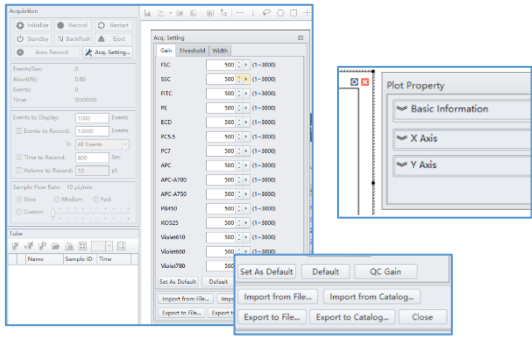


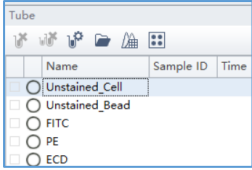


# Biovis CYTOFLEX QUICK INTRO v240130





This Quick intro includes

- A) a step wise description how to basically handle the Cytoflex S and LX
  - B) Optical Configuration of Cytoflex S, LX and SRT
  - C) Various screen shots, taken from BEC manual to visualize the workspace of CytXpert
- Note that CytXpert is freely available from Beckman Coulter

## A) Step wise Cytoflex Introduction

step	description
<p>1. Start up</p>	<ul style="list-style-type: none"> <li>- See extra Information seen “on wall”</li> <li>- Specific ON/OFF are instrument specific</li> <li>- Start up will take 10 minutes</li> <li>- Once started up make sure you are in Acquisition mode (see A) and check status bar (B) for information about various features</li> <li>- See overview of acquisition screen below (C)</li> </ul>
<p><b>General recommendation for your experiments:</b></p>	<ul style="list-style-type: none"> <li>- Open Acquisition panel to monitor/save/load gains</li> <li>- Open Plot properties to handle plot, by clicking on upper left wheel of plot</li> </ul> 
<p>2. Compensation file  <b>user/my documents/ “your folder”</b> for storage</p> <p> _create comp matrix</p> <p> _calculate comp matrix</p> 	<ul style="list-style-type: none"> <li>- → file/new compensation (or via icon, see left)</li> <li>- Choose channels (fluorophores) you want to use in the pop-up window</li> <li>- Read in data → initialize→Run (do not record yet)</li> <li>- Place population and peak as wished using “plot property “</li> <li>- Use “auto” to place populations in plots or the “hands”</li> <li>- If all placed good record on slow or medium mode</li> <li>- → recorded tubes will be marked GREEN (not blue)</li> <li>- Calculate compensation → settings/compensation calculation or icon (see left)</li> <li>- Save the comp file in your folder</li> <li>- Save the used gains as “default” or in your folder</li> </ul> <p><b>Recommendation:</b></p> <ol style="list-style-type: none"> <li>1) Have gains of different channels not changed between recording the single stains</li> <li>2) Do fast assessment of needed gains for all single stains under “unstained sample”, and changing gains here. Final gains of “Unstained” will be taken over than to single stain tubes.</li> <li>3) To speed up 2) - have a sample which contains all single stains pooled in one tube</li> <li>4) Have compensation and subsequent experiment using same gains (even though difference can be recalculated by CytXpert)</li> </ol>

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<p>3. Experiment set up</p> <p><b>user/my documents/ "your folder"</b> for storage</p>	<ul style="list-style-type: none"> <li>- Choose from pop-up window an old experiment or make a new one</li> <li>- choose the channels (fluorophores) you want to use under → settings/set channels</li> <li>- add one or more tubes</li> <li>- create plots (and gates)</li> <li>- close your experiment OR do 3. Comp file first</li> </ul>
<p>4. Experiment/Acquisition</p> <div style="display: flex; flex-direction: column; gap: 10px;"> <div data-bbox="204 613 309 703">  <p>use comp matrix</p> </div> <div data-bbox="204 748 309 837">  <p>hierarchy</p> </div> </div>	<ul style="list-style-type: none"> <li>- Open your experiment</li> <li>- Read (not record) in data</li> <li>- Use properties and hands to place peaks and populations</li> <li>- Apply compensation to first tube (see left)</li> <li>- Use the option which is marked</li> <li>- Apply the matrix on other tubes</li> <li>- Record all data</li> <li>- Make appropriate gating hierarchy</li> <li>- Show gating hierarchy via icon (see left)</li> <li>- To "all events" or "one population" right click on plot headline and choose</li> <li>- Record your data</li> <li>- If needed, save your experiment as a template</li> </ul>
<p>5. Statistic </p> <p>statistics</p>	<ul style="list-style-type: none"> <li>- Press statistics icon to get statistic</li> <li>- Right click on statistics window and chose settings to finetune your statistics</li> </ul>
<p>6. Export </p>	<ul style="list-style-type: none"> <li>- Export your data as .fcsfile, →file/export fcs file</li> <li>- Export your data as pdf (e.g. batch export to pdf file)</li> <li>See icon left</li> </ul>
<p>7. Daily shut down (if you are the last user)</p>	<ul style="list-style-type: none"> <li>- Follow guidelines seen on wall</li> <li>- Or →cytometer/daily clean</li> </ul>
<p>8. Logbook</p>	<ul style="list-style-type: none"> <li>- Make your entry in the logbook</li> </ul>

