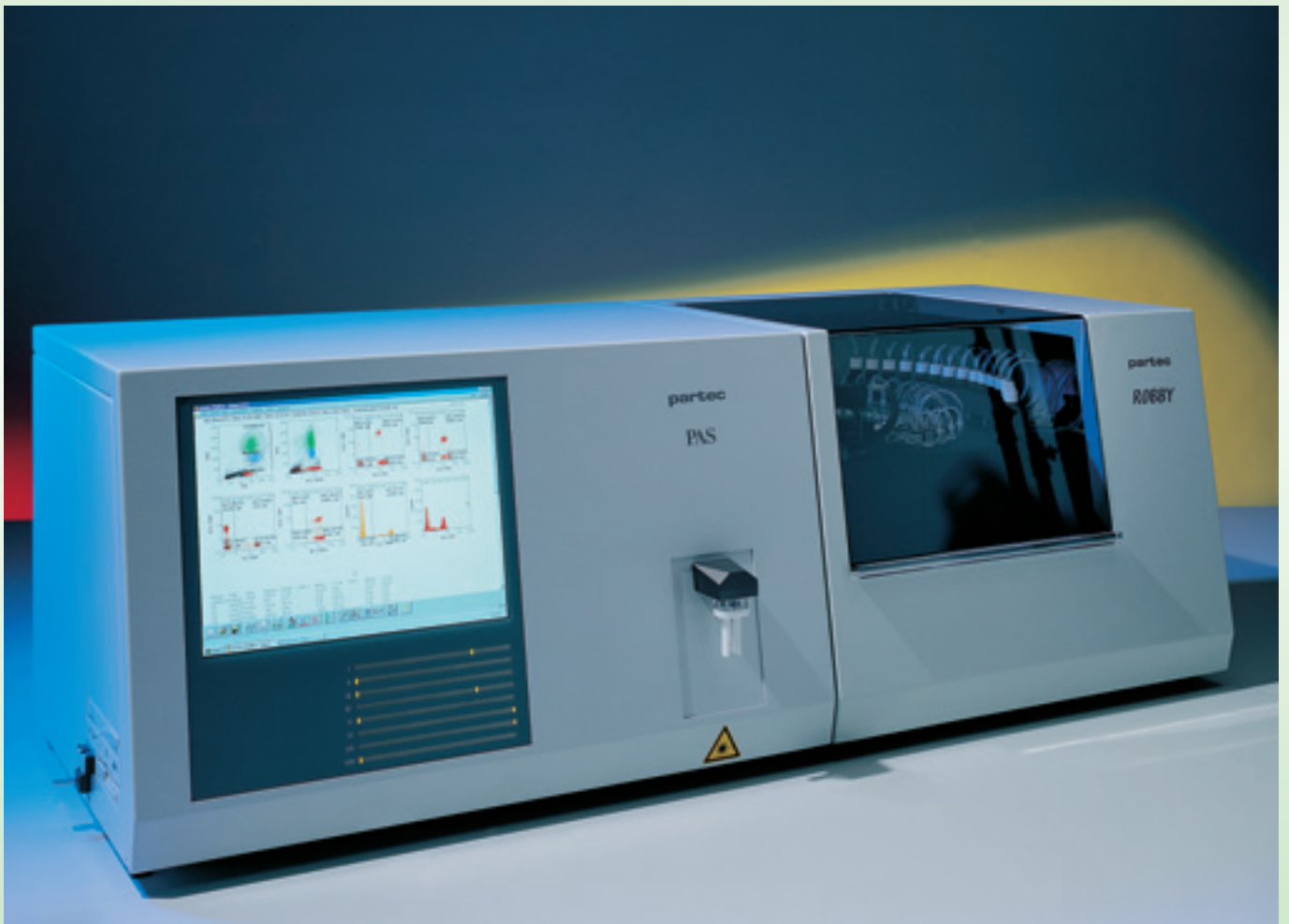


partec

# Partec PAS



**Your Flow Cytometer.**

## The Challenge...

New sophisticated applications and increasing requirements for reliable routine results in the shortest possible time continue to impose a challenge for flow cytometry instrumentation, automation and software.

## ... our Experience

Partec, pioneer in flow cytometry for more than 30 years, contributes to these requirements with the new generation of Windows™-based PAS Particle Analysing Systems. An innovative computer controlled flow system, the modular optical system with advanced photomultiplier tubes for all optical channels, recently available new computer and electronic technologies, for example new fast and precise 16 bit analog-to-digital converters, and the possibility for microsecond realtime data acquisition using the Windows™ operating system were combined in a completely new instrument and software design.

