BD Aria Fusion Standard Operation Protocol – Basic Operation

A. Laser

- 1. Check if the laser needed is turned ON.
 - * Turn off laser(s) not required may improve cell viability
 - ** Blue Laser MUST be turned ON.



B. User Login

2.

Login to FACSDiva Software with your username and password.
 *If you do not have account, please contact our staff for assistance.

Log In	BD	-
User Name:	🚴 Ocleaning	
Password:	ОК	Quit

Click Use CST Settings if the window below pops up.

9.00	Mismatch		
		from CST are different fr gs would you like to apply	om those currently in BD FACSDiva ?
-		w this message again for r my decision.	current login session.
	Details>>	Use CST Settings	Keep BD FACSDiva Settings

Centre for PanorOmic Sciences – Imaging and Flow Cytometry Core

C. Stream Optimization

- 1. Go to 100 micron window (upper monitor)
- 2. Adjust the Freq (Starting Value 29.2) so the "neck" of a drop is formed 4.
- 3. Adjust the Ampl (Starting Value 3.0) so the Drop 1 is close to 120 and Gap is close to 10

S AN AND

4. Turn ON the Sweet spot

100 micron 🛛 🔀

Stream

Sweet Spot

22

3.

2.

Ampl:

Freq:

Drop 1:

Gap:

3.1

29.2

127 117 9

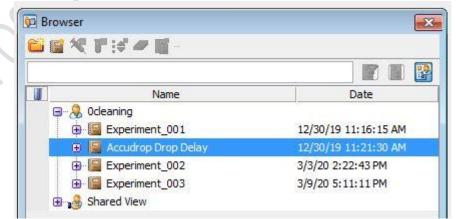
10

D. Accudrop Delay Assay

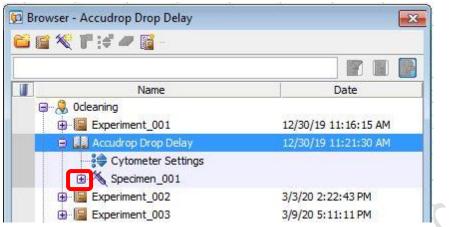
1. To Import Accudrop Drop Delay template, click *Experiment > New Experiment*. Select Accudrop Drop Delay, then click *OK*

<complex-block></complex-block>	BD FACSDiva Sof	tware - Ocleaning (Fusion 100	0u 488, 561	, 640, 4				
Iou mice New Tube Stream New Tube Upper Experiment Opper Experiment Ctrl+U Experiment Layout Compensation Setup								
100 micro New Tube Curlet New Global Worksheet Open Experiment Curlet Open Experiment Curlet Open Experiment Curlet Open Experiment Curlet Open Experiment Curlet Open Experiment Curlet Open Experiment Curlet Open Experiment Curlet Open Experiment Curlet Open Experiment Curlet Open Experiment Curlet Open Experiment Curlet Open Experiment Curlet Superiment Open Experiment Superiment Open Experiment	B *7	New Folder	Ctrl+N					
Streen Vew Tube Cutrint Cutrint Vew Tube Cutrint Vew Tube Cutrint Vew Tube Cutrint Vew Tube Cutrint Cutrint Vew Global Worksheet Open Experiment Cutrint Cutrint Curries Vew Global Worksheet Open Experiment Expout Compensation Setup Vew Tube Vew Global Worksheet Open Experiment Expout Compensation Setup Vew Tube Vew Global Worksheet Open Experiment Expout Compensation Setup Vew Tube Vew Global Worksheet Open Experiment Expout Compensation Setup Vew Tube Vew Global Worksheet Open Experiment Expout Compensation Setup Vew Tube Vew Global Worksheet Open Experiment Expout Compensation Setup Vew Tube Vew Global Worksheet Open Experiment Expouse Vew G		New Experiment	Ctrl+E					
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Image: Image	Stream 🦷	New Tube	Ctrl+T					
Image: Acudrop Drop Delay	🛛 Sweet Sp 🗐	New Cytometer Settings						
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Close Experiment Ctrl+W Experiment Layout Compensation Setup Image: Compen		New Global Worksheet						
Close Experiment Ctrl+W Experiment Layout Compensation Setup Image: Compen		Onen Evneriment	Ctrl+0					. (
Experiment Layout Compensation Setup Image: Co								
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Doublet Discrimination Gating 7/25/18 12:22 PM QC Experiment 7/25/18 12:22 PM ame: Accudrop Drop Delay Copies: 1 1 Copies: 1 C	Accudrop Drop Del	av 7/25/18 :	12:22 PM					
QC Experiment 7/25/18 12:22 PM	olaal. Execution on the							
Cancel	Name: Accudrop Dro	op Delay		Copies:				
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- OR -			O					
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		$\sim 0 $						

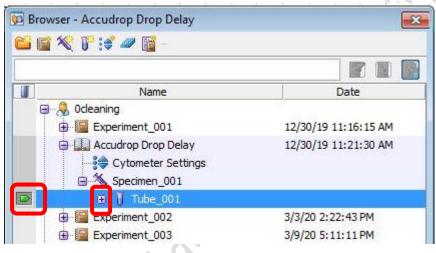
2. To open existing Accudrop Drop Delay experiment, double click *Accudrop Drop Delay* on the Browser window



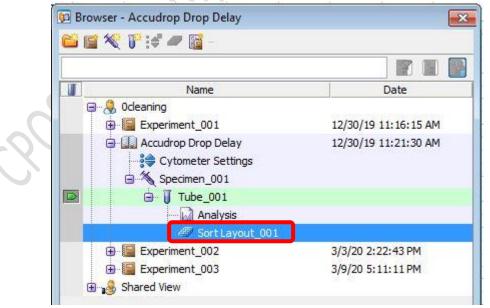
3. Expend Specimen_001



4. Click the tube pointer and expend Tube_001



5. Double click Sort Layout_001. Sort Layout window will pop up



6. Go to Sort Layout Window, Select *Precision > Initial*

Device:	Precision:	Target Events:		Save Sort Reports:		Save Conflicts	Index Sorting
2 Tube	ube 🔹 🖬 🛨 Co		👻 🗛 Ask User 🗸 🗸				
Left			Rig	ht			
P1	: Continuous						
Sort Rate:	0 evt/s		NA				
Confl. Cnt:	0 evt		NA				
Confl. Rate: 0 evt/s			NA				
Confl. Rate:	Efficiency: 0%			NA			

7. Go to 100 micron window (lower monitor), set the slider reading as 0 - 26 - 0 - 0

100 micron		X
Voltage 📉 Test Sort 🕜 Optica	l Filter 🔛 Attenuation 🗂 Waste Drawer Dro	p Delay: 25.00 🖨 Auto Delay
ACDU		
6 D=B	+	
0[26	······· 0 []	- 0 []
Voltage Center:		Plate Voltage: 3,000 🕃 🛉
2nd Drop: 17	3rd Drop: 7 💭 4th Drop: 3 💭	Phase: 0

