CyFlow® Cube 8

Instrument Operating Manual

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1 Introduction

The CyFlow® Cube 8 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 8 and its software. The confirmed flower will find an in-depth detail of the inner working and parameters of the Cube 8 to customize its use and obtain optimal performances.

CyView™ for Cube 8 is the instrument operation software for the **CyFlow® Cube 8.** Sysmex- Partec is continuously working on CyView™ to better fulfill 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 Sysmex Partec GmbH.

The present manual is valid as of CyView™ software version 1.5.

For more details about the reagent kits, suitable for use with the CyFlow® Cube 8**,** 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 Sysmex Partec subsidiaries, or Sysmex Partec in Germany (support@sysmex-partec.com).

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

- **www.sysmex-partec.com**

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

- **Serial number (serial No.) of the CyFlow® Cube 8**
- **Your complete contact address**

2 Presentation

2.1 Basics

What is the Sysmex Partec CyFlow® Cube 8?

The Sysmex Partec CyFlow® Cube 8 is a fully equipped desktop Flow Cytometer (FCM). CyFlow® Cube 8 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 8 allows easy optimization of the optics for any application by simple exchange of optical filters and mirrors. The CyFlow® Cube 8 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 8 can be used?

Together with the software, the CyFlow® Cube 8 offers automation for routine use and flexibility for research use for practically any flow cytometric application. The applications cover:

- Routine multi-color-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 8 Instrument Operating Manual covers the basic operation and maintenance of the CyFlow® Cube 8 instrument. This manual also covers details related to the software.

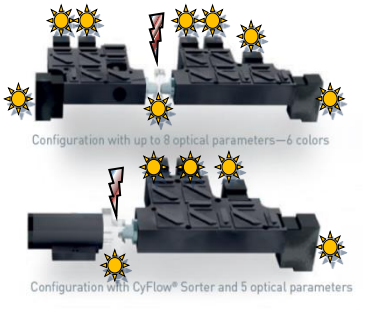
What other manuals are available?

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 8?

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).

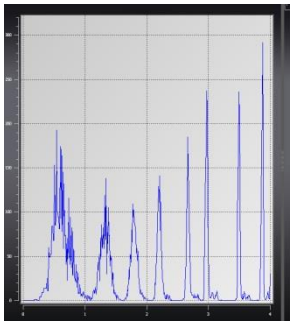
2.2 In flow cytometry, what is...



… 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 (16bit) 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 of a 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.

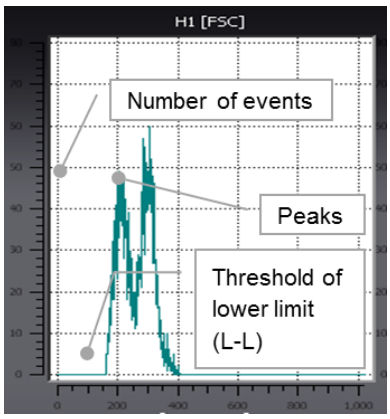
The lower level (L-L) or threshold?

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 colors (fluorescence parameters FL1 to FL6).

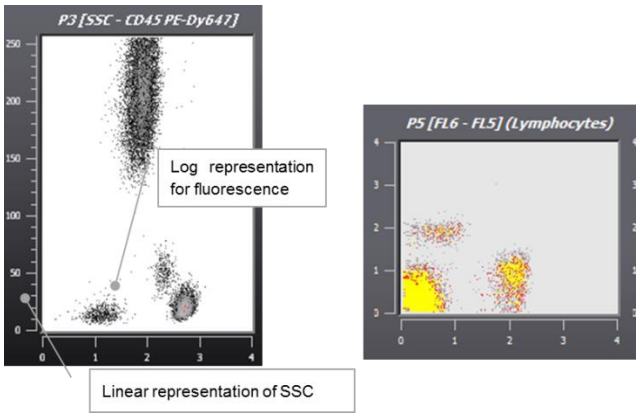
In this example, the dimension represented is the relative size (FSC) on a logarithmic scale in X, and the number of events on a linear scale in Y. Two peaks are visible.



Example of a dot plot

A 2D dot plot 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 yellow 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 color-coded in red.

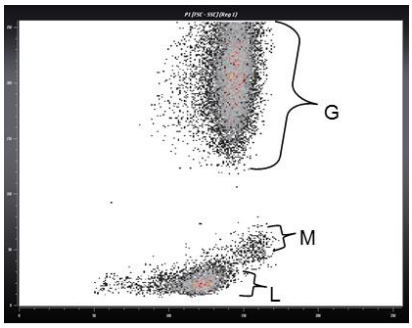


Histogram and dot plot in immunology

Lysed blood 2D dot plot

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**).

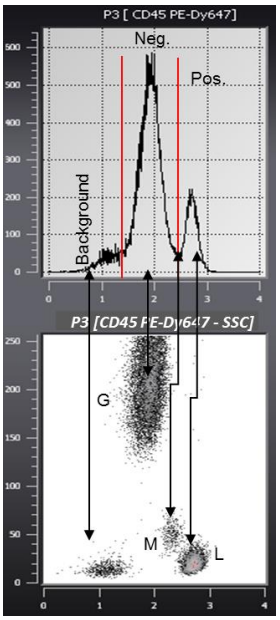


Histogram and dotplot of immunological staining

This histogram represents the spectrum of the cells presented in the previous dot plot stained with antibodies anti-CD45 conjugated to Phycoerythrin (PE) Dy647. 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.

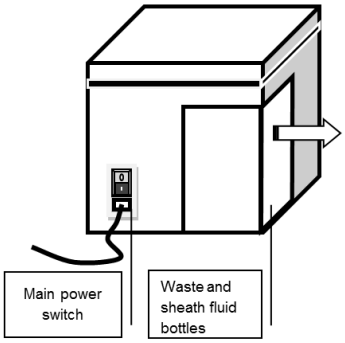
This dot plot presents the cells fluorescence in X on a logarithmic scale (CD45 PE-Dy647) versus SSC in Y on a linear scale.

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.



3. Instrument starting procedure

3.1 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 700 ml of clean, filtered, and degassed sheath fluid and is closed with the screw top.

Please notice: Higher level of sheath fluid could lead to unstable sample flow.

In order to guarantee highest quality of the measurements we highly recommend use Sysmex 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!

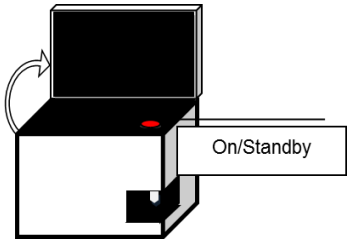
Make sure that the **WASTE bottle** is empty and the screw top is tightly closed.

