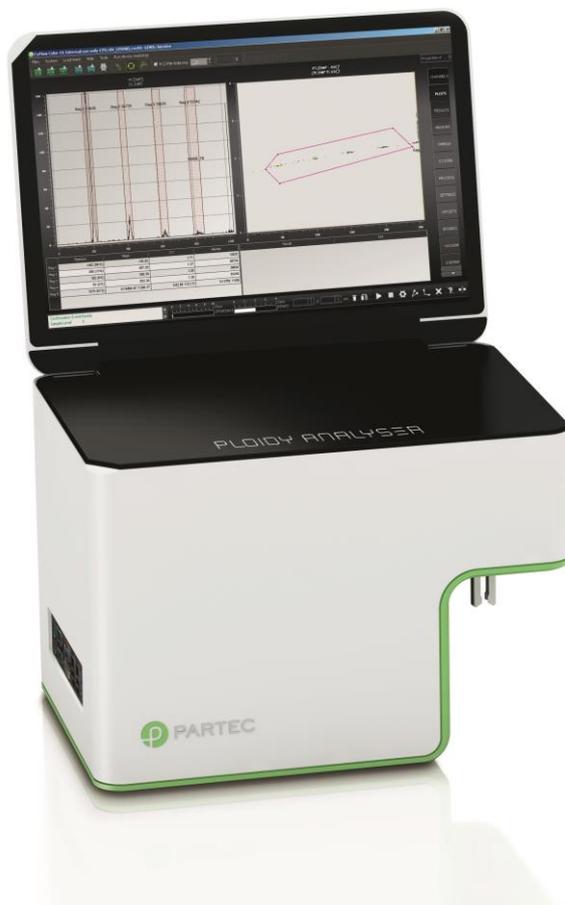




CyFlow[®] Ploidy Analyser

Instrument Operating Manual



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1. Introduction

The **CyFlow® Ploidy Analyser** is a compact Flow Cytometry (FCM) system produced by Sysmex Partec GmbH, Germany. It is designed to analyze the DNA content of intact plant and animal nuclei and of micro-organisms by fluorescence labeling of DNA by fluorochromes, propidium iodide (PI) or 4',6-diamidino-2-phenylindole (DAPI).

CyView™ for Ploidy Analyser is the instrument operation software of the **CyFlow® Ploidy Analyser**. Sysmex 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 Sysmex Partec GmbH.

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

For more details about reagent kits, suitable for use with the CyFlow® Ploidy Analyser, please refer to the product list in the chapter *Technical Specifications* and the respective product data sheets which can be requested by e-mail at Partec Support service@partec.com. 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@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® Ploidy Analyser**
- **Your complete contact address**

This manual contains references to names and products from Partec and other companies which are registered trademarks or protected by copyright.

Partec GmbH, CyFlow® Ploidy Analyser, CyView™ for Ploidy Analyser, Robby®.

Microsoft® Corp.: Windows, Word, Excel, PowerPoint, Paint.

Hewlett Packard®: Deskjet Laserjet.

2. Presentation

2.1 Basics

The CyFlow® Ploidy Analyser is available in three versions:

Order No. 11-01-1002

Sysmex Partec CyFlow® Ploidy Analyser "DAPI" one parameter (FL) | UV LED excitation

Order No. 11-01-1003

Sysmex Partec CyFlow® Ploidy Analyser "PI" two parameters (SSC/FL) | green 532nm laser

Order No. 11-01-1004

Sysmex Partec CyFlow® Ploidy Analyser "DAPI/PI" two parameters (SSC/FL) | green 532nm laser and UV LED excitation

The CyFlow® Ploidy Analyser is equipped with a UV-LED - 365 nm emission wavelength (11-01-1002) and/or a Nd-YAG green solid state laser - 532 nm emission line (11-01-1003 and 11-01-1004, respectively). For fluorescence detection of DAPI (DAPI-DNA) a sensitive blue PMT is used which analyses fluorescent light between 435 nm and 560 nm. The same detector is used to monitor 532 nm side scattered light from Nd-YAG laser excitation. Propidium iodide fluorescence (PI-DNA) is detected by a red-sensitive PMT at fluorescence wavelengths above 590 nm.

What are topics covered by this manual?

The CyFlow® Ploidy Analyser Instrument Operating Manual covers the operation and maintenance of the CyFlow® Ploidy Analyser. This manual also covers details related to the CyView™ software.

What other manuals are available?

Application Notes, reagent kit data sheets and a Service Manual are available on request at Sysmex Partec GmbH. They contain hints to achieve the best results.

What should I know before operating the CyFlow® Ploidy Analyser?

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).

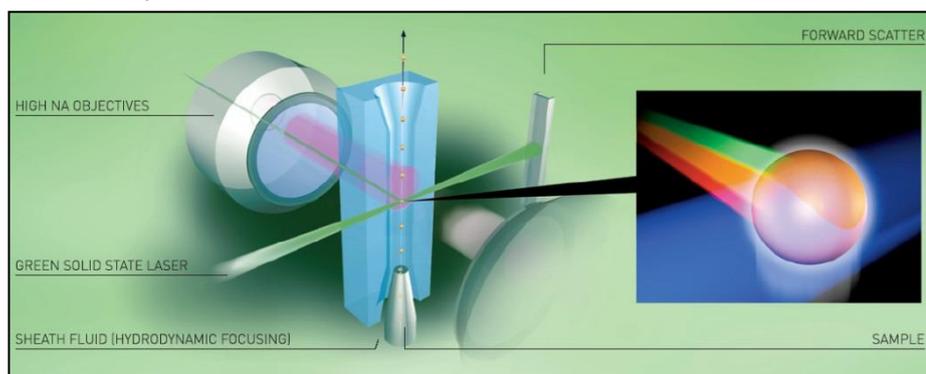
2.2 Introduction to Flow Cytometry

The term Flow Cytometry describes a technique for the analysis of cells – or particles – in aqueous suspension. Cells or other biological particles pass in a single cell stream through a measuring apparatus. In the most common scenario, one or more lasers interrogate each particle and, at a minimum, the system measures the degree and direction of scattered light – indicators of the particle's size, shape and structure. If particles have been stained with one or more fluorescent dyes – known as fluorochromes – the light source excites these dyes to provide additional biological information about each particle, such as the presence of specific cellular compounds. The unique advantage of Flow Cytometry is that it can rapidly and quantitatively measure multiple simultaneous parameters on individual live cells.

2.3 Flow Cuvette and Light Signals

Flow Cuvette

The main element of the CyFlow® Ploidy Analyser Flow Cytometer is a flow cuvette. This is made of quartz glass which contains a capillary with a diameter of 200 x 350 µm.



