

FLOW CYTOMETRY

PHONG DO

EE 494

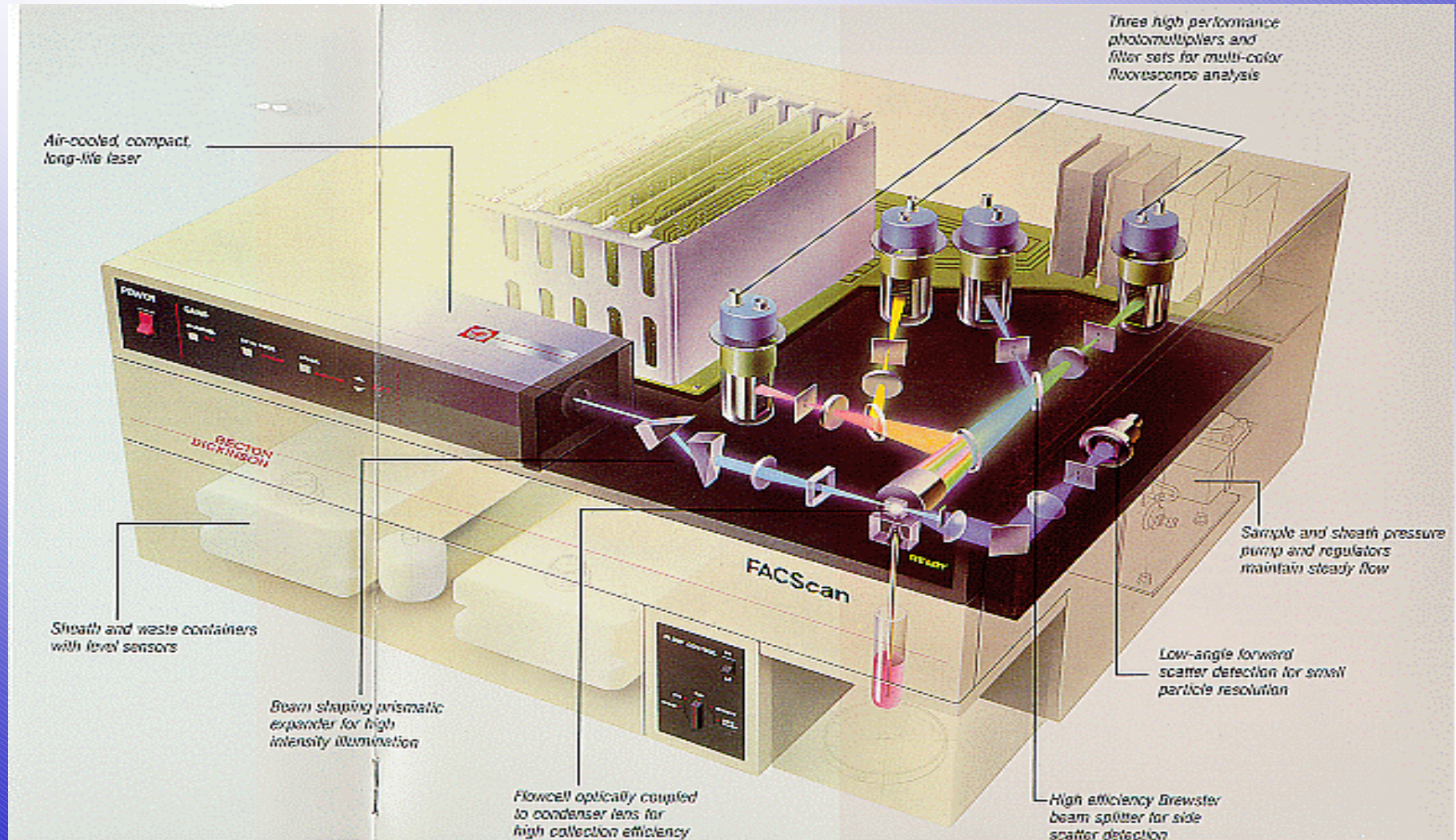
Motivation

- To familiarize those that are uncomfortable with these testing and thus helps reduce their fears of these testing.
- I always have a strong interest in researching how technology being applied into the medical field
- To complete my presentation project for EE494 class.

What is Flow Cytometry

- It is a method for quantitating components or structural features of cells primarily by optical means as the cells move single-file in a liquid suspension.
- Flow cytometer can process thousands of cells in a few seconds.
- different cell types can be distinguished by quantitating structural features, flow cytometry can be used to count cells of different types in a mixture.

