

SONY ID7000™ Training

Introduction to Spectral Flow Cytometry

Software & Workflow

Standardization Mode

Panel Design

João Monteiro

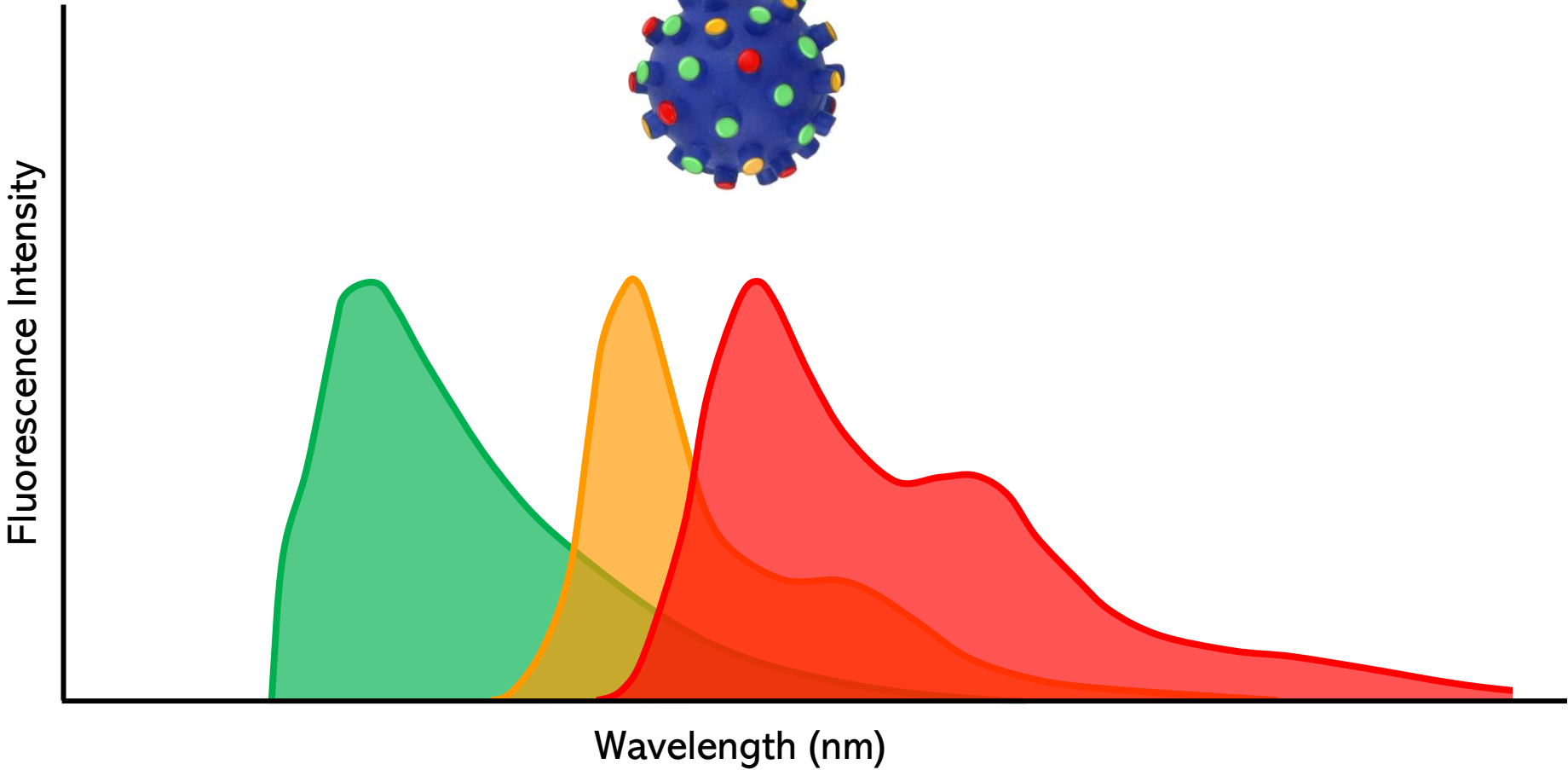
joao.monteiro@sony.com

Field Application Scientist - Nordics

Sony Biotechnology Europe

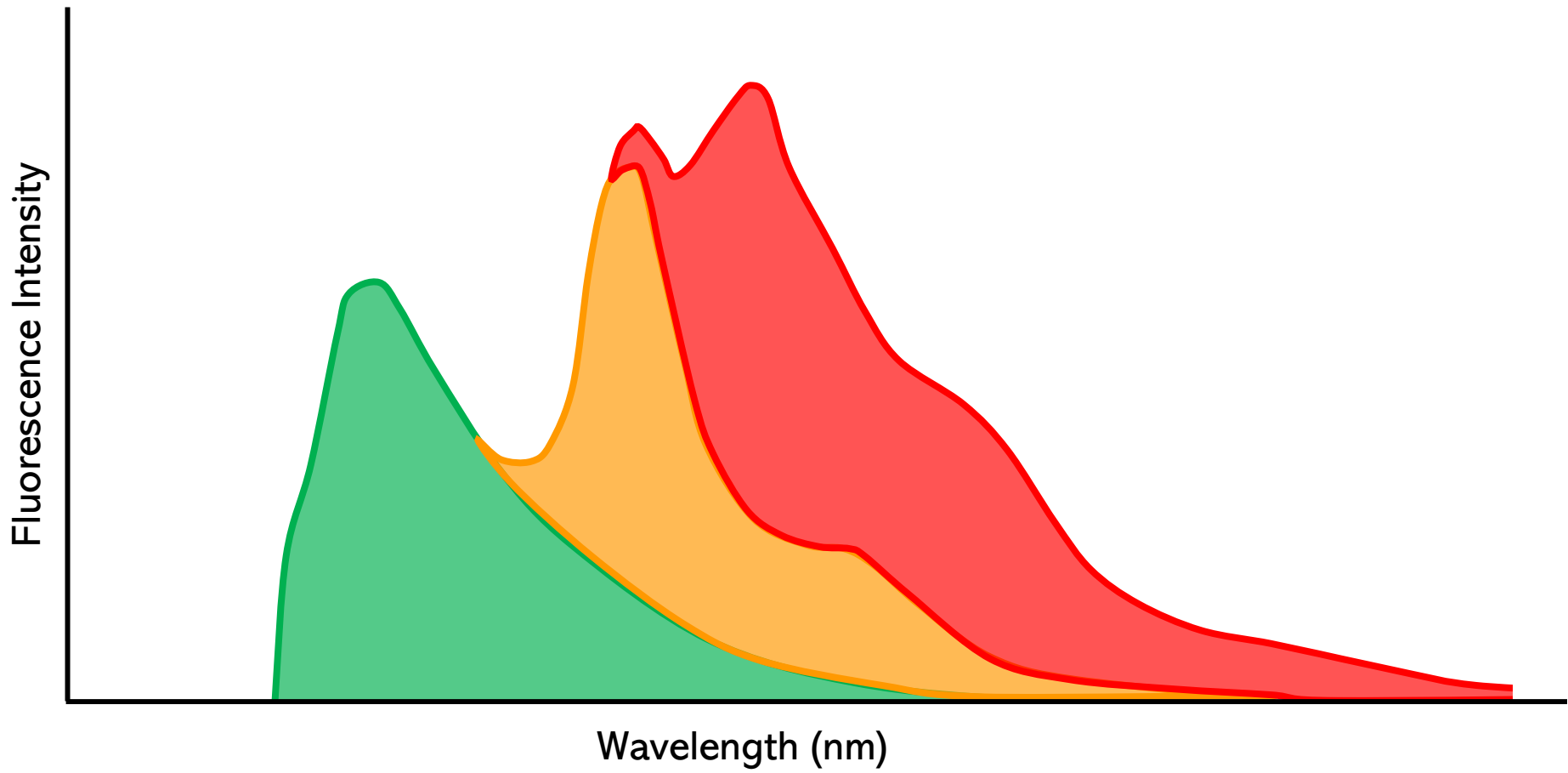
Spectral vs Conventional Flow Cytometry

Consider a cell stained with three fluorochromes.



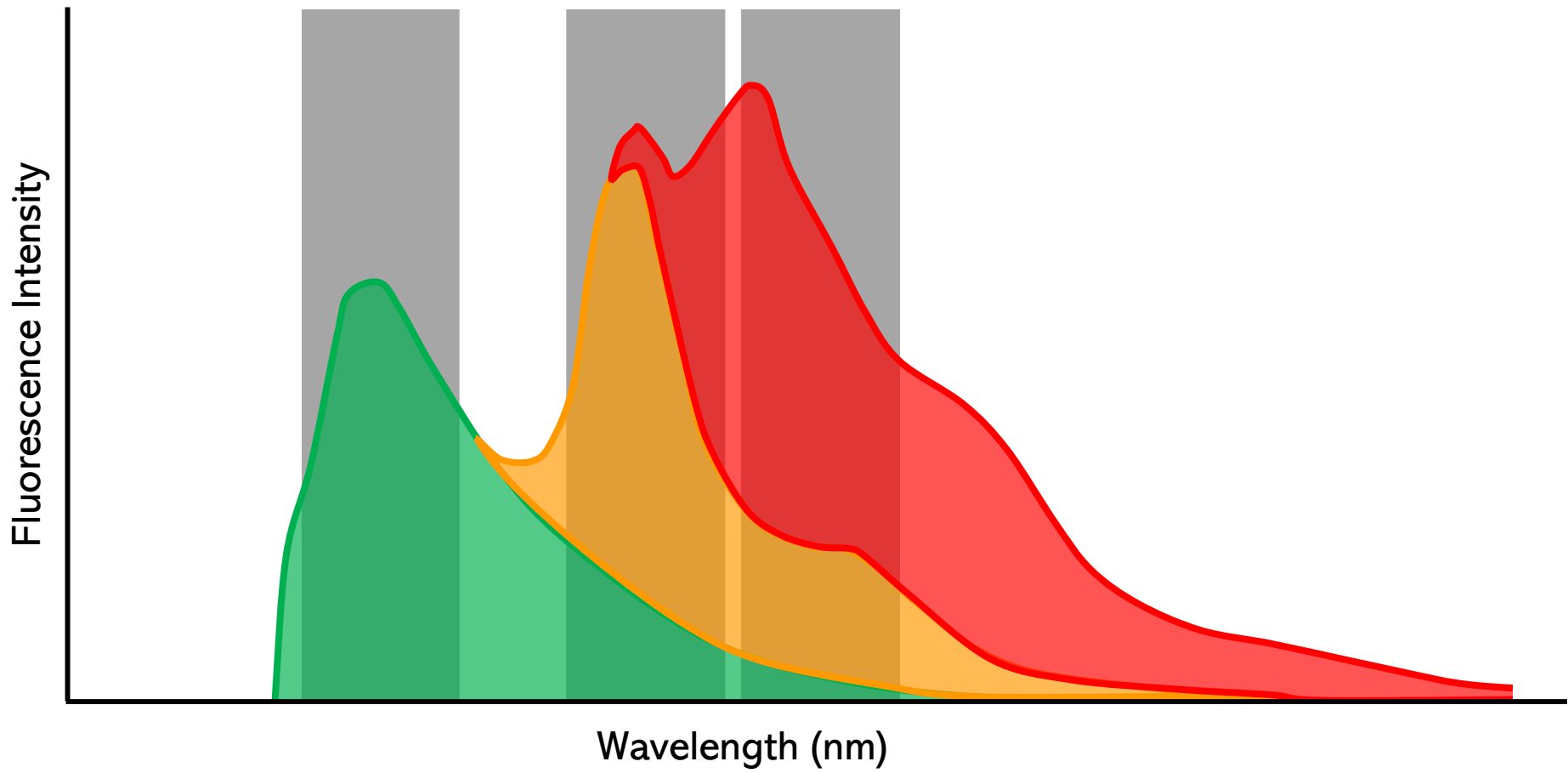
Spectral vs Conventional Flow Cytometry

The fluorescence intensity is additive, that is, it sums up wherever the spectra overlap.



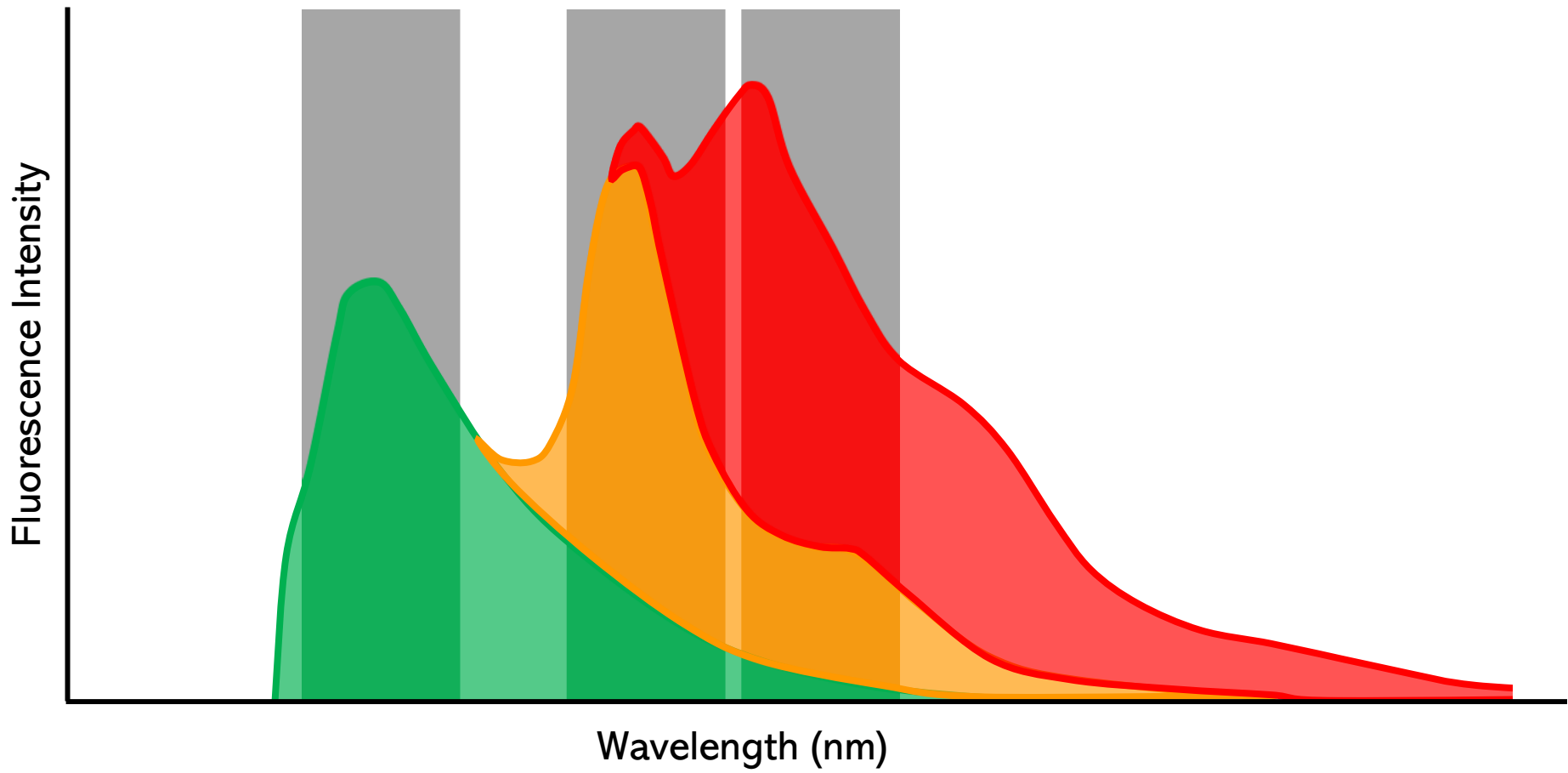
Conventional Flow Cytometry

Fluorescence is measured only in a few defined ranges, one detector for each fluorochrome.



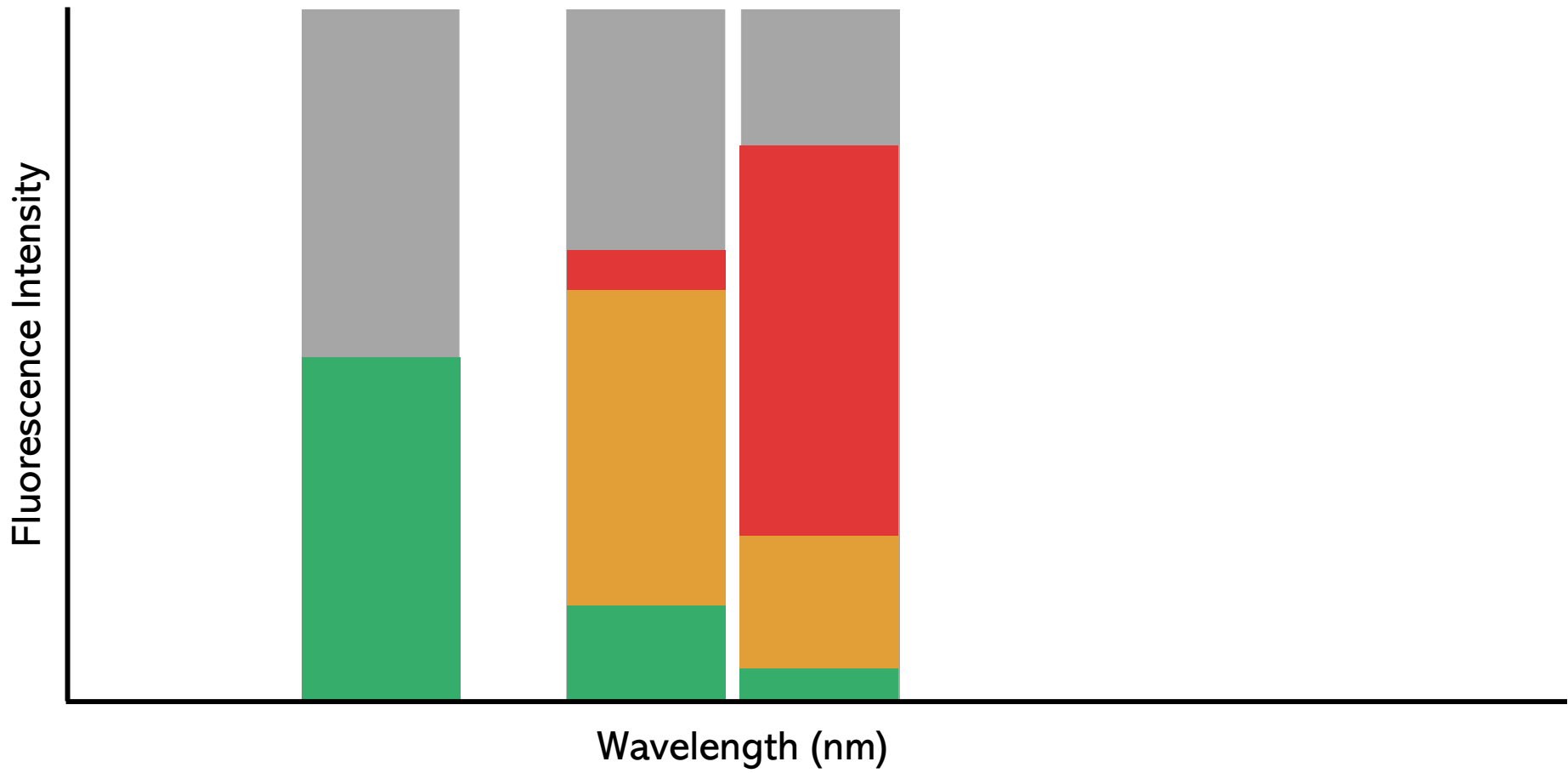
Conventional Flow Cytometry

Any fluorescence outside the defined detector ranges is not collected.



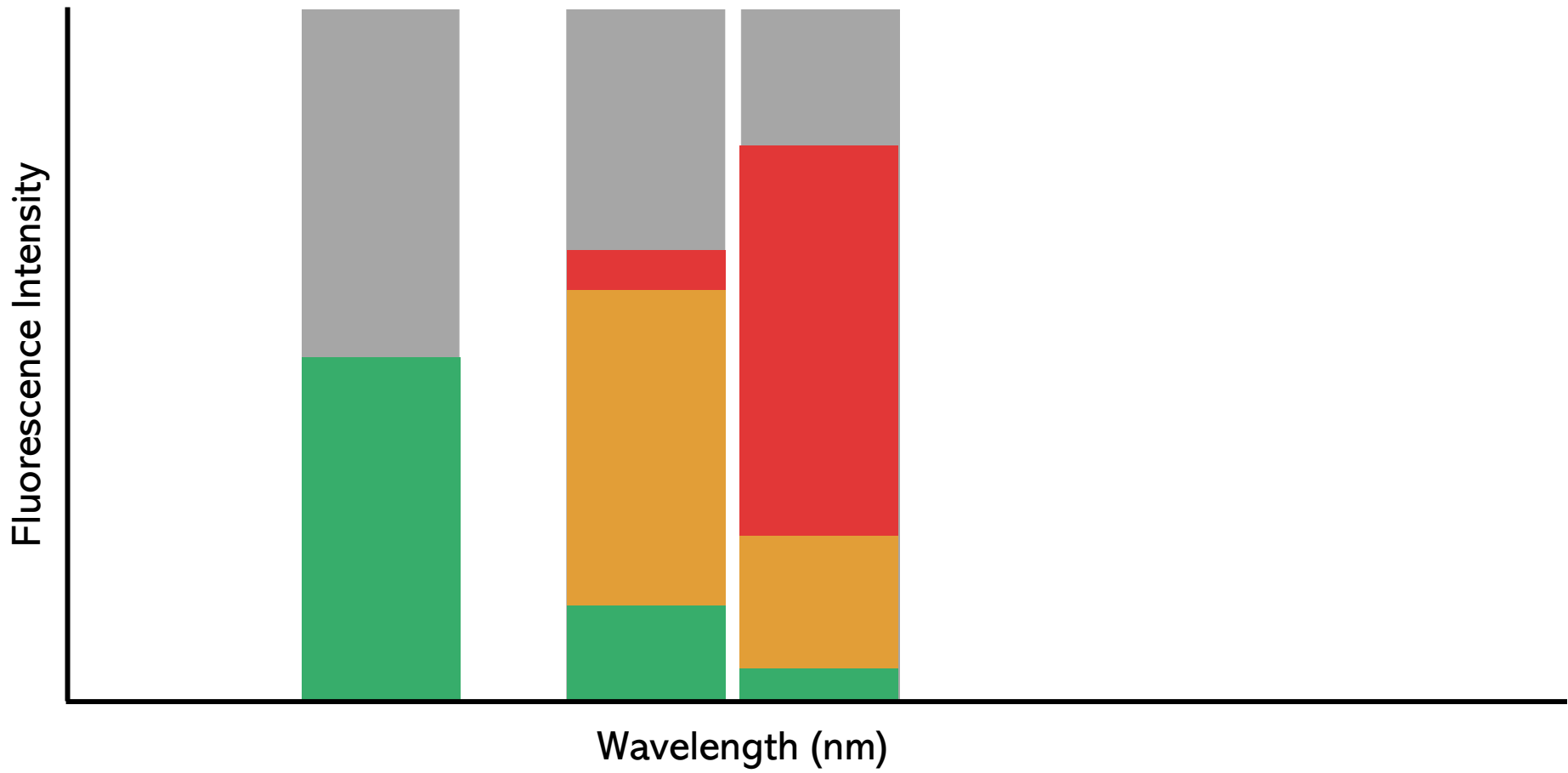
Conventional Flow Cytometry

The fluorescence measured in some detectors includes spillover from other fluorochromes.



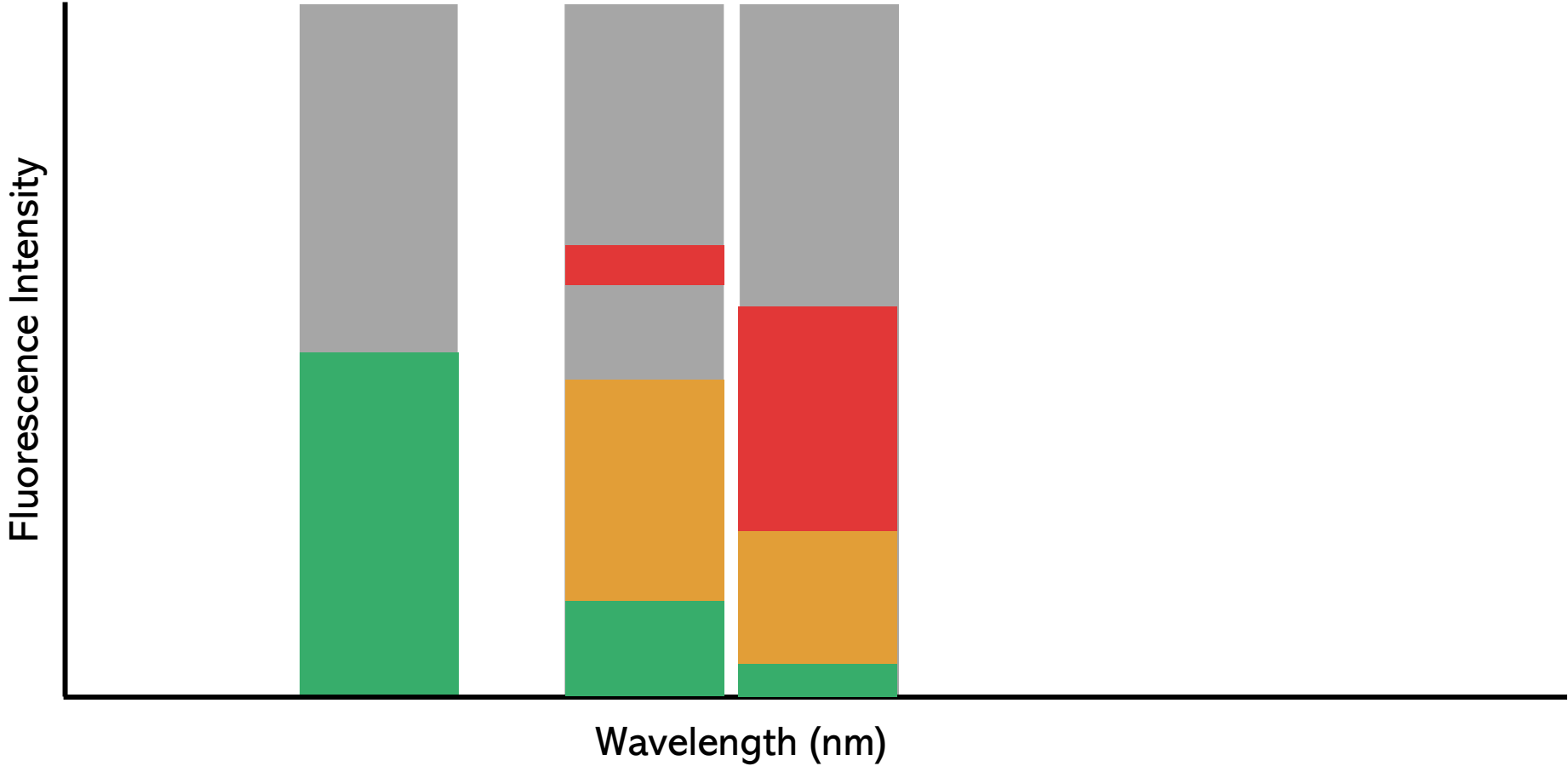
Conventional Flow Cytometry

The spillover is subtracted by colour compensation.



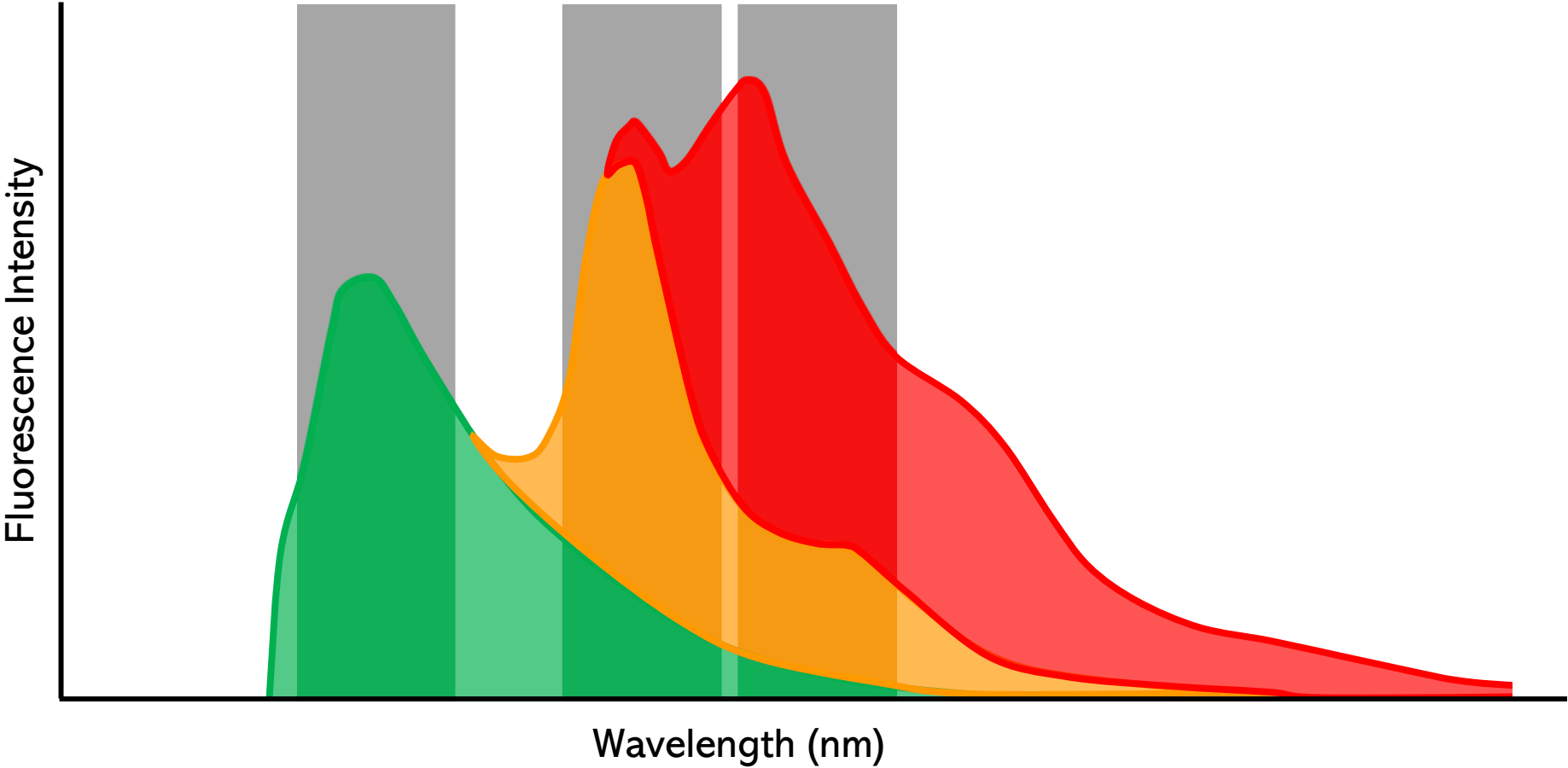
Spectral Flow Cytometry

There is no subtraction.



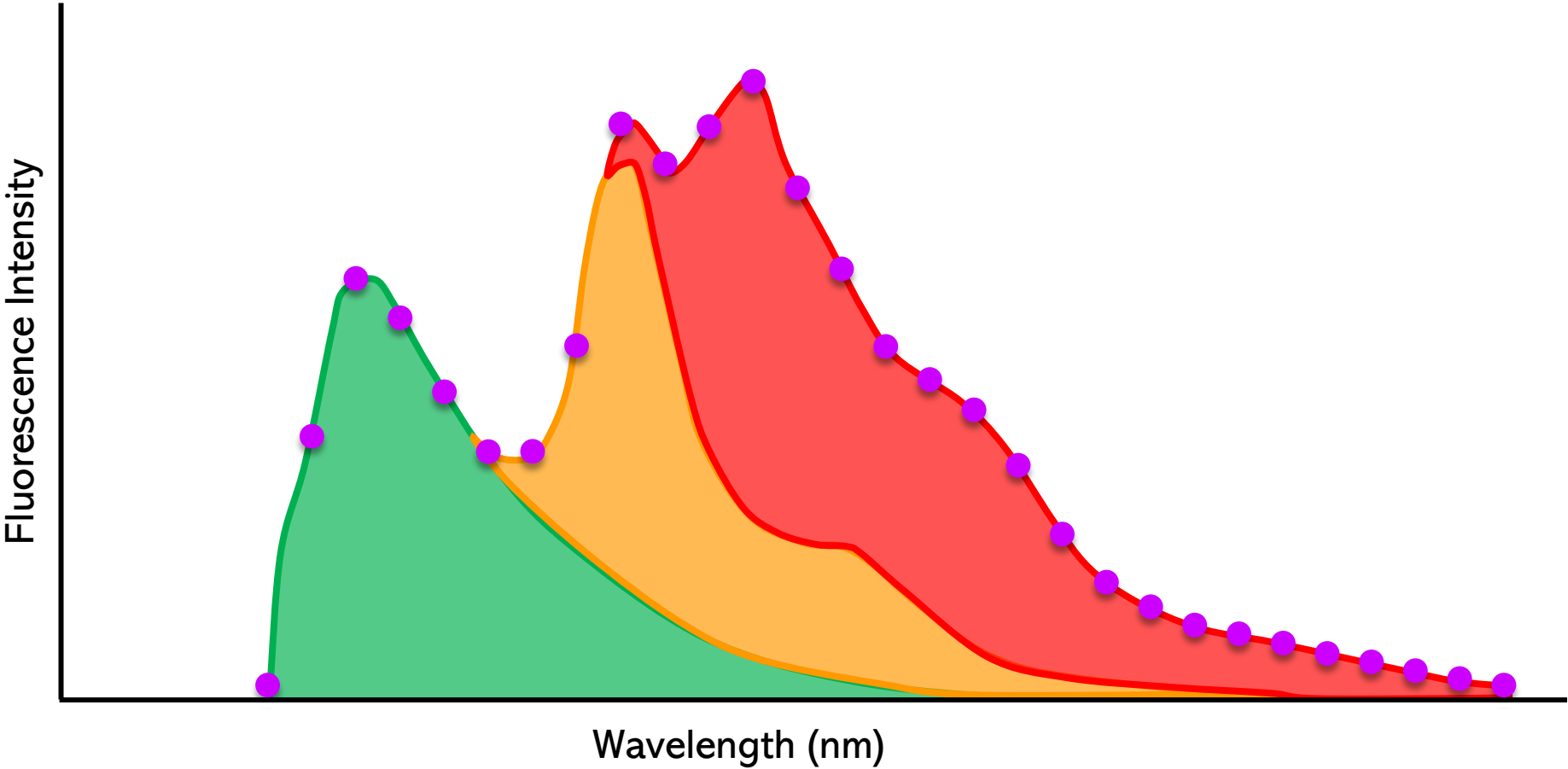
Spectral Flow Cytometry

All of the fluorescence is included.



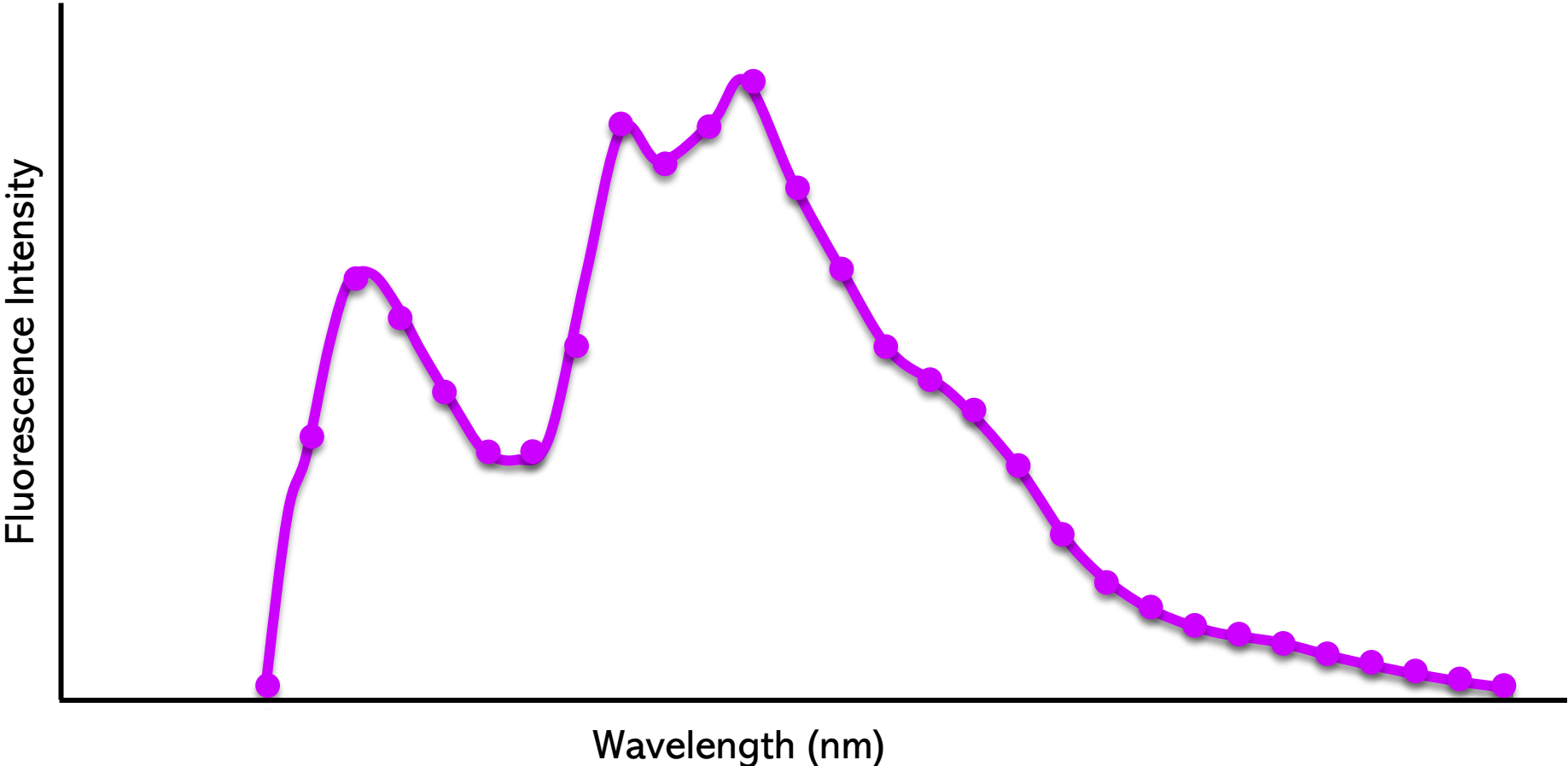
Spectral Flow Cytometry

The fluorescence from each cell is measured at many points throughout the spectrum...



Spectral Flow Cytometry

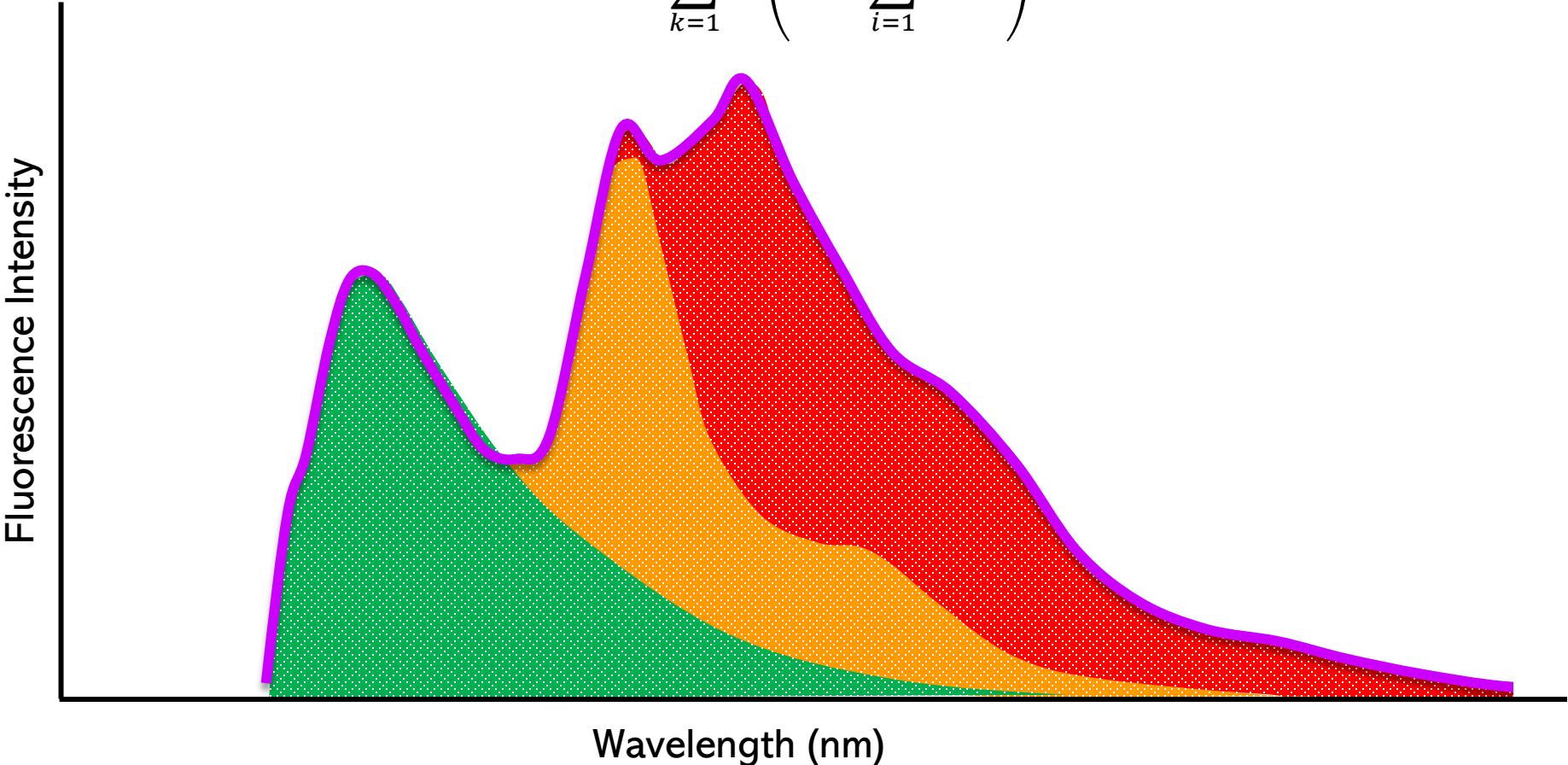
...to define the overall fluorescence of each cell, the sum of all fluorescence from all fluorochromes.



Spectral Flow Cytometry

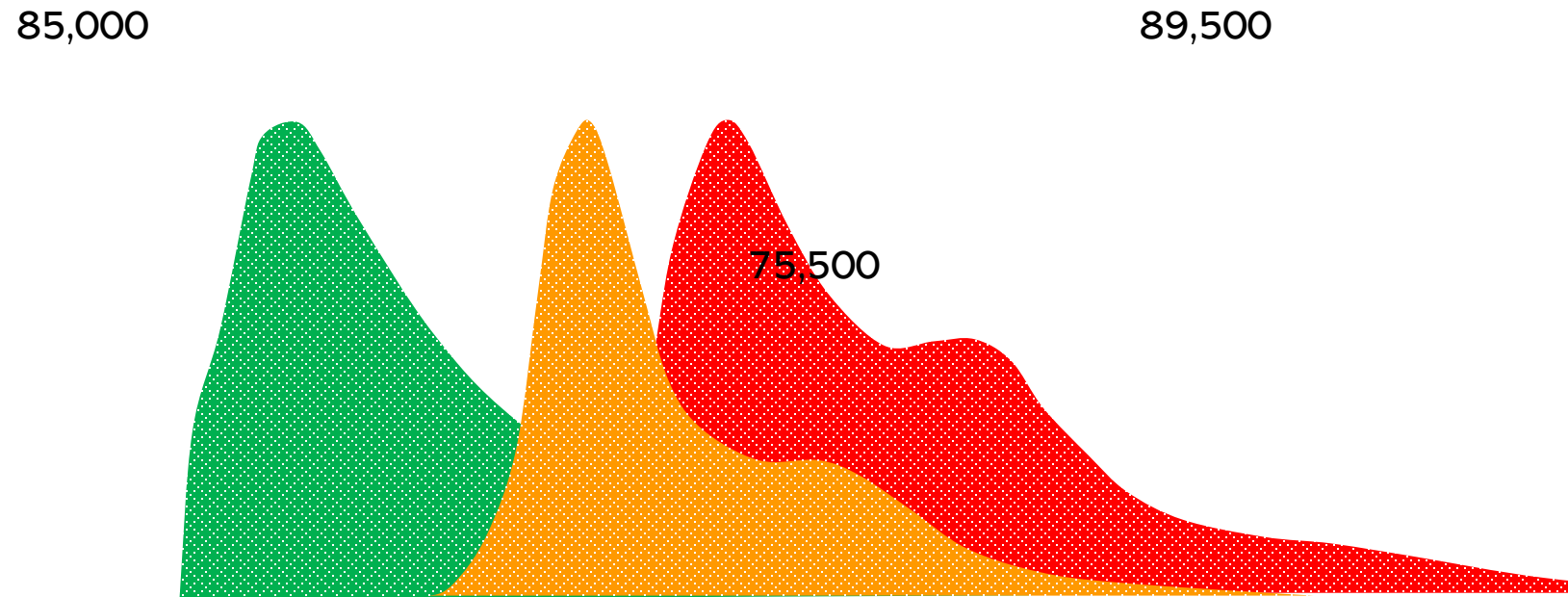
The Weighted Least Squares Method (WLSM) is applied...

$$\hat{\omega} = \operatorname{argmin}_{\omega} \sum_{k=1}^K \lambda_k \left(y_k - \sum_{i=1}^M \omega_i m_{ik} \right)^2$$



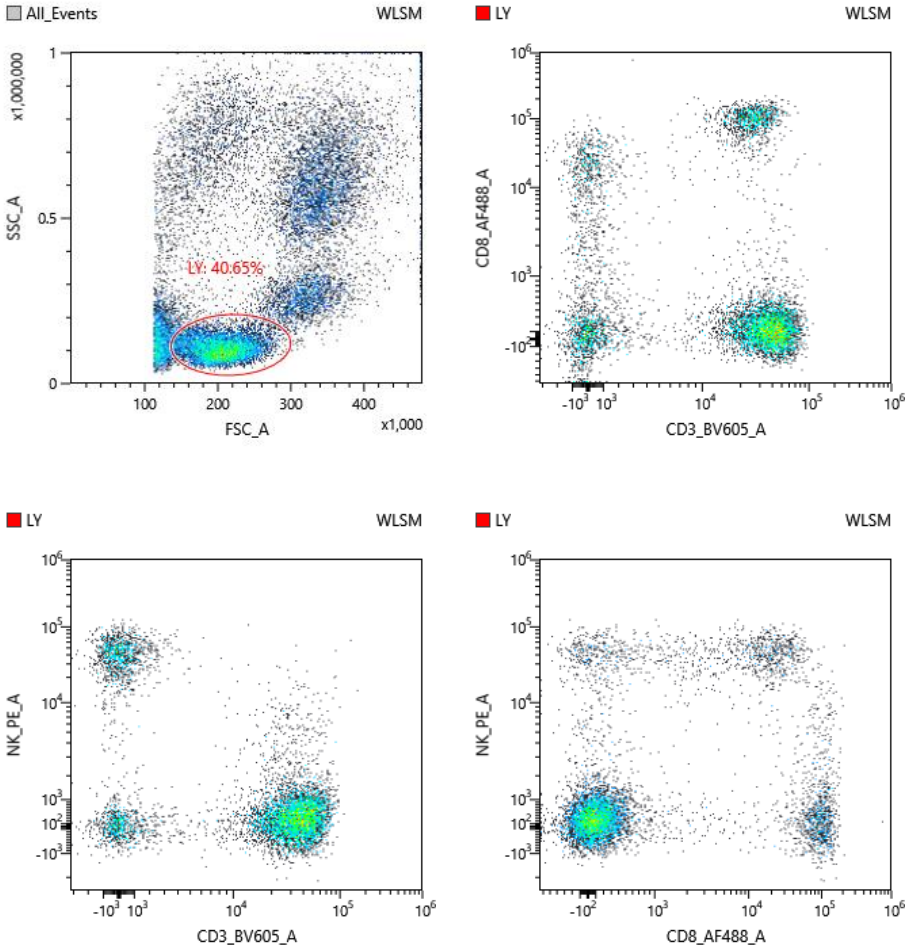
Spectral Flow Cytometry

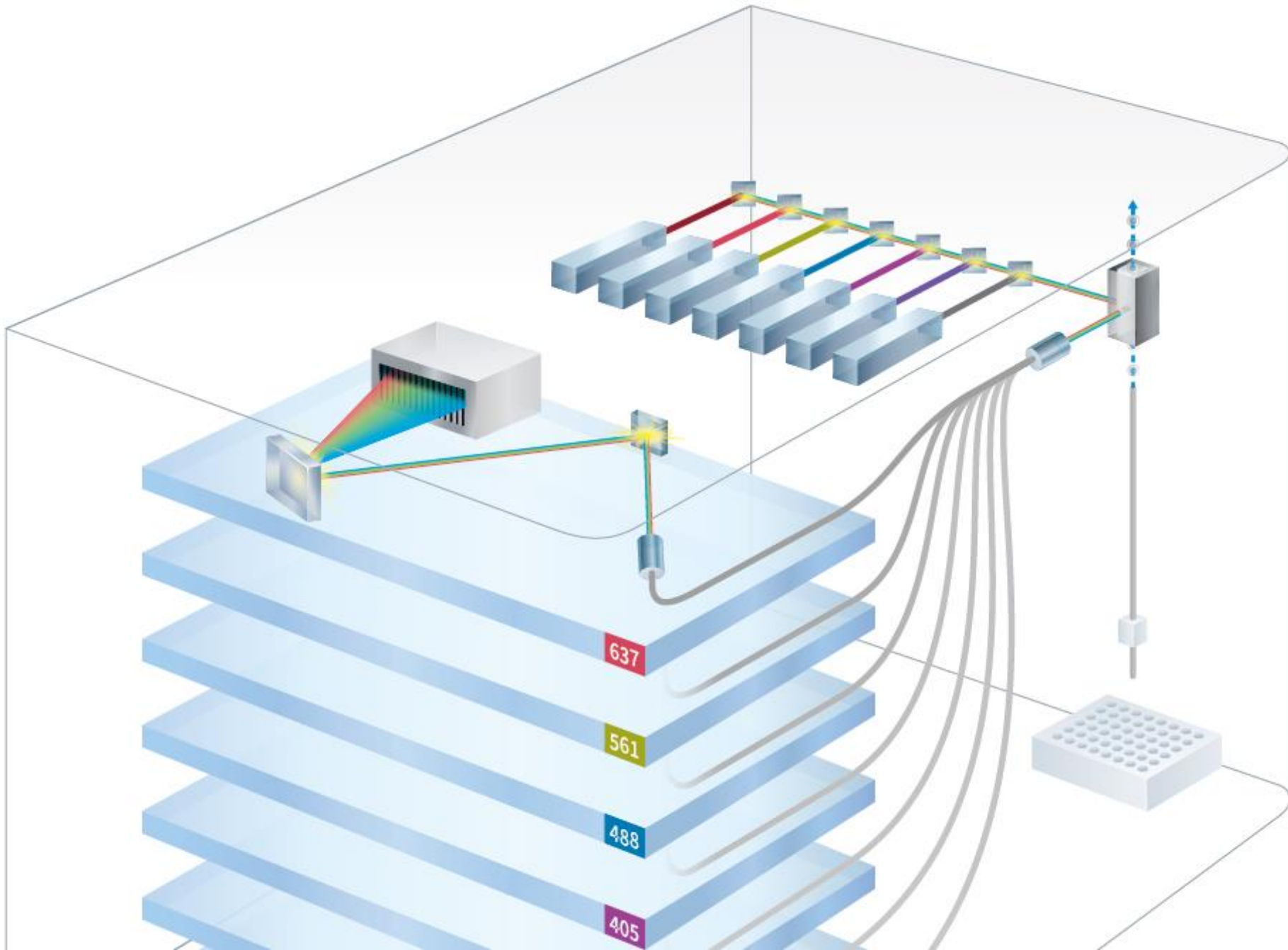
...to calculate the intensity of each fluorochrome on each cell.



