Particle Digestion Chamber User's Manual

May 20, 2019

Contents

1 Hardware

1.1 Chamber Assembly

Ensure all parts are clean and dry. A picture the different parts and attachments of the chamber are shown in Figure 1. Flip the chamber over so the bottom is facing upwards. Using a pair of plastic tweezers, carefully align the O-ring on top of the opening and then place the bottom glass on top as shown in Figure 2. Carefully place the metal lid on top so the holes on the lid are aligned with the ones on the chamber. When placing the screws into the holes, it is easiest to place your finger on top of the metal lid to hold it down. Using the other hand, place one screw in and use an Allen key to tighten the screw and lid in place. Do **NOT** tighten it too tight. Repeat with the other three screws. Once the screws are in place, carefully tighten them more until the metal lid is flushed with the chamber. Make sure the metal lid is evenly secured onto the chamber on all four sides.

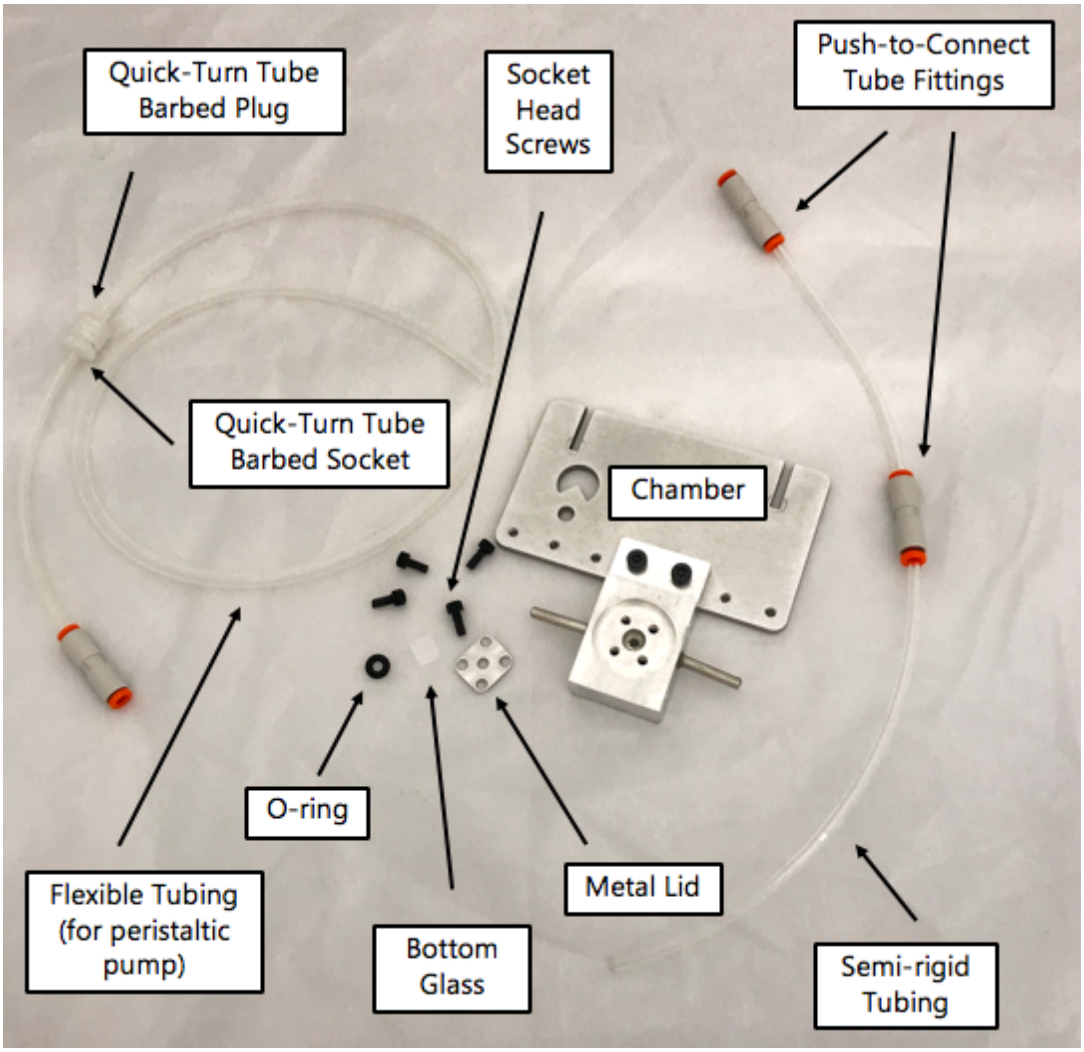

Figure 1: A collection of the different parts of the chamber and its attachments

1.1.1 Potential Problems

1. The o-ring, glass, and metal may experience static electricity sometimes. Use the Blue Milty Zerotat 3 Anti-static gun (located in the 'electical equipment' drawer) to eliminate the static charge on the surface. With the bottom of the chamber facing upwards, place the o-ring on top of the opening, point the gun at the opening, and slowly squeeze the trigger to release ions and remove built-up static charge. Place the glass on top of the o-ring and repeat to ensure all the static is removed.