8. Go to Acquisition Dashboard window, Set *Flow rate* to 1.0

Acquisition Dashboa	rd				×
Current Activity Active Tube/Well Tube 00	Threshol		Stopping Gate Eve 0 evt		osed Time
Basic Controls	00003		0011		
→ Next Tube	Loa	ad 📕 🗖	cquire Data	Record Data	Restart
Acquisition Setup Stopping Gate:	All Events	✓ Events To Record:	10000 evt	✓ Stopping Time (see	
Storage Gate:	All Events	✓ Events To Display:	1000 evt	Flow Rate:	140
Acquisition Status Processed Events: Threshold Count:			Electronic Abort Rate Electronic Abort Cour		

9. Close upper flow cell access door.



- 10. Load a tube of Accudrop beads (1 mL of PBS + 1 drop of stock) on the sample stage
- 11. Go to Acquisition Dashboard, Click Load

Current Activity Active Tube/Well	Threshold Rate	Stopping Gate Eve	ents Elapsed	Time
Tube_001	0 evt/s	0 evt	00:00:	00
Basic Controls				
♦ Next Tube	Load	Acquire Data	Record Data	Restart

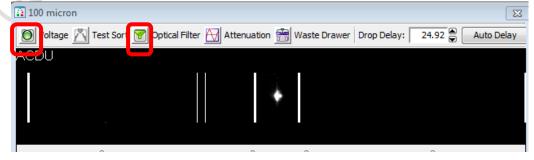
- 12. Adjust *Flow rate* if needed to obtain threshold rate constantly 1000-1500 events per sec
- 13. Go to Sort Layout window, click Sort

Precision:	Target Events:	Save Sort Repo	orts: Save Cont	flicts Index Sorting			
▼ Initial ▼	Continuous	Ask User	•				
		Right					
: Continuous							
0 evt/s			NA				
0 evt		NA					
0 evt/s		NA					
Efficiency: 0%			NA				
	Initial Continuous O evt/s O evt/s O evt/s	Initial Continuous Continuous Oevt/s O evt/s O evt/s	Initial Continuous Ask User Right Continuous O evt/s O evt O evt/s O evt/s	Initial Continuous Ask User Ask User Right Continuous NA 0 evt/s NA 0 evt/s NA			

14. Click Cancel on the confirm window



15. Go to 100 micron window (lower monitor), Click Voltage and Optical Filter



16. Adjust Drop Delay value (starting value: 25.00) so that the reading on the left reach 100

			on <u> </u> Waste Draw	ver Drop Delay: 25.00	Auto Dela
	100.0	*	0.0		
o[]	26		0 []	0 []	
Voltage Center:		0		Plate Voltage: 3	,000 S H

17. Go to Sort Layout window, Select Precision > Fine Tune

Device:	Precision:	Target Events:		Save Sort Reports:	Save Conflicts	Index Sorting		
2 Tube	Fine Tune	- Continuous	•	Ask User	-]			
Left			R	ight				
P1	: Continuous							
	NÁ			NA				
Sort Rate:	NÁ		L		NA			
	NA NA		1		NÁ NA			
Sort Rate: Confl. Cnt: Confl. Rate:	1992201				ANGEOR C			

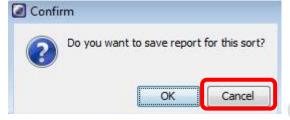
18. Adjust Drop Delay value bit by bit so that the reading on the left reach >97

Voltag	e 🖄 Test Sort 👔	👩 Optical Filter	Attenuatio	n 🗂 Waste Drawer	Drop Delay: 2	4.9	Auto Del
		98.0		2.0			
		90.0		2.0			
0]	26		0	0	0	
Volta	ge Center:				Plate Voltage:	3,000	
	2nd Drop:	17 🛢 3rd I	Drop: 7	🕄 4th Drop:	3 🛢 Phase:	0	

19. Go to Sort Layout Window, Click Sort again.

Device	Device: Precisio		1:	Target Events:		Save Sort Reports:		Save Conflicts	Index Sorting
2 Tube 👻		Initial 🔹		Continuous	✓ Ask User				
L	.eft				Rig	jht			
	P1:Cor	ntinuous							
Sort Rate:		0	evt/s		NA				
Confl. Cnt:		() evt		NA				
Confl. Rate:		0	evt/s		NA				
Efficiency:	ficiency: 0%			NA					

20. Click Cancel on the confirm window



21. Go to Acquisition Dashboard, Click Unload

Current Activity								
Active Tube/Well	Thre	shold	Rate	Stopping Gate Eve	ents	Elapsed Ti	me	
DAPI Stained Control		228 evt/s		5000 evt		00:00:19		
Basic Controls								
🛛 🖓 Next Tube		Unload	e 📃 si	Stop Acquiring		Record Data	🔞 Restart	
Acquisition Setup								
Stopping Gate:	All Events	۲	Events To Record:	5000 evt	•	Stopping Time (sec):	0	
Storage Gate:	Gate: All Events - Events To Display: 1000 evt		1000 evt	•	Flow Rate:	1.0		
Acquisition Status								
Processed Events:	2752	evt		Electronic Abort Rate	::			
Threshold Count:	3667	evt		Electronic Abort Cour	nt.			

ometry

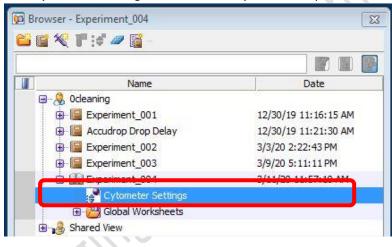
22. Return the Accudrop beads to 4 degree refrigerator

E. 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



1.3 Go to Inspector Window, highlight unwanted channels and click Delete.

arameters Threshold Ratio Con	npensation					
Parameter	Voltage	Log	A	н	W	
FSC	353					
SSC SSC	292			V		
THE	547	N.	v			
PerCP-Cy5-5	→ 483	V	V			
	1.0	(and the second s		- man		-
PE-Texas Red	414					
PE-Cy5	536	V				1.0
PE-Cy5-5	386	V				III
PE-Cy7	440	V				
APC	395	V				
Alexa Fluor 700	361	V				
APC-Cy7	368	V				
DAPI	343	V				
AmCyan	383	V				
BV605	567	V				
BV650	536	V	V			-
Add	Delete					

analieters Inreshold Ratio	Compensation						
Parameter	Voltage	Log	A	н	W		
FSC	353					~	
SSC	292	1		V	V		
THE	547	v	V		_		
PerCP-Cy5-5	483						
PE	446	V					
PE-Texas Red	414	V					
PE-Cy5	536	V					
PE-Cy5-5	386	V				E	
PE-Cy7	440	V					
APC	395	V					5
Alexa Fluor 700	361	V					
APC-Cy7	368	V					
DAPI	343	V					
AmCyan	383	V					
# BV605	567	V					
BV650	536	V				-	
Add			14	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 (for Multi-colour panel)
 - 2.1 Click Experiment > Compensation Setup > Create Compensation Control

- F	🔒 New Folder	Ctrl+N	-
Sweet 5	 New Specimen New Tube New Cytometer Settings Import Cytometer Settings 	Ctrl+E Ctrl+M Ctrl+T	Date
	Open Experiment Close Experiment	Ctrl+O Ctrl+W	12/30/19 11:16:15 AN 12/30/19 11:21:30 AN 3/3/20 2:22:43 PM 3/9/20 5:11:11 PM 3/11/20 11:57:10 AM
	Experiment Layout Compensation Setup	•	Create Compensation Controls
		v	Modify Compensation Controls Calculate Compensation

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".

