Technical Specifications

Optics

Excitation Optics

Optical Platform
Fixed optical alignment of all Class IIIb lasers with the cuvette flow cell. The 488-nm and 640-nm lasers are standard. All other laser choices are optional. All lasers are solid state.
Beam height: 9 ±3 µm
Beam width: 65 ±7 µm
Beam shaping: elliptical

Power Out of the Laser Head
355 nm: >15 mW
405 nm: >85 mW
488 nm: >50 mW
561 nm: >50 mW
640 nm: >100 mW

Steering Optics
488, 640, 561 and 405-nm laser: Fiber optics steer the fixed-alignment laser beams onto the expansion prisms to focus them on the cuvette flow cell.
UV (355-nm) laser: Air-launched and focused on the cuvette flow cell

Emission Optics

Optical Coupling
The quartz cuvette flow cell is gel-coupled by refractive index-matching optical gel to the fluorescence objective lens for optimal collection efficiency.
Numerical aperture: 1.2

Forward Scatter Detector and Filters
Photodiode with 488/10 bandpass filter for the 488-nm laser.

Side Scatter Detector
Photomultiplier with a 488/10 bandpass filter for the 488-nm laser.

Fluorescence Detectors and Filters
Five fixed-fiber apertures for the 488, 640, 561, 355 and 405-nm lasers. An octagon technology detector array enables user-defined detection configurations.
Filters and mirrors are user changeable.
Additional detectors up to a total of 18 wavelengths can be added to the arrays.

Wavelengths Detected from the 488-nm Laser without the 561-nm Laser Installed
530/30-nm FITC, Alexa Fluor® 488
585/42-nm PE
616/23-nm PE-Texas Red®, propidium iodide (PI)
695/40-nm PerCP-Cy™5.5 or PI,
or 675/20-nm PerCP
760/60-nm PE-Cy™7

Wavelengths Detected from the 488-nm Laser with the 561-nm Laser Installed
530/30-nm FITC
695/40-nm PerCP-Cy5.5 or PI,
or 675/20-nm PerCP

Wavelengths Detected from the 640-nm Laser
670/30-nm APC, Alexa Fluor® 647
780/60-nm APC-Cy7 or APC-H7

Wavelengths Detected from the 405-nm Laser
450/40-nm BD Horizon Brilliant™ Violet 421, V450, VPD450, Pacific Blue™, DAPI
525/50-nm BD Horizon Brilliant™ Violet 510 or V500, AmCyan
610/20-nm BD Horizon Brilliant™ Violet 605

Wavelengths Detected from the 561-nm Laser
582/15-nm PE, DsRed
610/20-nm PE-Texas Red®, mCherry, PI
670/14-nm PE-Cy™5 or 710/50-nm PE-Cy5.5
780/60-nm PE-Cy7

Wavelengths Detected from the 355-nm Laser
450/20-nm Hoechst Blue, DAPI
670 LP Hoechst Red
Optional BD optical filter (not provided)

Fluidics

General Operation
Sheath and cleaning fluid tanks, as well as waste collection, are located in an easy-to-reach drawer at the base of the system.
An auxiliary air input is designed to connect the cytometer to a lab's air filtration and drying system. If needed, a third-party compressor is available as an option.
Sheath pressure is adjustable from 5 to 75 psi.

Fluidic Reservoirs
Autoclavable 10-L sheath and waste containers and 5-L cleaning reservoirs are provided.

Sample Flow Rates
Adjustable dynamic range of sample flow rates

Fluidic Cleaning Modes Included (Software)
Automated startup and shutdown
Clean flow cell
Prepare for aseptic sort

Nozzles
70, 85, 100 and 130-µm nozzles are removable and can be sonicated.
A registered key-fit position at the bottom of the cuvette provides fixed stream alignment.

Bubble Detector
A bubble detector in the sample line detects air bubbles from the sample tube and stops sample flow when the sample tube is empty, to avoid air bubbles from reaching the nozzle. The bubbles are then purged when unloading the sample tube.

Sample Collection Cooling and Heating
Refrigerator/heater option is available to provide cooling or heating for sort collection into tube holders, multiwell plates and slides.

Automatic Cell Deposition Unit
Sorts into slides and 6, 24, 48, 96 and 384-well plates. Index sorting can be enabled when sorting single cells. This capability indexes the cell surface phenotype to the well containing that cell.

Performance

Fluorescence Sensitivity
Measurements performed at 70 psi and 90 kHz using SPHERO™ Rainbow Calibration Particles (RCP-30-5A).*
*MESF can vary lot-to-lot.

