- .375
	5	+/- 5	+/- 0.25	+/- 5	+/- .25
P300 Single Channel	300	+/-0.3	+/- 0.9	+/- .6	+/- 1.8
	150	+/- 0.4	+/- 0.6	+/- 1	+/- 1.5
	30	+/- 1.5	+/- 0.45	+/- 3	+/- 0.9
P1000 Single Channel	1000	+/- .15	+/- 1.5	+/- .7	+/- 7
	500	+/- 0.2	+/- 1	+/- 1	+/- 5
	100	+/- 1	+/- 1	+/- 2	+/- 2
P10 8-Channel	10	+/- 2	+/- 0.2	+/- 3	+/- 0.3
	5	+/- 5	+/- 0.25	+/- 5	+/- 0.25
	1	+/- 10	+/- 0.1	+/- 25	+/- 0.25
P50 8-Channel	50	+/- 0.6	+/- 0.3	+/- 1.2	+/- 0.6
	25	+/- 1.5	+/- 0.3.75	+/- 2	+/- 0.5
	5	+/- 5	+/- 0.25	+/- 5	+/- 0.25
P300 8-Channel	300	+/- 0.5	+/- 1.5	+/- 1.5	+/- 4.5
	150	+/- 0.8	+/- 1.2	+/- 2.5	+/- 3.75
	30	+/- 2.5	+/- 0.75	+/- 5	+/- 1.5

DATA DISCUSSION

When compared with other liquid handling robot performance data freely available online [5], 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.

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**
- **P10 8-Channel**
- **P300 Single Channel**
- **P300 8-Channel**

SECTION 6

Citations

1. Batista, E., Pinto, L., Filipe, E. and Van der Veen, A. (2007). Calibration of micropipettes: Test methods and uncertainty analysis. Measurement, 40(3), pp.338-342
2. US Geological Survey. (2017). The USGS Water Science School. [online] Available at: **https://water.usgs.gov/edu/density.html** [Accessed 23 Mar 2018]
3. TTE Laboratories (2015). ISO 8655 Pipette Standards. [online] Available at: **http://www.ttelaboratories.com/TTE-University/ISO-8655** [Accessed 23 Mar 2018].
4. Opentrons Labworks, Inc (2018). Opentrons Gravimetric Test Script. [online] Available at: **https://github.com/Opentrons/Volumetric_Testing_Scripts** [Accessed 25 Mar 2018]
5. Eppendorf AG (2008). EPMotion 5070 Technical Data. [online] Available at: **https://online-shop.eppendorf.us/US-en/eshopdownload/downloadbykey/101139_Operating-Manual_186** [Accessed 26 Mar. 2018]