Fluorophore	Label				
FITC	Generic				
PE	Generic				
PE-Cy5-5	Generic				
PE-Cy7	Generic				
APC	Generic				
Alexa Fluor 700	Generic				
APC-Cy7	Generic				
DAPI	Generic				
• AmCyan	Generic				
BV605	Generic				
e BV711	Generic	-			

2.3 Click OK

Fluorophore	Label	1		
* FITC	Generic			
• PE	Generic			
• PE-Cy5-5	Generic	-		
PE-Cy7	Generic			
# APC	Generic			
Alexa Fluor 700	Generic	2		
# APC-Cy7	Generic	-		
• DAPI	Generic			
• AmCyan	Generic			
# BV605	Generic	3		
• BV711	Generic			

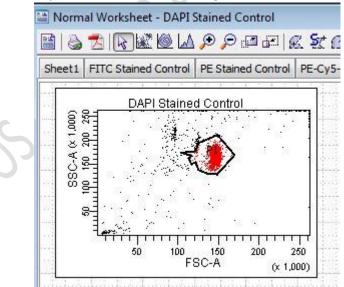
	Experiment_004 Gobal Worksheets Compensation Controls	3/11/20 11:57:10 AM	
	Cytometer Settings Cytometer Settings FITC Stained Control P Cytometer Settings P FITC Stained Control P PE-Cy5-5 Stained Control		
2.5	Click the tube pointer of the first tube		ا ح <i>ر</i>
	Cytometer Settings Compensation Controls Cytometer Settings Cytometer Settings Cytometer Settings	3/11/20 11:57:10 AM	P
	💌 😨 🗊 FITC Stained Control		

2.4 Expand the Compensation Control Specimen

- 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 Stained Control"
- 2.7 Go to Acquisition Dashboard, Click Load.

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

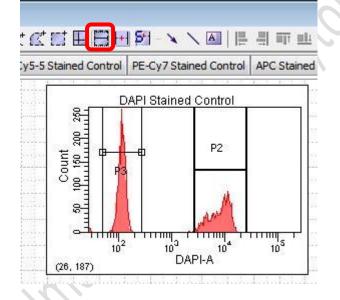
🖶 🗍 PE-Cv7 Stained Control



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

Status	Parameters	Threshold Laser	Compensation	Ratio				
Pa	arameter		Voltage	Log	A	н	W	
• FS	С		353					
. 55	C		292					
• 70	rc.		347					
· PE			440					
· PE	-Cy5-5		386					
· PE	-Cy7		440	1			(M)	
• AP	C		395	V				
• Ale	exa Fluor 700		361	V				
• AP	C-Cy7		368					
_								

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



2.12 Go to Acquisition Dashboard, Click Unload. *DO NOT Record Data at this point

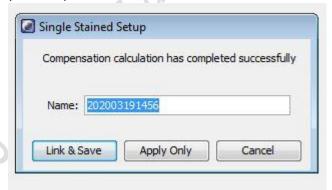
Current Activity Active Tube/Well	Thres	hold Rate		Stopping Gate Event	s Ela	psed Time	
DAPI Stained Co	ontrol 228	228 evt/s		5000 evt	00	00:00:19	
Basic Controls							
🛛 🖓 Next Tube		Unload	Stop .	Acquiring	Record Data		🙆 Restart
Acquisition Setup			1				
Stopping Gate:	All Events	✓ Events To F	lecord:	5000 evt	✓ Stopping Time (se	c):	0
Storage Gate:	All Events	✓ Events To D)isplay:	1000 evt	✓ Flow Rate:		1.0
Acquisition Status							
Processed Events:	2752 e	vt	1	Electronic Abort Rate:			
Threshold Count:	3667 e	out	1	Electronic Abort Count:			

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

- 2.14 After optimising the PMT voltage of ALL the fluorescence channel, load each single stained control and click *Record Data* for ALL single stain controls
- 2.15 Click *Experiment > Compensation Setup > Calculate Compensation*

FACSDiva	Soft	ware - Ocleaning (Fus	ion 100u 488, 561	, 640	, 405 (B3-YG5-R3-V6))
dit View (Expe	riment Populations	Worksheet Cytor	mete	r Sort Help
⊇₩₽		New Folder	Ctrl+N		-
100 micro		New Experiment	Ctrl+E		5
ml	×	New Specimen	Ctrl+M		2
Stream	\mathbf{I}^{n}_{-}	New Tube	Ctrl+T		
Sweet S	;€	New Cytometer Set	tings		
		Import Cytometer S	ettings		Date
		New Global Worksh	eet		12/30/19 11:16:15 AM
		Open Experiment	Ctrl+0	y	12/30/19 11:21:30 AM
		Close Experiment	Ctrl+W		3/3/20 2:22:43 PM
		crose experiment	Currw		3/9/20 5:11:11 PM
		Experiment Layout	-	ngs	3/19/20 2:43:59 PM
•		Compensation Setu	•		Create Compensation Controls
`			Gobar She		Modify Compensation Controls
-			🔛 Analys 🌌 Sort Li		Calculate Compensation
•			🥔 Sort Lay	/out_	002

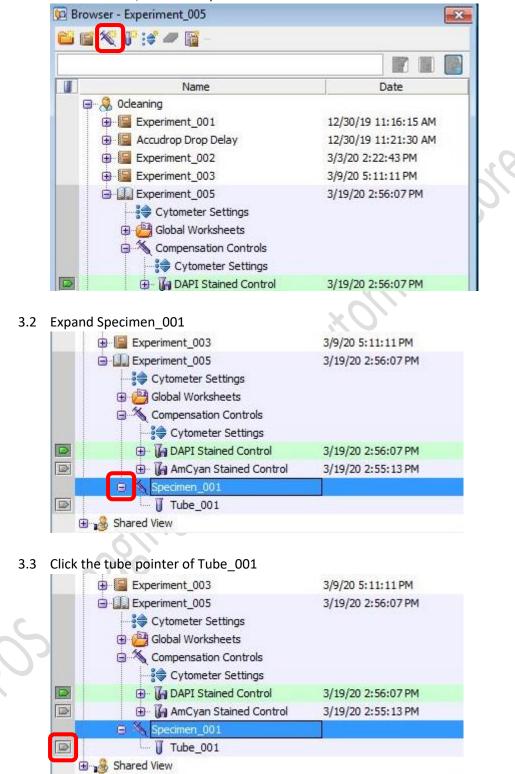
2.16 Click *Link and Save* for the most stringent practice, i.e. cannot adjust PMT voltage anymore **OR** Click *Apply Only* for some flexibility on PMT voltage adjustment of your samples.



