



## Acquiring samples in the BD LSRFortessa

Launch DIVA program and log into you session. When then CST mismatch window pops up click in: "Use CST Settings"



Identify the windows of Diva workspace:



If one of the windows is missing, go to the top menu bar, click on view and select the missing window.

File	Edit	View	Experiment	Populations	Worksheet	Cytometer	HTS	Help	
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Use the Browser window to create experiments:

Click in the folder icon to create a folder, right click to rename.



Now you could create a new experiment in the folder. Click in the icon: Double click to open the experiment and right click to rename it. ( 📄 )

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Name	Date
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Create a Specimen by clicking the Specimen icon:

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Tube_001	
🕒 😹 Shared View	

The specimen contains a tube, when clicking in the tube pointer you will visualise the list of available parameters in the cytometer window/parameter tab:

itatus	Parameters	Threshold	Laser	Compensation	Ratio				
P	arameter			Voltage	Log	А	н	W	
• FS	c			507					^
• 55	c			299		V			1
• 48	8-530/30			478	~	<b>v</b>			1
• 48	8-710/50			610	<b>V</b>	<b>V</b>			1
e 64	0-670/14			515	~	<b>V</b>			1
e 64	0-730/45			553	<b>V</b>	<b>V</b>			1
* 64	0-780/60			503	<b>~</b>	<ul> <li>Image: A start of the start of</li></ul>			1
· 405	5-450/50			424	<b>V</b>	<b>V</b>			1
<ul> <li>405</li> </ul>	5-525/50			533	<b>~</b>	<b></b>			1
• 405	5-540/30			596	<b>V</b>	<b>v</b>			1
· 405	5-586/15			665	~	<b></b>			1
• 405	5-610/20			765	<b>v</b>	<b>v</b>			1
· 405	5-670/30			648	<b>V</b>	<b>V</b>			1
• 35	5-450/50			405	~	<b>v</b>			1
• 353	5-530/30			623	<b>v</b>	<b>V</b>			1
• 56	1-586/15			581	<b>~</b>	<b>V</b>			1
<ul> <li>563</li> </ul>	1-610/20			663	~	<b>V</b>			1
<ul> <li>56.</li> </ul>	1-670/30			542	<b>V</b>	<b></b>			]
• 56	1-710/50			568	<b>V</b>	<b>V</b>			1
<ul> <li>56.</li> </ul>	1-780/60			619	<b>V</b>	<b>V</b>			
									~
		Add				Del	ete		



Erase the parameters that you do not need to measure in your experiment.

To delete parameters, click the selection button next to each unneeded parameter. Hold down the Ctrl key to select more than one. When you are finished selecting, click Delete.

Verify that Log mode is selected for all fluorescence detectors but not for FSC and SSC (Lin mode). Tick H and W for SSC parameter in order to discriminate doublets.

tatus Parameters Threshol	d Laser Compen	sation R	atio			
Parameter	Voltage	Log	A	н	W	
<ul> <li>FSC</li> </ul>	507		$\checkmark$			^
<ul> <li>SSC</li> </ul>	299		<b>V</b>	<b>~</b>	<b>V</b>	1
<ul> <li>488-530/30</li> </ul>	478					
<ul> <li>561-610/20</li> </ul>	663					
<ul> <li>355-450/50</li> </ul>	405					
						~

\*For cell cycle, don't forget to measure the parameter of your DNA dye in Lin mode and to tick H and W for doublet exclusion.

Add your specific labels to the parameters names.

Choose Experiment (top menu bar) > Experiment Layout, and define labels for each parameter.



The experiment layout window opens up:

2uidk Entry Label				Labels Name
Name	Label	Label	Label	List by user Daniel R-Q, BD Defined R-Q, SSungalee
Tube_001	488-530/30	561-610/20	355-450/50	Add to List Delete from List
				Assign or Remove Labels Assign Remove

Click on the parameter box and type in the label for each parameter.

label DAPI				Name
Name	Label	Label	Label	List by user
💷 Training Exp				B
Specimen_001				a a source
Tube_001	488-530/30 GFP	561-610/20 mCherry	355-450/50 DAPI	
				Add to List Delete from List
				Assign or Remove Labels

Create dot plots and histograms on the global worksheet to visualise the data: Click the Dot Plot button on the Worksheet toolbar and click in the worksheet, a plot of a default size is drawn.

🛗 Global Worksheet -	- Global Sheet1				
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Global Sheet1 Dot I	Plot				
Specime Spe	00-1-Tube_001				

Start with a FSC vs SSC plot, create a second plot SSC-A vs SSC-W to exclude doublets.

🚟 Global Worksheet - Global Sheet1	
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Global Sheet1	
Specimen_001-Tube_001	Specimen_001-Tube_001
	SSC-A
60 100 150 200 220 FSC-A (x 1.000)	50 60 100 160 200 250 550 (x 1.000)

To change the plot parameter, click the x-axis label and choose the parameter from the menu that appears. Notice that the parameter names include the labels you defined previously.



Create additional plots and/or histograms with the parameters that you need to measure in your samples. Do not forget to include a plot/histogram to exclude dead cells.



Place your sample tube in the SIP, close the arm and press RUN-Low in the cytometer control panel.



Press acquire data in the acquisition dashboard window as well.



Adjust FSC and SSC voltages in the parameter tab, to bring the population of interest onto scale. Press restart (acquisition dashboard window) after modifying the voltages to refresh the image and quickly visualise the effect of the changes.



Draw a gate to select the cell population and exclude debris. Click on the polygon gate tool at the worksheet menu and draw a polygon around the population, the gate will be automatically named P1. Right click inside the plot and choose Show Population Hierarchy. The population hierarchy is used to view defined populations and display the relationships between different gated populations.

