BD FACSymphony A5 SE Standard Operation Protocol

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Basic Operation – Tube mode

A. Log in the Window

- 1. Press Ctrl+Alt+Delete
- 2. Key in Password BDIS#2\$\$

*<mark>No need to switch user</mark>

B. Log in the BD FACSDiva Software

1. Key in user name and password and click OK to log in.

*Please contact the Technical Staff in charge if you do not have an account. **Please contact the Technical Staff in charge if you forget password.

< ⊖	B	D	-
User Name:		User Name	~
Password:	9	XXXXXXXXXX	
		OK	Quit

2. Click Use CST Settings when pop-up message as below is shown.

CST Mismate	h	
Do you wa	gs from CST are different int to use the CST values how this message again f iber my decision.	
Details>>	Use CST Settings	Keep Current Settings

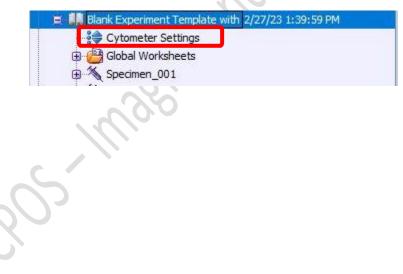
C. <u>General Experiment Setup</u>

1. Setup New Experiment

1.1 Go to Browser, Click **New Experiment** icon. A blank experiment template will be created.

			[
Name	D	ate	
🖏 Ocleaning			
🕀 📳 Blank Experiment Template with	8/23/22 3:11:	46 PM	
🗊 📔 Blank Experiment Template with	9/20/22 2:55:	04 PM	
😟 📔 Blank Experiment Template with	10/11/22 3:20	:21 PM	
🕀 📔 CST	1/13/23 8:55:	43 AM	
🕀 🔚 Training	12/29/22 3:04	1:36 PM	$\langle \rangle$
🕀 📔 Blank Experiment Template with	1/18/23 3:22:	21 PM	\sim
🕀 📔 Blank Experiment Template with	2/21/23 10:47	7:29 AM	9
🕀 📔 Blank Experiment Template with	2/21/23 11:52	2:55 AM	P
🕀 🔚 Blank Experiment Template with	2/21/23 3:07:	24 PM	
🕀 📔 Blank Experiment Template with	2/23/23 11:45	5:30 AM	
🗉 🛄 Blank Experiment Template with	2/27/23 1:39:	59 PM	

1.2 Click Cytometer Settings under the newly created Experiment



1.3 Go to Inspector Window, select **unwanted** channels and click **Delete**

		Settings		
arar	mete	rs Threshold Ratio Co	ompensation	
		Parameter	Voltage	sur un un
•		FSC	530	
•		SSC	280	
• E	3537	B537	540	
+ Y	·	YG585	690	
e Y	(YG670	630	
•		YG695	570	
•	6	YG730	570	
•	ſ	YG750 🗸	690	
e)		YG780	540	
• 1		YG825	630	
e F	ε	R660	690	
• F	ł	R675	600	
e F	ł	R680	600	
11.04	<u></u>	R710	570	
e F	ł	R730	540	
e F	ł	R780	480	

1.4 Check **H** and **W** boxes of FSC and SSC

ytom	eter	Set	tings							
Para	mete	ers	Thresho	bld	Ratio	Comp	ensatio	n		
		Pa	rameter	٧	oltage	Log	А	н	W	
•		FSC	2	53	30					1
		SSC	1	28	80					
+ 1	B537	B53	17	54	ю					1
•	Y	YGS	585	69	90					
	Y	YGE	570	63	30					1
	Y	YG	780	54	ю					

1.5 Keep Log boxes of FSC and SSC unchecked

1.6 Keep Log boxes of all fluorescence channels checked

* If you are doing <u>cell cycle</u> or <u>DNA content</u> analysis, please keep log box of your DNA specific fluorescence channel unchecked.

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2. Setup Compensation (for Multi-color panel)

2.1 Click *Experiment* > *Compensation Setup* > *Create Compensation Control*

Expe	riment Populations Works	heet Cyto	ometer HTS H	Help	
e i	New Folder	Ctrl+N			
F	New Experiment	Ctrl+E	g 🔀	Cytometer - LSRFortessa	
×	New Specimen	Ctrl+M	- <u> </u>		
1	New Tube	Ctrl+T		Status	.0
;¢°,	New Cytometer Settings			Time Event	
	Import Cytometer Settings		e		(\mathcal{O})
F	New Global Worksheet		:46 PM		
	New Plate	Ctrl+K	:04 PM		
	Open Experiment	Ctrl+0	0:21 PM		
	Close Experiment	Ctrl+W	:43 AM 4:36 PM		6
	close experiment	car. n	:21 PM		
	Experiment Layout		15 PM		
	Compensation Setup	;	Create C	Compensation Controls	
Glo	bal Worksheets		Modify	Compensation Controls	
Spe	ecimen_001		Calculat	e Compensation	
ed Viev	N		Calculat	e Spectral Unmixing	

2.2 If any one of your single stain controls is known to be 100% positive, i.e. no negative population, check the box "Include separate unstained control tube/well". Label the detectors and click "OK".

