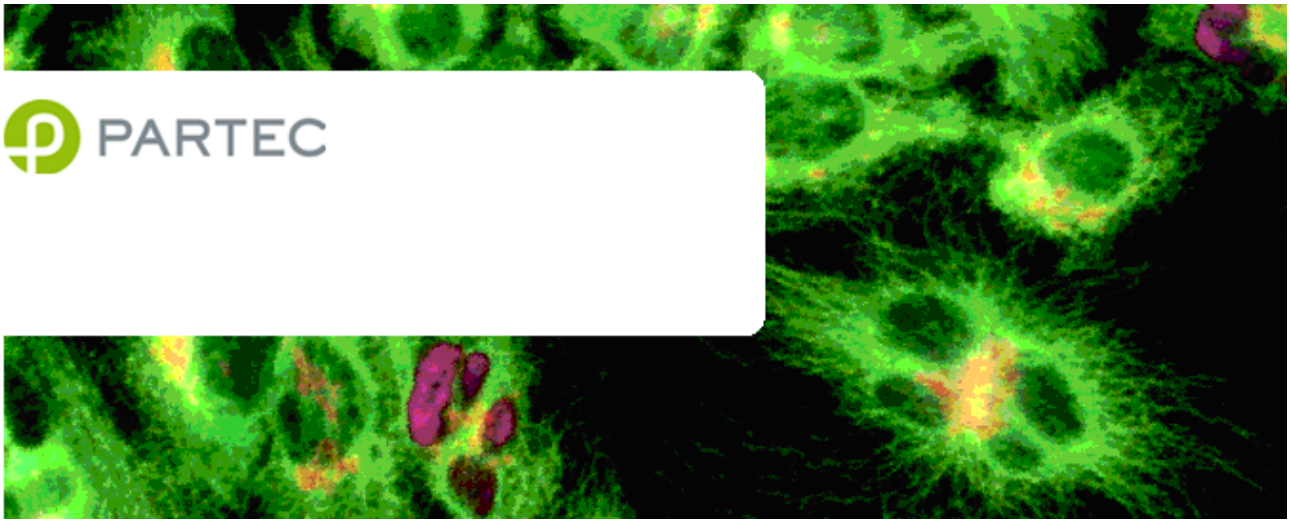


PARTEC

CyFlow® space

Instrument Operating Manual

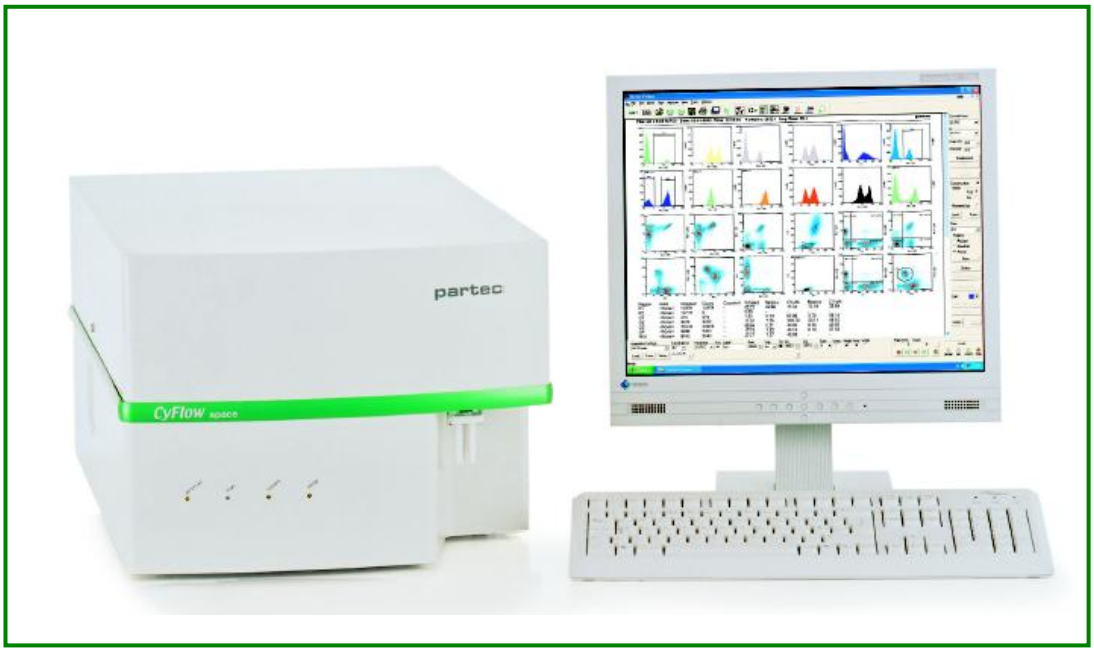


Table of contents

IVD

For in vitro diagnostic use with Partec recommended IVD reagents.

The Partec CyFlow® space flow cytometer complies with the European IVD Directive 98/79/EC and is therefore marked with the CE sign.

Otto-Hahn-Str. 32
D-48161 Münster
Germany

CE

Introduction to CyFlow® space

What...

… is the Partec CyFlow® space?

The Partec CyFlow® space is a fully equipped desktop Flow Cytometer (FCM). It features a modular optical concept which allows using up to three different laser light sources and between one and nine optical channels (parameters). The CyFlow® space allows easy adaption of the optics to any application by simple exchange of optical filters and mirrors.
The CyFlow® space runs with a standard PC or notebook. Data acquisition, instrument control, and data analysis are controlled and performed by the FloMax® software.

… are the applications for which the CyFlow® space can be used?

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

- Routine and research Immunophenotyping, Blood Cell Analysis, HIV monitoring
- Leukocyte Counting / Rare Event Analysis
- Microorganism Analysis
- Fermentation Control
- Particle Concentration Analysis with the feature «True Volumetric Absolute Counting»
- Particle Size and Fluorescence Distribution Analysis

… topics are covered by this manual?

The CyFlow® space Instrument Operating Manual covers the basic operation and maintenance of the CyFlow® space instrument. This manual does not cover details related to the software. Different software operating manuals are available.

… other manuals are available?

FloMax® – Acquisition and Instrument Control covers instrument control and multiparametric data acquisition.

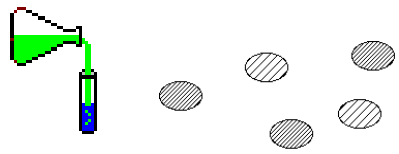
FloMax® – Data Analysis covers all aspects of on- and offline data analysis.

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

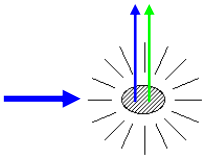
... should I know before operating the CyFlow® space?

This manual assumes you have basic knowledge about 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)

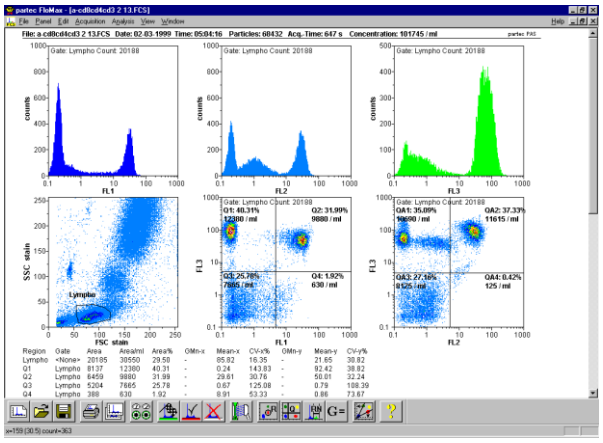
Typical Steps of Particle Analysis



1. Cells are separated in a suspension and stained with fluorescent markers.



2. Light from the laser(s) excites cell –bound fluorophores and is scattered by the cells.



3. Fluorescence signals are displayed and analysed in histogram- and/or dotplot-diagrams.

1. Preparation and Staining

For flow cytometric analysis, the cells (or other particles) must be separated from each other in an aqueous suspension. Typically, if not generating sufficient optical signals by themselves, the cells are then labelled by staining with one or more fluorescent dyes. The fluorescent molecules bind to the cell substance of interest, e.g. CD4- and CD45-antibodies bind cell surface antigens of leukocytes. The quantity of labelling cells with a specific dye is proportional to the number of antigen molecules of a single cell. A good preparation with suited reagents is the requirement for a precise analysis. A check of staining in a fluorescence microscope can un-reveal preparation problems.