The waste bottle must be emptied after and before each user session. When using bio-hazardous 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.

3.2 Switching on the CyFlow® Cube 8

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.

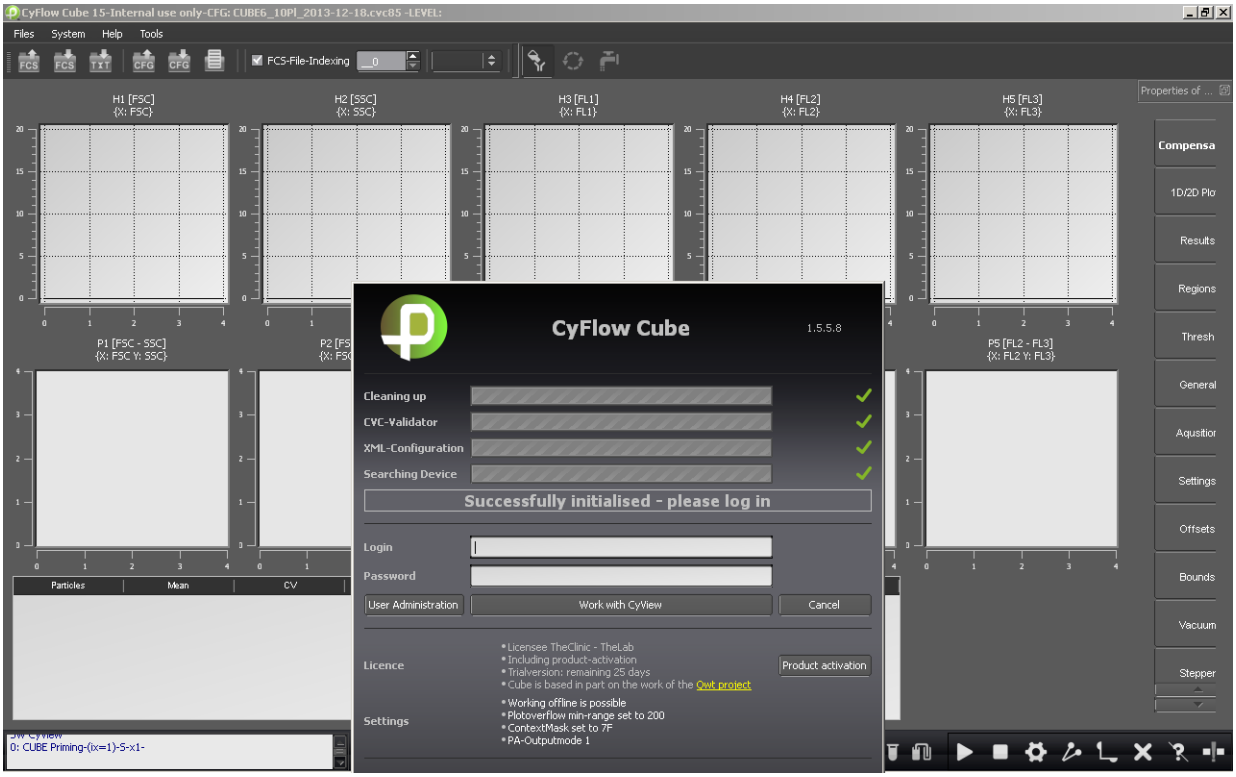
This will start the embedded computer, automatically start the CyView™ software and load the last employed configuration.



Casual/medium expertise user:

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

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:

Cleaning up and *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 all 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.

3.3 User levels

Three different user levels exist:

- **SERVICE/ADMINSTRATOR:**

  Restricted to authorized Sysmex Partec trained persons and for service purposes only

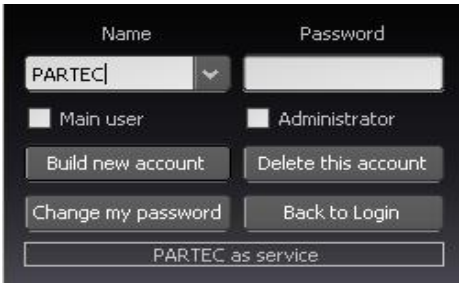
- **MAIN USER:**

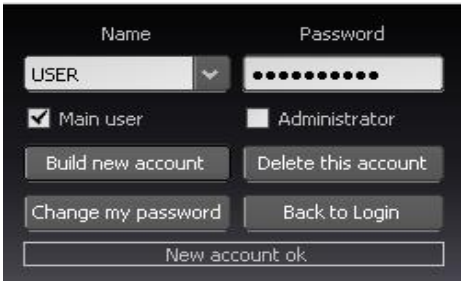
  Complete functionality of the instrument, method development

  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.

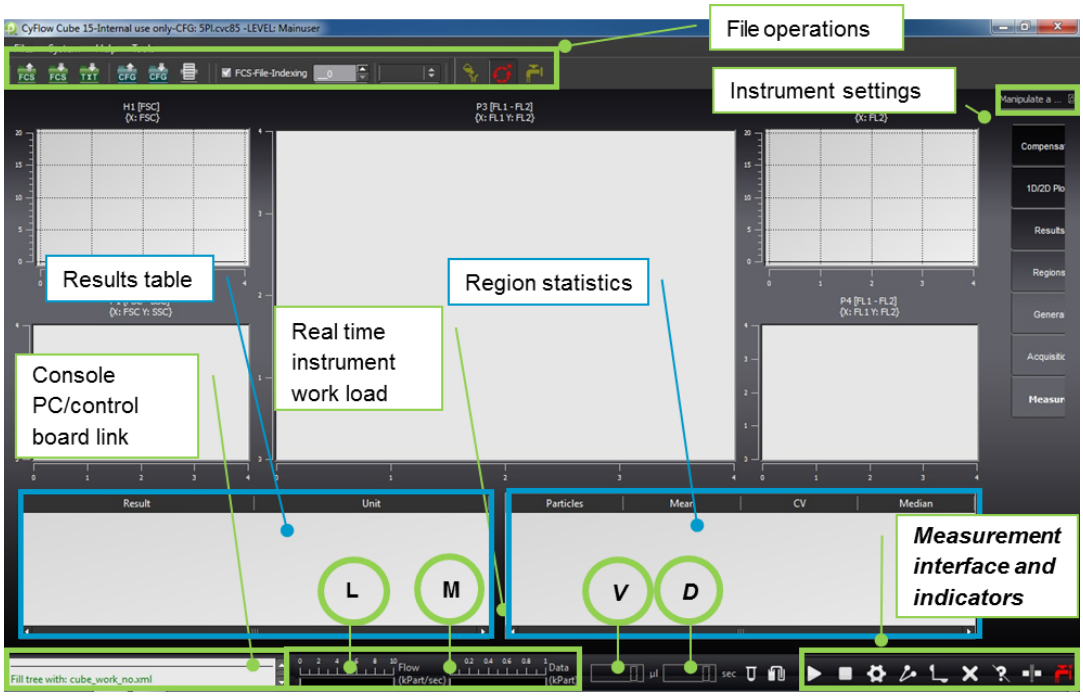
The default user account (Name: USER; Password: Cube1) can be deleted when logged in as Main user. Please make sure at least one Main User remains in the user list in order to guarantee complete functionality of the software.