	Detector	Label
B537		FITC
YG585		PE
YG670		PE Cy5
YG780		PE Cy7
9790		PE Cyr

2.4 Expand the Compensation Control Specimen

	🕀 🔚 Blank Experiment Template with 2/23/23 11:45:30 Al	М
	Blank Experiment Template with 2/27/23 1:39:59 PM	
	🖶 🚰 Global Worksheets	
	🕀 🌂 Specimen_001	
	💼 🌂 Compensation Controls	
	🕞 🌂 Compensation Controls	C
	🖶 🧃 Unstained Control	
	i FITC B537 Stained Contr	
	i PE YG585 Stained Contro	
	i PE Cy5 YG670 Stained C	
	进 🧻 PE Cy7 YG780 Stained C	
Click t	the tube pointer of the first tube	$'O_{j}$

ole of the

	🚊 📉 Compensation Controls	
	Cytometer Settings	
	🖶 🧻 Unstained Control	
	🇉 🐧 FITC B537 Stained Contr	
	🖶 🧻 PE YG585 Stained Contro	
	i PE Cy5 YG670 Stained C	
D	🛓 🥛 PE Cy7 YG780 Stained C	

2.5

2.6 Load the single stain controls on the sample stage according to the tube label, i.e. run FITC single stain when the tube pointer is pointing at "FITC B537 Stained Control"

2.7 Go to Acquisition Dashboard, Click *Acquire Data*.

Current Activity						
Active Tube/Well		Threshold Ra	te	Stopping Gate Events		Elapsed Tim
Tu	be_001	0 evt/s	0 evt/s 0 evt			00:00:00
Basic Controls						
ojj Next	Tube	Acquire D	sta	Record Data		Restart
Acquisition Setup						
Stopping Gate:	All Even	ts 🛩	Events To Record:	10000 evt	~	Stopping Time (sec):
		5			~	

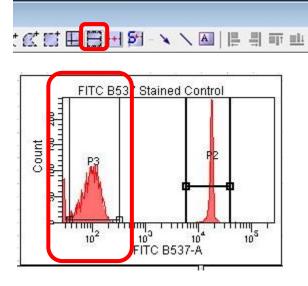
2.8 Go to **Normal Worksheet**, move the P1 gate to include major cluster. Adjust FSC and SSC PMT Voltage if needed.

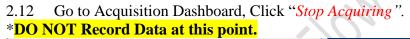
Sheet1	AutoF UV540 Stained Control	Unstained Control	FITC B537 Stained Control	PE YG585 Stained Control
	FITC B537 Sta	ined Control		
	2000) 2000) 2000)	*		
	SSC-A (K 1.000) 100 150 200 200 1 150 200 200 1 150 200 200	A. 4		
				h.
		OC A	,000)	X / I

2.9 Go to Cytometer window, fine tune the corresponding fluorescence PMT voltage to have best separation of negative and positive peak

	Parameter	Voltage	Log	Α	н	W	
	FSC	530				\square	1
	SSC	280					1
B537	B537	540					1
YG	YG585	690					1
YG	YG670	630					1
YG	YG780	540					1
							,
	8537 YG YG	FSC SSC B537 B537 YG YG585 YG YG670 YG YG780	FSC 530 SSC 280 B537 B537 540 YG YG585 690 YG YG670 630 YG YG780 540	FSC 530 SSC 280 B537 B537 540 Image: Comparison of the second	FSC 530 Image: Constraint of the sector	FSC 530 Image: Constraint of the system SSC 280 Image: Constraint of the system B537 B537 540 Image: Constraint of the system YG YG585 690 Image: Constraint of the system YG YG670 630 Image: Constraint of the system YG YG780 540 Image: Constraint of the system	FSC 530 Image: Constraint of the system Image: Constraint of the system SSC 280 Image: Constraint of the system Image: Constraint of the system Image: Constraint of the system B537 B537 540 Image: Constraint of the system B537 B537 540 Image: Constraint of the system YG YG670 630 Image: Constraint of the system YG YG780 540 Image: Constraint of the system YG YG780 540 Image: Constraint of the system YG YG780 540 Image: Constraint of the system YG YG YG <t< td=""></t<>

- 2.10 Move the interval gate (P2) to include the positive peak
- 2.11 Use interval gate to gate out negative peak (P3)





Current Activity							
Active Tube/Well	Thre	shold Rate	Stopping Gate Even	nts E	lapsed Time		
FULL	FULL 9 evt/s		0 evt		00:00:08		
Basic Controls							
🛛 🖓 Next Tube	Sto	p Acquiring	Record Data	🙆 Restart			
Acquisition Setup							
Stopping Gate:	All Events	Events To Record:	10000 evt	Stop Time (sec)):	0	
Storage Gate:	All Events	Events To Display:	5000 evt	$\overline{\mathbf{v}}$			
Acquisition Status							
Processed Events:	70 ev	t	Electronic Abort Rate:				
Threshold Count:	71 ev		Electronic Abort Count	. 16	evt		

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2.13 Repeat step 2.5 - 2.12 with all the single stain controls.

