BD FACSDiva Compensation Protocol

- 1. **Click Experiment > Compensation Setup > Create Compensation Control** BD FACSDiva Software - Ocleaning (Fusion 100u 488, 561, 640, 405 (B3-YG5-R3-V6)) ile Edit View Experiment Populations Worksheet Cytometer Sort Help - 🗊 🕶 🔎 New Folder Ctrl+N 32 1 New Experiment Ctrl+E 100 micro New Specimen Ctrl+M 🐹 Stream New Tube Ctrl+T Sweet S Br) New Cytometer Settings Date Import Cytometer Settings New Global Worksheet F 12/30/19 11:16:15 AM 12/30/19 11:21:30 AM Open Experiment Ctrl+O 3/3/20 2:22:43 PM **Close Experiment** Ctrl+W 3/9/20 5:11:11 PM 3/11/20 11:57:10 AM **Experiment Layout Compensation Setup** . **Create Compensation Controls** Modify Compensation Controls Calculate Compensation
- 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	
• BV711	Generic	

3. Click **OK**

4.

5.

Fluorophore	Label		1
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		
- PV711			
Add Delete Lab	IGeneric oels OK ontrol Specimen A 3/11/20 11:57:10 A Gettings	Cancel	
Add Delete Lab	IGeneric oels OK ontrol Specimen A 3/11/20 11:57:10 A Settings sheets on Controls	Cancel	
Add Delete Lat	IGeneric Dels OK OK OK OK OK OK OK OK OK OK	Cancel	
Add Delete Lat and the Compensation Co Experiment_004 Cytometer S Global Work Compensation Cytometer S Cytometer S Cytometer S Cytometer S Cytometer S	IGeneric Dels OK OK OK OK OK OK OK OK OK OK	Cancel	
Add Delete Lab	IGeneric Dels OK OK OK OK OK OK OK OK OK OK	Cancel	
Add Delete Lat and the Compensation Co Experiment_004 Cytometer S Global Work Cytometer S Cytometer Cytome	IGeneric Dels OK OK OK OK OK OK OK OK OK OK	Cancel	
Add Delete Lab	IGeneric Dels OK OK OK OK OK OK OK OK OK OK	Cancel	
Add Delete Lab Delete State Cytometer State Delete State Delete State Delete Lab Delete State Delete State Delet	IGeneric Dels OK OK OK OK OK OK OK OK OK OK	M	
Add Delete Lab Delete State Cytometer State Cytometer State Delete State Delete Lab Delete State Delete	IGeneric Dels OK OK OK OK OK OK OK OK OK OK	M M	
Add Delete Lab Delete Service Ser	IGeneric Dels OK OK OK OK OK OK OK OK OK OK	M	
Add Delete Lab	IGeneric Dels OK OK OK OK OK OK OK OK OK OK	M	
Add Delete Lab Delete Lab Delete Lab Delete Lab Delete Lab Delete Lab Delete Lab Delete Lab Delete Lab Delete Lab Cytometer S Cytometer S Delete Lab Cytometer S Delete Lab Cytometer S Delete Lab Delete Lab Cytometer S Delete Lab Delete Lab Delete Lab Delete Lab Delete Lab Delete Lab Delete Lab Delete S Delete Lab Delete S Delete Lab Delete S Delete Lab Delete S Delete Lab Delete S Delete S Delete Lab Delete S Delete S Delet	IGeneric Dels OK OK OK OK OK OK OK OK OK OK	M	

- 6. Load the single stained controls according to the tube label, i.e. run FITC single stain when the tube pointer is pointing at "FITC Stained Control"
- 7. Go to Acquisition Dashboard, Click Acquire Data or Load in AriaSORP or Fusion.

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



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

atus Parameters Threshold I	aser Compensation	Ratio				
Parameter	Voltage	Log	A	н	W	
FSC	353					
SSC	292			V		
FIIC	347	t) 🛛				
PE	110					
PE-Cy5-5	386					
PE-Cy7	440					
APC	395					
Alexa Fluor 700	361	1				
APC-Cy7	368	V				

- 10. Move the interval gate (P2) to include the positive peak
- 11. Use interval gate to gate out negative peak (P3) if there is no separate unstained control.



12. Go to Acquisition Dashboard, Click Stop Acquiring or Unload in AriaSORP or Fusion.

Acquisition Dashb	oard						
Current Activity							
Active Tube/Well		Threshold	Rate	Stopping Gate Ev	ents	Elapsed Ti	me
DAPI Staine	d Control	228 evt/	s	5000 evt		00:00:1	9
Basic Controls	C						
⇒ <mark>]</mark> Next Tub	e	📕 Unloa	d 📕 s	top Acquiring		Record Data	🔞 Restart
Acquisition Setup							
Stopping Gate:	All Eve	ents 👻	Events To Record:	5000 evt	•	Stopping Time (sec):	0 🛢 🕇
Storage Gate:	All Eve	ents 👻	Events To Display:	1000 evt	•	Flow Rate:	1.0
Acquisition Status		2752		Electronic Abort Rat	e:		
Acquisition Status Processed Events:		2/52 evt					

*DO NOT Record Data at this point

- 13. Repeat step 5 12 with all the single stained controls.
- 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
- 15. Click Experiment > Compensation Setup > Calculate Compensation

Soft	ware - Ocleaning (Fusion 1	00u 488, 561,	640), 405 (B3-YG5-R3-V6))
Expe	riment Populations Work	csheet Cytor	nete	r Sort Help
	New Folder	Ctrl+N		-
	New Experiment	Ctrl+E		
×	New Specimen	Ctrl+M		
\mathbb{T}^n	New Tube	Ctrl+T		
÷¢" ∣	New Cytometer Settings			
	Import Cytometer Setting	Is		Date
	New Global Worksheet			12/30/19 11:16:15 AM
	Open Experiment	Ctrl+O	y	12/30/19 11:21:30 AM
	Close Experiment	Ctrl+W		3/3/20 2:22:43 PM 3/9/20 5:11:11 PM
	Experiment Layout		ngs	3/19/20 2:43:59 PM
	Compensation Setup	Þ		Create Compensation Controls
	.	giobal Sher		Modify Compensation Controls
		Analys 		Calculate Compensation
		🥔 Sort Lay	out_	002

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



17. Switch Normal worksheet to Global worksheet by clicking the first icon on the left

	s 🔁 🖟	2 🖾 🔘 🔝	, p 🗗 🗗 📢
Sheet	FITC S	t <mark>a</mark> ined Control	PE Stained Control
	* <u>*</u> **** 00	DAPI Staine	d Control

- 18. Create a **new specimen**
- 19. Expend the new specimen and **click the tube pointer** of Tube_001
- 20. Run a sample that is fully stained
- 21. Visualise the compensated data by go to Cytometer > Compensation > Check the box of Enable Compensation

Status	Parameters	Threshold I	Laser Compens	sation Ratio
		Enable Cons	menten .	lear
FI	uorochrome	- % F	luorochrome	Spectral Overlap
+ PE		FITC		19.70
+ Per	CP-Cy5-5	FITC		2.20
. AP	c	FITC		0.00
	c	DE		1.00

22. Adjust the compensation value if needed

	1 Idol och onlo	no risorochi onio	spectral overlap
	PE	FITC	19.70
	PerCP-Cy5-5	FITC	2.20
•	APC	FITC	0.00
	FITC	PE	1.00

Spectral Overlap