FACS LSRFortessa Standard Operation Protocol Basic Operation

1. <u>Make sure the following actions have been taken before running your samples.</u>

- a. Make sure the waste tank is empty and refill with bleach if the fluid reaches RED line (Please refer to Page 14: Empty Waste Tank Procedure).
- b. Make sure the sheath fluid container is fully refilled with sheath fluid (Please refer Page 15: Refill Sheath Container Procedure).

2. Launching the BD FACSDiva Software

a. Log in FACSDiva software \rightarrow key in login name and password. Please contact the administrator to create a new user account.

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Jser Name:		User Name	~
Password:	R	XXXXXXXXX	

b. Click Use CST Settings when pop-up message as below is observed.

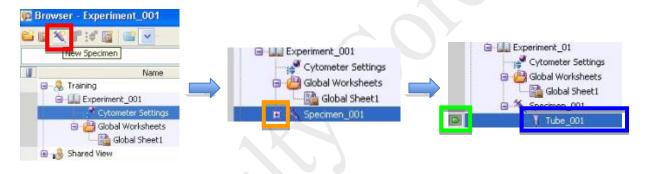


3. Creating and working with experiment in BD FACSDiva Software

a. *Browser* toolbar \rightarrow click New Experiment \rightarrow Rename the experiment if necessary.

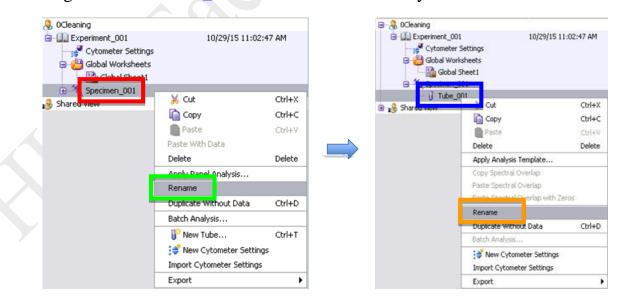


b. Select New Specimen → expands the Specimen to show Tube 001. Highlight the tube with the Tube Pointer.



c. Right click on **Specimen_001** \rightarrow **Rename** if necessary;

Right click on **Tube _001** \rightarrow **Rename** if necessary.



d. Select Cytometer Settings \rightarrow Parameters \rightarrow Delete unnecessary parameters on the

Inspector Window.

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	 SSC 	392		4		
	· FITC	562	2	~		
	· PE	536	4			
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	 PE-Cy7 	804				
	· APC	648				
	· APC-Cy7	507				
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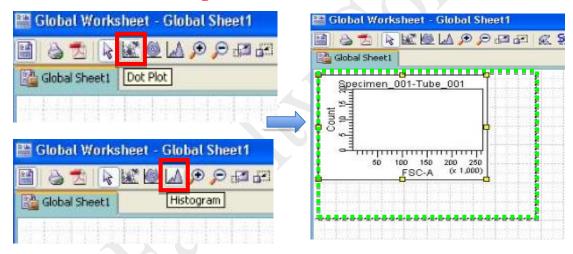
e. FSC (measure *Cell Size*) and SSC (measure *Cell Granularity*) are **MUST** for all kind of analysis and they should be kept in **linear scale**. Please check 'A'rea, 'H'eight and the 'W'idth of FSC and SSC. 'Log' and 'A'rea boxes should be checked for fluorescence channels except for cell cycle and/or DNA analysis.

Par	ameters Threshol	d Ratio Comp	ensation				
	Parameter	Voltage	Log	А	н	W	
	FSC	524			~		1
•	SSC	392		V	V	V	
,	FITC	562					
	PE	536	~				
	APC	648					

f. Choose Experiment → Experiment Layout and define labels for each parameter.
 Select the column of fluorescence channel and enter a label in the Quick Entry Label field.

New Foider	Ctrl+N	Labels k	eywords Acquisition		
👕 New Experiment	Ctrl+E		comoras negalitation		
餐 New Specimen	Ctrl+M	Ouick			
New Tube	Ctrl+T	Label	CD3 💌		
🖨 New Cytometer Settin	igs		Name	Label	Label
Import Cytometer Setting:	s			FITC	PerCP-Cy5-5
🎬 New Global Worksheel	t		🔚 Unstain	CD3	
🥌 New Plate	Ctrl+Y	-	FITC	FITC CD3	PerCP-Cy5-5
Open Experiment	Ctrl+0			FITC	PerCP-Cy5-5
Close Experiment	Chd+W		🔚 PE	CD3	
Experiment Layout				FITC CD3	PerCP-Cy5-5

g. Select **Dot Plot** or **Histogram** \rightarrow move the cursor onto the **blank worksheet**.