2.14 After optimizing the PMT voltage of ALL the fluorescence channels, load each single stained control and click *Record Data* for ALL single stain controls

2.15 Click *Experiment > Compensation Setup > Calculate Compensation*

File Edit View	Expe	riment Populations Works	heet Cyto	ometer HTS H	lelp
🔒 - 🔯 🏼 🗶	e i	New Folder	Ctrl+N		
👼 Browser - Bla		New Experiment	Ctrl+E	×	💌 Cytometer - LSRForte
	1	New Specimen	Ctrl+M		
	17	New Tube	Ctrl+T		Status
	:€	New Cytometer Settings			Time Event
		Import Cytometer Settings		e	
🖃 - 🦂 Oclea	1555	New Global Worksheet		:46 PM	
⊕- 🔚 B		New Plate	Ctrl+K	:04 PM	
⊕- (⊑ B		Open Experiment	Ctrl+0	0:21 PM :43 AM	
⊕- <u></u> ∎ T		Close Experiment	Ctrl+W	4:36 PM	
⊕- 🔚 B ⊕- 🗐 B		Experiment Layout		:21 PM 7:29 AM	
⊕- (⊒ 8		Compensation Setup	>	Create C	ompensation Controls
	Cyt	tometer Settings		Modify (Compensation Controls
		bal Worksheets		Calculate	e Compensation
⊕ 3		cimen_001		Calculate	e Spectral Unmixing

2.16 Click *Link and Save* for the most stringent practice, i.e. cannot adjust PMT voltage anymore OR Click *Apply Only* for some flexibility on PMT voltage adjustment of your samples.

	Compensation calculation has completed successfully
	Name: 202003191456
	Link & Save Apply Only Cancel
-	
7 Swite	ch <mark>Normal worksheet</mark> to <mark>Global worksheet</mark>
	ch Normal worksheet to Global worksheet

3. Spectral Unmixing Experiment Setup

* Do not delete any fluorescent parameters. Doing so will remove the ability to perform spectral unmixing.

3.1 Set up a new spectral experiment

3.1.1 Click *Experiment > New Experiment*

The Experiment Templates dialog is displayed. select the **Blank Experiment Template with**

eneral QC				
Name	Date	Name: Blank Experiment Template with 50P_Sat V		
lank Experiment				
lank Experiment Template with 50P_Sat V	7/15/22 3:53 PM			
ank Experiment Template with 50P_Sat V				
xperiment Template with 50P	5/11/22 9:35 AM			
ngrunying t cell function	11/4/22 5:28 PM			
ew Experiment Layout	10/13/22 1:10 PM			
T_04112022	11/4/22 3:46 PM			
aochun	11/14/22 4:21 PM			
me: eriment Template with 50P_Sat V		Copies: 1		

3.1.2 Click *Experiment > Experiment Layout* to assign parameter labels for each reagent to an individual primary detector.

*Typically, both the <u>dye</u> and <u>the antibody names</u> are added to the label, as shown in the following example for FITC and BB515 staining.

*To add an **autofluorescence** control to your experiment, assign the label "**AutoF**" to parameter <u>UV446</u>. If that parameter is already assigned to another fluorochrome in your experiment, select any of parameters <u>UV379–UV540 or V427–V540 for AutoF</u>.

per	iment Layout				
oels	Keywords Acquisition				
-					
	Name	Label	Label	Label	Label
•	Experiment_001				
•					
•	🔲 🥛 Tube_001	UV379	UV446 AutoF	UV515	UV540
	Qui Lat	Name Experiment_001 Specimen_001	Name Label Image: Specimen_001 Image: Specimen_001	vels Keywords Acquisition Quick Entry	Dels Keywords Acquisition Quick Entry

Note: The autofluorescence control is only supported in spectral workflows, not compensation workflows.

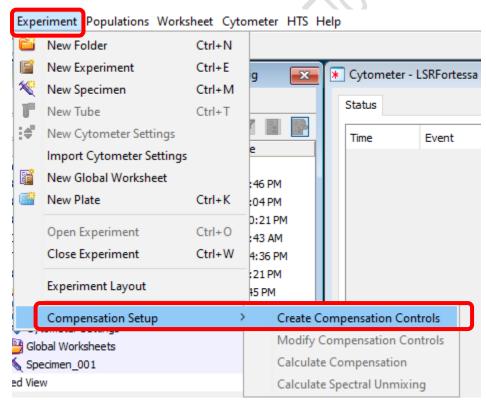
	ick Entry					
La	Label	Label	Label	Label	Label	Label
•						
•						
•	V750	V785	V845	B510 BB515	8537 FITC	B576
	eriment Layout	uisition				
Ou						
	abel 38515	~			MP 821010	
		Label	Label	Label	Label	Label
La	abel 38515	Label	Label	Label	Label	Label

