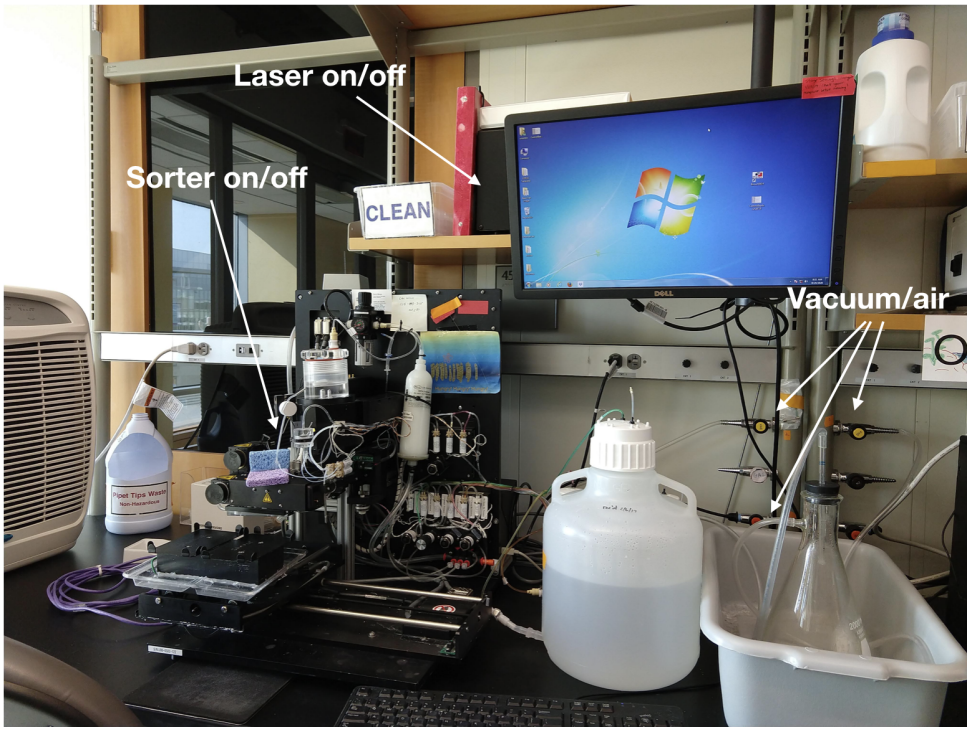
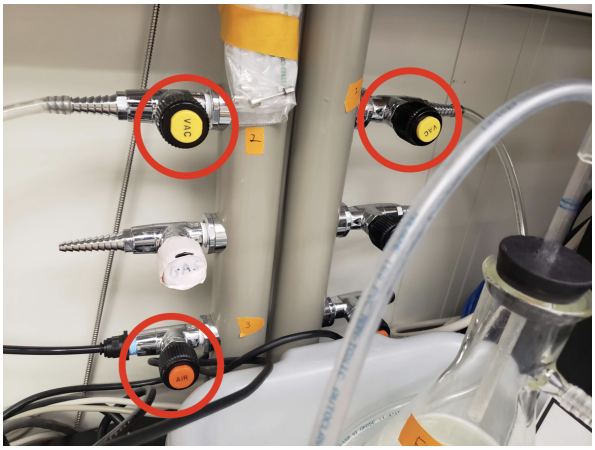
Sorter setup, shutdown, and sterilization