How it works

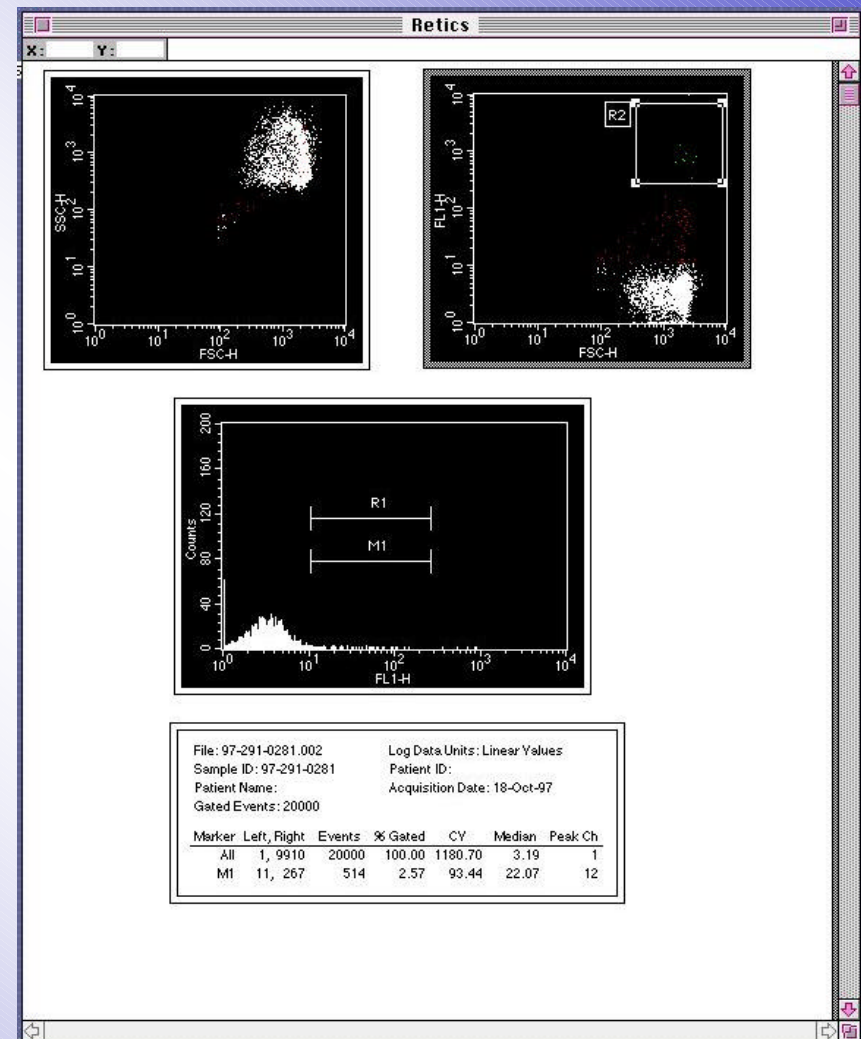


Measurable quantities

- Low angle forward scatter intensity, approximately proportional to cell diameter.
- Side light scatter intensity, approximately proportional to the quantity of granular structures within the cell.
- Fluorescence is the property of a molecule to absorb light of a particular wavelength and re-emit light of a longer wavelength. The wavelength change relates to the energy change that takes place in the process.