Experiment Layout Labels Keywords Acquisition Quick Entry Labels Label BB515 ~ Name List by user Label Label Label Label Label Label Ocleaning 🗄 🖓 BD Defined e. 🗄 - Dingtao . V750 V785 V845 8510 88515 B537 8576 e. FITC Add to List Delete from List Assign or Remove Labels Assign Remove < > OK Cancel

Centre for PanorOmic Sciences – Imaging and Flow Cytometry Core

3.2 Setting up the Spectral Unmixing algorithm

3.2.1 select *Experiment > Compensation Setup > Create Compensation Controls* from the main menu.



3.2.2 Leave the checkbox Include Generic in its default (deselected) state.

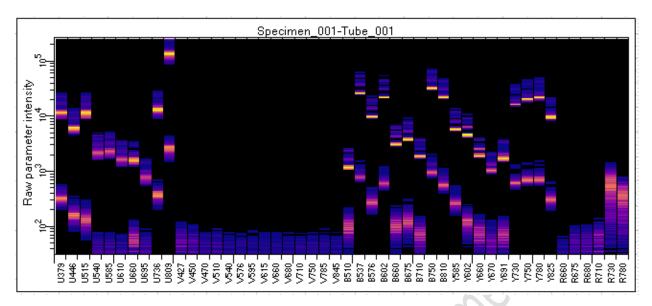
<i>S S</i>
c

3.2.3 Click OK to create the controls. If you assigned AutoF to a detector in the experiment layout, an AutoF control tube will be displayed from the Compensation Controls specimen from experiment Browser window.

3.2.4 Adjust the flow cytometer settings as needed for your experiment, including and PMT voltage and area scaling.

Note: For spectral experiments, create a spectral plot to assist with adjusting PMT voltage settings to ensure that the signal across all detectors is on-scale, not just the signal for the primary detector for each fluorochrome.





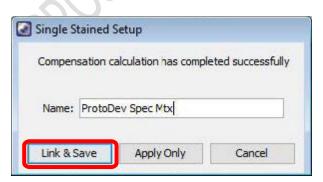
Note: The plot supports the display of specific populations or combinations of populations and displays live data during acquisition. The spectral plot does not support zoom, biexponential scaling, or gate drawing.

3.2.5 Record each of the single-color controls after voltage adjustments $by \pm 30$ according to the selected template after voltration.

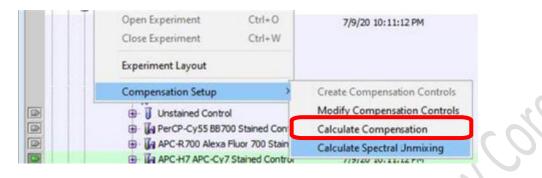
3.2.6 Select *Experiment > Compensation Setup > Calculate Spectral Unmixing*.

	Open Experiment Ctrl+O	7/9/20 10:11:12 PM
	Close Experiment Ctrl+W	
	Experiment Layout	
	Compensation Setup	Create Compensation Controls
	Unstained Control	Modify Compensation Controls
	PerCP-Cy55 BB700 Stained Cont	Calculate Compensation
	APC-R700 Alexa Fluor 700 Stain	Calculate Spectral Unmixing
0	APC-H7 APC-Cv7 Stained Control	1191-01-01-11-12-000

3.2.7 Click *Link and Save* to apply.



3.2.8 When the software calculates spectral unmixing, it also calculates <u>compensation</u> values (as if you had selected *Experiment* > *Compensation Setup* > *Calculate Compensation*).



<u>Compensation setup does not take autofluorescence into account</u>. By default, when you select spectral unmixing, Spectral is selected in the dropdown menu on the Compensation panel of the Cytometer window as shown in the following:

	pectral 🗸	Clear	
Detector	Fluorochrome	Spectral Overlap	
• U379	BB515		2
• U446	BB515	0.00	
+ U515	BB515	5.91	
• U540	BB515	2.82	
• U585	BB515	1.09	
• U610	BB515	0.55	
# U660	BB515	0.11	
• U695	BB515	0.00	
• U736	BB515	0.04	
• U809	BB515	0.00	
• V427	BB515	0.07	
• V450	BB515	0.03	
• V470	BB515	0.00	
 V510 	BB515	3.34	
• V540	BB515	2.03	
• V576	BB515	0.45	
• V595	BB515	0.26	
• V615	BB515	0.19	

You can elect to switch between <u>spectral unmixed</u> and traditional <u>compensation</u> values, by toggling between *Spectral* and *Compensated* in the dropdown menu, to compare the plot data in each case.