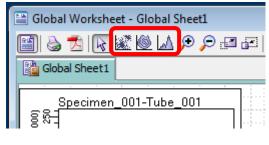
2.17 Switch Normal worksheet to Global worksheet



- 3. Setup Plots and Tables
 - 3.1 Go to Browser, Click New Specimen icon

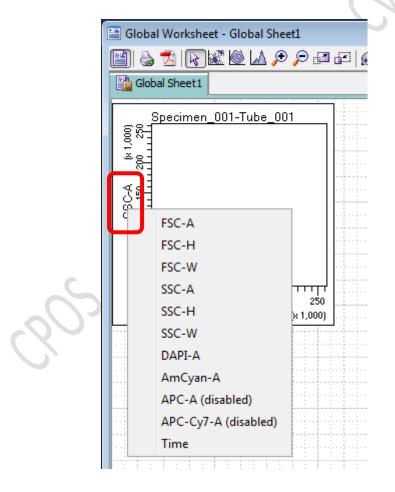


3.4 Go to Global Sheet Window, Click the graph type icon



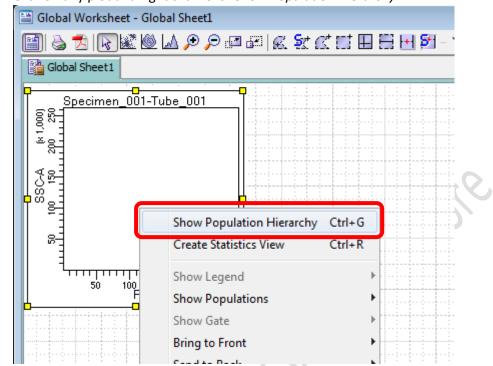
lcon	Туре
*	Dot Plot
	Contour Plot
	Histogram

- 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.



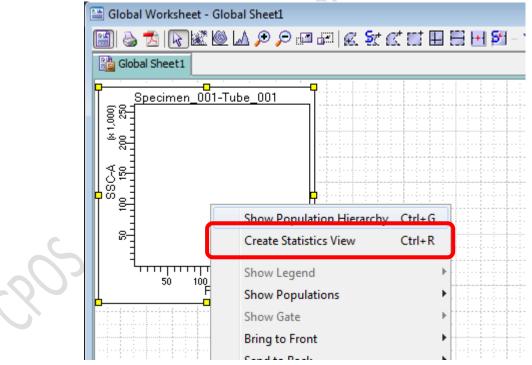
3.7 Repeat step 3.4 – 3.6 until all plots needed is created.

