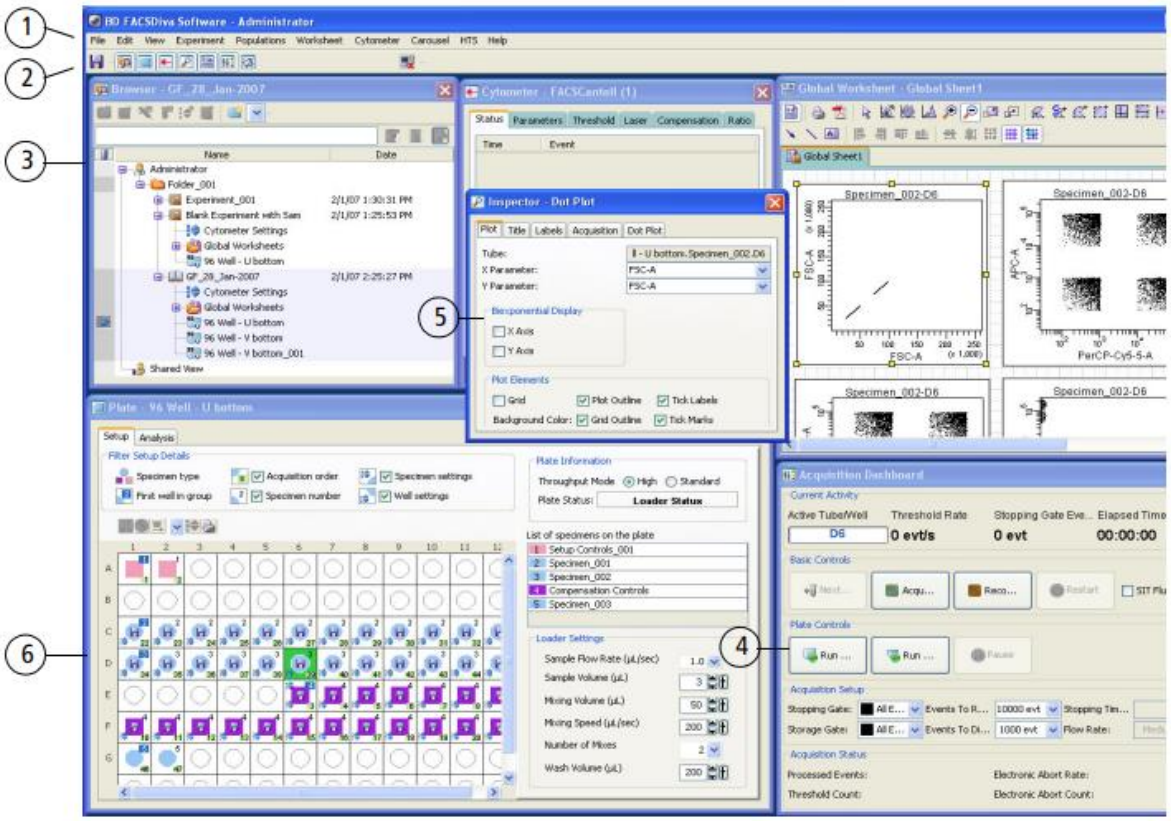
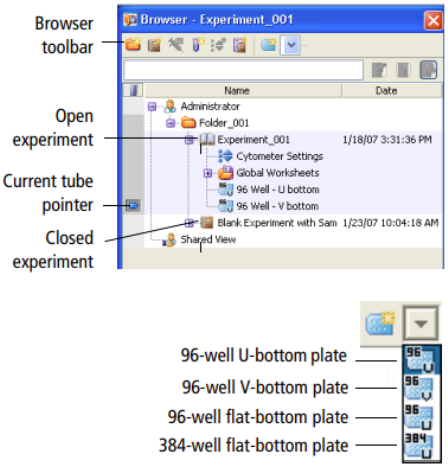
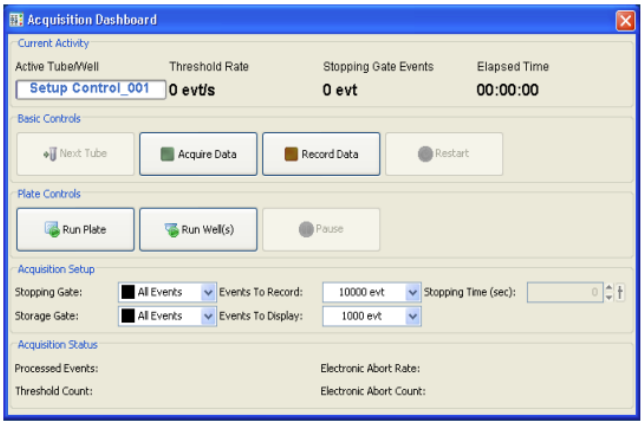
HTS info sheet

