SONY ID7000™ Training

Introduction to Spectral Flow Cytometry

Software & Workflow

Standardization Mode

Panel Design

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Field Application Scientist - Nordics
Sony Biotechnology Europe

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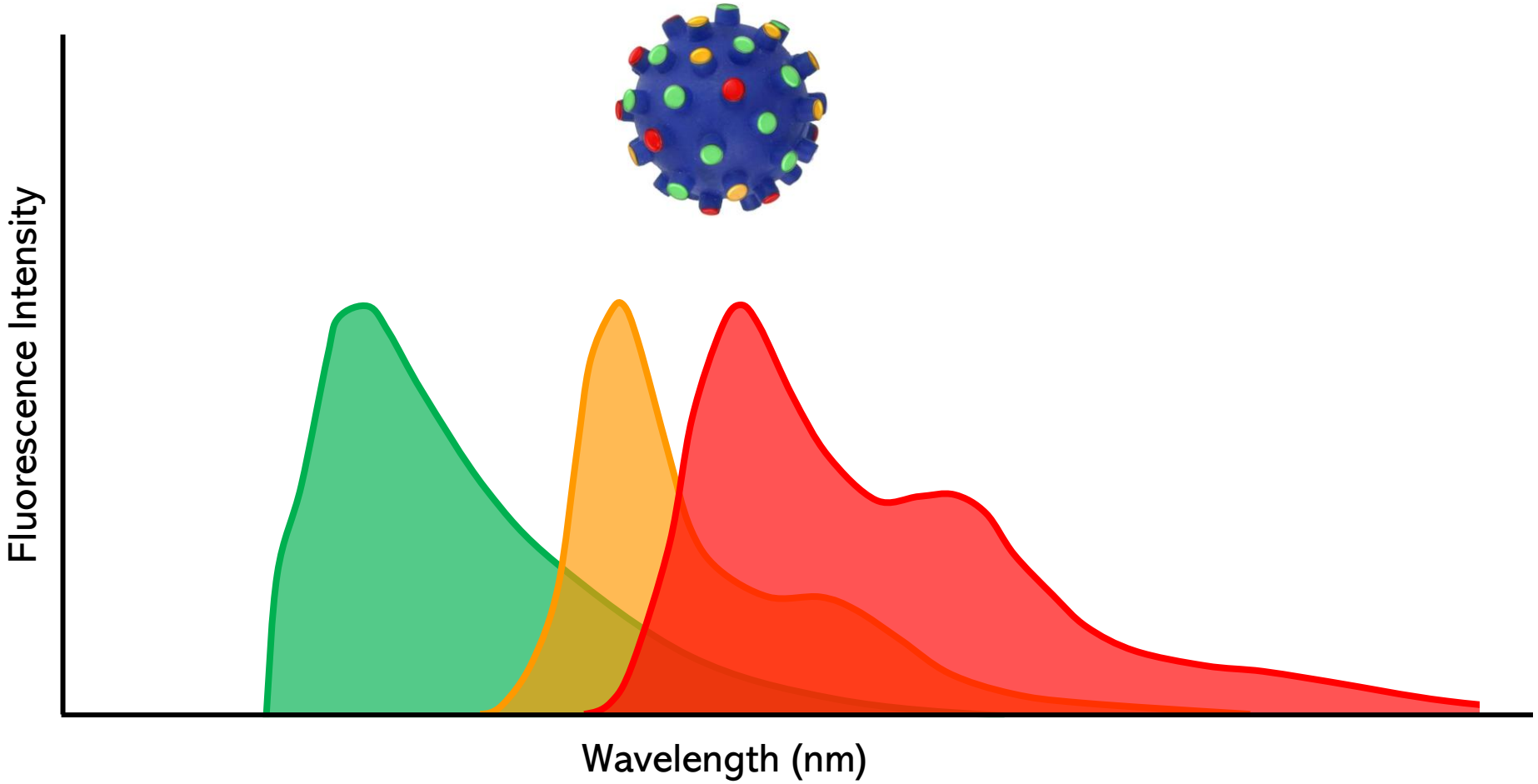
Spectral vs Conventional Flow Cytometry

Consider a cell stained with three fluorochromes.



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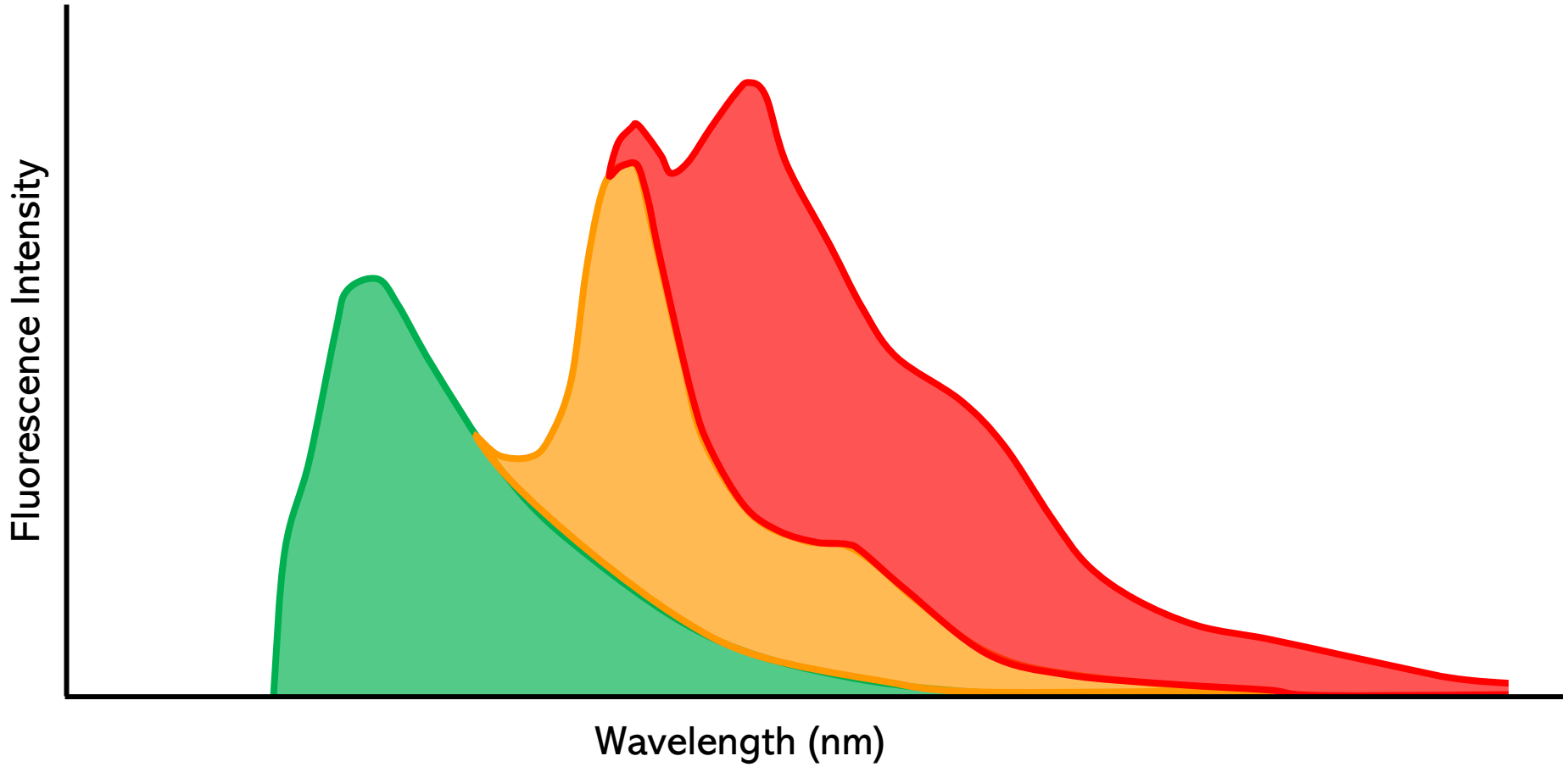
Spectral vs Conventional Flow Cytometry

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



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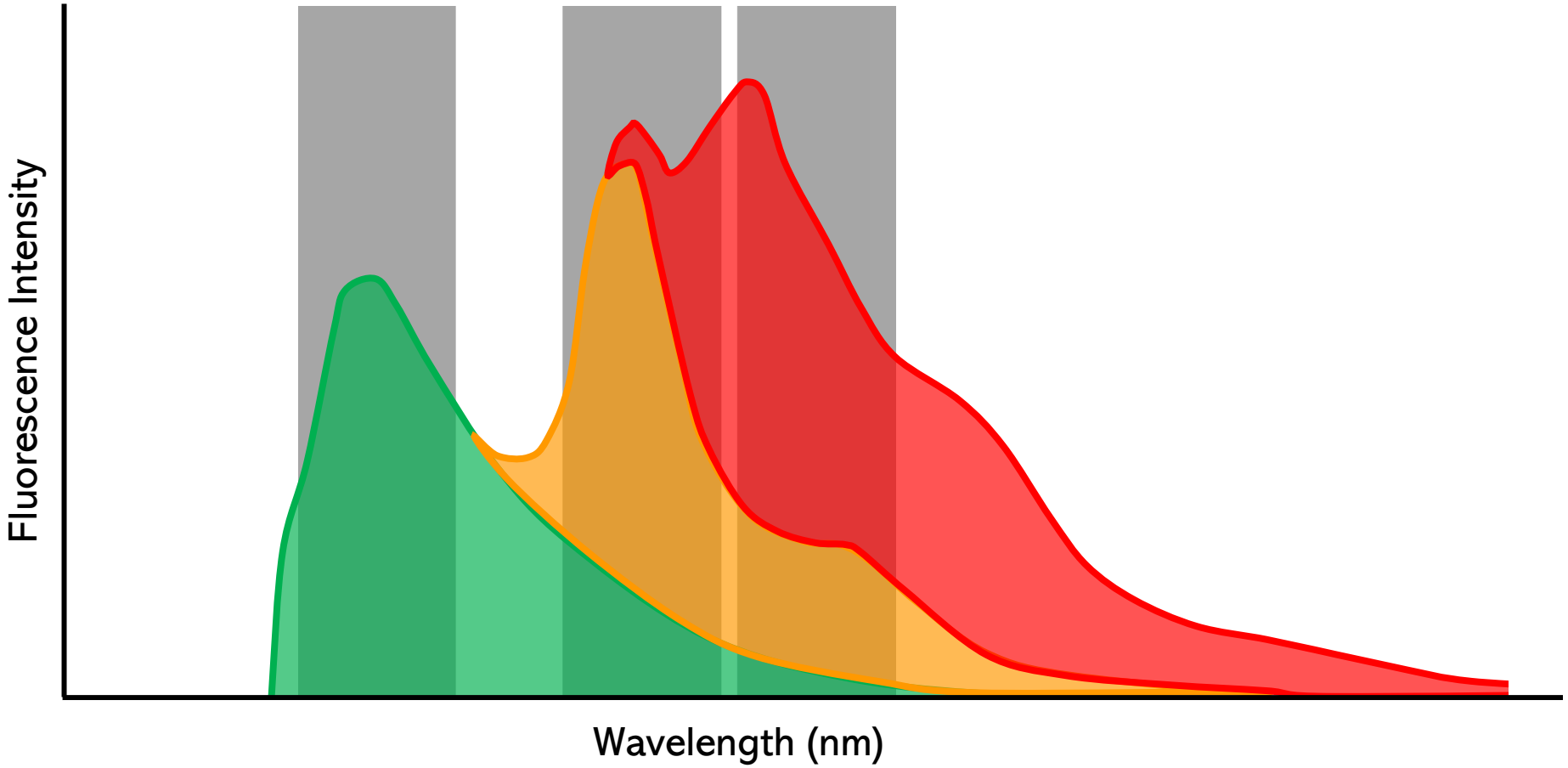
Conventional Flow Cytometry

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



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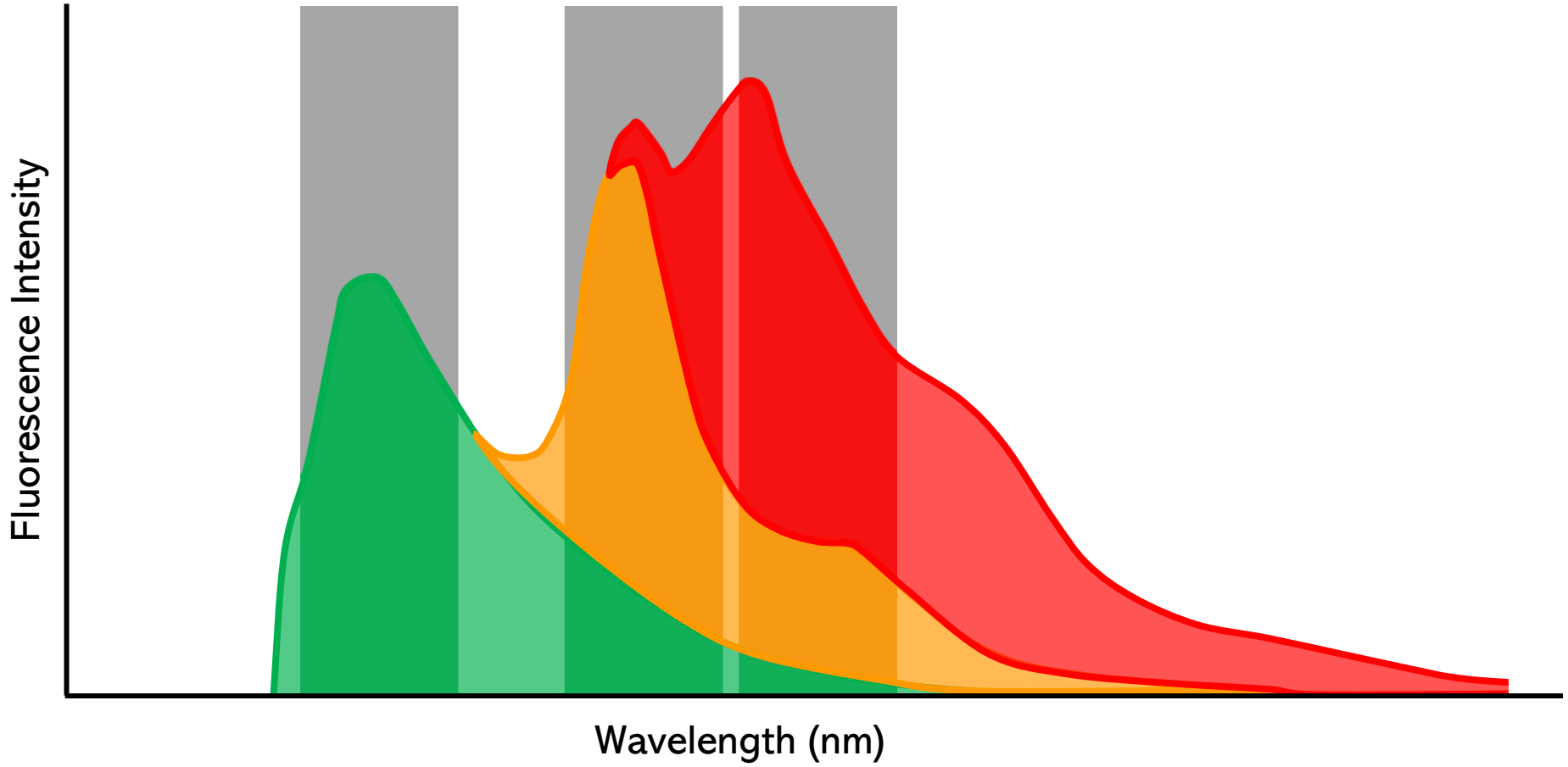
Conventional Flow Cytometry

Any fluorescence outside the defined detector ranges is not collected.



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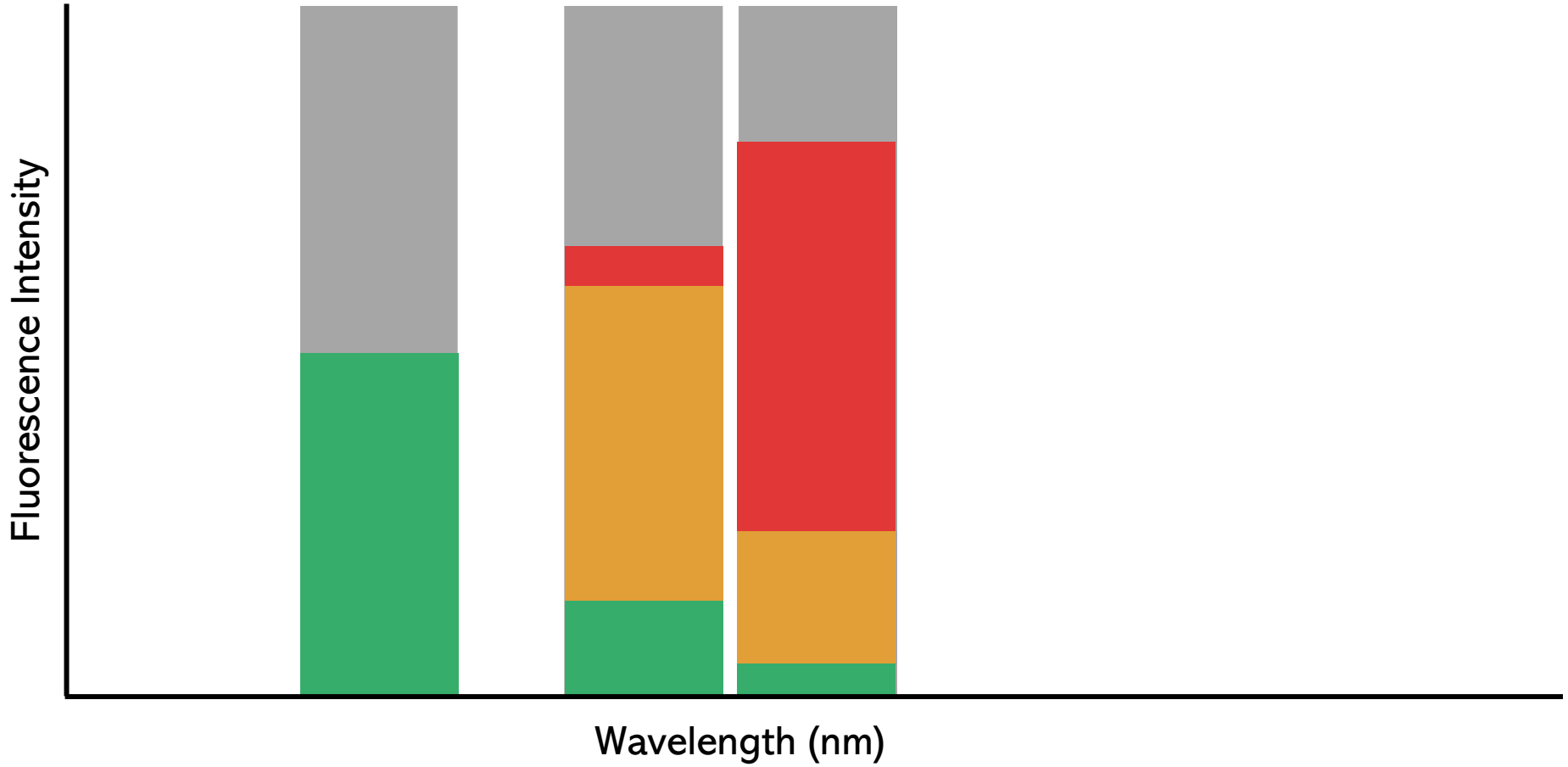
Conventional Flow Cytometry

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



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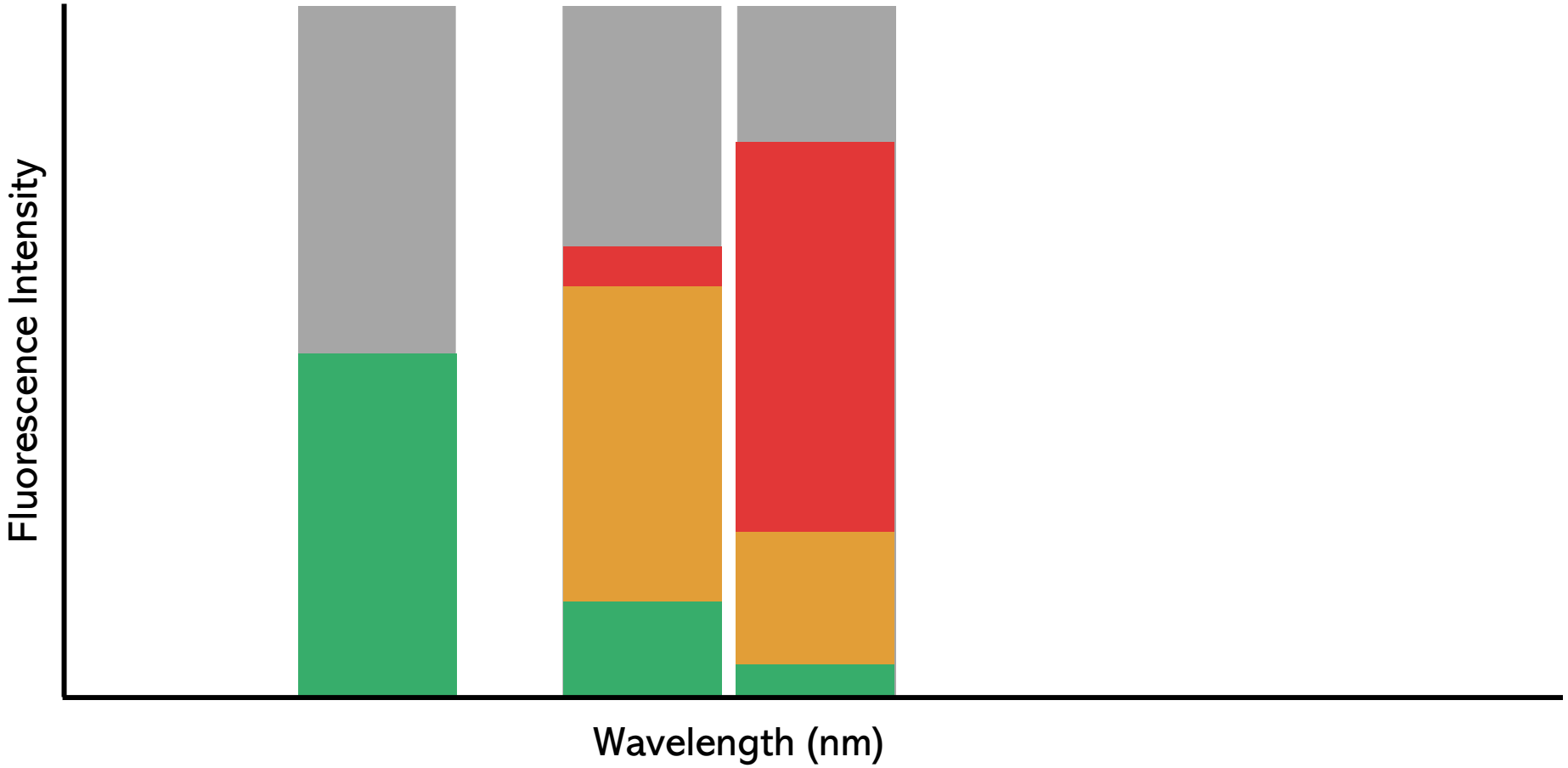
Conventional Flow Cytometry

The spillover is subtracted
by colour compensation.