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™.

4. CyView™ Controls

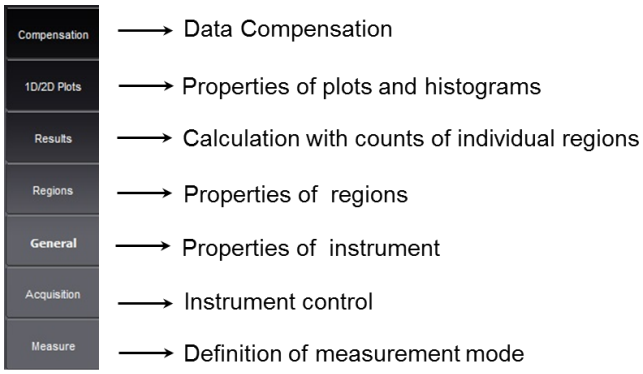
CyView™ Main Page – after successful log-in

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**).

4.1 CyView™ Instrument Setting Controls



Overview of instrument settings

4.1.1 1D/2D PLOTS– display options

The **1D/2D 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 File***.
As example: 12Pl → 6 Histograms + 6 dot plots

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

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

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

Switch between ***Lin*** Log On ☐ and ***Log*** scale Log On ☑ for ***X*** and ***Y*** axis and change the ***Z*** axis (scaling)

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

Select Dotplot **(DP)-Mode**

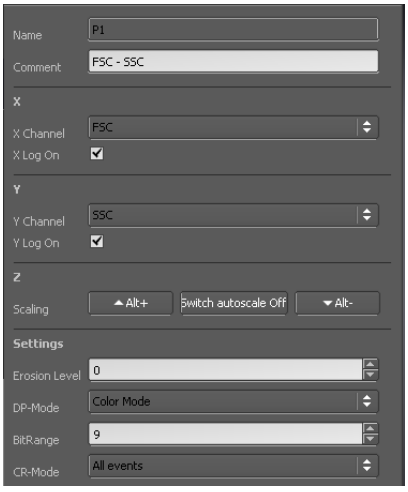
(Color Mode, Contour Mode or Color + Contour Mode)

Select histogram resolution as ***BitRange***,

(values 16bit to 12bit)

Select CR – Mode to show “All events” or “Regions only”

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



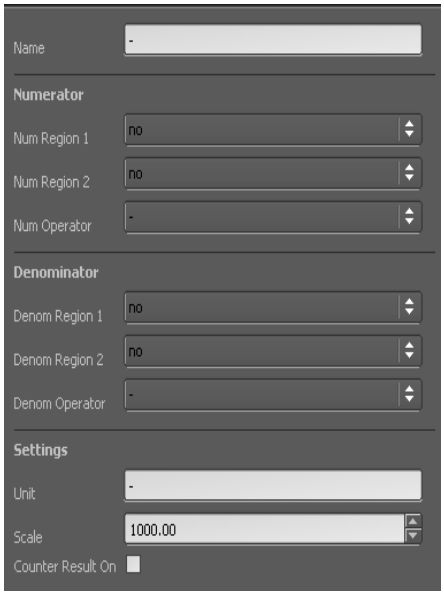
4.1.2 RESULTS

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

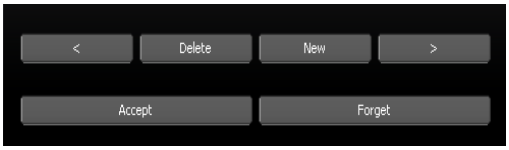
Properties of result calculations

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



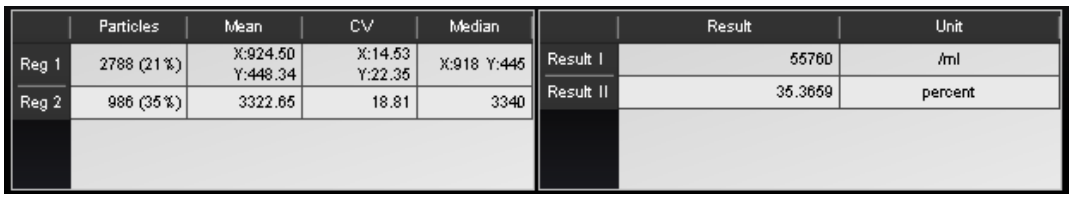
- **NumRegion1/2** defines numerators
- **DenomRegion1/2** defines denominators
- ***NumOperator*** defines operator between 2 numerators „+“, „-“, „x“ or „/“
- ***DenomOperator*** defines operator between 2 denominators „+“, „-“, „x“ or „/“
- ***Unit*** allows to add text to the result table
- ***Scale*** introduces a factor to the formula
- ***Counter results on*** refers to the result of a Volumetric counting



Create a result by pressing “**New**” or delete a result by pressing “**Delete**”

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

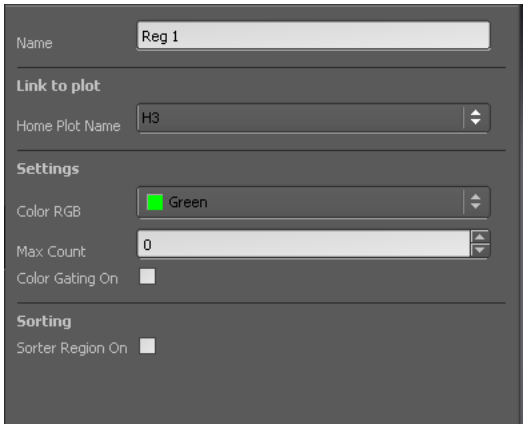
Result will be display in the “Results table” next to the “Region statistics”



	Particles	Mean	CV	Median
Reg 1	2788 (21%)	X:924.50 Y:448.34	X:14.53 Y:22.35	X:918 Y:445
Reg 2	986 (35%)	3322.65	18.81	3340

