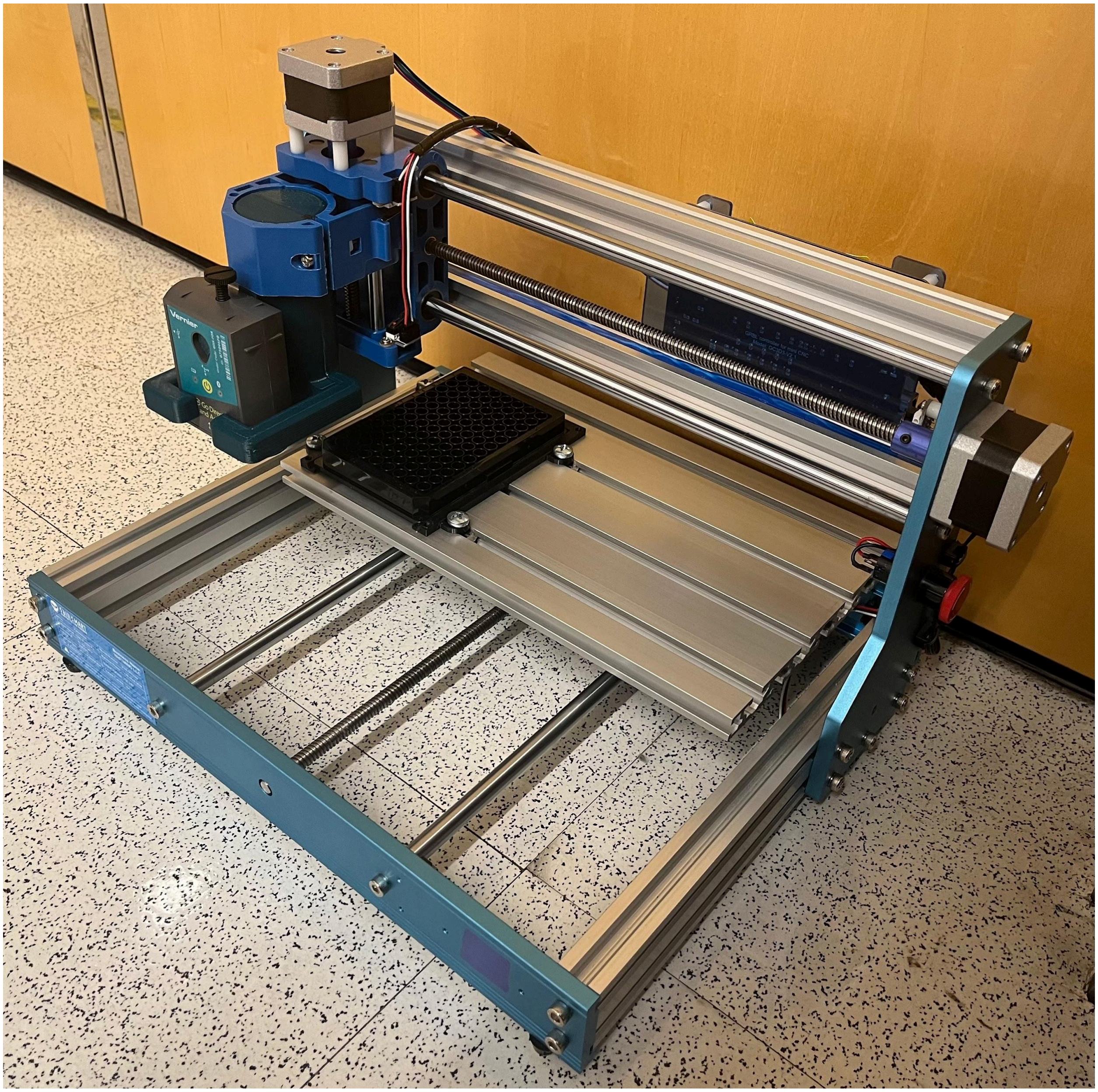

Automated Soft Matter Indenter (ASMI)