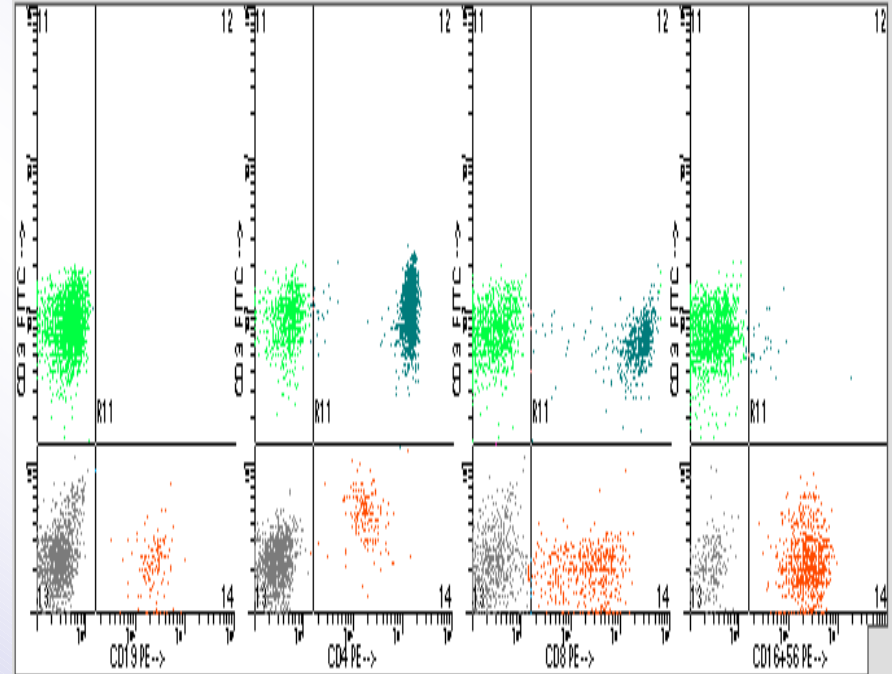
Data Presentation

- Single-parameter histogram with the x-axis representing fluorescence intensity and the y-axis representing the number of counts in each channel along the x-axis, usually in a logarithmic scale.



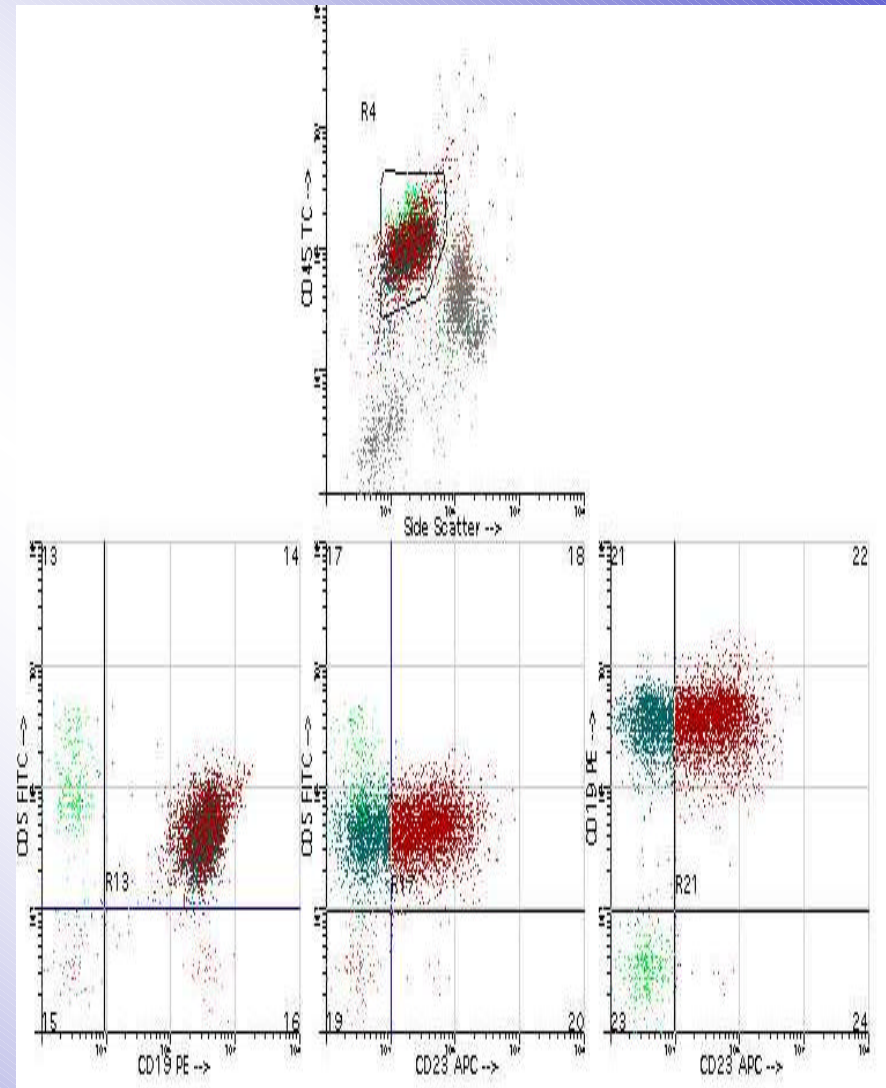
Data Presentation cont...

