

APPENDICES

Technique Specifications of flow cytometry

FACSCalibur

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
Fluorescence Resolution	Scatter performance is optimized for resolving lymphocytes, monocytes, and granulocytes
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- μ m elliptical beam for argon-ion laser. Nominally 15 x 61- μ m elliptical beam for red diode laser

Optical Coupling	Quartz cuvette is coupled to emission lens by refractive index matching optical gel for optimum collection efficiency
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 (APC) with FL4 option
General Operation	Front key panel control provides three modes: RUN, STNDBY, and PRIME; automatic standby mode conserves sheath fluid by stopping sheath flow when no sample tube is installed
Sample Flow Rates	Three selectable flow rates of 60 $\mu\text{L}/\text{min}$, 35 $\mu\text{L}/\text{min}$, and 12 $\mu\text{L}/\text{min}$. Pressure difference between sheath and sample is regulated and monitored; particle velocity in flow cell is approximately 6 meters/sec
Sample Concentration	Single-cell suspension of 10^5 to 2×10^7 particles/mL recommended range
Workstation Resolution	1024 channels on all parameters
Dynamic Range	Logarithmic amplifiers for SSC, FL1, FL2, FL3, and FL4 (with FL4 option) provide four log decade range

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

Partec PAS

- Sensitivity:<100 FITC molecules
- True volumetric absolute counting
- 1 to 8 parameters: FSC, SSC, FL1 to FL6
- 2 or 3 lasers, e.g.: Argon ion laser 488 nm, 488nm blue solid state lasers (20mW, 50mW, 100mW), 635nm red diode laser (25mW), 633nm HeNe laser, 405nm violet solid state lasers (20mW, 50mW, 100mW), 532nm green Nd:YAG lasers (50mW, 100mW), 561nm yellow solid state laser, UV lasers
- 200-600nm HBO 100W UV mercury arc lamp
- 16 bit ADC for each parameter
- Video flow monitor
- Windows® XP operation system
- Windows® XP based FloMax software
- Ethernet connection
- Built-in active LCD screen
- Optional external monitor
- Complete software package for lymphocyte subset, cell cycle, apoptosis, ploidy, microbiology, bacteria and yeast analysis
- Processor > 3.2 GHz
- Interchangeable filter combinations
- Realtime acquisition
- Automated immunology sample preparation and analysis
- Barcode Reader