ABSTRACT
The goal of this project was to create a semi-automated stand that will be used for the process of calibrating various micropipettes by Transcat of Rochester, NY. The project operated with the intent that the design should remove as much operator error from the calibration process as possible. The team achieved this goal by incorporating a pneumatic system with a rotating physical stand that is constrained by positioning sensors and a user-operated Graphical User Interface.

INTRODUCTION
The project was for the Rochester division (headquarter) of Transcat, Inc. Transcat is one of North America’s leading providers of calibration services and instruments. Transcat wished to increase the scope of their services by addressing the market of micropipette calibration for labs and medical facilities.

There are two main fields in the calibration of micropipettes: gravimetric and colorimetric calibration. With the gravimetric method, a certain amount of water is dispensed from the pipette onto a scale. Based on the mass and the known specific gravity of the fluid, one can find the volume of water. The colorimetric method involves the analysis of volumes of diluted dye in a cell of known path length. While the gravimetric method of calibration may be more widespread in use today, the design needed the use of colorimetric calibration due to the relatively large influence of environmental factors, including the amount of evaporation of fluids, in the gravimetric method.

The current method of colorimetric calibration is highly user-intensive, with significant amounts of training necessary before a technician would be able to effectively calibrate a micropipette. Even with this training, there are still operator factors that cannot fully be controlled. There are many factors that affect calibration process.

Technique
Pipettes should be held vertical during the aspiration of liquids. Holding a pipette 30deg. off vertical can cause as much as 0.7% more liquid to be aspirated due to the impact of hydrostatic pressure.

Release of Plunger
Releasing the plunger abruptly can cause liquid to be “bumped” inside the pipette during a liquid transfer application. This can cause liquid to accumulate inside the instrument which in turn can be transferred to other samples causing variability in sample volume and the potential for cross contamination. Smooth, consistent pipetting rhythm helps in increase both accuracy and precision.

Immersion Depth
The pipette tip should only be inserted into the vessel containing the liquid to be transferred about 1-3mm. If the tip is immersed beyond this; the results could be erroneously high. This is due the fact that liquid could adhere to the tip and be transferred along with the aliquot in the tip. If the tip is not immersed far enough then air could be drawn into the tip, which could yield results that are incorrect on the low end.
Thermal conductance
Thermal energy can be transferred from the operator’s hand to the air within the pipette (dead air) or even the internal components themselves. This can have a dramatic impact on the amount of liquid dispensed due to the effects of expansion and/or contraction. The scope of this project is to eliminate as much of these operator factors as possible. The scope does not include environmental factors due to the inability of the team to effectively control the factors, given the limited availability of laboratories and other resources.

NOMENCLATURE
All nomenclature is defined below, corresponding to the variables for each equation that is listed

PIPETTE DESCRIPTION
Pipette, which are calibrated, are positive displacement pipette. Pipettes have a piston in a cylinder or capillary tube that moves to the appropriate position once the volume is set. Sample-to-sample and cross-contamination are kept to a minimum by using micro syringe tips that are disposable. These pipettes are in range of 2uL-2mL. First, the piston moves to the appropriate position when the volume is set, so when the operating button is depressed to the first stop, the piston descends to the tip opening. When the tip is immersed into the liquid and the button is released, the plunger is raised creating a partial vacuum, which causes the liquid to enter the tip. Finally, when the operating button is depressed again, the piston descends, expelling liquid from the tip.

PRODUCT DESCRIPTION
The final design consists of a rotating stand, pneumatic actuators and controls, sensors for precise positioning the stand, a computer interface and the ARTEL PCS colorimetric calibrator.

THE ROTATING STAND
The Pneumatic Actuators (Items 1 and 4)
In our system, we have two pneumatic actuators. One is a 6” universally mountable double-acting pneumatic cylinder. The other is a 2” stud mount double-acting pneumatic cylinder. Both of the actuators have ¾” bores for the piston to move up and down in. The greatest force needed is the blowout force for the pipette. The smallest force needed is the force needed to provide the initial depression of the pipette. The initial depression needs at most 8.9N of force and the blowout force is at most 48.9 N.

We found the amount of pressure that needed to be supplied in order to get these forces by using Equation 2, which can be found in the equations section of the paper.

The Arm Assembly (Items 2 and 3)
The arm assembly consists of two parts: the cylindrical and the rectangular parts. The cylindrical portion of the assembly supports the pipette holder (Item 14). The rectangular part of the assembly lies on top of the cylinder and it allows the plunger depressing actuator to sit above the pipette plunger and line up axially with the center of the pipette (Item 13).

Flanged gaskets (Items 5 and 7)
These flanged gaskets are placed on top and bottom of pipe. These gaskets support the six rods that are instrumental in stabilizing the upper part of the design.