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### B) Optical Configurations

BioVis Platform Uppsala University  
 Optical Configuration of Cytoflex LX and S (Flow Cytometer Analyzer) and Cytoflex SRT (Cell Sorter)

Cytoflex LX			
UV Splitter			
Laser	Name	BP	Ch
355	U405	405/30	5
	U525	525/40	
	U675	675/30	
	empty	450/45	
	U(S)740	740/35	
	U(S)819	819/44	
405	empty	405/10	5
	V450	450/45	
	V525	525/40	
	V610	610/30	
	V660	660/10	
	V763	763/40	
488	SSC	488/8	3
	B525	525/40	
	B610	610/20	
	B690	690/50	
561	none	561/6	5
	Y610	610/20	
	Y763	763/45	
	Y585	585/42	
	Y675	675/43	
	Y710	710/50	
638	none	638/6	3
	R763	763/43	
	R660	660/10	
	R712	712/25	
808	not active		

Both instruments have 96 well plate reader, LX can also handle deep well plates. In case you work on both instruments use the "BioVis S&LX" config on the Cytoflex S (instead "Default"). BPs are the same.

The SRT is a cell sorter with a 100 µm nozzle, sorts into tubes (2,5/5/15ml) and plates like 96 and 384, deep well plates included

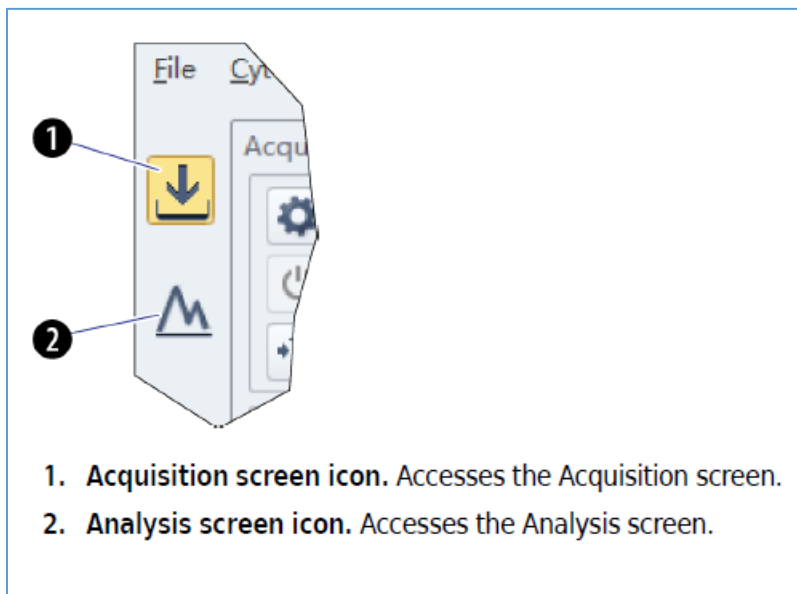
Cytoflex S			
Default			
Laser	Name	BP	Ch
405	none	405/10	4
	PB450	450/45	
	KO525	525/40	
	Violet610	610/20	
	Violet660	660/20	
	none	780/60	
488	SSC	488/8	2
	GFP	525/40	
	PerCP	690/50	
561	none	561/10	4
	mCherry	610/20	
	PE DsRed	585/42	
	PC5.5	690/50	
	PC7	780/60	
	none	none	
638	APC	660/20	3
	APC-A700	712/25	
	APC-A750	780/60	

