Standard Operating Procedure (SOP) for ARIA sorters

Startup

- Turn on Air-pressure.
- Open the hood (only for Aria Fusion) and start the blower and the light.
- Switch on the cytometer and the water bath (if needed).
- Turn on the PC, log in to Windows, log in to Diva, wait until change to connected status.
- Check the Fluidic tanks, that the Sheath and EtOH tanks are filled to the mark and the Waste tank is empty.
- Do fluidics startup (**Cytometer>Fluidics startup**) and follow the 2 steps wizard on the screen. Skip the nozzle change and keep the closed loop nozzle in the slot, just click OK.
- In the meantime, take out the deflection plates, inspect and if needed clean them with water and then 70% EtOH.
- Fill up one FACS tube with 30% Contrad and one with DI water. **Cytometer>Cleaning modes>Clean flow cell** 4X with 30% Contrad followed by 4X Flow cell clean with DI water. Make sure that liquid is taken up from the sample tube (otherwise the sample line is clogged).
- Replace closed loop nozzle with the nozzle that will be used for sorting.
- Make sure the configuration in Diva is matched with the nozzle size. Start the stream and rinse water into the waste drawer (all 3 waste compartments) and make sure it is drained.
- Leave the stream to stabilise for 20 minutes and acquire water meanwhile at flow rate 11 by the "cleaning template" experiment (record).
- Run CST performance check (**Cytometer>CST**). Use an already prepared tube with CST-beads (good for up to 5 days if stored cold) or prepare a new one according to instructions on the box (350uL FACS fluid + 1 drop CST Beads).
- Change the stream **Drop 1** (around 140-170 = 1 drop and a half before the Gap) and **Gap** (10-13) values by adjusting the amplitude, frequency must be kept (30.0 for 100um nozzle). Refer to the ARIA user's guide for recommended values.
- Enter the actual values for **Drop1** and **Gap** into the white boxes, Click **Sweet Spot.**

Accudrop

- Open the Accudrop Drop Delay template experiment.
- Open Specimen 001 and tube 001.
- Open the sort layout by double-clicking it (hidden under the + symbol of Tube 001).
- Turn on the voltage.
- Adjust the micrometre dial to obtain the brightest bead spots.
- Set the percentage of charge to be applied to the left stream by moving the corresponding slider to 25. Press Optical Filter. Two square boxes should appear, make sure the left stream and center stream are within the boxes.
- Load the accudrop tube beads and adjust the flow rate till 800-1200 event/sec for 100 um nozzle.
- Start sort (from the sort layout) and select cancel in the dialog box message.
- Click on the Auto Delay button.
- Select Start Run in the Auto Drop Delay dialog window and wait the algorithm will itinerate 4 times (pass 1 till 4). When done a gaussian curve will be depicted.
- If the drop delay is above 95% press exit.

Sorting

From now you are ready to run your samples (create an Experiment; Specimen; select parameters; gating strategy, depending in the panel it may require calculating the compensation).

Once you have checked your sample, set the gating strategy and defined the target population proceed to select the collection device (1,5 Eppendorf tube, 15ml falcon tubes, plates, etc) and consequently confirmed the side streams are hitting the target device. To start sorting:

- Open the **Sort layout**. Set device, precision, target event and save all, add population to be sorted out in the proper collecting tube.
- Load sample tube and activate sort in the sort layout and ok in the warning message.
- Sample line backflush is recommended between samples to prevent carryover. **Cytometer>cleaning modes>Sample line Blackflush** run for a few seconds.

Cleaning the instrument

- Daily cleaning template (flow rate 11 and record) 30% Contrad for 20 min and DI water for 10 min.

If you are the last user of the day follow the below steps, otherwise leave the instrument with stream on for the next user.

- Turn off the stream.
- Remove the nozzle and install the closed loop nozzle.
- Rinse the nozzle in DI water to remove potential clogs. In case of clog, sonicate for 1 minute. Immediately after, dry it and place it the the nozzle holder/box.
- **Cytometer>Cleaning modes>Clean flow cell** 4 times with 30% contrad and 4 times with DI water.

Fluidics Shutdown

- **Cytometer>Fluid Shutdown**: follow the steps in the wizard. (the nozzle was already replaced by the closed loop nozzle during flow cell cleaning) so dismiss this step by clicking “done”. At the last step place a tube with **Shut down solution** in the sample station.
- Clean the deflection plates by wiping with wated and then 70% EtOH.
- Depressurize and refill the **Sheath** and **Ethanol** tanks.
- Empty the waste in a Waste box.
- Exit Diva software.
- Turn off the computer.
- Switch off the instrument.
- Switch off the water bath.
- Close the pressure.