Fluidics

The fluid system of a flow cytometer is used to transport particles from a random three dimensional sample suspension to an orderly stream of particles travelling past one or more illuminating beams. The fluidic system uses air pressure regulation to ensure stable operation and consists of a sheath fluid line and a sample line feeding into the flow cell. As the sample enters the flow cell chamber, the outer, sheath fluid flow hydro-dynamically focuses the sample flow into a narrow core region within the jet and presents a single file of particles to excitation sources. This geometry provides increased positioning accuracy at the laser interrogation point for consistent excitation irradiance and greatly reduced particle blockage of the flow cell.

Scatter Signals

In the **CyFlow® Ploidy Analyser** 11-01-1003 and 11-01-1004 cells pass in a single file through the Nd-YAG laser beam focused into the capillary. This causes scattering of light providing information about different cellular characteristics. The side scattered light (side scatter, SSC) is emitted in an angle of about 90° and provides information about the “granularity” or refraction properties of the particles or cells. Particles/Cells with a high granularity/refraction create a strong signal in the SSC.

Fluorescence Signals

Fluorescence occurs when a molecule excited by light of one wavelength returns to a lower energy state by emitting light of a longer wavelength. Cell nuclei can be stoichiometrical labelled by fluorescent probes and fluorescence emission after sufficient excitation can be correlated to DNA content.

2.4 Optical Standard Setup – Parameters and Filters

The **CyFlow® Ploidy Analyser** is equipped with one (11-01-1002) or two (11-01-1003/11-01-1004) optical parameters for the detection of DAPI fluorescence (11-01-1002) and/or Propidium Iodide fluorescence and side scattered light (11-01-1003/1004). The fluorescence and side scattered light is collected by a microscope lens in an angle of 90°. The light is subdivided into two different wavelength bands by optical filter glasses.

Signal processing with Photomultiplier Tubes (PMT)

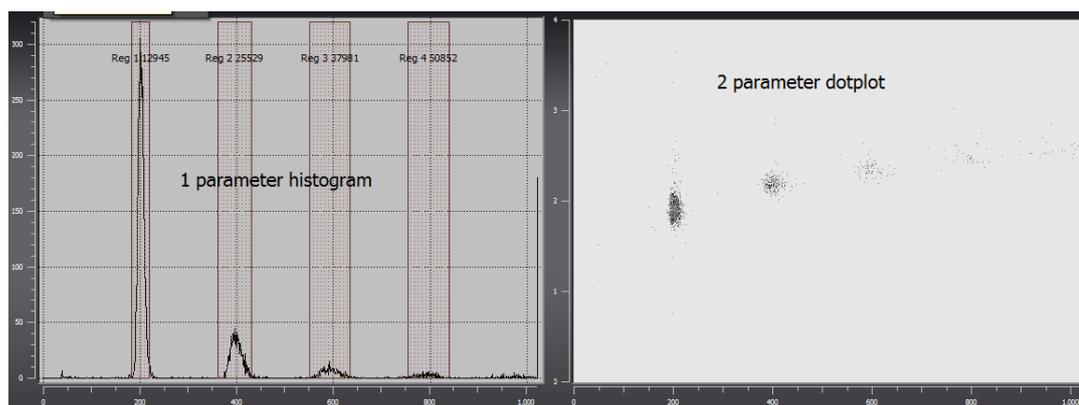
Photomultiplier tubes (PMT) collect fluorescence or scatter light signals, which generate an electronic pulse inside the PMT. The pulse amplitude is proportional to the number of incoming photons. Following linear or logarithmic amplification, the signals are converted into electronic signals by an Analog/Digital-Converter (ADC). As a particle of interest passes the focus, its fluorescence is detected by the PMT, and an electronic pulse is generated. The acquisition of a signal is triggered when the signal amplitude exceeds a predefined threshold level. The threshold is primarily used to reject events such as debris particles or noise from optical and electronic sources.

Optical filtration

Optical filters transmit a specific wavelength band of the visible light and block other wavelengths. Optical filtration is used to adapt the wavelength reaching the optical detector to the emission wavelengths of the used fluorochrome.

Display of a measurement

The flow cytometric data can be displayed either as a 1-parameter histogram (left plot next page) or as a 2-parameter dot plot (right plot next page). A 1-parameter histogram displays the distribution of cells among a specific property, e.g. how many cells contain a given quantity of DNA. A 2-parameter dot plot presents correlated data over 2 dimensions. In the CyFlow® Ploidy Analyser with 532 Nd-YAG lasers it displays DNA content versus side scatter signal (correlated to particles size and refraction properties, right plot next page).



The CyFlow® Ploidy Analyser with UV-LED displays one single 1-parameter histogram (left figure). The instrument with 532 nm laser displays both, 1 parameter histogram for DNA quantity and a 2 P-dot plot for DNA quantity versus side scatter property (right figure).

Gain

The gain is the value of the PMT amplification applied to a signal.

Regions and Gates

Regions are drawn to define a certain cell or particle population. In the 1-parameter histogram this is done by setting a *Histogram Region* (see *Reg1*, *Reg2*, *Reg3* and *Reg4*, figure above), within the 2-parameter dot plot a *Polygonal Region* is used. A gate is a region applied on a second plot as a data window. A gated plot displays only those data defined by the gating region.

Fluorescence staining

For the characterization of DNA content cell nuclei are marked with a specific fluorescent stain. DAPI based reagent protocols like *CyStain UV*, *CyStain Ploidy* or *CyStain precise* label the double-stranded DNA at A-T base-pairs. The DNA fluorochrome DAPI is optimally excited by the UV-LED at 365 nm and blue and green fluorescence is emitted (11-01-1002/1004).

Propidium iodide, a DNA fluorochrome, which is used in Sysmex Partec *CyStain absolute P* and *T* reagent kits, intercalates into the double-stranded DNA and is excited by green light (Nd-YAG laser). Propidium iodide emits orange and red

fluorescence. In the **CyFlow® Ploidy Analyser** the relevant fluorescence signals are collected in the DNA-DAPI channel and the DNA-PI channel, respectively. Next to the fluorescence scattered light is detected in the SSC channel only of **CyFlow® Ploidy Analysers** equipped with a Nd-YAG solid state laser.

A list of dedicated Sysmex Partec reagent kits is contained in the *Technical Specification* section of the Appendix of this manual.

2.5 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 2 parameters is equipped with 2 optical detectors.

... 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 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.

... background in a histogram?

Histograms sometimes show undesired signals in the lower channels, frequently called 'noise' or 'background'. These signals may originate from cell or nuclei 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.

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.