	Result	Unit
Result I	55760	/ml
Result II	35.3659	percent

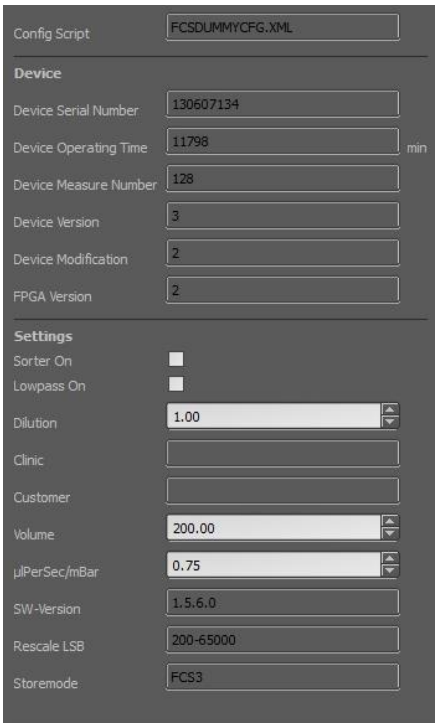
4.1.3 REGIONS



- ***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 “Cells in Region” particle limit (see also Pages 17-19)
- ***Sorter Region On*** activates region as sorter region (only in CyFlow® Cube Sorter)
- ***Color Gating On*** activates/deactivates the region
- Use ***Delete*** and ***New*** to delete and create new regions

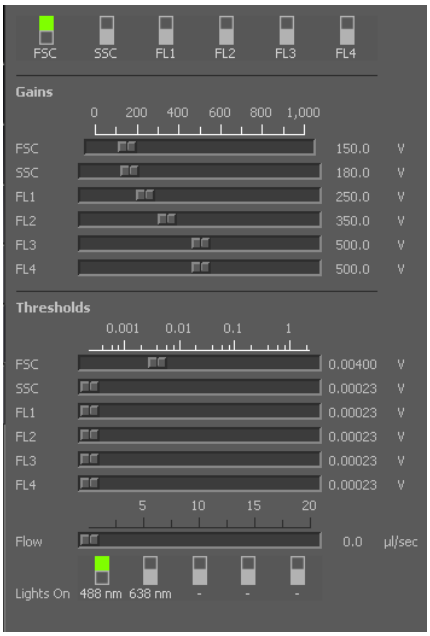
Use arrow keys to switch between regions
Confirm all modifications by pressing ***Accept***

4.1.4 GENERAL - properties of the instrument



- The active ***Configuration File***
- The instruments ***Serial Number***
- Total ***Operating Time*** of the instrument
- ***Measure Number*** shows total number of measurements
- ***Version*** of the instrument
- ***Modification*** of the instrument
- Activates ***Sorter*** function
- Activates **Lowpasses**
- Defines a factor for sample ***Dilution***
- ***Clinic/Customer*** display specific user information
- Defines sample port electrode ***Volume*** in µl
- ***µlPerSec/mBar*** defines a sheath fluid flow parameter
- ***SW-Version*** specifies current software version
- ***Rescale LSB*** shows area of automatic scaling
- **Storemode** defines FCS file format

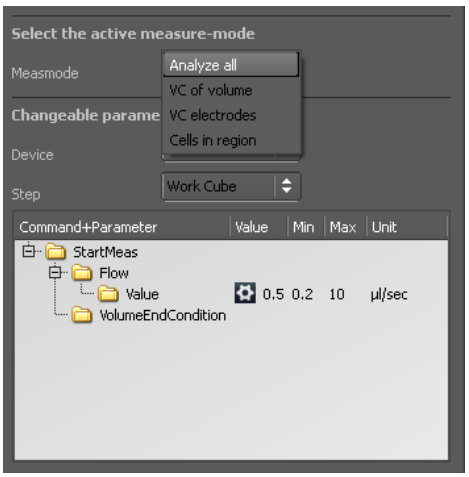
4.1.5 ACQUISITION – instrument control



- Select the trigger parameter
  multiple trigger parameters are logical “or” connections
- ***Gain*** values to change the 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

4.1.6 MEASURE – definition of measure modes

Measmode allows selection of:



Analyze all:
run sample until its levels reached the stop electrode

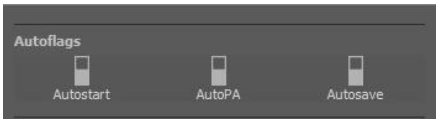
Volumetric counting (VC) with volume
a volume can be pre-selected

Volumetric counting (VC) with electrodes:
START and STOP electrodes of the sample port are used to define a fixed sample volume of 200µl

Cells in region
a particle number can be pre-selected

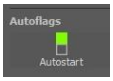
Speed values and ***Volume values*** can be edited and will be stored upon saving a configuration file.

Autoflags allows activation of specific functions:



Autostart: automatic start of the measurement

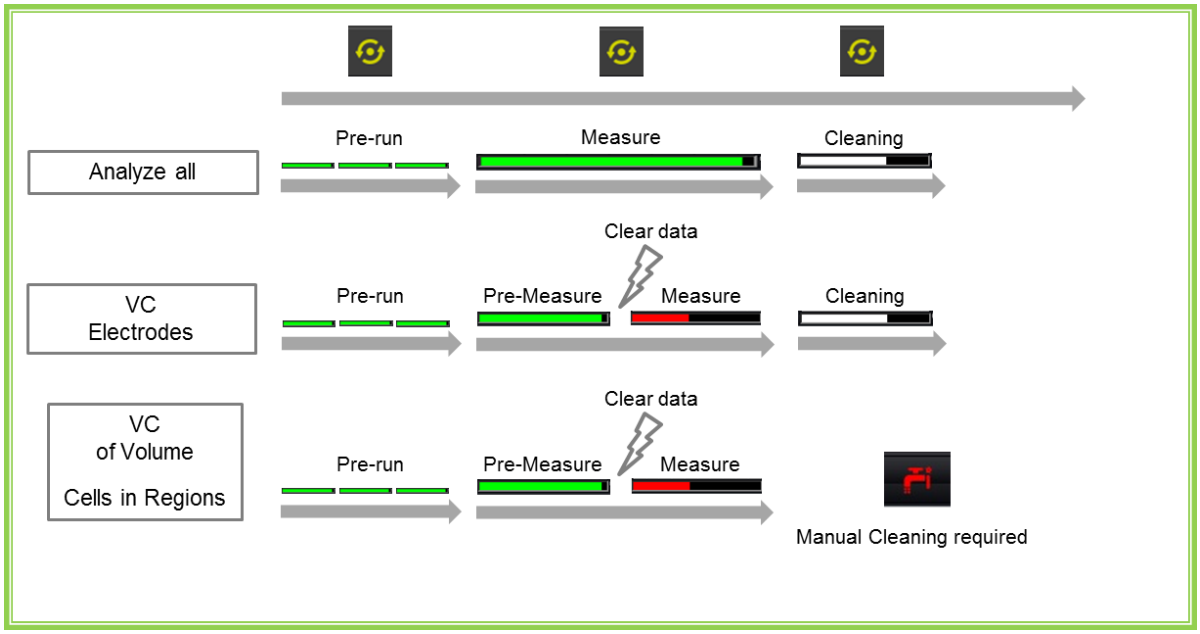
- Activate the AutoStart flag
- Press start (only once to confirm the process)
- Connect a sample tube



Autostart:
With each new sample tube the next measurement will be started until the STOP button is pressed.