User’s Manual

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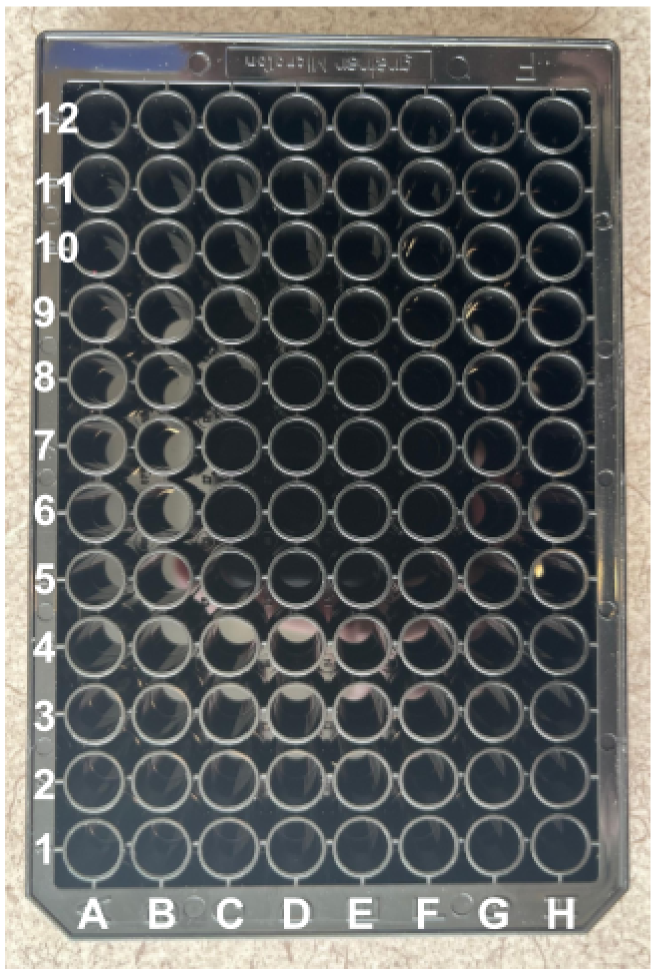
1. Introduction:

Thank you for using the ASMI. This automated materials testing device will measure the elastic moduli of your samples for you, so you can work on something else and come back to useful results. It functions best with materials which have elastic moduli between 5 kPa and 2 MPa, and can be used for samples placed in a standard well plate of 96 wells. On average, it takes 5 minutes to test each well, and at most, it takes 9.5 minutes per well, but this time is highly dependent on the height of the samples. The taller the samples, the quicker the results will be ready. If you have any questions, please feel free to email Dylan List at dlist@bu.edu.

2. User Guide:

Before using the device, please ensure that your samples meet the following criteria:

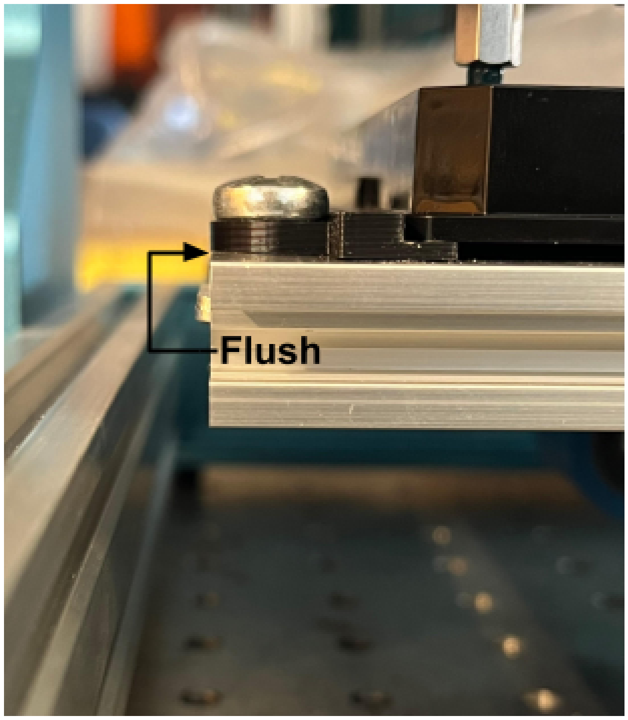
- The samples are in a standard 96 well SBS microplate. This well plate must fit snugly into the 3d printed well plate holder attached to the device. Please do not move or adjust this holder, as this will cause issues with testing the device! For examples of standard well plates, please see the examples below.