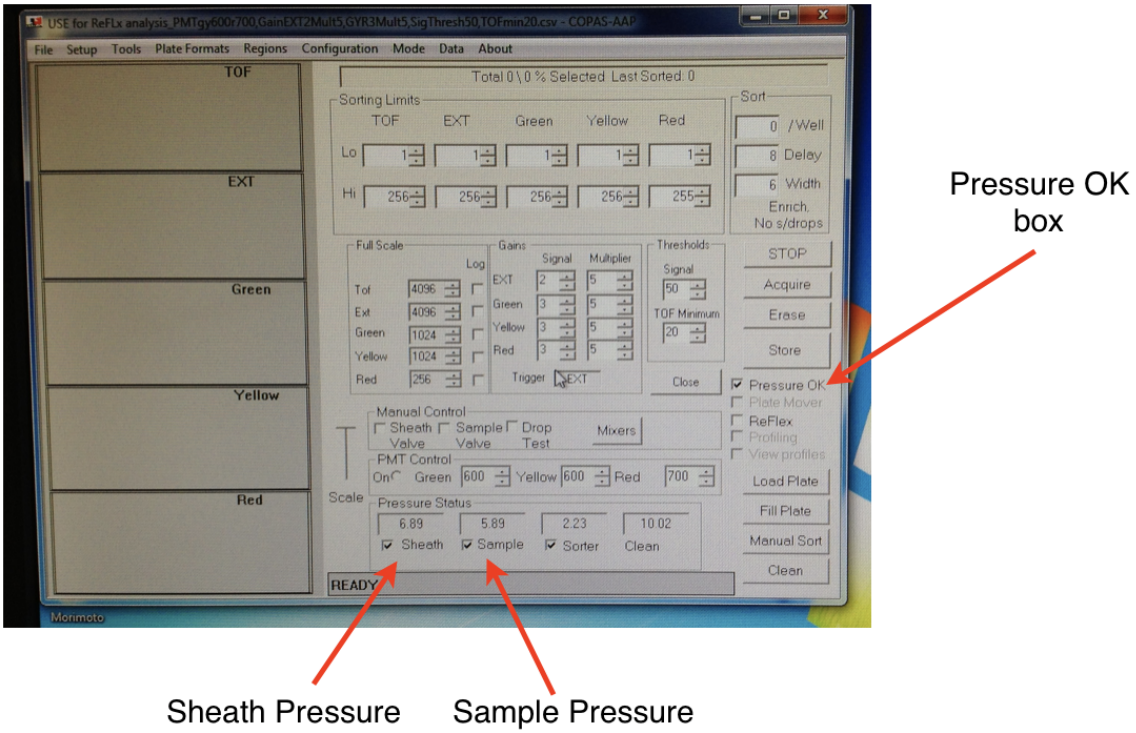
Start Up

1. Turn on **in the following order**:
    a. Sorter (left side of unit near bottom)
    b. Computer and monitor
    c. Laser, located on the shelf above the sorter (switch is to the left, on the front of the machine)
    d. Yellow vacuum traps (#1 and #2)
    e. Orange air pump (#3)
    f. Air purifier





2. Open sorter software (located on the Desktop).
3. In software, select the appropriate template (depending on what you are doing that day (sorting, scoring, etc.) from the Open -> COPAS templates folder.
4. Click “Start”.
5. When the laser control dialog box comes on, click “RUN”. Wait for the 90 seconds for the laser to power on.
6. While the laser is starting:
    a. Take a clean 96-well plate (from the “CLEAN” box) (i.e. - not a brand new plate)
    b. Fill one row as: 4 wells with ddH_2O, 4 wells with 10% filtered bleach, and 4 wells with cleaning solution. Pipette 250 µL in each well.
    c. Fill the next row (all 12 wells) with ddH_2O.
7. When the laser is powered on, click “Done”.
    a. Laser will be approximately 13.4 mW
8. The pressure comes on while the laser is warming up:
    a. “Sheath” pressure should be in the range of 5.7-6.0.
    b. “Sheath” and “Sample” should be ~1 unit from each other (Sample is always higher than Sheath.
    c. If the sheath pressure seems low, make sure that the sheath bottle cap is on **tightly** and the sheath liquid is at the correct level.



9. When the pressure is correct, check “Pressure OK” box.

a. Wait for 70 seconds while the ReFlex module primes itself.

10. After the ReFlex module primes, check the “Reflex” box (located two below the “Pressure OK” box). When prompted, select “Run without bubble trap.”
11. Uncheck sheath under Manual Control. Pop off the tubing coming from the top of the sample cup, and unscrew the lid. Aspirate out any liquid, and fill with 10% filtered bleach. Put the lid back on, put the tubing back on, and click the check box by “Sample” under the manual control area. This will prompt a warning that you will contaminate the flow cell. Select “OK”. This step will run bleach through the flow cell. Do not allow the sample cup to completely empty. Set a timer for three to four minutes so that the sample cup does not empty completely. Once almost empty, click the “Sample” check box again to stop the flow. Remove the tubing and lid, aspirate out the bleach, fill the sample cup halfway with cleaning solution, and run until almost empty. Once almost empty, click the “Sample” check box again to stop the flow. Remove the tubing and lid, aspirate out the cleaning solution and fill with autoclaved H_2O. Aspirate out the water and repeat for a total of three rinses. Fill with autoclaved H_2O again, replace the top and tubing, and run the water in the same manner as the bleach. Repeat for a total of three washes. On the final wash, don’t run the liquid down all the way; 1/2 or 3/4 of the way down is fine.
12. Click on the bottom right “Close” button to close the Parameters screen and you will see a screen that looks like a 96-well plate.
13. Go to Plate Formats, and in the drop down menu select “New 96 well template”. A box will say “Current polygon will be reset. Keep the current one?” Select “Yes”. Now select the same rows that you filled the 96 well plate with ddH_2O and bleach. This action will will allow the ReFlex to run those rows on the sorter, to clean out the ReFlex tubing. On whichever rows have the water, bleach, and cleaning solution, select those rows in this orientation.

