CyFlow® Cube 8

Operating Manual

RUO For Research Use Only. Not for use in diagnostic procedures.

Imprint

Original Operating Manual

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1 Introduction

1.1 About this operating manual

This operating manual will help you to operate your CyFlow® Cube device. Emphasis is placed on the installation, start-up and safe operation of the device.

The basic principles of flow cytometry can be found in the medical literature (e.g. Howard M. Shapiro, Practical Flow Cytometry, Wiley 2003).

This operating manual comes with the device. Read this operating manual thoroughly before using the device. Please keep this operating manual for future reference.

This operating manual is divided in two parts. The first part, chapter 1–11, gives the user all necessary information for safe handling, scope of delivery, to set up the device and how to operate the device properly. The general use of the software and the typical workflows are described in chapter 6.5. This chapter also is suitable to get to know the functioning of your device.

In the second part, chapter 12, you will find a description of all software functions and a detailed description of the user interface. This chapter is for reference.

There are product data sheets for the reagent kits and application notes available. Please also refer to these documents.

If you have any questions about the content of this operating manual or the use of the device, contact your local Sysmex representative.

1.2 Typographical conventions

In this operating manual, warnings are indicated by a signal word. Hazards are categorised into hazard levels with consequences of differing severity.

⚠DANGER

Type of hazard

This will result in death or have a serious impact on health.

■ Information about avoiding the hazard.

⚠WARNING

Type of hazard

This could result in death or have a serious impact on health.

■ Information about avoiding the hazard.

⚠CAUTION

Type of hazard

This may have a minor impact on health.

■ Information about avoiding the hazard.

NOTICE

Type of hazard

This could result in material damage.

■ Information about avoiding the hazard.

All warnings are structured according to the same model. The type of hazard is stated after the signal word. Then the consequences that may result from the hazardous situation are described. And finally, how the hazardous situation can be avoided.

1.3 General conditions

Ensure that the following conditions are met when working with the device:

- This operating manual must be read prior to using the device.
- Report any recurring faults or problems to your local Sysmex representative.

Sysmex Partec GmbH does not accept liability for any damage or personal injury arising from:

- Modifications or manipulation of the device;
- Improper use;
- Use of non-approved accessories;
- Improper handling;
- Non-compliance with this operating manual.

1.4 CE compliance

The device is marked with a CE mark of conformity, which confirms the compliance with the essential requirements of the following European Directives:

- 2014/35/EC on electrical equipment designed for use within certain voltage limits;
- 2014/30/EC on electromagnetic compatibility;
- 2011/65/EC on the restriction of the use of certain hazardous substances in electrical and electronic equipment.

2 Safety

2.1 Intended use

For Research Use Only. Not for use in diagnostic procedures.

The device is a flow cytometer, it offers automation for routine use and flexibility for research use in the field of flow cytometric applications.

The applications cover:

- Multi-colour analysis of immune cells for research purposes;
- Leukocyte counting/rare event analysis for research purposes;
- Microbiological analysis;
- Fermentation control;
- Bead-based assays for research purposes;
- Particle and cell concentration analysis based on True Volumetric Absolute Counting;
- Particle size and fluorescence distribution analysis.

2.2 Prohibited use

Never use the device with organic solvents as this can destroy the fluidic system. Only use particles with a diameter < 100 µm. Only use Sysmex approved fluids for sheath, cleaning and decontamination.

Never for use in diagnostic procedures.

2.3 Reasonably foreseeable misuse

Only use this device as a sensitive measuring device. It should:

- NOT be used as personal computer.
- NOT be used with chemicals harmful to the device (household sanitisers etc.).

2.4 User definition

The device may only be used by personnel properly instructed in its handling. The manufacturer, authorised distributor or a person authorised by the operator (and trained by either the manufacturer or an authorised distributor) must provide instruction in handling the device with the help of this operating manual.

The device is aimed for a large spectrum of users, from beginners to experts. In the best case an expert is around – so, if you are a beginner, let her/him help you.

Access to the device is not permitted to insufficiently qualified personnel.

2.4.1 User qualification groups

Instructed personnel

Instructed personnel have to be trained by qualified personnel or personnel authorised by Sysmex to operate the device within the scope of the intended use. They have to be able to assess if the device can be safely used and are proficient in handling hazardous substances.

Qualified personnel

Qualified personnel have in-depth knowledge of the device and the applications. They have been trained by personnel authorised by Sysmex to operate the device, to perform cleaning and certain maintenance operations. Qualified personnel have a scientific or technical education and several years of experience in flow cytometry and are proficient in handling hazardous substances.

Authorised service personnel

Personnel conducting technical service must be authorised by Sysmex. Please contact the service department of your local Sysmex representative.

2.4.2 User qualification table

Activity	Instructed personnel	Qualified personnel	Authorised service personnel
Transport		×	×
Installation			×
Operation	×	×	×
Cleaning	×	×	×
Decontamination		×	×
Maintenance	×[1]	×[1]	×
Technical service			×
Uninstalling		×	×
Packing		×	×
Disposal		×	×

× = activity can be performed by the user

[1] = please refer to the maintenance table for detailed instructions (see chapter *9 Maintenance* on page 44).

2.5 Environmental conditions

The device may only be used in laboratories. The specified ambient temperature and relative humidity conditions must be maintained at all times.
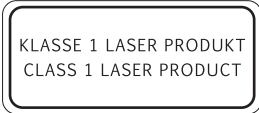
The laboratory environment must be clean. Direct sun light should be avoided.

CyFlow® Cube 8 Configuration	Operating temperature range
V1, V2, V4, V5, V6, V7, V8, V9, V10, V11	15 °C to 30 °C
N1	15 °C to 30 °C

■ Relative humidity: between 20 % and 85 %, non-condensing.

2.6 Prohibition and warning signs on the device

The following prohibition and warning signs have been affixed to the device.

Warning sticker	Meaning
(laser radiation symbol)	Laser radiation
(electrical hazard symbol)	Electrical hazards *Beware of electrical currents and electrical shocks*
KLASSE 1 LASER PRODUKT CLASS 1 LASER PRODUCT	Additional laser labelling

2.7 Personal protective equipment

The following personal protective equipment is recommended to operate the device.

(gloves symbol)	Gloves must be worn at all times.
(lab coat symbol)	Lab coat must be worn at all times.

2.8 Laser safety

The device is equipped with a class IIIb laser unit. If the housing of the device is closed, the laser class of the laser unit is classified as a laser class I (EN 60825-1:2015).

Never open the housing of the device.

Laser light of laser class IIIb can be emitted if the housing of the device is damaged or the laser protection is removed. Serious injuries of the eyes and the skin are the results of emission of laser light of laser class IIIb.

2.9 Electrical safety

The main electrical source used must be grounded or earthed due to local specifications.

Please place the device in such a way that the electrical power cord can easily be unplugged. Removable power cords should not be replaced by inadequate wiring.

Beware of possible voltage when changing the fuse (T 4.0 A 250 V).

2.10 Alterations to the device

Unauthorised alterations to the device can result in risks and hazards. Therefore, unless expressly permitted by the manufacturer, no alterations may be made to the device.

If the device is altered, appropriate tests and trials must be performed to ensure the continued safe use of the device.

3 Overview

Your CyFlow® Cube device is a fully equipped desktop Flow Cytometer (FCM). Which features a modular optical concept. This allows different lasers with different wavelength ranges be used as light sources and the detection of up to 8 optical channels (parameters).

The device allows the optics to be optimised for any application simply by exchanging optical filters and mirrors. The device runs with an internal PC. Data acquisition, device control, and data analysis are controlled and performed by the software.
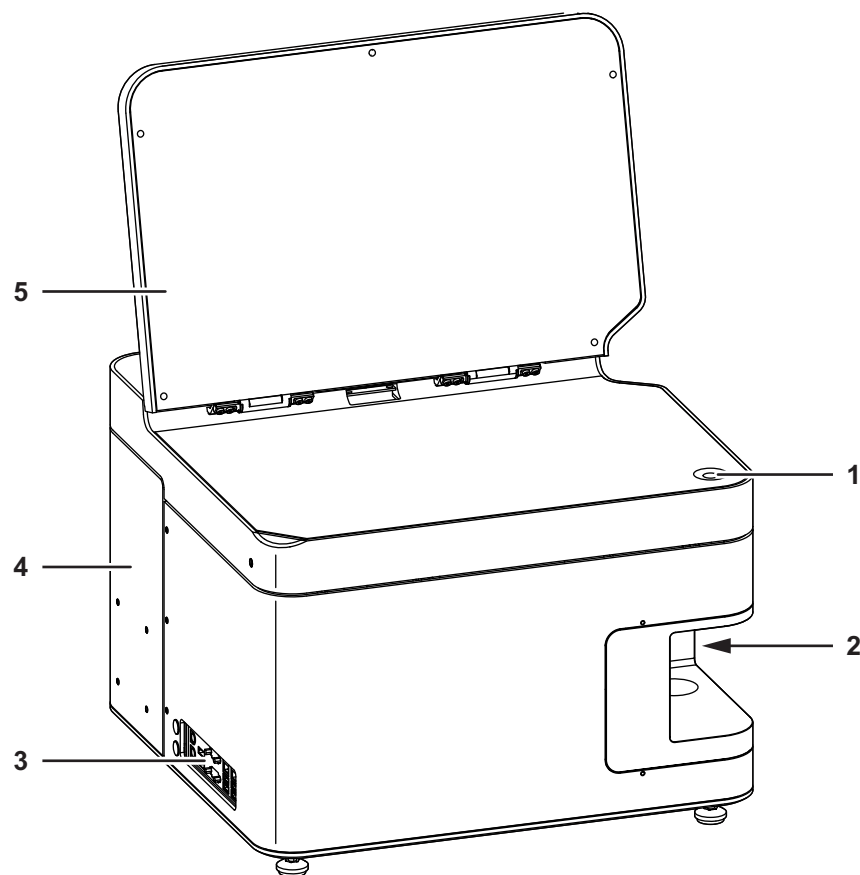
Fig. 3.1 Front and left side of the device

1	**On** switch	4	Sliding compartment
2	Sample port	5	Display
3	Ports for external devices		
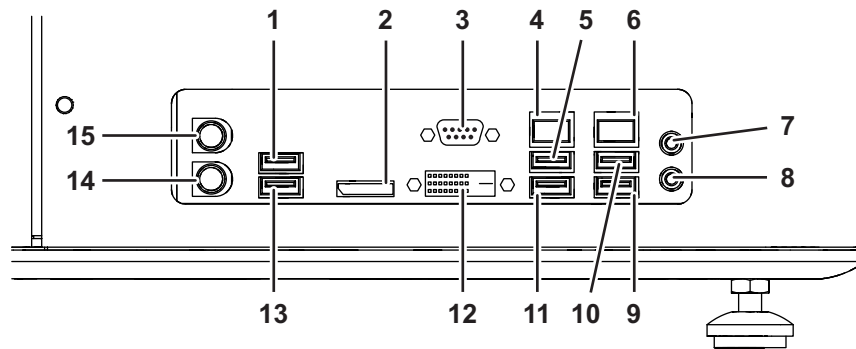
Fig. 3.2 Ports for external devices

1   USB port
2   HDMI port
3   VGA port
4   Ethernet port
5   USB port
6   Ethernet port
7   Audio port
8   Audio port

9   USB port
10 USB port
11 USB port
12 DVI port
13 USB port
14 Keyboard port
15 Mouse port
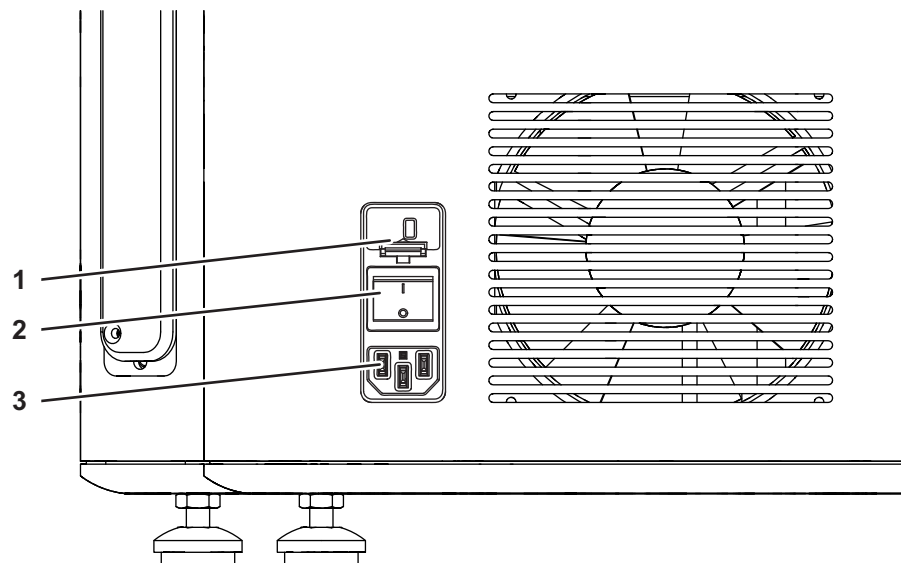
Fig. 3.3 Rear side of the device

1   Fuse insert (T 4.0 A 250 V)
2   Power switch
3   Connector socket for the power cord
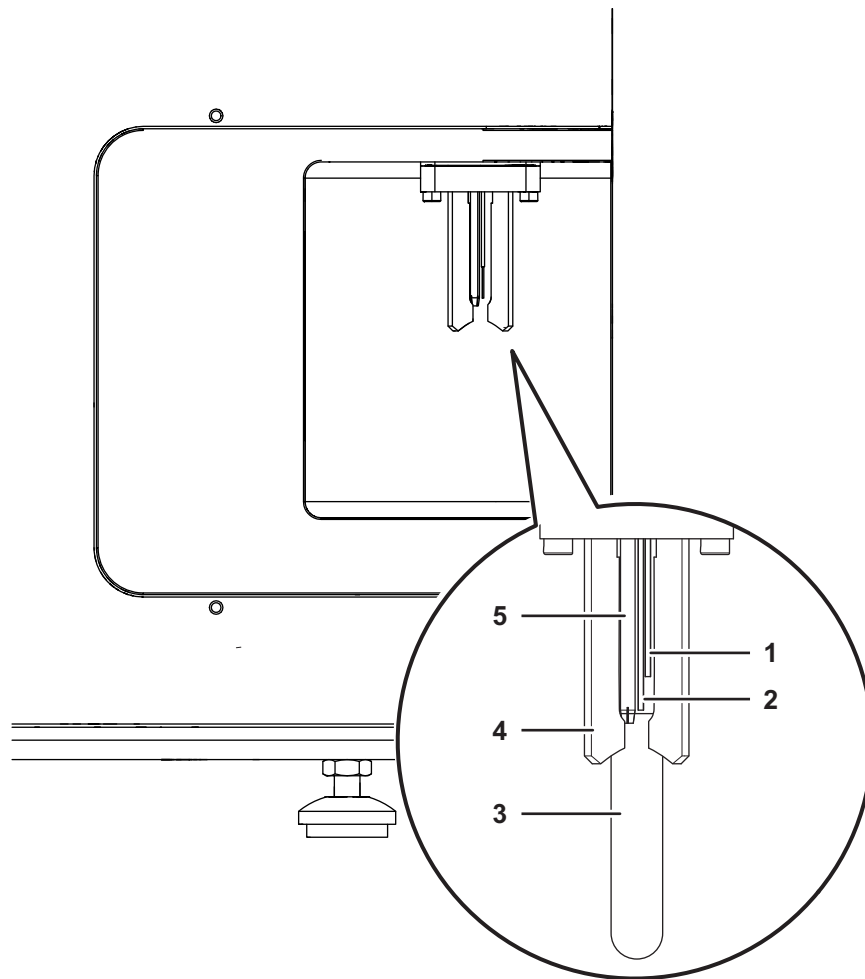
Fig. 3.4 Sample port at the right side of the device

1	Start electrode	4	Sample holder
2	Stop electrode	5	Sample port injection needle
3	Sample tube		

3.1 Components of the device

The device contains the flow cuvette, the optics, the fluidics, the computer unit and data acquisition electronics. No work steps inside the device are necessary.

The main components of the device are:

Control elements

The device is operated via keyboard and mouse. The **On** switch is located on the top, when the display of the device is lifted up. The power switch is located on the rear side of the device.

Computer interface ports

The computer interface ports connect the device with additional optional electronic devices. They are located on the left side of the device.

Sheath fluid bottle and waste bottle

The sheath fluid transports the sample flow during the measurement.

The connecting ports for the tubings of the sheath fluid bottle and waste bottle are located inside the device. The tubings are preinstalled. The sheath fluid bottle and waste bottle are located in the sliding compartment at the rear side of the device.

Sample port with electrodes

The sample port is located on the right side of the device.

The sample port holds the sample tube during the measurement. The start and stop electrodes of the sample port allow the independent determination of exact 200 µL volume for each sample. The method of True Volumetric Absolute Counting (TVAC) supported by CyFlow® Cube systems is based on the precise measurement of a fixed sample volume.

3.2 Type plate

The type plate is attached to the rear side of the device.

The content of the type plate varies according to the system configuration. The system configuration is represented by an addition to the REF number (e.g. CY-S-3068R_N1).
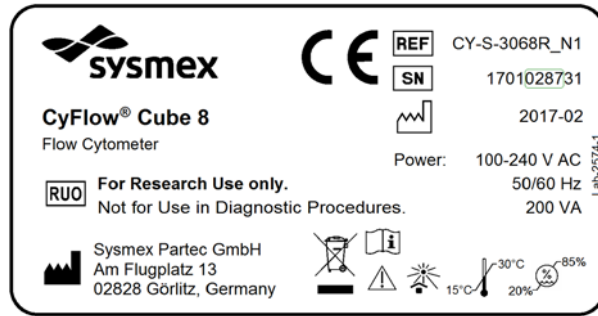
The following signs are part of the type plate which has been affixed to the device.

Symbol	Meaning
RUO	For Research Use Only
	Manufacturer
CE	Certification mark
REF	Reference Number
SN	Serial Number
	Manufacturing date
	Waste of Electrical and Electronic Equipment
	Operating manual
30°C / 15°C	Indicates the temperature limits to which the device can be safely exposed
	No direct sunlight at the installation location
85% / 20%	Indicates the range of relative humidity to which the device can be safely exposed
	Caution

3.3 Scope of delivery
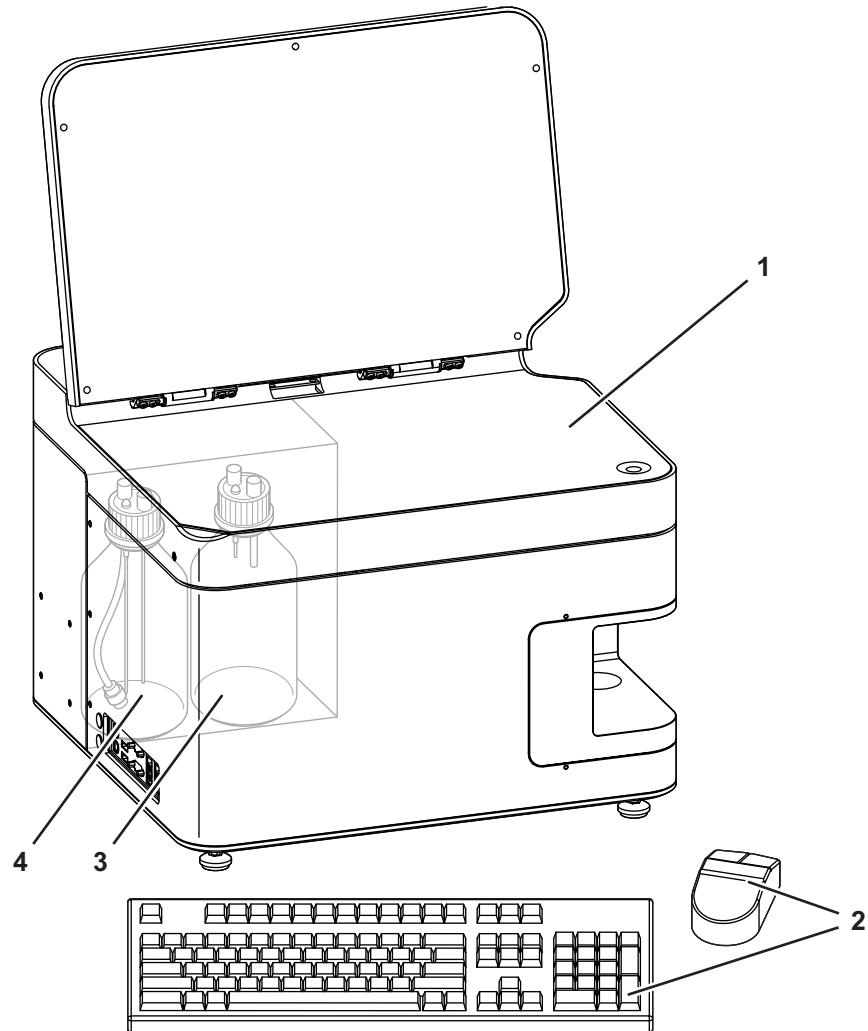
Fig. 3.5 Scope of operation

1	CyFlow® Cube device	3	Waste bottle
2	Keyboard and mouse	4	Sheath fluid bottle with inline filter for sheath fluid

The scope of delivery includes:

- 1 CyFlow® Cube device;
- 1 Power cord;
- 1 Software dongle;
- 1 Operating manual;
- 1 Laboratory bottle, 1 litre, labeled with sheath;
- 1 Laboratory bottle, 1 litre, labeled with waste;

- 1 Blue cap with electrodes, cabling and tubing for sheath including inline filter;
- 1 Red cap with electrodes, cabling and tubing for waste.

The scope of operation includes:

- 1 CyFlow$^®$ Cube device;
- 1 Power cord;
- 1 Software dongle;
- 1 Operating manual;
- 1 Keyboard and 1 mouse;
- 1 Laboratory bottle, 1 liter, labeled with sheath;
- 1 Laboratory bottle, 1 liter, labeled with waste;
- 1 Blue cap with electrodes, cabling and tubing for sheath;
- 1 Red cap with electrodes, cabling and tubing for waste.

3.4 Accessories and spare parts

Additional available application accessories and spare parts:

Product name	Content	Order No.
Sheath fluid	5000 ml	04-4007_R
Sheath fluid tap	1 piece	04-4006
Cleaning solution	250 ml	04-4009_R
Decontamination solution	250 ml	04-4010_R
Hypochlorite solution	250 ml	04-4012_R
Count Check Beads (low, medium, high)	50 tests	05-4010
Calibration Beads 1 μm Concentrate	1 ml	05-4007
Calibration Beads 3 μm, ready-to-use	30 ml	05-4018
DNA Control PI	25 ml	05-7303
DNA Control UV	25 ml	05-7302
Sheath fluid bottle 1l	1000 ml empty bottle	04-200-1041
Waste bottle 1l	1000 ml empty bottle	04-200-1042
Cap for 1 l glass sheath fluid bottle	1 piece	04-200-1056
Cap for 1 l glass waste bottle	1 piece	04-200-1057
Sample tube 3.5 ml	500 pieces	04-2000
Keyboard	1 piece	06-500-1040
Mouse	1 piece	06-500-1041
Spare fuses T 4,0 A	5 pieces	06-7-8006

Additional available upgrades:

- CyFlow® Robby 8 Autoloading Station
- CyFlow® Sorter for CyFlow® Cube

Visit our website http://www.sysmex-partec.com or contact your local Sysmex representative for further information about our products.

4 Transport and storage

Warning! The weight of the device is about 40 kg.

Always have at least two people carry or lift the device.

In order to transport the device to a different location all external data and supply connections have to be disconnected.

The device should be carried in an upright position.

The device is supplied in packaging that protects it against damage and contamination. Please retain the packaging. Place the device in the packaging for transport and storage.

The storage environment must be dry, clean and dust-free:

- Storage temperature: between 5 °C and 50 °C;
- Relative humidity: between 20 % and 85 %, non-condensing.

5 Installation

5.1 Positioning the device

> **⚠ WARNING**
>
> **Heavy weight**
>
> When moving the device (packed or unpacked) or removing it from its packaging, a person is exposed to a high risk of damaging his or her body due to the heavy weight of the device.
>
> ■ At least two persons are required for transport and installation.

Carefully select the place where to position and operate the device. Comply with the following criteria when positioning the device:

■ Place the device on a solid, dry and clean horizontal surface.

■ Avoid smoke, dust, vibrations, direct sunlight and any source of direct heat.

■ Leave a minimum distance of 15 cm between the back of the device and a wall to keep the ventilation effective.

■ Do not block the ventilation grid at the back of the device.

■ Do not place any objects on the device.

5.2 Installation and uninstallation

Installation, connection, disconnection and uninstallation may only be performed by authorised service personnel and in conformity with the applicable national rules and regulations.

6 Operation

Due to heavy use of software-related content in the following chapter please also refer to chapter *12 Software description* on page 50.

6.1 Inserting the Software dongle

Procedure

1. Insert the included Software dongle into an open USB port of the device.

Result

■ The software will check automatically in regular intervals if the dongle is plugged in. If the dongle is not plugged in, the software will not be functional.

6.2 Start-up procedure

NOTICE

Old sheath fluid

Old sheath fluid affects the sample flow through the flow cuvette.

■ Replace the sheath fluid at least once a week or before any daily use.

6.2.1 Switching on the device

Before switching on the device make sure the sheath fluid bottle is filled with 800 ml of clean, filtered and degassed sheath fluid and is closed with the blue screw cap. It is recommended to replace the sheath fluid at least once a week or before any daily use.

Requirements

■ The sheath fluid bottle is filled and connected to the device.

■ The waste bottle is empty and connected to the device.

Procedure

1. Switch on the device using the power switch on the rear side of the device.

2. Lift up the display of the device.

3. Push the **On** switch on the top of the device.

    – The device is switched on.

4. Start the software by double click the CyFlow® Software icon.

   – The log-in dialogue is displayed.

   – The device is, by default, set in a standard mode.

6.2.2 Logging in and logging out

Each user is assigned to a user role. Each user role has specific privileges. Some functions of the software are not available for each user role and availability depends on the assigned privileges.

User management

Role	Privileges
Service operator	User administration
	Worklist creation
	Worklist management
	Sample analysis
	Manage Workspace and settings
	Create comp setup
User administrator	User administration
	Parameter properties
Assay developer	Manage Workspace and settings
	Worklist creation
	Worklist management
	Sample analysis
	Create comp setup

Logging in

Requirements

■ The log-in dialogue is displayed.

■ User access must be set up and the user name and password must be known to log into the software.

Procedure

1. Enter the user name.

2. Enter the password.

3. Click the **Log-in** button.

   – The log-in dialogue closes.

   – The user role dialogue will be displayed.

4. Select the respective user role.

5. Click the **OK** button.

    –   If the log-in was successful the start screen is displayed.

> ⓘ **Information**
>
> After a specified number of unsuccessful login attempts (default 3), the account will be locked. If the account is locked, please contact a user with the "user administration" privilege in its user role, e. g. a user administrator.
>
> The default settings for logins can be changed in the user management.

Logging out

Procedure

1. On the *File* tab of the Ribbon (see chapter *12.4.1 File tab* on page 58) click the **Log-out** button.

    –   The current user is logged out.

    –   The log-in dialogue is displayed.

6.2.3 Performing a Prime

A Prime mode is available as a start-up process for the device. This process includes cleaning, filling the tubes with sheath fluid and controlling the device set-up.

The Prime should be performed in the following cases:

■   Starting the device for the first time of the day,

■   After the sheath fluid bottle has been filled,

■   As a trouble-shooting procedure (no/bad signals, blocking, etc.).

Requirements

■   Sample tube with 1.6 ml Decontamination Solution (Order No. 04-4010_R) connected.

Procedure

1. On the *Cytometer* tab of the Ribbon (see chapter *12.4.10 Cytometer tab* on page 105) or on the *Acquire* pane (see chapter *12.5.4 Acquire pane* on page 140) click the **Prime** button.

    –   The instruction dialogue for the Prime procedure is displayed.

2. Follow the instructions until the finish message is displayed.

    –   The device is ready for the sample measurements.

3. Remove emptied sample tube.

6.3 Performing a measurement

Requirements

- The device is primed.
- The respective Worklist is loaded.
- A Worklist item is active with a defined Workspace.
- The Worklist contains at least one defined item that does not contain a listmode file.
- The sample is prepared for the measurement.
- The sheath fluid is not low.
- The waste level is not high.

Procedure

1. Fill the sample tube with the sample fluid.
2. Insert the sample tube into the sample port of the device until the sample tube audibly clicks.
3. On the *Acquire* pane (see chapter *12.5.4 Acquire pane* on page 140) click the **Start** button.
   - Data acquisition starts. Progress is displayed in the status bar.
4. If a stop condition is set, wait until acquisition stops.

   If no stop condition is set, click the **Stop** button on the *Acquire* pane to stop acquisition.
   - A measurement is performed.
5. Remove emptied sample tube.

6.4 Performing an intermediate cleaning

A cleaning mode is available to clean the device when a different sample type is to be measured.

Requirements

■ Log-in as a user with the respective role.

■ The respective Worklist is loaded.

■ Sample tube with 1.6 ml Cleaning Solution (Order No. 04-4009_R) connected.

Procedure

1. On the *Cytometer* tab of the Ribbon (see chapter *12.4.10 Cytometer tab* on page 105) click the **Clean** button.

    – An instruction dialogue is displayed.

2. Follow the instructions until the finish message is displayed.

    – An intermediate cleaning was performed.

    – The device is ready for the next sample measurement.

6.5 Shutdown procedure

6.5.1 Cleaning for shutdown

Perform the shutdown cleaning procedure to close the software.

Requirements

- No acquisition is in progress.
- Sample tube with 1.6 ml Decontamination Solution (Order No. 04-4010_R) connected.

Procedure

1. On the *File* tab of the Ribbon (see chapter *12.4.1 File tab* on page 58) click the **Exit CyFlow Software** button.
   - If the Worklist or Playlist is currently open and has been modified, a save changes dialogue appears.
   - A dialogue asking for a shutdown cleaning procedure appears.
2. Select **Yes** in the dialogue.
   - The dialogue closes and initiates the shutdown cleaning procedure.
   - After the cleaning procedure is complete the software closes.
3. Remove emptied sample tube.

6.5.2 Switching off the device

Requirements

- The shutdown cleaning procedure was successful and the software is closed.

Procedure

1. In the Windows menu, click the **Shut down** button.
   - The device shuts down.
2. Switch off the device using the power switch on the rear side of the device.
   - The device is switched off.

6.6 Software operation

The software can be run in two different modes:

- In Worklist mode, data can be acquired. The hardware and software settings for each measurement are predefined and organised in a list called Worklist.

- In Playlist mode, acquired data can be analysed and settings for future measurements can be prepared. Files are organised and edited in a Playlist.

This chapter describes how to use a Worklist for preparing sample measurements and how to use a Playlist to analyse data. For a detailed description of software functions, see chapter *12 Software description* on page 50.

6.6.1 Preparing measurements using the Worklist

This chapter describes the typical sequence of steps when preparing a Worklist for future measurements.

A Worklist contains the predefinitions of hardware and software settings for data acquisition (see chapter *12.6 Worklist pane* on page 146). These predefinitions are set prior to measurements when preparing the Worklist.

Preparing the Worklist includes:

- Defining the Workspace that contains the plots, regions and gates to represent the cell populations of interest;

- Defining and adapting the instrument settings to reduce background and to position the populations of interest properly into the plots;

- Performing a manual compensation to compensate for fluorescence spillover as appropriate.

These settings can be preset prior to acquiring data and adapted while measuring samples.

Creating a new Worklist

Requirements

■ To create a Worklist for future measurements, login as an assay developer is necessary.

Procedure

1. On the *File* tab of the Ribbon (see chapter *12.4.1 File tab* on page 58) select "File > New > New Worklist".

   In Worklist mode, a new Worklist can also be created by clicking the **New** button in the *Worklist* pane (see chapter *12.6 Worklist pane* on page 146).

   – A new default Worklist containing 400 blank Worklist items is created and displayed in the *Worklist* pane.

   – The software is in Worklist mode.

Defining a new Workspace

A Workspace contains the plots, regions and gates of interest (see chapter *12.8 Workspace* on page 160). To prepare a new measurement, a Workspace can be prepared before acquiring data and further adapted while measuring a sample.

Procedure

1. Double-click a Worklist item to open it in the Workspace.

2. In the Worklist, enter a Lab # and sample ID for the Worklist item as desired.

3. Define which plots to be displayed in the Plots area of the Workspace:

   On the *Home* tab of the Ribbon (see chapter *12.4.2 Home tab* on page 65), click the **Density Plot** button or the **Histogram Plot** button to add a corresponding plot to the Workspace.

   Alternatively, select the desired plots by double-clicking on the plots in the Previews area of the Workspace.

4. To change the parameter for an axis of a plot, right-click on the axis and select the parameter from the drop-down list.

   Alternatively, select the parameter from the *Parameters* group of the *Home* tab of the Ribbon (see chapter *Parameters group* on page 66).

5. To change the scaling of the axes, select a plot, then select the scaling (linear or logarithmic) on the *Parameters* group of the *Home* tab.

6. To change the plot title, double-click the current plot title and enter a new title.

   – A new Workspace is defined.

Presetting instrument settings

Procedure

1. Select the *Settings* pane (see chapter *12.5.3 Settings pane* on page 139).

2. To select a parameter as trigger parameter, set a parameter to "ON" in the "Logic" column (only available if all other parameters are set to "OFF").

   Data of this parameter are acquired.

   If desired, combine other parameters for trigger by setting them to "AND" or "OR".

3. To predefine threshold values, enter a value in the "Threshold" column for the relevant parameters.

4. To predefine the signal intensity to be displayed for each parameter, enter a value in the "Voltage" column for the relevant parameters.

5. Select the *Acquire* pane (see chapter *12.5.4 Acquire pane* on page 140).

6. Select the flow rate for the measurement by clicking the **Low**, **Medium** or **High** button or by using the flow rate slider.

   – The selected flow rate is displayed next to the flow rate slider.

   – The instrument settings are preset.

Starting measurement of the sample

Requirements

- ■ The device is primed.

- ■ A Worklist item is active with a defined Workspace.

Procedure

1. Insert the sample tube containing the sample into the sample port of the device until the sample tube audibly clicks.

2. On the *Acquire* pane (see chapter *12.5.4 Acquire pane* on page 140) click the **Start** button.

   To refresh the view, click the **Restart** button on the *Acquire* pane.

   – Data acquisition starts. Progress is displayed in the status bar.

   – In the *Plots* tab of the Workspace (see chapter *12.8.1 Plots tab of the Workspace* on page 160) the plots display the acquired data.

Adapting instrument settings during data acquisition

Procedure

1. Select the *Settings* pane (see chapter *12.5.3 Settings pane* on page 139).

2. In the "Voltage" column, adapt the voltage until the desired cell populations are displayed in the proper range in the plots and can be clearly separated.

   When measuring an unstained control, edit the values for all fluorescence parameters until the auto-fluorescence signals display within the first/between the first and second decade of log scale.

3. In the "Threshold" column, adapt the threshold values to reduce background for each parameter triggered. Increase or decrease the threshold settings if the background is still too high or if the cell population of interest is cut off.

   – The instrument settings are adapted.

   – The plots will automatically refresh according to the new instrument settings.

Creating a region

To select the events of interest, regions can be created on the plots.

Procedure

1. Select the *Home* tab of the Ribbon (see chapter *12.4.2 Home tab* on page 65).

2. To preselect creating an elliptical region, a rectangular region, a polygonal region, a linear region or a quadrant region, click the corresponding button on the *Region* group (see chapter *12.4.2 Home tab* on page 65).

   – The button is highlighted in orange.

3. For elliptical, rectangular or linear regions (on single-parameter plots), draw out the region on the plot.

   For a polygonal region, click on a plot to determine the first point of the region, then click to define additional points. Complete the polygon by clicking on the start point or by right-clicking.

   For a quadrant region, click on the plot at the point you wish the quadrant's intercept point to appear.

4. To assign a name to the region, select the region and double-click the region name on the plot. Enter a region name in the edit field or select a name from the drop-down list.

   Alternatively, select the region, select the *Region Format* tab of the Ribbon (see chapter *12.4.15 Region Format tab* on page 128) and use

the "Region Name" box to enter a name or to select a name from the drop-down list.

– A region is created.

Setting a parent gate

A region that contains all events of interest can be applied as a parent gate to the other plots so that only the relevant events are displayed on these plots. This gating will allow to focus on a subpopulation of cells only.

Procedure

1. In the Plots area of the Workspace, select the desired plot.

   To select several plots, hold the **Ctrl** key while selecting or draw a selection rectangle.

2. On the *Home* tab of the Ribbon (see chapter *12.4.2 Home tab* on page 65) click the arrow on the "Gate" box to expand a drop-down list and select the desired region to be applied as a parent gate.

   – A parent gate will be automatically created for the selected region.

   – The parent gate will be applied to the selected plots, indicated by a gate-coloured frame around the plots.

Stopping the measurement

If all described settings on the sample are set up, the measurement can be stopped. When the sample is empty (the stop electrode is reached) or if a stop condition is defined, measurement will stop automatically.

Procedure

1. On the *Acquire* pane (see chapter *12.5.4 Acquire pane* on page 140) click the **Stop** button.

   – The measurement is stopped.

After finishing a measurement a dialogue box will appear. Press **OK** button to proceed with an intermediate cleaning step. Please note that the remaining sample will be aspirated during the cleaning step. Alternatively, remove sample tube and press **OK** button. The cleaning step will be performed with air. To skip the intermediate cleaning step press **Cancel** button. The device is ready for the next sample measurement.

Performing a manual compensation

A manual compensation can be performed online (during the measurement) or offline (after data acquisition).

Procedure

1. On the *Compensation* tab of the Ribbon (see chapter *12.4.6 Compensation tab* on page 84) click the **Manual Compensation** button and the **Plot Compensation** button.

2. On the plot, draw the desired population of events into the required area of the plot.

    If using a quadrant, statistics are displayed on the *Compensation* tab of the Ribbon and can be used to arrange the population. Negative events are displayed in the lower left quadrant. A population negative for a parameter on Y scale should be placed in the lower right quadrant to the same height as the negative events. A population negative for a parameter on X scale should be placed in the upper left quadrant to the same range as the negative events.

    For fine adjustment of compensation, use the arrow buttons on the *Compensation* tab of the Ribbon. The direction of the arrow icons indicates the way the plot populations move rather than the way the compensation values change.

    – A manual compensation is performed.

Saving the Workspace and the instrument settings

On completion of a sample measurement, an FCS file is automatically saved containing the acquired data, the Workspace, the instrument settings and the compensation. Workspace and instrument settings can additionally be saved in separate files to use them independently.

Procedure

1. To save the Workspace of a Worklist item, select the *File* tab of the Ribbon (see chapter *12.4.1 File tab* on page 58) and select "File > Save > Save Workspace" or "File > Save > Save Workspace As".

    Alternatively, right-click the Worklist item in the Worklist to open the context menu and select "Workspace > Save Workspace (As)".

    – The Workspace is saved.

2. To save the instrument settings of a Worklist item, right-click the Worklist item in the Worklist to open the context menu and select "Instrument Settings > Save Instrument Settings".

    – The instrument settings are saved.

Defining the Worklist for remaining samples

Defining a Worklist for future measurements includes defining the Workspace, instrument settings and stop conditions. Instrument settings and stop conditions are loaded from the Workspace by default but can be changed in the Worklist.

Procedure

1. In the Worklist, enter a Lab # and sample IDs for the Worklist items as desired.

2. To apply the Workspace developed from the sample to all Worklist items, right-click the Worklist item to open the context menu and select "Workspace > Apply To".

   – A dialogue appears.

3. Select whether to apply the Workspace to the current panel or to the entire Worklist.

4. If differing instrument settings are to be used, click into the "Instrument Settings" cell of the Worklist item, select "Load from file" and select the desired instrument settings file.