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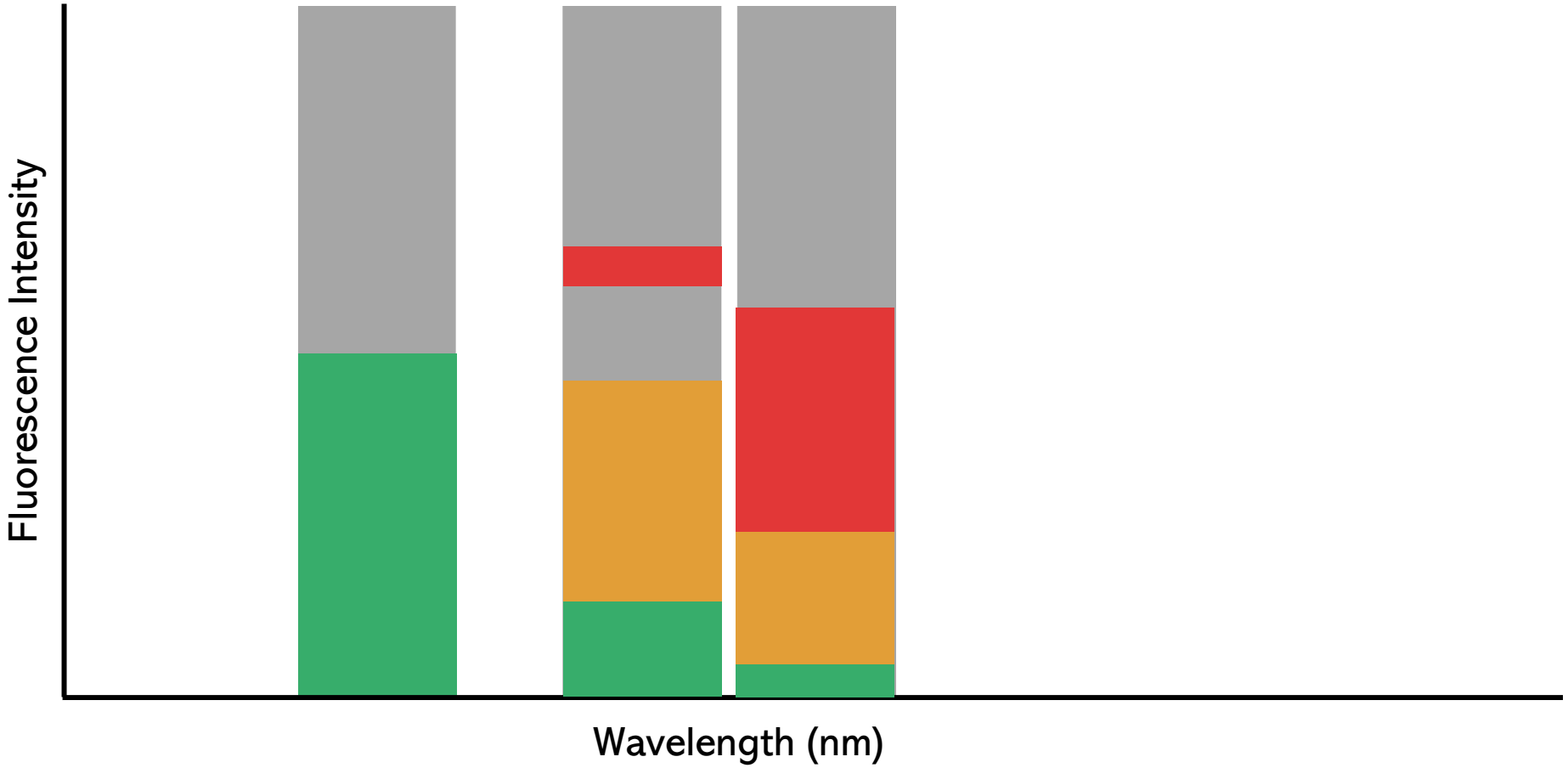
Spectral Flow Cytometry

There is no subtraction.



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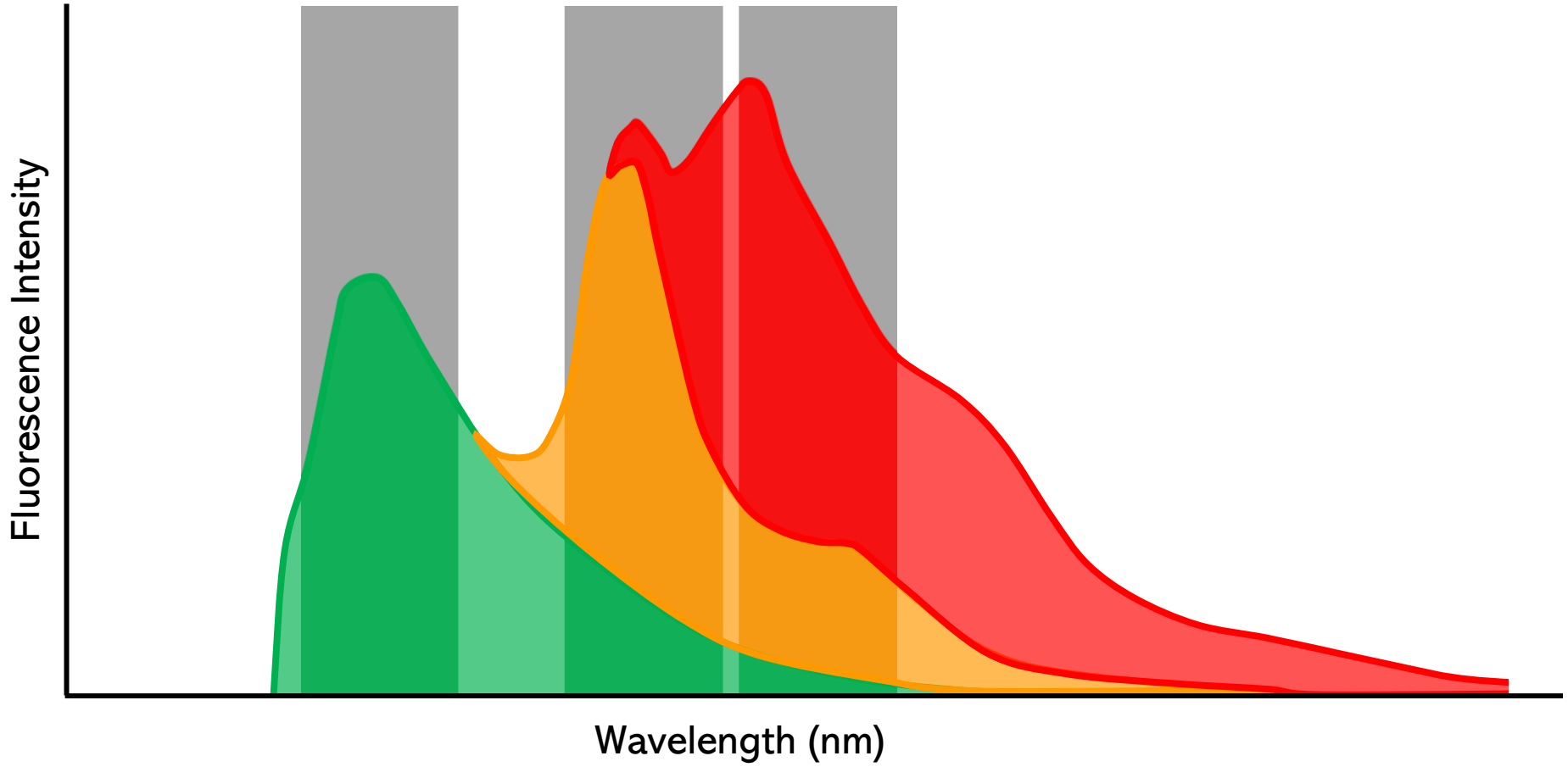
Spectral Flow Cytometry

All of the fluorescence is included.



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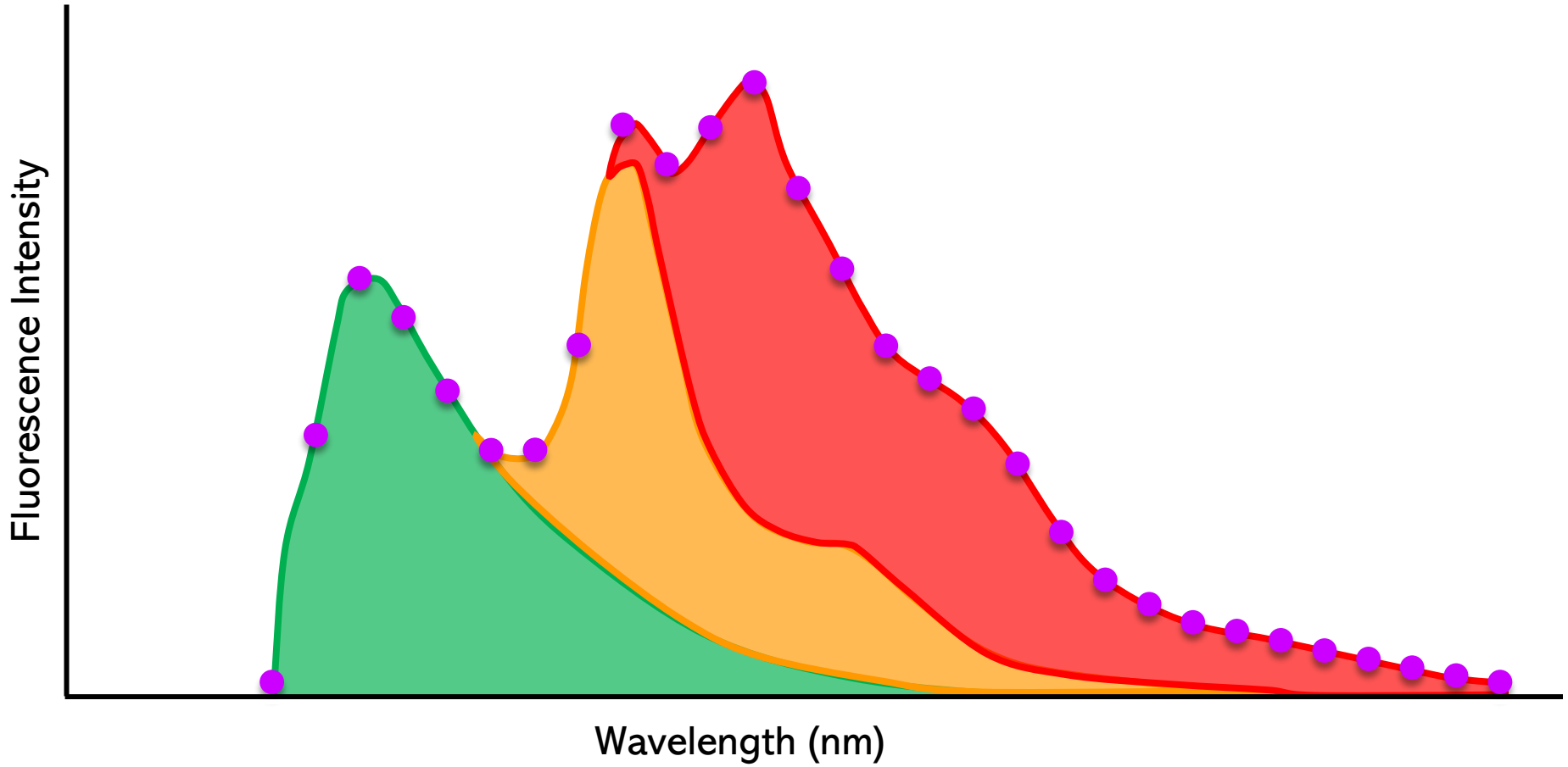
Spectral Flow Cytometry

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



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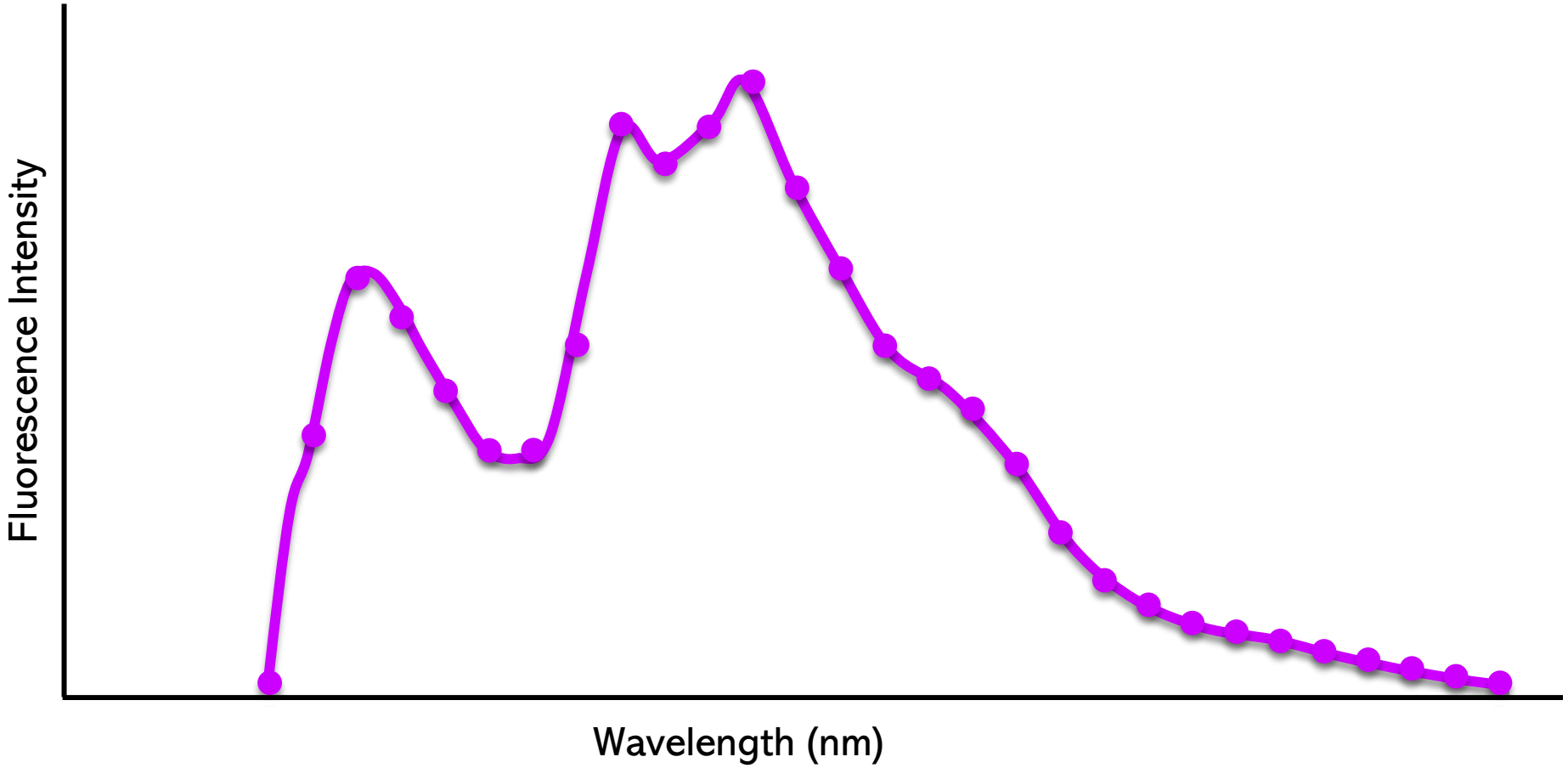
Spectral Flow Cytometry

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



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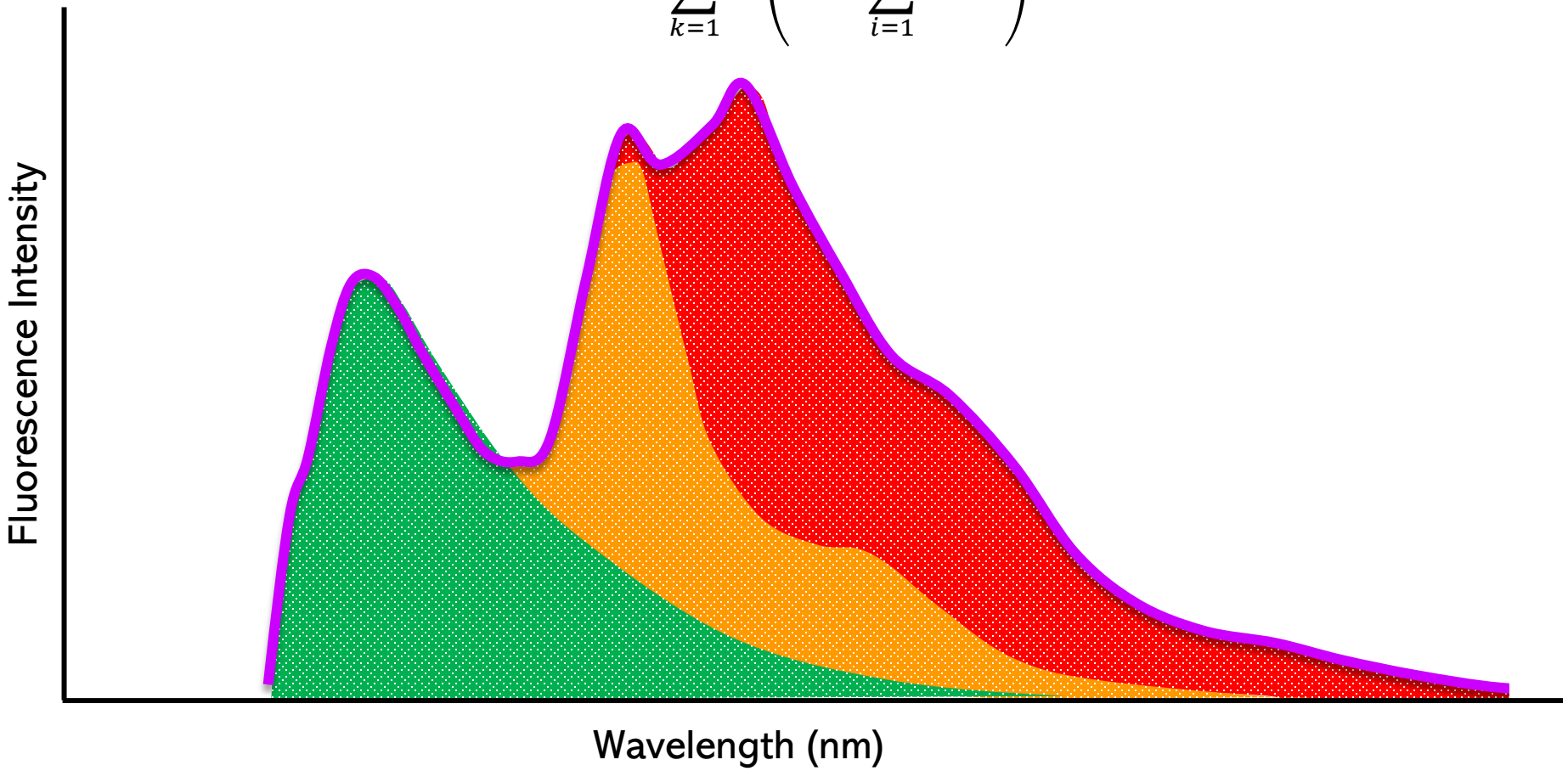
Spectral Flow Cytometry