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
24	**23**	**22**	**21**	**20**	**19**	**18**	**17**	**16**	**15**	**14**	**13**

14. Click the “Sample” button (near the bottom right corner of the COPAS program window) and you will be asked to title the file. Create a folder for the day’s data (the date, assay, sorting or scoring, etc.) and title the file “clean1”. Save, and the sorter will run the two rows you selected.
15. Load a different template in the program (file -> open). Do not save any changes. Click “Yes” to the question about keeping current polygon, if it asks. Loading the new template without saving changes to the old one allows you to re-open the old one, which you want to use, without having to re-program the template after doing the two clean rows.

16. Make sure the sheath fluid level is within the width of the piece of tape on the sheath bottle. This keeps the air pressure relatively constant, leading to constant flow rate through the flow cell. If the sheath level is too low, fill with M9 to an appropriate level and be sure the put the lid back on tightly.
17. Check the flow rate. First, place a paper towel beneath the flow cell/waste tray. Then manually open the sheath valve to start flow. Have a 15 mL conical tube ready, and then under “Pressure Status” uncheck the sorter pressure box. The diverter valve will shut off, so sheath fluid flowing through the flow cell will come out the bottom of the waste tray, onto the paper towel. Use a clock or stopwatch to time the amount of flow in one minute. Flow rate should be in the 9.7 to 10.2 mL/min range. If the flow is too high or low, get Erik. Be sure to note the sheath pressure and flow rate after one minute, and enter those data into the log notebook we keep on top of the sorter computer.
18. Now you are ready to run your plates. Place the first plate on the tray labeled “Sample”. If you are sorting to a new plate, put your target plate on the tray labeled “Dispense”.

19. Making sure the Sheath box is still checked, click the “Sample” button.
20. A dialog box will appear to name the file. Go into the same folder as the clean1 file you made and write a new file name. You must make a new file EVERY time you run the sorter. There are four components to naming a file on the sorter for the current data analysis pipeline. The general outline looks like: **pXX_strains_condition_control. pXX** corresponds to the plate number. Plate 1 should be p01. Plate 21 should be p21. **strains** correspond to the strains that you set up in the 96-well plates. **condition** corresponds to the condition in each individual plate. Abamectin, DMSO, copper and tunicamycin would all be examples of conditions. **control** is the appropriate control for the condition.

DMSO, None, water and DMSO would be the associated controls for the conditions listed above.

21. Sorter will start automatically once you save your new file.
22. If you are sorting, watch to make sure worms are being sorted into the appropriate wells using the booklight. Once the plate is finished running, check under the microscope that the correct number of worms were sorted into each well before starting a new plate. If they are not being sorted appropriately, there may be a clog.
23. Watch the sorter vigilantly while it runs for the day. The more you pay attention, the faster you can catch any problems that might arise, and the faster they get caught, the less data gets ruined.

Shut-Down

1. Clean the Reflex module
    a. Take the clean 96-well plate that you used when starting up the sorter.
    b. Fill one row as: 4 wells with ddH_2O, 4 wells with 10% filtered bleach, and 4 wells with cleaning solution. Pipette 250 µL of solution in each well.
    c. Fill the next row (all 12 wells) with ddH_2O.
2. Under the Plate Format drop down menu, select the new 96-well template. Click “No” to remove old formats. Do not save over the format file.
3. Select the wells for cleaning and run the Reflex by clicking Sample and saving the file as “clean2” (same manner as the way you did when starting the sorter).
4. While the clean plate is running, power down the laser.
    a. Select the “Tools” menu
    b. Click on Run external laser
    c. Click “Stop” and then “Done”
5. Turn off the laser power (on the box below the monitor).
6. Once the Reflex is cleaned, remove the lid of the sample cup (first pop out the tubing) and aspirate out any remaining fluid.
7. Clean the sample cup in the same manner as the start up procedure. Once with 10% filtered bleach, once with cleaning solution, then rinse out with autoclaved water. Then run three sample cups filled with autoclaved water. At the end, make sure that the sample cup is roughly half full.
8. Click “STOP” two times to shut off any valves and then to shut off pressure.
9. Close the software window.
    a. When the software asks to shut down with purging save, select “Shutdown without purging”.