5. To apply the differing instrument settings to other Worklist items, right-click the "Instrument Settings" cell to open the context menu and select "Apply to Panel".

6. To define differing stop conditions for the remaining samples, select the desired stop conditions from the *Stops* pane (see chapter *12.5.6 Stops pane* on page 144).

   Or right-click the "Stop Conditions" and "Stop Value" columns in the Worklist to open the context menu and select the desired stop conditions.

   – The Worklist for remaining samples is defined.

Saving the Worklist

The FCS data are saved automatically on completion of sample measurement. The Worklist can be saved separately.

Procedure

1. To save the Worklist, select the *File* tab of the Ribbon (see chapter *12.4.1 File tab* on page 58) and select "File > Save > Save Worklist" or "Save Worklist As".

   – The Worklist is saved.

6.6.2 Analysing data and creating reports using the Playlist

Data can be analysed offline by using a Playlist (see chapter *12.7 Playlist pane* on page 154).

All data created for analyses, including plots, statistics, compensation matrices and overlays, can be summarised in a page-based report that can be printed.

Analysing data using the Playlist

Procedure

1. To create a new Playlist, select the *File* tab of the Ribbon (see chapter *12.4.1 File tab* on page 58) and select "File > New > New Playlist".

   In Worklist mode, a new Playlist can also be created by clicking the **New** button in the *Worklist* pane (see chapter *12.6 Worklist pane* on page 146).

   Alternatively, drag and drop a Playlist file from the Windows Explorer into the Workspace or the *Playlist* pane.

   – A new blank Playlist is opened and displayed in the *Playlist* pane (see chapter *12.7 Playlist pane* on page 154).

   – The software is in Playlist mode.

2. To open a Playlist item in the Playlist, select the *File* tab of the Ribbon and select "File > Open > Open Listmode".

   Alternatively, click the **Open Listmode** button on the Quick Access Toolbar, or click the **Open Listmode** button on the *Home* tab of the Ribbon (see chapter *12.4.2 Home tab* on page 65), or drag and drop an FCS file from the Windows Explorer into the Playlist, or press **Ctrl** + **O**.

3. Plots, regions and gates can be edited in the *Plots* tab of the Workspace as desired (see chapter *12.8.1 Plots tab of the Workspace* on page 160).

4. Statistics can be displayed and edited in the *Results* tab of the Workspace (see chapter *12.8.2 Results tab of the Workspace* on page 171).

5. In Playlist mode, for setting compensations, the Compensation Wizard from the *Compensation* tab of the Ribbon can be used (see chapter *12.4.6 Compensation tab* on page 84).

6. To save the compensation, select the *File* tab of the Ribbon and select "File > Save > Save Compensation As".

7. For comparing plots to each other, overlay plots can be created manually (see chapter *12.8.3 Overlays tab of the Workspace* on page 174).

   In Playlist mode, the Overlay Wizard on the *Overlay* tab of the Ribbon can be used (see chapter *12.4.11 Overlay tab* on page 109).

   – The data are analysed.

Creating a report

A report can be created manually in the *Reports* tab of the Workspace (see chapter *12.8.4 Reports tab of the Workspace* on page 179). Alternatively, in Playlist mode, the Report Wizard from the *Report* tab of the Ribbon can be used (see chapter *12.4.12 Report tab* on page 114). When using the Report Wizard, first select the page orientation, page size and header and footer information (steps 1 to 4 in the following procedure).

Procedure

1. Select the *Reports* tab of the Workspace.

   – A blank report page is displayed.

   – The Ribbon switches to its *Report* tab.

2. From the *Page Setup* group on the *Report* tab of the Ribbon (see chapter *Pages group* on page 119), select the desired page orientation (portrait or landscape) and page size (A4 or letter).

3. From the *Insert* group on the *Report* tab of the Ribbon (see chapter *Insert group* on page 120select the items to be shown in the report.

4. Click on the desired section of the report page (header, body, footer) to insert the selected item in a default size or draw an insertion rectangle to get a custom size.

   If using the Report Wizard, select the items to be shown in the header and footer sections of all pages of the report here before starting the Wizard.

5. Use the options provided in the *Report* tab of the Ribbon to insert and organise pages, to edit text and other items and to arrange items.

   – A report is created.

7 Troubleshooting

If no data are displayed in the plots or histograms after acquisition process, air bubbles or contaminations may have blocked the flow cuvette.

To solve this problem perform a de-bubble or de-clog procedure.

7.1 Performing a de-bubble procedure

Requirements

- Log-in as a user with the respective role.

Procedure

1. On the *Cytometer* tab of the Ribbon, click the **De-bubble** button.
   - An instruction dialogue is displayed.
2. Follow the instructions until the finish message is displayed.
   - After successful de-bubble process the device is ready for the sample measurements.
   - If the de-bubble process was not successful, please contact your local Sysmex representative.

7.2 Performing a de-clog procedure

Requirements

- Log-in as a user with the respective role.

Procedure

1. On the *Cytometer* tab of the Ribbon, click the **De-clog** button.
   - An instruction dialogue is displayed.
2. Follow the instructions until the finish message is displayed.
   - After a successful de-clog process the device is ready for the sample measurements.
   - If the de-clog process was not successful, please contact your local Sysmex representative.

7.3 Sheath fluid, waste and fluids

Observation	Possible solution
Sample flow is very slow or is not running.	Check that the waste bottle is closed properly and that the bottle is not cracked.
	Check all visible tubes and make sure that they are not pinched.
	Make sure that no air bubbles are trapped in the yellow inline filter of the sheath fluid bottle.
	Check the connection of the sample tube to the sample port. Decant sample to another tube and measure again.
	Perform a priming procedure.
	Perform a cleaning procedure.
	Shut down the device, including the main switch on the back side of the device. Wait 3–5 minutes and re-start the system.
	Check the speed settings.
Sample flow is very fast even with low speed.	Change the yellow inline filter (50 µm) of the sheath fluid bottle.
	Check if the visible sheath tube is pinched or blocked.
	Shut down the device, including the main switch on the back side of the device. Wait 3–5 minutes and re-start the system.
	Check the speed settings.

7.4 Calibration and Count Check Beads (low, medium, high)

Observation	Possible solution
The results of the Count Check Beads (low, medium, high) measurements are not within ± 10% range of the lot-specific concentration stated on the bottle.	Shake the bottle vigorously (e.g. by vortexing) and repeat the measurement.
	Check the date of expiry of the Count Check Beads (low, medium, high).
	Perform a priming procedure.
	Perform a cleaning procedure.
The results of the Calibration Beads measurements are not in the preselected regions of the original configuration script.	Check in software if you have loaded the correct configurations for your measurement.
	Shake the bottle vigorously (e.g. by vortexing) and repeat the measurement.
	Check the date of expiry of the Calibration Beads.
	Perform a priming procedure.
	Perform a cleaning procedure.

7.5 Measurements and data acquisition

Observation	Possible solution
The device emits an unusual / noisy sound after starting the measurement.	Check that the waste bottle is closed properly and that the bottle is not cracked.
	Check all visible tubes and make sure that they are not pinched.
	Check that liquid (sheath fluid and sample) is dropping into the waste bottle after starting the measurement.
	Clean the two electrodes of the sample port carefully by wiping them with a soft tissue soaked in decontamination solution.
	Perform a de-clog procedure.
There is no data acquisition visible (peaks/dots in histogram/dot plot) during the RUN phase	Check in software if the laser/s is/are switched on.
	Check if the right trigger parameter has been selected (choose FSC trigger as default setting, select SSC for very small cells, e.g. bacteria).
	Check gain values and the threshold.
	Increase the flow rate.
	Perform a de-clog procedure.

8 Cleaning

> **NOTICE**
>
> **Contaminated sheath fluid bottle**
>
> A contaminated sheath fluid bottle disturbs the proper operation.
>
> ■ Clean the sheath fluid bottle regularly.

> **NOTICE**
>
> **Using incorrect cleaning solvents**
>
> Using incorrect cleaning solvents when cleaning the casing and screen could damage the device.
>
> ■ Do not use any organic solvents, nitro thinner, benzol, alcohol or highly concentrated bleach.
>
> ■ Only use special screen cleaner.

8.1 Cleaning the device

The following list gives an overview of the various essential cleaning work on the device.

■ Clean the device casing with a soft cloth on a regular basis carefully. Water should not enter the device or peripheral devices or come into contact with electric connections and switches.

■ Clean the screen with a soft cloth. Always use special screen cleaner.

■ Clean the sheath fluid bottle with distilled water and a brush. Flush with clean distilled water several times.

■ Empty the waste bottle after and before each user session. When using biohazardous samples, a volume of 50 ml of Hypochlorite Solution (Order No. 04-4012_R) should be introduced into the waste bottle after measurement or before emptying the waste.

If the device will not be used for longer periods, clean the flow system with distilled water. Put a sample tube half-way filled with distilled water in the sample port. Clean the waste bottle and the sheath fluid bottle, wipe top dry.

8.2 Performing a cleaning procedure

The device can be cleaned between sets of samples using a Cleaning Solution (Order No 04-4009_R) followed by a sample tube with sheath fluid. This procedure will allow you to significantly reduce cross contamination and reduce the background. There are three different levels of intensity available that can be set: Default, quick or deep.

Requirements

■ Log-in as a user with the respective role.

Procedure

1. On the *Cytometer* tab of the Ribbon, click the **Clean** button.

   – An instruction dialogue is displayed.

2. Follow the instructions until the finish message is displayed.

   – The device is ready for the sample measurements.

9 Maintenance

Servicing is to be performed by an authorised service engineer. Please contact your local Sysmex representative for further instructions.

Sequence	Activity	Minimum user qualification level
Daily maintenance	Prime the device by using the Prime function (see chapter *6.2.3 Performing a Prime* on page 26). Priming should be performed: – After starting the device; – After the sheath fluid was changed; – After the waste bottle was emptied.	Instructed personnel
	The system should not be left electrical switched on for several days without using it. Shut down the system completely each day.	Instructed personnel
Weekly maintenance	Clean the device according to cleaning instructions (see chapter *8 Cleaning* on page 42).	Instructed personnel
Quarterly maintenance	Inline filter exchange according to instructions.	Instructed personnel
Preventive maintenance	A preventive maintenance procedure needs to be performed on a regularly basis. Please contact the service department of your local Sysmex representative for further instructions.	Authorised service personnel

10 Disposal

Service life of the device is limited by aging of mechanical components. Regular cleaning, maintenance and service ensure high-quality function and prevent any premature end-of-life.

The device was developed and manufactured with high-quality materials and components which can be recycled.

NOTICE

Contamination of the environment

Electronic and electrical waste can contaminate the environment.

■ Decontaminate the device.

■ Dispose of electronic and electrical equipment separately opposed to regular waste according to local regulations and laws.

■ Dispose of this product by taking it to your local collection point or recycling centre for such equipment.

Electrical and electronic equipment must be disposed of separately from normal waste.

Dispose of this product by taking it to your local collection point or recycling centre for such equipment. This will help to protect the environment.

For further information, please contact your local Sysmex representative.

11 Technical data

⚠WARNING

Heavy weight

When moving the device (packed or unpacked) or removing it from its packaging, a person is exposed to a high risk of damaging his or her body due to the heavy weight of the device.

■ At least two persons are required for transport and installation.

ℹInformation

This is a Class A product. In a domestic environment, this product may cause radio interference in which case the user may be required to take adequate measures.

Hardware data	
Dimensions	Width: 500 mm
	Depth: 470 mm
	Height: 370 mm (670 mm with open display)
Weight	40 kg
Maximum sound	<70 dBA
Power	100 to 240 V AC
	50/60 Hz
	200 VA
Fuse	T 4.0 A 250 V
Overvoltage category	2/II
EMC class	Class A
Degree of protection	IP 20

Hardware data	
Operating environment	Temperature: Operation: 15 °C to 30 °C Transport and storage: 5 °C to 50 °C
	Relative humidity: Operation: 20 % to 85 %, non-condensing Transport and storage: 20 % to 85 %, non-condensing
	Room: Clean environment. Direct sun light should be avoided

Operation specifications	
Setup time	Max. 5 minutes
Parameters detected	Up to 8 optical parameters: FSC, SSC, FL1, FL2, FL3, FL4, FL5, FL6
Particle size range	0.1 µm to 100 µm
Maximum data	15000 events/second
Data acquisition	Max. 15000 events/second
Acquisition stop condition	Event-based or volume-based
Trigger	On all parameters, on multiple parameters or on single trigger parameter, selectable in software
Data resolution	16 bit

Application specifications	
Application	Immunophenotyping, DNA analysis, apoptosis, microbiology, industrial applications, 3 to 6 colour analysis. True Volumetric Absolute Counts = counting per volume
True Volumetric Absolute Counting	Based on precise counting and mechanical fluid volume measurement no need for reference sample or beads.

Optics (dependent on system configuration)	
Laser output	V1: Blue laser 488 nm 50 mW
	V2: Blue laser 488 nm 50 mW, Red laser 638 nm 25 mW
	V4: Blue laser 488 nm 50 mW, Red laser 638 nm 25 mW, UV-LED 365 nm
	V5: Violet laser 405 nm 100 mW, Blue laser 488 nm 50 mW, Red laser 638 nm 25 mW
	V6: UV laser 375 nm 60 mW, Blue laser 488 nm 50 mW, Red laser 638 nm 25 mW
	V7: Blue laser 488 nm 50 mW, Yellow laser 561 nm 100 mW, Red laser 638 nm 25 mW
	V8: Blue laser 488 nm 50 mW, Red laser 640 nm 40 mW
	V9: Blue laser 488 nm 50 mW, UV-LED 365 nm
	V10: Blue laser 488 nm 50 mW, Orange laser 594 nm 50 mW, Red laser 638 nm 25 mW
	V11: Violet laser 405 nm 100 mW, Blue laser 488 nm 50 mW
	N1: Blue laser 488 nm 50 mW, Red laser 640 nm 40 mW
Laser class	Laser class I
	(Laser class IIIb if device is opened and laser protection is removed)
Detectors	V1: 1 to 5 (FSC, SSC, FL1, FL2, FL3)
	V2: 1 to 6 (FSC, SSC, FL1, FL2, FL3, FL4)
	V4: 1 to 8 (FSC, SSC, FL1, FL2, FL3, FL4, FL5, FL6)
	V5: 1 to 8 (FSC, SSC, FL1, FL2, FL3, FL4, FL5, FL6)
	V6: 1 to 8 (FSC, SSC, FL1, FL2, FL3, FL4, FL5, FL6)
	V7: 1 to 8 (FSC, SSC, FL1, FL2, FL3, FL4, FL5, FL6)
	V8: 1 to 7 (FSC, SSC, FL1, FL2, FL3, FL5, FL6)
	V9: 1 to 6 (FSC, SSC, FL1, FL2, FL3, FL5)
	V10: 1 to 8 (FSC, SSC, FL1, FL2, FL3, FL4, FL5, FL6)
	V11: 1 to 8 (FSC, SSC, FL1, FL2, FL3, FL4, FL5, FL6)
	N1: 1 to 6 (FSC, SSC, FL1, FL2, FL3, FL4)
Filters	Standard setup and filters for all parameters according to laser configuration
Optical coupling	Standard objective mount with high numerical aperture objective, high numerical aperture immersion gel coupling, e.g. for detection of weak cytokines (option)
Excitation optics	Elliptical 15 μm × 100 μm at 488 nm, other beam geometries upon request

Fluids	
Fluid system	Completely closed system for sheath fluid and sample volumes; no fluid droplets or aerosols generated nor released from the device
Flow cuvette	Synthetic quartz flow cuvette with small centric flow channel (capillary diameter 350 µm x 250 µm) for laminar sample transport with sheath fluid.
Sample delivery	Computer controlled precision syringe pump for contamination-free sample transport. Built-in vacuum pump for waste bottle; vacuum pressure is adjustable (computer controlled)
Sample volume	Continuous up to 1200 µl Minimal content of sample volume 850 µl 200 µl for electrode based precision absolute counting (other counting volumes upon request) Minimal sampling volume 10 µl 5 µl to 1000 µl for syringe-based precision absolute counting
Flow rates	Sample volume speed adjustable continuously between 0.1 µl/s and 20 µl/s) Sheath fluid flow continuously adjustable in expert mode
Fluids volume	2 glass bottles (each litre) for sheath fluid and waste
Bio safety system	Avoids sample droplets and sample cross contamination (computer controlled)

12 Software description

The device is operated by means of the CyFlow® software. This chapter describes the software functions. Performing routine tasks using the software is described in chapter *6.6 Software operation* on page 30.

12.1 Overview

Data acquisition, device control and data analysis are controlled and performed by the integrated software.

The software can be run in two different modes:

- In Worklist mode, data can be acquired. The hardware and software settings for each measurement are predefined and organised in a list called Worklist.

- In Playlist mode, acquired data can be analysed and settings for future measurements can be prepared. Files are organised and edited in a Playlist.

Opening a Playlist sets the software to Playlist mode. Opening a Worklist sets the software to Worklist mode. Only one Worklist or Playlist can be open at a time.

When starting the device, the software is opened in Worklist mode.
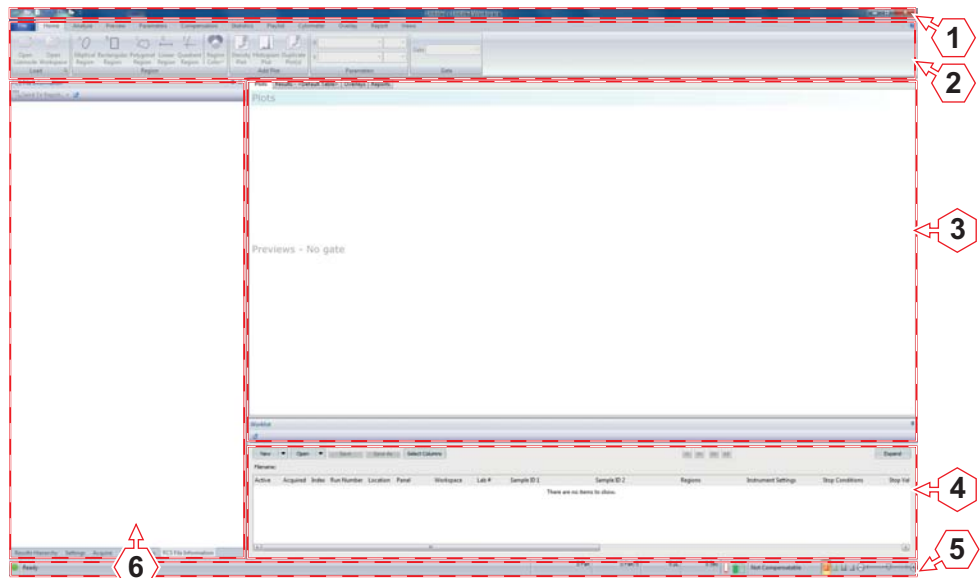
1	Quick Access Toolbar	4	Worklist pane
2	Ribbon	5	Status bar
3	Workspace	6	Panes

The default view of the software window contains the following elements:

- **Quick Access Toolbar:** The Quick Access Toolbar provides quick access to some of the most commonly used options in the software.

- **Ribbon:** The Ribbon provides access to the main functionality groups of the software. The Ribbon is organised into a set of tabs.

- **Workspace:** The Workspace contains four tabs for creating and editing plots, results, overlays and reports.

- **Worklist pane:** In the *Worklist* pane, a Worklist can be created or opened. The Worklist then can be edited.

- **Status bar:** The status bar displays status information. On the right, it contains functions for resizing objects in the Workspace.

- **Panes:** This area of the software window contains a set of panes for editing instrument settings, for acquiring data and for displaying information on FCS files.

If a Playlist is opened, the software changes to *Playlist* mode. The *Worklist* pane is no longer displayed. A *Playlist* pane is displayed on the left-hand side of the Workspace and nested with the other panes.

12.2 Views of the software window

The software window view can be adapted by displaying or hiding the panes and by docking and undocking the panes. Undocked panes can be positioned and docked as required.

The view settings are persisted between sessions.

Displaying and hiding the panes

By default, the panes are open and pinned to the left-hand side of the software window. The tabs of the panes are located at the bottom of the panes.
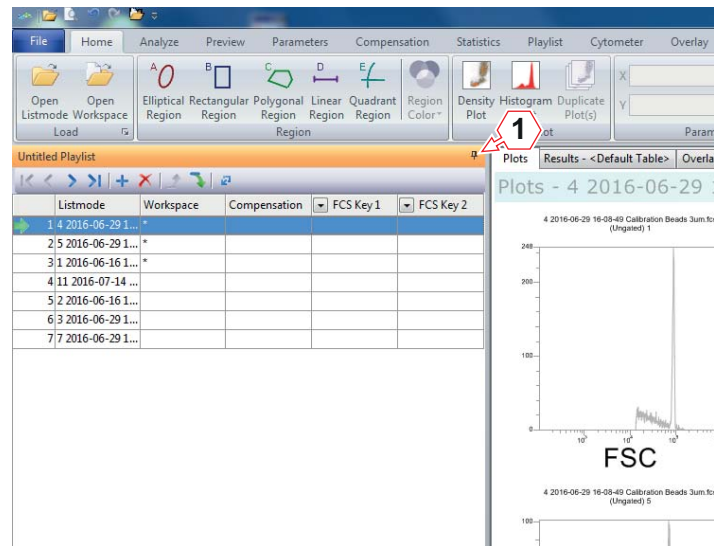
1   **Auto Hide** button

To hide the panes, click the pin-shaped **Auto Hide** button.

The panes are hidden and the tabs of the panes appear on the left-hand side of the software window.
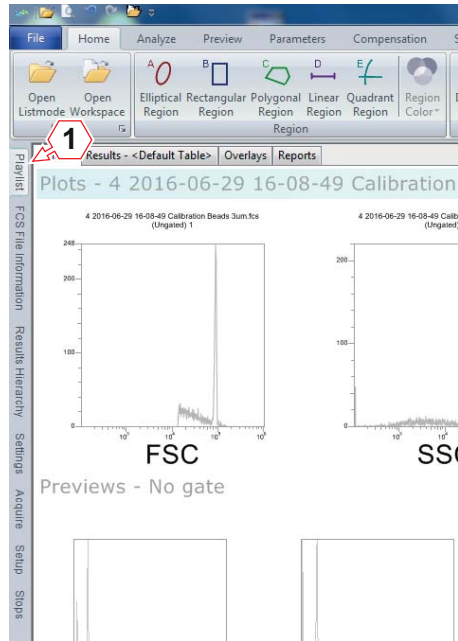
1 Tabs of the panes

To re-expand a pane, click the desired tab. The pane is visible as long as the cursor is over the pane.

To refix the panes in this open position so they are always visible, click the **Auto Hide** button on the expanded pane.

Undocking and positioning the panes

The panes, including the *Worklist* pane and the *Playlist* pane, can be positioned and docked as required.

To undock a pane, the following options are available:

- Click the title bar and relocate the pane by dragging and dropping. When a pane is selected, its title bar is highlighted in orange.

- Double-click the title bar.

- Click the **Float** button.

The pane can then be dragged to the desired position.
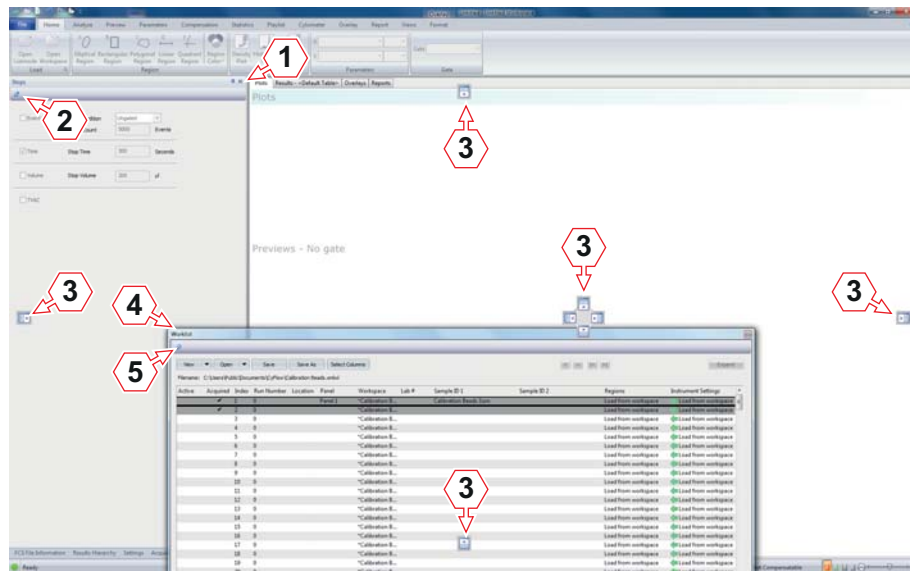
1	Title bar of docked pane	4	Title bar of undocked pane
2	**Float** button	5	**Dock** button
3	Relocation arrows		

To dock the pane to the previous position, double-click the title bar again or click the **Dock** button.

The pane can be docked to other positions by using the blue relocation arrows that are displayed while dragging the pane. To dock the pane to bottom, top, right or left of the software window, drag the cursor onto the corresponding relocation arrow. When the cursor is over a relocation arrow, the position where the pane will be located on dropping is highlighted in blue.

The *Worklist* pane can be docked and nested with the other panes by dragging it and placing the cursor in the centre of the relocation arrows on the left-hand side of the software window.
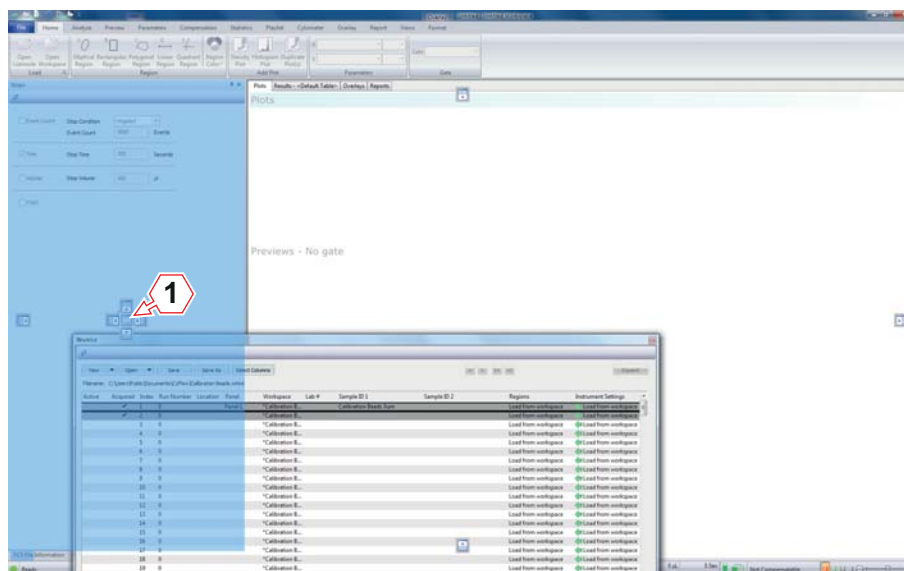
1   Centre of the relocation arrows

The size of docked panes can be adjusted by dragging the splitter bar to the desired position.

To get back to the default view of the software window, select the *Views* tab of the Ribbon and click the **Default Position** button.
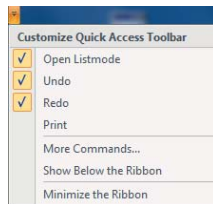
12.3 Quick Access Toolbar

The Quick Access Toolbar provides quick access to some of the most commonly used options in the software.

By default, the Quick Access Toolbar contains an **Open Listmode** button, an **Undo** button and a **Redo** button.

You can customise the Quick Access Toolbar. To do this, click on the arrow next to the buttons in the Quick Access Toolbar. A drop-down list appears.

Adding a button to the Quick Access Toolbar

The **Open Listmode**, **Undo**, **Redo** and **Print** menu items in the drop-down list allow you to add or remove the respective buttons. Check the menu item to add the corresponding button to the Quick Access Toolbar.

The **More Commands…** menu item allows you to add more buttons to the Quick Access Toolbar. When you select the menu item, a dialogue box appears. In this dialogue box you can choose further buttons for the Quick Access Toolbar.

Removing a button from the Quick Access Toolbar

Uncheck the **Open Listmode**, **Undo**, **Redo** or **Print** menu item in the drop-down list to remove the corresponding button from the Quick Access Toolbar.

If you want to remove a different button from the Quick Access Toolbar, use the **More Commands…** menu item.

Alternatively, right-click the button you wish to remove and select the **Remove from Quick Access Toolbar** menu item from the context menu.

Showing the Quick Access Toolbar below the Ribbon

Usually the Quick Access Toolbar is located above the Ribbon in the top left corner of the software window.

To place the Quick Access Toolbar below the Ribbon, click the **Show Below the Ribbon** menu item in the drop-down list.

When the Quick Access Toolbar is displayed below the Ribbon, the menu item in the drop-down list is named **Show Above the Ribbon**.

Minimising the Ribbon

Usually the Ribbon is fully visible. But if you need more place on the display, you have the possibility to minimise the Ribbon. Then, only the tabs are visible.

Check or uncheck the **Minimize the Ribbon** menu item in the drop-down list to toggle the Ribbon between minimised and restored.

Changing to another tab restores the Ribbon temporarily.

12.4 Ribbon

The Ribbon is the area at the top of the software window that presents an easy-to-browse main set of commands.

The Ribbon is organised into a set of tabs which represent the main functionality groups of the software.

12.4.1 File tab

The *File* tab contains file open, printing and export options. The *File* tab also provides access to the user management and to the software options.
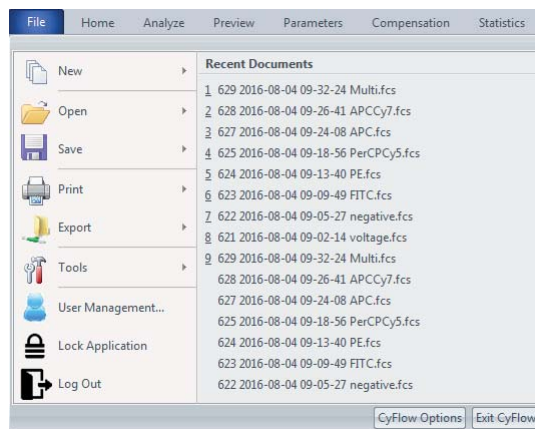
Recent documents

When clicking on the *File* tab, a list of recently used documents is displayed. In Worklist mode, recently used Worklist files are shown. In Playlist mode, the list comprises of recently used listmode, Workspace and Playlist files. A maximum of 10 files can be presented in this list.

Click a file name to open the file.

New

The *New* menu contains the following options:

■ **New Playlist:** Click the menu item to create a new Playlist. Selecting this option will clear the current Playlist or Worklist, and a new blank Playlist will be displayed in the *Playlist* pane.

■ **New Worklist:** Click the menu item to create a new Worklist. Selecting this option will clear the current Worklist or Playlist.

A new Worklist with 400 blank Worklist items is created.

Open

The *Open* menu contains the following options:

- **Open Worklist…:** Click the menu item to open a Worklist file (XMLWL file). Only one Worklist file can be open at a time.

- **Open Listmode…:** This menu item is only displayed in Playlist mode. Click the menu item to open one or more listmode files (LMD or FCS files) and select the desired files to add them to the Playlist. The Playlist item of the first added listmode file will be activated automatically in the Playlist.

- **Open Workspace…:** This menu item is only enabled if a listmode file of a Playlist is open in the Workspace. Click the menu item to open a Workspace file (XMLW file) and select the desired file to load it with the currently open listmode file.

- **Open Playlist…:** Click the menu item to open a Playlist file (XMLP file). Only one Playlist file can be open at a time.

- **Open Compensation File…:** This menu item is only enabled if a compensable listmode file of a Playlist is open in the Workspace. Click the menu item to open a compensation file (XMLC file) and select the desired file to load it into the currently open Playlist item. If a new compensation file is selected, this will replace the one currently loaded. Only one compensation file can be opened at a time and compensation is only available on high-resolution data files.

Save

In Worklist mode, the *Save* menu contains the following options:

- **Save Worklist:** Click the menu item to save the current Worklist with the current Worklist file name.

  When saving a Worklist, Workspace file paths and file extensions (if present) are removed and the Workspace files are saved within the associated panel within the Worklist file. If there are multiple Workspace files present within a panel with the same name, a unique identifier is added to the Workspace file name to differentiate the Workspace files.

- **Save Worklist As…:** Click the menu item to save the current Worklist with a new file name and an XMLWL extension.

- **Save Workspace:** Click the menu item to save the current Workspace with the current Workspace file name. A Workspace includes plots, regions, gates, colour precedence, selected statistics and displayed parameters. The Workspace file is always saved with an XMLW extension.

- **Save Workspace As…:** Click the menu item to save an untitled Workspace or the current Workspace with a new file name and an XMLW extension.

In Playlist mode, the *Save* menu contains the following options:

- **Save Workspace:** Click the menu item to save the current Workspace with the current Workspace file name. A Workspace includes plots, regions, gates, colour precedence, selected statistics and displayed parameters. The Workspace file is always saved with an XMLW extension.

- **Save Workspace As…:** Click the menu item to save an untitled Workspace or the current Workspace with a new file name and an XMLW extension.

- **Save Playlist As…:** Click the menu item to save the current Playlist with a new file name and an XMLP extension. For each Playlist item, the listmode file path, the Workspace and the compensation are saved. If there are multiple Workspace or compensation files within the Playlist with the same name, a unique identifier will be added to the name of the respective file. The report, software version and build number are also saved in the Playlist.

  If some of the listmode files in the Playlist have relative paths, a message indicating that the Playlist is an exported Playlist is displayed. You will have the option to export the Playlist or to save the Playlist as a regular Playlist.

- **Save Compensation As…:** This menu item is only enabled if a compensable listmode file is loaded. Click the menu item to save the current compensation settings with a new file name and an XMLC extension, or to apply the current compensation settings to other compensable files.

- **Save Plot(s) As…:** This menu item is only enabled if one or more plots are selected in the Plots area. Click the menu item to save these plots as image file. A dialogue box appears. Enter a name for the plot(s), select the file format (BMP, JPEG, GIF, TIFF or PNG), select the destination folder and click the **Save** button. The size of the plot in the Plots area will determine the size of the saved image. To increase the size of the saved image, increase the size of the plot using the resize slider in the status bar.

Print

The *Print* menu contains the following options:

- **Print…:** Click the menu item to print plots, statistics or reports. A dialogue box appears. Select the printer, define the print range and the number of copies and select what to print (plots and statistics for the current Workspace or the current report).

- **Print Preview:** Click the menu item to display a preview of the current plots in the Workspace or the current report to be printed. Modify the printing options if necessary and click the **Print…** button. A dialogue box appears. Change the printing options if necessary and click the **OK** button.

- **Print Setup…:** Click the menu item to select the required default setup for your printer.

Export

The *Export* menu is only available in Playlist mode except for the *Export Report to PDF* menu item which is also accessible in Worklist mode.

The menu contains the following options:

- **Export Statistics to CSV:** This menu item is only enabled when there are results present in the results table. The results exported to a CSV file are the columns displayed in the results table in the *Results* tab of the Workspace. The format of the export is either single row per file or one row per result, depending on the selection in the software options (see chapter *12.10 CyFlow Options* on page 187). Click the menu item to save a CSV formatted file containing the results of the currently open Playlist item only. A dialogue box appears. Enter a name for the export, select the destination folder and click the **Open** button.

  If the entered file name already exists and the format of the exported data matches the formatting of the data in the file, the results are appended to those already in the file.

  If the entered file name already exists but the headers in the two files do not match, you will have the option to overwrite the existing file. After the file has been saved, the Windows Explorer opens with the exported file highlighted.

- **Export All Statistics to CSV:** This menu item is only enabled when there are results present in the results table in the *Results* tab of the Workspace. Click the menu item to save a CSV formatted file containing the results of all files in the Playlist. A dialogue box appears. Enter a name for the export, select the destination folder and click the **Open** button.

  If the entered file name already exists and the format of the exported data matches the formatting of the data in the file, the results are appended to those already in the file.

  If the entered file name already exists but the headers in the two files do not match, you will have the option to overwrite the existing file. After the file has been saved, the Windows Explorer opens with the exported file highlighted.

- **Export Playlist:** This menu item is for exporting a Playlist for sharing with other users and colleagues or for archiving. Any modifications to the Playlist need to be saved before it can be exported.

  Click the menu item and select the destination folder for the export.

  Click the **OK** button to perform the export. A folder with the same name as the current Playlist is created inside the destination folder. The exported Playlist is placed in it. The exported listmode files are placed in a subfolder called "LMD". The exported image files are placed in a subfolder called "Images". Worklist and compensation files are stored as part of the Playlist file (XMLP file).

If the original Playlist (before export) contains the same LMD file in more than one of its items, only one copy of the file is in the export LMD folder. If the original Playlist (before export) contains LMD files that have the same name but are in different folders, they are all placed in the exported LMD folder with numbers in parenthesis appended to the file name, so they can be distinguished.

If a Playlist of the same name already exists in the selected folder, you will have the option to replace the existing Playlist. If you choose not to replace the existing Playlist, the new Playlist is saved with an appended number to the name to distinguish it from the existing one.

If some of the LMD or image files that are to be exported have the same name as the ones in the export LMD and images folders, the Windows Copy File dialogue is displayed and allows you to resolve the conflicts. When re-exporting a Playlist to the same folder it was exported before, you should use Copy & Replace in the Windows Copy File dialogue. Otherwise duplicate files will be created.

For an exported Playlist to open properly, the folder structure of the export folder has to be preserved.

- **Export Statistics to XML:** The menu item is enabled when a Playlist item containing a listmode file is active. Click the menu item to save an XML file containing the results of the currently open file only. A dialogue box appears. Enter a name for the export, select the destination folder and click the **Open** button.

  If the entered file name already exists and the format of the exported data matches the formatting of the data in the file, the results are appended to those already in the file. If the entered file name already exists but the headers in the two files do not match, you will have the option to overwrite the existing file.

  After the file has been saved, the Windows Explorer opens with the exported file highlighted.

- **Export All Statistics to XML:** The menu item is enabled if one or more Playlist items contain a listmode file. Click the menu item to save an XML file containing the results of all files in the Playlist. A dialogue box appears. Enter a name for the export, select the destination folder and click the **Open** button.

  If the entered file name already exists and the format of the exported data matches the formatting of the data in the file, the results are appended to those already in the file. If the entered file name already exists but the headers in the two files do not match, you will have the option to overwrite the existing file.

  After the file has been saved, the Windows Explorer opens with the exported file highlighted.

- **Export Report to PDF:** The menu item is enabled if there is an active item in the Worklist or the Playlist, the *Reports* tab of the Workspace is open and if acquisition is not in progress. Click the menu item to export a report as PDF file and select a name and a destination folder for the PDF.

When in Worklist mode, reports are exported by panels. If a Worklist contains several panels, a corresponding message is displayed and you will have the option to export only the current panel report or all panel reports.

On exporting, the software creates a subfolder inside the destination folder with a name containing the Worklist name and a time stamp. Each panel will be exported in a PDF file. The name of each PDF file contains the panel name, an index range, the index of the first item and the index of the last item of that panel.

Tools

The *Tools* menu contains the following option:

■ **Listmode File Keywords...:** This menu item is only enabled if a Worklist item or Playlist item containing a listmode file is open in the Workspace. Click the menu item to open the Keywords dialogue. The dialogue displays the keywords and values from the header of the active listmode file. Click the **Copy Keywords** button to copy the keywords and values displayed in the dialogue to the clipboard. This list can then be pasted into other applications (e.g. Microsoft® Excel).

User Management

Click the button to open the Task Pad. The Task Pad contains options to change your password, to lock the software or to view an Audit Log report.