The Weighted Least Squares Method (WLSM) is applied…

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



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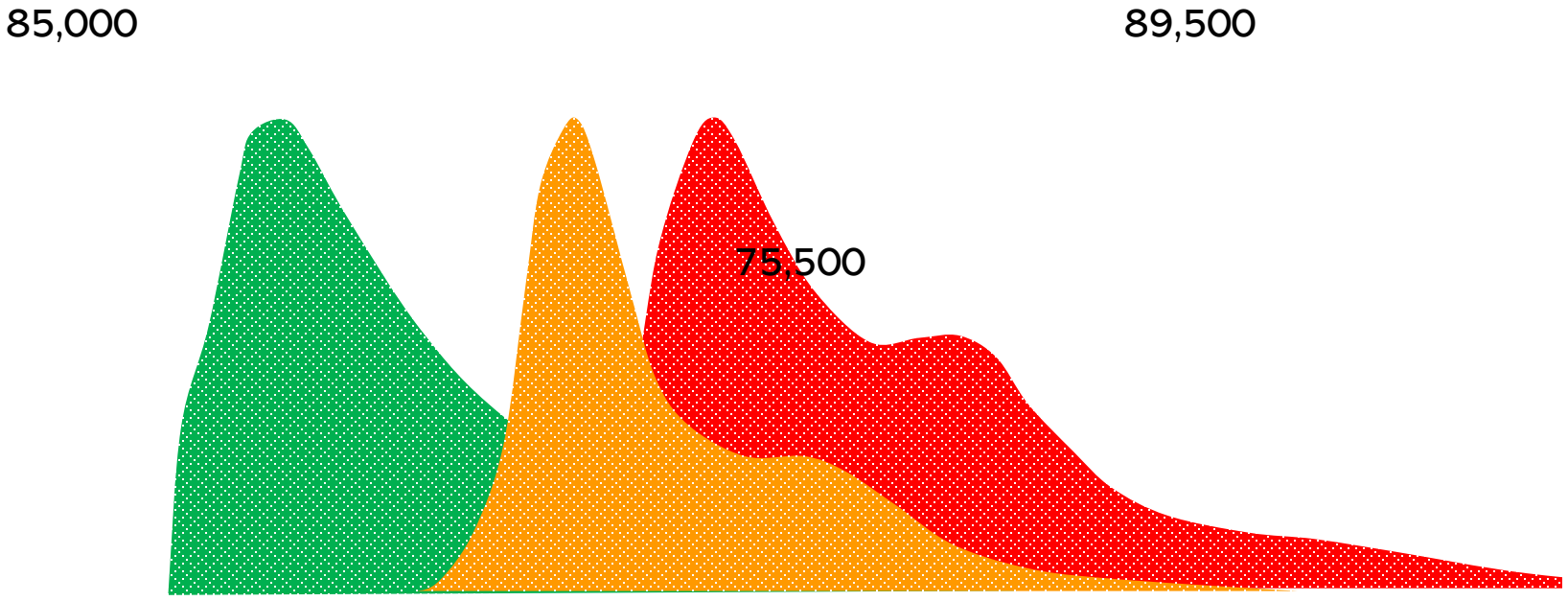
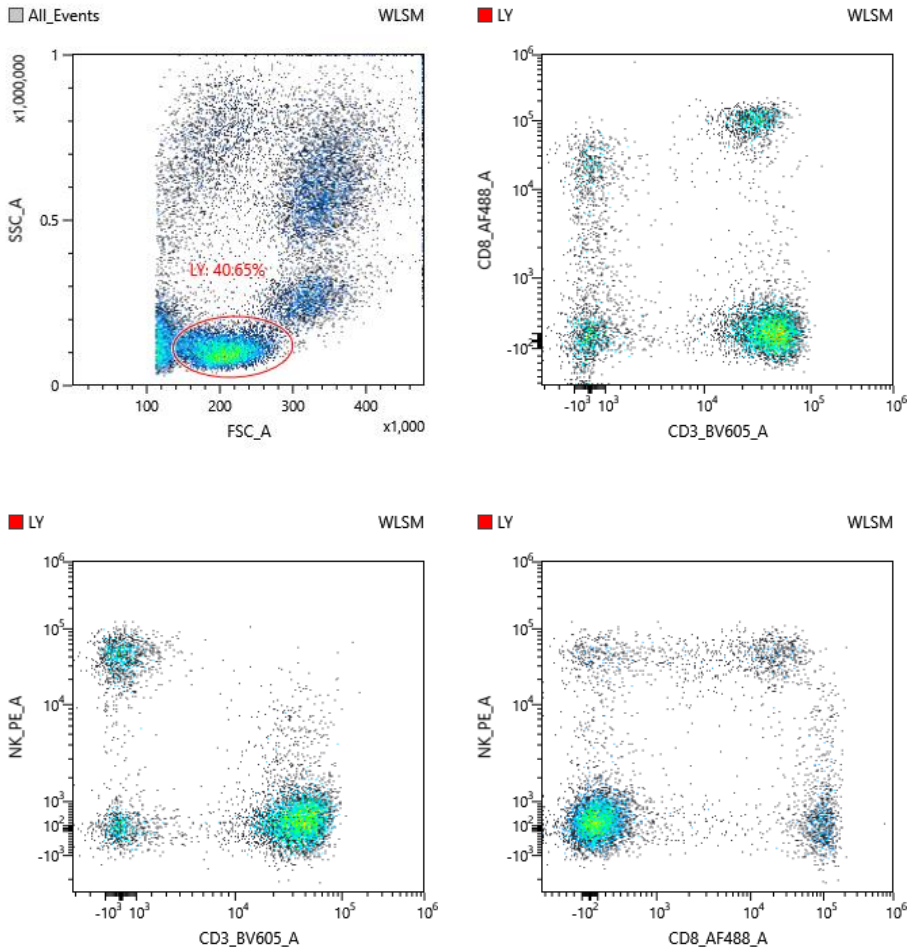
Spectral Flow Cytometry

…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**.



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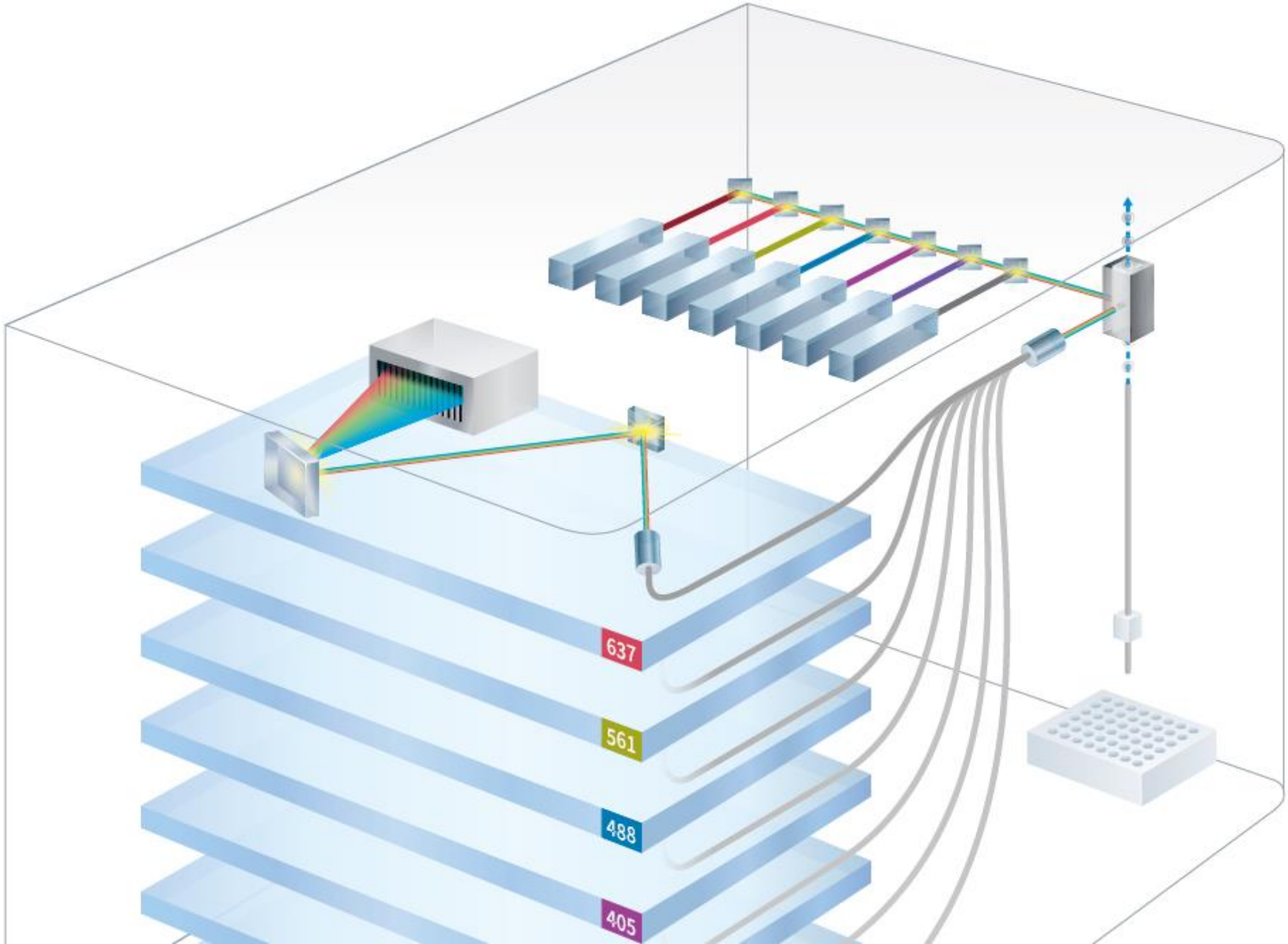

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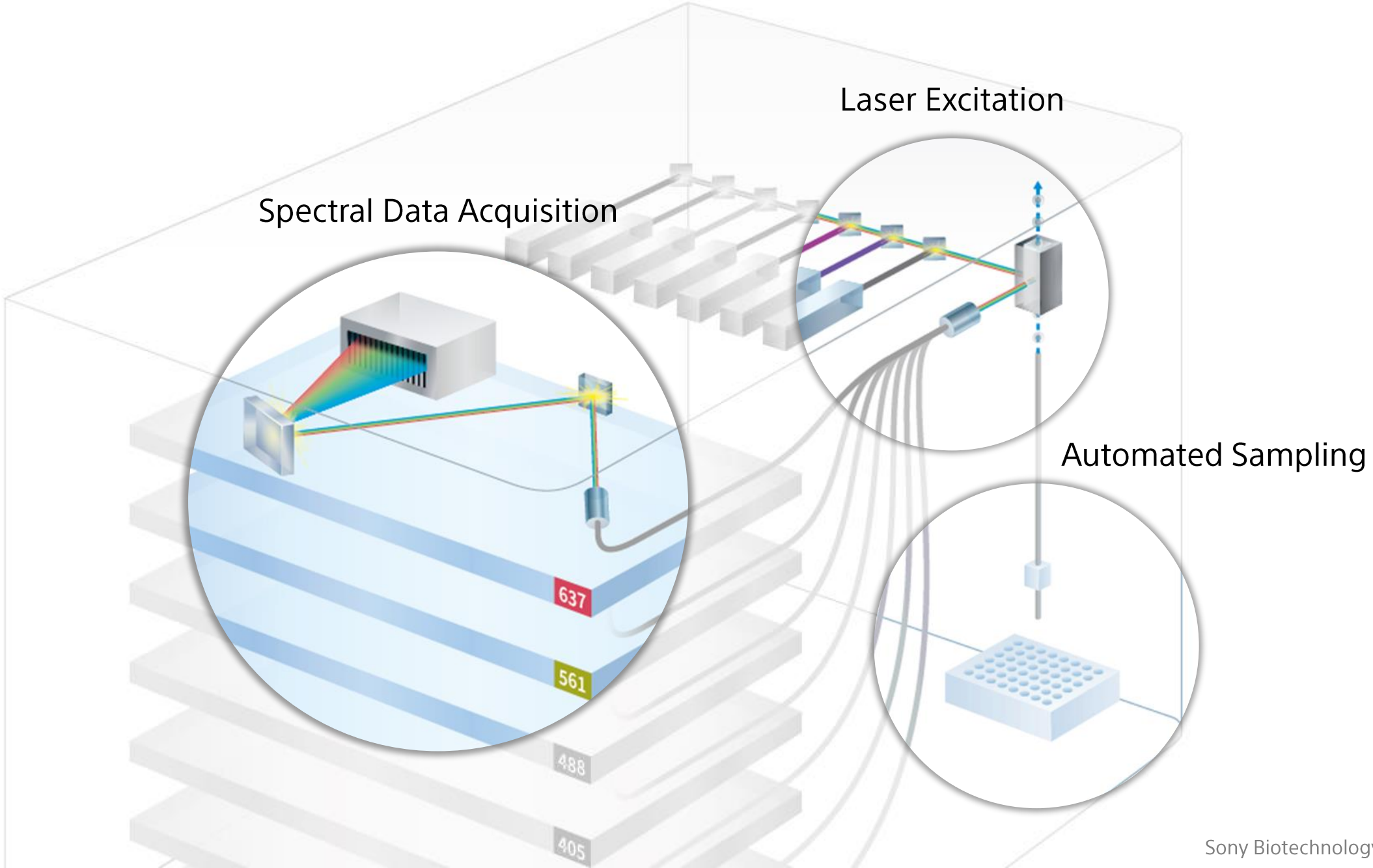
ID7000 Spectral Cell Analyzer



