



# BD Influx™ Cell Sorter

## Technical Specifications

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The BD Influx™ cell sorter offers an open, configurable platform ideal for life science research, including leading-edge applications in cell therapy research, drug discovery, and marine biology. The BD Influx system can be fully optimized for researchers' unique application-specific requirements. This level of choice and control is particularly important for biomedical discovery and other rapidly evolving cell research that can benefit from new sorting tools. The cell sorter's high degree of flexibility allows researchers to configure the instrument to meet routine requirements or tailor it for the changing needs of emerging applications.

## Excitation optics

Lasers are mounted on a standard optical breadboard. Laser beams are aligned independently by user-adjustable mirrors. Each laser beam has its own final focus lens mounted on a dedicated translational stage for fine adjustments.

### Standard laser options

Wavelength (nm)	Power (mW)
355	100
405	50
457	300
488	200
532	150
561	75
640	50

Optional beam shaping optics allow for round or elliptical beam shapes (3:1 ratio with a typical beam height of 15–20  $\mu\text{m}$ ).

## Emission optics

Emission light is collected through a 20X, 0.6 NA microscope objective (90 degrees). Light is focused on five spatially separated mirror pinholes. Modular detector blocks allow for a user-defined detection configuration (see *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  $>0.5 \mu\text{m}$  (measured using beads). Collection angle is 2–17°.

The Small Particle Option (SPO) incorporates a 20X, 0.42 NA microscope objective, a mirror pinhole, and a pinhole camera. Resolution for the SPO is  $>0.2 \mu\text{m}$  (measured using beads and 0.1- $\mu\text{m}$  filtered sheath fluid). Collection angle is 2–30°.

Side scatter is collected through the 90 degree collection lens and measured using a photomultiplier tube. Side scatter resolution is  $>0.2 \mu\text{m}$  (measured using beads and 0.1- $\mu\text{m}$  filtered sheath fluid).

## Fluorescence sensitivity

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

Sheath pressure: 33 psi  
 Drop drive: 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 chicken erythrocyte nuclei (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, even exceeding the droplet formation rate, can be achieved without affecting purity; however, yield will decrease based on Poisson statistics.

## Viability

As shown in published literature, sorts performed using murine<sup>1-4</sup> and human<sup>5</sup> cells and/or cell lines<sup>6</sup> demonstrated good recovery and viability in several experimental systems. Optimal sort conditions need to be established for different cell types.

## Nozzles

70, 100, 140, and 200  $\mu\text{m}$

## 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
- Plates and slides: 6, 24, 48, 96, and 384-well plates; slides; and user-defined collection devices
- Positional sorting: Slides or filters
- Proportional sorting: Slides or filters

## 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 through a semi-automated sort protocol on microscope slides using the CCDU. Inverted or upright fluorescence microscope necessary for manual bead counting (not included).

## Electronics and signal processing

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### Data acquisition channels

16 channels, usually 14 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 10<sup>8</sup> events

### Acquisition rate

System dead time is 5  $\mu$ s. This leads to a maximum throughput rate of 200,000 events/second, independent of the number of parameters.

### Fluorescence compensation

16 x 16 digital compensation matrix (DSP). Compensated parameters are being added to the bus as separate parameters.

### Pulse processing electronics

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

### Time

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

### Threshold channel

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

## Fluidics

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### 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 can be exchanged, including the nozzle assembly. There are no in-line 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.

## Data management system

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### 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

## Installation requirements

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### 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 need 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.

### 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.

## Size and weight

Configuration (without computer and table)	Height		Depth		Width	
	cm	in.	cm	in.	cm	in.
BD Influx	140	55.1	100	39.4	128	50.4
BD Influx with HEPA-filtered enclosure	218	85.8	125	49.2	128	50.4
BD Influx with HEPA-filtered enclosure (extended bench)	218	85.8	150	59.1	128	50.4

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

Weight does not exceed 220 kg (485 lb).

## Regulatory requirements

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

## Operational room/environment

Temperature: Stable, 18–22 ±3°C

Humidity: 55% ±10% RH

Room noise: <80 dBA, accumulated noise from all running equipment

Heat output: Maximum output estimated to be 6200 BTU/h, laser choice dependent

## References

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Class I (1) laser product.

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