If logged in with user administration privilege, the Task Pad contains options for administration of user accounts.
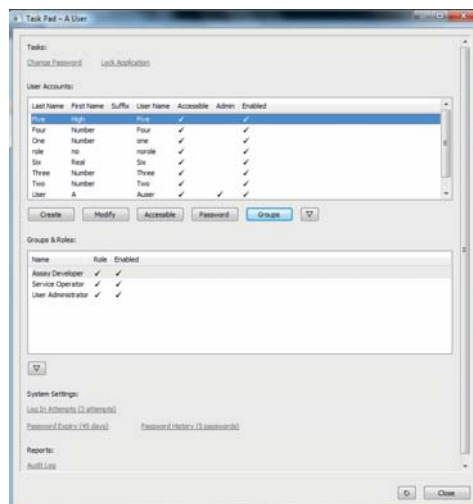
The table lists the current user accounts.

The following buttons are provided to create and modify the user accounts:

■ **Create:** Click the button to create a new user account.

■ **Modify:** Select a user account in the table and click the button to modify the user account.

- **Accessible:** Click the button to restore access for the selected user. Access is locked when a user enters a defined number of incorrect passwords consecutively. The number is defined in the "Log In Attempts" item.

- **Password:** Click the button to enter a temporary password for the selected user.

- **Groups:** Click the button to define the roles for the selected user. Click in the "Status" column to select or deselect the corresponding role for a user.

- **Sort Order:** Click the button to toggle the sorting order in the table between sorting by first name or sorting by last name.

- **Refresh:** Click the button to refresh the table.

The following options for system settings and reports are provided :

- **Log In Attempts:** Click the item to define the number of login attempts before the account will be locked (1 to 10 attempts).

- **Password Expiry:** Click the item to define the number of days before expiry of the passwords (1 to 90 days).

- **Password History:** Click the item to define the number of passwords that is stored in the password history (3 to 12 per user account).

- **Audit Log:** Click the item to view an Audit Log report.

Log Out

Click the button to log out. You are logged out, and a log-in dialogue is displayed.

If acquisition is in progress when logging out, a message is displayed and you will have the option to abort the acquisition.

CyFlow Options

Click the button to open the software options (see chapter *12.10 CyFlow Options* on page 187).

Exit CyFlow Software

Click the button to close the software. If changes have been made, you will be prompted to save the Playlist or Worklist before closing the software.

If acquisition is in progress, you will be prompted to stop the acquisition before exiting the software.

If acquisition is not in progress, a message is displayed, and you will have the option to perform a shutdown cleaning procedure. Click the **Yes** button to start the shutdown cleaning procedure. Once started, the software guides you through the process.

12.4.2 Home tab

The *Home* tab is the default tab and provides all the essential controls for analysing files in one convenient place. Here, files can be selected, regions created and plots created and gated.

Load group

The *Load* group contains controls for opening listmode files and Workspaces.
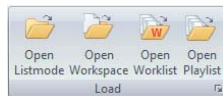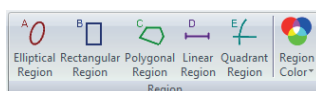

The *Load* group contains the following options:

- **Open Listmode:** The button is only enabled in Playlist mode. Click the button to open one or more listmode files (LMD or FCS files) and select the desired files to add them to the Playlist. The Playlist item of the first added listmode file will be activated automatically in the Playlist.

- **Open Workspace:** The button is only enabled if a listmode file of a Playlist is open in the Workspace area. Click the button to open a Workspace file (XMLW file) and select the desired file to load it with the currently open listmode file.

- **Most recently used:** The small button at the bottom right of the group is only enabled if a Playlist is open. Click the button to open a dialogue that lists the 10 most recently used listmode files and Workspace files.

  Select a listmode file listed under "Most Recent Listmode Files" to add that file to the end of the Playlist and open that file.

  Select a Workspace file listed under "Most Recent Workspaces" to assign that Workspace to the currently open file in the Playlist. This option is only available when a listmode file is open.

Region group

The *Region* group contains controls for creating and editing regions. You can create a maximum of 256 regions per Workspace. Once this number is reached, the region creation buttons in the *Region* group become disabled.

The *Region* group contains the following options:

- **Elliptical Region:** Click the button to preselect creating an elliptical region on a dual parameter plot.

- **Rectangular Region:** Click the button to preselect creating a rectangular region on a dual parameter plot.

- **Polygonal Region:** Click the button to preselect creating a polygonal region on a dual parameter plot.

- **Linear Region:** Click the button to preselect creating a linear region on a single parameter plot.

- **Quadrant Region:** Click the button to preselect creating a quadrant region on a dual parameter plot.

- **Region Color:** The button is only enabled if a region is selected on a plot. Click the button to display a colour control and select the desired colour for the region.

  The region colour on the plot will update. If the region has an associated gate, changing the region colour will not change the gate colour. The gate colour can be changed using the **Edit Gates** button in the *Analyze* tab (see chapter *Gate group* on page 69).

For further information on defining and editing regions (see chapter *Creating regions* on page 165).

Add Plot group

The *Add Plot* group contains controls for creating plots in the Workspace. The buttons are only available if a Playlist item or a Worklist item is open in the Workspace area.
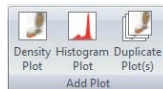
The *Add Plot* group contains the following options:

- **Density Plot**: Click the button to add a dual parameter density plot displaying the first two parameters of the current listmode file. This will normally display a plot showing Forward Scatter against Side Scatter.

- **Histogram Plot**: Click the button to add a single parameter histogram plot displaying the first parameter in the current listmode file. This will normally display the Forward Scatter.

- **Duplicate Plot**: Click the button to add another copy of the selected plot to the current Workspace. If the current plot has cell cycle mode enabled, the duplicate plot will display only the raw histogram with cell cycle disabled.

Parameters group

The controls in the *Parameters* group are used to change the X and Y parameters of the currently selected plots in the Workspace area. The scaling of parameters can be changed.

The *Parameters* group contains the following options:

- **X and Y Parameter Selection:**

  The X and Y parameter for the currently selected plots can be set using these boxes. Click the arrow to expand a drop-down list and select the desired parameter. All parameters enabled in the current file are available for selection.

  If all the selected plots have the same parameters, the boxes will be populated with the current parameter name. If all the selected plots do not have the same parameters, the boxes will be empty.

- **X and Y Parameter Scale Selection (Lin/Log):**

  This option allows changing the scaling of the chosen parameters for all selected plots. Click the arrow to expand a drop-down list and select the desired scaling. Lin, Log and V-Log options are available for selection. This option will not be available if the opened file is a low-resolution data file.

  If all the selected plots have the same parameter scaling, the boxes will be populated with the current parameter scale name. If all the selected plots do not have the same parameter scaling, the boxes will be empty.

Gate group

The *Gate* group options allow the selection of gating for the currently selected plots.

The *Gate* group contains the following option:

- **Gate:** To select a gate for the currently selected plot(s), click the arrow to expand a drop-down list and select the desired gate. All existing gates and regions are available for selection. If a region or quadrant is selected, a gate will be automatically created for that region or quadrant. Gates can be edited using the **Edit Gates** button in the *Analyze* tab (see chapter *Gate group* on page 69).

  Select the "Ungated" option to set selected plots to be ungated.

12.4.3 Analyze tab

The *Analyze* tab provides controls for analysing and modifying the plots in the Plots area of the Workspace.

Region group

The *Region* group contains options for creating and editing regions. You can create a maximum of 256 regions per Workspace. Once this number is reached, the region creation buttons in the *Region* group become disabled.

The *Region* group contains the following options:

- **Elliptical Region:** Click the button to preselect creating an elliptical region on a dual parameter plot.

- **Rectangular Region:** Click the button to preselect creating a rectangular region on a dual parameter plot.

- **Polygonal Region:** Click the button to preselect creating a polygonal region on a dual parameter plot.

- **Linear Region:** Click the button to preselect creating a linear region on a single parameter plot.

- **Quadrant Region:** Click the button to preselect creating a quadrant region on a dual parameter plot.

For further information on defining and editing regions, see chapter *Creating regions* on page 165.

Plots group

The *Plots* group contains options for adding a specific selection of plots to the Plots area of the Workspace.
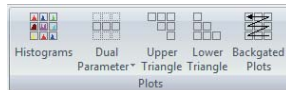
The *Plots* group contains the following buttons:

- **Histograms:** Click the button to add all the single parameter histogram plots displayed in the Previews area of the Workspace to the Plots area. The plots are gated with the same gate as the preview plots. All the added plots are colour-coded to illustrate the gating applied to the Previews area.

- **Dual Parameter:** Click the button to display a drop-down list of available parameters. Select a parameter from the drop-down list to add all the dual parameter combinations against this parameter to the Plots area. The selected parameter is fixed as the Y-axis. The plots are gated with the same gate as the preview plots. All the added plots are colour-coded to illustrate the gating applied to the Previews area.

- **Upper Triangle:** Click the button to add all the dual parameter plots that are displayed within the upper right triangle of the Previews area. All the added plots are colour-coded to illustrate the gating applied to the Previews area.

- **Lower Triangle:** Click the button to add all the dual parameter plots that are displayed within the lower left triangle of the Previews area. All the added plots are colour-coded to illustrate the gating applied to the Previews area.

- **Backgated Plots:** When you select the button, the software will assess the gate equation of the currently selected plot and identify the regions and parameters involved. The software then creates new plots with the parameters used by the regions within the gate equation, and gates these plots on the gate equation of the currently selected plot.

  For example, if a plot gated on B is selected and its expanded gate equation is A AND B, clicking the **Backgated Plots** button creates two plots gated on B, one for events in region A and one for events in region B.
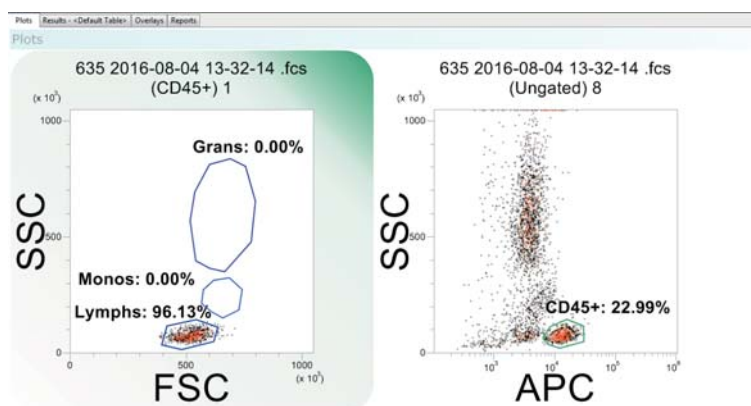
Gate group

The *Gate* group contains options for creating and managing the gates in the current Workspace.
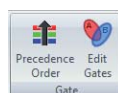
Using the **Precedence Order** button, you can edit the colour precedence order used when events are displayed on plots. You can also choose which gates are active within the precedence system.
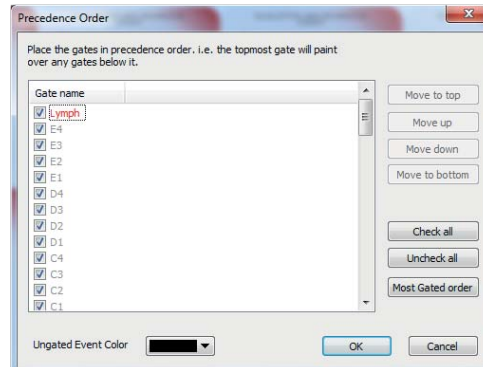
In the Precedence Order dialogue, a gates list displays all gates in the current Workspace. The text colour corresponds to their gate colour.

Adjacent to each gate name is a check box. Select the check boxes to include the corresponding gates in the colour precedence.

Gates positioned at the top of the list have higher precedence than the gates positioned at the bottom of the list. The precedence defines the appearance of the gates in the plots in the Workspace. The plots will display events in the top gate of the list on top of all other events in the plot. If a new gate is created, it will be added to the top of the preference list by default, and events associated with it will be painted on top of events in any other gates. To change the precedence, use the Precedence Order dialogue.

The Precedence Order dialogue contains the following options:

- **Move to top**, **Move up**, **Move down**, **Move to:** Select a gate and use these buttons to move the selected gate to the desired position within the gates list.

- **Check all**, **Uncheck all:** Use these buttons to select or unselect all gates in the list.

- **Most Gated order:** Click the button to place the selected gates in the precedence order ranked by the number of unique regions in each gate, using the expanded gate equations.

- **Ungated Event Color:** To change the current colour for ungated events on precedence plots. Click the arrow to display the colour control and select the desired new colour.

ⓘInformation

When the gate order is modified, always check the selected precedence order works as intended and no populations are masked by a colour of lower priority.

Always ensure that the colours assigned to the populations in the precedence system can be clearly distinguished from each other.

Using the **Edit Gates** button, you can create, edit and delete gates. In addition, you can view the Parent Gate and Expanded Equation information.
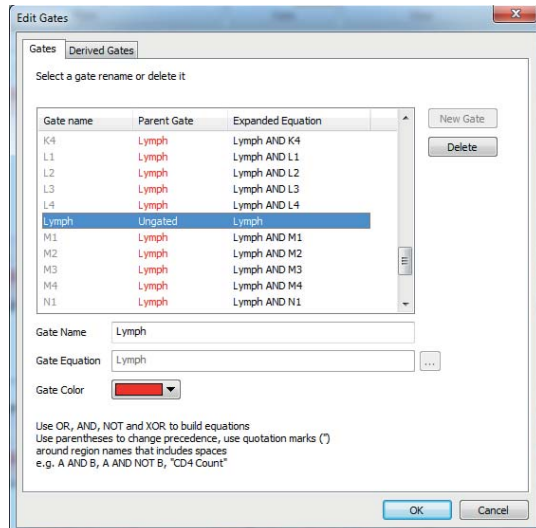
The Edit Gates dialogue displays a Gates list containing all the currently existing gates. In the *Gates* tab, you can rename and delete gates. In the *Derived Gates* tab, you can rename, delete and change the gate equation of derived gates. To edit a gate, first select it in the Gates list.

In the *Gates* tab, the Gates list contains the following columns:

- The "Gate name" column displays the name of the gate. The colour of the font is the colour of the gate.

- The "Parent Gate" column displays the parent gate for the gate. If the gate has no parent gate, this field displays "Ungated".

- The "Expanded Equation" column displays the full expanded equation for the gate. The parent gate equation is shown in full.
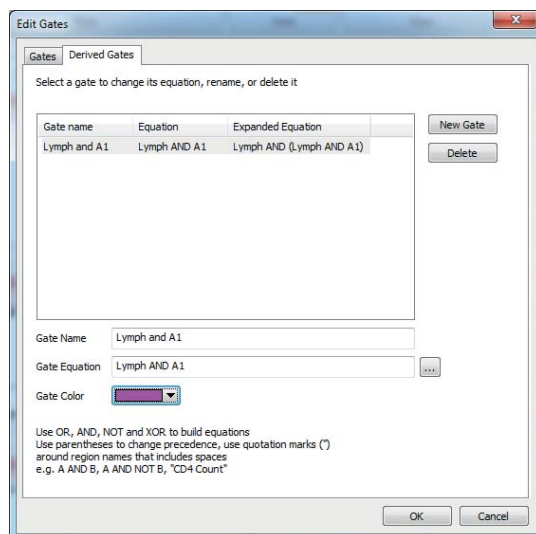
In the *Derived Gates* tab, the Gates list contains the following columns:

- The "Gate name" column displays the name of the gate. The colour of the font is the colour of the gate.

- The "Equation" column displays the equation for the gate. The contents of this field are added to the parent gate equation to create the expanded equation.

- The "Expanded Equation" column displays the full expanded equation for the gate. The parent gate equation is shown in full.

The Edit Gates dialogue contains the following options:

- **New Gate:** Click the button to add a new gate. A gate with a default name is added to the bottom of the gate list.

- **Delete:** Click the button to delete the currently selected gate. The deletion of the gate will be aborted if you press the **Cancel** button on the dialogue.

- **Gate Name:** To change the gate name, type in the new name in the field. You should use gate names that are significantly different.

- **Parent Gate:** The Parent Gate drop-down list is populated with the names of all available gates. To change the parent gate of the currently selected gate, click the arrow to expand the drop-down list and select a new parent gate from it.

  When creating a new gate, the Parent Gate field can be used to simplify gate equation creation. The field contains a list of all current gate names and you can select one of these gate names rather than having to specify the complete gate equation. If you wish to enter a full equation without using a parent gate, you should select "Ungated" as the parent and then enter the full equation into the Gate Equation field.

- **Gate Equation:** In this field, you can edit the gate equation. To display a larger Gate Equation dialogue, press the **...** button at the right of the field.

  The operators AND, OR, NOT, XOR can be used to help define a gate equation. The order of processing of these operators is NOT, AND, XOR, OR. Use parentheses () to change the order of processing. Region names and these operators are used to define a gate.

  Quotation marks ("") must be used around region names that include spaces, e.g. "CD4 Count" and around region names that use any of the shortcut symbols or words that are listed above, e.g. "A XOR B" and "A!B". The gate equation can include a maximum of 28 regions and 6000 characters.

- **Gate Color:** To change the gate colour, click the arrow to display the colour control and select the desired new colour.

  Always ensure that the colours assigned to the gates can be clearly distinguished from each other. You should avoid using system colours as they can be changed outside of the software.

View group

The *View* group contains options to modify the way in which plots are displayed in the Plots area of the Workspace.
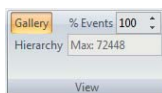
The *View* group contains the following options:

- **Gallery:** Click the button to choose the Gallery plot view. The plots in the Plots area are displayed next to each other.

- **Hierarchy:** Click the button to choose the Hierarchy plot view. The plots in the Plots area are displayed in a family tree style hierarchy format.

- **% Events:** To reduce the number of events displayed on screen, enter a value or use the up/down arrows to define the desired percentage of events to be displayed. The actual number of events displayed is shown in the "Max:" field below. Adjusting this value does not affect statistical calculations which are always performed on all events within a file.

Edit group

The *Edit* group contains options for copying images of the currently selected plots and statistics for pasting into other applications. These controls are also used for copying, cutting and pasting items within reports. Additionally, the fonts of text items in the current Workspace can be edited.

The buttons are only available if a Playlist item or a Worklist item is open in the Workspace area.

The *Edit* group contains the following buttons:

- **Paste:** Click the button to paste previously copied or cut report items back into the report. The button is only enabled on the *Reports* tab of the Workspace, after a report item has been copied or cut.

- **Cut:** Click the button to cut the selected report item and place it on the clipboard for pasting into the report. Report items cannot be pasted into other applications. The button is only enabled on the *Reports* tab of the Workspace, after a report item has been selected.

- **Copy:** When on the *Plots* tab of the Workspace, click this button to copy an image of the currently selected plot(s) or statistics. You can then paste the item into other applications (Microsoft® Word, PowerPoint, etc.).

  If you want to maintain the hierarchy view when copying and pasting plots displayed in the Hierarchy format, first select all plots in the hierarchy and then click the **Copy** button. If all plots in the hierarchy are not selected, when pasted, plots will be pasted in the standard Gallery format.

When on the *Report* tab of the Workspace, click the **Copy** button to copy the selected report objects for pasting into the report. Report items cannot be pasted into other applications.

■ **Delete:** Click the button to delete the currently selected plots or region. If the deleted plot has a region applied, deleting the plot also deletes the region and removes the region from any gating logic. In this case, a corresponding message box is displayed and you will have the option to confirm or abort deleting the plot.

■ **Select:** Click the button to select groups of plots from a drop-down list:

Click the **Select All** option to select all the plots in the Plots area.

Click the **Select Plots with Same Gate** option to select all the plots that have the same gate applied as the selected plot in the Plots area.

Click the **Invert Selection** option to select all the plots that are currently not selected in the Plots area.

Click the **Select Dual Parameter Plots** or **Select Histogram Plots** option to select all dual parameter plots or all histogram plots, respectively.

■ **Fonts:** Click the button to open the Edit Fonts dialogue. In the pages of this dialogue, you can change the font typeface and size for region labels, plot titles, cell cycle and parameter names and scale labels in the current Workspace. The pages are the same as in the software options (see chapter *Font pages* on page 192).

12.4.4 Preview tab

The *Preview* tab provides controls for modifying the plots displayed in the Previews area of the Workspace.

Preview group

The *Preview* group contains viewing options for the Previews area. The buttons in this group are only available if a Worklist item or a Playlist item is open in the Workspace. Selected buttons are highlighted in orange.

The buttons in the lower row of the *Preview* group contain filter options. Filters affect which parameters, and therefore plots, are displayed in the Previews area. The filters are used to selectively reduce the number of plots displayed in the Previews area.
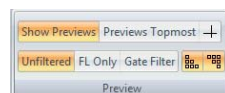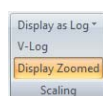

The *Preview* group contains the following buttons:

■ **Show Previews:** By default the button is selected, and previews of all parameters are displayed in the Previews area. Clicking the button

switches between hiding and showing the previews. The setting of this button will be persisted between sessions.

- **Previews Topmost:** By default, the Plots area is positioned above the Previews area in the Workspace. Clicking the button switches between displaying the Previews area above and below the Plots area.

- **Quadrants on Previews:** Clicking the button switches between displaying and hiding a quadrant region on all of the dual parameter preview plots. Only one quadrant can be displayed on each plot.

- **Unfiltered:** Click the button to show preview plots for all currently selected parameters. The Previews area is unfiltered by default.

- **FL only:** Click the button to only show preview plots of fluorescence parameters. Scatter and other parameters will not be displayed. Click the **Unfiltered** button to re-show all preview plots.

- **Gate Filter:** When this filter is applied, any preview plots containing parameters that are being used as a part of the gate equation for the selected plot or region are excluded from the preview. This can be used to reduce the number of preview plots. Click the **Unfiltered** button to re-show all preview plots.

- **Show Lower Triangle:** Click the button to show or hide the plots displayed on the lower left triangle of the Previews area.

- **Show Upper Triangle:** Click the button to show or hide the plots displayed on the upper right triangle of the Previews area.

Scaling group

The *Scaling* group contains options to specify the scaling for parameters in the Previews area. When no LMD file is open, or if a low-resolution file is open, the controls in the *Scaling* group are disabled.

The *Scaling* group contains the following options:

- **Display as Log:** The parameters displayed in the Previews area can be shown with either linear or logarithmic scaling. Click the arrow to expand a drop-down list and select the parameters to be displayed as logarithmic.

- **V-Log:** V-Log is an axis transformation that is similar to logarithmic for the upper three decades, but has a linear section around the axis and allows compensated events that have a "negative" fluorescence value to be better visualised. Click the button to change all the logarithmic parameters of the preview plots to V-Log scaling.

   The V-Log scaling properties can be changed from the *Scales* group on the *Parameters* tab of the Ribbon (see chapter *Scales group* on page 80).

■ **Display Zoomed:** If zoom levels are set for individual parameters, click the button to select or deselect displaying those parameter zoom settings in the Previews area. The default is to display parameters in the Previews area zoomed. For details on setting parameter zoom values, see chapter *Scales Properties dialogue* on page 81.

View group

The *View* group contains options for selecting the preview plot type.
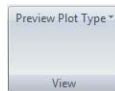
The *View* group contains the following control:

■ **Preview Plot Type:** The dual parameter preview plots can be displayed in a choice of three formats: as density plots, colour precedence plots or precedence density plots (see chapter *Types of plots* on page 161). Click the arrow to expand a drop-down list and select the desired preview format. The three choices are mutually exclusive.

12.4.5 Parameters tab

The *Parameters* tab provides controls for selecting the parameters displayed. The number of available parameters depends on the configuration of your device.

Some files contain a large number of parameters. Each parameter may be present with multiple signal types. To simplify parameter selection, the software provides options to select parameters by signal type (area, height and width) and further classifies parameters into "Scatter", "Fluorescence" and "Other" groups based on the signal source. The software will automatically assign parameters to these groups. If the classification is incorrect, you can manually reclassify parameters in the *Classification* tab of the Parameter Properties dialogue (see chapter *Parameter Properties* on page 76).

Parameter Properties

The Parameter Properties dialogue provides the custom naming and parameter classification functionality of the software. To open the dialogue, click the **Parameter Properties** button or double-click the parameter name area of a plot in the Plots area of the Workspace.

The Parameter Properties dialogue contains two tabs:

■ On the *Customize* tab, you can select which parameters are displayed in the Previews area. You can also choose a display name and assign custom names to individual parameters.

■ On the *Classification* tab, you can re-classify the group and type of a parameter.

When the Parameter Properties dialogue is launched, the *Customize* tab is displayed by default.
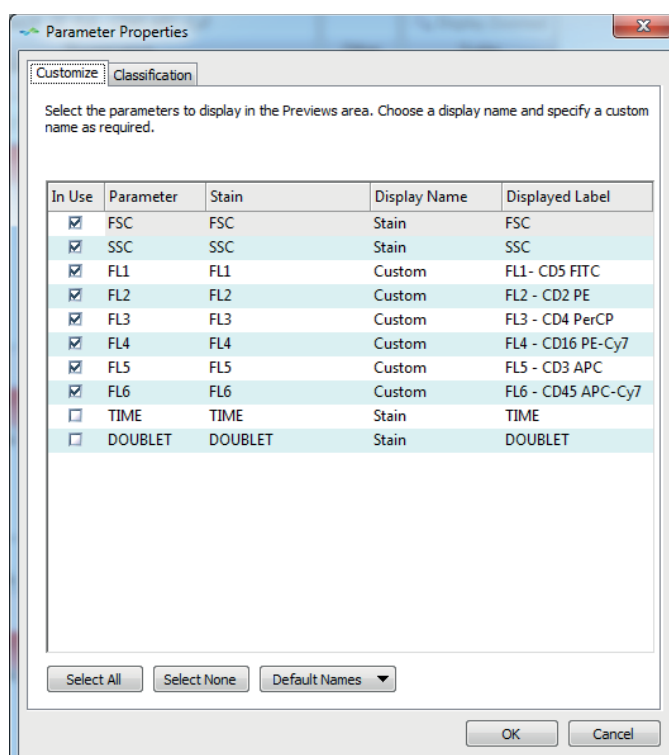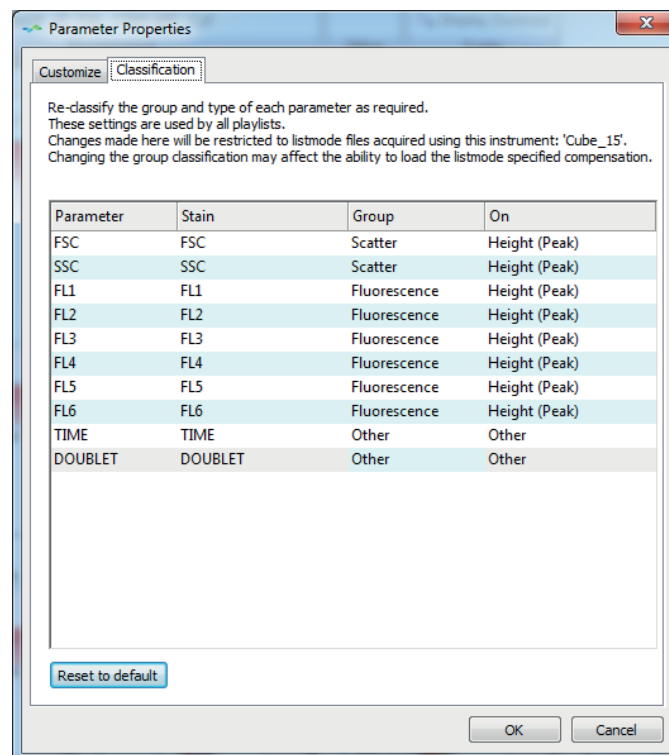

The *Customize* tab contains the following columns:

■ The "In Use" column contains check boxes. Select a check box to display the corresponding parameter in the Previews area or deselect it to hide the parameter. Selecting and deselecting parameters here does not affect plots already present in the Plots area.

■ The "Parameter" column displays the parameter name for each of the parameters available in the currently loaded listmode file.

■ The "Stain" column displays the stain name for each parameter.

■ The "Display Name" column allows you to select whether parameter name, stain name, parameter - stain name combination or a custom name is used as the display label for the parameter. Double-click in the cell or press F2 to open a drop-down list and select the desired display name. Once the dialogue closes, the display label is used throughout the system. When a new LMD file is loaded, the custom display label will be used only if both the parameter name and stain name for the new file match those in the Workspace. If they don't match, the stain name from the new file is used as the display label.

■ The "Displayed Label" column displays the name as selected in the "Display Name" column. In the "Displayed Label" column, you can also enter a custom label for the parameter. The default used for the custom name is the Stain name ($PnS). The custom name has to be unique. It cannot start with a space, and it has to be a minimum of 1 character and can be a maximum of 50 characters.

The *Customize* tab contains the following buttons:

■ **Select All:** Click the button to select all parameters.

■ **Select None:** Click the button to de-select all parameters.

■ **Default Names:** Click the button to reset the display names for all parameters to Stain.

On the *Classification* tab of the Parameter Properties dialogue, you can re-classify the group and type of a parameter. This classification is based on cytometer type. Modifications made here will be stored for future use of files from that cytometer type.

The *Classification* tab contains the following columns:

■ The "Parameter" column displays the parameter name for each of the parameters available in the currently loaded listmode file.

■ The "Stain" column displays the stain name for each parameter.

■ The "Group" column allows you to select whether the parameter belongs in the Scatter, Fluorescence or Other group. Double-click in the cell or press F2 to open a drop-down list and select the desired group for the parameter.

- The "On" column allows you to select whether the parameter belongs in the Height (Peak), Width (Time of Flight), Area (Integral) or Other type. Double-click in the cell or press F2 to open a drop-down list and select the desired type for the parameter.

Click the **Reset to default** button to reset all parameters for the currently loaded file to their default values.

View group

The *View* group contains options to select all parameters of a specific signal type for display in the Previews area.

The *View* group contains the following buttons:

- **Area (Integral):** The button toggles the selection of all Area / Integral parameters, i.e. parameters that represent the area under the curve from a pulse.

- **Height (Peak):** The button toggles the selection of all Height / Peak parameters, i.e. parameters that represent the peak height of a pulse. If the file does not contain this type of signal or if the software is unable to determine the signal type, this button will be disabled.

- **Width (Time Of Flight):** The button toggles the selection of all Width / Time Of Flight parameters, i.e. parameters that represent the width of a pulse. If the file does not contain this type of signal or if the software is unable to determine the signal type, this button will be disabled.

Scatter group, Fluorescence group and Other group

Individual parameters are classified into three groups: Scatter, Fluorescence and Other. A parameter that is selected here will appear within the plots in the Previews area. A parameter that is deselected will not appear within the plots in the Previews area. Plots already displayed in the Plots area are not affected by selections made here.

Parameter and stain names must be unique. If this is not the case, the software will automatically append either the stain or parameter name to ensure uniqueness.

ⓘ**Information**

Parameters deselected here are hidden from other parts of the software. You should be careful when hiding parameters which have compensation values applied as these values will not be visible in the compensation matrix.

Hovering the cursor over a check box will open a tooltip containing the display name followed by the stain name for the parameter if a stain name is available.

The *Scatter* group lists the scatter parameters, forward scatter (FSC) and side scatter (SSC).

The *Fluorescence* group lists all fluorescence parameters.

If a file has a large number of fluorescence parameters, they will not all fit on the Ribbon. In this case, a Fluorescence drop-down arrow is displayed. Click the arrow to display a window listing all the available fluorescence parameters.

The Other group lists parameters that are not scatter or fluorescence parameters. Examples of parameters that would belong to this group are TIME and RATIO. The displayed parameter names are the parameter name, stain name or custom as selected in the *Customize* tab of the Parameter Properties dialogue (see *Parameter Properties* on page 76).

Scales group

The *Scales* group contains options for adjusting the scaling and zooming of parameters. The Scales group is only enabled if a Worklist item or a Playlist item is open in the Workspace.

⊡**Information**

If you change the zoom status on a plot with a region, the region position does not change. You may need to reposition any regions set on zoomed parameters.

The *Scales* group contains the following options:

■ The **Scaling** button opens the Scales Properties dialogue that is used for presetting scaling of parameters for display in the Workspace (see chapter *Scales Properties dialogue* on page 81).

- The **Zoom** tool allows you to modify the zoom level for parameters of a plot. To set or change a zoom level for parameters of the selected plot, select the tool and then use it to define a zoom region. On a single parameter plot, the tool draws a linear zoom region. On a dual parameter plot, the tool draws a rectangular zoom region.

  The button is not available when a low-resolution LMD file is currently opened in the Workspace. The button is only enabled when at least one plot is added to the Plots area of the Workspace.

  Any change in scale via the Zoom tool will update the settings in the *Parameter Zoom* tab of the Scales Properties dialogue. Any plots in the Plots and Previews areas that are already zoomed will update accordingly.

- **Display Zoomed:** If zoom values are defined with the Zoom tool or in the *Parameter Zoom* tab of the Scales Properties dialogue, clicking the button toggles between the zoomed and un-zoomed view of the currently selected plots.

Scales Properties dialogue

The **Scaling** button opens the Scales Properties dialogue that is used for presetting scaling of parameters for display in the Workspace.

The Scales Properties dialogue contains two tabs:

- In the *Default Scaling* tab, you can modify the Linear, Logarithmic and V-Log scaling options for parameters in the Workspace

- In the *Parameter Zoom* tab, you can edit the displayed scale values for each parameter.
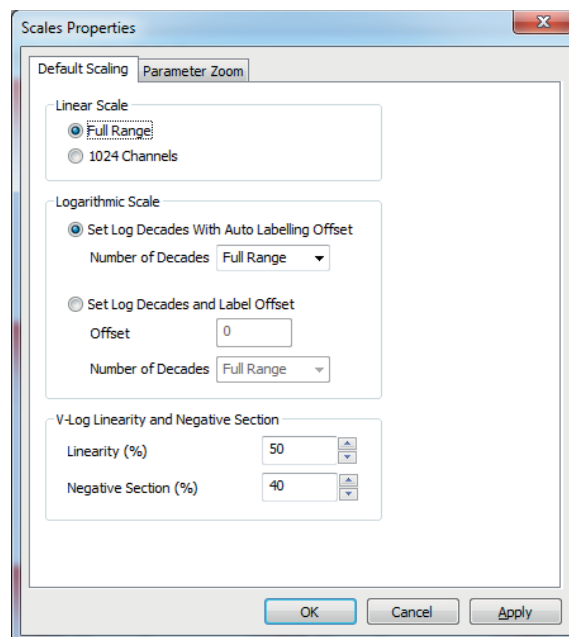
The *Default Scaling* tab provides the following options:

- **Full Range:** Select this option to scale all linear parameters in the Workspace and reports to the full range of data.

- **1024 Channels:** Select this option to scale all linear parameters in the Workspace and reports to 1024 channels, regardless of their full range.

  When you change the linear scale, all associated statistics and results will be updated so they are calculated on the selected range of the linear scale.

  For FC500 format files, the default is 1024 channels. For all other file types, the default selection is determined by the $PnR Keyword in the LMD file.

- **Set Log Decades with Auto Labeling Offset:** Select this option to scale all logarithmic parameters in the Workspace and reports to a scale based on the number of decades set and the resolution of data within the file.

- **Set Log Decades and Label Offset:** Select this option to scale all logarithmic parameters in the Workspace and reports to a scale based on the offset and number of decades specified.

- **Number of Decades:** This drop-down list is disabled for low-resolution data (less than 12-bits). For low-resolution files, the log parameter scaling is set according to the $PnE keyword. Click the arrow to expand the drop-down list and select the desired number of decades. You can also enter a numerical value into the input field.

  When **4 Decades** or **5 Decades** is selected, the data for all logarithmic scaled parameters in the Workspace and reports will scale to display 4 or 5 decades down from the highest value in the LMD file, respectively. When **Full Range** is selected, the data for all logarithmic scaled parameters in the Workspace and reports will scale to the full range of the data in the LMD file. Statistics and results will update accordingly.

- **Offset:** The number entered in this field determines the offset of the data. The number is converted to a Log scale when displayed on the plots e.g. 0.1 in dialogue = 10^{-1} on Log scale; 10 in dialogue = 10^{1} on Log scale; 100 in dialogue = 10^{2} on Log scale.

- **Linearity (%):** This control is used to enter a value for the linear section of the V-Log scale as a percent. The default value is read from the scaling in the software options (see chapter *Scaling page* on page 189).

  Enter the desired value or use the arrows to increase or decrease the value in the field. For example, when the linearity is set at 0%, the V-Log scale has a minimal linear part around 0. When the linearity is set at 50% half of the V-Log scale is linear. The value changes the number of data channels which appear within the linear section of the V-Log scale.

- **Negative Section (%):** This control is used to enter a value which determines the range of data displayed in the negative section of the scale. By default, the value is read in from the software options (see chapter *Scaling page* on page 189).

Enter the desired value or use the arrows to increase or decrease the value in the field. For example, when set at 100%, the number of decades displayed on the negative section of the scale will be the same as the number of decades displayed on the positive side of the scale. When set at 0% the number of decades displayed on the negative section of the scale will be 0.

- **OK:** Click the button to apply the changes made in the dialogue to the Workspace and to close the dialogue.

- **Apply:** Click the button if you wish to apply the changes made without closing the dialogue.

- **Cancel:** Click the button to close the dialogue and cancel all changes. Note that only changes made after the **Apply** button was clicked will be cancelled.
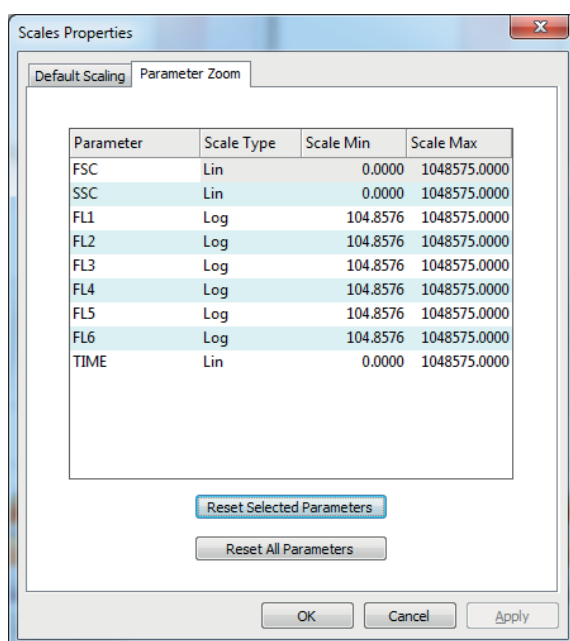
The *Parameter Zoom* tab lists all the parameters in the current file and allows you to edit the displayed scale values for each parameter in order to optimise the way in which your data is displayed. The selected range is only applied to the selected scale type (Lin, Log or V-Log). The selected zoom values are only applied to plots that have been selected to display zoomed parameters.

The Parameter Zoom tool is not available when a low-resolution LMD file is currently opened in the Workspace.

ⓘInformation

If you change the zoom status on a plot with a region, the region position does not change. You may need to reposition any regions set on zoomed parameters.

The *Parameter Zoom* tab contains the following columns:

- The "Parameter" column contains the display name for each parameter in the listmode file. This column has a tooltip detailing the acceptable range for the Scale Min and Scale Max with respect to the scale type selected.

- The "Scale Type" column contains a box that allows the selection of the scale type (Lin, Log or V-Log) to which the range will be applied. The default scale type is set from the current Workspace. For low-resolution LMD files, only the scale type specified in the LMD file header is available. It is displayed in the "Scale Type" column and the box is read only.

- The "Scale Min" and "Scale Max" columns display the current minimum and maximum values for the scaling of a parameter. You can manually change these values. The values allowed for Scale Min and Scale Max are limited to the range indicated by the settings in the *Default Scaling* tab. When editing the "Scale Min" and "Scale Max" fields, if an error occurs, the corresponding values will be highlighted in red. Hover over the value to display a tooltip indicating the reason for error.