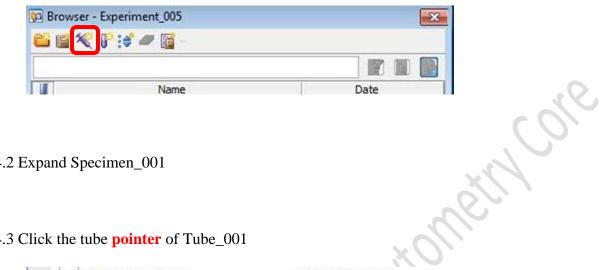
	Threshold Laser C	lear			
Fluorochrome	- % Fluorochrome				
V710	B537	0.11	~		
V750	B537	0.11			
V785	B537	0.07			
V845	B537	0.03			
B510	B537	61.39			
B576	B537	11.76			
B602	B537	22.30			
B660	B537	5.20			
B675	B537	1.51			
B710	B537	2.01			\sim
B750	B537	2.44			\sim
B810	B537	0.26			
YG585	B537	0.00		\sim	
YG602	B537	0.00			
YG660	B537	0.00			
YG670	B537	0.00			
YG695	B537	0.01			
YG730	B537	0.01	~		

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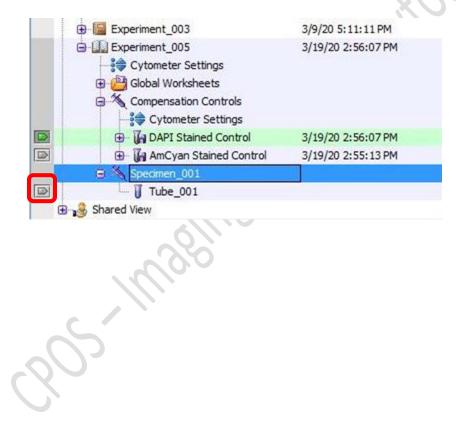
Cytometer Connected

4. Setup Plots and Tables

4.1 Go to Browser, Click New Specimen icon



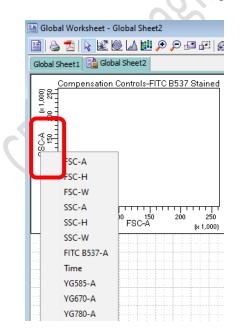
- 4.2 Expand Specimen_001
- 4.3 Click the tube **pointer** of Tube_001



🔛 Global Worksheet - Global Sheet2 🕊 🅲 🔎 🔎 🕮 🖉 👷 🥵 🖬 🖽 🖽 🛏 🗶 🔪 🕒 📕 🖶 🗮 🔛 🖉 🔍 🖉 🔡 🍐 🗾 Global Sheet1 Global Sheet2 Compensation Controls-FIT Compensation Controls-FITC B537 Stained Compensation Controls-FI FSC-N 1,000) 100 150 200 250 SSC-141,000) 100 150 200 250 250) x g SSC-A ജ 8 цип THE 50 100 1 ESC 150 200 250 50 100 1 SSC 150 C-A 200 250 (x 1,000) (x 1,000) S 50 200 100 150 FSC-A 250 (x 1,000) Туре Icon Dot Plot Contour Plot 6 Histogram A Spectral plot

4.4 Go to Global Sheet Window, Click the graph type icon

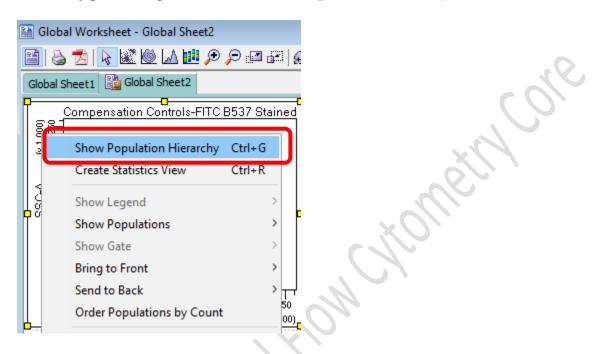
- 4.5 Click on the blank area of Global Worksheet window to create a new plot.
- 4.6 Mouse over the axis label and right click. Select the parameters of interest from the list.



4.7 Repeat step 4.4 - 4.6 until all plots needed is created.

* Essential Plots: FSC-A vs SSC-A; FSC-H vs FSC-W; SSC-H vs SSC-W

4.8 Click on any plot and right click. Click **Show Population Hierarchy**

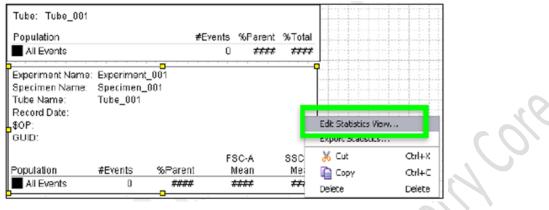


4.9 Click on any plot and right click. Click Create Statistics View

1	🚰 Glob	al Worksheet - Global Sheet2	
	::] 🎍	, 🔁 📐 🗟 🌋 🎯 🖾 📜 🔎	a 🗗 🖉
	Global S	Sheet1 🖺 Global Sheet2	
		Compensation Controls-FITC B53	7 Stained
	(k 1,000) 0 250	Show Population Hierarchy	Ctrl+G
	Ř.	Create Statistics View	Ctrl+R
	20A 150A	Show Legend	>
	စြီးရ	Show Populations	>
		Show Gate	>
		Bring to Front	>

4.10 Right Click on Statistics View table, select Edit Statistics View to select statistics of

interest to be shown in the table.