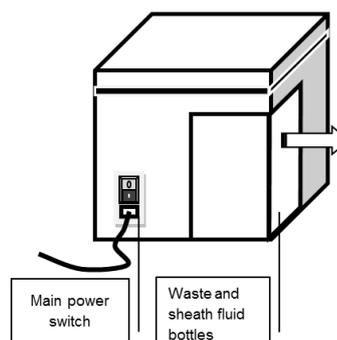
In order to guarantee highest quality of the measurements we highly recommend use Sysmex Partec Sheath Fluid (order no. 04-4007).

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.

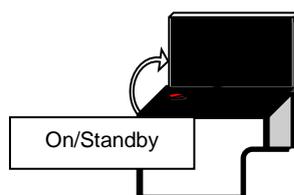
The waste bottle must be emptied after and before each user session. When using bio-hazardous samples, a volume of 50 ml of hypochlorite 0.5% (Order No 04-4012) should be introduced into the empty waste bottle for initial disinfection.



3.2 Switching on the CyFlow® Ploidy Analyser

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

The image shows two screenshots of the user administration interface. The left screenshot shows the 'PARTEC' user level with 'Main user' and 'Administrator' options. The right screenshot shows the 'USER' user level with 'Main user' checked and 'Administrator' unchecked.

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.

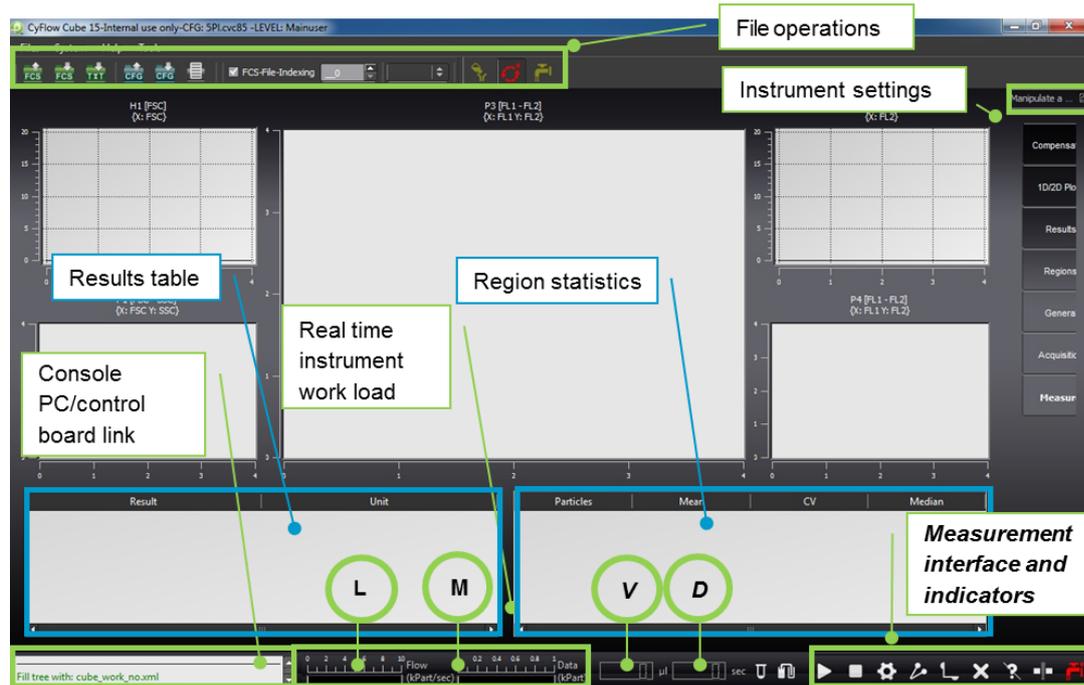
The default user account (Name: USER; Password: Cube1) can be deleted when logged in as Main User. Please make sure at least one Main User remains in the user list in order to guarantee complete functionality of the software.

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

Compensation	→ Data Compensation
1D/2D Plots	→ Properties of plots and histograms
Results	→ Calculation with counts of individual regions
Regions	→ Properties of regions
General	→ Properties of instrument
Acquisition	→ Instrument control
Measure	→ Definition of measurement mode

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: 12PI → 6 histograms + 6 dot plots

Plots are named **H1 – Hx** for histograms and **P1 – Px** for dot plots. 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** scale 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 dot plot **(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:

$$\frac{\text{NumReg1 (+ - x /) NumReg2}}{\text{DenomReg1 (+ - x /) DenomReg2}} \times \text{Scale}$$

- **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



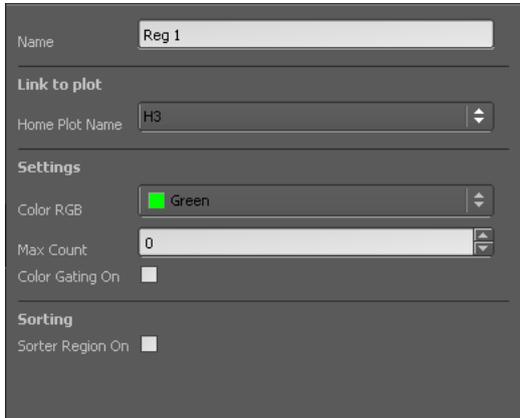
Create a result by pressing “**New**” or delete a result by pressing “**Delete**”

Confirm all modifications by pressing **Accept**

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

	Particles	Mean	CV	Median		Result	Unit
Reg 1	2788 (21%)	X:924.50 Y:448.34	X:14.53 Y:22.35	X:918 Y:445	Result I	55760	/ml
Reg 2	986 (35%)	3322.65	18.81	3340	Result II	35.3659	percent

4.1.3 REGIONS



Name: Reg 1

Link to plot: H3

Settings

Color RGB: Green

Max Count: 0

Color Gating On:

Sorting

Sorter Region On:

- **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
- **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
- **$\mu\text{lPerSec/mBar}$** defines a sheath fluid flow parameter
- **SW-Version** specifies current software version
- **Rescale LSB** shows area of automatic scaling
- **Storemode** defines FCS file format

The screenshot displays the configuration interface for the instrument. It is organized into several sections:

- Config Script:** FCSDummyCFG.XML
- Device:**
 - Device Serial Number: 130607134
 - Device Operating Time: 11798 min
 - Device Measure Number: 128
 - Device Version: 3
 - Device Modification: 2
 - FPGA Version: 2
- Settings:**
 - Sorter On:
 - Lowpass On:
 - Dilution: 1.00
 - Clinic: [Empty field]
 - Customer: [Empty field]
 - Volume: 200.00
 - $\mu\text{lPerSec/mBar}$: 0.75
 - SW-Version: 1.5.6.0
 - Rescale LSB: 200-65000
 - Storemode: FCS3

