

## Archimedes Quick Start Guide and SOP for bubbles analysis

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1. Before turning the instrument on check if the sample vial contains MilliQ water (do not overtighten glass vials, finger-tighten all vials to ensure a good seal. All vials must be in place for the fluidics to work properly).
2. Check if the reference vial contains MilliQ water/D2O mixture (H2O:D2O 15:1).
3. If the waste vials are more than half full dispose the waste down the drain.
4. Turn on the instrument using the power On/Off switch.
5. Turn on the computer and monitor. Start the Archimedes software by clicking the desktop icon.
6. The main menu in the upper left shows two choices for “Home” windows, Setup and Experiment. The Setup window is used to prepare the system to acquire data. The Experiment window is used for data acquisition and analysis. Click on Setup.
7. If you need to replace the sensor go to Setup and press Replace Sensor, if not proceed to step 15.
8. A reminder to rinse the sensor will appear.
9. Loosen the two knurled screws on the sensor holder 1 ½ to 2 turns so that a gap appears between the metal faceplate and the black frame and gently pull out the sensor.
10. Use compressed air in a can to blow air into sensor inlet/outlet ports to remove liquid, and store the sensors as appropriate. Be sure that the sensor holder is dry. Gently blow dry with compressed air if necessary.
11. Insert the new sensor into the notched gap in the black frame. Be sure to orient the sensor according to arrows, and with the sensor facing the optics. Gently but firmly push the sensor all the way in while keeping it straight.
12. Finger-tighten the knurled screws until snug; the gap will close. Slide the optics cover forward.
13. Select the sensor type from the drop-down menu, and enter the sensor serial number.
14. Click OK. Pressure will be applied to fill the sensor channels with fluid. The sensor installation is complete.
15. Set the Video to Sensor View and If needed, focus the camera view by adjusting the focus barrel until the image is as sharp as possible.
16. Use the Sensor Y and X knobs to position the light spot on the sensor. For Micro Sensors, position the spot along the center of the sensor long axis, and about 1/4 to 1/3 back from the end. For Nano Sensors, center the spot on “paddle” at the end of the sensor. See the screenshots on the PC desktop for examples.
17. Align the photodetector using the Det X and Det Y knobs to center the diamond-shaped cursor in the Alignment graph inside the central blue square;
18. Click the Optimize Amplitude button.
19. The system is ready to acquire data when all of the Status features are “green,” please refer to screenshots of the examples of Setup for micro and nano sensor.

20. Click Sneeze to check if any clogging is present in the sensor. Please refer to screenshots for “good sneeze”.
21. Put a sample with bubbles in the sample vial (you may use a centrifuge tube inside glass vial). If using in-house bubbles made according to the Dr. Exner’s group protocol use dilution 1:500 for nanobubbles and 1:2000 for microbubbles. Targeting concentrations of bubbles in the samples are  $10^7$  for nanobubbles and  $10^6$  for microbubbles.
22. Select Experiment mode and click Acquire.
23. Enter data in New Experiment dialog including Experiment and Operator names.
24. Check Allow positive buoyancies and Recompute after acquisition
25. Enter values of 1.34 g/cc for Negative buoyancy particle (Lipids) density, 0.08 g/cc for Positive buoyancy particles, Nanobubbles, and 0.8 g/cc for Microbubbles (still working on the physics behind this) and 1 cP for Viscosity
26. Select Auto load, Load for 90 s, every 300 s
27. Stop when the Number of particles reaches 1000
28. Uncheck Default Pressure [psi] and enter values of 5, 4, and 5, and use conc vs dia template. Click OK. The software will prompt you to load a sample. Load the sample for 90s.
29. The Acquisition Preview pane will show the real-time signals caused by bubbles passing through the sensor, and other information such as sample concentration, pressure values on sample and reference side and status of the Auto load function.
30. Data is acquired and displayed in real time in the Experiment pane.
31. To save a data set in a .CSV file suitable for Excel and Matlab click Export and choose Particle Distribution.
32. Remove the vial with sample, rinse the feed tube with MilliQ water, and install a glass vial with fresh MilliQ water.
- 33. Use Load function to rinse the sample from the chip and load it with MilliQ water. Never leave a sample with bubbles or particles in the sensor and sample vial because it might cause the sensor and tube clogging.**
34. Close the software and shut the computer down and turn the instrument off using the Power On/Off button when you are finished with it.

## Useful Tips

1. Use small sample vials. Any centrifuge vial, Eppendorf tube, etc., can be used so long as it fits completely inside one of the standard 20 ml glass vials supplied with ARCHIMEDES. After loading the sample into a small vial, place it inside one of the 20 ml glass vials. Load this vial as you would normally, while making sure the “sipper” tube is positioned inside the insert.
2. Before doing any measurements you must confirm that sensor is not blocked using “sneeze” function in the setup tab. If you have full or partial clog use some cleaning agent. This could be MilliQ water or Pluronic solution near the instrument. For stubborn clogs you may use also 0.1M NaOH . Do NOT expose sensor for more than 10 minutes! To prepare 0.1M NaOH – from solid NaOH, dissolve 0.4 g NaOH in 100 mL water, use syringe filter to filter the solution. You can also try gentle feed tube snapping while loading some cleaning fluid or sneezing. This is very effective method for unclogging.