2. The bottom glass may shatter if the screws are tightened too tight. To avoid this problem, it is recommended to use a slightly bigger Allen key to tighten the screws.

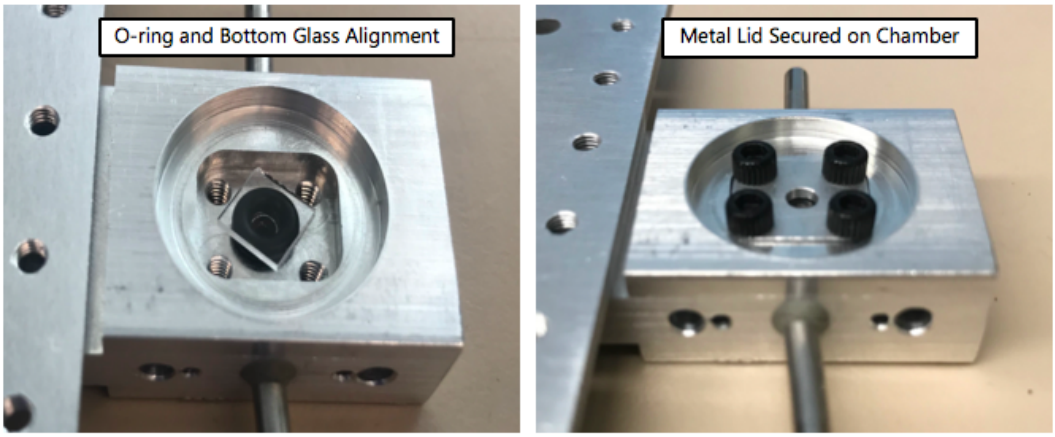

Figure 2: Assembly of bottom glass window

1.2 Operation

1.2.1 Set-up

Once the bottom of the chamber is assembled, attach tubing with **Push-to-Connect** tube fittings on both sides of the chamber (refer to Figure 1). Attach a **Quick-Turn Tube Barbed Socket** to one end of the tubing. This will be used to easily attach the syringe to load the food material into the chamber. Before loading the food material in, the chamber should be flushed with de-gased distilled water. Turn the chamber to its vertical position and use a 12 mL syringe to fill the chamber with water. Visually inspect the glass opening to ensure there are no visible bubbles adhered to the sides, or within the opening of the chamber. If bubbles are present, push more water into the chamber with force until the opening is clear.

Before attaching the chamber to the microscope, it is important to make sure the outside of the chamber is completely dry. Use compressed air (located beside the fume hood) to remove any residual water.

Attach the chamber to the light microscope using the two metal screws on the microscope stage. Turn the microscope on and turn the light on to the maximum setting. This is done so that the light setting will be consistent for every experiment. The brightness of the image will be adjusted using imaging software described in section 2.2 and 2.3.

1.2.2 Finding the Correct Field of View

There are four layers of view that the light microscope can detect for this version of the chamber:

1. The surface of the top glass;
2. The bottom of the top glass;
3. The surface of the bottom glass;
4. and the bottom of the bottom glass

The food material will settle onto the top of bottom glass. It is hard to detect the top of the bottom glass when there is just water in the chamber, so use the coarse adjustment knob on the microscope to find the bottom of the top glass, and then adjust it a little more further down. If bubbles are observed in the microscope view, detach the chamber and push more water into the chamber until no bubbles are present.

