BD FACSAriaFusion™ basic SOP

KEY SPECIFICATIONS

Low to high sample pressure (10 L/min).
Amplitude: intensity of the drop pulse
Frequency: drops formed per second
Drop 1: pixels from the top of the first disconnected drop
Gap: number of pixels at the breakoff
Data displayed on 262,144 scale

Fluid cart:
5L tanks

BD FACSAriaFusion™ basic SOP

SYSTEM STARTUP

1.	Check fluids, empty waste, fill sheath and attach sheath fluid li
2.	Check the waste container baffle for pressure problems
3.	Turn on BSC and compressor.
4.	Turn on computer and cytometer in that order.
5.	Start FACSDiva software, select Use Current CST settings
6.	Cytometer > Fluidics startup if a Fluidics Shutdown was perfor otherwise perform a 2 Flow Cell with DI.
7.	Clean the plates, stream camera, breakoff camera, the small s for behind the nozzle.

SYSTEM SHUTDOWN

1.	Stop stream, remove nozzle, clean and sonicate in DI, store dr Install closed loop nozzle.

[redacted]

cells).

EXPERIMENT SETUP

- Large or new cells, changes in pressure, smaller, large particles require different area scaling, area scaling should be performed on cells of inte (large cells have larger area). Draw Area vs. Height plot and draw diag FSC and each FL. Change on Cytometer window > Laser tab. Use app settings to preserve ASF and LD.
- Area >= Height, important for sorting!
- Sheath pressure > velocity > pulse width > area scaling > voltages > compensation.
-

SORTING

- Enrich with a high threshold > re-sort with a lower threshold.
- Donot use biexponential display or snap-to gates for sorting. Also events below 0 in biexp in a sort gate get sorted into the gate, even those outside of gate.
- Rare events > use storage gate > sort to far right stream >.less fanning
- Yield mask = drop sort 1 or 2, reduce to 1 drop sort for fanning.
- Purity mask = coincidence
- Phase mask = cell positioning, use to correct for fanning, yields are re
-

Fusion sort collection camera and stream targeting

1. Open the StCamSWare application for camera view. Use camera in th
   or upper position with the light deflector to monitor the collection
2. Open the IconSortDeviceControl application to open the Sort Alignm
   Software.
3. Change the sort device as required.

ACDU

1. Install the splash guard.
2. Clear the sort chamber, and eject the ACDU stage.
3. Choose the sort device or create a custom device. Adjust the home po
   and apply.
4. Sc

FAQ & Troubleshooting

- Most problems will be fluidics (air in sheath, waste – orange, sheath – blue).
- Bubbles, turn stream on and off, perform Fluidics Startup.
- Sort > backflush.
-

SYSTEM MAINTENANCE

(performed by Flow Cyt Lab technician or designated alternate)

At least TWICE WEEKLY maintenance

1. Deflection plates – Kimwipes and DI, EtOH ok
2. Cytometer>Cleaning modes>Prepare for aseptic shutdown (45 min, bleach clean).
3. Change the O-ring if required (temporary solution until new integrated nozzle purchased).
4. Clean nozzle before adjusting the sort block.

- Optional: Clean Flow Cell 3X with BD Detergent solution and 3X DI
- Full fluidics shutdown with bleach.
- Turn off stream, purge filters.
- Prime tanks after refill or changing filters.

Monthly maintenance

- Change waste lid white cap if necessary.

BD FACSAriaFusion™ basic SOP

EXTERNAL AIR REQUIREMENTS

Air supply 6.6.9 Bar (95100 psi) regulated. The source of the compressed
must deliver clean (<5 ppm) dry (-40 filtered (oil free) air at stable pressures. The wo
case air consumption, based on 100 tubes per hour, is approximately 0.5

Recommended Sort Settings/Parameters for BD FACSAria Fusion

	Nozzle		
	70µm	85µm	100µm
PSI	70	45	20
Amplitude	60	32	12
Frequency	87	45	30
Drop 1	150	150	150-210
Gap (Max)	6 (14)	7 (17)	10 (21)
SSI ΔGap	2-3	3	4
Events per sec	18'000	18'000	6'000