4.11 Click Statistics Tab, check the boxes of interested statistics and then click OK

Parameters	Al	Min	Max	Geo M	Mean	Median	50	- 150		□ %eCV	Mode
5C-A					V						
SC-H					V						
sc-w											
SC-A					v						
SC-H											
sc-w											
ITC-A					V						
E-A					2						
PC-A					V						
ime											
ecimal Places		0	0	0	0	0	0	0	1	t	D
			-	nt by Peren lav Range) Sort by Fo					

D. Sample Acquisition

1. Gently tab or pulse vortex your sample and put your sample tube on SIP (Sample injection port).

*Run the unstained/negative control sample before stained samples.

!! DO NOT return the support arm to the center without putting a tube filled with liquid on SIP!!

2. Press **RUN** and **LOW** on the fluidics control panel.



3. Go to Acquisition Dashboard, Click Acquire Data

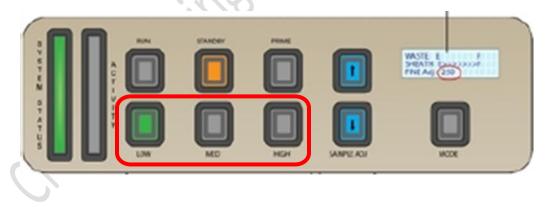
Active Tube/Well Tube_0	001	Threshold Ra	ite	Stopping Gate Ev O evt	ents	Elapsed Tim 00:00:00
Basic Controls		Acquire D	ata	Record Data		Restart
Acquisition Setup Ropping Gate:	All Events		Events To Record:	10000 evt	~	Stopping Time (sec):
Rorage Gate:	All Events	~	Events To Display:	1000 evt	~	

4. When Threshold rate > 0, go to Cytometry window and Click **Parameters** tab. Adjust PMT Voltage accordingly.

	Parameter	Voltage	
	FSC	351	- DVD
	SSC	222	
	88515	379	
	BB630	341	200
	B8660-P	260	
	88700-P	410	
•	88790-P	510	
	APC	370	V V V
	APC-R700	430	
	APC-H7	430	V / / /

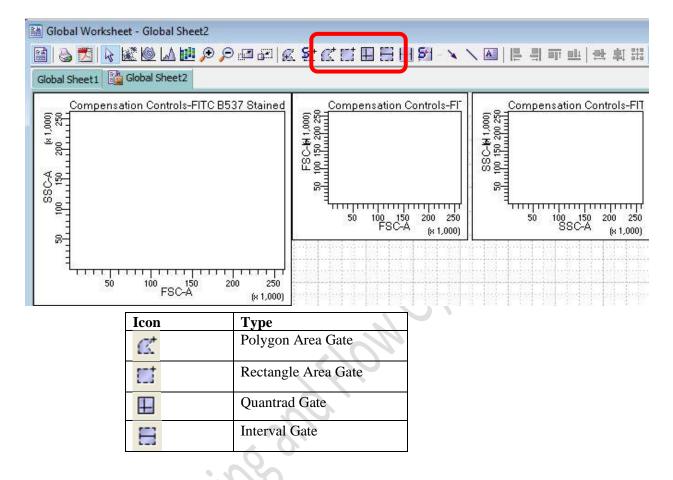
Channel	Suggested Voltage range for mammalian cells
FSC	400-700
SSC	250-310
Fluorescence	300-850