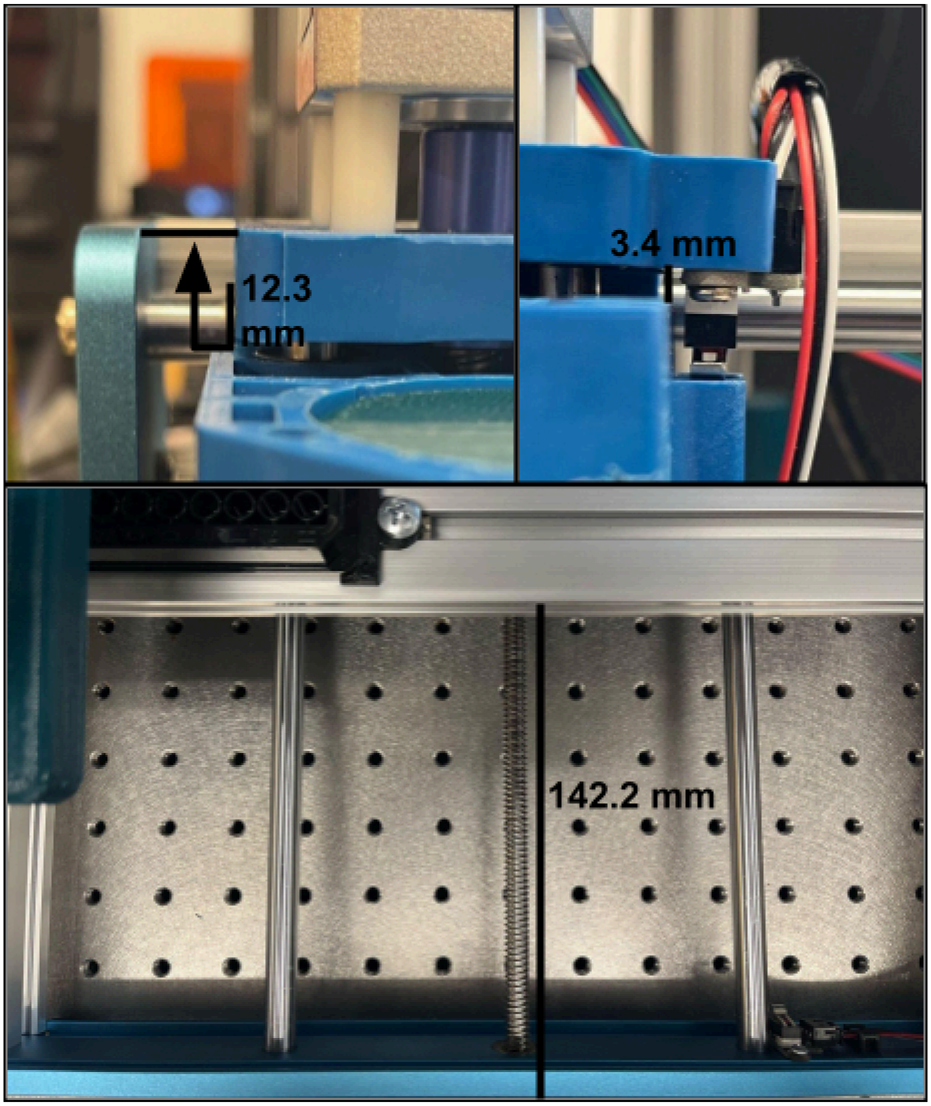
- The height of the wells in the holder is approximately 11 mm and the height of the top of the well plate above the metal floor of the device is approximately 16 mm. For standard well plates like the one shown above, these values should be the same.
- The height of the samples are between 3 mm and 11 mm tall. Samples that are at improper heights may damage or break the machine or lead to poor quality measurements.
- The estimated elastic moduli of the samples are between 5 kPa and 2 MPa. Samples that are too soft may not be able to be measured, and samples that are too hard may lead to poor results due to the limit on how far this sensor is able to indent them.

