PARTEC

CyFlow® Cube

Instrument Operating Manual

CyFlow® Cube 6 | CyFlow® Cube 8 | CyFlow® Cube 8 Sorter

For in vitro diagnostic use with Partec recommended IVD reagents.

IVD

The Partec CyFlow® Cube Flow Cytometer complies with the European IVD Directive 98/79/EC and is therefore CE marked.

CE

For *in vitro* diagnostic use with Partec recommended IVD reagents.

- Certified Management System
- EN ISO 9001
- EN ISO 13485

Contact Information:
Partec GmbH • Am Flugplatz 13 • D-02828 Görlitz • Germany
Tel +49 3581 8746 0 • Fax +49 3581 8746 70
E-mail: service@partec.com

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Foreword

The CyFlow® Cube Instrument Operating Manual is aimed for a large spectrum of users, from beginner up to the most skilled flower. The beginner or casual user will find the key functions and concepts to use the Cube and its software. The confirmed flower will find an in-depth detail of the inner working and parameters of the Cube to customize its use and obtain optimal performances.

CyView™ for Cube 8 is the instrument operation software for the **CyFlow® Cube 8 (CY-S-3068)**. New software versions and you profit from your requests: new features and software improvements. Partec is continuously working on CyView™ to better fulfil your demands. If you have questions concerning this manual or the software, find problems associated with CyView™ or you have a good suggestion to be included in a new version, please let us know by sending an email or a note to Partec GmbH.

For more details about the reagent kits, suitable for use with the CyFlow® Cube**,** please refer to the respective product data sheets. There are also several Application Notes available.

If you have questions, please contact your local distributor, one of the Partec subsidiaries, or Partec in Germany (service@partec.com).

Further details and addresses can be found on our website at:

- **www.partec.com/distributors**

Please do not forget to add in your request the following information:

- **Serial number (serial No.) of the CyFlow® Cube**
- **Your complete contact address**

Presentation

Basics

What is the Partec CyFlow® Cube?

The Partec CyFlow® Cube is a fully equipped desktop Flow Cytometer (FCM). CyFlow® Cube features a modular optical concept. This allows using different lasers as light sources and the detection of up to 8 optical channels (parameters). The CyFlow® Cube allows easy optimization of the optics for any application by simple exchange of optical filters and mirrors. The CyFlow® Cube runs with an internal PC. Data acquisition, instrument control, and data analysis are controlled and performed by the CyView™ software.

What are the applications for which the CyFlow® Cube can be used?

Together with the software, the CyFlow® Cube offers automation for routine use and flexibility for research use for practically any flow cytometric application. The applications cover:

- Routine multi-colour immuno-phenotyping
- Blood Cell Analysis/HIV monitoring (e.g. CD4 cell count)
- Leukocyte Counting/Rare Event Analysis
- Microorganism Analysis
- Fermentation Control
- Particle Concentration Analysis
- True Volumetric Absolute Counting
- Particle Size and Fluorescence Distribution Analysis

What are topics covered by this manual?

The CyFlow® Cube Instrument Operating Manual covers the basic operation and maintenance of the CyFlow® Cube instrument. This manual also covers details related to the software.

What other manuals are available?

Application Notes and **Service Manuals** are available to get started. They contain hints to achieve the best results.

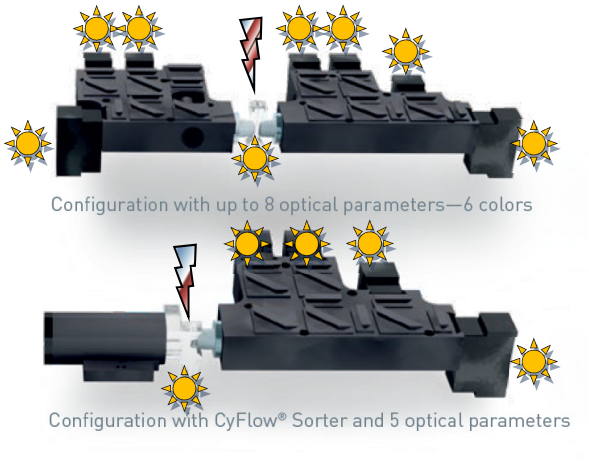
What should I know before operating the CyFlow® Cube?

This manual assumes that you have basic knowledge on flow cytometry. In the best case a well experienced "flower" is around - so let her/him help you. Basic books are available about flow cytometry which may help you as well (e.g. Howard M. Shapiro, Practical Flow Cytometry. Wiley 2002).

In flow cytometry, what is ...

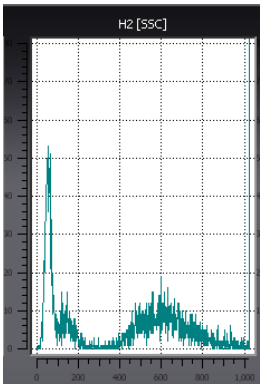
... a parameter?

In flow cytometry, parameter denotes a measured property of the particles. Frequently, a parameter is synonymous to an optical channel. E.g. an instrument with 6 parameters is equipped with 6 optical detectors.



... a one-parameter histogram?

A one- parameter histogram displays the distribution of cells among a specific property, e.g. how many cells contain a given quantity of DNA or bind a given number of antibody molecules.



... a histogram channel?

The measured signal intensity is assigned to one of 65536 quantity classes or channels. In a one-parameter histogram the channels are represented on the x-axis.

... the count in a histogram?

The number of cells being assigned to a given channel is referred to as channel content or simply count. In a one-parameter histogram, the count is shown on the y-axis.

... a peak?

All cells having about equal characteristics among the analysed cell property (e.g. content of a specific constituent like DNA), form a peak. In the case a of typical DNA histogram one peak represents the G1 and another peak (with twice the channel value) represents the G2/M phase of the cell cycle.

In case of immunolabelled cells often one peak for unlabelled (negative) and one peak for labelled (positive) cells can be detected. Peaks can be analysed by identifying them with region markers.

… background in a histogram?

Histograms sometimes show undesired signals in the lower channels, frequently called ´noise´ or ´background´. These signals may originate from cell fragments or other particles resulting from sample preparation. In case of high signal amplification, background can also be caused by particle contaminated sheath fluid.

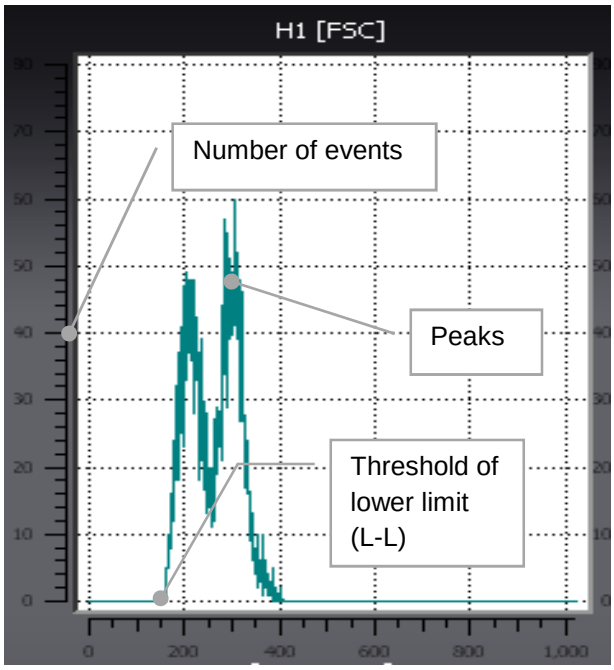
The lower level (L-L) or threshold?

The lower level (L-L) threshold is a mean to suppress background signals. Signals below the lower level are rejected from the signal acquisition. To exclude noise from a histogram already acquired, a region-gate can be used.

Example of a histogram

A histogram represents a distribution of measured signals (events) over 1 dimension. Data can be presented on the dimensions of relative particle size or optical particle structure (Forward Scatter (FCS) or Side Scatter (SSC)), resp. or on their relative fluorescence intensities in different light colours (fluorescence parameters FL1 to FL6).

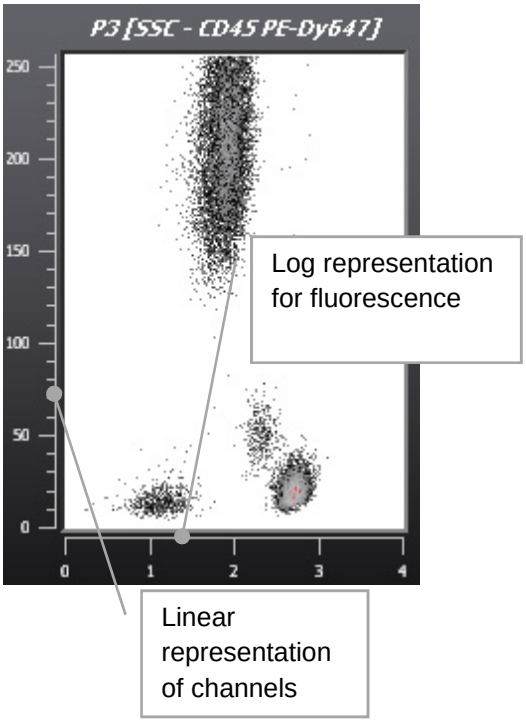
In this example, the dimension represented is the relative size (FSC) on a logarithmic scale in X, and the number of events on a linear scale in Y. Two peaks are visible.