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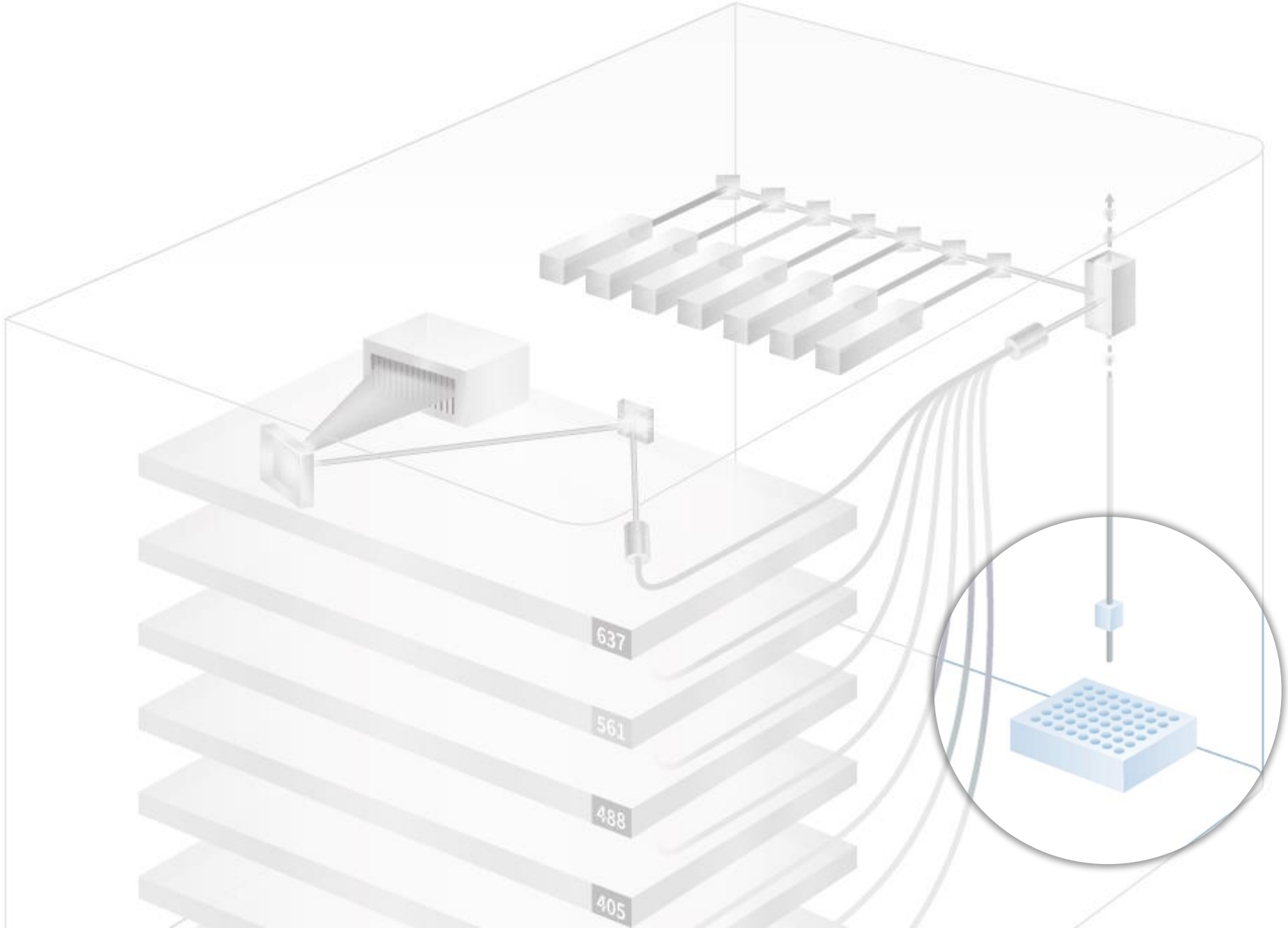
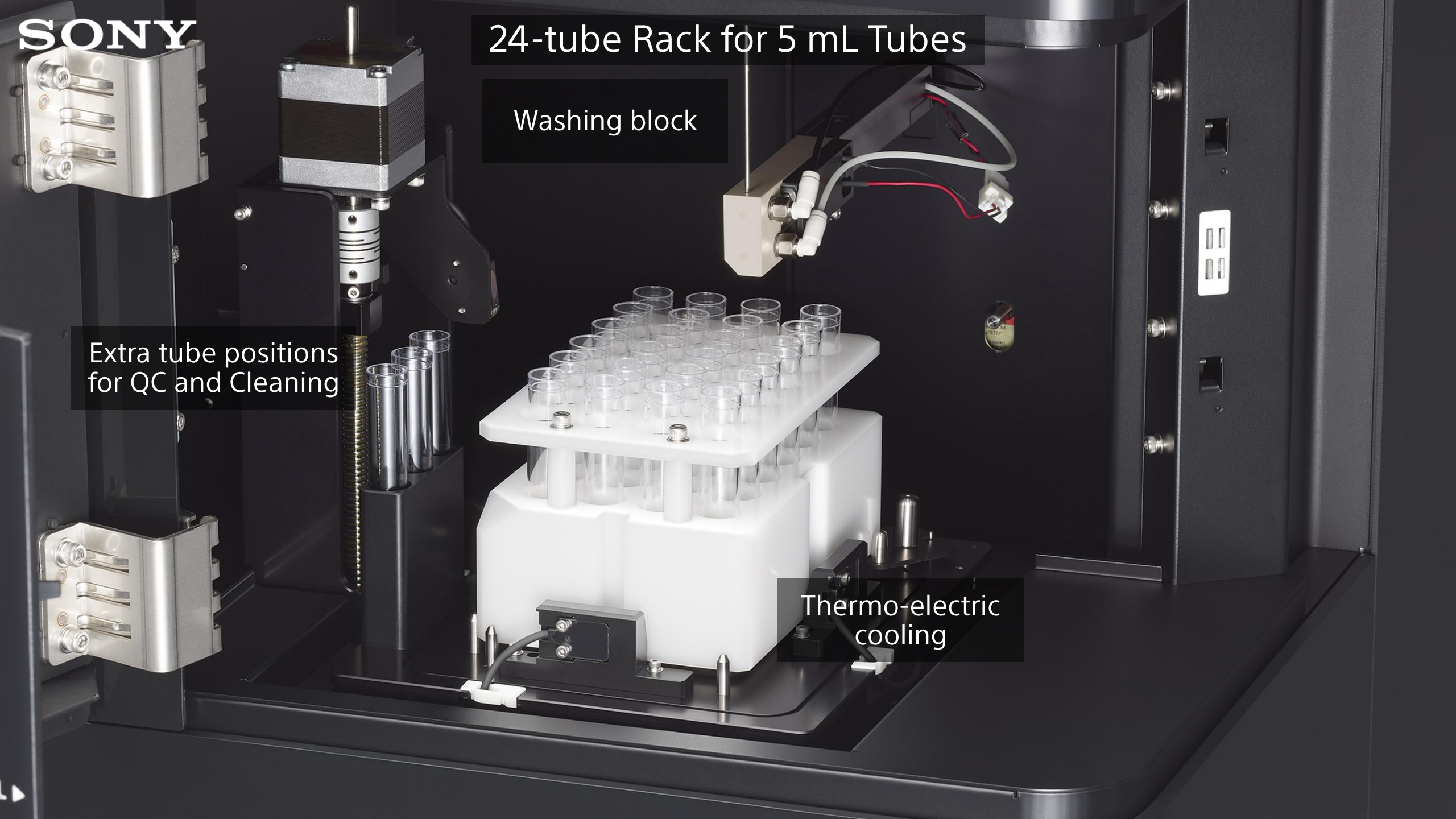
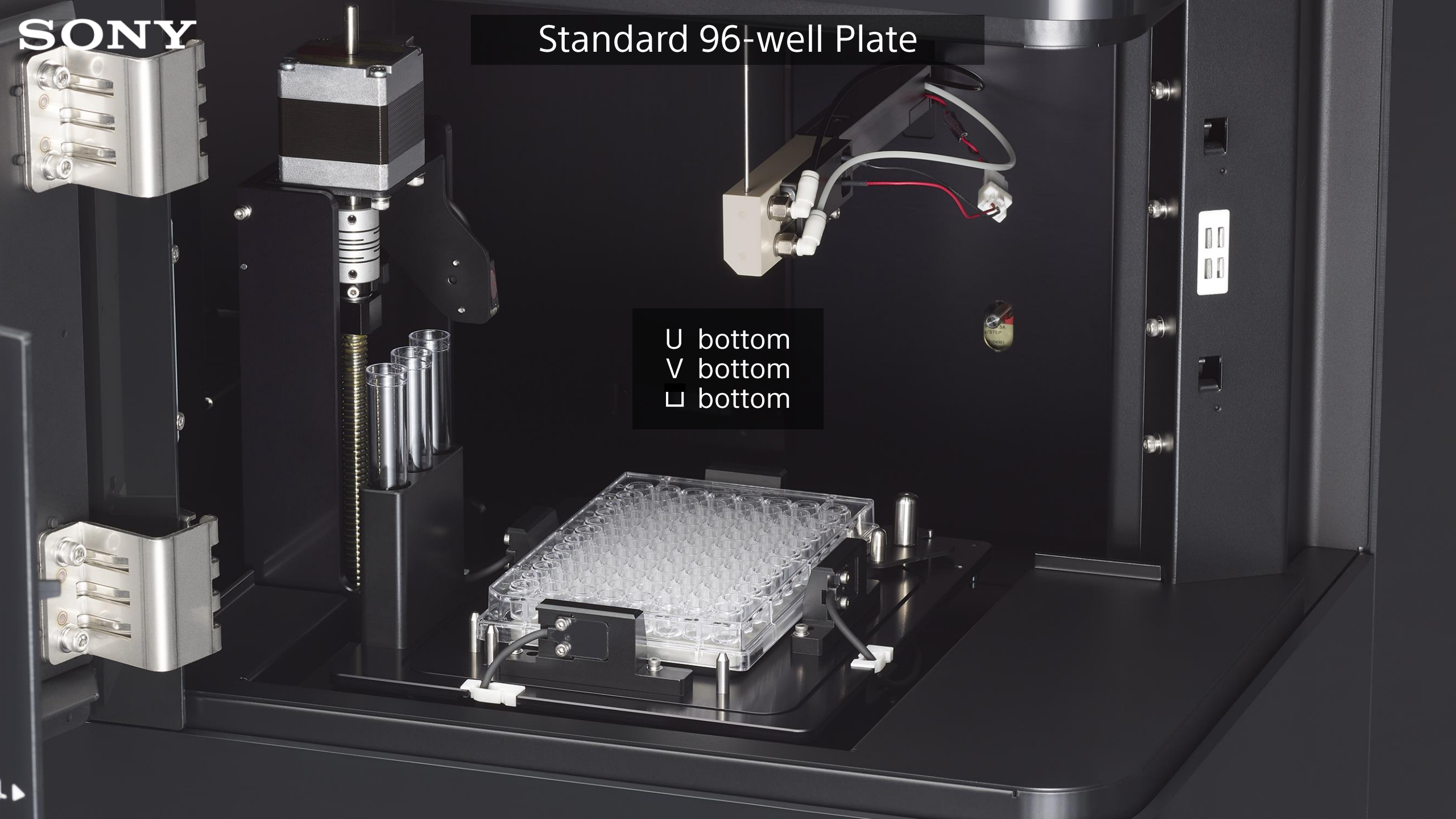
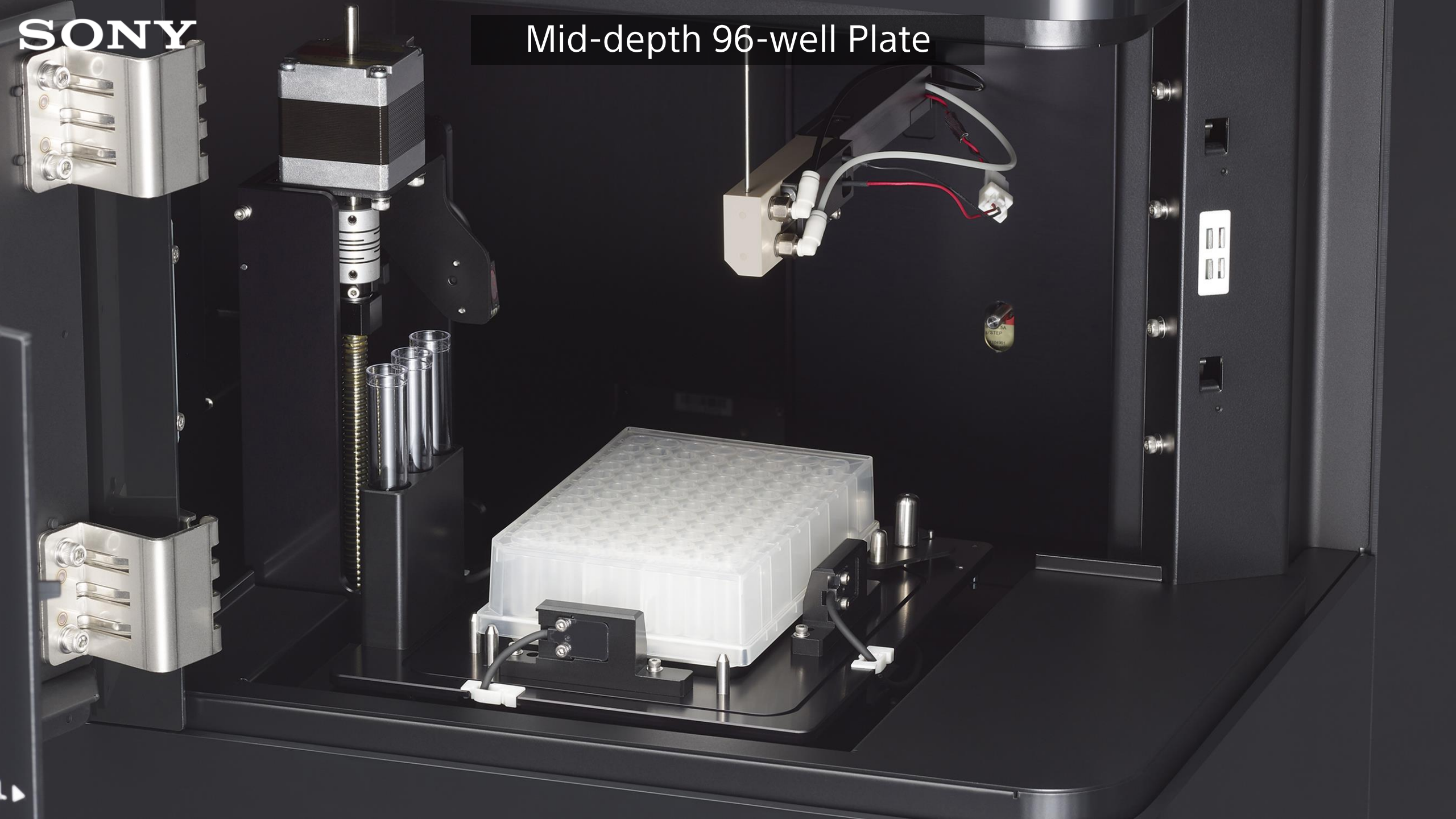
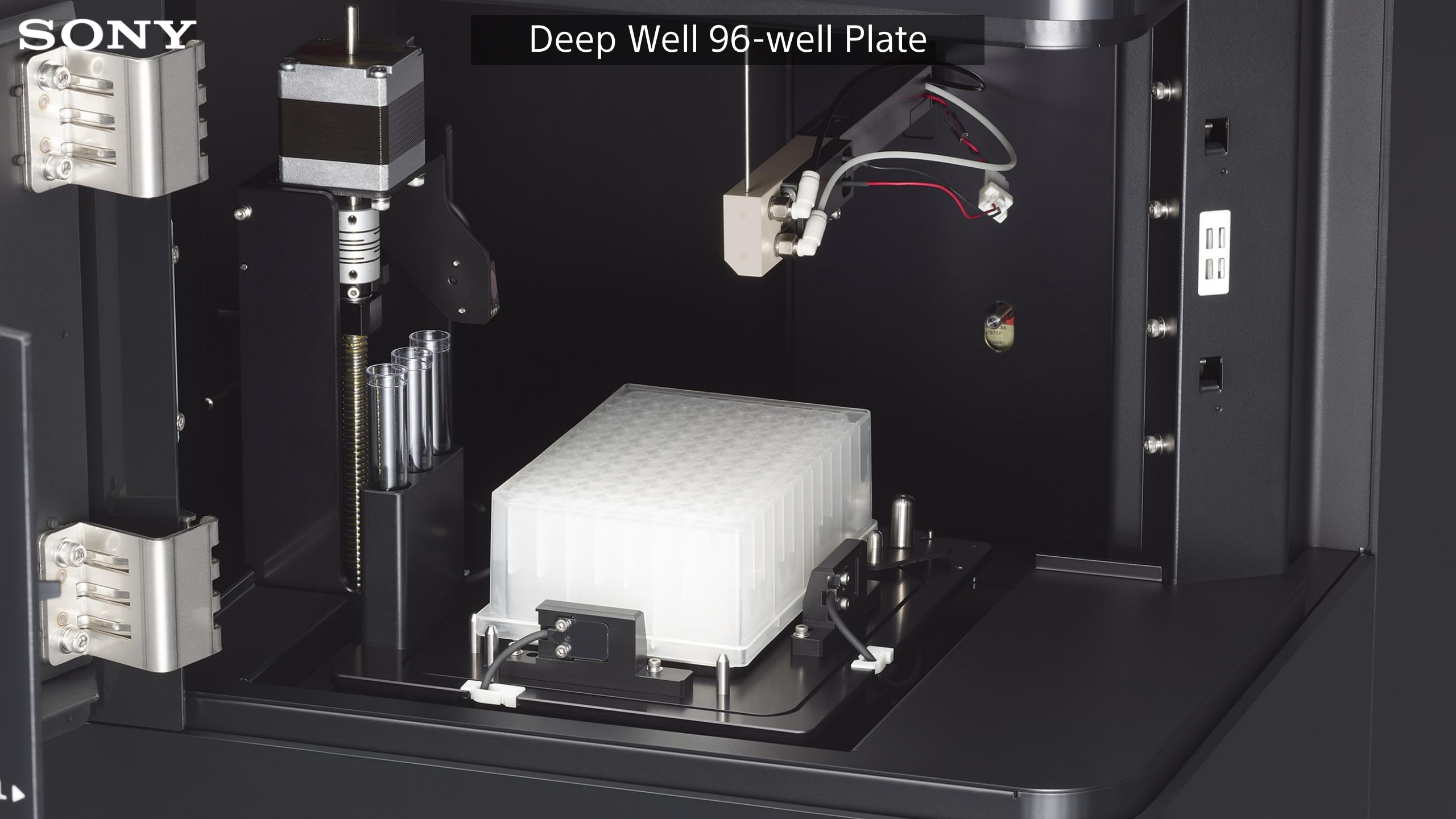
Automated Sampling

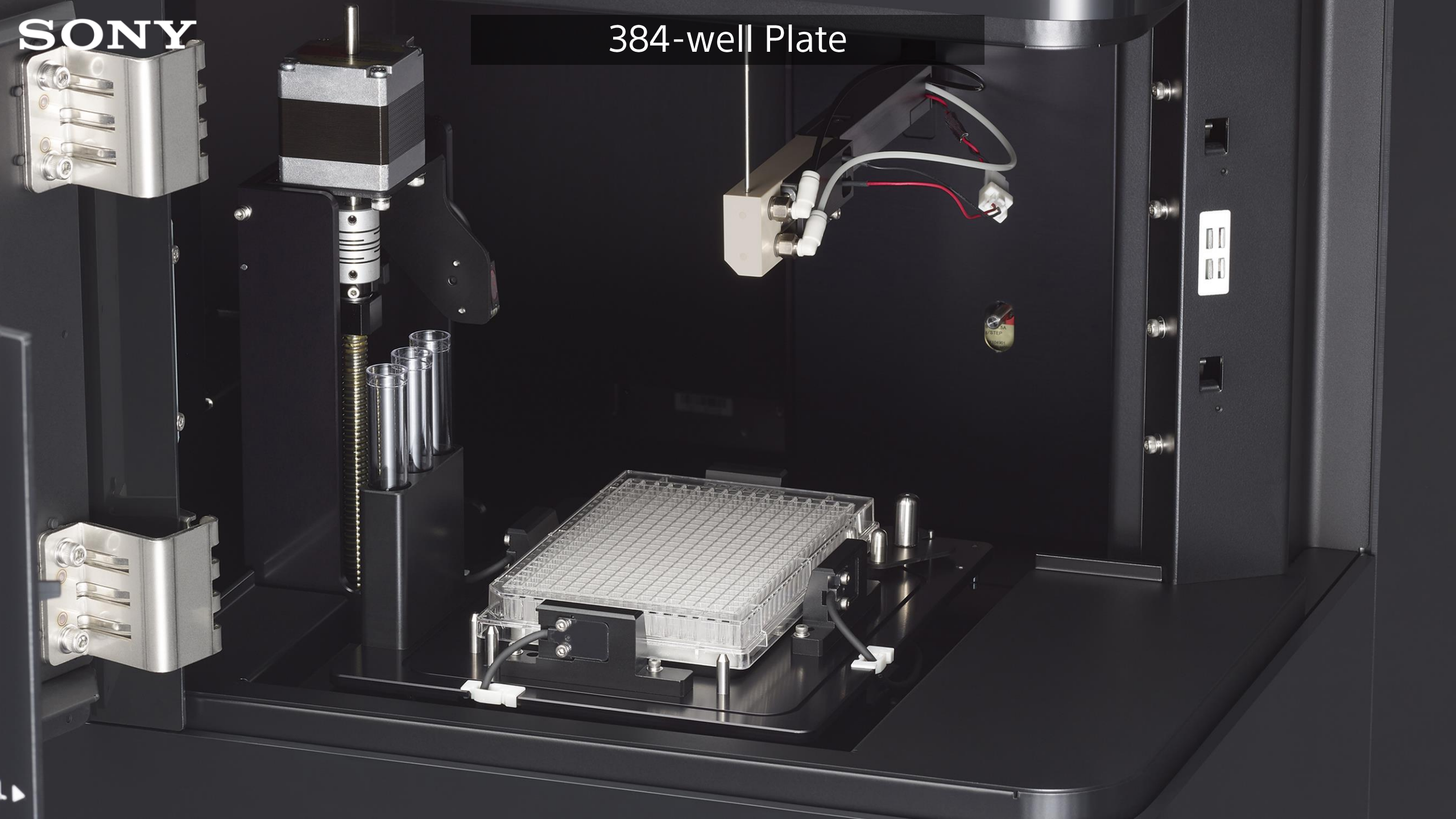



SONY
24-tube Rack for 5 mL Tubes
Washing block
Extra tube positions
for QC and Cleaning
Thermo-electric
cooling


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

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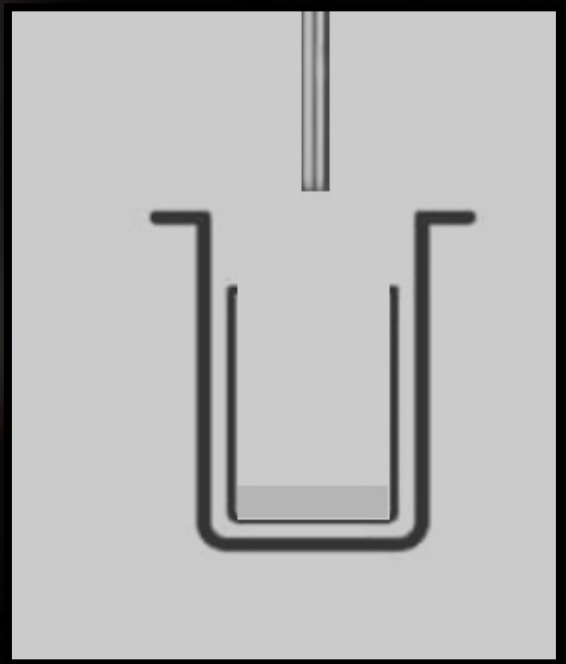
Configurable on a single well level:

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



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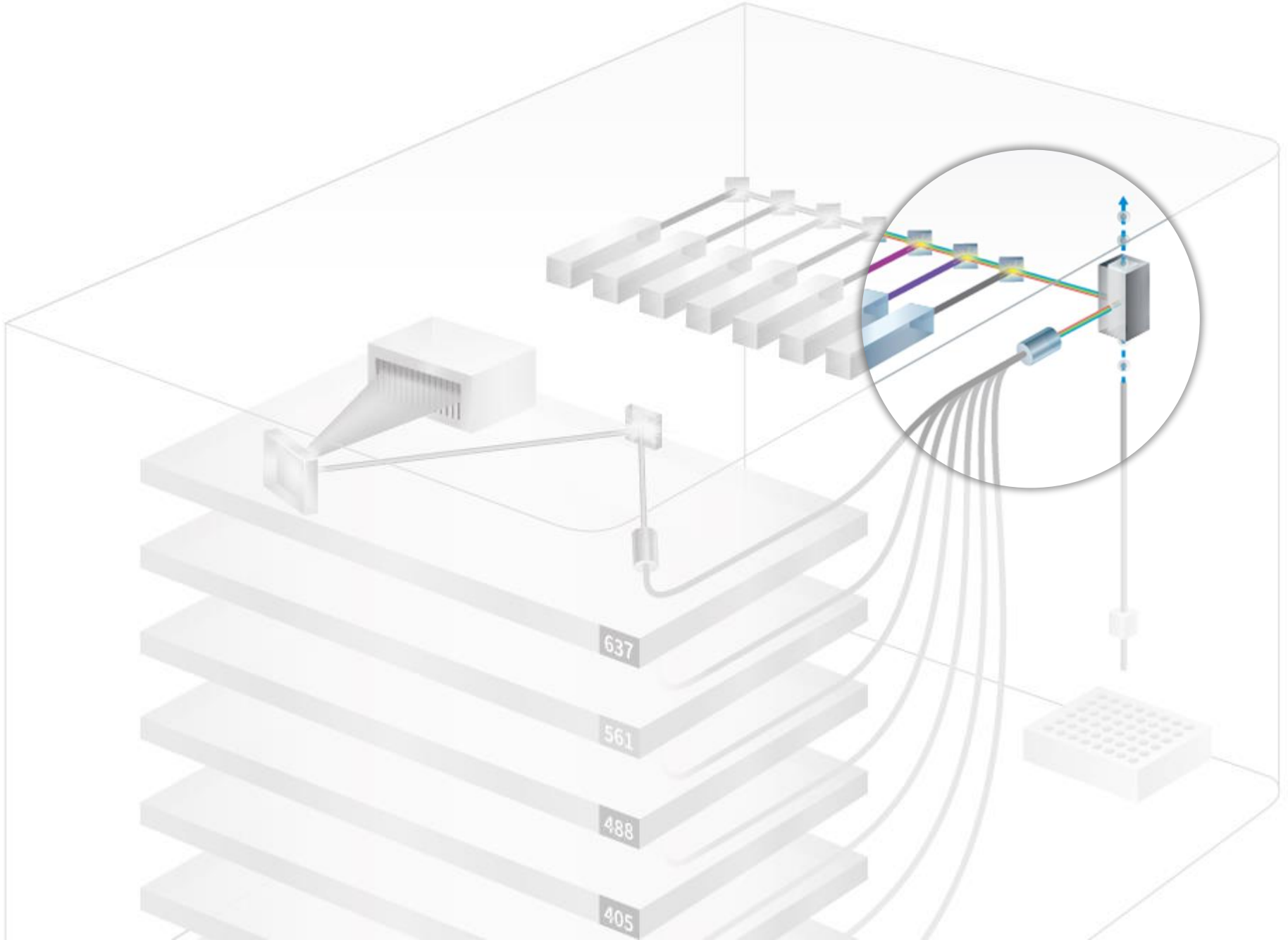
Laser Excitation



