

CANTO REFERENCE GUIDE

IF THE MACHINE IS OFF OR YOU ARE THE FIRST USER OF THE DAY

- 1. The computer should be on.
 - a. To log into the PC: Username: BDAdmin Password: BDIS
- 2. Unlock the screen with your PPMS Account.
- 3. Launch Tera Term.



4. Click on Serial, make sure the port is on COM1, and click OK.

📒 Tera	a Term - [disconnected] VT					23
File E	Edit Set	up Control Wi	ndow Help				
		Tera Term: New c	onnection		— ×		
		© <u>T</u> CP/IP	H <u>o</u> st; 192.168.	20.57	-		
			TCP port#: 23	Protoco <u>l</u> ; L	JNSPEC -		
			√ T <u>e</u> lnet				
		Serial		ommunications Port			
			OK COM23:	ommunications Port USB Serial Port (CO Standard Serial ove Standard Serial ove	M23J r Bluetooth lin		
					Blactoour in	in (001130)	Ŧ

- 5. Turn on the cytometer by pressing the green button on the side of the instrument.
- 6. Turn on <u>DIVA Software</u> and log in using the credentials you received after training.





- 7. Check the sheath and waste tank.
 - **a.** If you are emptying waste, add a layer of bleach to the bottom of the container before placing it back in its designated space.
- 8. Perform Fluidics Startup:
- 9. Prepare the cleaning carousel:
 - **a.** Place a tube of bleach in the first position.
 - **b.** Place a tube of Contrad in the second position.
 - c. Place a tube of shut down solution in the third position.
- **10.** Once fluidics startup has finished, insert the carousel, and go to <u>Carousel</u> \rightarrow <u>Clean</u>.

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- File Edit View Experiment Populations Worksheet Cytometer Carousel HTS Help
- a. Select 10 minutes for each tube.

Clean with card	ousel tubes
Selections and S	ettings
Tubes	Time (min)
🗹 Cleaning	10 ~
🗹 Rinse 1	10 ~
🗹 Rinse 2	10 ~
ОК	Cancel

11. After cleaning, go to <u>Cytometer</u> \rightarrow <u>CST</u>.

BD FACSDiva Software - Administrator (Current 1	
File Edit View Experiment Populations Workshe	eet Cytometer Sort Help
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Rrowser - Experiment 001	

- Take a clean FACS tube and add two drops of CST beads with ~ 300uL of FACS flow.
- **13.** Verify that the lot number on the side of the bead's container matches the one on the screen.



Setup Bead	s		
Lot ID:	20297 (RUO)		2
Product:		CST Setup Beads	
Part #:		910858	
Expiration	Date:	02-29-2024	

- 14. Take out the cleaning carousel and replace the tube in position 1 with the CST tube.
- 15. Press Run on setup control.

Setup Control						
Load a tube with beads and click Run button to start setup.						
Characterize: Check Performance						
🕞 Run 🔘 Abort						

- 16. Wait for CST to pass.
 - **a.** If it fails, try the following:
 - i. Re-run CST.
 - ii. Make new CST beads and re-run CST.
 - iii. Run another cleaning cycle, then re-run CST.
 - iv. Shut down the cytometer, restart the computer, and run CST.

ANY USER OF THE DAY

- 1. Log into PPMS and your DIVA account.
- 2. Prepare the cleaning carousel:
 - a. Place a tube of bleach in the first position.
 - b. Place a tube of Contrad in the second position.
 - c. Place a tube of shutdown solution in the third position.
- 3. Insert the carousel and go to <u>Carousel</u> \rightarrow <u>Clean</u>.

BD FACSDiva Software - Administrator (Canto RUO Special Order 10-color (5B-3R-2V))

File Edit View Experiment Populations Worksheet Cytometer Carousel HTS Help

a. Select two minutes for each tube, click OK.