4.1.5 ACQUISITION – instrument control

- Select the trigger parameter
Trigger Parameter should be always “DNA”
- **GAINS** of the individual optical parameters
(0 volt - 999 volts) defines PMT signal amplification
- **Thresholds** defines the trigger signals cut-off level
(should be at least 0.1)
- **Flow** defines the speed of sample injection in $\mu\text{l/s}$ (for regular analysis between 0.2 and 4 $\mu\text{l/s}$)
- **Lights on** switches light sources on / off

The screenshot displays the acquisition control interface. It features a sidebar on the left with the following sections:

- Compensation
- 1D/2D Plots
- Results
- Regions
- General
- Acquisition
- Measure

The main area shows the following controls and data:

- Manipulate the running process here:** Includes checkboxes for SSC and DNA, with DNA selected.
- Gains:** A horizontal scale from 0 to 1,000. Current values are 236.0 V for SSC and 418.0 V for DNA.
- Thresholds:** A horizontal scale from 0.001 to 1. Current values are 0.06006 V for SSC and 0.02186 V for DNA.
- Flow:** A horizontal scale from 0.0 to 20 $\mu\text{l/sec}$.
- Lights:** A row of five light indicators. The first is labeled "Lights On 532 nm" and is currently on.

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

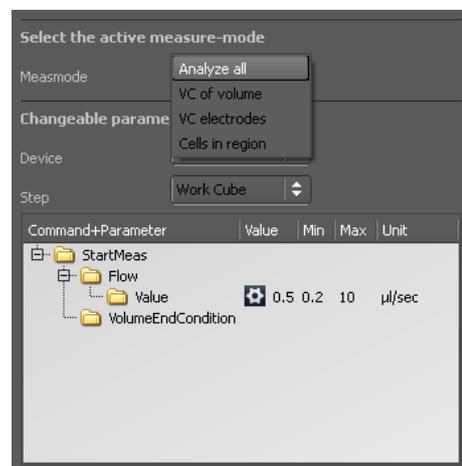
a volume can be pre-selected

Volumetric counting (VC) with electrodes:

START and STOP electrodes of the sample port were are used to define a fixed sample volume of 200µl

Cells in region

a particle number can be pre-selected

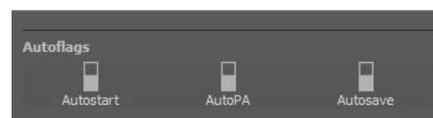


Speed values and **Volume values** can be edited and will be stored upon saving a configuration file.

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:

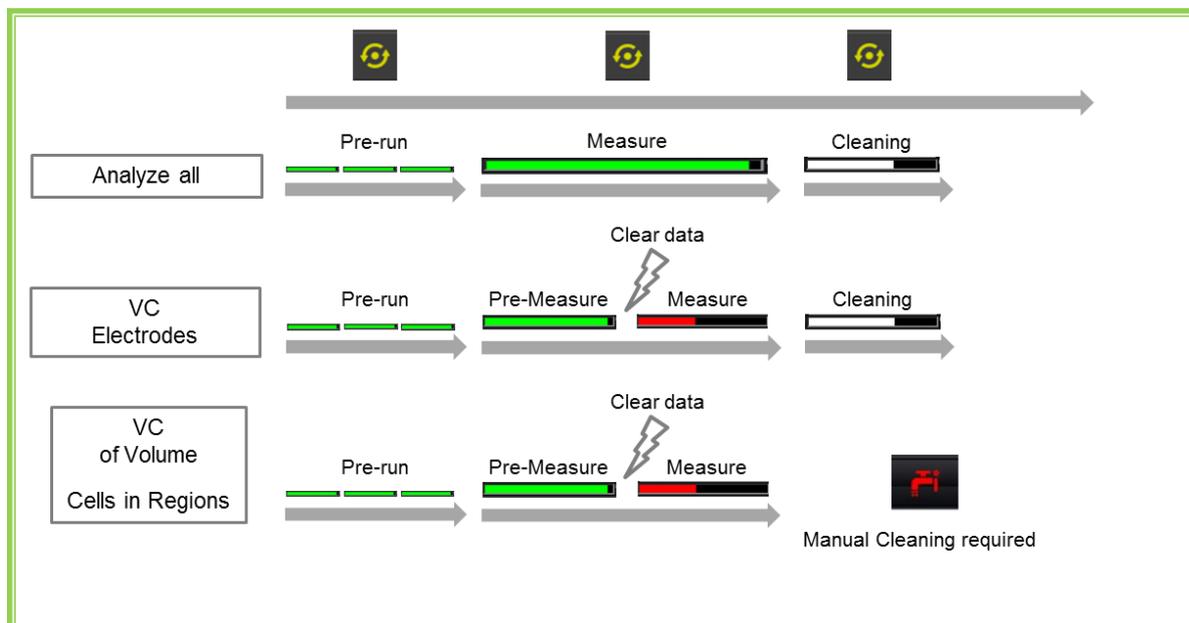
With each new sample tube the next measurement will be started until the STOP button is pressed.

AutoPA:

Automatic peak analysis, at the end of the measurement → only if an automatic Peak Analysis is pre-selected

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)

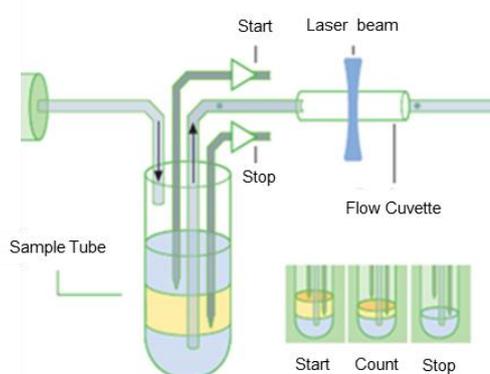
This default mode will allow you to run your sample until its levels reached the stop electrode.