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

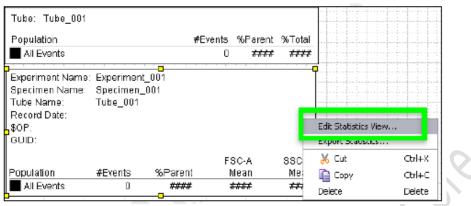


3.8 Click on any plot and right click. Click Show Population Hierarchy

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



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



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



205. Mare

4. Sample Acquisition

4.1 Load your sample on the sample stage

4.2 Go to Acquisition Dashboard, Click Load

				×
Threshold Rate	Stopping Gate E	Events	Elapsed Tir	ne
0 evt/s	0 evt		00:01:34	l I
Load	Acquire Data	📕 Re	cord Data	Restart
~~~				
✓ Events	To Record: 10000 evt	✓ Sto	pping Time (sec):	0 🗣
Events	To Display: 1000 evt	▼ Flow	w Rate:	2.0
13433 evt	Electronic Abort R	ate:		
25095 evt	Electronic Abort C	ount:	10 evt	
	■ Load ■ Events Events ■ Events 13433 evt	O evt/s     O evt     Load     Acquire Data     Events To Record:     10000 evt     1000 evt     13433 evt	0 evt/s 0 evt	O evt/s     O evt     O0:01:34       Load     Acquire Data     Record Data       • Events To Record:     10000 evt     Stopping Time (sec):       Events     Events To Display:     1000 evt     Flow Rate:       13433 evt     Electronic Abort Rate:

4.3 When you start seeing dots appear on the plot, Go to Cytometry window and Click *Parameters* 

ta	tus Parameters hreshold Laser C	Compensation	Ratio				
	Parameter	Voltage	Log	A	н	W	1
•	FSC	179					
•	SSC	240					
•	DAPI	343					
	AmCyan	383					

#### 4.4 Adjust PMT Voltage accordingly

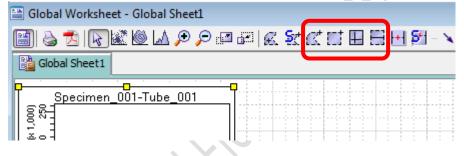
Status Parameters Threshold	Laser Compensation	Ratio				
Parameter	Voltage	Log	A	н	W	
• FSC	179	St 🗉		<b>V</b>		1.
• SSC	240			1		
• DAPI	343					
• AmCyan	383	<b>V</b>	V		(E)	

Channel	Suggested Voltage range for mammalian cells
FSC	180-300
	*If you sample cell size is too big to visualise with FSC voltage 180, you may change the FSC ND filter
	from 1.5 to 2.0
SSC	230-330
Fluorescence	300-850

4.5 Adjust Flow rate if needed (optimum Threshold rate 2000 – 5000 evt/s)

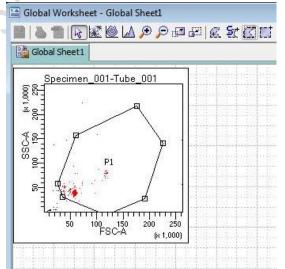
Acquisition Dashboar	u				E
Current Activity					
Active Tube/Well	Threshol	d Rate	Stopping Gate Events	s Elapsed T	ime
Tube_00	1 0 evt/s		0 evt	00:01:3	4
Basic Controls					
¢∬ Next Tube	Loa	ad D	Acquire Data	Record Data	Restart
Acquisition Setup					
Stopping Gate:	P2	✓ Events To Record:	10000 evt	<ul> <li>Stopping Time (sec):</li> </ul>	0
Storage Gate:	All Events	✓ Events To Display:	1000 evt	Flow Rate:	2.0
Acquisition Status					
Processed Events:	13433 evt		Electronic Abort Rate:		
Threshold Count:	25095 evt		Electronic Abort Count:	10 evt	

- * If you perform sorting, DO NOT set flow rate > 5.0 or threshold rate > 5000 event/s
- 5. Create Gates
  - 5.1 Go to Global Sheet Window, Click the type of gate needed

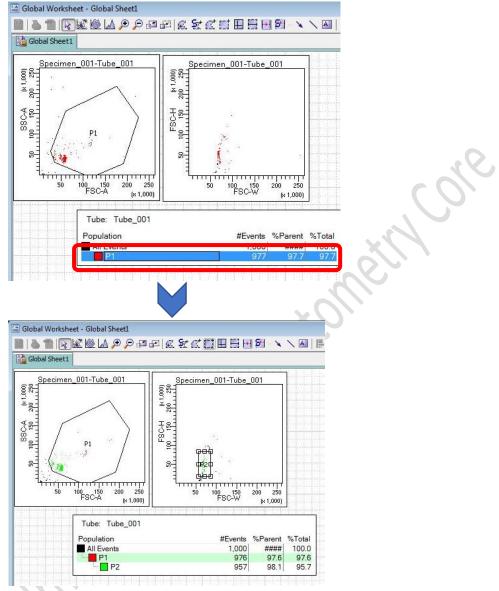


lcon	Туре
K	Polygon Area Gate
	Rectangle Area Gate
	Quantrad Gate
	Interval Gate

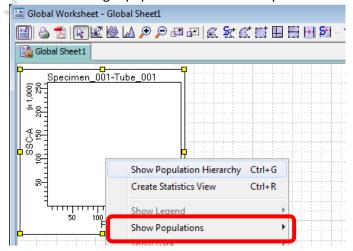
5.2 Draw the gate on the plot of interest to gate out target cluster /peak



5.3 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.



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



#### 6. Data Recording

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

Current Activity					
Active Tube/Well	Threshold Rate		Stopping Gate Event	ts Elapsed T	ïme
Tube_001	0 evt/s		0 evt	00:01:3	4
Basic Controls					
الَّ	Load	A	cquire Data	Record Data	Restart
Acquisition Setup					
Stopping Gate:	P2 👻 vents 1	Fo Record:	10000 evt	✓ Stopping Time (sec):	0
Storage Gate:	All Events	Fo Display:	1000 evt	▼ Flow Rate:	2.0
Acquisition Status					
Processed Events:	13433 evt		Electronic Abort Rate:		
Threshold Count:	25095 evt		Electronic Abort Count:	10 evt	

6.2 Set the Storage gate to All Events

Active Tube/Well	Threshold Rate		Stopping Gate Events	Elapsed Ti	ime
Tube_001	0 evt/s		0 evt	00:01:3	4
Basic Controls					
¢∏ Next Tube	Load	Ac	quire Data	Record Data	Restart
Acquisition Setup					
topping Gate:	P2	To Record:	10000 evt	<ul> <li>Stopping Time (sec):</li> </ul>	0
itorage Gate:	All Events 👻 vents	To Display:	1000 evt	▼ Flow Rate:	2.0
			Electronic Abort Rate:		
Acquisition Status	13433 evt				

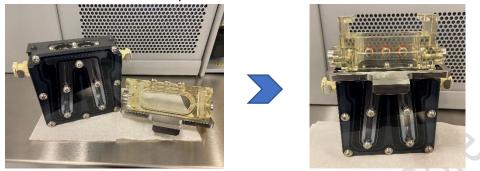
6.3 Set Events To Record, i.e. events number out of stopping gate to be recorded (suggested number 10,000 – 50,000)

Current Activity						
Active Tube/Well	Threshol	d Rate	Sto	pping Gate Event	s Elap	sed Time
Tube_001	0 evt/s		0 6	evt	00:	01:34
Basic Controls						
ø <b>∏</b> Next Tube	Loa	ad	Acquire (	Data	Record Data	Restart
Acquisition Setup				C		
Stopping Gate:	P2	· Events To Rec	ord:	10000 evt	• Sopping Time (sec	):
Storage Gate:	All Events	Events To Disp	blay:	1000 evt	✓ Flow Rate:	2.
Acquisition Status						
Processed Events:	13433 evt		Elec	tronic Abort Rate:		
riocesses events.						

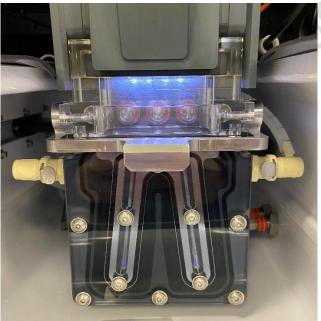
