Canto II Operation Protocol

Make sure the following actions have been taken before running your samples.

- Make sure the waste tank is empty and refilled with 500 ml clean (see page 15 for details)
- ✤ Make sure the FACS Flow container is fully filled (see page 16 for details)

1. Turn on the external pressure (on the opposite wall)

- 2. Turn on the Canto II
- 3. Turn on the Computer
- Log into Windows using User Name Admin and password: BDIS#1

4. Launch the BD FACSDiva Software

Log in FACSDiva software with your personal login name and password.

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User Name:	User Name	
Password: 🦷	XXXXXXXXXX	

Always click "Use CST Settings" when pop-up message as below appears.



5. Start Fluidics Startup

Starting the Fluidics startup (7 min)

- Choose Cytometer > Fluidics Startup.
- 6. Creating and working with experiments in BD FACSDiva Software
- Browser toolbar click New Experiment & rename the experiment if necessary or import an old one and duplicate without data.



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

Browser - Experiment_001	Cytometer Settings	⊟ I Experiment_01 ✓ Cytometer Settings ☐ I Gobal Worksheets
Name Nam Name Name Name Name Name Name Name	Global Sheet1	Global Sheet1
Gobal Worksheets		

Right click on Specimen_001 > Rename if necessary; Right click on Tube _001 & Rename if necessary.



Click the New Tube buttom to create new tubes. Rename them if necessary.



Select Cytometer Settings > Parameters & Delete unnecessary parameters on the Inspector Window.
Inspector - Cytometer Settings

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our workshoots	Parameter	Voltage	Log	A	н	W	
Global Sheet1	1 P.C.	524		14			^
STORES OF FORMA	• SSC	392		4			
	• FITC	562	~	~			
	* PE	536	4				
	PerCP-Cy5-5	668	~				
	PE 0.7	004	1221	100	0	-	
	APC	648					
	APC-Cy7	507	~				1
	APC APC-Cy7	648 507					

FSC (measure *Cell Size*) and SSC (measure *Cell Granularity*) are a 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.

arameters	Threshold	Ratio Com	pensation				
Parame	ater	Voltage	Log	А	н	W	
FSC		524			~		^
• SSC		392		V	~		
FITC		562	~	~			
• PE		536	~				
APC		648	~				

- 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

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billonooc e		C Experin	nent Layout		
Ctrl+N		Labels V-			
Ctrl+E		Labels Ke	yworas Acquisition		
Ctrl+M		-Ouick E	otry		
Ctrl+T		Label	CD3 🖌		
s			Name	Label	Label
				FITC	PerCP-Cv5-5
			🔚 Unstain	CD3	
Ctrl+Y				FITC CD3	PerCP-Cy5-5
Ctrl+O				FITC	PerCP-Cv5-5
CERTERN			🔚 PE	CD3	
			🔓 PerCP	FITC CD3	PerCP-Cy5-5
	Ctrl+N Ctrl+E Ctrl+M Ctrl+T s Ctrl+Y Ctrl+Y Ctrl+O Ctrl+W	Ctrl+N Ctrl+E Ctrl+M Ctrl+T s Ctrl+Y Ctrl+O Ctrl+W	Ctrl+N Ctrl+E Ctrl+M Ctrl+T s Ctrl+Y Ctrl+O Ctrl+W	Ctrl+N Ctrl+E Ctrl+M Ctrl+T s Ctrl+Y Ctrl+Q Ctrl+Q Ctrl+W Ctrl+O Ctrl+W Ctrl+O Ctrl+W Ctrl+O Ctrl+W Ctrl+D	Ctrl+N Ctrl+E Ctrl+M Ctrl+T s Ctrl+Y Ctrl+Y Ctrl+Q

Select Dot Plot or Histogram & move the cursor onto the blank worksheet.

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Global Sheet1	Histogram



Right click on a plot & **Duplicate** to create another plot of the same type.



Select each individual axis, and choose from a list of offered parameters the preferred one.







- Right click on the plot > Show Population Hierarchy
 - Right click on the plot > Create Statistics View & 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

Parameters	Min	May	Geo M	Mean	Median	SD				[M
FSC-A					Healan			70007	701 C Y	[
FSC-H										
FSC-W										[
SSC-A										1
SSC-H										[
SSC-W										[
FITC-A										[
PE-A				v						[
APC-A										[
Time										[
Decimal Places	0	0	0	0	0	0	0	1	1	
		⊙ So ⊡ Disp	ort by Parar lay Range	neter C) Sort by Fo	ormula 100) 💙				

7. Procedures for sample acquisition

Gently tab the tube to mix your sample & put your sample tube on SIP

(sample injection port) & Run the unstained/negative control sample before other sample tubes.