Example of a dotplot

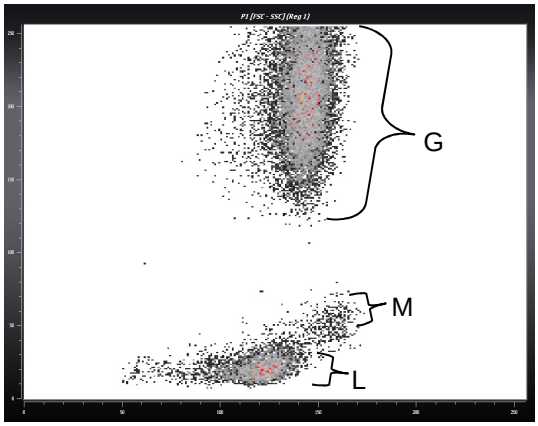
A 2D dotplot presents correlated data over 2 dimensions. In the image on the right a sample of leukocytes (after lyse of the red blood cells) is plotted with their relative light scattering (SSC) property against the intensity of the CD45 antigen.

The Z value represents the number of events that have the same coordinates. 1 event will be represented by a black point; if 10 events have the same coordinates, the point representing them will be grey. It will be red if more than 20 points are overlaying one another. The Z scale is dynamic and will adapt during the measurement to a scale of 1 (black) to the maximum overlaying event coordinate colour coded in red.



Histogram and dotplot in immunology

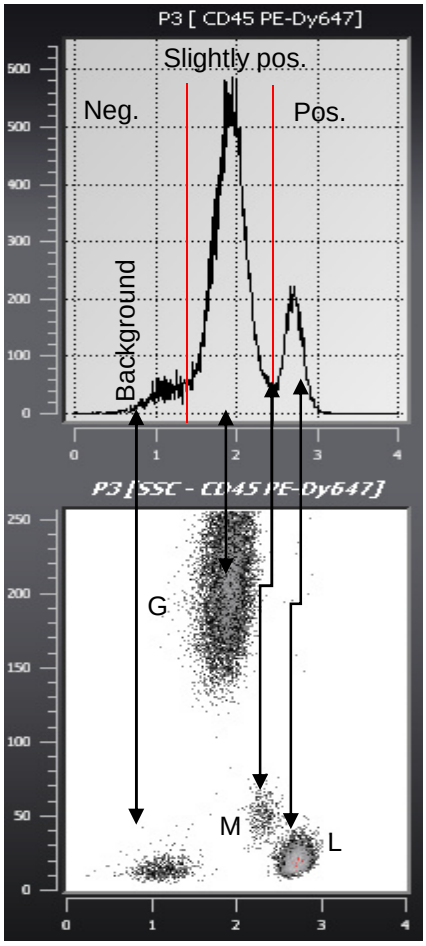
Lysed blood 2D dotplot



Lysed blood is represented in a dot plot presenting the FSC in X axis and SSC in Y axis. Both axes are in a linear scale.

Three distinct groups are visible; they represent the granulocytes (**G**), monocytes (**M**) and lymphocytes (**L**).

Histogram and dotplot of immunological staining



This histogram represents the spectrum of the cells presented in the previous dot plot stained with antibodies anti-CD16 conjugated to PhycoErytrin (PE). The X axis displays the fluorescence in a 4-dec logarithmic scale and the Y axis displays the number of events in a linear scale.

This dot plot presents the cells fluorescence in X on a logarithmic scale (CD45 FITC) versus SSC in Y on a linear scale.

This data display allows an easier interpretation of 2 parametric data compared to the histogram. Concluded from this example, the lymphocytes are strongly stained with the anti-CD45 antibody, the monocytes are slightly stained and the population of granulocytes is negative.

This example is a simplification of an immune-staining analysis by flow cytometry. It is directed to new users, and aims to link the histogram to the dotplot representation. To complete the analysis, gating and statistics are required.

Instrument starting procedure

Sheath fluid level control and/or refill

Before switching on the machine, it is recommended to check the levels of the sheath fluid and waste bottles. They can be found at the back-left of the apparatus in a sliding compartment.

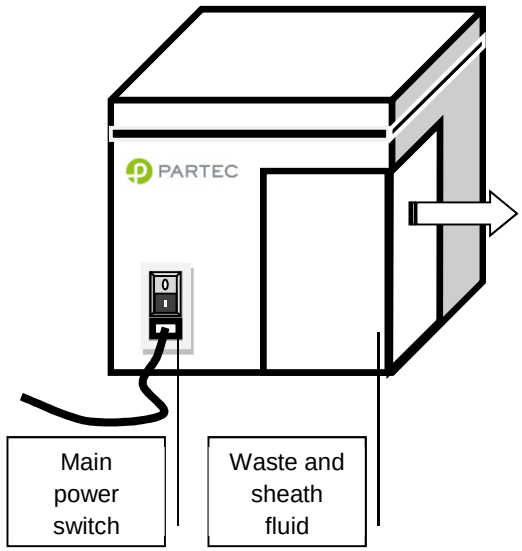
Make sure **SHEATH bottle** is filled with 800 ml of clean, filtered, and degassed sheath fluid and is closed with the screw top. In order to guarantee highest quality of the measurements we highly recommend use Partec Sheath Fluid (order no. 04-4007).

It is recommended to replace the sheath fluid at least once a week or before any daily use.

When filling up the sheath fluid bottle make sure no air bubbles are trapped in the yellow filter unit inside the bottle!

Make sure **WASTE bottle** is empty and the screw top is tightly closed.

The waste bottle must be emptied after and before each user session. When using bio-hazardeous samples, a volume of 50 ml of hypochlorite 0.5% (Order No 04-4012) should be introduce into the empty waste bottle for initial disinfection.



Rear side of CyFlow® Cube showing the main power switch and the sheath fluid and waste bottle compartment.

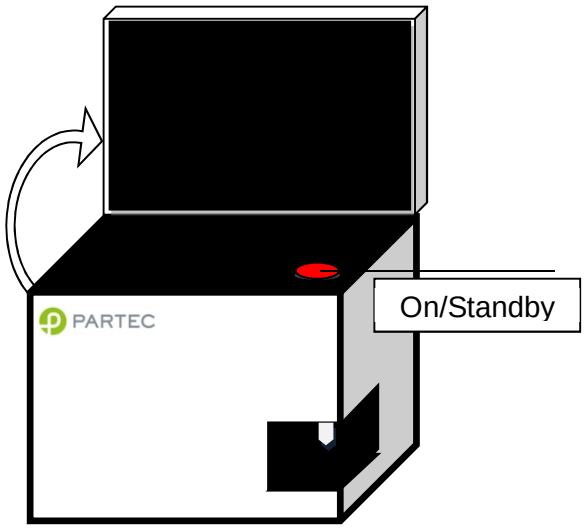
Casual/medium expertise user:

Once the Cube started no further steps are necessary. You can directly start your measurements!

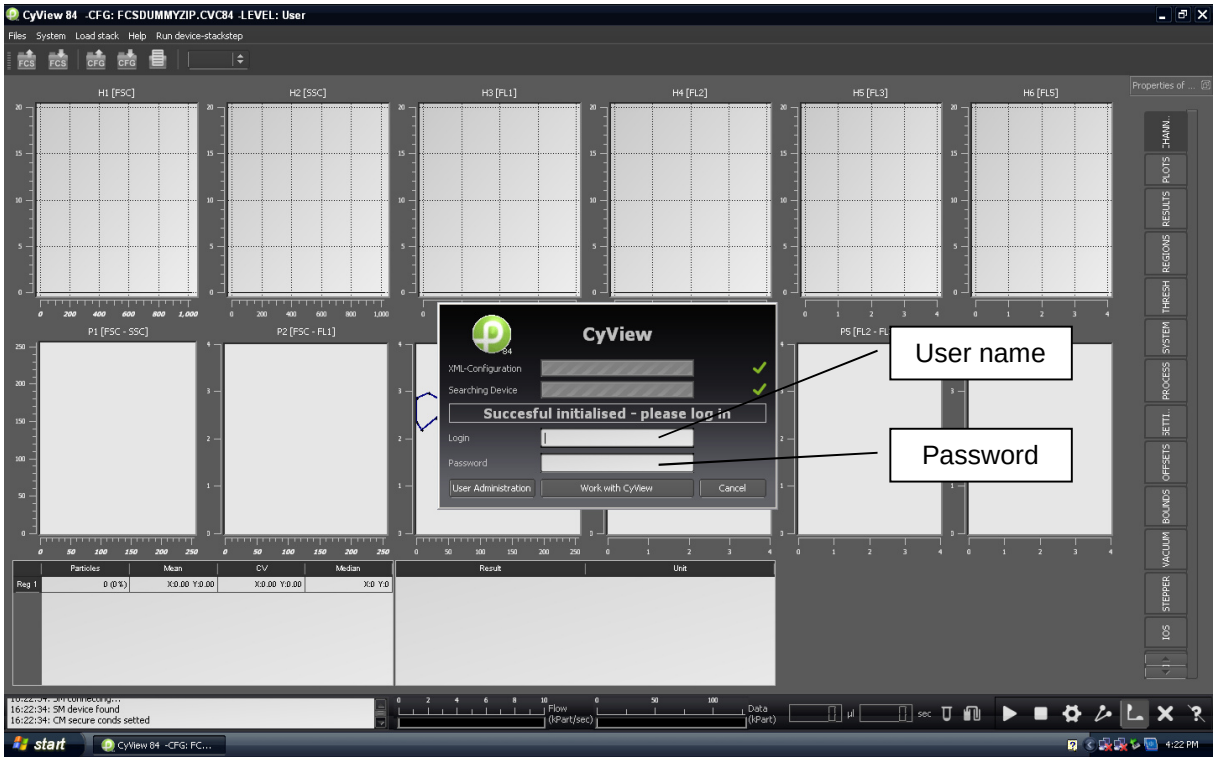
Switching on the CyFlow® Cube

The power supply switch is found at the back of the Cube next to the main supply cable. The Cube is, in default, set in a stand-by mode. The full activation of the Cube requires pressing lightly the on/off tactile button on the top of the machine. The display screen must be first lift up to access it.