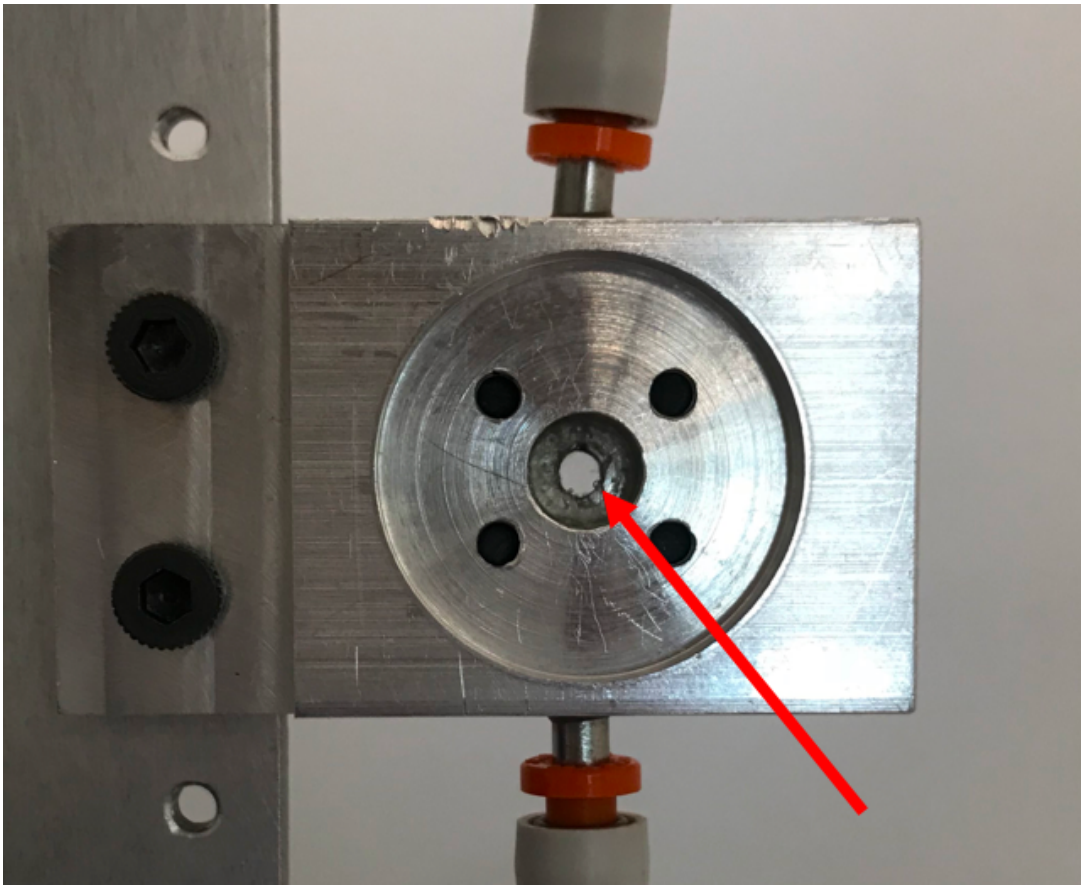

Figure 3: Bubbles attached to sides of opening

1.2.3 Loading of Food Materials

An example of the sample preparation for starch is described in section 3.2. Before detaching the syringe from the barbed socket, turn the tubing on the opposite side upwards so that the water does not leak out. Remove the syringe containing water and replace it with a syringe with the desired food material in aqueous solution. Inject at a slow rate. Look into the microscope lens to watch as the material sinks towards the bottom glass window. Adjust the focus using the coarse knob to find the top of the bottom glass. Load more sample if necessary.

1.2.4 Potential Problems

1. If one side of the view is blurry compared to the other side (ie. the left side is not clear, while the right side is clear) the bottom glass may not be evenly secured. To fix this, ensure that the metal lid is fixed onto the chamber evenly by adjusting and tightening the screws.

2. Sometimes the food material (ie. starch granules) all clump towards the centre or the edge of the view, as shown in Figure 4. The cause of this problem is still unknown but some possible solutions include:

    - Utilizing the Anti-static gun as discussed in section 1.1.1
    - Using de-gased, de-ionized water to flush the chamber and to disperse the food material
    - Ensuring there are no bubbles in the chamber
    - Making eusre the metal lid is placed on evenly by adjusting the screws

3. If the bottom of the chamber, specifically the metal lid and glass bottom is not completely dry, condensation will form on the outside of the bottom glass during heating. This will significantly impact the focus of the image (Figure 4).

4. Leaving bubbles in the chamber will cause issues with imaging when the chamber is heated. The bubbles will enlarge may disrupt the recording by: pushing the food material away from the field of view, producing a shadow along the edges of the image, or appear in the image recording.

