Salk Flow Core Reference Sheet: BD FACS Aria Fusion

CITING USAGE IN PUBLICATIONS: This instrument was acquired through the NIH Shared Instrumentation Grant (S10) with additional funding support from Larry and Carol Greenfield Technology Fund. ***In addition to the Salk's Cancer Center grant NIH-NCI CCSG: P30 014195, please include S10-OD023689 as required by NIH for reporting purposes.***

DESCRIPTION: The BD Biosciences FACSAria™ Fusion is a high-end Special Order Research Product (SORP) cell sorter configured with 5 high power lasers, 13 fluorescence detectors and 2 light scatter detectors. It is equipped with a Class II Type A2 biosafety cabinet.

This latest version of the Aria platform has the same highly efficient optical design of its predecessors, redesigned into a smaller footprint. The Aria design integrates fully fixed stream and optical alignment with a patented quartz cuvette flow cell and optical gel-coupled optics. Laser interrogation of the sample occurs within the cuvette before the sample is accelerated through the sorting nozzle. This design provides opportunity for maximal photon collection to occur while the sample is traveling at relatively slower flow rates, which in turn allows for high detection sensitivity. The fixed alignment optical design minimizes startup time and provides reproducibility with reduced variability from run to run.

The Aria Fusion runs on the same Diva software as the Canto and LSRII analyzers. It has several automated processes: there are software-controlled options for Fluidics Start Up, Shut Down, and cleaning modes. The Diva software also features sort monitoring and clog detection. Taken together, the fixed alignment and automated processes make this an ideal instrument for learning to operate. The core has a training program available for Salk researchers wanting to learn how to sort autonomously on this instrument: please enquire at fccf@salk.edu

HOW THE SORTING WORKS: *The Aria Fusion is a droplet generating cell sorter. These have a pair of high voltage plates and use electrostatic deflection to direct charged droplets (containing individual cells) into collection vessels. Droplets not marked for sorting will end up in the waste aspirator.* After laser interrogation in the cuvette (where fluorescent labels are analyzed and populations of interest can be defined in the software for sorting), the sample exits the flow cell through a vibrating nozzle. Acoustic vibrations from a piezo device close to the nozzle breaks the stream up into saline droplets containing cells. At the time of formation, if a droplet was expected to contain an individual cell to be sorted, a charge is applied momentarily to the sample stream to produce a charged droplet (+ or -, up to two magnitudes for each to yield 4 possible collection streams). Non-sorting droplets are not charged. The droplets fall downward between a pair of high voltage plates in the sort chamber. Depending on their charge, they are either deflected left or right to a designated collection vessel or else continue their path directly falling into the waste aspirator.

OPERATING SOFTWARE: FACS Diva

NOZZLES: 70, 85, 100, 130-um

SORT COLLECTION: Up to 4 sorting streams can be generated (i.e. can sort up to "4-way") while a single sample is being run in the system. Up to 4-way sorting possible for microscope slides, PCR, 1.5ml eppendorf or 5ml tubes. 15ml tubes are limited to 2-way sorting. The sorter is equipped with the Automated Cell Deposition Unit (ACDU) module: This allows for sorting into a variety of multi-well plate formats for collection (384w-, 96-, 48-, 24-, and 6- well). *When sorting into plates, only 1 population at a time can be sorted into a well.*

TEMPERATURE CONTROL: Both sample and collection

EXCITATION: 5 air launched lasers - 488nm (100mW), 640nm (100mW), 405nm (100mW), 561nm (100mW), 355nm (65mW)

CONFIGURATION:

*** Scatter parameters are off the 488nm laser**

Laser Name	Detector	Filter	Dichroic Mirror	Examples of Fluorescent Labels that can be used
488nm 100mW	A	710/50	690 LP	PerCP-Cy5.5, BB700
	B	530/30	505 LP	FITC, AF488, GFP, YFP, Zombie-Green
640nm 100mW	A	780/60	750LP	APC-Cy7, Zombie NIR
	B	670/30	665LP	APC, AF647
	Option*			Optional 710/50 with 690LP for switching A or B detector to AF700 filter
561nm 100mW	A	780/60	750 LP	PE-Cy7
	B	610/20	600 LP	PE-TR, mCherry, mKate, 7-AAD, PI, PE-Dazzle, Texas-Red, LD Red
	C	586/15	570LP	PE, tdTomato, AF594, dsRed, RFP, TMRE, TMRM, Cy3, PE-Dazzle
355nm 65mW	A	740/35	690LP	BUV737
	B	379/28	370 LP	BUV395
405nm 100mW	A	780/60	750 LP	BV785, BV786
	B	670/30	635 LP	BV650
	C	525/50	505 LP	BV510, LD Aqua, CFP
	D	540/50	410 LP	DAPI, Pacific Blue, BV421, Hoechst, Zombie Violet, BFP, Dylight 405

SORTED VOLUME, AND SORT SPEEDS: Sort speed "in theory" can be estimated on the interplay of nozzle size, sheath tank pressure and number of droplets that can be generated (smaller nozzle + higher pressure = more drops). In practice, a big part of it is also the quality and nature of the sample biology (clumping, size, viability, type/"fragileness" and debris amount), which impacts the trade off on speed vs recovering cells with reasonable loss. Sort volume is linked to the nozzle size and sort mode we can use for your sort. The core has a wealth of experience working with difficult sample types. Contact us for more information on optimizing your sort for a particular application: fccf@salk.edu