- Two-parameter dot plot is display in four quadrants, which are described by numbers and/or locations (LL, UL, LR, UR).
 - LL (lower-left) represents cells negative for the descriptors on both the x- and y-axis.
 - UR (upper-right) represents cells dual-positive for the descriptors on both the x- and y-axis.
 - UL (upper-left) represents cells positive for the y-axis descriptor, but negative for the x-axis descriptor.
 - LR (lower-right) represents cells positive for the x-axis descriptor, but negative for the y-axis descriptor.



Data Presentation cont...

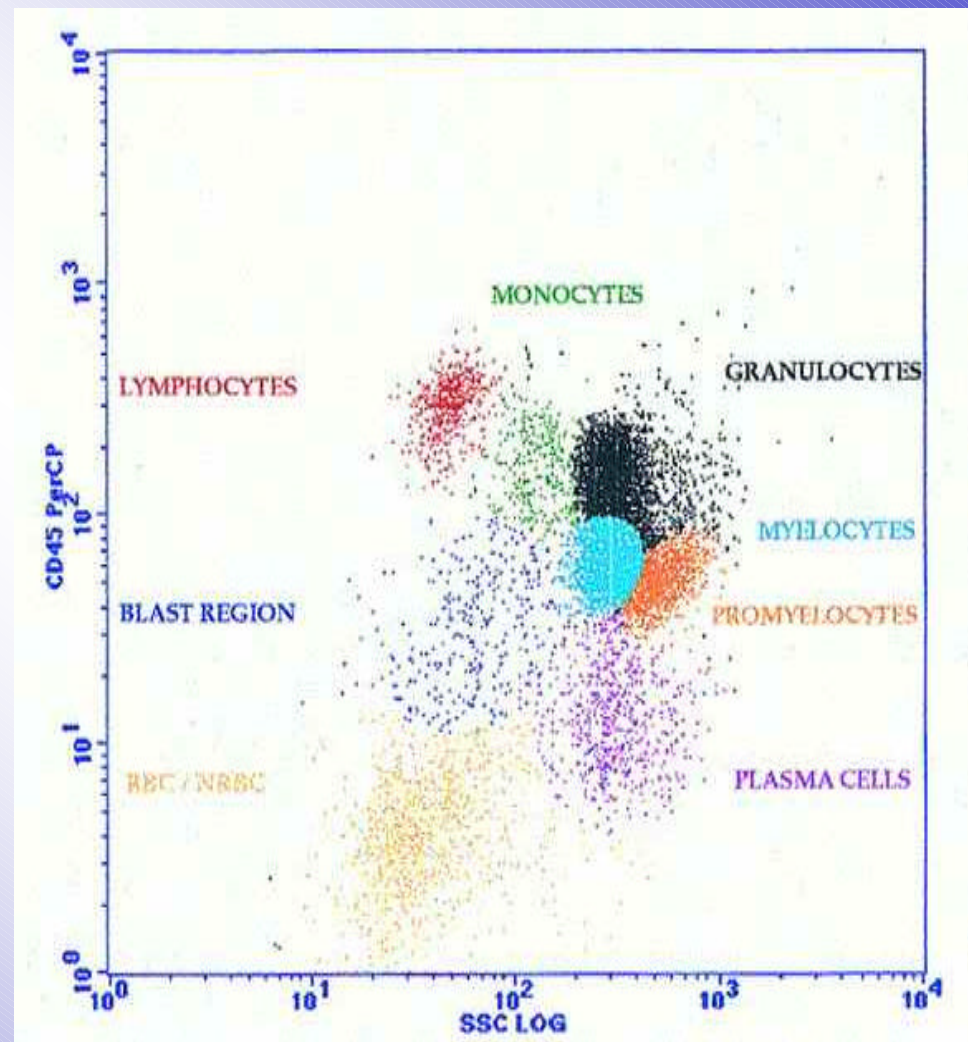
- Two-parameter contour plots display qualitative assessment of the position of clusters within the plots, denoted by expressing reactivity in terms of positive or negative, qualified by the relative fluorescence intensity (i.e., weak, moderate, strong) of each discernible distribution on the plot rather than displaying percentages within each quadrant.



Data Presentation cont...

