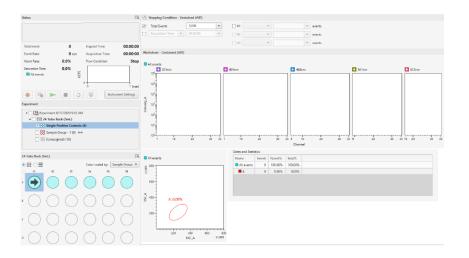
ID7000 Unmixing Settings

1. Preview and Acquire the Unstained Sample (beads or cells)

a. Go to the Status panel (upper left part of the screen) and click on the Load button.



b. Go to the Rack/Plate Layout panel (lower left). Ensure that the current position (black arrow) is on the unstained sample (A01). To change the current position, right click on the new location and click Set Current Position on the quick pick menu.



- c. Optional: One can adjust the axis scale or zoom the plot, as required, if the gate is difficult to draw. Right-click on the FSC SSC plot, then click on Zoom to enlarge your population.
- d. Go to the Status panel (upper left) and click on the Instrument Settings button.
- e. Go to the Instrument Settings window and click on the Flow Control tab.

Close

 \times ID Instrument Settings Sample:Unstained Mode:Normal Laser Detector & Threshold Flow Control Short Boost: Ŧ Sample Flow Rate: 4.0 ⊩♥⊣ Event Check None The software does not detect air/low event rate. 🔘 Stop When air/low event rate is detected, stop data acquisition and initiate automatic cleaning to recover the system. Continue When the air/low event rate is detected, stop data acquisition and initiate automatic cleaning to recover the system then CONTINUE auto acquisition.

_

Advanced Settings

Boost: Short is recommended unless you have lots of sample you can waste.

Sample Flow Rate: 4.0 this can be adjusted while previewing a sample, but not during data acquisition or auto-acquisition.

Event Check: ON is recommended during unattended auto-acquisition.

		Sam	ple Consun	nption Rate	(µL/min) at	each Samp	le Flow Rat	e Setting				
		1	2	3	4	5	6	7	8			
		33	50	67	80	100	133	167	200			
-Ď.	Sample	Time to run the indicated Sample Volume at each Sample Flow Rate Setting										
	Volume	1	2	3	4	5	6	7	8			
Ŧ	50 µL	01:30	01:00	00:45	00:37	00:30	00:22	00:18	00:15			
	100 µL	03:00	02:00	01:30	01:15	01:00	00:45	00:37	00:30			
	150 µL	04:30	03:00	02:15	01:52	01:30	01:07	00:55	00:45			

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ID7000 Unmixing Settings

- h. Go to the Status panel and click on the Preview button
- i. Go to the Instruments Settings window and click on Detector & Threshold tab.

Laser Detector & Th	eshold Flow Control	
FSC Gain: 12	SSC Voltage (%): 30.0	
355nm PMT Voltage(%	: 62.0 -V -	
405nm PMT Voltage(%	: 61.0 	
488nm PMT Voltage(%	: 66.0 	
561nm PMT Voltage(%	: 75.0 -U -1	
637nm PMT Voltage(%	: 75.0 F V-1	
Threshold CH 1: FS	 Threshold Value 1 (%): 20. 	0 +
Threshold CH 2: No	e Threshold Value 2 (%):	P.
Window Extension:	ormal 🔻	

Adjust the FSC Gain and SSC Voltage settings to properly position the cell or bead population on the FSC SSC plot.

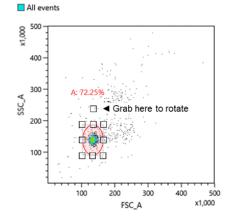
Click on the Restart button as needed to refresh the data.

Ensure that the right Instrument Settings is loaded

Adjust Threshold Value to eliminate excess debris downscale from the cell or bead population in FSC SSC plot.

j. Go to the FSC SSC plot. Adjust the size and position of Gate A to be tightly around the cell or bead population.

Close



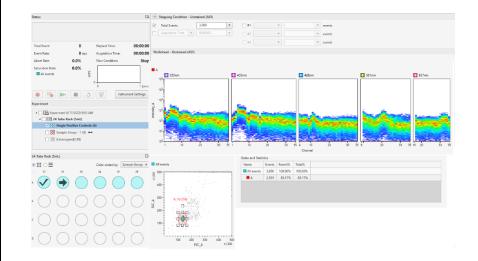
Click inside Gate A to select.