The *Parameter Zoom* tab provides the following buttons:

- **Reset Selected Parameters:** Click the button to reset the values for the currently selected parameters to their defaults.

- **Reset All Parameters:** Click the button to reset the values for all parameters to their defaults.

12.4.6 Compensation tab

The *Compensation* tab provides controls allowing you to calculate fluorescence compensation.

The correct compensation for a file can be obtained in a number of ways. Compensation values can be restored from the LMD file header values if the values were stored with the file. You can also calculate the compensation within the software and save these calculated values for future use. By default, the software will automatically load compensation values from the listmode file header. The status of the compensation settings of the current file is displayed in the status bar.

In Worklist mode, compensation modifications are applied to the compensation settings stored in the Workspace.

Wizard group

The Compensation Wizard is only available in Playlist mode. The Compensation Wizard simplifies the process of generating a full fluorescence compensation matrix.

Before starting the Compensation Wizard, you need to load all of the single stained samples and (if appropriate) the negative control sample into the Playlist. For ease of use, you should order the files with the negative control first, followed by the single stained samples in parameter order, e.g. FL1,

FL2, FL3, etc. You will be able to reallocate files later if required. If a single LMD file contains more than one positive stain, you must load in the file once for each stain present. The Wizard will allocate files based on the Playlist starting from the first file. Additional files can be present in the Playlist but will not be recognised by the Wizard.

Once the Playlist contains the required compensation setup files, click the **Compensation Wizard** button to launch the Wizard.

The Compensation Wizard is made up of four types of pages:

- Configuration Page

- Background Fluorescent Page (only if a negative control file is used)

- Compensation Pages (one for each selected compensation parameter)

- Review Page

Compensation Wizard: Configuration Page

The first page of the Wizard, the Configuration page, is displayed when the Wizard is launched. On this page, you can select the parameters you wish to compensate and allocate an LMD file to each of them. You can specify how negative control values should be calculated. You can also define the plot type required for definition of positive and negative populations.
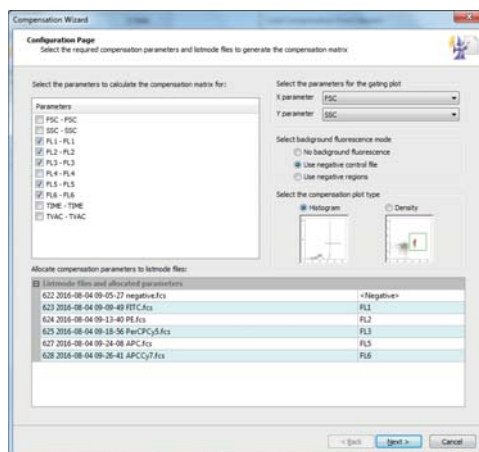
The page contains the following options:

- **Parameters:** The list displays all the parameters from the first LMD file in the Playlist in the format parameter name ($PnN) - stain name ($PnS). Parameters identified as fluorescence are automatically selected. You can deselect the ones you do not want to compensate. Parameters that are not recognised as fluorescence parameters cannot be selected. For details on parameter classification and how to reclassify parameters, see chapter *Parameter Properties* on page 76.

- **Select the parameters for the gating plot:** Select the parameters for the primary bead / cell gating plot using the "X parameter" and "Y parameter" boxes. They are populated with all the parameters shown in the "Parameters" list.

- **Select background fluorescence mode:** If no background fluorescence control is present, select the "No background fluorescence" option. If a separate background fluorescence control is being used, select the "Use negative control file" option. If you wish to use negative events within a positive sample to determine the background for each sample, select the "Use negative regions" option.

- **Select the compensation plot type:** Select whether histogram plots or dual parameter density plots should be used for the definition of positive and negative events for each parameter.

- **Allocate compensation parameters to listmode files:** The list allows you to allocate one of the parameters selected for compensation to each LMD file. The listmode files (left-hand) side of the list shows the files from the Playlist, in their current order, starting from the first file. The list contains one LMD file for each selected compensation parameter. If the "Use negative control file" option is selected, the list also contains a file to be used as the negative control file. When the selection of parameters changes, the list updates accordingly.

  The allocated parameters (right-hand) side of the list shows the allocated compensation parameters. Each row consists of a drop-down list that contains all the fluorescence parameters from the "Parameters" list selected for compensation. Selecting an item from the drop-down list which is already selected for a different file clears the setting for that file. The default allocation will be the negative control file first (if selected) followed by the selected parameters in the order of the "Parameters" list. If the listmode files list is updated (parameters selection or changing the "Use negative control file" selection), the allocated parameters return to their default allocations.

- **Next:** Click the button to proceed to the next page of the Wizard. If the "Use negative control file" option is selected, the next page is the Background Fluorescence page, otherwise it is the first Compensation page. If the selections made on the Configuration page are incorrect, you will not be allowed to proceed to the next page, and an appropriate error message will be displayed.

Compensation Wizard: Background Fluorescent Page

> The Background Fluorescence page of the Compensation Wizard is only displayed if the "Use negative control file" option is selected on the Configuration page. On this page, you will define a default gate position for subsequent pages and then use a region to define the negative events for each parameter.
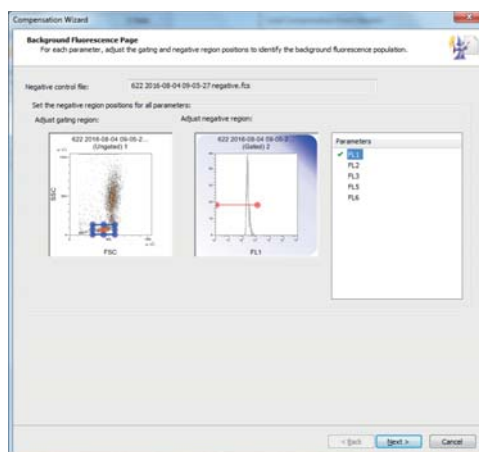
> The page contains the following options:
>
> ■ **Negative control file:** The field displays the name of the negative control LMD file that will be used for the background fluorescence computation.
>
> ■ **Adjust gating region:** The gating region plot is an ungated dual parameter density plot. The X and Y parameters on the plot are those selected on the Configuration page. The plot contains a single rectangular blue coloured region that can be moved and resized as required to define the gating region. Any adjustment of the region automatically updates the background fluorescence plot.
>
> ■ **Adjust negative region:** The negative region plot is gated on the gating region and used to define the negative population for the X parameter of the current plot. A region needs to be defined for each parameter to be compensated. The plot will be either a histogram or dual parameter density plot depending on the selection on the Configuration Page. The plot contains a single red coloured region that can be moved and resized as required to define the negative events.
>
> ■ **Select Y parameter:** If the negative region plot is a dual parameter plot, you can change the Y parameter in this box.
>
> ■ **Parameters List:** The list contains all fluorescence parameters that are selected for compensation on the Configuration page. The currently selected parameter is highlighted in the list. Parameters which have had the negative region configured are indicated by a tick in the check box.
>
> ■ **Next:** If not all of the parameters listed in the Parameters list have been configured, clicking the button will select the next unconfigured parameter in the list. When all parameters have been configured, the button will proceed to the first Compensation page of the Wizard.

Compensation Wizard: Compensation Pages

A Compensation page will be displayed once for each selected compensation parameter.
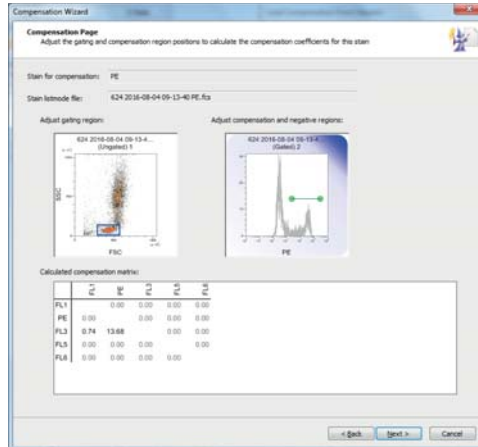
The page contains the following options:

- **Stain for compensation:** The field displays the stain that is being currently compensated.

- **Stain listmode file:** The field displays the name of the listmode file used for the compensation calculation.

- **Adjust gating region:** The gating region plot is an ungated dual parameter density plot. The X and Y parameters on the plot are those selected on the Configuration page. The plot contains a single rectangular blue coloured region that can be moved and resized as required to define the gating region. Any adjustment of the region automatically updates the compensation plot and the calculated compensation matrix.

- **Adjust compensation and negative regions:** The compensation plot is a single or dual parameter plot depending on the selection on the Configuration Page. The plot is gated on the gating region plot. The X parameter on the plot is the current compensation parameter.

  The compensation plot contains a green coloured region used to specify the positive population for that stain. If you have selected the "Use negative regions" option on the Configuration page, the compensation plot also contains a red coloured region which is used to specify the background fluorescence population. You can move and resize the regions. Adjusting the regions updates the calculated compensation matrix.

- **Select Y parameter:** If the compensation plot is a dual parameter plot, you can change the Y parameter in this box.

- **Calculated compensation matrix:** The matrix displays the currently calculated compensation coefficients. The top and bottom channels of the plot are excluded from the calculations.

- **Back:** Selecting the button returns you to the previous page of the Compensation Wizard. The **Back** button on the first Compensation page is disabled if the "Use negative control file" option was not selected.

- **Next:** Selecting the button proceeds to the next Compensation page or to the Review page when on the last Compensation page.

Compensation Wizard: Review Page
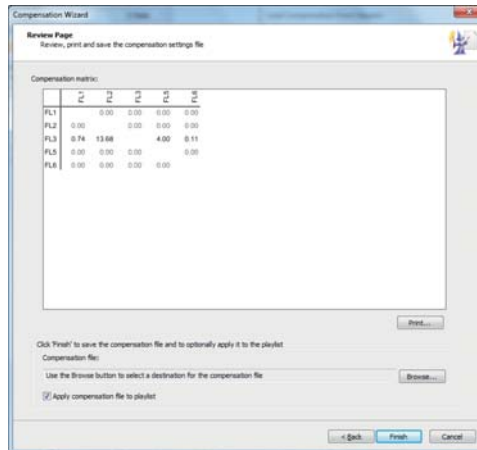
The page contains the following options:

- **Compensation matrix:** The matrix displays the completed matrix of compensation values.

- **Print...:** Click the button to print the compensation matrix.

- **Compensation file:** The field displays the destination path and file name for the compensation file. Click the **Browse** button to specify the destination and name for the compensation file. The compensation file is not saved at this time.

- **Apply compensation file to playlist:** Select the check box if you wish to replace the current compensation values in the Workspace with the newly calculated compensation coefficients.

- **Finish:** Click the button to complete the Compensation Wizard and save the compensation file with the specified name.

Plot Compensation group

The *Plot Compensation* group contains options to adjust the compensation on the current sample using plots in the Plots area. Populations can be dragged into position or accurately adjusted using the arrow buttons. Mean or Median values for the current plot can be used to help set the correct compensation.

The *Plot Compensation* group contains the following options:

■ **Plot Compensation:** To enable plot compensation, select a plot in the Plots area that contains the parameters required for compensation and then click the button. The button is highlighted, and the arrow buttons and manual dragging of populations on plots in the Plots area are enabled. The **Plot Compensation** button will be unavailable if a plot is selected which does not contain compensable parameters, for example Time or Scatter parameters.

When Plot Compensation is enabled, the selected plot shows a diagonal line from bottom left to top right to assist with drag/drop compensation. Then either click and drag a population into the correct position or use the arrow buttons to move the population to the required position.
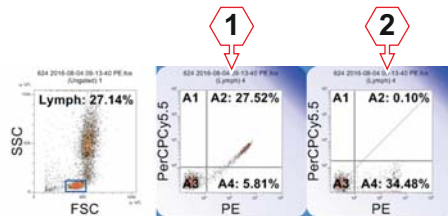
1 Original plot (gated)
2 Plot after manual compensation

■ **Comp %:** The fields display the current compensation values for the pair of parameters present on the currently selected plot. The top (X) field shows the subtraction of the Y-parameter from the X-parameter. The bottom (Y) field shows the subtraction of the X-parameter from the Y-parameter. The fields allow the entry of compensation values. Up to six characters can be entered into the fields in the range 0.00 to 100.00. Entered values are rounded to 2 decimal places.

■ **Compensation Arrows:** The arrow buttons allow the fine adjustments of compensation % values. Use the top row of arrows to increase/decrease the X compensation value by 0.1/by 0.01. Use the bottom row of arrows to increase/decrease the Y compensation value by 0.1/by 0.01. Compensation values are limited to the range 0.00 to 100.00. The direction of the arrow icons indicates the way the plot populations move rather than the way the compensation values change.

■ **Quadrant Region:** Click the button and then click onto a dual parameter plot to insert a quadrant on a dual parameter plot. For information on quadrant regions, see chapter *Creating regions* on page 165.

■ **Quadrant Statistics:** Statistics are displayed when the current listmode file is compensable, a dual parameter plot displaying compensable parameters is selected, and the plot contains a quadrant region. If the statistic cannot be calculated due to insufficient events, the edit field will show "ERROR". If multiple quadrant regions are present on the plot, the statistics are only displayed for the quadrant region set created first.

Click the **Mean/Median** button to select whether the mean or median statistic is displayed for the quadrant region (default: "Median").

Click the **X Stats/Y Stats** button to select whether the displayed statistics are for the X parameter or the Y parameter (default: "X Stats").

Preview Details group

Controls in the *Preview Details* group contains options to manually adjust the compensation of the current sample using the preview plots and also to display the compensation percentage on the preview plots. The buttons in the group are only enabled when a compensable LMD file is loaded in the Workspace.

The *Preview Details* group contains the following option:

- **Show Compensation %:** Click the button to display or hide the compensation coefficients for each parameter combination on all dual parameter preview plots. Compensation coefficients are only displayed on preview plots that have two relevant fluorescence parameters.

Compensation group

The *Compensation* group contains options for displaying the compensation matrix, for clearing the current compensation matrix and for loading the compensation settings from the current file header.

The *Compensation* group contains the following options:

- **Show Matrix:** Click the button to display the Compensation Matrix dialogue. The dialogue shows the compensation matrix associated with the current file.

Click the **Print** button to print the compensation matrix. The default font size for printing the matrix is 10 pt. If the matrix does not fit in the printing area, the font size for the print out of the matrix will be reduced. If the minimum font size is reached and the matrix still cannot fit in the printing area, the printout switches layout (from portrait to landscape or vice versa) and the fitting procedure is applied again. If the matrix still cannot

fit, the printout of the matrix is cropped, and a corresponding message is displayed at the end of printing.

Click the **Size to Fit** button to resize the Compensation Matrix dialogue to fit the size of the current matrix. This is restricted by the screen size of your monitor.

■ **Clear Compensation:** Click the button to clear the active compensation. All coefficients in the currently active matrix will be reset to 0.00%. This action will not clear the calculated compensation matrix stored in memory. The button is only enabled when a compensable listmode file is open in the Workspace.

■ **Load Compensation From Headers:** Click the button to retrieve the compensation matrix from the current LMD file headers and apply it as the active compensation matrix. The button is only enabled when a compensable LMD file is loaded in the Workspace.

■ **Display Calculated Compensation:** The button is only enabled when a compensation setup panel is active and acquisition is not in progress. Click the button to display the calculated compensation matrix.

12.4.7 Statistics tab

The *Statistics* tab provides controls for selecting which statistic is displayed on each plot in the Plots area and which statistic is presented in the associated results table in the Workspace.

The *Statistics* tab behaves differently depending on which tab in the Workspace area is selected:

■ When in the *Plots* tab of the Workspace, only one statistic can be selected at any time. The currently selected statistic is displayed adjacent to the region for all regions on all plots in the Plots area. If you select a new statistic, the previously selected one is automatically cleared. By default, the %Gated statistic is displayed on plots. The controls in the *General* group and the *DNA Statistics* group are disabled as these statistics cannot be displayed on plots.

■ When in the *Results* tab of the Workspace, any number of statistics can be selected and the statistical values for the selected statistics are displayed in the results table. For details of the display of statistics in the *Results* tab, see chapter *12.8.2 Results tab of the Workspace* on page 171.

■ When in the *Reports* tab of the Workspace, the *Statistics* tab is only enabled when a Stats Box is selected. Statistical values for the selected statistics are displayed in the Stats Box.

Results Tables group
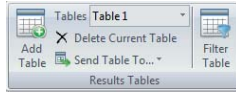
The *Results Tables* group provides options for working with tables on the *Results* tab of the Workspace.

The *Results Table* group contains the following options:

- **Add Table:** Click the button to create a new results table.

- **Tables:** To show an existing results table, click the arrow to expand a drop-down list and select the desired table.

- **Delete Current Table:** Click the button to delete the current results table.

- **Send Table To...:** Click the arrow to expand a drop-down list and select a destination to send the current results table to.

- **Filter Table:** Click the button to filter the current results table. The Listmode Files dialogue is displayed.
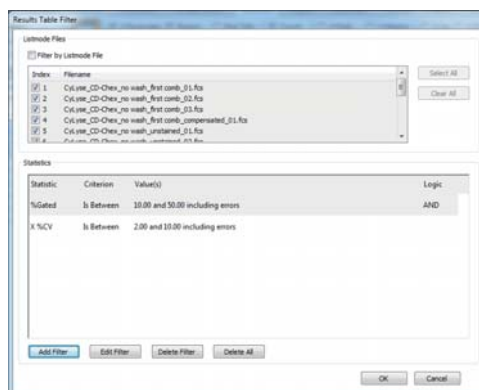
To filter by listmode files, select the file names of the listmode file(s) to filter the statistics.

To add a new filter, click the **Add Filter** button. The Add/Edit Filter dialogue is displayed. Select the statistics to filter on, select the type of filter and select or enter the desired filter value(s).

General group

The items selected in this group are displayed in the results table and in selected Stats Boxes on the reports.
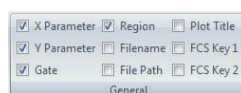
The following items can be selected:

- **X Parameter** displays the X parameter of the plot containing the region.

- **Y Parameter** displays the Y parameter of the plot containing the region.

- **Gate** displays the name of the gate on which the region is gated.

- **Region** displays the name of the region or the DNA plot name.

- **Filename** displays the LMD file name.

- **File Path** displays the LMD full path and file name.

- **Plot Title** displays the title of the plot.

- **FCS Key 1** and **FCS Key 2** displays the respective FCS keywords. The items are disabled in Worklist mode.

Events group

The items selected in this group are displayed on Plots, in the results table and in selected Stats Boxes on the reports.

The following items can be selected:

- **Count** displays the number of events in the region.

- **% Gated** displays the percentage of events in the region compared to all the events in the parent gate. Results will not be truncated, so any statistic that is too large to appear on a plot will be hidden. You can display just the region name by ensuring all check boxes are deselected.

- **% Total** displays the percentage of events in the region compared to all the events in the file.

Intensity group

The items selected in this group are displayed on Plots, in the results table and in selected Stats Boxes on the reports. The *Intensity* group displays statistical options that express the intensity of the generated populations.
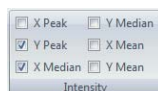
The following items can be selected:

- **X Peak** displays the peak channel (mode) for the X-axis histogram of a region.

- **Y Peak** displays the peak channel (mode) for the Y-axis histogram of a region. Where the region is only a single parameter region, the entry on the results table is left blank.

- **X Median** displays the median average for the X-axis histogram of a region.

- **Y Median** displays the median average for the Y-axis histogram of a region. Where the region is only a single parameter region, the entry on the results table is left blank.

- **X Mean** displays the mean average for the X-axis histogram of a region.

- **Y Mean** displays the mean average for the Y-axis histogram of a region. Where the region is only a single parameter region, the entry on the results table is left blank.

Variation group

The items selected in this group are displayed on Plots, in the results table and in selected Stats Boxes on the reports. The *Variation* group displays options that express the variation of populations.
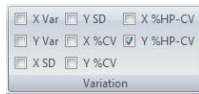
The following items can be selected:

- **X Var** displays the variance for the X-axis histogram of a region.

- **Y Var** displays the variance for the Y-axis histogram of a region. Where the region is only a single parameter region the entry on the results table is left blank.

- **X SD** displays the standard deviation for the X-axis histogram of a region.

- **Y SD** displays the standard deviation for the Y-axis histogram of a region. Where the region is only a single parameter region the entry on the results table is left blank.

- **X %CV** displays the Coefficient of Variation for the X scale range of events. This allows comparison of the variation of populations that have significantly different mean values. The X %CV is calculated by dividing the standard deviation by the arithmetic mean and is displayed as a percentage.

- **Y %CV** displays the Coefficient of Variation for the Y scale range of events. This allows comparison of the variation of populations that have significantly different mean values. The Y %CV is calculated by dividing the standard deviation by the arithmetic mean and is displayed as a percentage.

- **X %HP-CV** displays the Half-Peak Coefficient of Variation for the X scale range of events. The X %HP-CV uses a standard deviation calculated from the scale width between the half peak points either side of the Peak. Once the standard deviation is calculated, it is divided by the arithmetic mean and displayed as a percentage.

- **Y %HP-CV** displays the Half-Peak Coefficient of Variation for the Y scale range of events. The Y %HP-CV uses a standard deviation calculated from the scale width between the half peak points either side of the Peak. Once the standard deviation is calculated it is divided by the arithmetic mean and displayed as a percentage.

DNA Statistics group

The *DNA Statistics* group displays the statistical options associated with the DNA cell cycle plots.

The items selected in this group are displayed in the results table and in selected Stats Boxes on the reports. When a cell cycle plot is present in the Plots area, the statistics in the *DNA Statistics* group are selected by default.
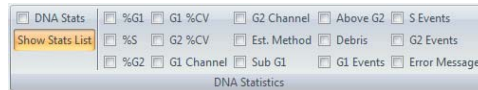
The following items can be selected:

- **DNA Stats:** Select the check box to display all DNA statistics for all regions in the results table.

- **Show Stats List:** Click the button and open the "DNA Statistics" drop-down list to access the individual DNA statistic settings:

- **%G1** displays events in the G1 phase, represented as a percentage of the total of events in G1, G2 and S phase.

- **%S** displays events in the S phase, represented as a percentage of the total of events in G1, G2 and S phase.

- **%G2** displays events in the G2 phase, represented as a percentage of the total of events in G1, G2 and S phase.

- **G1 %CV** displays the Coefficient of Variation for the G1 phase. This is calculated by dividing the G1 phase standard deviation by the arithmetic mean and is displayed as a percentage.

- **G2 %CV** displays the Coefficient of Variation for the G2 phase. This is calculated by dividing the G2 phase standard deviation by the arithmetic mean and is displayed as a percentage.

- **G1 Channel** displays the position of the G1 calculated peak channel.

- **G2 Channel** displays the position of the G2 calculated peak channel.

- **Est. Method** displays the estimate method used. This can be Automatic or User.

- **Sub G1** displays the number of events above the lower threshold divider and below the G1 phase.

- **Above G2** displays the number of events below the upper threshold divider and above the G2 phase.

- **Debris** displays the number of DNA debris events. These are the events outside the threshold limits.

- **G1 Events** displays the number of events in the G1 phase.

- **S Events** displays the number of events in the S phase.

- **G2 Events** displays the number of events in the G2 phase.

12.4.8 Worklist tab

The *Worklist* tab provides controls for modification of all aspects of a Worklist including Worklist items, panels, Workspace files and instrument settings.

The *Worklist* tab is only available in Worklist mode.

General group

The *General* group contains options to open, save and edit a Worklist.
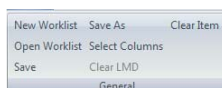
The *General* group contains the following buttons:

- **New Worklist:** Click the button to create a new Worklist
- **Open Worklist:** Click the button to open an existing Worklist.
- **Save:** Click the button to save the current Worklist with the same name.
- **Save As:** Click the button to save the current Worklist with a new name.
- **Select Columns:** Click the button to open a dialogue to customise the Worklist:

  To display or hide the columns in the Worklist, select or deselect the check boxes next to the columns. Some check boxes are disabled, the corresponding columns are always displayed.

  Columns can be reordered by dragging and dropping except for the first three columns.

  The "Lab #" and "Sample ID" columns can be renamed by double-clicking on the name and entering a new name of up to 30 characters. A "locked" icon on the right of a column name indicates that the name cannot be changed.

  To get back to the default settings, click the **Defaults** button in the dialogue.

  For a description of the columns, see chapter *12.6 Worklist pane* on page 146.

- **Clear LMD:** Click the menu item to clear the listmode data from the Worklist item. The check mark in the "Acquired" column is no longer displayed indicating that new data can be acquired on this Worklist item. This option is only available if data are acquired on this Worklist item.

- **Clear Item:** The option is only enabled if a single Worklist item is selected that is the first or last item of a panel. Click the menu item to clear all settings from the Worklist item. This option is only available if no data are acquired on this Worklist item.

Panel group

The *Panel* group contains options to edit a panel of the Worklist.
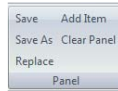
The *Panel* group contains the following buttons:

- **Save:** Click the button to save the panel with the same name.

- **Save as:** Click the button to save the panel with a new name.

  When saving a panel, the full path and file extension are stripped and the Workspace name is used as an identifier. If there are multiple Workspace files present within the panel with the same name, a unique identifier is added to the Workspace file name to differentiate the Workspace files.

- **Replace:** Click the panel to replace the panel. When replacing a panel, the selected panel in the Worklist will be removed, and the chosen panel will be inserted into the Worklist, with the first item of the panel at the same position the first item of the previous panel was at.

- **Add Item:** Click the button to add an item to the panel. The option will duplicate the last row in the panel. The option is only enabled when at least one undefined item is directly below the panel.

- **Clear Panel:** Click the button to clear the panel from the Worklist and to reset the corresponding Worklist items to an undefined state. The option is disabled if LMD files are present in any of the selected panels.

Workspace group

The *Workspace* group contains options to edit the Workspace.
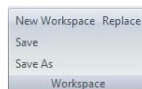
The *Workspace* group contains the following buttons:

- **New Workspace:** Click the button to create a new Workspace file. The option will create a default Workspace file for the selected Worklist item. The option is disabled when more than one Worklist item is selected or when a compensation setup panel is active.

- **Save:** Click the button to save the Workspace file with the same name.

- **Save As:** Click the button to save the Workspace file with a new name.

- **Replace:** Click the button to replace a Workspace file.

Instrument Settings group

The *Instrument Settings* group contains options to save or replace instrument settings files.
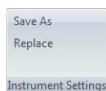
The *Instrument Settings* group contains the following buttons:

- **Save:** Click the button to save the instrument settings file with the same name. The threshold, threshold logic, voltages and compensation settings will be saved to the target file.

- **Save As:** Click the button to save the instrument settings file with a new name.

- **Replace:** Click the button to replace an instrument settings file.

Calculation group

The *Calculation* group contains options to switch between automatic background calculation and manually triggered calculation.
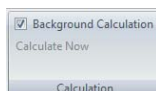
The *Calculation* group contains the following options:

- **Background Calculation:** Select the check box to turn on the automatic background calculation. All statistics in the *Results* tab and objects in the *Reports* tab of the Workspace are calculated in the background as the active panel is modified. This applies to all items within the active panel.

  Deselect the check box to turn off the automatic background calculation. You may want to turn off background calculation when you are using a Worklist containing very large files which are taking a long while to process.

- **Calculate Now:** The button is enabled when the "Background Calculation" check box is deselected. Click the button to start calculation of statistics in the *Results* tab and objects in the *Reports* tabs of the Workspace for all items in the current panel.

  The calculation progress is displayed in a corresponding message box. Closing the message will stop the calculations.

The calculation progress is indicated by the calculation indicator in the status bar (see chapter *12.9 Status bar* on page 184).

12.4.9 Playlist tab

The *Playlist* tab provides controls for modification of all aspects of a Playlist including Playlist items, Playlist files, Workspace files and compensation files.

The *Playlist* tab is only available in Playlist mode.

Listmode group

The *Listmode* group contains options to modify the listmode files in the Playlist.

The *Listmode* group contains the following buttons:

- **Replace:** The button is enabled when one or more Playlist items are selected in the Playlist. To replace the selected Playlist items, click the button and select the desired replacement listmode file(s).

  If the number of selected items in the Playlist does not match the number of the selected replacement listmode files, a confirmation message is displayed. To replace as many files as possible, click the **Yes** button.

  Check the Playlist after replacement to ensure all the selected files have been inserted into the expected positions.

  If any of the selected Playlist items have modified compensation, a message is displayed and you will have the option to keep the modified compensation files in the Playlist. They are then applied to the replaced listmode files.

- **Clear:** The button is only enabled when one or more Playlist items are selected and at least one of them has an associated listmode file. Click the **Clear** button to replace the listmode files in the selected items with a blank listmode item. If the currently open listmode file is amongst the files selected for clearing, plot data will be removed. The Workspace and compensation files set in the Playlist are not affected.

When replacing listmode files, mismatches can occur leading to error notes:

- **Parameter Mismatch:** A parameter mismatch can occur between the listmode file and the Workspace file of an item. To successfully replace the listmode file, the parameter mismatch must be resolved. If the mismatch is not resolved, the listmode file is not replaced and the error state remains displayed in the Playlist.

  If the Playlist item is open in the Workspace, the Parameter Resolver dialogue is displayed allowing you to resolve the mismatch.

  If the Playlist item is not open in the Workspace, an error state is displayed in the Playlist for that Playlist item. The Playlist item text is displayed in red and contains a corresponding tooltip. Open the Playlist item in the Workspace to open the Parameter Resolver dialogue.

- **Scaling Mismatch:** A mismatch between listmode and Workspace scale can occur. In this case, the LMD file is replaced, and the Workspace is modified to match the LMD file settings. Any zoom settings that have been applied are reset. A corresponding message is displayed as soon as the LMD file is opened in the Workspace. As long as mismatched LMD files are not open in the Workspace, they are coloured orange in the Playlist and contain a corresponding tooltip.

- **Compensation Values When Replacing Listmode:** If the Playlist item has no associated compensation file, compensation values will be obtained from the new listmode file. If the Playlist item has an associated compensation file, compensation values will be obtained from the compensation file.

  If a parameter mismatch occurs, opening the LMD file will remove the compensation file applied from the Playlist. A corresponding message is displayed. Select **Yes** to replace the listmode and remove the compensation file applied. Select **No** if you do not wish to replace the listmode file.

  If the Playlist item has a modified compensation, a corresponding message is displayed. Select **Yes** to keep all modified compensation files in the Playlist and apply them to the replaced LMD file. Select **No** to clear the modified compensation values. The compensation values in the replacement LMD file will be used instead. Select **Cancel** to cancel the replacement of the listmode file.

Workspace group

The *Workspace* group contains options to modify the Workspace file associated with Playlist items.
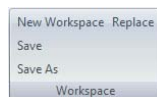
The *Workspace* group contains the following buttons:

- **Replace:** The button is enabled when one or more Playlist items are selected in the Playlist. To replace the Workspace for the selected Playlist items, click the button and select the desired replacement Workspace.

  When replacing Workspace files, a parameter mismatch may occur between the listmode file of the Playlist item and the Workspace file. If the Playlist item is open in the Workspace, the Parameter Resolver dialogue is displayed allowing you to resolve the mismatch. If the Playlist item is not open in the Workspace, an error state is displayed in the Playlist for that Playlist item. The Playlist item text is displayed in red and contains a corresponding tooltip. Open the Playlist item in the Workspace to open the Parameter Resolver dialogue.

- **Save as:** The button is enabled when a single Playlist item containing a Workspace is selected in the Playlist. Click the button to save the current Workspace file with a new file name.

- **Clear:** The button is enabled when one or more Playlist items are selected in the Playlist. Click the button to replace the Workspace files in the selected items with the default blank Workspace. The listmode and compensation files are not affected. Clearing the Workspace file of an open Playlist item will clear the Plots area only.

- **Copy:** The button is enabled when one or more Playlist items are selected and all of them contain a Workspace. Click the button to copy the Workspace files of the selected Playlist items to the clipboard. They can only be pasted to other Playlist items.

- **Paste:** The button becomes enabled when there is a Workspace file copied to the clipboard.

  If a single Workspace file is copied, clicking the **Paste** button will paste the clipboard's Workspace file into all selected items in the Playlist.

  If multiple Workspace files are copied, clicking the **Paste** button will paste the clipboard's Workspace files into the selected items in the Playlist. Pasted Workspace files will be pasted in the same order in which they appeared in the Playlist when copied. If you attempt to paste more items than are currently selected in the Playlist, as many items as possible will be pasted, any excess items will be ignored. If the target selection contains more Playlist items than the clipboard contains copied Workspace files, the pasted Workspace files will be repeated until all selected Playlist items are pasted a Workspace file.

  After pasting Workspace files, make sure that the positions of regions are correct for the individual listmode files.

Compensation group

The *Compensation* group contains options to modify compensation files in the Playlist.
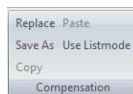
The *Compensation* group contains the following buttons:

- **Replace:** The button is enabled when one or more Playlist items are selected in the Playlist and at least one of the selected Playlist items is compensable. To replace the compensation file(s) of the selected items, click the button and select the desired replacement compensation file. If the Playlist item contains a low-resolution LMD file, the compensation file is not opened for this item, and a corresponding message is displayed.

  If the compensation for the current file is replaced with a file that is not compatible with the listmode file (e.g. parameter mismatch), the compensation file is not opened, and a corresponding message is displayed.

  If the compensation for a file not currently open is replaced with a file that is not compatible with the listmode file, the item turns red and contains a tooltip. If you open this Playlist item in the Workspace, a message is

displayed and you will have the option to abort opening the listmode file or to open the listmode file and remove the compensation file from the Playlist item.

- **Save As:** The button is enabled when a single compensable Playlist item is selected in the Playlist. Click the button to save the current compensation file with a new file name.

- **Copy:** The button is enabled when at least one of the selected items contains a compensable listmode file and/or compensation file. Click the button to copy the compensation files of the selected item(s) to the clipboard. They can then be pasted into the Playlist.

- **Paste:** The button becomes enabled when there is a compensation file from the Playlist copied into the clipboard and at least one of the selected items is compensable.

  If a single compensation file is copied, clicking the **Paste** button will paste the clipboards' compensation file into all selected items in the Playlist.

  If multiple compensation files are copied, clicking the **Paste** button will paste the clipboards' compensation files into the relevant selected items in the Playlist. Pasted compensation files will be pasted in the same order in which they appeared in the Playlist when copied. If you attempt to paste more items than are currently selected in the Playlist, as many items as possible will be pasted, any excess items will be ignored. If the target selection contains more Playlist items than the clipboard contains copied compensation files, the pasted compensation files will be repeated until all selected Playlist items are pasted a compensation file.

  When pasting a single or multiple compensation files, the parameters in the compensation files may not match those in the listmode file. In this case, the copied compensation files will be applied to the compatible items in the Playlist but not to the incompatible ones, and a corresponding message is displayed.

  If the parameters do not match and the item is not currently open, the message will only be displayed when you try to open the item containing the incompatible listmode and compensation files.

- **Use listmode:** The button is enabled when at least one of the selected Playlist items is compensable.

  Click the button to replace the compensation files of all items containing a high-resolution data file with the compensation settings stored in the FCS file headers. Any selected items that don't contain high-resolution data will not be updated, and a corresponding message will be displayed.

Calculation group

The *Calculation* group contains options to switch between automatic background calculation and manually triggered calculation.
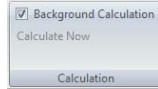
The *Calculation* group contains the following options:

- **Background Calculation:** Select the check box to turn on the automatic background calculation. All statistics in the *Results* and *Reports* tabs of the Workspace are updated constantly in the background as the Playlist is modified. Whilst the software calculates plots, statistics, compensation matrix and fields, the corresponding report item will display a question mark. As soon as the new or updated plots, statistics, compensation matrix and/or field are calculated, they will be displayed on the report. Uncalculated statistics do not appear on the results table. Any printing operations will wait for background calculation to be completed.

  Deselect the check box to turn off the automatic background calculation. You may want to turn off background calculation when you are using a Playlist containing very large files which are taking a long while to process.

- **Calculate Now:** The button is enabled when the "Background Calculation" check box is deselected. Click the button to start calculation of all statistics in the *Results* tab and objects in the *Reports* tab of the Workspace. The calculation progress is displayed in a corresponding message box. Closing the message will stop the calculations.

The calculation progress is indicated by the calculation indicator in the status bar (see chapter *12.9 Status bar* on page 184).

12.4.10 Cytometer tab

The *Cytometer* tab provides controls to start cleaning procedures of the device, to sort cells if a cell sorter is connected, and to prepare compensation settings for measurements.

Cleaning group

The *Cleaning* group contains options for starting priming and cleaning procedures.
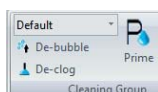
The *Cleaning* group contains the following options:

- **Script Selection:** In this box, the intensity of the cleaning procedure to be performed can be preselected. Click the arrow to expand a drop-down list containing the scripts specified for the level of cleaning functions and select the desired intensity. The "Default" option is the generally used cleaning level. The "Deep" option preselects a thorough procedure, the "Quick" option preselects a short cleaning procedure.

- **De-bubble:** Click the button to start a procedure to clear bubbles from the flow cuvette. Once started, the software guides you through the process.

- **De-clog:** Click the button to start a procedure to clear residual particles from the device lines when clogged. Once started, the software guides you through the process.

- **Prime:** Click the button to start a priming procedure. Once started, the software guides you through the process.

- **Clean:** Click the button to start a cleaning procedure. Once started, the software guides you through the process.

Sort group

> ⓘ **Information**
>
> The *Sort* group is only displayed if the necessary sorter upgrade is connected and ready to use.

Compensation Settings group

The *Compensation Settings* group contains options to create a fluorescence compensation setup panel in the Worklist. The options are only available if an empty Worklist item is selected but not opened in the Workspace.

Click the **Create Comp Panel** button and select a Workspace file (CyFlow acquisition Workspace files only) to use as a source for the compensation setup panel. From this Workspace file, the parameters and instrument settings (voltages, trigger logic and threshold information) are derived.

The Create Compensation Panel dialogue is displayed.
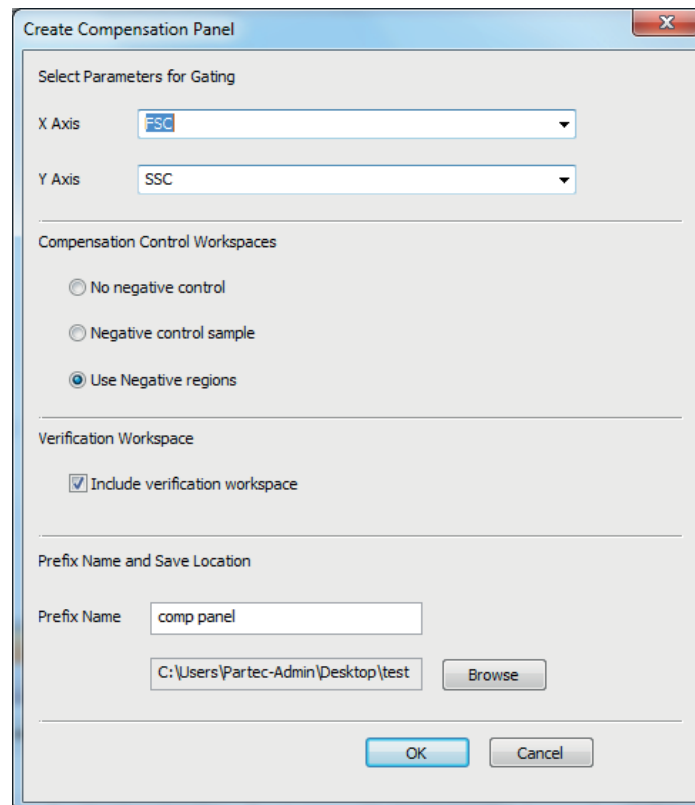
The Create Compensation Panel dialogue contains the following options:

- **Select Parameters for Gating:** Click the buttons to display drop-down lists and select the desired X and Y parameters for gating. These parameters will be used for the X-axis and Y-axis of gating plots within the negative control sample (if selected), the compensation calculation Workspaces and the verification tube (if selected). The drop-down lists contain all parameters in the selected Workspace file. By default, the first parameter of the list is used for the X-axis, the second parameter of the list is used for the Y-axis.