2. Flow Cytometry Analysis

While passing through a flow cuvette one-by-one, the cells are individually illuminated by the light spot of the laser. Due to the excitation, the dye molecules emit fluorescence of characteristic colour (emission wavelength spectrum). This fluorescence light is separated into colour ranges by means of optical filters. The intensity of each colour range is analysed for each single cell.
Besides fluorescence, the intensity of light scattered by each cell can be measured. Scatter light is measured in forward direction from the light source (forward scatter, FSC) and sideward direction (side scatter, SSC). The scatter intensity is a measure of cell size and morphology. Scatter light can be used to identify a cell before analysing its fluorescence, but a cell can also be identified by fluorescence before analysing its scatter properties.

3. Realtime Data Processing and Results

The light intensity of each parameter, and with it the quantity of substance of interest, is assigned to one of up to 2^{16} or 65536 quantity classes (channels). The classification is performed in realtime, while the cells are passing the flow cuvette. Single parameter histograms show the number of cells within the channels. Two parameter dotplots show the correlation between two cell properties.

4. Absolute Cell Counting

Since the CyFlow® space analyses all cells passing through the flow cell while precisely monitoring the fluid volume of the sample, it allows volumetric counting during the analysis, i.e. the determination of concentration of any cell subpopulation. Cell subpopulations can even be defined and their concentration analysed at a later date, after reloading data from a file.

Operating Basics

Switching on the CyFlow® space

Notes

As sheath fluid you may use optimized Partec Sheath Fluid or prepare it yourself.
To prepare sheath fluid, fill destilled water into a glass bottle or an Erlenmeyer flask (refer to Application Note: "Sheath fluid pre-paration"), through a small size filter mesh (< 0.2 µm). Degas the water by connecting it to vacuum (e.g. a water jet pump). Place the bottle onto a heated stirrer for about 15 minutes:

Remember: The smaller the particles in your analysis are, the more critical clean sheath fluid becomes!

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

For information how to work with Windows® please consult the manual "Introducing Microsoft Windows" or books available in your local book shop.

1. Check SHEATH and WASTE bottle

Make sure **SHEATH bottle is filled** with no more than 1600 ml of Sheath Fluid and is closed tightly with the screw top. **Tilt sheath bottle in order to release air bubbles trapped in the yellow in-line filter!**
Make sure the **WASTE bottle is empty**.

2. Switch on the instrument

The CyFlow® space Flow Cytometer is operated with 100 - 240 V AC.
Switch on **main power** at the left side of the instrument. The 488nm laser has its own power button closeby, please also switch on the 488 nm laser.
All other lasers are software controlled (for details please refer to next page)

3. Control of the 488nm laser output power (for 200 mW high power laser only)

The 200 mW high power laser can be adjusted in its operation power between 50 and 200 mW. For power selection please doubleclick the icon shown on the left (488-200)

To change selected laser power please type in:
p=value →enter

To inquire about actual selected laser power type in:
?p →enter

3. Switch on peripheral devices

Switch on **printer**.

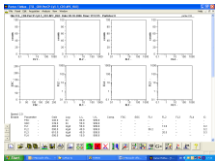
4. Switch on computer

Finally, switch on computer by **Computer Power switch**. After some ten seconds, the display shows the **Windows® desktop**.

5. Start instrument operating software

Move mouse pointer (arrow on display) onto FloMax® or other instrument control **program icon**.

Doubleclick on the icon with left mouse button.
- after a few seconds, the display shows the **FloMax® Welcome window**. By confirming with "OK" the CyFlow® space is initialized and the last used instrument settings are loaded.



The display shows empty histograms.

6. Cleaning

Use speed 4. Run 1.6 ml Partec *Cleaning Solution* until sample tube is empty. Activate CLEAN. Run a second tube with Partec *Sheath Fluid* for 2 minutes.

Now, the **CyFlow® space is ready for acquisition**.

DANGER
Warning: Biohazards!
The waste may contain biohazardeous and cancerogenic material from the samples (infectious cells, dyes). To minimize biohazards, fill about 25 ml of Partec *Hypochlorite Solution* into the waste container. When emptying the container, be sure not to come in contact with the fluid. In case of accidental contact, wash your skin throughoutly with soap and disinfectant.

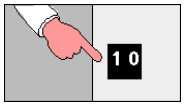
Multi laser Measurements

Notes

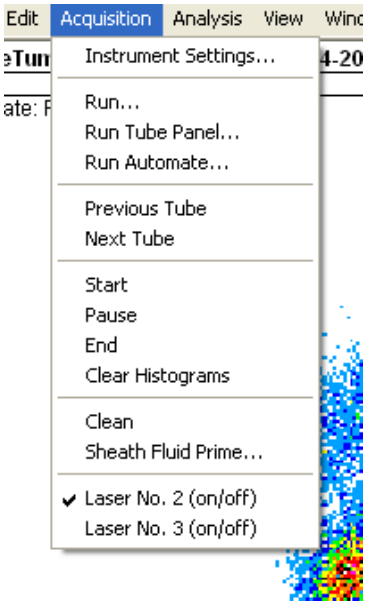
Alignment of the lasers should only be done by Partec representatives or by your local distributor.

The CyFlow® space Flow Cytometer can be equipped with up to 3 laser light sources according to individual customer demands. Actual setup of your instrument may therefore vary. For questions concerning the laser setup of this instrument please contact Partec or your local distributor.

In order to minimize crosstalk between multiple light sources, laser beams from different lasers are focussed on different positions (spots) of the flow cuvette. The CyFlow® space Flow Cytometer supports either 2 laser spots (when employing FloMax version 2.6 or 2.7) or 3 laser spots (when employing FloMax version 3.0). The blue 488 nm laser which is the leading laser of the CyFlow® space is always located at spot 1. Additional lasers if present are located on spot 2 and spot 3.



While the blue 488 nm laser is switched on with the button on the left side of the instrument all other lasers are controlled by software:



Select “Acquisition” from the upper command line and click on Laser No. 2 and / or Laser No. 3 as required. The tick indicates that a laser is switched on. Laser no. 2 would be a red laser (if present), laser no. 3 would be a UV or violet laser (if present).

Laser status will automatically be saved upon creation of Instrument settings files (please refer to the manual **FloMax® – Acquisition and Instrument Control**).

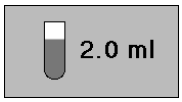
In the standard configuration all particles pass through laser spot no. 1 of the 488nm laser – the leading laser – first and then through the laser(s) at spot no. 2 and no. 3 (if present).

Therefore, all parameters derived from a laser located at a spot other than no. 1 require a time delay. Please refer to Parameter Setup Dialog Box for details (Fig. 5, page 13).

Starting a Measurement

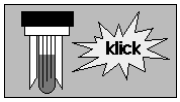
Notes

See FloMax® - Acquisition and Instrument control manual on how to change instrument settings.



1. Make sure your flow cytometer is ready for analysis and the operating software is prepared for the measurement.

2. Prepare sample according to the Application Notes and preparation procedures, resp. Use not less than 830 µl for true volumetric absolute counting and not more than 2.8 ml. For smaller amounts of sample use Partec “Small Volume Sample Tubes” (Product no. 04-2010, however these tubes are not compatible with the True Volumetric Absolute Counting option, page 10).



3. Insert sample tube onto the sample port until you recognize a "click". The sample should be fully mounted within a second.