The Pipe Encasement (Item 6)
This pipe encasement served two main purposes. The first purpose is to protect the 6” pneumatic actuator. The most obvious way that this is achieved is by encasing the cylinder itself, protecting it from most potential outside contact. The other way that the pipe protects the actuator is by creating a space between the actuator and the arm assembly. To be specific, it supports the topmost flange, upon which the arm assembly sits. This is beneficial, seeing as the pneumatic actuator has a thin wall thickness that is made of stainless steel, making it susceptible to buckling factors. The flange, on the other hand, translates the downward force through the pipe. While the pipe may be hollow, the wall thickness is greater, large enough to make up for the stress concentrations made by the milled holes, which are necessary for access to the ports of the 6” pneumatic actuator.

The bottom assembly (Items 8 and 11)
The bottom assembly is comprised of two parts, which are held together by use of four 8-32 cap screws. Bottom assembly grips onto plastic base to hold structure down and lets the plastic base absorb the weight of the structure in the form of shear stress.
Thrust bearings and washers (Items 9 and 10)
These bearings are seated between the bottom assembly and the base. The bearing itself consists of a disc of metal that has an annular ring of small rollers, each of which are arranged so that their axes are all on radial lines from the center of the bearing. Washers have been added to ensure the bearings do not harm the surfaces they are compressed against.

Control Rods (Item 12)
These rods are supported by the flanged gaskets and serve the purpose of stabilizing the arm assembly as it travels vertically.

PNEUMATIC CONTROLS

Solenoid Valves (Item D)
All of the solenoid valves are three-way, two-position valves. The three ways in the valve are as follow: the inlet pressure, the exhaust, and the outlet going to a port on the pneumatic actuator. The two positions are dependent on whether or not a 24V signal is sent to the solenoid valve. There are five solenoid valves in the pneumatic system: two for extending and retracting the 6” pneumatic actuator rod and three valves for controlling the 2” pneumatic actuator through the initial depression, blowout and retraction stages of plunger depression.

The Solenoid Manifolds (Item E)
There are two manifolds used in the pneumatic system. One, which has four solenoid valves on it, has a common pressure of 137.89 Kilopascal running through it. The other manifold, which has only one solenoid valve, has a pressure of 68.94 Kilopascal running through it.

The Air Regulator (Item F)
The air regulator serves the sole purpose of decreasing the pressure of the compressed air from 140 Kilopascal to 70 Kilopascal.

The Shuttle Valve (Item G)
The shuttle valve is a valve that will direct air coming from either of two sources to a single destination. The two sources for our system are 140 Kilopascal and 70 Kilopascal, both of which are used to extend the rod of the 2” pneumatic actuator. First, the 70 Kilopascal feed goes through. Then, both of the feeds go through at the same time and the higher pressure of the 140 Kilopascal feed pushes the ball so that only the 140 Kilopascal feed goes through. This is necessary so that there is minimal interruption in the feed of pressure as there is a transition in the pressure. After that, the 70 Kilopascal feed is exhausted so there is no backpressure. This is necessary so that there will be no chance of the 70 Kilopascal feed bleeding off after the 140 Kilopascal feed is exhausted out.

MOVEMENTS OF THE SYSTEM

![Fig 2: Vertical movement of pneumatic actuator on arm assembly](image1)

This figure shows the movement of the smaller pneumatic actuator’s rod as it depresses the plunger of the micropipette so it can load and dispense the liquid in pipette.

![Figure 3: Vertical movement of pneumatic actuator on bottom assembly](image2)

This figure shows movement of the larger pneumatic actuator’s rod as it raises and lowers the arm assembly, along with the attached micropipette, so the micropipette can clear obstacles as it moves between the calibrator (A), home (B) and dye (C) positions.

![Figure 4: Radial movement of calibration stand and a schematic of the pneumatic system](image3)
Figure 4 shows movement of the calibration stand as it rotates between calibrator, home, and dye positions.

**THE ELECTRICAL CONTROLS**

**Micro-controller**
The Basic Stamp was chosen for this project due to its ease of use and cost. The specifications for the Basic Stamp 2 Module, which were found in *Microcontroller Projects Using the Basic Stamp*, are:

- 24 pin DIP
- Microcontroller: PIC16C57
- Processor speed: 20 MHz
- Program Execution Speed: 4000 instructions/sec.
- RAM size: 32 Bytes (6I/O, 26 Variable)
- EEPROM (Program) Size: 2K Bytes, 500 instructions
- No. Of I/O pins: 16+ 2 Dedicated Serial
- Current Draw @ 5V: 3mA Run / 50uA Sleep

The electrical system has five inputs and five outputs. The inputs are in the form of sensors. The five outputs go to the h-bridges, which control the solenoid valves for airflow management. After studying *Labview GUI Essential Techniques*, we found that the Labview software would be very feasible for graphical user interface, which uses serial communication to provide the initialization for the micro-controller as well as feedback and instructions to the user. The Stamp 2 Board has a built in RS232 interface, as well as EEPROM.

