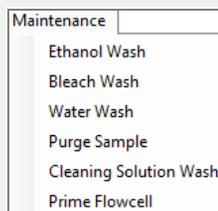


Start up and pre-clean the system

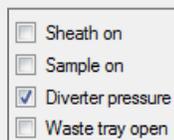
1. On the fluid cart, fill all fluid containers with appropriate solution and reconnect all fittings to instrument. Empty Waste container.
2. Ensure FOCA (fluidics and Optics Core Assembly) and an empty sample cup are in place on the system and all fluid tubings are connected correctly.
3. Turn on the computer, compressor and instrument main power.
4. Open FlowPilot software.
5. Select cleaning scripts from the **MAINTENANCE** menu; run each automatic cleaning sequence to completion in the following order: **Bleach Wash, Cleaning Solution Wash, Water Wash** followed by **Prime Flow Cell**.



Checking Instrument Performance

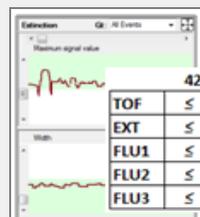
Check the flow rate

1. Check waste tray alignment while sheath is not diverted to waste. (Open the SHEATH Valve and waste tray, turn off diverter, adjust micrometer on waste tray to bring it close to the sheath stream). Then close waste tray and turn on diverter.
2. Place 15-50ml culture tube below the waste tray and Select Flow Rate from the **MAINTENANCE** menu. (Standard flow rates for each FOCA: 250um=10ml/min, 500um=25ml/min, 1000um=45ml/min, 2000um= 55ml/min).
3. Adjust SHEATH rate (%) if necessary.



Run Control Particles

4. Open Control Particle template (control particle.csv).
5. **Select Control particle mode (TOOLS menu)** and ensure the correct optical filter is in place in front of the extinction detector.
6. Fill sample cup with 20ml Control particles (order P/N: 310-5071-001), close sample cup, press **ACQUIRE** and process at least 500 events to produce statistics for a coefficient of variation (CV's).



	42um CP	500um CP
TOF	≤ 10.00%	≤ 15.00%
EXT	≤ 10.00%	≤ 30.00%
FLU1	≤ 15.00%	≤ 50.00%
FLU2	≤ 15.00%	≤ 50.00%
FLU3	≤ 15.00%	≤ 50.00%

7. If CV's are within specs continue to step 9. Otherwise it is necessary to optimize laser focus on the FOCA.

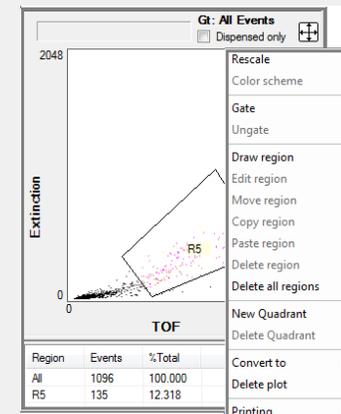
Optimize/Focus the laser on the FOCA

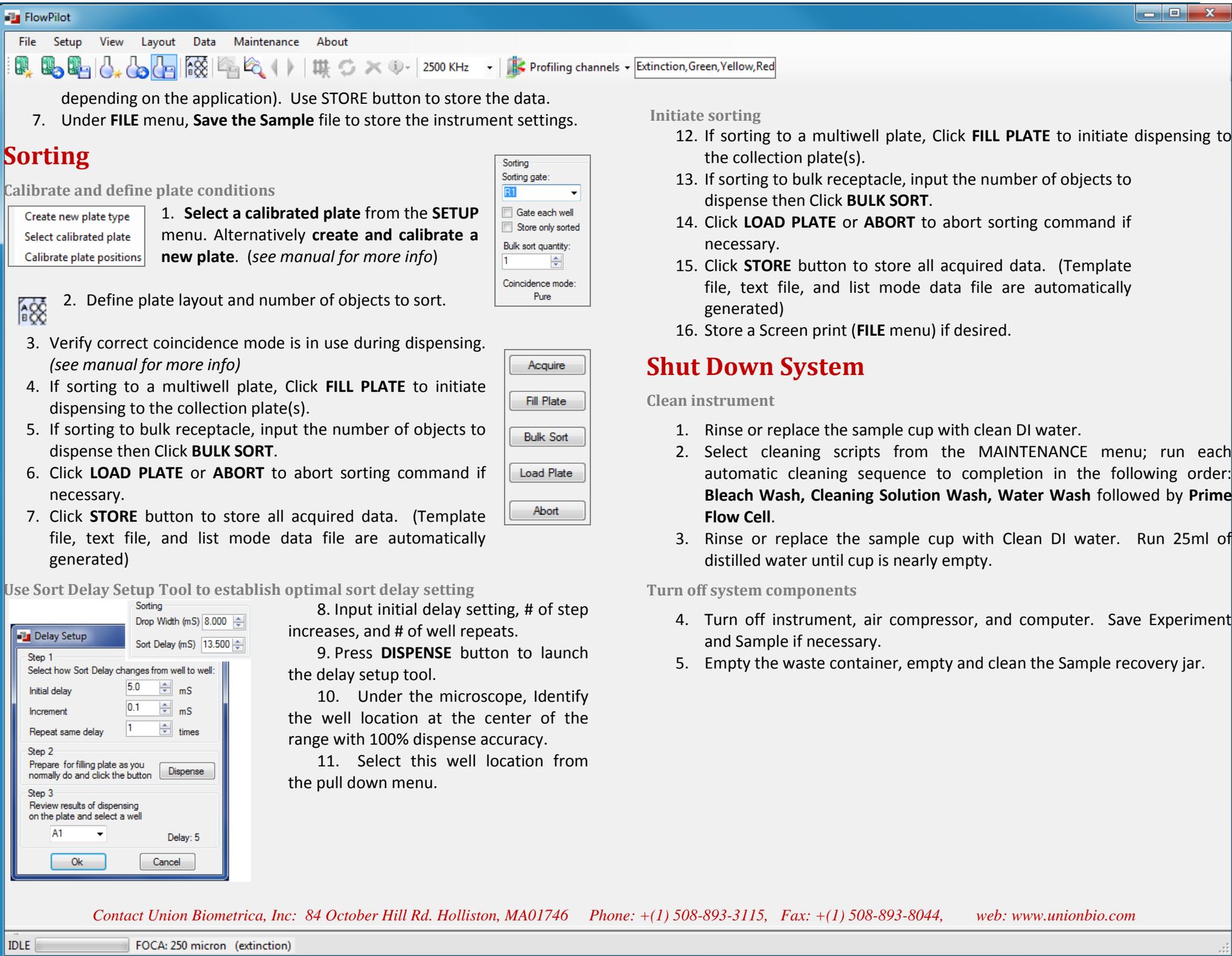
Either set micrometers to previous FOCA specific settings OR adjust the alignment manually in the following manner.