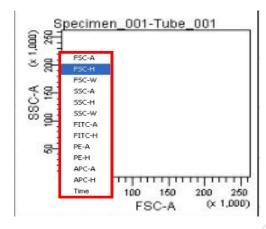


h. Right click on a plot \rightarrow **Duplicate** to create another plot of the same type.

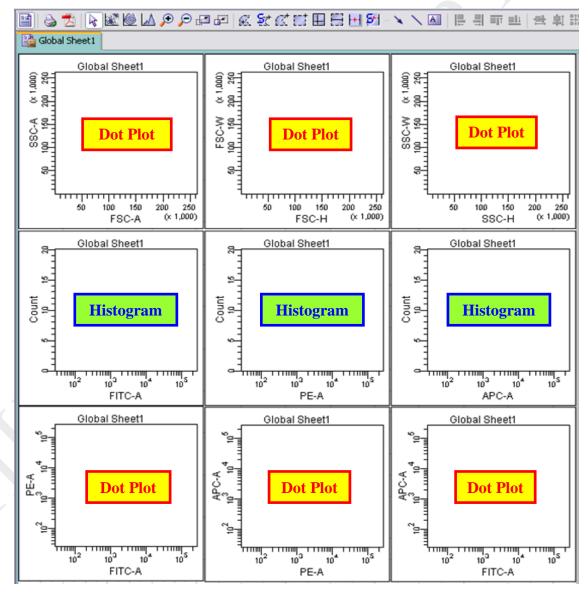
		PRECT	
Global Sheet1			
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- 39	Show Population Hiera Create Statistics View	rchy Ctrl+G Ctrl+R	
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2	Show Gate	•	
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	💥 Cut	Ctrl+X	
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	Delete	Delete	

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i. Select each individual **axis**, and opt from a list the preferred parameter.



j. Below shows a template of plots used in routine analysis.

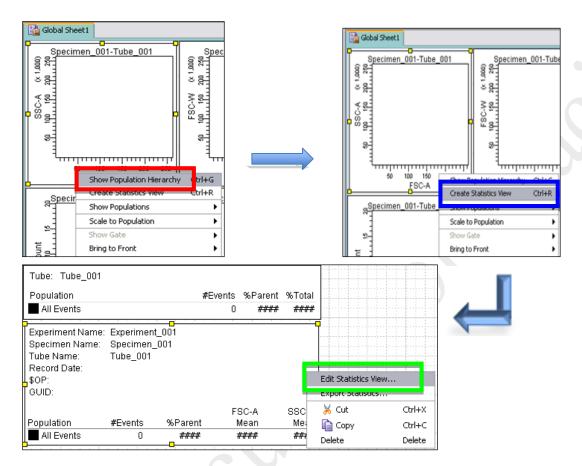


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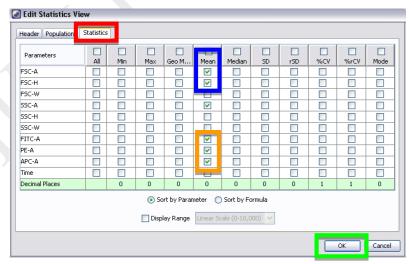
k. Right click on the plot \rightarrow Show Population Hierarchy

Right click on the plot \rightarrow Create Statistics View \rightarrow right click on the statistics view

→ Edit Statistics View



 Select *Statistics* tab → tick mean of FSC-A and FSC-H → tick the mean of the 'A'rea of the parameters → click OK



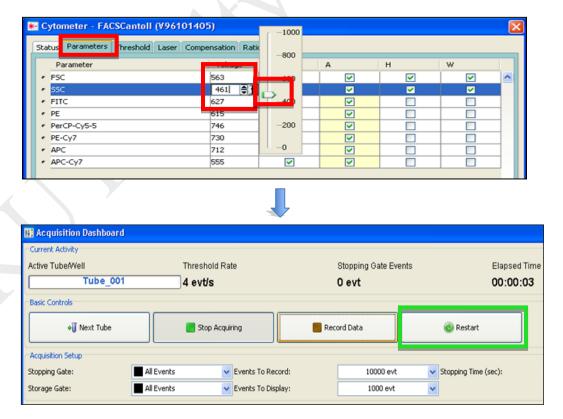
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4. <u>Procedures for sample acquisition</u>