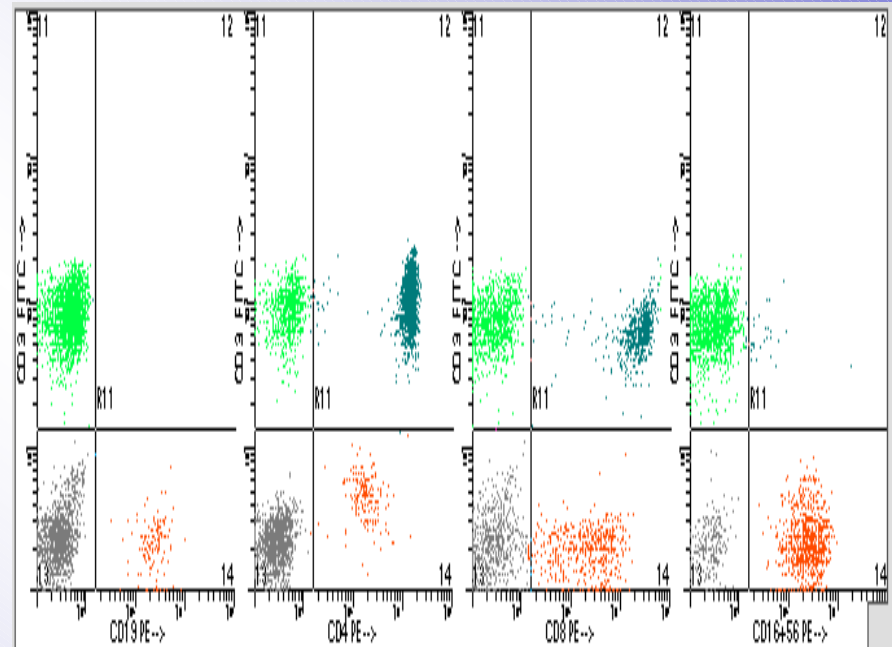
Bone Marrow

- Gating specifies the cells of interest or, in other words, specifies the denominator of all subsequent calculated results.
 - Cells have traditionally been gated according to physical characteristics, as identified by forward and side scatter signals, but, current gating strategies utilize a fluorescence parameter along with a scatter parameter; usually side scatter is combined with the fluorescence associated with an anti-CD45 conjugate.
 - **CD45 versus side scatter (linear scale) can be used to set gates around CD45-bright lymphocytes in peripheral blood for CD4**