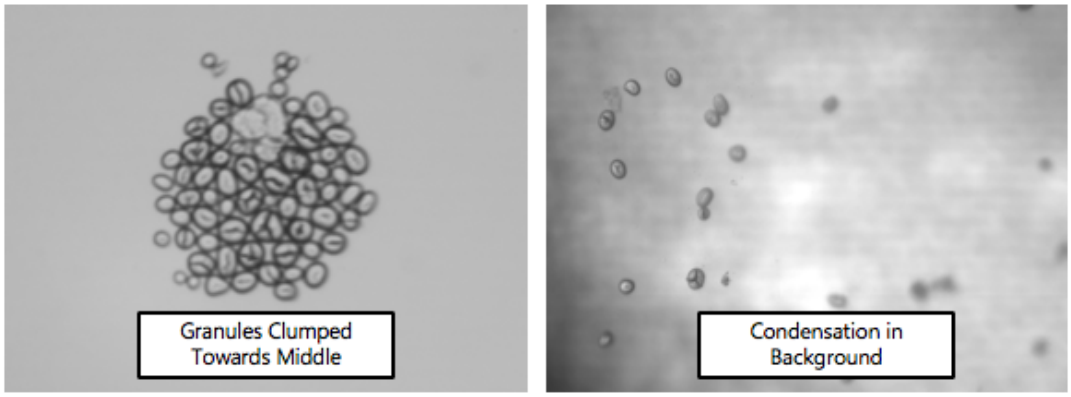

Figure 4: Examples of potential problems that may be encountered

1.3 Heating

To heat the chamber up, insert the heating and temperature probe into the sides of the chamber, as shown in Figure 5. To change the set point, press the "<" button and use the 'up' and 'down' arrows to select a desired temperature in Celsius. Hit "SET" to start. The device will beep once the temperature is near the set point. To stop, change the set point to a lower temperature and hit "SET" again.

1.4 Cleaning

Make sure the chamber has cooled down to about room temperature before detaching from the microscope stage. Detach the chamber, loosen the screws, and remove the metal lid. Wash the bottom glass, o-ring, and metal lid with warm, soapy water. Be careful not to lose them in the sink! Dry with a Kimwipe and ensure the glass is completely free of residue by rinsing with ethanol. Wash the chamber with warm, soapy water and scrub with a Proxabrush to clean the inside of the chamber. Make sure to scrub inside the openings where the fluid flows through, as well as the top and bottom of the glass window. The chamber can be left to air dry or byusing compressed air.

Note: Do **NOT** use acetone to clean the chamber as it will dissolve the superglue used to attach the top glass window.

2 Software

2.1 Raspberry Pi

Raspberry pi is the small, single-board computer that is plugged into the monitor (Figure 6). Turn the monitor on. If the computer isn't on, check to see if the power cord is plugged into the computer/wall. The video imaging software (Sec. 2.3) uses a significant amount of memory and can overheat the computer; a **thermometer icon** will appear on the **top right** of the screen when the computer is getting hot. If the compressed air is not in use, attach the longer cord of compressed air (located on the right side of the fume hood) to cool the heat sensors while running experiments.

2.2 uEye

uEye is the imaging software that allows us to adjust the settings (ie. exposure, colour, etc) of the image projected by the microscope camera. Attach the chamber to the microscope and find