FOR A NEW EXPERIMENT

1. Select Experiment → New Experiment → Blank Experiment.

BD FACSDiva Software - Administrator (Current 100-20 UT_SW_Aria 2B-3R-3V-2UV-5YG)

File	Edit	View	Experiment	Populations	Worksheet	Cytometer	Sort	Help
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	Brown	er - Fv	periment 00	1				

Experiment Templates	
General Automation	

4/7/21 11:49 PM 2/6/20 2:32 PM	^	
2/6/20 2:32 PM		
1/20/22 6:38 PM		
10/14/20 4:29 PM		
8/8/22 6:08 PM		
10/20/20 8:59 PM		
1/20/22 7:02 PM		
8/31/20 7:18 PM		
8/30/21 7:30 PM		
7/11/22 8:06 PM	v	
	10/14/20 4:29 PM 8/8/22 6:08 PM 10/20/20 8:59 PM 1/20/22 7:02 PM 8/31/20 7:18 PM 8/30/21 7:30 PM	10/14/20 4:29 PM 8/8/22 6:08 PM 10/20/20 8:59 PM 1/20/22 7:02 PM 8/31/20 7:18 PM 8/30/21 7:30 PM

2. Click on the <u>Parameters</u> tab in the cytometer window.

🔭 Cytometer - FACSAriallI (1)	23
Status Parameters Threshold Laser Compensation Ratio	

3. Delete all fluorochromes and add the fluorochromes you will be working with.

Add Delete		×
Add Delete		
	Add	Delete

4. Create a new specimen.

		Experiment_001
6 f	×	(* :\$ # 🍯 -



5. Expand the specimen and place the acquisition pointer on Tube_001.

The second		
🖨 🏭 Experiment_002	9/2/22 2:43:09 PM	
Cytometer Settings		
🕀 📴 Global Worksheets		
Specimen_001		
🕞 👘 🗍 Tube_001		
🗄 🥵 Shared View		

CALCULATING COMPENSATION:

1. Select Experiment \rightarrow Compensation Setup \rightarrow Create.

ب	rowser - Experiment_001				×
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			and a	0.00	
J	Name	Date			
	🗐 🧏 Administrator				
	🖹 🛄 Experiment_001	8/26/22 8:50:44 AM			
	🕀 📴 Global Worksheets				
	🕀 🚿 Specimen_001				
	🗈 🍰 Shared View				

a. Verify compensation controls coincide with your chosen fluorochromes, then press <u>OK</u>.

Create Compensation Controls						
 Tubes 						
Include separate unstained control tube/w	Label					
Fluorophore	Generic					
• Alexa Fluor 430	Generic					
• Alexa Fluor 488	Generic					
e PE	Generic					
• PE-Alexa 610	Generic					
• PE-Cy5	Generic					
• PE-Cy7	Generic					
 Alexa Fluor 647 	Generic					
 Alexa Fluor 680 	Generic	~				
Add Delete Labels	OK Cancel					



2. Open the Compensation Controls specimen in your experiment.

J	Name	Date				
	🗐 🖓 Administrator					
		8/26/22 8:50:44 AM				
	🕀 🚰 Global Worksheets					
	🕀 🔨 Specimen_001					
[🚊 🌂 Compensation Controls					
	👾 💝 Cytometer Settings					
	🕀 🧃 Unstained Control					
	🕀 🧻 🗍 Alexa Fluor 405 Stained Control					
	🕀 🧻 🗍 Alexa Fluor 430 Stained Control					
	Alexa Fluor 488 Stained Control					

3. Make sure the tube pointer is on your unstained control.

Name	Date
🗐 🖓 Administrator	
🖮 💷 Experiment_001	8/26/22 8:50:44 AM
😪 😌 Cytometer Settings	
🕀 📇 Global Worksheets	
🕀 🖄 Specimen_001	
🚊 🌂 Compensation Controls	
🖓 Cytometer Settings	
🕀 🧻 Unstained Control	
🕀 🧻 🗍 Alexa Fluor 405 Stained Control	
🕀 🧻 Alexa Fluor 430 Stained Control	
🕀 🧻 Alexa Fluor 488 Stained Control	

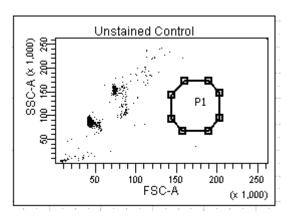
4. Load your unstained tube and hit <u>Acquire Data</u>.