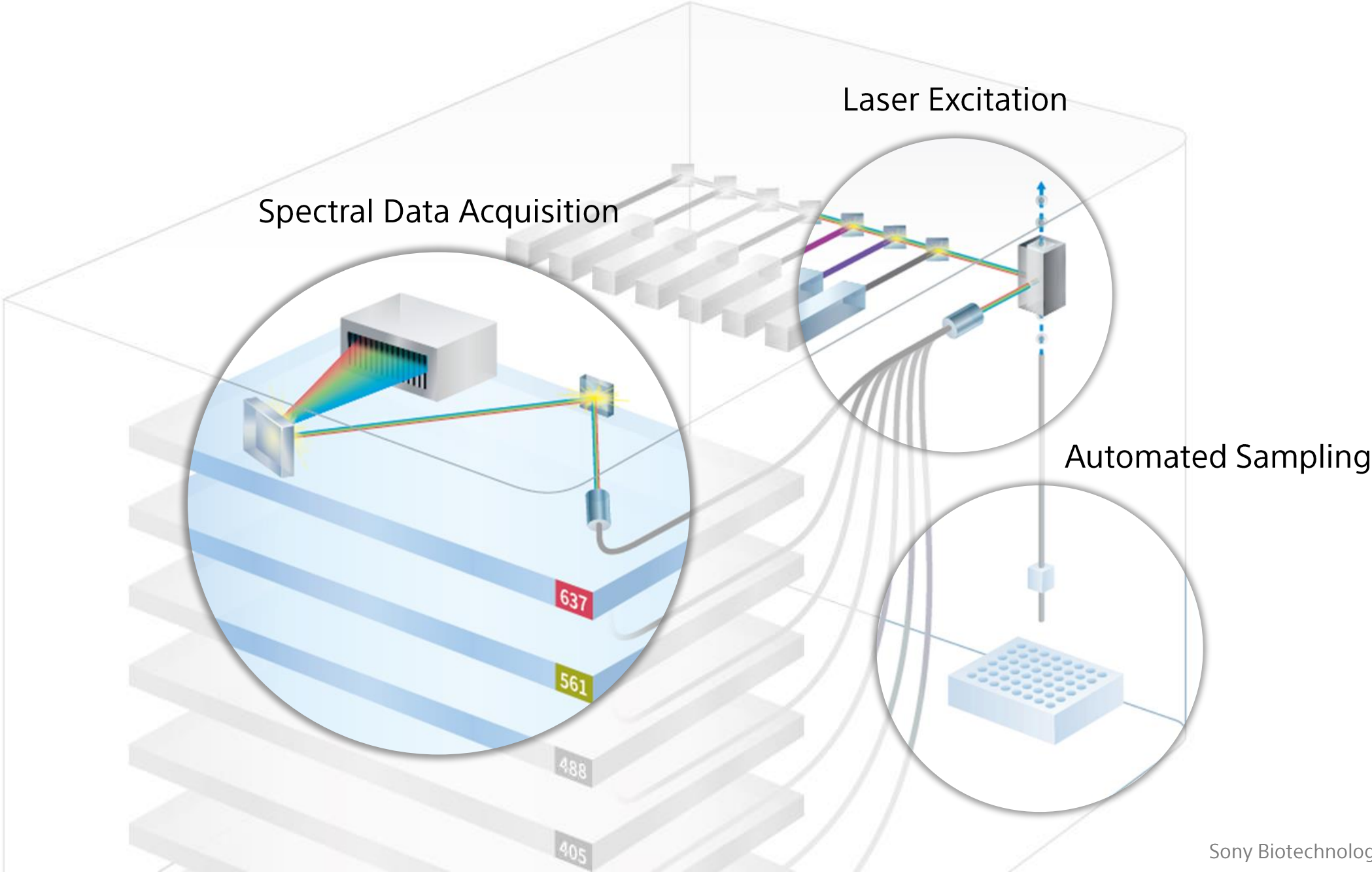
Spectral Flow Cytometry

The intensity of each fluorochrome on each cell is the **Parametric Data**.

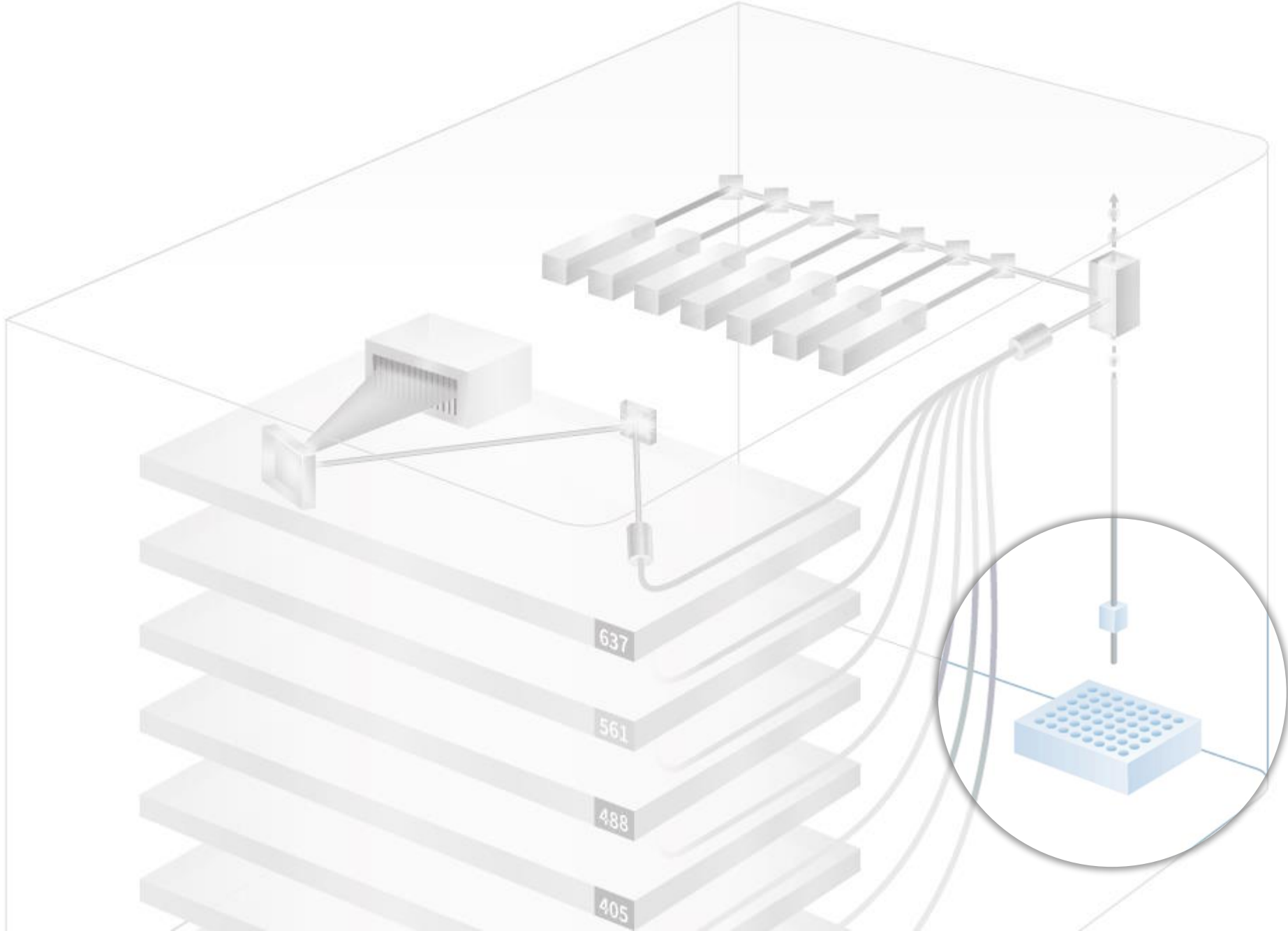




ID7000 Spectral Cell Analyzer



Automated Sampling



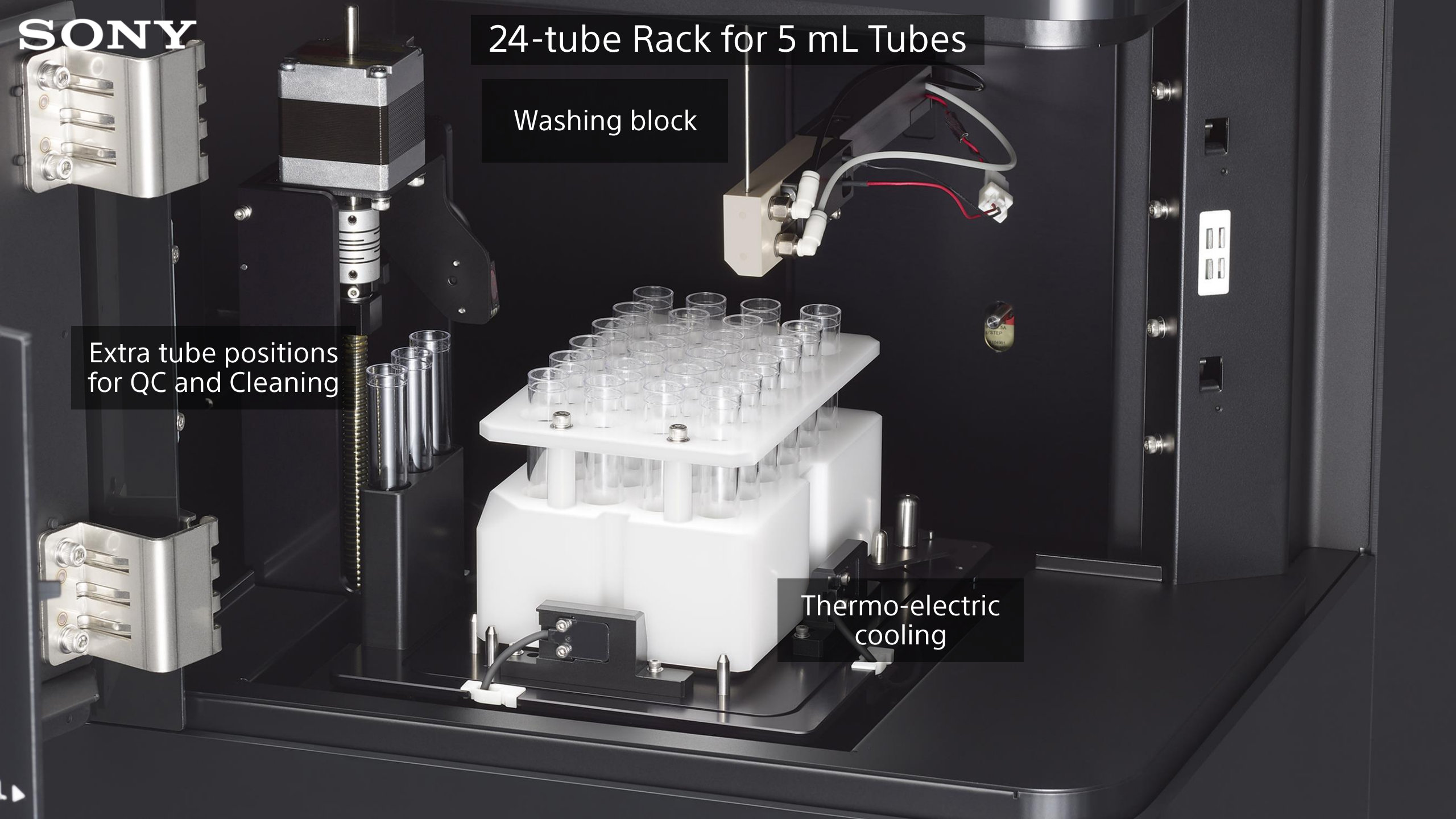
SONY

24-tube Rack for 5 mL Tubes

Washing block

Extra tube positions
for QC and Cleaning

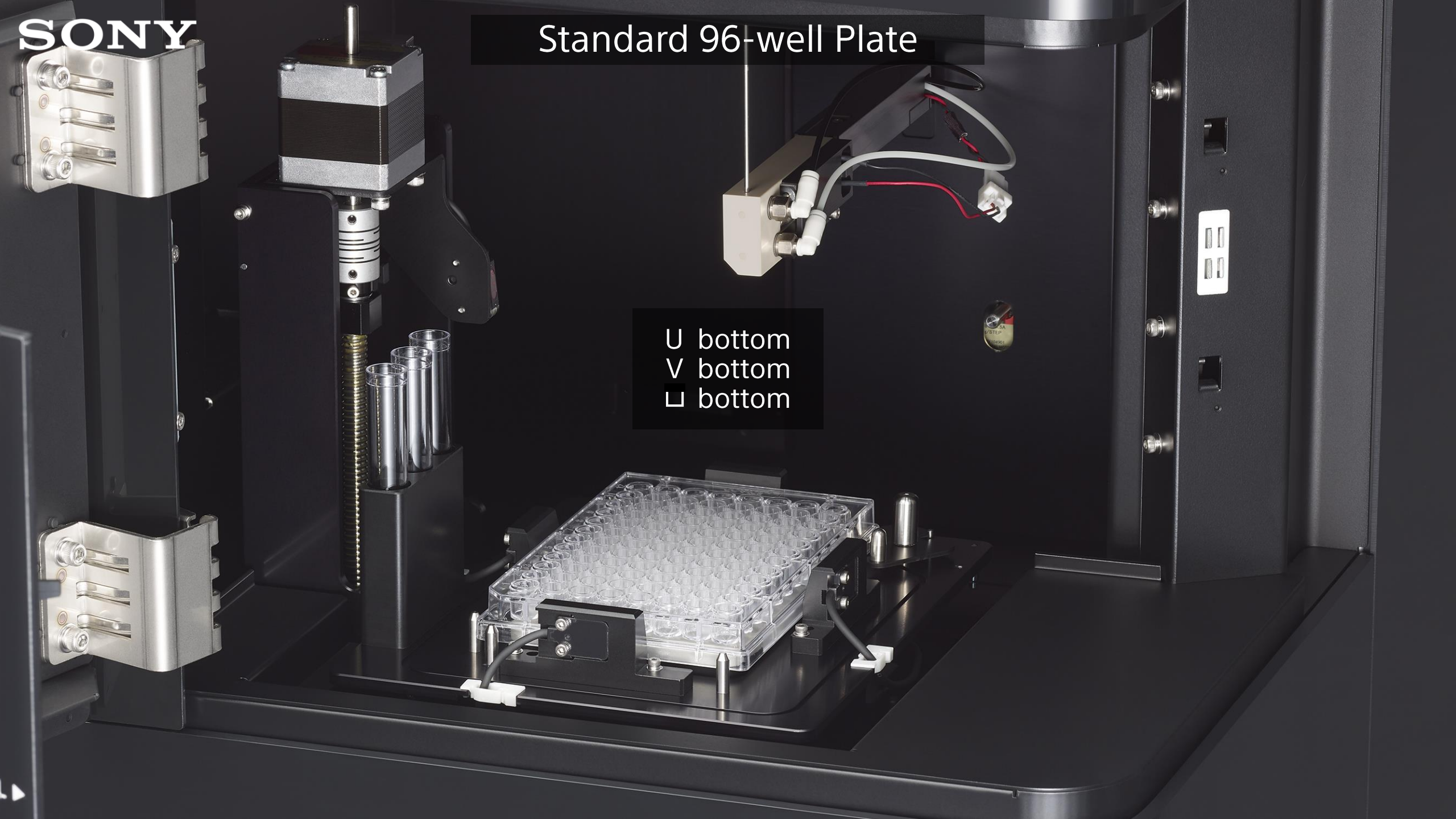
Thermo-electric
cooling



SONY

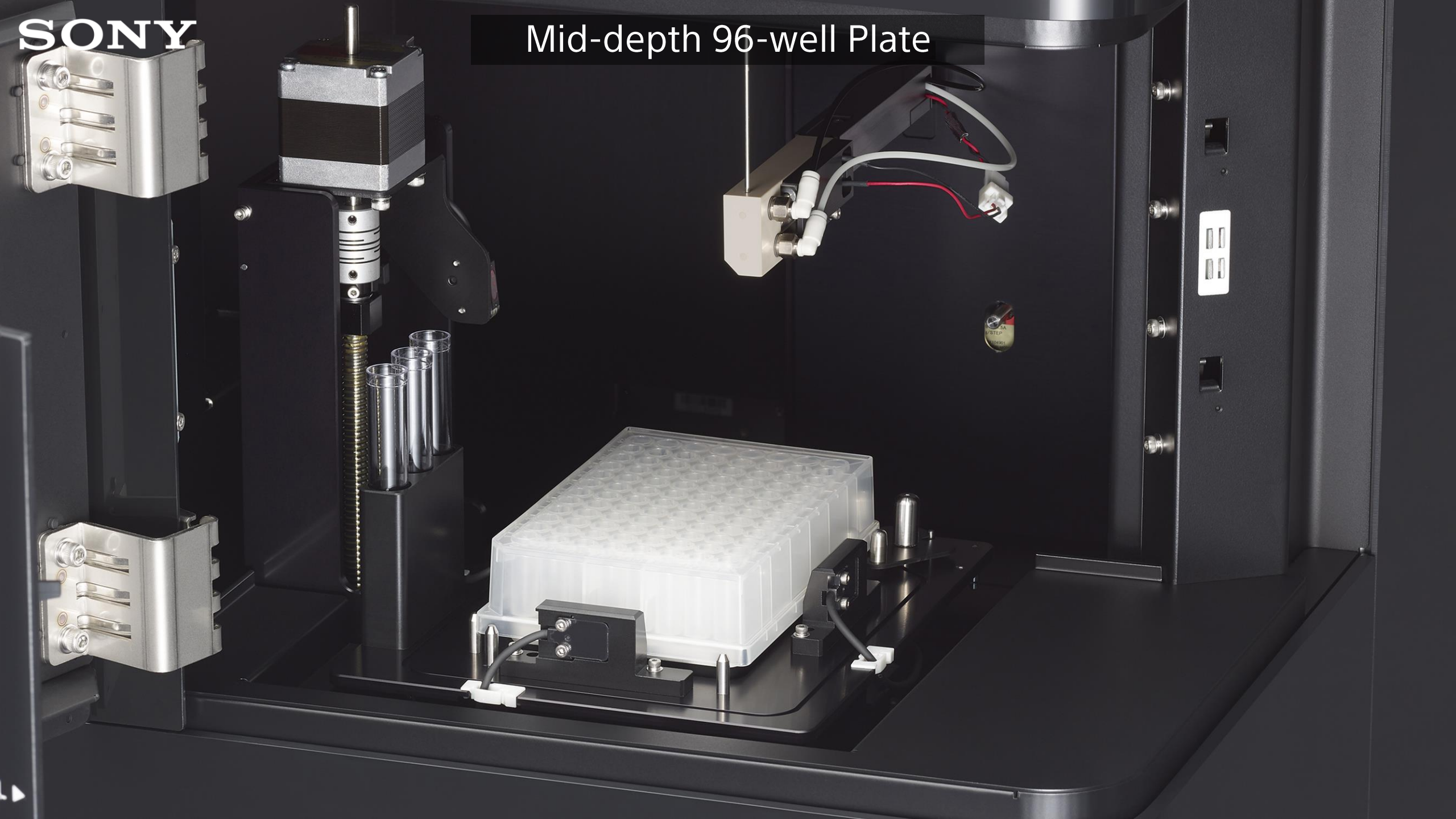
Standard 96-well Plate

U bottom
V bottom
□ bottom



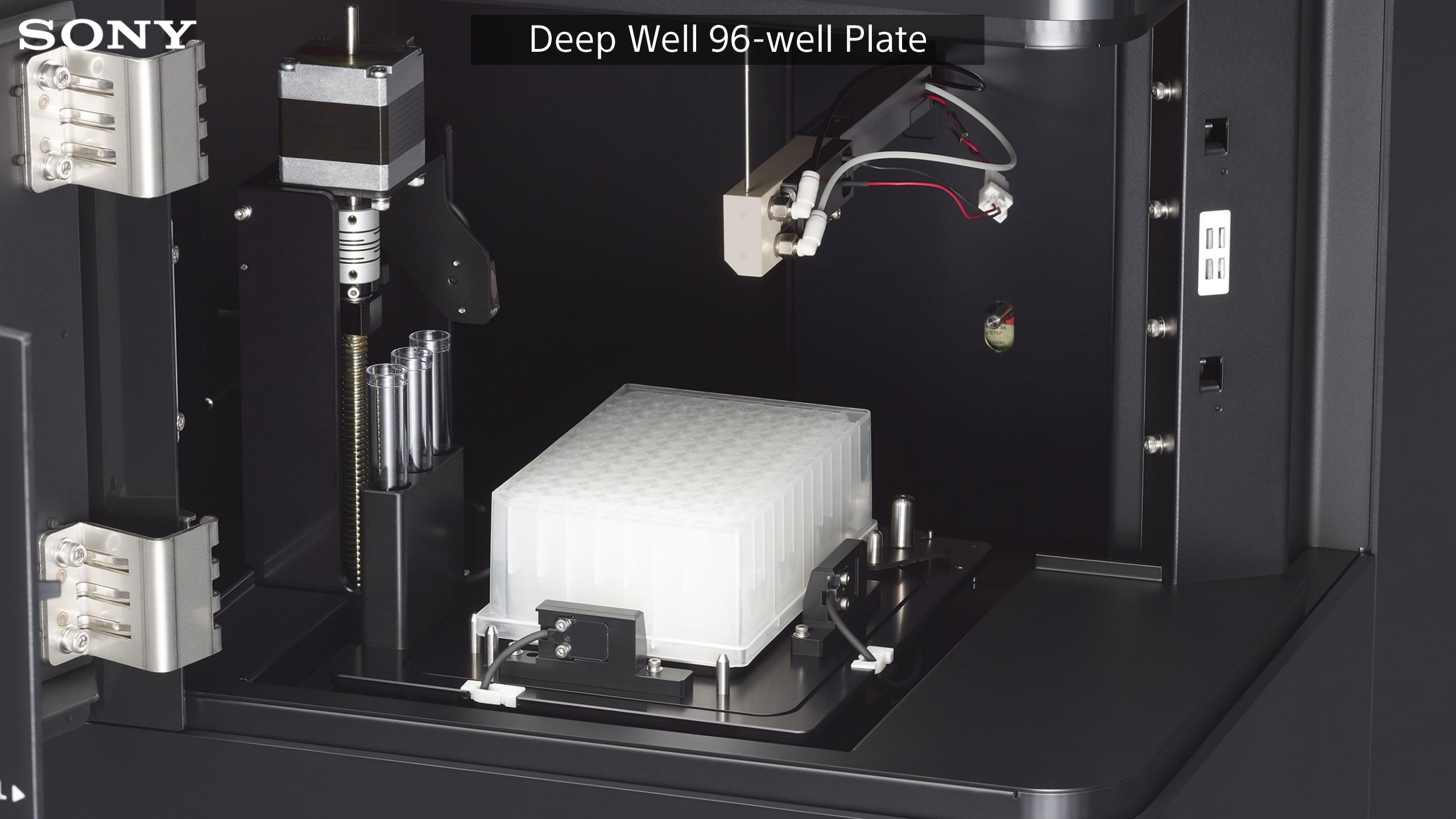
SONY

Mid-depth 96-well Plate



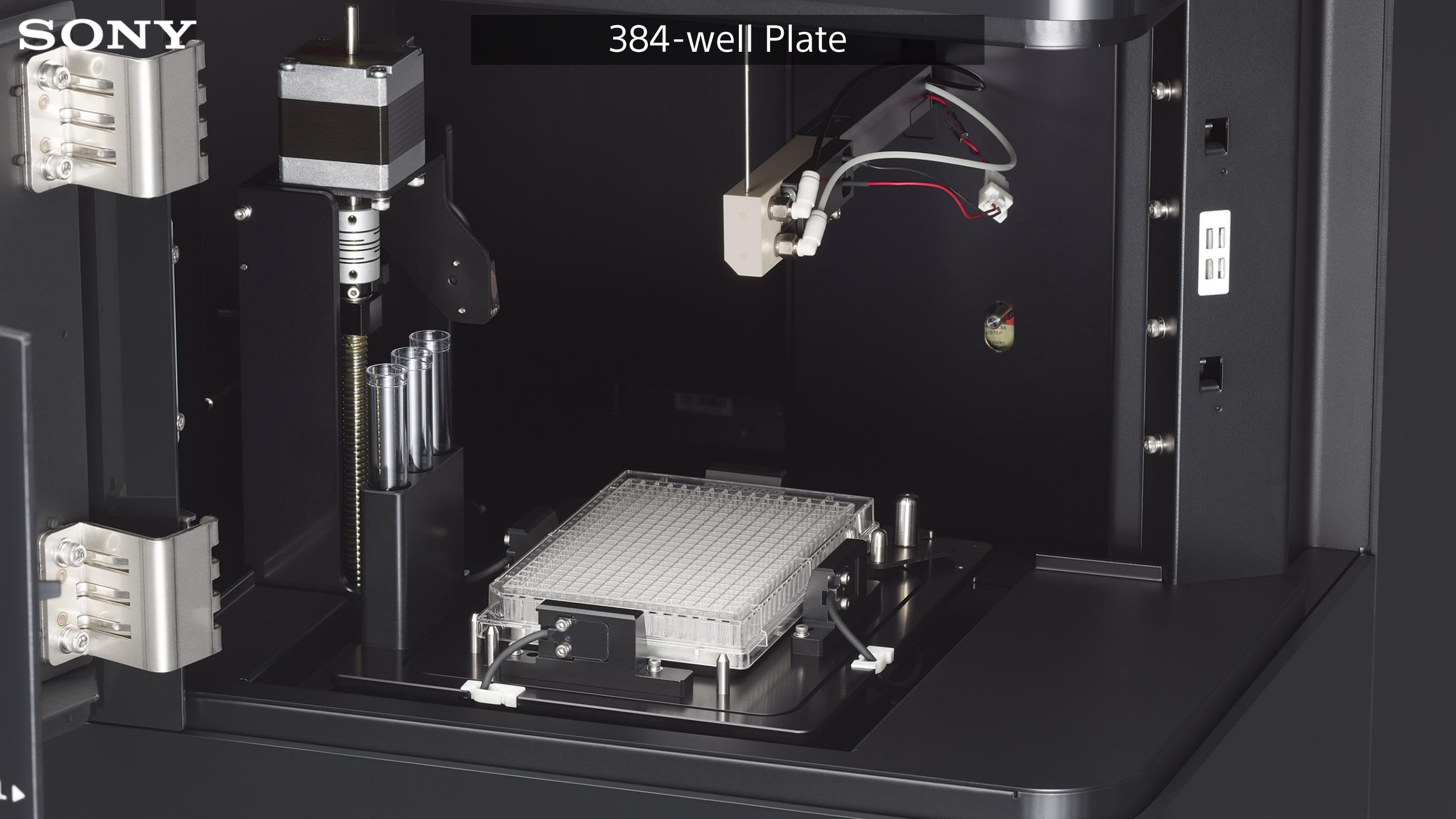
SONY

Deep Well 96-well Plate



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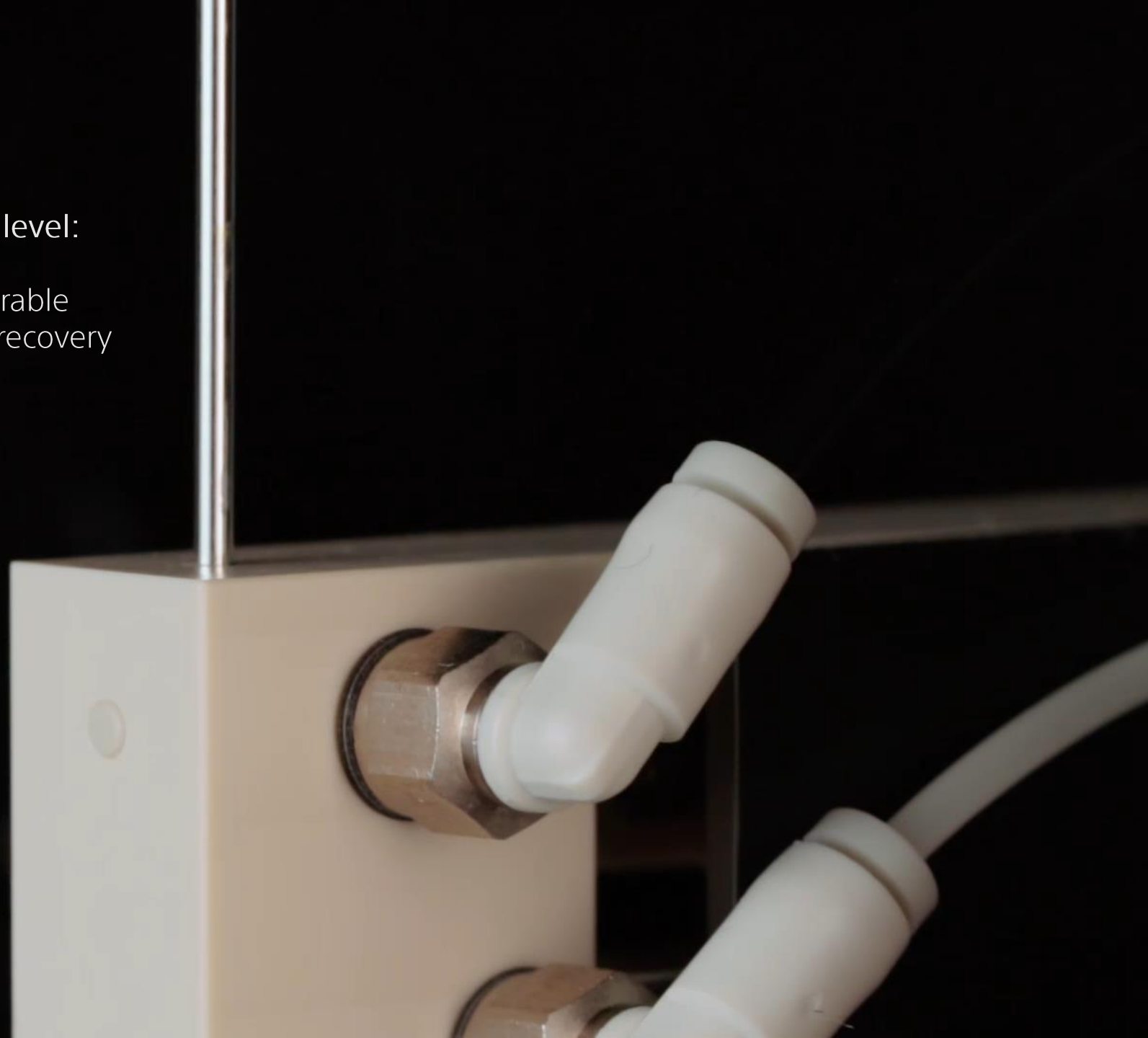
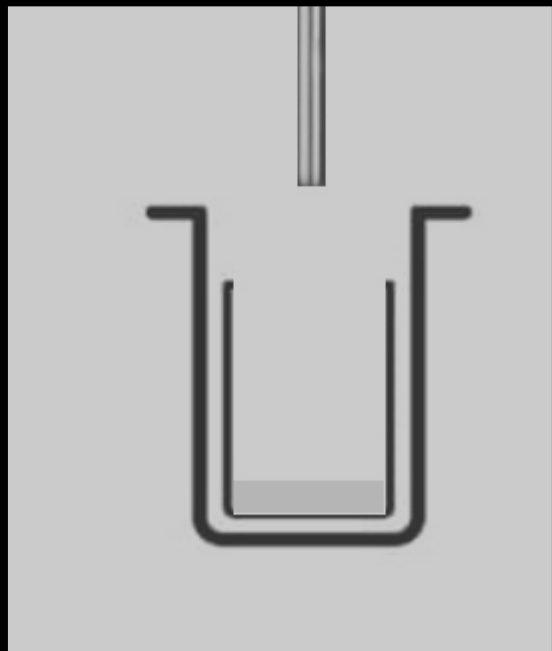
384-well Plate



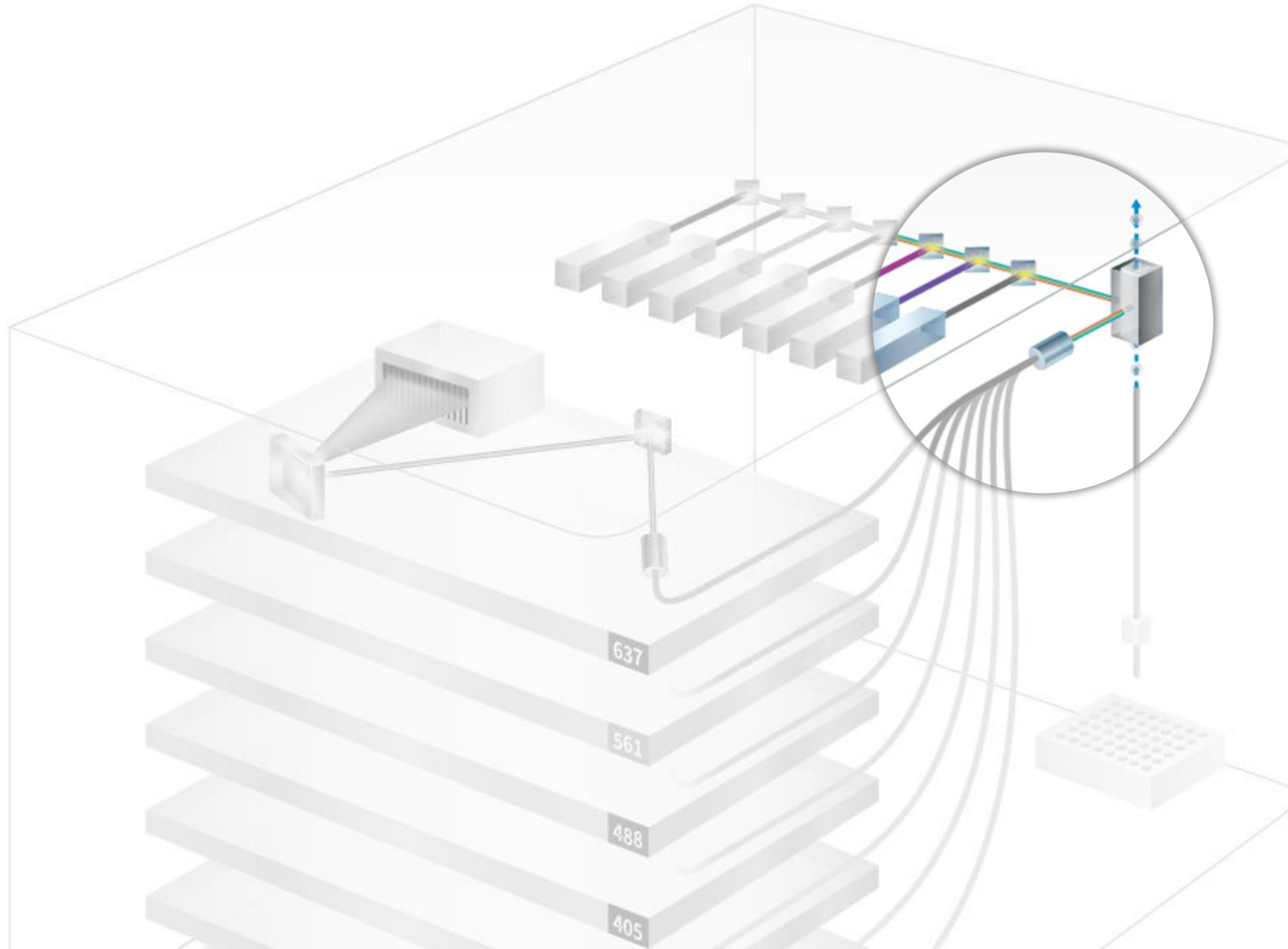
SONY

Configurable on a single well level:

- Up to 200uL/min, 40k eps
- Agitation, washing - configurable
- Clog/bubble detection and recovery
- Low Dead Volume mode



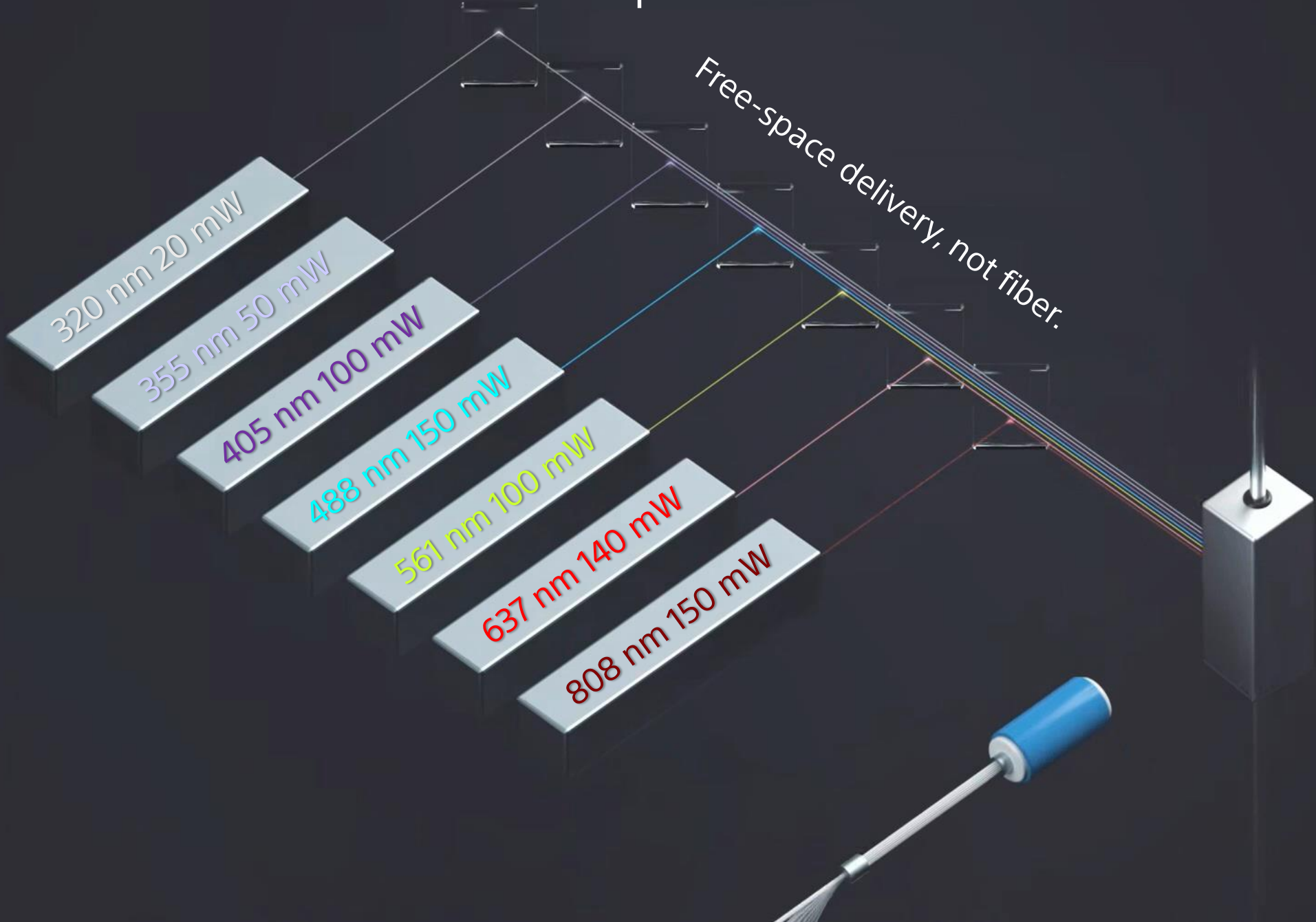
Laser Excitation



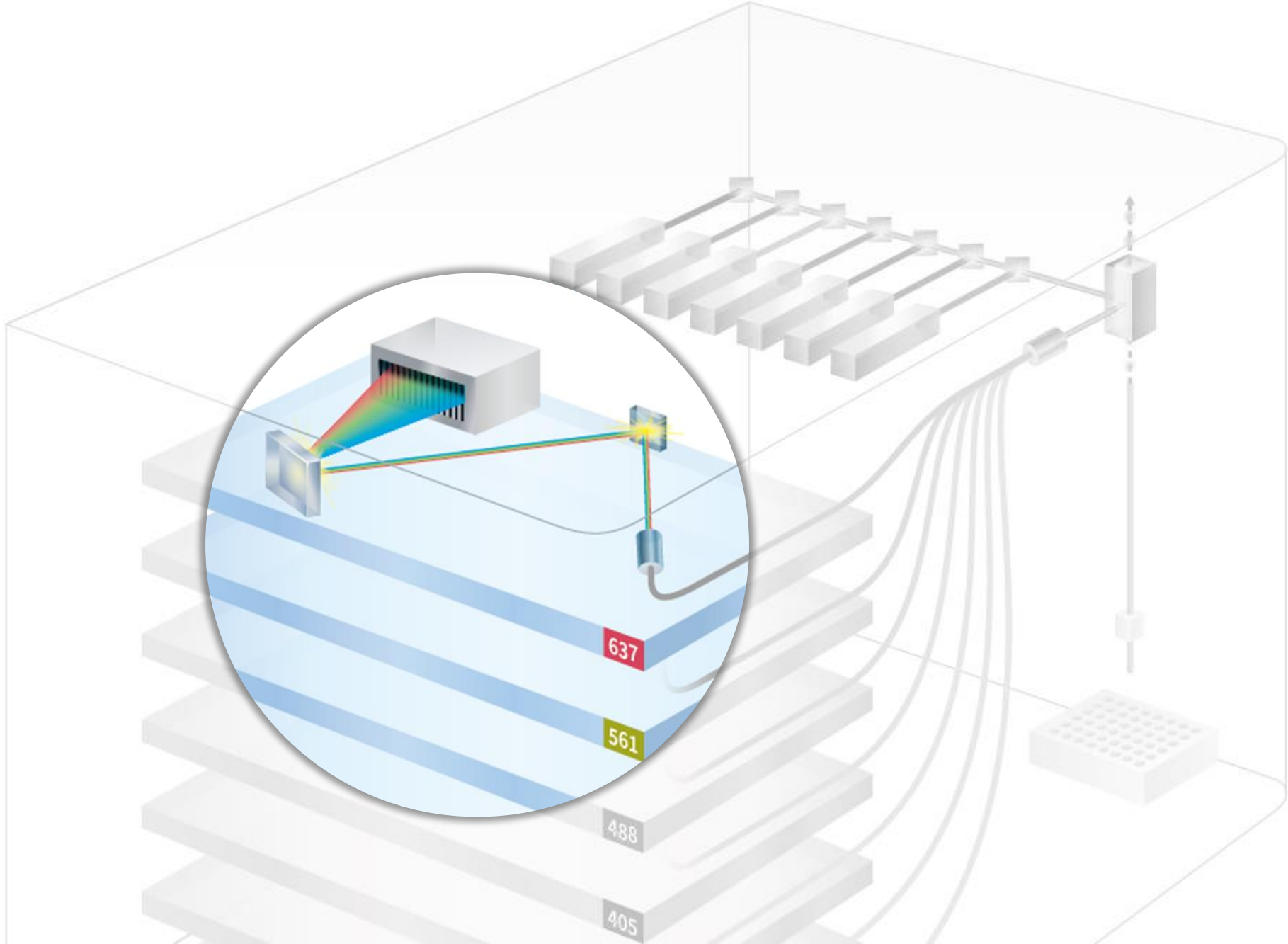
SONY

Up to Seven Lasers

Free-space delivery, not fiber.

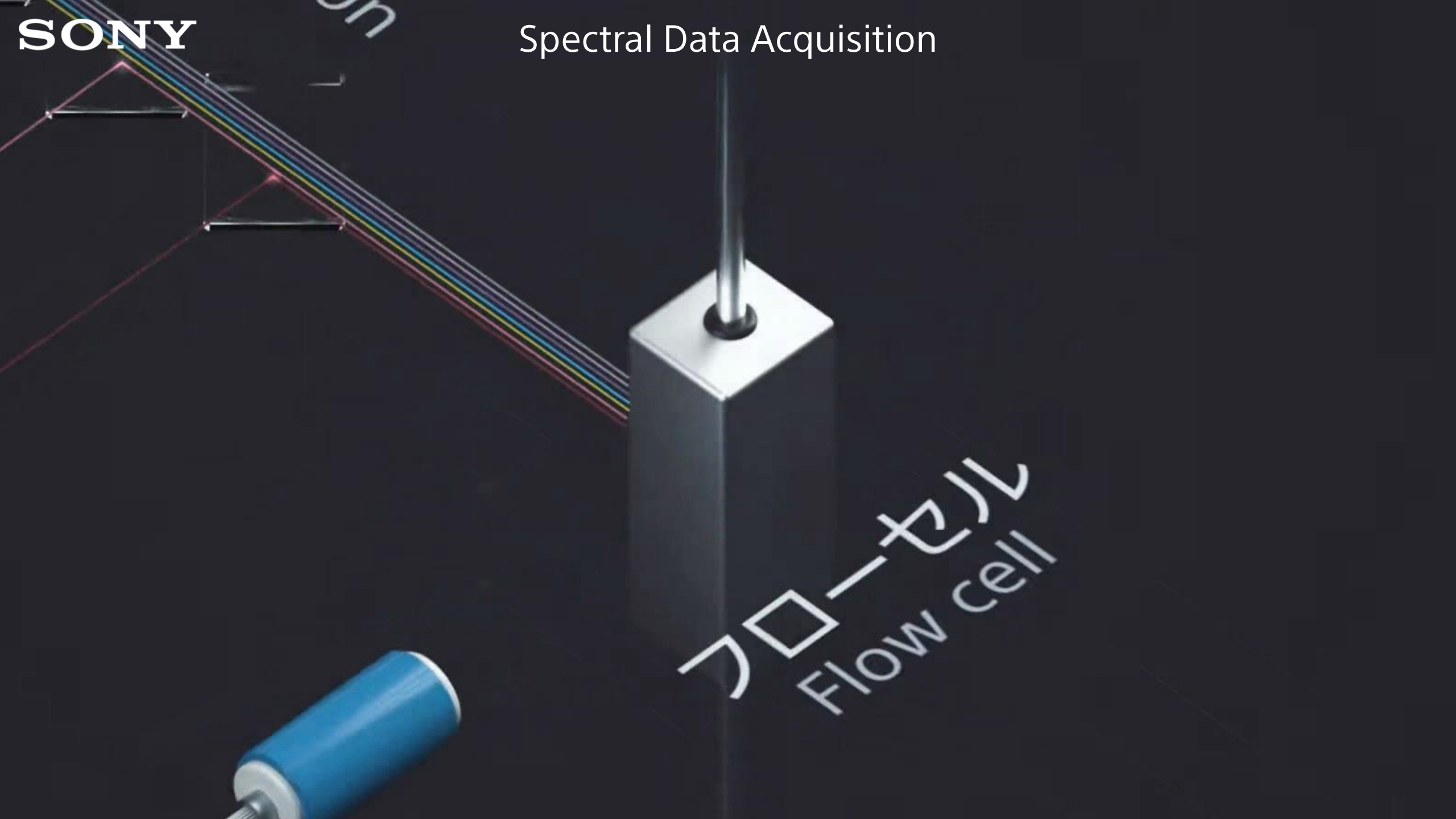


Spectral Data Acquisition

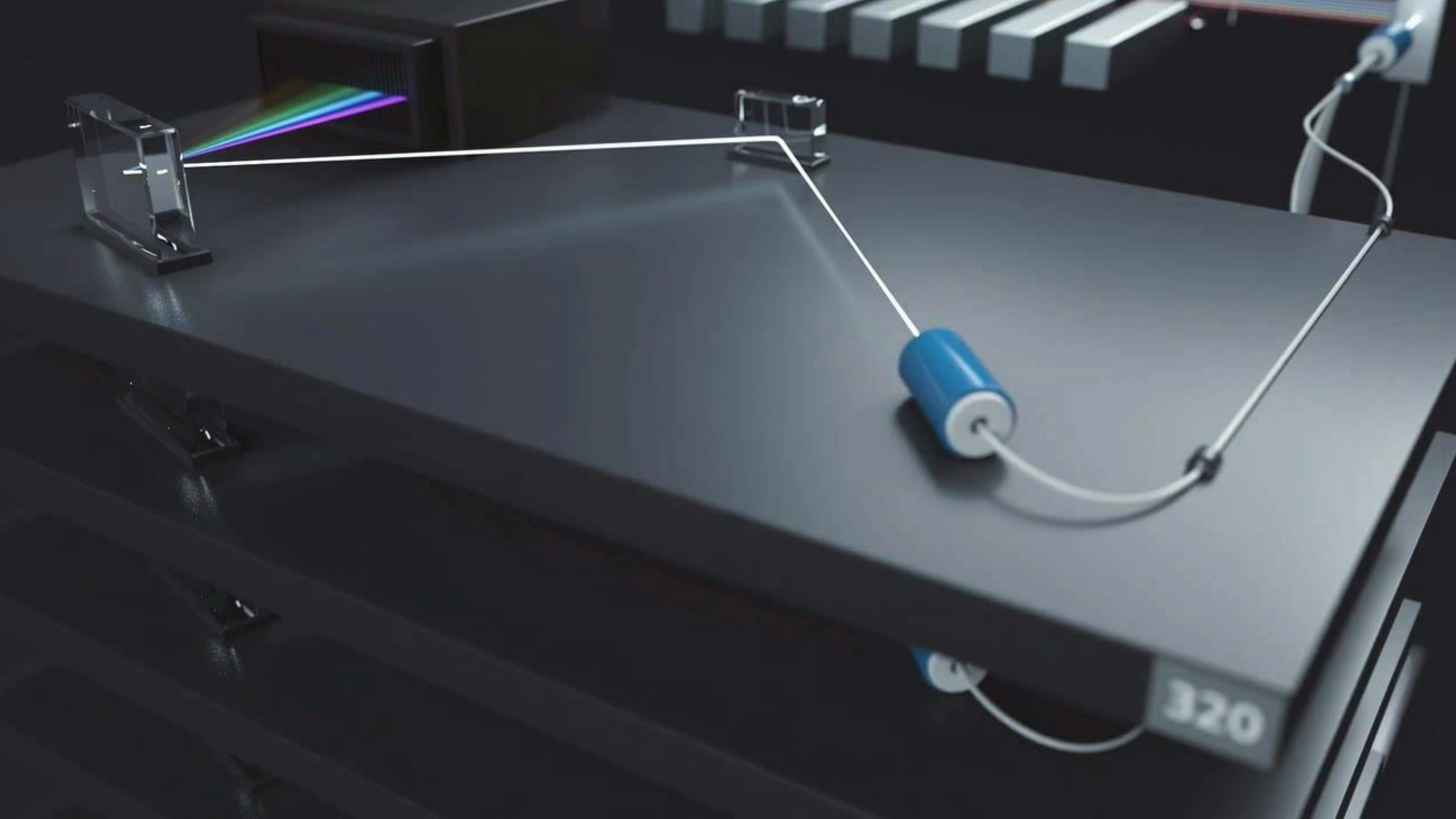


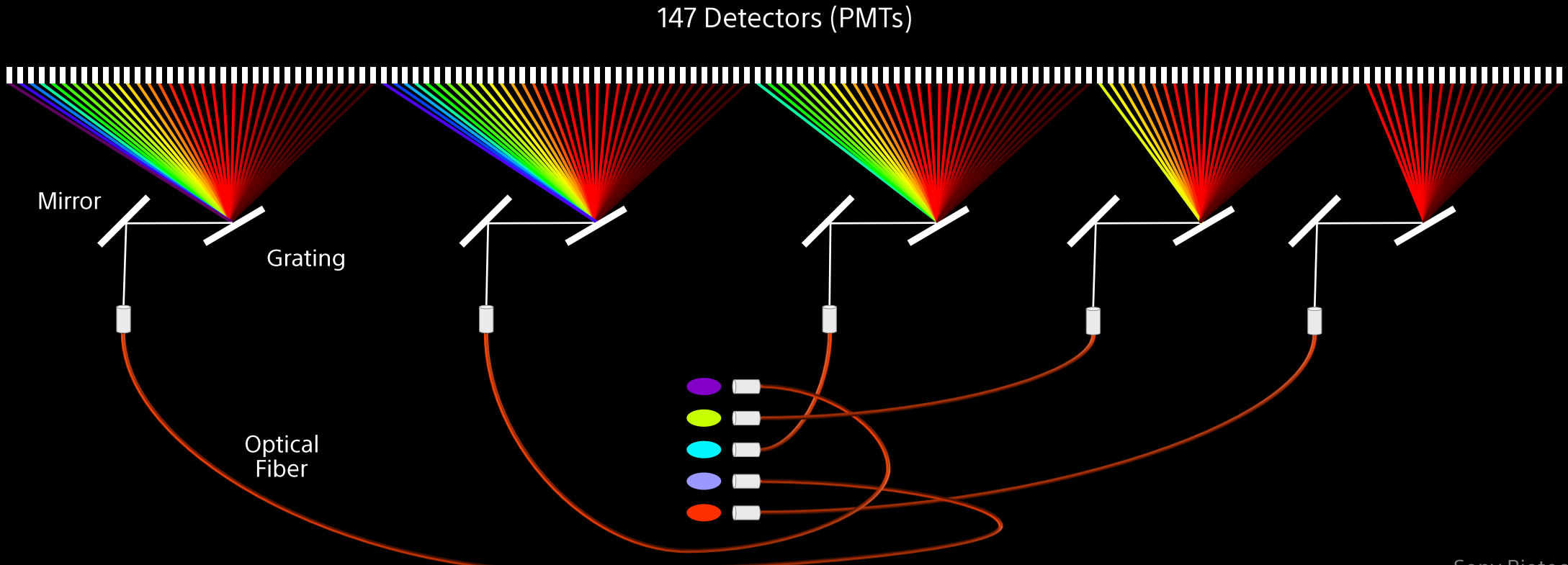
SONY

Spectral Data Acquisition



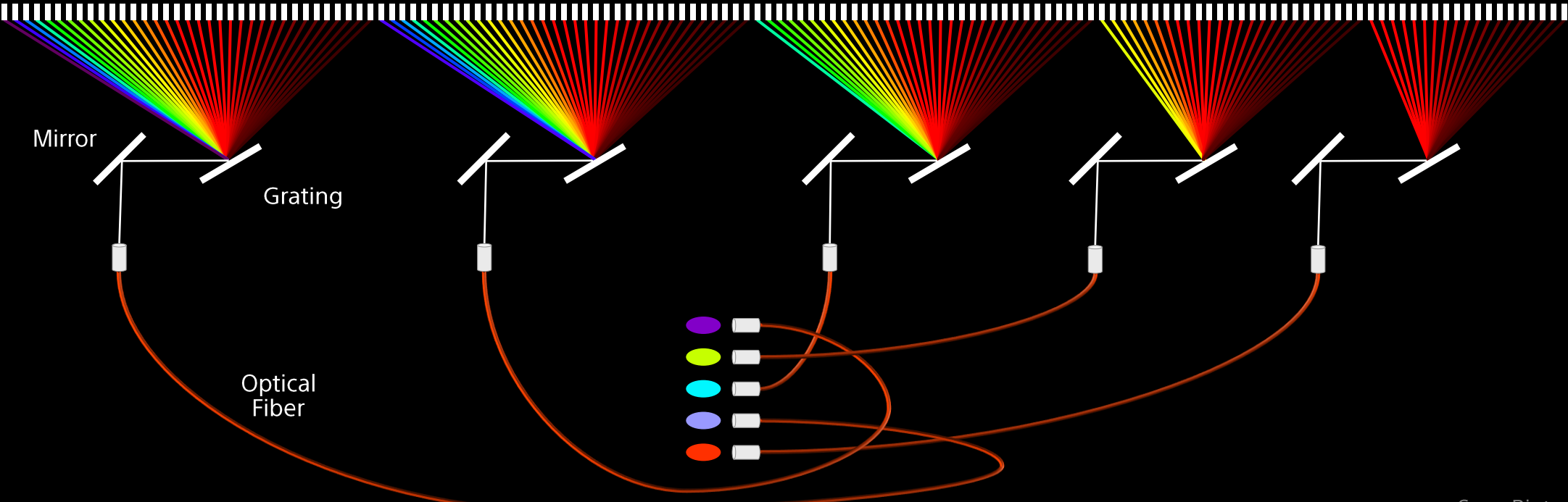
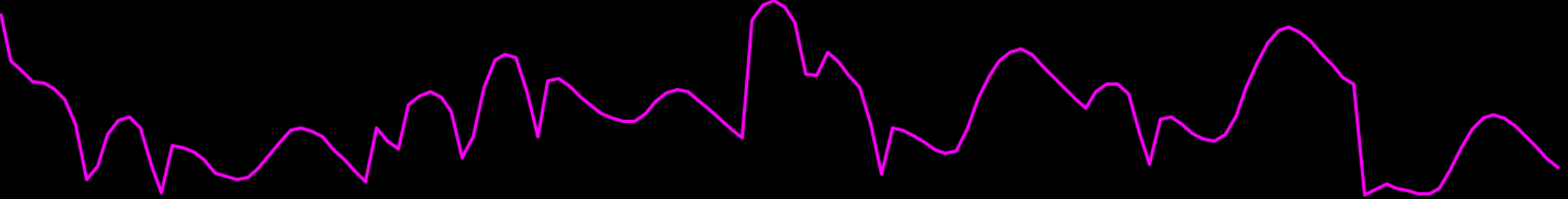
フローセル
FLOW CELL





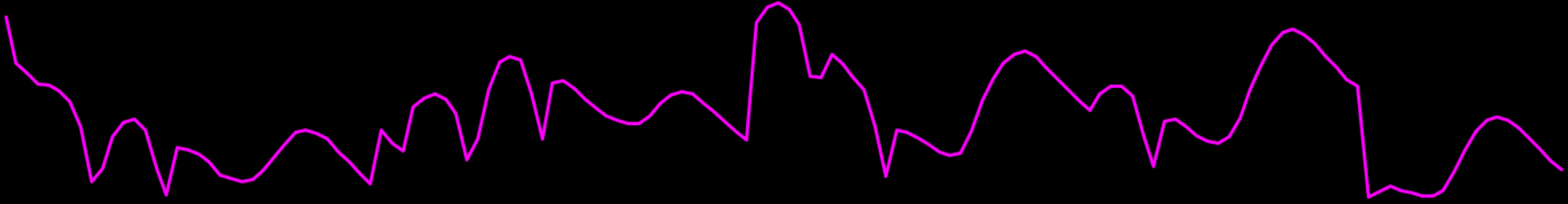
Each cell produces a unique set of intensity values, a unique **spectral signature**.