All flow cytometry applications in healthcare, microbiology, food and cosmetics control, plant and animal cell analysis benefit from the unique features of this design - and this brochure tries to give a short overview about some of the striking features and results with the new PAS.

We invite you to take part in the fascinating investigations the PAS offers us today. And we guarantee to offer you qualified scientific and technical application support and service, now and in the future.



Partec Headquarters in Münster, Germany

## The PAS Particle Analysing System - Your Fully Equipped Bench-Top Flow Cytometer

Available with a range of light sources, e.g. Argon lasers, ranging from 20 mW to 100 mW, 100 W mercury UV lamp, 25 mW red laser diode and solid state lasers, and 1 to 8 parameters, the optical configuration of the PAS is the optimum prerequisite for routine cell analysis applications, covering microbiology and small particle analysis, 4-colour immunophenotyping, precise volumetric absolute counting and DNA analysis. New upcoming applications, employing multiple light source excitation with UV and lasers and involving high precision DNA analysis with DAPI or Hoechst together with surface markers, e.g. for no-lyse immunophenotyping techniques, are now available with the PAS.



PAS-III: Expandable by many light sources

Additional larger external laser sources like NdYAG, HeCd or HeNe lasers can be added to the modular optical system by means of a stable optical bench support for both the instrument and extra light sources, thus offering a compact and rigid solution for the most demanding applications.

Eight fully parallel optical parameters, each with its own photomultiplier tube (PMT), including the forward scatter PMT, give you the most flexible choice and offer the best sensitivity and enough space for future applications. Due to the built-in Windows™ computer and ergonomic TFT LCD display, fully equipped and side-by-side with the Robby™ sample automat and autoloader, the PAS requires no more space than a normal laboratory table and a normal electrical line connection, thus reducing your laboratory requirements to a minimum. By featuring standardized PC-computer components, the PAS can be directly integrated into your laboratory information system via barcode reader, automated Word / Excel reports and a fast Ethernet network connection.

## The Flow

The heart of the PAS is the unique quartz flow cuvette, ensuring that particles cross the UV and visible excitation light with best possible precision, well one after the other. The design of the flow cuvette incorporates more than 30 years of experience in handling fluids with sub-micrometer and nanoliter precision. Thanks to the optical and mechanical precision of the flow cuvette, superior results are guaranteed for all parameters, e.g. coefficients of variation (CVs) of better than 1% with DAPI staining - a prerequisite e.g. for high precision DNA measurements for tumor or chromosome analysis.

The sample is transported with help of a computer controlled digital syringe pump, part of a virtually cross-contamination-free fluid system (< 0.1% cross-contamination). This system allows continuous flow absolute counting, displaying concentration results a few seconds after starting a sample analysis.

## Optics and Light Sources

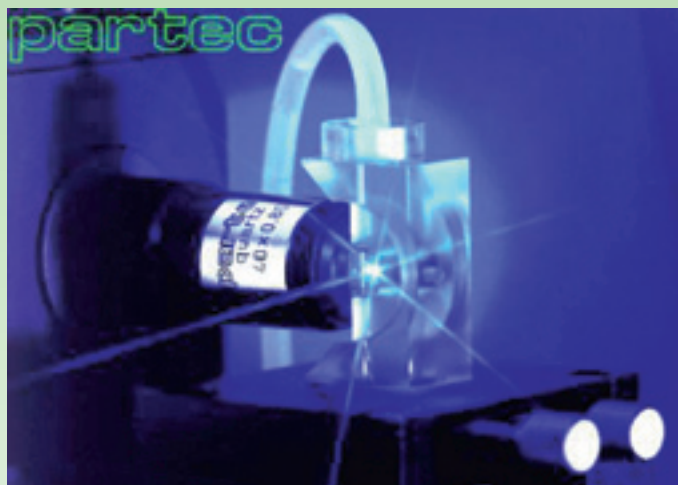
The 8-parameter optical system features a clear optical pathway, built upon Partec's legendary optical cubes. All optical elements, including the UV mercury lamp and the lasers, are mounted onto a single rigid optical bench, which guarantees optimum mechanical stability and avoids problems when moving the instrument to another place.

By means of the built-in colour CCD camera, the stability of the sample flow and the sorting process (in conjunction with the closed system piezo-sorter) can be controlled in a video flow monitor on the LCD screen.