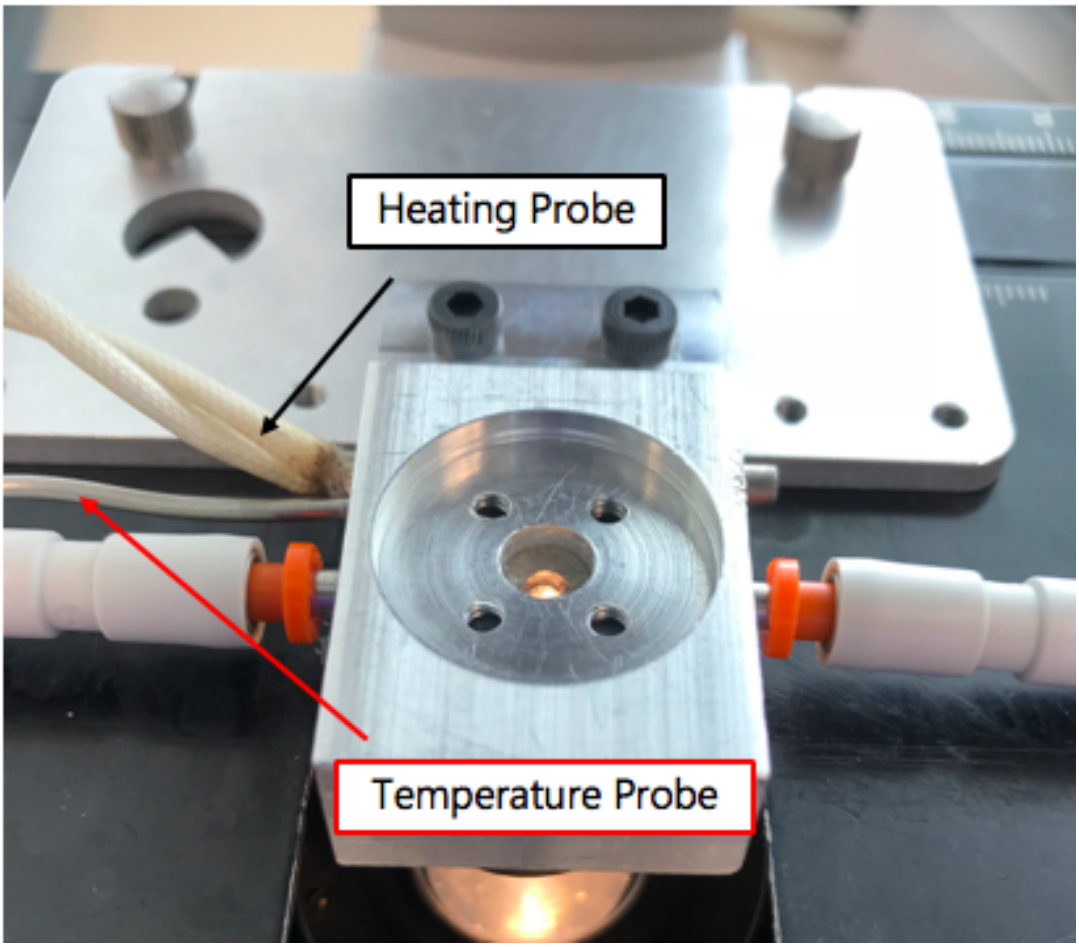

Figure 5: Chamber attached to microscope with heating and temperature probe inserted

the desired field of view before opening this program. Any adjustments made on the microscope will display on the computer screen instantly.

Steps to run uEye are described below:

1. To run this program, open the terminal and type **sudo ueyedemo** into the command line, as shown in Figure 7. Open the live camera to view the image displayed by the microscope camera.

2. Since the light on the microscope is turned to its maximum setting in order to ensure the lighting is consistent throughout each experiment, the settings on uEye need to be adjusted or loaded before each experiment. Instead of manually adjusting the settings each time, a pre-saved parameter called **iniFile.ini** can be loaded. This is done by clicking **File** > **load parameter** > **from file...** > **iniFile.ini**

3. iniFile.ini is located in the Digestion Chamber folder on the Desktop: */home/pi/Desktop/Digestion Chamber/Code/*.

4. To adjust the image preferences, click the **wrench** as shown in Figure 8.

5. Once the desired preferences are chosen, save the parameter and replace the existing iniFile: **File** > **save parameter** > **iniFile.ini**