5. Adjust Sample flow rate on the fluidics control panel if needed (optimum Threshold rate 2000 – 5000 evt/s)

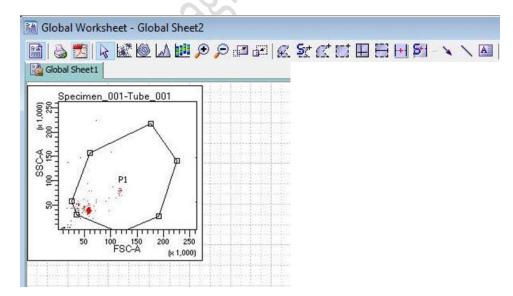


E. Create Gates

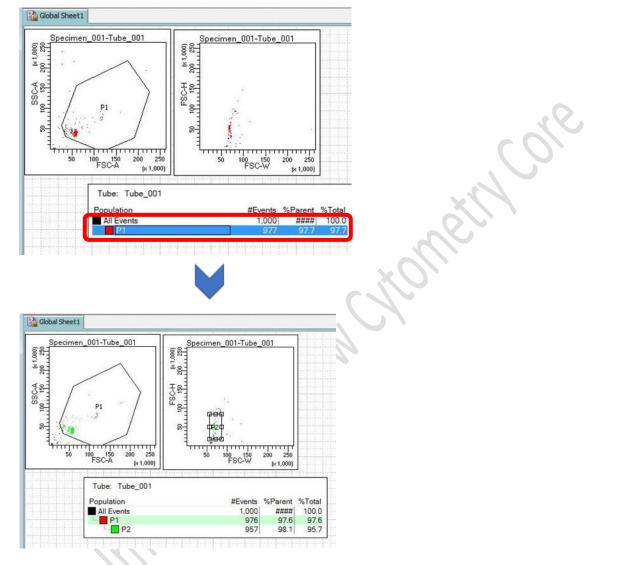
1. Go to Global Sheet Window, Click the type of gate needed



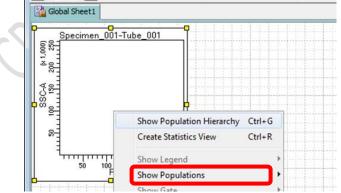
2. Draw the gate on the plot of interest to gate out target cluster /peak



3. If you want to create a new population out of particular parent population, highlight the parent gate on the hierarchy table first and then create the gate.



4. Click on target plot and right click, Click *Show Population* > *Target population* to visualize the target population ONLY in that plot.



F. Data Recording

1. Go to Acquisition Dashboard, set Stopping gate to singlet gate or live cell gate

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				
Stopping Gate:	P2 vents To Reco	ord: 20000 evt ~	Stopping Time (sec):	°‡†
Storage Gate:	All Events V Events To Displ	lay: 1000 evt 🗸	•	
Acquisition Status				
Acquisition Status Processed Events:		Electronic Abort Rate:		

2. Set the Storage gate to All Events

Current Activity Active Tube/Well	Thre	eshold Rate	Stopping Gate	Events	Elapsed Time	
Tube_0	01 0 e	vt/s	0 evt		00:00:00	
Basic Controls						
🛛 🗸 🖓 Next Tube	A	cquire Data	Record Data	Resta	rt	
Acquisition Setup Stopping Gate:	□ P2	Events To Reco	ord: 20000 evt	✓ Stopping	Time (sec):	0 🋱 t
Storage Gate:	All Events	Events To Disp	lay: 1000 evt	~		
Acquisition Status						
Processed Events:			Electronic Abort	Rate:		
Threshold Count:			Electronic Abort	-		

3. Set Events To Record, i.e. events number out of stopping gate to be recorded

Acquisition Dashbo	ard						×
Current Activity Active Tube/Well	ТІ	hreshold Rate		Stopping Gate Ev	ents	Elapsed Time	
Tube_0	01 0	evt/s		0 evt		00:00:00	
Basic Controls							
¢نَآ Next Tube		Acquire Data	Re	cord Data	Restar	t	
Acquisition Setup Stopping Gate:	□ P2	Events To I	Record:	20000 evt	Sppping	Time (sec):	0 ‡ †
Storage Gate:	All Events	✓ Events To I	Display:	1000 evt	~		
Acquisition Status							
Processed Events:				Electronic Abort Rat	e:		
Threshold Count:				Electronic Abort Cou	unt:		

4. Click Record Data

Acquisition Dash	board					×
Current Activity Active Tube/Well		Threshold	Rate	Stopping Gate E	vents	Elapsed Time
Tube_	001	0 evt/s		0 evt		00:00:00
Basic Controls						
🖓 Next Tub	e	Acquire D	ata 📕	Record Data		Restart
Acquisition Setup						
Stopping Gate:	2 P2	~	Events To Record:	20000 evt	~	Stopping Time (sec): 0
Storage Gate:	All Even	nts ~	Events To Display:	1000 evt	~]
Acquisition Status						
Processed Events:				Electronic Abort Ra	ate:	
Threshold Count:				Electronic Abort Co	ount:	

5. Click **Next Tube** to create a new sample

II: Acquisition Dashbo	ard						×
Current Activity							
Active Tube/Well		Threshold	Rate	Stopping Gate E	vents	Elapsed Time	
Tube_0	01	0 evt/s		0 evt		00:00:00	
Basic Controls							
¢آن Next Tube		Acquire D	lata 📕	Record Data	(Restart	
Acquisition Setup							
Stopping Gate:	P2	~	Events To Record:	20000 evt	~	Stopping Time (sec):	0 🗘 🕇
Storage Gate:	All Even	ts v	Events To Display:	1000 evt	~		
Acquisition Status							
Processed Events:				Electronic Abort R	ate:		
Threshold Count:				Electronic Abort C	ount:		
20							

H. <u>Data Export</u>

- 1. FCS file
- 1.1 Go to Browser window, Select the Tubes / Specimen of interest.
- 1.2 Right Click over the selection and click *Export* > *FCS file*