For most applications, you will not need to change the standard configuration for side scatter (SSC), forward scatter (FSC) and 4-colour fluorescence analysis. The standard setup is optimized for all common dyes, e.g. FITC, GFP, PI, PE, PE-CY5, SR, APC, FDA, DAPI, and Hoechst. In its standard configuration, the PAS allows spatially separated 2-wavelength excitation analysis with UV + laser, laser diode + Ar laser or UV + laser diode, optimized by diaphragms in several intermediate image planes.

However, the modular optical system lets you easily configure the PAS for special applications and other fluorochromes - simply by changing dichroics/filter blocks and diaphragms without time-consuming readjustments.

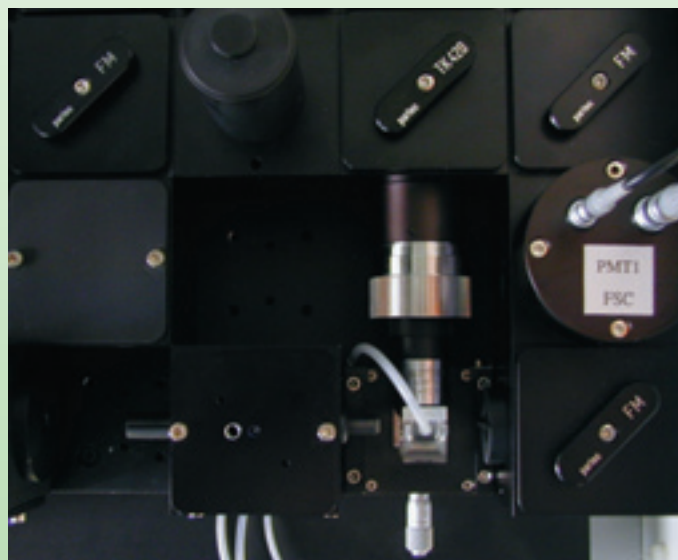
For your routine and new applications, Partec offers you the assistance you need.



*The „heart“ of the PAS: the synthetic quartz flow cuvette. Quartz optics guarantee high transmission for UV applications without temporal degradation.*



*The PAS integrates an Ar-laser, red laser diode, and UV mercury arc lamp.*



*Modular PAS optics built upon optical cubes for 8 parameters. For the PAS-III external lasers, e.g. NdYAG, HeCd or HeNe, can be mounted onto a common rigid support.*

## Computer and Electronics

Each of the optical channels is equipped with an independent processor controlled pulse analyser for parallel pulse processing in order to minimize signal losses due to coincidence and dead-time effects. The logarithmic amplifiers, specified for a 6-decade range, provide safe dynamics for 4-decade operation. 16 bit (65536 channels) analogue-to-digital converters for each parameter are the fundament for software-based colour crosstalk compensation algorithms and other numerical operations, e.g. fluorescence ratio measurements, without artifacts.

The computing power of the Pentium allows signal analysis, processing, and display of each event generated by a particle in real-time, while offering the convenience of the 32 bit Windows™ operating system. Real-time is a prerequisite for precise volumetric absolute counting and high speed and high purity sorting based on complex software-gates.

The PAS is equipped with a 650 MB CD-Writer for convenient data storage and Ethernet network connection with 10 MB/s and 100 MB/s for an easy connection to your laboratory information system (LIS).

## Software

The Windows™ FloMax® software integrates instrument control, including acquisition, cell sorting, on- and offline data analysis into one single software package. Ready prepared and freely adaptable instrument settings and panels facilitate switching between different applications. FloMax® is optimized for microbiology analysis, immunophenotyping, cell-cycle, DNA ploidy, and scientific flow cytometric analysis.

Data is always stored as original data in FCS flow cytometry standard file format for easy exchange with other analysis software.

One of the unique features is the on- and offline colour crosstalk compensation of the spectral overlap of fluorescence from simultaneously analysed dyes. The N-colour compensation algorithm allows a correction of the crosstalk between any parameters without need to rerun a sample.

FloMax® optimally supports the volumetric absolute counting feature of the PAS, displaying particle concentrations for any subsets of cells, even if defined by a gate at a later time after the acquisition.

The FloMax® Panel system allows automated analysis of repeating sample series employing different dyes or instrument settings.