	Global Worksheet - Global Sheet1							
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1					Global S	Sheet1		
-1	Specimen_001-Tube_001	Show Population Hierarchy	Ctrl+G					
		Create Statistics View	Ctrl+R		- 8 8-1	Specimen_001-Tube_001		
	8	Show Populations	•				201	<b>.</b>
	- 퀵 - 그는 그 그 그 가슴을 가슴다.	Scale to Population	•		- 8			Tube: Tube
		Show Gate	•					Population
	★뮉 김 김 강경 관광장이	Bring to Front	•		S-A	P1		
	0 P1	Send to Back	•		1 % <u>i</u>	1000	199	
		Order Populations by Count				1 / St. J		
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		🛅 Copy	Ctrl+C		· · ·	FSC-A	(x 1,000)	
	50 100 150 200 250	Delete	Delete					
	FSC-A (× 1,000)							

Right click inside the SSC-A vs SSC-W plot and choose Show Populations > P1.



Only events within the P1 gate are shown in the plot. Notice that all events in the plot are now coloured red, the color of the P1 population.

Make a new gate to select the singlet population, named P2.



To make a new population from P1, make sure P1 population is highlight in the population hierarchy. When a population is highlighted in the population hierarchy, any new populations will be a subset of the population that is highlighted.

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Parent #### 43.4 The name of the populations could be modified in the population hierarchy list: double click in the name to enter a new one, or in the inspector window.

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R	Global Sheet1							
	Specimen 001-Tube 001	88C-A (+1400) 88C-A (+1400) 140 200 120	<u>men_00</u>	P2				
•••	60 100 160 200 250 FSC-A (x 1,000)	50	100 S	150 200 250 SC-W (× 1,000)				
	Inspector - Cells  Color:			Tube: Tube_001 Population All Events Cels P2		#Events 3,396 1,473 1,332	%Parent #### 43.4 90.4	%Total 100.0 43.4 39.2

Click on the coloured square to choose a new colour for the population.

Population	#Events	%Parent	%Total
All Events	3,396	####	100.0
Cells	1,473	43.4	43.4
Singlets	1,332	90.4	39.2

Modify the title of the dot plots to display a selected population: Select the plot, go to the inspector >Title tab: Tick populations

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Global Sheet1	Dispector - Det Plet	
	mspector - bot Plot	
Specimen 001-Tube 001	Plot       Title       Labels       Acquisition       Dot Plot         Title       Content       Specimen       Title       Custom Title:         Title       Fork       Face:       SanSerif       Size:       12       Color:         Italic       Bold	

Select the viable cells from your Singlets population (DAPI negative) and restrict you further analysis to events contained in Live gate.



Once the FSC and SSC voltages are set-up, do the same for the fluorescent detectors. Load your negative/unstained sample and adjust the voltages while the same is running. Negative population should be placed in the lower left quadrant of a plot. When using an histogram place the negative population on the left.





If the population appears below the axes (in the dot plot), enable Biexponential display to view populations with negative events. Click on the plot and check the boxes in the Inspector window.

Adding a quadrant (worksheet menu) or grid onto the plot and histogram respectively, will help you to place the negative population correctly.



Establish in the acquisition dashboard the amount of events that you want to acquire: Storage gate: keep all events

For stopping gate: choose live/P3 and record 10.000 events, enough for most of the applications. If you population of interest is very rare and you need to analyse additional parameter within, choose it as a stopping gate an make sure to collect at least 2000 positive events per population.

🔢 Acquisition Dasl	iboard			
Current Activity Active Tube/Well Tube_001	Threshold Rate	Stopping Gate Events <b>0 evt</b>	Elapsed Time 00:00:21	
Basic Controls				
Stopping Gate:	Live Vents To Re All Events Vents To Dis	cord: 10000 evt 💌 Stopp play: 5000 evt 💌	ing Time (sec): 0 🖨	
Acquisition Status Processed Events: Threshold Count:	846 evt 5458 evt	Electronic Abort Rate: Electronic Abort Count:	25 evt	

Rename tube: Right click on the tube > Rename



Now that all your settings have been established press: Record in the acquisition dashboard window. Note that after acquire the samples the tube icon changed indicating that events have been recorded in the tube.



Add new tubes, rename them and start the acquisition:

Make sure to select the tube pointer and verify in the dashboard the name of the tube where events will be recorded.

(			🔛 Acquisition Dashboard	
	a Training Exp a Training Exp a ⇒ Cytometer Settings a ⊖ Global Worksheets b ≤ Society 001	8/9/16 12:05:01 PM	Current Activity         Current Activity           Active Tube/Well         Threshold Rate         Stopping Gate Events         Elapsed Time           Treatment 2         0 evt/s         0 evt         00:00:05	
		Basic Controls		
	🔳 🕕 Treatment 2			

Follow the steps below when loading a new tube:

- a. Press acquire in the acquisition control
- b. Visualise the sample in the plots, allow the number events to stabilise (Threshold Rate)
- c. Press Record Data

The acquisition will be stopped automatically when the sample reach the events stated in the stopping gate. If you do not have enough sample, stop manually first the recording and then the acquisition.

🔢 Acquisition Dashboard 🛛 🛛 🔀				
Current Activity				
Active Tube/Well	Threshold Rate	Stopping Gate Events	Elapsed Time	
Treatment 2	0 evt/s	0 evt	00:00:05	
Basic Controls	2	1		
<b>∳</b> Next Tube	Stop Acqu	🧧 Stop Reco	art	

Once you finish, make sure to export your FCS files to the FCS folder and to transfer them to the fccf server or the server of you group. Once your files are safely storage, please remove them from the fccf server.



## For more info check the **data transfer protocol** and the protocol to **create experiment templates**.

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