BD LSRFortessa Standard Operation Protocol

Basic Operation – Tube mode

A. Log in the Window

- 1. Press Ctrl+Alt+Delete
- 2. Key in Password **BDIS#3\$\$** *No need to switch user

B. Log in the BD FACSDiva Software

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.



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



C. Experiment Setup

- 1. Setup New Experiment
 - 1.1 Go to Browser, Click New Experiment icon. A new experiment will be created

Name	Date
🗐 🧏 Ocleaning	
🕀 🔚 Experiment_001	12/30/19 11:16:15 AM
🕀 📔 Accudrop Drop Delay	12/30/19 11:21:30 AM
Experiment_002	3/3/20 2:22:43 PM
🕀 🔚 Experiment_003	3/9/20 5:11:11 PM
🖻 🛄 Experiment_004	3/11/20 11:57:10 AM
Cytometer Settings	
🕀 🤷 Global Worksheets	
🕀 🦂 Shared View	

1.2 Click Cytometer Settings under the newly created Experiment

😡 Bro	owser - Experiment_004	×
	🖬 餐 🏴 📝 🜌 📓 -	
	Name	Date
(⊒… 🚴 Ocleaning	
	🕀 🔚 Experiment_001	12/30/19 11:16:15 AM
	🖶 🔚 Accudrop Drop Delay	12/30/19 11:21:30 AM
	🕀 🔚 Experiment_002	3/3/20 2:22:43 PM
	🕀 🔚 Experiment_003	3/9/20 5:11:11 PM
	🖨 🛄 Experiment_004	3/11/20 11:57:10 AM
	Cytometer Settinos	
	🕀 👸 Global Worksheets	
(nared view	

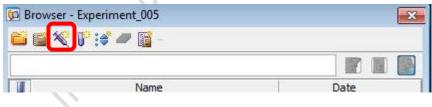
1.3 Go to Inspector Window, select unwanted channels and click Delete

rameters Threshold Ratio C	ompensation						
Parameter	Voltage	Log	A	н	w		
FSC	353					-	
SSC	292	1			V		
ETTO	247			-			
PerCP-Cy5-5	483		V				
PE	446	V	V				
PE-Texas Red	414	V					
PE-Cy5	536	V					
PE-Cy5-5	386					8	
PE-Cy7	440	V					
APC	395	V					
Alexa Fluor 700	361	V					
APC-Cy7	368	V					
DAPI	343	V	V				
AmCyan	383	V					
BV605	567	V					
BV650	536			(m)			
Add			240	Delete	- V2		

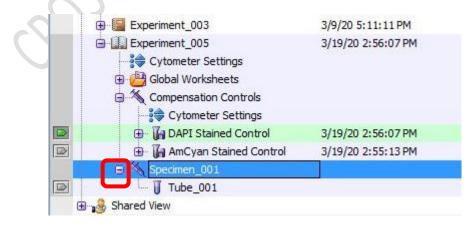
arameters Threshold Ratio							
Parameter	Voltage	Log	A	Н	W		
FSC	353		V	V			
SSC	292	E	V	V	V		
- me	547	V	V				
PerCP-Cy5-5	+ 483	V	V				
PE	446	V					
PE-Texas Red	414	V	V				
PE-Cy5	536	V					
PE-Cy5-5	386	V				E	
PE-Cy7	440	V					
APC	395	1					J
Alexa Fluor 700	361	V					
APC-Cy7	368	V					
e DAPI	343	V					
 AmCyan 	383	1					
# BV605	567	V					
BV650	536	V	V				
Add			lde -	Delete			

1.4 Check H and W boxes of FSC and SSC

- 1.5 Keep *Log* boxes of FSC and SSC **unchecked**
- 1.6 Keep Log boxes of all fluorescence channels checked
 - * If you are doing **cell cycle or DNA content** analysis, please keep **log box** of your DNA specific fluorescence channel **unchecked**.
- 2. Setup Compensation Please refer to FACSDiva compensation automated protocol
- 3. Setup Plots and Tables
 - 3.1 Go to Browser, Click New Specimen icon