6. To save an image, click the floppy disk icon indicated in Figure 8.

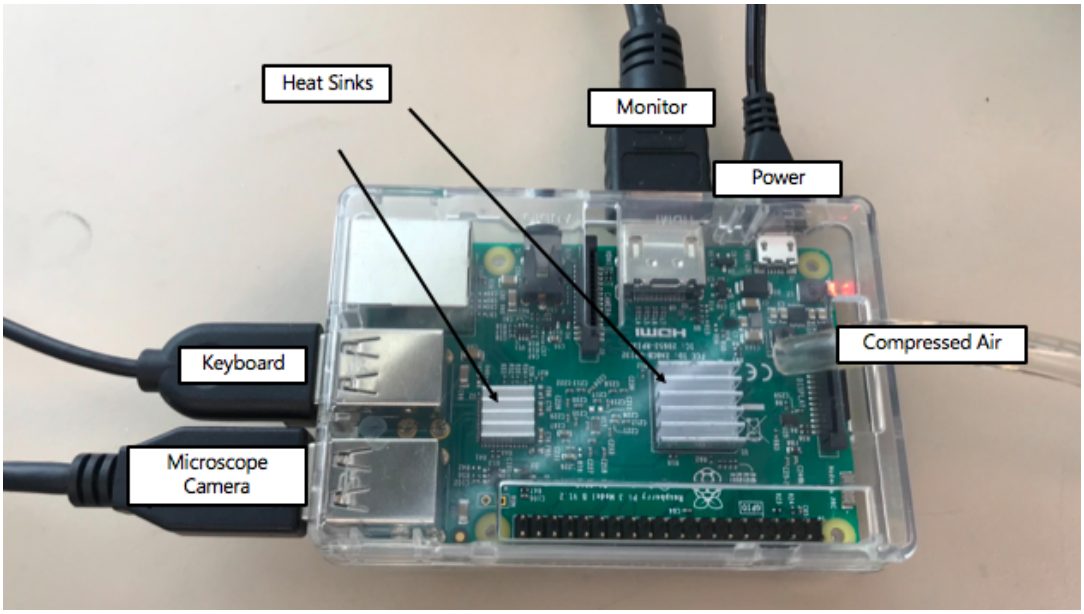

Figure 6: Raspberry Pi computer

2.3 Video Time Lapse

Video Time Lapse is the software used to record images from the microscope camera. This program is located in the **Code** subfolder of the **Digestion Chamber** folder on the Desktop. The desired microscope and image preferences with uEye should be selected prior to starting this program, as the image preview on Video Time Lapse has a delay of 1 second. If the image needs to be re-adjusted, close video Time Lapse and re-open uEye for further adjustments.
Note: uEye and Video Time Lapse cannot be open at the same time.

Steps on running Video Time Lapse are described below:

1. To open, type the list of commands as demonstrated in Figure 7.
   Note: Instead of typing out the whole code, you can type out the first few letters and hit "tab" to auto-complete, **OR** use the "up" arrows on the keyboard to select previous commands.
2. The default frequency of image capture is every 1 second. To change this, the frequency must be changed before selecting the saving folder or it will default to 1 second. If the saving folder was selected prior to changing the desired frequency, close and re-open Video Time Lapse to restart.
3. Select an appropriate folder for the images to be saved. To save on Raspberry Pi, select */home/pi/Desktop/Digestion Chamber/Data* To access an external hard drive, select */media/pi/[NAME OF USB ie. KINGSTON]* in the drop-down menu.
4. Click **Preview** to display the microscope image.
5. When ready to start experiments, tape the microscope in place (one tape on each side of the chamber is sufficient) so the chamber does not move during image recording. Select **START Recording** to begin capturing images.
6. Photos will automatically save in the following format: **[date]-[time started recording]-[Unix time (ms)]**. For example, for the image named "2019-03-27_17;44_1553734318630ms.jpg:"
   - The images were captured on March 3rd, 2019
   - The recording started at 5:44pm
   - The image was captured at 1553734318630ms = 5:51:59pm.
7. Check to see if images are saving to the right folder.

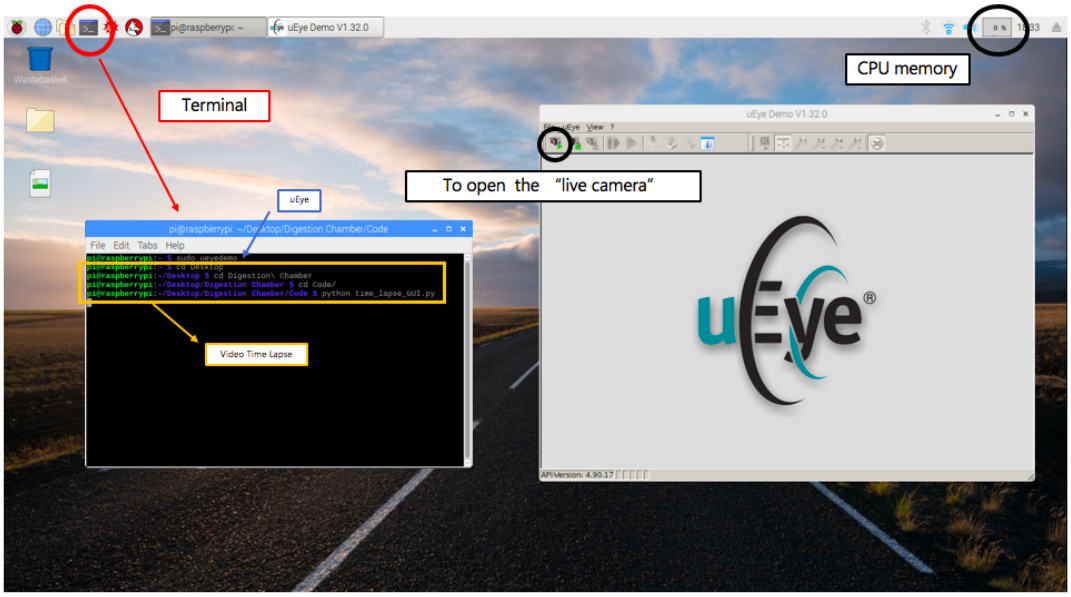

Figure 7: Raspberry Pi user interface and commands to open imaging software

2.3.1 Potential Problems

The Video Time Lapse Software uses a significant amount of CPU memory. The CPU memory percentage is displayed on the **top right** of the Desktop screen, as shown in Figure 7. To minimize CPU usage, close (or minimize) the folder in which the images are being saved once it is confirmed that the images are saving properly during recording. Oftentimes, the images will be saving but the "preview" appears to be frozen. To ensure that the preview is not frozen, cover the microscope light once in a while to see if it shows up on the computer screen. It may also be ideal to re-start the Video Time Lapse Software in between digestion steps and to re-start the computer after each digestion experiment in order to prevent this problem.