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

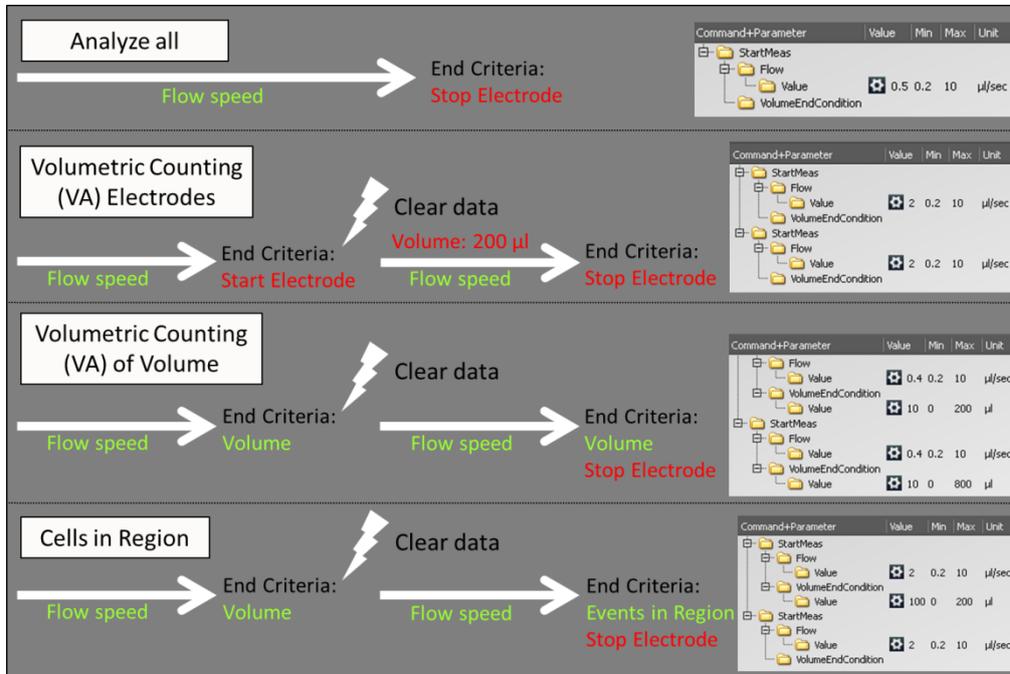
This measuring mode uses the START and STOP electrodes of the sample port to define a fixed sample volume. In a standard sample port this “counting volume” is 200µl. In a pre-counting phase the sample is acquired normally as in **continuous acquisition mode**. Reaching the START electrode the data are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

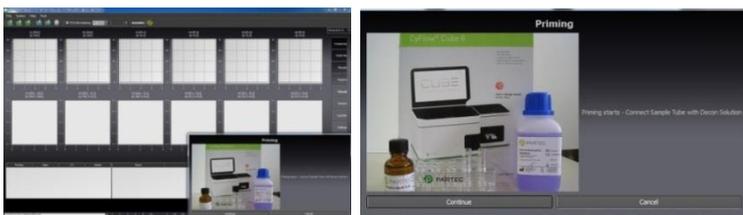
- Open a configuration file and select the initialization modus by clicking on the initialization batch symbol (Sysmex Partec Master Cfg-file: Prime mode is pre-set).

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

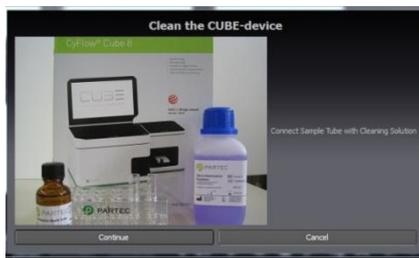


Choose the work batch for measure and acquire of data.

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:

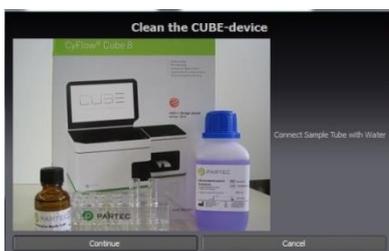
- Select the “CLEAN” batch by clicking on the “CLEAN” batch symbol 
- After pressing Start  the system will start the priming process automatically and guide through the program.
- Connect a sample tube with Cleaning Solution (green solution, Order No. 04-4009) and press “Continue”



- 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”



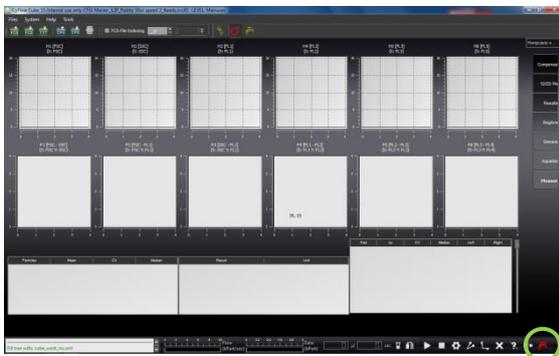
- After finishing the cleaning process CyView™ will be shut down automatically. Laser sources will also be switched off.
- Switch off the PC
- Close Windows (Start – Exit- Switch Computer Off)
- To power off completely, use the main power switch at the back panel

5.4 Intermediate cleaning process

The Ploidy Analyser 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 procedure 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 Instrument setting properties

The **GAIN (G)** and **THRESHOLD (T)** sliders must be adjusted to obtain the optimal signal amplification at minimum background. Typically, the major DNA signals of the sample will be adjusted to channel 200 on a scale of 1024 channels (e.g. a diploid sample 2n is on channel 200, a 4n sample can be expected to give a peak at channel 400).

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.

6.1.1 Threshold settings (T)

The threshold allows cutting off background (fragmented nuclei or DNA segments) 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.

6.1.2 Flow speed (S)

This slider allows 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



6.1.3 Light source (L)

This option allows to switch on/off the light sources. UV-LED which excites DAPI or 532nm laser which excites Propidium Iodide. Light source configuration of the system according to the model

6.2 1D/2D Plot properties

6.2.3 Plot name

A default plot name (Hx, Px) 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.

6.2.4 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.

The screenshot shows a configuration panel for a plot. It includes the following fields and controls:

- Name:** A text input field containing 'H1'.
- Comment:** A text input field containing 'FSC'.
- X Channel:** A dropdown menu showing 'FSC'.
- X Log On:** A checked checkbox.
- Y Channel:** A dropdown menu (partially obscured).
- Scaling:** Three buttons: '▲ Alt+', 'Switch autoscale Off', and '▼ Alt-'.
- Settings:** A section containing:
 - BitRange:** A dropdown menu showing '10'.
 - CR-Mode:** A dropdown menu showing 'All events'.

6.2.5 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.

6.2.6 X/Y Channel

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

6.2.7 BitRange

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

6.2.8 CR-Mode

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

- All events
- Region only
- Color gating

6.3 Region/ROI properties

6.3.3 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.

6.3.4 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 nx, successive region: region nx+1

Compensation	Name	DNA Control
1D/2D Plots	Link to plot	
Results	Home Plot Name	H1
Regions	Settings	
General	Color RGB	Black
	Max Count	0
	Color Gating On	<input type="checkbox"/>

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

This allows choosing 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

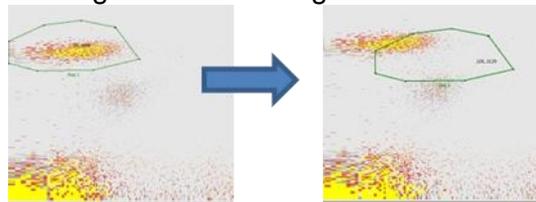
To set the ROI/region as a sorting gate, just by selecting the box.