If all these criteria are met, then the samples are okay to test.

Load the well plate into the holder, ensuring that it is pushed as far back as possible. The well plate holder should be positioned so that the outer leftmost edge of the circular parts of the holder are flush with the left side of the metal floor of the device as shown below.



Check the position of the cnc machine. The metal tray should be far back, and the end effector should be up and to the left so that it barely touches the limit switches. When at its home position, the plastic blue sliding piece which moves in the x-direction should be 12.3 mm to the right of the metal blue wall, the blue holder which moves in the z direction should be 3.4 mm below the plastic blue sliding piece, and the front face of the metal tray should be 142.2 mm from the front of the bottom blue plate.



Before proceeding, check that the device is on and that everything is properly plugged in. The switch on the back of the cnc controller should be set up so that the machine is on when the line is pushed down. Additionally, the USB-B cable should be connected to a computer and the power cable should be plugged into a nearby outlet. If anything is unplugged, turn the machine off before plugging it back in. Also, check that the red emergency stop button on the right side of the device is not pushed in and that the force sensor is plugged in. When the sensor is powered properly, there should be either a green or red light under the left battery indicator. If the sensor is unplugged, please ensure the right bluetooth light is not flashing before replugging it in. If this light is flashing, hold the power button on the sensor for a few seconds so that it stops, and then replug in the cable. Lastly, make sure that there are no cables which can get caught in the machine, especially in the back area.

If the device is clearly not positioned as shown above, please run the homing script from the computer. Ensure you are in the ASMI folder, and run *python3 home.py*. If the device does not move, or moves to an unexpected location, it needs to be exteriorly homed. Please see the troubleshooting section of this manual.

Once properly homed, the device is ready to take measurements. Ensure that you are in the ASMI folder on the computer, and run *python3 measure.py*. The script may take a couple seconds to load. If there is an error during the loading of this script, please refer to the common errors in the troubleshooting section.

Once properly loaded, the script will ask you to enter in the wells that you would like to be tested. Wells can be entered in 4 different modes: 1 well at a time, 1 row at a time, 1 column at a time, and the entire plate. Wells are identified by a letter corresponding to a column and a number corresponding to a row (A1-H12) as shown earlier, and should be entered as such. To enter a row, use the corresponding number and to enter a column, use the corresponding letter. Depending on which wells you would like to be tested, you can mix and match these different entry modes to fit your needs. When you are done entering wells using 1 mode, when asked for another input, press enter. The screen will then show the most updated list of wells which will be tested, and ask you if these are correct. If they are not, enter n or N for no, and the well entry process will restart. If they are, then the script will ask you if all wells have been entered. Please respond to these prompts using y or Y for yes and n or N for no. Please note it is not possible to delete a well once it has been entered except if the whole process is restarted. It is recommended that wells are entered 1 at a time if at most ¾ of a row or a column have wells which need to be tested. If not, empty wells will be tested, taking up time in order to get no result. Also note that wells will be tested in the order they are entered, and if a well is entered twice, it will only be tested once.

Once all wells are entered, the device will start testing them. Before each well it will estimate the time remaining, though this time is highly dependent on the height of the samples. The taller the samples, the quicker the tests. The machine will keep going, so at this point, feel free to leave and come back when the testing is done.

If you need to stop the machine in the middle of a test, or if you return to the device and either an error message is displayed on the screen or the machine is not back at its home position, please see the troubleshooting section.