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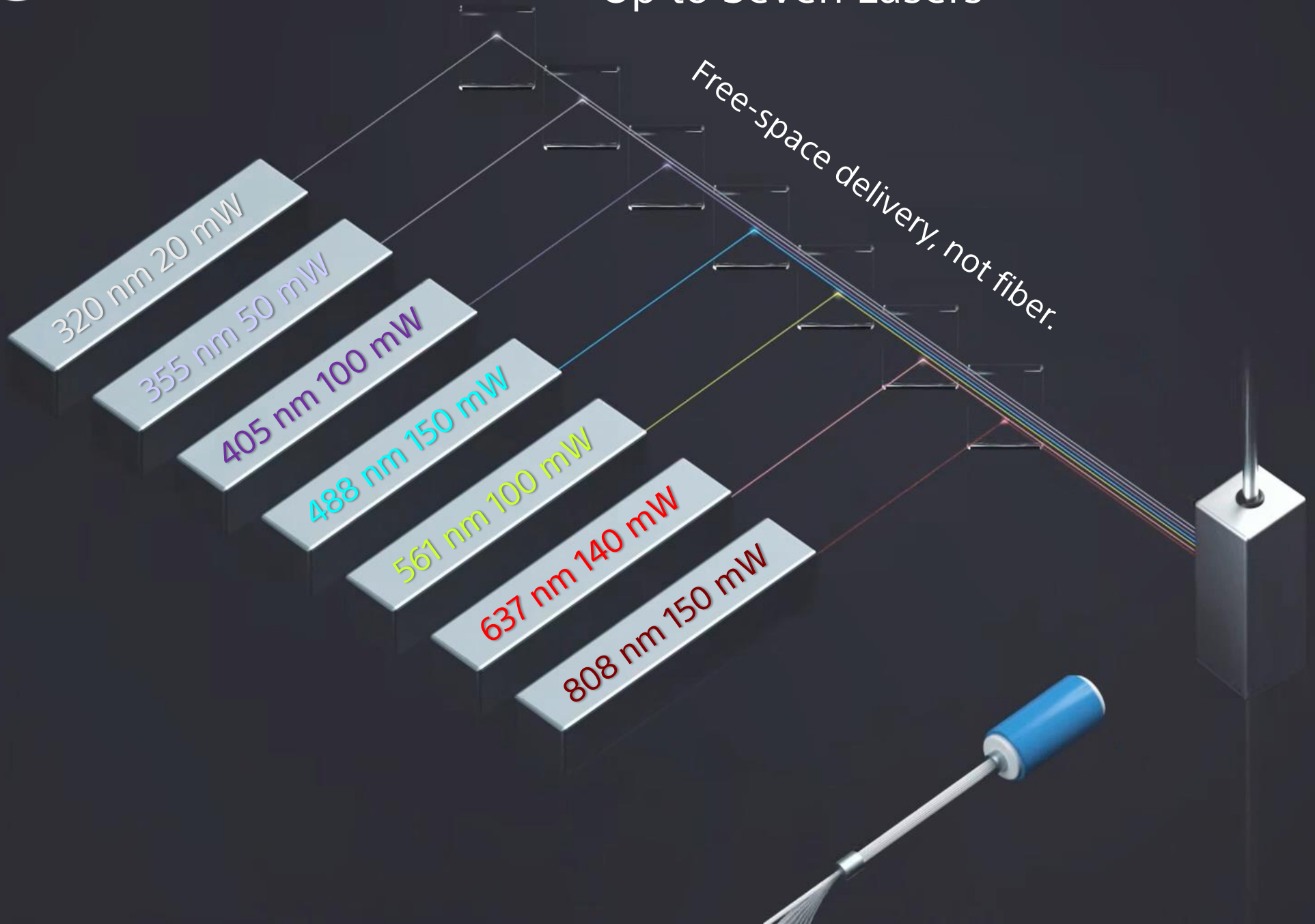
Up to Seven Lasers



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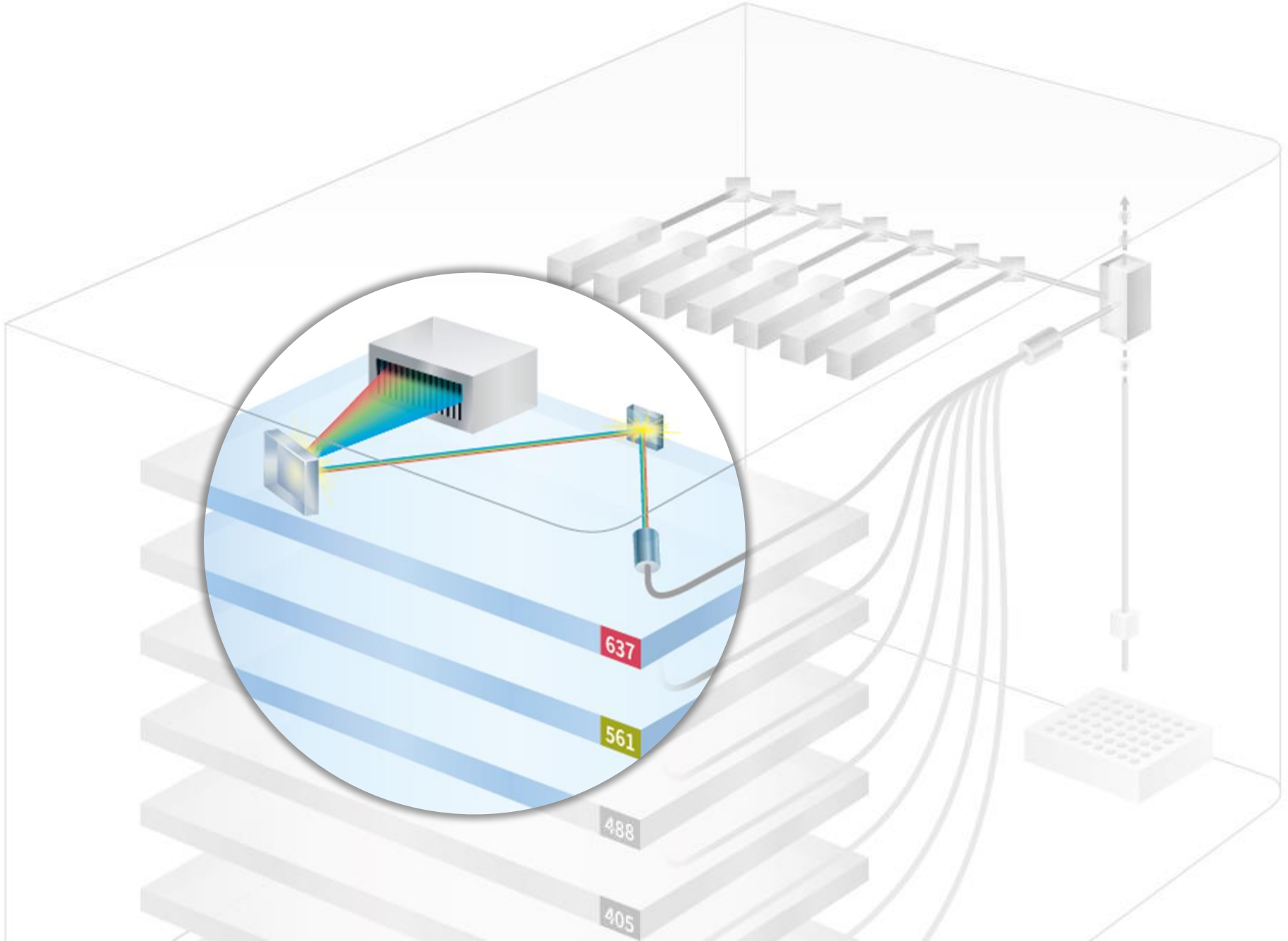
Spectral Data Acquisition



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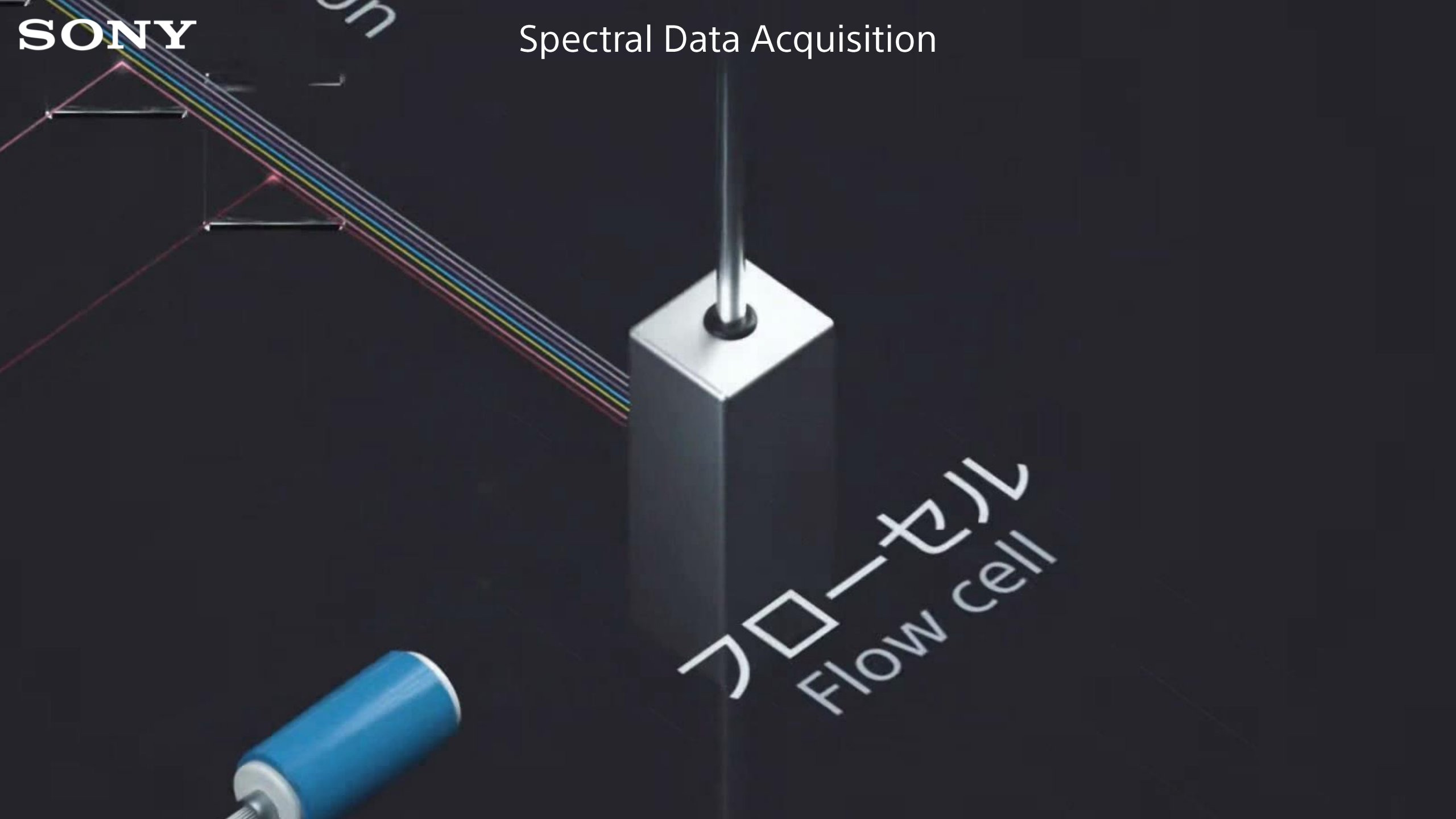
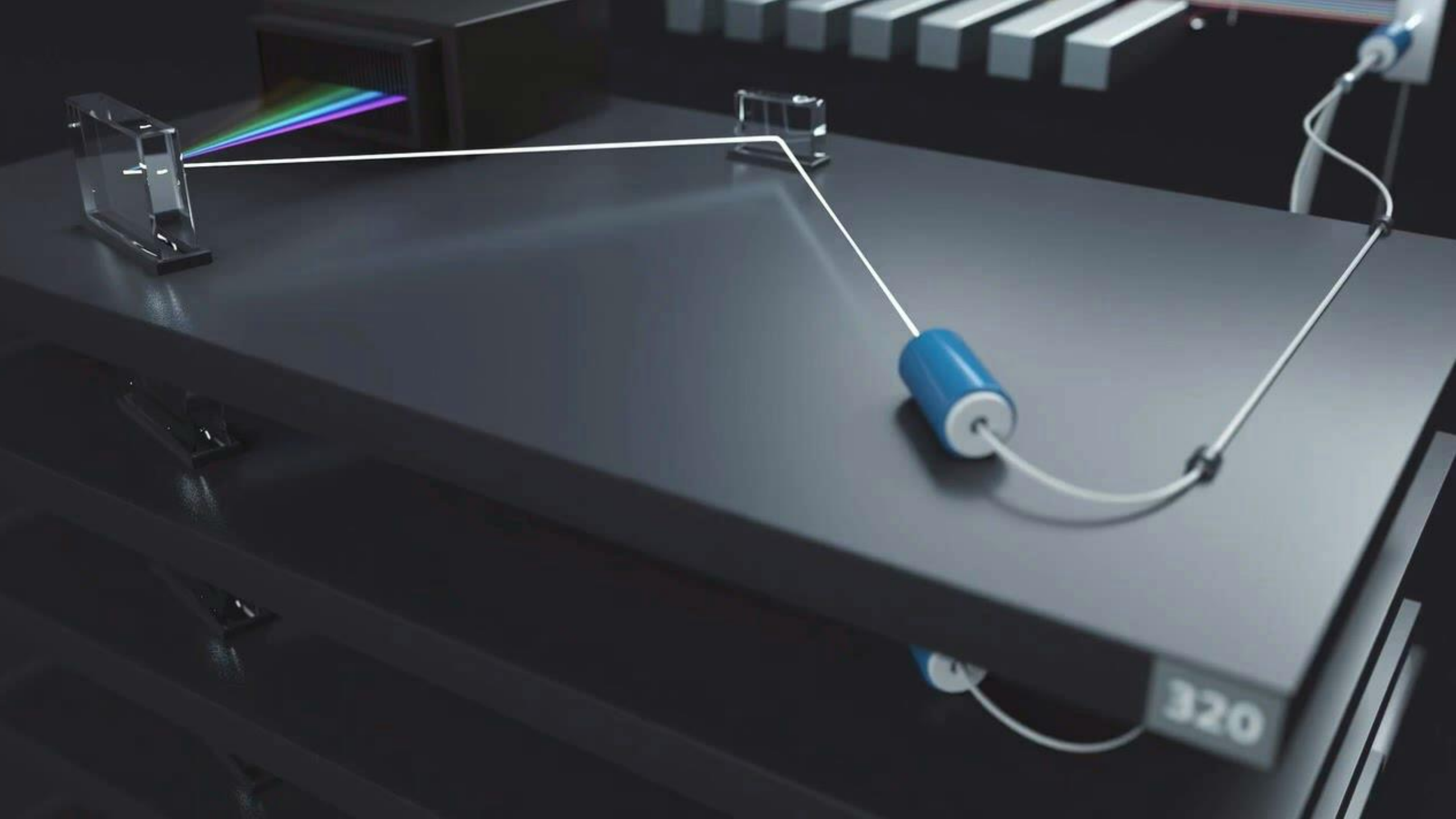
Spectral Data Acquisition




320

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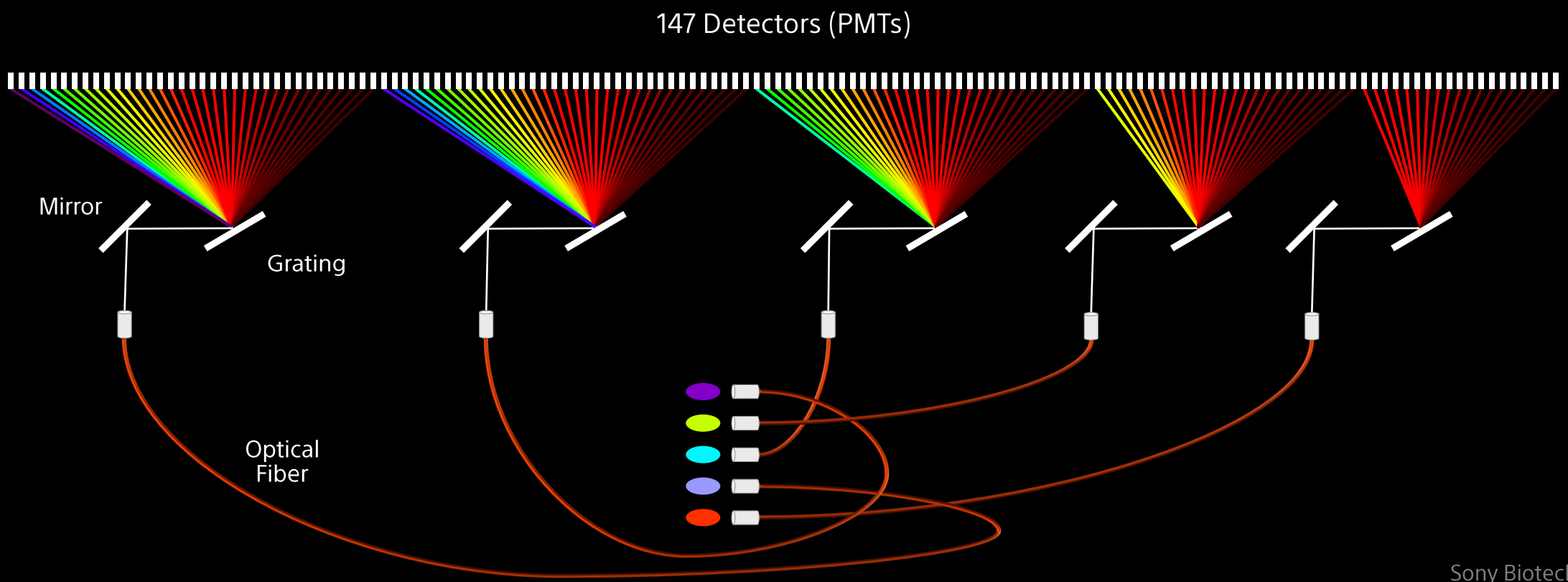
Spectral Data Acquisition