Now the measurement (acquisition) starts automatically – the operating software indicates the **Prerun**, **Stabilize**, **Run** and **Count** status. Instrument status is also highlighted by LEDs on the front side of the CyFlow® space.

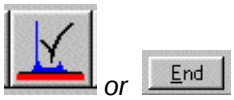
- In the **Prerun** phase, cells are quickly transported to the position of analysis, the flow cuvette.

- During the **Stabilize** phase, the system waits a given time for the flow to reach slow speed required for analysis.

- In the **Run** phase, cells are analysed and classified into histograms on the display in realtime.

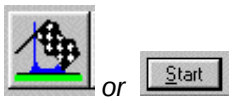
- In the **Count** phase, cells are counted for a given volume. Do not change instrument settings in the count phase.

When removing a tube from the sample port, the BioSafety system is activated to prevent dropping and contamination by hazardeous material. Simultaneously, a cleaning cycle is performed to avoid cross-contamination between individual samples.



After the count phase, the acquisition finishes automatically. To finish the acquisition before this manually, click the end icon or end button, or simply remove the sample tube from the sample port.

- The BioSafety cycle is started.
- Status changes to **Clean** and then **Ready**.



To restart an acquisition without removing the sample tube, click onto start.

4. To save results, click on the disk icon.

Switching off the CyFlow® space

Make sure data from the last acquisition have been saved.

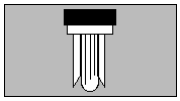
Note

Cleaning
Final cleaning with sheath fluid is a must to avoid clogging of the flow cuvette.


or

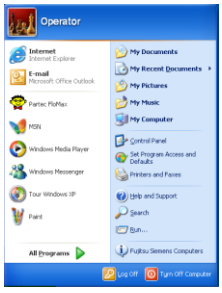

1. Clean the flow cuvette and connecting tubes
Use speed 15.
a) Daily claning procedure:
Run 1.6 ml of Partec *Cleaning Solution* (Product no. 04-4009) until sample tube is empty. Run 1.6 ml of Partec *Decontamination Solution* (Product no. 04-4010) for one minute. Press CLEAN button once to start manually a cleaning cycle. Run 1.6 ml Partec *Sheath Fluid* (Product no. 04-4007) for two minutes. Press STOP.

b) Weekly cleaning procedure:
Run 1.6 ml of Partec *Decontamination Solution*. Pinch the sheath fluid tube for 5 seconds. Stop the instrument while keeping the tube pinched. Incubate for 15 minutes. Re-start the system by pressing START and let it run to the end. Run the system with 1.6 ml Partec *Cleaning Solution* until the sample tube is empty. Run 1.6 ml Partec *Sheath Fluid* for two minutes. Press STOP.

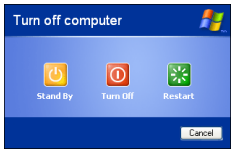


2. Protect the sample port
Leave the final sample tube connected to the sample port – this avoids drying and cristallizing of any remaining material.

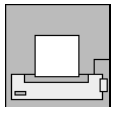
3. Quit the operating software
Click onto the close button [**X**] in the upper right corner of the window (or select File...Exit in the menu).



4. Shut down the computer
Click with the left mouse button onto the Windows® "Start" button in the taskbar - the Windows menu is displayed.
Click "Turn Off Computer..." - the shut down window appears.



Click "Turn Off" in the Shut Down Window.



5. Switch off printer

6. Switch off instrument
Switch off main power and blue laser at the left side of the instrument.

True Volumetric Absolute Counting – Overview

Analysis of concentrations of samples, loosely called Absolute Counting, can be of significant interest for medical diagnostics or monitioring the status of cells in cell cultures or biotechnological processes.

Traditional Flow Cytometric Techniques for Absolute Counting

i) Dual Platform Technique

To determine the concentration of a sample flow cytometers (FCM) are frequently used in combination with Hematology Counters.

Disadvantages:
- Two instruments required
- This technique relies on the assumption that the number of cells seen by the FCM and counted by the Hematology Counter are identical.

ii) Single Platform Technique with Reference Beads

An alternative method employes reference beads of known concentration which are added to the sample of unknown concentration.

Disadvantages:
- Depends on the accuracy of the specified beads concentration
- Constant running costs for the reference beads

Partec True Volumetric Absolute Counting

To overcome the drawbacks of the Dual- and Single-Platform Technique with reference beads, Partec intruments additionally offer an alternative way of absolute cell counting, which is based directly on the basic definition of a concentration

$$c = N / V ,$$

by precisely counting the number *N* of cells suspended in a purely mechanically defined volume *V*.

i) Precise Counting: Determination of *N*

For True Volumetric Counting the precise detection of cells is an essential. This requires fast recognition and analysis of the events by electronics and computer. All Partec instruments are specifically designed to minimize counting losses by providing direct connection between computer and electronics, which avoids dead-times involved in traditional FCM designs and instrument interfaces. This reduces the probability of a counting loss for typical event rates below 2%.

ii) Electrode-Principle: Determination of *V*

The method for True Volumetric Absolute Counting supported by the CyFlow® space is based on the precise measurement of a fixed sample volume by means of two electrodes.

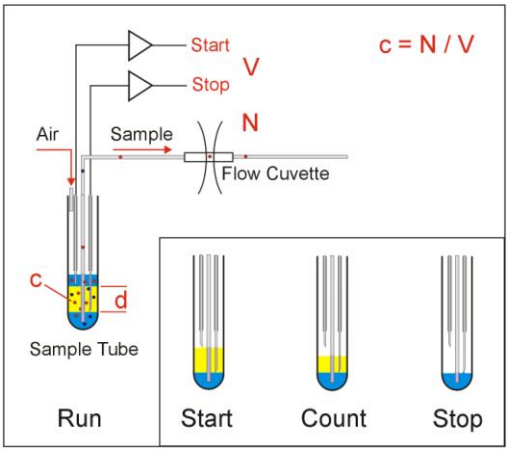


Fig. 1: True Volumetric Absolute Counting by the Electrode Principle.

During analysis by the CyFlow® space, the sample liquid loses contact first from the upper and then from the lower electrode. These events trigger a START and a STOP signal, respectively which is stored by software.
The volume *V* of sample liquid analysed between the START and STOP signal is physically defined by the distance of the two electrodes and the diameter of the sample tube. The analysed volume is exactly 200 µl (= counting volume).

Effects of the sample meniscus in the tube are eliminated because the START and STOP electrodes are arranged symmetrically. The sample conductivity does not influence the volumetric measurement, as long as the fluids can be detected electrically.

In some cases, larger or smaller counting volumes *V* are advantageous, either to improve the counting statistics (e.g. for rare events < 10/ml) or to decrease the analysis time. This can be accomplished by replacing the standard 200 µl volumetric sample port by one with a different volume (Please contact Partec or your local distributor for details).

Precision and Reproducibility
Precision and reproducibility of the Partec instruments can be demonstrated and checked with Partec *CountCheck beads* (Product no. 05-4010).

Benefits of True Volumetric Absolute Counting
Partec instruments can perform traditional Dual- and Single-Platform counting and unique True Volumetric Absolute Counting, the latter offering unique benefits:

i) Precision
A high precision of better than 5% is guaranteed by precise counting and mechanical volume measurement. The counting reproducibility is better than 2% rel. standard deviation.

ii) No Errors Due to Calibration
Unstability of beads suspensions over time or counting statistics of beads cannot influence the counting results, since no beads are used.

iii) Less Preparation or Setup Time
The sample concentration is directly analysed by the FCM without any instrument calibration or additional sample preparation step.

iv) Less Analysis Time
Concentration results for subpopulations, as defined by gates, are immediately displayed on the instrument screen. No additional analysis steps, e.g setting gates for beads, are required.

v) Less Expenses
No reference beads required.