Fluorescence Intensity



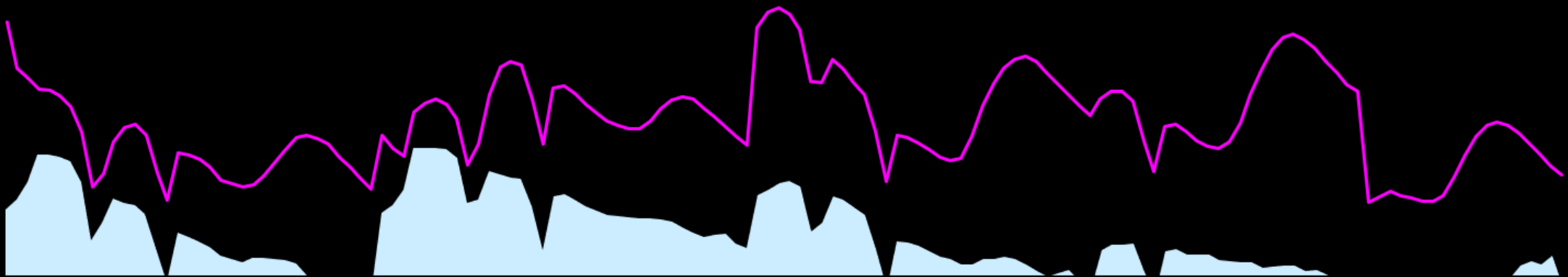
Spectral Data

The shape of each cell's **spectral signature** is determined by the combination of fluorochromes and autofluorescence in or on that cell.




Spectral Data

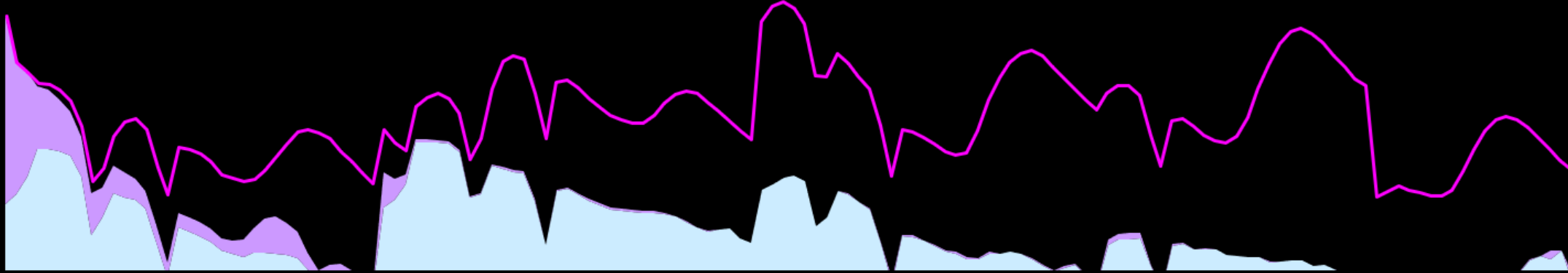
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AF
colour 1

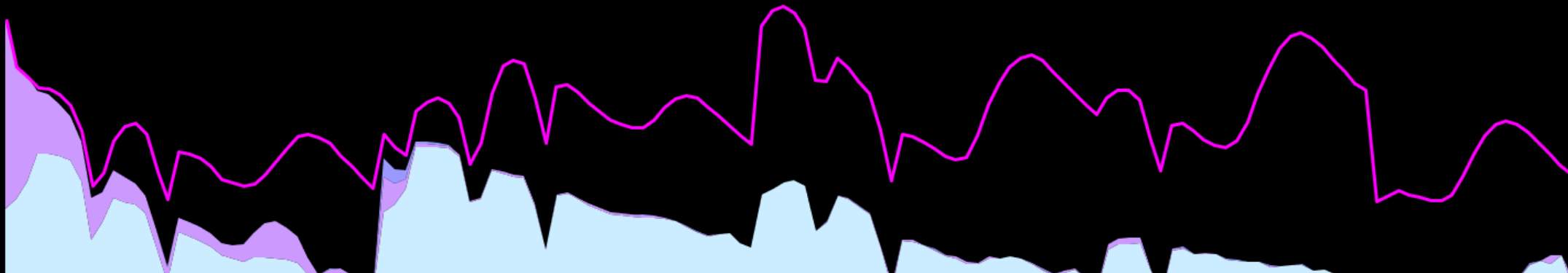


The shape of each cell's **spectral signature** is determined by the combination of fluorochromes and autofluorescence in or on that cell.



AF colour 1	CD45RA BUV395
	

The shape of each cell's **spectral signature** is determined by the combination of fluorochromes and autofluorescence in or on that cell.



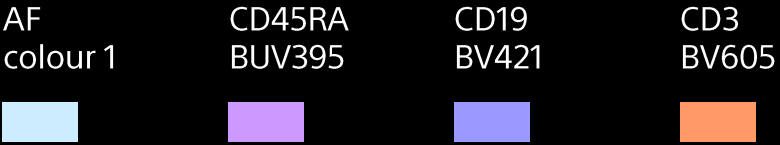
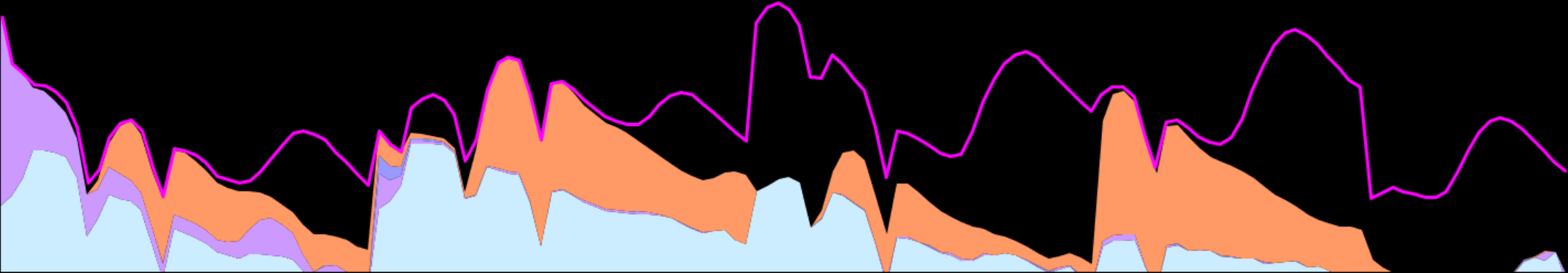
AF
colour 1

CD45RA
BUV395

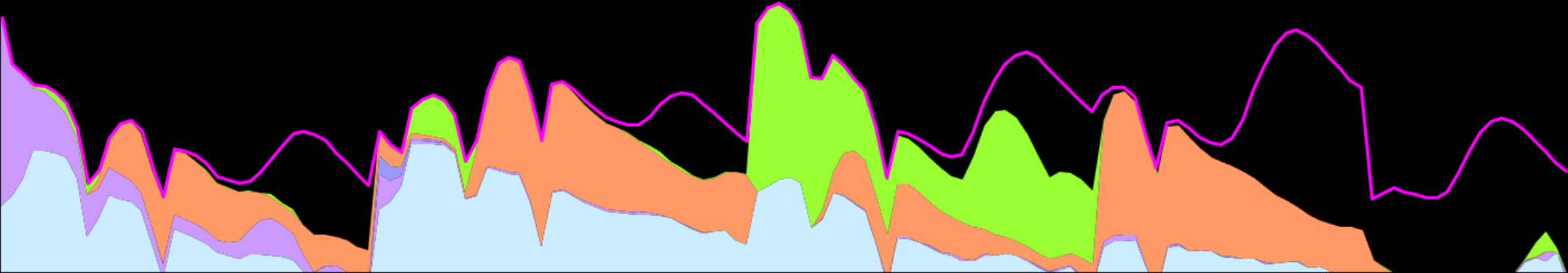
CD19
BV421



The shape of each cell's **spectral signature** is determined by the combination of fluorochromes and autofluorescence in or on that cell.

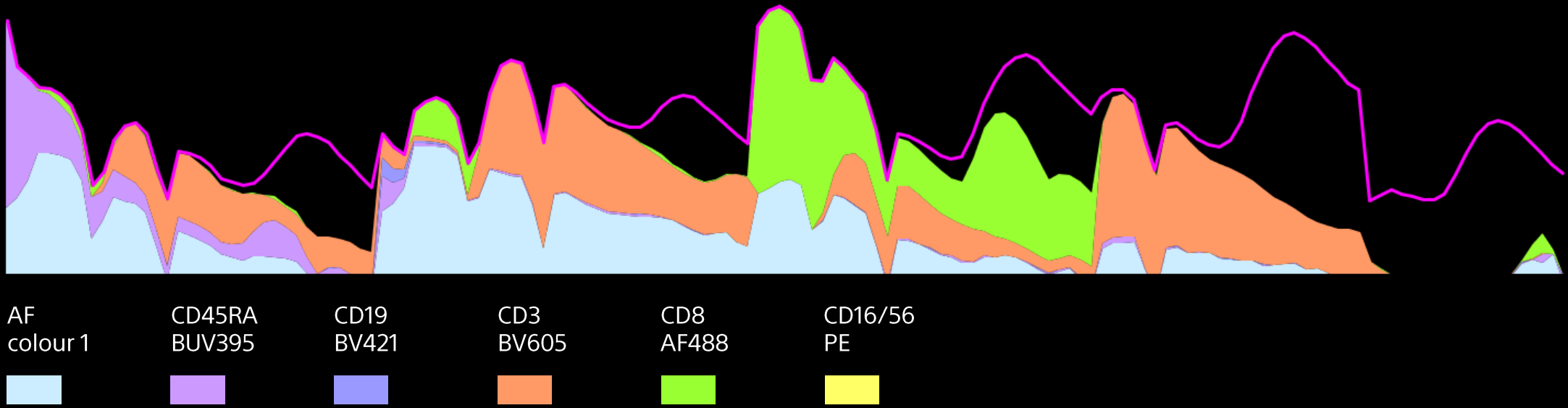


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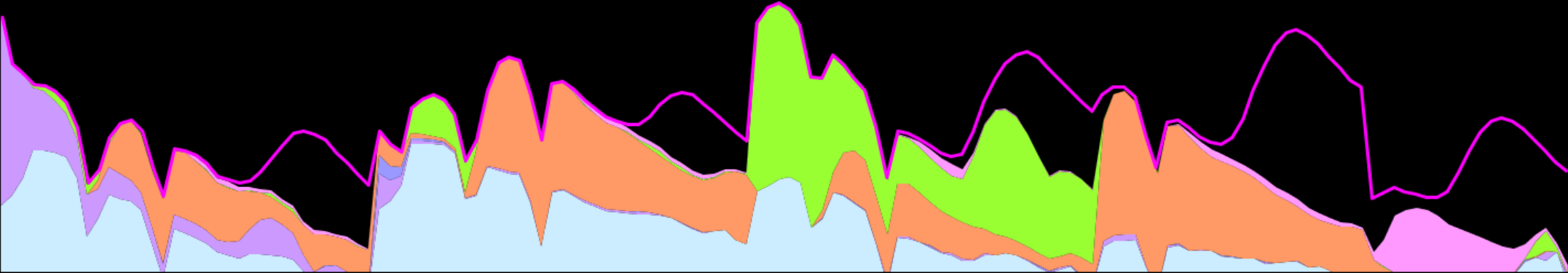


- AF colour 1
- CD45RA BUV395
- CD19 BV421
- CD3 BV605
- CD8 AF488

The shape of each cell's **spectral signature** is determined by the combination of fluorochromes and autofluorescence in or on that cell.

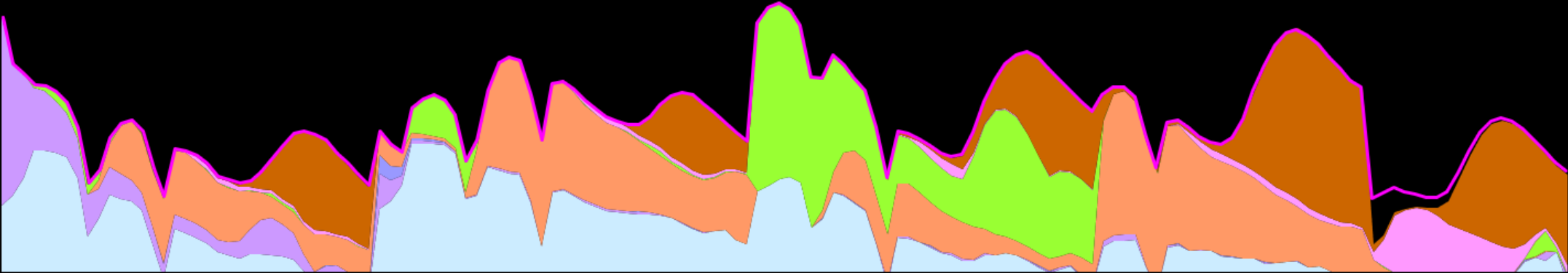



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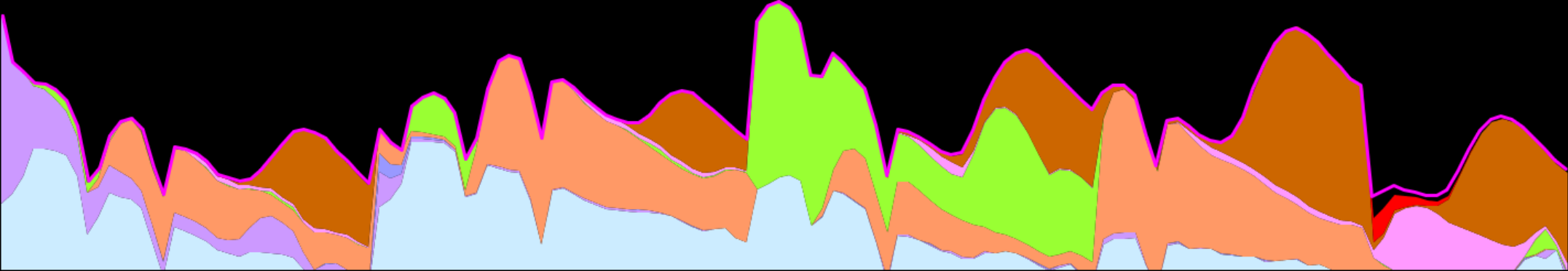
- AF colour 1
- CD45RA BUV395
- CD19 BV421
- CD3 BV605
- CD8 AF488
- CD16/56 PE
- CD45RO PerCP-Cy5.5

The shape of each cell's **spectral signature** is determined by the combination of fluorochromes and autofluorescence in or on that cell.



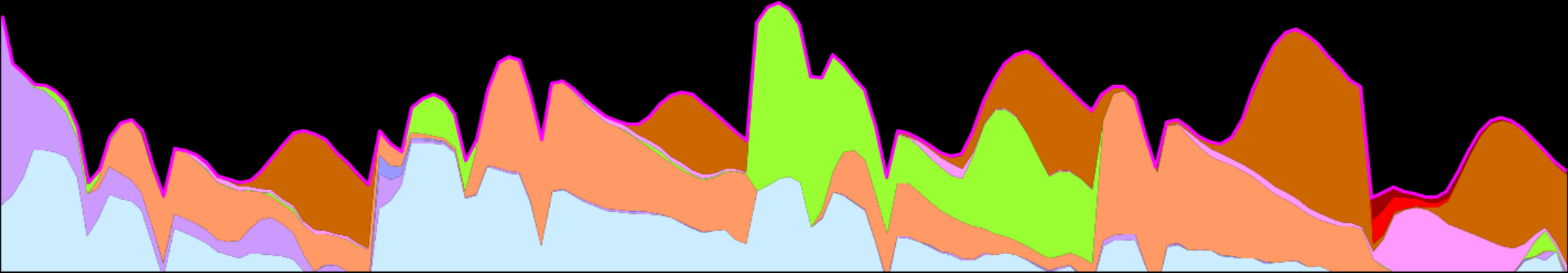
AF colour 1	CD45RA BUV395	CD19 BV421	CD3 BV605	CD8 AF488	CD16/56 PE	CD45RO PerCP-Cy5.5	CD7 PE-Cy7
							

The shape of each cell's **spectral signature** is determined by the combination of fluorochromes and autofluorescence in or on that cell.

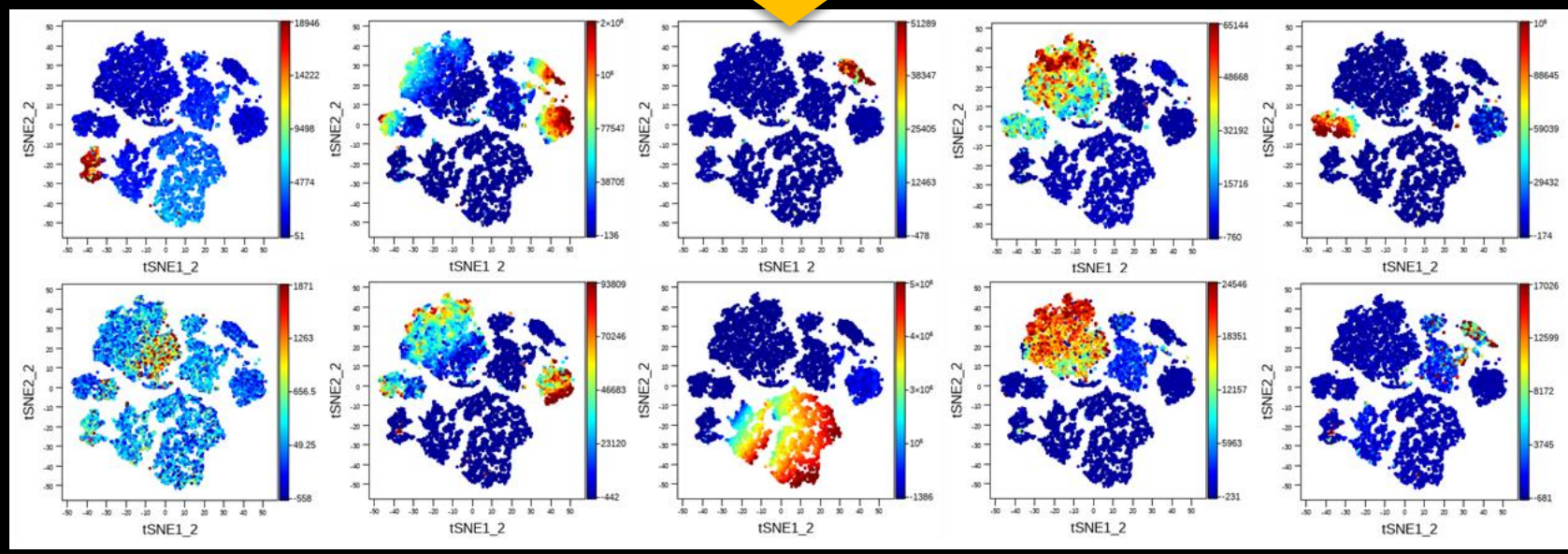
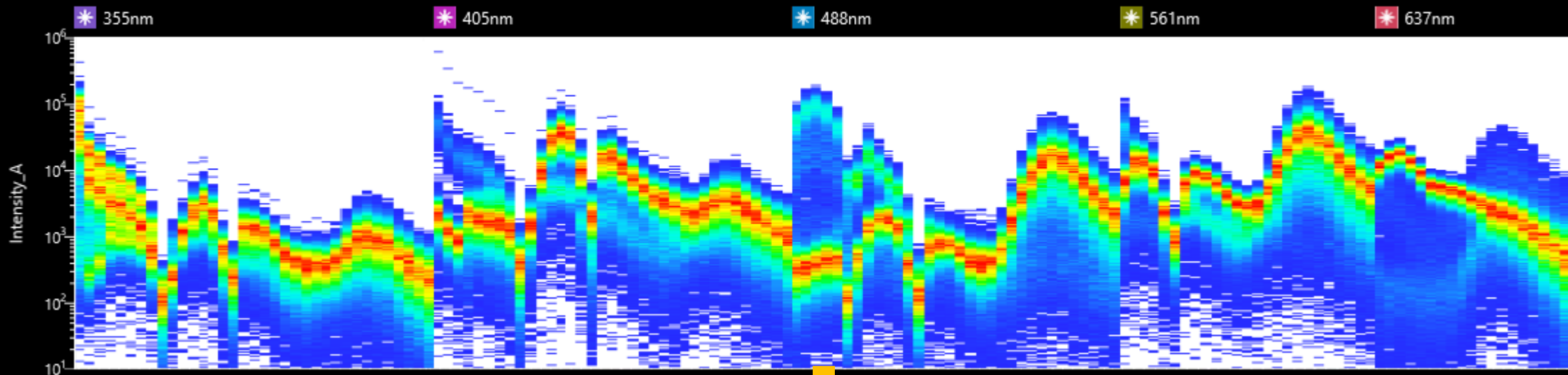


AF colour 1	CD45RA BUV395	CD19 BV421	CD3 BV605	CD8 AF488	CD16/56 PE	CD45RO PerCP-Cy5.5	CD7 PE-Cy7	CD4 AF647
								

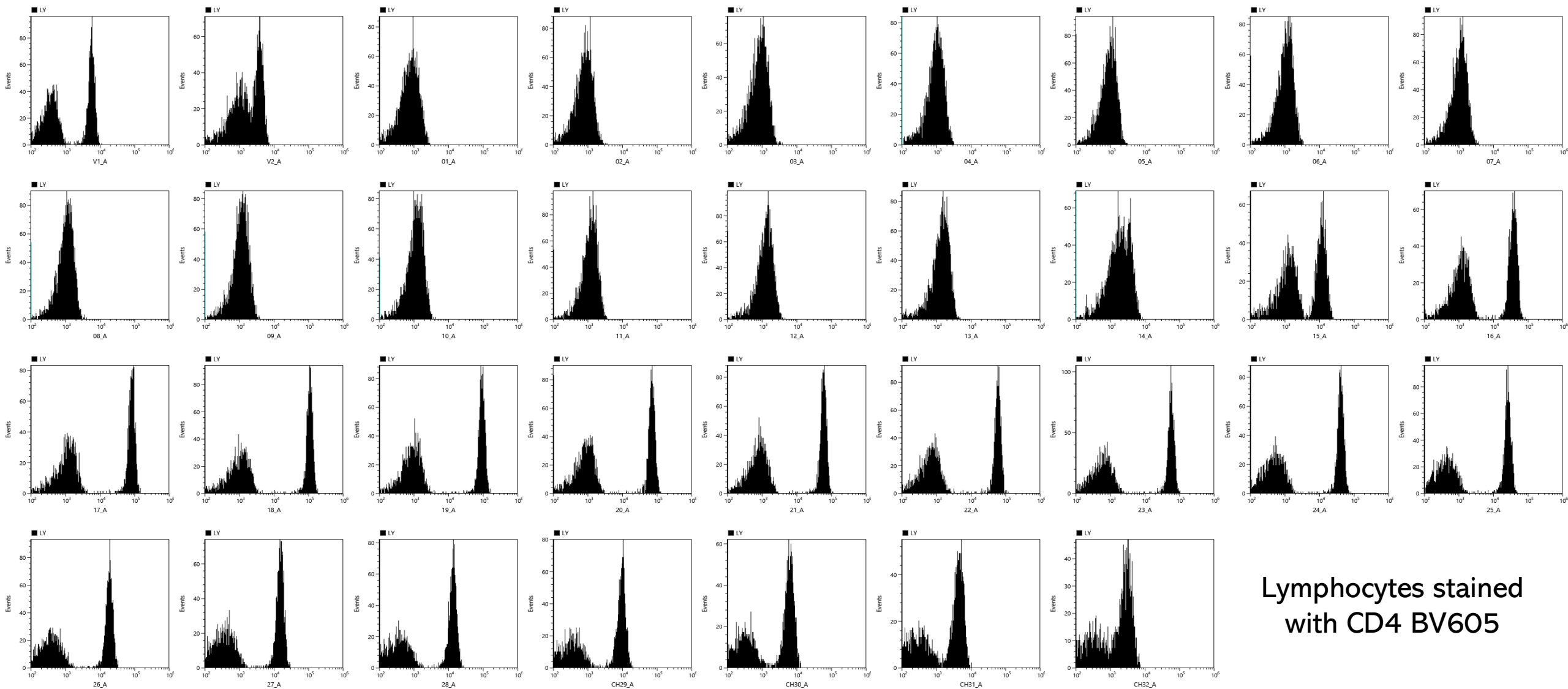
The shape of each cell's **spectral signature** is determined by the combination of fluorochromes and autofluorescence in or on that cell.



AF colour 1	CD45RA BUV395	CD19 BV421	CD3 BV605	CD8 AF488	CD16/56 PE	CD45RO PerCP-Cy5.5	CD7 PE-Cy7	CD4 AF647	HLADR APC-Cy7
									