Cytoflex SRT			
Default			
Laser	Name	BP	Ch
405	none	405/10	4
	V450	450/45	
	V525	525/40	
	V610	610/20	
	V660	660/20	
	none	780/60	
488	SSC	488/8	2
	B525	524/40	
	B690	690/50	
561	none	561/10	5
	Y610	610/20	
	Y780	780/60	
	Y585	585/42	
	Y710	710/50	
638	Y675	675/30	3
	R660	660/20	
	R712	712/25	
	R780	780/60	

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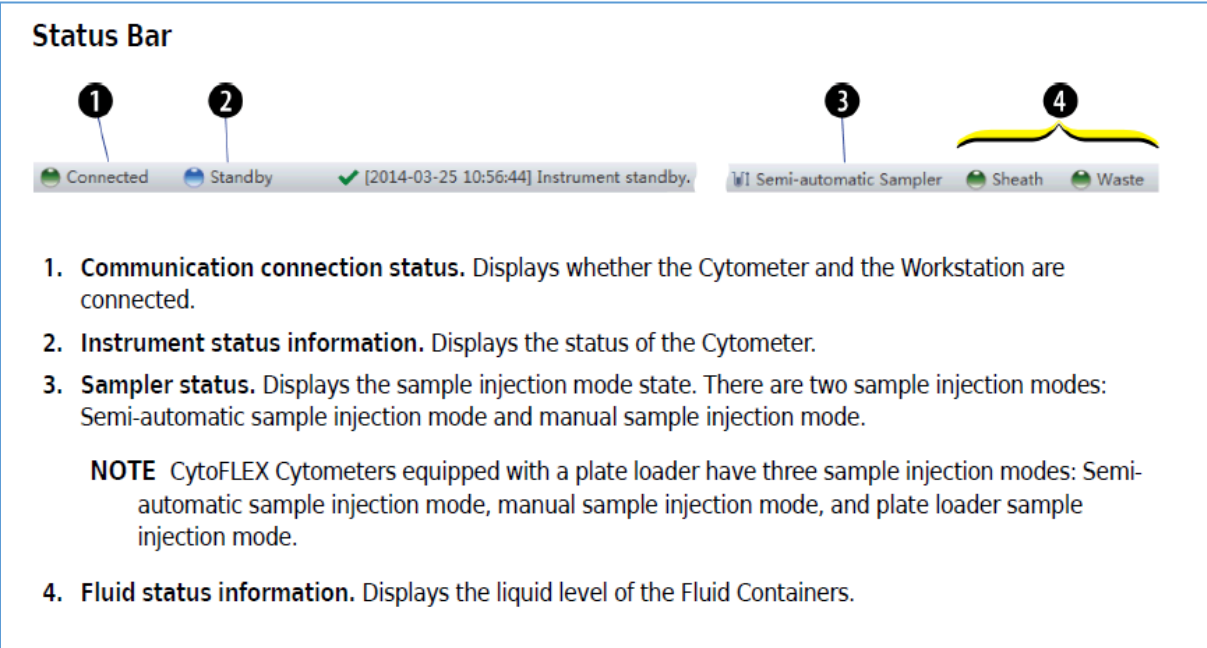
### C) Workspace of CytXpert

#### C.1 Use the Acquisition



#### C.2 check Status bar for errors or messages

### Status Bar




The screenshot shows a status bar with several indicators:

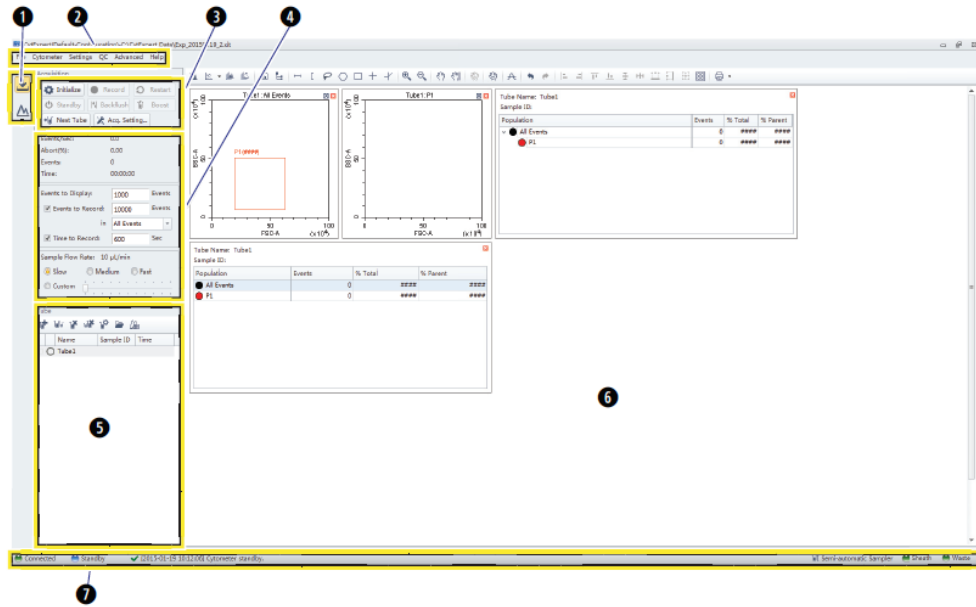
- 1. Communication connection status:** A green circle with a white checkmark, labeled "Connected".
- 2. Instrument status information:** A blue circle with a white checkmark, labeled "Standby".
- 3. Sampler status:** A green circle with a white checkmark, labeled "[2014-03-25 10:56:44] Instrument standby." and "Semi-automatic Sampler".
- 4. Fluid status information:** A yellow bracket above two green circles with white checkmarks, labeled "Sheath" and "Waste".

- 1. Communication connection status.** Displays whether the Cytometer and the Workstation are connected.
- 2. Instrument status information.** Displays the status of the Cytometer.
- 3. Sampler status.** Displays the sample injection mode state. There are two sample injection modes: Semi-automatic sample injection mode and manual sample injection mode.  
  
**NOTE** CytoFLEX Cytometers equipped with a plate loader have three sample injection modes: Semi-automatic sample injection mode, manual sample injection mode, and plate loader sample injection mode.
- 4. Fluid status information.** Displays the liquid level of the Fluid Containers.

C.3. Overview of Acquisition Screen

**Acquisition Screen**

Selecting **New Experiment**, **New Experiment From Template**, or **Open Experiment** automatically opens the Acquisition screen. The Acquisition screen can be accessed by selecting  on the left side of the page.



1. **Navigation.** Gives the option of accessing the acquisition screen or analysis screen.
2. **Menu.** Allows you to configure settings for sample acquisition, instrument operation, and software options.
3. **Instrument Operation Controls.** Controls sample loading/unloading and data acquisition and recording.
4. **Collection.** Establishes control over data recording options and displays the acquisition status.
5. **Test tubes.** Allows you to configure and duplicate sample tubes, set display attributes, manage experimental data and compensation.

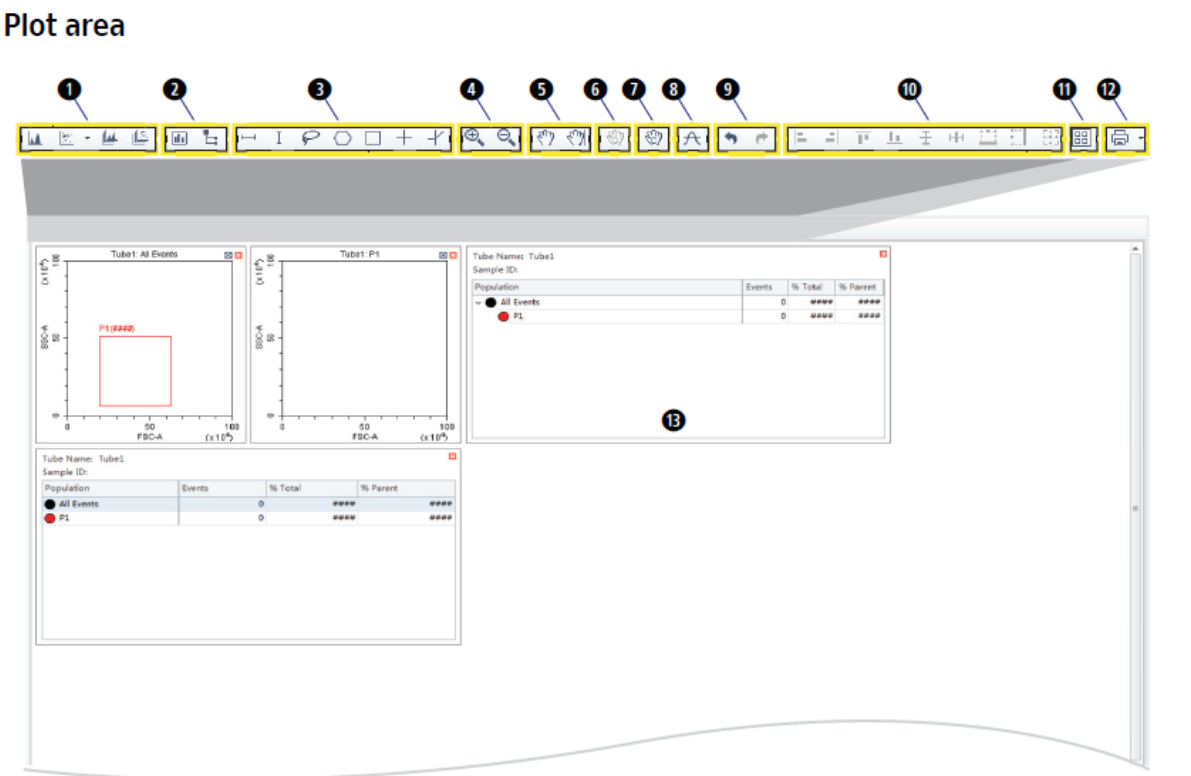
**NOTE** The Tube section of the screen can be expanded or retracted by dragging the top border of the Tube section of the screen. Expanding this section covers other elements of the screen, including: Events to Display, Events/Sec, and the Acquisition buttons.