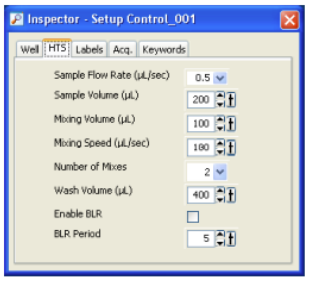
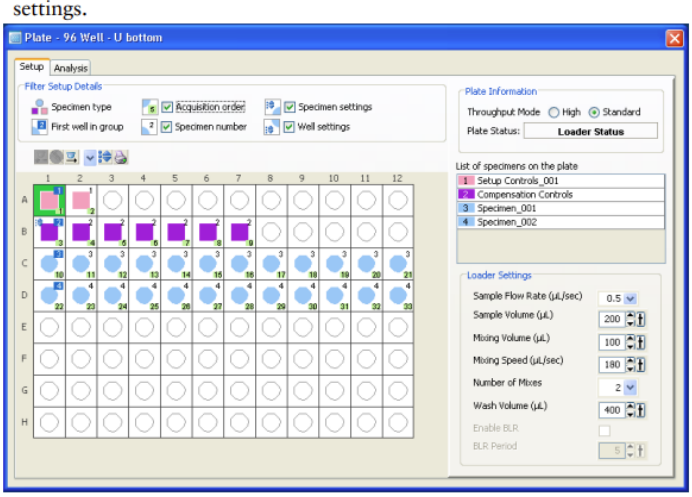
Figure 2-1 BD FACSDiva™ workspace displaying an open experiment and the Plate window