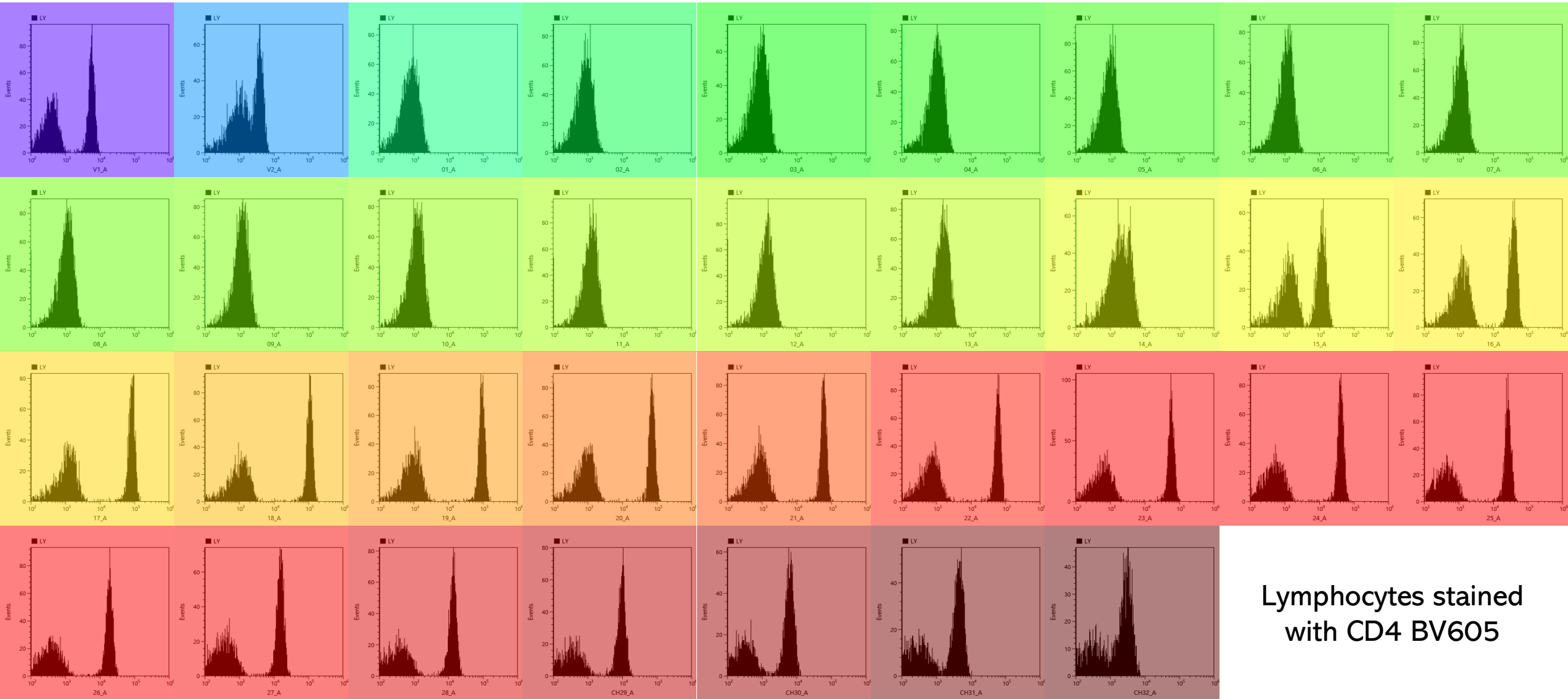


Data from the 405nm laser

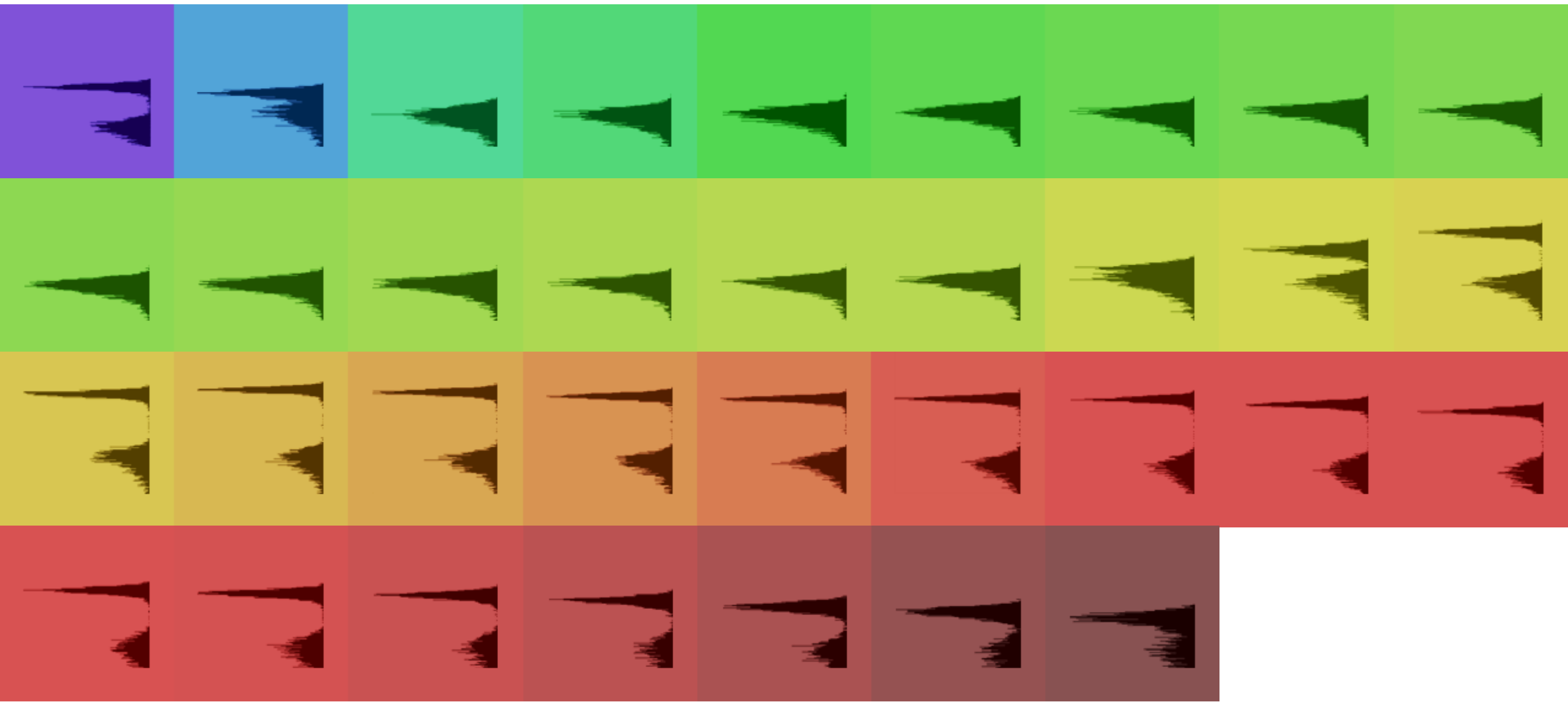


Lymphocytes stained
with CD4 BV605

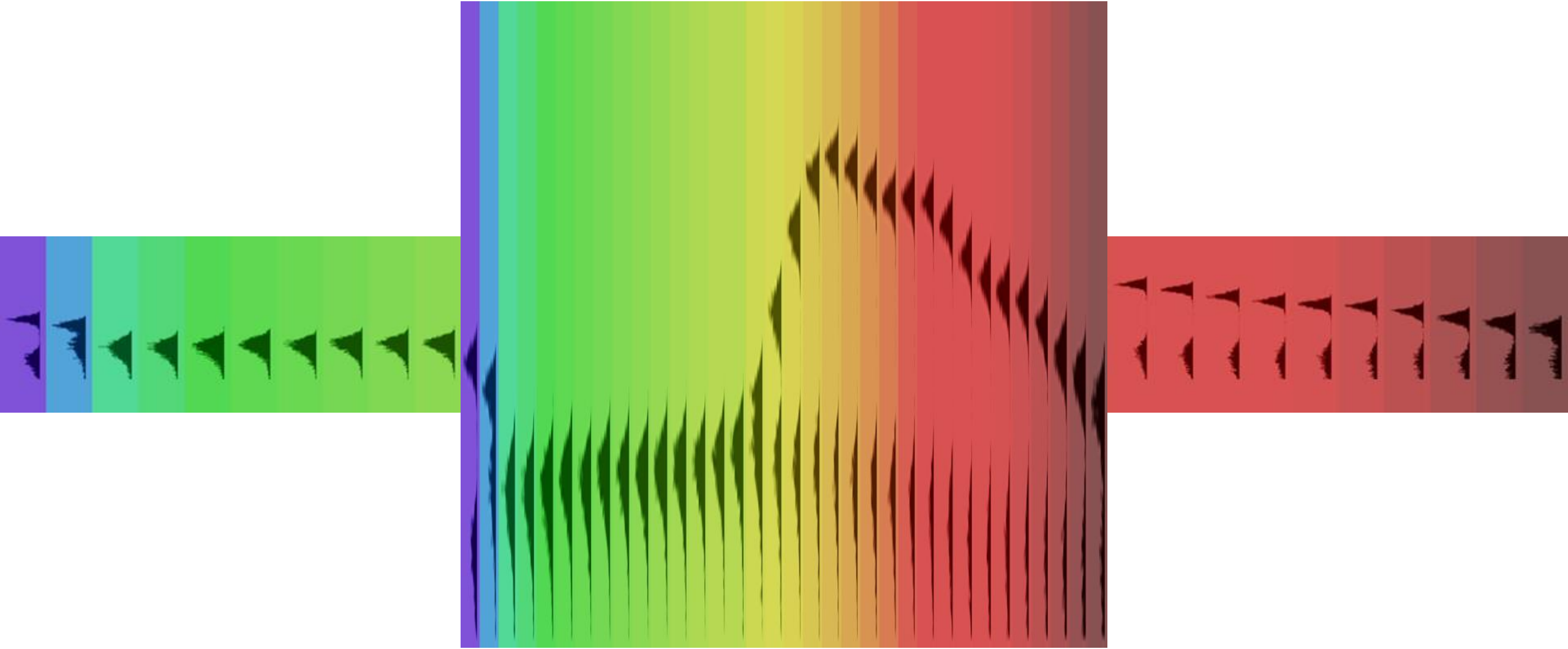
Data from the 405nm laser



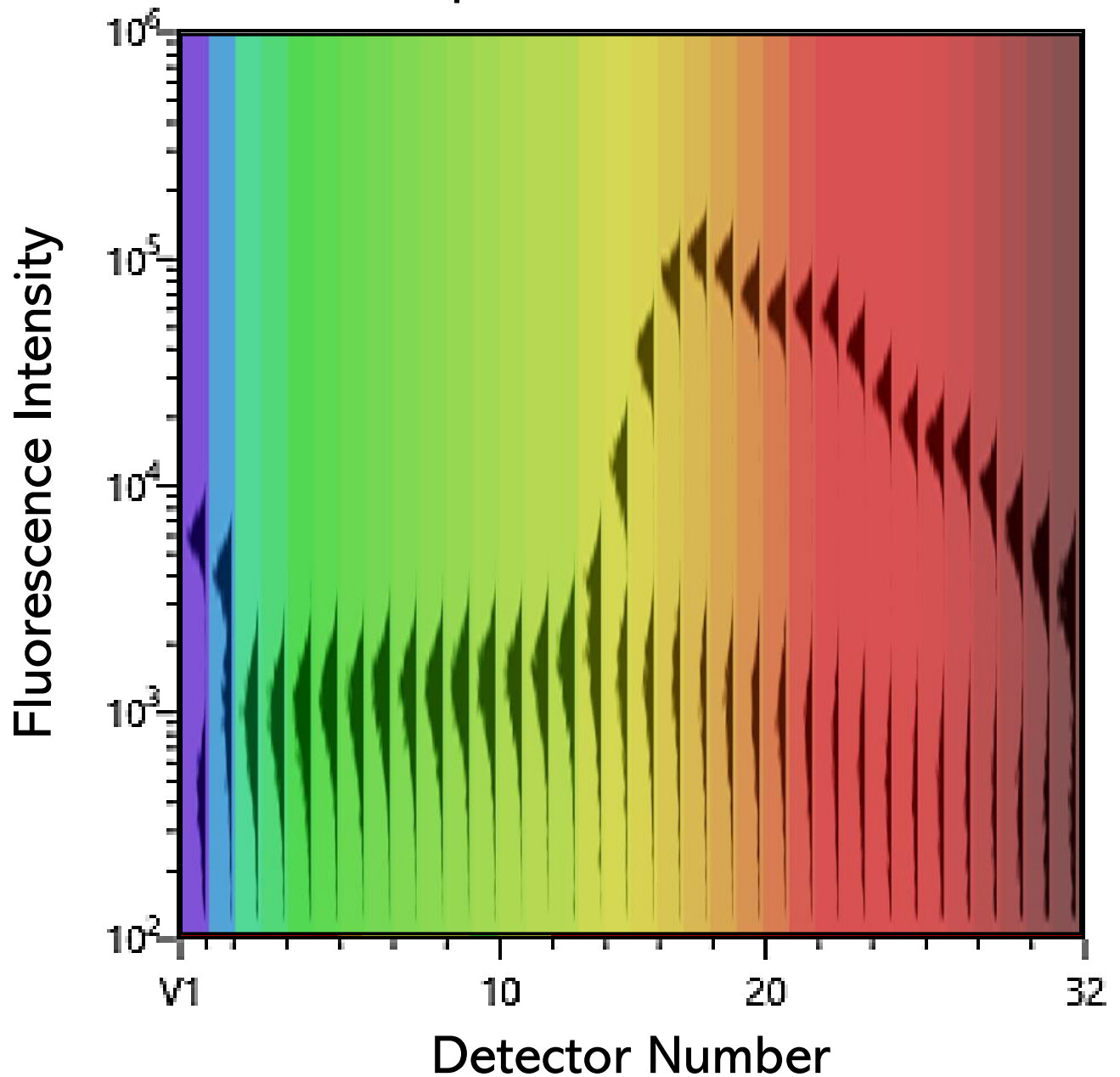
Lymphocytes stained
with CD4 BV605



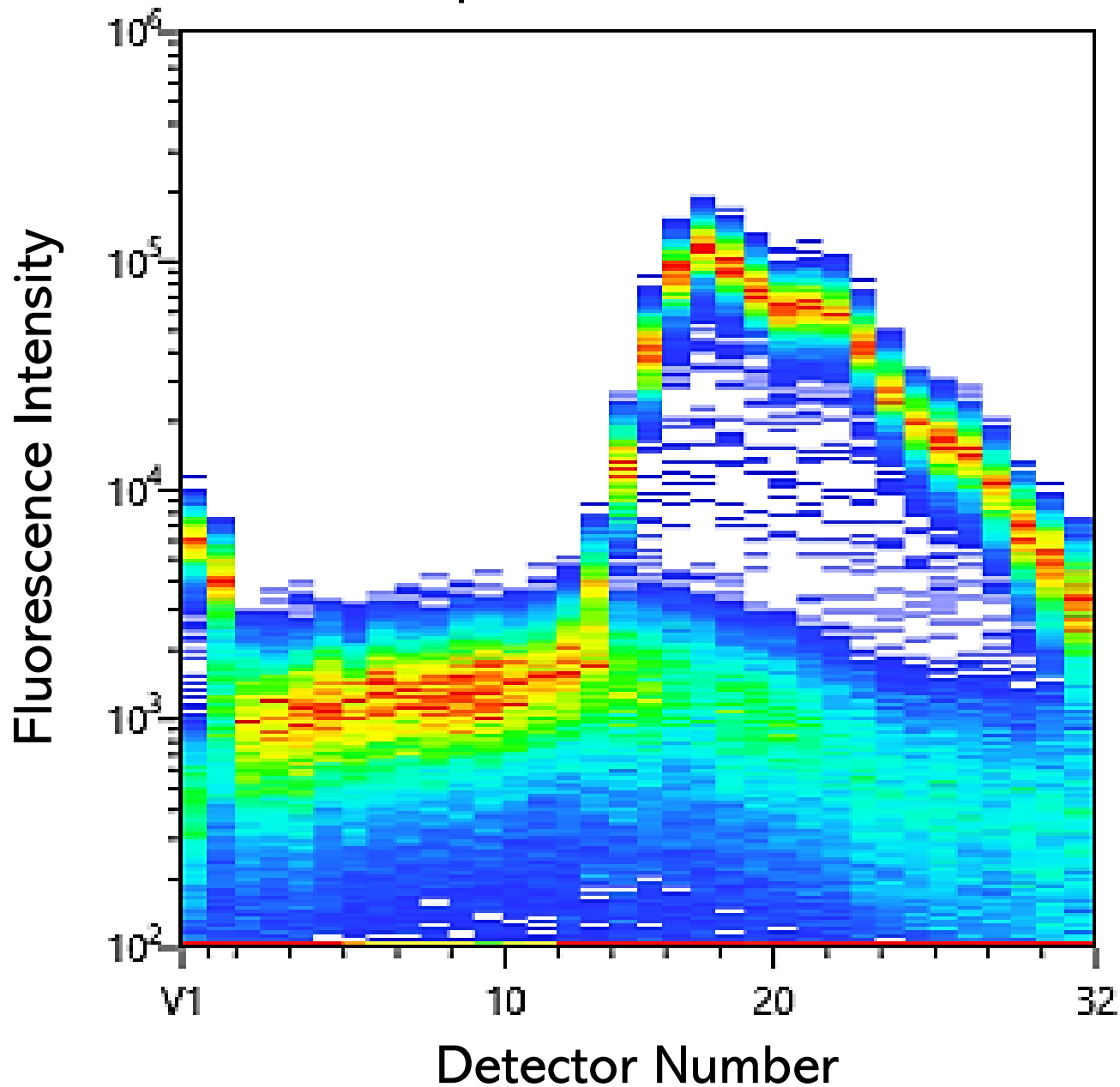
SONY



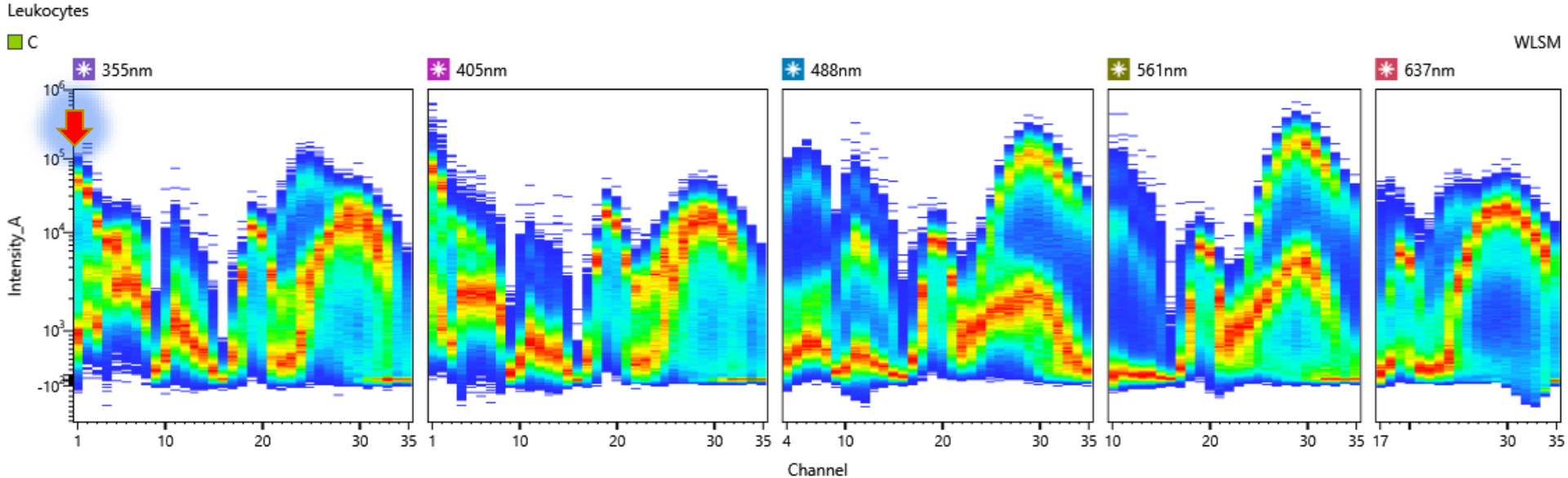
Spectral Data Plot



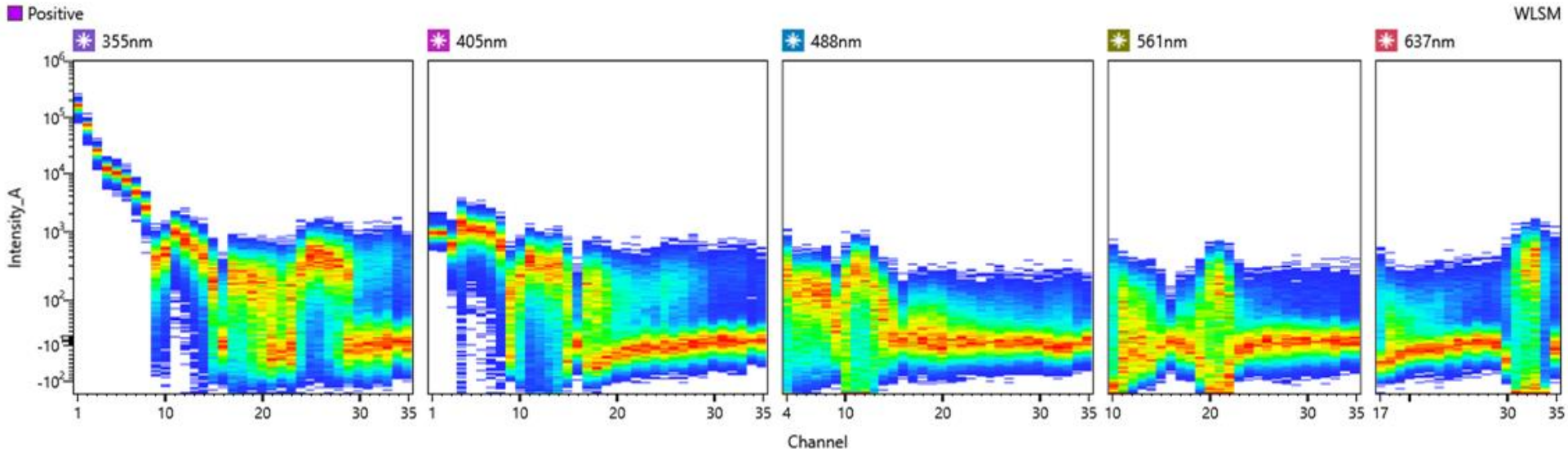
Spectral Data Plot



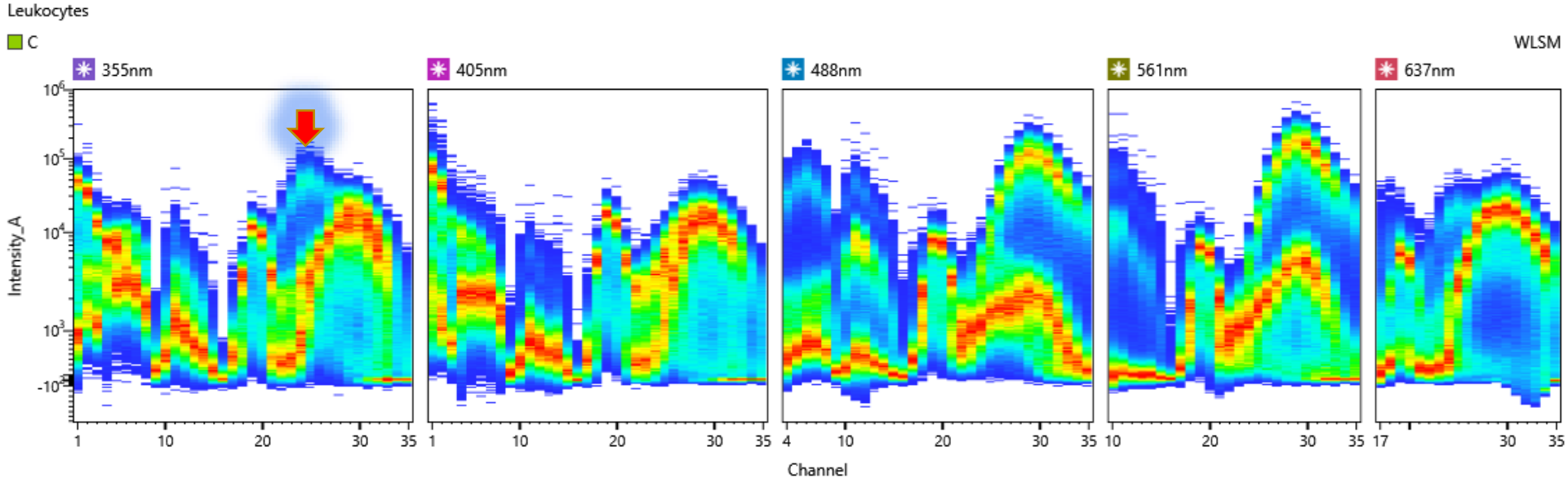
10c PBMCs: Raw Data



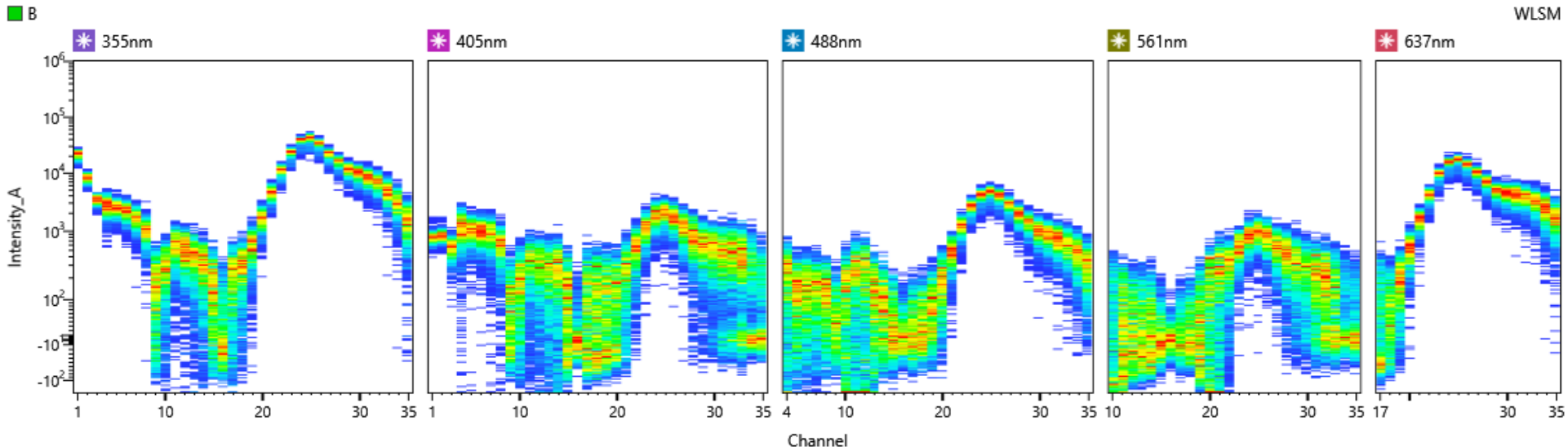
BUV395 - beads



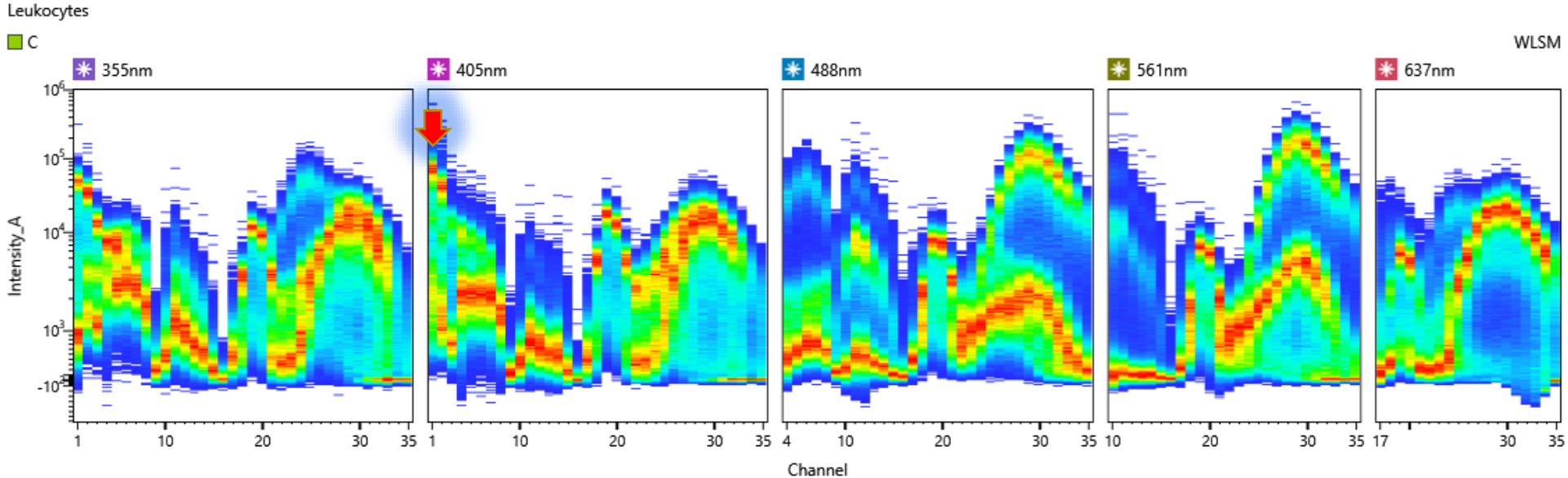
10c PBMCs: Raw Data



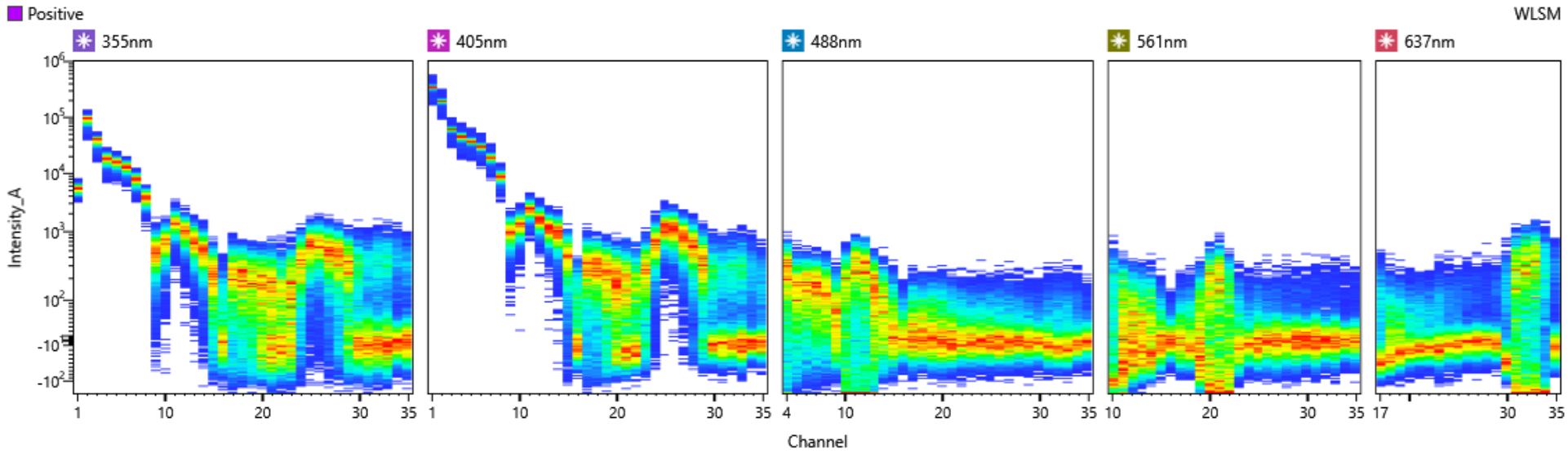
BUV737 - beads



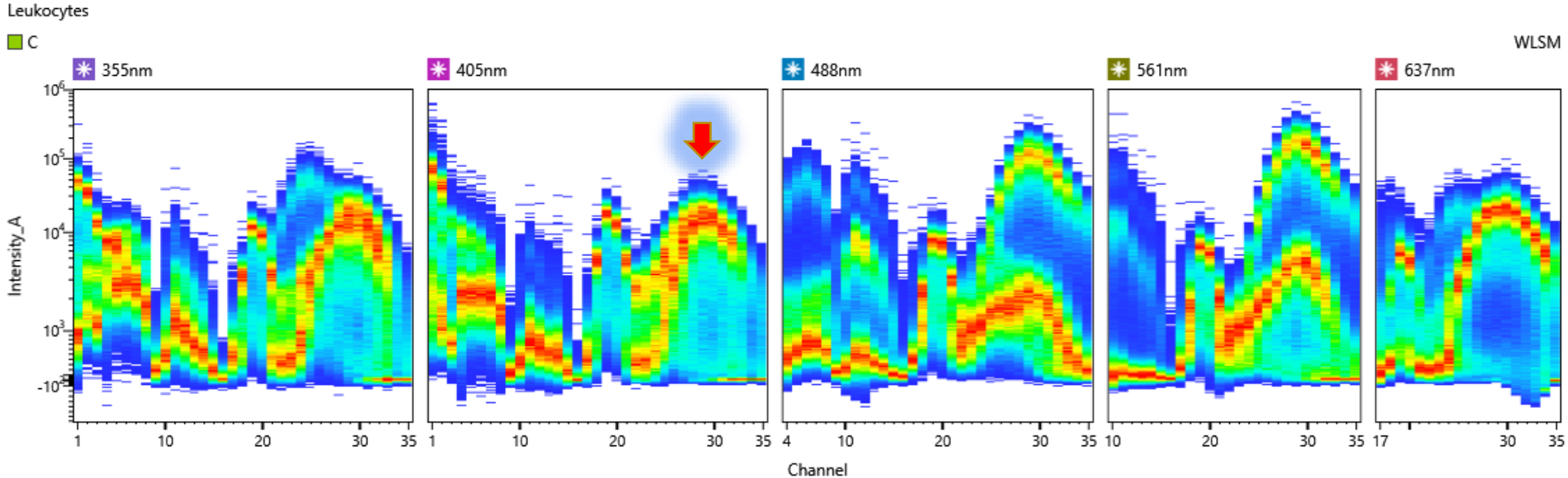
10c PBMCs: Raw Data



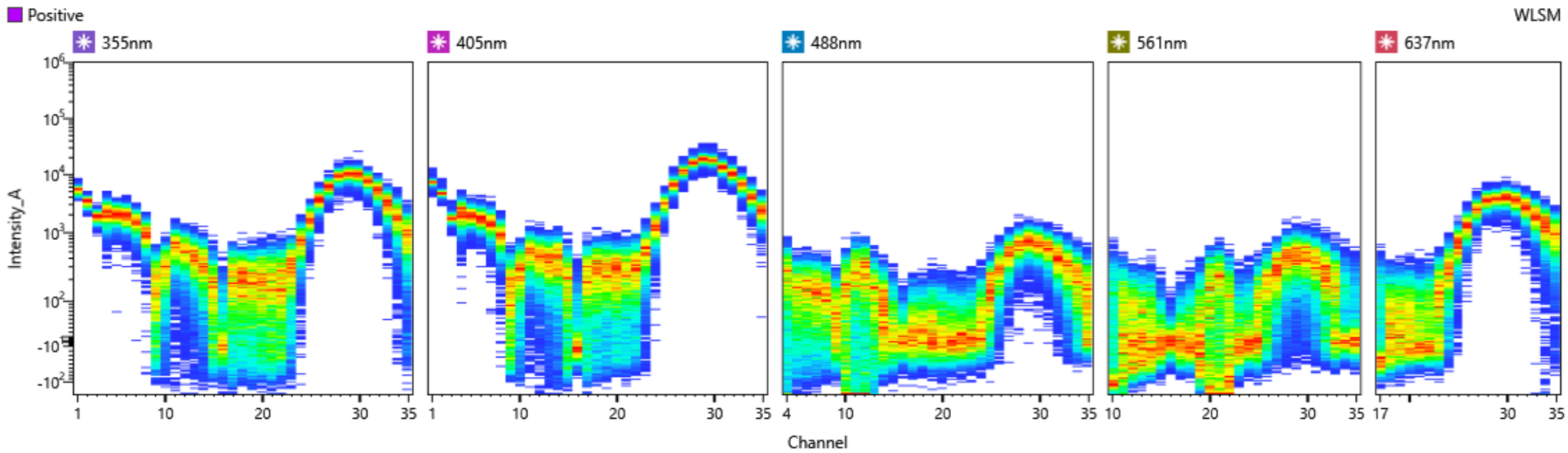
BV421 - beads



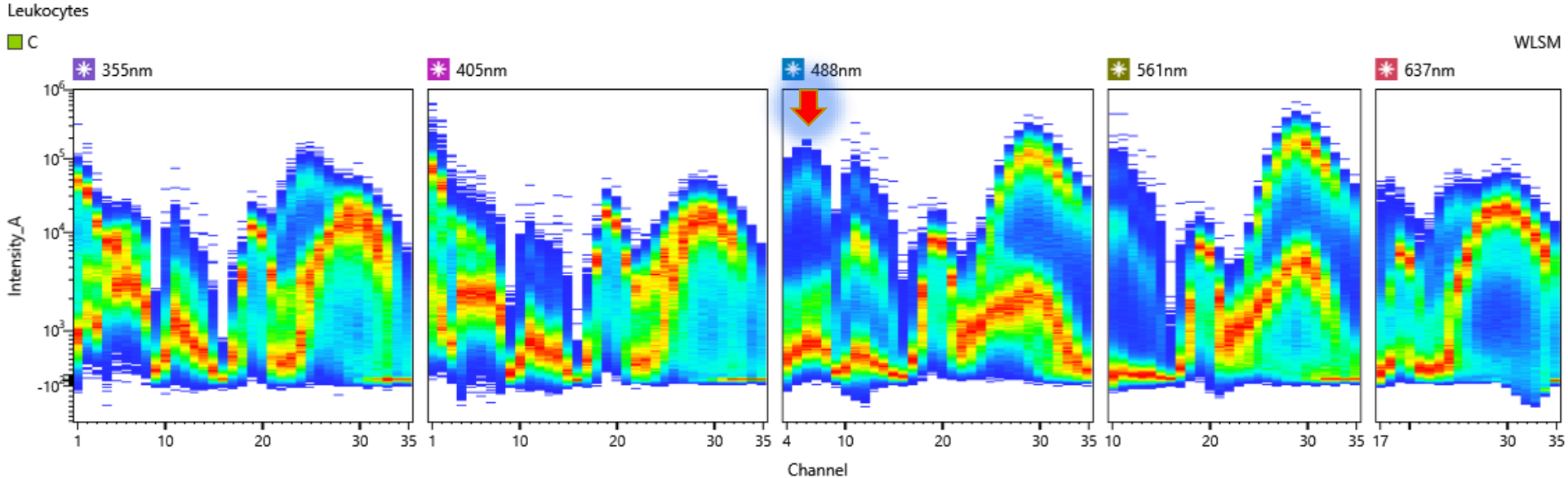
10c PBMCs: Raw Data



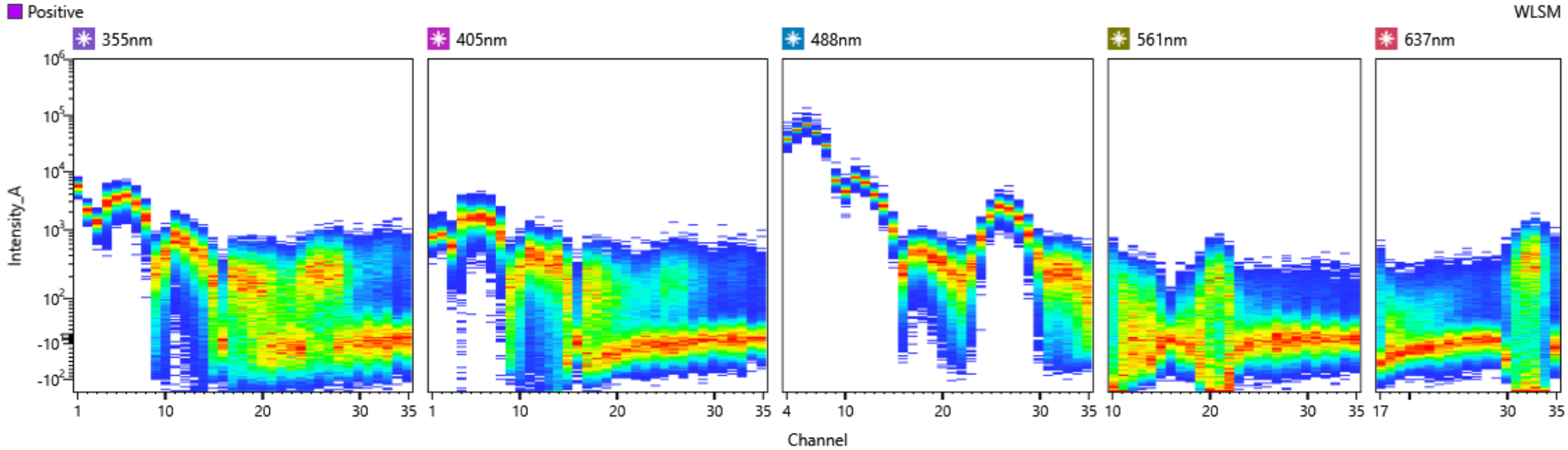
BV785 - beads



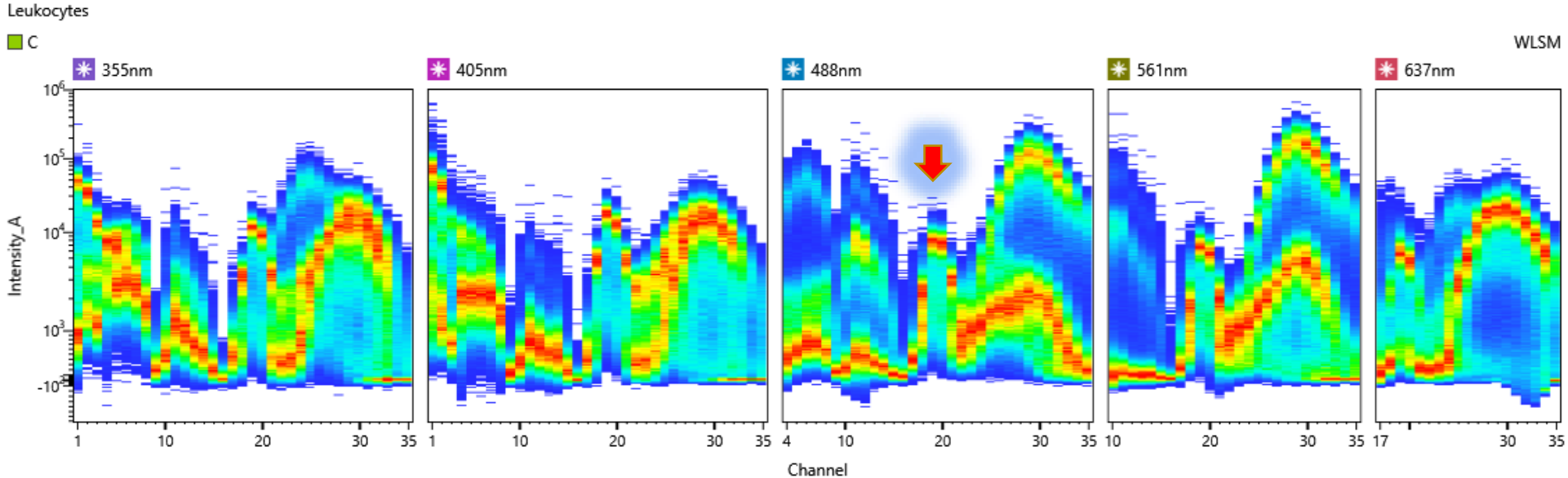
10c PBMCs: Raw Data



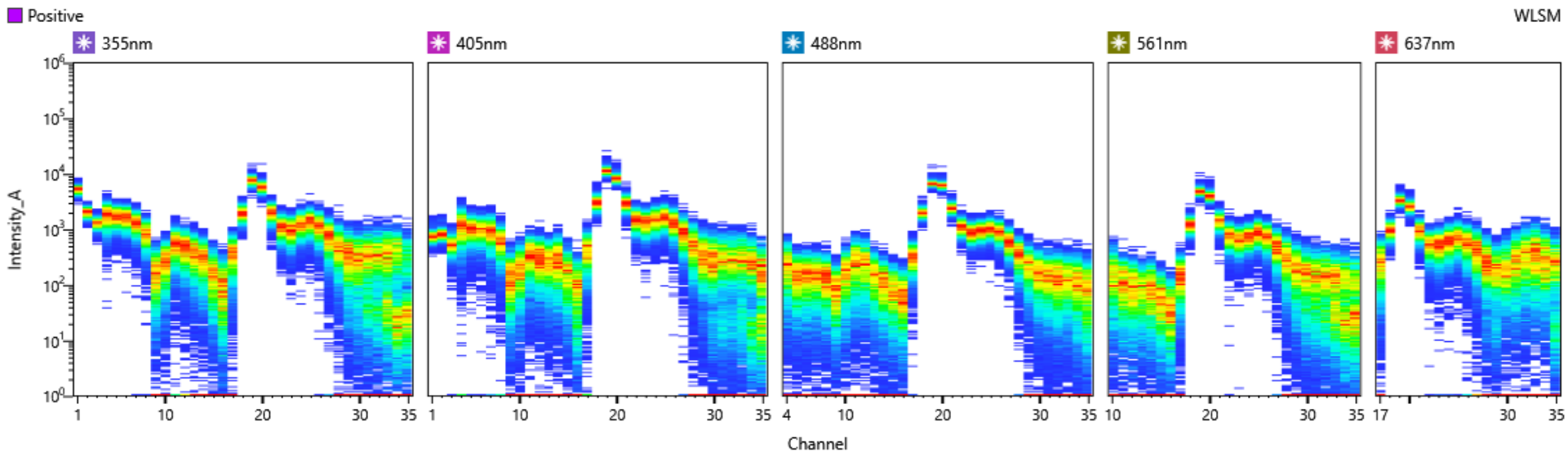
AF488 - beads



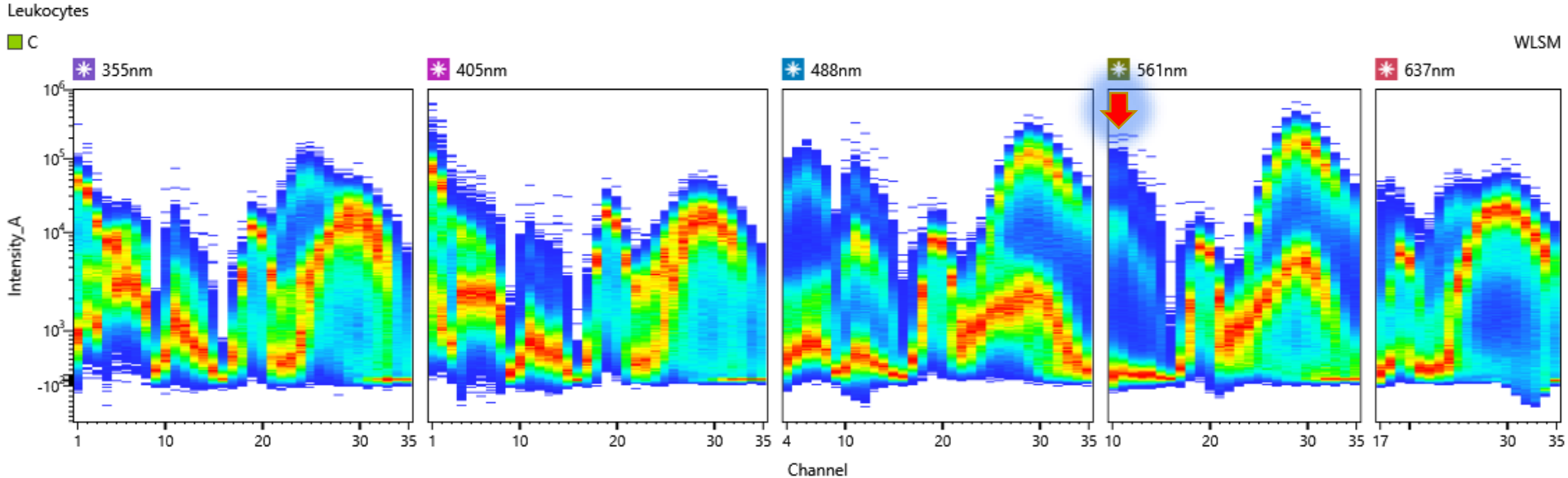
10c PBMCs: Raw Data



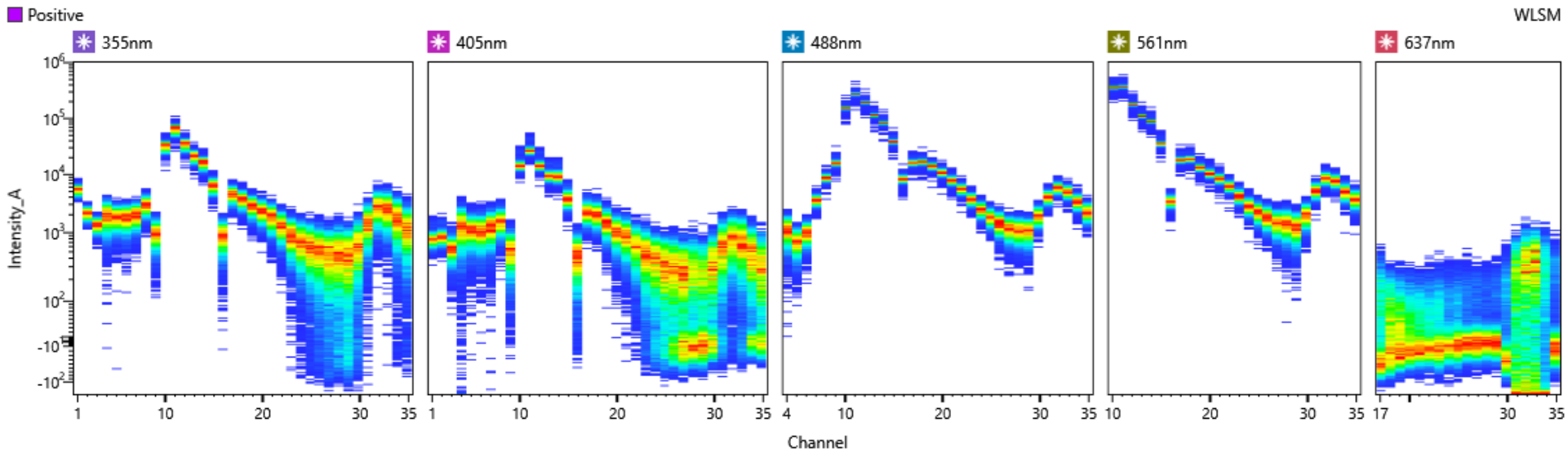
PerCP - beads



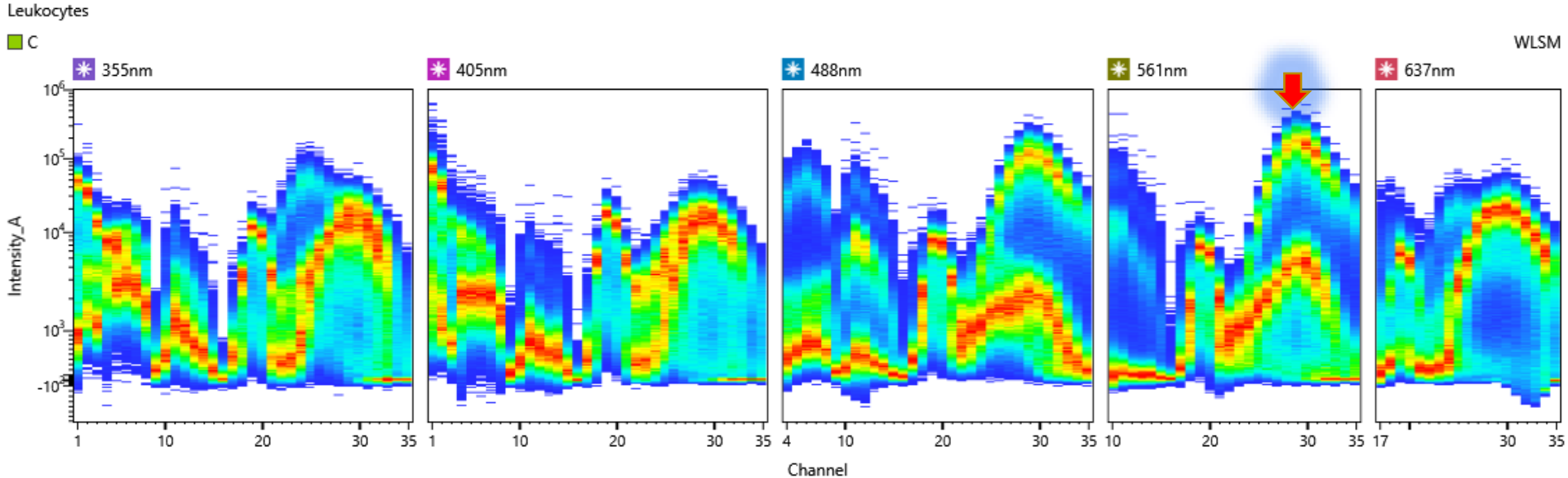
10c PBMCs: Raw Data



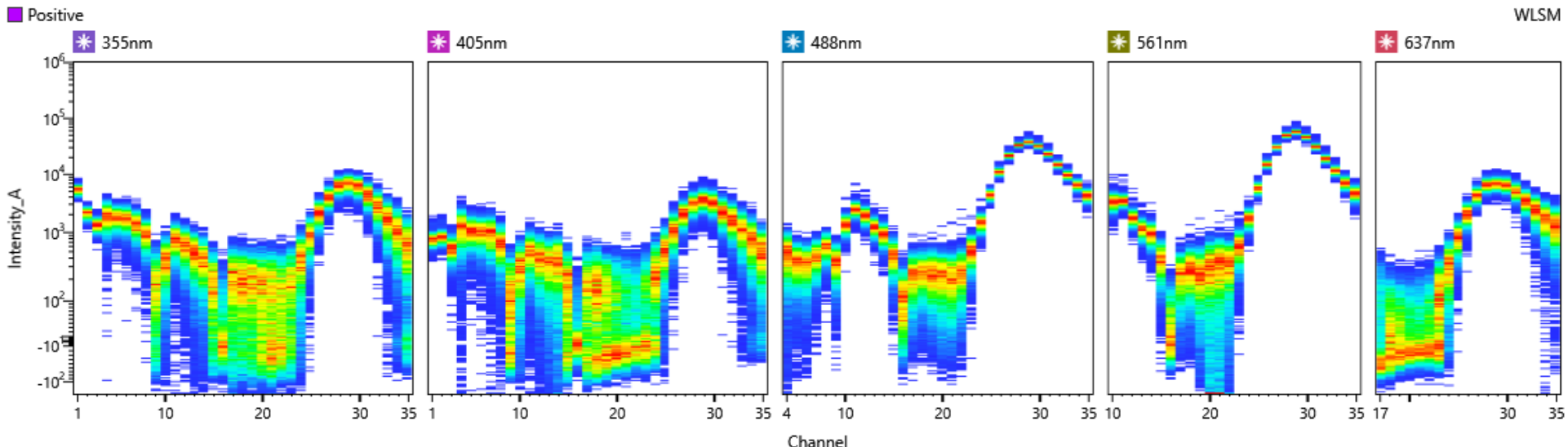
PE - beads



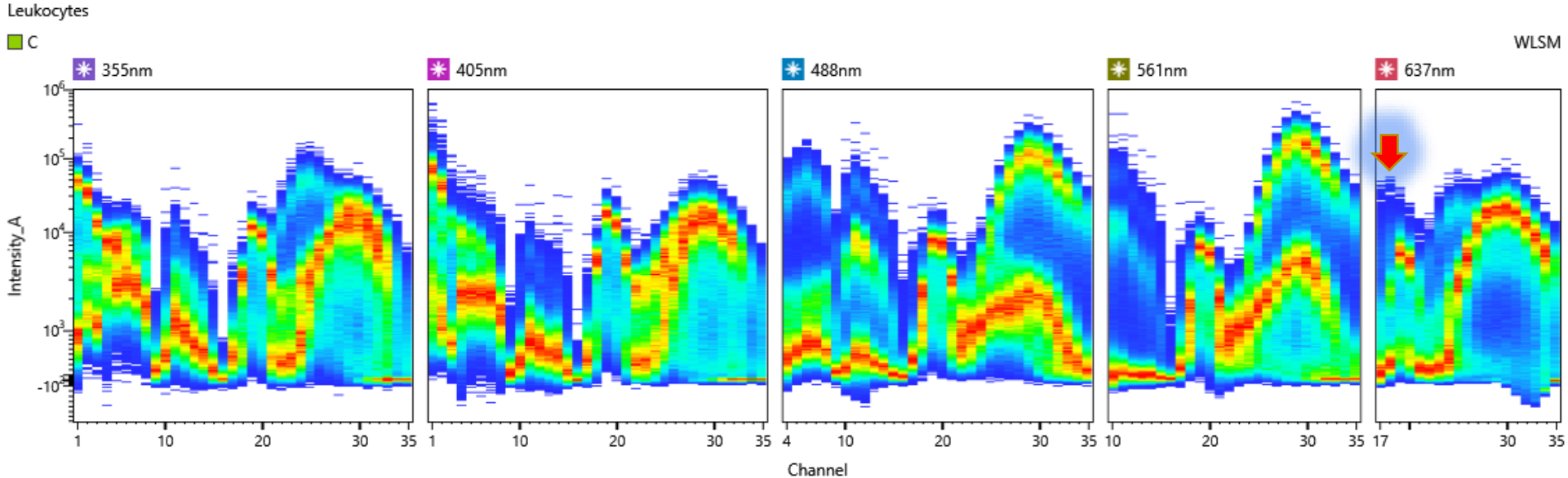
10c PBMCs: Raw Data



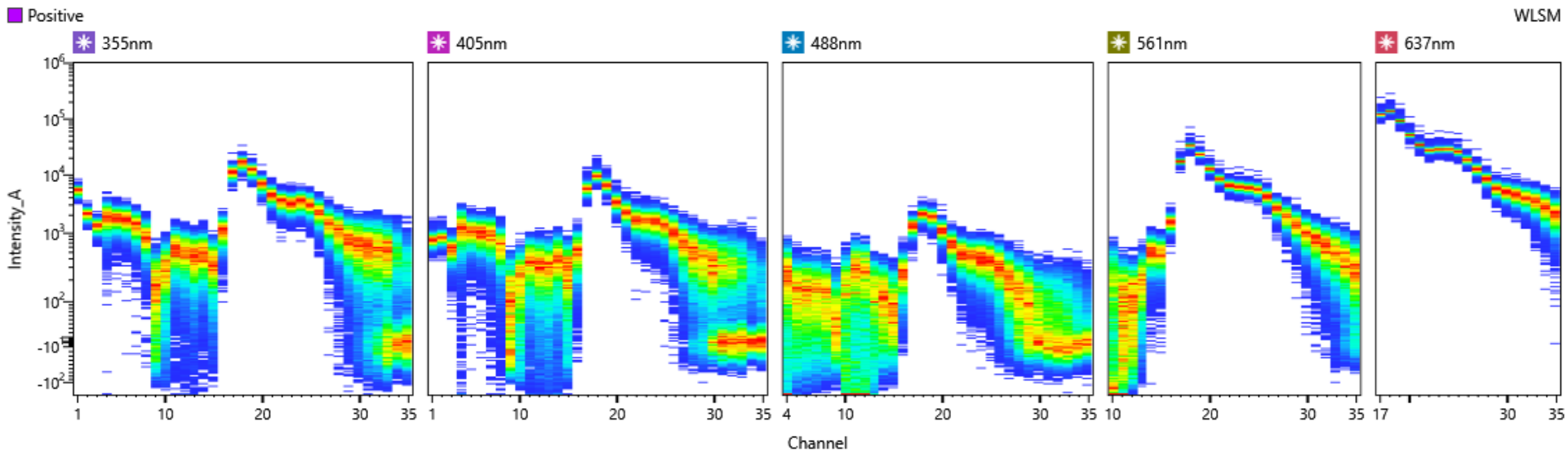
PE-Cy7 - beads



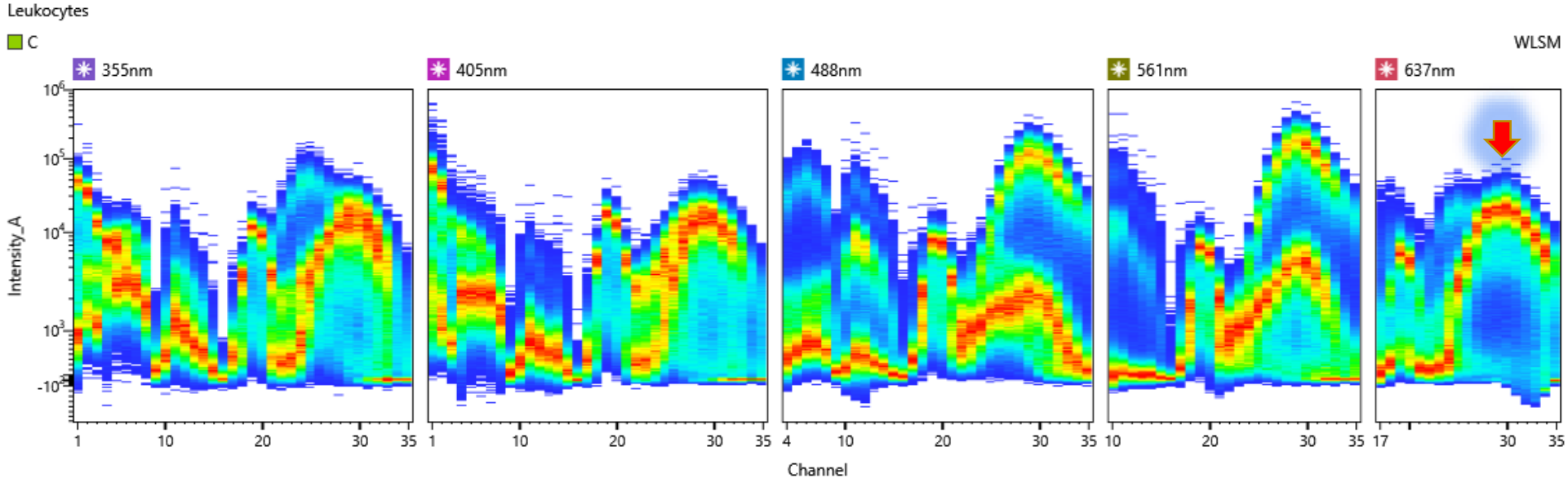
10c PBMCs: Raw Data



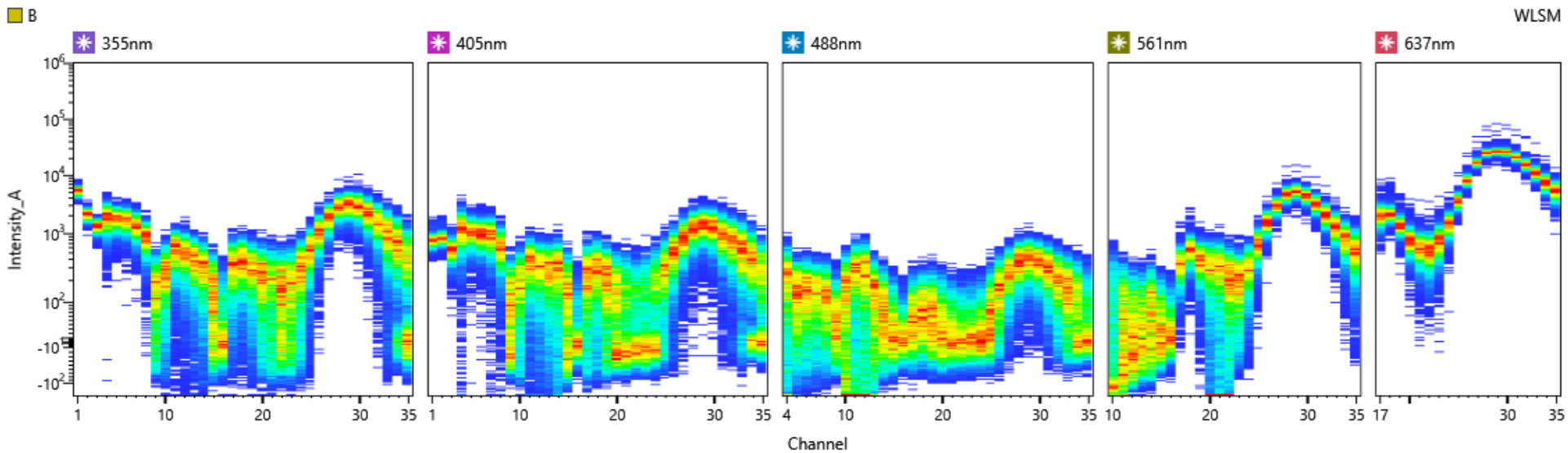
APC - beads



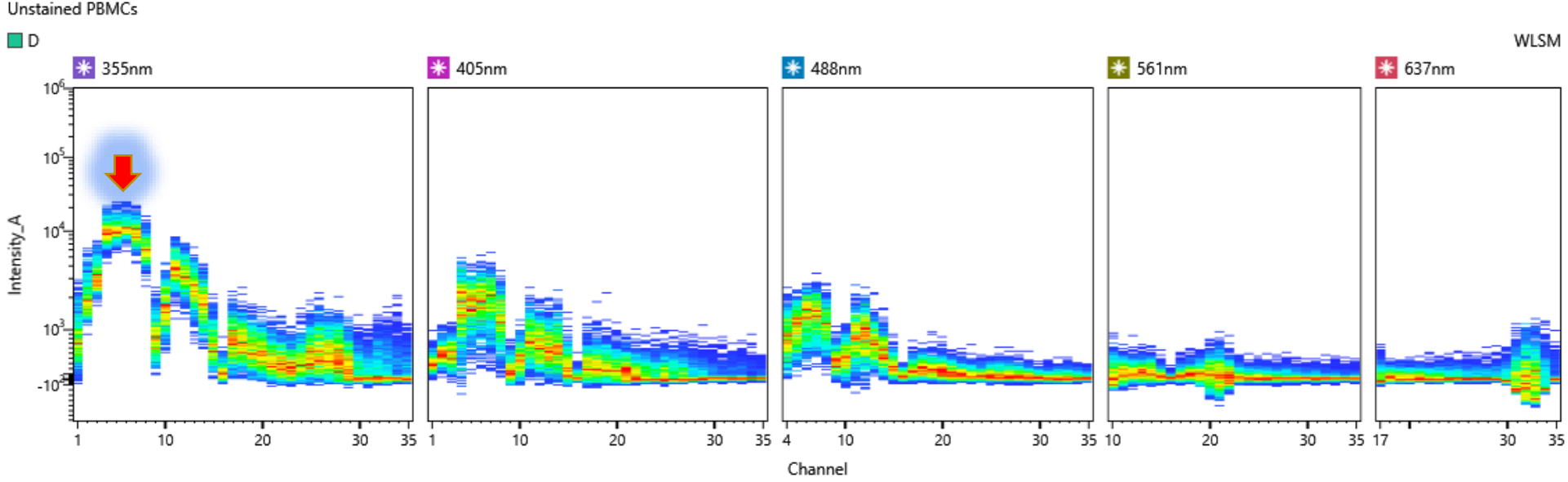
10c PBMCs: Raw Data



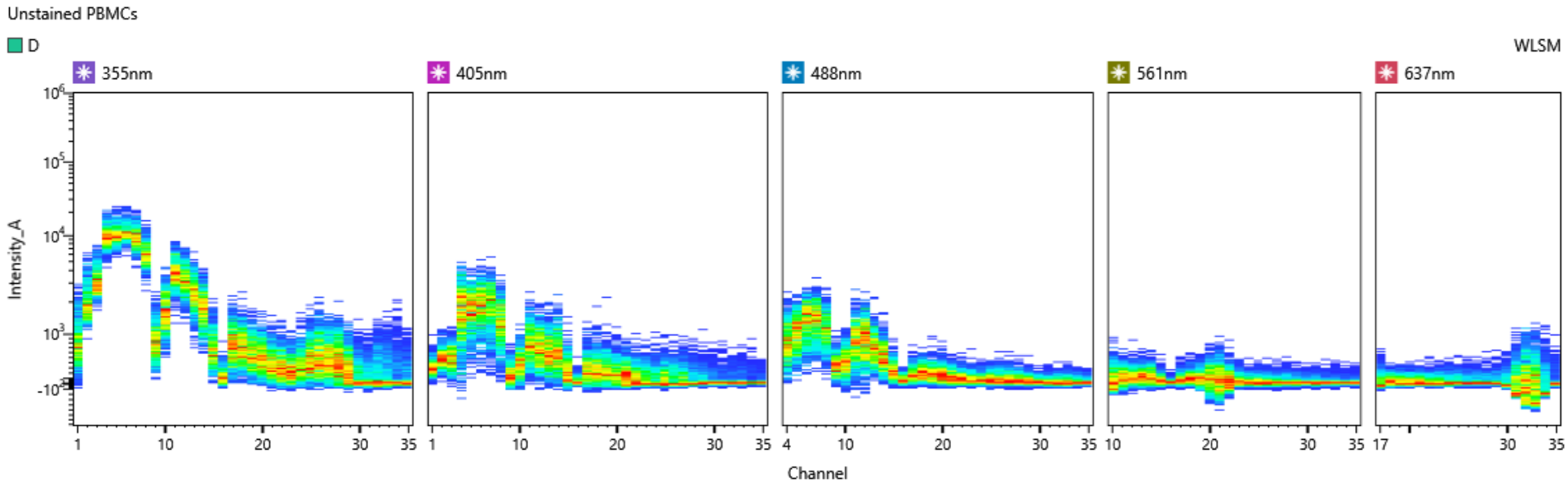
APC-Cy7 - beads




10c PBMCs: Raw Data



Unstained PBMCs





SONY

Software & Workflow

ID7000 Workflow

1. QC
2. Experiment
3. Colour Panel (colour + marker)

or

Reference Spectra

4. Instrument & Acquisition Settings
5. Reference Spectra
6. Autofluorescence
7. Unmixing QC

ID7000 Workflow

1. QC
2. Experiment
3. Colour Panel (colour + marker)

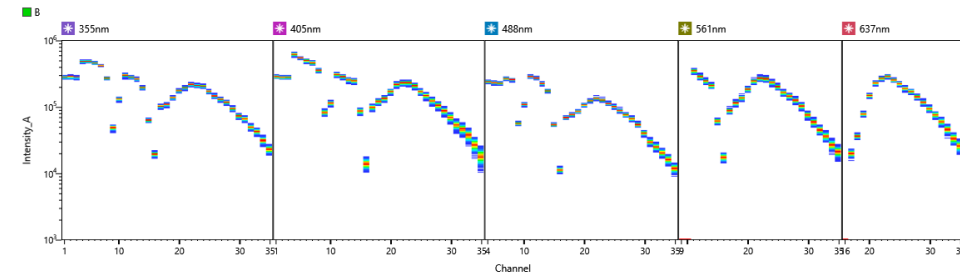
or

Reference Spectra

4. Instrument & Acquisition Settings
5. Reference Spectra
6. Autofluorescence
7. Unmixing QC

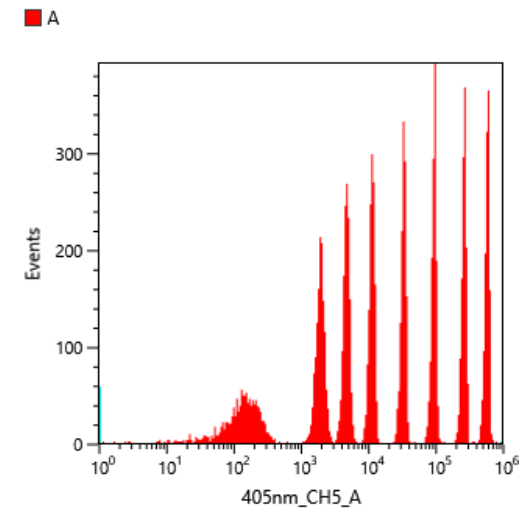
Daily:

- Align Check Beads
- Every day:
 - Fluidics
 - Laser Delay
 - Standardization



Performance:

- 8-peak beads
- Periodically:
 - Linearity
 - MESF
 - Background



Print
 Import Bead Lot File
 Export QC Results to CSV
 Export QC Results to XML
 Import QC Results from XML
 QC Criteria Settings

Daily QC

Performance QC

Control

Status

Date: 12/22/2020 10:07:01 AM
 Operator: administrator
 Result: Pass

Start Daily QC

Start Daily and Performance QC

View

History

Trend

Date	Operator	Result
12/22/2020 10:07:01 A	administrator	Pass

Daily QC History

QC Information

QC Type: Daily QC
 Result: Pass
 Model: LE-ID7000C
 Serial: 0803005
 Date: 12/22/2020 10:07:01 AM
 Operator: administrator
 Mode: Normal
 Bead Lot: YAL01toYAL03

Fluidics Initialization Results

Step	Result
1. Initialize Pressure	
2. Fluidics Adjustment	

Unit Calibration Results

Step	355nm	405nm	488nm	561nm	637nm
3. PMTV Adjustment					
4. PMT32 Adjustment					
5. A/H Ratio Calculation					

Calculation Results

Step	355nm	405nm	488nm	561nm	637nm
6. PMTV Standardization					

Evaluation Results

Step	355nm	405nm	488nm	561nm	637nm
7. Evaluation					

Details

Name	Height	Area	rCV	ΔrCV	PMT V	ΔPMT V
355-CH1	37,030	37,046	3.22 %	-0.81 %	4600h	-200h
355-CH2	74,366	74,340	3.03 %	-0.87 %	4D80h	-200h
355-CH3	37,294	37,294	3.07 %	-0.87 %	5200h	-200h
355-CH12	30,833	30,836	3.69 %	-0.70 %	6500h	-200h
405-CH1	67,801	68,033	2.13 %	0.16 %	5900h	0h
405-CH2	67,800	68,002	1.94 %	0.04 %	5C00h	0h
405-CH3	35,318	35,397	1.94 %	-0.02 %	5900h	0h
405-CH12	27,045	27,039	2.32 %	-0.17 %	7300h	0h
488-FSC	114,652	114,460	2.35 %	---	---	---
488-SSC	30,456	30,389	3.18 %	---	4680h	0h
488-CH12	29,998	29,915	1.61 %	-0.13 %	6D00h	0h
561-CH12	31,593	31,566	1.88 %	0.01 %	7900h	0h
637-CH23	28,401	28,339	2.05 %	-0.04 %	6A80h	0h

Comments

No issue found.