    b. When the software asks to “Save changes to (whatever file you were using)”, select “No”.
10. Turn off the sorter power (located on the left face of the sorter, if looking at the front of the machine).
11. Turn off the air pump.
12. Turn off the computer (Start -> Shutdown).
13. Turn off the monitor.
14. Make sure the purple ethernet cable is not plugged in.
15. Turn off the two vacuum flasks
16. Empty the liquid in the waste flask to the right of the sorter. If we have been running toxic compounds, empty the flask into the large drum that is on the floor near the sorter (large funnel in the top) and add the name of the toxic compound to the attached sheet. If we have only been sorting, or running bacterial food plates, dump down the sink).
17. Check the waste flask to the right of the sorter, if full empty it too. If toxic, into the drum. If not, in the sink.
18. Fill the Clean and ReFlex bottles (the smaller bottles with red mesh on them) with autoclaved H_2O to the fill line. Fill the sheath bottle to the tape line with M9. If we are bleaching the bottles the next day (without sorting or scoring anything beforehand), then there is no need to fill any bottles.

Sterilizing the sorter (complete with replacement of parts)

This procedure includes how to remove all of the parts and tubes connected to the sheath fluid and replace them. If you do not intend to replace these parts, start this process at ***Step 4*** *and ignore the steps about cleaning the parts you removed.*

1. Disconnect (using LT Quick Connectors) the tubes from the sheath bottle and the two clean bottles. Empty all liquids from the three bottles.
2. Completely disconnect and disassemble the current sheath bottle set up.
    a. Remove 1/2 inch Tygon tube from the sheath bottle and from the 1/2-to-3/8 connector. Throw away.
    b. Remove 1/2-to-3/8 connector from the 3/8 inch tube. Set aside.
    c. Remove the 3/8 inch Tygon tube from the male end of the 3/8-to-1/16 inch LT Quick Connector. Throw away.
    d. Disconnect the male and female parts of the 3/8-to-1/16 inch LT Quick Connector. Set aside.

    e. Remove the 1/16 inch Tygon tube from the female end of the 3/8-to-1/16 inch LT Quick Connector. Set aside.
    f. Follow the 1/16 inch Tygon tube all the way to where it connects to the sorter (second valve from the left, right-hand attachment site on that valve, orange tape is closest) and remove the tube from that connection. Set aside.
    g. Remove the sheath bottle from the sorter area, set aside with other pieces to be bleached and autoclaved.
3. Get an autoclaved sheath bottle, and reassemble a new sheath bottle set up. All pieces (besides the new bottle) should be located in the metal cabinet underneath the fume hood closest to the sorter.
    a. Attach a new length of 1/16 inch Tygon tube to the sorter (second valve from the left, right hand attachment site one that valve, orange tape is closest).
    b. Attach female end of the 3/8-to-1/16 inch LT Quick Connector to the 1/16 inch Tygon tube.
    c. Attach male end of the 3/8-to-1/16 inch LT Quick Connector to the female end.
    d. Attach 3/8 inch Tygon tube to male end of the 3/8-to-1/16 inch LT Quick Connector.
    e. Attach 1/2-to-3/8 connector to 3/8 inch Tygon tube
    f. Attach 1/2 inch Tygon tube to 1/2-to-3/8 connector.
    g. Attach 1/2 inch Tygon tube to the boss connection onto the sheath bottle.

Most days, start here:

4. Disconnect the new sheath bottle and two clean bottles (at LT Quick Connector) rinse them each one time with 10% filtered bleach (use plain Clorox).
5. Fill the sheath bottle with a ~liter of 10% filtered bleach.
6. Fill the clean bottles with ~200 mL of 10% filtered bleach.
7. Fill the sample cup with 10% filtered bleach
8. Reconnect all bottles.
9. Power on the sorter. Start all pressures.
10. Open the sample valve, run almost all the liquid through the sample cup, and then close the sample valve.
11. Open the sheath valve and leave it open for 10-30 minutes.
12. During that time, press the clean button 25-30 times.
13. Take a clean 96-well plate, fill every well with 10% filtered bleach.
14. After 10-30 minutes with the sheath valve open, run the Reflex for an entire plate.
15. Once the bleach steps are complete, stop the flow of any liquid by making sure Sheath is unchecked.