Performing True Volumetric Absolute Counting

Sedimentation and Count Time
Cells or other particles may tend to sediment inside the sample tube depending on their density in relation to the surrounding suspension solution. Depending as well on the particle size, significant sedimentation can take place in terms of tens of seconds or many minutes. The CyFlow® space analyses the concentration at the location of the sample uptake, where it can vary over time due to sedimentation or desedimentation effects. Consequently, avoid too long count phase due to too low sample speeds. Count Time typically should not exceed 2-4 minutes.

During the count phase, user interference must be avoided!

1. Fill 830 µl of ready prepared sample into a sample tube (instrument setup should be set to: prerun 2 sec, stabilize 2 sec).

2. Shake sample for resuspension of particles, but avoid air bubbles.

3. Insert sample tube onto sample port.

- the acquisition starts and performs
  prerun
  stabilize
  run
  count.
- particles are counted during the *count* phase.
- the acquisition stops automatically and **ready** is displayed.

4. Define subpopulations by regions and gates to analyse concentrations

- the concentration is displayed for each subpopulation.

Optical Standard Setup – The Parameters

Note
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 readjustments.

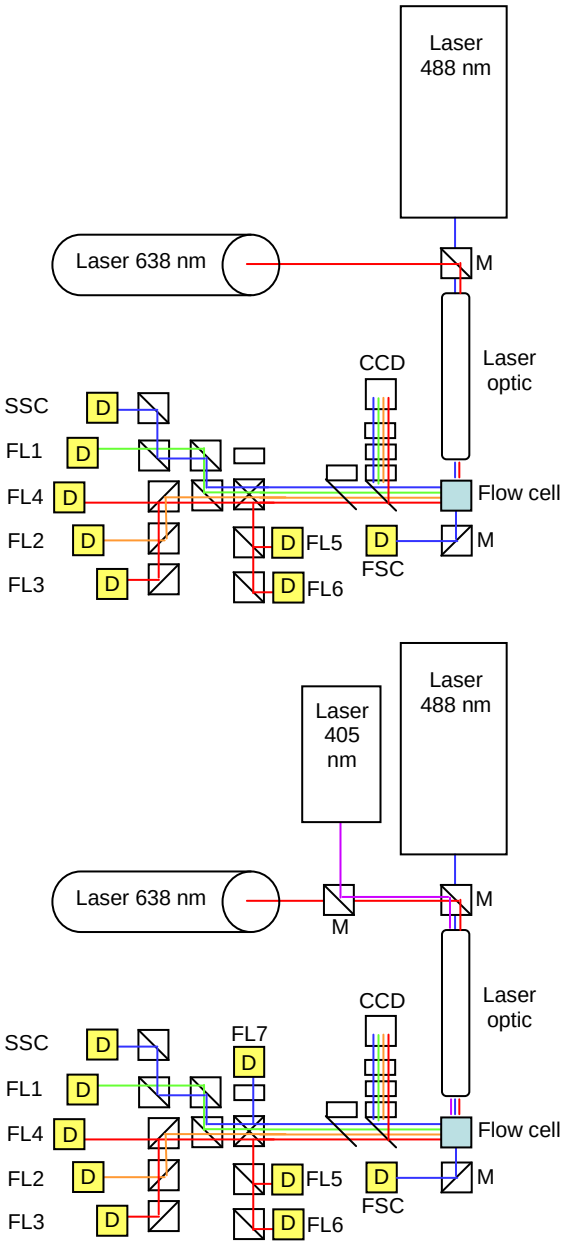


Fig. 2: Examplary demonstration of the optical bench of a CyFlow® space with 2 lasers and 8 parameters (top) and 3 lasers and 9 parameters (bottom). Other laser light sources and parameter allocations possible. D detector (photo multiplier)

The CyFlow® space flow cytometer can be equipped with various laser light sources (1, 2 or 3 lasers), different laser power intensities and up to nine optical parameters.
Due to its modular concept the optical configuration can be adopted to many different clinical and scientific purposes. The main standard configurations include:

2 Laser instrument: This instrument is equipped with a blue diode pumped solid-state Laser (20 mW) at 488 nm and a red laser diode at 638 nm (25 mW). Up to eight optical parameters can be included (colours reflect laser origin of the signal).

FSC: forward scatter
SSC: side scatter
FL1: green fluorescence **(FITC)**
FL2: orange fluorescence **(PE)**
FL3: red fluorescence I **(PE-Cy5)**
FL4: far red fluorescence I **(PE-Cy7)**
FL5: red fluorescence II **(APC)**
FL6: far red fluorescence II **(APC-Cy7)**

Other parameter allocations possible

3 Laser instrument, high power laser 488 nm: This instrument is equipped with a blue diode pumped solid-state Laser (200 mW) at 488 nm, a violet laser diode at 405 nm (100 mW) and a red laser diode at 638 nm (25 mW). Up to nine optical parameters can be included (colours in the table below reflect laser origin of the signal).

FSC: forward scatter
SSC: side scatter
FL1: green fluorescence **(FITC)**
FL2: orange fluorescence **(PE)**
FL3: red fluorescence I **(PE-Cy5)**
FL4: far red fluorescence I **(PE-Cy7)**
FL5: red fluorescence II **(APC)**
FL6: far red fluorescence II **(APC-Cy7)**
FL7: blue fluorescence **(CFP, Alexa 405)**

Other parameter allocations possible

Other lasers available:

532 nm green solid state laser (50 or 100 mW)
375 nm UV laser diode (16 mW)
More lasers on request

The instrument is equipped with a CCD camera in order to monitor the particle flow and to check the focus of the objective.

Optical Flow Geometry

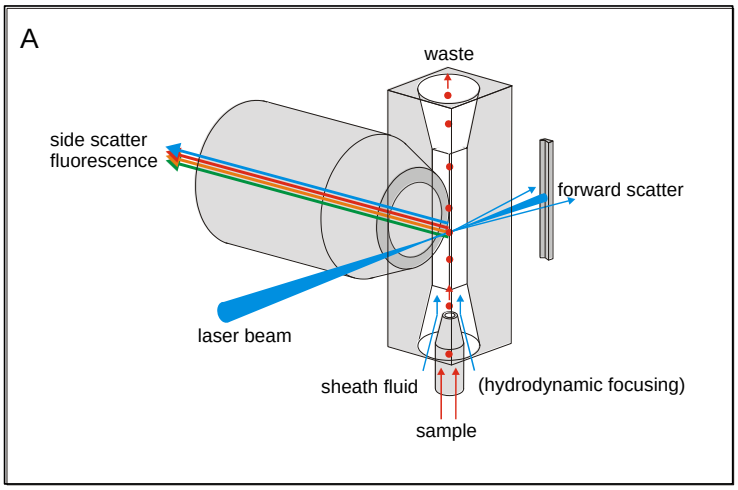


Fig. 3: Optical geometry at the flow cuvette (schematics).

Laser light is focused into the flow cell. Laser light scattered from the particles is detected in the forward direction range (forward scatter). Side scatter and fluorescence light is collected by the objective at a right angle.

Instrument Settings

Instrument settings are used to optimize the CyFlow® space acquisition for the particles of interest. The adjustments cover the gains of the optical detectors, e.g. the photomultiplier high voltages, the amplification mode (lin, 3 or 4 decade logarithmic), lower and upper level thresholds and sample speeds. Instrument settings can be once set up for a given application and then be saved and reloaded for later use.
The following pages will describe the instrument settings that can be made.
Please also refer to the software manual for details on how to change the instrument settings.

