

BD Influx Cell Sorter

Technical Specifications

The BD Influx™ cell sorter is a flexible flow cytometry platform that adapts to a researcher's application requirements. A modular architecture and a combination of detection capabilities, hands-on controls, and high performance allow researchers to configure the BD Influx system to various site and application needs.

The BD Influx system can handle a throughput rate of up to 200,000 events per second.

Optics

Excitation Optics

Optical Platform

Lasers are mounted on a standard optical breadboard. Laser beams are aligned independently by adjustable mirrors. Each laser beam has its own final focus lens mounted on a dedicated translational stage for fine adjustments. For six- and seven-laser systems, one or two laser paths have collinear beams through the steering optics and focusing lens, but intercept the stream independently.

Power Out of the Laser Head

355 nm: >100 mW 405 nm: >50, >100 mW 457 nm: >300 mW 488 nm: >200 mW 532 nm: >150 mW 561 nm: >150 mW 640 nm: >120 mW

Optional beam shaping optics allow for round or elliptical beam shapes (3:1 ratio with a typical beam height of 15–20 µm).

Emission Optics

Emission light is collected through a 20X, 0.6 NA microscope objective (90°). Light is focused on three, five, or seven spatially separated mirror pinholes depending on the number of lasers. Modular detector blocks allow for a user-defined detection configuration (see the BD Influx Cell Sorter Filter Guide).

Simultaneous video observation of the stream, the pinholes, and the laser intercepts allows for fast and intuitive alignment.

Regular forward scatter is detected using 75- and 50-mm lenses, an aperture, and a photomultiplier tube. Resolution for the standard forward scatter detector is $>0.5 \mu m$ (measured using beads). Collection angle is $2-17^{\circ}$.

Side scatter is collected through the 90° collection lens and measured using a photomultiplier tube. Side scatter resolution is >0.2 μ m (measured using beads and 0.1- μ m filtered sheath fluid).

Fluidics

General Operation

- Laboratory air pressure and/or vacuum can be used for operation (regulated at 90 psi, 6.2 bar).
- An optional air pressure supply and/or vacuum pump is available.
- Sheath pressure is adjustable from 1–90 psi (0.07–6.2 bar).

Fluidics Reservoirs

Autoclavable 7-L sheath and waste containers, equipped with pressure and vacuum readout, are provided.

Fluidics Control

- Sheath, sample, and boost pressure can be individually adjusted.
- A sample flow fine adjustment is provided for precise regulation of sample flow.
- Purge, pulse, rinse, and run buttons are provided for quick stream startup and bubble removal.

Replaceable Fluidics Path

- The fluidics path, including the nozzle assembly, can be exchanged. There are no inline valves. Only pinch valves are used.
- The sample line can also be exchanged.

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, preventing air bubbles from reaching the nozzle assembly.

Sample Input

12 x 75-mm tubes, polypropylene

Temperature Control

Sample tubes can be cooled or heated by an optional circulating water bath.

Performance

Fluorescence Sensitivity

Measured using SPHÉRO™ Rainbow Calibration Particles (RCP-30-5A) according to the manufacturer's specifications:

Sheath pressure: 33 psi

Drop dive: On, ~68 kHz
Excitation: 488 nm, 200 mW
Emission: 530/40 nm for FITC
580/30 nm for PE

FITC: 125 molecules of equivalent soluble fluorochrome (MESF-FITC) PE: 125 molecules of equivalent soluble fluorochrome (MESF-PE)

Fluorescence Resolution

Measured using propidium iodide (PI)-stained chicken erythrocyte nuclei (CEN):

Sheath pressure: 33 psi

Drop drive: On, ~68 kHz Excitation: 488 nm, 200 mW Emission: 610/20 nm for PI

Coefficient of variation (CV) of PI: <3%, full G_0/G_1 peak

Fluorescence Linearity

Measured using propidium iodide PI-stained CEN:

Sheath pressure: 33 psi

Drop drive: On, ~68 kHz Excitation: 488 nm, 200 mW Emission: 610/20 nm for PI

Doublet/singlet ratio: 1.95-2.05

Sort Performance

Drop Drive Frequency Adjustable 9–180 kHz

Purity and Yield

At 60 psi and 100 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 for all four populations. Higher threshold rates of up to 70,000 events per second can be achieved without affecting purity; however, yield will decrease based on Poisson statistics.

Viability

As shown in published literature, sorts performed using murine¹⁻⁴ and human⁵ cells and/or cell lines⁶ demonstrated good recovery and viability in several experimental systems. Optimal sort conditions need to be established for different cell types.

Nozzles

Supplied: 70, 86, 100, and 140 μm

Optional: 200 µm

Sort Collection Devices

All collection devices are designed to fit on the Computerized Cell Deposition Unit (CCDU). The CCDU is standard on all instruments.