16. Disconnect and remove all bottles and rinse three times with dH_2O.

NOTE: Before proceeding to steps with cleaning solution (1% unscented Tide), make sure the vacuum flask connected to the waste tray is NO MORE than a quarter full. The cleaning solution makes lots of bubbles that should not be allowed to back up into the vacuum system. Watch closely during this step.

17. Fill the sheath bottle with ~500 mL of cleaning solution. Because it is a small volume, tilt the sheath bottle so that the liquid is at the tube. Use a styrofoam block to hold the bottle in position.
18. Fill the clean bottles with ~100 mL of cleaning solution. Because it is a small volume, tilt the sheath bottle so that the liquid is at the tube. Use a styrofoam block to hold the bottle in position.
19. Fill the sample cup with cleaning solution.
20. Reconnect all bottles.
21. Repeat steps 10 through 16 with cleaning solution instead of bleach.
22. Once the cleaning solution steps are complete, stop the flow of any liquid.
23. Remove all bottles and rinse three times with dH_2O.
24. Fill the sheath bottle with a ~liter of dH_2O.
25. Fill the clean bottles with ~500 mL of dH_2O.
26. Fill the sample cup with dH_2O.
27. Reconnect all bottles.
28. Repeat steps 10 through 16 with autoclaved water.
29. Once the water steps are complete, stop the flow of any liquid.
30. Disconnect and remove all bottles.
31. Rinse the the clean bottles once with with dH_2O and then fill with dH_2O up to the fill lines
32. Rinse the sheath bottle once with dH_2O and then fill the sheath bottle with ~8 liters of M9 (to the green tape fill line on the sheath bottle).
33. Reconnect all of the bottles.
34. Take a clean 96-well plate, fill with dH_2O.
35. Run the Reflex for the entire plate.
36. Run a bead test
    a. Use bead solution supplied by Union Biometrica (under hood). Make a 1:10 dilution with M9.
    b. Aspirate out any remaining liquid in the sample cup and add the bead solution to the sample cup.
    c. You MUST use the protocol 42UControlBeads
    d. Click Tools -> Run Control Particles
    e. Uncheck “Reflex” and then click Acquire

f. Wait for the sorter to run 500 control beads (with Bead Saver check boxes clicked).
g. After the operation has completed, save the screen image under file. Name the file for the current date (YYYYMMDD) into the Bead Images folder on the Desktop.
h. If the peaks are not tight and 42 µm average TOF, please tell Erik ASAP. (compare to control bead image from Kevin on Desktop)
i. Aspirate beads and run water through the sample cup once or twice to clean the system.

37. Power down the system. Shut down the laser ("Tools">"Run External Laser">"Stop"), press STOP one time to shut off the pressure, and proceed from step 9 of the shut down procedure.
38. Slide the waste tray out from under the flow cell chamber (WEAR GLOVES!). Spray out the accumulated salts from the waste tray with a spray bottle of water. Try not to move the teflon ring. If the teflon ring gets moved, simply place it back where it should be and press it down so it fits snugly. After washing out the waste tray, reattach the waste tray to the Tygon tubing and slide the waste tray back into position beneath the flow cell. Be sure to do this step in a way that you don't spray any water towards the sorter.

If you are not replacing parts (most days), stop here, you are done! Otherwise finish these steps:

39. Clean the previously set aside pieces of the initial sheath bottle set up. The parts you should have left are a length of 1/16 inch Tygon tube, male and female ends of the LT Quick Connector, the 1/2-to-3/8 inch connector, and the initial sheath bottle.
40. Rinse out the sheath bottle with 10% bleach.
41. Rinse out sheath bottle with ddH_2O three times.
42. Let dry, then cover boss connection and top with tin foil and store until next use.

43. Cut new pieces of Tygon tubing; two inches of 3/8 inch and three inches of 1/2 inch tubing.
44. Soak 1/16 inch Tygon tube, male and female ends of the LT Quick Connector, the 1/2-to-3/8 inch connector, and new pieces of 3/8 and 1/2 inch Tygon tubes in 10% bleach for 10 minutes.
45. Rinse 1/16 inch Tygon tube, male and female ends of the LT Quick Connector, the 1/2-to-3/8 inch connector, and new pieces of 3/8 and 1/2 inch Tygon tubes with ddH_2O.
46. Wrap all pieces in tin foil, and put on autoclave tape.
47. Send all pieces to the autoclave, and sort them into the appropriate bags in the metal cabinet when they come back.