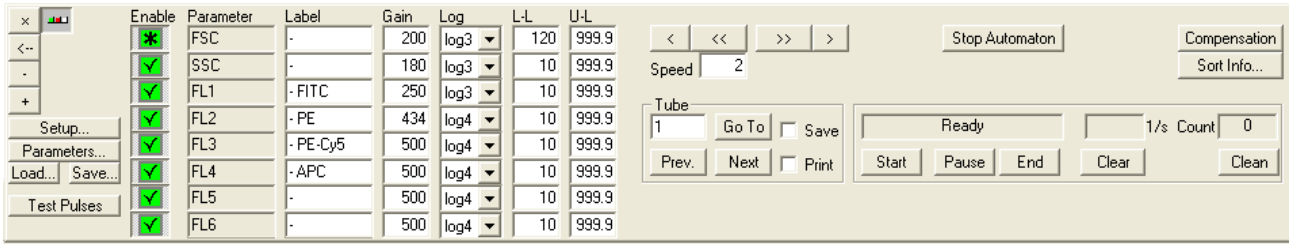
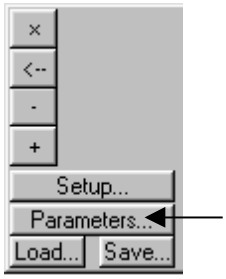


Fig. 4: Multiparameter instrument settings box

The Parameter Setup Dialog Box: Pulse Height, Area and Width

Opening the Parameter Setup



Before starting an acquisition, assure the parameters of interest are selected and set up according to your analysis.

Click the "Parameters..." button in the Instrument Settings box.

The Parameter Setup Dialog Box appears.

Fig. 5: The Parameter Setup Dialog Box

Note
Parameter names cannot be changed after an acquisition.

Parameter names

Check if the parameter names are according to your optical setup. Typical names are shown in the example above. The parameter names are displayed on the histogram axis together with a parameter label. To change parameter names, click into the fields and enter the names by using the keyboard.

Pulse Property Selections

For each optical channel, you may analyse three different pulse properties: 1. The pulse height, 2. the pulse area, and 3. the pulse width. Typically, only the pulse height is used for analysis. In case of a DNA analysis, the pulse area of the DNA parameter may be selected in order to discriminate cell doublets from single cells, which show up in the same DNA peak.

Low Pass

The electrical low pass filter smoothes the signals from the detectors by averaging the signal over a given time. This feature is only required for lamp light sources (not realized for the CyFlow® space).

2 Wavelengths Signal Delay

Light beams from different lasers are directed to different positions (spots) of the flow cuvette (please refer to page 6). Therefore, all laser derived signals from a laser at a spot other than spot no. 1 need to be analyzed with a time delay of 50 µsec (2-Wavelengths Signal Delay activated, see Fig. 5)

Trigger

FSC, SSC, FL1, FL2, FL3 and FL4 are selected for detection. FSC is the leading trigger parameter.

Leading Trigger
In order to discriminate particles of interest e.g. cells from other particles e.g. cell fragments or nutrition particles in cell culture, a proper trigger has to be chosen. In the CyFlow® space, any parameter can be used as leading trigger.

Frequently, the forward scatter parameter (FSC) or, preferently for smaller particles, the side scatter parameter (SSC) is used to trigger on all particles above a certain size range. However, especially for very small particles, e.g. microorganisms, triggering on a fluorescence parameter can be more efficient.

Selecting a parameter as leading **trigger parameter** means: Only particles that deliver a sufficient signal above the lower level threshold on that parameter will be acquired. Other non-triggering parameters are aquiring signals only for particles that generated a valid trigger signal (above treshhold). All other particles will not be "recognized" by the instrument. The trigger parameter can be used for an efficient exclusion of unwanted particles from the analysis.

Example: Assume vertebrate leukocytes in full blood are to be analysed. Triggering on a scatter parameter would be difficult due to the high number of erythrocytes in the same sample. Staining with a DNA dye (e.g. PI) and triggering on the DNA parameter will discriminate the leukocytes containing a nucleus from erythrocytes without a nucleus. This works even though there are orders of magnitutes more erythrocytes than leukocytes in the sample.

Trigger All
The CyFlow® space offers an additional method for triggering which allows to trigger on all parameters.

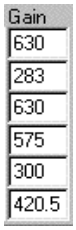
Example: Assume a part of the particles of interest are exclusively red and another part exclusively green fluorescing. Triggering on the green fluorescence would exclude the red particles from the analysis, triggering on red would exclude the green. If the interest is in both subpopulations, it is required to trigger on green and red simultaneously. This can be done with Trigger All.

Trigger parameter(s) and mode can be selected in the instrument settings box. Refer to the software operating manual for details.

PMT High Voltage, Gain, and Log Amplification

Controls

Gain



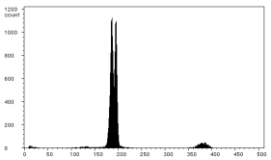
Note
Gain and lin/log amplification mode cannot be adjusted after the acquisition.

Note
Be sure not to use too high gain values. Increasing the gain values too much can cause a decrease of amplification or a loss of signals due to saturation effects.

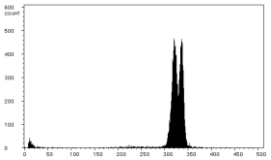
Note
The gain value does not necessarily be equal to the PMT high voltage nor does it be proportional to the amplification.

Log

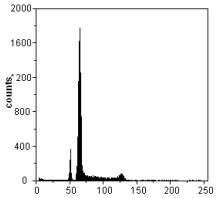
Typical effect of change



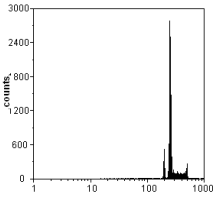
DNA distribution of bull sperms with X- and Y-chromosomes. **Gain = 445**.



DNA distribution of bull sperms with X- and Y-chromosomes. **Gain = 476.5**.



EAT cells, linear amplification



EAT cells, log 3 (3 decade) amplification

Adjustment

By means of the gain, the signal amplification can be individually adjusted in a wide range for each parameter. For the used photomultiplier tubes (PMTs) by changing the gain value, the PMT's high voltage is adjusted.

The gain value of a parameter can be selected by clicking into the corresponding gain value field in the instrument settings box (see Fig. 4). By using the Right (Left) buttons gain values are increased (decreased).

In case linear amplification is used, peaks are expanded to the right (compressed to the left) when increasing (decreasing) the gain (see example on the left).
In case logarithmic amplification is used, peaks are moved to the right (left) when increasing (decreasing) the gain.

Use the gain to move the peaks of interest to a suited position in the histograms.

Start with low gain values around 250. When increasing the gain, make sure the most intense signals still appear in the histogram and are not moved out of scale.
Increasing (decreasing) the gain by 50 approximately causes an increase (decrease) of amplification by a factor of two. Gain values between 250 and 600 are a good start for adjustment.

For each parameter, you can choose between linear or logarithmic amplification. In logarithmic amplification mode, the signal range can be set to 3 (log3) or 4 decades (log4), corresponding to a range of 1...1000 or 0.1...1000. Generally, a logarithmic amplification is advisable if particles with a broad range of intensities above 1:10 are to be analysed.

Click the little arrow, select one of the following

lin (linear)
log3 (logarithmic, 3 decade range)
log4 (logarithmic, 4 decade range)

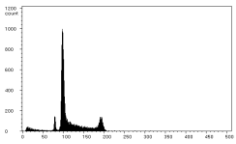
and click it.

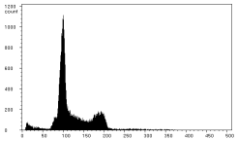
Sample Speed

Speed

Note
For a high accuracy measurement, a low speed smaller than 1.0µl/s is advisable.

Typical effect of change


EAT cells, Speed = 3µl/s


EAT cells, Speed = 20µl/s

Adjustment

By means of the speed value, the sample speed (in terms of µl/s) is set.

The speed value can be increased (decreased) by clicking into the corresponding field in the instrument settings box and using the Right (Left) buttons (see Fig. 4).

The count rate increases with elevated speed values. If the speed is increased too much, peaks in the histograms may become wider as a result of decreasing accuracy.

If the speed is too low, particle sedimentation effects can influence a counting result.