🔢 Acquisition Dashboard				×		
Current Activity						
Active Tube/Well	Threshold Rate	Stopping Ga	ate Events Elaps	sed Time		
Unstained Contro	0 evt/s	0 evt	00:0	00:00		
Basic Controls						
🛛 🖥 Next Tube	🔞 Remove Tube	Acquire Data	Record Data	Restart		
Carousel Controls						
🚱 Run Carousel	🐌 Run Single 1	Tube 👘 Mix	🏞 Skip 🚦	R P		
Acquisition Setup						

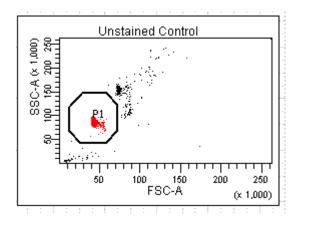


🖹 Cytometer - FACSAriallI (1)								
Status Parameters Threshold Laser Compensation Ratio								
	Parameter	Voltage	Log	A	н	w		
•	FSC	566		~			^	
	SSC	278		~				

5. Adjust the FSC and SSC voltage until your cells are on-scale in the plot.

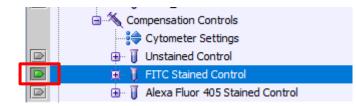


- 6. Stop acquiring.
- 7. Move the P1 gate until it encompasses the population of interest.



- **8.** Right-click the P1 gate \rightarrow apply to all compensation controls.
- 9. Acquire data again and <u>Record</u>.
- 10. Unload your unstained tube.
- 11. Load your first compensation tube.
- **12.** Make sure that your tube pointer is on the correct tube.





13. Click on the next worksheet panel.

Mormal Worksheet - FITC Stained Control										
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Sheet1	Sheet1 Unstained Control 🔠 FITC Stained Control Alexa Fluor 405 Stained Control									
		ned Control			8.,	FITC Stai	ined Con	trol		_
SSC-A & 1.0	200 250 200 250 200 250 200 250	P1 P1 F1 F50 FSC-A	200 250 (× 1,000)		Count 0 10 20 30 40 50 50 70 80 90 100		FITC-A	P2	ן יייייין וס ^ג	

- 14. Click on your histogram plot.
- 15. Click the X-axis <u>Biexponential Display</u> in the inspector window.

ρ	Insp	ector	- Histog	gram			
1	Plot	Title	Labels	Acquisition	Histogram		
	Tube:						:xpe FITC
		X Axis		lay			
	Plo	Y Axis					



16. Click Acquire Data.

Acquisition Dashboard	d			×		
Current Activity						
Active Tube/Well Unstained Control	Threshold Rate 0 evt/s	Stopping G 0 evt		osed Time 00:00		
Basic Controls						
🛛 🖥 Next Tube	🔞 Remove Tube	Acquire Data	Record Data	Restart		
Carousel Controls						
🚱 Run Carousel	🐌 Run Single	Tube 👘 Mix	🏞 Skip 🚦	9 R		
Acquisition Setup						

17. Adjust the voltage of the fluorochrome until the negative population is close to zero.

Cytometer - FACSCanto Status Lifter Parameters	(V07300503)	aser Com	nensa
Status Linter Fordineters		aser [Con	pense
Parameter		Voltage	Lo
 FSC 		170	
 SSC 		352	
 FITC 		467	
 Alexa Fluor 405 		364	
FITC Stained C	P2	10 ⁵	

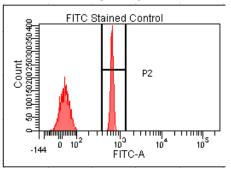
a. It's important to make sure you can see both ends of your population. If it bleeds into the side of the graph, increase the voltage until you can see the bottom left of the peak.