- **Compensation Control Workspaces:** The option selected here defines the type of Workspaces that are created for the compensation setup panel.

  The "No negative control" option is the default selection. This will create a compensation control Workspace for each of the classified fluorescence parameters present within the original Workspace selected.

  The "Negative control sample" option will add a negative control Worklist item and Workspace within the compensation setup panel which will run a negative control for baseline background measurement.

  The "Use negative regions" option will add a negative region to each of the fluorescence plots within the compensation control Workspaces.

- **Verification Workspace:** Selecting the "Include Verification Workspace" check box will add a verification item to the compensation setup panel. The verification item will include a verification Workspace and the instrument settings file of the compensation setup panel.

- **Prefix Name and Save Location:** If desired, enter a prefix name for the Workspace, panel and settings file created by the compensation setup procedure. The default name will be the name of the original Workspace file selected. The default save location will be the last location used. If desired, click the **Browse** button to select another file for the created compensation settings file to be saved.

On clicking the **OK** button, the required compensation setup panel, Workspaces and instrument settings file will be created. The created panel will be inserted into the Worklist.

The created files will be named as follows:

- Panel: <prefix name>-SetupPanel.xml

- Voltage adjust Workspace: <prefix name>voltage.xmlws

- Negative Control Workspace: <prefix name>negative.xmlws

- Compensation Workspace: <prefix name>compX.xmlws

- Compensation Workspace with negative regions: <prefix name>compXNegRegion.xmlws

- Verification Workspace: <prefix name>verification.xmlws Instrument

- Settings File: <prefix name>settings.xml

Compensation setup panel

The compensation setup panel will be created using the selections made in the Create Compensation Panel dialogue. The created Workspaces are placed in the compensation setup panel. The parameters selected will show scaling as dictated by the original Workspace file. The panel is set to load the instrument settings from the associated instrument settings file. The instrument settings file contains the voltages, trigger logic and threshold information from the selected source Workspace file.

The compensation setup panel is made up of the following components and always appears in this order:

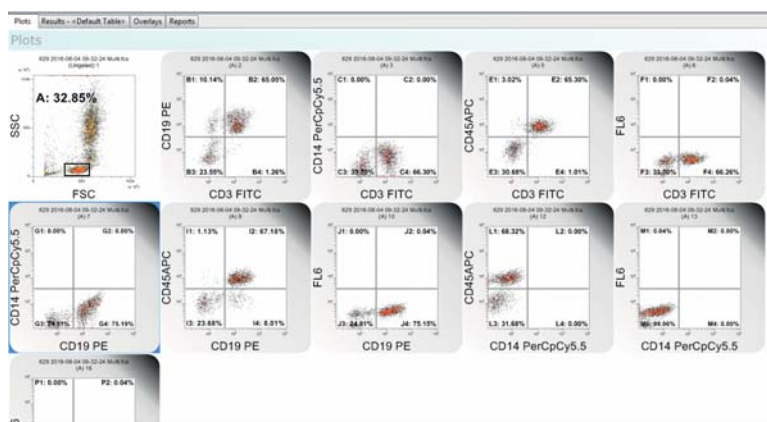- Voltage adjust Workspace (always present):
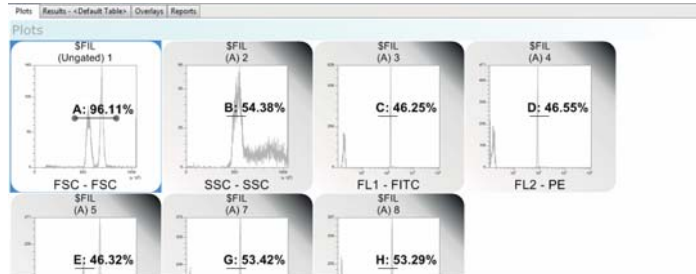
- Negative control sample Workspace (optional):
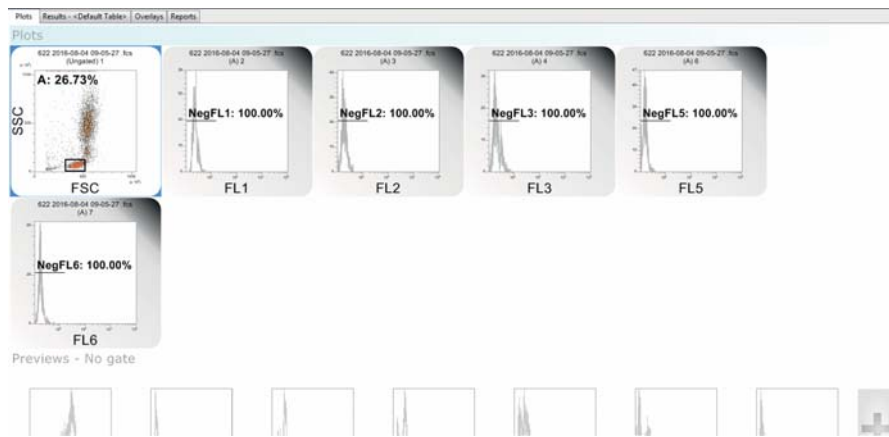
- Compensation control Workspaces – one per fluorescence parameter (always present):
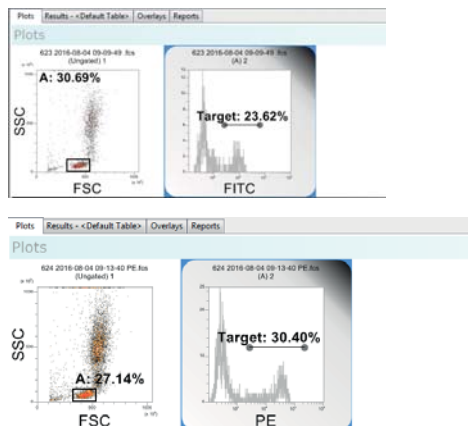
■ Verification Workspace (optional):

When acquisition is completed on the last compensation Workspace or, if existing, on the verification sample, the calculated compensation matrix is displayed.

12.4.11 Overlay tab

The *Overlay* tab provides controls for creating single and dual parameter plot overlays. Overlay plots can be used to compare plots. The overlay plots are displayed in the *Overlays* tab of the Workspace (see chapter *12.8.3 Overlays tab of the Workspace* on page 174).

Wizard group

The Overlay Wizard is only available in Playlist mode. The Overlay Wizard simplifies the process of generating overlay plots from a Playlist of samples.

Before starting the Overlay Wizard, make sure Workspaces in the Playlist are the same for all files.

Click the **Overlay Wizard** button to launch the Wizard.

The Overlay Wizard is made up of four types of pages:

■ Welcome Page,

■ Plot Selection Page,

■ Overlay Layout Page,

■ Overlay Labels Page.

Overlay Wizard: Welcome Page

The Welcome page introduces you to the Wizard's functionality.
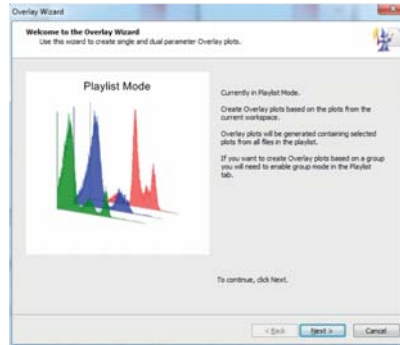
Click the **Next** button to proceed to the Plot Selection page of the Wizard.

Overlay Wizard: Plot Selection Page

The Plot Selection page contains options to select the required plots to create an overlay plot.
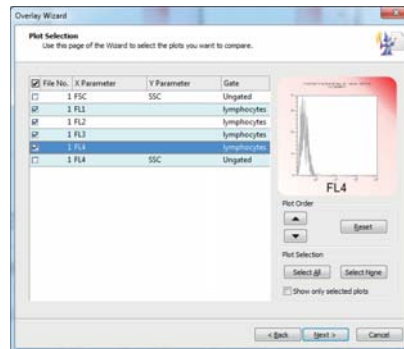
A list of the plots from the current Workspace is displayed. By selecting a line of the list, the line is highlighted and a preview of the corresponding plot is displayed.

For each plot, the list displays the item number for the Workspace containing the plot, the X and Y parameters and the gate.

To add a plot to the overlay plot, select the check box next to the relevant plot in the list.

The page contains the following options:

■ **Plot Order:** Plots appear in the created overlay plot in the order they appear in the plot list. To change the order, click the up/down arrows to move selected plots up and down in the list. To reset the plot order, use the **Reset** button.

■ **Plot Selection:** Click the **Select All** or **Select None** buttons to easily select or deselect all plots. To display only the plots which you have selected for the overlays, select the "Show only selected plots" check box.

■ **Next:** Click the button to proceed to the Overlay Plot Selection page.

Overlay Wizard: Overlay Layout Page

The Overlay Layout Page contains options to define how the plots you have selected will be added to the overlay plot.
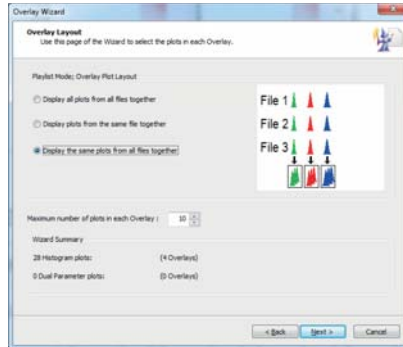
The page contains the following options:

- **Display all plots from all files together:** Select the option to display all the selected plots from all the files in the Playlist on one overlay plot. If you have selected both single and dual parameter plots, these are arranged on separate overlay plots.

- **Display all plots from the same file together:** Select the option to display all the selected plots from each file in the Playlist on one overlay plot. Each file in the Playlist is displayed on a new overlay plot. If you have selected both single and dual parameter plots, these are arranged on separate overlay plots.

- **Display the same plots from all files together:** Select the option to display the same plot from all the files in the Playlist on one overlay plot. Different plots are displayed on separate overlay plots.

- **Maximum number of plots in each overlay:** Enter a maximum number of plots on one overlay plot. If the number of plots selected exceeds this number, additional overlay plots are created as required.

- **Wizard Summary:** This section displays the number of single parameter and the number of dual parameter plots selected and details how many overlay plots are created based on the selections made above.

Overlay Wizard: Overlay Labels Page

This page contains options to define titles and legends to be displayed in the *Overlays* tab of the Workspace.
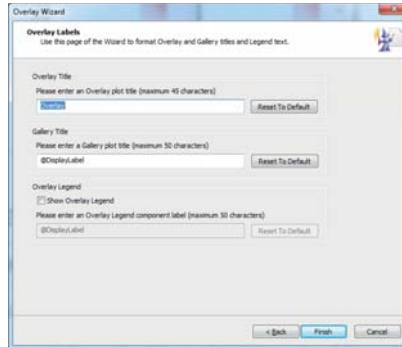
The page contains the following options:

- **Overlay Title:** Enter a title for the overlay plot.

- **Gallery Title:** Enter a title for the Galleries area of the Workspace.

- **Overlay Legend:** Select the "Show Overlay Legend" check box and enter a label to display a legend next to the overlay plot in the Overlays area of the Workspace.

- **Reset to default:** Click the button to reset the corresponding title or legend to its default name.

Region group

The *Region* group contains options for creating and editing regions. You can create a maximum of 256 regions per Workspace. Once this number is reached, the region creation buttons in the *Region* group become disabled.

The *Region* group contains the following options:

- **Elliptical Region:** Click the button to preselect creating an elliptical region on a dual parameter plot.

- **Rectangular Region:** Click the button to preselect creating a rectangular region on a dual parameter plot.

- **Polygonal Region:** Click the button to preselect creating a polygonal region on a dual parameter plot.

- **Linear Region:** Click the button to preselect creating a linear region on a single parameter plot.

- **Quadrant Region:** Click the button to preselect creating a quadrant region on a dual parameter plot.

- **Region Position:** Select a region on a plot and click the button to modify the position of the selected region as described for the *Region Format* tab (see chapter *Position group* on page 129).

For further information on defining and editing regions, see chapter *Creating regions* on page 165.

Plots group

The *Plots* group contains options to insert plots from the Plots area of the Workspace into overlay plots.

The *Plots* group contains the following button:

- **Insert Plot:** The button is only enabled when an overlay plot is selected in the Overlays area of the Workspace. Click the button to display a drop-down selection of all the plots currently open in the Plots Workspace. The plots appear in the order they appear in the Workspace when in Gallery view. When hovering the cursor over a plot, plots that are not part of the current overlay plot will be highlighted. Click on the desired highlighted plot to insert it into the current overlay plot.

  Plots that are not highlighted are already part of the current overlay plot and cannot be added again.

Galleries View group

The *Galleries View* group contains options to modify the way in which plots are displayed in the Galleries area.

The *Galleries View* group contains the following button:

- **Wrap Galleries:** The button controls the display of gallery plots for each overlay plot in single or multiple rows. Click the button to display the gallery plots on multiple rows if necessary to fit the current window width.

Edit group

The *Edit* group contains options to copy, delete and select plots in the Plots area and to edit the fonts of text items in the current Workspace.

The *Edit* group contains the following buttons:

- **Copy:** Click the button to copy an image of the currently selected plot(s) onto the clipboard. You can then paste them into other applications (Microsoft® Word, PowerPoint, etc.).

- **Delete:** Click the button to delete the currently selected plots or region. If the deleted plot has a region applied, deleting the plot also deletes the region and removes the region from any gating logic. In this case, a corresponding message box is displayed and you will have the option to confirm or abort deleting the plot.

- **Select:** Click the button and select an option for selecting plots:

  Click the **Select All Overlay Plots** option to select all the plots in the Overlays area.

  Click the **Select All Gallery Plots** option to select all the gallery plots for the currently selected Overlay Plot. This option is only available when one overlay is active.

  Click the **Invert Selection** option to select all the plots that are currently not selected in the Overlays area or the Galleries area. This option is only enabled if either a selection is made or when only one overlay is active.

  Click the **Select All Dual Parameter Overlay Plots** or **Select All Histogram Overlay Plots** to select all dual parameter or all histogram overlay plots respectively.

- **Fonts:** Click the button to open the Edit Overlay Fonts dialogue. In the pages of this dialogue, you can change the font typeface and size for the region labels, plot titles, parameter names, legend/stats text and scale labels displayed in the *Overlays* tab of the Workspace. The pages are the same as in the software options (see chapter *Font pages* on page 192).

12.4.12 Report tab

The *Report* tab provides controls for creating page-based reports from a Playlist or from a panel of a Worklist. The *Report* tab allows you to add and set up report pages, insert objects and text into the report and edit and organise the objects on the report. The reports are displayed in the *Reports* tab of the Workspace (see chapter *12.8.4 Reports tab of the Workspace* on page 179).

Wizard group

The Report Wizard is only available in Playlist mode. The Report Wizard simplifies the process of creating a report.

When creating a report via the Wizard, the current report page size and orientation will be used, and any existing header and footer content will be retained. Therefore, before starting the Report Wizard, set the report page size and orientation using the *Page Setup* group of the *Report* tab and insert the desired header and footer content using the *Insert* group of the *Report* tab.

The Wizard retains settings until you open or create a new Playlist.

Click the **Report Wizard** button to launch the Wizard. The button is disabled when there are no plots in the Workspace.

The Report Wizard is made up of five types of pages:

- Welcome Page,
- Plots Selection Page,
- Statistics Layout Page,
- Statistics Selection Page,
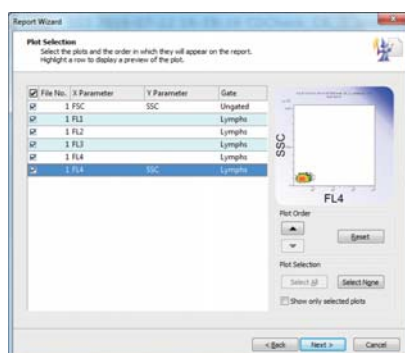- Report Layout Page.

Report Wizard: Welcome page

The Welcome page introduces you to the Wizard's functionality.
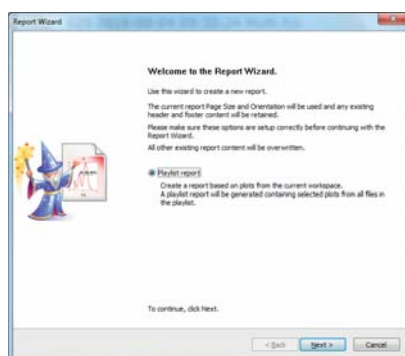
Click the **Next** button to proceed to the Plots Selection page of the Wizard.

Report Wizard: Plots Selection page

The Plots Selection page enables you to pick the required plots for the report that will be created by the Wizard.

A list of the plots of the current Workspace is displayed. By selecting a line of the list, the line is highlighted and a preview of the corresponding plot is displayed.

For each plot, the list displays the item number for the Workspace containing the plot, the X and Y parameters and the gate.

To add a plot to the report, select the check box next to this plot. During a session of using the same Playlist when rerunning the Wizard, previously checked plots will be remembered. Changing the Playlist or closing the software loses these settings.

The page contains the following options:

- **Plot Order:** Plots appear in the report in the order they appear in the plot list. To change the order, click the up/down arrows to move selected plots up and down in the list. To reset the plot order, use the **Reset** button.

- **Plot Selection:** Click the **Select All** or **Select None** buttons to select or deselect all plots. To display only the plots which you have selected for the report, select the "Show only selected plots" check box.

- **Next:** Click the button to proceed to the Statistics Layout page.

Report Wizard: Statistics Layout page

The Statistics Layout page enables you to select the required statistics for the report and their layout on the report. A preview of the layout of the statistics on the page is displayed.
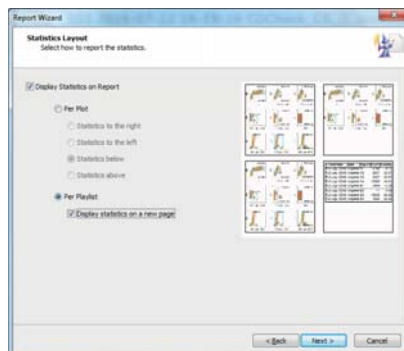
The page contains the following options:

- **Display Statistics on Report:** Select the check box to display statistics on the generated report.

- **Per Plot:** Select the option to display the statistics next to the associated plot. Select the desired position for the display of statistics (to the right, to the left, below or above the plot).

- **Per Playlist:** Select the option if you would like all statistics displayed together at the end of the Playlist report. To start statistics on a new page of the report, select the "Display statistics on a new page" check box.

- **Next:** Click the button to proceed to the next page of the Wizard.

Report Wizard: Statistics Selection page

This page is only available if the "Display Statistics on Report" check box was selected on the Statistics Layout page. The Statistics Selection page enables you to select the required statistics and the order in which they are displayed on the report.
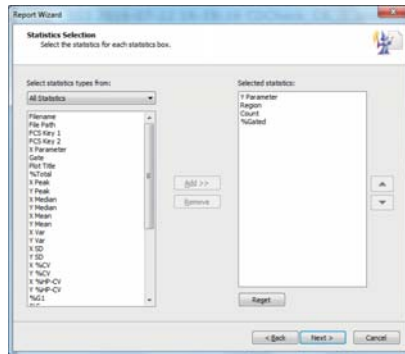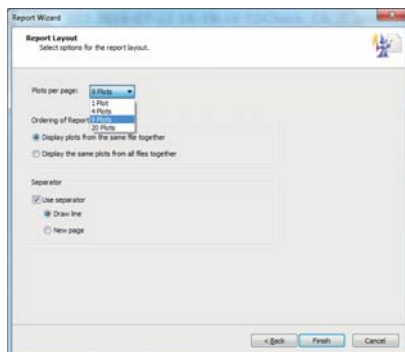

The page contains the following options:

- **Select statistics types from:** By default, all statistics are available for selection. Use the box to view a reduced group of statistics.

- **Add:** Click the button to add a selected statistic to the "Selected statistics" list. By default, the "Selected statistics" list is populated only with the statistics that are selected in the *Results* tab of the Workspace.

- **Remove:** Click the button to remove a selected statistic from the "Selected statistics" list.

- **Arrow buttons:** Statistics appear in the generated report in the order they appear in the "Selected Statistics" list. To change the order, click the up/down arrows to move selected statistics up and down in the list.

- **Reset:** Click the button to reset the selected statistics to their state when the page was first displayed.

Report Wizard: Report Layout page

The Report Layout page enables you to select the required report layout.

- **Plots per page:** Use the drop-down list to specify the number of plots per page in the report. When not reporting statistics or when statistics are aggregated at the end of the report, you can have 1, 4, 9 or 20 plots per page. When reporting statistics, the options are 1, 2 or 3 plots per page.

- **Display plots from the same file together:** Select the option if you would like plots from the same file to be kept together in the report. Each section has all the plots belonging to the same file.

- **Display the same plots from all files together:** Select the option if you would like the same plots from all files displayed together. Each section has all the plots with the same parameters.

- **Use separator:** Select the check box to separate sections in the generated report and select whether to separate them with a line or starting on a new page.

- **Finish:** Click the button to complete the Wizard. The Wizard closes and the report is generated. If the report requires more than 800 pages, you are warned via a message box. Select OK to close the message. The Report Wizard remains open to enable you to make changes to the required report.

Edit group

The *Edit* group contains options to select and delete objects on the report.

The *Edit* group contains the following options:

- **Delete:** Click the button or press the **Delete** key on the keyboard to delete all currently selected objects.

- **Select:** Click the button to display a drop-down list and select the desired option:

  Click the **Select All** menu item to select all objects from the body sections of all pages in the report. Objects in the headers and footers are not selected.

  The **Select All of Same Type** menu item is only enabled when a single object is selected in the report. Click the menu item to select all objects of the same type as the currently selected item from the body sections of all pages in the report. If an object in the header or footer is selected, clicking the menu item will select all corresponding instances of that object in the body sections of all pages in the report. The object originally selected in the header or footer will no longer be selected.

  Click the **Select All Header and Footer** menu item to select all objects in the header and footer sections of all pages in the report.

Pages group

The *Pages* group contains options to add new pages to the report and to organise the pages of the report.
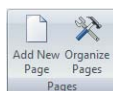
The *Pages* group contains the following options:

- **Add New Page:** Click the button to add a new blank page to the end of the report. All new pages are assigned a unique number, which is displayed at the top left-hand corner. All new pages display a "Page X of Y" in the bottom right-hand corner. Up to 800 pages can be added to the Report. When the maximum number of pages is reached, the **Add New Page** button is disabled.

- **Organize Pages:** Click the button to display the "Organize Report Pages" dialogue. A list of all pages is displayed. Select the desired page and click the corresponding button to insert a page before or after the selected page, to move the page up or down, to delete the page or to delete all pages.

  A newly inserted page is given the next available unique number (between 1 and 800) and has the suffix "(new)" in the dialogue. The naming is a temporary name which will be updated when the dialogue is closed and reopened.

  A maximum of 800 pages can be inserted into each report. When this limit is reached, the **Insert Page Before** button is disabled.

  To preview a page in the Workspace, double-click on the page in the list.

  Click the **OK** button to close the "Organize Report Pages" dialogue and accept the changes made to the report pages. Click the **Cancel** button to close the "Organize Report Pages" dialogue and cancel any changes made to the report pages.

Page Setup group

The *Page Setup* group contains options to change the orientation and size of the pages in the report.
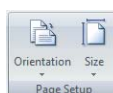
The *Page Setup* group contains the following options:

- **Orientation:** Click the button to display a drop-down list and select the desired page orientation (portrait or landscape). The page orientation to all pages in the report is changed. All header, footer and body objects that lie outside the bounds of the new page are automatically resized to fit the new orientation. If the orientation is then switched back, any resized objects are not restored to their original size or position. In order to restore pages exactly as they were, click the **Undo** button in the Quick Access Toolbar.

The option selected here will take precedence over that selected in the "Print Setup" dialogue.

- **Size:** Click the button to display a drop-down list and select the desired page size (A4 or letter). The page size for all pages in the report is changed. All header, footer and body objects that lie outside the bounds of the new page size are automatically resized to fit the new size. If the size is then switched back, any resized objects are not restored to their original size or position. In order to restore pages exactly as they were, click the **Undo** button in the Quick Access Toolbar.

  If there is a mismatch between the paper size set in the "Print Setup" and the size selected here, the page will be automatically scaled to fit the paper when printing.

Insert group

The *Insert* group contains options to insert objects into the report. The controls are only enabled when you are on the *Reports* tab of the Workspace. To insert an object into the report, first select it with the buttons described below, then click on the report to insert the object in default size or draw out a rectangle of the desired size. For more information on inserting, resizing and moving objects in the report, see chapter *12.8.4 Reports tab of the Workspace* on page 179.
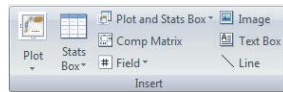
The *Insert* group contains the following buttons:

- **Plot:** Click the Plot button to display a drop-down gallery of all the plots currently open in the Workspace. The order in which the plots appear matches the order they appear in the Workspace when in Gallery view. To select a plot for insertion into the report, click on the desired plot.

- **Stats Box:** Click the button to display a drop-down gallery of all the plots currently open in the Workspace. The order in which the plots appear matches the order they appear in the Workspace when in Gallery view. Click on a plot to select the corresponding statistics box for insertion into the report.

- **Plot and Stats Box:** Click the button to display a drop-down gallery of all the plots currently open in the Workspace. The order in which the plots appear matches the order they appear in the Workspace when in Gallery view. Click on a plot to select the plot and the corresponding statistics box for insertion into the report.

- **Comp Matrix:** The button is only enabled when an LMD file is open. Click the button to select the compensation matrix for insertion into the report.

- **Field:** The button is only enabled when an LMD file is open. Click the button to open a drop-down list and select a report item for insertion into the report.

In the "Report Item" drop-down list, the page number, date and time, user name, file names and/or file paths with file names of the currently open files can be selected.

In the "Primary Keyword" and "Secondary Keyword" submenus, the primary and secondary keywords from the currently open LMD file can be selected.

- **Image:** Click the button and navigate to the desired folder to select an image file for insertion into the report. Only image files with the extension ".jpg", ".gif", ".bmp" and ".png" are supported. If required, use the "Save as Type" drop-down list to display pictures of a viable file type.

  Click the **Open** button or double-click on the image file to insert the selected image file into the report. If the inserted image lies outside the bounds of the page, it is automatically resized. Images are inserted into the body section of a page by default. They can, however, be moved into the header or footer.

- **Text box:** Click the button to select a text box for insertion. Click on the report page at the point where you wish to place the text box. Double click on the text box to enter the desired text. You may need to resize the text box.

- **Line:** Click the button to select a line for insertion. Click on the report page at the point where you wish to place the line.

Text group

The *Text* group contains controls for editing and organising of text.

Select a text box, statistics box, compensation matrix and/or field and use the options in the *Text* group to edit the font typeface, font size, font style (bold, italic, underlined) and colour of the selected object.

Select a text box and/or a field and use the alignment buttons (align left, centre, align right) to align the text.

Format group

The *Format* group contains options to format items in the report.
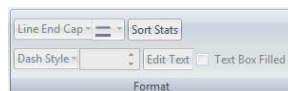
The *Format* group contains the following options:

- **Line End Cap:** The box is enabled when one or more line objects are selected in the report. Click the arrow to expand a drop-down list and select the desired option for the line end (uncapped, solid arrowhead or line arrowhead).

■ **Line Colour:** The button is enabled when one or more line objects are selected in the report. Click the button and select the desired colour for the currently selected lines.

■ **Sort Stats:** The button is enabled when a statistics box is selected in reports. It is also enabled when multiple statistics boxes are selected provided the selected statistics boxes contain exactly the same statistics.

Click the button to display the "Sort Statistics" dialogue. Use the up/down arrows to define the left to right column order. Select the statistic to sort rows by and whether to sort rows in ascending or descending order as required.

■ **Dash Style:** The box is enabled when one or more line objects are selected in the report. Click the arrow to display a drop-down list and select the desired dash style.

■ **Line Width:** The control is enabled when one or more line objects are selected in the report. The default line width for inserted lines is 1.00 pt. Type in the desired width or use the up/down arrows to adjust the width. The line width must be between 0.25 pts and 50 pts.

■ **Edit Text:** The button is only enabled when a single text box is selected in the report. Click the button and enter the desired text into the edit field. The maximum number of characters is 8000.

■ **Textbox Filled:** The check box is only enabled when one or more text boxes or fields are selected in the report. Select the check box to show the selected text boxes filled.

Arrange group

The *Arrange* group contains options to move, align and resize report items.

The *Arrange* group contains the following options:

■ **Bring To Front:** The button is enabled when one or more objects are selected on the same page and section (header, footer, body). Click the button to move the selected objects to the front of the view, making them appear on top of non-selected objects.

■ **Send To Back:** The button is enabled when one or more objects are selected on the same page and section. Click the button to move the selected objects to the back of the view, making them appear behind non-selected objects.

■ **Align:** The button is enabled when two or more objects on the same page and section are selected. Click the button to display a drop-down list and select how to align the selected objects. When aligning objects, they are subject to a bounds check. If an object lies outside the bounds

of the page after it has been aligned, it is automatically repositioned to fit on the page.

- **Resize:** The button allows for adjusting two or more objects to the same height, width or size. First select the object that displays the target size, then select the objects to resize. Click the button to display a drop-down list and select whether to make the objects the same height, the same width or the same size as the first selected object.

  When plots are selected in the objects to resize, their aspect ratios are always maintained. Using the "Make Same Size" option, the plots are sized to the smallest dimension (height or width) of the first selected object.

Zoom

The *Zoom* group contains options to zoom in and out on the page using predefined and custom percentages.
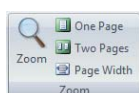
Click the **Zoom** button to display a dialogue and use the dialogue options to zoom in and zoom out as required.

12.4.13 Views tab

The *Views* tab provides controls to define the panes that are available.

Click the corresponding button to select or deselect viewing the following panes:

- *Acquire* pane,
- *Stops* pane,
- *Parameter Settings* pane,
- *Setup* pane,
- *FCS File Information* pane,
- *Results Hierarchy* pane.

A button is highlighted in orange if the corresponding pane is available for viewing.

Default Position: Click the button to view all panes in their default position. The default position for the Worklist is docked to the bottom of the Workspace. The default position for the Playlist is docked and pinned to the left-hand side of the Workspace. The default position of the other panes is docked, pinned and nested together on the left-hand side of the Workspace.

The view settings selected here are persisted between sessions.

12.4.14 Plot Format tab

The *Plot Format* tab is only available when at least one plot is selected in the Plots area of the Workspace (see chapter *12.8.1 Plots tab of the Workspace* on page 160).

The *Plot Format* tab contains controls to adjust the settings of selected plots in the Plots area.

Plot Type group

The *Plot Type* group contains options to format dual parameter plots as density plots, colour precedence plots or precedence density plots. For information on the plot types, see chapter *12.8.1 Plots tab of the Workspace* on page 160.

The group also contains options for formatting single parameter plots as histogram or cell cycle plots.
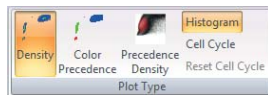
The *Plot Type* group contains the following options:

- **Density:** Select one or more dual parameter plots and click the button to display the selected plots as density plots. The dual parameter plots in the Plots area are density plots by default.

- **Color Precedence:** Select one or more dual parameter plots and click the button to display the selected plots as colour precedence plots.

- **Precedence Density:** Select one or more dual parameter plots and click the button to display the selected plots as precedence density plots.

- **Histogram:** Select one or more single parameter plots and click the button to display the selected plots as histograms. The single parameter plots in the Plots area are standard histogram plots by default.

- **Cell Cycle:** Select one or more single parameter plots and click the button to display the selected plots as DNA cell cycle plots (see chapter *DNA cell cycle plots* on page 169). Each cell cycle is identified numerically with the title "Cell Cycle #".

- **Reset Cell Cycle:** The button is only enabled when a cell cycle plot is selected. If the G1 and/or G2 markers have been manually adjusted, clicking the **Reset Cell Cycle** button returns the markers to their automatically calculated positions.

Gate group

The *Gate* group contains an option to choose a gate to be applied to selected plots.

Click the drop-down arrow positioned on the *Gate* group to display a list of all the available gates. Click the desired gate from this list to apply the gate to selected plots.

X-Axis group

The *X-Axis* group contains options to change the X-axis of the selected plots to the linear, logarithmic or V-Log scale type.

If a low-resolution file is open, the axis parameters are set by the information within the LMD file header and cannot be changed. The X-axis buttons are disabled for low-resolution files and if cell-cycle plots have been selected.

For information on the available plot scaling types, see chapter *Scaling* on page 168.

The *X-Axis* group contains the following options:

- **Linear, Logarithmic, V-Log:** Select the desired plots in the Plots area. If the selected plots have different axis types, multiple axis buttons will be highlighted. Click the desired button to apply the axis selection to the selected plots.

Y-Axis group

The *Y-Axis* group contains options to change the Y-axis of the selected plots to the linear, logarithmic or V-Log scale type.

If a low-resolution file is open, the axis parameters are set by the information within the LMD file header and cannot be changed. The Y-axis buttons are disabled for low-resolution files and if single parameter histogram plots have been selected.

For information on the available plot scaling types, see chapter *Scaling* on page 168.

The *Y-Axis* group contains the following options:

- **Linear, Logarithmic, V-Log:** Select the desired plots in the Plots area. If the selected plots have different axis types, multiple axis buttons will be highlighted. Click the desired button to apply the axis selection to the selected plots.

Histogram Scaling group

The *Histogram Scaling* group contains options for scaling of the selected histograms for optimal plot visualisation. By default, the software scales the histogram plots to the maximum value on the plot. The automatic scaling ignores events in the first and last sixteen channels, unless there are no events in the remaining channels.
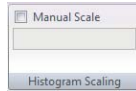
The *Histogram Scaling* group contains the following option:

- **Manual Scale:** The check box is enabled if at least one histogram plot is selected. Check the check box to turn off the automatic scaling. For manual scaling, enter the desired scaling value into the edit field and press **Enter**.

Histogram Smoothing group

The purpose of smoothing is to improve the visual display of histograms for presentation purposes. The underlying data and statistics are not altered. Smoothing can only be applied to single parameter histograms.

Two methods of smoothing are available: Bin Reduction and Moving Average. In the Bin Reduction method of smoothing, the values of consecutive bins are averaged to produce a histogram with a reduced number of bins. The software implements 4 levels of bin reduction. Each level has the number of bins displayed on the plot: Level 1 (bin count 1024; no smoothing), Level 2 (bin count 512), Level 3 (bin count 256), Level 4 (bin count 128).

In the Moving Average method of smoothing, the average value of a number of consecutive bins (window size) of the raw histogram is used in the first bin in the window. This is repeated for the next bin until the whole histogram has been covered. The process can be repeated a number of times to produce an increasingly smoothed graph. There are ten levels of smoothing available by this method. The level 1 setting is the same as no smoothing.
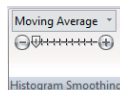
The *Histogram Smoothing* group contains the following options:

- **Histogram Smoothing:** The button is enabled when one or more single parameter plots are selected. The button displays the currently active smoothing method. To change the smoothing method, click the arrow to open the drop-down list and select the desired option (none, bin reduction, moving average). The selected method is applied to all currently selected plots. Plot smoothing is displayed on the reports, print preview and printout. The smoothing settings are saved as part of the Workspace whenever the Workspace or the Playlist are saved. Applying smoothing to plots does not affect statistical results.

■ **Histogram Smoothing Level Selection Slider:** The slider is only visible if a smoothing method (bin reduction, moving average) is selected.

When Bin Reduction is selected, the slider has 4 tick marks representing levels 1 – 4. Level 1 (1024 bins) is no smoothing. When Moving Average is selected, the slider has 10 tick marks, representing levels 1 – 10. Level 1 is no smoothing, level 10 is the greatest level of smoothing. The currently selected level in the slider is marked by an arrow.

Click the "minus" button or to the left of the arrow on the slider to decrease the level by one. Click the "plus" button or to the right of the arrow on the slider to increase the level by one. The level can also be changed by dragging and dropping the arrow with the mouse.

The smoothing level selected is applied to all currently selected single parameter histograms and cell cycle plots. The default setting is level 1 (No smoothing). The level setting for each method is persisted for each plot and saved in the Workspace.

Histogram Styles group

The *Histogram Styles* group contains options to choose colour and filling of the selected histograms. By default, histograms are displayed using the colour specified in the software options (see chapter *General page* on page 187).
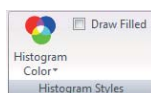
The *Histogram Styles* group contains the following options:

■ **Histogram Color:** Click the button and select the desired colour to modify the line and fill colour of the selected histogram(s) in the Plots area.

■ **Draw Filled:** Check the check box to fill the selected histograms of the current file with the colour set with the **Histogram Color** button. The "Draw Filled" selection is saved in the Workspace.

12.4.15 Region Format tab

The *Region Format* tab is only available when a region is selected in the Plots area.

You can use the options on the *Region Format* tab to modify the appearance and type of a selected region. The region label can also be repositioned and regions can be tracked to other regions.

Appearance group

The *Appearance* group contains options to change region colour, rename a region and reselect the automatic positioning of region names.
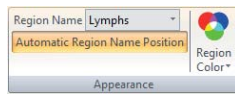
The *Appearance* group contains the following options:

- **Region Name:** The box displays the name of the currently selected region. To change the region name, enter a name or select a name from the drop-down list. The region name on the plot and in the box will update.

  The drop-down list is populated with the names provided on the Region page in the software options (see chapter *Region page* on page 188).

  A region name can also be changed by double-clicking on the region name on the plot.

  Try to avoid applying long region names. If a name is too long, the region label will not be displayed on the plot. You should use region names that are significantly different.

- **Region Color:** Click the button to display a colour control and select the desired colour for the region.

  The region colour on the plot will update. If the region has an associated gate, changing the region colour will not change the gate colour. The gate colour can be changed using the **Edit Gates** button in the *Analyze* tab (see chapter *Gate group* on page 69).

- **Automatic Region Name Position:** Once a region has been created, a unique name for the region is automatically generated. The region name and associated statistic are displayed adjacent to the region. You can position the name as desired within the limits of the plot. To return a region name to its default position, select the region and click the **Automatic Region Name Position** button.

Type group

The *Type* group contains options to change the type of a selected region.
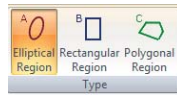
The region type of the currently selected region is highlighted. To change the region to another type (elliptical, rectangular or polygonal region), click the respective button.

Position group

The positioning of created elliptical, rectangular, linear and quadrant regions can be modified by selecting a region and clicking the **Region Position** button. The button is disabled if a polygonal region is selected.

A dialogue is launched that allows for precise definition of position and size of the region.

Depending on the type of the selected region, the corresponding dialogue is displayed:

- **Ellipse:** To position the centre of the elliptical region, use the "Center X" and "Center Y" input fields. A lower value positions the centre to the left or down, respectively. The values must be between -1500 and 1500.

  To define the width of the X-axis of the ellipse, use the "Major Axis size" input field. To define the width of the Y-axis of the ellipse, use the "Minor Axis size" input field. The size values must fall between 0 and 1023.

  To rotate the ellipse, enter the desired number of degrees into the "Rotation(degrees)" input field. Rotation limits are -360° to 360°.

- **Rectangle:** Use the input fields to define the positions of the left side ("Lower X"), the bottom side ("Lower Y"), the right side ("Upper X") and the top side ("Upper Y") of the rectangle. A lower value positions the side of the rectangle to the left or down, respectively. The values must be an integer between 0 and 1023.