- a. Press "**RUN**" and "**LO**" on fluid control panel.
- b. Gently tab the tube to mix your sample \rightarrow put your sample tube on SIP \rightarrow Run the unstained/negative control sample before other sample tubes.
- c. Acquisition Dashboard → Acquire Data

🄢 Acquisition Dashboard			
Current Activity			
Active Tube/Well	Threshold Rate	Stopping Gate Events	Elapsed Time
Tube_001	0 evt/s	0 evt	00:00:00
Basic Controls			
			-
📲 Next Tube	Acquire Data	Record Data	Restart
Acquisition Setup			
	All Events To Re	cord: 10000 evt	Stopping Time (sec):
	All Events V Events To Dis		
Storage Gate:	Events Events to Dis	inay. Too eve	×

d. Identify the population of interest by adjusting the voltage of FSC and SSC on
 Parameters → Press Restart to accelerate the changes



e. Cytometer \rightarrow Laser \rightarrow Adjust the FSC Area Scaling until the mean of FSC-A and FSC-H are <u>APPROXIMATELY SAME</u>.

Return to *Parameters* tab and finely re-adjust voltage of **FSC** and **SSC**.

Status Parameters Threshod L	aser Compensation Ratio		
Status	Measured	Reference	
Blue Laser Current	1.26	1.28	
Blue Laser Power	20,20	20.19	
Red Laser Power	17.10	18.00	
FSC Area Scaling: 0.50 🖨 🛉			
Name	Delay	Area Scaling	
Blue	0.00	1.40	
Red	31.39	1.28	

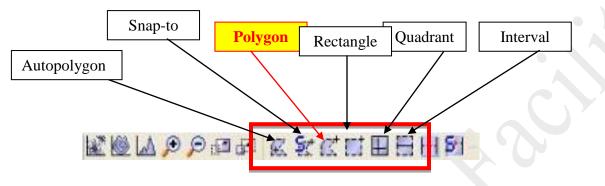
f. Adjust voltage of each of the fluorescent channels; preferably the peak of population of interest is greater than **ZERO** but less than 10^2 .

Stal	IS Parameters Theshold Laser	Compensation	Ratio		-1000	
	Parameter	Voltage	Log	A	-000	
•	FSC	526			-600	
	SSC	395			400	1
	FITC	555	4		100	
•	PE	534	~	5	200	
	APC	643	V	1		

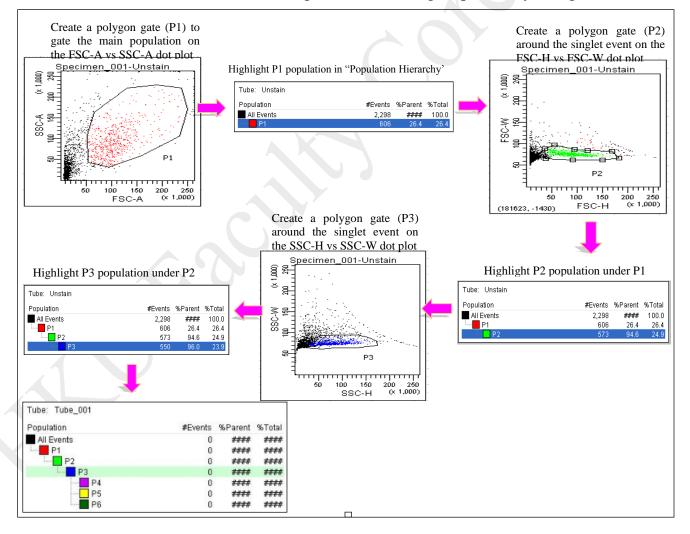
- g. Click Stop Acquiring on Acquisition Dashboard and replace your sample with DI H2O.
- h. Repeat above steps with the positive control sample tubes. Adjust the voltage of corresponding channels if their signal peaks are outside the limit of the histograms.

5. Creating Gates

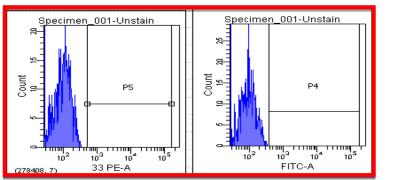
a. Set the current tube pointer to the following tube and use **Polygon Gate** to gate the targeted cells.