Grab and drag any corner or side handle to re-size the shape.

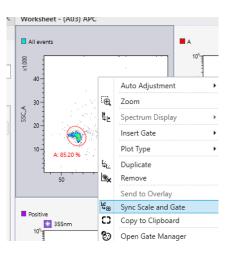
Grab and drag the top handle to rotate the shape.

Grab and drag the label to move it.

k. Click on the Acquire button • to record data from the unstained sample.



I. To apply the position and scaling of Gate A to all the single-color controls, right click on the FSC SSC Plot, and click on Sync Scale and Gate (Synchronizes the scale and gates of all plots within a sample group).

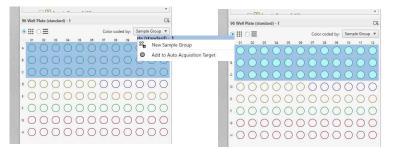


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ID7000 Unmixing Settings

3. Auto Acquire the Single-color Controls

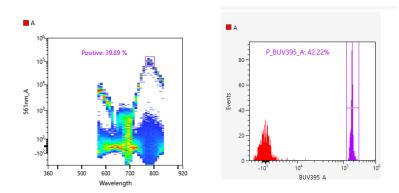
Select the wells that you want to acquire automatically in the [Plate] panel, and right click [Add to Auto Acquisition Target]



- a. Stopping conditions: Select for Compensation Beads: 5000 events
- b. click on the Auto Acquire button



- c. check is positive gate sits tightly around the positive peak in the Spectral Blot or histogram.
- d. Repeat step b for each single-color control.



4. Calculate the Unmixing Settings

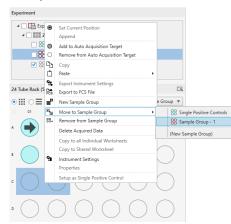
a. Go to the Unmixing tool ribbon (top of screen) and click on Unmixing Settings.
Cave: Make sure the correct gate is selected for each marker
b. Click on the Calculate button.

	Matrix Ur	nmixing-1 👻		De 🛍	tit.	1	<u></u>	₩.	1		
1	Use com	non matrix	Calculate	Apply Unmixing	Spectral R	eference	Load Spectral	Add to	Autofluorescence	Fluoroch	
			Matrix	Settings	Adjus		Reference	Library	Finder	Datab	
		Unm	ixing			Spectro	al Reference		Autofluorescence	Fluoroch	rome
mining	Settings										
mixing	Settings									_	Ч
iorochr	rome Autof	uorescence Matrix									
										Add	h
											*
Index	Marker	Fluorochrome	Ex. Laser	Mode	SR	Negativ			ositive		^
	CD45	BUV395	355nm	Normal(Adv.)	0		nstained)				
2	CD45RA	BUV737	355nm	Normal(Adv.)	0	A (U)	Instained)	•	P_BUV737_A (BUV73)	37)	
3	CD19	BV421	405nm	Normal(Adv.)	0	A (U)	instained)		P_BV421_A (BV421)		
1	CD3	BV605	405nm	Normal(Adv.)	0	A (U	nstained)	•	P_BV605_A (BV605)		
5	CD8	AF488	488nm	Normal(Adv.)	0	A (U	instained)	•	P_AF488_A (AF488)		
5	CCR7	PE	488nm	Normal(Adv.)	0	이 A (U	instained)	•	P_PE_A (PE)		
7	HLADR	BB700	488nm	Normal(Adv.)	0	🔵 A (U	instained)	(P_BB700_A (BB700)		
В	CD7	PE-Cy7	488nm	Normal(Adv.)	0	🔵 A (U	nstained)	•	P_PE-Cy7_A (PE-Cy	7)	
Э	CD4	AF647	637nm	Normal(Adv.)	0	🔵 A (U	Instained)	•	P_AF647_A (AF647)		
10	CD56	APC-Cy7	637nm	Normal(Adv.)	0	O A (U	Instained)		P_APC-Cy7_A (APC		
											v.
								Co	or Panel Spectral	Reference	•

c. Click on yes to confirm applying the unmixing settings to Sample Group - 1.