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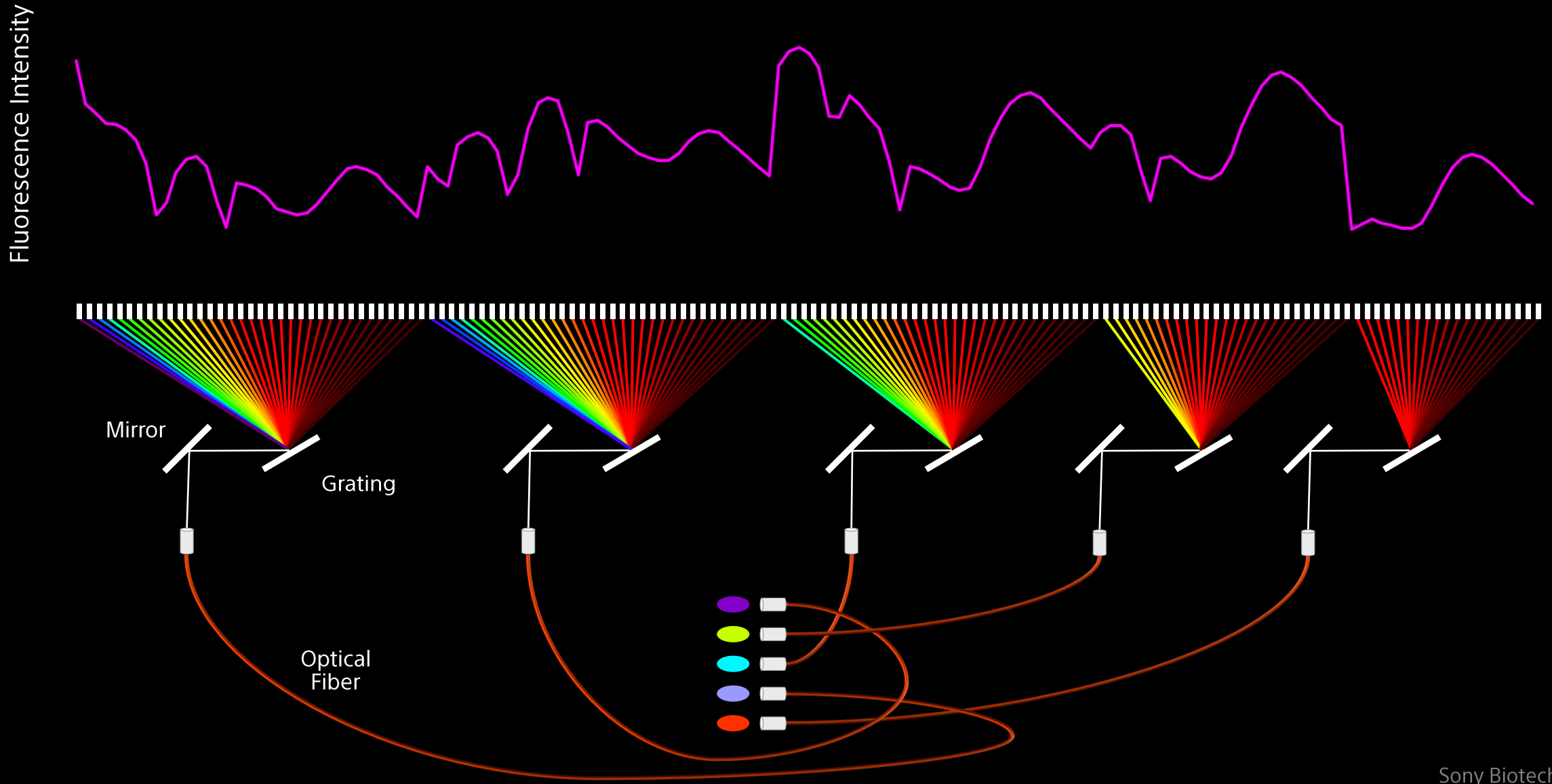
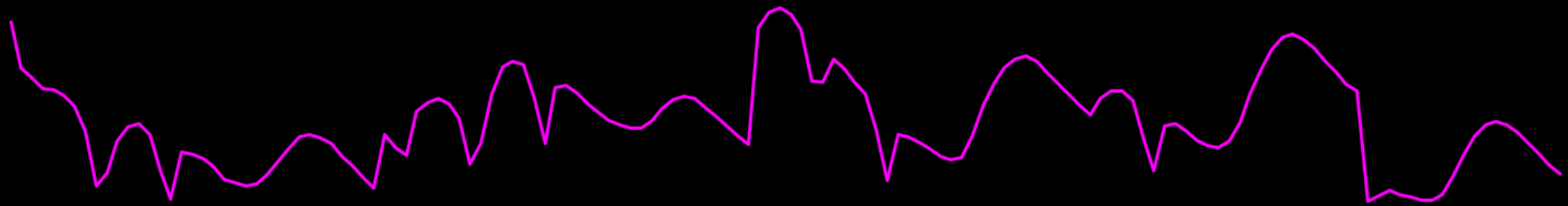
Spectral Data

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



Spectral Data

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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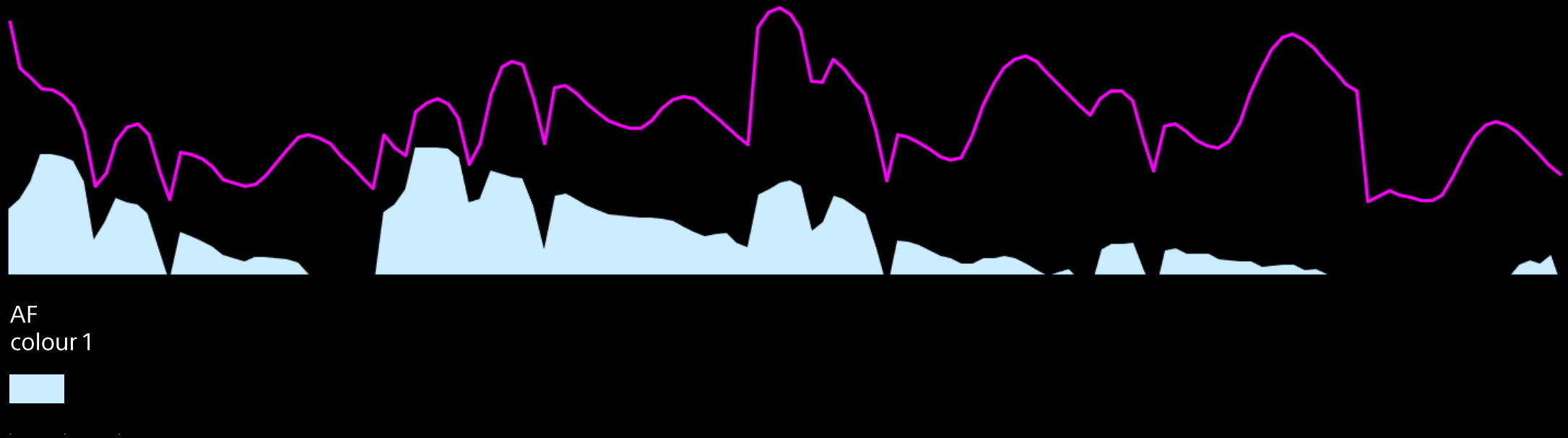

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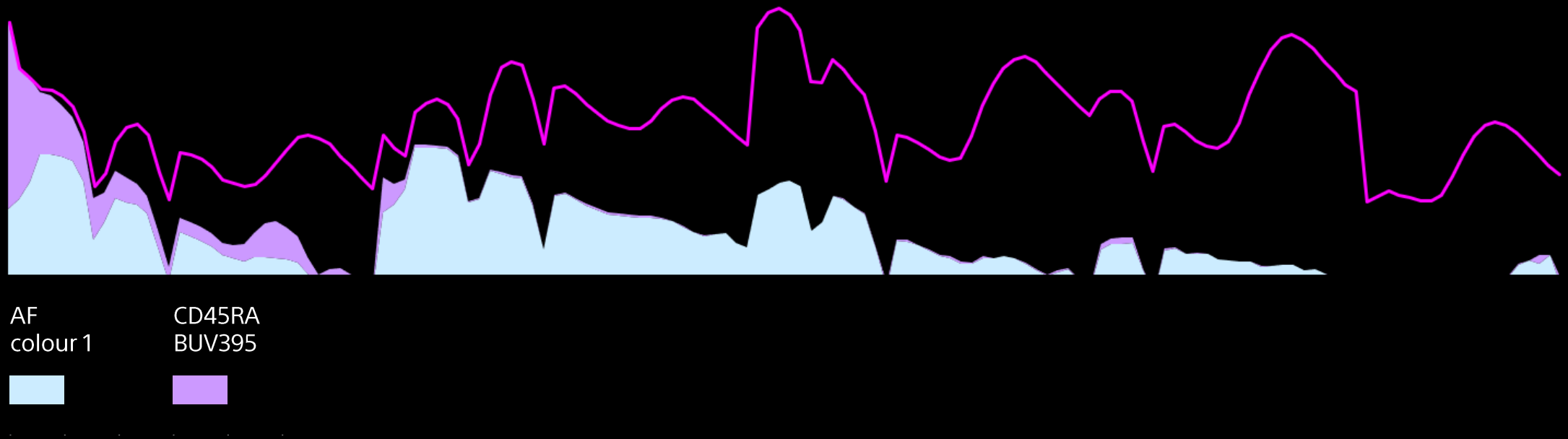

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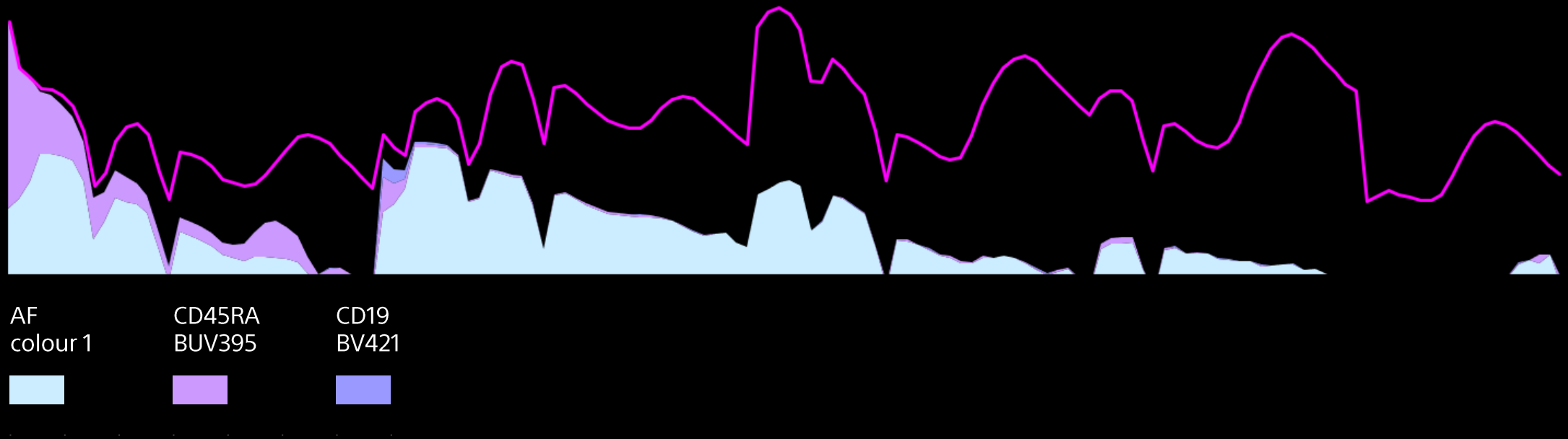

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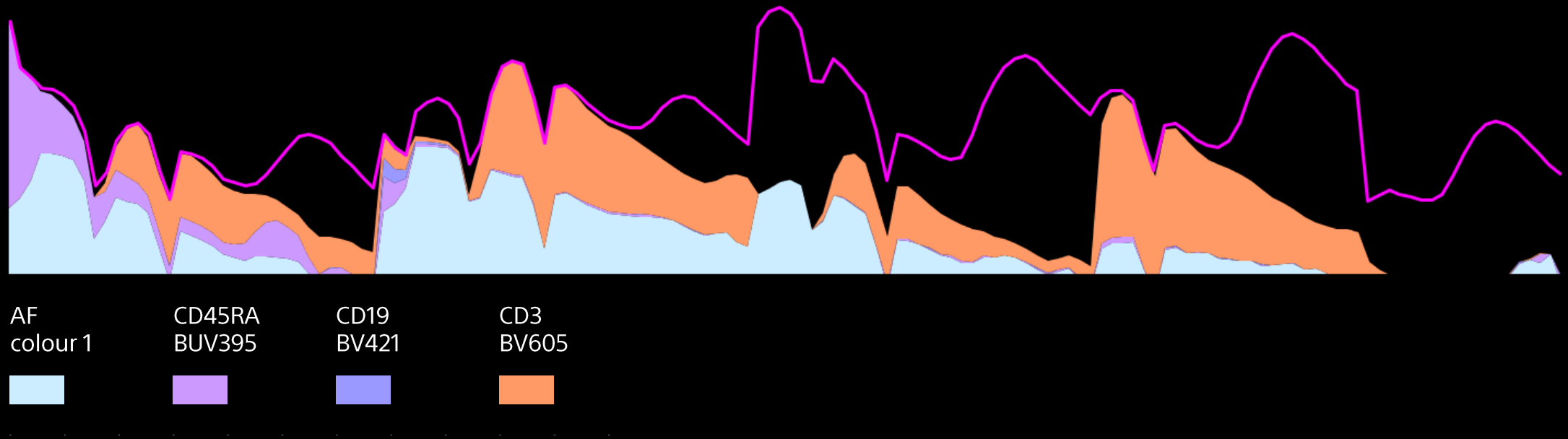

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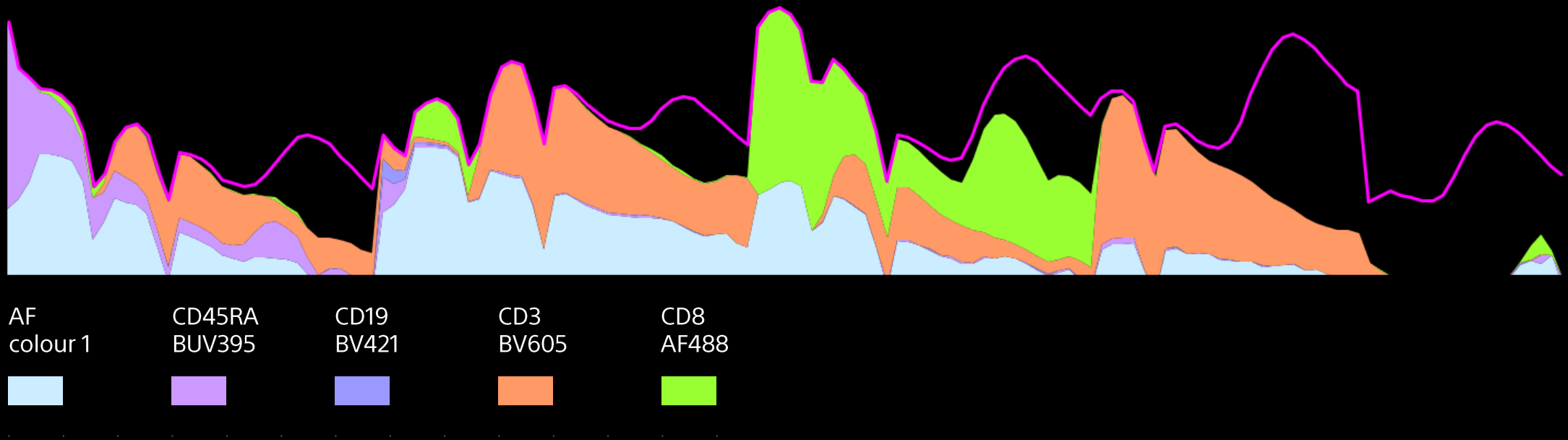

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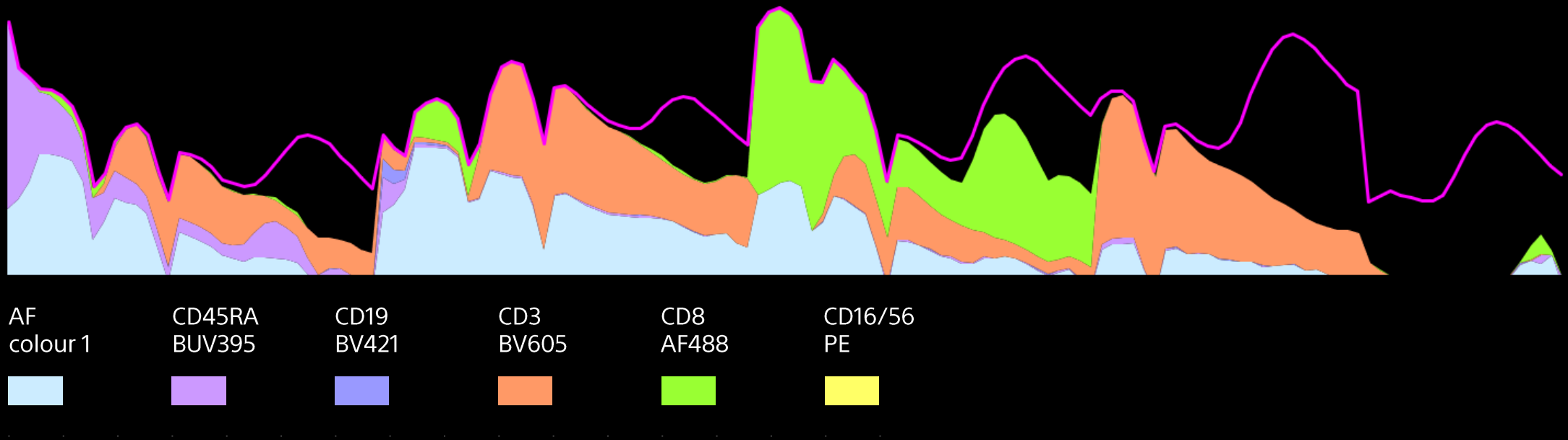

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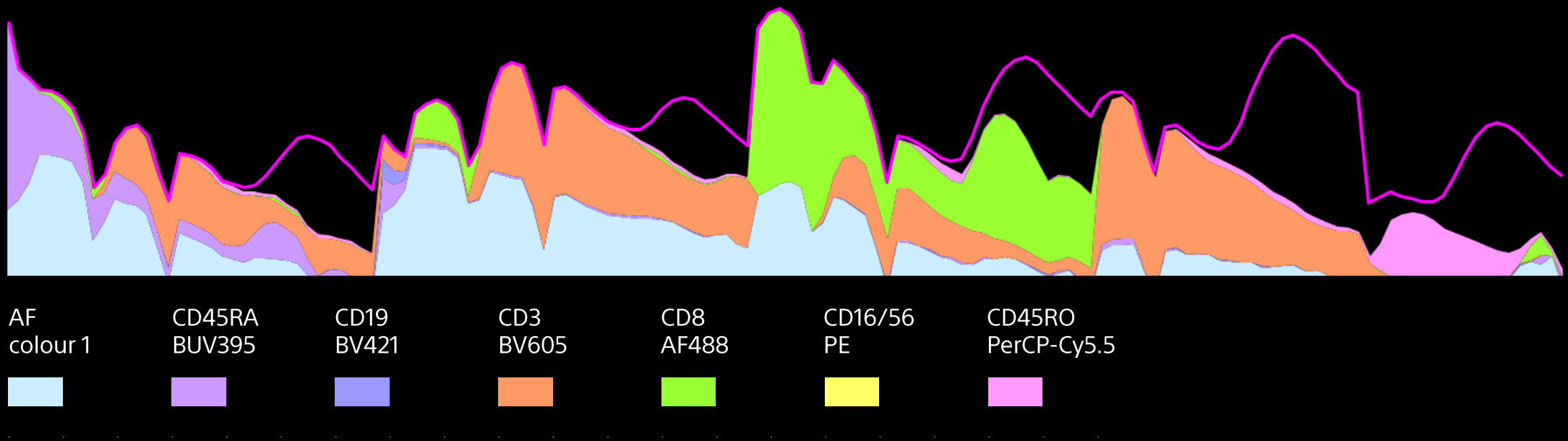

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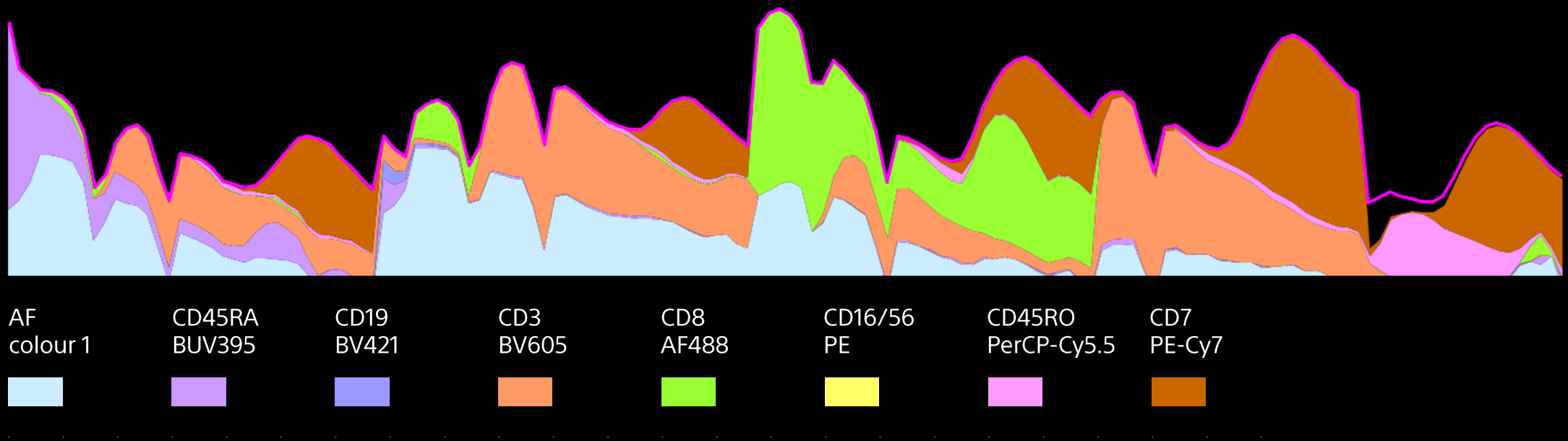