- **Linear:** Use the input fields to define the positions of the left point ("Lower bound") and of the right point ("Upper bound") of the linear region. A lower value positions the respective point to the left. The values must be an integer between 0 and 1023.

- **Quadrant:** To choose between straight and bendy quadrant region, select the corresponding option (see chapter *Creating regions* on page 165).

  Use the "Center X" input field to position the quadrant intercept on the X-axis. Increase the value to shift the intercept to the right. Decrease the value to shift the intercept to the left.

Use the "Center Y" input field to position the quadrant intercept on the Y-axis. Increase the value to shift the intercept upwards. Decrease the value to shift the intercept downwards.

The "Bendy X" and "Bendy Y" input fields are disabled when the "Straight" option is selected.

Use the "Bendy X" input field to position the 'bent' upper divider edge on the X-axis. Increase the value to shift the divider edge to the right. Decrease the value to shift the divider edge to the left. "Bendy X" values must be greater than the "Center X" value.

Use the "Bendy Y" input field to position the 'bent' right divider edge on the Y-axis. Increase the value to shift the divider edge upwards. Decrease the value to shift the divider edge downwards. "Bendy Y" values must be greater than the "Center Y" value.

All values must be an integer between 0 and 1023.

Tracking group

The *Tracking* group contains an option to link region positions on plots that display the same parameters. If one of the linked regions is changed, all the linked regions are adjusted simultaneously.
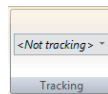
The *Tracking* group contains the following option:

■ **Region Tracking:** To link one region to another, first select the region and then click the drop-down arrow of the box. If the currently selected region can't be linked to any regions, the only option in the box is "(Not tracking)". If there are regions to which the currently selected region can be linked, the drop-down list displays them. Select the region you would like the currently selected region to be linked to. The selected region inherits its position from the "linked to" region and is now displayed in the "Region Tracking" field.

A region is only linked to one region and once it has been linked it will be removed from the "Region Tracking" list.

Moving any linked region will move all tracking regions.

To unlink linked regions, select each region and select "(Not Tracking)" from the "Region Tracking" list.
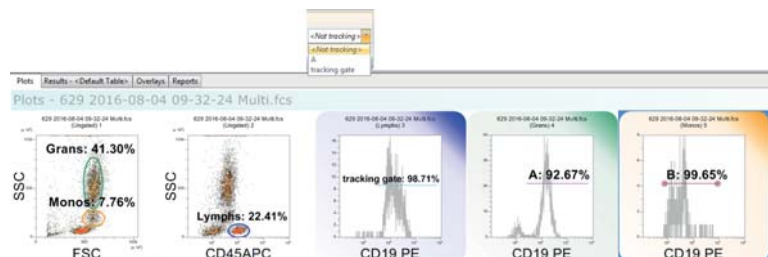
The rules to link regions are:

- Only regions that are on plots that display the same parameters can be linked.

- A maximum of 256 regions can be linked for each opened Workspace.

- A linear region can only be linked to another linear region.

- For linked linear regions, only movement in a horizontal direction of the selected region is reflected in the rest of the region in the linked group, i.e. they can be set to different vertical positions.

- Elliptical, rectangular and polygonal regions can be linked to one another, however, if regions are of different types, linking them changes them to the same type as the "linked to" region.

- Quadrant regions cannot be part of a linked region.

- Once regions are linked, only the "linked to" region appears in the drop-down list when other compatible regions are selected.

- When a "linked to" region is deleted, the regions linked to it change to "(Not Tracking)". Deleting a linked region has no effect on the linked status of the remaining regions.

12.4.16 Overlay Format tab

The *Overlay Format* tab is only available when at least one plot is selected in the *Overlays* tab of the Workspace (see chapter *12.8.3 Overlays tab of the Workspace* on page 174).

The *Overlay Format* tab provides controls to edit overlay plots.

Format group

The *Format* group contains options to adjust line and fill styles of overlay and gallery plots, also options for the display of plots, scaling and legend.

┌─────────────┐
│ ⓘ Information │
└─────────────┘

Use colour selection with caution as it is possible to choose the same colour for data, background and filling, most importantly if white is used.
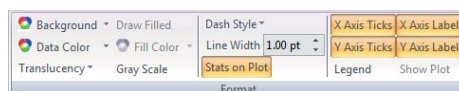
The *Format* group contains the following options:

- **Background:** Select this option to choose a background colour for all selected plots. If overlay plots are selected, this option also affects the corresponding gallery plots.

- **Data Color:** Select this option to choose a colour for all data displayed on the selected plots. If overlay plots are selected, this option also affects the corresponding gallery plots.

- **Translucency:** Select this option to choose a translucency percentage for all data on the selected plots. Translucency can improve the visualisation of overlapping populations. The option displays a translucency percentage selection control where 0% is non-translucent and 80% is the maximum level of translucency.

- **Draw Filled:** Click the button to show the selected plots filled.

- **Fill Color:** The option is available when filled plots are selected. Select this option to choose a fill colour for the selected plots.

- **Gray Scale:** The button toggles the colour display of selected plot(s) between the standard colour palette and a grey scale. When selected, the button is highlighted in orange. Then, the "Data Color" and "Fill Color" options display a grey scale palette instead of a colour palette. The "Background" option always displays a colour palette.

- **Dash Style:** Select this option to choose a dash style for the selected plots. If overlay plots are selected, this option also affects the corresponding gallery plots.

- **Line Width:** Enter a value or use the up/down arrows to adjust the line width in the selected plots. If overlay plots are selected, this option also affects the corresponding gallery plots.

- **Stats on Plot:** The button toggles between displaying the statistics on a gallery plot and at the side of the plot. If not all results appear in the legend, a warning icon is displayed.

- **X-axis Ticks, Y-axis Ticks:** Select the options to display the tick marks of the respective axis of the currently selected plot(s). Scale tick marks are only displayed on the overlay plot if the scaling is identical for all plots.

- **X-axis label, Y-axis label:** Select the options to display the labels of the respective axis of the currently selected plot(s).

- **Legend:** Select this option to display the plot legend at the side of the selected overlay plots. For each plot, the overlay line colour and a description are displayed. The default description is the X-axis label. Dual parameter overlay plots have both X- and Y-axis labels displayed.

  To modify the legend label, double-click on the label and enter the required text or change the plot axis labels in the Galleries area. If there are too many items present in the legend and not all legend text appears, a warning icon is displayed.

- **Show Plot:** The button is available when one or more plots are selected in the Galleries area. Select this option to display the selected plots in the overlay plot. Deselect this option to remove selected plots from the overlay plot. Any plots in the gallery which are not hidden will display a "Not Displayed" icon in the Galleries area. This icon is not visible in reports or copied and pasted images.

Overlays group

The *Overlays* group contains buttons to switch the available views for the overlay plot from overlay mode to 3D mode. The buttons are available when one or more plots are selected in the Overlays area. The highlighted button indicates the current mode of the selected plot(s). When different modes are present in the selected plots, no button will be highlighted.

The *Overlays* group contains the following options:

- **Overlay Mode:** Click the button to change all selected overlay plots to display in overlay mode.

- **3D Mode:** Click the button to change all selected overlay plots to display in 3D mode.

Histogram Scaling group

The *Histogram Scaling* group contains options to control the scale settings for histograms in both the Overlay and Galleries areas. These options are mutually exclusive. Select an overlay plot to apply the selected scale option to all gallery plots of the selected overlay plot. Select one or more gallery plots to apply the selected scale option to only the selected plots.
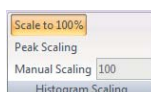
The *Histogram Scaling* group contains the following options:

- **Scale to 100%:** The option scales all selected plots to their maximum values. If a control plot is present in the Galleries area, the control and test histograms are each scaled to their maximum values.

- **Peak Scaling:** The option is only useful if a control plot is present in the Galleries area. When this option is selected, the control and test histogram are both scaled to the highest scale factor of either histogram. If no control plot is set, "Scale to 100%" and "Peak Scaling" have the same values.

- **Manual Scaling:** The option will apply the selected custom value to all selected plots. Enter a value and press **Enter** to apply that value to all selected plots.

Controls group

The options in this group are available for single parameter overlay plots only. They allow one plot within a gallery to be displayed as control on all other plots within the gallery of the current overlay plot.

The *Controls* group contains the following options:

- **Set Control:** The button is available when a single gallery plot is selected. Click the button to display the plot as a control on all gallery plots of the current overlay plot. The gallery plot set as a control displays a dashed border.

  If a control plot is already set, selecting another plot as a control will replace the currently set control with the new selection on all plots within the gallery.

- **Remove Control:** The button is available when a plot is set as a control for the gallery. This option removes the control plot from all plots in the gallery.

- **Control Front:** Select this option to display the control plot in front of the plot data. Deselect the option to display the control plot in the background of the plot data.

Calculations group

The buttons in this group are available when one or more single parameter plots are selected in either the Overlay or Galleries area and the current plot has a control already set.

When a control is set, two calculation options are available to compare test and control plots: Overton or K-S comparison statistics. The selected calculation will be applied to all plots within the selected overlay plot.

The *Calculations* group contains the following options:

- **None:** The button is highlighted by default indicating that no calculation is being displayed for the current plot.

- **Overton mode:** The option shows the positive result after subtraction of the control histogram from the test. The subtraction algorithm is a cumulative subtraction routine based on that published by Roy Overton in *Cytometry 9:619-626 (1988)*.

- **K-S:** The Kolmogorov–Smirnov test (K–S test) is a nonparametric test method for comparing two samples, the control and test plots. The maximum vertical deviation between the two curves is reported as the statistic D.

Histogram Smoothing group

The options in the *Histogram Smoothing* group are available when single parameter plots in a gallery or an overlay plot containing single parameter plots are selected.
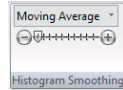
The options allow smoothing to be applied to histogram plots to improve the visual appearance without affecting statistical results. For information on histogram smoothing options, see chapter *Histogram Smoothing group* on page 126.

3D group
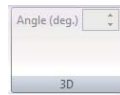
The *3D* group contains the following option:

- **Angle (deg.):** The option is available when an overlay plot in 3D mode is selected. Enter a value or use the up/down arrows to rotate the 3D overlay plot between 5 and 85 degrees.

12.5 Panes

In the panes of the software window, the functions for acquiring data are provided, and informations concerning the currently open Worklist item or Playlist item are displayed.

The panes that are available in the panes area can be selected and deselected in the *Views* tab of the Ribbon (see chapter *12.4.13 Views tab* on page 123). To get back to the default view, click the **Default Position** button in the *Views* tab of the Ribbon.

The panes can be positioned on the screen as required (see chapter *12.2 Views of the software window* on page 52). To get back to the default view, click the **Default Position** button in the *Views* tab of the Ribbon.

The *Playlist* pane is only displayed in Playlist mode and is described in a separate chapter (see chapter *12.7 Playlist pane* on page 154). The *Playlist* pane is displayed in the panes area by default.

The *Worklist* pane is only displayed in Worklist mode and is described in a separate chapter (see chapter *12.6 Worklist pane* on page 146). The *Worklist* pane is docked to the bottom of the Workspace area by default.

The other panes are described in this chapter and comprise:

- *FCS File Information* pane,
- *Results Hierarchy* pane,
- *Settings* pane,
- *Acquire* pane,
- *Setup* pane.

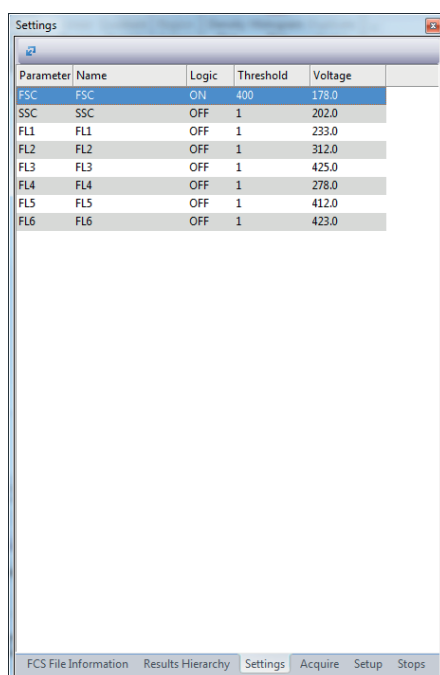### 12.5.1 FCS File Information pane

This pane displays as a title the name of the currently active FCS file. In Worklist mode, the title is only displayed if an FCS file is present.

Beneath the title, the pane displays information on the currently open FCS file. The file information comprises file path, header, parameters, compensation and other information.

In Worklist mode, the "compensation" section displays the compensation matrix of the listmode file associated with the currently active Worklist item.

The pane contains the following option:

- **Send to Report...:** To send file information to the report, click the button to open a drop-down list. Select the desired information to be sent to the report: header, parameters, compensation, other information or all information.

12.5.2 Results Hierarchy pane

This pane displays as a title the name of the currently active FCS file. In Worklist mode, the title is only displayed if an FCS file is present.

Beneath the title, the pane displays the count of ungated events and count and percentage of gated events in a hierarchical view.
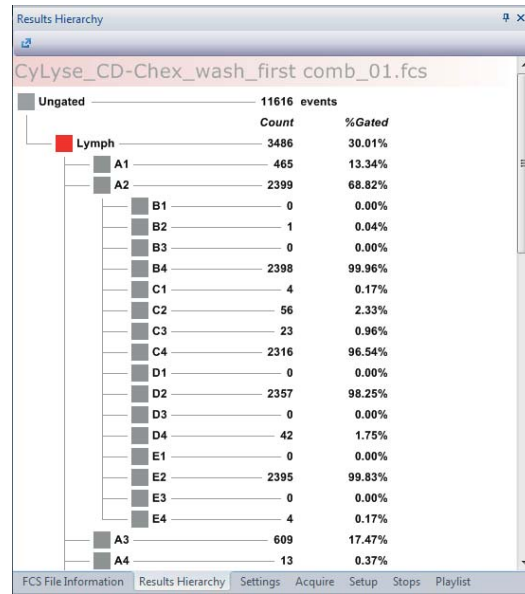
12.5.3 Settings pane

This pane displays a table of settings for the parameters. In Playlist mode, the table is empty and the options are disabled. In Worklist mode, the table entries can be edited for the samples to be measured. For samples already measured, the table is read only.

Parameter	Name	Logic	Threshold	Voltage
FSC	FSC	ON	400	178.0
SSC	SSC	OFF	1	202.0
FL1	FL1	OFF	1	233.0
FL2	FL2	OFF	1	312.0
FL3	FL3	OFF	1	425.0
FL4	FL4	OFF	1	278.0
FL5	FL5	OFF	1	412.0
FL6	FL6	OFF	1	423.0

FCS File Information   Results Hierarchy   Settings   Acquire   Setup   Stops

The table contains the following columns:

- **Parameter:** The column displays the default parameter name for each parameter.

- **Name:** The column displays the parameter name as selected in the *Customize* tab of the Parameter Properties dialogue (see chapter *Parameter Properties* on page 76). The name of a parameter can be manually edited by double-clicking on the manual entry field.

- **Logic:** The column defines which parameter data are triggered. By default, the first parameter (FSC) is selected ("ON"), the other parameters are deselected ("OFF").

  To edit, click the desired row of the column and click the arrow to expand a drop-down list. Select one parameter to be triggered ("ON").

  For the other parameters, select "AND" to record data of both the "ON" parameter and the current parameter. Select "OR" to trigger data of either the "ON" parameter or the current parameter. Select "OFF" to exclude data of the current parameter from the trigger.

- **Threshold:** The column defines the threshold value for an event to be recorded. The threshold allows cutting off background by setting a lower limit of the acquired data in the corresponding parameter. To edit, enter a

value or use the up/down arrows. Threshold limits are between 1 and 2000. Threshold value can only be set when logic is not deactivated (off).

▪ **Voltage:** The voltage of the individual optical parameters defines the photomultiplier signal amplification and therefore the signal intensity to be displayed. To edit, enter a value or use the up/down arrows. Voltage limits are between 1 V and 1000 V.

12.5.4 Acquire pane

The *Acquire* pane displays the acquisition controls which run the flow cytometer and controls for priming and cleaning procedures.

The options within the *Acquire* pane are disabled in Playlist mode and when the software is offline.
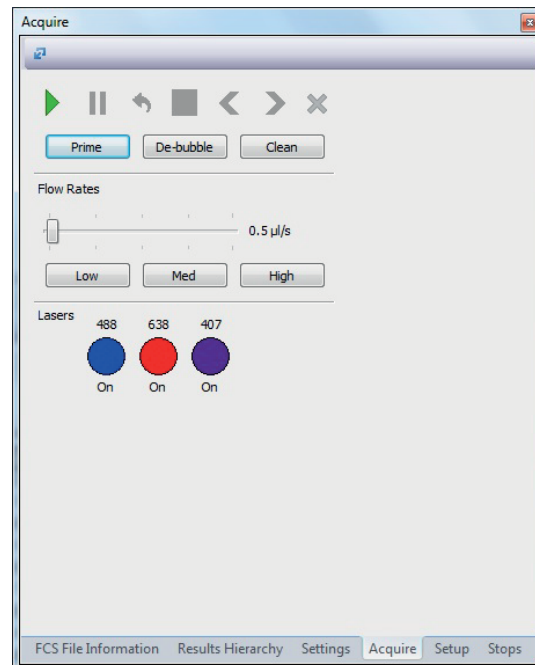
The *Acquire* pane contains the following buttons for acquiring data:

▪ **Play:** Click the button to start measuring a sample.

The acquisition will start.

▪ **Pause:** The button is only enabled if acquisition is in progress. Click the button to interrupt the acquisition process. The sample flow is interrupted while the sheath flow is continued. The data already acquired are held in memory but not saved until acquisition is completed or stopped. When clicked, the pause button is disabled.

To resume acquisition from the paused state and continue the collection of data, click the **Play** button.

To restart the acquisition discarding any previously held data for the sample, click the **Restart** button.

To stop the acquisition process for that sample and save the acquired data, click the **Stop** button. Acquisition will move on to the next defined Worklist item to be acquired. If the current sample is the last in the acquisition sequence, acquisition will terminate.

▪ **Restart:** The button is only enabled if acquisition is in progress. Click the button to restart the acquisition process for that sample. The data acquired up to this point are discarded.

▪ **Stop:** The button is only enabled if acquisition is in progress. Click the button to stop the acquisition process for that sample. The data acquired up to this point are saved and acquisition will move on to the next defined Worklist item to be acquired. If the current sample is the last in the acquisition sequence, acquisition will terminate.

▪ **Previous:** The button is disabled if acquisition is in progress. Click the button to activate the previous Worklist item in the Worklist.

▪ **Next:** The button is disabled if acquisition is in progress. Click the button to activate the next Worklist item in the Worklist.

▪ **Abort:** The button is only enabled if acquisition is in progress. Click the button to stop acquisition and discard data from the current sample. You will have the option to clear the acquired data and proceed to the next Worklist item or to clear the acquired data and stop measuring.

The *Acquire* pane contains the following buttons for performing predefined procedures:

▪ **Prime:** Click the button to start a priming procedure. Once started, the software guides you through the process.

▪ **De-bubble:** Click the button to start a procedure to clear bubbles from the flow cuvette. Once started, the software guides you through the process.

▪ **Clean:** Click the button to start a cleaning procedure. Once started, the software guides you through the process.

The "Flow Rates" section of the *Acquire* pane contains the following controls:

▪ Above the flow rate slider, the acquisition flow rate (events per second) and the absolute event count are displayed during acquisition.

▪ **Flow Rate Slider:** The slider allows to adjust the sample injection speed into the flow cuvette. Low speed values result in a better precision and accuracy. A higher speed can be used when the particle concentration is measured and accuracy is of lower relevance. The scale ranges from 0.1 µl/s to 19.8 µl/s. The value (µl/s) is displayed at the right-hand side of the slider.

The flow rate can be changed in the following ways:

Mouse drag and drop of the slider will increase/decrease the flow rate in steps of 0.1 µl/s.

Using the **PgUp** and **PgDn** keys of the keyboard will increase/decrease the flow rate in steps of 0.5 µl/s.

Using the up/down arrow keys of the keyboard will increase/decrease the flow rate in steps of 0.1 µl/s.

Scrolling the mouse scroll wheel will increase/decrease the flow rate in steps of 0.3 µl/s.

- **Low:** Click the button to set a low flow rate (0.5 µl/s).
- **Medium:** Click the button to set a medium flow rate (2 µl/s).
- **High:** Click the button to set a high flow rate (5 µl/s).

The "Lasers" section of the *Acquire* pane can contain between 1 and 6 buttons that represent the number of lasers detected in the connected device. The wavelengths of the lasers are displayed on top of the buttons.

Clicking a button toggles the on/off state which will switch the associated laser on or off. The current on/off state of the lasers is displayed below the buttons.

ⓘInformation

After switching on a non-active laser, it will be fully operational after booting for about 60 seconds. Switch on the desired lasers a few minutes before starting an acquisition.

12.5.5 Setup pane

The *Setup* pane displays the setup options of the flow cytometer. You can select the number of events and the parameters to be displayed in the current Workspace.

The options within the *Setup* pane are disabled when the software is in Playlist mode.
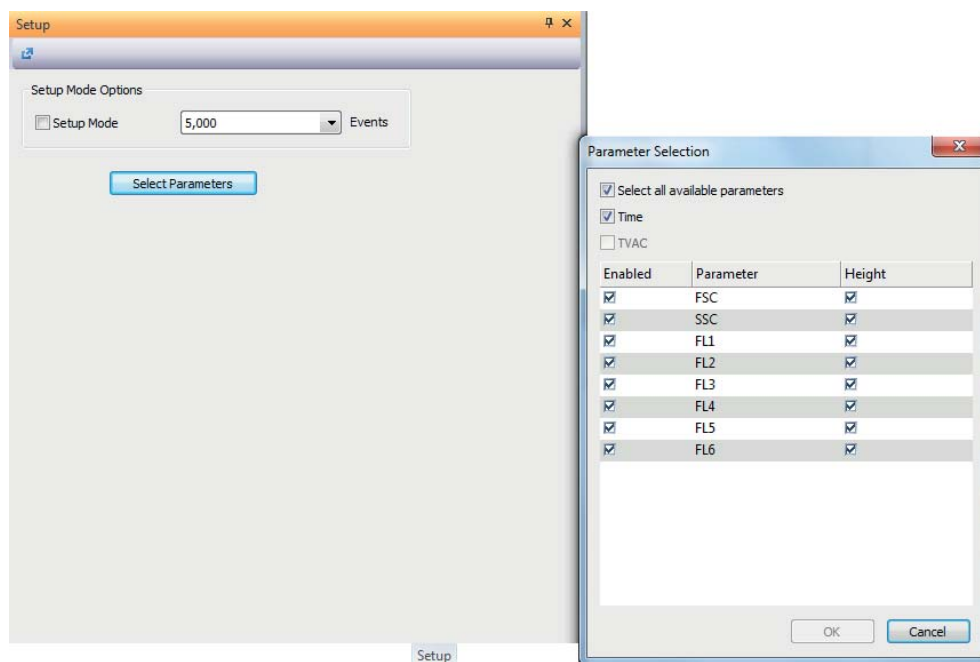
The pane contains the following options:

- **Setup Mode:** Select the check box to store only the latest events in the buffer. Only these events are displayed in the Workspace.

  If "Setup Mode" is selected during acquisition, the acquisition will restart in setup mode and any previously acquired data will be discarded.

  If "Setup Mode" is deselected during acquisition, the acquisition will restart, not in setup mode, and any previously acquired data will be discarded.

- **Events:** This box displays the number of events to store in the buffer and to display in the Workspace. Click the arrow to expand a drop-down list and select the desired number of events to be displayed. The default value is 5,000 events, the available values range from 500 to 100,000 events.

- **Select Parameters:** To display selected parameters only, click the button and select the desired parameters from the Parameter Selection dialogue.

  Selecting the "Time" check box will create and enable a time parameter to be recorded during acquisition. This is an available parameter on the device, but is not listed within the parameter table.

- The "TVAC" check box is selected when the TVAC stop condition has been set in the *Stops* pane. Using TVAC (True Volumetric Absolute Counting), a fixed volume of 200 µl between the start and stop electrodes in the sample is measured. This setting creates and enables the TVAC parameter to be recorded during acquisition. The TVAC parameter is not a parameter defined by the device configuration.
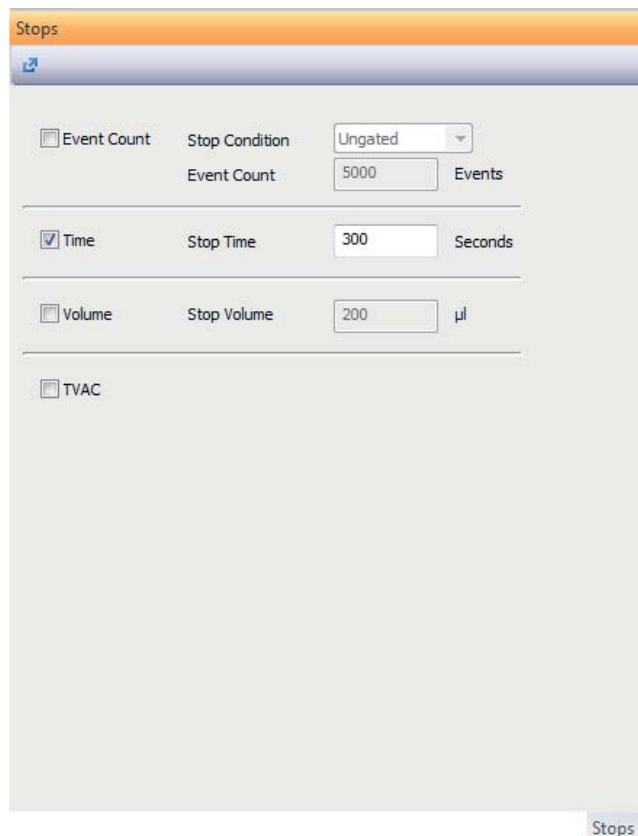
12.5.6 Stops pane

This pane provides options for setting stop conditions. In default mode, the sample is run until its level reaches the stop electrode.

ⓘInformation

- The sample analysis automatically stops when the sample is empty (the stop electrode is reached) even if the selected stop condition is not yet realised.

- If the Worklist contains differing stop conditions, the Worklist conditions will override the stop conditions set in the *Stops* pane.

The options within the *Stops* pane are disabled when the software is in Playlist mode.

The pane contains the following options:

- **Event count:** Select the check box to measure a defined number of events. In the "Stop Condition" box, click the arrow to expand a drop-down list and select the desired condition (ungated or the required gate) to base the stop event count on. The default option is "ungated".

  In the "Event Count" box, enter the desired number of events. During acquisition, once this entered acquisition event count is reached, acquisition will stop. The default event count is 5,000 events. The event count range is 1 to 100,000,000 events.

- **Time:** Select the check box to define a stop time and enter the desired value (in seconds) for the acquisition stop time after which the acquisition of the sample is to be stopped. The default value for the stop time is 300 seconds. The stop time range is 1 – 999,999 seconds.

- **Volume:** Select the check box to define a volume to be measured and enter the desired value (in µl) for the volume. During acquisition, once this stop volume is reached, acquisition will stop. For manual acquisition, the default stop volume is 200 µl and the range is 1 to 1200 µl.

- **TVAC:** The check box is only enabled for manual acquisition. Select the "TVAC" check box (True Volumetric Absolute Counting) to measure a fixed volume of 200 µl between the start and stop electrodes in the sample. When the TVAC check box is selected, all other Workspace stop settings will be ignored. The TVAC parameter in the Parameters list of the *Setup* pane will be checked automatically.

  The TVAC parameter sorts events into two different channels for the different stages of TVAC: All events acquired prior to the sample passing the start electrode will be placed in the "preTVAC" channel of the TVAC parameter. All events acquired proceeding the sample passing the start electrode will be placed in the "TVAC events" channel of the TVAC parameter. Acquisition will be stopped when the sample passes the stop electrode.

12.6 Worklist pane

The *Worklist* pane is displayed in Worklist mode and by default docked to the bottom of the Workspace.

A Worklist contains the predefinitions of hardware and software settings for data acquisition. Each Worklist item contains the definitions for a single sample measurement. These definitions comprise information on Workspace, regions, compensation, instrument settings, stop conditions and stop values.

The Worklist is used to define a set of samples for acquisition and to acquire the data. Up to 400 Worklist items can be provided in the Worklist.

When measuring a sample is completed, the Worklist item information is stored in an FCS file and can be opened in a Playlist.

The Worklist can be saved as a Worklist file.
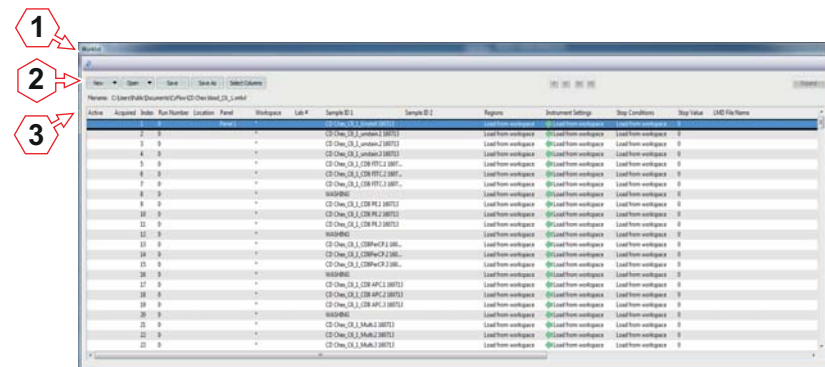
1   Worklist title bar
2   Worklist buttons
3   Worklist

Worklist title bar

The Worklist title bar displays the name of the Worklist file.

Worklist buttons
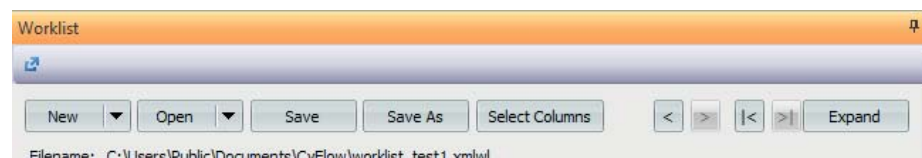
The Worklist buttons are disabled during acquisition except for the **Expand/Restore** button.

The functionality of the **New**, **Open**, **Save** and **Save as** buttons is the same as on the *File* tab of the Ribbon.

The Worklist buttons are:

- **New:** Click the button to create a new Worklist or a new Playlist.

- **Open:** Click the button to open an existing Worklist or Playlist.

- **Save:** Click the button to save the current Worklist with the same name.

- **Save As:** Click the button to save the current Worklist with a new name.

- **Select Columns:** Click the button to open a dialogue to customise the Worklist:

  To display or hide the columns in the Worklist, select or deselect the check boxes next to the columns. Some check boxes are disabled, the corresponding columns are always displayed.

  Columns can be reordered by dragging and dropping except for the first three columns.

  The "Lab #" and "Sample ID" columns can be renamed by double-clicking on the name and entering a new name of up to 30 characters. A "locked" icon on the right of a column name indicates that the name cannot be changed.

  For a description of the columns (see chapter *Worklist rows and columns* on page 147).

  To get back to the default settings, click the **Defaults** button in the dialogue.

- **Previous Sample:** Click the button to activate the previous sample in the Worklist.

- **Next Sample:** Click the button to activate the next sample in the Worklist.

- **First Sample:** Click the button to activate the first sample in the Worklist.

- **Last Sample:** Click the button to activate the last sample in the Worklist.

- **Expand/Restore:** The button is disabled if the Worklist is undocked. Clicking the **Expand** button sets the *Worklist* pane to the expanded position which fills the current Workspace area. The button changes its name to **Restore**. Clicking the **Restore** button resets the *Worklist* pane to the previous docked position.

Worklist rows and columns

Each row of the Worklist is a Worklist item and represents one prepared test from a given sample.

In the columns, the definitions for acquisition can be edited. The columns displayed in the Worklist can be customised using the **Select Columns** button.

If the column width does not allow the contents of a cell to be displayed in full, the text will be truncated and ellipses ("…") will be displayed at the end of the text. When hovering over this, the full name will show as a tooltip.

The columns are width adjustable. To adjust the width, drag the column divider to the right of the column. If you double-click a column divider, the column will resize to display its contents optimally.

The Worklist contains the following columns and associated context menus:

- **Active:** A green arrow displayed in this column indicates the currently active Worklist item.

- **Acquired:** A check mark displayed in this column indicates that the Worklist item has been acquired and has associated listmode data. No more data can be acquired on this Worklist item.

- **Index:** This column displays the sequential number representing the Worklist order. The column is automatically filled and cannot be changed.

- **Run Number:** The run number is an updating incremental cycle count per system. The column is automatically filled when acquisition begins. When acquisition is aborted by the user and a new acquisition has been started for that Worklist item, the run number is incremented.

- **Location:** This column specifies the location of a sample within the source type (plate or tube rack) if an autoloader is used. In manual mode, the column is blank.

- **Panel:** To combine several Worklist items, they can be organised in a panel. This is typically used for series of measurements of the same sample with different settings.

  The column displays the panel name for defined Worklist items. When a Workspace is defined within the Worklist, a panel name is created. The default names will ascend numerically ("Panel 1", "Panel 2", etc.) and are unique to the Worklist.

  In the context menu of the "Panel" column, options are provided to save the panel, to insert or replace a panel, to add an item to the panel and to clear the panel, i.e. to delete all Worklist items associated with this panel.

- **Workspace:** The column displays the name of the Workspace file associated with the Worklist item.

  When a Workspace is defined within the Worklist, a Workspace name is created. The default names will ascend numerically ("Workspace 1", "Workspace 2", etc.) and are unique to the Worklist.

  If a Workspace has been modified since its last save, an asterisk is displayed in front of the Workspace name indicating that the corresponding file contains unsaved changes.

  In the context menu of the "Workspace" column, options are provided to open a new Workspace file, to save the Workspace file, to replace the Workspace file or to apply the Workspace file to a panel or to the complete Worklist.

- **Lab #:** This column is provided for entering the laboratory barcode number or blood tube identification number. When data acquisition is finished, the column is disabled.

- **Sample ID 1** to **Sample ID 12:** These columns are provided for entering sample or patient information. When data acquisition is finished, the column is disabled.

- **Regions:** This column displays the source of the region positions for Worklist item containing a Workspace. By default, the region information is loaded from the Workspace file.

  From a drop-down list in the cell, the "Carry" option can be selected instead. Then, the region information will be loaded from the Workspace file of the row above at the point of acquisition. The "Carry" option will only work if the previous Worklist item has acquired data. Otherwise, the Worklist item will be reset to "load from Workspace". In case a region is carried across to a different Workspace and the regions do not match the new Workspace, information on which regions could not be carried is displayed prior to acquisition.

- **Instrument Settings:** This column displays the source of the instrument settings for the Worklist item.

  To edit, click into the cell, click the arrow to expand a drop-down list and select the desired option:

  Load from Workspace: By default, the instrument settings information is loaded from the Workspace file. At the point of acquisition, the threshold, voltage, compensation and threshold logic instrument settings will be applied.

  Load from file...: This option is disabled when creating a Worklist offline. Click the option to select an instrument settings file (XMLIS file). At the point of acquisition, the Workspace instrument settings for the Worklist item will be updated to match those in the selected file.

  Carry: This option is disabled for use in a compensation setup panel. Select this option to carry the instrument settings information from the Workspace file of the row above at the point of acquisition. The "Carry" option will only work if the previous Worklist item has acquired data. Otherwise, the Worklist item will be reset to "load from Workspace".

  In the context menu of the "Instrument Settings" column, options are provided to save or replace the instrument settings and to apply the instrument settings to the panel.

- **Stop Conditions:** In this column, stop conditions can be set. The default option is to load the stop conditions from the Workspace that are set in the *Stops* pane.

  By clicking into the "Stop Conditions" cell, a deviating stop condition can be chosen. This setting will override the setting in the *Stops* pane. If the "TVAC" check box in the *Stops* pane is checked, the column will display "TVAC" and be read only. For information on stop conditions (see chapter *12.5.6 Stops pane* on page 144).

  In the context menu of the "Stop Conditions" column, the option is provided to apply the stop condition to the panel.

■ **Stop Value:** If a deviating stop condition is set in the "Stop Conditions" column, the stop value can be entered here.

■ **LMD File Name:** This column displays the LMD file name and path created at the successful termination of acquisition. The column is automatically filled and cannot be changed. The file name consists of the run number, the timestamp at the end of data acquisition and the sample ID.

Activating Worklist items

To activate a Worklist item, double-click on the item. The Worklist item is then marked by a green arrow in the "Active" column in the Worklist. Settings and results associated with the active Worklist item are displayed in the Workspace area. If no Workspace file was associated with the Worklist item, a default Workspace file is created.

Only one Worklist item can be active at a time.

When acquisition is in progress, the acquired Worklist item is active. Activating another Worklist item is not available.

Selecting Worklist items

To edit Worklist items, they need not to be activated but must be selected. Selected Worklist items are highlighted in blue.

The following options for selecting Worklist items are available:

■ Single click on a Worklist item to select it.

■ To select consecutive items, drag the mouse cursor over the Worklist rows.

■ To select multiple items, hold down the **Ctrl** key while selecting the items.

■ To select all the items in the Worklist, press **Ctrl** + **A**.

■ To select all items from the currently selected one to the end of the list, press **Ctrl** + **Shift** + **End**.

■ To select all items from the currently selected one to the beginning of the list, press **Ctrl** + **Shift** + **Home**.

Worklist context menu

Context menus within the Worklist allow for easy access to the most commonly used functions. The context menus are disabled during acquisition.

If no specific context menu is defined for the region the cursor is placed, the general context menu is displayed on right-clicking. In the "Panel", "Workspace" and "Instrument Settings" columns of the Worklist, only the relevant submenu is displayed.

New	▶
Open	▶
Save	
Save As	
Insert Panel	
Replace Workspace	
Panel	▶
Workspace	▶
Instrument Settings	▶
Stop Conditions	▶
Clear LMD	
Clear Item	
Copy	
Paste	
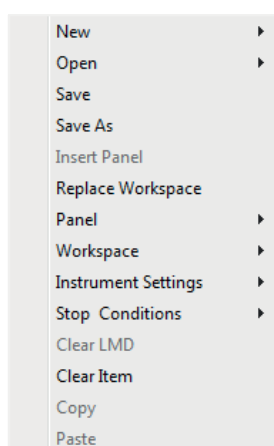
The functionality of the *New*, *Open*, *Save* and *Save as* menu items is the same as on the *File* tab of the Ribbon.

The general Worklist context menu contains the following options:

- **New:** Click the menu item to create a new Worklist or a new Playlist.

- **Open:** Click the menu item to open an existing Worklist or Playlist.

- **Save:** Click the menu item to save the current Worklist with the same name.

- **Save As:** Click the menu item to save the current Worklist with a new name.

- **Insert Panel:** To insert a previously saved panel, click the menu item and select a panel (XMLPA file) from the Windows Open dialogue. The panel is added to the Worklist starting at the selected row. The option is only available if the Worklist item is not yet assigned to a panel.

- **Replace Workspace:** To replace the current Workspace file, click the menu item and select a Workspace file (XMLWS file) from the Windows Open dialogue. Only a single Workspace file can be opened. If multiple Worklist items are selected, the Workspace files for all selected items are replaced with the chosen Workspace file, and are automatically grouped into a panel. A panel name is created by default

  The option is disabled when a compensation setup panel is active.

- **Panel:** In this submenu, options are provided to save the panel, to insert or replace a panel, to add an item to the panel and to clear the panel, i.e. to delete all Worklist items associated with this panel.

  When saving a panel, the full path and file extension are stripped and the Workspace name is used as an identifier. If there are multiple Workspace files present within the panel with the same name, a unique identifier is added to the Workspace file name to differentiate the Workspace files.

  When replacing a panel, the selected panel in the Worklist will be removed, and the chosen panel will be inserted into the Worklist, with the first item of the panel at the same position the first item of the previous panel was at.