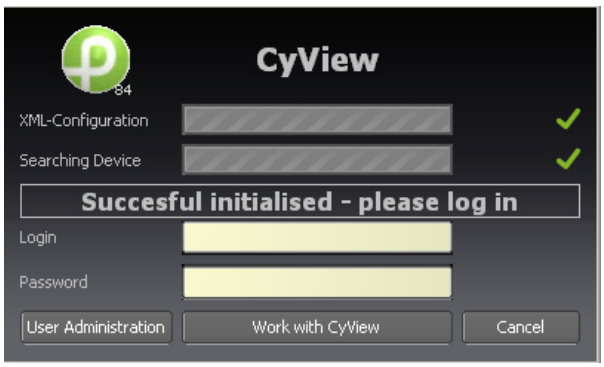
This will start the embedded computer, automatically start the CyView™ software and load the last employed configuration.



Main CyView™ login window



The log in window allows to start the software at the USER, MAIN USER or SERVICE level.



During start of the instrument the automated self-testing procedures are processed:

XML-configuration: displays the correct loading of the setting files

Searching Device: display of the correct recognition of the connection between the computer and the embedded electronics

Please verify that both operations are confirmed by a green tick.

Lost your login details?

Important: On each new instrument a Main User login is already established

Login: **USER** (case sensitive)
Password: **Cube1** (case sensitive)

Login as a standard USER:

The code will be given to you by the main user(s) of the instrument. The main user has the rights to define or delete users.

User levels

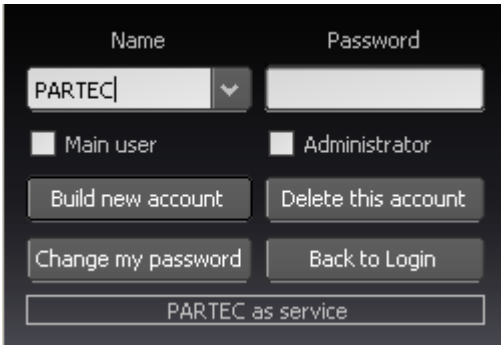
Three different user levels exist:

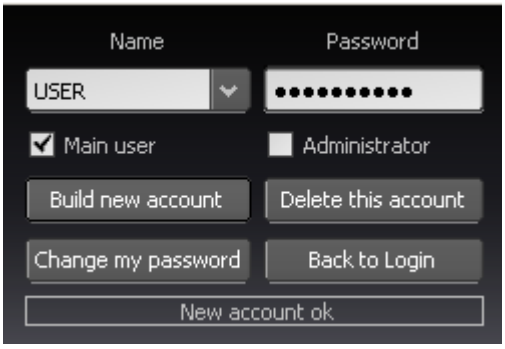
- **SERVICE:** Restricted to authorized Partec trained persons and for service purposes only
- **MAIN USER:** Complete functionality of the instrument, method development

Main users can create new accounts of the Main User and User level

- **User:** Applying standardized methods only

Users cannot create new accounts.





As a main user in order to create new accounts type in your Login name and password and select “User Administration”.

To create a new MAIN USER account type in Name and Password and activate “Main user” followed by “Build new account”.

To create a new USER account type in Name and Password followed by “Build new account”. (Main user should be deactivated).

As User the own password can be changed by selecting the Name and placing a new Password followed by “Change my password”.

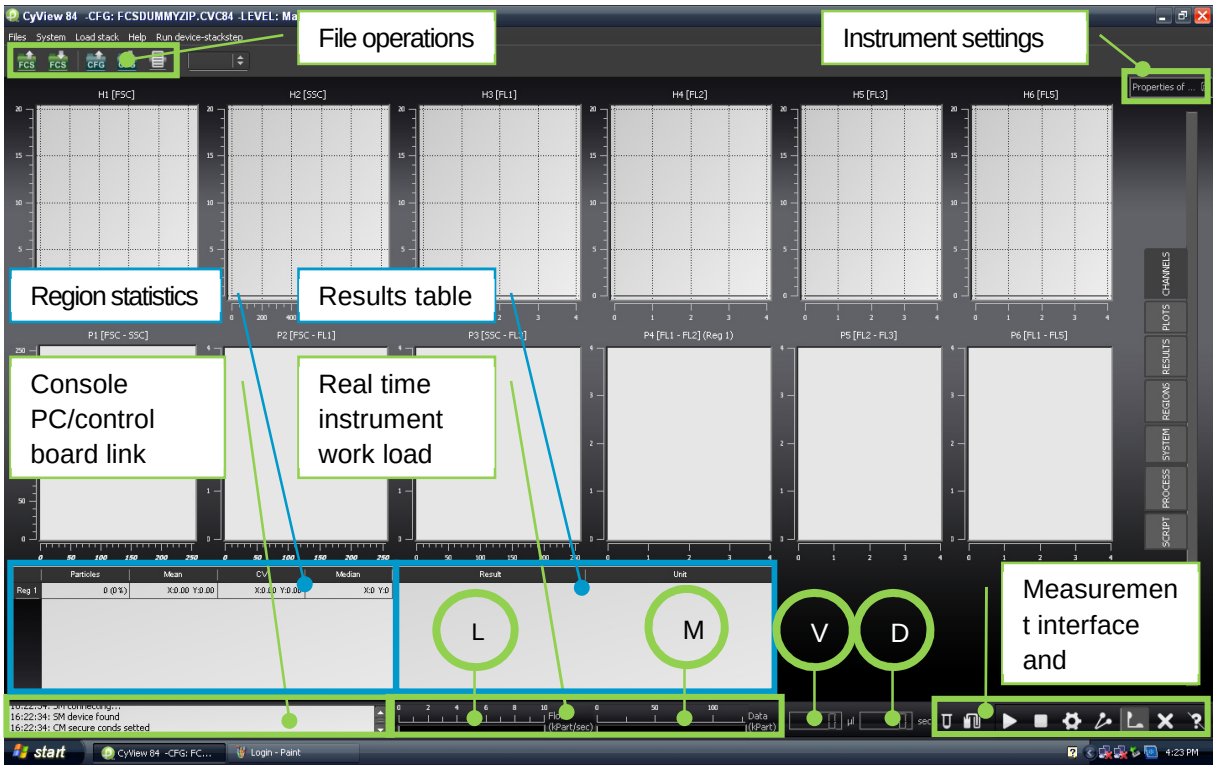
As Main User the own password can be changed by selecting the Name and placing a new Password followed by “Change my password”. As Main User any User account can be deleted by selecting the Name followed by “Delete this account”. The Main User does not require the respective password to delete any User account.

The default user account (Name: USER; Password: Cube1) can be deleted when logged in as Main User. Please make sure at least one Main User remains in the user list in order to guarantee complete functionality of the software.

To enter the CyView™ software from the user administration level select “Back to login”, login with your personal Login name followed by “Work with CyView™.

CyView™ Main Page

Presentation



The main window will be your interface to acquire, save, re-load and analyse your data.

Instrument real time display of workload (**L**), on-board memory status (**M**), analysis volume (**V**) and analysis duration (**D**).

Buttons allowing to ***load and save*** data files (.fcs data files).

Buttons dedicated to ***saving and retrieving instrument settings*** (.xml data files or configuration scripts).

Button for the generation of a ***PDF report*** of the acquired data.

Console displaying the control board status.

Status of sample (high, counting phase, empty) and of waste and sheath fluid bottles levels.

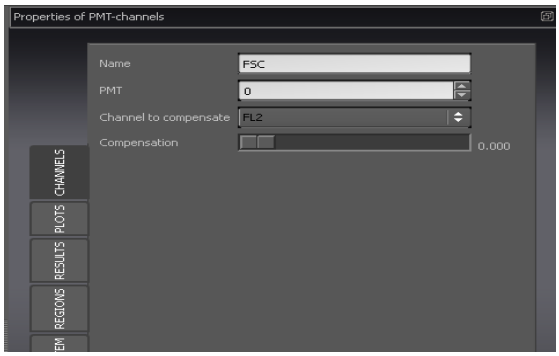
Start and Stop measure buttons.

Compensation button and random bias button.