3.2 Expand Specimen_001





Globa	al Worksheet - Global Sheet1 Image: Specimen_001-Tube_001 Icon Type Icon Type Dot Ph		
Globa	bal Sheet1		
Globa	bal Sheet1 Specimen_001-Tube_001		
Globa	bal Sheet1	KOMPE'L'	
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		e e	
	obal Sheet Window, Click the gra	pri type icon	
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		()	2
€8			Yr.
	🕀 🖟 AmCyan Stained Control	3/19/20 2:55:13 PM	
	General DAPI Stained Control	3/19/20 2:56:07 PM	
	- 😝 Cytometer Settings		
	🖨 📉 Compensation Controls		
	🕀 🥶 Global Worksheets		
	Cytometer Settings		
1	Experiment 005	3/19/20 2:56:07 PM	
	Experiment_005	3/19/20 2:56:07 PM	

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

Image: Solution of the solut	
	.
_ <u>Specimen_001-Tube_001</u>	

Ison	Turne
Icon	Туре
	Dot Plot
6	Contour Plot
	Histogram

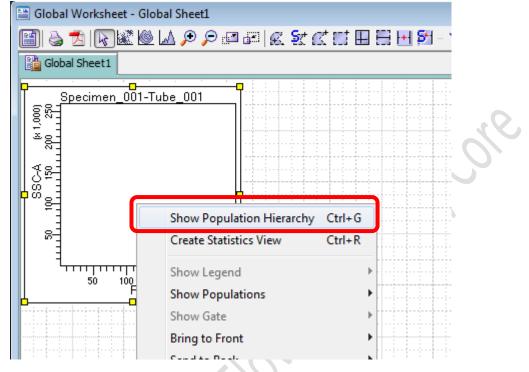
- 3.5 Click on the blank area of Global Worksheet window to create a new plot.
- 3.6 Mouse over the axis label and right click. Select the parameters of interest from the list.

🗎 Global Worksheet - Global Sheet1	
i 🖾 😞 🔁 💽 🌌 🕲 🖾 🗩 🔎 i 🗖	21
Global Sheet1	
Specimen_001-Tube_001	
200 K 1000	
Kara Sector Sec	
FSC-H	
FSC-W	
SSC-A TTTT	
SSC-H × 1,000	
SSC-W	

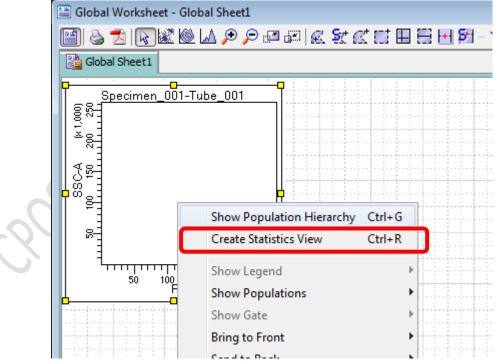
3.7 Repeat step 3.4 - 3.6 until all plots needed is created.

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

3.8 Click on any plot and right click. Click Show Population Hierarchy



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



3.10 Right Click on Statistics View table, select **Edit Statistics View** to select statistics of interest to be shown in the table.

Tube: Tube_001							
Population			#Events	%Parent	%Total		
All Events			0	****	****		
Experiment Name:	• •	-				1	
Specimen Name:	Specimen_I	001					
Tube Name:	Tube_001						
Record Date:							
\$OP:						Edit Statistics Vie	
GUID:						Export Statistics	
			F	SC-A	SSC	🔏 Cut	Ctrl+X
Population	#Events	%Parent	1 1	Mean	Me	🗈 Сору	Ctrl+C
All Events	D	_ <i>****</i>	: ;	****	##	Delete	Delete

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

Parameters	Al	Min	Max	Geo M	Mean	Median	50	150	200	2 %/CV	Mode
PSC-A											
PSC-H					V						
PSC-W											
SSC-A					¥						
SSC-H											
99C-W											
FITC-A					V						
PE-A					Image: A start and a start						
APC-A					V						
Time											
Decimal Places		0	0	0	0	0	0	0	1	t	0
	 Sort by Perameter Sort by Formula Display Range Linear Scale (0-10,000) 										

S. Mar

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 centre without putting a tube filled with liquid on SIP!!