6. **Plot area.** Includes plot and gating controls, as well as an area for drawing plots and generating graphs.
7. **Status bar.** Displays instrument connection status and system information.

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### C.4. Overview of Plot Screen

**Plot area**

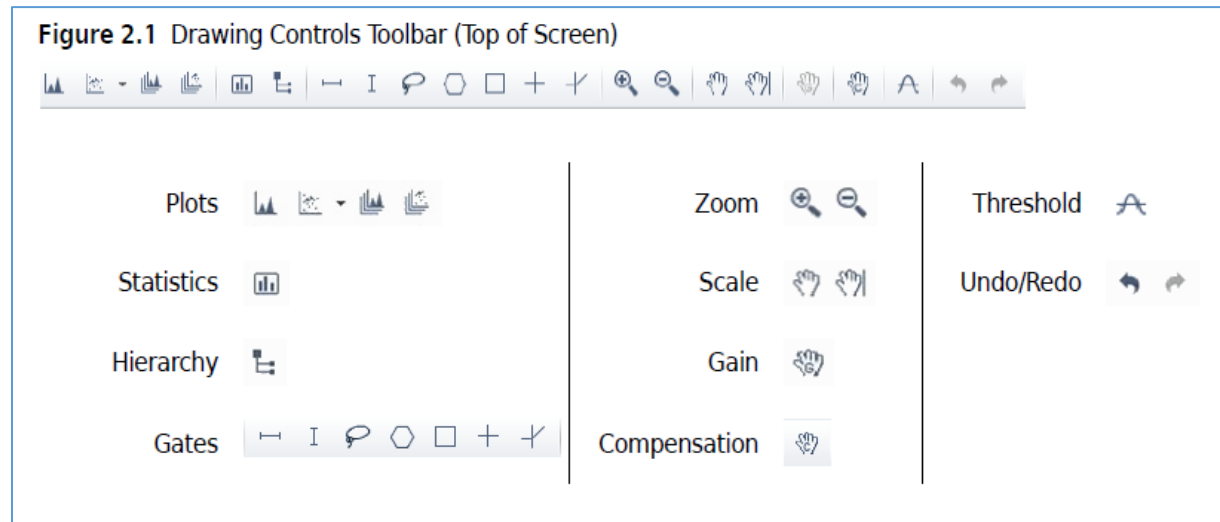


1. **Plot controls.** For creating single or multiple plots, such as scatter plots, histograms, density plots, pseudocolor plots, and contour plots.
2. **Statistics and hierarchy controls.** For creating statistical and hierarchical charts.
3. **Graphical gating controls.** For graphical gating of plots that have already been drawn.
4. **Zoom controls.** For zooming in and out.
5. **Axis display controls.** For scaling axis ranges in the plots.
6. **Gain adjustment control.** For increasing and lowering gain adjustments on the plots.
 