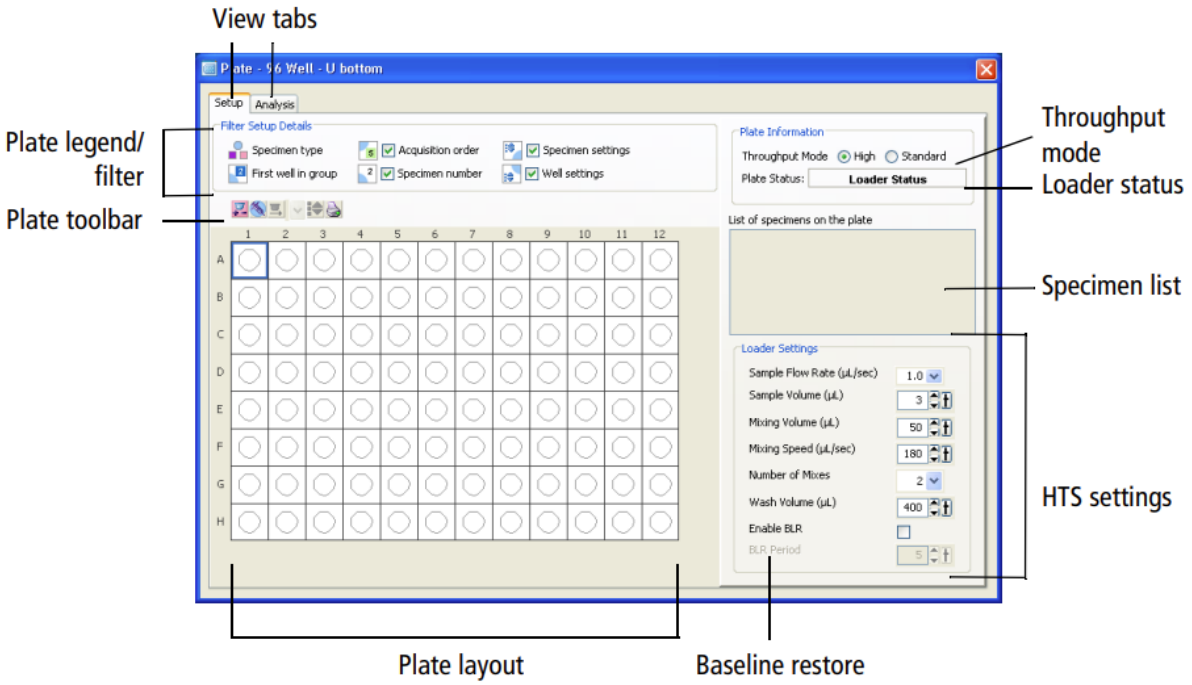


Number	Common Workspace Components
1	Use the commands in the menu bar to operate the software. File Edit View Experiment Populations Worksheet Cytometer HTS Help • Use the Experiment menu to add a new experiment to the Browser or open a template. A new experiment automatically contains global cytometer settings and a global worksheet. For information, see Creating Experiments on page 72. • Use the commands in the HTS menu to perform maintenance procedures on the HTS. For information, see Maintenance on page 111.
2	Click a button on the Workspace toolbar to hide or show the corresponding window. Windows can be resized by dragging a border or corner. Click the **Plate** button ( ) to view the **Plate** window.

First things first: Go to File / User Preferences / FCS: click Auto Increment, so that each file gets the appendix _001, _002 etc. This is important for later evaluation in another software (f.e. flowjo, fcsexpress).

3	• Use the Browser window to create and set up experiments and view experimental data hierarchically. In the **Browser**, double-click an experiment to open it. • Click the **New Experiment** button ( ) on the **Browser** toolbar to create a new experiment. • Click the **New Plate** button ( ) on the **Browser** toolbar to add a default 96-well U-bottom plate to the open experiment. • Click the arrow next to the **New Plate** button ( ) to select a new plate type to add to the experiment. The type you select becomes the default.  	Create a new experiment as usual. Select the respective plate format here. U and V plates are usually better because the cells are mixed much more thoroughly.
4	Use the Acquisition Dashboard to acquire and record well data. Use the Plate controls to acquire and record wells in sequence using the selected throughput mode. Run Plate runs the wells from the current position to the end of the plate. Run Well(s) runs the selected wells only. Use the Basic Controls to manually acquire or record selected wells in standard mode using the current loader settings.  You can expand (show) and contract (hide) the Acquisition Dashboard. To show or hide the Plate Controls, Acquisition Setup, or Acquisition Status sections of the Acquisition Dashboard, right-click the Acquisition Dashboard in any blank area (except for Basic Controls) and select a section to show or hide from the shortcut menu. You can resize the Acquisition Dashboard using standard Windows methods.	If the plate controls are not displayed, simply right-click “Show plate controls”.

5 	 Use the Inspector to view or modify the attributes of a single object or set of objects on the worksheet or plate, or in the Browser. The contents of the Inspector change depending on what is selected in the workspace. The Inspector can be used to view or change HTS (Loader) settings. See Loader Settings on page 51.	
6 	Set up, acquire, and analyze plate-based experiments in the Plate window. Use this window also to select the throughput mode and adjust loader settings. 	
In the inspector window: Use Global Cytometer Settings can be deactivated there. This means that different settings can be used for different wells. Many commands can also be accessed by right-clicking on the respective well.		