ID7000 Workflow

1. QC
2. Experiment
3. Colour Panel (colour + marker)
- or
- Reference Spectra
4. Instrument & Acquisition Settings
5. Reference Spectra
6. Autofluorescence
7. Unmixing QC



Spectral Reference

- Experiment Designer
- Experiment Template
- Reopen Experiment**

Experiments

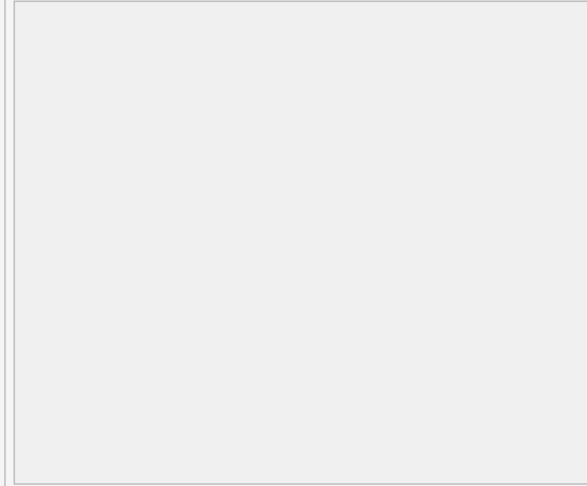
Experiment List

From: To: Keyword:

- Public
- administrator
- comparison
- melio
- sony-service

Details

Sample List



Select plate or sample group to show information

ID7000 Workflow

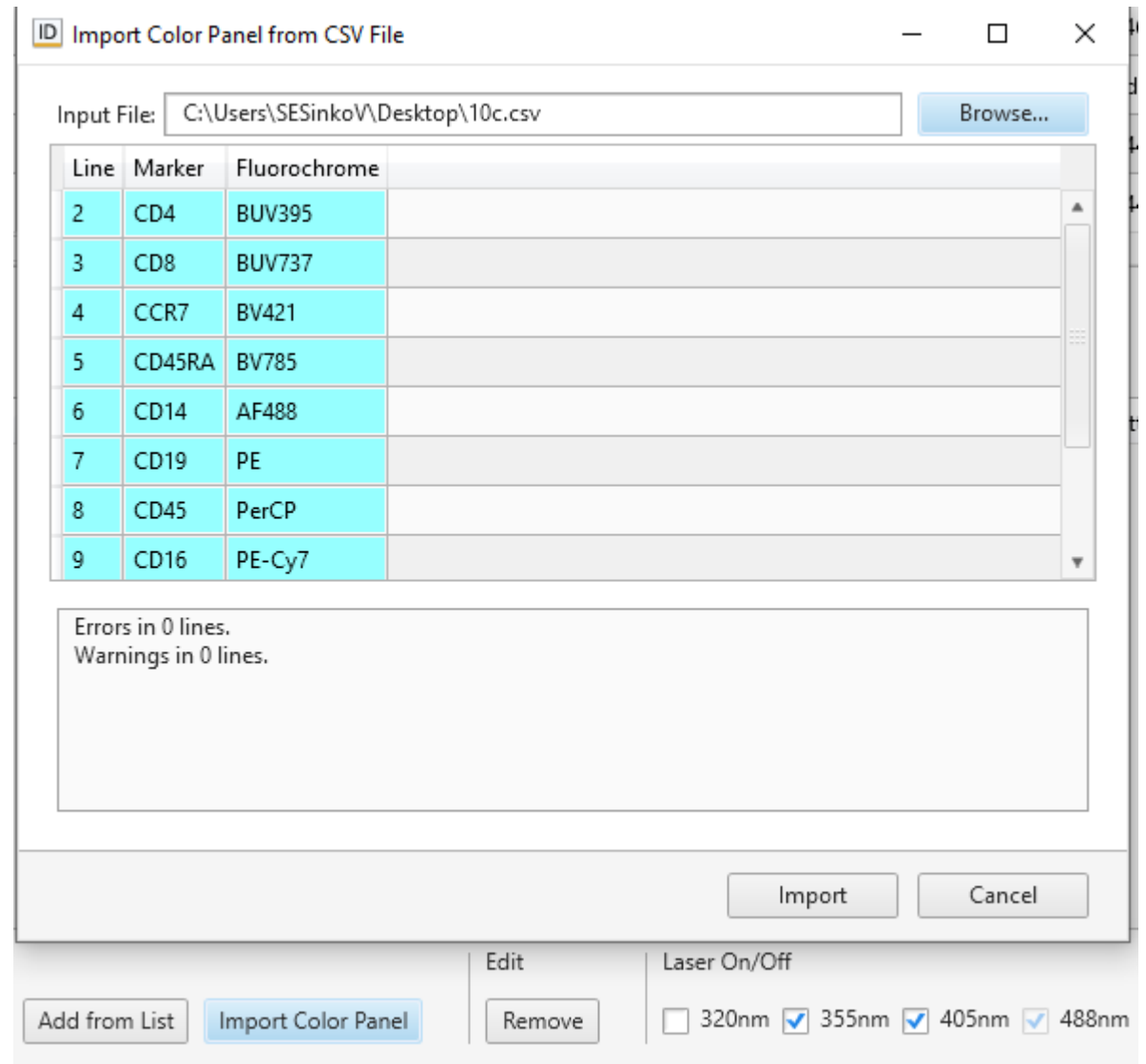
1. QC
2. Experiment
3. Colour Panel (colour + marker)

or

Reference Spectra

4. Instrument & Acquisition Settings
5. Reference Spectra
6. Autofluorescence
7. Unmixing QC

From .csv



ID7000 Workflow

1. QC
2. Experiment
3. Colour Panel (colour + marker)

or

Reference Spectra

4. Instrument & Acquisition Settings
5. Reference Spectra
6. Autofluorescence
7. Unmixing QC

From the List

Number of selection 3 / 100 Ex. Laser: ALL

- BB660
- BB700
- BB755
- BB790
- BFP
- BODIPY
- BODIPY-FL
- BUV395
- BUV496
- BUV563
- BUV615
- BUV661
- BUV737
- BUV805
- BV421
- BV480
- BV510
- BV570
- BV605
- BV650
- BV711
- BV750
- BV785

320nm

355nm

405nm

488nm

561nm

637nm

808nm

Fluorochrome Information

Laser: 355nm Emission Spectrum

CH25-CH28

733.6-775.2nm

OK Cancel

ID7000 Workflow

1. QC
2. Experiment
3. Colour Panel (colour + marker)

or

Reference Spectra

4. Instrument & Acquisition Settings
5. Reference Spectra
6. Autofluorescence
7. Unmixing QC

From the library

Spectral Reference Settings

Filters Search Results Ex. Laser: ALL

Keyword Search

Label

Laser Setting

- 320nm
- 355nm
- 405nm
- 488nm
- 561nm
- 637nm
- 808nm

Fluorochrome

- [Zombie-UV]
- AF488
- AF532
- AF700
- APC
- APC-H7
- BB700
- BUV395
- BUV496
- BUV563
- BUV615
- BUV661
- BUV737
- BUV805
- BV421
- BV510
- BV605
- BV650
- BV711
- BV785
- PE
- PE-Cy5
- PE-Cy7

Warning	Label	Marker	Fluorochrome	Ex. Laser	320nm	355nm	405nm	488nm	561nm	637nm	808nm	Mode	Instrument Settings ID	Spectral Index	Saturation
	23 SC	CD56	BUV395	355nm								Normal	d28f434b	477	0.0%
	23 SC	CD25	BV421	405nm								Normal	d28f434b	1,410	0.0%
Training panel	Live Dead	[Zombie-UV]	355nm									Normal	b701ffbe	Not Available	0.0%
	23 SC	CD38	BUV496	355nm								Normal	d28f434b	188	0.0%
	23 SC	CD194	BV510	405nm								Normal	d28f434b	216	0.0%
	23 SC	CD8a	AF488	488nm								Normal	d28f434b	654	0.0%
	23 SC	CD20	AF532	488nm								Normal	d28f434b	158	0.0%
	23 SC	CD14	BUV563	355nm								Normal	d28f434b	403	0.0%

Selected Spectral References Ex. Laser: ALL

Warning	Label	Marker	Fluorochrome	Ex. Laser	320nm	355nm	405nm	488nm	561nm	637nm	808nm	Mode	Instrument Settings ID	Spectral Index	Saturation
	23 SC	CD56	BUV395	355nm								Normal	d28f434b	477	0.0%
	23 SC	CD25	BV421	405nm								Normal	d28f434b	1,410	0.0%
	23 SC	CD194	BV510	405nm								Normal	d28f434b	216	0.0%
	23 SC	CD8a	AF488	488nm								Normal	d28f434b	654	0.0%
	23 SC	CCR7	PE	488nm								Normal	d28f434b	725	0.0%
	23 SC	CD24	PE-Dazzle594	488nm								Normal	d28f434b	1,038	0.0%
	23 SC	CD3	BV605	405nm								Normal	d28f434b	389	0.0%
	23 SC	CD27	APC	637nm								Normal	d28f434b	451	0.0%

Add New Spectral Reference

Laser On/Off 320nm 355nm 405nm 488nm 561nm 637nm 808nm

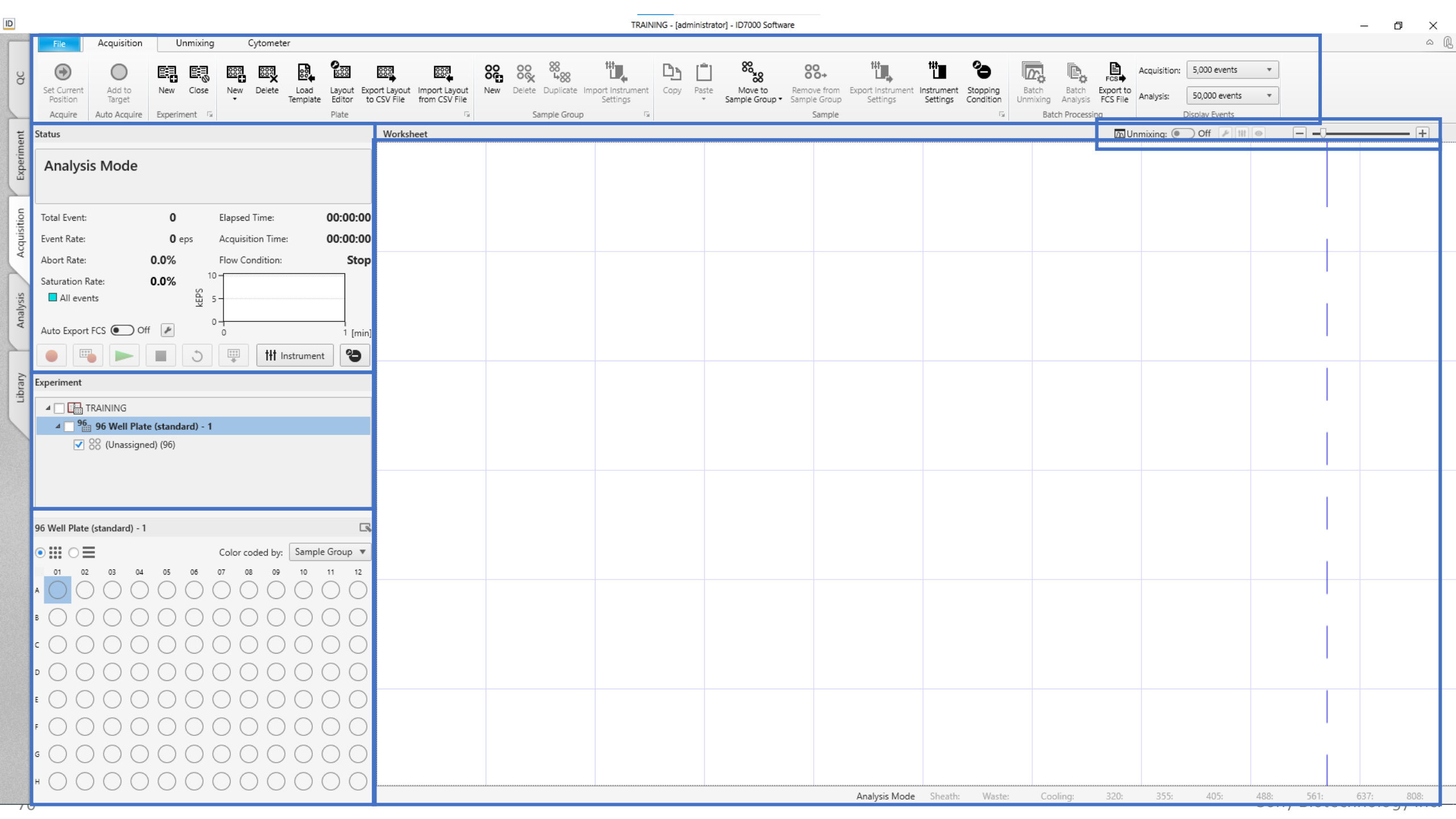
ID7000 Workflow

1. QC
2. Experiment
3. Colour Panel (colour + marker)

or

Reference Spectra

4. Instrument & Acquisition Settings
5. Reference Spectra
6. Autofluorescence
7. Unmixing QC



File Acquisition Unmixing Cytometer

Toolbar with icons for: Acquire, Auto Acquire, Experiment, New, Delete, Load Template, Layout Editor, Export Layout to CSV File, Import Layout from CSV File, Sample Group, Sample, Instrument Settings, Stopping Condition, Batch Processing, Acquisition: 5,000 events, Analysis: 50,000 events, Display Events.

Status Analysis Mode

24 Tube Rack (5mL)
 96 Well Plate (standard)
 96 Well Plate (half deep)
 96 Well Plate (deep)
 384 Well Plate (standard)

Acquisition Statistics:

- Total Event: 0
- Event Rate: 0 eps
- Abort Rate: 0.0%
- Saturation Rate: 0.0%
- Flow Condition: Stop
- Elapsed Time: 00:00:00
- Acquisition Time: 00:00:00

Graph: kEPS vs [min] (0 to 1)

Auto Export FCS: Off

Instrument controls: Stop, Start, Refresh, Instrument, Stop Condition

Experiment Library

- TRAINING
 - 96 Well Plate (standard) - 1
 - (Unassigned) (96)

96 Well Plate (standard) - 1

Color coded by: Sample Group

	01	02	03	04	05	06	07	08	09	10	11	12
A	○	○	○	○	○	○	○	○	○	○	○	○
B	○	○	○	○	○	○	○	○	○	○	○	○
C	○	○	○	○	○	○	○	○	○	○	○	○
D	○	○	○	○	○	○	○	○	○	○	○	○
E	○	○	○	○	○	○	○	○	○	○	○	○
F	○	○	○	○	○	○	○	○	○	○	○	○
G	○	○	○	○	○	○	○	○	○	○	○	○
H	○	○	○	○	○	○	○	○	○	○	○	○

Worksheet

SAMPLE GROUP:

Instrument Settings

- Detectors & threshold

Unmixing | Spectral Reference | Autofluorescence | Fluorochrome

Load Spectral Reference | Add to Library | Autofluorescence Finder | Fluorochrome Database

Shared Worksheet (A01) Well - A01 - (Sample Group - 1)

Unmixing: Off



Instrument Settings

Sample: Well - A01
Mode: Normal

Detector & Threshold

Laser: 355nm, 405nm, 488nm, 561nm, 637nm

FSC Gain: 5
SSC Voltage (%): 25.7

PMT Voltage (%)

355 nm:	43.8
405 nm:	48.4
488 nm:	43.1
561 nm:	49.7
637 nm:	46.5

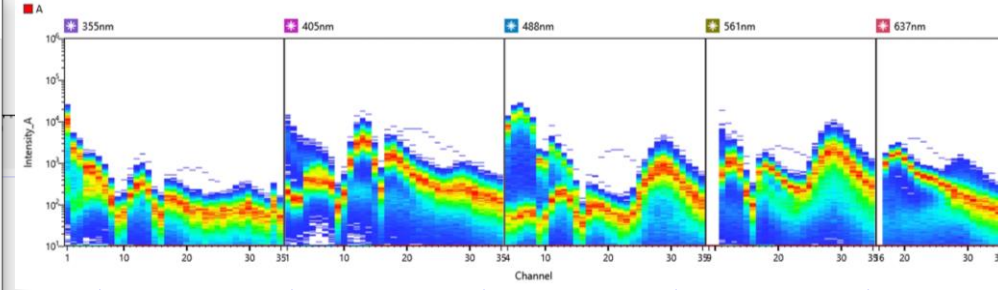
Threshold

CH1: FSC Value1(%): 11.1
CH2: None Value2(%):

Window Extension: Normal

Advanced Settings

Close



Analysis Mode

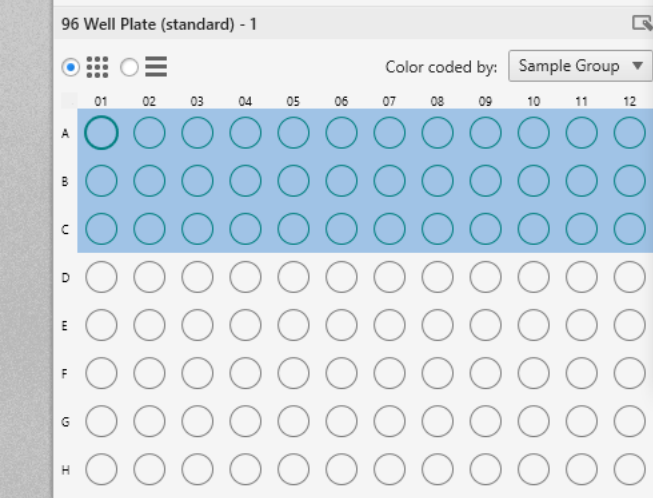
Total Event: 0 Elapsed Time: 00:00:00
Event Rate: 0 eps Acquisition Time: 00:00:00
Abort Rate: 0.0% Flow Condition: Stop
Saturation Rate: 0.0%

All events

Auto Export FCS: Off

Experiment

- TRAINING
- 96 Well Plate (standard) - 1
 - Sample Group - 1 (36/36)
 - (Unassigned) (60)



SAMPLE GROUP:

Instrument Settings

- Detectors & threshold
- Flow control

Experiment

Acquisition

Analysis

Library

Experiment

96 Well Plate (standard) - 1

Color coded by: Sample Group

	01	02	03	04	05	06	07	08	09	10	11	12
A	●	○	○	○	○	○	○	○	○	○	○	○
B	○	○	○	○	○	○	○	○	○	○	○	○
C	○	○	○	○	○	○	○	○	○	○	○	○
D	○	○	○	○	○	○	○	○	○	○	○	○
E	○	○	○	○	○	○	○	○	○	○	○	○
F	○	○	○	○	○	○	○	○	○	○	○	○
G	○	○	○	○	○	○	○	○	○	○	○	○
H	○	○	○	○	○	○	○	○	○	○	○	○

TRAINING - [administrator] - ID7000 Software

Shared Worksheet (A01) Well - A01 - (Sample Group - 1)

Unmixing: Off

All events

355nm 405nm 488nm 561nm 637nm

SSC-A x1,000,000

FSC-A x1,000,000

Instrument Settings

Sample: Well - A01

Mode: Normal

Laser Detector & Threshold Flow Control Agitation Event Check Cleaning

Boost: Short

Sample Flow Rate: 1.0

Acquisition Offset Time: 0 sec

Advanced Settings

Close

Analysis Mode Sheath: Waste: Cooling: 320: 355: 405: 488: 561: 637: 808:

SAMPLE GROUP:

Instrument Settings

- Detectors & threshold
- Flow control
- Agitation

TRAINING - [administrator] - ID7000 Software

Unmixing: Off

Shared Worksheet (A01) Well - A01 - (Sample Group - 1)

All events: 355nm, 405nm, 488nm, 561nm, 637nm

Analysis Mode

Total Event: 0 Elapsed Time: 00:00:00
Event Rate: 0 eps Acquisition Time: 00:00:00
Abort Rate: 0.0% Flow Condition: Stop
Saturation Rate: 0.0%
Auto Export FCS: Off

Experiment

- TRAINING
 - 96 Well Plate (standard) - 1
 - Sample Group - 1 (36/36)
 - (Unassigned) (60)

96 Well Plate (standard) - 1

Color coded by: Sample Group

	01	02	03	04	05	06	07	08	09	10	11	12
A	●	●	●	●	●	●	●	●	●	●	●	●
B	●	●	●	●	●	●	●	●	●	●	●	●
C	●	●	●	●	●	●	●	●	●	●	●	●
D	○	○	○	○	○	○	○	○	○	○	○	○
E	○	○	○	○	○	○	○	○	○	○	○	○
F	○	○	○	○	○	○	○	○	○	○	○	○
G	○	○	○	○	○	○	○	○	○	○	○	○
H	○	○	○	○	○	○	○	○	○	○	○	○

Instrument Settings

Sample: Well - A01
Mode: Normal

Laser | **Detector & Threshold** | Flow Control | Agitation | Event Check | Cleaning

Low Dead Volume: Enable low dead volume mode

Agitation

Enable sample agitation

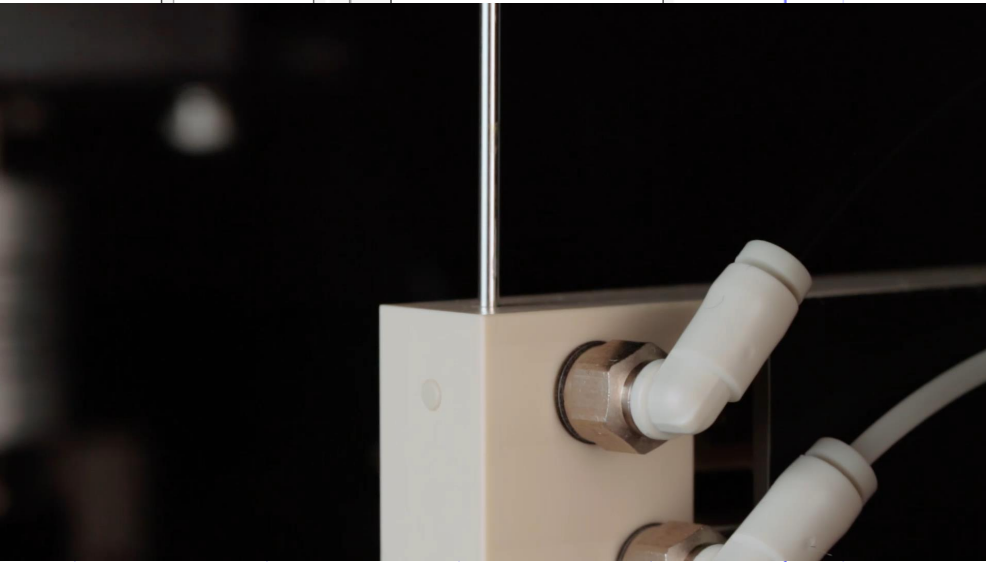
Pattern: Once Cyclic Continuous

Mode: Normal Short

Advanced Settings

Close

Analysis Mode Sheath: Waste: Cooling: 320: 355: 405: 488: 561: 637: 808:



SAMPLE GROUP:

Instrument Settings

- Detectors & threshold
- Flow control
- Agitation

TRAINING - [administrator] - ID7000 Software

Shared Worksheet (A01) Well - A01 - (Sample Group - 1) Unmixing: Off

All events

All events

All events

All events

All events

All events

ID Instrument Settings

Sample: Well - A01
Mode: Normal

Low Dead Volume: Enable low dead volume mode

Agitation

Enable sample agitation

Pattern

Once Cyclic Continuous

Mode

Normal Short

Advanced Settings

96 Well Plate (standard) - 1

Color coded by: Sample Group

01	02	03	04	05	06	07	08	09	10	11	12
A	●	●	●	●	●	●	●	●	●	●	●
B	●	●	●	●	●	●	●	●	●	●	●
C	●	●	●	●	●	●	●	●	●	●	●
D	○	○	○	○	○	○	○	○	○	○	○
E	○	○	○	○	○	○	○	○	○	○	○
F	○	○	○	○	○	○	○	○	○	○	○
G	○	○	○	○	○	○	○	○	○	○	○
H	○	○	○	○	○	○	○	○	○	○	○

Analysis Mode Sheath: Waste: Cooling: 320: 355: 405: 488: 561: 637: 808:

SAMPLE GROUP:

Instrument Settings

- Detectors & threshold
- Flow control
- Agitation
- Event check

TRAINING - [administrator] - ID7000 Software

Shared Worksheet (A01) Well - A01 - (Sample Group - 1) Unmixing: Off

All events

355nm

All events

405nm

All events

488nm

All events

561nm

All events

637nm

All events

Acquisition

Total Event: **0** Elapsed Time: **00:00:00**

Event Rate: **0 eps** Acquisition Time: **00:00:00**

Abort Rate: **0.0%** Flow Condition: **Stop**

Saturation Rate: **0.0%**

All events

Auto Export FCS Off

Experiment

TRAINING

 96 Well Plate (standard) - 1

 Sample Group - 1 (36/36)

 (Unassigned) (60)

96 Well Plate (standard) - 1

Color coded by: **Sample Group**

	01	02	03	04	05	06	07	08	09	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Instrument Settings

Sample: Well - A01
Mode: Normal

Event Check:

Off
The system will not detect air bubbles or low event rate.

On
If the system detects air bubbles or low event rate data acquisition is paused. The system will then clean itself and recover automatically of stop acquisition based on the Auto Acquire Preference Setting.

Auto Acquire Preferences:
Set Preference for the system action when an error is detected during Auto Acquire.

Stop
System will stop Auto Acquire.

Continue
System will continue Auto Acquire.

Advanced Settings

Analysis Mode Sheath: Waste: Cooling: 320: 355: 405: 488: 561: 637: 808:

SAMPLE GROUP:

Instrument Settings

- Detectors & threshold
- Flow control
- Agitation
- Event check
- Cleaning

TRAINING - [administrator] - ID7000 Software

Shared Worksheet (A01) Well - A01 - (Sample Group - 1) Unmixing: Off

Instrument Settings

Sample: Well - A01
Mode: Normal

Cleaning Mode: Inner and Outer

Advanced Setting Inner Only

No Wash

96 Well Plate (standard) - 1

Color coded by: Sample Group

	01	02	03	04	05	06	07	08	09	10	11	12
A	○	○	○	○	○	○	○	○	○	○	○	○
B	○	○	○	○	○	○	○	○	○	○	○	○
C	○	○	○	○	○	○	○	○	○	○	○	○
D	○	○	○	○	○	○	○	○	○	○	○	○
E	○	○	○	○	○	○	○	○	○	○	○	○
F	○	○	○	○	○	○	○	○	○	○	○	○
G	○	○	○	○	○	○	○	○	○	○	○	○
H	○	○	○	○	○	○	○	○	○	○	○	○

Analysis Mode Sheath: Waste: Cooling: 320: 355: 405: 488: 561: 637: 808:

SAMPLE GROUP:

Instrument Settings

- Detectors & threshold
- Flow control
- Agitation
- Event check
- Cleaning

Unmixing Settings

- Colour panel
- Spectral References
- Autofluorescence

96 Well Plate (standard) - 1

- Sample Group - 1 (36/36) ↔
- (Unassigned) (60)

96 Well Plate (standard) - 1

Color coded by: Sample Group

	01	02	03	04	05	06	07	08	09	10	11	12
A	●	●	●	●	●	●	●	●	●	●	●	●
B	●	●	●	●	●	●	●	●	●	●	●	●
C	●	●	●	●	●	●	●	●	●	●	●	●
D	○	○	○	○	○	○	○	○	○	○	○	○
E	○	○	○	○	○	○	○	○	○	○	○	○
F	○	○	○	○	○	○	○	○	○	○	○	○
G	○	○	○	○	○	○	○	○	○	○	○	○
H	○	○	○	○	○	○	○	○	○	○	○	○

Unmixing Settings

Color Panel Matrix

Fluorochrome Add

Universal Negative --- Ex. Laser: ALL

Index	Marker	Fluorochrome	Ex. Laser	Mode	SR	Negative	Positive
1	CD56	BUV395	355nm	Normal(Adv.)	●	○ ---	●
2	CD38	BUV496	355nm	Normal(Adv.)	●	○ ---	●
3	CD14	BUV563	355nm	Normal(Adv.)	●	○ ---	●
4	CD4	[BUV615]	355nm	Normal(Adv.)	●	○ ---	●
5	CD11c	BUV661	355nm	Normal(Adv.)	●	○ ---	●
6	CD45RA	BUV737	355nm	Normal(Adv.)	●	○ ---	●
7	IgD	BUV805	355nm	Normal(Adv.)	●	○ ---	●
8	CD19	BV421	405nm	Normal(Adv.)	●	○ ---	●
9	CCR4	BV510	405nm	Normal(Adv.)	●	○ ---	●
10	CD3	BV605	405nm	Normal(Adv.)	●	○ ---	●
11	CD25	BV650	405nm	Normal(Adv.)	●	○ ---	●
12	CCR6	BV711	405nm	Normal(Adv.)	●	○ ---	●
13	CXCR2	BV785	405nm	Normal(Adv.)	●	○ ---	●
14	CD8	AF488	488nm	Normal(Adv.)	●	○ ---	●
15	CD20	AF532	488nm	Normal(Adv.)	●	○ ---	●
16	CCR7	PE	488nm	Normal(Adv.)	●	○ ---	●
17	CD24	PE-Dazzle594	488nm	Normal(Adv.)	●	○ ---	●
18	CD123	PE-Cy5	488nm	Normal(Adv.)	●	○ ---	●
19	HLA-DR	BB700	488nm	Normal(Adv.)	●	○ ---	●
20	CD45RO	PE-Cy7	488nm	Normal(Adv.)	●	○ ---	●
21	CD27	APC	637nm	Normal(Adv.)	●	○ ---	●
22	CD127	APC-R700	637nm	Normal(Adv.)	●	○ ---	●

Color Panel Settings Fluorochrome Settings

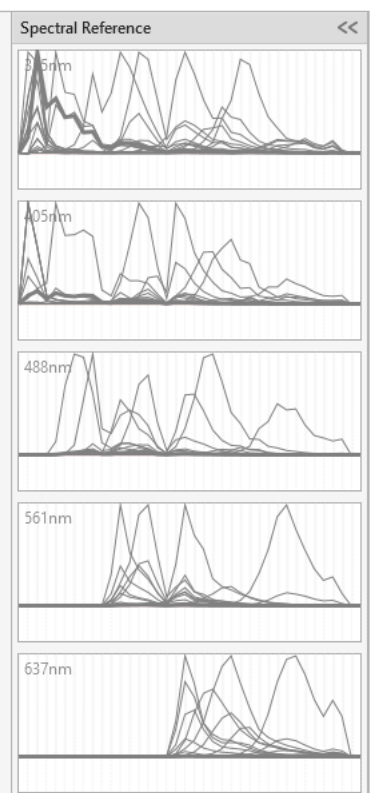
Autofluorescence

Add

Index	Use as AF	Autofluorescence	Ex. Laser	Mode	SR	Negative	Positive
1	<input checked="" type="checkbox"/>	[AF color 1]	405nm	Normal(Adv.)	●	● Zero Reference	● AF-B (Well - A01)

Autofluorescence

Calculate Apply



Sample groups (shared)

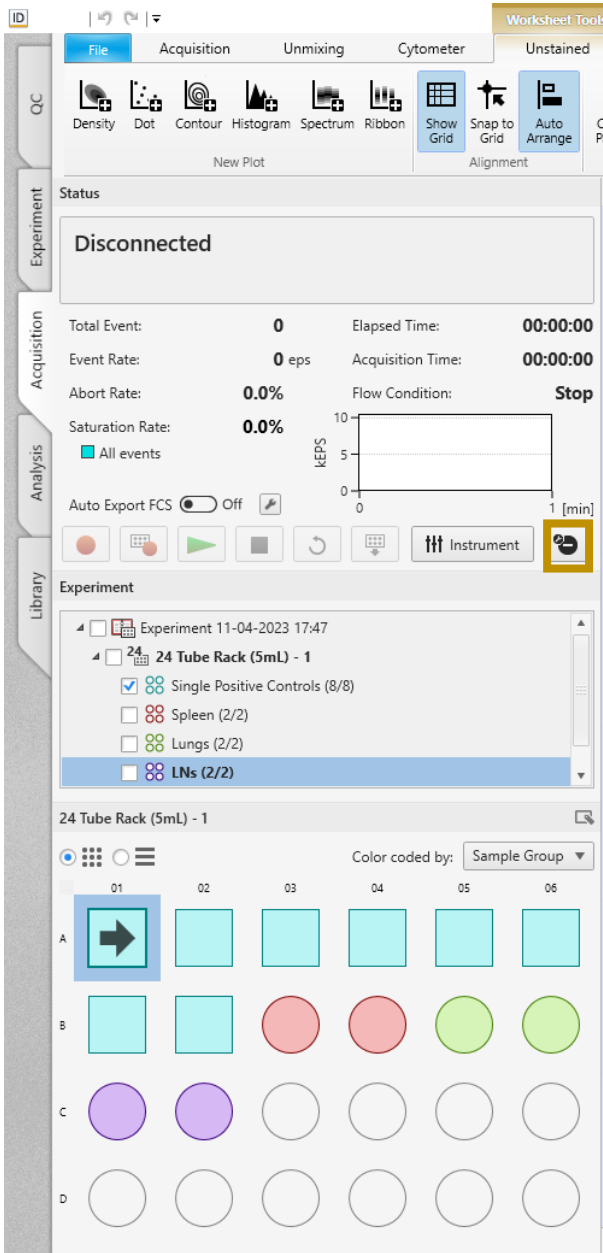
- Instrument settings
- Unmixing settings
- Shared worksheet

The screenshot shows the Sony Flow cytometry software interface. The top menu bar includes File, Acquisition, Unmixing (highlighted), Cytometer, and Unstained. Below the menu is a toolbar with icons for Density, Dot, Contour, Histogram, Spectrum, Ribbon, Show Grid, Snap to Grid, and Auto Arrange. The main window is divided into several sections: Status (Disconnected), Acquisition (Total Event: 0, Elapsed Time: 00:00:00, Event Rate: 0 eps, Acquisition Time: 00:00:00, Abort Rate: 0.0%, Flow Condition: Stop), Analysis (Saturation Rate: 0.0%, Auto Export FCS: Off), and Library (Experiment 11-04-2023 17:47, 24 Tube Rack (5mL) - 1). The 24 Tube Rack (5mL) - 1 section shows a grid of 24 tubes (A-D, 01-06) color-coded by Sample Group. Tube A01 is highlighted with a blue box and a right-pointing arrow.

The screenshot shows the Sony Unmixing Settings dialog box. The top section is for Fluorochrome settings, with a table listing 7 fluorochromes: BUV737, BV421, BV650, FITC, NovaFluorBlue585, PE, and APC. Each row includes columns for Index, Marker, Fluorochrome, Ex. Laser, Mode, SR, Negative, and Positive. The bottom section is for Autofluorescence settings, with a table listing 1 autofluorescence setting: [AF color 1]. The right side of the dialog box shows Spectral Reference settings for 320nm, 355nm, 405nm, 488nm, 561nm, and 637nm. The Calculate and Apply buttons are at the bottom right.

Index	Marker	Fluorochrome	Ex. Laser	Mode	SR	Negative	Positive
1		BUV737	355nm			<input type="radio"/> A (Unstained)	<input checked="" type="radio"/> Positive (BUV737)
2		BV421	405nm			<input type="radio"/> A (Unstained)	<input checked="" type="radio"/> Positive (BV421)
3		BV650	405nm			<input type="radio"/> A (Unstained)	<input checked="" type="radio"/> Positive (BV650)
4		FITC	488nm			<input type="radio"/> A (Unstained)	<input checked="" type="radio"/> Positive (FITC)
5		NovaFluorBlue585	488nm			<input type="radio"/> A (Unstained)	<input checked="" type="radio"/> Positive (NovaFluo...)
6		PE	561nm			<input type="radio"/> A (Unstained)	<input checked="" type="radio"/> Positive (PE)
7		APC	637nm			<input type="radio"/> A (Unstained)	<input checked="" type="radio"/> Positive (APC)

Index	Use as AF	Autofluorescence	Ex. Laser	Mode	SR	Negative	Positive
1	<input checked="" type="checkbox"/>	[AF color 1]	488nm			<input type="radio"/> Zero Reference	<input checked="" type="radio"/> A (Unstained)

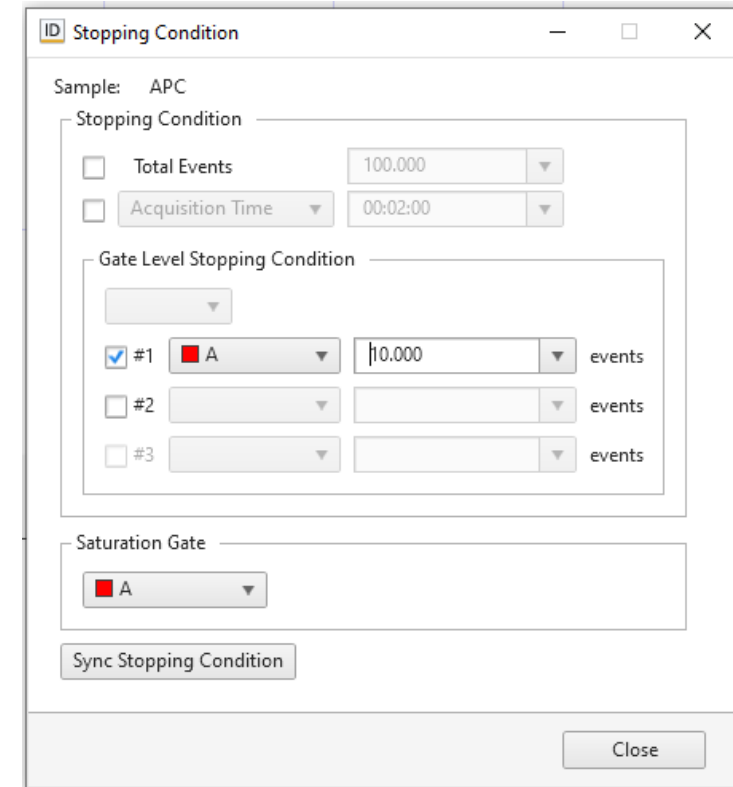


Sample groups (shared)

- Instrument settings
- Unmixing settings
- Shared worksheet

Sample groups (not shared)

- Stopping condition
- Individual worksheet



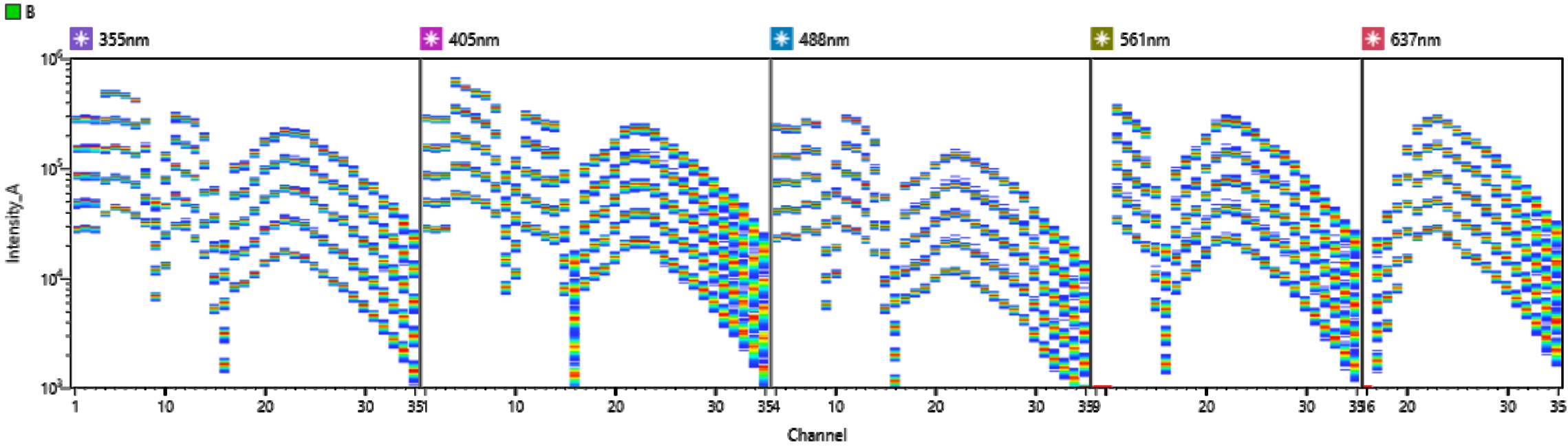
SONY

Standardization Mode

Standardization Mode (ST Mode)

Performed during daily instrument QC using Align Check beads.

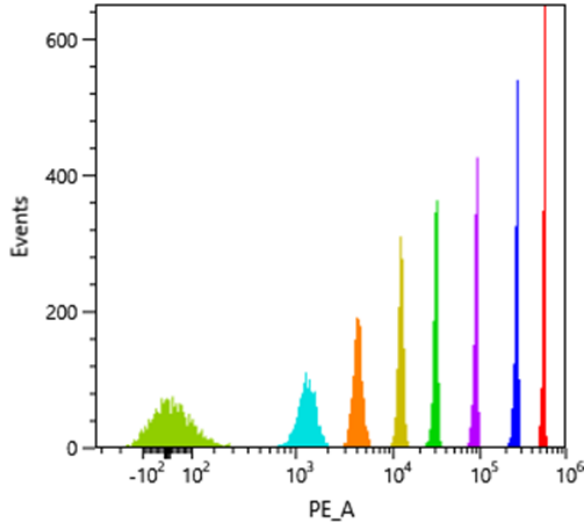
AlignCheck beads produce consistent broad spectrum fluorescence.



Std. PMTV Setting 5.00

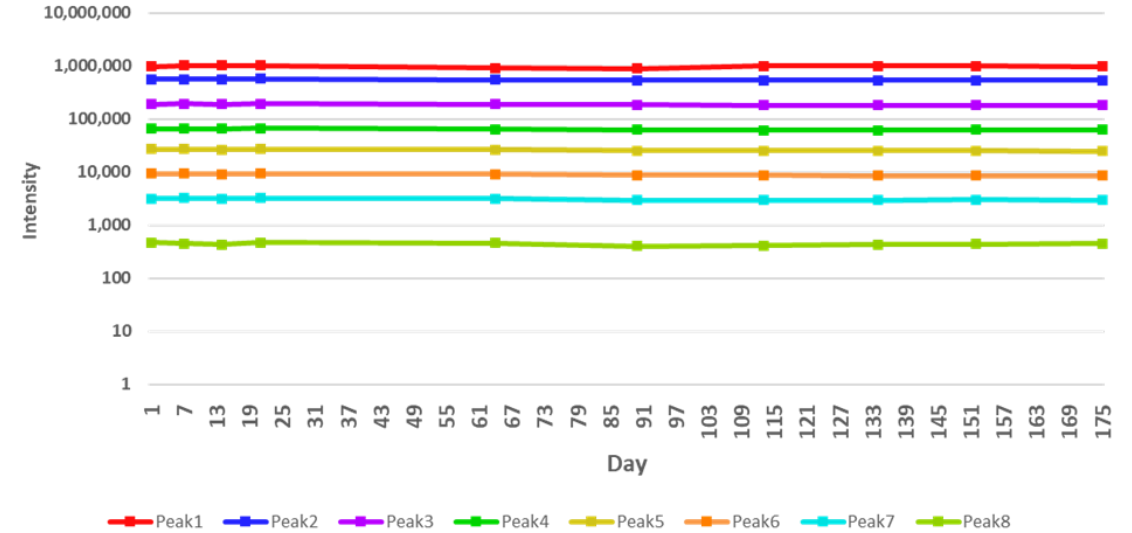
Measurement stability over time

8-peak beads



Peaks	CV(%)
Peak1	4.2 %
Peak2	2.2 %
Peak3	2.6 %
Peak4	2.8 %
Peak5	3.1 %
Peak6	3.4 %
Peak7	3.7 %
Peak8	4.8 %

Stability over 175 days at ST value of 5

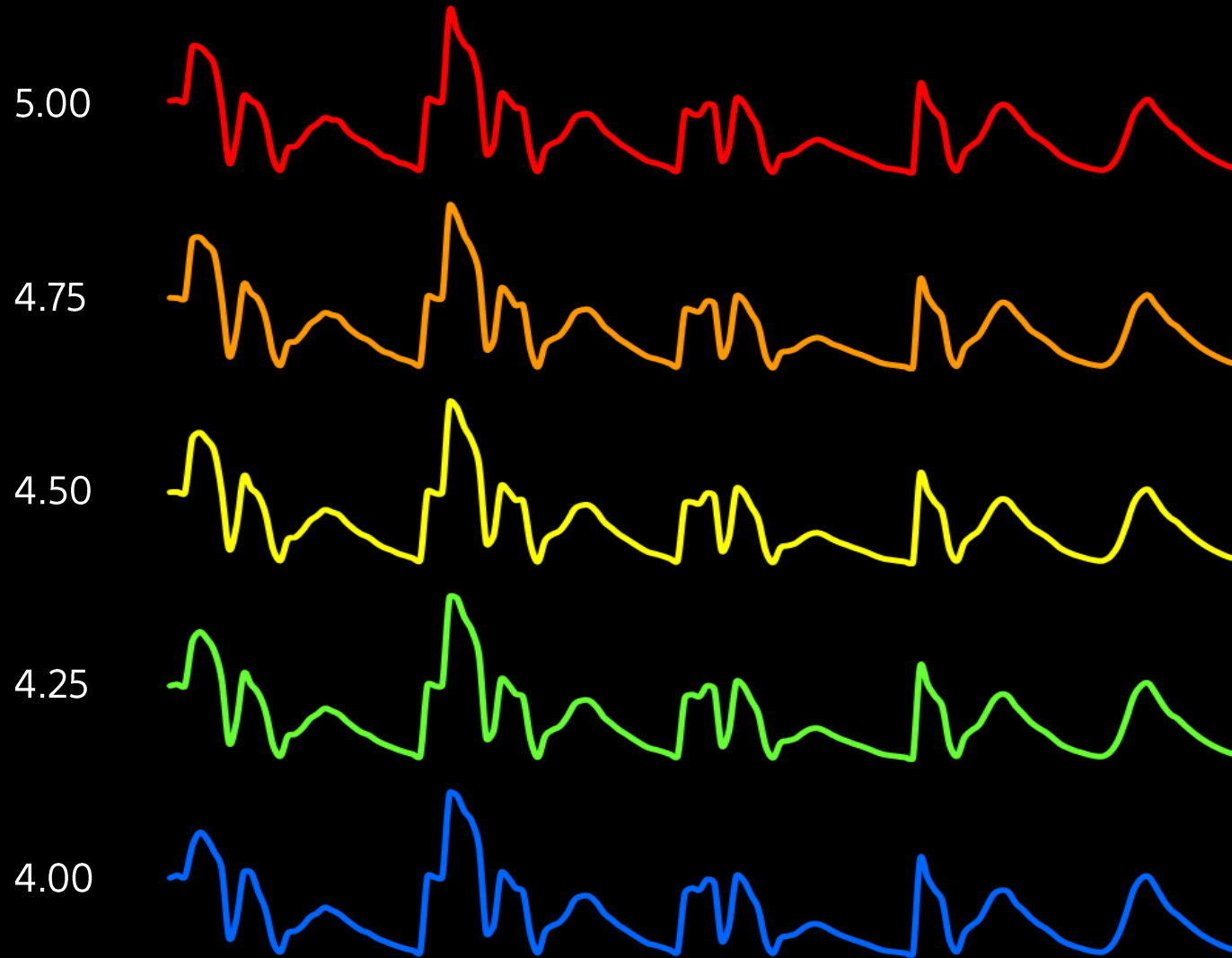


The median intensities of the PE region of 8 peak bead profiles were calculated and tracked

The Coefficient of Variation (CV) of intensity values being less than 5% for each peak between the time points

ST Mode - PMT voltage validation

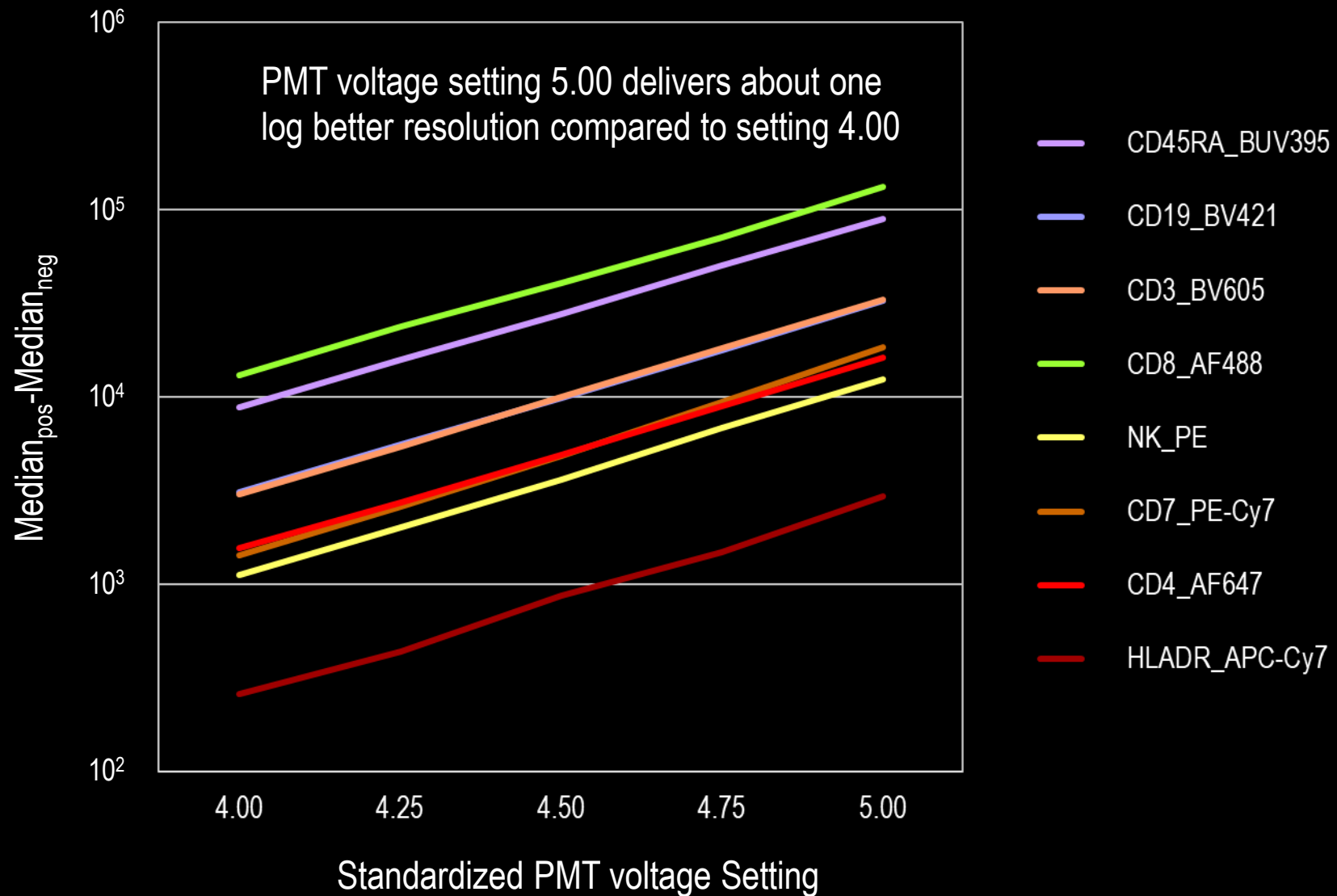
Normalized fluorescence distributions from Align Check beads acquired at five standardized PMT array voltages