ID7000 Unmixing Settings

- 5. Acquire Data from the Unstained Cell Sample
- a. Go to the Rack/Plate Layout panel (lower left) and select positions C01 and C02.
- b. Right-click on the selected positions, hover over Move to Sample Group and click on Sample Group – 1.



- c. Right-click on position C01 and click on Set Current Position.
- d. Copy and paste the Worksheet settings from A01 to C01: click on A01, then rightclick, and click on Copy. Click on C01, then right-click, hover over Paste, and click on Worksheet.

e. Click to change from Map view to List view and overwrite the sample names:

24 Tube	Rack (5	imL) >	Sample 0	Group - 1		La
0	•			Color	r coded by: Sample Gro	oup 🔻
Target	Status	Row	Column	Sample Name	Sample Group	Events
	\odot	с	01	Unstained Cells	Sample Group - 1	
	0	с	02	Stained Cells	88 Sample Group - 1	

f. Change the Stopping Condition to A and 5,000:

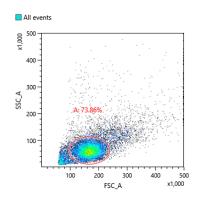


g. Go to the Status panel and click on the Preview button

- h. Go to the Instruments Settings window and click on Detector & Threshold tab.
- Adjust the FSC Gain and SSC Voltage settings to properly position the cell population on the FSC SSC plot.

Click on the Restart button 3 as needed to refresh the data.

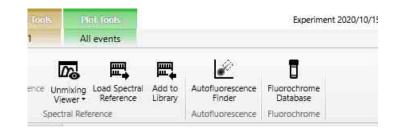
- k. Adjust FSC Threshold Value 1 to eliminate excess debris downscale from the cell population on the FSC SSC plot.
- I. Move, size, and rotate gate A around the cell population.



- m. Click on the Acquire button et arcord data from the unstained cell sample.
- 6. Add a Reference Spectrum for Cellular AF to the Unmixing Settings

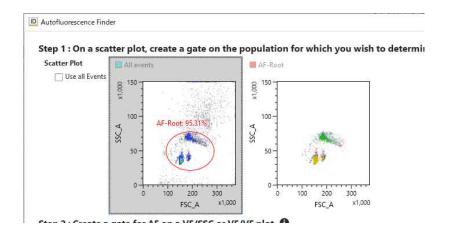
Do this section if you used capture beads for your single-color controls. You may skip this section if you used the cells for your single-color controls.

- a. Make sure you are viewing the worksheet for unstained cells.
- b. Click [Autofluorescence Finder] in the [Autofluorescence] group on the [Unmixing] tab of the ribbon.Go to the Autofluorecence Finder ribbon (top of screen) NOTE: The Unmixing Settings needs to be OFF.



1: In [Step 1], add the appropriate gates to the FSC/SSC plot. The added gate automatically is reflected as the parent gate in [Step 2].

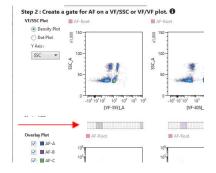
Note: Gate the corresponding populations to make later adjustment easier



2: In [Step 2], search for the autofluorescence population while changing the virtual filter ranges.

Note: You can change the wavelength band of the virtual filters to any range using the mouse.

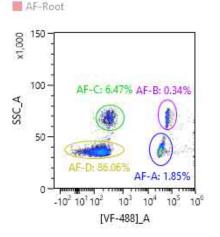
If you want to change the Y-axis of the plot to the wavelength range selected by the virtual filter, select the virtual filter of the desired laser axis from [Y Axis].



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3. Add a gate for the population to register as the autofluorescence population.



Note: Up to eight autofluorescence components can be registered as fluorochromes named "AF color 1" up to "AF color 8." 4. Click [OK].

7. Apply the Unmixing

- a. Go to the Unmixing tool ribbon (top of screen) and click on Apply.
- b. **WLSM** will appear in the upper-right corner of each data plot to indicate that the unmixing is applied to the data.

8. Acquire Data from the Stained Cell Sample

- a. Go to the Rack/Plate Layout panel (lower left) and make sure the current position (black arrow) is on C02.
- b. Click on C02 to view the worksheet for C02.
- c. Change the stopping conditions to A and 10,000.
- d. Click on the Acquire button or Autoacquire button to record data from the stained cell sample.