**Inputs**
- Hall Effect sensors for Piston Up and Piston Down
- Optical interrupter for Dye and Calibrator Positions
- Magnetic switch for Calibrator lid

**Outputs**
- Piston Up/Down
- 1" Stroke of Pipette
- Blowout Stroke of Pipette

**Serial**
- Receive Command to Begin Run from user
- Send feedback messages to user (i.e. “Please move the pipette to the Home Position”)

**Hall Effect Sensor**
24V is supplied to Hall Effect Sensor with a 2K load, which supplies current of 12 mA. Hall Effect sensor acts as a switch. The output is connected to 100 K and 5V, which provide 0.005 mA of current to the Stamp board. Hall Effect sensors are mounted to the side of the stand and keep track of any magnetic fields around them. When the piston is at sensor position, the Hall Effect sensor provides output.

**Optical Interrupter**
The Optical Interrupter keeps track of any objects in its limited vicinity by seeing if any infra red light reaches the receiver. If not, something is in the path of the infrared light. The optical interrupter is mounted so as to allow the encoder disc to be equidistantly between the transmitter and receiver. As the shaft rotates, the disc rotates and lines in the disc rotate as well. Optical Interrupter is 2-channel, which allows it to use 2-bit logic to locate up to four positions, including the home, dye and calibrator positions. As infra red light comes in contact with receiver, output is taken.

**Magnetic Switch**
The magnetic Switch is mounted on calibrator lid. When lid is closed, it makes contact and when lid is open, there is no contact.

**H-Bridge**
The H-Bridge (L293D) chips provide 24V and 30 mA of current to each solenoid when 5V is supplied to the input. Instead of two L292D chips, three L293D chips are used in order to prevent the L293D chips from overheating due to excessive current when all solenoids are ON.
Data and Analysis:

As was stated before, the main factor of pipette calibration error is the human interference and therefore if that can be diminished then the error may be able to also be diminished. In the tables below the experiment was held using different types of strategies to operate the pipette. The following table contains the experimental readings for Fisherbrand pipette SN# N30775, which has a range of 20 - 200 uL. The column labeled “percent error based on averages” is based upon the average reading value, which can be found in Table 2. The motive for computing this value is to calculate the error based on how much is actually in the pipette as opposed to approximate volume setting, which is set by the operator.

<table>
<thead>
<tr>
<th>Reading (uL)</th>
<th>Load Speed/ Dispense Speed/ Angle</th>
<th>Actual Reading (uL)</th>
<th>% Error</th>
<th>Percent Error based on averages</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>slow/slow/vertical</td>
<td>99.41</td>
<td>0.61</td>
<td>0.3258</td>
</tr>
<tr>
<td>100</td>
<td>slow/slow/vertical</td>
<td>99.81</td>
<td>0.21</td>
<td>0.0752</td>
</tr>
<tr>
<td>100</td>
<td>fast/fast/vertical</td>
<td>100.11</td>
<td>0.11</td>
<td>0.3760</td>
</tr>
<tr>
<td>100</td>
<td>slow/fast/vertical</td>
<td>99.61</td>
<td>0.41</td>
<td>0.1253</td>
</tr>
<tr>
<td>45</td>
<td>slow/slow/vertical</td>
<td>44.26</td>
<td>1.64</td>
<td>0.5560</td>
</tr>
<tr>
<td>45</td>
<td>slow/slow/vertical</td>
<td>44.49</td>
<td>1.13</td>
<td>0.0393</td>
</tr>
<tr>
<td>45</td>
<td>fast/fast/vertical</td>
<td>44.65</td>
<td>0.78</td>
<td>0.3201</td>
</tr>
<tr>
<td>45</td>
<td>slow/fast/vertical</td>
<td>44.63</td>
<td>0.82</td>
<td>0.2752</td>
</tr>
<tr>
<td>20</td>
<td>slow/slow/vertical</td>
<td>19.66</td>
<td>1.71</td>
<td>0.2410</td>
</tr>
<tr>
<td>20</td>
<td>slow/slow/vertical</td>
<td>19.72</td>
<td>1.41</td>
<td>0.0634</td>
</tr>
<tr>
<td>20</td>
<td>fast/fast/vertical</td>
<td>19.84</td>
<td>0.81</td>
<td>0.6723</td>
</tr>
<tr>
<td>20</td>
<td>slow/fast/vertical</td>
<td>19.61</td>
<td>1.95</td>
<td>0.4947</td>
</tr>
</tbody>
</table>

Table 1: Tests for the Fisherbrand Pipette

<table>
<thead>
<tr>
<th>Reading (uL)</th>
<th>Average Reading (uL)</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>99.73</td>
<td>0.27</td>
</tr>
<tr>
<td>45</td>
<td>44.51</td>
<td>1.09</td>
</tr>
<tr>
<td>20</td>
<td>19.71</td>
<td>1.45</td>
</tr>
</tbody>
</table>

Table 2: Average Errors for each Data Level

The different pipettes were tested to examine what amount of mass was needed to actuate both levels of the plunger.