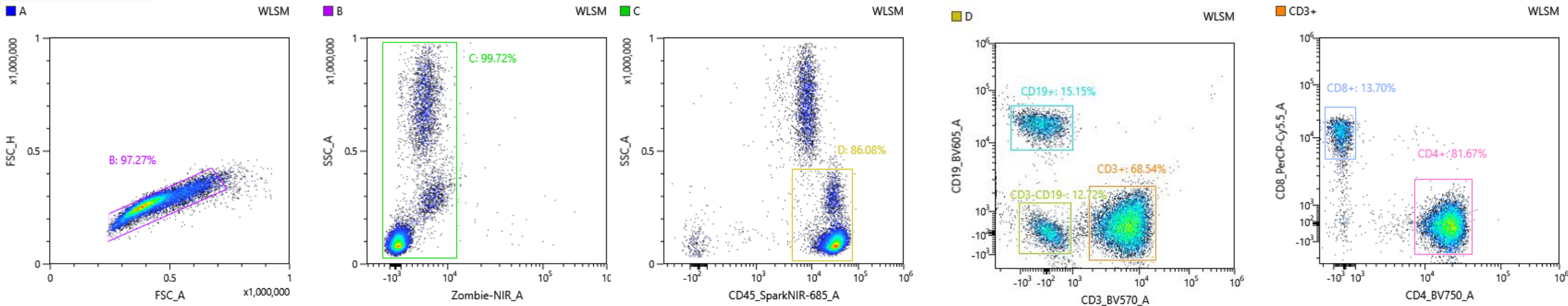
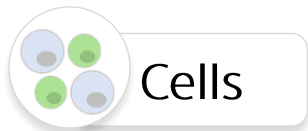
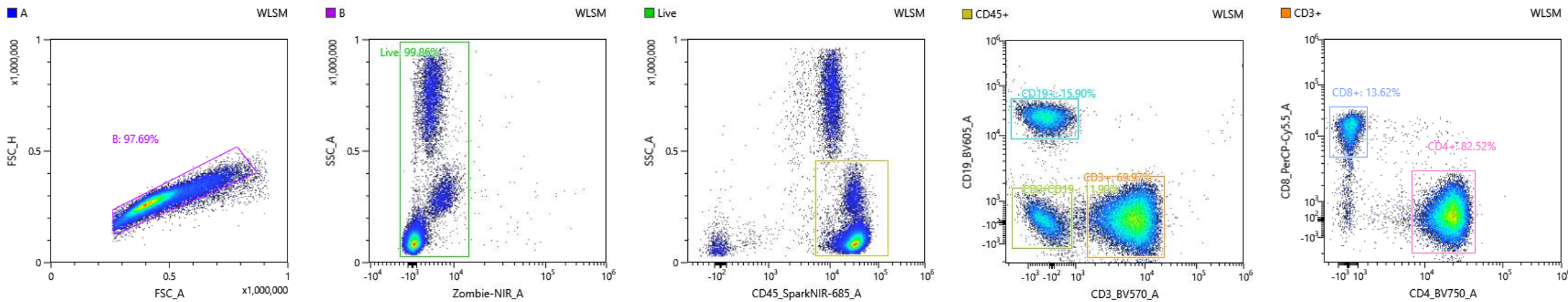


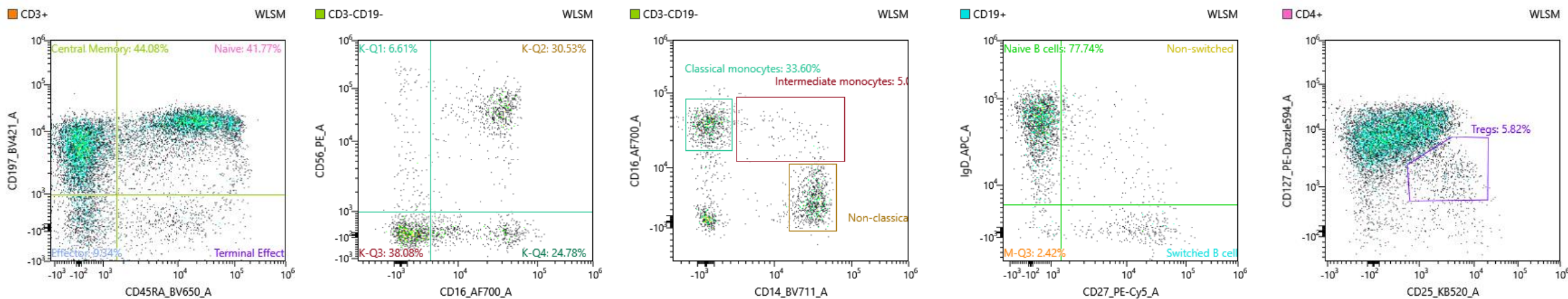
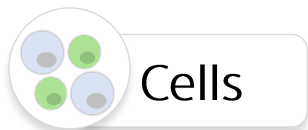
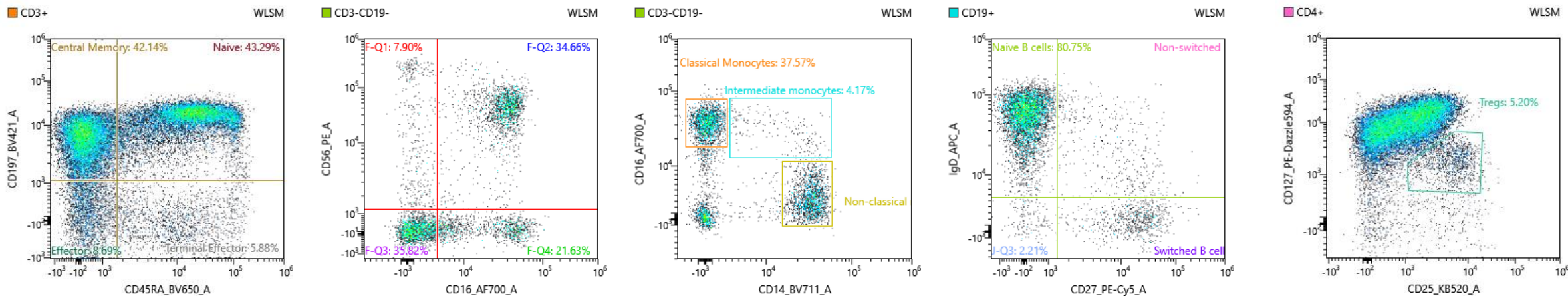
R-squared analysis of Align Check at five Standardized PMT Array Voltages

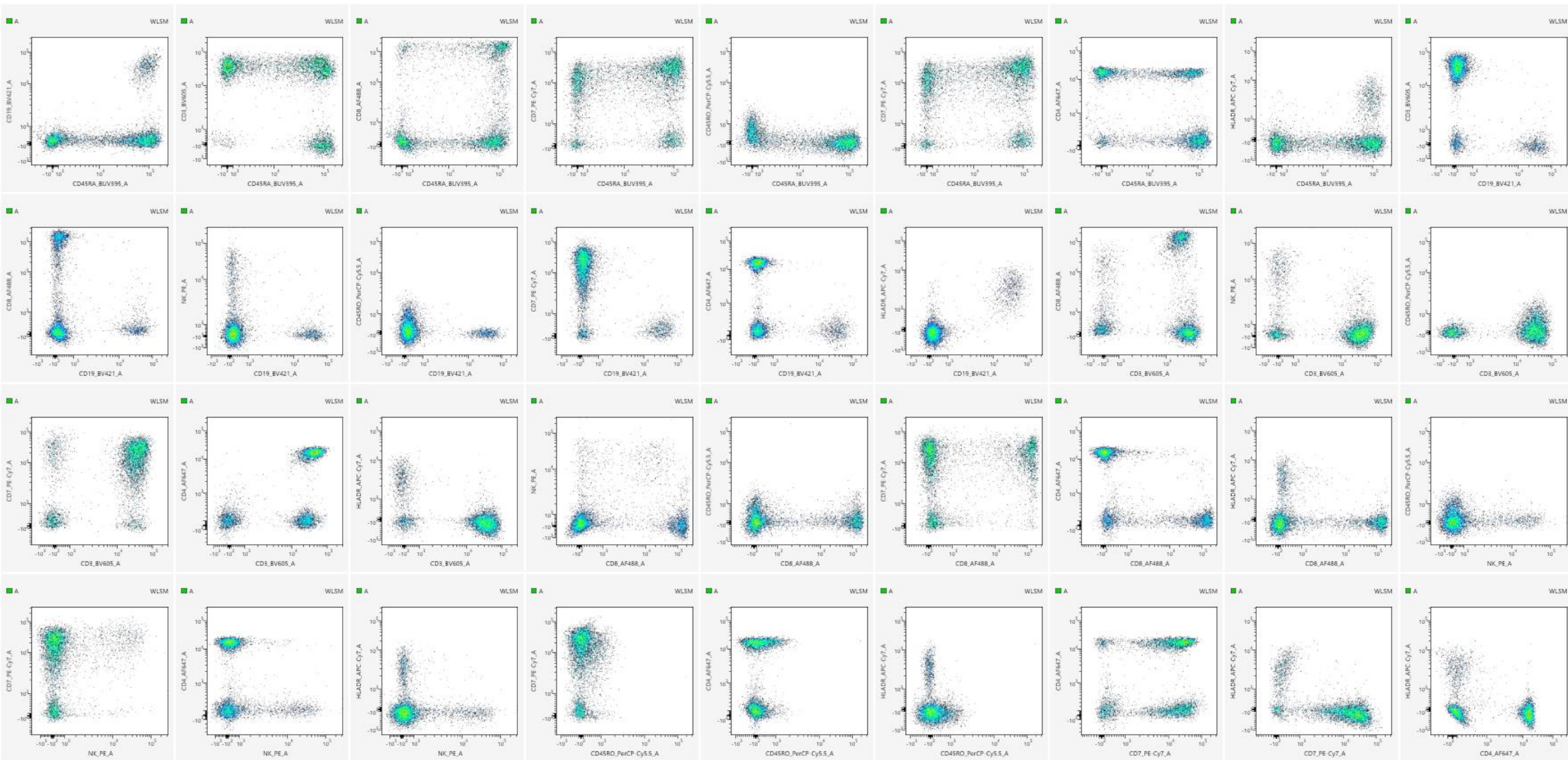
	4.00	4.25	4.50	4.75	5.00
4.00		0.996	0.987	0.980	0.981
4.25	0.996		0.997	0.992	0.991
4.50	0.987	0.997		0.999	0.996
4.75	0.980	0.992	0.999		0.997
5.00	0.981	0.991	0.996	0.997	

Median_{pos}-Median_{neg} at ST Mode PMT voltage settings 4.00 to 5.00

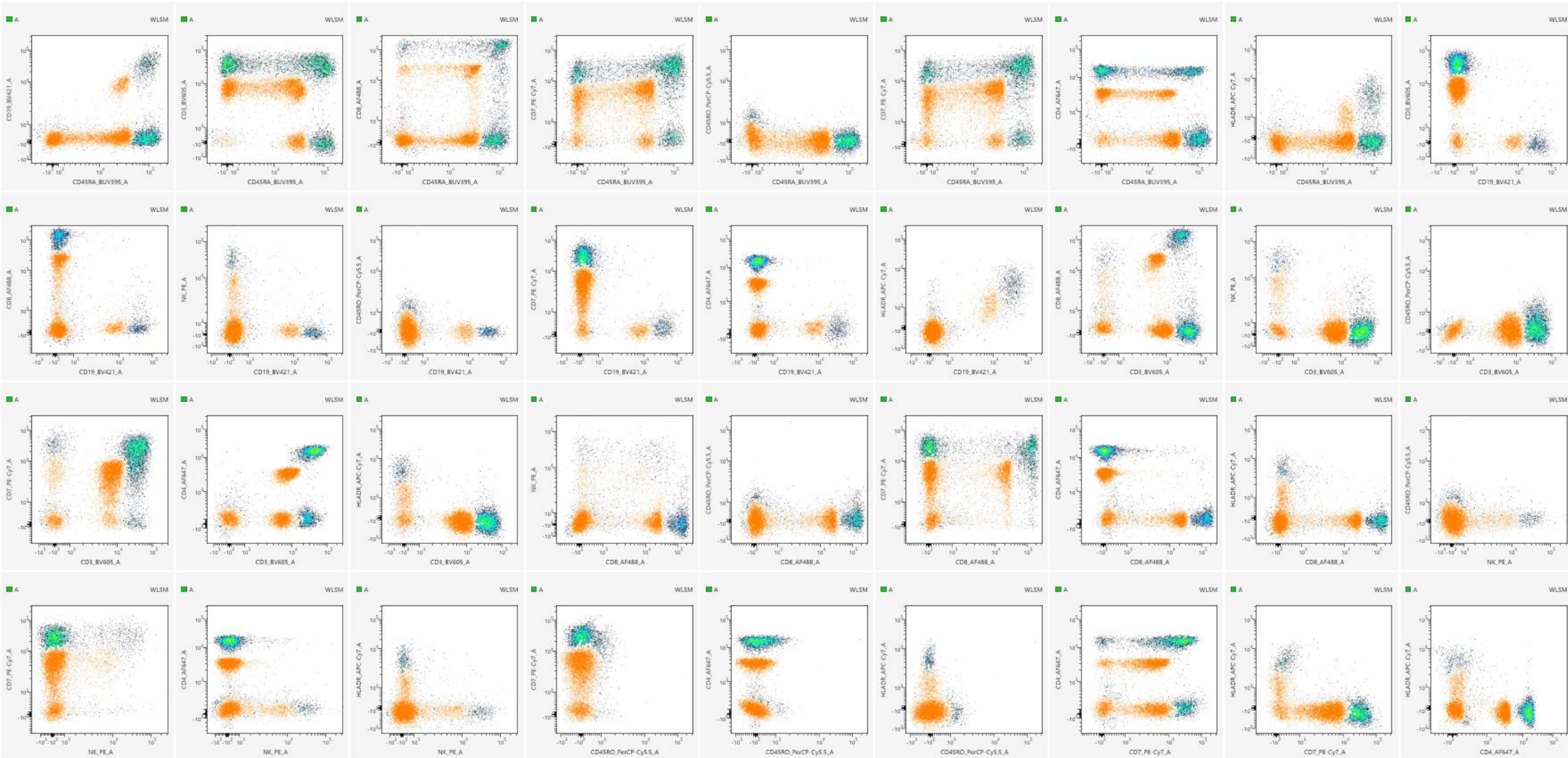


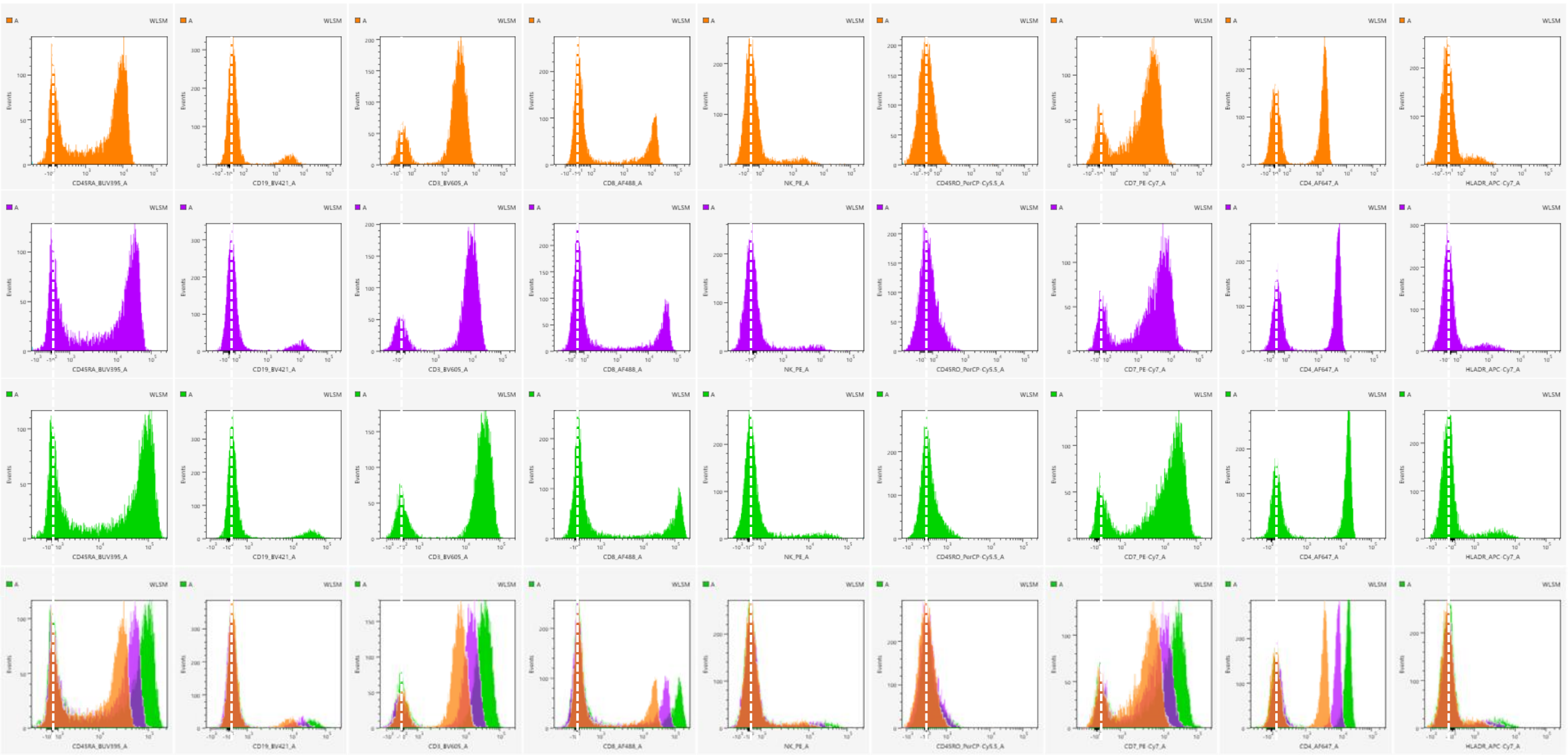




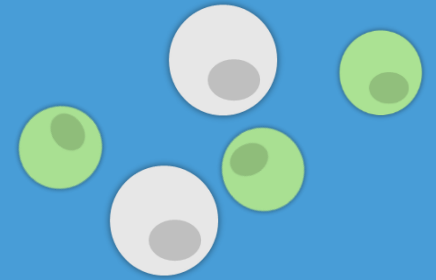


Overlay PMT Voltage 4.00 and 5.00



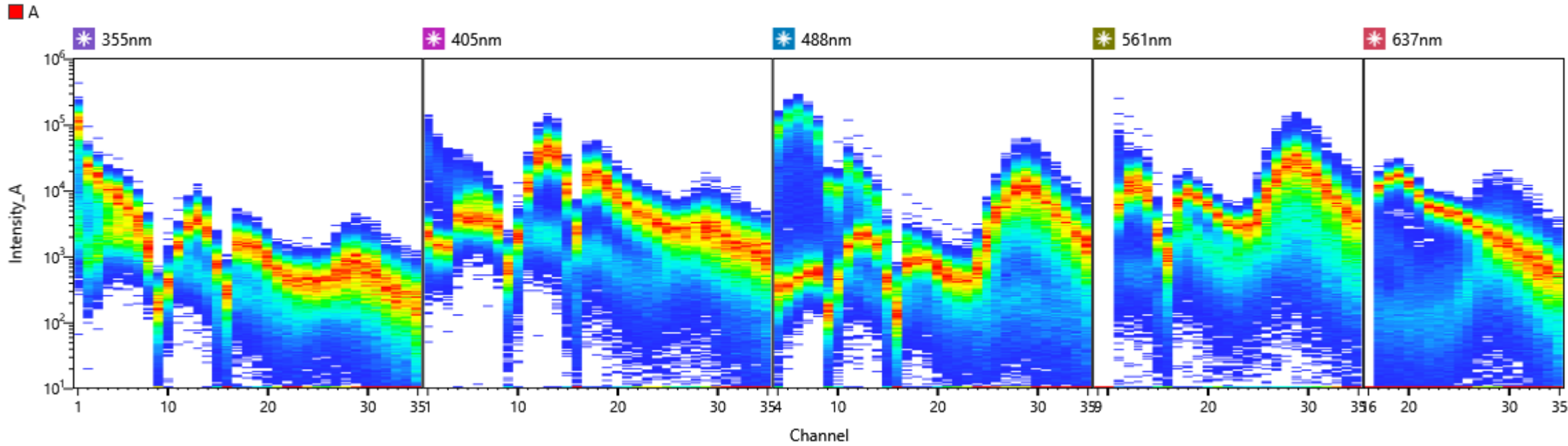


ACQUISITION
Workflow



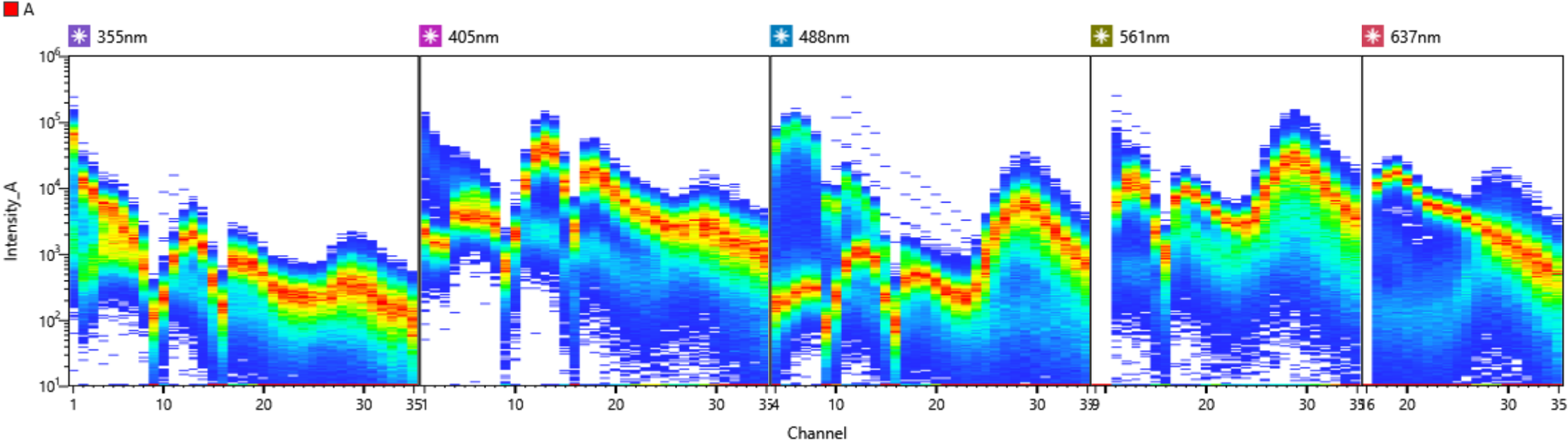
Standardized Mode

All the detectors in the entire array with a single PMT adjustment:
The sensitivity of all detectors increases and decreases in synchrony.



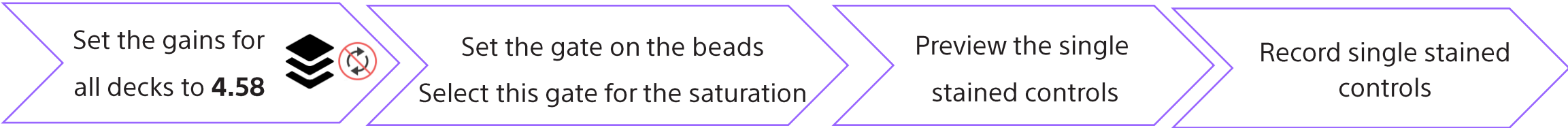
Std. PMTV Setting 5.00

Or change the gain of each detector deck separately by a single PMT adjustment:



Step 1

Single stain controls



Instrument Settings

Sample: Well - A01
Mode: Standardization

Laser Detector & Threshold Flow Control Agitation Event Check Cleaning

FSC Gain: 17
SSC Voltage (ST): 3.78 (1.00 - 5.00)

PMT Voltage (ST)

320 nm:	4.58	(1.00 - 5.00)
355 nm:	4.58	(1.00 - 5.00)
405 nm:	4.58	(1.00 - 5.00)
488 nm:	4.58	(1.00 - 5.00)
561 nm:	4.58	(1.00 - 5.00)
637 nm:	4.58	(1.00 - 5.00)
808 nm:	4.58	(1.00 - 5.00)

Synchronous Voltage Adjust

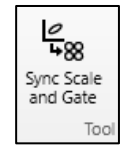
1.00 - +
0.10 - +
0.01 - +

Threshold

CH1: FSC Value1(%): 11.1
CH2: None Value2(%):

Window Extension: Normal

- Synchronize across the sample group
- Sync. Stopping conditions



Exceptions:



Sample: Well - A01

Stopping Condition

Total Events 2,000
 Acquisition Time 00:02:00

Gate Level Stopping Condition

#1 A 2,000 events
 #2 events
 #3 events

Saturation Gate

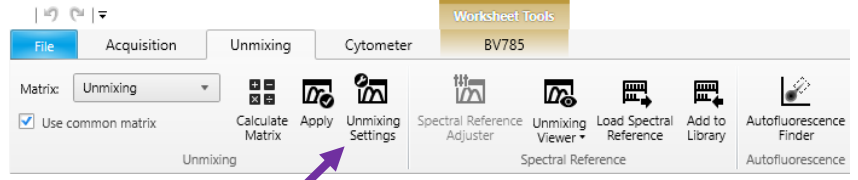
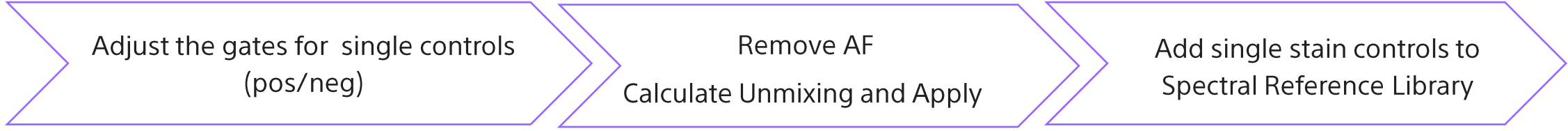
A

Sync Stopping Condition

Step 2

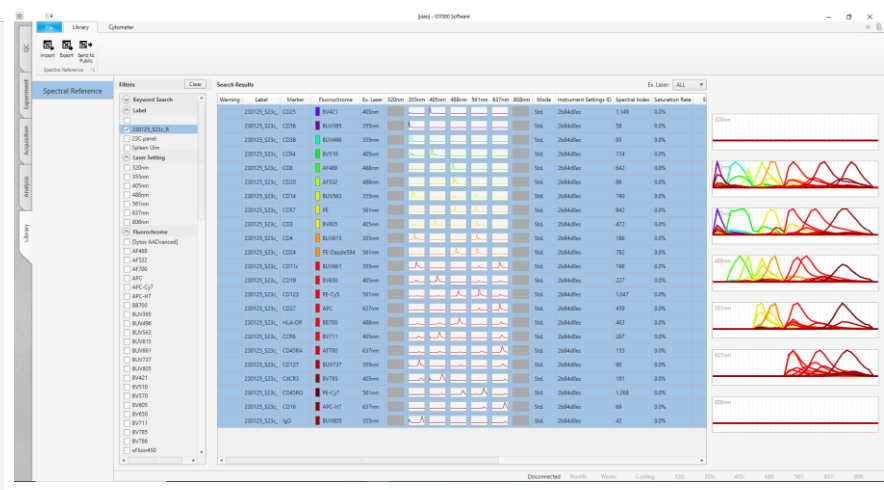


Unmixing Settings



Fluorochrome							
Index	Marker	Fluorochrome	Ex. Laser	Mode	SR	Negative	Positive
1	CD45RO	BUV395	355nm	Std.		A (Unstained)	Positive (BUV395)
2	CD16	BUV496	355nm	Std.		A (Unstained)	Positive (BUV496)
3	CD45RA	BUV737	355nm	Std.		A (Unstained)	Positive (BUV737)
4	CD56	BV421	405nm	Std.		A (Unstained)	Positive (BV421)
5	CD8	BV510	405nm	Std.		A (Unstained)	Positive (BV510)
6	CD4	BV750	405nm	Std.		A (Unstained)	Positive (BV750)
7	CD20	BV785	405nm	Std.		A (Unstained)	Positive (BV785)
8	CD45	PerCP	488nm	Std.		A (Unstained)	Positive (PerCP)
9	CD3	APC	637nm	Std.		A (Unstained)	Positive (APC)
10	CD19	APC-Cy7	637nm	Std.		A (Unstained)	Positive (APC-Cy7)

Autofluorescence							
Index	Use as AF	Autofluorescence	Ex. Laser	Mode	SR	Negative	Positive
1	<input checked="" type="checkbox"/>	[AF color 1]	405nm	Std.		Zero Reference	AF-A (Well - C01)



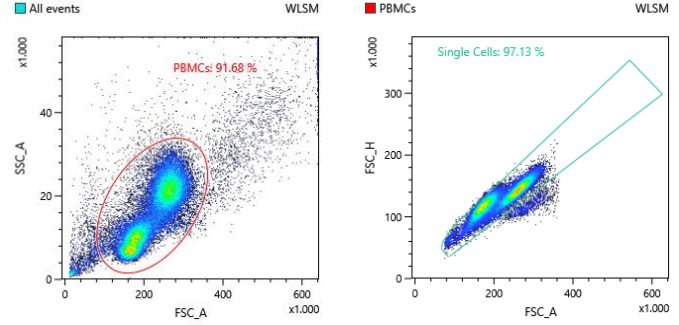
Step 3



Full stained samples

Preview sample

Draw gate on FSC/SSC and select singlets (FSC-H/FSC-A)



Stopping Condition

Sample: Lung 2

Stopping Condition

Stop Gate1: ■ A 500,000 events

Saturation Gate

■ Singlets

Close

In Stopping Conditions:
Select in Saturation Gate – Singlets

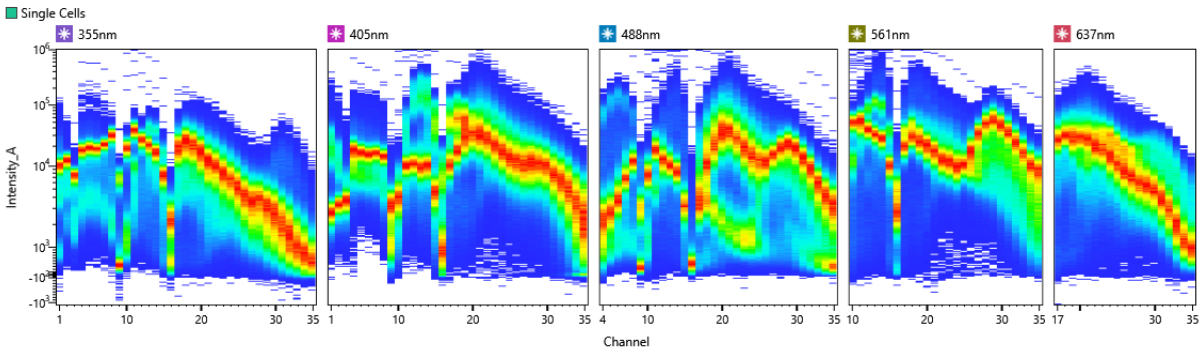
One Max

Maximize gains in synchrony 2



PMT Voltage (ST)

355 nm	5.38
405 nm	5.38
488 nm	5.38
561 nm	5.38
637 nm	5.38



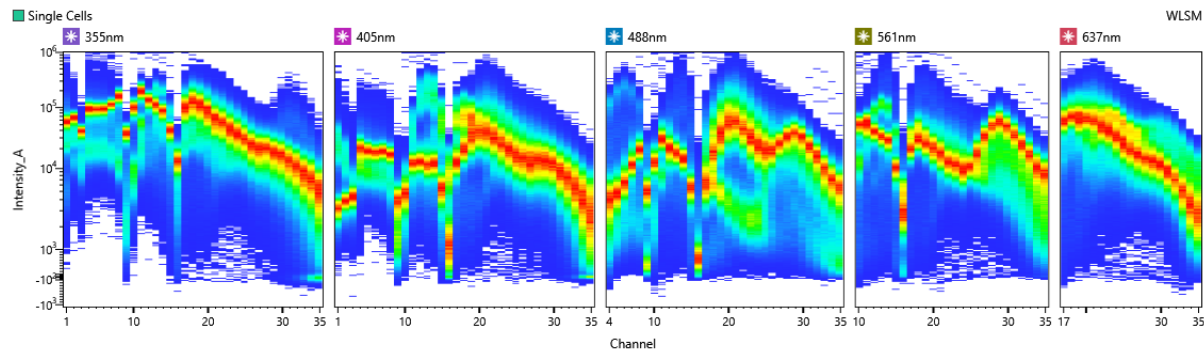
All Max

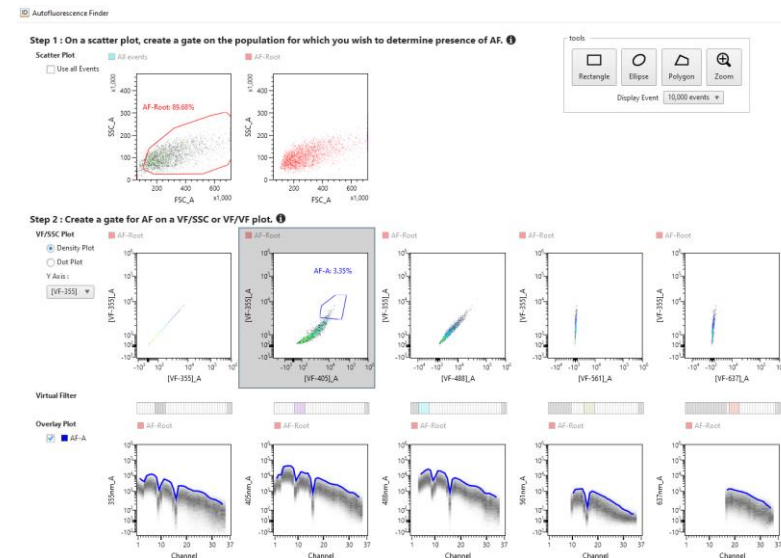
Maximize gains across the individual decks 3



PMT Voltage (ST)

355 nm	6.10
405 nm	5.45
488 nm	5.60
561 nm	5.38
637 nm	5.75





One Max



Maximize gains in synchrony **2**

Maximize gains while viewing the Ribbon plot
Monitor saturation rate

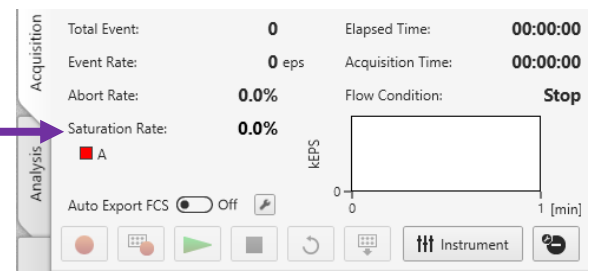
Acquire the unstained sample with the same settings

Use the unstained sample for the calculation of AF signal

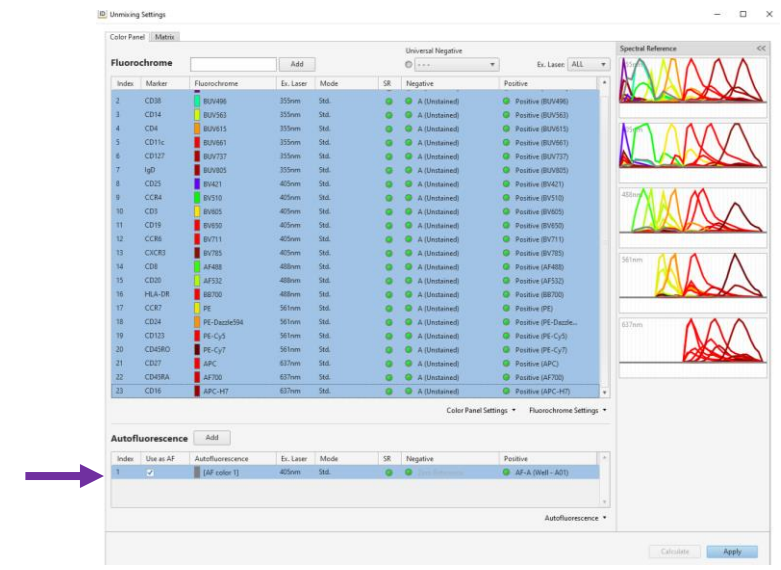
All Max



Maximize gains across the individual decks **3**



Note: Keep saturation rate < 3%





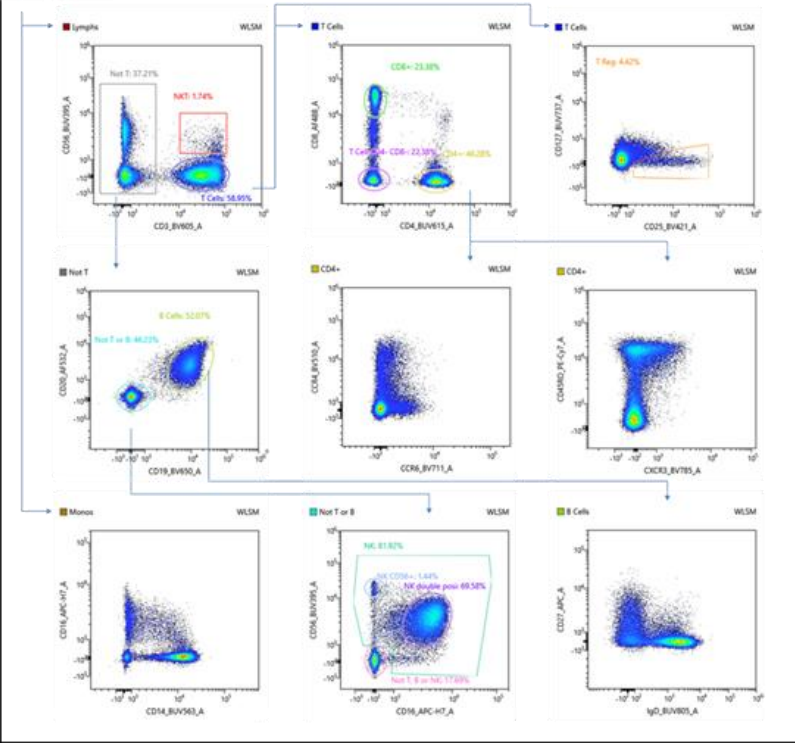
One Max



All Max



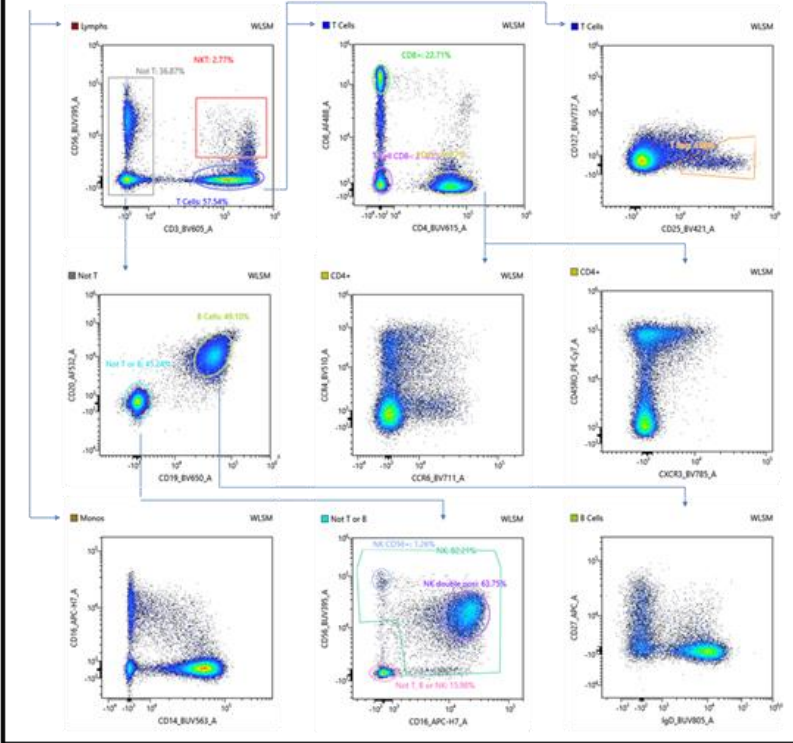
Setting 1



PMT Voltage (ST)

355 nm	4.58
405 nm	4.58
488 nm	4.58
561 nm	4.58
637 nm	4.58

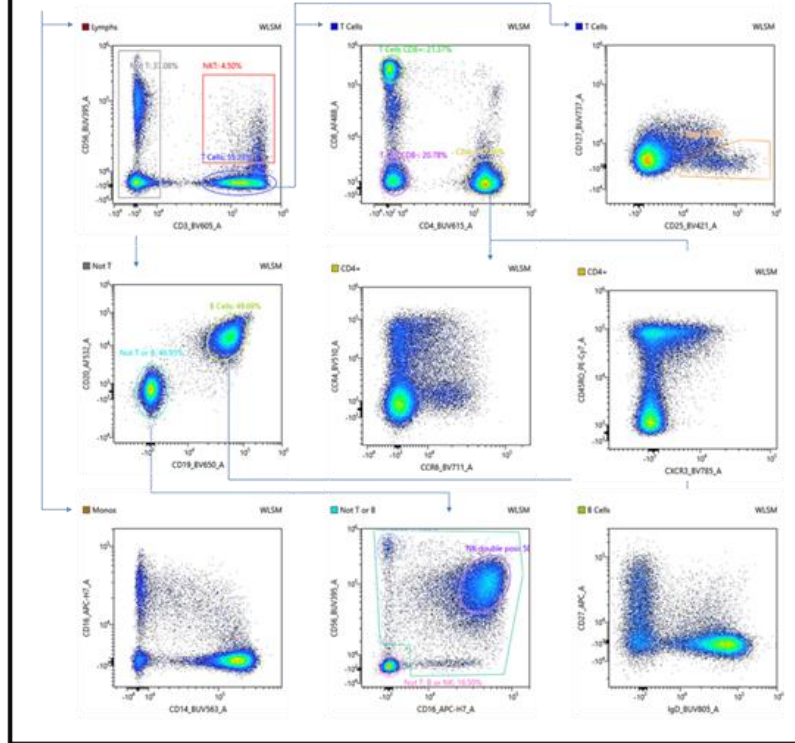
Setting 2



PMT Voltage (ST)

355 nm	5.38
405 nm	5.38
488 nm	5.38
561 nm	5.38
637 nm	5.38

Setting 3



PMT Voltage (ST)

355 nm	6.10
405 nm	5.45
488 nm	5.60
561 nm	5.38
637 nm	5.75



Based on Daily QC

Minimizes subjectivity and instrument variability

Single stain controls acquired at one PMT voltage (ST)

PMT voltage (ST) for samples can be optimized/maximized without the need to match the single stain controls

Spectral references (controls) can be reused * to unmix data at different voltage settings

Ideal for longitudinal studies

Multi-instrument standardization

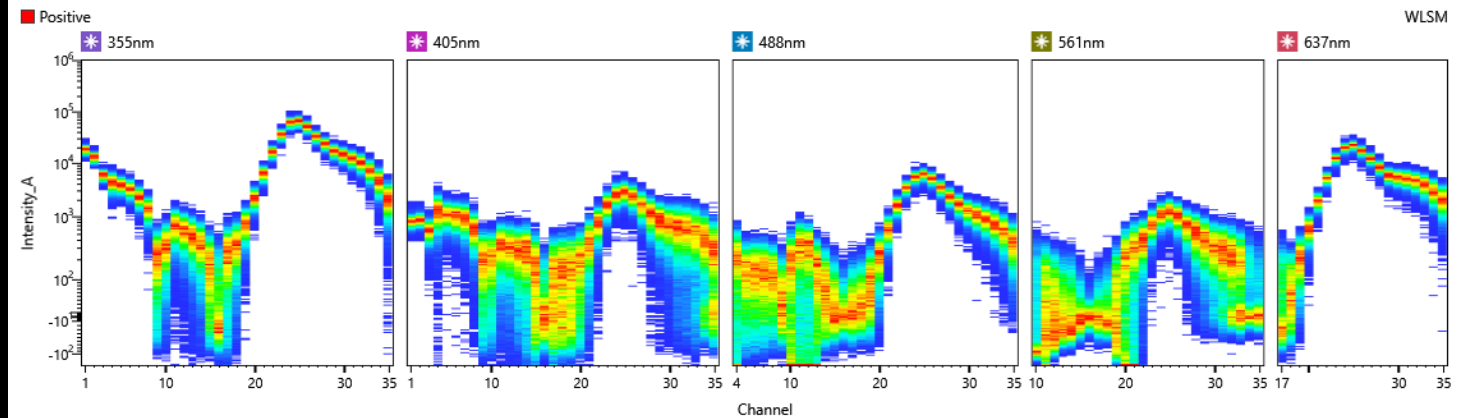
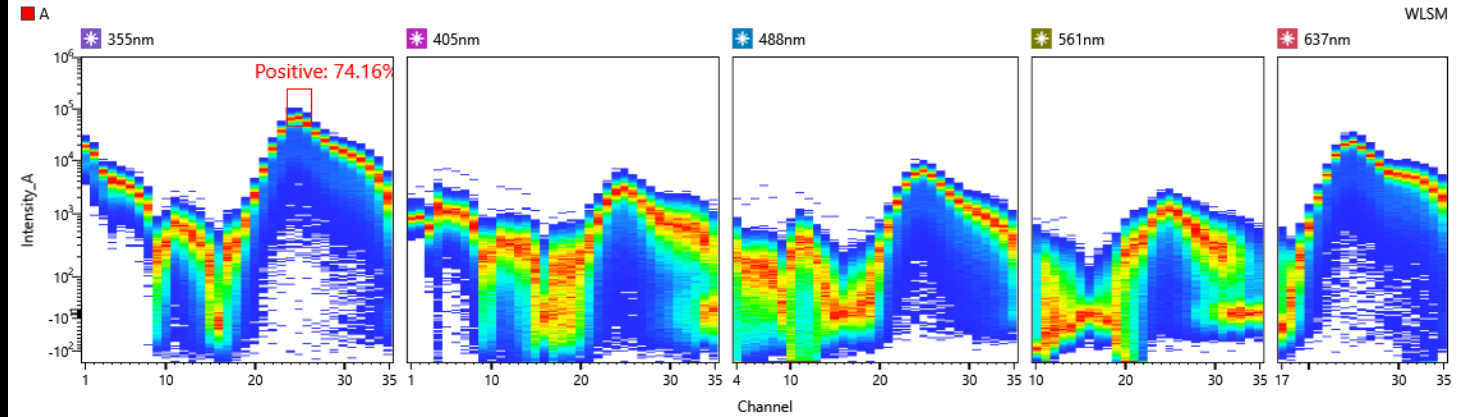


* check for tandem dyes stability over time

ID7000 Workflow

1. QC
2. Experiment
3. Colour Panel (colour + marker)
or
Reference Spectra
4. Instrument & Acquisition Settings
5. Reference Spectra
6. Autofluorescence
7. Unmixing QC

Single Colour Control



Sinle Colour Control

ID7000 W...
Unmixing Settings



Color Panel Matrix

Fluorochrome

Add

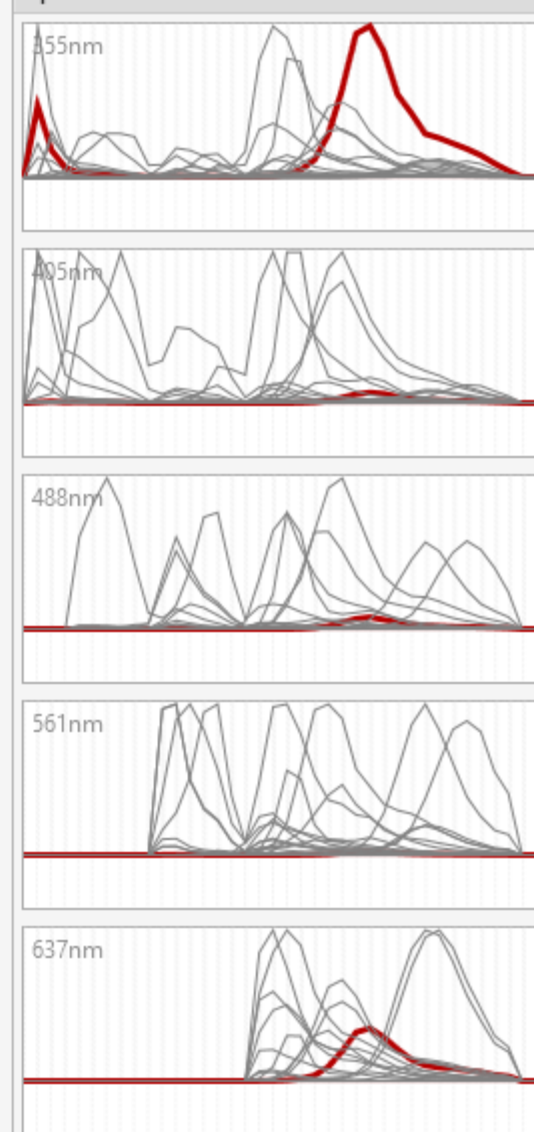
Universal Negative

E (Unstained) ▼

Ex. Laser: ALL ▼

Index	Marker	Fluorochrome	Ex. Laser	Mode	SR	Negative	Positive
1	CD8	BUV395	355nm	Normal	<input checked="" type="radio"/>	B (BUV395)	Positive (BUV395)
2	CD11c	BUV661	355nm	Normal	<input checked="" type="radio"/>	B (BUV661)	Positive (BUV661)
3	CD4	BUV737	355nm	Normal	<input checked="" type="radio"/>	E (BUV737)	Positive (BUV737)
4	CCR7	BV421	405nm	Normal	<input checked="" type="radio"/>	B (BV421)	Positive (BV421)
5	CD123	SB436	405nm	Normal	<input checked="" type="radio"/>	B (SB436)	Positive (SB436)
6	IgD	BV480	405nm	Normal	<input checked="" type="radio"/>	B (BV480)	Positive (BV480)
7	HLA-DR	SparkViolet-538	405nm	Normal	<input checked="" type="radio"/>	C (Unstained)	Positive (SparkViol...
8	CD27	BV650	405nm	Normal	<input checked="" type="radio"/>	B (BV650)	Positive (BV650)
9	CD45RO	BV711	405nm	Normal	<input checked="" type="radio"/>	B (BV711)	Positive (BV711)
10	CD14	AF488	488nm	Normal	<input checked="" type="radio"/>	B (AF488)	Positive (AF488)
11	CD19	PE	488nm	Normal	<input checked="" type="radio"/>	B (PE)	Positive (PE)
12	CXCR2	PE-Dazzle594	488nm	Normal	<input checked="" type="radio"/>	B (PE-Dazzle594)	Positive (PE-Dazzle...
13	CD25	PE-Cy5	488nm	Normal	<input checked="" type="radio"/>	B (PE-Cy5)	Positive (PE-Cy5)
14	CD45	PerCP	488nm	Normal	<input checked="" type="radio"/>	B (PerCP)	Positive (PerCP)
15	TCRgd	PerCP-eFluor710	488nm	Normal	<input checked="" type="radio"/>	C (Unstained)	Positive (PerCP-eF...
16	CD16	PE-Cy7	488nm	Normal	<input checked="" type="radio"/>	C (Unstained)	Positive (PE-Cy7)
17	CD39	PE-Fire810	488nm	Normal	<input checked="" type="radio"/>	B (PE-Fire810)	Positive (PE-Fire810)
18	CD20	SparkYG-570	561nm	Normal	<input checked="" type="radio"/>	B (SparkYG-570)	Positive (SparkYG-...
19	CD127	PE-Fire700	561nm	Normal	<input checked="" type="radio"/>	B (PE-Fire700)	Positive (PE-Fire700)
20	CD56	APC	637nm	Normal	<input checked="" type="radio"/>	D (APC)	Positive (APC)
21	CCR6	AF647	637nm	Normal	<input checked="" type="radio"/>	B (AF647)	Positive (AF647)

Spectral Reference

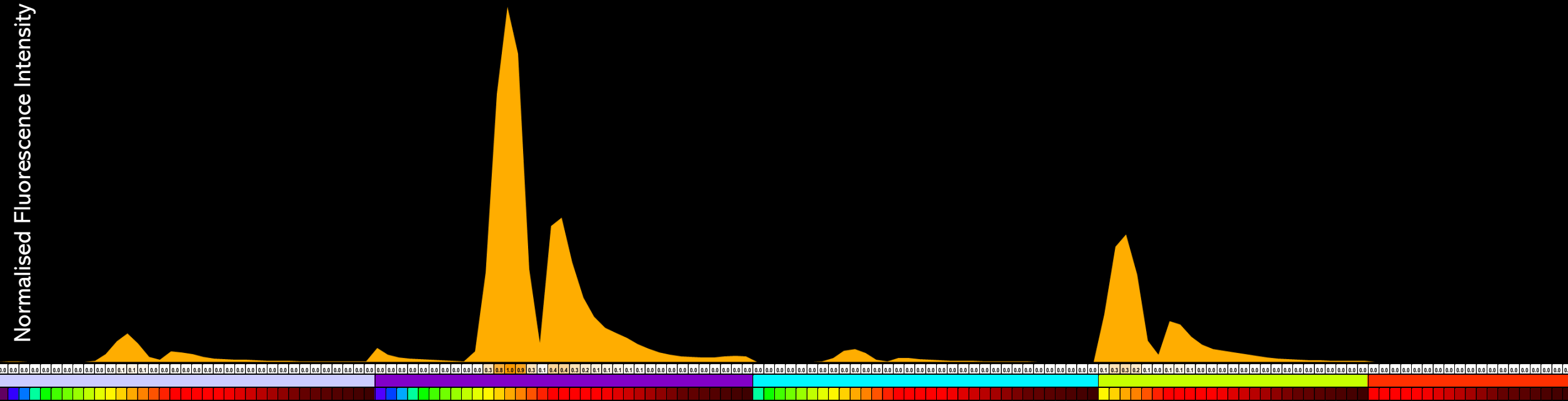


1.
2.
3.
4.
5.
6.
7.