Clear button – deletes all data during a measurement

CyView™ Controls

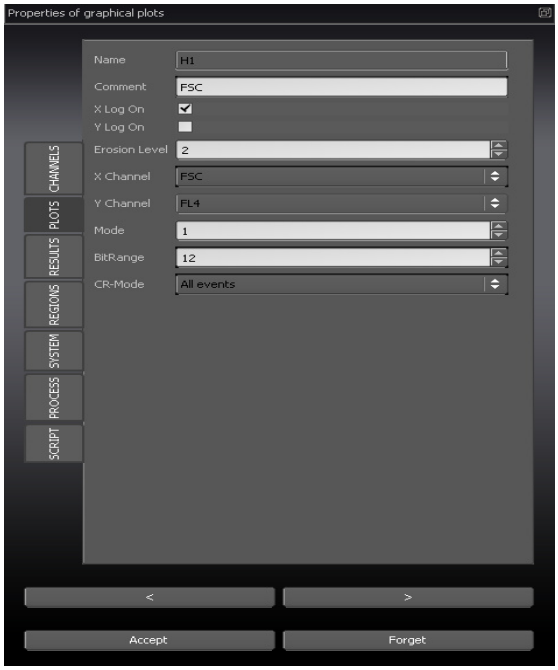
CHANNELS – parameter definition



The CHANNELS register defines properties of PMT channels (the parameters) present in the systems (PMT0 – PMT7). It names the parameters (FSC, SSC, FL1 …FLx) and defines the Color Crosstalk Compensation.

Confirm all modifications by pressing ***Accept***

PLOTS – display options



The PLOTS register defines properties of the graphical plots. The basic layout in terms of number of plots, type of plots (histograms, dot plots) and position of the plots is defined in the used ***Configuration Script***.

Plots are named ***H1 – Hx*** for histograms and ***P1 – Px*** for dotplots. As specific plot is selected with the arrow keys.

Define a ***Comment*** characterizing the plot e.g. *FSC*

Switch between ***Lin*** and ***Log*** scale for ***X*** and ***Y*** Axis

Define an ***Erosion level*** for dot to be displayed (z-axis level), e.g. an ***Erosion level*** of 2 shows only dots representing 3 and more signals

Select ***X-axis*** channel and ***Y-axis*** channel in dot plots or ***X-axis*** channel only for histograms

Mode (function inactive)

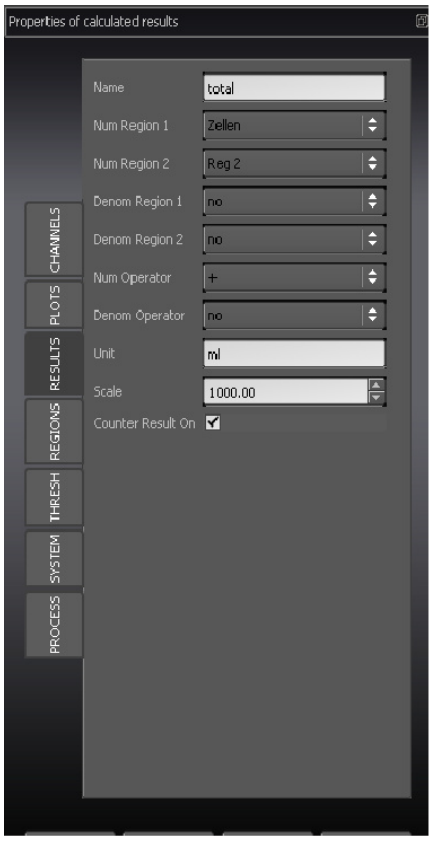
Select histogram resolution as ***Bitrange***, (values 6 bit to 12 bit)

Select CR – Mode

Confirm all modifications by pressing ***Accept***

RESULTS

The RESULTS register defines properties of calculated results displayed in the RESULTS table.



Properties of result calculations
It is possible to set up calculations with the COUNT of individual regions according to the specified formula:

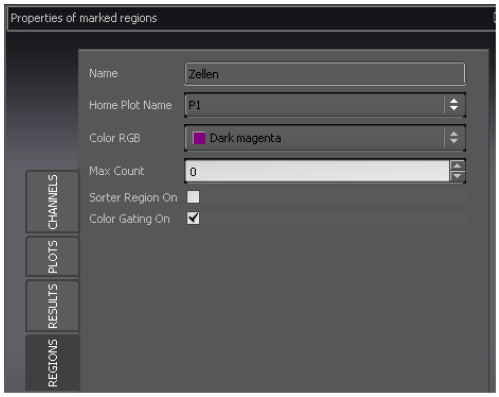
NumReg1 (+ - x /) NumReg2
----------x Scale
DenomReg1 (+ - x /) DenomReg2

- ***NumOperator*** defines operator between 2 numerators „+“, „-“, „x“ or „/“
- ***DenomOperator*** defines operator between 2 denominators „+“, „-“, „x“ or „/“
- ***Unit*** allows to add text to the result table
- ***Scale*** introduces a factor to the formula
- ***Counter results on*** refers to the result of a volumetric counting

Confirm all modifications by pressing ***Accept***

	Particles	Mean	CV	Median
Zellen	5517 (95%)	X:1250.19 Y:7811.99	X:17.66 Y:18.35	X:1230 Y:7536
Reg 1	5014 (86%)	12845.66	4.20	12926
Reg 2	332 (5%)	24890.92	8.46	24963

	Result	Unit
total beads	110.34	per ml
single beads	100280	beads ml
doublett beads	6640	doubletts ml
total	116980	ml

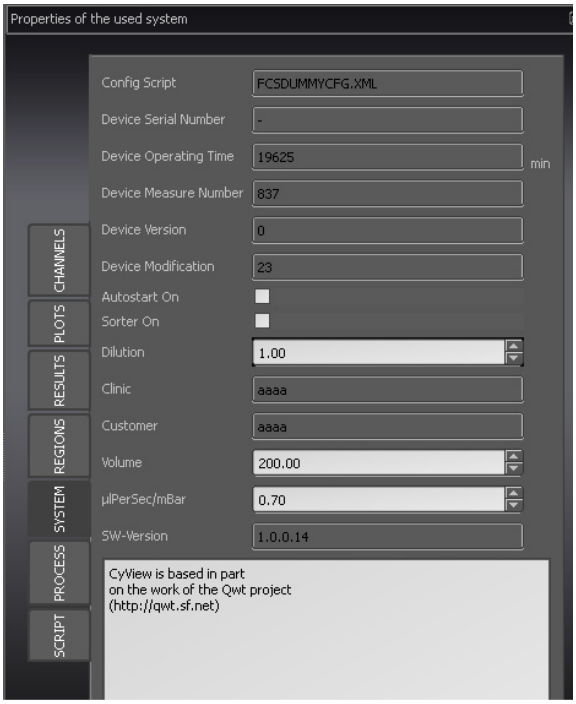


- ***Home Plot Name*** defines the plot the region refers to
- ***Color RGB*** defines the region´s color and its color in color gating
- ***Max Count*** defines a maximum count for an “Events in Region” particle limit
- ***Sorter Region On*** activates region as sorter region (only in CyFlow® Cube Sorter)
- ***Color Gating On*** activates color gating for this region
- Use ***Delete*** and ***New*** to delete and create new regions

Use arrow keys to switch between regions

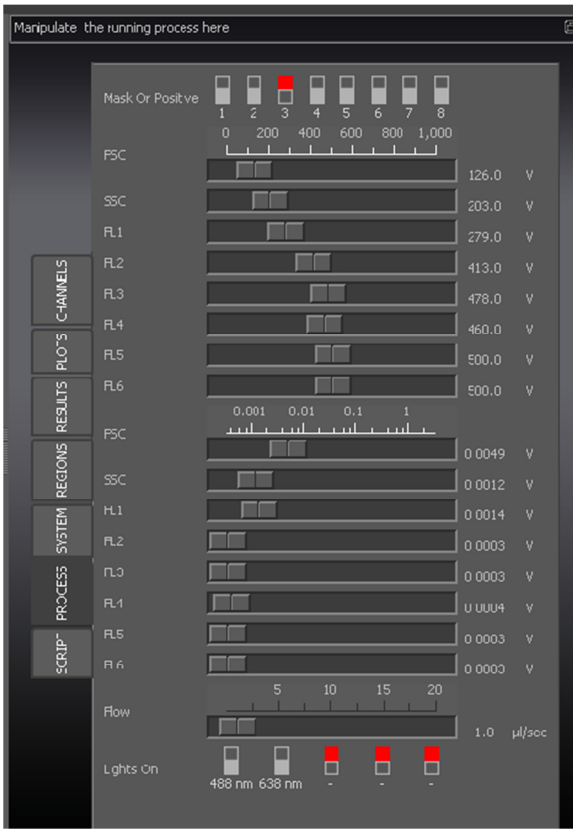
SYSTEM - properties of the instrument

- The active ***Configuration Script***
- The instruments ***Serial Number***
- Total ***Operating Time*** of the instrument
- ***Version*** of the instrument
- ***Modification*** of the instrument
- Activate ***Autostart*** function
- Activates ***Sorter*** function
- Defines a factor for sample ***Dilution***
- ***Clinic/Customer*** defines specific User information
- Defines sample port electrode ***Volume*** in µl
- ***µlPerSec/mBar*** defines a sheath fluid flow parameter
- ***SW-Version*** specifies current software version