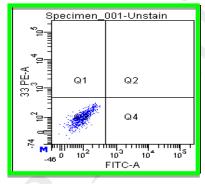


b. Gate the cells of interest according to the following sequence by using:

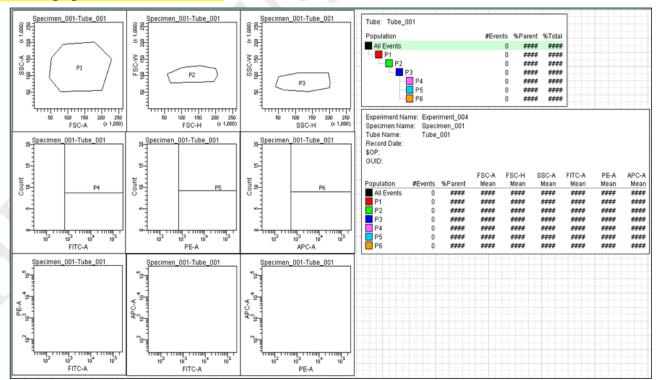


c. To define fluorescence positive signal, interval gate (P4, P5,...) beyond negative peak of fluorescent channels in histogram plot of unstained samples can be created. For over multiple fluorescence channels, quadrant gate could be created to define single/double positive signals (Q1; Q2; Q3; Q4....).





* Note that *P1* is the children of *All Events* and the parent of *P2* population; *P2* population is the children of *P1* population and the parent of *P3* population and the grandparent of *P4*, *P5*, and *P6* populations. Thus, <u>on the hierarchy table, users should highlight the *P1* population when a gate for *P2* population is drawn, the *P2* population when a gate for *P3* population when gates for *P4*, *P5*, and *P6* population is drawn, and the *P3* population when gates for *P4*, *P5*, and *P6* population is drawn.</u>



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6. <u>Recording data for all samples</u>

- a. <u>Gently tab the tube to mix your sample</u>, then put your sample tube on SIP (sample injection position); Run the unstained sample before other sample tubes.
- b. Press "RUN" and "LO" on fluid control panel.
- c. Acquisition Dashboard \rightarrow Acquire data \rightarrow Record data

Current Adbity Active Tube/Well Tube_001		hreshold Rate	Stopping Gate Eve	nts Elapsed Time
) evt/s	0 evt	00:00:00
Basic Controls	_	oor oo hadha		
📲 Next Tu	be	📓 Acquire Data	Record Data	Restart
Acquisition Setup				
Ropping Gate:	Al Events	Events To Re	cord: 10000 evt	Stopping Time (sec):
Storage Gate:	Al Events	Vents To Dis	play: 1000 evt	~

- d. Please pay attention to the sample tube to make sure it will not run dry!
- e. Click "Stop Acquiring" to stop acquire the data if necessary then unload your sample.
- f. Repeat above steps for each sample.

7. Machine Cleaning

- a. Cleaning procedure between each user is required.
- a. Prepare **3** mL of each cleaning solution (FACS Clean, FACS Rinse, Milli-Q H2O).
- b. Press "RUN" and "HIGH" on fluid control panel.
- c. Install a tube of FACS Clean solution on the SIP with the support arm to the side (vacuum on) and let it run for **1** minute.
- d. Move the tube support arm under the tube (vacuum off) and allow the cleaning solution to run for **5** minutes with the sample flow rate set to HI.
- e. Repeat steps 2 and 3 with BDTM FACSRinse solution.
- f. Repeat steps 2 and 3 with Milli-Q water.
- g. Make sure the fluidics control panel shows **LO** and **STANBY** when the entire process of cleaning is complete.

8. Export FCS Data / Experiment

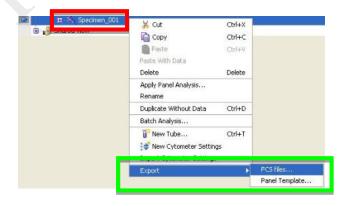
1. To save Experiment, right click on the **Experiment** \rightarrow **Experiment** \rightarrow **Experiment** \rightarrow **Browse** to choose the destination folder.