AutoPA:
automatic peak analysis, at the end of the measurement → only for Ploidy

Autosave:
Automatic storage at the end of the measurement. Autosave does not function when the measurement is finished by the “end” button.



Schematic overview of the acquisition process for all measure modes

Green and red colored bars refer to particle count indicator of CyView.

Please note: In the measure mode “VC of Volume” or “Cells in Regions” there is no cleaning cycle. Subsequently the data can be stored. In the measure modes “VC electrodes” and “Analyze all” the cleaning cycle will occur automatically as soon as the sample is finished.

No cleaning cycle will be started if the measurement is terminated by the “end” button.

Beware: for all measure modes the sample analysis automatically stops when the sample is empty (the stop electrode is reached) even if the selected end criterion is not yet realized.

4.1.7 Measure modes

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

- Analyze all
- Volumetric counting (VC) of volume
- Volumetric counting (VC) electrodes
- Cells in region

Analyze all (default selection)

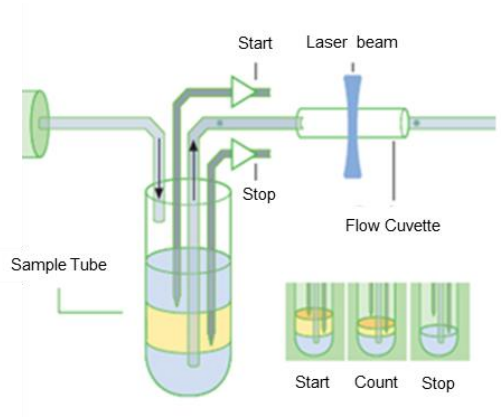
This default mode will allow you to run your sample until its levels reached the stop electrode.

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.

Volumetric Counting with Electrodes

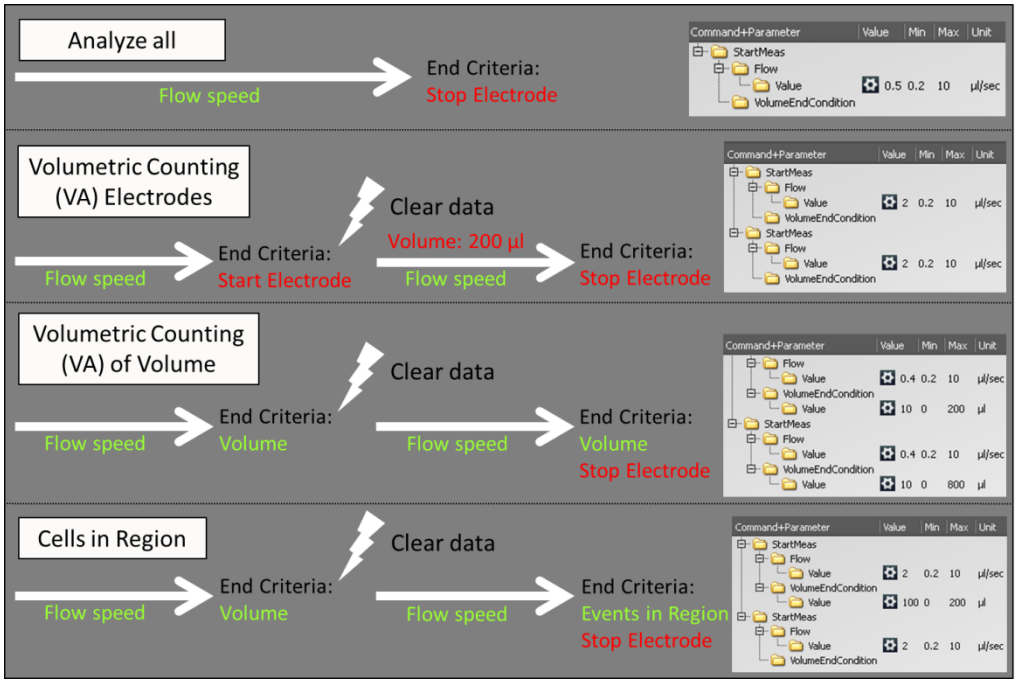
This measuring mode uses the START and STOP electrodes of the sample port to define a fixed sample volume. In a standard sample port this “counting volume” is 200µl. In a pre-counting phase the sample is acquired normally as in ***continuous acquisition mode***. Reaching the START electrode the data 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 Electrodes

Cells 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).



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

5. CyView™ Instrument/Measurement Settings

Prime/Work/Clean

There are 3 different possible modes available: The “PRIME” mode for a priming/initialization process, the “WORK” mode for measurements and the “CLEAN” mode for cleaning of the system.

Prime, Work and Clean mode

5.1 Priming / Initialization process

There is a PRIME mode available as a Start Up process for the CUBE instrument. This process includes cleaning, filling the tubes with sheath fluid and control of the instrument set up. If a new original CFG-file will be loaded the PRIME mode is always selected. User defined configuration files can start in PRIME or WORK mode, depend on the status during saving.

In the following cases the PRIME mode should be performed:

- Starting the instrument for the first time → Daily initialization
- After the sheath fluid bottle was filled
- As trouble-shooting procedure (no/bad signals, blocking, etc.)

To start the priming/initialization process, please follow the instructions below:

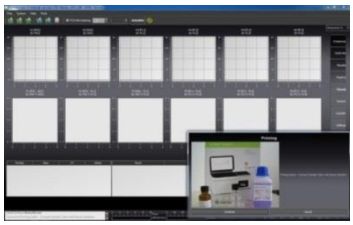
- Open a configuration file and select the initialization modus by clicking on the Initialization batch symbol (Sysmex Partec Master Cfg-file: Prime mode is pre-set).