  The "Add Item" option will duplicate the last row in the panel. The option is only enabled when at least one undefined item is directly below the panel.

  The "Clear Panel" option is disabled if LMD files are present in any of the selected panels. The option will clear the selected panel(s) from the Worklist and reset the Worklist items to an undefined state.

- **Workspace:** In this submenu, options are provided to create a new Workspace file, to save the Workspace file, to replace the Workspace file or to apply the Workspace file to a panel or to the complete Worklist.

  The "New Workspace" option will create a default Workspace file for the selected Worklist item. The option is disabled when more than one Worklist item is selected or when a compensation setup panel is active.

- **Instrument Settings:** In this submenu, options are provided to save or replace the instrument settings and to apply the instrument settings to the panel.

  When saving the instrument settings, the threshold, threshold logic, voltages and compensation settings will be saved to the target file.

- **Stop conditions:** In this submenu, the option to apply the selected stop conditions to the panel is provided.

- **Clear LMD:** Click the menu item to clear the listmode data from the Worklist item. The check mark in the "Acquired" column is no longer displayed indicating that new data can be acquired on this Worklist item. This option is only available if data are acquired on this Worklist item.

- **Clear Item:** The option is only enabled if a single Worklist item is selected that is the first or last item of a panel. Click the menu item to clear all settings from the Worklist item. This option is only available if no data are acquired on this Worklist item.

- **Copy:** The menu item is only available if the context menu is launched from a manual entry field ("Lab #", "Sample ID" or "Stop Value" columns). Click the menu item to copy the text from the column of the selected Worklist items to the clipboard.

■ **Paste:** The menu item is only available if the context menu is launched from a manual entry field ("Lab #", "Sample ID" or "Stop Value" columns). Click the menu item to paste the previously copied text to the column of the selected Worklist items. If only one text was copied, it will be pasted to all selected Worklist items. If text from several Worklist items was copied, select the same number of Worklist items before pasting.

Compensation setup panel

For defining compensation using control samples, a compensation setup panel can be created in the Worklist (see chapter *Compensation Settings group* on page 106 and chapter *Compensation setup panel* on page 107).

12.7 Playlist pane

The *Playlist* pane is displayed in Playlist mode and by default docked to the left of the Workspace, nested with the other panes.

The *Playlist* pane contains a Playlist. Each row of the Playlist is a Playlist item and represents one acquired sample. A Playlist item contains a listmode file (FCS file) with associated Workspace and compensation files. Up to 400 Playlist items can be provided in the Playlist. The Playlist can be used to analyse data and to predefine settings for future measurements by editing the Workspaces and compensations associated to the LMD files.

The *Playlist* pane allows you to create a list of files that you wish to analyse and contains controls for navigating through the files. Multiple files can be selected for simultaneous analyses. Files can be reordered and Workspace and compensation files can be applied to each file as desired.

The Playlist can be saved as a Playlist file.

When loading a Playlist, the Workspace and compensation are read from the Playlist (not from their original files). The report is also read from the Playlist.
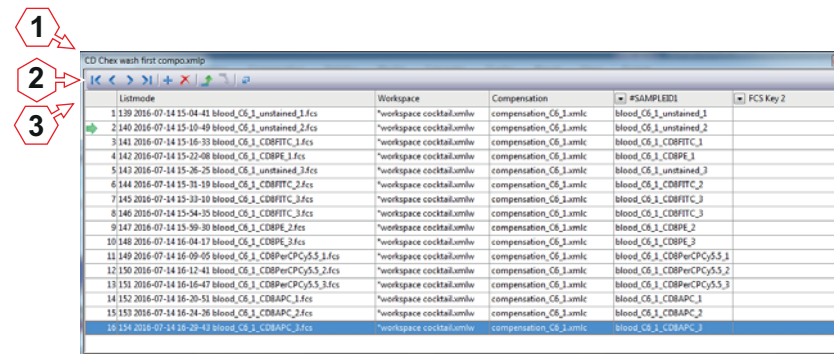
1   Playlist title bar
2   Playlist toolbar
3   Playlist

Playlist title bar

The Playlist title bar displays the name of the Playlist file. When a Playlist has not been saved, it displays "Untitled Playlist".

When the *Playlist* pane is undocked, right-clicking on the Playlist title bar displays a context menu providing the following options:

- **Move** changes the cursor to a move cursor. The *Playlist* pane can then be moved using the arrow keys on the keyboard.

- **Size** changes the cursor to a size cursor when over the edges of the pane. The *Playlist* pane can then be resized using the arrow keys on the keyboard.

- **Hide** is always disabled.

Playlist toolbar

The options in the Playlist toolbar allow you to navigate through a Playlist, to reorder or to delete items in the Playlist.

The Playlist toolbar contains the following buttons:

- **First File:** Click the button to skip to the first file in the Playlist and open the file in the Workspace.

- **Previous:** Click the button to skip to the previous file in the Playlist and open the file in the Workspace.

- **Next:** Click the button to skip to the next file in the Playlist and open the file in the Workspace.

- **Last File:** Click the button to skip to the last file in the Playlist and open the file in the Workspace.

- **Insert Item:** The button is enabled when a single Playlist item is selected. Click the button to insert a new blank Playlist item above the currently selected item. The newly inserted line will become selected. The button is disabled if the maximum number of 400 Playlist items in the Playlist is reached.

- **Remove Item:** The button is enabled when one or more Playlist items are selected in the Playlist. Click the button to remove the selected Playlist items from the Playlist. Removing an item removes the item's results from the results table and invalidates any report items for the removed Playlist item. If the item is currently open, the Workspace will be cleared.

- **Move Up:** Click the button to move the currently selected items one position up in the Playlist. If multiple Playlist items are selected, they will be grouped together and then moved.

- **Move Down:** Click the button to move the currently selected items one position down in the Playlist. If multiple Playlist items are selected, they will be grouped together and then moved.

- **Float/Dock:** Clicking the **Float** button undocks the *Playlist* pane. The *Playlist* pane can then be relocated as required (see chapter *12.2 Views of the software window* on page 52). The button changes its name to **Dock**. Clicking the **Dock** button resets the *Playlist* pane to the previous docked position.

Playlist columns

The rows of the Playlist represent the Playlist items. Each Playlist item comprises a listmode file (FCS file) with associated Workspace and compensation files.

The Playlist contains the following columns:

- **First column:** This column displays a sequential number or the Playlist item. A green arrow displayed in this column indicates the active Playlist item, i.e. the Playlist item that is currently open in the Workspace.

- **Listmode:** This column displays the name of the listmode file of the Playlist item.

- **Workspace:** This column displays the name of the Workspace file associated with the Playlist item. In the context menu, options are provided to replace the Workspace, to assign a new Workspace, to save, copy and paste the Workspace file and to apply the Workspace file to the Playlist.

- **Compensation:** This column displays the name of the compensation file associated with the Playlist item. In the context menu, options are provided to replace, save, copy and paste the compensation file or to use the listmode compensation settings.

- **FCS Key 1** and **FCS Key 2:** In these columns, FCS keywords can be displayed. Click the arrow to expand a drop-down list and select the desired primary or secondary keyword from the list. The drop-down list displays all keywords available from the listmode file of the first Playlist item in the Playlist. On selection of a keyword, the column title is changed to the keyword designation. If a keyword cannot be found in a listmode file, the column of the respective Playlist item displays "ERROR".

  To remove the current keyword selection, select "None" from the drop-down list.

  If the listmode file of the first Playlist item of the Playlist is cleared, replaced, moved or a new Playlist item is inserted, the keyword selection is cleared in the Playlist.

The first column is not width adjustable. All other columns are width adjustable. To adjust the width, drag the column divider to the right of the column. If you double-click a column divider, the column will resize to display its contents optimally.

If the column width does not allow the contents of a cell to be displayed in full, the text will be truncated and ellipses ("…") will be displayed at the end of the text.

Activating Playlist items

To activate a Playlist item, double-click on the item. The Playlist item is then marked by a green arrow in the first column of the Playlist. The listmode, Workspace and compensation files of the Playlist item are opened in the Workspace.

Only one Playlist item can be active at a time.

Selecting Playlist items

To edit Playlist items, they need not to be activated but must be selected. Selected Playlist items are highlighted in blue.

The following options for selecting Playlist items are available:

- Single click on a Playlist item to select it.

- To select multiple items, hold down the **Ctrl** key or **Shift** key while selecting the items.

- To select all the items in the Playlist, press **Ctrl** + **A**.

- To select all items from the currently selected one to the end of the list, press **Ctrl** + **Shift** + **End**.

- To select all items from the currently selected one to the beginning of the list, press **Ctrl** + **Shift** + **Home**.

- To invert the selection, press **Ctrl** + **Shift** + **A**.

Playlist context menus

Context menus within the Playlist allow for easy access to the most commonly used functions.

For editing, context menus are available throughout the Playlist.

By right-clicking on the blank area of the *Playlist* pane, options for opening a new Playlist, opening an existing Playlist and saving the Playlist as are provided. The functionality is the same as in the *File* tab of the Ribbon.

If the cursor is placed in the "Listmode" column, the main context menu described here is displayed on right-clicking. In the "Workspace" and "Compensation" columns of the Playlist, only the relevant submenu is displayed.

The main context menu provides the following options:

- **Insert Item:** Click the menu item to insert a new Playlist item above the currently selected Playlist item.

- **Remove Item:** Click the menu item to remove the selected Playlist item.

- **Replace Listmode:** To replace the selected Playlist items, click the menu item and select the desired replacement listmode file(s).

- **Clear Listmode:** Click the menu item to clear the listmode file from the Playlist item.

- **Workspace:** In this submenu, options are provided to replace the Workspace, to assign a new Workspace, to save, copy and paste the Workspace file. The functionality of these submenu items is the same as in the *Workspace* group in the *Playlist* tab of the Ribbon (see chapter *12.4.9 Playlist tab* on page 100).

   The "Apply to Playlist" option copies the Workspace file of the selected Playlist item and pastes it to all Playlist items in the Playlist.

The "Use Listmode" option is enabled if the listmode file is a CyFlow® file with an embedded Workspace. Selecting this option restores the embedded Workspace for the selected file.

- **Compensation:** In this submenu, options are provided to replace, save, copy and paste the compensation file or to use the listmode compensation settings. The functionality of these submenu items is the same as in the *Compensation* group in the *Playlist* tab of the Ribbon (see chapter *12.4.9 Playlist tab* on page 100).

- **New Playlist:** Click the menu item to create a new Playlist. The functionality is the same as in the *File* tab of the Ribbon.

- **Open Playlist:** Click the menu item to open an existing Playlist. The functionality is the same as in the *File* tab of the Ribbon.

- **Save Playlist As:** Click the menu item to save the current Playlist with a new name. The functionality is the same as in the *File* tab of the Ribbon.

Adding files to the Playlist

A listmode file can be opened in many ways including:

- using the **Open Listmode** button in the Quick Access Toolbar,

- using the **Open Listmode** button in the *Home* tab of the Ribbon,

- from the *Open* menu in the *File* tab of the Ribbon,

- by pressing **Ctrl** + **O**,

- by drag and drop from the Windows Explorer into the *Playlist* pane.

Each listmode file is added to the end of the Playlist as a new Playlist item displaying the listmode file name in the "Listmode" column.

If one or more listmode files are added to the Playlist, the first of the newly added listmode files is automatically opened in the Workspace and selected in the Playlist.

If a Workspace file is opened, it is added to the currently opened active Playlist item.

If a compensation file is opened, it is added to the currently active Playlist item.

Playlist tooltips and error display

When moving the cursor over the "Listmode" column of a Playlist item, a tooltip containing the corresponding LMD file path is displayed. On "Workspace" or "Compensation" columns, the tooltip contains the Workspace or compensation file path if the Playlist is not yet saved. If the Playlist is saved, the tooltip contains only the Workspace or compensation file name.

If an error occurs, the Playlist item text is coloured, and moving the cursor over the text displays a corresponding tooltip. If a Playlist item has multiple errors/warnings associated with it, the tooltip only displays one error at a time with errors taking precedence over warnings.

The following colours are used:

- Playlist items that have no errors are displayed in black.

- Orange text indicates a Workspace file warning. Playlist items have LMD files which do not match their associated Workspace file concerning scaling information, number of parameters, region and gate definition or zoom settings.

- Red text indicates a listmode file, Workspace file or compensation file error. Playlist items are invalid, files are not compatible with the current Workspace or compensation coefficients in the LMD file or files cannot be accessed.

- Grey text indicates a processing issue. Playlist items are not yet processed or unable to process.

Modified file indicators

If a Workspace or compensation file has been modified since its last save, an asterisk is displayed in front of the Workspace or compensation name in the Playlist indicating that the corresponding file contains unsaved changes.

If you save a modified Workspace file or compensation file and that file occurs more than once in the Playlist, a corresponding message is displayed and you will have the option to apply the changes to all Playlist items with the same Workspace file or compensation file.

Reordering Playlist items

Playlist items can be reordered in the Playlist by dragging them to a new position. While a Playlist item is being dragged, a red line appears denoting where the item will be relocated when dropped. If multiple Playlist items are selected, they will be grouped together and then moved.

Alternatively, use the **Move Up** and **Move Down** buttons of the Playlist toolbar.

12.8 Workspace

In the Workspace area of the software window, the Workspace of the currently active Playlist item or Worklist item is displayed and can be edited. A Workspace includes plots, regions, gates, colour precedence, selected statistics and displayed parameters. A Workspace can be saved as a Workspace file.

The Workspace contains four tabs:

■ The *Plots* tab contains two areas: Plots and Previews. The Previews area automatically displays plots once a listmode file is opened. Plots can be added to the Plots area as required for further analysis. The *Plots* tab is open by default.

■ The *Results* tab displays the statistics selected in the *Statistics* tab of the Ribbon.

■ The *Overlays* tab allows the creation of single and dual parameter overlay plots.

■ The *Reports* tab allows you to create and modify reports.

12.8.1 Plots tab of the Workspace

Plots are displayed in the *Plots* tab of the Workspace.

The *Plots* tab of the Workspace consists of two areas:

■ **Plots area:** Selected plots are displayed in the Plots area and can be analysed and modified in this area.

■ **Previews area:** When an LMD file is opened, the Previews area of the Workspace automatically displays all single parameter histogram plots and all dual parameter colour density plots associated with the opened LMD file. The axis displays the stain name taken from the FCS header of the opened LMD file.
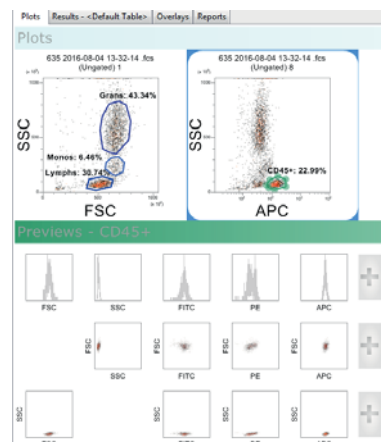
Types of plots

The software can display four plot types:

- **Single Parameter Histogram Plots** display the 1024 bin histogram for a single parameter. The plot may be gated or ungated.

- **Dual Parameter Colour Density Plots** are two dimensional 256 x 256 bin histograms which use colour to represent the level of events within each bin. An algorithm is used to calculate the levels based on the number of events. The levels have logarithmic intervals.

  Six levels of colour are used for the Density Plots: Black for outlier singles and, in order of increasing density: blue (lowest density), green, yellow, orange and red (highest density).

- **Dual Parameter Colour Precedence Plots** are two dimensional 256 x 256 bin dot plots which use colour to represent the gating events that fall within specific gates. Where an event may be in multiple gates, the precedence order determines which colour is chosen. Where events from multiple gates fall within a single bin, the highest precedence gate colour is chosen. Ungated events are displayed black. Precedence order can be changed via the Precedence order dialogue (see chapter *Gate group* on page 69).

- **Dual Parameter Precedence Density Plots** are displayed as colour precedence plots but include density information as well as colour precedence.

Properties of individual plots, including changing of the plot type, can be modified using the *Plot Format* tab of the Ribbon (see chapter *12.4.14 Plot Format tab* on page 124).

Adding plots to the Plots area

For adding plots to the Plots area, you can select plots from the Previews area or use buttons of the Ribbon.
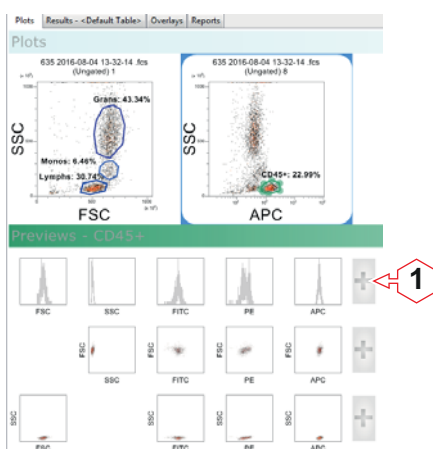
1   **+** button

The following options are available:

- To add a single plot from the Previews area to the Plots area, double-click the desired plot in the Previews area.

- To add several plots from the Previews area, use the **+** buttons in the Previews area:

  To add a row of plots, click the **+** button at the right of the respective row in the Previews area.

  To add a column of plots (excluding histograms), click the **+** button at the bottom of the respective column in the Previews area.

  To add the upper right triangle or lower left triangle of plots, use the split **+** button at the bottom right corner of the Previews area.

  When the mouse is hovered over any **+** button, the plots that will be added when the button is clicked become highlighted in a blue outline.

- Further possibilities to add a specific selection of plots to the Plots area are provided in the *Plots* group on the *Analyze* tab of the Ribbon (see chapter *Plots group* on page 68).

- To add a plot displaying the first parameter(s) of the current listmode file or to duplicate plots, use the buttons in the *Add Plot* group of the *Home* tab of the Ribbon (see chapter *Add Plot group* on page 66).

Gallery and hierarchy view

When you add plots to the Plots area, they are added next to each other in a gallery plot view. You can change the display to a family tree style hierarchy format by clicking the **Hierarchy** button in the *View* group of the *Analyze* tab (see chapter *View group* on page 73). In this format, the plots are displayed in a tree-like structure with a separate branch for each gate. Selecting the **Gallery** button will return you to the gallery plot view.
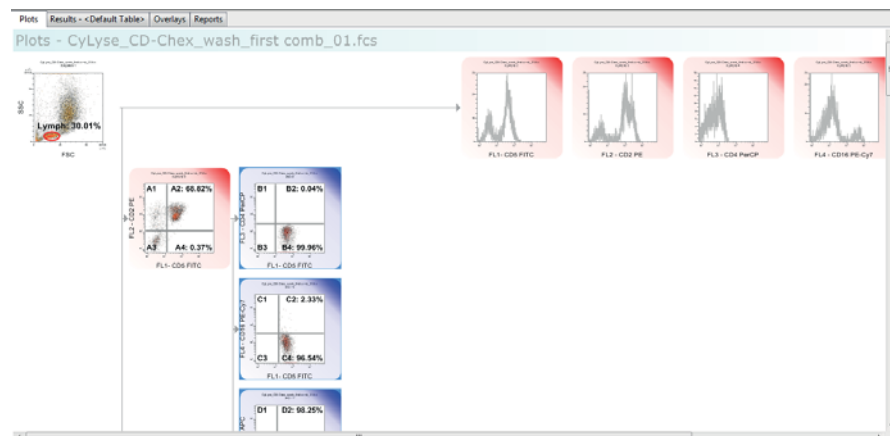
A plot containing a region which is part of a gate equation is called a classifier plot. A plot that has no gating regions on it is called an outcome plot. In hierarchy view, a pathway is drawn between each classifier plot and between other classifier plots and the subsequent gated outcome plots. Ungated plots with no gating regions drawn on them appear outside the hierarchy as orphaned plots.

Selecting plots

Selected plots are surrounded by a blue selection rectangle.

The following options are available:

- To select a single plot within the Plots area, click on the plot.

- To select or deselect multiple plots, hold **Ctrl** while clicking on plots.

- Plots may also be selected by dragging out a selection rectangle around the desired plots. The rectangle selects everything it touches. **Ctrl** dragging a selection area around plots will toggle that selection of the plots.

- To select all plots, press the **Ctrl** + **A** keys.

- Plot selection options are also available from the *Edit* group on the *Analyze* tab of the Ribbon (see chapter *Edit group* on page 73).

On selecting a plot, the *Plot Format* tab becomes available on the Ribbon providing options to edit type, gating and axis scales of plots and to set histogram smoothing (see chapter *12.4.14 Plot Format tab* on page 124).

Resizing plots

For resizing plots, select the **Resize Plots** or **Resize Previews** button from the status bar and use the resize slider on the status bar to adjust the size of the plots (see chapter *Resize options* on page 186).

If a mouse with a central wheel is used, plots can also be resized by selecting plots and pressing the **Ctrl** key while scrolling the mouse wheel.

Selecting parameters for display

If the Previews area does not display the required parameters, you can select or deselect parameters from those present on the *Parameters* tab of the Ribbon (see chapter *12.4.5 Parameters tab* on page 76).

Analysing and modifying plots in the Plots area

Plots are selected from the Previews area and added sequentially to the Plots area.

An ungated plot displays no colour border and "[Ungated]" as the plot heading.

If the Previews area has a gate applied, the plots added to the Plots area will also have this gate applied. This process will automatically create a gate if required. A gated plot is surrounded by a coloured border which denotes the gate colour applied. By default, the gate name is displayed as the plot heading.

The following options for editing plots in the Plots area are provided:

- **Editing the Plot Title:** To customise the title of a plot, double-click in the top margin of the plot and enter a custom title into the edit field. The title is added to the heading of the plot.

- **Moving Plots:** Plots can be rearranged within the Plots area by dragging and dropping. A vertical blue insert cursor highlights the new location of the plots. When multiple plots are selected and dragged, they are grouped together and moved as a group. After they are released, they remain together. The plots displayed in the hierarchy view cannot be moved.

- **Deleting Plots:** Plots may be removed from the Plots area by selecting them and pressing the **Delete** key. Selected plots can also be deleted using the **Delete** button in the *Edit* group on the *Analyze* tab.

  If the deleted plot has a region applied, deleting the plot also deletes the region and removes the region from any gating logic. In this case, a corresponding warning message is displayed and you will have the option to cancel deleting the plot.

Changing parameter properties

To change parameter properties, double-click in the area of the plot displaying the parameter name. The Parameter Properties dialogue is launched (see chapter *Parameter Properties* on page 76).

Changing Plot Parameters

The software provides functionality for changing the parameters of the plots in the Plots area.

The following options are available:

- To change the parameter of an axis of a plot, right-click in the area of the plot displaying the parameter name. A context menu is displayed that contains all the parameters currently selected on the *Parameters* tab of the Ribbon. Select the required parameter to be displayed in the plot.

  When a different parameter is selected, the plot is updated to use the new parameter. The statistics and report are automatically updated as well. The following settings are retained when a parameter is changed: Zoom status of the plot, smooth settings for histogram plots, gating of the plot, cell cycle options.

  If there are regions on the plot, they will be deleted on the plot and in any gate equations if parameters are changed. In this case, a corresponding warning message will be displayed and you will have the option to cancel changing the parameters.

- To flip the axes of a dual parameter plot, right-click in the area of the plot displaying the parameter name. From the context menu, select the "Flip Axes" option. Any regions on the plot will be retained and flipped as well. Statistics and reports will automatically update.

When flipping a plot containing a quadrant region, the quadrant dividers are flipped, but the names of the quadrants are persisted. Any gate equations using these quadrant regions will be updated if required.

Any linking of regions will be removed if you flip plot axes. In this case, a warning message will be displayed and you will have the option to cancel flipping the axes.

- Alternatively, use the "Parameters" options of the *Home* tab to change the parameters of selected plots (see chapter *Parameters group* on page 66).

Creating regions

Regions can be defined on single or dual parameter plots. The maximum number of regions that can be created in a Workspace is 256. Quadrant regions are not included in this count.

Regions are created using the buttons in the *Region* group on the *Home* tab (see chapter *Region group* on page 65) or on the *Analyze* tab (see chapter *Region group* on page 68).
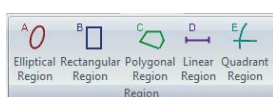
To create a region on a plot, click the required region type button. The button is highlighted. Then either click on a plot to set the region at the default size, or draw out the required region size on the desired plot.

To cancel the creation of a region, press the **Esc** key before or during the dragging of that region.

Once the maximum number of 256 regions is reached, the buttons in the *Region* group become disabled.

Only one region can be drawn per button selection for the Elliptical, Rectangular and Polygonal region types. Linear regions can be applied to multiple selected single parameter plots by pressing the **Ctrl** key while clicking to add the region. Quadrant regions can be applied to multiple selected dual parameter plots by pressing the **Ctrl** key while clicking to add the region.

To create a region on a plot, first click the required region type button on the *Home* tab or the *Analyze* tab. Five region types can be created:

- **Elliptical Region:** Click the region type button. Then either click on a dual parameter plot to set an ellipse of the default size, or draw out the required ellipse on the desired plot.

- **Rectangular Region:** Click the region type button. Then either click on a dual parameter plot to set a rectangle of the default size, or draw out the required rectangle on the desired plot.

- **Polygonal Region:** Click the region type button. Click onto a dual parameter plot to determine the first point of the region. Then click on the plot to add additional points to the polygon. The polygon is then

completed by clicking on the start point. If there are at least three points, the polygon is also completed by right-clicking (which will complete the polygon by adding a line from the last to first point). Double-clicking on a line inserts a new point which can then be moved to create a modified region.

■ **Linear Region:** Click the region type button. Then either click on a single parameter plot to set a default sized linear region, centred on the point where the plot was clicked. Alternatively click onto a single parameter plot and drag out a line to determine the region size. The same linear region can be applied to multiple selected single parameter plots by pressing the **Ctrl** key while clicking to add the region.

■ **Quadrant Region:** Quadrant regions are formed from two divider lines (one vertical, one horizontal). Click the Quadrant Region button, and then click onto a dual parameter plot at the point on the plot where you wish the quadrant's intercept to appear. By hovering the cursor over the quadrant intercept, a statistic is displayed for each quadrant. The same quadrant region can be applied to multiple selected dual parameter plots by pressing the **Ctrl** key while clicking to add the region.

When a broad distribution of events is observed, you may need to create non-linear quadrant dividers. The control point on the right and top can be used to move the horizontal and vertical dividers respectively to a higher channel than the intercept point, creating a "Bendy Quad" region. If the "Bent" divider is moved to a lower value than the intercept point, the divider returns to its straight form.

Editing regions

On selecting a region, the *Region Format* tab becomes available on the Ribbon providing options to edit region name and colour, region type and position and to link regions (see chapter *12.4.15 Region Format tab* on page 128).

Additionally, the region orientation, size and position can be edited by selecting a region on a plot and dragging the handles displayed on the selected region, as indicated by the cursor.

The region can be moved by selecting between the handles, as indicated by the move cursor, then dragging the region to a new position.

If you change the region size or position unintentionally, click the **Undo** button in the Quick Access Toolbar to restore the previous size or position.

The options for the different region types are:

■ **Ellipse:** Elliptical regions have four control points that will be displayed when you click on a region to select it. You can use these control points to resize, rotate and move the elliptical region. While an elliptical region is being moved or resized, the coordinates of the centre, the axes length and the rotation angle in degrees are displayed.

■ **Rectangle:** Rectangular regions have eight control points that will be displayed when you click on a region to select it. You can use these

control points to resize and move the rectangular region. While a rectangular region is being moved or resized, the coordinates of the top left and bottom right corners are displayed.

- **Polygon:** The polygonal region cannot be rotated and cannot be moved outside the plot axes. Each vertex of the polygon is a control point. Dragging the vertex will move that vertex only within the polygon. Dragging a line of the polygon will move the region whilst maintaining the shape of the region. While the vertex of a polygonal region is being moved or resized, its coordinates are displayed.

- **Linear:** Linear regions have control points situated at each end of the region. You can drag the control points to resize the region. Dragging the line allows you to move the region. While a linear region is being moved or resized, its coordinates are displayed.

- **Quadrant:** Quadrant regions have five control points, one situated at the divider intercept point and one situated at each end of the two dividers. Each of the control points can be used to modify the quadrant region. The quadrant region cannot be moved outside the plot axes. While a control point is moved, the channels of the intercept point are displayed in the status bar.

  When a broad distribution of events is observed, you may need to create non-linear quadrant dividers. The control point on the right and top can be used to move the horizontal and vertical dividers respectively to a higher channel than the intercept point, creating a "Bendy Quad" region. If the "Bent" divider is moved to a lower value than the intercept point, the divider returns to its straight form.

Deleting regions

To delete a region, select it and press the **Delete** key or the **Delete** button in the *Edit* group on the *Analyze* tab. If deleting a region affects gating logic, a warning message will be displayed and you will have the option to cancel deleting the region.

Region names

Once a region has been created, a unique name for the region is automatically generated. The region name and associated statistic are displayed adjacent to the region on the plot. The size of the text is auto-adjusted. The associated statistic is not displayed if there is not enough space. If the name is too long to fit on the plot, it is not displayed. By hovering the cursor over the region, a tooltip is displayed stating the name and the statistic associated with that region.

If a statistical value cannot be calculated, "ERROR" will be displayed instead of a numerical value.

To change the automatically assigned name, select the region and double-click on the region name on the plot. An edit field appears. Enter a name or select a name from the drop-down list. The drop-down list is populated with the names provided on the Region page in the software options (see chapter *Region page* on page 188).

The region name can also be changed from the *Appearance* group of the *Region Format* tab (see chapter *Appearance group* on page 128).

The region name can be repositioned but cannot be dragged outside the plot.

Region colour

When a region is created (except for quadrant regions), it is automatically assigned a colour. There is a set of ten predefined region colours within the software.

The region colour can be changed in the *Region* group of the *Home* tab (see chapter *Region group* on page 65) or from the *Region Format* tab (see chapter *Appearance group* on page 128).

Regions and gates

Any existing plot can be gated by selecting the required region or gate from the "Gate" drop-down list on the *Home* tab (see chapter *Gate group* on page 67).

When a region on a plot is selected, the plots in the Previews area are gated on the selected region. The heading of the Previews area displays the name of the region and is highlighted with its colour.

If a plot gated on a region is added to the Plots area, a gate associated with this region is automatically created with the same name and colour as the region. The plot in the Plots area is marked with this colour.

If a region is added to one of the gated plots and then selected, plots in the Previews area will become gated on the new region. If you now add a new plot to the Plots area, it will be gated on a compound gate consisting of both the original gate of the plot (the parent gate) and the newly added region.

The gate name and colour can be changed in the Edit Gates dialogue of the *Analyze* tab (see chapter *Gate group* on page 69).

Scaling

For high-resolution data files, a parameter can be displayed in three different formats: linear, logarithmic and V-Log. This is selectable from the *Home* tab (see chapter *Parameters group* on page 66) or from the *Plot Format* tab (see chapter *X-Axis group* on page 125).

The scaling of parameters can be customised in a number of ways using the scaling and zoom options available in the *Scales* group of the *Parameters* tab (see chapter *Scales group* on page 80).

ⓘ**Information**

If you change the zoom status on a plot with a region, the region position does not change. You may need to reposition any regions set on zoomed parameters.

Plots added to the Plots area will display their scales on the X and Y axes. Fluorescence parameters default to Log scaling. Scatter or other parameters default to Lin scaling.

The scaling options are:

- **Linear Scales:** Linear scaled parameters display linear tick marks and numbering. The default values are determined from information in the LMD file. Linear scaled data always starts at 0. The minimum and maximum values are always displayed at the ends of the scale.

- **Log Scales:** Logarithmic scaled parameters display logarithmic tick marks and numbering. The default values are determined from information in the LMD file. When no scaling information is available in the opened LMD file nor in the Workspace, the scaling is determined by the values found in the Scaling page of the software options (see chapter *Scaling page* on page 189).

- **V-Log Scales:** V-Log scaled parameters display a combination of linear and logarithmic sections. Linear tick marks are displayed around the origin and logarithmic tick marks are displayed towards the end of a scale. When V-Log is selected, the full range of data is displayed.

To toggle between zoomed and unzoomed displays:

- If one or more of the parameters associated with the plot have non-default zoom settings, a **Plot Zoom Toggle** button is displayed on the top left corner of the plot. Click the button to toggle between the zoomed and unzoomed view of the plot.

- Alternatively, use the **Display zoomed** button on the *Parameters* tab of the Ribbon.

DNA cell cycle plots

You can perform DNA cell cycle analysis of single parameter linear data plots. To display DNA cell cycle information, first select one or more histograms and then press the **Cell Cycle** button on the *Plot Format* tab of the Ribbon (see chapter *Plot Type group* on page 124).

If there are regions on any of the selected histograms, cell cycle cannot be activated and the **Cell Cycle** button is disabled. You have to delete the regions or deselect the plots containing them before cell cycle can be activated. The DNA cell cycle plots are saved as part of the Workspace. Each cell cycle plot is identified numerically with the title "Cell Cycle n", where "n" is a number.

When hovering the cursor over a cell cycle plot, a tooltip is displayed listing the cell cycle number, statistics and the error message associated with the plot.

The three phases of the DNA cell cycle are coloured on the cell cycle plot as follows:

- **Red:** Denotes the G0/G1 phase,

- **Blue:** Denotes the S phase,

- **Green:** Denotes the G2/M phase.

Additional regions cannot be applied to the cell cycle plot.

The following markers are displayed on the plot:

- **Blue Markers** are debris markers allowing to set noise thresholds and to exclude cellular debris. Events that occur below or above these noise markers will be presented on the plot, but not analysed by the software.

- The **Red Marker** marks the estimated mean channel of the G0/G1 peak.

- The **Green Marker** marks the estimated mean channel of the G2/M peak.

If the calculated phases are not as expected, the G0/G1 and/or G2/M estimates can be adjusted by clicking on the marker and dragging/dropping it to the desired position to generate a successful fit for the DNA cycle. The estimate markers cannot be crossed over. The markers can only be moved to within 10 channels (based on a 1024 channel histogram) from each other. Noise markers cannot be moved outside the limits of the histogram.

Moving any of the markers triggers recalculation on the DNA cell cycle plot. When the noise markers are moved, the automatically estimated G1/G0 and G2/M peak values will be used. When the G0/G1 and/or G2/M are moved, the adjusted values of these markers will be used.

Following a manual adjustment of the estimates, you can return the calculated estimates to their original calculated positions. Click the **Reset Cell Cycle** button on the *Plot Format* tab of the Ribbon to reset the cell cycle with the automatically calculated G0/G1 and G2/M position estimates.

12.8.2 Results tab of the Workspace

In the *Results* tab of the Workspace, a results table is displayed. Here, you can view statistics associated with the current Playlist or the current *Worklist* pane. You can copy statistics for use in other applications, export statistics to CSV and XML files and print statistics using the options on the *File* tab of the Ribbon.

When you switch to the *Results* tab of the Workspace, the Ribbon automatically switches to its *Statistics* tab providing the options to select the statistics for the results table.

X Parameter	Y Parameter	Gate	Region	Count	%Gated
FSC - FSC		Ungated	A	12379	96.23
SSC - SSC		A	B	6681	53.97
FL1 - FITC		A	C	5677	45.86
FL2 - PE		A	D	5711	46.13
FL3 - PerCPCy5		A	E	5685	45.92
FL5 - APC		A	G	6660	53.80
FL6 - APCCy7		A	H	6643	53.66
FSC	SSC	Ungated	A	2386	32.85
CD3 FITC	CD19 PE	A	B1	205	8.59
CD3 FITC	CD19 PE	A	B2	1589	66.60
CD3 FITC	CD19 PE	A	B3	561	23.51
CD3 FITC	CD19 PE	A	B4	31	1.30
CD3 FITC	CD14 PerCpCy5.5	A	C1	0	0.00
CD3 FITC	CD14 PerCpCy5.5	A	C2	13	0.54
CD3 FITC	CD14 PerCpCy5.5	A	C3	766	32.10
CD3 FITC	CD14 PerCpCy5.5	A	C4	1607	67.35
CD3 FITC	CD45APC	A	E1	45	1.89
CD3 FITC	CD45APC	A	E2	1585	66.43
CD3 FITC	CD45APC	A	E3	721	30.22
CD3 FITC	CD45APC	A	E4	35	1.47
CD3 FITC	FL6	A	F1	0	0.00
CD3 FITC	FL6	A	F2	1	0.04
CD3 FITC	FL6	A	F3	766	32.10
CD3 FITC	FL6	A	F4	1619	67.85
CD19 PE	CD14 PerCpCy5.5	A	G1	0	0.00
CD19 PE	CD14 PerCpCy5.5	A	G2	13	0.54
CD19 PE	CD14 PerCpCy5.5	A	G3	592	24.81
CD19 PE	CD14 PerCpCy5.5	A	G4	1781	74.64
CD19 PE	CD45APC	A	I1	27	1.13
CD19 PE	CD45APC	A	I2	1603	67.18
CD19 PE	CD45APC	A	I3	565	23.68

Results table rows and columns

A results table generates result rows based on regions, plot gates (if selected) and derived gates from all items in a Playlist or from all items in the current panel of a Worklist which have a listmode file. Each region created in the Workspace generates a row in the results table. For a quadrant region, four rows are created, one for each quadrant. Each DNA cell cycle plot also generates a results row in the table.

The columns in the results table represent the individual statistics as selected from the *Statistics* tab of the Ribbon (see chapter *12.4.7 Statistics tab* on page 92). For a cell cycle plot, an additional "Error Message" column is added to the results table. The message reported by the cell cycle

analysis, denoting whether the algorithm experienced any problems, is displayed in this column.

When background calculation has completed, results for all valid Playlist items will be available to be displayed in the results table. For a Worklist, the results for all items of the current panel with a listmode file are available to be displayed in the results table.

If a statistical value cannot be calculated, "ERROR" will be displayed instead of a numerical value.

If a value is not applicable to the row, it is left blank. Not applicable items are:

- DNA statistics for non-cell cycle regions,

- Y statistics for linear regions,

- Events, intensity and variation group statistics for cell cycle plots,

- X and Y parameter, region, plot title, intensity group, variation group and DNA statistics group statistics for derived gates.

Columns will default to automatically adjust their width to fit the widest text in the column. The width of a column can be manually changed by dragging the column divider at the right of the column to be adjusted. Double clicking the column divider at the right of a column will set that column back to automatically adjust its width.

The following options for editing the results table are available:

- **Reordering columns:**To reorder the columns, drag and drop their headers.

- **Adding columns:** To add a column, select its check box in the *Statistics* tab of the Ribbon. Alternatively, click a column header to display a drop-down list of all statistics and select the desired statistics.

- **Removing columns:** To remove a column, deselect its check box in the *Statistics* tab of the Ribbon or drag the column header off the column header area. Alternatively, click a column header to display a drop-down list of all statistics and deselect the desired statistics.

- **Sorting by columns:** The table can be sorted by any column in an ascending or descending order by clicking the column title.

Selecting table rows

Selected rows are highlighted in blue. Selected rows can then be copied for pasting in to other applications, e.g. Microsoft® Excel.

The following options for selecting rows are available:

- Click on a results row to select it.

- To select multiple rows, hold down the **Ctrl** or **Shift** key while selecting the rows.

- To select all rows, press **Ctrl** + **A**.

- To select all rows from the currently selected one to the end of the table, press **Ctrl** + **Shift** + **End**.

- To select all rows from the currently selected one to the beginning of the table, press **Ctrl** + **Shift** + **Home**.

Grouping results by column headers

Results can be grouped by the values displayed in the column headers. You can do this by dragging a column header from the results table onto the grey field above the table labeled "Drag a column header here to group by that column". When the header is dragged, two red arrows indicate where the column header will be placed on the field.

The following options are available:

- To group by a column and remove the column from the results table, drag the desired column header into the grey field.

- To group by a column without removing the column from the results table, hold the **Ctrl** key while dragging the desired column header into the grey field.