Reference Spectra

A reference spectrum is a set of values that defines the distribution of a fluorochrome's fluorescence in the detector array.

All of the fluorescence from all of the lasers and all of the detectors is included in each reference spectrum.



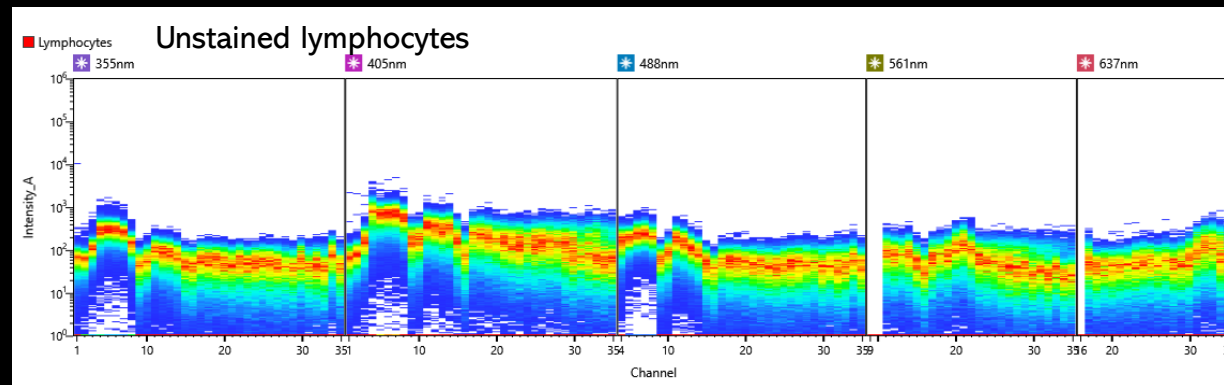
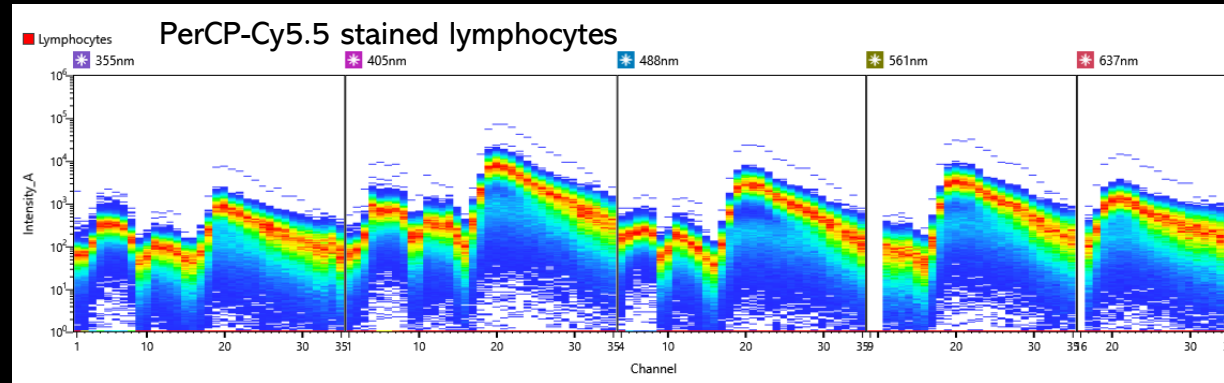
SONY

Single Colour Control Best Practices

- Cells or beads will both work fine.
- Treat the controls the same way the sample will be treated.
- Antibody capture beads are preferred when possible.

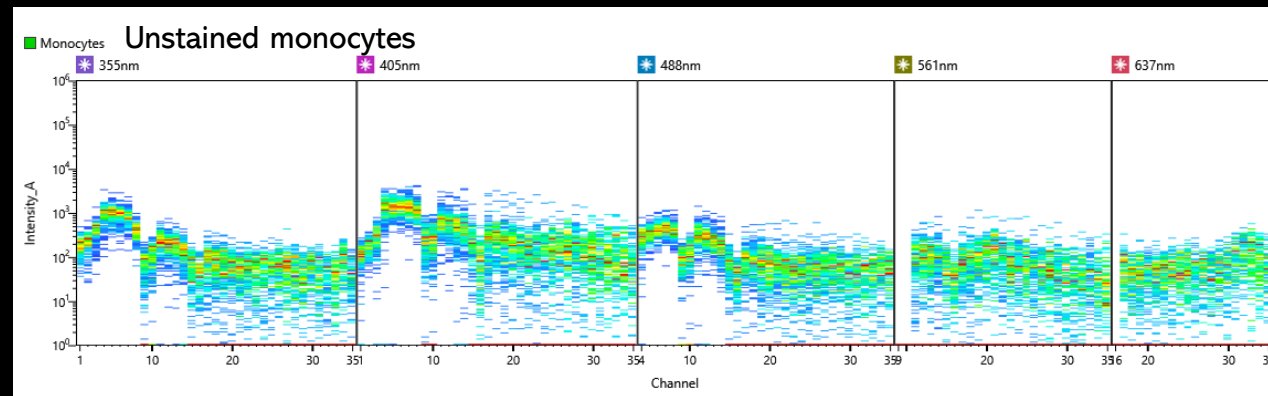
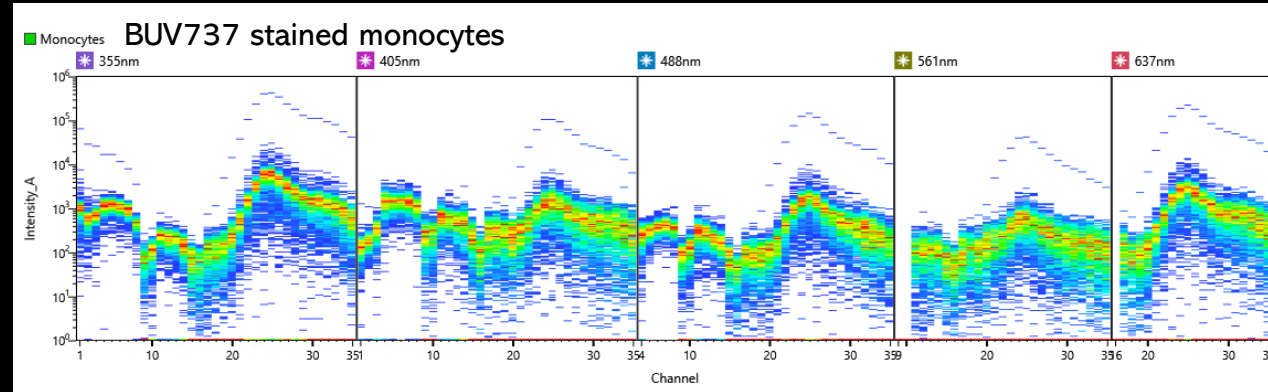
Single Colour Control Best Practices

- If using cells for single colour controls, you will need a corresponding negative control.



Single Colour Control Best Practices

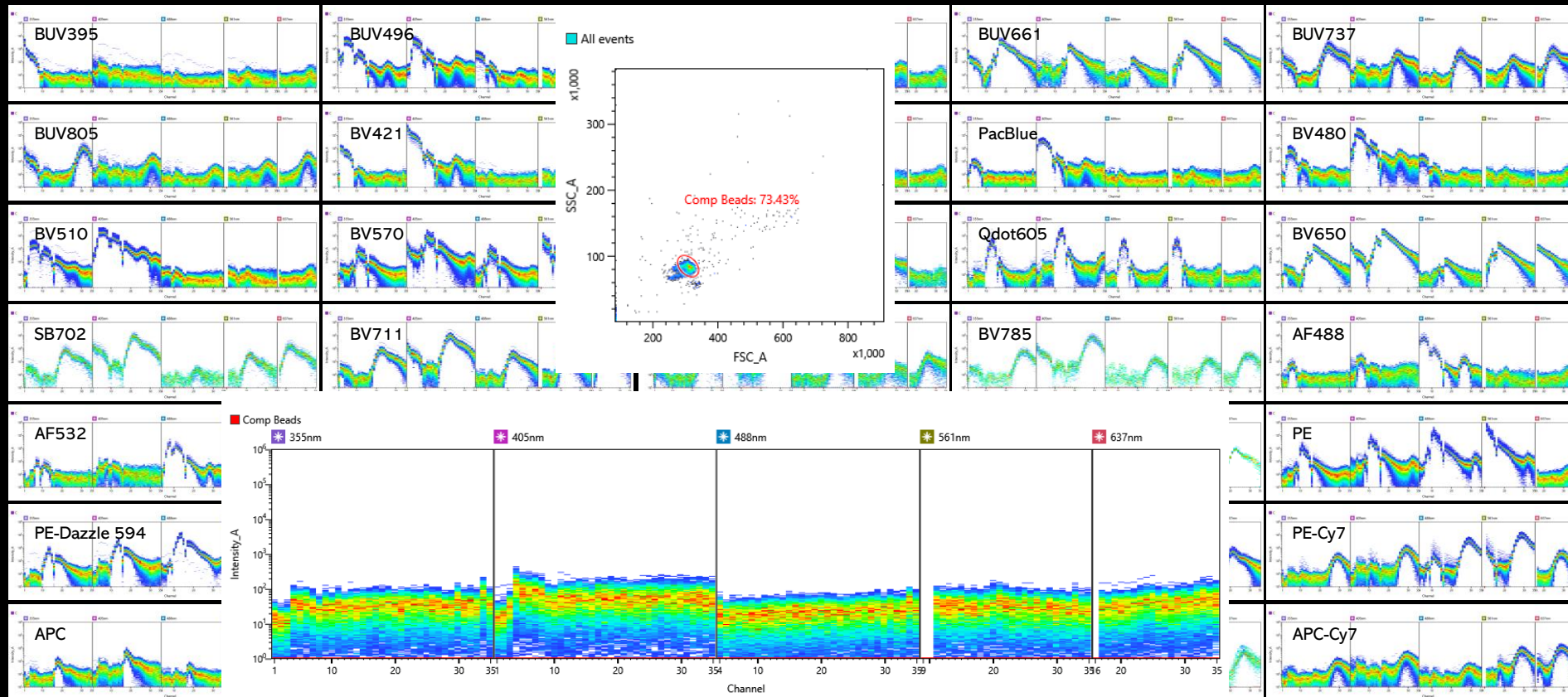
- If using cells for single colour controls, you will need a corresponding negative control.



Single Colour Control Best Practices

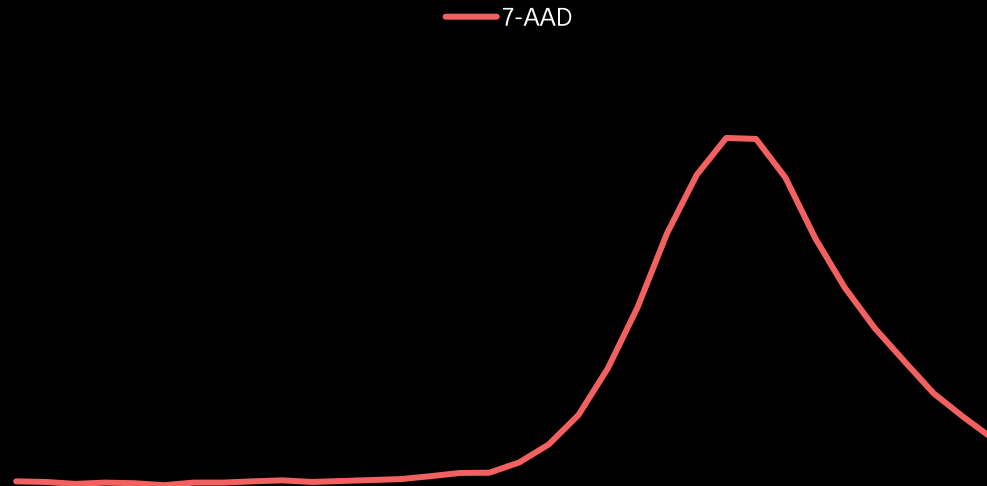
Compensation beads

- Bright, well-defined signature
- Require only 1 negative control



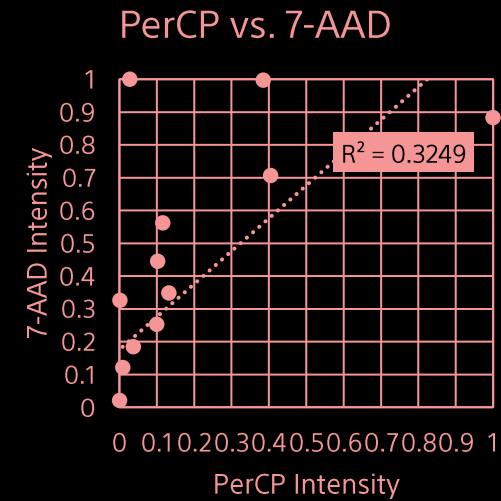
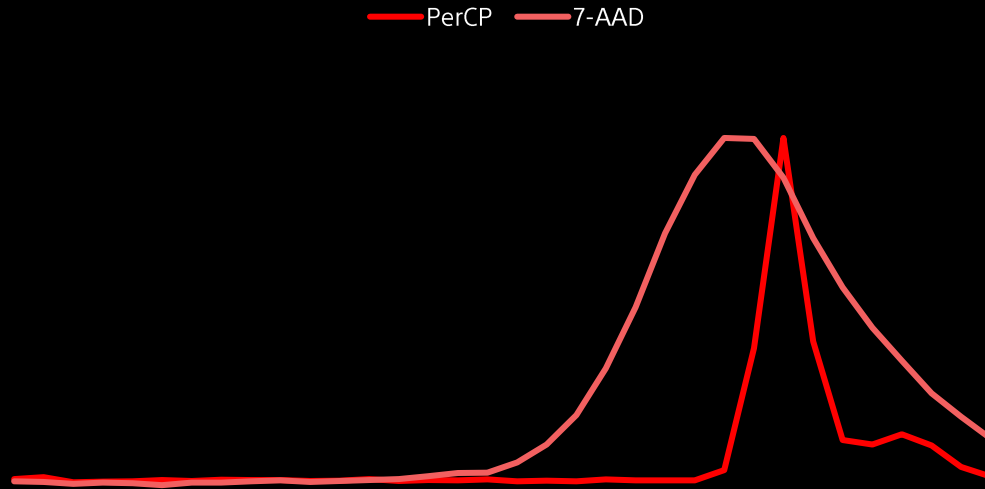
Single Colour Control Best Practices

- Do not substitute similar fluorochromes for what is in your sample.



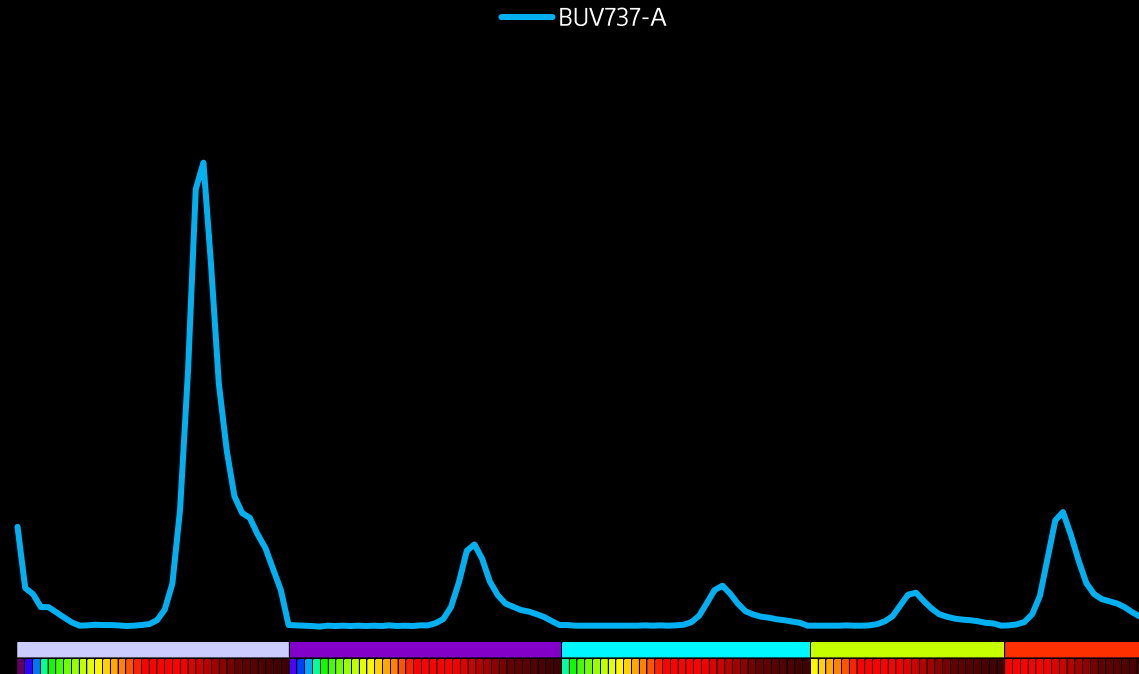
Single Colour Control Best Practices

- Do not substitute similar fluorochromes for what is in your sample.
- This results in incorrect unmixing.



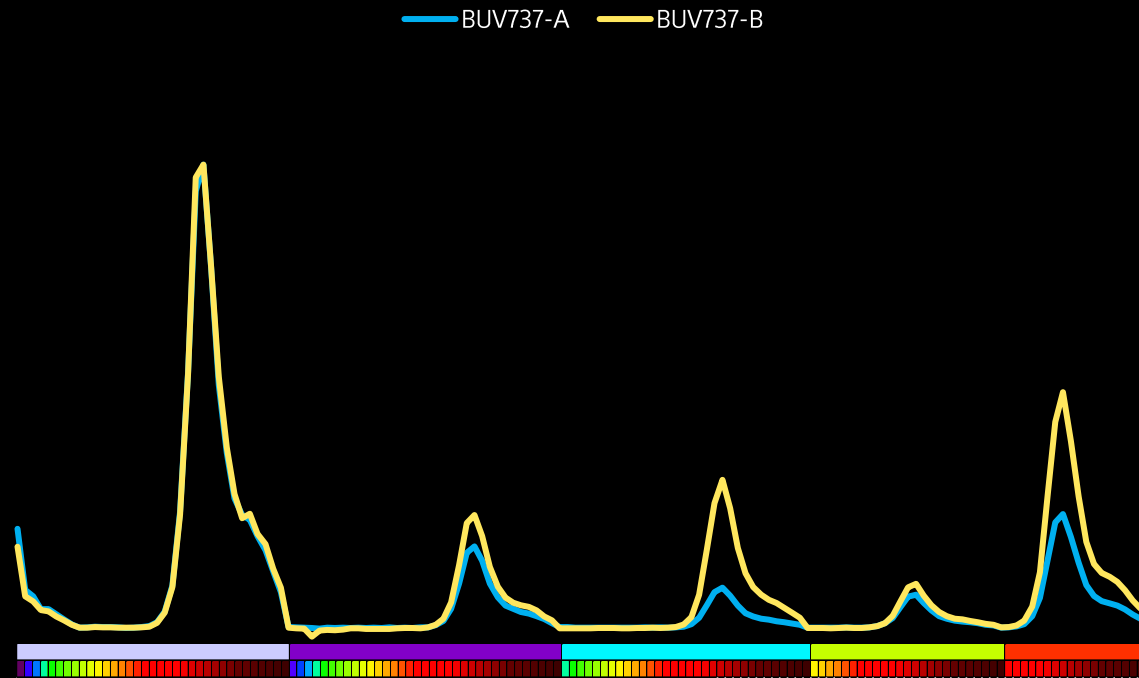
Single Colour Control Best Practices

- Tandem dyes may have different degradation states.



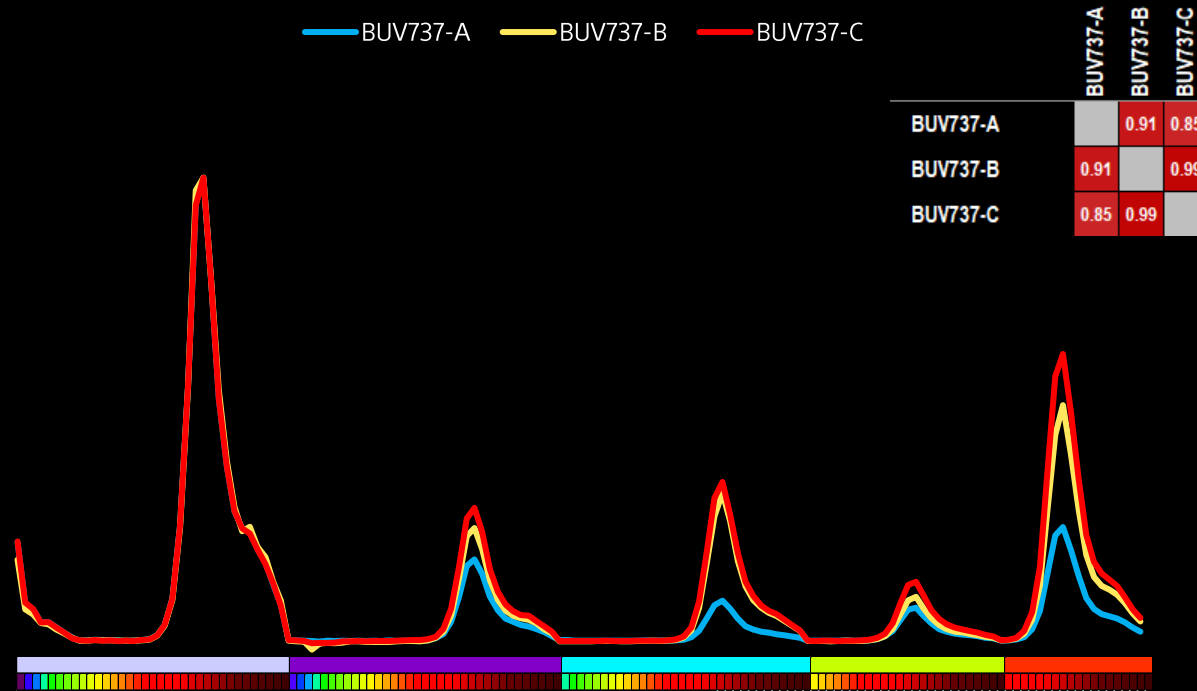
Single Colour Control Best Practices

- Tandem dyes may have different degradation states.



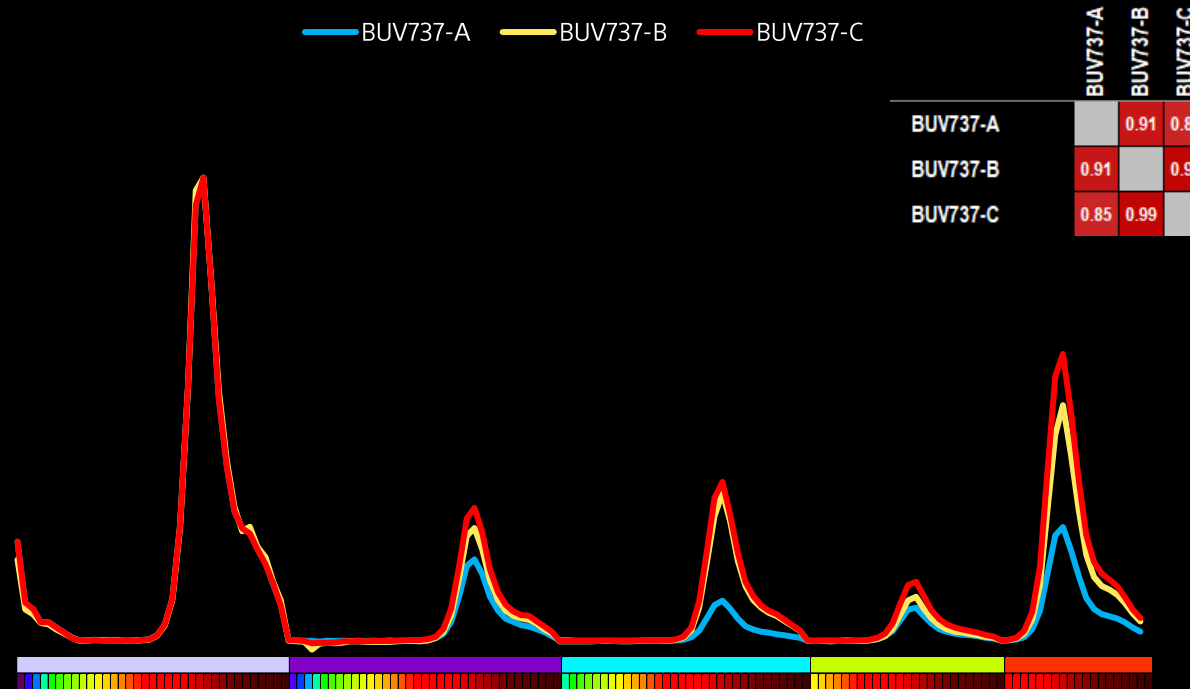
Single Colour Control Best Practices

- Tandem dyes may have different degradation states.



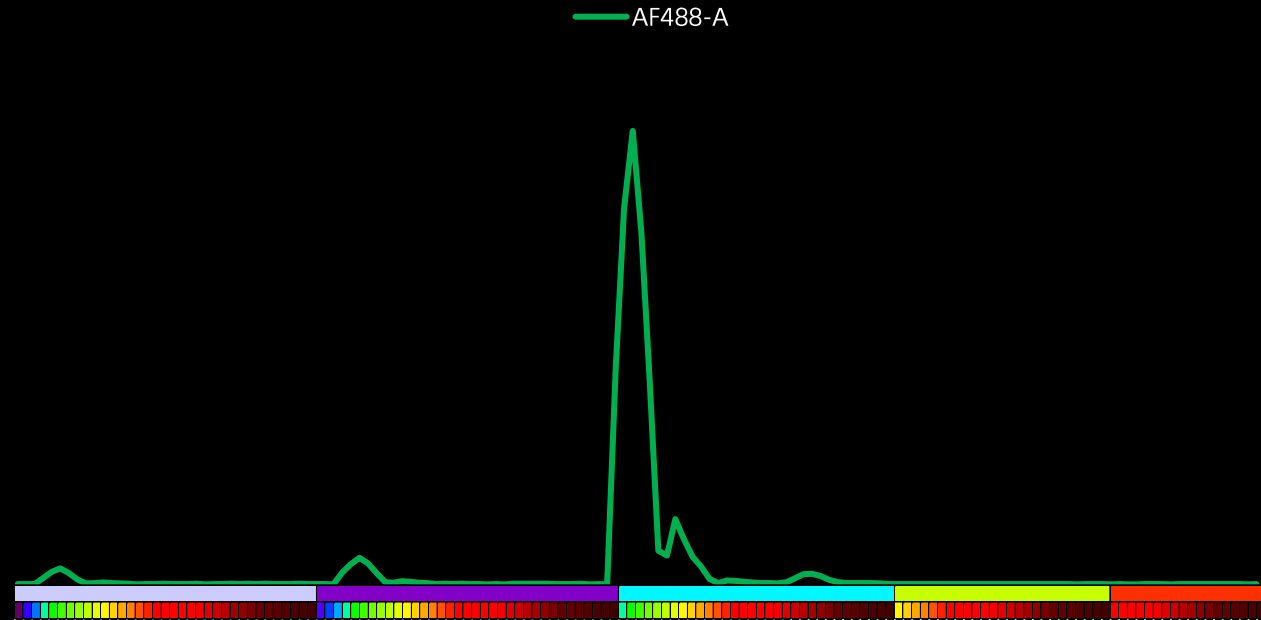
Dump Channels

- Because of tandem variability, it is not recommended that you use them for dump channels.



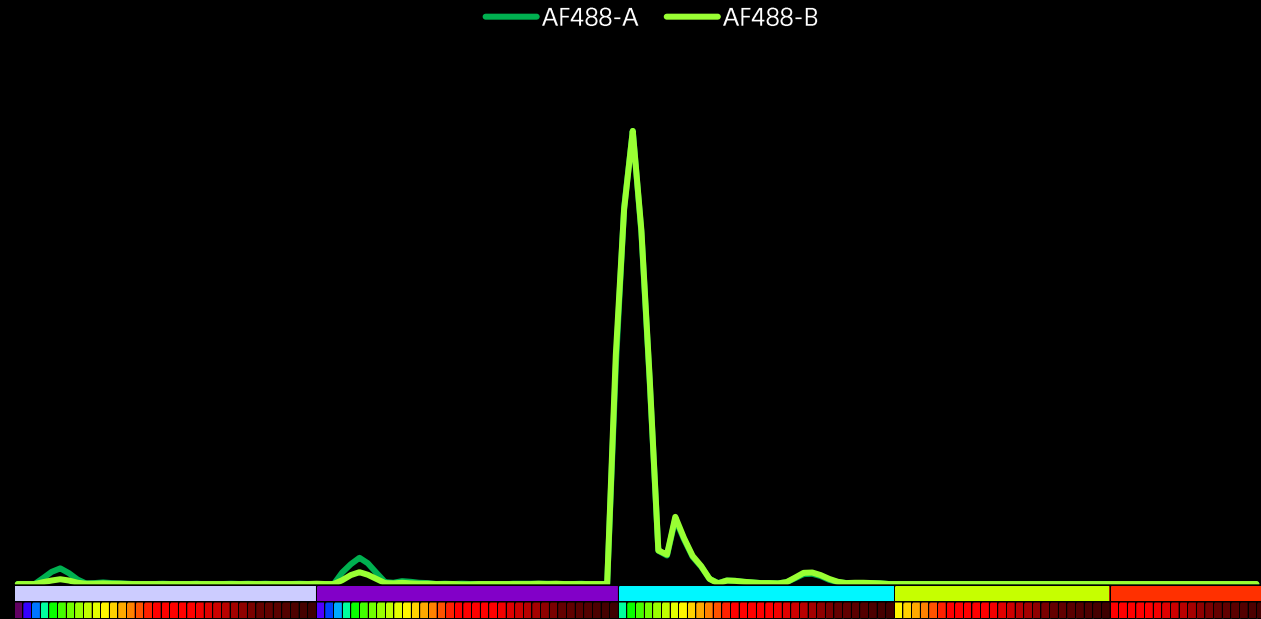
Dump Channels

- Single molecule dyes are much more consistent with each other.



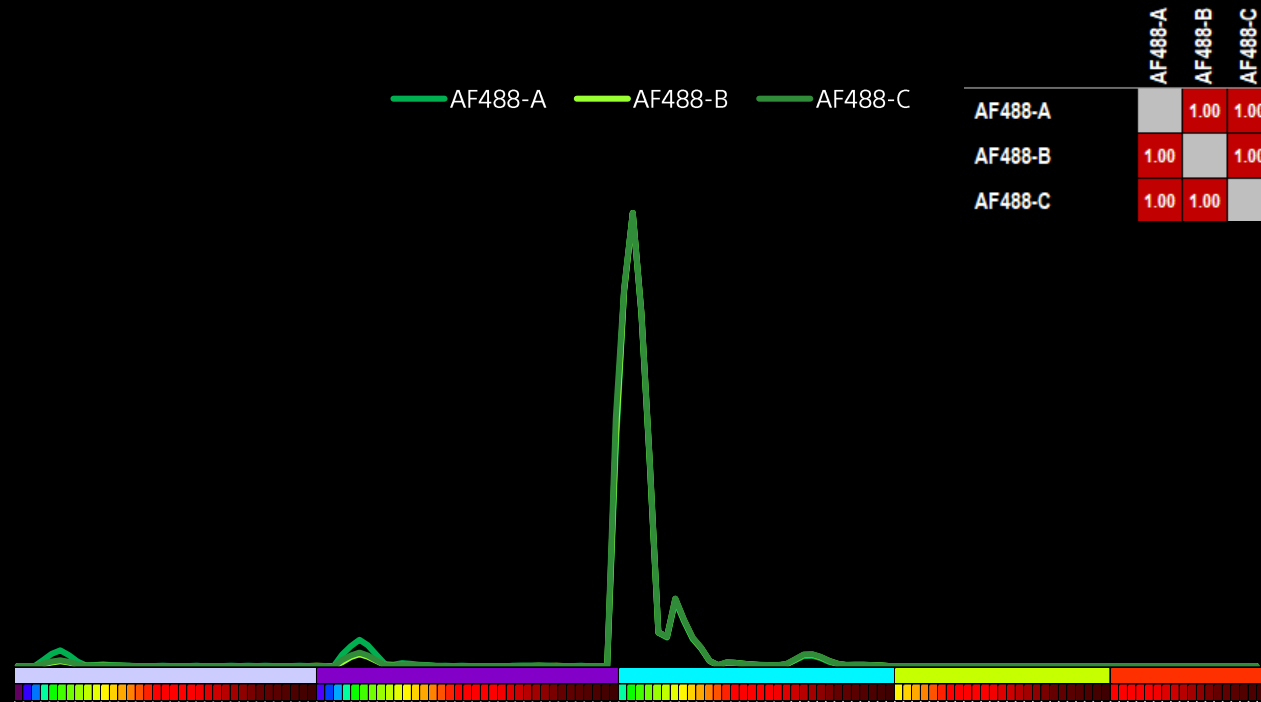
Dump Channels

- Single molecule dyes are much more consistent with each other.



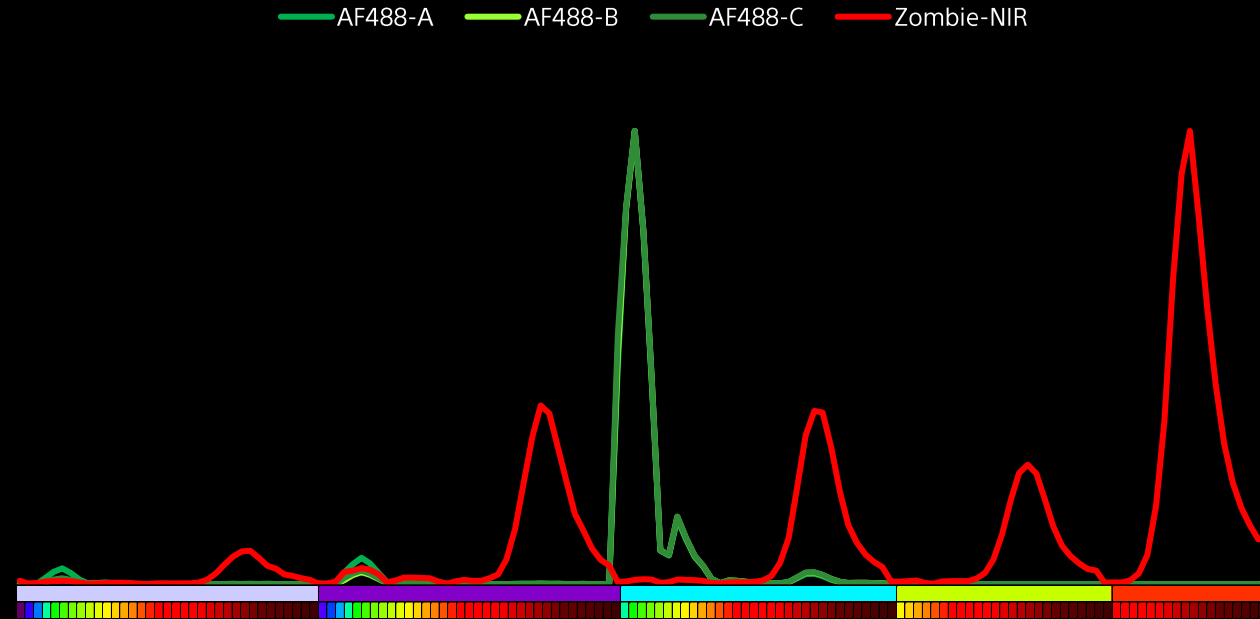
Dump Channels

- Single molecule dyes are much more consistent with each other.



Dump Channels

- Don't try to match your viability dye to your dump channel.

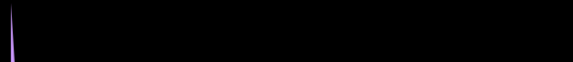
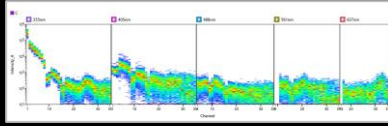


Single Colour Controls

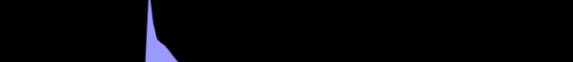
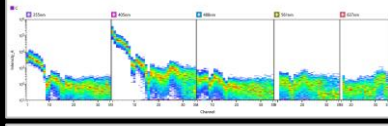
Reference Spectra

Spectral Reference Library

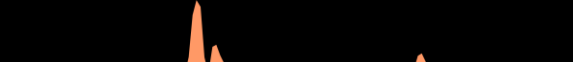
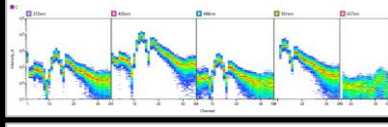
BUV395



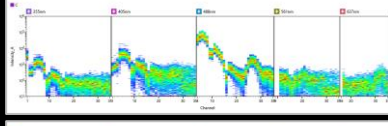
BV421



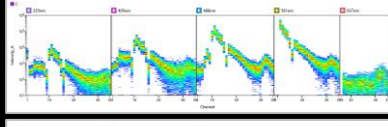
BV605



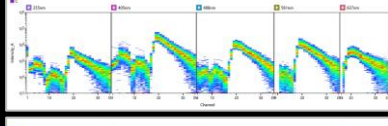
AF488



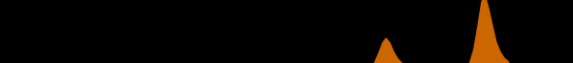
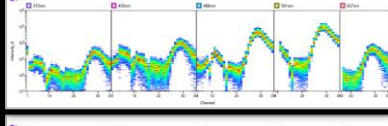
PE



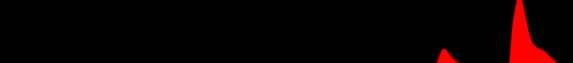
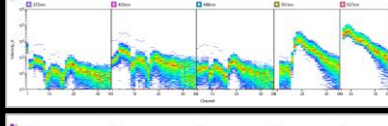
PerCP-Cy5.5



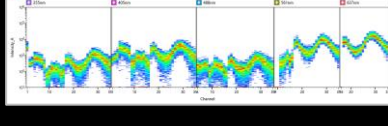
PE-Cy7



AF647



APC-Cy7



The screenshot shows the 'Spectral Reference Library' window with a list of markers and their associated spectra. The interface includes a 'Filters' panel on the left and a 'Search Results' table on the right.

Varning	Marker	Fluorochrome	Ex. Laser	320nm	355nm	405nm	488nm	561nm	637nm	808nm
	CD8	AF488	488nm							
	CD8	AF488	488nm							
		AF488	488nm							
	CD8	AF488	488nm							
	CD8	AF488	488nm							
	CD4	AF647	637nm							
	CD4	AF647	637nm							
		AF647	637nm							
	CD4	AF647	637nm							
	CD4	AF647	637nm							
		APC-Cy7	637nm							
	HLADR	APC-Cy7	637nm							
	HLADR	APC-Cy7	637nm							
	HLADR	APC-Cy7	637nm							
	HLADR	APC-Cy7	637nm							
	CD45RA	BUV395	355nm							
	CD45RA	BUV395	355nm							
	CD45RA	BUV395	355nm							
	CD45RA	BUV395	355nm							
		BUV395	355nm							
		BUV395	355nm							
	CD19	BV421	405nm							
		BV421	405nm							
	CD19	BV421	405nm							
	CD19	BV421	405nm							
		BV421	405nm							

ID7000 Workflow

1. QC
2. Experiment
3. Colour Panel (colour + marker)

or

Reference Spectra

4. Instrument & Acquisition Settings
5. Reference Spectra
6. Autofluorescence
7. Unmixing QC

Unstained (autofluorescent) Control

- Consider autofluorescence to be an additional colour(s) in your panel.
- Unstained control will be used to define the spectral reference for autofluorescence colours.
- Unstained control needs to be treated the same way as the sample.
- **Autofluorescence Finder Tool**

[administrator] - ID7000 Software

Worksheet Tools

File Analysis Unmixing Cytometer unstained

Open in Acquisition Send to Public Delete Layout Editor Export Layout to CSV File Import Layout from CSV File Delete Copy Paste Move to Sample Group Remove from Sample Group Export Instrument Settings Instrument Settings Stopping Condition Batch Unmixing Batch Analysis Export to FCS File Analysis: Full events Display Events

Experiment Explorer

Experiment List

From: [] To: []

Keyword: [] Search

Public administrator

- with 561 std
- 22c webinar
- 96 Well Plate (standard)**
 - Single Positive Controls (28/28)
 - PBMCs panel 1 (1/1)
 - (Unassigned) (67)
- 384 Well Plate (standard) - 1
 - (Unassigned) (384)
- 24 Tube Rack (5mL) - 1
 - (Unassigned) (24)
- without 561

96 Well Plate (standard)

Color coded by: Sample Group

	01	02	03	04	05	06	07	08	09	10	11	12
A	✓	✓	✓	○	○	✓	✓	✓	✓	✓	✓	✓
B	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C	✓	✓	✓	○	○	○	○	○	○	○	○	○
D	○	○	○	○	○	○	○	○	○	○	○	○
E	○	○	○	○	○	○	○	○	○	○	○	○
F	○	○	○	○	○	○	○	○	○	○	○	○
G	✓	○	○	○	○	○	○	○	○	○	○	○

Worksheet - (G01) unstained

All events

AF-Root: 66.39%

AF-Root

CD19_PE_A

CD123_SB436_A

AF-Root

IgD_BV480_A

CD8_BUV395_A

AF-Root

CD14_AF488_A

CCR7_BV421_A

Unmixing: Off

Autofluorescence Unmixing

SONY

Worksheet Tools [administrator] - ID7000 Software

File Analysis Unmixing Cytometer unstained

Open in Acquisition Send to Public Delete Layout Editor Export Layout to CSV File Import Layout from CSV File Delete Copy Paste Move to Sample Group Remove from Sample Group Export Instrument Settings Instrument Settings Stopping Condition Batch Unmixing Batch Analysis Export to FCS File Analysis: Full events Display Events

Experiment Explorer Experiment List From: To: Keyword: Search

Public administrator 96 Well Plate (standard) Single Positive Controls (28/28) PBMCs panel 1 (1/1) (Unassigned) (67) 384 Well Plate (standard) - 1 (Unassigned) (384) 24 Tube Rack (5mL) - 1 (Unassigned) (24) without 561

96 Well Plate (standard) Color coded by: Sample Group

	01	02	03	04	05	06	07	08	09	10	11	12
A	✓	✓	✓	○	○	✓	✓	✓	✓	✓	✓	✓
B	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C	✓	✓	✓	○	○	○	○	○	○	○	○	○
D	○	○	○	○	○	○	○	○	○	○	○	○
E	○	○	○	○	○	○	○	○	○	○	○	○
F	○	○	○	○	○	○	○	○	○	○	○	○
G	✓	○	○	○	○	○	○	○	○	○	○	○

Worksheet - (G01) unstained

All events WLSM AF-Root WLSM AF-Root WLSM AF-Root WLSM AF-Root

SSC_A x1,000 AF-Root: 66.39% FSC_A x1,000

CD19_PE_A CD123_SB436_A

lgD_BV480_A CD8_BUV395_A

CD14_AF488_A CCR7_BV421_A

Autofluorescence

Administrator - ID7000 Software

Unmixing Settings

Color Panel Matrix

Fluorochrome Add

Universal Negative --- Ex. Laser: ALL

Index	Marker	Fluorochrome	Ex. Laser	Mode	SR	Negative	Positive
1	CD8	BUV395	355nm	Normal(Adv.)	●	○ ---	●
2	CD11c	BUV661	355nm	Normal(Adv.)	●	○ ---	●
3	CD4	BUV737	355nm	Normal(Adv.)	●	○ ---	●
4	CCR7	BV421	405nm	Normal(Adv.)	●	○ ---	●
5	CD123	SB436	405nm	Normal(Adv.)	●	○ ---	●
6	IgD	BV480	405nm	Normal(Adv.)	●	○ ---	●
7	CD4	SparkViolet-538	405nm	Normal(Adv.)	●	○ ---	●
8	CD27	BV650	405nm	Normal(Adv.)	●	○ ---	●
9	CD45RO	BV711	405nm	Normal(Adv.)	●	○ ---	●
10	CD45RA	BV785	405nm	Normal(Adv.)	●	○ ---	●
11	CD14	AF488	488nm	Normal(Adv.)	●	○ ---	●
12	CD19	PE	488nm	Normal(Adv.)	●	○ ---	●
13	CXCR2	PE-Dazzle594	488nm	Normal(Adv.)	●	○ ---	●
14	CD45	PerCP	488nm	Normal(Adv.)	●	○ ---	●
15	CD25	PE-Cy5.5	488nm	Normal(Adv.)	●	○ ---	●
16	TCRgd	PerCP-eFluor710	488nm	Normal(Adv.)	●	○ ---	●
17	CD16	PE-Cy7	488nm	Normal(Adv.)	●	○ ---	●
18	CD39	PE-Fire810	488nm	Normal(Adv.)	●	○ ---	●
19	CD20	[SparkYG-593]	561nm	Normal(Adv.)	●	○ ---	●
20	CD127	PE-Fire700	561nm	Normal(Adv.)	●	○ ---	●
21	CCR6	AF647	637nm	Normal(Adv.)	●	○ ---	●
22	CD56	APC	637nm	Normal(Adv.)	●	○ ---	●

Color Panel Settings Fluorochrome Settings

Autofluorescence Add

Index	Use as AF	Autofluorescence	Ex. Laser	Mode	SR	Negative	Positive
1	<input checked="" type="checkbox"/>	[AF color 1]	405nm	Normal(Adv.)	●	● Zero Reference	● AF-A (Well - A01)
2	<input checked="" type="checkbox"/>	[AF color 2]	405nm	Normal(Adv.)	●	● Zero Reference	● AF-B (Well - A01)
3	<input checked="" type="checkbox"/>	[AF color 3]	405nm	Normal(Adv.)	●	● Zero Reference	● AF-C (Well - A01)

Autofluorescence

Calculate Apply

Spectral Reference

Unmixing: On

Administrator - ID7000 Software

Unmixing Settings

Color Panel Matrix

Fluorochrome Add

Universal Negative --- Ex. Laser: ALL

Index	Marker	Fluorochrome	Ex. Laser	Mode	SR	Negative	Positive
1	CD8	BUV395	355nm	Normal(Adv.)	●	○ ---	●
2	CD11c	BUV661	355nm	Normal(Adv.)	●	○ ---	●
3	CD4	BUV737	355nm	Normal(Adv.)	●	○ ---	●
4	CCR7	BV421	405nm	Normal(Adv.)	●	○ ---	●
5	CD123	SB436	405nm	Normal(Adv.)	●	○ ---	●
6	IgD	BV480	405nm	Normal(Adv.)	●	○ ---	●
7	CD4	SparkViolet-538	405nm	Normal(Adv.)	●	○ ---	●
8	CD27	BV650	405nm	Normal(Adv.)	●	○ ---	●
9	CD45RO	BV711	405nm	Normal(Adv.)	●	○ ---	●
10	CD45RA	BV785	405nm	Normal(Adv.)	●	○ ---	●
11	CD14	AF488	488nm	Normal(Adv.)	●	○ ---	●
12	CD19	PE	488nm	Normal(Adv.)	●	○ ---	●
13	CXCR2	PE-Dazzle594	488nm	Normal(Adv.)	●	○ ---	●
14	CD45	PerCP	488nm	Normal(Adv.)	●	○ ---	●
15	CD25	PE-Cy5.5	488nm	Normal(Adv.)	●	○ ---	●
16	TCRgd	PerCP-eFluor710	488nm	Normal(Adv.)	●	○ ---	●
17	CD16	PE-Cy7	488nm	Normal(Adv.)	●	○ ---	●
18	CD39	PE-Fire810	488nm	Normal(Adv.)	●	○ ---	●
19	CD20	[SparkYG-593]	561nm	Normal(Adv.)	●	○ ---	●
20	CD127	PE-Fire700	561nm	Normal(Adv.)	●	○ ---	●
21	CCR6	AF647	637nm	Normal(Adv.)	●	○ ---	●
22	CD56	APC	637nm	Normal(Adv.)	●	○ ---	●

Color Panel Settings Fluorochrome Settings

Autofluorescence

Index	Use as AF	Autofluorescence	Ex. Laser	Mode	SR	Negative	Positive
1	<input type="checkbox"/>	[AF color 1]	405nm	Normal(Adv.)	●	● Zero Reference	● AF-A (Well - A01)
2	<input type="checkbox"/>	[AF color 2]	405nm	Normal(Adv.)	●	● Zero Reference	● AF-B (Well - A01)
3	<input type="checkbox"/>	[AF color 3]	405nm	Normal(Adv.)	●	● Zero Reference	● AF-C (Well - A01)

Autofluorescence

Calculate Apply

Spectral Reference

Unmixing: On

Administrator - ID7000 Software

Unmixing Settings

Color Panel Matrix

Fluorochrome Add

Universal Negative --- Ex. Laser: ALL

Index	Marker	Fluorochrome	Ex. Laser	Mode	SR	Negative	Positive
1	CD8	BUV395	355nm	Normal(Adv.)	●	○ ---	●
2	CD11c	BUV661	355nm	Normal(Adv.)	●	○ ---	●
3	CD4	BUV737	355nm	Normal(Adv.)	●	○ ---	●
4	CCR7	BV421	405nm	Normal(Adv.)	●	○ ---	●
5	CD123	SB436	405nm	Normal(Adv.)	●	○ ---	●
6	IgD	BV480	405nm	Normal(Adv.)	●	○ ---	●
7	CD4	SparkViolet-538	405nm	Normal(Adv.)	●	○ ---	●
8	CD27	BV650	405nm	Normal(Adv.)	●	○ ---	●
9	CD45RO	BV711	405nm	Normal(Adv.)	●	○ ---	●
10	CD45RA	BV785	405nm	Normal(Adv.)	●	○ ---	●
11	CD14	AF488	488nm	Normal(Adv.)	●	○ ---	●
12	CD19	PE	488nm	Normal(Adv.)	●	○ ---	●
13	CXCR2	PE-Dazzle594	488nm	Normal(Adv.)	●	○ ---	●
14	CD45	PerCP	488nm	Normal(Adv.)	●	○ ---	●
15	CD25	PE-Cy5.5	488nm	Normal(Adv.)	●	○ ---	●
16	TCRgd	PerCP-eFluor710	488nm	Normal(Adv.)	●	○ ---	●
17	CD16	PE-Cy7	488nm	Normal(Adv.)	●	○ ---	●
18	CD39	PE-Fire810	488nm	Normal(Adv.)	●	○ ---	●
19	CD20	[SparkYG-593]	561nm	Normal(Adv.)	●	○ ---	●
20	CD127	PE-Fire700	561nm	Normal(Adv.)	●	○ ---	●
21	CCR6	AF647	637nm	Normal(Adv.)	●	○ ---	●
22	CD56	APC	637nm	Normal(Adv.)	●	○ ---	●

Spectral Reference

355nm

405nm

488nm

561nm

637nm

Color Panel Settings Fluorochrome Settings

Autofluorescence Add

Index	Use as AF	Autofluorescence	Ex. Laser	Mode	SR	Negative	Positive
1	<input checked="" type="checkbox"/>	[AF color 1]	405nm	Normal(Adv.)	●	● Zero Reference	● AF-A (Well - A01)
2	<input type="checkbox"/>	[AF color 2]	405nm	Normal(Adv.)	●	● Zero Reference	● AF-B (Well - A01)
3	<input type="checkbox"/>	[AF color 3]	405nm	Normal(Adv.)	●	● Zero Reference	● AF-C (Well - A01)