- 6.4 if the sample is Unload or Acquisition is stopped, Click Load or Acquire Data
- 6.5 Click Record Data
- 6.6 Click Next Tube to create a new sample

### F. Sort Device Alignment

- 1. Tube Holder
  - 1.1 Assemble the tube holder as picture below

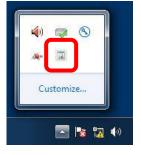


- 1.2 Put dummy tubes into the position
- 1.3 Slide the tube holder right under the sort chamber. Plug in water tubing if cooling is needed.



1.4 Click *Select Device* icon on the lower right corner of the upper monitor





1.5 Select collection device of interest



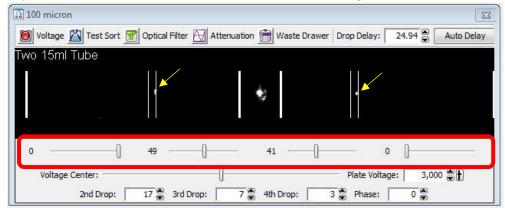
1.6 Go to 100 micron window (lower monitor). Adjust the Slider

Device	Suggested Slider reading
2-tube 15 ml	0-49-41-0
4-tube 1.5ml / 2.0ml	71 - 30 - 25 - 68
4-tube 5 ml	80 - 30 - 25 - 74

1.7 Click Voltage. Wait 2 seconds and then Click Test Sort.

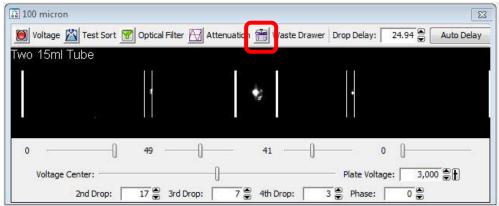
100	) micron				23
	A STATE OF	Optical Filter	Attenuation 🗂 Waste Drav	wer Drop Delay: 24.94	Auto Delay
TWO	15mi rupe				
18		•	÷	•	
Ľ		bil bi	s I a d	l _{as} ta	
0		49	41[	) o [}	
	Voltage Center:		-0	Plate Voltage:	3,000 🛢 🚹
	2nd Drop:	17 🔵 3rd Drop:	7 🝧 4th Drop:	3 🛢 Phase: 0	

#### 1.8 Adjust the slider so the side stream dot lines within the target lines

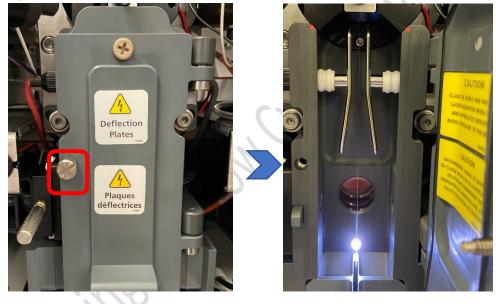


-Ort

1.9 Click Waste Drawer



1.10 Open the sort chamber door



- 1.11 Check if the side streams can entre the dummy tubes. Adjust the slider if needed
- 1.12 Click Waste Drawer and then Voltage

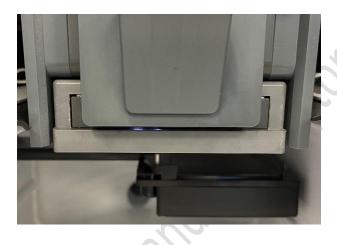
100 micron				
🔘 /oltage 脳 Test Sort	Optical Filter	🗑 Attenuation 🗂 Vaste D	rawer Drop Delay: 24.94 曼 🗛	uto Dela
Two 15ml Tube				
1				
- U	50 K-	#		
0]	49		-[ 0 [	
Voltage Center:		0	Plate Voltage: 3,000	Ð
2nd Drop:	17 🛢 3rd Dro	op: 7 🚔 4th Drop:	3 🖨 Phase: 0	

- 1.13 Close the sort chamber door
- 1.14 Slide out the tube holder and Put collection tubes into position

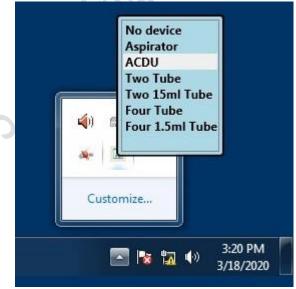
- 2. ACDU (Culture plate)
  - 2.1 Slide the ACDU adaptor right under the sort chamber



ACDU adaptor



2.2 Select the device (ACDU) on the lower right corner of the upper monitor



2.3 Go to 100 micron window (lower monitor). Adjust the Slider

Device	Suggested Slider reading
96-well plate	33-0-0-0

etry CO.

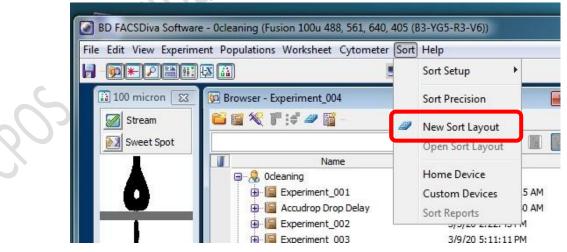
100 micron			8
🔘 'oltage 📓 Test Sort 👔	Optical Filter 🔛 A	ttenuation 🗂 Waste Dra	wer Drop Delay: 24.94 🕤 Auto Delay
ACDU			
	61 E		
33	0	o [	0 []
Voltage Center:		0	Plate Voltage: 3,000 🕃 🛉
2nd Drop:	17 💭 3rd Drop:	7 🔮 4th Drop:	3 🖨 Phase: 0 🛎

2.4 Click Voltage. Wait 2 seconds and then Click Test Sort

2.5 Adjust the slider so the side stream dot lines within the target lines

100 micron	🐨 Optical Filter 🔛 Attenuation 醟 Waste Drawer Drop Delay: 24.94 🔮	Auto Dela
33	0	
Voltage Center:	Plate Voltage: 3	,000 🕃 🕇
2nd Drop:	17 💭 3rd Drop: 7 💭 4th Drop: 3 💭 Phase: 0	

- 2.6 Click Voltage again to stop test sort
- 2.7 Click Sort > New Sort Layout



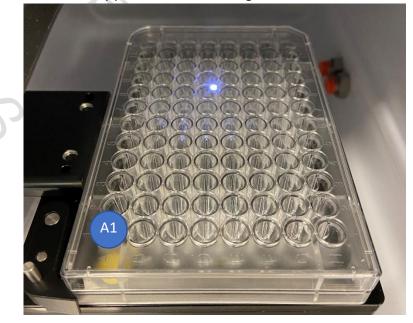
Devi	ce:	Precision:		Target Events:		Save Sort Report	s:	Save Conflicts	Index Sorting
2 Tube	•	4-Way Purity	•	Continuous	•	Ask User	•		
	Left				Rig	pht			
Sort Rate:		NA					1	NA	
Confl. Cnt:	[	NA	9		1		1	NA	
Confl. Rate:		NA	(				1	NA	
Efficiency:		NA	0	_			3	NA	

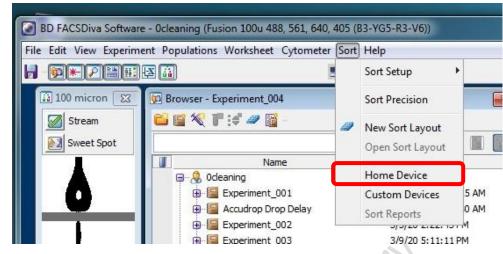
2.8 Click the *Eject* button on the Sort Layout window

2.9 Plug in water tubing if cooling of the ACDU stage is needed



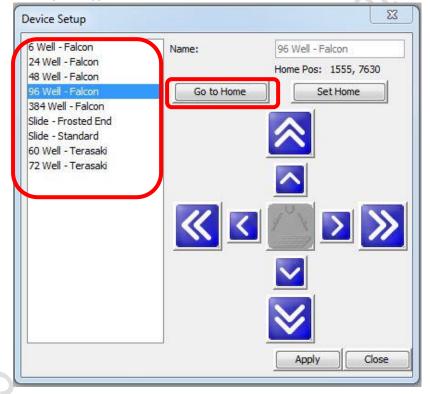
2.10 Load a dummy plate on the ACDU stage with A1 on the outer left corner



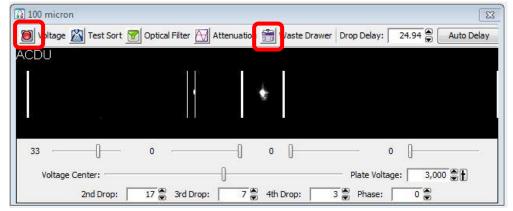


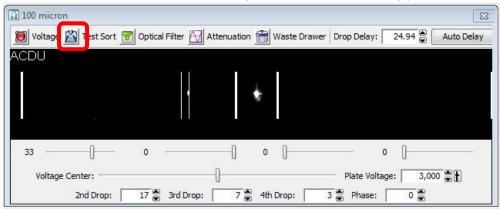
2.11 Click Sort > Home Device





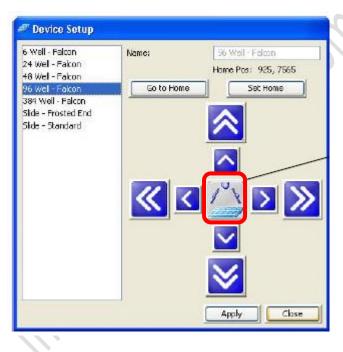
2.13 Go to 100 micron window (lower monitor), Click Voltage and Waste Drawer





2.14 Double Click Test sort to shot a small drop of sheath on the dummy plate cover





- Device Setup 6 Well - Falcon 56 Well - Falcon Name: 24 Well - Falcon Home Pos: 925, 7565 48 Wel - Fakon Go to Home Set Home 96 Wel - Fakon 384 Well - Falcon Side - Frosted End in Side - Standard > » left right out Close Apply
- 2.15 Move the stage accordingly in order to shot the drop of sheath on A1 position

2.16 Go to Home Device Window, Click Set Home and Apply

6 Well - Falcon 24 Well - Falcon	Name:	96 Well - Falcon
48 Wel - Fakon		Home Bass 935, 755
96 Wel - Fakon	Go to Home	Set Home
384 Well - Falcon		
Side - Frosted End Side - Standard		
pros promoting		
	$\ll$	
		$\mathbf{i}$
		Apply Ck

2.17 Remove Dummy plate from the stage and Load the collection plate

# G. Sort Setup

1. Click Sort > *New Sort Layout* 

File Edit View Experime	ent Populations Worksheet Cytometer	Sort	Help	
	¥ 🚻		Sort Setup	•
100 micron 🔀	Discover - Experiment_004		Sort Precision	
Stream	E E X F :# # E -	2	New Sort Layout	