8. Ensure beads are moving through the system; slowly adjust the Amplitude(X)-micrometer to maximize the height of the extinction peak (traced by height tool and laser power reading at the detector).
9. Very minimally adjust the width(Y)-micrometer to minimize the extinction peak width WITHOUT sacrificing the peak height.
10. Reduce the acquisition rate to 10/sec by changing the set sample pressure.
11. After final adjustments have been made, erase current data and collect fresh data (at least 500 events) to produce statistical coefficient of variation (CV).
12. If CV's are within specifications save a screenshot (**FILE** menu) and record the current date in the file name.

Setting up the experiment

1. Remove remaining control particle solution and rinse or replace the sample cup with fresh DI water.
2. Run a Water Wash from the **MAINTENANCE** menu.
3. **Open** an existing Sample (**FILE** menu) or open appropriate histograms, dot plots and profiler windows from the **LAYOUT** menu.
4. Fill the sample cup with sample and click **ACQUIRE** button.
5. Draw regions and modify graphs or sample settings appropriately. (Change parameter headings by clicking the name, draw/modify regions or change parameter settings by right clicking on the body of the graph. See manual for more info)
6. Acquire sufficient events of the population of interest (number will vary





depending on the application). Use STORE button to store the data.

- Under **FILE** menu, **Save the Sample** file to store the instrument settings.

Sorting

Calibrate and define plate conditions

- Create new plate type
- Select calibrated plate
- Calibrate plate positions

1. **Select a calibrated plate** from the **SETUP** menu. Alternatively **create and calibrate a new plate.** (see manual for more info)



2. Define plate layout and number of objects to sort.

3. Verify correct coincidence mode is in use during dispensing. (see manual for more info)
4. If sorting to a multiwell plate, Click **FILL PLATE** to initiate dispensing to the collection plate(s).
5. If sorting to bulk receptacle, input the number of objects to dispense then Click **BULK SORT**.
6. Click **LOAD PLATE** or **ABORT** to abort sorting command if necessary.
7. Click **STORE** button to store all acquired data. (Template file, text file, and list mode data file are automatically generated)

Sorting

Sorting gate:

Gate each well

Store only sorted

Bulk sort quantity:

Coincidence mode:

Acquire

Fill Plate

Bulk Sort

Load Plate

Abort

Use Sort Delay Setup Tool to establish optimal sort delay setting

Delay Setup

Drop Width (mS)

Sort Delay (mS)

Step 1
Select how Sort Delay changes from well to well:

Initial delay mS

Increment mS

Repeat same delay times

Step 2
Prepare for filling plate as you normally do and click the button

Step 3
Review results of dispensing on the plate and select a well

Delay: 5

8. Input initial delay setting, # of step increases, and # of well repeats.

9. Press **DISPENSE** button to launch the delay setup tool.

10. Under the microscope, Identify the well location at the center of the range with 100% dispense accuracy.

11. Select this well location from the pull down menu.

Initiate sorting

12. If sorting to a multiwell plate, Click **FILL PLATE** to initiate dispensing to the collection plate(s).
13. If sorting to bulk receptacle, input the number of objects to dispense then Click **BULK SORT**.
14. Click **LOAD PLATE** or **ABORT** to abort sorting command if necessary.
15. Click **STORE** button to store all acquired data. (Template file, text file, and list mode data file are automatically generated)
16. Store a Screen print (**FILE** menu) if desired.

Shut Down System

Clean instrument

1. Rinse or replace the sample cup with clean DI water.
2. Select cleaning scripts from the **MAINTENANCE** menu; run each automatic cleaning sequence to completion in the following order: **Bleach Wash, Cleaning Solution Wash, Water Wash** followed by **Prime Flow Cell**.
3. Rinse or replace the sample cup with Clean DI water. Run 25ml of distilled water until cup is nearly empty.

Turn off system components

4. Turn off instrument, air compressor, and computer. Save Experiment and Sample if necessary.
5. Empty the waste container, empty and clean the Sample recovery jar.