  If you will perform the daily check-up please select the “Calibration Beads” configuration-file. Only in this configuration file you will find the correct settings for the calibration beads. These settings are crucial for a correct verification of the instrument performance.

Initialization batch, Work batch and Clean batch

- After pressing Start  the system will start the priming process automatically and guide through the program.

- Connect a sample tube with Decontamination Solution (violet solution, Order No. 04-4010), press “Continue” and wait until the system has finished.




- Remove the sample tube, press “Continue” and wait until the system has finished.

  - Connect the sample tube again, press “Continue” and wait until the system has finished

- At the end press “Continue”. The instrument will be cleaned and the priming process ends.

- Switch to “WORK” batch

5.2 Work process

Choose the work batch for measure and acquire of data.

5.3 Shut down process

For the cleaning of the sample port a separate cleaning mode is available. The detail procedure would be the following:

- Select the “CLEAN” batch by clicking on the “CLEAN” batch symbol 
- After pressing Start  the system will start the priming process automatically and guide through the program.
- Connect a sample tube with cleaning solution (green solution, Order No. 04-4009) and press “Continue”

- Connect a sample tube with decontamination solution (violet solution, Order No. 04-4010) and press “Continue”

- Connect a sample tube with sheath fluid (Order No.04-4007) and press “Continue”

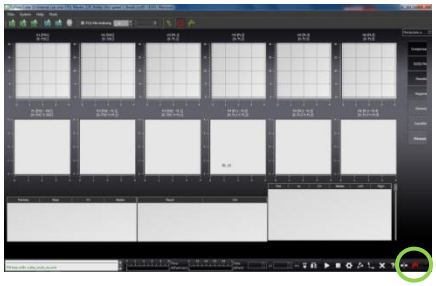
- After finishing the cleaning process CyView™ will be shut down automatically.
  - Laser sources will also be switched off.
- Switch off the PC
- Close Windows (Start – Exit- Switch Computer Off)
- To power off completely, use the main power switch at the back panel

5.4 Intermediate cleaning process

The Cube can be cleaned between sets of samples using cleaning solution (green solution, Order No 04-4009) followed by a sample tube with sheath fluid. This procedures will allow you to reduce significantly the cross contamination and reduce the background.

For this procedure, connect a sample tube with cleaning solution and clean the instrument by clicking on the “Clean” symbol.

The instrument will perform one cleaning cycle. Repeat the procedure with a sample tube filled with sheath fluid.



Please notice the differences between the clean-modes. If you choose the Clean-symbol display at right lower corner, the system performs one cleaning cycle. In contrast to the Clean-symbol display in the upper part, if this symbol will be select the complete shutdown process will be performed.

5.5 Sheath and Waste bottle

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.

Sheath bottle can be cleaned with sheath fluid or hypochlorite solution 0.5% (Order No. 04-4012)

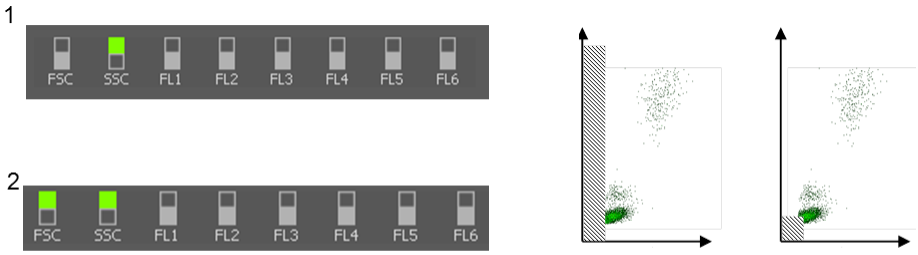
6. Measurement parameters

The Acquisition register can be opened by clicking on the button Meas ( ) Gains settings

6.1 Trigger properties (P)

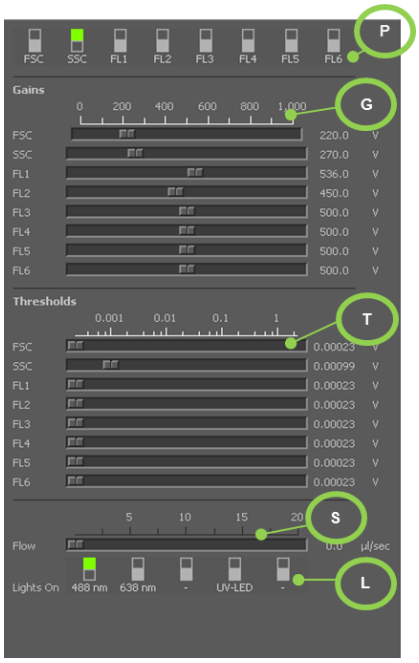
- ***Single trigger:***
  The trigger is the parameter defining if a signal gets recorded. Only if the trigger parameter detects a signal, other parameters of the system will record signals. In other words, if a trigger is set in FSC to record only bigger particles (e.g. threshold set at 0.3871V with 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



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

6.2 Instrument setting properties



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.

6.2.1 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.

6.2.2 Flow speed (S)

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

6.2.3 Light source (L)

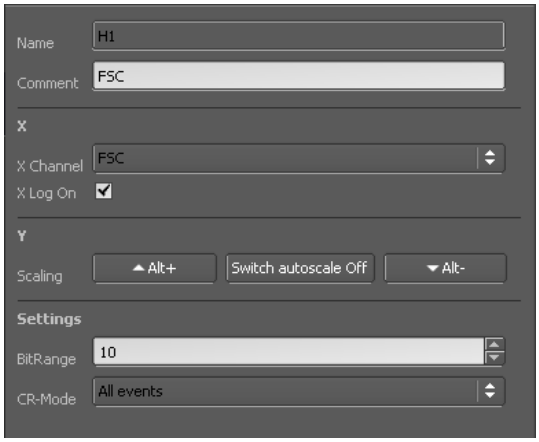
This option allows to switch on/off the light sources.

Please notice Laser needs a few minutes before active.

6.3 1D/2D Plot properties

6.3.1 Plot name

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



6.3.2 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.

6.3.3 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).

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

6.3.4 X/Y Channel

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

6.3.5 BitRange

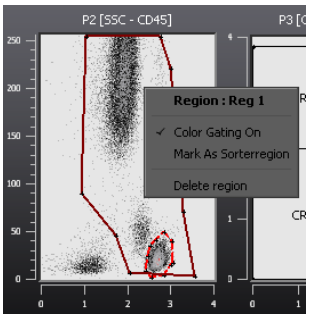
The values are ranging from 6 to 12. It sets the channel resolution from 16bit (64channels) up to 12bit (4096 channels).