FITC[†]: <87 molecules of equivalent soluble fluorochrome (MESF-FITC)

PE[†]: <29 molecules of equivalent soluble fluorochrome (MESF-PE)
[†]Average MESF from four instruments.

Qr[‡] (x1000)
Measurements performed at 70 psi and 90 kHz. 488-nm laser excitation.

FITC[§]	(BD FACSAria Fusion):	97
	(BD FACSAria™ III):	40
PE[§]	(BD FACSAria Fusion):	669
	(BD FACSAria III):	333

[‡]Qr is the relative fluorescence detection efficiency, used for describing the light collection efficiency of a detector, measured in assigned BD units (ABD units). One ABD unit, for a given fluorochrome, is defined as the fluorescence of one antibody bound to a $CD4^+$ cell. The higher the Qr value, the better the relative fluorescence detection efficiency.

[§]Qr values from the same lot of beads were taken from one BD FACSAria Fusion and one BD FACSAria III. Qr values can vary between instruments and instrument configurations. The BD FACSAria III was used for comparison.

Fluorescence Resolution
Coefficient of variation (CV)
PI: Area, <3.0%, full G0/G1 peak for PI-stained chicken erythrocyte nuclei (CEN)
Hoechst: Area, <3.5%, full G_0/G_1 peak for Hoechst-stained CEN

Fluorescence Linearity
Doublet/singlet ratio
CEN stained with PI: 1.95–2.05 (488-nm laser) or
Hoechst: 1.95–2.05 (405-nm laser)

Forward and Side Scatter Sensitivity
Sensitivity enables separation of fixed platelets from noise, identification of bacteria and detection of 0.5-µm beads.

Forward and Side Scatter Resolution
Scatter performance is optimized for resolving lymphocytes, monocytes and granulocytes.

Sample Acquisition Rate
Maximum acquisition rate (events per second) with 12 compensation pairs and 8 parameters: 70,000

Sort Performance

Drop Drive Frequency
Range: 1–100,000 Hz

Purity and Yield
At 70 psi and 87 kHz with an average threshold rate of 25,000 events per second, a four-way sort achieved a purity of >98% and a yield >80% of Poisson's expected yield. Higher threshold rates up to 70,000 events per second can be achieved without affecting purity. However, yield will decrease based on Poisson's statistics.

Functionality
Dendritic cells (myeloid and plasmacytoid, mDs and pDCs, respectively) were isolated from the peripheral blood mononuclear cells of three donors and sorted on the BD FACSAria III system (one sort per donor) which uses the same cuvette-based flow cell design as the BD FACSAria Fusion. Post-sort cell viability was assessed using a live/dead exclusion marker and functionality was assessed by intracellular cytokine staining after 6 or 18 hours of stimulation with the TLR 7 & 8 agonist R848. Post-sort viability at 6–18 hours was >90% for all three donors. Both pDCs and mDCs for all three donors produced IFN-α TNF-α and IL-12 stimulation, demonstrating post-sort functionality.

Sort Collection Devices
Two-way sorting: 12 x 75-mm and 15-mL tubes
Four-way sorting: 1.5-mL microtubes and 12 x 75-mm tubes

Sort Collection Cooling
Water recirculator for refrigeration or heating (optional)

BD FACS™ Accudrop
Red diode laser provided for fully automated drop-delay determination
Automated drop breakoff monitoring
Automated clog detection and sort tube protection system using Sweet Spot technology

Signal Processing

Converter
10-MHz analog-to-digital converter. Pulse sampling is precisely matched to the particle flow rate in the cuvette. Particles travel slower compared to conventional stream-in-air sorters. This increases the light collected, resulting in better sensitivity. High-speed sorting is achieved by accelerating the stream through the nozzle, achieving drop rates comparable to stream-in-air sorters. The flow cell design and electronics are matched to maximize signal while maintaining maximum sort speed, purity and yield.

Workstation Resolution
262,144 channels

Data Acquisition Channels
20 parameters (18 fluorescence and 2 scatter)

Fluorescence Compensation
No limit to inter- and intra-beam compensation

Pulse Processing
Height, area and width measurements available for any parameter. Ratio measurements are also available.

Time
Time can be correlated to any parameter for kinetic experiments or other applications.

Channel Threshold
Available for any parameter from any laser, with the ability to use multiple thresholds from different lasers simultaneously.

Loading

Sample Input Sizes
Microtubes, 12 x 75-mm and 15-mL tubes
Polystyrene or polypropylene tubes can be used.