If the machine functions properly, when you return, you should see the results for each well. In order to obtain the results from a specific well, you can run the analysis script using *python3 analysis.py*. This will ask you for the specific well you would like the results for and will provide those values. This script will only provide results on the data from the most recent tests. Once a new test is started, all old data will be deleted!

3. Troubleshooting:

- Running the measure script results in "No Bluetooth Adapters Found" or no force sensor found:

  The force sensor is not properly plugged in. Unplug it, reconnect it, and try again. You can also use *python3 button.py* to check that the sensor is properly taking measurements. This script will move the device back and forth each time the force sensor reads a measurement with an absolute value greater than 0.1 N.

- Running the measure script results in "[Errno 2] could not open port …":

  Check that the device is plugged in and properly powered. Also, make sure it is connected to the correct USB port on the computer.

- The device doesn't move after inputting wells in the move, measure, or custom measure program, or after applying force to sensor in button program:

  Turn the CNC off using the switch on the back, unplug everything, plug it all back in, and turn the machine on again. Also check that the CNC is plugged into the proper port on the computer. To test that the CNC works properly, run *python3 move.py* to move the CNC to a few locations to make sure it is running properly. Then, restart whatever you were doing before.

- The device stays still for a while:

This issue typically resolves itself in a minute if it occurs. If the device stays still for longer than that, press Control + C and follow the directions for if the CNC is frozen when you return to it.

- The CNC is frozen when you return with no error on the screen:
  Something might have happened during the test which caused either the computer or the device to lose power. Press Control + C. Run *python3 home.py* and check that the device has returned to its proper home location. If it has not, the device needs to be rehomed following the procedure in this section of the manual. Gather any data that was collected using *python3 analysis.py*, and then test the wells that weren't tested before using *python3 measurement.py*.

- You need to stop the device after starting a test:
  Wait for the device to stay still for a moment and press Control + C. Run *python3 home.py* to move the device back to its home position.

- The machine needs to be rehomed:
  Either open the Candle software program on the connected computer or download and open it on an alternate computer by following the directions at: https://docs.sainsmart.com/article/7c20d7zaw3-how-to-install-candle-grblcontrol-for-windows. If you are rehoming the device on an external computer, you will need to connect to the USB-B port on the back of the CNC. Make sure that Candle is accessing the

correct port, and press the home button. Once homed, reconnect the device to the original computer and run *python3 reset_home.py*.

- Using the *home.py* program doesn't return the device to its proper home position: Follow the rehoming directions above.

- The indenter keeps crashing into a wall:
  The well plate you are using does not follow the same dimensions as a standard 96 well SBS microplate, however, if your well plate still has uniform distances between the wells, it can still be tested. Run *python3 custom_measure.py* which allows you to specify the locations of wells and the distances between them. Follow the directions on screen to move the indenter to 3 wells, and using the coordinates from those wells, the device can determine where each well is located. The more accurate the placement of the indenter above the original 3 wells, the better the test results will be.

4. How it Works:

All of the code for the ASMI can be found at https://github.com/dlist26/ASMI.git. The main *measure* script can be broken down into three interdependent processes: moving the device, taking measurements, and analyzing the data. Below is a flow chart overview of the main code that the device uses to take measurements.