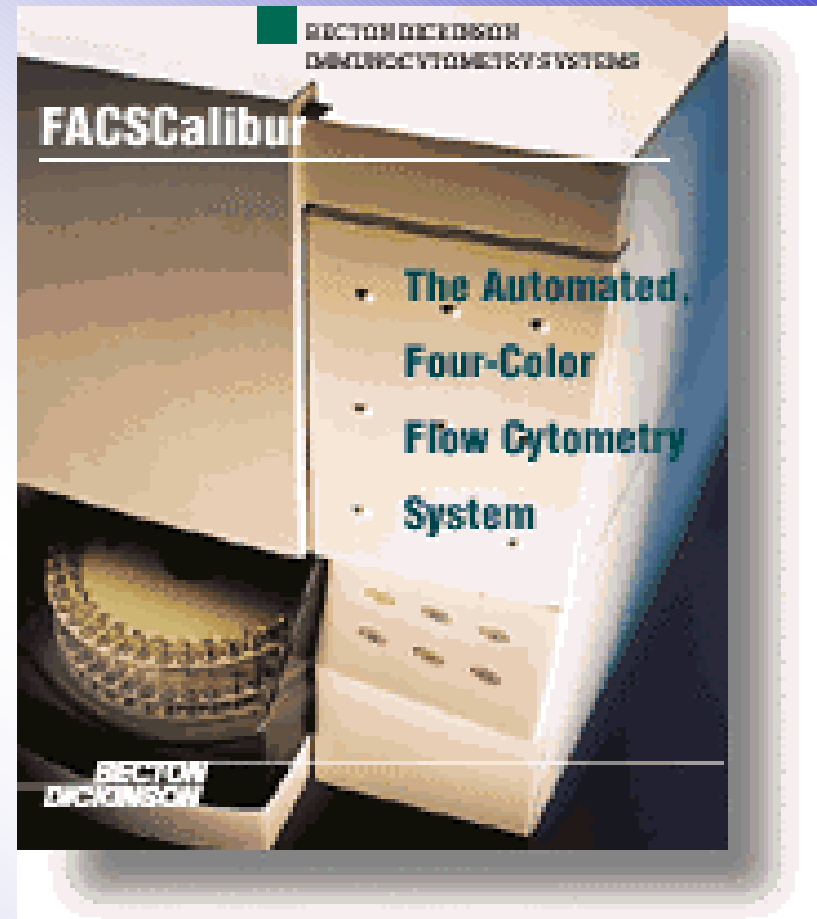
Applications

- CD4 enumeration refers to the quantification of CD4-positive T-lymphocytes in peripheral blood. An overwhelming proportion of requisitions for this test occur in the context of monitoring disease course in HIV-infected individuals.
- The second panel (CD3/CD4) that there are two CD4+ populations: one in the upper-right quadrant that is CD3+ , and one in the lower right quadrant that is CD3-. The latter represents CD4 binding to monocytes that are contaminants in the lymphocyte gate and are not included in the calculation for CD4 positivity, since we are only interested in CD4+ **T-lymphocytes**.
- As with any other applications, the data generated are



Commercial Products

- FACSCalibur is another BD first: the only four-color, *dual-laser* benchtop flow cytometry system available today capable of both analyzing and sorting.
- The ease of use of the FACSCalibur flow cytometer is enhanced by its user-friendly data management system. FACStation takes advantage of the intuitive Macintosh environment. It performs all the computing tasks required for fast, accurate results, including instrument setup, data collection, analysis, and management. FACStation's automated software and high-performance acquisition and analysis tools make plotting, gating, statistical analysis, and reporting easier.
- FACSCalibur is the only benchtop flow cytometry system available today capable of sorting cells of interest.



Facscalibur's Performance

- **Fluorescence Sensitivity**

- Estimated detection limit is 750 molecules of equivalent soluble Fluorescein.
-

- **Fluorescence Resolution**

- Coefficient of variation in FL2-Area of <3%, full peak for propidium iodide-stained chicken erythrocyte nuclei
-

- **Forward and Side Scatter Sensitivity**

- Sensitivity enables the separation of fixed platelets from noise
-

- **Forward and Side Scatter Resolution**

- Scatter performance is optimized for resolving lymphocytes, monocytes, and granulocytes

Excitation Optics

- **Optical Platform:** Fixed optical assembly.
- **Lasers:** 15 mW 488 nm, air-cooled argon-ion laser; life expectancy >5,000 hours Optional second red diode laser: nominally 635 nm.
- **Beam Geometry:** Prismatic expander and achromatic spherical lens provide 22 x 66-? elliptical beam for argon-ion laser. Nominally 15 x 61-? elliptical beam for red diode laser.

Emission Optics

- **Optical Coupling:** Quartz cuvette is coupled to emission lens by refractive index matching optical gel for optimum collection efficiency
- **Background Rejection:** Obscuration blade and slit minimize unwanted laser radiation at the detector
- **Forward Scatter Detector and Filter:** High-performance solid-state silicon detector; 488 nm bandpass filter for clear signal detection and red diode laser signal rejection
- **Side Scatter Detector:** Photomultiplier using Brewster-angle beam splitter in the emission optical train
- **Fluorescence Detectors and Filters:** High performance, high dynamic range photomultipliers with bandpass filters: 530 nm (FITC), 585 nm (PE/PI), 661 nm (APC), and >650 nm (PerCP) with base unit, >670 nm (PerCP) with FL4 option

Scatter Optics

- **Forward Scatter Range:** Obscuration blade and optics aperture limit the forward scatter radiation collection to a half-cone angle range of ~ 0.7 to 10 degrees
- **Background Reduction:** Obscuration blade and 100- μ m slit minimize unwanted laser radiation at the detector. 488 nm bandpass filter in front of FSC and SSC detectors
- **Forward Scatter Detector:** Solid state silicon detector with spectral response from 300 nm to 1100 nm
- **Side Scatter Detector:** High-performance photomultiplier using Brewster angle beam splitter in the emission optical train

Sorting Specifications

- **Sorting Purity:** >95%
- **Capture Rate:** 300 cells/sec
- **Sort Modes:** Three modes (all aerosol-free): single cell, exclusion, and recovery
- **Recovery:** Depends upon sample and sorting conditions, >50%
- **Sterile Sorting:** System design allows for aerosol-free sterile sorting

References

- <http://www.isac-net.org/>
- http://www.bdfacs.com/literature/catalog/facscan_edu.shtml
- <http://www.biotech.ufl.edu/~fccl/agronomy/flowdesc.html#definition>
- <http://www.bio.umass.edu/mcbfacs/intro.htm>