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



3. Go to Acquisition Dashboard, Click Acquire Data

Current Activity							
Active Tube/Well		Threshold Rate	Stoppin	ng Gate Events	Elapsed Tir		
Tube	_001	0 evt/s		0 evt		0:00:00	
Basic Controls	_						
eij Next Tube		Acquire Data	Record Data			Restart	
Acquisition Setup							
Stopping Gate:	All Events	V Events To Re	cord:	10000 evt	~	Stopping Time (sec):	
Storage Gate:	All Events	V Events To Dis	nlav:	1000 evt	~		

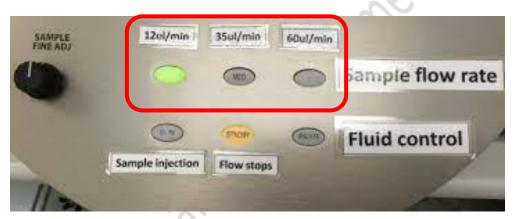
4. When Threshold rate > 0, go to Cytometry window and Click **Parameters** tab.

Statu Parameters Threshold Laser	Compensation	Ratio				
Parameter	Voltage	Log	A	н	W	-1
+ FSC	179	Ð 🗆		V		1
• SSC	240			1		
• DAPI	343					
• AmCyan	383	1	V		100	

5. Adjust PMT Voltage accordingly

Status Parameters Thresho	ld Laser Compensation	Ratio				
Parameter	Voltage	Log	A	н	W	
• FSC	179					
• SSC	240			V	V	
• DAPI	343					
• AmCyan	383	1			0	
Channel	Suggested V	oltage ra	nge for n	nammalia	n cells	
SC	450-600					5
SC	250-310				- ()	
luorescence	300-850					

6. 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

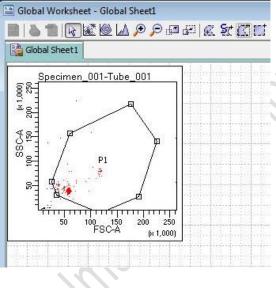
 Global Worksheet - Global Sheet1

 Image: Second Sheet1

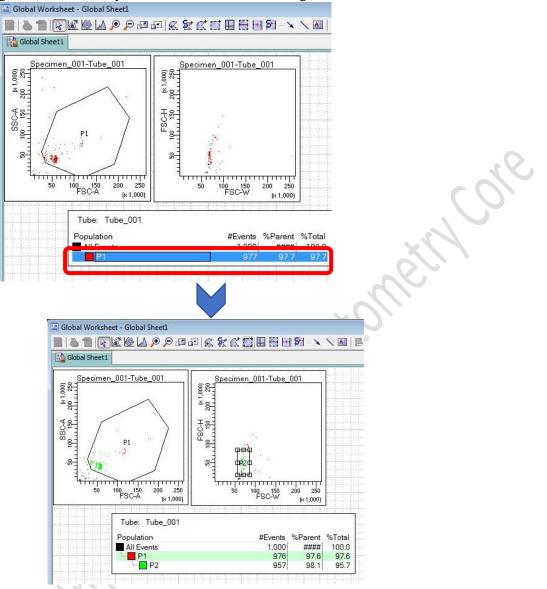
 Image: Sheet1

Icon	Туре
€¢	Polygon Area Gate
EET	Rectangle Area Gate
	Quantrad Gate
8	Interval Gate

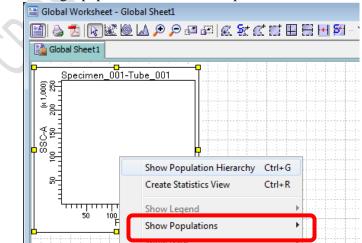
2. Draw the gate on the plot of interest to gate out target cluster /peak



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	start	
Acquisition Setup				
Stopping Gate: XP2	vents To Record:	20000 evt 🗸 Stopp	ing Time (sec):	0
Storage Gate:	Events 🗸 Events To Display:	1000 evt \sim		
Acquisition Status				
Processed Events:		Electronic Abort Rate:		
Threshold Count:		Electronic Abort Count:		

2. Set the Storage gate to All Events

🔢 Acquisition Dashboard			
Current Activity			
Active Tube/Well	Threshold Rate	Stopping Gate Event	s 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	v Events To Record:	20000 evt	✓ Stopping Time (sec): 0
Storage Gate: All Ev	ents 🗸 vents To Display:	1000 evt	~
Acquisition Status			
Processed Events:		Electronic Abort Rate:	
Threshold Count:		Electronic Abort Count:	