(This option is not applicable on the Ploidy Analyser).

6.3.5 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.



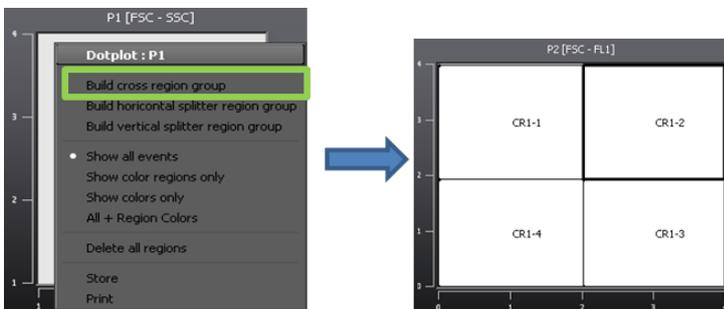
6.3.6 Create a vertical or horizontal histogram splitter

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.



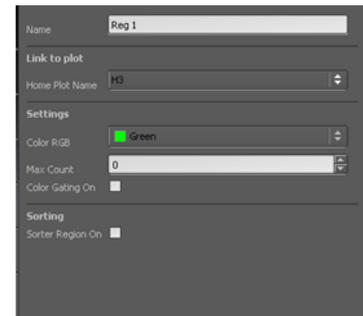
6.3.7 Create a quadrant assembly

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.



6.3.8 Change layout for regions

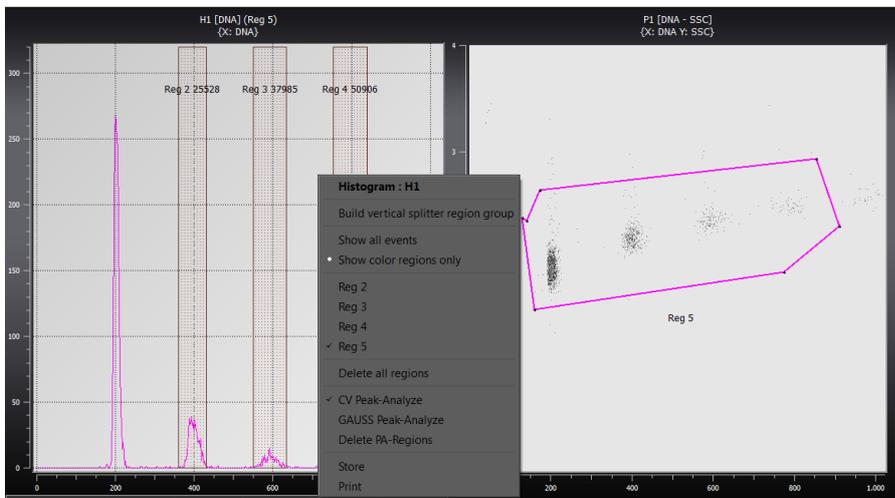
In the register chart file select “Regions”. Individual regions can be selected with the <> arrows. Regions can be transferred to other plots by the function “Home plot name” or modified in their color with “Color RGB”. To activate changes press “Accept”.



6.3.9 Applying regions to other plots → Gating Function

Clicking into the 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 region color and black
- Show color regions only → shows the events in the selected region(s) in region color



6.4 Compensation

The function of compensation is not applicable to the use of the instrument for DNA analysis.

7 A 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-script **DNA-DAPI** for analysis of DAPI-stained samples or **DNA-PI** for analysis of Propidium Iodide stained samples or any customized own configuration file. Saved instrument settings will be loaded. In case you want to continue with the same instrument set-up as before you can skip this action as the instrument will automatically load the last used system configuration.



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™ Start Pre-run
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 “Spacebar” 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

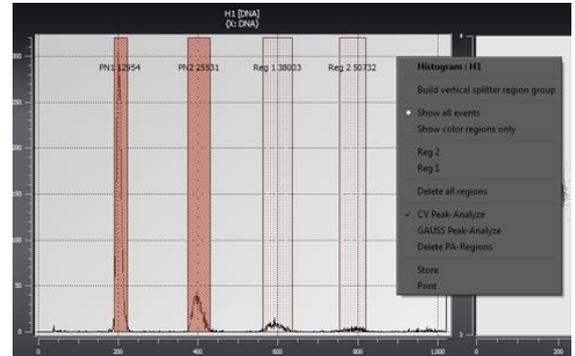


Besides the **Analyze all** measure mode other measure modes can be selected:

- 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 Automatic Peak Analysis

CyView™ offers two pre-defined algorithms to automatically find signal peaks in 1-parameter histograms and calculate relevant statistical data (automatic peak analysis). According to the log-in user level the automatic peak analysis function runs in a routine mode (MAIN USER/USER) or an expert mode (SERVICE/ADMINISTRATOR).



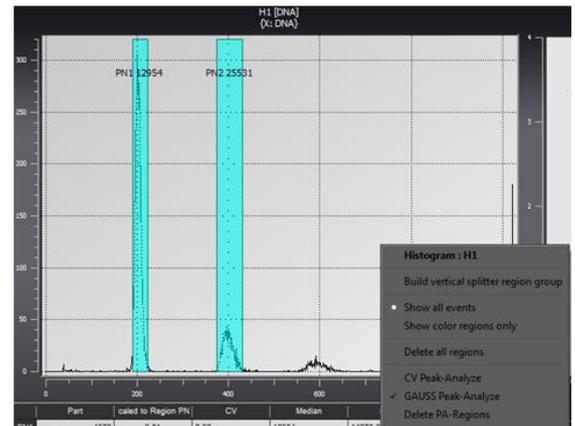
CV Peak-Analyze

8.1 MAIN USER/USER Mode

To activate a Peak Analysis use right mouse click on the 1-parameter histogram to open the histograms context menu. Peak Analysis options **CV Peak-Analyze** and **GAUSS Peak-Analyze** can be selected by a right mouse click and are labelled by tick.

CV Peak-Analyze will run an algorithm for peak recognition and label the recognized peak area in red.

GAUSS Peak-Analyze will run an algorithm for a Gaussian data fit, peak recognition and label the recognized peak area in blue.