Typical Speed Values
a) High accuracy measuremens
(e.g. DNA with < 1.5% CV):
Speed = 0.5 µl/s.

b) Fast measurements and absolute counting:
Speed = 4.0 - 10.0 µl/s

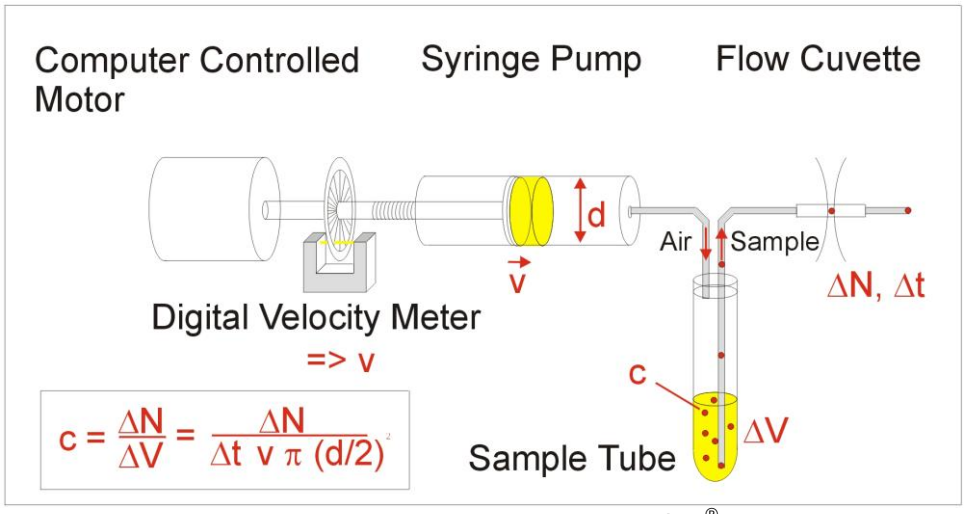
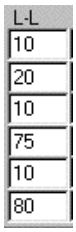


Fig. 6: Sample Transporting System of the CyFlow® space.

Threshold: Lower Level (L-L)

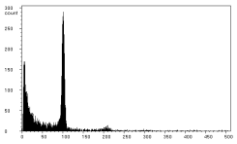
Lower Level (L-L)

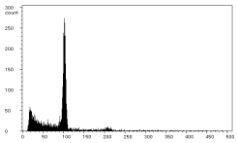


Note
L-L and U-L cannot be adjusted after the acquisition.
After acquisition, you may use 1P ranges or 2P polygons to gate out undesired signals instead.

Note
L-L values do not directly correlate with axis values.

Typical effect of change


L-L = 10


L-L = 40

Adjustment

Lower Level L-L
Setting a proper lower level (L-L) avoids the acquisition of small and unwanted background or "noise" signals below a threshold.

The L-L value of a parameter can be increased (decreased) by clicking into the corresponding L-L value field in the instrument settings box and using the Right (Left) buttons (see Fig. 4).

Start with a low L-L (e.g. 10). Increase the L-L until no more of the small "noise" signals appear in the histograms. Make sure not to remove signals from particles of interest by a L-L being too high.

A L-L range of 0…999.9 corresponds to the full histogram scale, independent of the channel resolution actually selected.

If a leading trigger is selected, then the L-L can only be effectively set on this trigger parameter.

Appendix

Installation Requirements

Line Power

CyFlow® space Basic Unit	AC 100-240 V 50-60 Hz, 250 VA max

Instrument Size

CyFlow® space Basic Unit	560 mm x 650 mm x 300 mm (W x D x H)

Operating Environment

The CyFlow® space should be placed on a solid base, e.g. a laboratory table. It must be placed horizontally. Reduce smoke, dust, vibrations, direct sunlight and direct neighbourhood of heatings as possible. The installation room must be well ventilated and dry.

Temperature	15-30°C
Humidity	20-85% relative (non-condensing)
Room	Clean environment. Direct sun light should be avoided.

Minimum Recommended Work Space

Table dimensions and space on desktop	120 cm x 90 cm x 80 cm (W x D x H)