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

🚺 Acquisition Dashboard				—
Current Activity				
Active Tube/Well	Threshold Rate	Stopping Ga	te Events Elaps	ed Time
Tube_001	0 evt/s	0 evt	00:0	0:00
Basic Controls				
∳] Next Tube	📕 Acquire Data	Record Data	Restart	
Acquisition Setup			_	
Stopping Gate:	P2 V Events	To Record: 20000 ev	/t 🗸 Stopping Time (sec)	: • • • •
Storage Gate:	All Events 🗸 Events	s To Display: 1000 ev	t v	
Acquisition Status				
Processed Events:		Electronic Abo	rt Rate:	
Threshold Count:		Electronic Abo		

4. CHER RECOLU Data	1.	Click Record	Data
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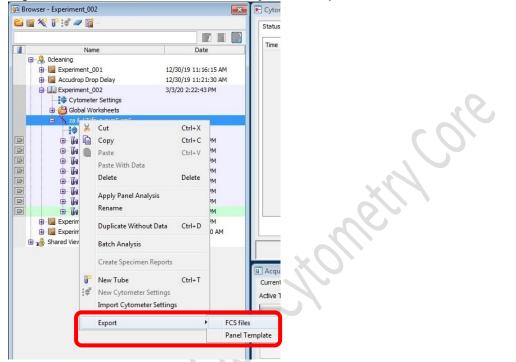
II: Acquisition Dashboard				×
Current Activity				
Active Tube/Well	Threshold Rate	Stopping Gate	Events Elapse	ed Time
Tube_001	0 evt/s	0 evt	00:00	0:00
Basic Controls				
ø investor value valu	Acquire Data	Record Data	Restart	
Acquisition Setup				
Stopping Gate:	P2 v Events To	Record: 20000 evt	Stopping Time (sec):	0 🖨 🕇
Storage Gate:	All Events V Events To	Display: 1000 evt	~	
Acquisition Status				
Processed Events:		Electronic Abort	Rate:	
Threshold Count:		Electronic Abort	Count:	

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

Active Tube/Well Tube_001 Basic Controls	Threshold Rate 0 evt/s	Stopping Gate E 0 evt	Events Elapsed Time 00:00:00
Basic Controls	0 evt/s	0 evt	00:00:00
7			
ø <mark> </mark> Next Tube	📕 Acquire Data	Record Data	Restart
Acquisition Setup			
Stopping Gate:	P2 V Events To	Record: 20000 evt	Stopping Time (sec):
Storage Gate:	All Events V Events To	Display: 1000 evt	~
Acquisition Status		L	
Processed Events:		Electronic Abort R	ate:
Threshold Count:		Electronic Abort C	ount:
11	06)		

G. Data Export

- 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*



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

Gated Events				
File Version	FCS3.1			
Parameter	Parameter Type	U Log		
FSC-H	Linear	🕐 Log	🔘 None	
FSC-W	Linear	🕐 Log	🔘 None	
SSC-A	Iinear	🕐 Log	None	
ssc-H	Iinear	🕐 Log	None	
SSC-W	 Linear 	🕑 Log	None	
FITC-A	inear	🖱 Log	None	
DAPI-A	Linear	🔘 Log	🔘 None	
APC-Cy7-A	Linear	🔵 Log	🔘 None	
PE-Cy7-A	inear	🖱 Log	None	
Alexa Fluor 700-A	Linear	🕐 Log	🔘 None	
Time	Linear	O Log	None	

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

1.5	Click	Save
1.5	CIICK	Save

irectory Path		
D:\User\SCM\LinX		Browse
Save	Details>>	Cancel

- 2. PDF file
 - 2.1 To export pdf of multiple tubes, Click the tube pointer of any tubes

I	Name	Date
G	∋ 😓 Ocleaning	
	🖶 📔 Experiment_001	12/30/19 11:16:15 AM
	 Accudrop Drop Delay Cytometer Settings Specimen_001 	12/30/19 11:21:30 AM
	🖽 🔰 Tube_001	
_	🕀 🔚 Experiment_002	3/3/20 2:22:43 PM
	🕀 🔚 Experiment_003	3/9/20 5:11:11 PM