6.3.6 CR-Mode

This option allows selecting the part of the data to be displayed:

- All events
- Region only
- Color gating

6.4 Region/ROI properties

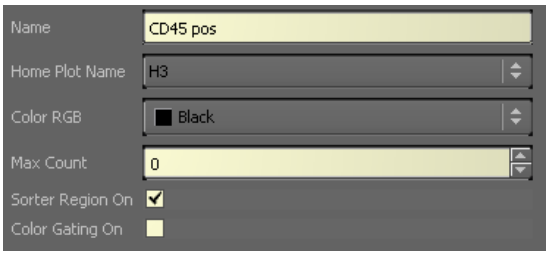


6.4.1 Create a polygonal region

By 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.

6.4.2 Region properties

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

Home Plot name

This defines the physical plot where the region is located.

Color RGB

Allows to choose the color of the region. This color will be reported to each plot selected as ***color gating only***.

Max count

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

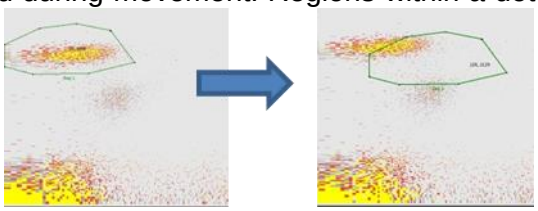
Sorter region

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).

6.4.3 Moving of regions within a histogram

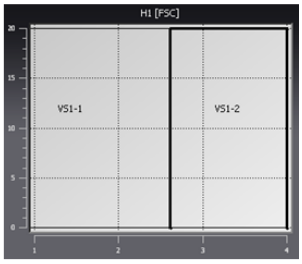
Move the cursor into the region and keep the left mouse button pressed while moving the position. Individual points of a region can be changed by approaching with the cursor to the point and keep the left mouse button pressed during movement. Regions within a dot plot can be changed in size by selecting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

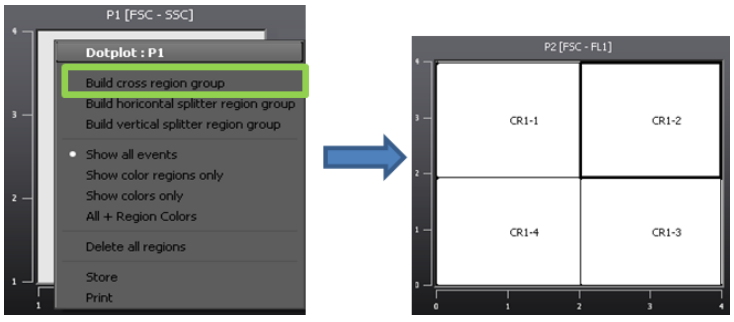
6.4.4 Create a vertical or horizontal histogram splitter

To create a vertical or horizontal histogram splitter, please move the cursor into the histogram and press the right mouse button. Select ***“Built vertical splitter region group”***/***“Built horizontal splitter region group”*** 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.



6.4.5 Create a quadrant assembly

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



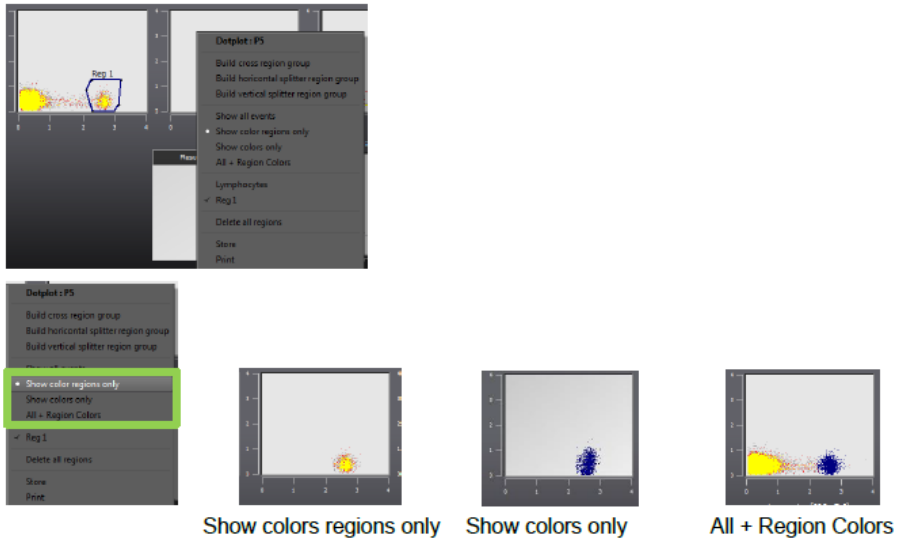
6.4.6 Change layout for regions

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”.

6.4.7 Applying regions to other plots → Gating Function

Clicking into any histogram or plot with the right mouse button (outside a region area) allows to select any existing region (Reg1-Regx) 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) in color together with all events in pseudo 3D color



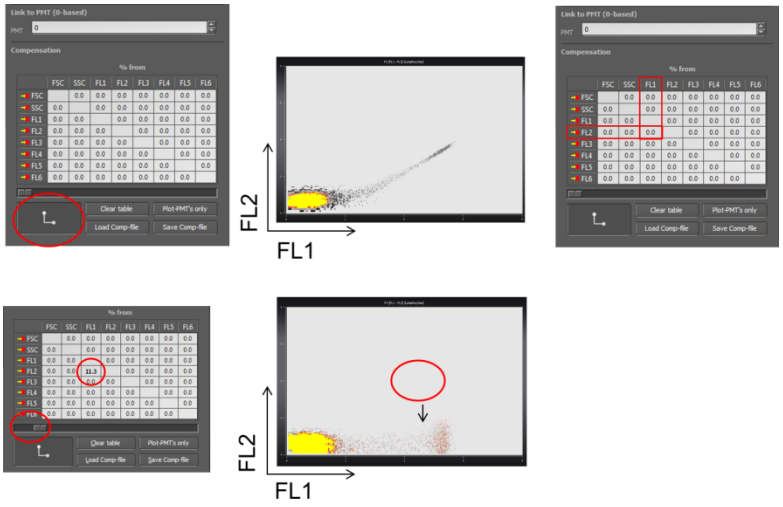
6.5 Compensation

Press **Compensation button** to open the compensation

Compensation:
Select a parameter combination to compensate.
Use the slide bar to set the compensation.

Clear/Save and Load Compensation

Confirm by clicking Accept



Example of a color-crosstalk compensation of FL1 into FL2.

7. Typical Sample Analysis

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

Starting a measurement

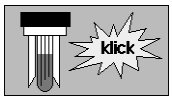
Load a sample file. The respective instrument settings are automatically loaded.