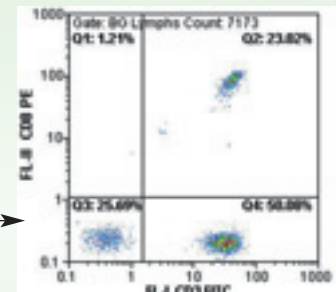
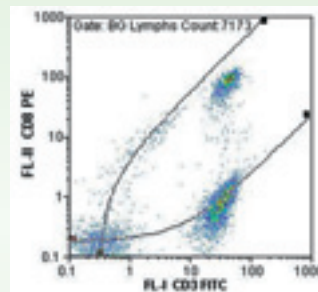
The FloMax® Report system generates easy-to-adapt result reports based on Microsoft® Word templates (.dot - files) or Excel Macros written in Visual Basic.



Built-in CD-Writer, Ethernet network connection and Barcode Reader facilitate data archive and transfer to laboratory information systems.



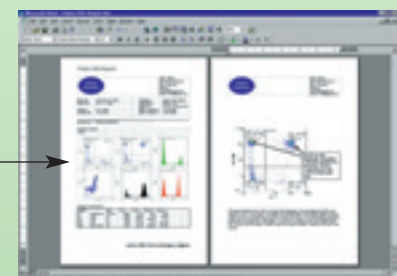
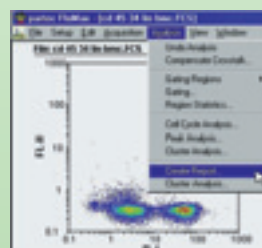
FloMax®



FloMax® graphical N-colour crosstalk compensation supports matrix-based on- and offline adjustments at any time without need to rerun samples.



The flexible multi-tube panel system of FloMax® automates routine processes.



FloMax® Report generates automated single or multi-tube panel and measurement series reports in Word or Excel.

## True Volumetric Absolute Counting

The PAS analyses concentrations of any particle subpopulations of interest using true volumetric absolute counting. This unique method is solely based on the fundamental definition of a particle concentration  $c$ , namely the counted number  $N$  of particles in a given volume  $V$ ,  $c = N / V$ . In the PAS, the volume is measured directly by mechanical means, rather than by calibration with expensive beads with a (sometimes doubtful) „given“ nominal concentration. Thus, the precision of volume measurement is defined by a fixed mechanical design, eliminating any errors related to varying bead concentrations. The PAS allows analysis of either a fixed volume as defined by the distance  $d$  between two platinum electrodes reaching into the sample tube with a given diameter  $2r$ , or, alternatively, a volume of free choice involving the digital sample speed control.

Benefits of true volumetric absolute counting:

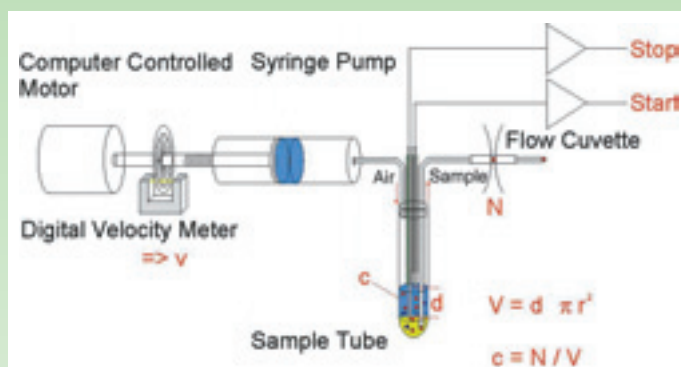
- i) digital volumetric precision by mechanical design: better than 2% reproducibility, 5% precision,
- ii) no errors related to calibration,
- iii) no additional preparation time for reference beads,
- iv) no expenses for calibration beads.

## Sample Automation

Barcodes are a significant automation component in many routine laboratories. Today, due to the variety of different systems, a flexible barcode system is most important. The PAS Barcode supports all common barcodes used for laboratory automation. Barcode reading is well integrated into the software. Besides for identifying samples and reagents, barcodes also serve as a safe selection among predefined panel protocols or instrument settings.

For optimum sample automation, the PAS can be equipped with the Robby™ sample preparation automat and autoloader for carrousel of 36 sample tubes. Samples are prepared according to predefined protocols that can be freely adapted for special purposes. Controlled by the Windows™ RobbyPrep software, the preparation covers staining with up to 16 reagents, incubation, sample shaking and cell lysis. The analysis of complete carrousel is automated by FloMax®, which combines the panel system with the Robby™ automat control.