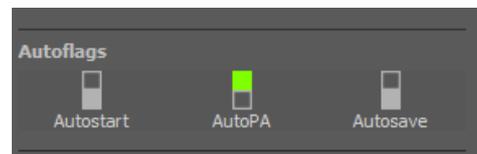


GAUSS Peak-Analyze

Detected peak areas will be labelled PN1, PN2, PN3... . Additional regions can be added manually to a 1-parameter histogram (Reg1, Reg2, RegX ...).and will be displayed light red and blue, respectively.

Delete PA-Regions deletes all regions.

With an active Autoflag on **AutoPA** (register card **Measure**) the selected peak analysis mode (**CV Peak-Analyze** / **GAUSS Peak-Analyze**) will be automatically triggered after a measurement is started.



In the **Peak Analysis Result Table** statistical data of automatically detected and manually selected peak areas (PNx, Regx) are displayed.

- **Part** – no. of particles in the detected peak area
- **Mean / DI (Reg x)/ VI (xxxxx)** – arithmetic mean of signals / DNA index scaled to Region X / DNA index scaled to channel xxxxx
to switch in between parameters use left mouse click on columns header to switch scale use slider right of the result table
- **CV** – coefficient of variation in % (CV%)
- **Median** – median value of signals
- **Left / Right** – left and right margin of the peak area

	Part	Mean	CV	Median	Left	Right
PN1	3196	12828.99	1.63	12833	11904	13440
PN2	629	25418.18	1.38	25403	24320	26432
Reg 1	198	37974.10	1.15	37992	0	0
Reg 2	114	50432.02	1.25	50359	0	0

	Part	DI (PN1)	CV	Median	Left	Right
PN1	3196	1.00	1.63	12833	11904	13440
PN2	629	1.98	1.38	25403	24320	26432
Reg 1	198	2.96	1.15	37992	0	0
Reg 2	114	3.93	1.25	50359	0	0

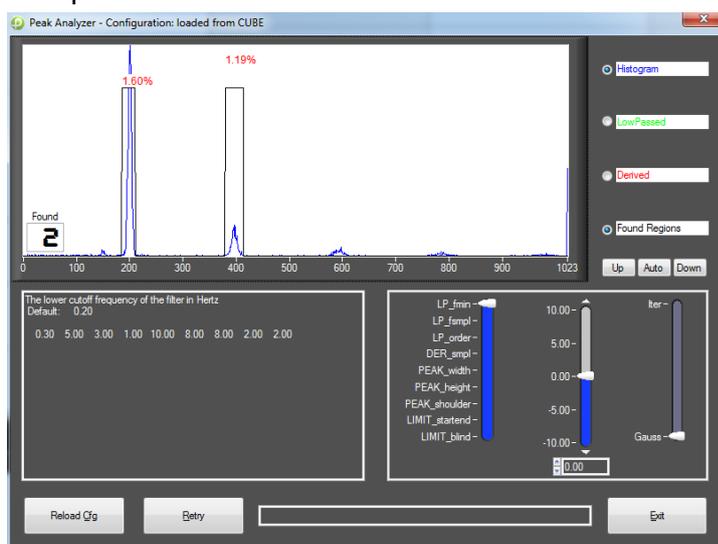
	Part	VI (25002)	CV	Median	Left	Right
PN1	3196	0.51	1.63	12833	11904	13440
PN2	629	1.02	1.38	25403	24320	26432
Reg 1	198	1.52	1.15	37992	0	0
Reg 2	114	2.02	1.25	50359	0	0

Peak Analysis Result Table

Version Mean /DI/PI

8.2 SERVICE/ADMINISTRATOR Mode

Running CyView™ in the **SERVICE/ADMINISTRATOR** mode allows adjusting essential parameters of the peak analysis algorithms. After triggering **Peak Analysis** the Peak Analysis configuration will be loaded (figure below). Here the algorithm can be manipulated.



In the upper right area display options *Histogram / LowPassed / Derived / Found Regions* can be switched on/off. In the algorithm parameter box below *Gaussian* or *Iterative* mode can be selected (right slider) as well as the following parameters can be adapted (select with left slider, modify with central slider):

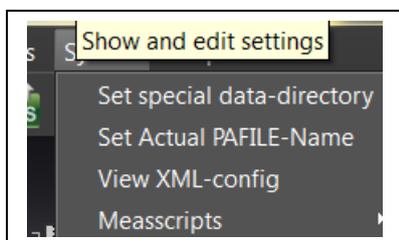
- *Data filter LP_Fmin* – Lower border frequency
- *Data filter LP_fmpl* – Filter sample frequency
- *Data filter LP_order* – order of the low pass filter (“0” = without low pass filter)
- *Derivation (optional) DER_fmpl* – Sample interval of the differentiation (“0” = without derivation)
-
- *Peak Finding PEAK_width* – no. of consecutive points for the evaluation
- *Peak Finding PEAK_height* – minimal height of a peak for the evaluation
- *Peak Finding PEAK_shoulder* – width of background shoulder not taken into evaluation
- *StartEnd Search Limit_startend* – amplitude relation in % to search start- and endpoints of the peak

- *StartEnd Search Limit_blind* – distance to peak to start peak search

Reload Cfg loads the current peak analysis back from the configuration to memory. *Exit* closes the Peak Analysis configuration and continues to apply the modified peak analysis parameters. Saving a configuration script  will store the modified peak analysis parameters so that the modified algorithm can be recalled by loading the same configuration script  again.

8.3 Export of Peak Analysis and 1-parameter histograms

CyView™ exports Peak Analysis data and histograms as well as additional information on the data file to txt-formatted tables. The default name of the export file (PAFILE) is *pa.txt*.



Export file name and file location can be set in the **System** menu under **Set Actual PAFILE-Name** and **Set special data-directory**.

Peak Analysis data will be added to the export file until a new export file is defined. Saving the FCS data file is triggering the export of the current PA-data set. If a data file is not saved, no PA-data set will be added to the current export file. If a data file is saved repeatedly a new PA-data set will be added to the export file each time.

The export file can be formatted in 3 versions. The export file version is defined by a command line argument in the program fetch of CyView™. Version 1 is default, version 2 and 3 require

-p <exportversion of pa> as command line argument. In case of problems to activate version 2 or 3 please contact your local support.