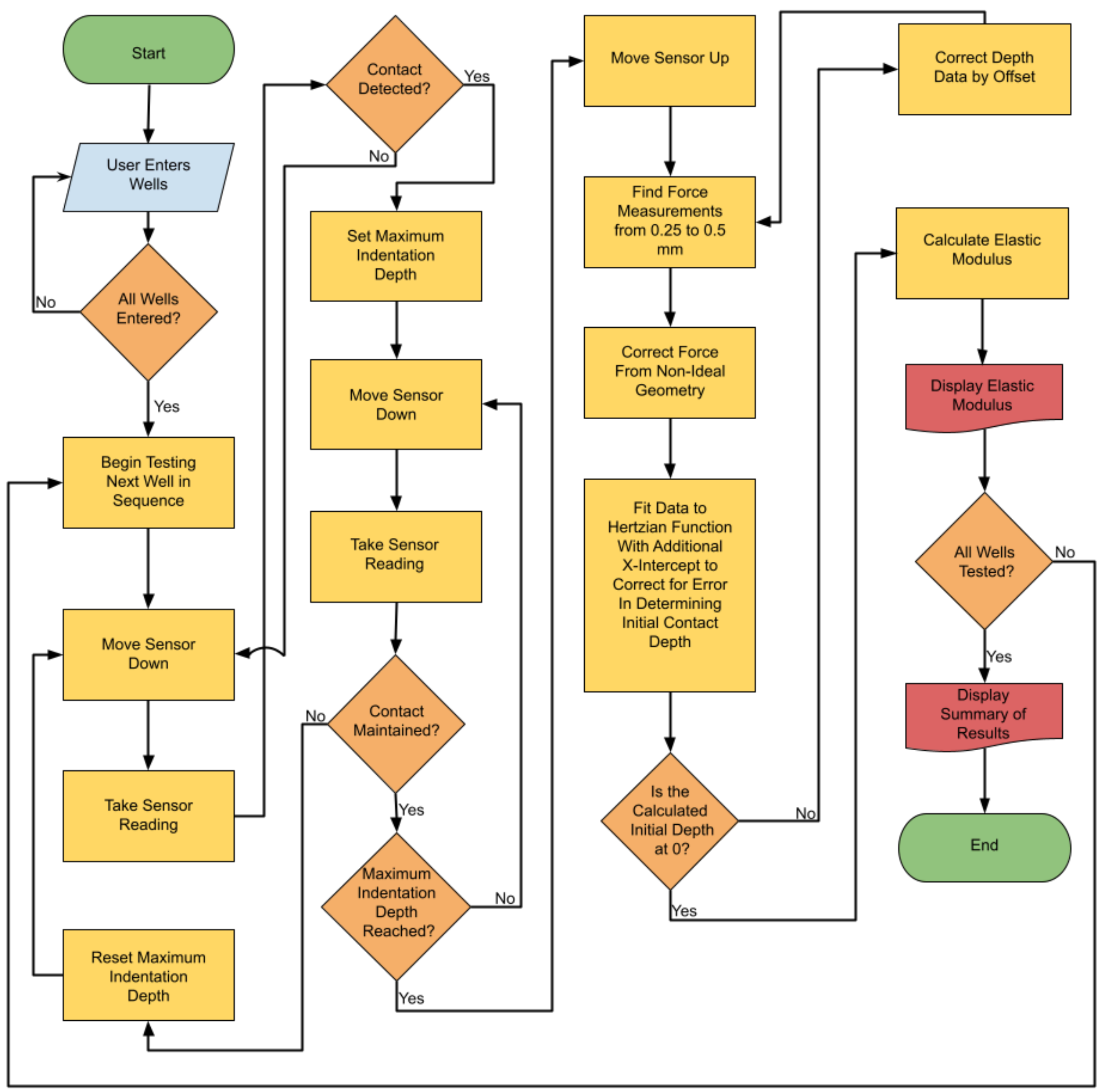


Fig. 3. A flow chart overview of the code used to take measurements.

The device is moved through the use of g-code, a programming language which the CNC understands. At a basic level, it tells the device what coordinates to move to and how quickly to move there.

This aspect of the code begins with asking users to enter which wells in the well plates they would like to test, giving them the ability to input the desired wells one at a time, in rows, in columns, or the entire plate. Then, for each well, g-code is sent to the device to move it to the x-y position of the well so testing can begin. It is at this point that this aspect of the code begins to interact with the part of the code responsible for collecting measurements from the force sensor.