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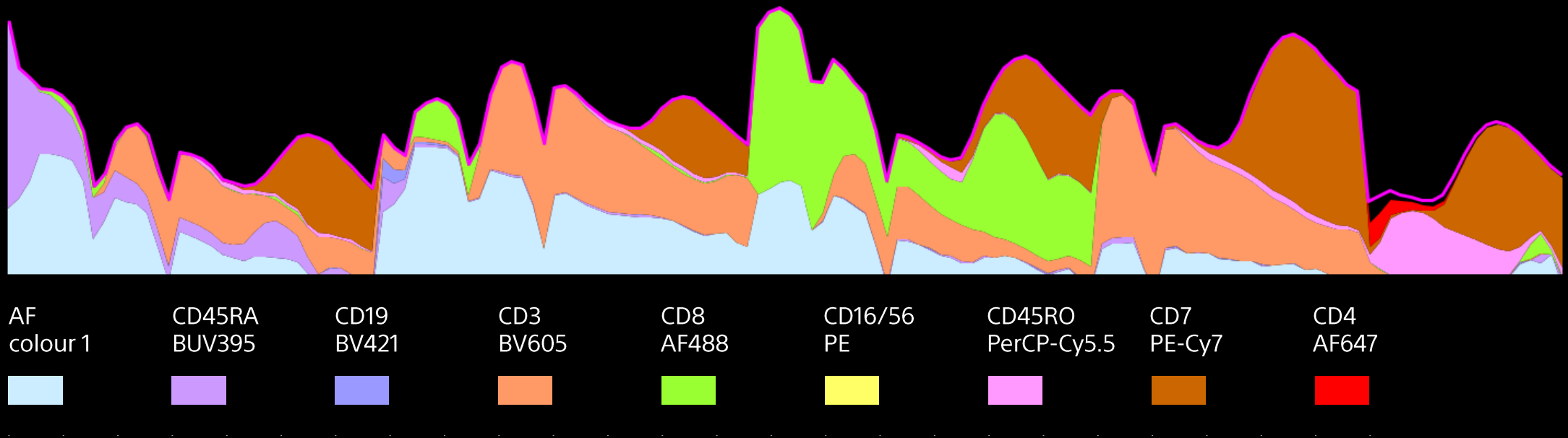

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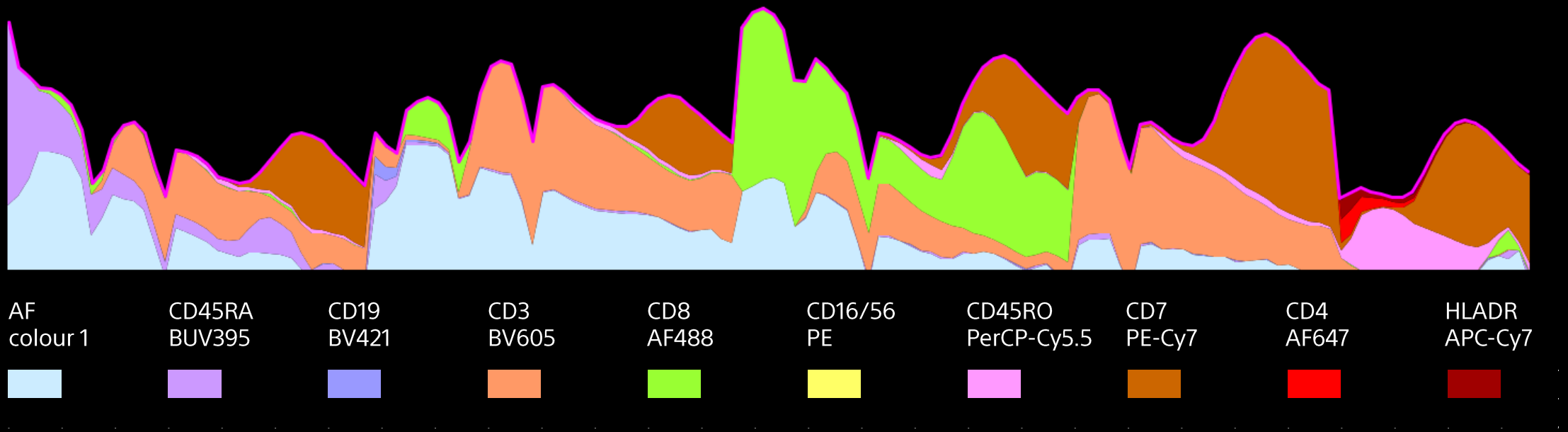

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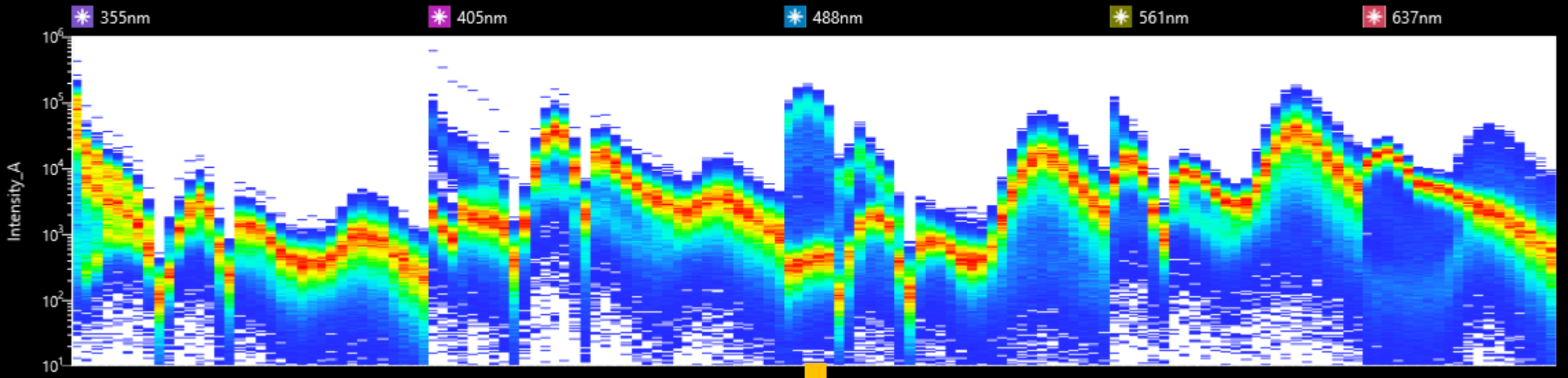
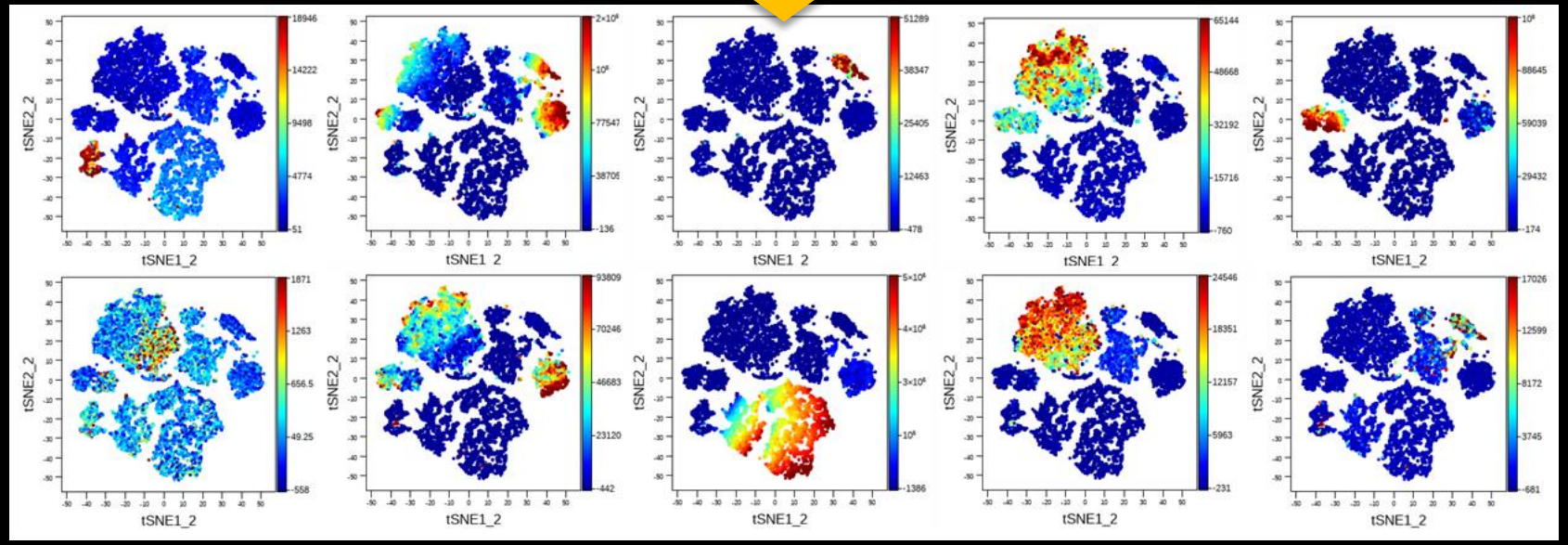
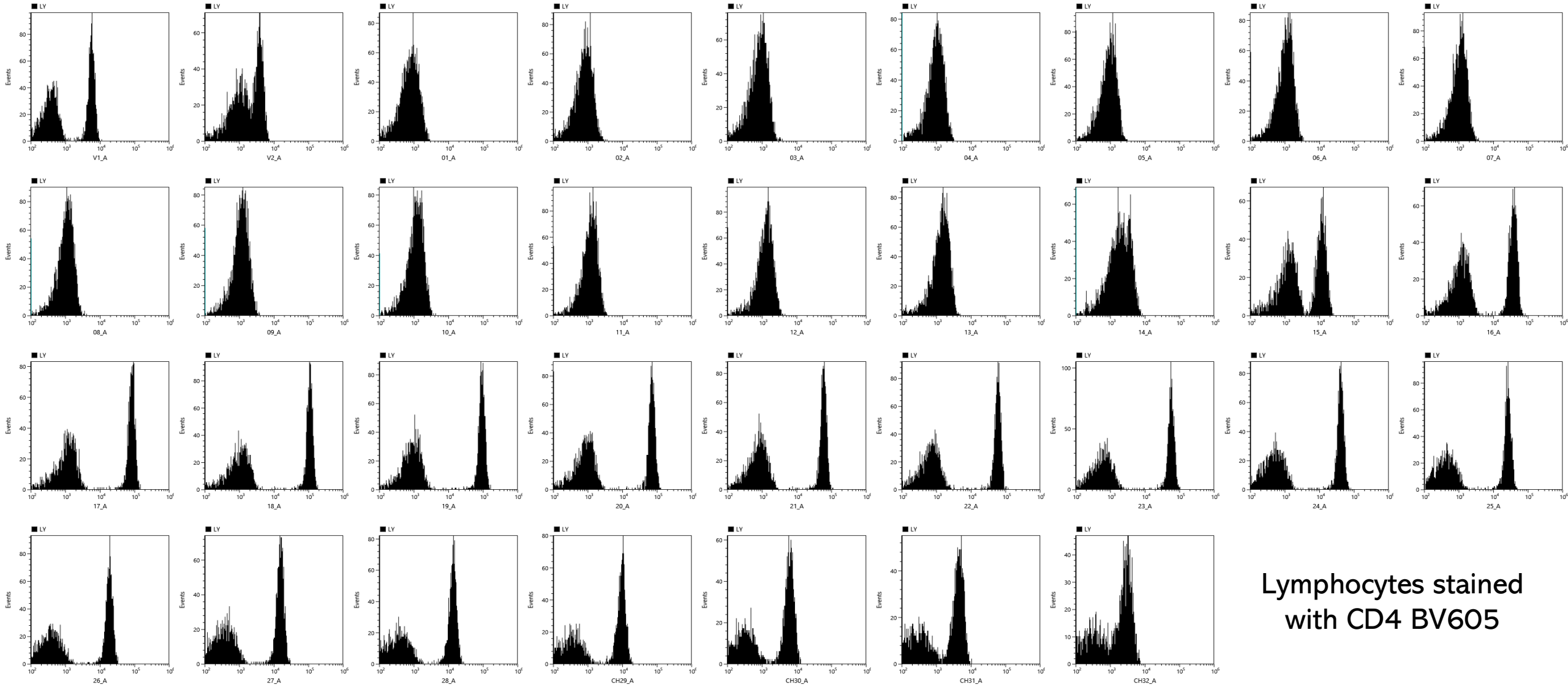
Spectral Data