Sample Input Agitation
Adjustable through the software to keep the sample constantly suspended

Temperature Control
Sample input: 4ºC, 20ºC, 37ºC and 42ºC (adjusted in the software)
Sample output: water recirculation unit (optional)

Data Management

Workstation
PC workstation with at least an Intel® Xeon® processor, 3.0 GHz or faster

Memory
>4 GB of RAM

Data Storage
250-GB and 500-GB hard drives
16x DVD +/- RW, dual layer
Floppy drive

Networking
10/100/1000 Ethernet
FireWire® serial bus

Monitor
Two 19-inch LCDs, 2,560x1,024 resolution (standard)
One 20-inch LCD, 1,600x1,200 resolution (optional)
One 23-inch LCD, 1,920x1,200 resolution (optional)

Printer
Color network laser printer

Data File Structure
Flow Cytometry Standard (FCS) 3.0 or 2.0

Software
BD FACSDiva™ software v8.0 or later

Operating System
Microsoft® Windows® 7

Installation Requirements

Instrument Dimensions (H x W x D)
BD FACSAria Fusion without BSC:
142 x 128 x 87 cm
(56 x 50 x 34 in.)
Weight: 475 kg (1,047 lb)

BD FACSAria Fusion with BSC:
220 x 127 x 89 cm
(87 x 50 x 35 in.)
Weight: 765 kg (1,687 lb)
See Site Preparation Guide for space and clearance requirement.

Temperature Operating Range
Without BSC: 17.5ºC–27.5ºC (63.5ºF–81.5ºF)
With BSC: 17.5ºC–22.5ºC (63.5ºF–72.5ºF)

Workspace Table (Optional)
94 x 71.1 x 66 cm
(37 x 28 x 26 in.)

Heat Dissipation
Without BSC: 1,965 BTU/hour (maximum, depends on the choice and number of lasers)
With BSC: 4,422 BTU/hour (5-laser system)

Power

Operation at 100/115/230 VAC and 50 or 60 Hz
Maximum power: 1,800 watts

Water Supply
None required

Air Supply
95–100 psi, regulated air, clean (<5 ppm) and free of oil
Approximately 0.52 standard cubic meters per hour

Options

Integrated Biosafety Cabinet (BSC) Option
The BD FACSAria Fusion can be equipped either at the time of purchase or as a field upgrade with a fully integrated custom-tailored BSC designed in collaboration with The Baker Company.

The BSC has been verified by The Baker Company to meet personnel and product protection standards: for a Class II Type A2 biosafety cabinet, the National Sanitation Foundation International Standard 49, the European Standard 12469 and the Australian Standard AS 2252.2–2009. No verification to other aspects of this or other standards has been made.

While the BSC protects the operator from aerosol exposure during a sort, the built-in aerosol management system also evacuates aerosols and operates independently of the cabinet for an added measure of safety.

The system can be easily field upgraded with the cabinet at a later date.

Aerosol Management Option (AMO) for Systems Without the BSC Option
The BD FACSAria Fusion features an enclosed pathway from the sample injection chamber to the sort collection tubes. For an added level of aerosol management, the BD™ Aerosol Management Option (AMO) evacuates the sort collection chamber and traps aerosolized particles during sorting. It is equipped with a 0.01-µm size ultra-low penetrating air (ULPA) filter to trap aerosolized particles.

When operated under normal and stressed conditions (mimicking a clog), <3 Glo Germ™ particles were identified outside the automated cell deposition unit (ACDU) sort collection chamber. Glo Germ particles, developed by Glo Germ in Moab, Utah, have been shown to provide good visualization of aerosol deposition in normal and mock failure modes by Oberyszyn and Robertson (*Cytometry*. 43:217-222, 2001).

Temperature Control Option
This option can be used for both sort tube and plate temperature regulation during a sort and includes:
Recirculating water bath
Specially designed collection tube holders with ports for recirculating water:
- Two-way 15 mL
- Four-way 12 x 75 mm
- Four-way 1.5-mL Eppendorf style

Lasers Available for Upgrade
355 nm
405 nm
561 nm
BD optical filters for BD Horizon Brilliant™ polymer conjugates available for the BD FACSAria™ Family.

Compliance with Safety Standards

UL 61010 (US)
IEC 61010 and IEC 60825 (Europe)
CAN/CSA - C22.2 No. 61010 (Canada)
Class 1 Laser Product per CDRH regulations and EN/IEC 60825

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