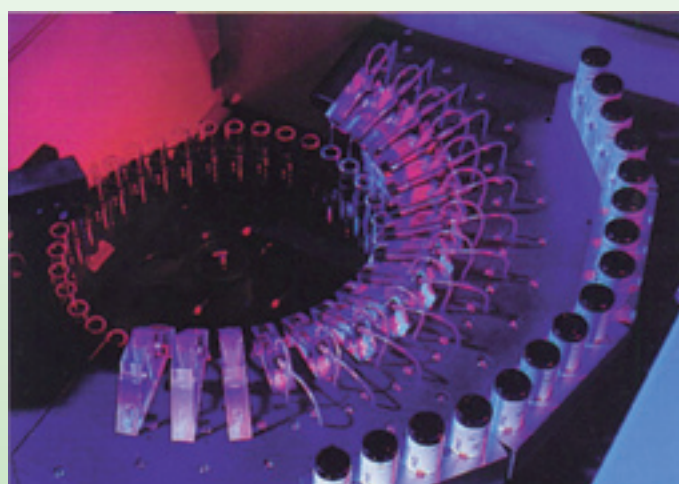
As a result, a fully automated process generates complete multi-page panel or routine series reports in Word or Excel format.



*Schematics of true volumetric absolute counting, based on real-time acquisition and counting of the number  $N$  of particles passing the flow cuvette and mechanical measurement of volume  $V$ .*

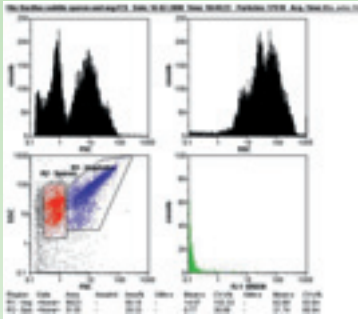


*BioSafety Sample Port for true Volumetric Absolute Counting.*

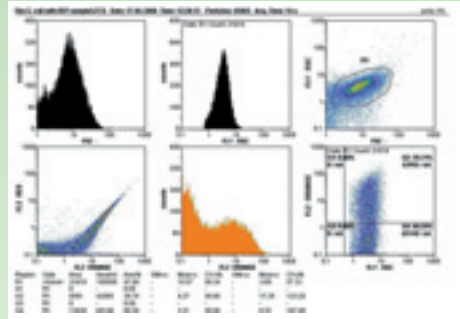


*Full walk-away automation including staining, lysing and sample analysis by the Windows™ controlled Robby™ sample preparation automat and autoloader.*

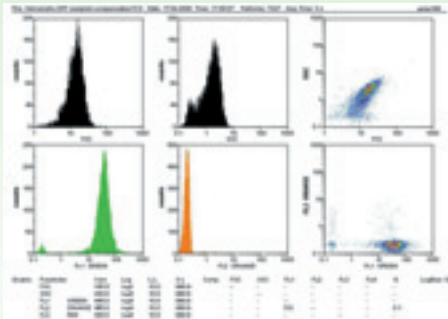
## Bacteria Analysis



FSC and SSC plots of spores and vegetative cells of *Bacillus subtilis*. Excitation with 488 nm by an argon laser (20mW).

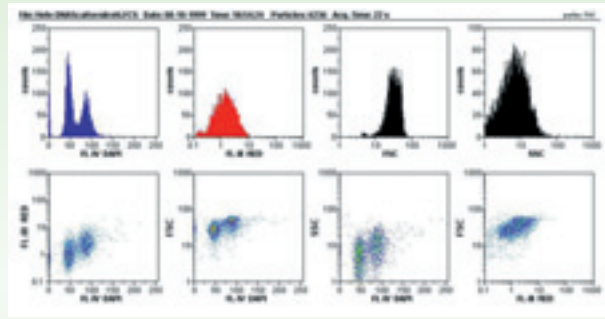


Absolute counting and measurement of RFP-expression (FL2: orange-red) of an *E. coli* population after excitation by a NdYAG-laser (532 nm, 50 mW): 40% of the *E. coli* population show the red fluorescence due to RFP-expression.



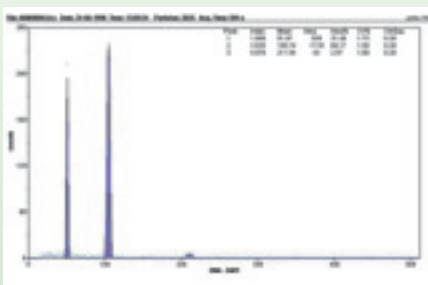
Measurement of GFP-expression (FL1 GREEN) of a *Salmonella sp.* population after excitation by an argon laser (488 nm, 20 mW).