6	Name       Image: Constraint of the second seco		Home Device Custom Devices Sort Reports	5 AM 0 AM
	⊕ 🔚 Experiment_002 ⊕ 🔚 Experiment 003		3/9/20 5:11:1:	

# 2. Select appropriate *Device*:

Device:	Precision:	Target Events:	Save Sort Reports:	Save Conflicts	Index Sorting
2 Tube	🔹 🖣 4-Way Purity 👻	Continuous 👻	Ask User 🗸		
Left		Righ	t		
					÷
Sort Rate:	NA			NA	
Confl. Cnt:	NA		1	NA	
Confl. Rate:	NA			NA	
Efficiency:	NA			NA	

Name	Supported Device			
2 tube	2-way 15 mL, 5.0 mL, 2.0 mL, 1.5mL			
4 tube	4-way 5.0 mL, 2.0 mL, 1.5 mL			
96-well Falcon	96-well culture plate			
200				

# 3. Select appropriate *Precision*:

Device:	Precision:	Target Events:		Save Sort Reports:	Sa	ve Conflicts	Index Sorting
2 Tube	👻 🖣 🕂 🗣 🗣 🗣	Continuous	•	Ask User	•		
Left		F	Righ	t			
							1
Sort Rate:	NA				NA		
	NA NA				NA NA		
Sort Rate: Confl. Cnt: Confl. Rate:							

Name	Suitable Application
Purity	Sorting target population higher than 20%
Yield	Sorting target population less than 20%
Single Cell	Single cell sorting into 96-well plate / Single cell sequencing
4-way Purity	4-way sorting target population higher than 20%

- Global Sheet1: Sort Layout_001 X Device: Precision: Target Events: Save Sort Reports: Save Conflicts Index Sorting 2 Tube -Continuous Ask User 100 --Left Right 1 P1 Add Delete P2 NA NA Sort Rate: P3 Confl. Cnt: NA Clear All NA P4 Confl. Rate: NA NA Efficiency: NA NA Sort Pause View Counters
- 4. Assign target population to position by *clicking the position > Add > Target gate*

5. Input Target Events (sorting will stop when the sorted cell number reached the target event) for each target population if needed.

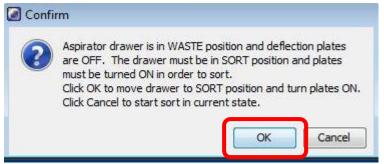
Precision:	Target Events:	Save Sort Reports:	Save Conflicts	Index Sorting		
🔹 🛛 4-Way Purity 🔹	10,000,000 -	Ask User 👻				
	Rigi	nt				
10000000				\$		
NA		NA				
NA		NA				
NA		NA				
NA			NA			
	[4-Way Purity     [0000000     NA     NA     NA     NA	4-Way Purity     10,000,000     Rig      NA     NA     NA     NA     NA	Alght     Ask User     Ask	4-Way Purity     10,000,000     Ask User     Image: Constraint of the second		

Select *Continuous* for unlimited number.

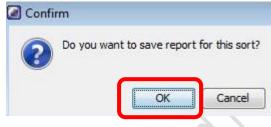
- 6. Load your Sample onto the sample stage.
- 7. Go to Acquisition Dashboard window, click Load
- 8. Go to Sort layout window, Click Sort

Device:		Precision:	Target Events:		Save Sort Reports:	Save Conflicts	Index Sorting
2 Tube	•	4-Way Purity 🗸 🗸	10,000,000	¥	Ask User 🔫		
Lef	:			Righ	nt		
	1:100	00000					
Sort Rate:		NA				NA	
Confl. Cnt:		NA		2		NA	
Confl. Rate:		NA				NA	
Efficiency:		NA		1		NA	

9. Click OK on Confirm window



- 10. During the sort keep monitoring Threshold Rate and Drop 1 value
- 11. Click *Pause* if you wish to pause the sort and replace new collection tube. Click *Resume* after finish replacement.
- 12. Click Sort to end the sort
- 13. Click OK to save sort report

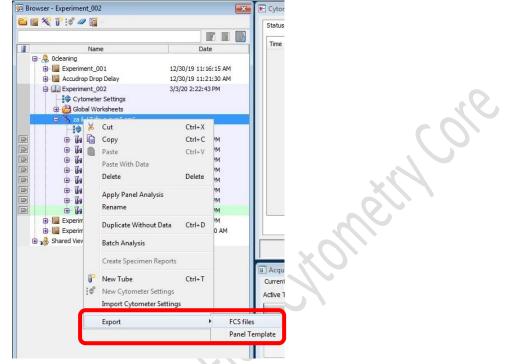


14. Go to Acquisition Dashboard window, click Unload.

Active Tube/Well	Threshold	Rate	Stopping Gate Events	Elapsed Ti	me
Tube_001	0 evt/s		0 evt	00:00:00	1
Basic Controls					
<b>∳</b> Next Tube	🗾 Unioa	ad 🗾 St	op Acquiring	Record Data	🎯 Restart
Acquisition Setup					n
topping Gate:	All Events	Events To Record:	10000 evt 👻	Stopping Time (sec):	0
torage Gate:	All Events	• Events To Display:	1000 evt 🗸	Flow Rate:	1.0
Acquisition Status					
rocessed Events:			Electronic Abort Rate:		
Inreshold Count:			Electronic Abort Count:		

#### H. 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

File Version				
	FCS3.1			
Parameter	Parameter Type			
1.30 A		Orog		-
FSC-H	Linear	🕐 Log	None	10
FSC-W	Linear	🕐 Log	🔘 None	
SSC-A	Iinear	🕐 Log	None	
SSC-H	Linear     Lin	🕐 Log	None	
SSC-W	Inear	🕑 Log	None	
FITC-A	Iinear	🖱 Log	None	
DAPI-A	Linear	🔘 Log	🔘 None	
APC-Cy7-A	Linear	🔿 Log	🔘 None	
PE-Cy7-A	Linear     Lin	🕐 Log	None	
Alexa Fluor 700-A	Linear     Lin	🕐 Log	🔘 None	
Time	(inear)	0100	None	

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

irectory F	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

Brow	ser - Accudrop Drop Delay	
<u>e</u>	🌂 🧊 😂 🜌 🖥 –	
J	Name	Date
9	🚴 Ocleaning	
	🖶 📔 Experiment_001	12/30/19 11:16:15 AM
	🖨 🛄 Accudrop Drop Delay	12/30/19 11:21:30 AM
	😑 🌂 Specimen_001	
	⊞ 🔰 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.

Browse	er - Experiment	.002				💽 🗶 Cyt
🛍 📑 🖄	C 🗗 :# 🥔	<b>1</b>				Stat
II.	1	lame		Dat	e	Tim
	Ocleaning					
	Experimen			/30/19 11:1		
	Accudrop			/30/19 11:2		
	Experimen	t_002 eter Settings		3/20 2:22:43	SPM	
	Global					
	🖯 🖂 za i	· · · · · · · ·				
	÷.	6 Cut		Ctrl+X		
		👌 Сору		Ctrl+C	PM	
	🕀 🚺 🚺	Paste		Ctrl+V	PM	
	· ·	Paste W	ith Data		PM	
	⊕- <b>6</b> a	Delete		Delete	PM PM	
					PM	
	• <b>1</b>		anel Analysis		PM	
	😐 🐻	Rename			PM	
	Experim	Duplicat	e Without Data	Ctrl+D	M D AM	
	Shared Viev	Batch A	nalysis			
		Create 5	pecimen kepons		1	