<table>
<thead>
<tr>
<th>Pipette</th>
<th>Volumetric Setting</th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fisherbrand (0-200)</td>
<td>20 uL.</td>
<td>830g</td>
<td>2.4kg</td>
</tr>
<tr>
<td></td>
<td>50 uL.</td>
<td>900g</td>
<td>2.4kg</td>
</tr>
<tr>
<td></td>
<td>100 uL.</td>
<td>900g</td>
<td>2.5kg</td>
</tr>
<tr>
<td></td>
<td>150 uL.</td>
<td>900g</td>
<td>2.4kg</td>
</tr>
<tr>
<td></td>
<td>200 uL.</td>
<td>900g</td>
<td>2.4kg</td>
</tr>
<tr>
<td>Ranin (0-20)</td>
<td>5 uL.</td>
<td>900g</td>
<td>3.8kg</td>
</tr>
<tr>
<td></td>
<td>10 uL.</td>
<td>700g</td>
<td>4kg</td>
</tr>
<tr>
<td></td>
<td>15 uL.</td>
<td>700g</td>
<td>4kg</td>
</tr>
<tr>
<td>Oxford (0-100)</td>
<td>10 uL.</td>
<td>700g</td>
<td>2.8kg</td>
</tr>
<tr>
<td></td>
<td>50 uL.</td>
<td>700g</td>
<td>2.5kg</td>
</tr>
<tr>
<td></td>
<td>100 uL.</td>
<td>700g</td>
<td>2.6kg</td>
</tr>
</tbody>
</table>

Table 3: Mass Requirements
The level one actuation is the amount of force used to inject the tip with the appropriate amount of fluid and then eject the fluid. The level two is the clean out stroke ensures that all of the fluid is ejected from the tip after the first actuation has taken place. Approximately 8.9 N of force would be required to actuate level one and to make sure that the entire clean out stroke was actuated a force of 48.9 N will be used. The masses in Table 3 were found using calibrated masses that were set on top of the pipette plunger. The forces were computed by using equation 1.

**EQUATIONS**

**Optimizing Pressure:**

In order to figure out how much pressure is needed for the rods to accomplish their tasks, one first needs to find the force that the actuator has to exert. In our case, we merely had to find how much vertical force is needed to depress the pipette plunger through its various phases. We originally only used masses in our experiments, so the following equation was needed in order to find the minimum vertical force that will be needed to actuate the plunger:

\[
F_p = m \cdot g
\]  

(1)

Once this was found, we found the pressure needed to exert this force by using this simple relationship between force and pressure:

\[
P = \frac{F_p}{A}
\]  

(2)

**Dynamics of the Arm Assembly:**

In order to simplify the system, it was decided by the team that the pressure used by the larger pneumatic actuator should be the same as one of the two feed pressures used by the smaller pneumatic actuator. After consulting the *Manual of Pneumatic Systems Optimization*, we decided which pressure to use with this equation:

\[
F_v = P \cdot A - M \cdot g
\]  

(3)

If the force computed was negative, the pressure was too small. After finding the pressure and the force that would push the arm assembly up, we found that it would be useful to find the acceleration of the arm assembly:

\[
a = \frac{F_v}{M}
\]  

(4)

We found that the acceleration was a bit too high for our purposes, so we sought to restrict the terminal velocity (the velocity where acceleration is no longer possible) by restricting the flow rate of the air. We found the velocity by using the following equation as a guideline:

\[
v = \frac{Q}{A}
\]  

(5)

**TERMS USED:**

- $F_p$: Pneumatic Force
- $m$: Mass
- $g$: Local Gravitational Constant
- $P$: Pressure
- $A$: Surface Area of the piston inside the pneumatic actuator
- $a$: Acceleration of the arm assembly
- $v$: Velocity
- $Q$: Air Flow Rate

**ACKNOWLEDGMENTS**

The team would like to thank Transcat corp., for sponsoring this project. We wish to extend our appreciation to Howard Zion and Rainer Stellrecht and all of the members of Transcat’s production staff for their time and assistance in our quest to gather data.

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**REFERENCES**