## Yeast Analysis



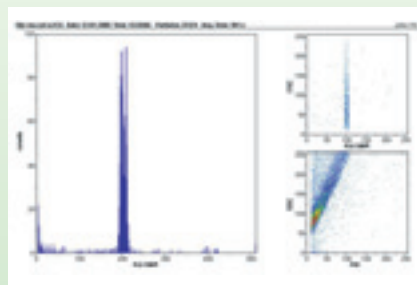
DAPI (DNA, FL-IV) and Nile red staining (neutral lipids, FL-III) of brewery yeasts after double wavelength excitation by HBO-lamp (UV) and argon ion laser (488 nm, 20 mW).

## DNA Ploidy Analysis with DAPI and PI



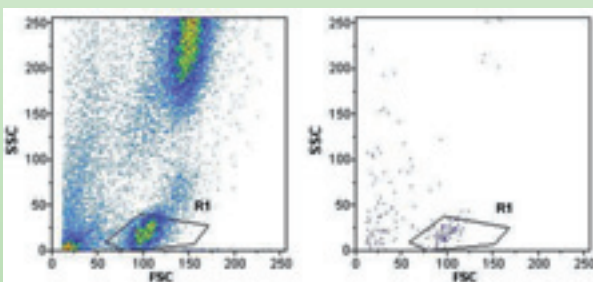
Cells of potato plant, 2n + 4n, stained with Partec CyStain UV (DAPI).

## Tumor Analysis

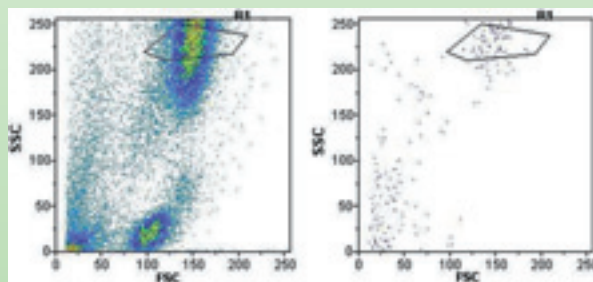


Mamma carcinoma biopsy - laser scatter (488 nm, 20 mW) + DAPI excitation by HBO lamp.

## Cell Sorting

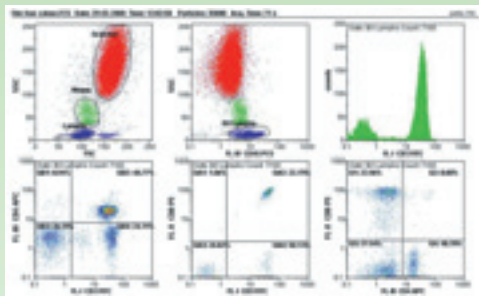


Sorting of lymphocytes - selection of the sort window (left) and reanalysis (right).



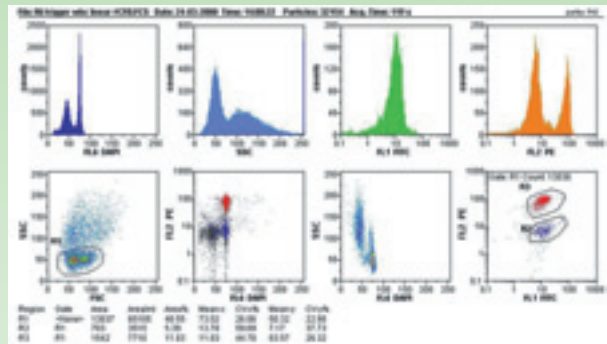
Sorting of a granulocyte subset - selection of the sort window (left) and reanalysis (right).

### Immunophenotyping: 6 parameter, 4 Colour Fluorescence Analysis with ColorGating



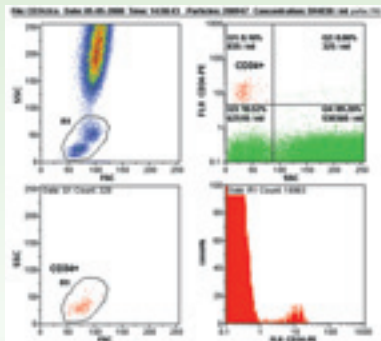
Simultaneous analysis of four antibody surface markers by argon laser and red laser diode excitation.