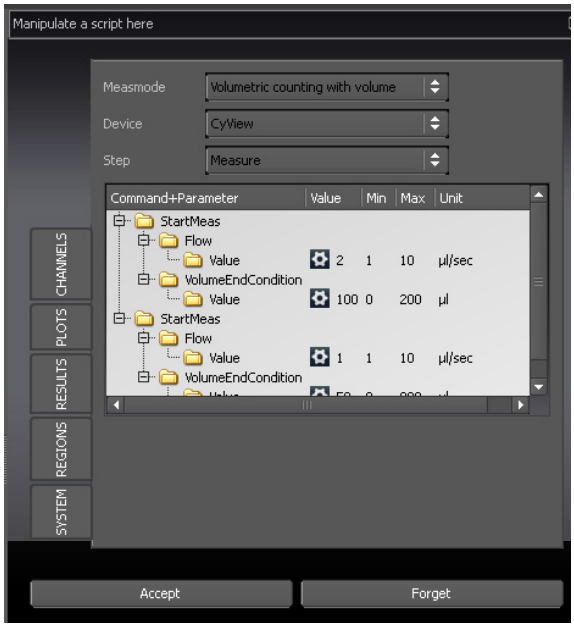


PROCCESS – instrument control

- ***Mask or Positive*** selects the trigger parameters, multiple trigger parameters are logical “or” connections
- ***Voltage*** of the individual optical parameters (0 volt - 999 volts) defines PMT signal amplification
- ***Threshold*** defines the trigger signals cut-off level (on 4 dec log scale)
- ***Flow*** defines the speed of sample injection in µl/s
- ***Lights on*** switches light sources on / off



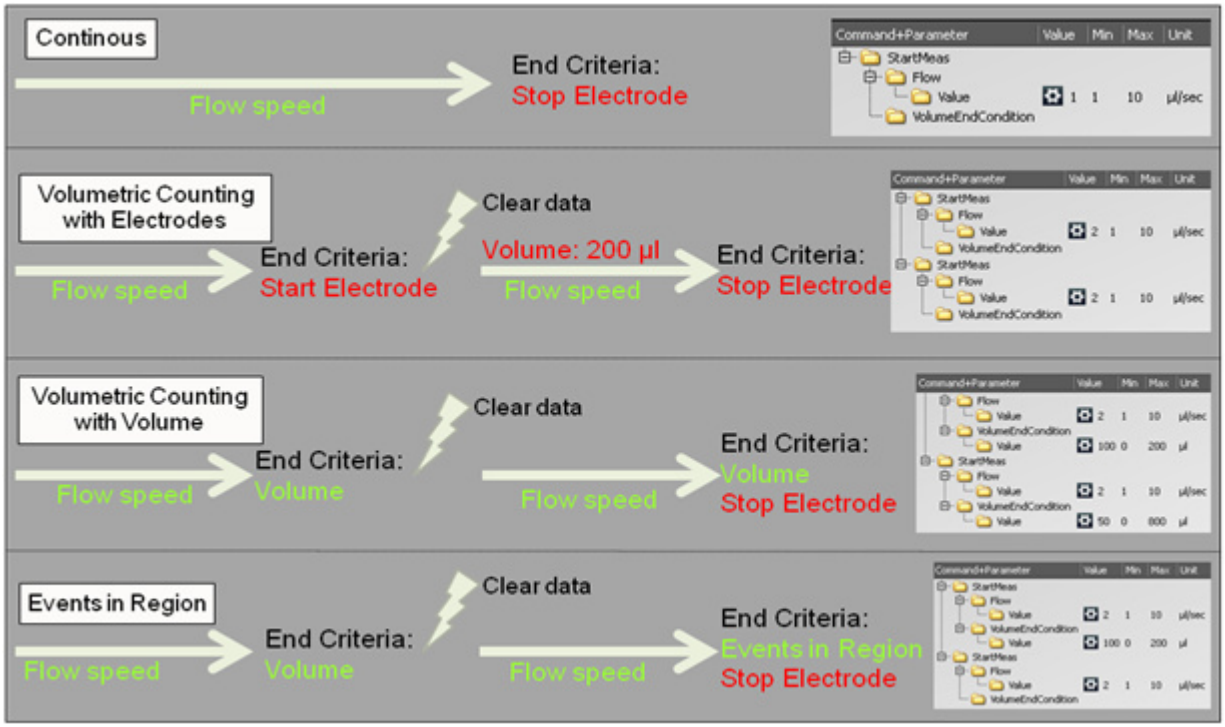
SCRIPT – definition of measure modes



Measmode allows selection of:
- Continuous analysis
- Volumetric Counting with Electrodes
- Volumetric Counting with Volume
- Events in Region

Speed values and ***Volume values*** can be edited and stored in FCS file and Config script

Confirm all modifications by pressing ***Accept***



Schematic overwiew of the measure modes with. A flash indicates clearing of the data. User selectable criteria in green, software-defined values in red.

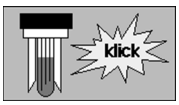
Typical Sample Analysis

A step by step procedure and what are the important steps in the data acquisition.

Starting a measure

Load a sample demo file (data files can be selected from the folder (C:/CyView_85\data\xxxxx.fcs). The respective instrument settings are automatically loaded.

Fill a sample tube with 1.2 ml of calibration beads. Check the tube for eventual imperfection or contaminants (cracks, aggregates, hair) and replace or remove, resp. if necessary.



Insert the tube into the sample port, push it up until a distinct click is heard

Press the start button. In the console window, information on the status of the measurement is displayed. The script work is proceeding step by step.

CyView™ StartPrerun
Prerun and stabilization of sample flow
Start Measure
Data acquisition

Console displaying control board status

During the data acquisition the total particle count and the analysis rate is indicated as a sliding bar.

During the acquisition phase it is possible to manipulate the instruments set-up e.g. by changing samples speed, PMT voltage and threshold levels. Use ***CLEAR*** button

to erase data after manipulations of the instruments set-up.

By default, the ***continuous*** measure mode is pre-selected. In this case the measurement will only stop automatically when the sample is consumed. An earlier stop can be realized by pressing the ***STOP*** button  . Data can be saved with

The configuration file can be saved with

Besides the ***Continous*** measure mode other measure modes can be selected:

- Volumetric counting with volume
- A volume can be pre-selected
- Volumetric counting with electrodes
- The volumetric counting is based on the electrode status
- Events in region
- A particle number can be pre-selected

Measure modes

The following measure modes can be selected prior to start of an analysis:

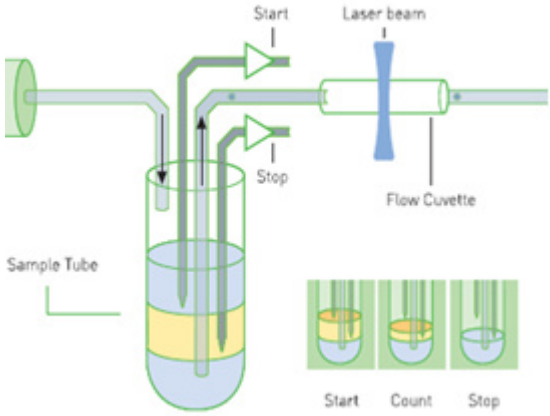
- Continuous
- Volumetric counting with electrodes
- Volumetric counting with volume
- Events in region

Continous (default selection)

This default mode will allow you to run your sample until its levels reached the stop electrode.

Volumetric Counting with Electrodes

This measuring mode uses the START and STOP electrodes of the sample port to define a fixed sample volume. In a standard sample port this “counting volume” is 200 µl. In a pre-counting phase the sample is acquired normally as in ***continuous acquisition mode***. Reaching the START electrode the data are cleared and the volumetric counting phase starts. Reaching the STOP electrode the counting procedure will be terminated and a system cleaning cycle will be initiated automatically



Working principle of the Absolute Volumetric Counting with Electrode

Volumetric Counting with Volume

In the measure mode ***volumetric counting with volume*** the counting volume is flexible and can be pre-selected by the user. In a first analysis phase the sample is acquired normally as in continuous acquisition mode. Reaching the pre-selected volume the data are cleared and the volumetric counting phase starts and the pre-selected volume will be analyzed. The volume can be used as the basis for concentration determination.

Events in Region

The ***Events in Region*** measure mode allows to define a number of particles within a specified region to operate as STOP condition (select the respective ***MaxCount*** function in the ***REGIONS*** register).

Advice: for the measure modes *Volumetric counting with volume* and *Events in Region* there will be a remaining sample volume left-over within the sample tube. In this case the cleaning cycle will only be triggered when the START button ▶ is pressed. Subsequently the data can be saved.

Be aware: for all measure modes the sample analysis automatically stops when the sample is finished (the stop electrode is reached) even if the selected end criteria is not yet realized.

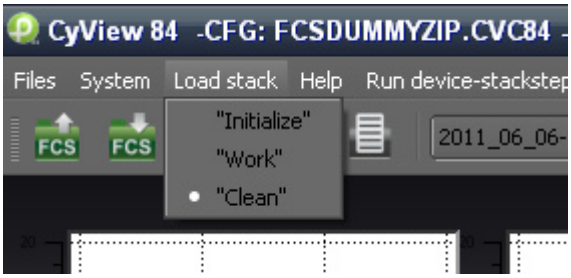
Cleaning Procedures

Cleaning procedure during Cube running

The Cube can be cleaned between sets of samples using the cleaning and rinsing solution (green solution, Order No 04-4009). This procedures will allow you to reduce significantly the cross contamination and reduce the background.

The cleaning procedure should be set as follows: connect a sample tube with distilled water to the sample port of the cube and press START three times. The cleaning tube will be entirely aspired by the instrument and used for cleaning.

Cleaning procedure for switching off



Before switching off the Cube, it is necessary to run the decontamination solution (violet solution, Order No 04-4010) using the clean function ***(Menu -> stack -> clean).*** Then, the same operation must be repeated with distilled water. The Cube can be put in standby by closing CyView™ ***(Menu -> Exit***) and closing WindowsXP (start-> Exit-> Switch Computer Off). To power off completely the Cube, use the main power switch at the back panel.

The waste bottle content must be discarded accordingly to the relevant biohazard regulations.