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🕀 📒 Blank Experiment Ter	-		Cui+v		J
🕀 📒 Blank Experiment Ter		Paste With Data			
🕀 🍰 Shared View		Delete	Delete		
		Apply Panel Analysis			
		Rename			
		Kename			
		Duplicate Without Data	Ctrl+D		
		Batch Analysis			
		Create Specimen Reports			
	1 ¹⁰	New Tube	Ctrl+T		
	:4	New Cytometer Settings			
		Import Cytometer Settings			
		, , <u>,</u>			
Acquisition Dashboard		Export	>	FCS files	
Current Activity				Panel Template	

1.3 Select FCS 3.0 and keep all parameters Linear. Click OK

Gated Events		
File Version	FCS3.1	
Parameter	Parameter Type	2
		^
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FSC-W	🔘 Linear 🕐 Log 🛞 None	
SSC-A	🔘 Linear 💮 Log 🔘 None	
ssсн	🕘 Linear 💿 Log 💿 None	
SSC-W	🖲 Linear 🔵 Log 🔘 None	
FITC-A	🖲 Linear 💿 Log 💿 None	
DAPI-A	🕘 Linear 💿 Log 💿 None	
APC-Cy7-A	🖲 Linear 💮 Log 🔘 None	
PE-Cy7-A	🕘 Linear 🕘 Log 🔘 None	
Alexa Fluor 700-A	🕘 Linear 🕐 Log 🕐 None	
Time	() Linear () Log () None	

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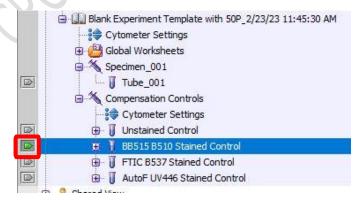
1.4 Click **Browse** to choose the destination (D:/User/Department/PersonalFolder)

1.5 Click Save

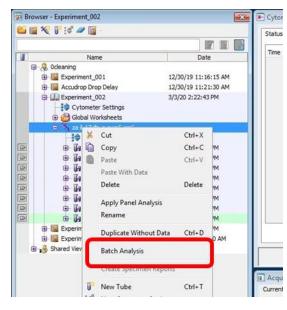
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, loser lo	Citit		Drowse
•			
	Save	Details>>	Cancel

2. PDF file

2.1 To export pdf of multiple tubes, Click the tube pointer of any tubes or select the wanted Specimen



2.2 Right Click over the selection and click **Batch Analysis**



2.3 Check the boxes as picture below

G Good	WORKSHICE LS								
C 201	Cut Copy Paste Paste With Da Delete Apply Panel A Rename Duplicate Wit Batch Analysi Create Specin New Tube	Analysis hout Data (s hear Reports	Ctrl+X Ctrl+C M M M M M Ctrl+D Ctrl+T Ctrl+T		B Acqu Current			er er	519
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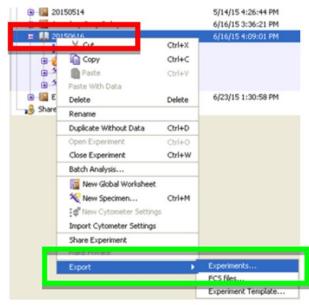
2.4 Click **Browse** to choose the destination (D:/User/Department/PersonalFolder)

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2.5 Click **Start** and then **OK**.

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- 3. Experiment
- 3.1 Right click on the **Experiment > Export > Experiments**



3.2 Click **Browse** to select the file destination

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	Delete experime Oirectory Export	nts after export	
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Experime	nt	Date	
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		ОК	Cancel

3.3 Select the file destination and click Export.



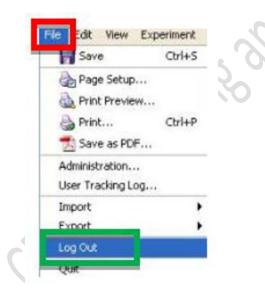
ometrycore

3.4 Click **OK** to export

	 Delete experiment Directory Export 			
Directory:	D:\USER\Anatomy\E	Emily Pang\today	Browse	
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I. <u>Software Log out</u>

1. To log out of FACSDiva software, go to File and click Log Out.



J. System Cleaning

*Cleaning procedure will not interfere with the data export and analysis.

1. Prepare 3ml of each cleaning solution (Solution1: FACSClean; Solution 2: FACSRinse; Solution 3: MilliQ water)

- 2. Press **HI** and **RUN** on fluidics control panel
- 3. Load the tube with Solution 1 on the SIP with the support arm on the side for 1 minute
- 4. Move the support arm under the tube and run for another 4 minutes
- 5. Repeat step 3 and 4 with Solution 2 and Solution 3

*If PI stain is used, please clean the system 9 minutes instead of 4 for Solution 2

6. After cleaning with Solution 3, press LOW and Standby

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