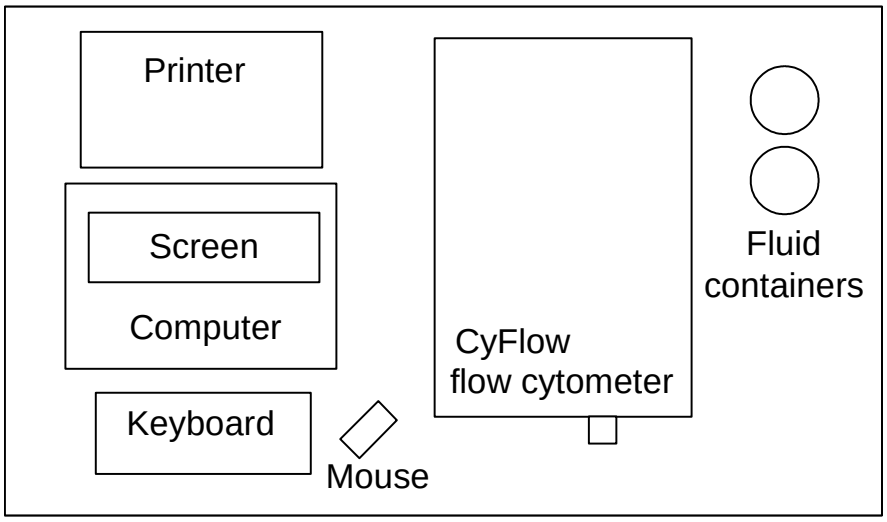


Fig. 7: Recommended placement of CyFlow® space components

Instrument Setup – Overview

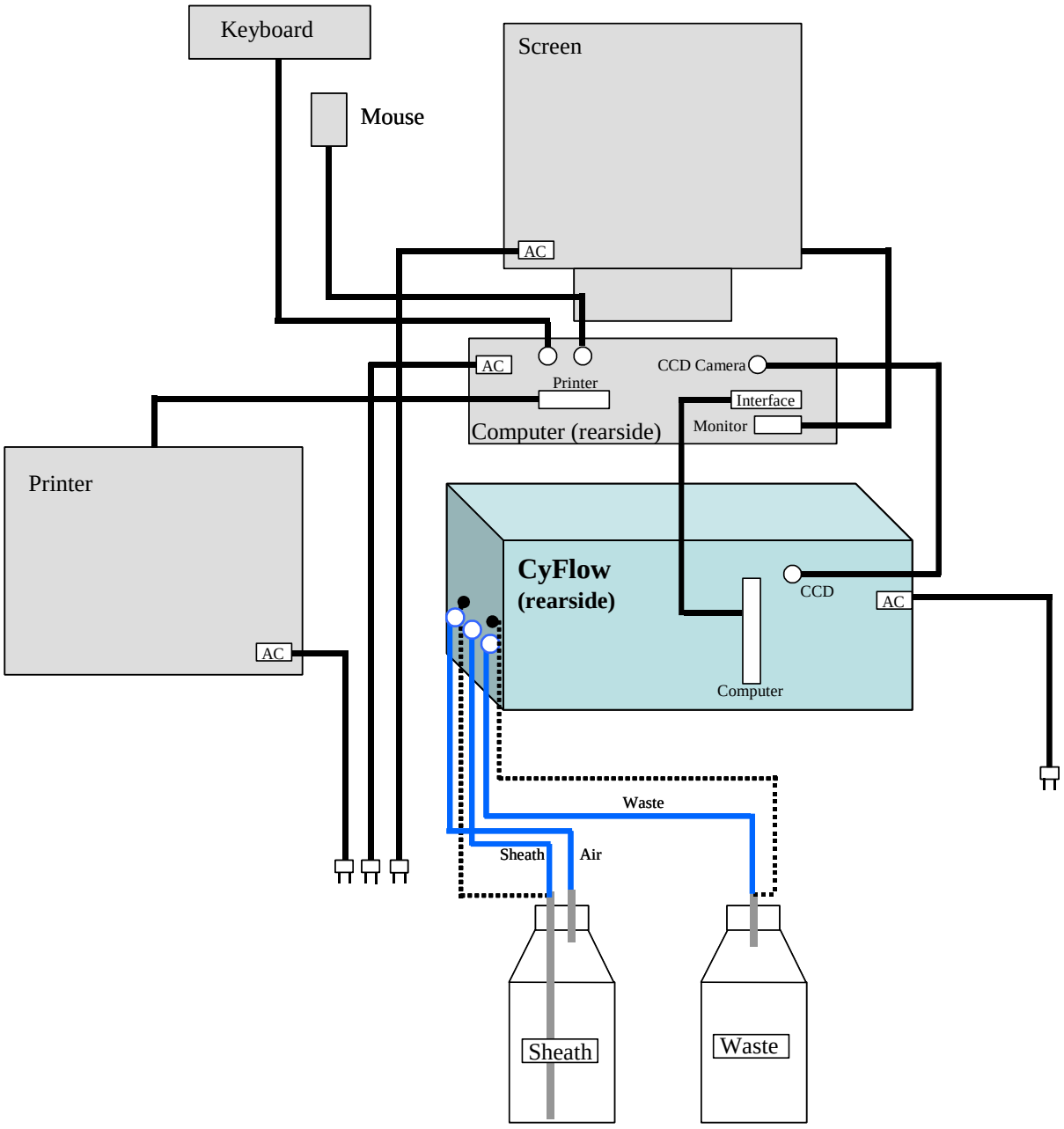


Fig. 8: Instrument Setup and periphery (CyFlow® space shown from the rearside).

Instrument Setup – Step by Step

Fig. 9: Computer connection on the CyFlow® space rearside.

Fig. 10: Power connection and laser switch on the left side of the CyFlow® space.

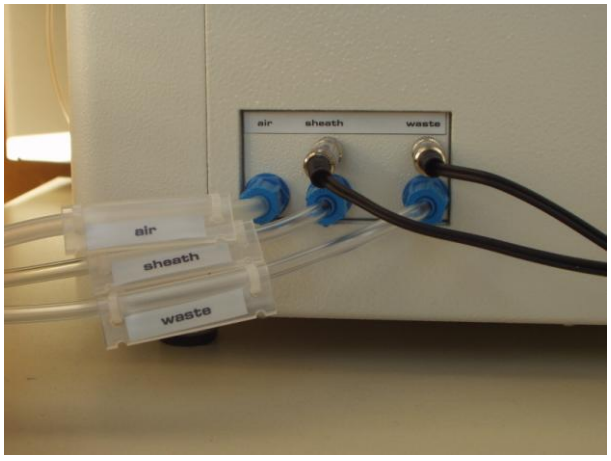


Fig. 11: Tube connections for sheath and waste containers and cable connections for the fluid level sensors on the right side of the CyFlow® space.

Note: Before first operation remove protection material from inside the system!

1. Setting up the Computer

Connect printer, screen, keyboard and mouse to the computer with the appropriate connection cables.
Connect computer, screen and printer to AC power line.

2. Connecting the CyFlow® space to power line

The CyFlow® space must be operated with AC 100/240 V 50/60 Hz.

3. Connecting the CyFlow® space to a Computer

The CyFlow® space runs with a standard Windows® PC or notebook (optional). This makes the latest computer technology available at any time. The integrated FloMax® software controls all functions of the CyFlow® space, e.g. instrument settings, sample supply, sheath pressure and sample flow rate.

The Computer connections of the CyFlow® space are located at the rear side of the instrument. **Connect the CyFlow® space to the computer with the interface cable.** The laser connection is only required for the **200 mW** 488 nm laser.

There is a CCD camera inside the CyFlow® space optical bench to monitor the focus of the objective and the sample flow inside the cuvette. **Connect the camera signal outlet (CCD) to the Video In connection of the computer with the video connection cable**.

Network support of the CyFlow® space is provided through a standard ethernet connection by the Windows® computer. Please refer to the Windows® manuals for details on how to set up a network connection.

4. Connecting the CyFlow® space to sheath and waste containers

Connect the sheath tube and the air tube of the sheath fluid container to the corresponding connectors at the right side of the CyFlow® space.

Connect the waste fluid container to the waste outlet at the right side of the CyFlow® space.

All tubes are labeled accordingly.

Note
Make sure that the sheath and waste fluid bottles stand on the same level as the instrument to prevent that hydrostatic pressure is influencing the sheath fluid pressure.

The Flow Cuvette

Note
The flow cuvette is “the heart” of your flow cytometer. It is responsible to guide cells and particles through the center of the illumination spots with a micrometer precision. The flow cell is manufactured with highest possible precision and should be treated like that. Any deformation will degrade the flow precision and make it irrepairably unusable.

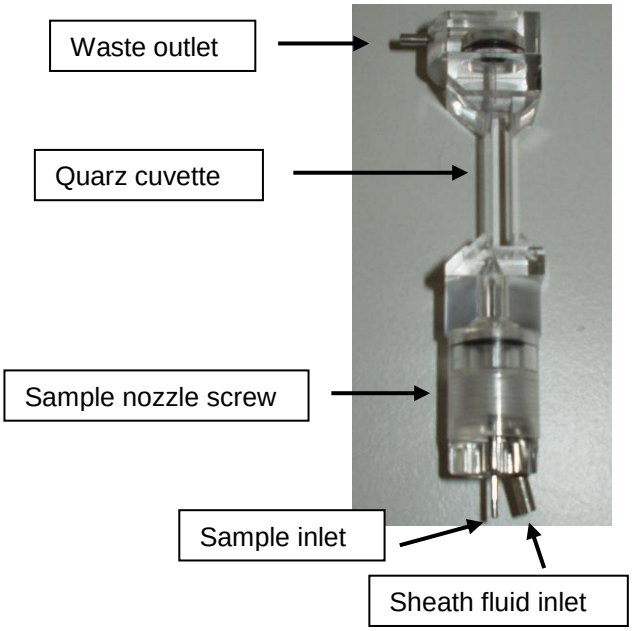


Fig. 12: Flow cuvette with sample, sheath fluid and waste connections.

Note
Air bubbles usually enter the flow cuvette through the sheath fluid inlet. Bubbles might stick inside the conical section above the sample nozzle and do not enter the flow channel of the quartz cuvette segment. The laminar flow of sheath fluid and sample flow as well as hydrodynamic focusing are disturbed and broad peaks appear in the histograms.

Note
Do not readjust the laser or laser optics.

DANGER
Warning: Do not look into the laser beam. Use eye protection glasses of filters or block the laser beam.

Cleaning of the flow cuvette

For **cleaning the flow channel** use Partec *Cleaning Solution* (Product no. 04-4009) filled into a standard sample tube and connect to the sample port 2-3 times. Then perform additional cleaning cycles with destilled water filled into a fresh sample tube.
Do not use mechanical wires or tools for cleaning or declogging the flow cell.

Removing Air Bubbles from Flow Cuvette

If you notice unsufficient peak resolution during sample run remove your sample and activate CLEAN several times. In this way the flow cuvette is back flushed with sheath fluid. Restart the sample and observe peak resolution again. If the results are still insufficient, run Partec Calibration Beads (e.g. 3 µm Calibration Beads). Compare the result with the exemplary data files located in the FloMax/QC or FloMax/Calibration data directory and observe the flow monitor (CCD camera). A single bright spot should appear in the centre of the visible field. If this is not the case air bubbles or dirt inside the flow cell may cause the disturbance. As first easy approach to release bubbles **during system run** attach a tube with Partec Cleaning Solution and **pinch first the sheath and then the waste tubing** several times to remove air bubbles through the flow channel into the waste.