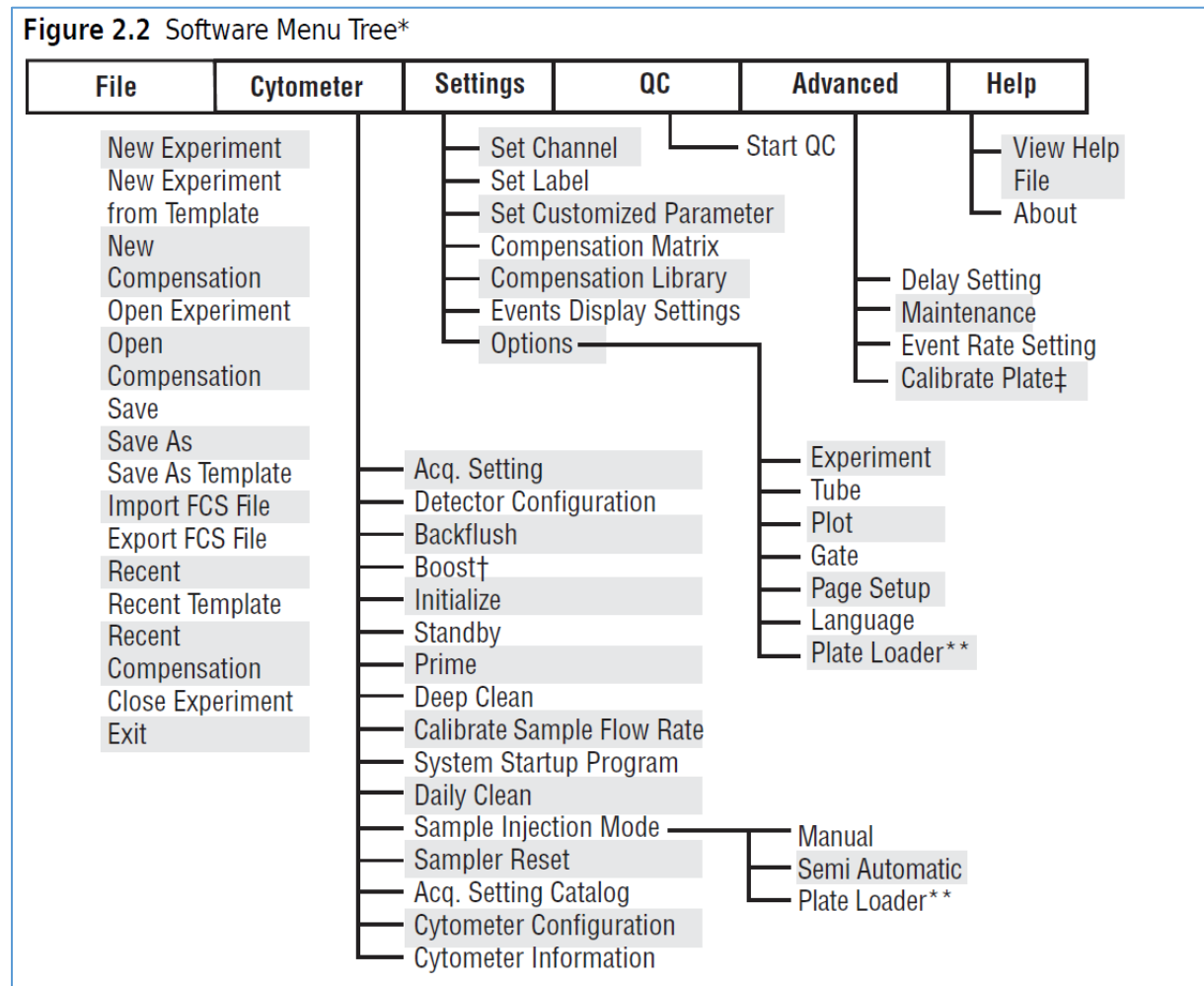
**NOTE** The gain adjustment control only works when a sample is running.
7. **Adjust compensation control.** For adjusting compensation of either of the parameters on a 2-D histogram.
8. **Threshold control.** For setting the minimum particle size limit or fluorescence intensity that acquisition will allow.
9. **Undo and redo controls.** For undoing or redoing an action in the drawing area.
10. **Display controls.** For controlling how plots and tables are aligned and arranged.
11. **Rearrange.** For restoring the plots to the default positions.
12. **Printing controls.** For printing and previewing the plot area.
13. **Plot area.** For drawing plots and displaying statistics and hierarchy tables.

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### C.5. Overview of different drawing tools



### C.6. Overview of available menus



END