3 Sample Preparation

3.1 Starch Isolation

The starch was isolated from the beans using the procedure of Marquezi *et al.* (2016) with modifications. Detailed steps are described below:

1. White navy beans (200g) were steeped in de-ionized water containing 0.5 percent sodium bisulfite for 20h at 4C. The addition of sodium bisulfite assists in breaking down the protein-starch matrix and restricts microbial growth.

2. The steep water was drained off and the beans were rinsed with de-ionized water. The beans were blended in a commercial blender for 5 min with the addition of 500 mL deionized water.

3. The slurry was separated into four parts and then filtered through a 120mesh/125micron sieve with approximately 400 mL of distilled water after each filtration.

4. The resulting solution was poured into 6 x 300 mL centrifuge bottles and centrifuged at 1500 $x\ g$ for 15 min. De-ionized water was added to each centrifuge bottle to resuspend and purify the starch. The bottles were weighed on a scale in order to ensure they were the same weight to balance in the centrifuge.

5. The supernatant was discarded and the solution was condensed into 4 centrifuge bottles. Distilled water was added, the starch solution was re-suspended, and centrifuged at 1500 $x\ g$ for 15 min.

6. The supernatant was removed, de-ionized water was added, the solution was re-suspended, transferred to 6 x 50 mL Falcon tubes, and centrifuged at 1500 $x\ g$ for 15 min.