- To group by more than one column, drag the desired column headers into the grey field. Drag and drop the headers in the field to sort them in the desired sort order.

The information displayed in the groups can be collapsed and expanded by clicking the +/- buttons.

A column header can be removed from the grouping by dragging it back to its position in the results table. When a column header is dragged back to the results table, two red arrows indicate where the header will be placed. The header can also be dragged off the table and it will be removed from the group control.

12.8.3 Overlays tab of the Workspace

In the *Overlays* tab of the Workspace, you can create overlay plots of single or dual parameter plots. Overlay plots can be used to compare plots.

In Playlist mode, there is an overlay Workspace per Playlist. In Worklist mode, there is an overlay Workspace per panel.

On selecting the *Overlays* tab in the Workspace, a blank area is displayed. The *Overlay* tab of the Ribbon automatically becomes active providing options to edit the overlay Workspace (see chapter *12.4.11 Overlay tab* on page 109).

Overlay plots are created by sending plots from the *Plots* tab of the Workspace to the *Overlays* tab.

In Playlist mode, you can also use the Overlay Wizard on the *Overlay* tab of the Ribbon to create overlay plots.

Each overlay consists of an overlay plot and an associated gallery of its source plots. An overlay can contain a maximum of 400 source plots.

The *Overlays* tab of the Workspace consists of two areas:

- The **Overlays area** displays a 2D or 3D view of available overlay plots.

- The **Galleries area** displays the individual source plots that make up the overlay plots. For each overlay plot, a gallery of individual plots is displayed with the name of the overlay plot as a title.
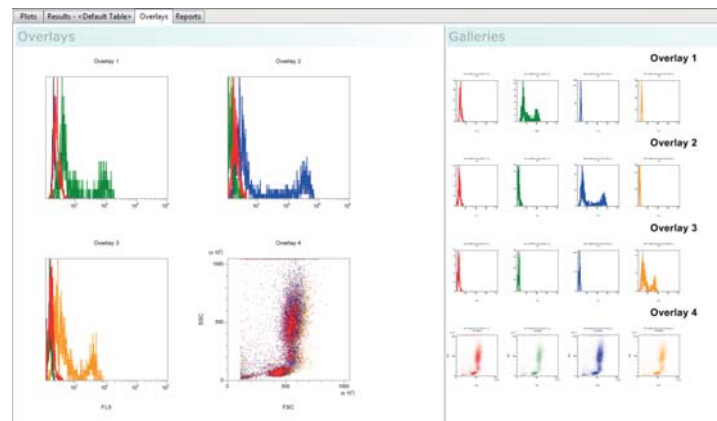
Creating overlay plots

A new overlay plot is created by sending plots from the *Plots* tab of the Workspace to the *Overlays* tab. Select the required plot(s) in the Plots area of the Workspace. Right-click to open a context menu and select "Send selected Plot(s) to Overlay Plot". A context submenu is displayed.
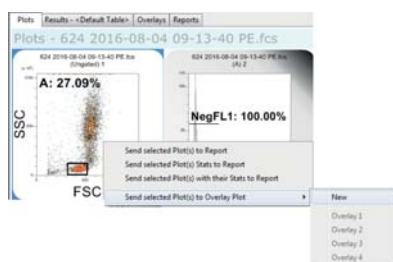
From the submenu, select the desired option:

- To add the plots to a new overlay plot, select "New". An overlay plot cannot contain both single and dual parameter plots. If you have selected both types of plot, a new overlay plot will be created for each type.

- To add new plots to an existing overlay plot, select the desired overlay plot in the context submenu. More than one instance of a plot cannot exist in an overlay plot, therefore if the currently selected plot(s) already exist in an overlay plot, that plot is disabled in the context submenu. An overlay plot cannot contain both single and dual parameter plots. Any overlay plots to which the currently selected plots cannot be copied are disabled in the context submenu.

Plots can also be added to a selected overlay plot by using the **Insert Plot** button on the *Overlay* tab of the Ribbon (see chapter *Plots group* on page 113).

Plot views

If zoom and scale settings exist in the plot from the Plots area, these will be persisted in the overlay plots.

If any of the source plots are unavailable after the associated item has been processed, an exclamation mark will be displayed on the overlay plot in front of all other successfully calculated data sources within the overlay.

If background calculation is turned off, a warning icon will be displayed in the bottom right-hand corner of any overlay plot that contains a source plot in an unknown state. Hovering over it will display a tooltip indicating that background calculation needs to be turned on.

Overlay plots can be displayed in 3D using the **3D Mode** button on the *Overlay Format* tab of the Ribbon (see chapter *Overlays group* on page 133). In 3D mode, plots are displayed as three-dimensional at a specified angle and will be equally distributed. The plot can be rotated using the **Angle (deg.)** button on the *Overlay Format* tab of the Ribbon.
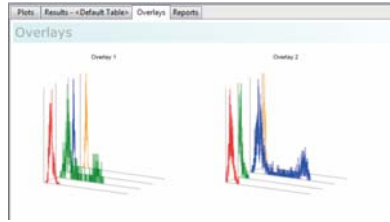
Selecting plots

Selected plots are surrounded by a blue selection rectangle.

If an overlay plot is selected in the Overlays area, the associated title in the Galleries area is also highlighted.

In the Galleries area, plots from only one gallery can be selected at a time.

The following options are available:

■ To select a single plot within the Overlays area or Galleries area, click on the plot.

■ To select or deselect multiple plots, hold **Ctrl** while clicking on plots.

■ Plots may also be selected by dragging out a selection rectangle around the desired plots. The rectangle selects everything it touches. **Ctrl** dragging a selection area around plots will toggle that selection of the plots.

■ To select all plots, press the **Ctrl** + **A** keys.

On selecting an overlay plot, the *Overlay Format* tab becomes available on the Ribbon providing options to change the format of all plots that are available to that overlay plot (see chapter *12.4.16 Overlay Format tab* on page 131).

Reordering plots

Selected plots can be reordered within the Overlays area or the Galleries area by drag and drop. Changing the order of the overlay plots will also change the order of the associated galleries.

When changing the order of individual plots in the Galleries area, the plots will be displayed on the overlay plot in that new order.

Resizing plots in the Overlays or Galleries area

For resizing plots, select the **Resize Overlays** or **Resize Galleries** button from the status bar and use the resize slider on the status bar to adjust the size of the plots (see chapter *Resize options* on page 186).

If a mouse with a central wheel is used, plots can also be resized by selecting plots and pressing the **Ctrl** key while scrolling the mouse wheel.

Editing plot titles, legend and axis labels

The following options are available:

- **Plot title:** Each overlay plot has a plot title which defaults to the overlay number, e.g. "Overlay 1". To change a plot title, double-click on the current plot title and enter a custom plot title into the edit field. Gallery plot titles can be edited in the same way.

- **Legend:** A legend can be optionally displayed on an overlay plot using the **Legend** button on the *Overlay Format* tab. The legend can be edited by double-clicking a label in it. A warning icon containing a tooltip is displayed below the legend area of the plot if all the legends cannot be displayed.

- **Overlay axis label:** Axis labels are displayed on an overlay plot if all parameter names of the individual plots have the same label. Otherwise, no axis label will be displayed for the overlay plot. To change the axis label, double-click on the axis and enter a label into the edit field. To return the label to the default setting, press the **Reset to Default** button of the edit field. The default setting is the file name from the listmode file.

- **Gallery axis label:** Each gallery plot can have an axis label displayed, by default this label is the parameter name from the listmode file. To change the axis label, double-click on the axis label and enter a label into the edit field.

  The **Keywords** button of the edit field allows you to select from a list of available FCS keywords present in the file header to display as the axis label. You may combine multiple FCS headers and text to create a customised axis label.

  The **Apply To All** button applies the current axis label to all of the plots in the gallery for the current overlay plot. If the plot is a dual parameter plot, this button only sets the current axis (X or Y). Press the **Reset to Default** button to return the label to the default setting.

Regions on overlay plots

Regions can be defined on overlay plots using the *Region* group on the *Overlay* tab of the Ribbon (see chapter *Region group* on page 112) and will be rendered black. When a region is moved or resized on any plot in the overlay, it is updated accordingly in all the rest of the plots in this overlay. Regions on the *Overlays* tab of the Workspace do not affect the source plots on the *Plots* tab of the Workspace. Statistics for regions are only displayed in gallery plots. Hovering the cursor over a region on a gallery plot displays a tooltip stating the name and the statistic associated with that region.

Regions are created and edited the same way as on plots in the *Plots* tab of the Workspace (see chapter *Creating regions* on page 165).

Sending plots to the report

To send plots to the report, select the desired plots in the Overlays area or the Galleries area, right-click and select the "Send selected Plot(s) to Report" option. The currently selected plots are sent to a new page at the end of the current report. For information on reports, see chapter *12.8.4 Reports tab of the Workspace* on page 179.

Using control plots

Single parameter gallery plots can be selected to be control plots. A control plot is displayed on all the plots in its gallery.
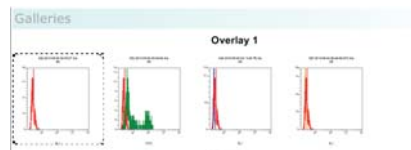
To define a control plot, select the plot from the Galleries area and right-click to open the context menu.

The following options are available:

- **Set as Control Plot** is available when a single gallery plot is selected. Select the option to display the plot as a control on all gallery plots of the current overlay plot. The gallery plot set as a control displays a dashed border.

  If a control plot is already set, selecting another plot as a control will replace the currently set control with the new selection on all plots within the gallery.

- **Remove Control Plot** is available when a plot is set as a control for the gallery. This option removes the control plot from all plots in the gallery.

- **Control in Front** is available when a plot is set as a control for the current gallery. Select this option to display the control plot in front of the plot data. Deselect the option to display the control plot in the background of the plot data.

Alternatively, the buttons in the *Controls* group of the *Overlay Format* tab of the Ribbon can be used (see chapter *Controls group* on page 134).

Moving and copying plots from the Galleries area to an overlay plot

To add plots to an overlay plot from the Galleries area, select the plot(s) from the Galleries area and right-click to open the context menu.

The following options are available:

- **Move selected Plot(s) to Overlay Plot** moves the currently selected plots to a new selected overlay plot. If all of the selected plots already exist in an overlay plot, that overlay plot is greyed out in the list. If some

of the selected plots already exist in the target overlay plot, any new plots will be added but all selected plots will be removed from the current plot.

■ **Copy selected Plot(s) to Overlay Plot** takes a copy of the selected plots and adds them to a new selected overlay plot. If all of the selected plots already exist in an overlay plot, that overlay plot is greyed out in the list. If some of the selected plots already exist in the target overlay plot, any new plots will be added but existing plots will not be added to the target overlay plot.

12.8.4 Reports tab of the Workspace

In the *Reports* tab of the Workspace, you can create page-based reports from a Playlist or from a panel of a Worklist. Reports can be used to create a customised page-based summary of plots, statistics and other information. A report can comprise up to 800 pages.

In Playlist mode, there is a single report per Playlist. In Worklist mode, there is a single report per panel of the Worklist.

On selecting the *Reports* tab in the Workspace, a blank report page is displayed. The *Report* tab of the Ribbon automatically becomes active providing options to edit the report (see chapter *12.4.12 Report tab* on page 114).

Reports are created by inserting objects into the report page. Objects can be inserted via the *Insert* group on the *Report* tab of the Ribbon or sent to the report from the *Plots* tab of the Workspace.

In Playlist mode, you can also use the Report Wizard on the *Report* tab of the Ribbon to create reports.
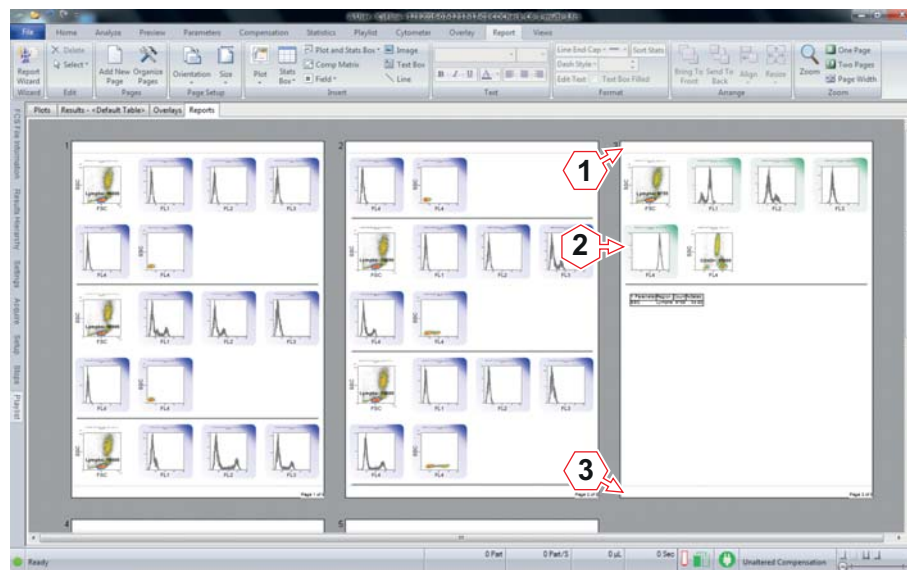
1 Header of a report page
2 Body of a report page
3 Footer of a report page

The following objects can be inserted into reports:

- plots,

- statistics boxes,

- compensation matrices,

- fields (objects containing derived contents such as file names, file paths, etc.),

- images,

- text boxes (boxes provided for entering free text),

- lines.

Each report page consists of three separate sections: header, body and footer. All elements of the header and footer sections are repeated on all pages of the report.

If the source information for an inserted object is unavailable, a red exclamation mark will be displayed on the report. If the source information of the associated Playlist item is unknown, a blue question mark will be displayed on the report. If a field has been inserted and the value is not present in the file or if a file is not present, "ERROR" will be displayed instead of the value.

Inserting objects into a report

In Playlist mode, objects from any item of the current Playlist can be inserted into a report. In Worklist mode, objects from the current panel of the Worklist can be inserted.

There are several possibilities to insert objects into a report:

- From the *Insert* group of the *Report* tab of the Ribbon, click the desired button to display a drop-down list and select the desired object.

  To insert this object in a default size, click into the desired page section (i.e. header, footer or body) of the report. The object is bounds checked to the section and resized and/or repositioned as necessary.

  To insert the object in a custom size, drag out an insertion rectangle to the required size of the object and then release to insert the object at the desired size. Starting a drag in a particular page section (i.e. header, footer or body) restricts the insertion rectangle to the bounds of that section.

  An object can be inserted into a report if the cursor is displayed as a crosshair when moved over a reports page. If an object cannot be inserted, a "no entry" sign is displayed. The cursor remains a "no entry" sign until the object is inserted in a valid location or the **Esc** key is pressed.

- Plots and statistics can also be inserted into a report by sending them from the Plots area of the Workspace. For this purpose, select one or more plots in the Plots area of the Workspace. Right-click to display a

context menu and select whether to send the selected plot(s) and/or their associated statistics to the report. If the last page of the report contains any objects, sent objects start on a new blank page in the report. If the last page is blank, sent objects start on this page.

■ Results tables from the *Results* tab of the Workspace can be inserted by clicking the **Send Table To** button on the *Statistics* tab of the Ribbon.

■ FCS file information can be inserted from the *FCS File Information* pane using the **Send To Report...** button.

ⓘInformation

Ensure objects do not obscure each other when inserted to a report.

Make sure statistics boxes contain enough information to clearly identify which files the results come from. You may need to add file name or gate name to ensure this. You should also make sure that enough space has been allocated for the statistics box so that the full results are visible.

When inserting a compensation file name, the name of the compensation file present in the Playlist will be displayed even if you have cleared compensation, manually modified the compensation or loaded compensation from header.

Selecting objects on the report page

The options for selecting objects on a page are:

■ To select a single object, click on it.

■ To select multiple objects, hold the **Ctrl** key while selecting the objects.

■ To select objects on a per page basis, draw a blue selection rectangle around the desired objects. The rectangle can only be drawn when the cursor displays the selection arrow (i.e. the cursor is not selecting an object). The rectangle selects everything it touches. Objects on multiple pages cannot be selected via a selection rectangle.

To select objects inserted into the header and footer regions with the rectangle, start drawing the rectangle in the header or footer regions as desired.

■ To select all body section objects within a report, press **Ctrl** + **A**.

■ To select all body section objects of the same type within a report, press **Ctrl** + **Shift** + **A**.

Moving objects

Moving the cursor over an object on a report page will change the cursor to a move icon. The object can then be dragged and dropped to new locations within the pages of a report. If objects are selected within different sections (header, footer, body) or different pages, the move cursor is not displayed and objects cannot be moved.

Objects can be moved between the header, footer and body sections of a page and are subject to bounds checks. If an object (or group of objects) is too big to fit in the new section, a "no entry" sign is displayed. Trying to drop the object will move the object back to its original position on the page.

Resizing objects

When an object is selected on a report page, it can be resized using the resize handles. When resizing an object, it is bounds checked to its current section on the page and cannot be resized outside of these bounds.

The options for resizing objects are:

- Moving the cursor over one of the handles will change the cursor to a double-headed arrow indicating which way the object will be resized.

- The aspect ratio of a plot is fixed and is always square.

- When moving corners of images, their aspect ratio is maintained.

- When moving corners, the aspect ratio of the statistics box, compensation matrix, text box and field is not fixed, i.e. it can be stretched horizontally or vertically.

- For all objects, holding **Shift** whilst resizing using a corner handle will maintain the aspect ratio.

- When moving the top or bottom centre of the statistics box, compensation matrix, text box, field and image, its height can be resized.

- When moving the right or left middle of the statistics box, compensation matrix, text box, field and image, its width can be resized.

The options for resizing lines are:

- Moving the cursor over either handle will change the cursor to a crosshair, indicating the handle can be moved in any direction.

- To resize or change orientation of the line, drag either of the handles. The line can be dragged to any size and orientation around 360 degrees. The line is limited to the current section on the page.

- To fix the position of a line to horizontal and vertical positions, hold the **Shift** key and drag the handle. To relocate the line to its new position, first release the mouse and then let go of the **Shift** key.

Resizing headers and footers

To resize a header or footer, click and drag the divider of the header or footer up or down as required. The size of the header and footer is applied to all pages in the report.

If the header or footer contains an object, the divider cannot be dragged beyond the border of that object.

If the body section contains an object close to the header or footer boundary, you can still drag and drop the divider as required. Upon releasing the drag, the object will be bounds checked, moved and, if necessary, resized to accommodate the new header or footer size.

Editing inserted text

To edit text, double-click the desired text box and edit the text as required.

Deleting objects

To delete an object from the report, select it and press the **Delete** key or the **Delete** button in the *Edit* group of the *Report* tab of the Ribbon.

Using the report context menu

Right-click on the report page brings up a context menu allowing to paste, cut or copy selected items in the report. When pasting, objects are pasted cascaded (down and to the left) from their original position on the page.

12.9 Status bar

The status bar is located at the bottom of the software window. The status bar displays the current status of calculations, of the current measurement and of compensation settings. When regions are modified, it displays the coordinates. The status bar also provides a resize slider for plots and overlays.
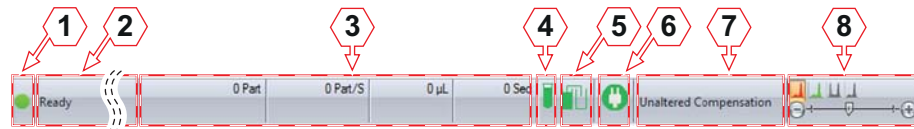
1	Calculation indicator	5	Sheath fluid and waste level indicator
2	Miscellaneous messages	6	Status of the device
3	Status of acquisition	7	Status of compensation settings
4	Sample level indicator	8	Resize options

Calculation indicator

The Calculation indicator indicates if the processing of results has been completed:

- When all items are up to date, a green icon is displayed.

- When background calculation is in progress, an orange icon is displayed.

- When background calculation is off and some items have not been calculated, a red icon is displayed. This icon acts as a button. Clicking it is equivalent to clicking the **Calculate Now** button of the *Playlist* tab of the Ribbon and will recalculate all results.

Miscellaneous messages

In this area of the status bar, the following messages are displayed:

- Ribbon controls: For each Ribbon control, a status bar message is displayed describing the function of the control.

- Window controls: For the Quick Access Toolbar icons and the File menu options, a status bar message is displayed describing the function of the option.

- Print preview: When the print preview is displayed, the status bar will show the current displayed page or page range.

- Region coordinates display: When a control point of a region on a plot is moved, the coordinates of the region are displayed.

- Cell cycle analysis: While a noise threshold estimate marker is being repositioned, information regarding the noise threshold, G1 and G2 estimates and the upper noise threshold is displayed.

- Device functions or fluid level errors: During a device function, a status bar message stating the name of the function currently being performed will be displayed. If a fluid level error occurs, the appropriate error message is displayed.

Status of acquisition

During acquiring, the current status of the acquisition process is displayed:

- **Part** displays the absolute number of particles recorded.
- **Part/S** displays the rate of measurement in particles per second.
- **µL** displays the absolute volume.
- **Sec** displays the absolute time.

If a stop condition is in use, a progress bar under the relevant counter(s) will be displayed.

Fluid level indicators

The icons indicate the fluid level of a tube on the manual sample probe and that of the sheath and waste bottles as detected by the respective electrodes:

- **Sample level:** The icon is green when the sample is full or low. The icon is red if there is no sample tube attached or the sample is below both electrodes. Hover over the icon to display a corresponding tooltip on the sample level.
- **Sheath and waste level:** The icon is green when the sheath and waste levels are sufficient. The left part of the icon is red if the sheath level is too low. The right part of the icon is red if the waste level is too high. Hover over the icon to display a corresponding tooltip on the levels.
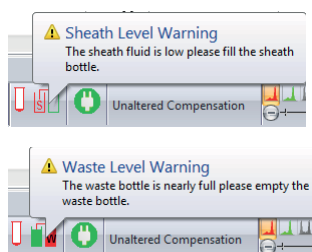
On change of fluid level status, a warning balloon will be displayed above the status bar icon. When the sheath fluid bottle is empty or the waste bottle is full, a warning balloon pops up automatically, to signalise the critical fluid level. If the device is measuring a sample when a fluid level error occurs, a timer will start, and you will be allowed to continue acquiring the current sample for up to 10 minutes. If the error condition is not corrected, the warning balloon will be redisplayed at 5 minutes remaining, 3 minutes, 2 minutes and 1 minute remaining. After that, acquisition will automatically be stopped and the appropriate fluid level error message displayed

If an instrument function is not taking place and a sheath or waste level error is detected, the fluid level error message is displayed immediately. If any other instrument function is taking place, that function will be allowed to complete and the error displayed at completion.

Status of the device

This symbol indicates the status of the device.

■ Green: The device is operational.

■ Yellow: Maintenance is necessary.

■ Red: A problem occurred.

■ Caution symbol: The DLI is not connected.

Status of compensation settings

The status of the compensation settings of the current file is displayed indicating if the file is not compensable, if the compensation is unaltered or if the compensation has been modified.

If the **Manual Compensation** button on the *Compensation* tab of the Ribbon is active, a warning icon and the text "Manual Compensation" is displayed.

Resize options
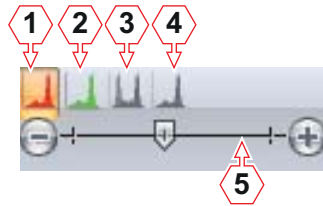
1	**Resize Plots** button	4 **Resize Galleries** button
2	**Resize Previews** button	5 Resize slider
3	**Resize Overlays** button	

To resize plots displayed in the Workspace, first select the type of plots to resize using the plot type buttons:

■ **Resize Plots** (enabled in the *Plots* tab of the Workspace),

■ **Resize Previews** (enabled in the *Plots* tab of the Workspace),

■ **Resize Overlays** (enabled in the *Overlays* tab of the Workspace),

■ **Resize Galleries** (enabled in the *Overlays* tab of the Workspace).

Then use the resize slider to adjust the size of items as required. Move the slider to the desired position or click to the left or right of the slider to decrease or increase the size of the selected plot type, respectively.

12.10 CyFlow Options

The CyFlow Options dialogue contains controls to set various default options for the software. It is launched from the **CyFlow Options** button on the *File* tab of the Ribbon (see chapter *12.4.1 File tab* on page 58).

The CyFlow Options dialogue has several pages accessed by selecting the options in the list to the left of the dialogue. The button for the currently selected page is highlighted in the list.

General page

The General page contains options to specify how statistics displayed on plots are to be rounded and to set the default colours used for regions, gates, overlay plot histograms and histogram plots.
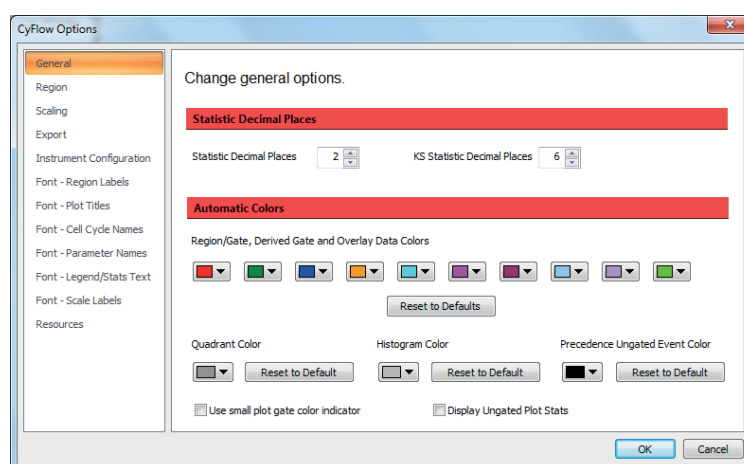
The page contains the following options:

- **Statistic Decimal Places:** This control allows you to set the number of decimal places to which the statistics displayed on the plots and results table are rounded. The default is 2 decimal places. To edit, enter a value or use the up/down arrows to select a number. The acceptable range is between 0 and 10 decimal places.

- **KS Statistic Decimal Places:** This control allows you to set the number of decimal places to which the statistics displayed on gallery plots of overlays and results are rounded. The default is 6 decimal places. To edit, enter a value or use the up/down arrows to select a number. The acceptable range is between 0 and 10 decimal places.

- **Region/gate and overlay data colors:** The ten-colour index displayed here controls the default order in which colours are used when creating regions, gates and overlay plots. To modify a colour used in this default index, click the corresponding arrow button to display a colour control and select the desired colour. The colour assigned to the existing regions is not changed.

  Click the **Reset to Default** button to return the region colours to their default settings.

■ **Quadrant Color:** The box displays the default colour for the quadrant divider lines and is used when quadrant regions are created. This will not affect the colour of quadrant gates. The default colour is Grey-40%. To edit, click the arrow and select a colour. The colours of previously created quadrant divider lines are not changed.

Click the **Reset to Default** button to return the quadrant region colour to its default setting.

■ **Histogram Color:** The box displays the colour for histograms displayed in the Plots area. The default colour is coloured Gray-25%. To edit, click the arrow and select a colour.

Click the **Reset to Default** button to return the histogram colour to its default setting.

■ **Precedence Ungated Event Color:** The box displays the colour for ungated events on precedence plots. The default colour is black. To edit, click the arrow and select a colour.

Click the **Reset to Default** button to return the ungated event colour to its default setting.

■ **Use small plot gate color indicator:** This option is checked by default. When checked, the gate colour indication present on the plot is reduced to a 3D colour circle in the top right-hand corner of the plot. When unchecked, the plot border will be a gradient fill based on the plot gate colour.

■ **Display Ungated Plot Stats:** This option is unchecked by default. Selecting the check box will display plot gate statistics in all results tables.

Region page

The Region page contains options to specify the available default region names.
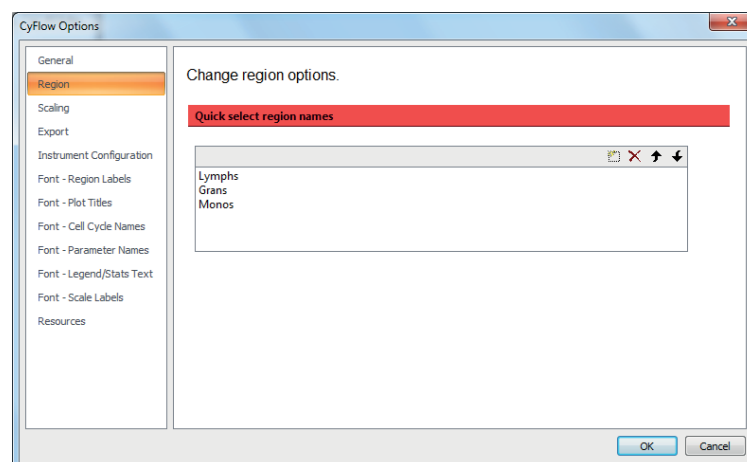
- **Quick select region names:** By default, the software offers three region names that can be designated to regions: Lymphs, Grans, Monos. To edit, double-click on a region name. To add new region names, click the **New** button or double-click below the last name in the list. To delete, select the name and click the **Delete** button. Regions can be reordered using the arrow buttons.

  The region names listed here can be applied to a selected region by clicking the "Region Name" drop-down list in the *Region Format* tab (see chapter *Appearance group* on page 128).

Scaling page

The Scaling page contains options to specify the default options used for Log and V-Log scaling. The setting here is only used if there is no Workspace file or if the open Workspace file and the current listmode file do not contain any defined scaling. As soon as a Workspace or listmode file which contains scaling values is opened, those values are used instead of the default values.
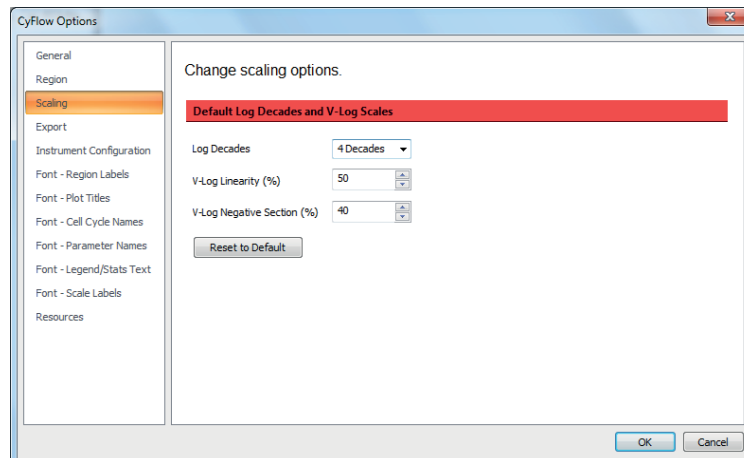
The page contains the following options:

- **Log Decades:** The value set here determines the number of decades shown when logarithmic data is displayed and is used for scaling all relevant plots and statistics. Click the arrow to display a drop-down list and select the desired value (4 decades, 5 decades or full range) or enter a value.

  When full range is selected, the data for all logarithmic scaled parameters in the Workspace defaults to the full range of data in the listmode file. This is the default selection.

  The accepted range for log decades is between 0 and 10.00. The number that you enter here should match the number of decades provided by your device. If you enter a value that is higher than the maximum range in the listmode file, the data will be offset from its correct position on the scale. The number of decades selection has no effect on low-resolution files with less than 12 bit data resolution.

- **V-Log Linearity (%)** and **V-Log Negative Section (%):** These options allow you to select the default values for scaling of V-Log parameters. The accepted range is between 0 and 100%. To edit, enter a value or use the up/down arrows.

  The values entered here will be the default values displayed in the Default Scaling tab of the Scales Properties dialogue (see chapter *Scales Properties dialogue* on page 81). The *V-Log* group is disabled for low-resolution data (i.e. less than 12 bits).

- **Reset to Default:** Click the button to return the scaling options to their default settings.

Export page

The Export page contains options to specify how results are to be exported to CSV files and to specify default options used when images are exported.
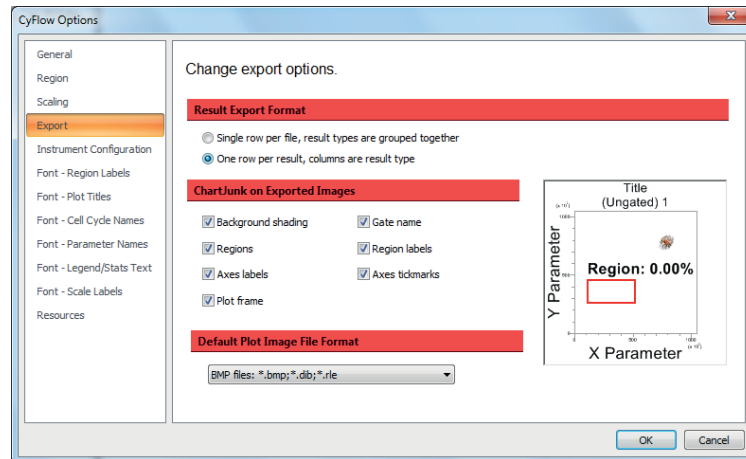
The page contains the following options:

- **Result Export Format:** Select the "Single row per file, result types are grouped together" option to display all the results from the results table, grouped together and exported in a single line. If multiple files are exported, a new line is created for each file. When using Microsoft® Excel 2003 the maximum number of columns is limited to 256. If you need to generate tables with more than 256 columns, you should upgrade to Excel 2007.

  Select the "One row per result, columns are result type" option to display all the results from the results table, exported in the order they appear in the table

- **ChartJunk on Exported Images:** This option allows you to select the detail displayed on plot images when copied and pasted to other applications (e.g. Microsoft® PowerPoint). Uncheck any item that is not required for the pasted plot images.

- **Default Plot Image File Format:** This option sets the default file format for the "Save Plot(s) as…" menu item. Options include BMP, JPEG, GIF, TIFF and PNG. The default setting is BMP.

Instrument Configuration page

The Instrument Configuration page contains options to set the configuration of your device when using an offline PC to create a Worklist.
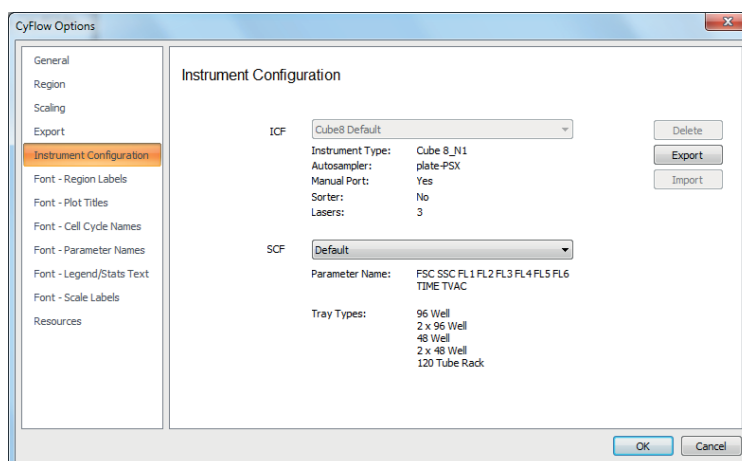
The page contains the following options:

- **ICF:** The ICF (instrument capabilities file) drop-down list contains the title tag from each of the ICF's held in the InstrumentConfigurations.xml file in addition to the default configuration option which appears at the top of the list. Select an ICF to use the ICF configuration when a new Worklist is created.

  The ICF information contains the type of device, autoloader type, manual port availability, sorter availability and lasers. The lasers defined in the ICF will determine the laser options displayed in the *Acquire* pane. The sources defined in the ICF will determine the available options in the Source Selection dialogue when creating a new Worklist.

- **Delete:** Click the button to delete the currently selected ICF (and associated SCF's) from the InstrumentConfigurations.xml file and from the ICF drop-down list. The default configuration will be automatically selected in the drop-down list.

- **Export:** Click the button to save the currently selected ICF (and associated SCF's).

- **Import:** The button is only enabled when the workstation has not been connected to a DLI (device layer interface) before. Click the button to import an ICF (and associated SCF's) into the InstrumentConfigurations.xml file. The ICF will be present and automatically selected in the ICF drop-down list.

- **SCF:** The SCF (system configuration file) drop-down list contains the title tag from each of the SCF's (of which there will be one or more) defined under the currently selected ICF. The SCF information contains the parameters and tray types.

At the point of opening a Worklist or connecting to a DLI, a validation process is started checking the configuration of the Worklist against the ICF and SCF contents. Also, when loading a Workspace file into a Worklist, the Workspace file is checked against the ICF and SCF contents that are held within the Worklist. If validation fails, an error message is displayed.

Font pages

There are six Font pages containing options to select the default font typeface and size of text displayed in the Workspace. On the right of each page, a preview plot is displayed.
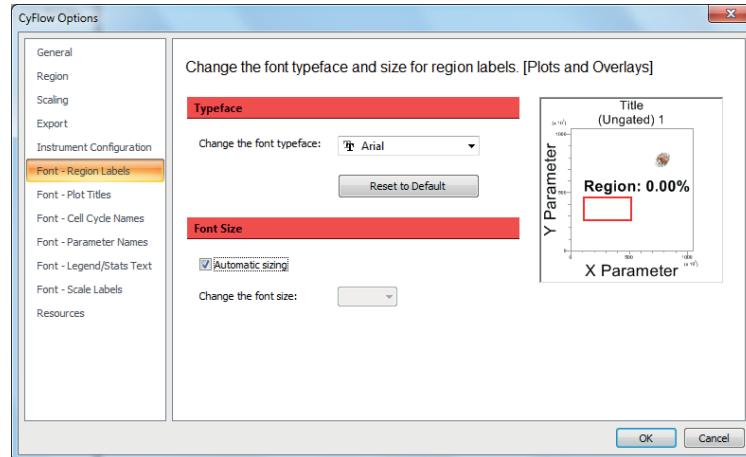
The following pages are available:

- **Font - Region Labels** to select the font typeface and size for region labels in plots and overlays.

- **Font - Plot Titles** to select the font typeface and size for plot titles in plots and overlays.

- **Font - Cell Cycle Names** to select the font typeface and size for cell cycle names in plots.

- **Font - Parameter Names** to select the font typeface and size for parameter names in plots and overlays.

- **Font - Legend/Stats Text** to select the font typeface and size for the legend and gallery statistics text in overlays.

- **Font - Scale Labels** to select the font size of scale labels in plots and overlays. On this page, only three options are available: small (4 pt), medium (5 pt) and large (6 pt) font size.

The pages contain the following options:

- **Reset to Default:** Click the button to reset the font typeface to its default setting, which is Arial.

- **Automatic sizing:** Select the check box to achieve automatic sizing of texts. This is the default setting. The check box is not available for cell cycle names.

Resources page

The Resources page contains options to access license and software version information.
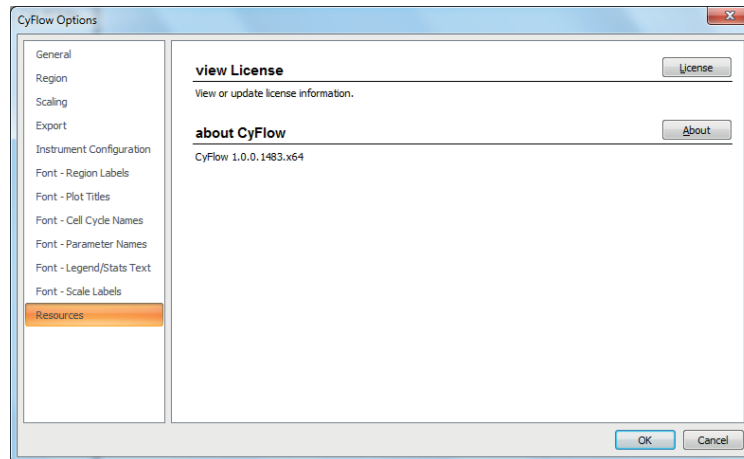
The page contains the following options:

- **License:** Click the button to access the license information and update your user license.

- **About:** Click the About button to view software information, version number and copyright details.

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