- 2.2 Select the wanted tubes/ Specimen.
 - Browser Experiment_002 🗶 Cytor ビ 🝯 餐 🧗 📝 🜌 📓 Status Time J Date Nam 🖃 🚴 Ocleaning 12/30/19 11:16:15 AM Experiment_001
 Accudrop Drop Delay 12/30/19 11:21:30 AM Experiment_002 3/3/20 2:22:43 PM Cytometer Settings Cut Ctrl+X X :0 🕀 🐻 🗋 Сору Ctrl+C - 6 PM Paste Ctrl+V PM Paste With Data ÞΜ Delete Delete M • **h** Apply Panel Analysis • 6 M Rename M 🕀 🔚 Experim Duplicate Without Data Ctrl+D 🕀 📔 Experim MAC Batch Analysis 🗄 🍰 Shared View Acqu 📔 New Tube Ctrl+T Curren
- 2.3 Right Click over the selection and click Batch Analysis

2.4 Check the boxes as picture below

 Auto View Time: Manual 	10 👻	Output To Printer Save as PDF Save as XML Add Report	Sec. and	s Biexponentia ferred Globa	
PDF Filename:	t_003-B	atch_Analysis_19032020	143013.pdf	Browse	View PDF
(ML Filename;	t_003-B	atch_Analysis_19032020	143013.xml	Browse	
Stats Filename:	t_003-B	atch_Analysis_19032020	143013.csv	Browse]
			10		

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

 Auto View Time: [Manual 	10 👻	 Output To Printer Save as PDF Save as XML Add Report 	Sec. and	:s Biexponentia ferred Globa	
PDF Filename:	t_003-B	atch_Analysis_19032020	143013.pdf	Browse	View PDF
XML Filename:	t_003-B	atch_Analysis_19032020	143013.xml	Browse	
Stats Filename:	t_003-B	atch_Analysis_19032020	143013.csv	Browse	
Status:		0°	/0		

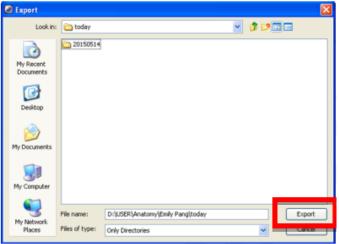
2.6 Click **Start** and then **OK**.

 Auto View Time: Manual 	Output To Pr Output To Pr Save as PDF Save as XML Add Report	🔽 Fr	atistics eeze Biexponent e Preferred Glob	
PDF Filename:	t_003-Batch_Analysis_190	032020143013.p	odf Browse	View PDf
XML Filename:	t_003-Batch_Analysis_190)32020143013.>	cml Browse	Ĩ
Stats Filename:	t_003-Batch_Analysis_190)32020143013.c	sv Browse	ī.
Status:		0%		
	Start	Pause	Continue	Close
Tube_(Tube_(Tube_(02	al worksheet:		

- 3. Experiment
 - 3.1 Right click on the **Experiment > Export > Exerpiments** 3 20150514 5/14/15 4:26:44 PM 6/16/15 3:36:21 PM E 1 20150616 6/16/15 4:09:01 PM Ctrl+X Сору Ctrl+C) ی 0.3 Paste Ctrl+V 0.5 Paste With Data 🕀 🔚 E 6/23/15 1:30:58 PM Delete Delete 🔒 Share Rename Duplicate Without Data Ctrl+D Open Experiment Ctrl+O Close Experiment Ctrl+W Batch Analysis... 👔 New Global Worksheet X New Specimen... Ctrl+M New Cytometer Settings Import Cytometer Settings Share Experiment Export Exp FCS files... Experiment Template...
 - 3.2 Click **Browse** to select the file destination

🙆 Export	Experiments	
	Delete experiments after export	
	⊙ Directory Export ○ Zip File Export	
Directory:	D:\USER\Anatomy\Emily Pang\today	Browse
Experime	nt Date	
20150616	6/16/1	5 4:09:01 PM 🔨
		×
	ОК	Cancel

3.3 Select the file destination and click **Export**.



Cole

3.4 Click **OK** to export

Directory:	Delete experiment Directory Export D:\USER\Anatomy\Em	O Zip File Export	Browse	
Experime		Date	Croinsen.	
20150616		6/16/15 4:0	09:01 PM	
			~	$\langle O \rangle$
		ОК	Cancel	

H. Software Log out

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

Save	Ctrl+S
Page Setup	
A Print Preview	
la Print	Ctrl+P
🛃 Save as PDF	
Administration	
User Tracking Log	
Import	٠
Export	•
Log Out	
Quit	

I. 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 (60ul/min) and RUN (Sample Injection) 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 LO (12ul/min) and Standby (Flow Stop)