Version 1 (default):

This version exports the 1-parameter histogram as a png file to the current data directory and adds the following data set to the export file:

- A header with file reference to the correlated data and png file
- A metadata section with information on the peak analysis parameters
- A regions section with the results of the peak analysis

Beispiel										
FCS-FILE C:/Cube_15/data/MS/15.fcs										
PLOT_FILE15_PAI_H1_13_12_02_12_43_34.PNG										
METADATA										
Timestamp 10.04.201313_28_18										
LP_fmin	LP_fsmpl	LP_order	DER_smpl	PEAK_width	PEAK_height	PEAK_shoulder	LIMIT_startend	LIMIT_blind		
0.1	5	4	1	10	2	8	5	0.3		
PA-Nick PAI										
Histogram H3										
REGIONS										
ix	Name	Part	CV%	Median	Mean	Left	Right			
1PN1	84182.749	95089525.46	934410944							
2PN2	378127.714	1278212789.41254414208								
3PN3	121192.407	2485024848.42393626304								
4PN4	76	21.871	3675836613.23449638336							

Version 2:

- A metadata section with information on the peak analysis parameters
- A histogram section with the histogram as table including indices for the detected peaks
- A regions section with the results of the peak analysis

Beispiel										
METADATA										
Timestamp 10.04.2013 13_28_18										
LP_fmin	LP_fsmp1	LP_order	DER_smp1	PEAK_width	PEAK_height	PEAK_shoulder	LIMIT_start	LIMIT_end	LIMIT_blind	
0.1	5	4	1	10	2	8	5	0.3		
PA-Nick	PAI									
Histogram H3										
143	1.48									
144	5.19									
145	8.15									
146	10.37									
147	13.33	1								
148	14.07	1								
149	13.33	1								
REGIONS										
ix	Name	Part	CV%	Median	Mean	Left	Right			
1PN1	84182.749	95089525.46	934410944							
2PN2	378127.714	1278212789.41254414208								
3PN3	121192.407	2485024848.42393626304								
4PN4	76	21.871	3675836613.23449638336							

Version 3:

Exporting png files as well as histogram tables.

9 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
Region	click and hold left mouse	Moves a region in standard steps
	Ctrl+ click and hold left mouse	Moves a region in small steps
	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

10 Appendix

10.1 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

10.2 Maintenance



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

Clean the CyFlow® Ploidy Analyser casing on a regular base carefully with soft cloth. Water should not enter the CyFlow® Ploidy Analyser 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® Ploidy Analyser 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.

10.2.3 Service

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

For further information, please consult our web site:

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

And much more ...

www.sysmex-partec.com

10.2.4 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

10.2.5 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.

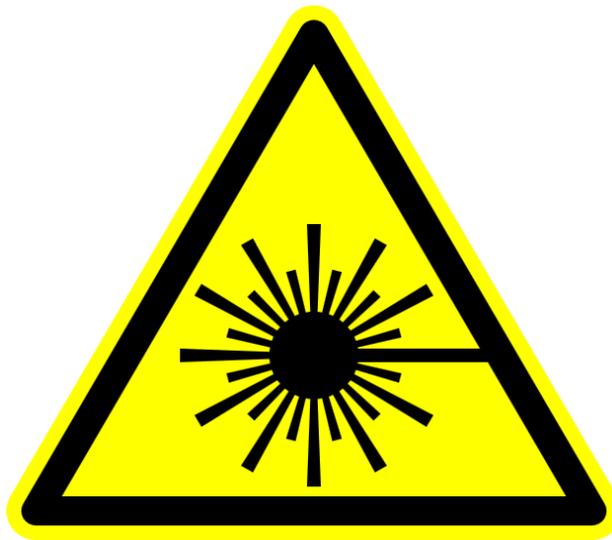
10.3 Laser Safety



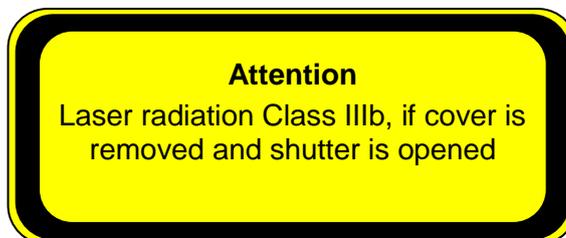
Warning: It is prohibited to open the instrument as it is equipped with a class 3b laser unit.

The CyFlow® Ploidy Analyser order code 11-01-1003 and 11-01-1004 are class I laser products 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

10.4 Technical Specifications

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

10.4.3 Optical Standard Setup

The Parameters

SSC: Side Scatter SSC (11-01-1003/11-01-1004)

DNA: PI fluorescence (11-01-1003/4), **DAPI** fluorescence (11-01-1002)

10.4.4 CyFlow® Ploidy Analyser System

Size	Dimensions: 385 mm L x 282 mm D x 287 mm H (530 mm with open display)
Weight	appr. 18 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	DNA Genome Size Analysis, Ploidy Analysis
True Volumetric	Based on precise counting and mechanical fluid volume measurement
Absolute Counting	No need for reference sample or beads
Instrument Check	Calibration Beads UV (Sysmex Partec No. 05-4022) Calibration Beads green (Sysmex Partec No. 05-4006) DNA Control UV (Sysmex Partec No. 05-7302) DNA Control PI (Sysmex Partec No. 05-7303)
Instrument Cleaning	Cleaning Solution (Sysmex Partec No. 04-4009) Decontamination Solution (Sysmex Partec No. 04-4010) Hypochlorite 0.5% (Sysmex Partec No. 04-4012)

Staining reagents	CyStain UV Ploidy (Sysmex Partec No. 05-5001) CyStain UV Precise P (Sysmex Partec No. 05-5002) CyStain UV Precise T (Sysmex Partec No. 05-5003) CyStain PI Absolute P (Sysmex Partec No. 05-5022) CyStain PI Absolute T (Sysmex Partec No. 05-5023) CyStain DNA 1step (Sysmex Partec No. 05-5004) CyStain DNA 2step (Sysmex Partec No. 05-5005)
Set-up Time	Max. 5 minutes
Parameters	11-01-1002 1 optical parameters DAPI 11-01-1003 2 optical parameters: SSC, PI 11-01-1004 2 optical parameters: SSC, PI/DAPI
Particle Size Range	0.1 µm – 50 µm (standard cuvette)
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)
Service	1-3 year service contracts
Warranty	12 months on all parts except filters, mirrors, other quartz or glass parts, disposables and cuvettes

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

10.4.5 CyFlow® Ploidy Analyser Optics

Laser / Output	Green NdYAG: 30 mW to 100 mW (option) at 532 nm Ultra-Violet Diode (UV-LED): 3 W at 365 nm
Detectors	1 to 2 (SSC, 1 FL)
Filters	Standard setup and filters for all for DAPI, Propidium Iodide and side scatter analysis
Excitation Optics	Elliptical 15 µm x 100 µm at 532 nm
Excitation Optics	Elliptical 15 µm x 100 µm at 488 nm Other beam geometries upon request

10.4.6 CyFlow® Ploidy Analyser Fluidics

Completely closed system for sheath water and sample volumes; no fluid droplets or aerosols generated nor released from the instrument.