Next, the sensor takes measurements when specified. Once the machine has moved to the position of each well, the sensor takes readings to determine a baseline force which can be compared against throughout the test. Then, g-code is sent to the device which moves it downwards in small steps of 0.02 mm at a time. After each movement, a measurement is taken to determine if contact has been made with the sample. If the sensor determines that it has contacted the material, then a final indentation height is set for the well 1 mm below the current height. If not, the sensor continues moving down. In the event of a false detection, the final height is reset until the sensor once again determines that contact has been made again.

Once contact has been firmly established, the device will indent the sample to a depth of 1 mm below the surface of the sample. This ensures the sample is being elastically deformed for proper measurements. Once the sensor has reached this depth, the machine moves it upwards and the process continues for each well.

Additional error prevention is also done throughout these parts of the code to prevent damaging the device or its components. If the sample is too stiff or so short that the sensor may hit the bottom of the plate if it indents it to the full depth, the device will automatically stop lowering the sensor and will analyze the data that could be collected. Additionally, in the event that something causes the device to stop running in the middle of a series of tests, its position is always externally updated which allows it to be rehomed using the *home* program so more tests can be performed.

The last part of the code analyzes the data that could be collected. For each well, the force data corresponding to the indentation of the sample is collected and zeroed based on the average force detected before indentation. Next, a correction is performed on the force values due to the non-ideal Hertzian setup of testing these samples in a well plate. Ideal Hertzian indentation occurs on a sample which can be modeled as an infinite half-space. However, the walls of the wells create conditions which amplify the measured force compared to what is expected based on the depth and material properties of the sample. To correct for this, simulations were performed in COMSOL Multiphysics to determine how much this force was amplified at different indentation depths. This amplification factor was found to follow Equation 1.

$$F_{Measured} = F_{Expected} * 1.5364 * d^{0.1113} \quad (1)$$

Where:

$F_{Measured}$ = The force measured by the sensor

$F_{Expected}$ = The true force the sensor should measure under ideal conditions

d = The depth below the surface of the sample the sensor has indented in mm

By adjusting the force data by this depth dependent factor, Hertzian equations could be used to extract the true elastic modulus of the sample.

Next, the data corresponding to depths between 0.25-0.5 mm is chosen to calculate the elastic modulus because these depths are deep enough that the slight fluctuations in force which occur at the surface of the part are no longer an issue, but shallow enough that the sample is still being indented elastically. This data is then fit to the Hertzian contact mechanics equation for a spherical indenter and an infinite half-space which is shown in Equation 2. A diagram of this scenario is also displayed in Figure 2.

$$F = 0.75 * E^* * R^{0.5} * d^{1.5} \quad (2)$$

Where:

F = The force being applied to the sample

E^* = The reduced elastic modulus

R = The radius of the indenter

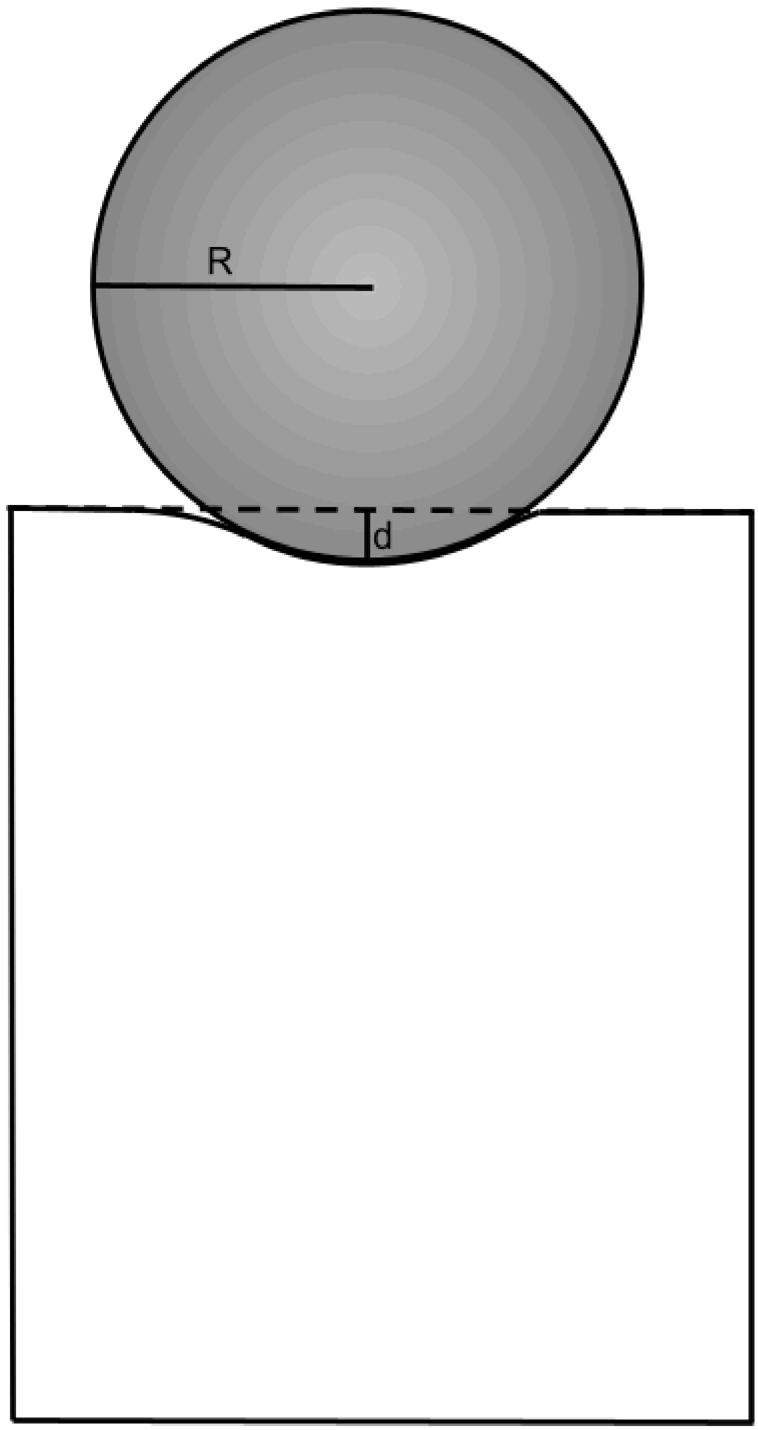


Fig. 2. Diagram of contact between spherical indenter and sample.

The data is fit to Equation 2, except for a modification to account for errors in determining the true depth at which contact was established since some samples are soft enough to cause fluctuations in the force measurements at small indentation depths. Thus, the data is actually fit to Equation 3.

$$F = A * (d - d_0)^{1.5} \qquad (3)$$

Where:

A = A coefficient combining the elastic modulus with constants

d_0 = a depth offset if the determined initial contact point differs from the expected initial contact point

This curve fit shown in Equation 3 is performed in case the true depth at which contact was made differs from the depth at which the sensor determined contact was made. While this difference is often small, this ensures that the best possible calculations are done using the data that was collected.

If the difference in depth is large enough, the d_0 value will be subtracted from all of the depth data. The data between 0.25 and 0.5 mm from the updated depths will again be selected and the previous calculations will be performed until this difference is small enough.

Then, using the A value from the curve fit described in Equation 3, the elastic modulus can be determined using equations 4 and 5.

$$A = 0.75 * E^{*} * R^{0.5} \tag{4}$$

$$(E^{*})^{-1} = \frac{1-(\nu_s)^2}{E_s} + \frac{1-(\nu_i)^2}{E_i} \tag{5}$$

Where:

ν_s = The poisson ratio of the sample, assumed to be 0.5

E_s = The elastic modulus of the sample

ν_i = The poisson ratio of the indenter

E_i = The elastic modulus of the indenter

Using equations 4 and 5 together allow the elastic modulus to be determined for each sample. The values are then reported to the user after testing each well and an additional summary is provided once the testing is complete. An uncertainty is also calculated based on the uncertainty of the curve fit.