		New Tul	he	Ctrl+T		Si Aco Curre

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

2.4 Check the boxes as picture belo	2.4	Check the	boxes as	picture	belov
-------------------------------------	-----	-----------	----------	---------	-------

<ul> <li>Auto</li> <li>View Time:</li> <li>Manual</li> </ul>	10 🔻	Output To Printer  Save as PDF  Save as XML  Add Report	and the second	Biexponentia	al Scales Il Worksheet
PDF Filename:	t_003-8	atch_Analysis_19032020	143013.pdf	Browse	View PDF
(ML Filename;	t_003-8	atch_Analysis_19032020	143013.xml	Browse	
Stats Filename:	t_003-E	atch_Analysis_19032020	143013.csv	Browse	]
Status:		09	10		_

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

	and the second second	
_003-Batch_Analysis_19032020143013.pdf	Browse	View PDF
_003-Batch_Analysis_19032020143013.xml	Browse	
_003-Batch_Analysis_19032020143013.csv	Browse	
0%		
	Save as XML       ✓ Use Provide ProvideProvide Provide Provide Provide ProvideProvideProvide	Save as XML       ✓ Use Preferred Global W         ✓ Add Report         _003-Batch_Analysis_19032020143013.pdf       Browse         _003-Batch_Analysis_19032020143013.xml       Browse         _003-Batch_Analysis_19032020143013.csv       Browse

2.6 Click Start and then OK.

<ul> <li>Auto</li> <li>View Time:</li> <li>Manual</li> </ul>		Biexponentia	al Scales al Worksheet	
PDF Filename:	t_003-Batch_Analysis_19032020143013.pdf	Browse	View PDF	Confirm
XML Filename:	t_003-Batch_Analysis_19032020143013.xml	Browse		Below tubes do not have preferred global wo Tube 001
Stats Filename:	t_003-Batch_Analysis_19032020143013.csv	Browse	j	Tube_002
Status:	0%			Tube_003 Would you like to continue?

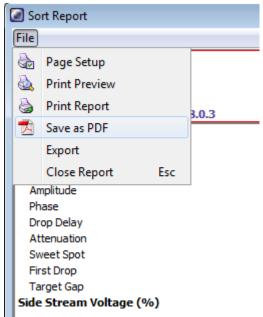
- 3. Sort Report
  - 3.1 To export sort report, select Sort Layout and then right click. Click Sort Reports

😥 Browser - Experiment_002			
🖴 🗃 餐 T 😥 🖉 -			
Name		Da	
🗐 🧏 Ocleaning			
🕀 🔚 Experiment_001		12/30/19 11:	
🕀 🔚 Accudrop Drop Dela	y	12/30/19 11:	
Experiment_002		3/3/20 2:22:4	
Cytometer Settin	ngs		
🖨 🔁 Global Workshee			$(\mathcal{O})$
🖻 🛗 Global Sheet			
🔛 Analysis			
Sort Law			
😟 🔬 🕺 za il-17 ifn- 🥇	Cut	Ctrl+X	
🕀 🔚 Experiment_00	Сору	Ctrl+C	
Experiment_00	Paste	Ctrl+V	
⊕ · · · · · Experiment_00 ⊕ · · · · · · · · · · · · · · · · · · ·	Delete	Delete	
	Rename		
	Open Sort La	ayout	
	Sort Reports		

3.2 Select the sort from the list and then click OK

Mon Feb	24	16:1	9:54	CST	2020
Mon Feb	24	16:1	2:55	CST	2020
Mon Feb	24	16:0	2:03	CST	2020
Mon Feb	24	15:5	3:55	CST	2020
Mon Feb	24	15:4	9:12	CST	2020
Mon Feb	24	15:3	4:06	CST	2020
Mon Feb	24	15:2	6:05	CST	2020
Mon Feb	24	15:2	2:46	CST	2020
Mon Feb	74	15.1	6.00	CST	2020

3.3 Click File > Save As PDF



3.4 Choose the destination (D:/User/Department/PersonalFolder) and click Save

end integration

#### I. Cleaning

1. Go to Browser window, click the tube pointer of any tube

Name	Date
🗐 😓 Odeaning	
🕀 📔 Experiment_001	12/30/19 11:16:15 AM
<ul> <li>Accudrop Drop Delay</li> <li>Cytometer Settings</li> <li>Specimen_001</li> </ul>	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. Load a tube of 2 mL of cleaning solution No. 1 (FACSClean) on the sample stage
- 3. Go to Acquisition Dashboard window, set *Flow rate* to 11.0

Active Tube/Well	Threshold Rate	Stopping Gate Eve	ents Elapsed 1	lime	
	0 evt/s	0 evt	00:00:0	00:00:00	
Basic Controls					
<b>≫</b> Next Tube	Load	Acquire Data	Record Data	Restart	
Acquisition Setup					
topping Gate:	<ul> <li>Events To Record</li> </ul>	1: 5000 evt	Stopping Time (sec):	0 8	
torage Gate:	👻 Events To Display	r: 1000 evt	Flow Rate:	11.0	
Acquisition Status					
rocessed Events:		Electronic Abort Rate	:		
hreshold Count:		Electronic Abort Cour	nt:		

4. Click Load.

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		L	201
	Load	Acquire Data	Record Data

5. Acquire the solution for **5 minutes**.

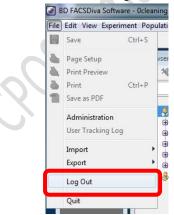
6. Click Unload.

Current Activity Active Tube/Well	Threshold	Rate	Stopping Gate Events	Elapsed Ti	me
Tube_001	0 evt/s		0 evt	00:00:07	1
Basic Controls					
<b>≫</b> Next Tube	🗾 Unioa	d 📃 s	top Acquiring	Record Data	🕐 Restart
Acquisition Setup					
Stopping Gate:	All Events 🗸	Events To Record:	10000 evt 👻	Stopping Time (sec):	0
Storage Gate:	All Events 👻	Events To Display:	1000 evt 🗸	Flow Rate:	1.0
Acquisition Status					
Processed Events:			Electronic Abort Rate:		
Threshold Count:			Electronic Abort Count:		

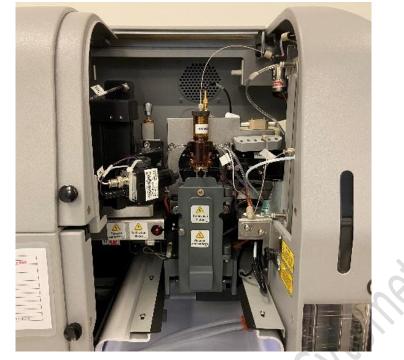
- 7. Load a tube of 2 mL of cleaning solution No.2 (FACSRinse) on the sample stage
- 8. Repeat step 3-5.
- 9. Load a tube of 2 mL of cleaning solution No.3 (MilliQ water) on the sample stage
- 10. Repeat step 3-5.
- J. User Logout
  - 1. TURN OFF the Sweet Spot



2. Click File > Logout



3. Open the upper flow cell access door of the system before you leave.



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