In case air bubbles are still persistent please apply the following procedure:

1. Select the instrument settings:
   Sample prerun = 15 sec
   Stabilizing time = 5 sec
   Automatic stop = 15 sec
2. Place an **empty** sample tube on sample port
3. Press START and wait till automatic stop
4. Place a tube with 1.5 ml Partec Cleaning Solution on the sample port and repeat step 3.
5. Reset instrument settings to normal values

If analysis with Partec Calibration Beads does not result in acceptable signal distributions an incubation of the flow cell with Partec Decontamination Solution is required. Run the system with 1.6 ml Partec *Decontamination Solution*. During system RUN pinch the sheath fluid tube for 5 seconds. Stop the instrument while keeping the tube pinched by pressing STOP.

Incubate for at least 30 minutes. Re-start the system by pressing START and let it run to the end. The Decontamination Solution may incubate even over night! Wash the flow cuvette by attaching a tube with 1.6 ml of Partec Sheath Fluid and run it for at least 2 minutes.

In case your flow cytometer shows degraded performance even after thorough cleaning, please contact your local distributor or Partec.

Maintenance and Service

Maintenance

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

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

For cleaning of flow cuvette, refer to page 21. Do not use tools to clean the flow cuvette. In case the flow cuvette is blocked, enquire Partec for rapid exchange.

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

Clean sheath reservoir with destilled water and a clean brush and flush with clean destilled water several times. Remember cleanliness of sheath fluid reservoir is critical for proper operation.

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

Service

All service is to be made from an authorized service engineer. Please contact your supplier or partec for service requests.

Transport and Storage

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

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

Disposal

In case of product disposal, please proceed according to the Partec standard operating procedure (SOP) for decontamination.
After decontamination, the system has to be disposed according to the local regulations and laws.
For further information, please contact your local distributor or Partec.

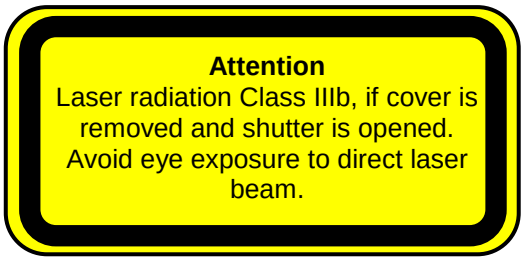
Laser Safety

The CyFlow® space is a class I laser product according to the EN 60825-1 : 2001.

Warning:

Laser light can be emitted if the protection cover for the laser beam is removed and the beam shutter is opened. Therefore, the system is marked with the following laser safety labels:

Warning: laser radiation



Additional explanation

CyFlow® space - Technical Specifications

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

1. CyFlow® space System

Size	Flow cytometer: 560 mm x 650 mm x 300 mm (W x D x H)
Weight	ca. 37 kg
Maximum sound power level	< 70 dBA
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	Research applications, Routine and Research Immunophenotyping, DNA Analysis, Apoptosis, Blood Cell Analysis, HIV monitoring, Leukocyte Counting / Rare Event Analysis, Microorganism Analysis (live / dead, others), Fermentation control, Particle Concentration Analysis, True Volumetric Absolute Counting, Particle Size and Fluorescence Distribution Analysis
True Volumetric Absolute Counting	Based on precise counting and mechanical fluid volume measurement. No need for reference sample or beads
Instrument Check	Blue laser: Product no. 05-4018 Calibration Beads 3 µm Product no. 05-4010 CountCheck Beads Red laser: Product no. 05-4012 Red Flourescent Particles UV laser: Product no. 05-4020 Calibration Beads UV Violet laser: Product no. 05-4018 Calibration Beads 3 µm
Set-up Time	10 minutes
Parameters	Up to 9 optical parameters: FSC, SSC, FL1, FL2, FL3, FL4, FL5, FL6, FL7 Time parameter Pulse height, pulse area, pulse width for each optical parameter
Particle Size Range	0.1 µm - 50 µm (standard cuvette)
Maximum Acquisition Speed	25,000 events/sec
Acquisition Stop Time	Time-, event- or volume-based
Trigger	On all parameters or on specific trigger parameter, selectable by software
Data Resolution	65,536 channels (16 bit)
Service	1-3 years service contracts

Warranty	12 months on all parts except filters, mirrors, other quartz or glass parts, disposables and cuvettes

2. CyFlow® space Optics

Laser / Output	Red Diode Laser:	25 mW or 40 mW at 638 nm
	Green solid-state Laser:	30 mW to 100 mW at 532nm
	Blue solid-state Laser:	20 mW or 200 mW at 488 nm
	Violet Diode Laser	100 mW at 405 nm
	Ultra Violet Diode Laser	16 mW at 375 nm
	Laser specifications may vary. Other lasers on request.	
Detectors	1 to 9 (FSC, SSC, FL1, FL2, FL3, FL4, FL5, FL6, FL7)	
Filters	Standard setup and filters for FSC, SSC, FL1-FL7	
Video Flow Monitor	Colour CCD camera for video flow monitor	
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 70 µm at 488 nm Other beam geometries upon request	

3. CyFlow® space Fluidics

Flow Cuvette	Synthetic quartz flow cuvette (350x 200 µm) for laminar sample transport with sheath fluid for fluorescence, forward and side scatter light detection
Sample Delivery	Computer controlled precision syringe pump for contamination-free sample transport. Built-in air pressure for sheath fluid. Sheath fluid pressure is adjustable from 0-300 mbar (Computer controlled). Default setting: 200 mBar
Sampling Volume	Continuous up to 1500 µl 200 µl for precision absolute counting Other counting volumes upon request Free sampling volume with syringe counting method
Flow Rates	1) Sample volume speed adjustable continously between 0 and 50 µl/s 2) Sheath fluid pressure continuously adjustable
Fluidics Volume	2 x 2-litre reservoirs for sheath fluid and waste
BioSafety System	Avoids sample droplets and sample cross contamination (computer controlled)

4. CyFlow® space Electronics and Computer

Electronics	Parallel realtime signal processing for each of the optical channels with selectable linear, 3- or 4-decade logarithmic amplification (6-decade log amplifiers), pulse height, area and width analysis for doublet discrimination, 16 bit analog-to-digital converters, trigger on any parameter or all parameters
Computer Desktop: (specifications continiously updated according to improved performance of available hardware and software components)	Processor ≥ Pentium 4 3.0 GHz, ≥ 512 MB RAM (or equivalent) Harddisk ≥ 160 GB Floppy disk drive 1.44 MB and DVD CD-RW 19" TFT monitor Keyboard and mouse Microsoft Windows XP® professional Microsoft Office® 2007 Basic

5. FloMax® Software

Software	32 bit Windows™ FloMax® software for routine and research applications All parameters stored in FCS 2.0 or FCS 3.0 standard listmode format Time parameter for kinetic studies Realtime acquisition Multiparameter N-colour compensation (online and offline) Multiparameter gating, MultiColor gating Peak and cluster analysis and statistics DNA-cell cycle analysis DNA peak analysis Report module: automated multi-tube report generation as MS Word or MS Excel document Copy & paste to desktop publishing software Ratio measurements Parameter arithmetics
Acquisition Gating	Lower-/upper-level hardware thresholds for event triggering parameters, adjusted by software Real-time software gating: 32 regions can be combined to 32 gates in free logical combinations Regions, quadrants, 1P-ranges
Gating / Crosstalk Compensation	On- or offline gating and crosstalk compensation provide adjustments without need to rerun samples Different gating and compensation adjustments can be stored and reloaded
Protocols	Individual instrument setups and acquisition displays can be saved in setup-files The plot display, axis labels, statistics and cell concentrations are saved in documents together with the acquisition data
Panels	Multi-tube analysis can be predefined in panels Automated panel acquisition
Report Generation	Report modul for Microsoft Word, directly called by the FloMax® software: Single- or Multi-Page Report templates include institute logo(s), addresses, graphs and statistical results. Templates can be individually adjusted in a desktop publishing manner Individual calculations can be defined in spreadsheet formulas Copy & Paste / export to desktop publishing software Multi-tube reports
Optional Software	Other software upon request