Loader Settings	
Sample Volume	50 µl dead volume, which means if I want to measure 200 µl of my sample, I need to put 250 µl of sample into my well.
Mixing Volume	½ of the total volume is a good benchmark.
Mixing Speed	220 default value for PBMCs, f.e. for dendritic or thawed cells you should choose a lower value.
Carry over	You should incorporate cleaning wells with H_2O or PBS
BLR	Baseline Restore Period, goes from 5 to 150. The value x10 is the delayed recording in milliseconds, f.e. is the value 5, the recording starts after 50 milliseconds and not immediately.

Volume type	Definition
Well volume	Volume that well can hold filled to the brim
Dispensed volume	Volume pipetted into well minus aspirated excess volume
Aspirated excess volume	Standard mode = 20 µL
Available volume	Volume pipetted into well minus aspirated excess volume minus dead volume
Minimum volume	50 µL for both standard and high-throughput modes for 96-well plates
Mixing volume	Approximately one-half the available volume **NOTE** A mixing volume that is larger than the available volume introduces air bubbles into the sample
Dead volume	Volume in the bottom of the well that the probe cannot reach
Sample volume	Amount of sample requested for analysis in BD FACSDiva™ software

<table>
<tr><th colspan="2">Other information</th></tr>
<tr><td>Which cell density is recommended?</td><td>Fresh lympocytes: max. 10 million cells / mL
Dendritic cells: 1-2 million cells / mL</td></tr>
<tr><td>Additives</td><td>By adding EDTA, BSA etc. the cells remain intact.</td></tr>
<tr><td>thawed PBMCs</td><td>Clogging of the sample line possible. Solution: lower cell density, lower sample flow rate, adding f.e. BSA to the sample.</td></tr>
<tr><td>Standard Mode
High-Throughput Mode</td><td>In contrast to the standard mode, both pumps are working in the High-Throughput Mode at the same time. (this could be useful when checking a GFP signal on the whole plate → quick measurement)
For everything else the standard mode is recommended!
The sample flow rate goes from 0,5-3µl/sec. (which equals to MED – HIGH). SO with the HTS we simply don´t have a “LOW” (which means it is not suitable for f.e. cell cycle analyses).
<table>
<tr><th rowspan="2">Setting</th><th colspan="2">Standard Mode</th><th colspan="2">High-Throughput Mode</th></tr>
<tr><th>Default</th><th>Range</th><th>Default</th><th>Range</th></tr>
<tr><td>Sample flow rate (µL/sec)</td><td>1</td><td>0.5–3.0</td><td>1</td><td>0.5–3.0</td></tr>
<tr><td>Sample volume (µL)</td><td>10</td><td>2–200</td><td>2</td><td>2–10</td></tr>
<tr><td>Mixing volume (µL)[a]</td><td>100</td><td>5–100</td><td>50</td><td>5–100</td></tr>
<tr><td>Mixing speed (µL/sec)</td><td>180</td><td>25–250</td><td>200</td><td>25–250</td></tr>
<tr><td>Number of mixes (cycles)</td><td>2</td><td>0–5</td><td>2</td><td>0–5</td></tr>
<tr><td>Wash volume (µL)</td><td>400</td><td>200–800</td><td>200</td><td>200–800</td></tr>
</table>
a. We recommend a mixing volume that is one-half the available volume. See Loader Settings on page 35, Sample Well Volumes on page 35, and Mixing on page 37.</td></tr>
<tr><td>Which plates?</td><td>384 well or 96 well plate
Important: define in advance which plate size is used, otherwise the sample needle may be damaged in the process since the device knows the exact position of the wells for each plate format.</td></tr>
<tr><td>The lid must be on correctly!</td><td>The device detects if the lid is not properly seated.</td></tr>
<tr><td>Create settings</td><td>Settings should be created beforehand with tubes instead of the plate. This has the advantage that you have more time when adjusting the PMTs etc.</td></tr>
<tr><td>Cleaning plate at the end!!</td><td>After the measurement, insert a cleaning plate.
F.e.
3 wells BD FACSClean or BD FACSRinse
3 wells Aqua dest.
Repeat 3 times in a row</td></tr>
</table>