Or

Load a CFG-file, saved instrument settings will be load.

Perform PRIME process

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



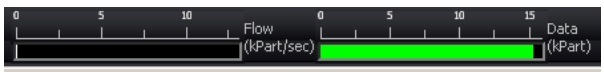
Insert the tube into the sample port, push it up until a distinct click is heard

Press the start button. In the console window, information on the status of the measurement is displayed. The following 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 on the real-time count meters



During the acquisition (Pre-Measure) 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 or “Space” at the keyboard

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 ***Analyze all*** measure mode other measure modes can be selected:

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

8. Keyboard/Mouse combinations

Context	Action	Effect
Graphic Plot/Histogram	Alt + Mouse left-click	Opens Plot/Histogram (big) or close (small)
Create Region	Right Mouse-click	Ends creation of a region
	Left Mouse-click	Sets dot of a region
	click and hold left mouse	Moves a region in standard steps
	Ctrl+ click and hold left mouse	Moves a region in small steps
Region	Shift+ click and hold left mouse	Moves a region to the original position
	+Shift+Ctrl click and hold left mouse	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
	Left mouse +Alt	increased/decreased Plot
Region	Central mouse	Marks the region and moves it in all regionplots in the visual area

9. Appendix

9.1 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
PPE (personal protection equipment) must be met for all users. Therefore, the system is marked with the following biohazard label:

Warning: biohazards

9.2 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 should 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 the 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 Sysmex 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-way filled with distilled water at the sample port. Clean waste and sheath reservoir, wipe top dry.

9.2.1 Service

All service is to be made from an authorized service engineer. Please contact your local contact.

9.2.2 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 Sysmex 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

9.2.3 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 Sysmex Partec standard operating procedure (SOP) for decontamination.

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

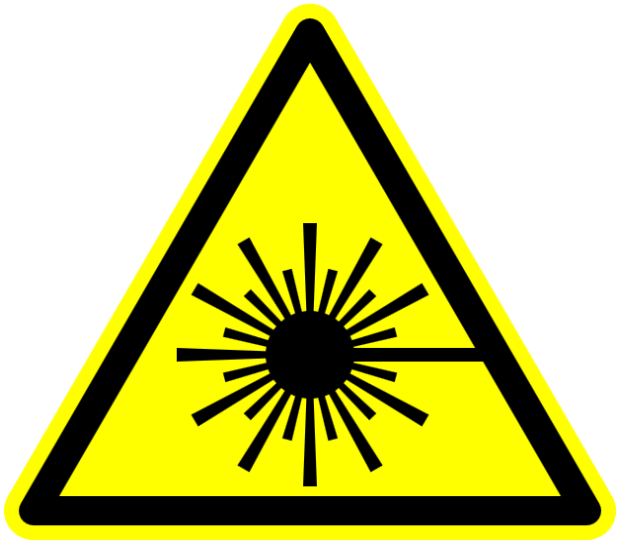
For further information, please contact your local distributor or Sysmex Partec.

9.3 Laser Safety

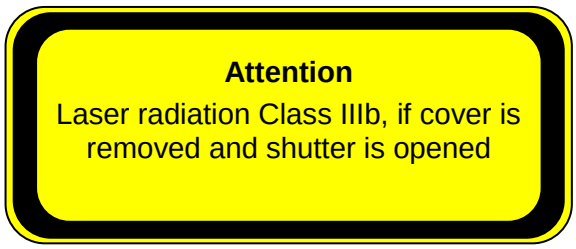
Warning: It is prohibited to open the instrument as it is equipped with a class 3b laser unit.

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

9.4 Technical Specifications

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

9.4.1 Example for 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)

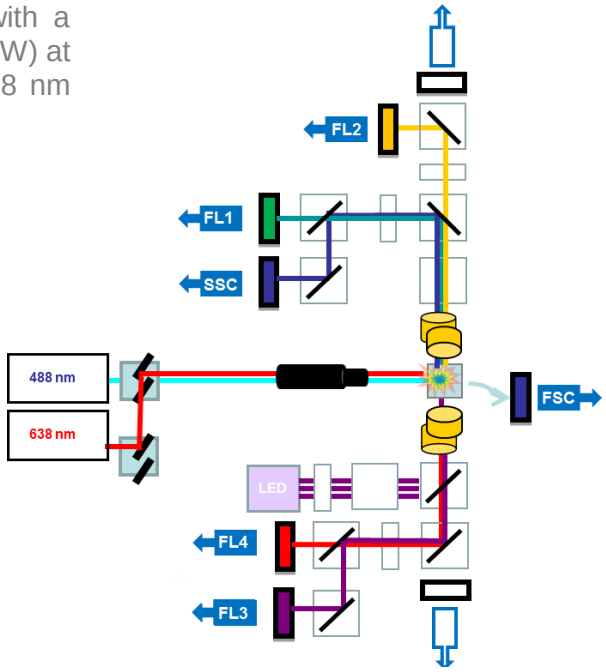
FL3: blue fluorescence
fluorescence origin: UV-LED (DAPI)

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 (25 mW) at 638 nm and a 365 nm UV-LED.



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

9.4.2 CyFlow® Cube 8 System

Size	Dimensions: 500 mm L x 470 mm D x 355 mm (670 mm with open display)
Weight	appr.40 kg
Maximum sound	<70 dBA
Power level	200 VA
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, Apoptosis, Microbiology, Industrial applications, 3 to 6 Color 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	Sysmex Partec Count Check Beads (No. 05-4010) Sysmex Partec Calibration Beads 1µm (No. 05-4007) Sysmex Partec Calibration Beads 3µm (No. 05-40018) Sysmex Partec DNA Control PI (No. 05-7303) Sysmex Partec DNA Control UV (No. 05-7302)
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 – 100 µm
Maximum Data	15.000 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)

The **CyFlow® Cube** System is a completely closed system not producing any aerosols during cellular analysis.

9.4.3 CyFlow® Cube 8 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 laser diode	50 mW at 488 nm/200 mW at 488 nm
	Violet Diode Laser:	100 mW at 405 nm
	Near 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	

9.4.4 CyFlow® Cube 8 Fluidics

Fluidic System	Completely closed system for sheath water and sample volumes; no fluid droplets or aerosols generated nor released from the instrument.
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 1200 ml.
	200 µl for electrode based precision absolute counting, Other counting volumes upon request
	5 –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	2x 1-litre integrated reservoirs for sheath fluid and waste
BioSafety System	Avoids sample droplets and sample cross contamination (computer controlled).

Notes