A regular thorough cleaning of the sheath fluid bottle and exchange of the yellow filter will keep the background in the measurements to a minimum level.

No cross contamination between samples

To minimize the carry-over of one sample to the next, a cleaning cycle between sample can be done.

Start your measurement.

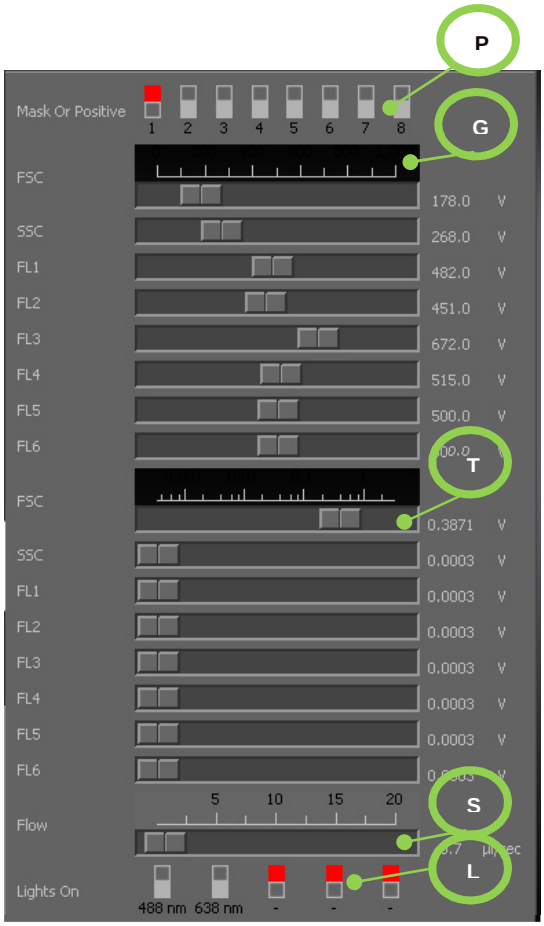
Click Pause, remove you sample from the sample port, introduce a sample tube filled with distilled water

Click twice on play, the sample port will be cleaned and the distilled water removed from the tube. Your Cube is now ready for your next measurement with no cross contamination from the previous sample.

Instrument settings

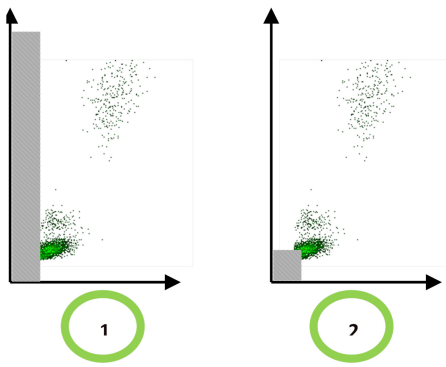
Measurement parameters

The PROCESS register can be opened by clicking on the button ***Meas*** (⚙)



Flow speed (S)

This slider allows you to change the sample injection speed into the flow cuvette. Low speed values results in a better precision and accuracy. A higher speed can be used when the particle concentration is measured and accuracy is of lower relevance.



Example of single (1) and multiple trigger (2) in FSC, and FSC+SSC.

Gains settings

The ***GAIN* (G)** and ***THRESHOLD (T)*** sliders must be adjusted to obtain the optimal gain (maximum signal and minimum background). Typically, the Forward Scatter (FSC) will be the first gain tuned to adjust the size of the studied particles.

To move a slider, click mouse left on the slider, keep mouse button down and move mouse left – right or use the scroll wheel to adjust the value.

Threshold settings (T)

The threshold allows cutting off background by setting a lower limit of the acquired data in the trigger parameter. This tool allows to increase the accuracy and precision of the acquired data.

Light source (L)

This option allows to switch on/off the light sources.

Trigger properties (P)

- ***Single trigger:***
  The trigger is the parameter defining if a signal gets recorded. Only if the trigger parameter detects a signal, other parameters of the system will record signals. In other words, if a trigger is set in FSC to record only bigger particles (e.g. threshold set at 0.3871V with a gain value of 170V), e.g. only intact mammalian cells will be acquired. Cell debris and smaller particles will be excluded as long as their FSC signal remains below the trigger threshold.

- ***Multiple trigger:***
  The option allows to use multiple trigger parameters for signal acquisition.

Plot properties

Plot name

A default plot name (H*x*, P*x*) is defined in the script. Default names of the Parameters will be displayed. The user can modify the parameter name (line ***comment)*** enter a parameter name better matching the experiment.

X Log On/Y Log On

This option will allow the user to change the plot's scaling. Note that changing the scale will require you to adapt the gains of the PMTs!

Erosion levels

The erosion level will set a threshold on the data displayed (not the acquired data). Some of the low frequency points will not be displayed allowing a better visual discrimination of the higher frequency data (signal against background).

X/Y Channel

The displayed parameters can be chosen from the drop down menu; giving the user the list of the activated parameters.

Mode

Graphical model for the data (line histogram, filled area histogram, overlay,..)

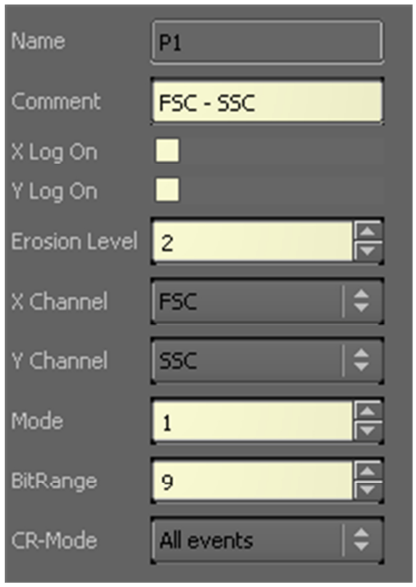
BitRange

The values are ranging from 6 to 12. It sets the channel resolution from 6 (64channels) up to 12 (4096channels).

CR-Mode

This option allows you to select the part of the data you want to be displayed:

- All events
- Region only
- Colour gating



To obtain the properties of a plot area, press the **Ctrl** + right click on the plot.

Once the parameters set, the change must be **validated**, the back and forth arrows will allow you to navigate from one plot to the next.

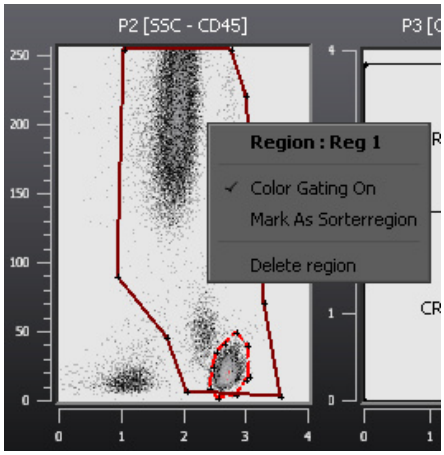
Region/ROI properties

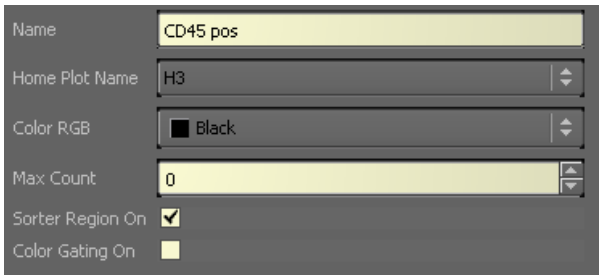
Create a polygonal region

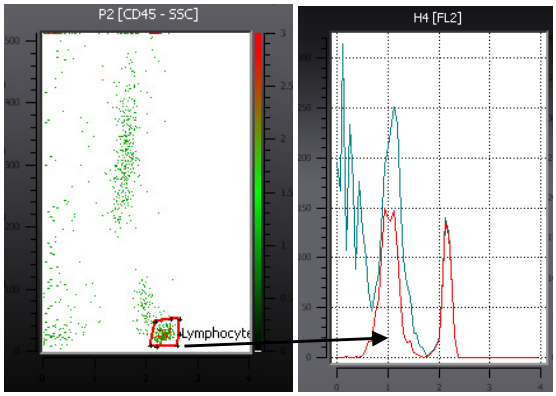
Double left click on the plot where the gate/region is required, a first point will be set; a second click will set a further point, and so on. Label as many points as required with the left mouse button. To close the gate surface, click mouse right.

Region properties

To access the region property, **Ctrl+right-click** on the gate to edited. On the ***PLOTS*** register on the right of the screen a selection of region options are displayed.







Region name

As default, the gate will be named: region n*x*, successive region: region n*x+1*

The user can also define a name. Remember to validate by ***Accept*** before to move on another region.

Home Plot name

This defines the physical plot where the region is located.

Colour RGB

Allows to choose the colour of the region. This colour will be reported to each plot selected as ***colour gating only***, illustrated in the graph on the left hand side with an overlay of the staining of lymphocytes in red overlaying the blue histogram presenting all events.

Max count

This option allows to fix the maximum number of events from a particular ROI (Region Of Interest) to be analysed (see CyView™ 8 SCRIPT register).

Sorter region

To set the ROI/region as a sorting gate, just by selecting the box.

(This option is only available on the Cube equipped with Sorter flow cuvette).

Colour Gating On

Selecting this option willallow the user to apply this particular gate to another plot.

