

# Flow Cytometry

Research Administration
Seattle, WA ● 501(c)(3) Nonprofit

Fred Hutch's Shared
Resources are catalysts for
lifesaving discoveries. This
uniquely centralized program
of 15 specialized core facilities
and scientific services drives
advances by integrating
dedicated experts and
cutting-edge technologies
across the entire research
pipeline, from basic science to
clinical trial.



Spectral Imaging Cell Sorter

## **Excitation Optics**

The optics layout includes five lasers.

349 nm: 30 mW 405 nm: 50 mW 488 nm: 100 mW\* 561 nm: 50 mW 637 nm: 100 mW

\*100 mW is split to enable BD CellView™ Image Technology

### **Scatter detectors**

Blue laser: Forward side scatter (FSC), Side scatter (SSC), Axial light loss (ALL) Violet laser: Axial light loss (ALL), Side

scatter (SSC)

## **Fluorescent detectors**

Spectral arrays, 78 algorithmically optimized fluorescent detectors covering the full spectrum:

22 ultraviolet (UV) detectors

20 violet detectors 16 blue detectors

12 yellow-green detectors

8 red detectors

## Fluorescence detectors for imaging

FL1: LP505: 534/46 FL2: LP570: 600/60 FL3: LP675: 788/225

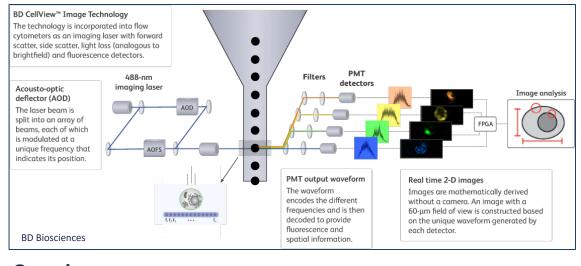
# LEARN MORE

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# Overview

BD FACSDiscover™ S8 Cell Sorter with BD CellView™ Image Technology is the first spectral instrument with sort-capable image analysis, expands the power of cell analysis and sorting to new dimensions by combining spectral flow cytometry with real-time spatial and morphological insights—empowering scientists to address previously impossible-to-answer questions.

- Obtain insights on cell populations and characteristics that can be visually confirmed in real time during analysis and sorting
- Enhance spectral flow cytometry with spatial and morphological insights to interrogate and sort cell types that previously could not be identified or isolated
- Create a comprehensive profile of highly complex diseases and systems with correlation of downstream analysis with imaging and traditional flow data

# **Examples of Imaging features**

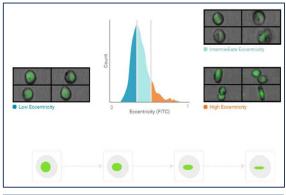
## **Eccentricity**

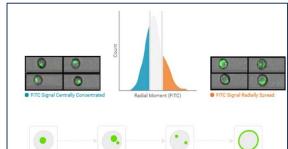
Eccentricity is a ratio of the shortest to the longest axis of the identified particle (as identified by the region of analysis)

**Usage examples:** Doublet discrimination, cluster

identification

Availability: All imaging channels





#### **Radial Moment**

Radial moment is the average distance of the pixels from the centroid within the region of analysis.

Usage examples: Doublet discrimination (with Eccentricity), cell—cell interactions (cellular synapse)

Availability: All imaging channels

## Correlation

Correlation is the degree to which the location of two imaging channels are the same within the region of pixels defined by the region of analysis.

**Usage example:** Translocation assay **Availability:** Any two imaging fluorescence channels

## **Additional Imaging features**

- Center of mass X
- [
- Center of mass Y
- Delta center of mass
- Diffusivity
- Max intensityMoment (long)
- Moment (short)
- Size
- Total intensity

### **Cell sorting features**

The Discover S8 is a full spectral sorter capable of running highly complex panels. The sorter has 3 nozzle sizes, 85um, 100um, and 130um all use lower pressure than other similar sorters which helps to mitigate loss of cellular function due to sorting induced cellular stress. <sup>1</sup>

Researchers at Fred Hutch have designed and successfully run 50 color experiments for deep immune profiling, developed assays to better characterize immune synapse between antigen presenting cells (APC's) and T-cells, and obtained highly purified populations using imaging features to better discriminate single cells, while being able to visually confirm each cell with index sorting. Novel sorting applications are continuing to be developed as research incorporate the spectral and imaging capabilities of the Discover S8 into their research.



## References

1. Ryan K, Rose RE, Jones DR, Lopez PA. Sheath fluid impacts the depletion of cellular metabolites in cells afflicted by sorting induced cellular stress (SICS). Cytometry. 2021;99:921–929. https://doi.org/10.1002/cyto.a.24361