18. Click Record Data

III Acquisition Dashboa	rd				×	
Current Activity						
Active Tube/Well	Threshold Rate	Stopping (Gate Events	Elaps	ed Time	
FITC Stained Cor	trol 0 evt/s	5000 ev	rt	00:0	0:06	
Basic Controls						
🛛 🖓 Next Tube	🔞 Remove Tube	📕 Acquire Data	Record I	Data	Restart	
Carousal Controls				_		



19. Move the histogram gate until it surrounds your positive population.



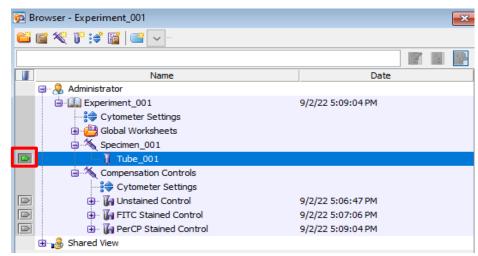
- 20. Repeat steps 10-17 until all your compensation tubes have been completed.
- 21. Select Experiment → Compensation Setup → Calculate Compensation → Apply Only.

BD FACSDiva Software - Administrator (Canto RUO Special Order 10-color (5B-3R-2V)) File Edit View Experiment Populations Worksheet Cytometer Carousel HTS Help

	Single Stained Setup						
	Compensation calculation has completed						
ľ							
	Warningspectral overlap over 100%!						
L	FITC Stained Control: spectral overlap of FITC in PerCP						
	v						
	Name: 202209021709						
	Link & Save Apply Only Cancel						

ACQUISITION

1. Place the Acquisition Pointer on Tube_001.





2. Create a new global worksheet.



3. Create your graphs, dot plots, and gates to run your experiment.



- a. Hover over each item in the toolbar for more information.
- **b.** Once you have your graphs set up, right-click one and press <u>Show</u> <u>Population Hierarchy.</u>

Tube: Tube_001			
Population	#Events	%Parent	%Total
All Events	0	####	####
- P1	0	####	####
	0	####	####
	0	####	####
P4	0	####	####

4. Add additional tubes to the experiment, if needed.

📴 Browser	📴 Browser - Experiment_001							
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5. Select Experiment \rightarrow Experiment Layout.

BD FACSDiva Software - Administrator (Canto RUO Special Order 10-color (5B-3R-2V)) File Edit View Experiment Populations Worksheet Cytometer Carousel HTS Help

uick Entry abel			
Name	Label	Label	
Experiment_001			
-× Specimen_001			
↓ Tube_001	FITC	PerCP	
Compensation Controls			
🔤 🥡 Unstained Control	FITC	PerCP	
FITC Stained Control	FITC	PerCP	
PerCP Stained Control	FITC	PerCP	



- 6. In the Labels tab:
 - **a.** Change the names of the tubes and the axis labels.
 - **b.** Highlight the name you want to change and press the Shift button.
- 7. In the Keywords tab, Add Patient ID and Sample ID.

Experiment Layout	
Labels Keywords Acquisition	
Quick Entry	
Value	System Defined Keywords

- 8. In the Acquisition tab:
 - a. Change the number of events to record/acquire.
- 9. Run a fully stained sample and set up your gates.
 - a. Check to make sure the gates are collecting the desired populations.

IF USING SINGLE TUBES

- 1. Make sure the probe is in "single tube" mode.
 - a. Move the tube guide to the left.
 - **b.** Place the probe in the upward position.
- 2. Run tube.
- 3. Record data.