- Acquisition Dashboard & Acquire Data
- Choose Flow Rate low, medium or high to acquire your samples

Current Activity			
Active Tube/Well	Threshold Rate	Stopping Gate Events	Elapsed Tim
Tube_001	0 evt/s	0 evt	00:00:00
∳iji Next Tube	Acquire Data	Record Data	Restart
Acquisition Setup			
	Events V Events To De	cord: 10000 evt 🗸	Stopping Time (sec):
Stopping Gate:	LVEIKS VEIKS TO RE		

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

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1553		A	-	W	
1 4 4 1 1 A 12					
461	<u>ن</u>				
627					
615	200				-
746	-200				_
730	-				_
712					_
	↓				
	+				
Threshold Rate	+	Stopping	Gate Events		Elaps
Threshold Rate 4 evt/s	•	Stopping 0 evt	Gate Events		Elaps 00:0
Threshold Rate 4 evt/s	•	Stopping O evt	Gate Events		Elaps 00:0
Threshold Rate 4 evt/s Stop Acquiring		Stopping O evt	Gate Events	© Restart	Elapso 00:0
	615 746 730 712 555	615 746 730 712 555 ☑	615 746 730 712 555 V	615 746 730 712 555	615 746 730 712 -0 V 555

- Cytometer > Laser & Adjust the FSC Area Scaling until the mean of FSC-A and FSC-H are <u>APPROXIMATELY THE SAME</u>.
- Return to the *Parameters* tab and finely re-adjust voltage of FSC and SSC.

Status Parameters Thresho	Laser 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 Scainor 0.50			
Name	Delay	Area Scaling	
Name	Delay	Area Scaling	
Name Blue	Delay 0.00	Area Scaing 1.40	

Adjust voltage of each of the fluorescent channels; preferably the peak of the "negative" population (mean in statistic view) of interest is greater than ZERO but less than 10².

at s Parameters Theshold Lase	r Compensation	Ratio	_	900
Parameter	Voltage	Log	A	-800
• FSC	526		1	-600
• SSC	395		1	4
• FITC	555		1	400
PE	534	~	E	200
• APC	643		R	

- Click Stop Acquiring on Acquisition Dashboard.
- Repeat the steps above with the positive control sample tubes. Adjust the voltage of corresponding channels if their signal peaks are **outside** the limit of the histograms.

8. Creating Gates

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



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



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



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

Specimen_001-Tube_001	Specimen_001-Tube_001	Specimen 001-Tube 001	Tube: Tube: <td< td=""></td<>
50 100 150 200 250 FSC-A (* 1,000)	50 100 150 200 200 FSC-H (x 1000)	00 100 100 200 200 SSC-H (x 100)	Experiment Name: Experiment_004 Specimen Name: Specimen_001 Tube Name: Tube_001 Record Date: \$0P: OUID: FSC-A FSC-A Population #Events %Parent Mean Mean Mean MIEvents 0 #### P2 0 #### P3 0 #### P4 0 #### P5 0 #### P6 0 #### ####
Specimen_001-Tube_001	Specimen_001-Tube_001	Specimen_001-Tube_001	

9. Recording data for all samples

- Gently tab the tube to mix your sample, then put your sample tube on SIP (sample injection port); Run the unstained sample before other sample tubes.
- Acquisition Dashboard > Acquire data > Record data

Active Tube/Well	т	hreshold Rate	Sto	opping Gate Events		Elapsed Time	
Tube_001		evtis	0 (0 evt		00:00:00	
Basic Controls	_	er on ander					
📲 Next Tub		📓 Acquire Data	Record	Data	01	start	
Acquisition Setup							
	Al Fuerte	Puents To R	ecord:	10000 evt	Stoppin	g Time (sec):	
Stopping Gate:	MICTORS	and a route to route				PERSONAL PROPERTY AND ADDRESS OF ADDRESS OF ADDRESS ADDRESS ADDRESS ADDRESS ADDRESS ADDRESS ADDRESS ADDRESS ADD	

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

10. Machine Cleaning

Cleaning procedure between each user is required.

- Prepare 3 mL of each cleaning solution (FACS Clean, FACS Rinse, Milli-Q H₂O).
- Install a tube of FACS Clean solution on the SIP and let it run for 5 minutes with the flow rate set to high.
- ♦ Repeat this step with BD^m FACSRinse solution & Milli-Q H₂O.
- Remove the tube from the SIP.

Note: Please be sure in all the cases not to exceed the maximum allowed volume

of 2 ml in the tube.

11. Export FCS Data / Experiment

To save Experiment, right click on the Experiment Export > Experiment > Browse to choose the destination folder (D/year/AG/name).



Create a new folder and rename, then click Export > OK

Export				🛛 🚺 Expor	t Experiments
Look in My Recent Documents	: 👝 today			Directory:	Delete experiments after export Directory Export Divectory Export Divectory Empire Export Divectory Empire Export Browse Browse
Desktop				Experime	ant Date
My Documents				20150616	6/16/15 4:09:01 PM
My Computer					~
My Network Places	File name: Files of type:	D:\USER\Anatomy\Emily Pang\today Only Directories	Expo		OK Cancel

To save FCS files, right click on Experiment > Export > FCS Files



✤ Select FCS 3.0 > OK > Browse to choose destination folder (D/year/AG/name).