### No-Lyse Leukocyte Analysis



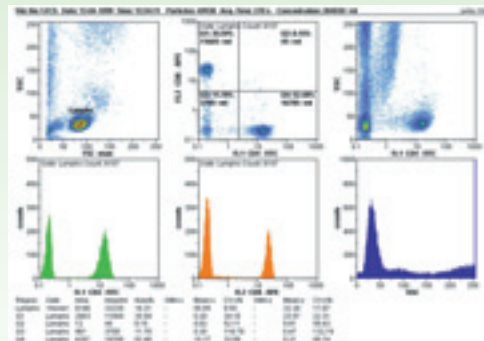
Dual wavelength excitation by UV lamp and laser. By using DAPI as trigger signal, full blood analysis is limited to the leukocytes.

### CD34+ Volumetric Absolute Counting



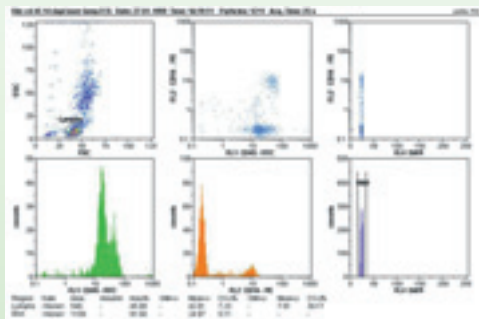
Rare event concentration analysis on CD34+ cells. (patient sample).

### CD4/CD8 Volumetric Absolute Counting



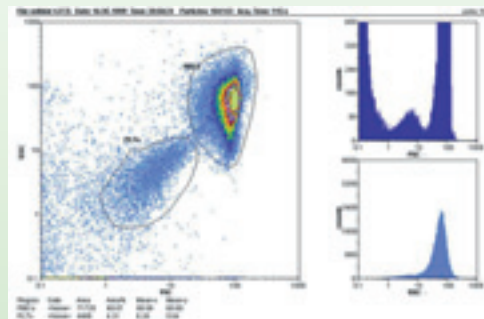
Routine concentration analysis on CD4/CD8 for HIV monitoring.

### UV plus Ar-laser 2 Wavelength Excitation



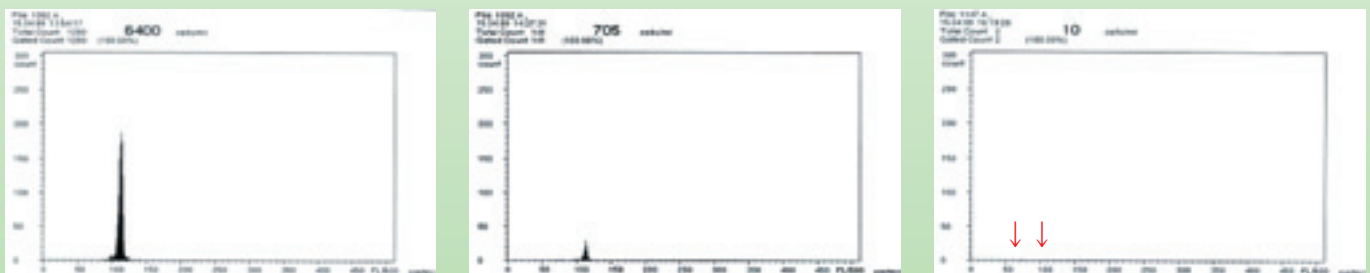
Simultaneous analysis of DNA (DAPI) and antibody surface markers.

### Platelet Analysis



Supported by the use of a PMT for the FSC parameter, the small platelets (PLT) can be easily discriminated from red blood cells (RBC) in the scatterplot.

### Leukocyte Depletion Process Control



Analysis of leukocyte concentration based on CyStain (DAPI) staining and volumetric absolute counting. Due to the digital nature (high signal to noise ratio) of the bright DAPI staining of the leukocyte nuclei, concentrations down to 0.01 cells/μl (10 cells / ml) can be safely determined.