- Two-way sorting: Microtubes; 12 x 75-mm, 15-mL, and 50-mL tubes
- Three-way sorting: One 50-mL tube and two 12 x 75-mm tubes
- Four-way sorting: Microtubes, 12 x 75-mm tubes
- Six-way sorting:* 0.4-mL tubes
- Plates and slides: 6, 24, 48, 96, and 384-well plates; slides; and userdefined collection devices

Sample Collection Cooling

Water recirculator for refrigeration or heating of collection tubes (optional).

Sort Monitoring

- Live video feed of waste collection and side streams.
- Live video feed of breakoff point.
- Drop-delay determination is achieved with BD FACS™ Accudrop technology. The drop-delay value can be adjusted while viewing BD Accudrop beads which are illuminated by a red diode laser in the center and side sort streams.

Signal Processing

Data Acquisition Channels

- Up to 5-laser systems: 16 channels, usually 14 colors plus forward and side scatter
- 6- and 7-laser systems: 24 channels,* usually 22 colors plus forward and side scatter

Signal Processing

- 16-bit analog-to-digital conversion, 65,536 channels
- Parallel data stream with channel ID and integrity check
- Less than 1 correlation error per 10⁸

Acquisition Rate

Dead time is 0 µs. The maximum throughput rate is 200,000 events per second, independent of the number of parameters.

Fluorescence Compensation

- Up to 5-laser systems: 16 x 16 digital compensation matrix.
- 6- and 7-laser systems: 24 x 24 digital compensation matrix.*
- Compensated parameters are being added to the bus as separate parameters.

Pulse Processing

- All signals are height (peak) by default.
- Optional pulse processor electronics add area and width measurements for a maximum of 8 parameters to the
- Width measurement on the trigger parameter is standard.

Time

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

Channel Threshold

- Any parameter can be used as the threshold from the primary laser.
- Lasers and detectors can easily be switched to change laser sequence.

Workstation

Workstation

PC workstation with at minimum Intel® Core2 Duo, 2.40 GHz or faster, USB (2 ports), Windows® XP professional operating system

Memory

1 GB

Data Storage

- 2 x 80 GB hard drives, RAID 1 (mirrored) configured
- 16x CD/DVD +/- RW
- Floppy drive

Networking

10/100/1000 Ethernet

Monitor

20-inch LCD, 1280x1024 resolution

Data File Structure

Flow cytometry standard (FCS) 3.0

Software

BD Spigot v6.1.4 or later

^{*} Enabled with new software currently in development.

Installation Requirements

Dimensions (H x D x W)

BD Influx: 55.1 x 50.4 x 39.4 in. (140 x 128 x 100 cm)

BD Influx with HEPA-filtered enclosure:* 85.8 x 50.4 x 49.2 in. (218 x 128 x 125 cm)

BD Influx with HEPA-filtered enclosure (extended bench):* 85.8 x 50.4 x 59.1 in. (218 x 128 x 150 cm)

Weight does not exceed 485 lb (220 kg).

* A minimum of 102.4 in. (260 cm) floor-to-ceiling height is required during installation of the HEPA-filtered enclosure.

Size and Weight

On all sides, 18 in. (46 cm) is required for service access. A minimum of 102.4 in. (260 cm) floor-to-ceiling is required for installation with the HEPA-filtered enclosure.

Temperature Operating Range $18-22 \pm 3^{\circ}C$

Heat Dissipation

6,200 BTU/h maximum, dependent on laser choice

Power

Two (2) dedicated 120-VAC, 50/60-Hz, 15-A lines. Multiple peripherals may require an additional line. An appropriate transformer will be delivered depending on region-specific settings. Optional air and vacuum supply require an additional line. Power requirements are between 1,080 watts per hour (3 lasers, no HEPA-filtered enclosure) and 1,800 (5 lasers with HEPA-filtered enclosure). Standby status is not applicable.

Humidity

55% ±10% RH

Room Noise

< 80 dBA from all running equipment

Water Supply

Not required unless water-cooled lasers are used. Specifications defined by laser manufacturer.

Air Supply

90 psi (6.2 bar) regulated. The source of compressed air must deliver clean (less than 5 ppm), dry-filtered (oil-free) air and stable pressures.

Vacuum Supply

A vacuum supply is delivered with the system. A laboratory vacuum supply between 5 and 15 in. Hg at 1 CFM can also be used.

Options

Small particle

Small particle with polarization

HEPA-filtered enclosure

Aerosol evacuation

Sample temperature control

Polarization (without small particle)

Air compressor

Regulatory Status

CE marked for electrical safety (Europe)
UL Standard for electrical safety (USA)
CSA for electrical safety (Canada)

Class I (1) laser product per CDRH regulations and EN/IEC 60825-1

References

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Class I (1) laser product.

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