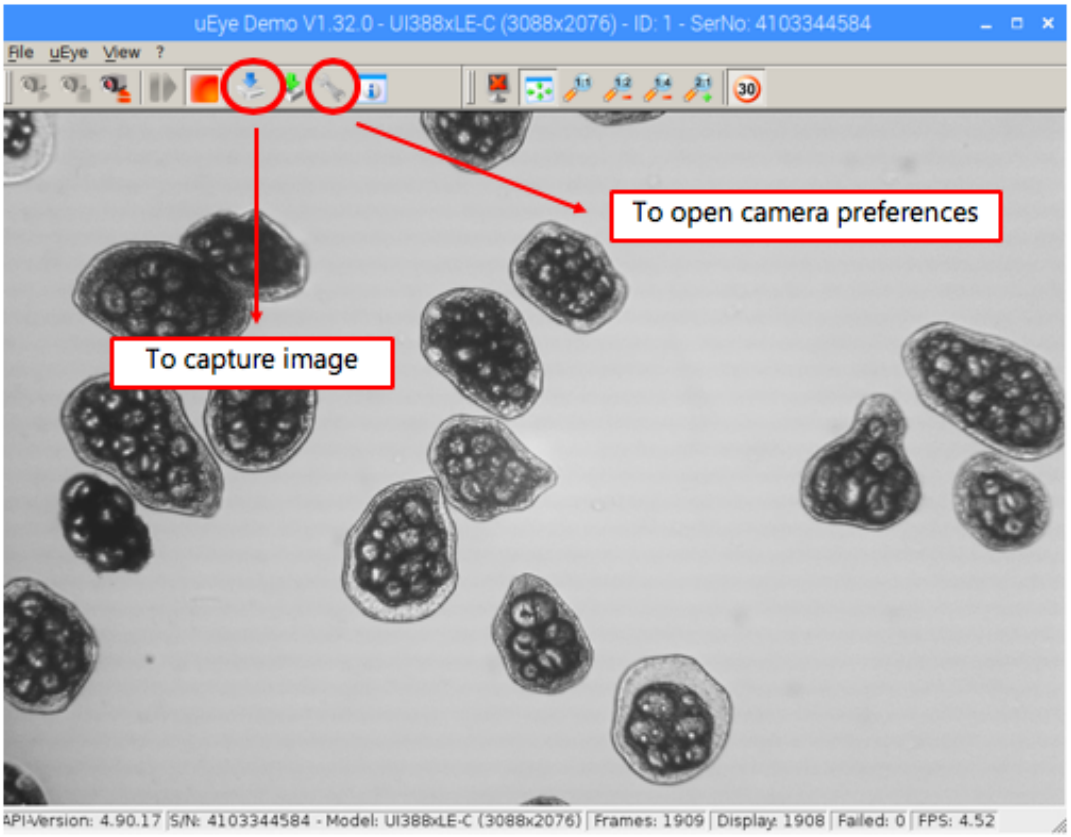

Figure 8: Ueye interface displaying navy bean starch cells on the live camera

7. The supernatant was discarded and upper non-white tailing starch layer was separated from the bottom prime starch using a spatula. The starch was condensed into 4 Falcon tubes and the prime starch was further purified by washing, re-suspending, and centrifuging at at 1500 $x\ g$ for 15 min. This procedure was repeated three more times.

8. The resulting prime starch was scraped out of the Falcon tubes and placed ona watch glass. The starch was dried in a 40C oven for 24h.

9. The starch was then ground up using a mortar and pestle and stored in a 50 mL Falcon tube.

3.2 Starch Solution

It is difficult to track the food particles if too many are loaded into the chamber. It is recommended to make up a small solution of food particles and de-ionized water at a concentration of **0.1mg/mL**. For example, for navy bean starch granules, approximately 0.002 g of starch powder was suspended in 17 mL water in a small glass vial. The food particles will settle to the bottom of the glass over time so it may be helpful to use a vortex to re-suspend the solution before injecting the food material into the chamber. Remember to inject at a slow rate to prevent over-filling the chamber.

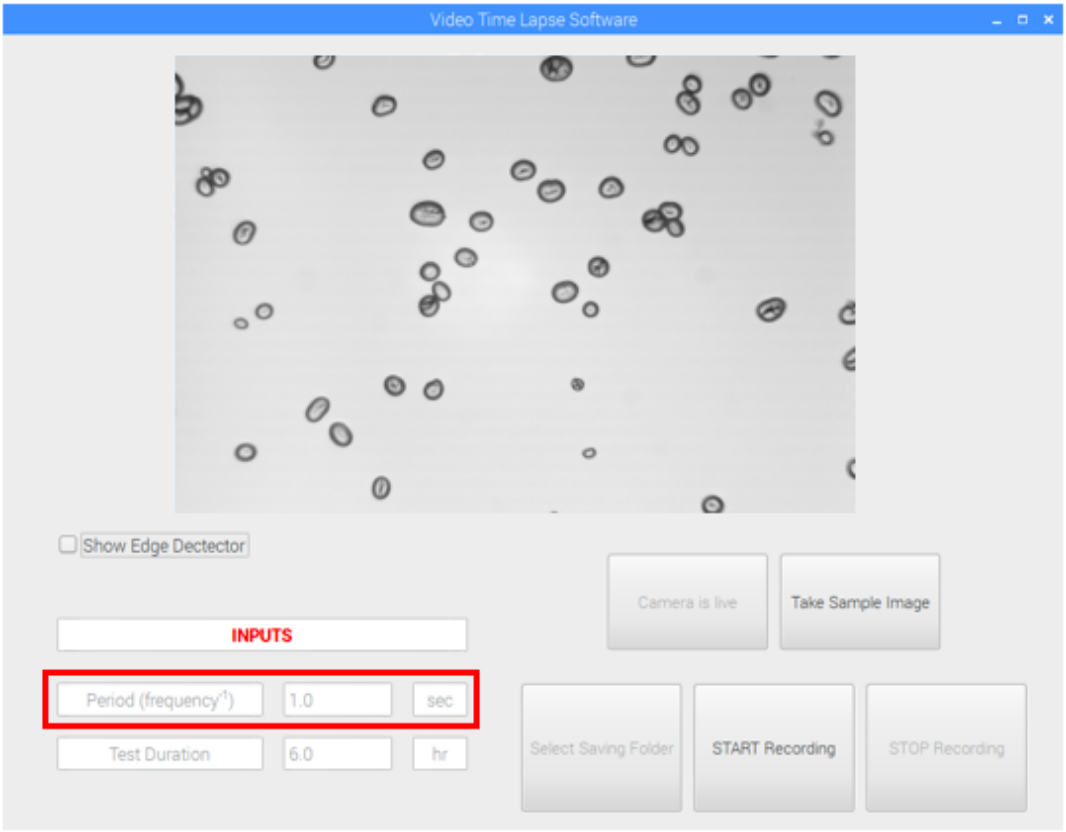

Figure 9: Video Time Lapse Software displaying navy bean starch granules