Gated Events		
File Version O FCS2.0 O FCS3.0		
Parameter	Parameter Type	
FSC-A	S Linear ○Log ○ None	^
FSC-H	● Linear ○Log ○ None	
FSC-W	S Linear ○Log ○ None	
SSC-A	● Linear ○Log ○ None	
SSC-H	Linear ○Log ○ None	
SSC-W	● Linear ○Log ○ None	
FITC-A	● Linear ○Log ○ None	
FITC-H	● Linear ↓Log	
PE-A	€ Linear ◯Log ◯ None	
PE-H	Linear ○Log ○ None	
APC-A	● Linear ○Log ○ None	
	0.000	~

Umen	y Path ts and Settings\Adr	ministrator\Deskto		wse	
	Save	Details>>	Cancel		
		<u></u>			1
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Create a new folder and rename > Choose Directory and save the file.

Look in:	🚞 user		🖌 🕼 🛤 🖬 👘	
My Recent Documents Desktop	Anatomy Biochemistr Partedicine Pathology SCRMC	Y		
My Network Places	File name: Files of type:	C:\Documents and Settings\Administrator\Desktop\user	Choose Directory Cancel	

- Duplicate the experiment if needed again:
 - Select the experiment; right click and select duplicate without data. This retains the mask, the labeled samples and the stored cytometer settings
 - o It is not allowed to store more than 3 experiments without data
- Mandatory: Delete the measured experiment experiment exported.
- Mandatory: remove your data (within 7 days) from the hardcopy drive D e.g. by transfer to (S/year/AG/name).

12. Shut down

Before you leave please be sure to switch the instrument off if required. **Policy of keeping instruments "ON" or "OFF" depends on the PPMS schedule:**

- **During the daytime**, we keep the analyzers **"ON" except** there is a gap between users longer than 3 hours;
- If you finish your BD Canto II experiment after 6 pm on the weekday or any time on the weekend and if the next person is not physically present at the site we request to switch "OFF" the BD Canto II by Starting the Fluidics shutdown (7 min)
- Choose Cytometer > Fluidics Shutdown.
- Turn-off the instrument
- Turn-off the pressure of the external air supply drain (valve on the opposite wall)
- Empty the waste,
- check and fill up Clean or shut-down solution if necessary

13. Log Out

To log out of FACSDiva software, go to File > Log Out



- Please be sure to logoff Windows and shutdown the computer before you leave FCF Berg to avoid unnecessary charges.
- 14. Sign in the labbook (next to the computer) for documentation

Note: Please report all the problems/concerns to FCF Berg Staff:

During business hours — get to Room 581/527 and tell the FCF Berg staff member.

After hours — e-mail to fcf-berg@med.uni-tuebingen.de; leave the note about the problem on the instrument's keyboard;

FCF-Berg

Empty Waste Tank Procedures (before and during measurement)



- Ensure the cytometer is not acquiring events.
- Detach the waste container's sensor and fluid line from the fluidics cart waste port.
- Pull the sensor straight out.
- Press the metal clip on the fluid line.



The waste tank can become pressurized when the cytometer is running. Always disconnect the tank from the fluidics cart before you empty it. Wait at least 30 seconds for pressure to dissipate before you remove the waste cap or level sensor cap.

• Remove the disposable waste cap and attached trap from the container; place the assembly on the bench label-side up.

Do not wet the cap. If you see liquid inside the trap, remove the drain plug and fully drain the liquid before you replace the plug.

• Empty the waste.

• Add approximately 500 ml of clean to the empty waste container (10 L container).

- Screw the cap assembly onto the tank and hand-tighten until it is fully closed.
- Re-attach the sensor and fluid lines.

Refill Flow/Clean or shut down solution Containers (before and/or during

measurement)



- Ensure the cytometer is not acquiring events.
- Detach the sensor and fluid line from the cart.
- Pull the sensor straight out.







- Press the metal clip on the fluid line.
- Unscrew the cap on the container.
- Remove the cap and sensor assembly and set it aside.



- Put a new container onto the fluidics cart.
- Replace the cap assembly and hand-tighten it until it is fully closed.
- Reattach the sensor line and fluid line to the cart.
 - To attach the sensor line, gently rotate until the connection aligns, and then push.
 - To attach the fluid line, push the coupling into the port until it clicks.
- Prime the fluidics when you refill a tank during measurement.
 - Use the "Prime After Tank Refill" command to remove air from the fluidics lines after you changed a container.
 - Choose Cytometer (Instrument) > Cleaning Modes > Prime After Tank Refill.
 - Select the checkboxes for the containers you changed; click OK.

Tank Prime	
Please select the checkboxes for the tanks that need to be primed.	
FACSFlow F Shutdown Solution Cleaning Solution	
OK Cancel	

BD FACSDiva software