IF USING CAROUSEL

1. Select <u>Carousel</u> \rightarrow <u>Carousel Setup</u>.

BD FACSDiva Software - Administrator (Canto RUO Special Order 10-color (5B-3R-2V))

File	Edit	View	Experiment	Populations	Worksheet	Cytometer	Carousel	HTS	Help
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	ousel Setup	E			-	_		
	anment							
Assign a carousel number for each specimen. To create multiple carousels, select a row, dick 'New Carousel', and select the carousel number from the drop-down list. To combine carousels, change the carousel number to 'None', select the break line, and dick 'Delete Carousel Break'.								
	Specimen	# of Tubes	ubes Location Sta		us Carousel ID	Export Folder		
		4	1-1	1	None	🔯 Undefined		
1	Specimen_001	1						
1 2	Specimen_001 Compensation Co	3	2 - 4		None	Didefined		



2. Under the <u>Carousel ID</u> tab, select the carousel number.

a re	sign a carousel numb ow, click 'New Carou	sel', and select	the carouse	l number f	from the drop-de	own list.
	combine carousels, o d click 'Delete Carous		r to 'None'	, select the bre	ak line,	
	Specimen	# of Tubes	Location	Status	Carousel ID	Export Folder
1	Specimen_001	1	1 - 1		5	🗊 Undefined
2	Compensation Co	. 3	2 - 4		5	💯 Undefined
~						
-						•
-						-
						-
2						-
2						-

3. Select the appropriate settings and press <u>OK</u> when done.

Run Settings	
Recording Delay time	Mix Settings
Seconds 3 \sim	Start of carousel mix
	Interim mix: After every 4 \sim tubes
Tube Pressurization Error Ha	indling (Current Run)
◯ Show error and wait	
Skip to next tube	
Skip to next specimen	

- 4. Make sure the probe is in <u>Carousel mode</u> when using the carousel.
 - a. Ensure the tube guide is on.
 - **b.** Place the probe in the downward position.

5. Click on Run Carousel.

Acquisition Dashboar	rd				X
Current Activity					
Active Tube/Well	Threshold Rate	Stopping G	ate Events	Elapsed Tim	e
Tube_001	0 evt/s	0 evt		00:00:00	
Basic Controls					
≫ IJ Next Tube	🔞 Remove Tube	Acquire Data	Record Da	ata	Restart
Carousel Controls					
🚱 Run Carousel	🐌 Run Single T	ube 😗 Mix	î Skip	B R	• P
Acquisition Setup					



EXPORTING DATA

- 1. BioHPC
- 2. Cytobank

a.

***NOTE:** The flow core deletes experiments off DIVA on the first of every month. They are stored in a cloud backup. Please make sure that you are saving your experiments after every session.*

CLEANING BETWEEN USERS

- 1. Prepare the cleaning carousel:
 - **a.** Place a tube of bleach in the first position.
 - **b.** Place a tube of Contrad in the second position.
 - c. Place a tube of shut down solution in the third position.
- 2. Insert the carousel and go to <u>Carousel</u> \rightarrow <u>Clean</u>.
 - BD FACSDiva Software Administrator (Canto RUO Special Order 10-color (5B-3R-2V))
 - File Edit View Experiment Populations Worksheet Cytometer Carousel HTS Help
 - b. Select 5 minutes for each tube.

Clean with card	ousel tubes
Selections and S	ettings
Tubes	Time (min)
Cleaning	5 ~
Rinse 1	5 ~
Rinse 2	5 ~
ОК	Cancel

- 1. Wipe down workspace.
- 2. Check sheath and waste levels.
- 3. Log out of FACS DIVA.
- 4. Log out of PPMS.

DAILY SHUTDOWN FOR THE LAST USER OF THE DAY

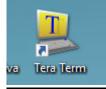
- 1. Prepare the cleaning carousel:
 - **a.** Place a tube of bleach in the first position.
 - **b.** Place a tube of Contrad in the second positon.
 - c. Place a tube of shutdown solution in the third position.
- **2.** Insert the carousel and go to Carousel \rightarrow Clean.



a. Select 5 minutes for each tube.

Clean with care	ousel tubes
Selections and S	Settings
Tubes	Time (min)
Cleaning	5 ~
Rinse 1	5 ~
Rinse 2	5 ~
ОК	Cancel

- 3. Wipe down the workspace.
- 4. Check sheath and waste levels.
- 5. Log out of DIVA.
- 6. Exit out of TeraTerm.



- 7. Log out of PPMS.
- 8. Turn off the cytometer.