Autofluorescence

Calculate Apply

Unmixing: On

AF Root WLSM

CD123_SB436_A

CD8_BUV395_A

CCR7_BV421_A

Administrator - ID7000 Software

Worksheet Tools Plot Tools

ID Unmixing Settings

Color Panel Matrix

Fluorochrome Add

Universal Negative --- Ex. Laser: ALL

Index	Marker	Fluorochrome	Ex. Laser	Mode	SR	Negative	Positive
1	CD8	BUV395	355nm	Normal(Adv.)	●	○ ---	●
2	CD11c	BUV661	355nm	Normal(Adv.)	●	○ ---	●
3	CD4	BUV737	355nm	Normal(Adv.)	●	○ ---	●
4	CCR7	BV421	405nm	Normal(Adv.)	●	○ ---	●
5	CD123	SB436	405nm	Normal(Adv.)	●	○ ---	●
6	IgD	BV480	405nm	Normal(Adv.)	●	○ ---	●
7	CD4	SparkViolet-538	405nm	Normal(Adv.)	●	○ ---	●
8	CD27	BV650	405nm	Normal(Adv.)	●	○ ---	●
9	CD45RO	BV711	405nm	Normal(Adv.)	●	○ ---	●
10	CD45RA	BV785	405nm	Normal(Adv.)	●	○ ---	●
11	CD14	AF488	488nm	Normal(Adv.)	●	○ ---	●
12	CD19	PE	488nm	Normal(Adv.)	●	○ ---	●
13	CXCR2	PE-Dazzle594	488nm	Normal(Adv.)	●	○ ---	●
14	CD45	PerCP	488nm	Normal(Adv.)	●	○ ---	●
15	CD25	PE-Cy5.5	488nm	Normal(Adv.)	●	○ ---	●
16	TCRgd	PerCP-eFluor710	488nm	Normal(Adv.)	●	○ ---	●
17	CD16	PE-Cy7	488nm	Normal(Adv.)	●	○ ---	●
18	CD39	PE-Fire810	488nm	Normal(Adv.)	●	○ ---	●
19	CD20	[SparkYG-593]	561nm	Normal(Adv.)	●	○ ---	●
20	CD127	PE-Fire700	561nm	Normal(Adv.)	●	○ ---	●
21	CCR6	AF647	637nm	Normal(Adv.)	●	○ ---	●
22	CD56	APC	637nm	Normal(Adv.)	●	○ ---	●

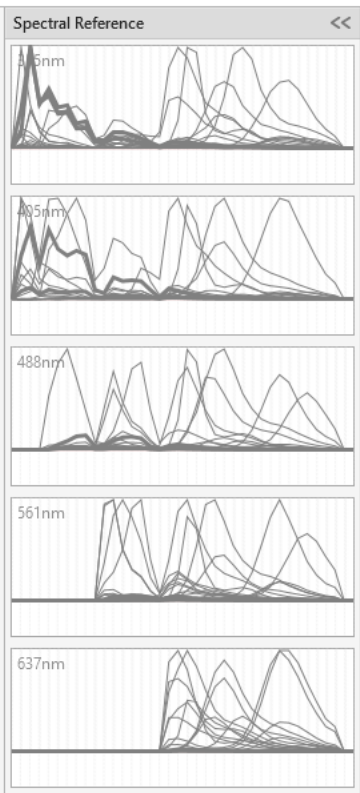
Color Panel Settings Fluorochrome Settings

Autofluorescence

Add

Index	Use as AF	Autofluorescence	Ex. Laser	Mode	SR	Negative	Positive
1	<input checked="" type="checkbox"/>	[AF color 1]	405nm	Normal(Adv.)	●	● Zero Reference	● AF-A (Well - A01)
2	<input checked="" type="checkbox"/>	[AF color 2]	405nm	Normal(Adv.)	●	● Zero Reference	● AF-B (Well - A01)
3	<input type="checkbox"/>	[AF color 3]	405nm	Normal(Adv.)	●	● Zero Reference	● AF-C (Well - A01)

Autofluorescence



Unmixing: On

Calculate Apply

Autofluorescence

Administrator - ID7000 Software

Unmixing Settings

Color Panel Matrix

Fluorochrome Add

Universal Negative --- Ex. Laser: ALL

Index	Marker	Fluorochrome	Ex. Laser	Mode	SR	Negative	Positive
1	CD8	BUV395	355nm	Normal(Adv.)	●	○ ---	●
2	CD11c	BUV661	355nm	Normal(Adv.)	●	○ ---	●
3	CD4	BUV737	355nm	Normal(Adv.)	●	○ ---	●
4	CCR7	BV421	405nm	Normal(Adv.)	●	○ ---	●
5	CD123	SB436	405nm	Normal(Adv.)	●	○ ---	●
6	IgD	BV480	405nm	Normal(Adv.)	●	○ ---	●
7	CD4	SparkViolet-538	405nm	Normal(Adv.)	●	○ ---	●
8	CD27	BV650	405nm	Normal(Adv.)	●	○ ---	●
9	CD45RO	BV711	405nm	Normal(Adv.)	●	○ ---	●
10	CD45RA	BV785	405nm	Normal(Adv.)	●	○ ---	●
11	CD14	AF488	488nm	Normal(Adv.)	●	○ ---	●
12	CD19	PE	488nm	Normal(Adv.)	●	○ ---	●
13	CXCR2	PE-Dazzle594	488nm	Normal(Adv.)	●	○ ---	●
14	CD45	PerCP	488nm	Normal(Adv.)	●	○ ---	●
15	CD25	PE-Cy5.5	488nm	Normal(Adv.)	●	○ ---	●
16	TCRgd	PerCP-eFluor710	488nm	Normal(Adv.)	●	○ ---	●
17	CD16	PE-Cy7	488nm	Normal(Adv.)	●	○ ---	●
18	CD39	PE-Fire810	488nm	Normal(Adv.)	●	○ ---	●
19	CD20	[SparkYG-593]	561nm	Normal(Adv.)	●	○ ---	●
20	CD127	PE-Fire700	561nm	Normal(Adv.)	●	○ ---	●
21	CCR6	AF647	637nm	Normal(Adv.)	●	○ ---	●
22	CD56	APC	637nm	Normal(Adv.)	●	○ ---	●

Color Panel Settings Fluorochrome Settings

Autofluorescence Add

Index	Use as AF	Autofluorescence	Ex. Laser	Mode	SR	Negative	Positive
1	<input checked="" type="checkbox"/>	[AF color 1]	405nm	Normal(Adv.)	●	● Zero Reference	● AF-A (Well - A01)
2	<input checked="" type="checkbox"/>	[AF color 2]	405nm	Normal(Adv.)	●	● Zero Reference	● AF-B (Well - A01)
3	<input checked="" type="checkbox"/>	[AF color 3]	405nm	Normal(Adv.)	●	● Zero Reference	● AF-C (Well - A01)

Autofluorescence

Calculate Apply

Spectral Reference

Unmixing: On

Autofluorescence

Administrator - ID7000 Software

Unmixing Settings

Color Panel Matrix

Fluorochrome Add

Universal Negative --- Ex. Laser: ALL

Index	Marker	Fluorochrome	Ex. Laser	Mode	SR	Negative	Positive
1	CD8	BUV395	355nm	Normal(Adv.)	●	○ ---	●
2	CD11c	BUV661	355nm	Normal(Adv.)	●	○ ---	●
3	CD4	BUV737	355nm	Normal(Adv.)	●	○ ---	●
4	CCR7	BV421	405nm	Normal(Adv.)	●	○ ---	●
5	CD123	SB436	405nm	Normal(Adv.)	●	○ ---	●
6	IgD	BV480	405nm	Normal(Adv.)	●	○ ---	●
7	CD4	SparkViolet-538	405nm	Normal(Adv.)	●	○ ---	●
8	CD27	BV650	405nm	Normal(Adv.)	●	○ ---	●
9	CD45RO	BV711	405nm	Normal(Adv.)	●	○ ---	●
10	CD45RA	BV785	405nm	Normal(Adv.)	●	○ ---	●
11	CD14	AF488	488nm	Normal(Adv.)	●	○ ---	●
12	CD19	PE	488nm	Normal(Adv.)	●	○ ---	●
13	CXCR2	PE-Dazzle594	488nm	Normal(Adv.)	●	○ ---	●
14	CD45	PerCP	488nm	Normal(Adv.)	●	○ ---	●
15	CD25	PE-Cy5.5	488nm	Normal(Adv.)	●	○ ---	●
16	TCRgd	PerCP-eFluor710	488nm	Normal(Adv.)	●	○ ---	●
17	CD16	PE-Cy7	488nm	Normal(Adv.)	●	○ ---	●
18	CD39	PE-Fire810	488nm	Normal(Adv.)	●	○ ---	●
19	CD20	[SparkYG-593]	561nm	Normal(Adv.)	●	○ ---	●
20	CD127	PE-Fire700	561nm	Normal(Adv.)	●	○ ---	●
21	CCR6	AF647	637nm	Normal(Adv.)	●	○ ---	●
22	CD56	APC	637nm	Normal(Adv.)	●	○ ---	●

Color Panel Settings Fluorochrome Settings

Autofluorescence Add

Index	Use as AF	Autofluorescence	Ex. Laser	Mode	SR	Negative	Positive
1	<input checked="" type="checkbox"/>	[AF color 1]	405nm	Normal(Adv.)	●	● Zero Reference	● AF-A (Well - A01)
2	<input checked="" type="checkbox"/>	[AF color 2]	405nm	Normal(Adv.)	●	● Zero Reference	● AF-B (Well - A01)
3	<input checked="" type="checkbox"/>	[AF color 3]	405nm	Normal(Adv.)	●	● Zero Reference	● AF-C (Well - A01)

Autofluorescence

Open Positive Sample
Clear Gates
Clear Spectral Reference
Edit Virtual Filter
Remove Fluorochrome

Calculate Apply

Unmixing: On

CD123_SB436_A
CD8_BUV395_A
CCR7_BV421_A

Autofluorescence

Administrator - ID7000 Software

Unmixing Settings

Color Panel Matrix

Fluorochrome Add

Universal Negative --- Ex. Laser: ALL

Index	Marker	Fluorochrome	Ex. Laser	Mode	SR	Negative	Positive
1	CD8	BUV395	355nm	Normal(Adv.)	●	○ ---	●
2	CD11c	BUV661	355nm	Normal(Adv.)	●	○ ---	●
3	CD4	BUV737	355nm	Normal(Adv.)	●	○ ---	●
4	CCR7	BV421	405nm	Normal(Adv.)	●	○ ---	●
5	CD123	SB436	405nm	Normal(Adv.)	●	○ ---	●
6	IgD	BV480	405nm	Normal(Adv.)	●	○ ---	●
7	CD4	SparkViolet-538	405nm	Normal(Adv.)	●	○ ---	●
8	CD27	BV650	405nm	Normal(Adv.)	●	○ ---	●
9	CD45RO	BV711	405nm	Normal(Adv.)	●	○ ---	●
10	CD45RA	BV785	405nm	Normal(Adv.)	●	○ ---	●
11	CD14	AF488	488nm	Normal(Adv.)	●	○ ---	●
12	CD19	PE	488nm	Normal(Adv.)	●	○ ---	●
13	CXCR2	PE-Dazzle594	488nm	Normal(Adv.)	●	○ ---	●
14	CD45	PerCP	488nm	Normal(Adv.)	●	○ ---	●
15	CD25	PE-Cy5.5	488nm	Normal(Adv.)	●	○ ---	●
16	TCRgd	PerCP-eFluor710	488nm	Normal(Adv.)	●	○ ---	●
17	CD16	PE-Cy7	488nm	Normal(Adv.)	●	○ ---	●
18	CD39	PE-Fire810	488nm	Normal(Adv.)	●	○ ---	●
19	CD20	[SparkYG-593]	561nm	Normal(Adv.)	●	○ ---	●
20	CD127	PE-Fire700	561nm	Normal(Adv.)	●	○ ---	●
21	CCR6	AF647	637nm	Normal(Adv.)	●	○ ---	●
22	CD56	APC	637nm	Normal(Adv.)	●	○ ---	●

Color Panel Settings Fluorochrome Settings

Autofluorescence Add

Index	Use as AF	Autofluorescence	Ex. Laser	Mode	SR	Negative	Positive
1	<input checked="" type="checkbox"/>	[AF color 1]	405nm	Normal(Adv.)	●	● Zero Reference	● AF-A (Well - A01)
2	<input checked="" type="checkbox"/>	[AF color 2]	405nm	Normal(Adv.)	●	● Zero Reference	● AF-B (Well - A01)

Autofluorescence

Spectral Reference

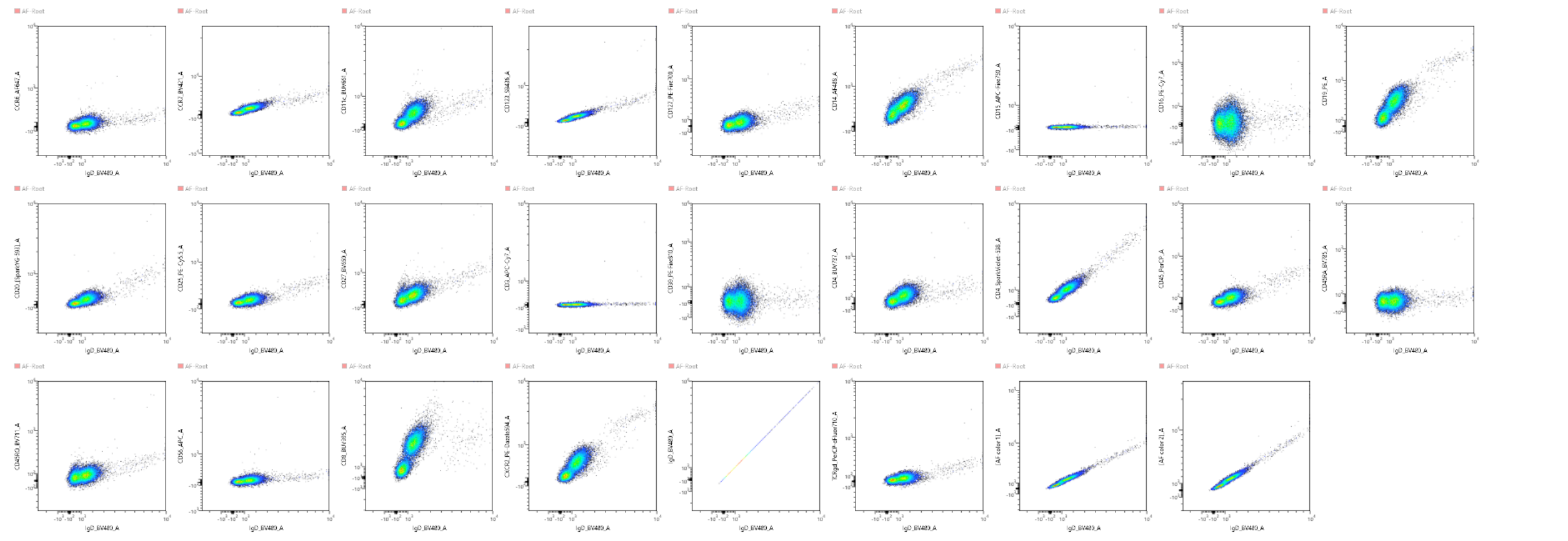
Unmixing: On

Calculate Apply

Autofluorescence

SONY

X Axis: IgD_BV480_A Prev Next Gate: AF-Root Unmixing: Off Matrix: Unmixing Scale: Auto Adjust X Auto Adjust Y Auto Adjust XY Display Events: 50,000 events

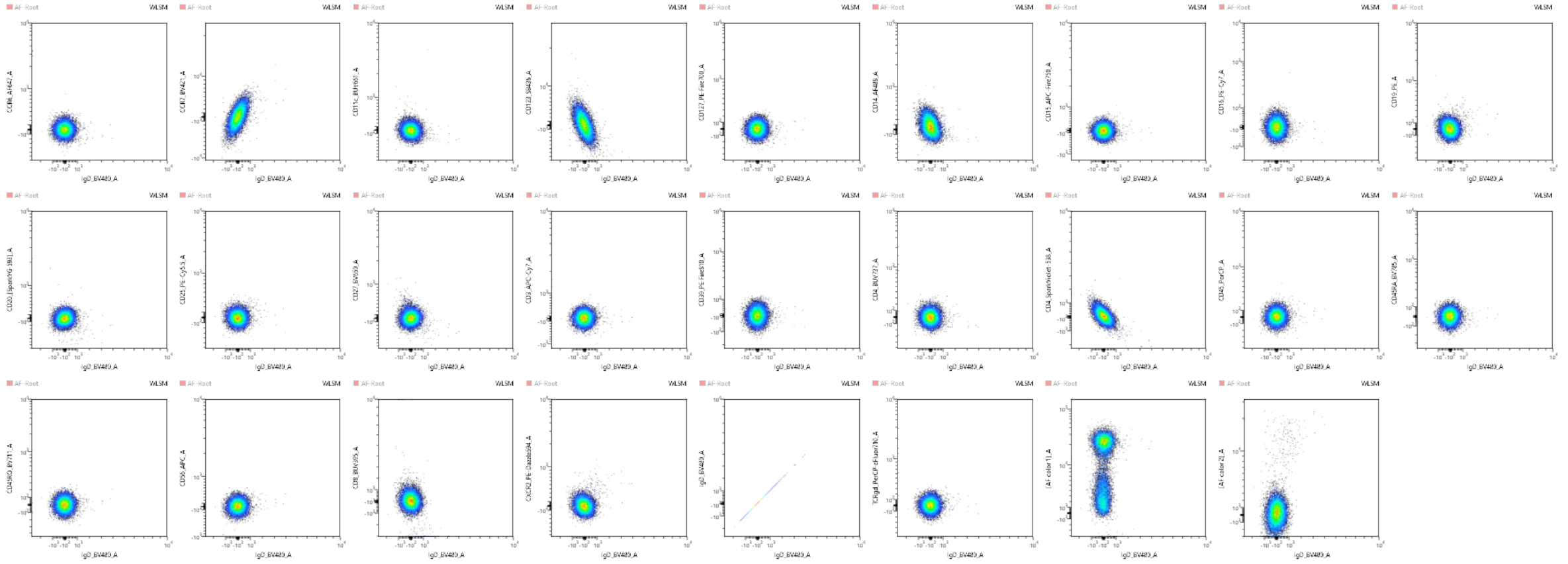


Autofluorescence

SONY

ID Unmixing Viewer

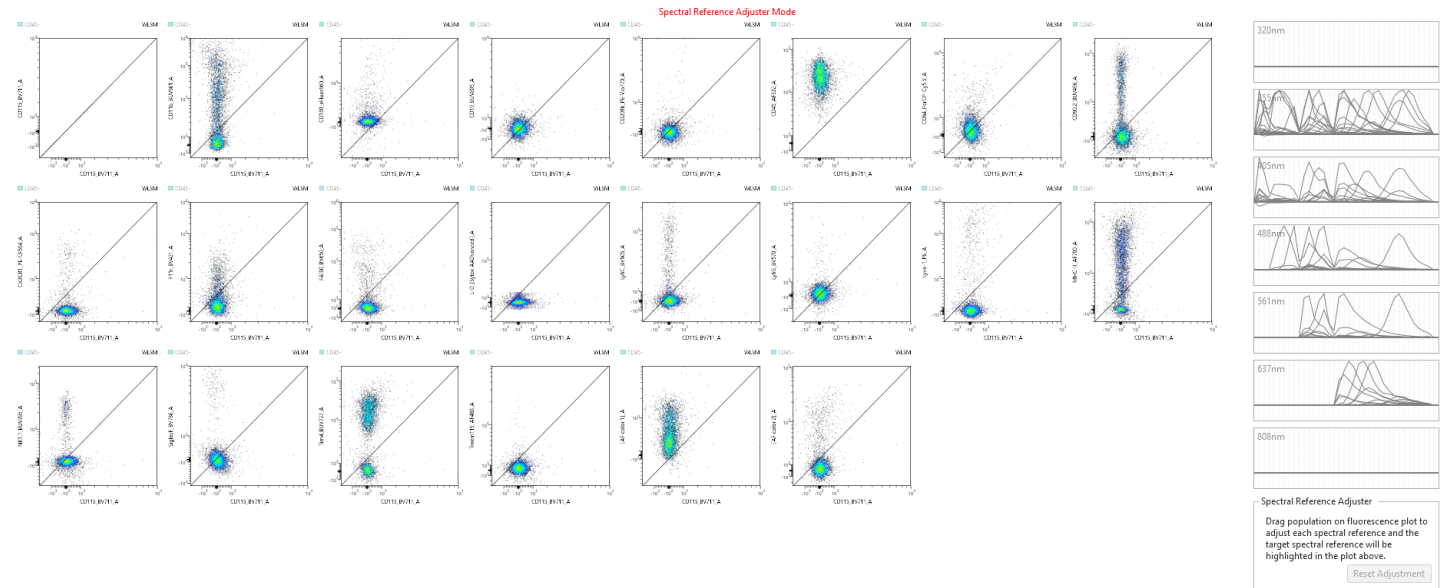
X Axis: IgD_BV480_A Prev Next Gate: AF-Root Unmixing: On Matrix: Unmixing Scale: Auto Adjust X Auto Adjust Y Auto Adjust XY Display Events: 50,000 events



ID7000 Workflow

1. QC
2. Experiment
3. Colour Panel (colour + marker)
or
Reference Spectra
4. Instrument & Acquisition Settings
5. Reference Spectra
6. Autofluorescence
7. Unmixing QC

Unmixing Viewer & Adjuster



3. Spectral Reference Adjuster

PE-Cy7

The screenshot displays the Unmixing Viewer software interface. At the top, the X-axis is set to 'CD45RO_PE-Cy7_A'. The interface includes a 'Spectral Reference Adjuster Mode' section with a 'Matrix' dropdown set to 'Unmixing-Adjusted' and 'Scale' buttons for 'Auto Adjust X', 'Auto Adjust Y', and 'Auto Adjust XY'. The 'Display Events' is set to 50,000. The main area contains a 3x10 grid of flow cytometry plots. The central plot in the second row is highlighted with a blue border and a double-headed arrow, indicating it is the active reference. To the right, the 'Spectral Reference Adjuster' panel shows fluorescence spectra for various wavelengths: 420nm, 455nm, 488nm, 561nm, 637nm, and 808nm. The 561nm spectrum is highlighted in red. Below the spectra, there are instructions: 'Drag population on fluorescence plot to adjust each spectral reference and the target spectral reference will be highlighted in the plot above.' and a 'Reset Adjustment' button. At the bottom right, there is an 'Add to Worksheet' button and a 'Close' button.

3. Spectral Reference Adjuster

PE-Cy7

The screenshot displays the Unmixing Viewer software interface. At the top, the X-axis is set to 'CD45RO_PE-Cy7_A'. The interface includes a 'Gate' dropdown set to 'NOT GRANS', 'Unmixing' and 'Adjuster' toggle buttons, a 'Matrix' dropdown set to 'Unmixing adjusted', and 'Scale' options for 'Auto Adjust X', 'Auto Adjust Y', and 'Auto Adjust XY'. The 'Display Events' is set to 50,000. The main area contains a 3x10 grid of plots. The central plot is highlighted with a blue border and a dropdown menu showing 'Unmixing adjusted', 'Unmixing', and 'Unmixing adjusted'. The right panel, titled 'Spectral Reference Adjuster', shows fluorescence spectra for various wavelengths: 420nm, 455nm, 485nm, 498nm, 561nm, 637nm, and 808nm. A red line in the 498nm and 561nm plots indicates the target spectral reference. Below the spectra are instructions: 'Drag population on fluorescence plot to adjust each spectral reference and the target spectral reference will be highlighted in the plot above.' and 'Reset Adjustment'. At the bottom of the panel is an 'Add to Worksheet' button. A 'Close' button is located at the bottom right of the entire window.



Panel Design

Panel Design

1. Know your fluorochrome

- Similarity/ R^2 value
- Brightness
- Availability

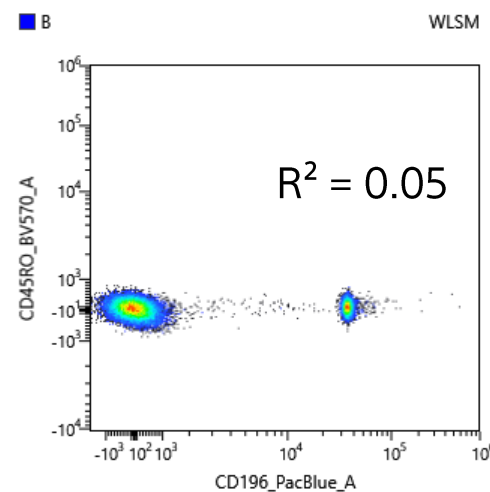
2. Know your sample

- Primary: ON/OFF (lineage)
- Secondary: continuum
- Tertiary: low/unknown
- Gating Strategy

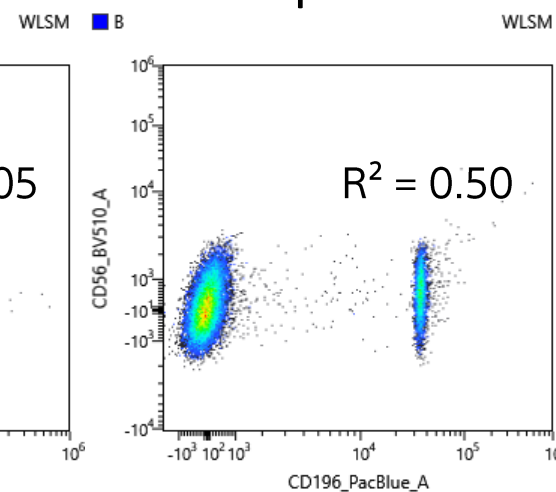
R^2 Value

	BV421	eF450	PacBlue	BV480	BV510	PacOrange	BV570
BV421		0.59	0.41	0.12	0.07	0.03	
eF450	0.59		0.95	0.51	0.34	0.15	
PacBlue	0.41	0.95		0.70	0.49	0.23	
BV480	0.12	0.51	0.70		0.88	0.53	
BV510	0.07	0.34	0.49	0.88		0.82	0.05
PacOrange	0.03	0.15	0.23	0.53	0.82		0.23
BV570					0.05	0.23	

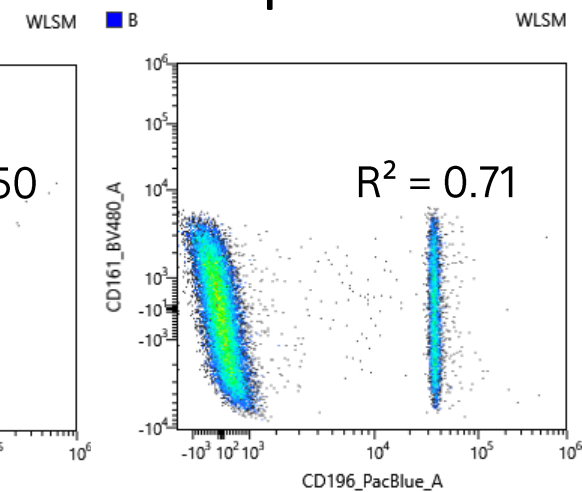
spread



spread



spread



Panel Design

1. Know your fluorochrome

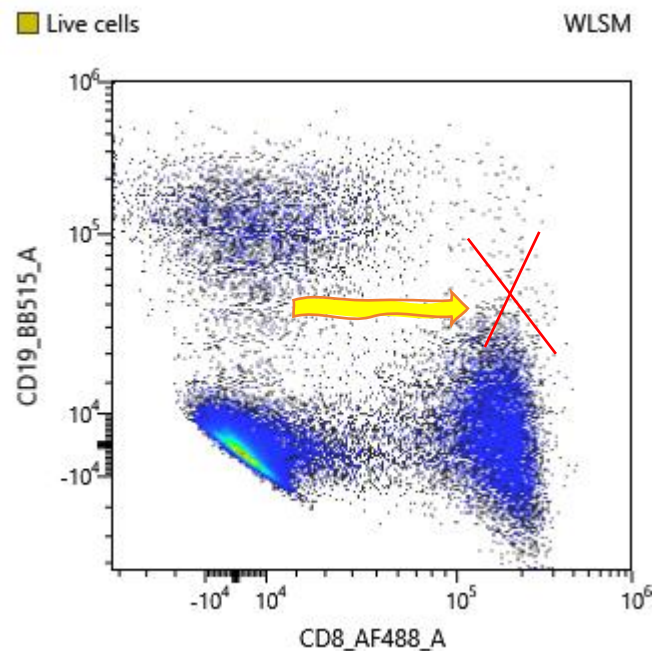
- Similarity/ R^2 value
- Brightness
- Availability

2. Know your sample

- Primary: ON/OFF (lineage)
- Secondary: continuum
- Tertiary: low/unknown
- Gating Strategy

Should I use similar dyes in my panel?

Only with careful panel design!



Panel Design

1. Know your fluorochrome

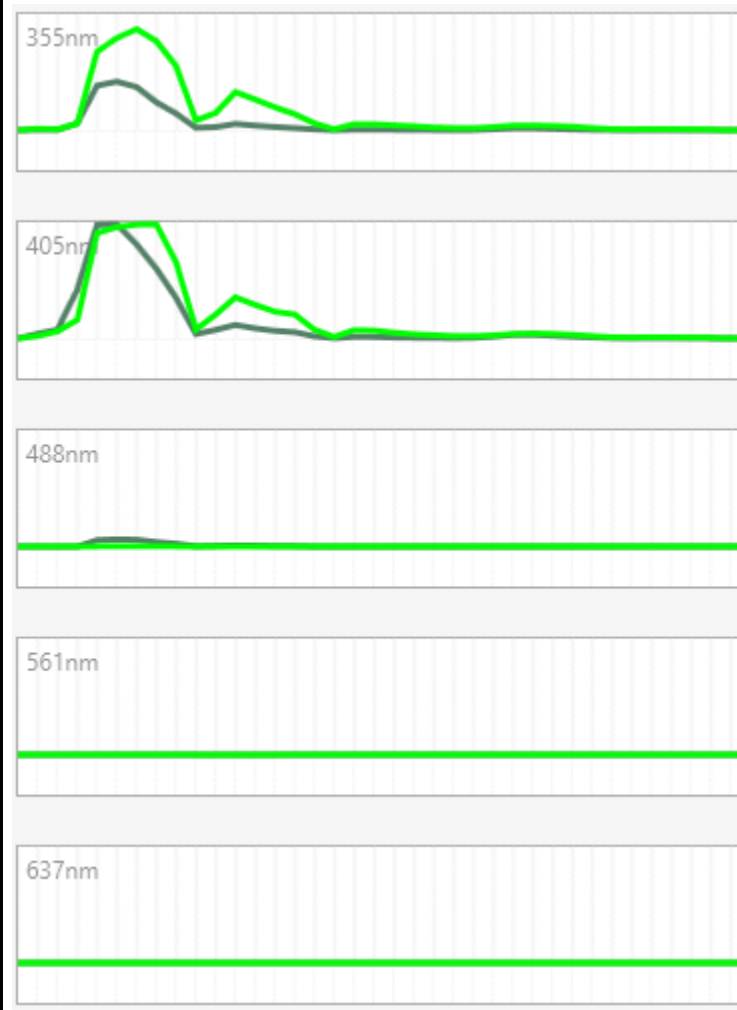
- Similarity/ R^2 value
- Brightness
- Availability

2. Know your sample

- Primary: ON/OFF (lineage)
- Secondary: continuum
- Tertiary: low/unknown
- Gating Strategy

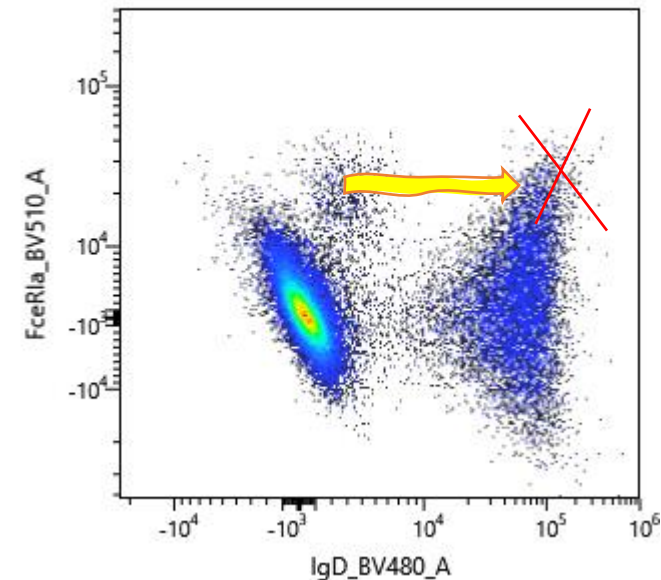
Should I use similar dyes in my panel?

Only with careful panel design!



CD3 NEG

WLSM



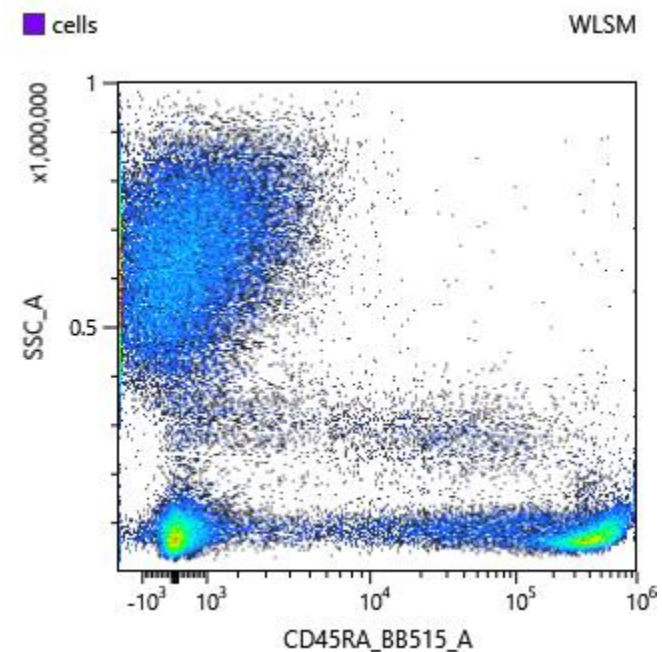
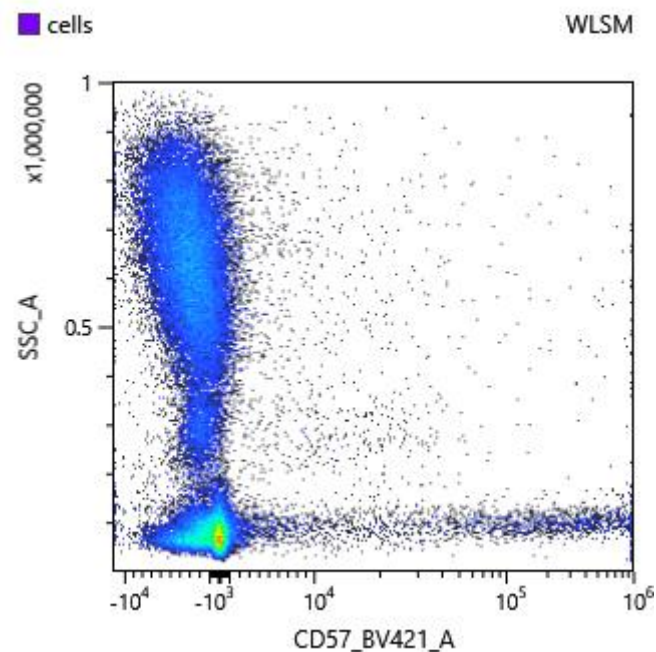
Panel Design

1. Know your fluorochrome

- Similarity/ R^2 value
- Brightness
- Availability

2. Know your sample

- Primary: ON/OFF (lineage)
- Secondary: continuum
- Tertiary: low/unknown
- Gating Strategy



Fluorochrome Brightness Guide

Sorted by the average of stain index rank on cells and beads

Very Bright

Fluorochrome	On Beads	On Cells
BV421	●	●
BYG584	●	●
PE-AF610	●	●
DL650	●	●
BB515	●	●
PE-Cy5	●	●
PE-eFluor610	●	●
DL550	●	●
QD655	●	●
PE-Dazzle594	●	●
PE-CF594	●	●
CF568	●	●
BB700	●	●
AF647	●	●
PE	●	●
AF568	●	●
Cy5	●	●
SB436	●	●
BB660	●	●
eFluorYG584	●	●
DL594	●	●
PE-Cy7	●	●
DL405	●	●
DL633	●	●
NR700	●	●
AF488	●	●

Bright

Fluorochrome	On Beads	On Cells
BUV661	●	●
NY610	●	●
AF514	●	●
APC-R700	●	●
QD605	●	●
DL680	●	●
NY660	●	●
PE-AF700	●	●
AF405	●	●
BUV615	●	●
PE-Fire640	●	●
BB790	●	●
SparkNIR-685	●	●
BV650	●	●
BB630	●	●
BUV395	●	●
NR685	●	●
APC	●	●
PE-Fire810	●	●
NB660	●	●
PerCP-eFluor710	●	●
BV711	●	●
cFluor450	●	●
cFluorR720	●	●
BV480	●	●

Moderate

Fluorochrome	On Beads	On Cells
BB755	●	●
PE-Cy5.5	●	●
NB610	●	●
BUV737	●	●
QD585	●	●
SB702	●	●
BUV563	●	●
NY690	●	●
BV605	●	●
PacBlue	●	●
BV785	●	●
QD565	●	●
NY700	●	●
BV750	●	●
NB660	●	●
SparkBlue-550	●	●
BV786	●	●
NY570	●	●
cFluorB548	●	●
NR660	●	●
APC-Cy7	●	●
AF555	●	●
BUV805	●	●
QD525	●	●
FITC	●	●
QD705	●	●

Dim

Fluorochrome	On Beads	On Cells
VioletFluor450	●	●
BV510	●	●
NB610	●	●
BUV496	●	●
AF700	●	●
Cy3	●	●
NB510	●	●
BV510	●	●
APC-H7	●	●
PerCP-Cy5.5	●	●
APC-eFluor780	●	●
AF532	●	●
AF750	●	●
eFluor506	●	●
BV570	●	●
APC-Fire810	●	●
eFluor455UV	●	●
Cy2	●	●
NB555	●	●
CF430	●	●
NB530	●	●
PacOrange	●	●
DL800	●	●
DL350	●	●
PerCP	●	●
QD800	●	●

Panel Design

1. Know your fluorochrome

- Similarity/ R^2 value
- Brightness
- Availability

2. Know your sample

- Primary: ON/OFF (lineage)
- Secondary: continuum
- Tertiary: low/unknown
- Gating Strategy

Panel Design

1. Know your fluorochrome

- Similarity/ R^2 value
- Brightness
- Availability

2. Know your sample

- Primary: ON/OFF (lineage)
- Secondary: continuum
- Tertiary: low/unknown
- Gating Strategy

	B cell	T cell	Monocyte
CD3	-	+	-
CD19	+	-	-
CD14	-	-	+

- Highly overlapping fluorochromes
- Dim fluorochromes

Panel Design

1. Know your fluorochrome

- Similarity/ R^2 value
- Brightness
- Availability

2. Know your sample

- Primary: ON/OFF (lineage)
- Secondary: continuum
- Tertiary: low/unknown
- Gating Strategy

	B cell	T cell	Monocyte
CD3	-	+	-
CD19	+	-	-
CD14	-	-	+

	B cell	T cell	Monocyte
CD45RA	+	+	-

- Can often be co-expressed
- Dim/med fluorochromes

Panel Design

1. Know your fluorochrome

- Similarity/ R^2 value
- Brightness
- Availability

2. Know your sample

- Primary: ON/OFF (lineage)
- Secondary: continuum
- Tertiary: low/unknown
- Gating Strategy

“Good” fluorochromes:

- Low overlap (e.g. BUV395)
- Bright

Panel Design

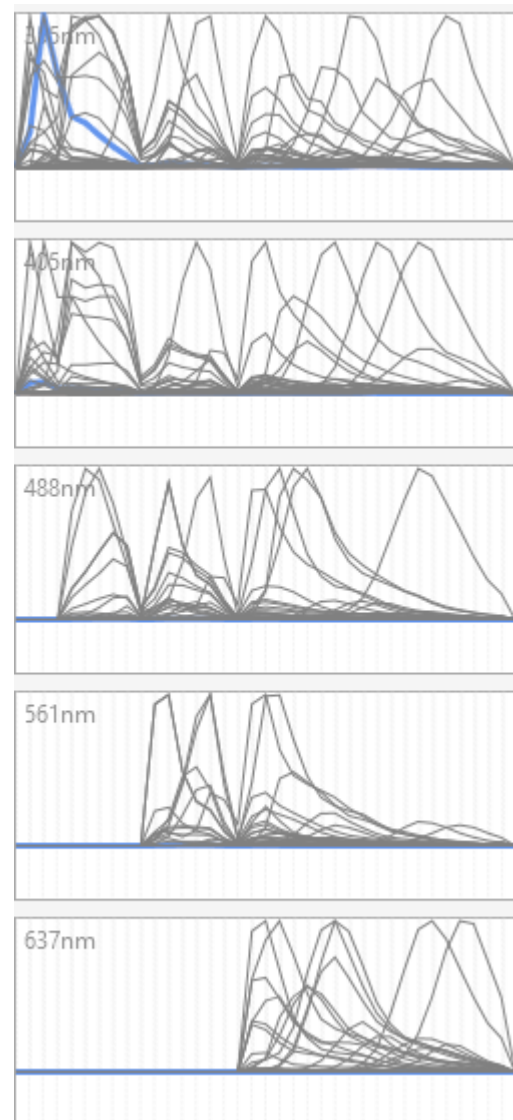
1. Know your fluorochrome

- Similarity/ R^2 value
- Brightness
- Availability

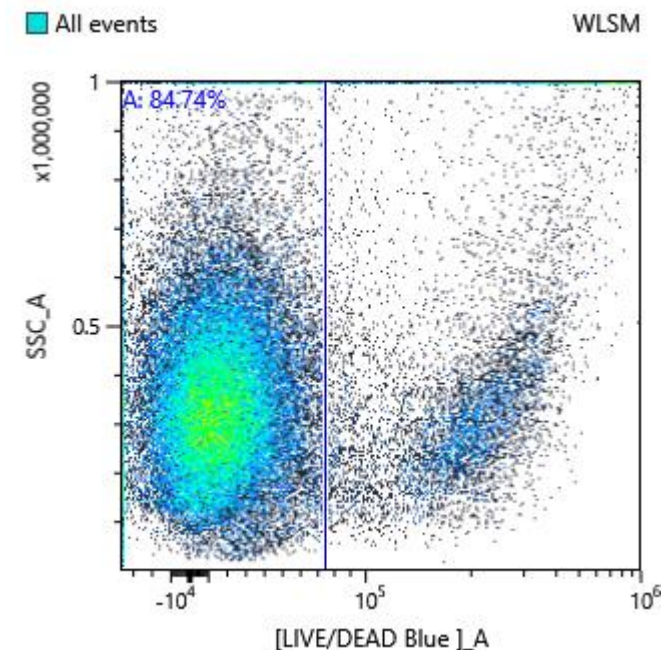
2. Know your sample

- Primary: ON/OFF (lineage)
- Secondary: continuum
- Tertiary: low/unknown
- Gating Strategy?

Live/Dead



Live gate = 1st gate



Panel Design

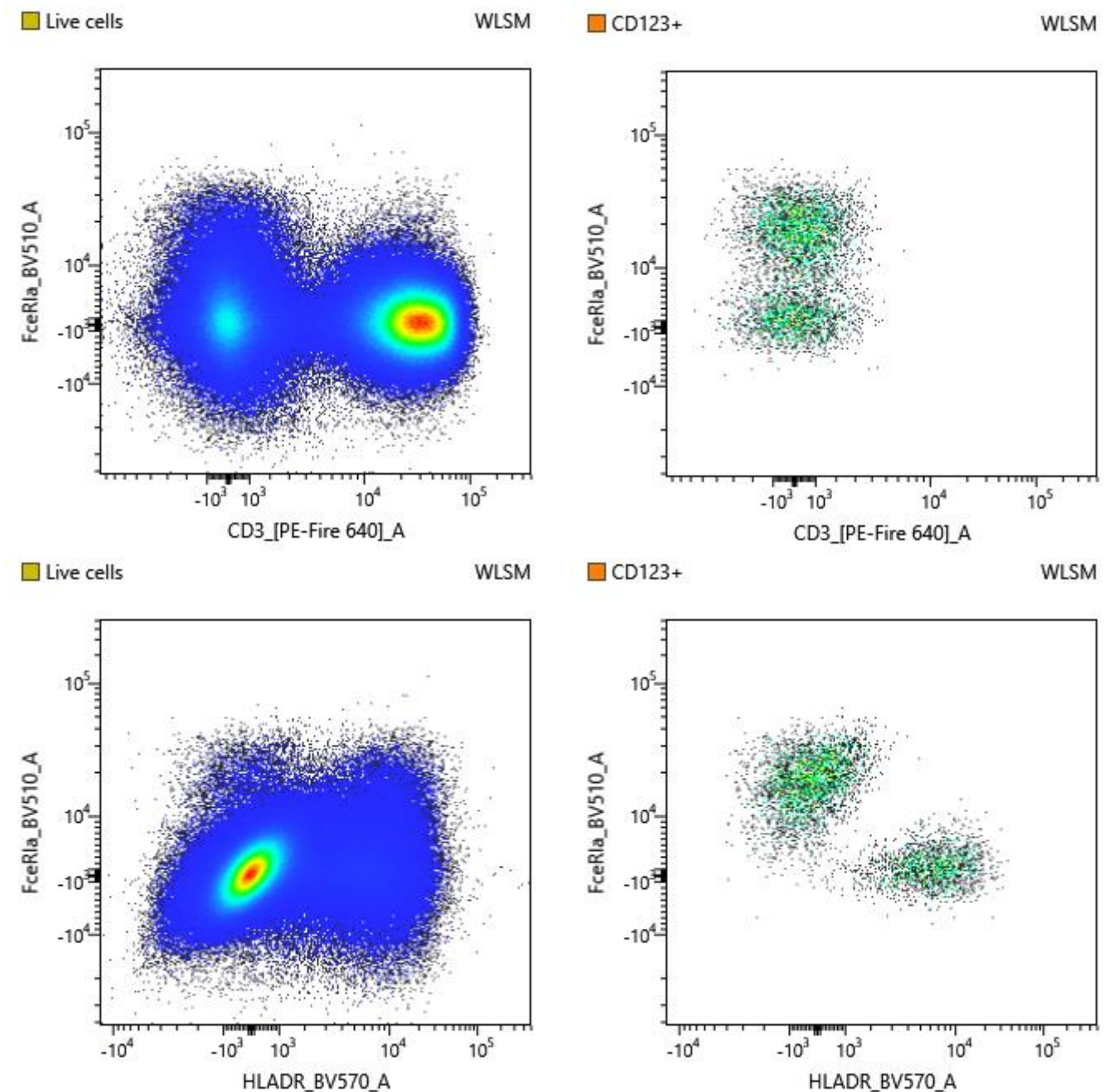
1. Know your fluorochrome

- Similarity/ R^2 value
- Brightness
- Availability

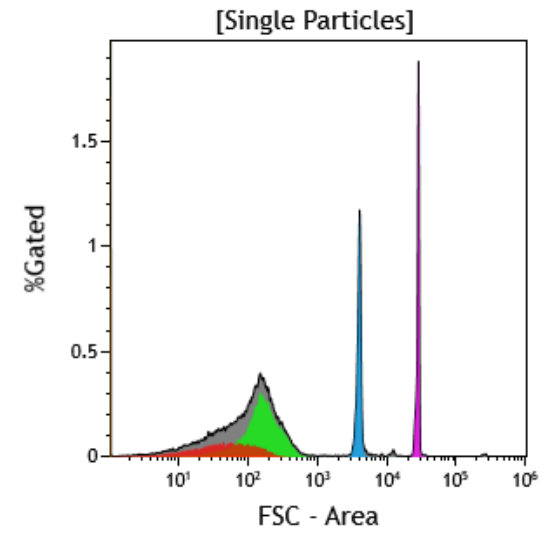
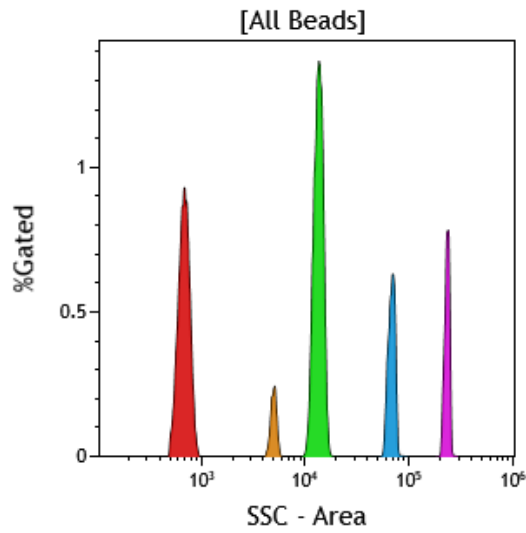
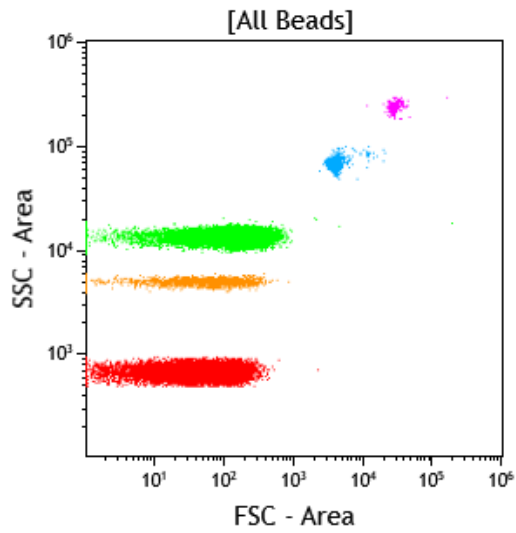
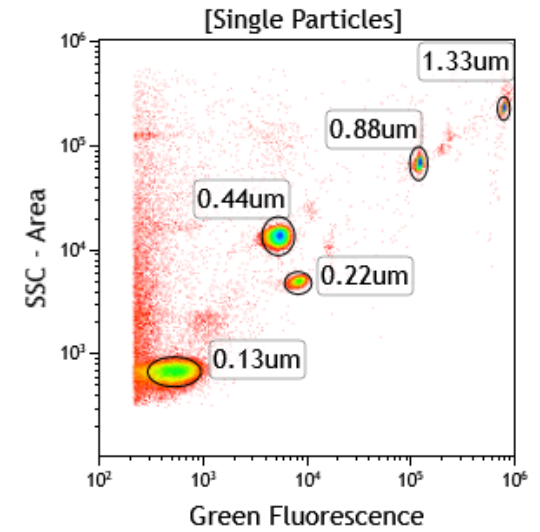
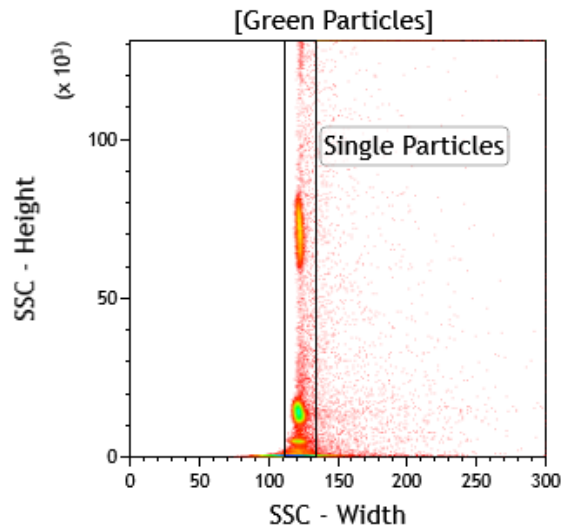
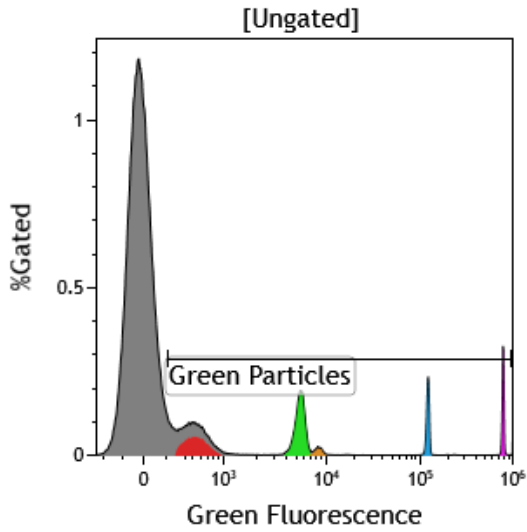
2. Know your sample

- Primary: ON/OFF (lineage)
- Secondary: continuum
- Tertiary: low/unknown
- Gating Strategy?

Lineage gating



SPHERO™ Flow Cytometry Nano Fluorescent Size Standard Kit



Our Team

Nordics Regional Team



Regional Sales Manager
Michelle Jackson



Nordics Sales Account Manager
Sebastian Hedlund



Field Application Scientist
João Monteiro



Field Service Coordinator
Rudolf Bichele



Field Service Engineer
Joris Jansen



Field Service Engineer
Paulo Urbano



Field Service Engineer
Edwin de Haas



Webinar

Fundamentals of Advanced Spectral Cell Analysis Using the New ID7000™ System



Webinar

High-Dimensional High-Throughput Rare Event Immunophenotyping on the ID7000™ Spectral Cell Analyzer



Webinar

Panel Design Considerations for Spectral Flow Cytometry



Webinar

Software Workflows and Tools that Enable High Parameter Flow Cytometry using the ID7000™ Spectral Cell Analyzer



Questions?