😽 OCleaning		
Experiment_001		4/29/15 3:44:41 PM
20150514		5/14/15 4:26:44 PM
		6/16/15 3:36:21 PM
1 20150616		6/16/15 4:09:01 PM
	Ctrl+X	
a 🧃 🚺 Copy	Ctrl+C	
🔋 🐴 💼 Paste	Ctrl+V	
Paste With Data		
E Delete	Delete	6/23/15 1:30:58 PM
Share Rename		
Duplicate Without Data	Ctrl+D	
Open Experiment	Ctrl+O	
Close Experiment	Ctrl+W	
Batch Analysis		
👔 New Global Worksheel	t	
K New Specimen	Ctrl+M	
New Cytometer Settin	qs	
Import Cytometer Setting		
Share Experiment	-	
Marc Elivate		
Export	•	Experiments
	_	FCS files
	_	Experiment Template

2. Create a new folder and rename, then click **Export** \rightarrow **OK**

Export						Export Experiments	
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My Computer	File name:	D:\USER\Anatomy\Emily Pang\today		Export	1		OK Cancel
Places	Files of type:	Only Directories		Cancer			

3. To save FCS files, right click \rightarrow **Specimen** \rightarrow **Export** \rightarrow **FCS Files**



4. Select FCS 3.0 \rightarrow OK \rightarrow Browse to choose folder destination.

Gated Events All Events File Version FICS2.		
Parameter	Parameter Type	Save Export
FSC-A	O Linear ○Log ○ None	- Directory Path
FSC-H	€ Linear └og ◯ None	uments and Settings(Administrator(Desktop)user
FSC-W	O Linear ○Log ○ None	
SSC-A	G Linear ◯ Log ◯ None	Save Details>> Cancel
SSC-H	O Linear ○Log ○ None	
ssc-w	⊙ Linear ◯ Log ◯ None	
FITC-A	G Linear ○Log ○ None	
FITC-H	O Linear ○Log ○ None	
PE-A	O Linear ○Log ○ None	
PE-H	O Linear ○Log ○ None	
APC-A	G Linear ○ Log ○ None	
APC-H	🕢 Linear 🔤 🔛	

5. Create a new folder and rename \rightarrow Choose Directory and save the file.

Anatomy Biochemistr medicine Pathology SCRMC		
e name:	Categometry and Settinosi Administrator/Desktooluser	Choose Directory
		Cancel
	name: s of type:	

6. Delete experiment after having FCS data / experiment exported.

9. Log Out

1. To log out of FACSDiva software, go to **File** \rightarrow **Log Out**

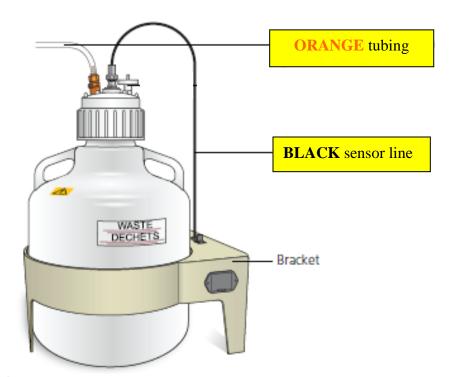
File	Edit	View	Experiment
- P	Sav	Ctrl+5	
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Empty Waste Tank Procedures

1. Make sure the flow cytometer is in **Standby** mode.



- 2. Diva Software \rightarrow "Cytometer" \rightarrow "Standby".
- 3. Disconnect the **ORANGE** tubing and **BLACK** sensor line from the waste container.



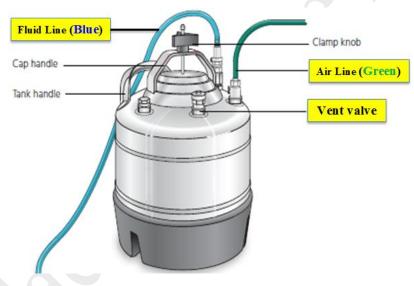
- 4. Take waste tank to the sink \rightarrow Remove the lid \rightarrow Empty the waste tank.
- 5. Add about 0.5L of bleach into the waste tank \rightarrow Place the lid back to the waste tank.
- 6. Reconnect the **ORANGE** tubing and **BLACK** sensor line to the waste tank.

Refill Sheath Container Procedures

1. Make sure the flow cytometer is in **Standby** mode.



- 2. Disconnect Diva Software: "Cytometer" → "Standby".
- 3. Disconnect the Air Line (Green) and Fluid Line (Blue) from the sheath tank.



- 4. Depressurize the sheath tank by pulling up the **vent valve**.
- Unscrew the clamp knob and sheath tank → push down to loosen the lid → Tilt the lid to the side to remove it from the tank.
- 6. Refill the sheath fluid using an air pump until the maximum level.
- 7. Tilt the lid and close the sheath tank by tightening the clamp knob to finger-tight.
- 8. Reconnect the Air Line (Green) and Fluid Line (Blue).
- 9. Release the bubbles that trap in the filter if necessary.
- 10.Reconnect Diva Software: "Cytometer" → "Connect".