**Author: Colin Fischer**

**Date Last Modified: 25 Jan 2020**

**Balch Flowcam Simplified Procedure**

**Section 1: Calibration**

1. Assemble flowcam parts, secure syringe on mount, secure tubing to pump and waste.
2. Clean flowcell with ethanol and lens paper, gently wet and clean, then gently dry with another piece of lens paper.
3. Place flowcell in mount (shorter tube should face up), lightly screw top of mount while keeping flowcell centered, tighten until flowcell can only move slightly or gives resistance to the push of a finger.
4. Mount flowcell, connect to syringe on upper end and pump on the other.
5. Fill syringe with 10 mL DI.
6. In Visual Spreadsheet (Visp) select Preferences > Filters > Load a Filter Layout > Edge Gradient for Focusing
7. Under Setup select Context > Load Context File > Focusing.
8. Select Setup > Pump > Prime System, type in 1 mL of fluid and 1 mL/min and select Aspirate.
9. Exit Window and select Flush System, type 1 and press Enter key.
10. Refill syringe with 10 mL DI.
11. Take flowcell calibration beads (10, 15, and 20um) from fridge, vortex beads and drop into syringe (5 drops for 10um, 4 drops for 15um, and 5 drops for 20um beads). Use disposable dropper pipettes from Flowcam supply box for 10um and 20um beads.
12. Prime system at 0.5mL for 1min.
13. Exit Pump controls, select Setup/Focus Icon. Use top knob on lens to move the camera left and right, find center of flowcell. Start pump and focus with focus knob until you see beads.
	1. If Mean Intensity is < 80, go to File > Camera and increase the Flash Duration until Mean Intensity is > 80.

14. Under Setup select Autoimage Mode (No Save) and try to focus beads as best as possible.

15. Exit out and select the AutoImage icon. Save in a focus folder and the run will start. Once 1000 particles have been counted or 20 minutes passes, a particle count will generate. If it is 60% or higher you may proceed with sampling. If lower than 60%, refocus and rerun.

16. Rinse syringe by priming and flushing with fresh DI.

**Section 2: Running Samples:**

1. Open Visual Spreadsheet (Visp).
2. Create a folder on Desktop with the cruise name, cast/event number, and create a sub folder for each depth/sample.
3. In Visp select Setup > Context > Load Local Default Context
4. Under Preferences select Filters > Load Filter Layout > ABD sizes and open.
5. Fill syringe to 10mL with DI. Under Setup select Pump > Prime system, type 0.5mL at 1mL/min and click aspirate.
6. Flush system.
7. Pipette 7mL of sample into syringe.
8. Prime system, 1 mL at 1 mL/min. Select Aspirate.
9. Exit pump controls, in main menu select Trigger Icon and save run in proper folder.
10. Run lasts 20 min, check up briefly but frequently to assure that no bubbles have caught in the flowcell.
11. After run has completed, go to Setup > Pump > Prime System and Aspirate at 1mL more than what is left in syringe, at 2mL/min.
12. Fill syringe with DI to 10mL. Prime 0.5mL at 1mL/min. Flush system. This will rinse syringe for the next sample (no need to repeat steps 5-6 after first sample).

**Troubleshooting tips:**

1. The biggest issue the flowcam faces is bubbles getting stuck in the flowcell. To avoid this, pipette slowly when filling the syringe. If a bubble gets stuck, don’t panic. Under pump controls select pause pump, then go to Tools > Recalibrate. This will recalibrate the background. This is much faster and easier than starting a new run, even if a lot of duplicate images have been taken I can sort them later.
2. The troubleshooting tip above can also be used in the case that the camera mistakes the background for flowing sample. This usually occurs when the flowcell is bumped or moved significantly, so avoiding contact with the flowcell or syringe when operating the flowcam helps avoid this. When pipetting try not the touch the syringe, and only touch the flowcell setup if it is absolutely necessary.