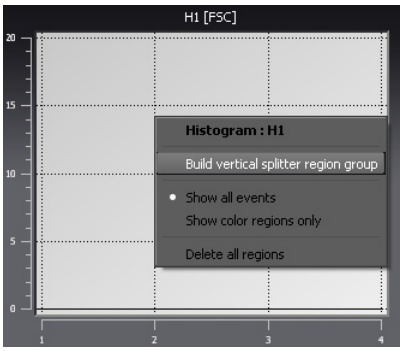
Moving of regions within a histogram

Move the cursor into the region and keep the left mouse button pressed while moving the position. Individual points of a region can be changed by approaching with the cursor to the point and keep the left mouse button pressed during movement. Regions within a dotplot can be changed in size by selecting the region with the cursor and pressing the “Shift” button during cursor movement

Each region can be selected with the right mouse button for deletion.

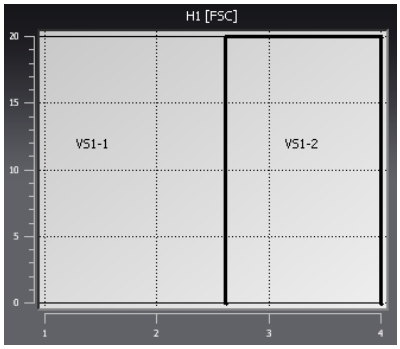
Create a vertical histogram splitter

To create a vertical histogram splitters please move the cursor into the histogram and press the right mouse button. Select ***"Built vertical splitter region"*** to divide the histogram into two sections (VS1-1 and VS1-2). The intersection can be modified by moving the cursor into the plot and keeping the left mouse button pressed.



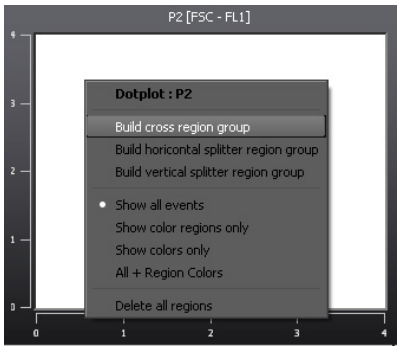
Create a vertical dotplot splitter

To create a ***vertical dotplot splitter***s move the cursor into the dotplot and press the right mouse button. Select ***"Built vertical splitter region"*** to divide the dotplot into two sections (VS1-1 and VS1-2). The intersection can be modified by moving the cursor into the dotplot and keeping the left mouse button pressed.



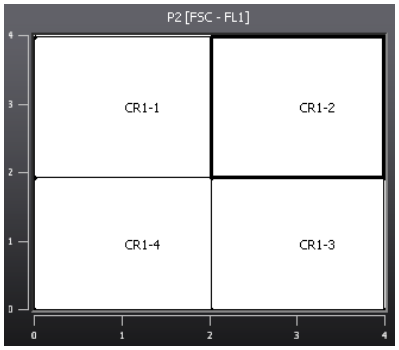
Create a horizontal dotplot splitter

To create a horizontal dotplot splitters move the cursor into the dotplot and press the right mouse button. Select ***"Built horizontal splitter region"*** to divide the dotplot into two sections (HS1-1 and HS1-2). The intersection can be modified by moving the cursor into the dotplot and keeping the left mouse button pressed.



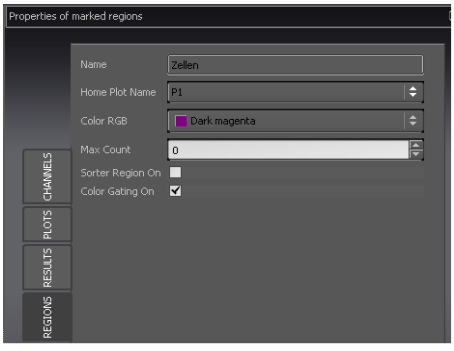
Create a quadrant assembly

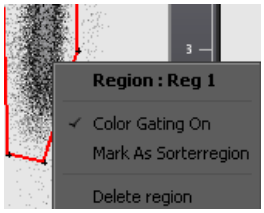
To create a quadrant assembly within a dotplot move the cursor into the dotplot and press the right mouse button. Select ***"Built cross region"*** to divide the dotplot into four sections (CR1-1, CR1-2, CR1-3 and CR1-4). To change the quadrants move the cursor into the dotplot and keep the left mouse button pressed. To create asymmetric quadrants please approach individual points (at the border of the dotplot or at the intersection point of the quadrants) with the left mouse button and keep the left mouse button pressed.



Change layout for regions

In the register chart file select "Regions". Individual regions can be selected with the <> arrows. Regions can be transferred to other plots by the function "Home plot name" or modified in their color with "Color RGB". To activate changes press "Accept".

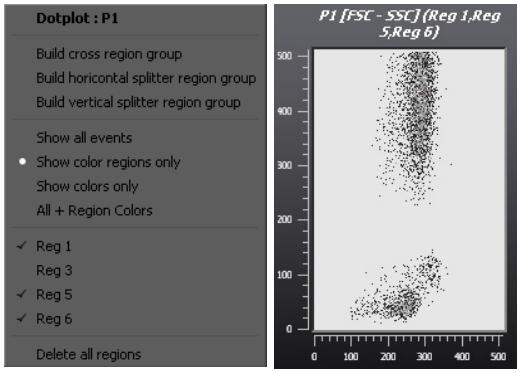




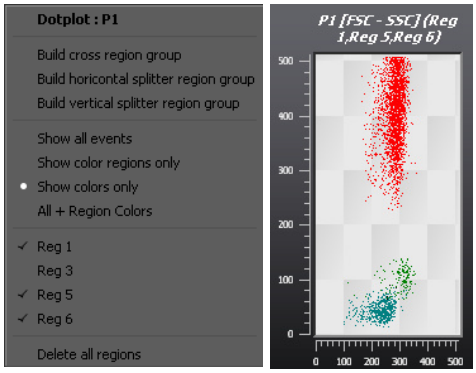
Applying regions to other plots → Gating Function

To select an existing region for gating select the respective region with the right mouse button and select ***"Color gating on".***

Clicking into any histogram or plot with the right mouse button (outside a region area) allows to select any existing region which is labeled with "Color gating on". Display options are:



- Show all events → shows all events in pseudo 3D color
- Show color regions only → shows the events in the selected region(s) in pseudo 3D color
- Show colors only → shows the events in the selected region(s) in the region color
- All + Region colors→ shows the events in the selected region(s) together with all events



Multi color display options are possible by selecting multiple gates.

Keyboard/Mouse combinations

Context	Action	Effect
Graphic Plot	Mouse double-click	Opens edit function for regions
Create Region	Right Mouse-click	Ends creation of a region
	Left Mouse-click	Sets dot of a region
Region	click and hold left mouse	Moves a region in standard steps
	click and hold left mouse +Ctrl	Moves a region in small steps
	click and hold left mouse +Shift	Moves a region to the original position
	click and hold left mouse +Shift+Ctrl	Moves the region by addition of an interval to the original position in very small steps
	click and hold left mouse +M	Move the regions name over 5 positions (N,E,S,W,central)
	Right mouse +Alt	Opens mouse menu
Axis of a plot	Right mouse +Alt	Opens the Channel register
Plot	Right mouse +Alt	Opens Plot register
Region	Central mouse	Marks the region and moves it in all regionplots in the visual area

Access to contest menus and keyboard short cuts

Appendix

Biohazards

Warning: The Waste may contain biohazardous and carcinogenic material from the samples (infectious material, dyes).

Please note: **Strict guidelines, international, as well as national regulatory standards such as GLP (good laboratory practice) must be met for all users. Therefore, the system is marked with the following biohazard label:**

Warning: biohazards

Maintenance

Warning: The Waste may contain biohazardous and carcinogenic material from the samples (infectious material, dyes).

Clean the CyFlow® Cube casing on a regular base carefully with soft cloth. Water must not enter the CyFlow® Cube or peripheral devices or come into contact with electric connections and switches. For cleaning the screen, always use special screen cleaner and soft cloth.

Do not use any organic solvents, nitro thinner, benzol, alcohol, highly concentrated bleach etc!

For cleaning of flow cuvette, refer to the described cleaning procedure. Do not use tools to clean the flow cuvette. In case the flow cuvette is blocked, enquire Partec for rapid exchange.

Regularly empty the waste bottle and clean with warm detergent solution and a brush.

Clean sheath reservoir with distilled water and a clean brush and flush with clean distilled water several times.

Remember: a clean sheath fluid reservoir is critical for proper operation.

If the CyFlow® Cube will not be used for longer periods, clean flow system by using distilled water. Put a sample tube half-ways filled with distilled water at the sample port. Clean waste and sheath reservoir, wipe top dry.

Service

All service is to be made from an authorized service engineer. Please contact your local supplier or Partec (service@partec.com).

For further information, please consult our web site:

- Products
- Application tips
- Instrument and software support
- News and events

And much more ...

www.partec.com

Transport and Storage

For the transport of the system to a different location it will be necessary to disconnect all external data and supply connections. In case of use with potentially bio-hazardous material, please see Partec standard operating procedure (SOP) for decontamination. The system should be carried in upright position. During transport or storage please take care that the system will be stored under the following conditions:

Temperature	5-50°C
Humidity	20-85% relative (non-condensing)
Room	Clean environment, no direct sun light

Disposal

Warning: The Waste may contain biohazardous and carcinogenic material from the samples (infectious material, dyes).

In case of product disposal, please proceed according to the Partec standard operating procedure (SOP) for decontamination.

After decontamination, the system has to be disposed according to the local regulations and laws.