## PAS Specifications

### General

Compact flow cytometer for automated sequential analysis of single cells and microscopic particles  
 Scatter particle size range 0.2  $\mu\text{m}$  - 200  $\mu\text{m}$   
 Fluorescence sensitivity: 100 MEFS (FITC) or better  
 Fluorescence resolution (CV): 1% or better (DAPI)  
 Configurations with 1 to 8 optical parameters + time parameter

### Light Sources

Built-in air-cooled Ar-ion laser with 20 mW at 488 nm (PAS and PAS-III) and 50 or 100 mW (PAS-III only) at 488 nm, 458 and 515 nm  
 HBO mercury arc lamp, 100 W, for UV excitation and other wavelengths  
 Diode laser 25 mW at 635 nm, temperature controlled  
 Diode laser, 430 nm  
 NdYAG 50 mW at 532 nm  
 Instrument size PAS: 770 mm x 500 mm x 550 mm (WxHxD) - Instrument size PAS-III: 1400 mm x 500 mm x 550 mm (WxHxD)  
 Recommended laboratory table size (PAS): 1600 mm x 800 mm  
 External lasers on optical bench support (PAS-III):  
 HeCd 325 nm, 354 nm  
 HeNe 30 mW at 633 nm  
 Other lasers upon request

### Optics

Modular optical system with 1 to 8 optical parameters with selected PMTs with integrated electronic preamplifier for each parameter, including FSC  
 Standard setup and filters for FSC, SSC, FL1-FL6  
 Colour CCD camera for video flow monitor  
 Köhler illumination by HBO lamp  
 UV transmitting quartz optics  
 Standard objective mount with high numerical aperture objective, high numerical aperture immersion gel coupling, e.g. for detection of weak cytokines (option)  
 Various intermediate image planes for optimized spatial filtering by diaphragms

### Flow System

Synthetic quartz flow cuvette for laminar sample transport with sheath fluid  
 Sample port with computer controlled BioSafety cleaning system, avoids sample droplets and minimizes cross contamination  
 True Volumetric Absolute Counting based on mechanical volume measurement, no need for reference particles  
 Contamination-free computer controlled precision syringe pump for sample transport and volumetric absolute counting, pump speed continuously adjustable from 0-50  $\mu\text{l}$  / s, sheath fluid pressure continuously adjustable from 0-300 mbar, easily accessible 5 l sheath fluid and waste reservoirs with filling level sensors

### Cell Sorter

Closed piezo sorting system  
 Minimizes sample hazards  
 Analysis speed up to 15 000 particles / s, sorting speed up to 500 sorted particles / s for sort purities > 95% (depending on sample), video flow monitor for monitoring of sorting process - easy and reliable sorting setup.

### Robby sample automat and autoloader

36 tube carousel  
 Up to 16 reagents  
 Sample shaker  
 Windows™ RobbyPrep software

### Electronics and Computer

Parallel signal processing for each of the optical channels with selectable linear, 3 or 4-decade logarithmic amplification (6-decade log amplifiers), pulse height, area and width analysis for doublet discrimination, 16 bit analog-to-digital converter, trigger on any parameter or all parameters  
 Upgradable built-in industry standard computer, Pentium processor (at least 800 MHz), 128 MB RAM, >30 GB harddisk, built-in 15" TFT LCD display, CCD camera video flow monitor on LCD window, external 17" or 21" monitor for dual screen setup (option), 650 MB CD-Writer, 3.5" Floppy Disk Drive, 100 MB ZIP drive optional, keyboard, mouse, barcode reader, 10 MB/s / 100 MB/s Ethernet connection, DeskJet colour printer, b&w or colour laser printer optional, printing via network

### Software

Microsoft® Windows™ 98 or 2000 operating system with full network support - Apple®/Macintosh network connection optional  
 32 bit Windows™ FloMax® software for routine and research applications  
 1-8-parameter real-time data acquisition and analysis  
 Dual wavelength acquisition from spatially separated light sources with time-window delay-system  
 Internal floating point numerics with double precision  
 One and two parameter histograms and dotplots  
 64 - 32768 channels resolution for 1P histograms  
 32/32 - 1024/1024 channels for 2P dotplots  
 Multiparameter N-colour crosstalk compensation, settings may be corrected any time  
 Multiparameter ColorGating  
 Ratio measurement  
 Doublet discrimination based on pulse height, area and width analysis  
 Peak and cluster analysis and statistics  
 Robby™ sample automat control  
 DNA cell cycle analysis  
 DNA peak analysis  
 Multitube panel system with automated acquisition  
 Flow Cytometry Standard data format FCS 2.0 for storage of original and evaluated data  
 Storage of gates and screen layout  
 Automated data transfer of graphics, statistics and instrument settings to Word, Excel, other desktop publishing systems or laboratory information systems (LIS).



### Instruments, Reagents and Accessories

*Partec offers a broad range of instruments, protocols and reagents for microorganism detection, red blood cell lysing, DNA cell cycle and ploidy analysis, disposable CellTrics™ cell separation filters, sample tubes for volumetric absolute counting, UV objectives and more. Please inquire for any information for your application.*