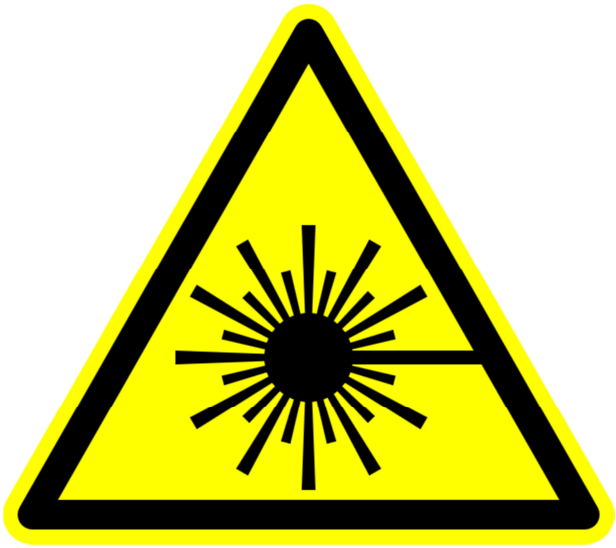
For further information, please contact your local distributor or Partec.

Laser Safety

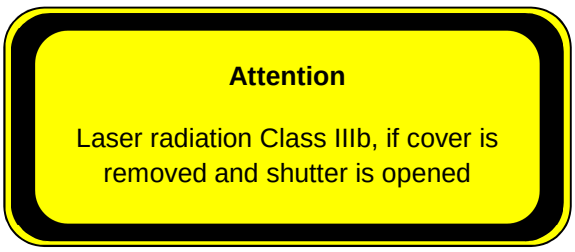
Warning: It is prohibited to open the instrument as it is equipped with a class 3b laser unit.

The CyFlow® Cube is a class I laser product according to the EN 60825-1:2007.

Please note: **Laser light can be emitted if the housing of the device is damaged and the protection cover for the laser beam is removed. Therefore, the system is marked with the following laser safety labels:**



Warning: laser radiation



Additional explanation

Technical Specifications

Note: Due to fast technological improvements, specifications herein are subject to change. For details, please inquire information from your local supplier.

Optical Standard Setup

The Parameters

FSC: forward scatter

SSC: side scatter

FL1: green fluorescence
fluorescence origin: 488 nm laser (GFP)

FL2: orange fluorescence
fluorescence origin: 488 nm laser (PE)

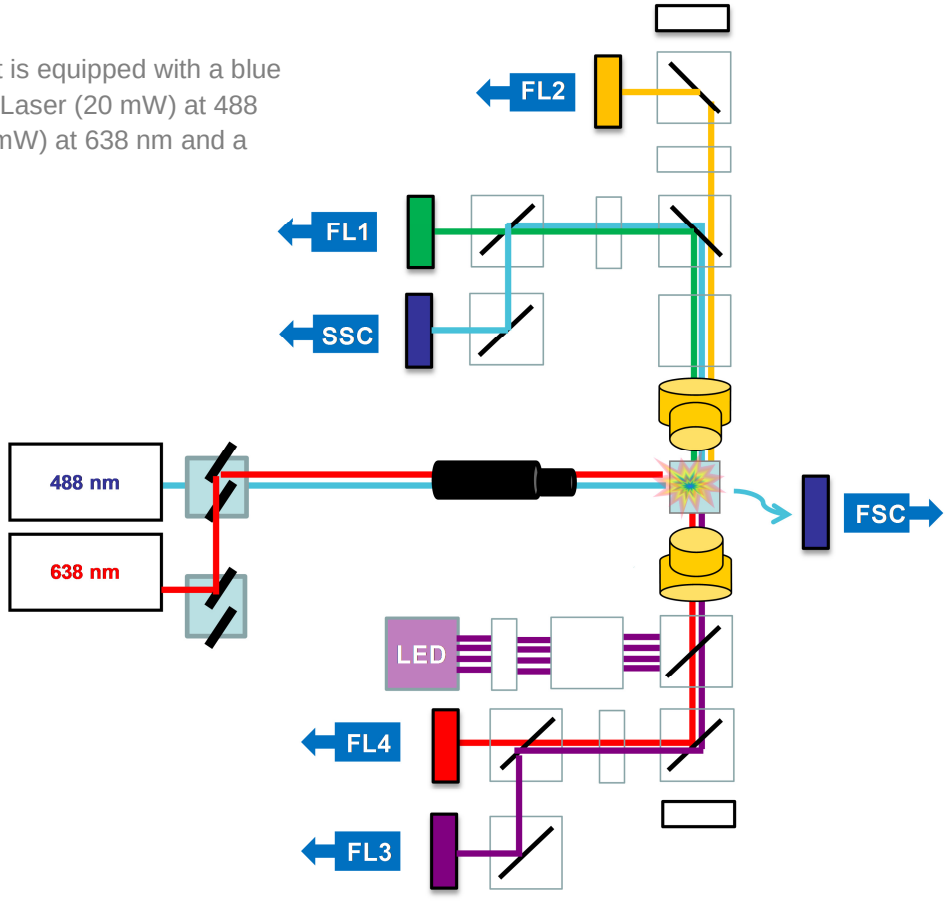
FL3: blue fluorescence
fluorescence origin: UV-LED (DAPI)

FL4: red fluorescence
fluorescence origin: 638 nm laser (APC)

If required by a specific application, the optical standard setup can be optimized by exchanging preassembled removable mirror/filter blocks. This is a matter of seconds and does not require any re-adjustment.

The CyFlow® Cube flow cytometer can be equipped with various light sources and up to eight optical parameters. Depending on the number of light sources and optical parameters different optical benches are available.
Due to its modular concept the optical configuration can be adopted to many different clinical and scientific purposes. Standard configurations are presented below:

Example: This instrument is equipped with a blue diode pumped solid-state Laser (20 mW) at 488 nm, a red laser diode (25mW) at 638 nm and a 365 nm UV-LED.



Optical bench of a CyFlow® Cube with 2 lasers, UV-LED and 6 parameters.

1. CyFlow® Cube System

Size	Dimensions: 500 mm L x 470 mm D x 355 mm H (670 mm with open display)
Weight	appr.40 kg
Maximum sound	< 70 dBA
Power level	
Installation/overvoltage category	2/II
Degree of protection	IP 20
Operating Environment	Temperature:15-30°C
	Humidity: 20-85% relative (non-condensing)
	Room: Clean environment. Direct sun light should be avoided.
Applications	Immunophenotyping, DNA Analysis, Ploidy Analysis, Apoptosis, Microbiology, Industrial applications, 3 to 6 Colour Analysis. True volumetric absolute counts = counting per volume
True Volumetric	Based on precise counting and mechanical fluid volume measurement
Absolute Counting	No need for reference sample or beads
Instrument Check	Partec Count Check Beads
	Partec Calibration Beads 1 µm and 3 µm
	Partec DNA Control PI
Set-up Time	Max. 5 minutes
Parameters	Up to 8 optical parameters: FSC, SSC, FL1, FL2, FL3, FL4, FL5, FL6
Particle Size Range	0.1 µm - 50 µm (standard cuvette)
Maximum Acquisition Speed	25,000 events/sec
Acquisition Stop Time	Event- or volume-based
Trigger	On all parameters, on multiple parameters or on single trigger parameter, selectable in software
Data Resolution	65,536 channels (16 bit)
Service	1 - 3 years service contracts
Warranty	12 months on all parts except filters, mirrors, other quartz or glass parts, disposables and cuvettes

2. CyFlow® Cube Optics

Laser / Output	Red Diode Laser:	25 mW at 635 nm / 40 mW at 640 nm
	Green NdYAG:	30 mW to 100 mW at 532 nm
	Blue solid-state Laser:	20 mW at 488 nm
	Blue laser diode:	50 mW at 488 nm
	Violet Diode Laser:	100 mW at 405 nm
	Ultra-Violet Diode Laser:	16 mW at 375 nm
	Yellow Diode Laser:	100 mW at 561 nm
	Orange Diode Laser:	50 mW at 594 nm
Detectors	1 to 8 (FSC, SSC, FL1, FL2, FL3, FL4, FL5, FL6)	
Filters	Standard setup and filters for all parameters according to laser configuration	
Optical Coupling	Standard objective mount with high numerical aperture objective, high numerical aperture immersion gel coupling, e.g. for detection of weak cytokines (option)	
Excitation Optics	Elliptical 15 µm x 100 µm at 488 nm	

Other beam geometries upon request

3. CyFlow® Cube Fluidics

Flow Cuvette	Synthetic quartz flow cuvette (350x 200 µm) for laminar sample transport with sheath fluid fluorescence, forward and side scatter light detection
Sample Delivery	Computer controlled precision syringe pump for contamination-free sample transport.
	Built-in vacuum pump for waste container. Vacuum pressure is adjustable (Computer controlled).
Sampling Volume	Continuous up to 1500ml.
	200 µl for electrode based precision absolute counting, Other counting volumes upon request
	50 – 1000 µl for syringe based precision absolute counting
Flow Rates	1) Sample volume speed adjustable continuously between 0.1 and 20 µl/s
	2) Sheath fluid flow continuously adjustable in expert mode
Fluidics Volume	2 x 1-litre integrated reservoirs for sheath fluid and waste
BioSafety System	Avoids sample droplets and sample cross contamination (computer controlled)

Notes

PARTEC

Excellence in Flow Cytometry and Cell Analysis