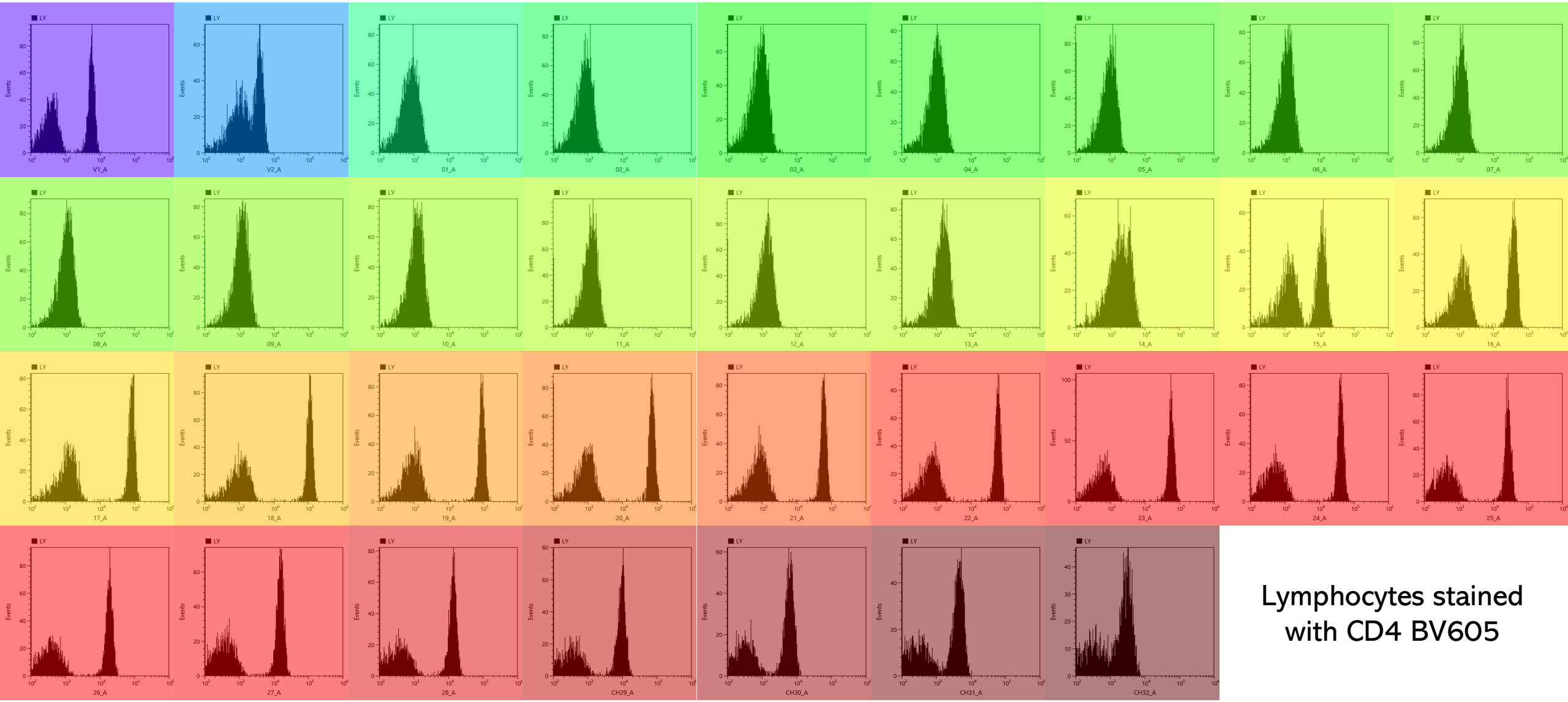


Data from the 405nm laser

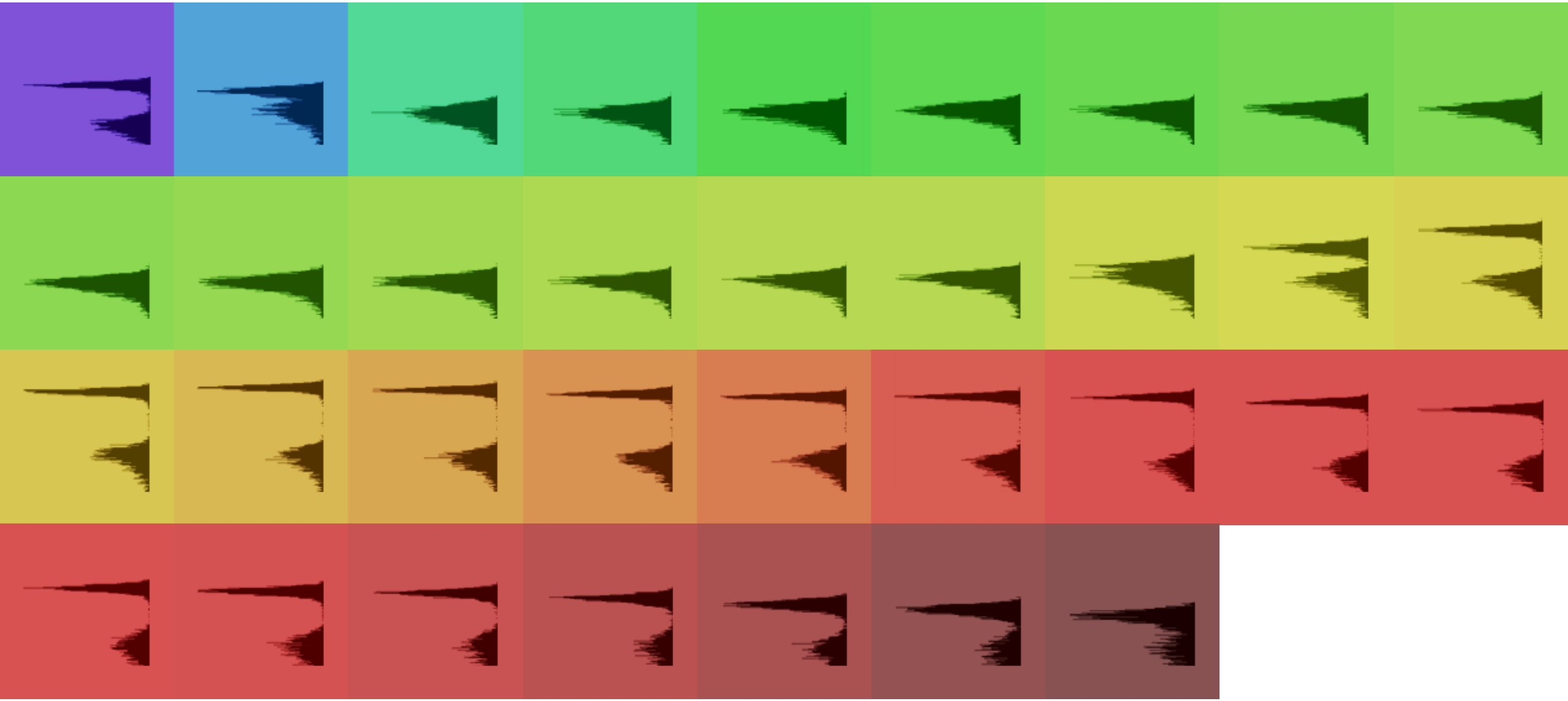


Lymphocytes stained with CD4 BV605

Data from the 405nm laser



Lymphocytes stained with CD4 BV605



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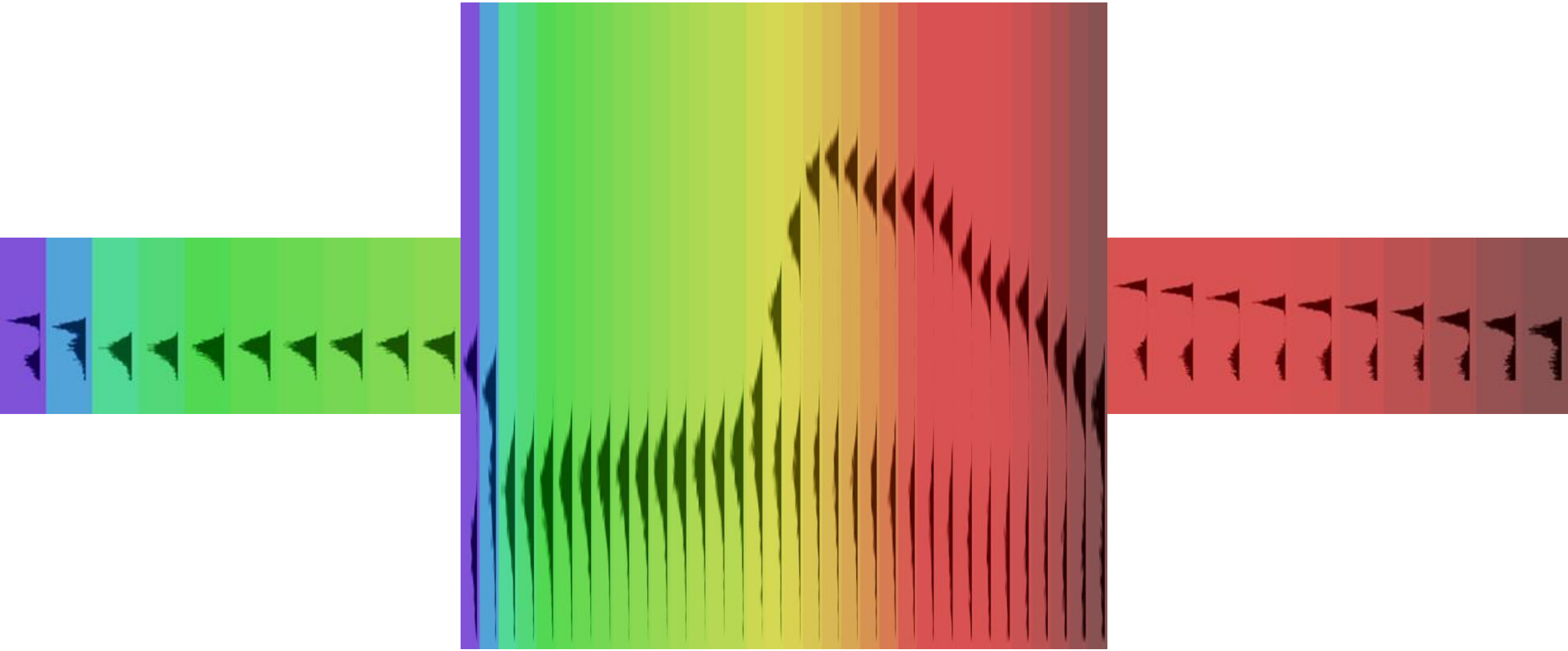

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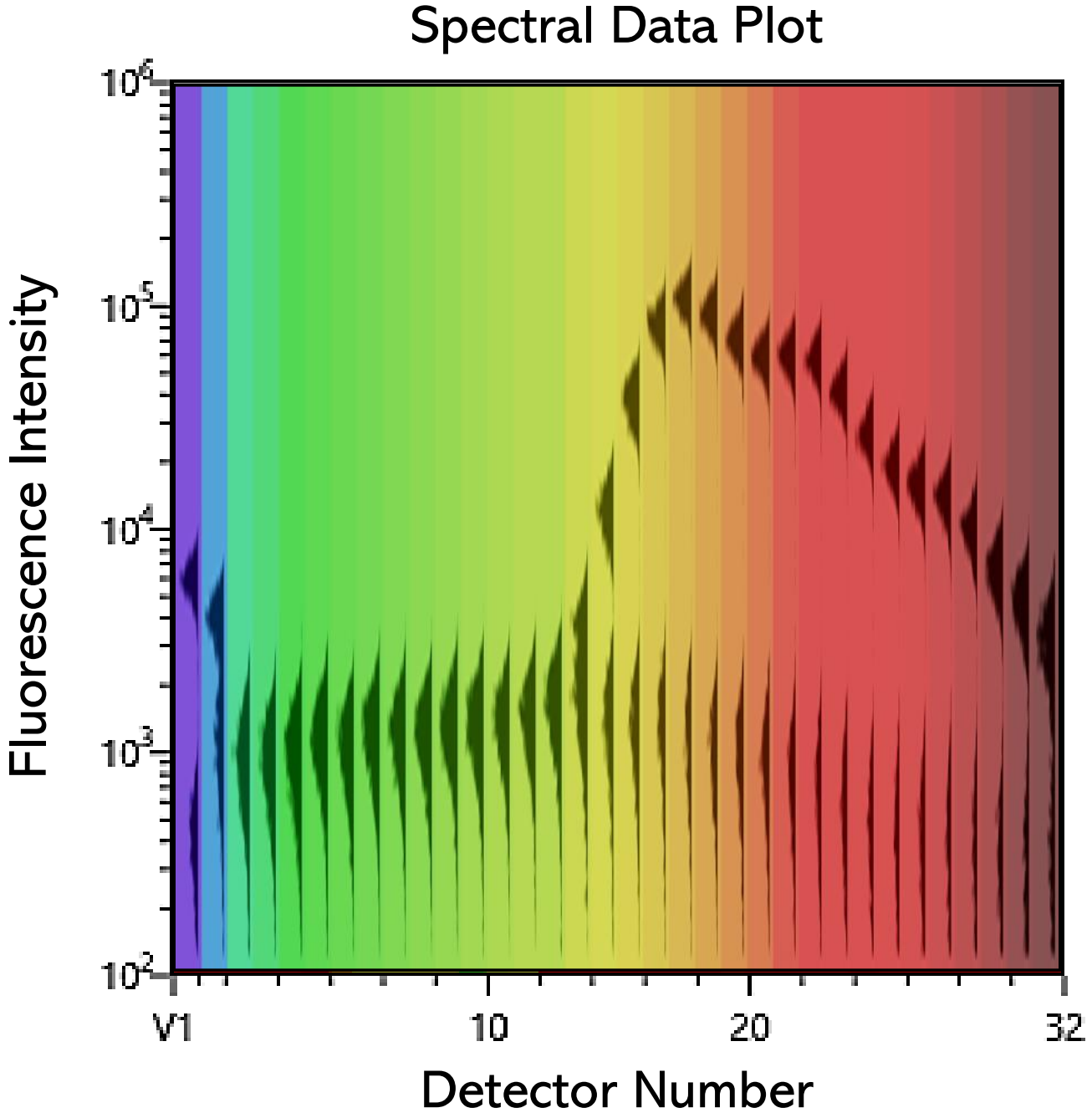
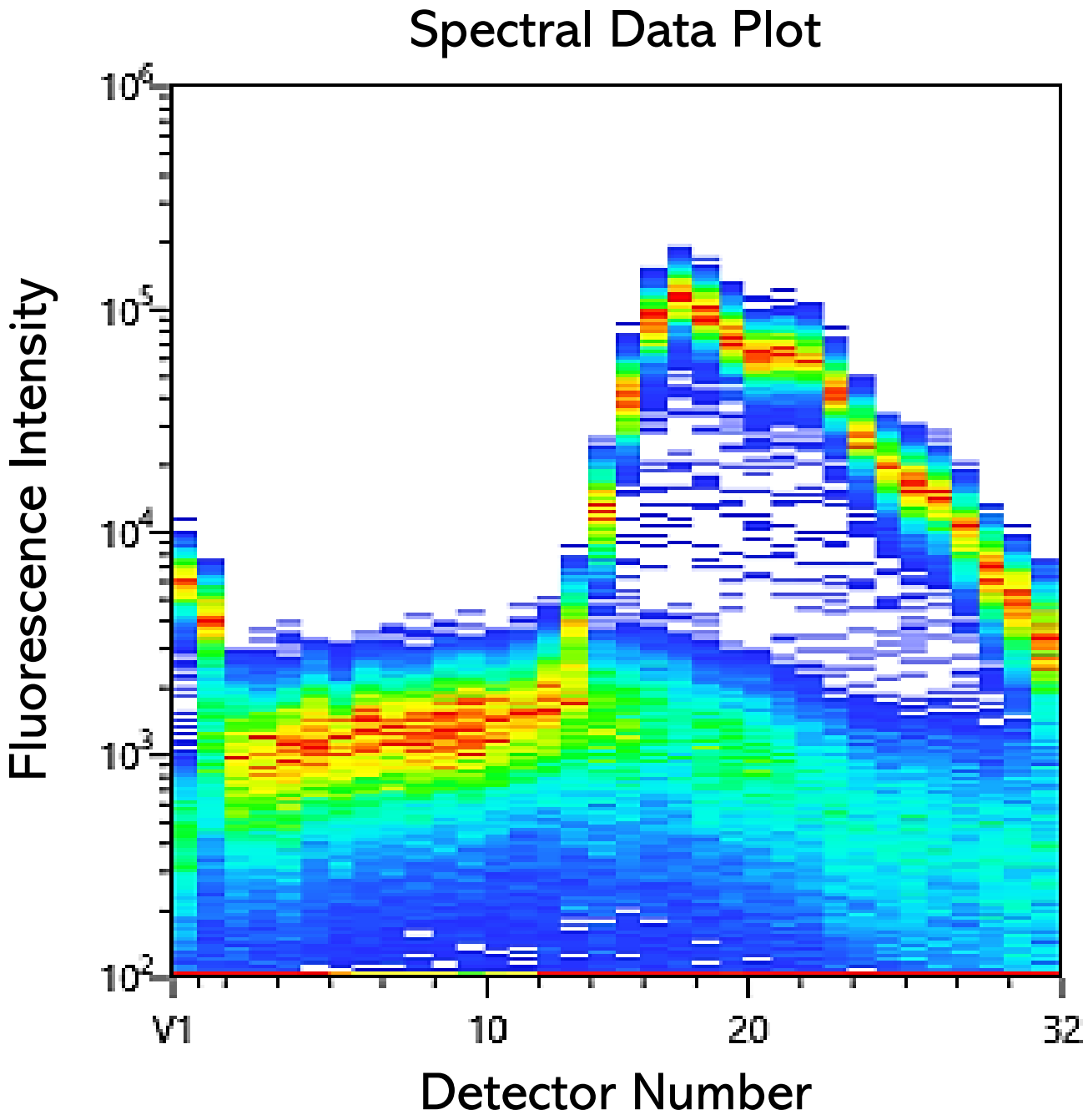

Spectral Data Plot
Fluorescence Intensity
10^6
10^5
10^4
10^3
10^2
V1
10
20
32
Detector Number

SONY


Spectral Data Plot
Fluorescence Intensity
10^6
10^5
10^4
10^3
10^2
V1
10
20
32
Detector Number

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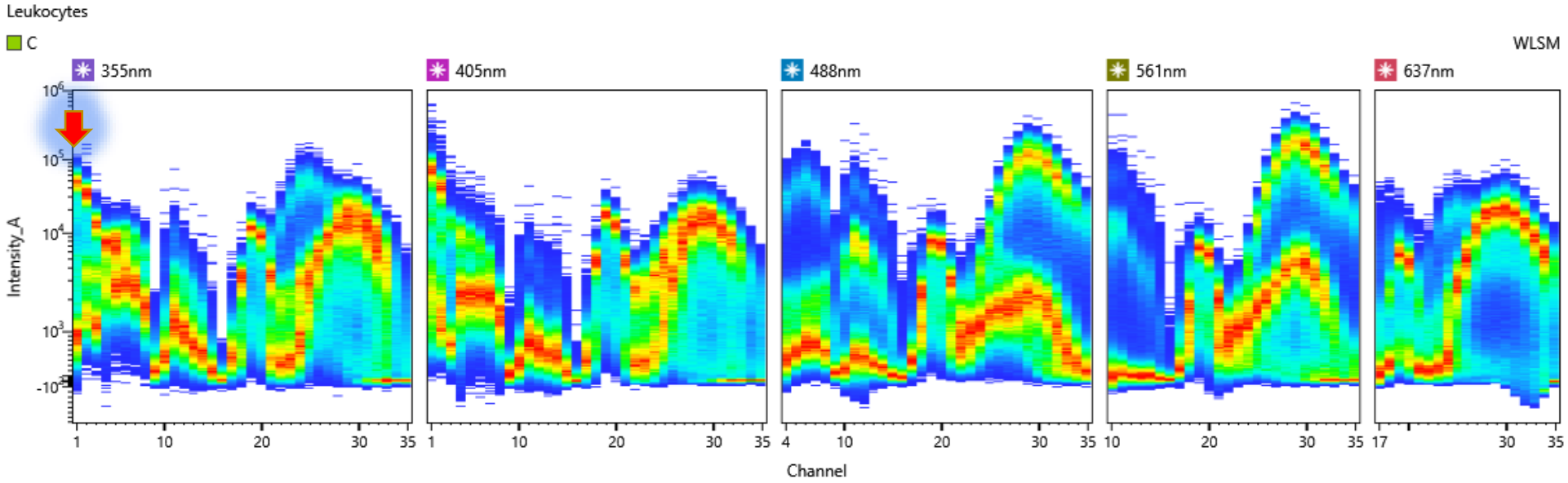
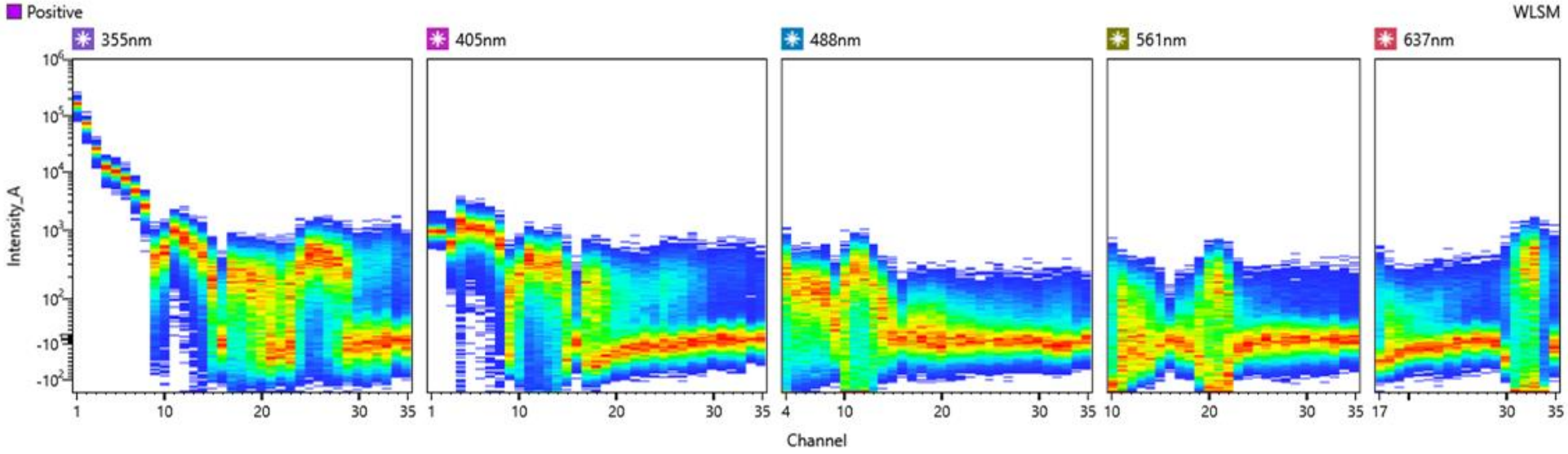
10c PBMCs: Raw Data

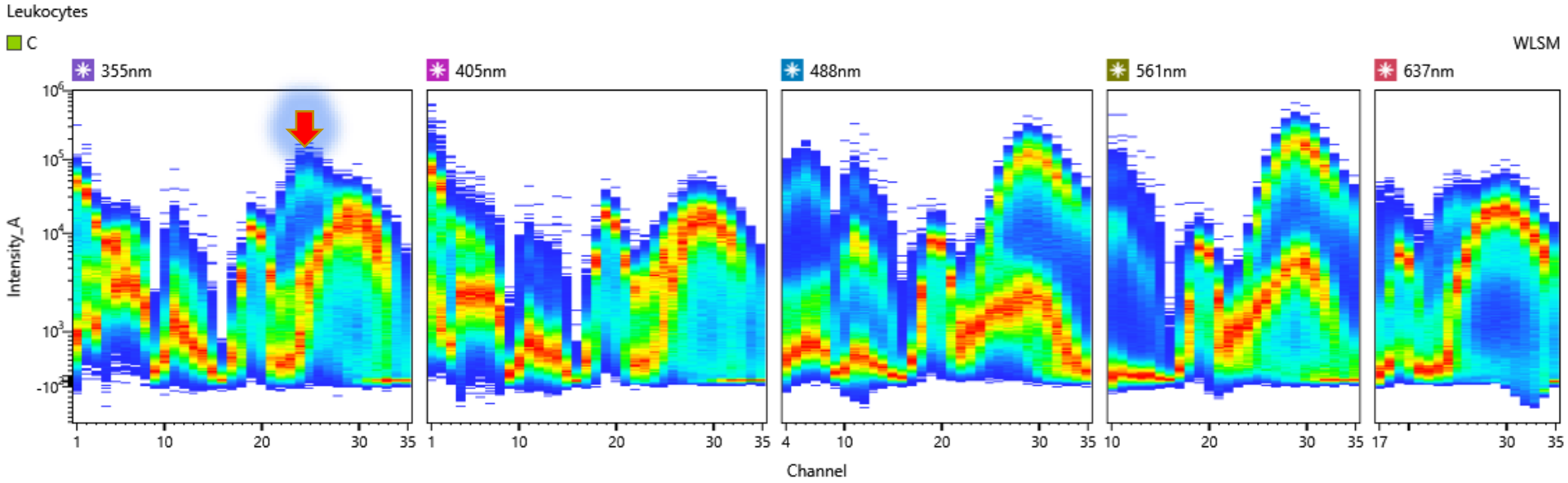


BUV395 - beads

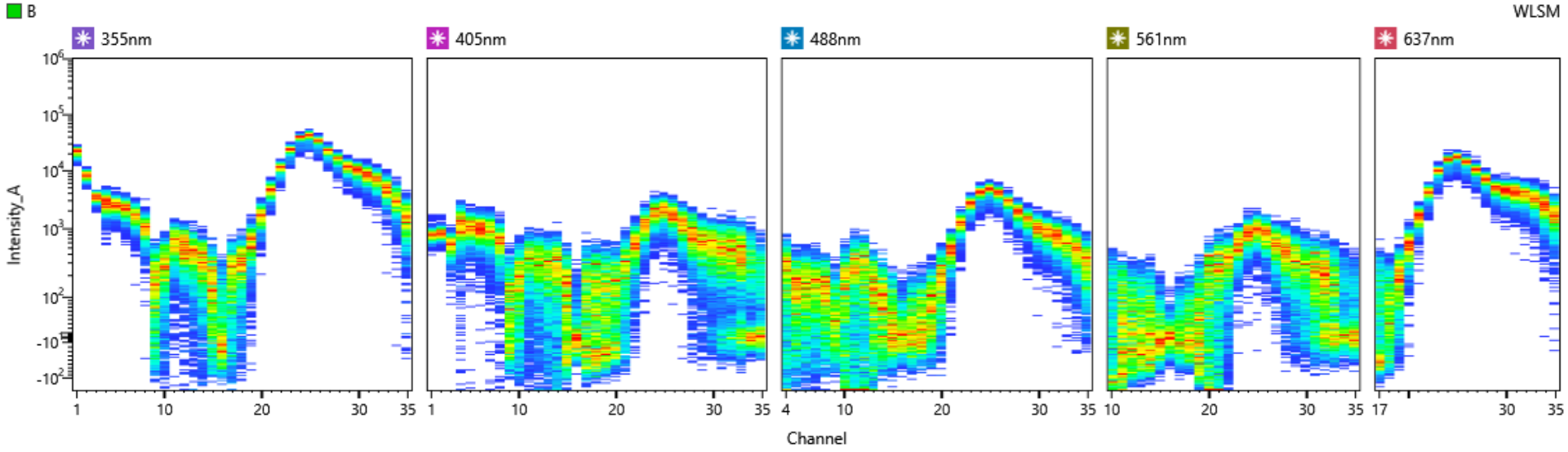


SONY

10c PBMCs: Raw Data

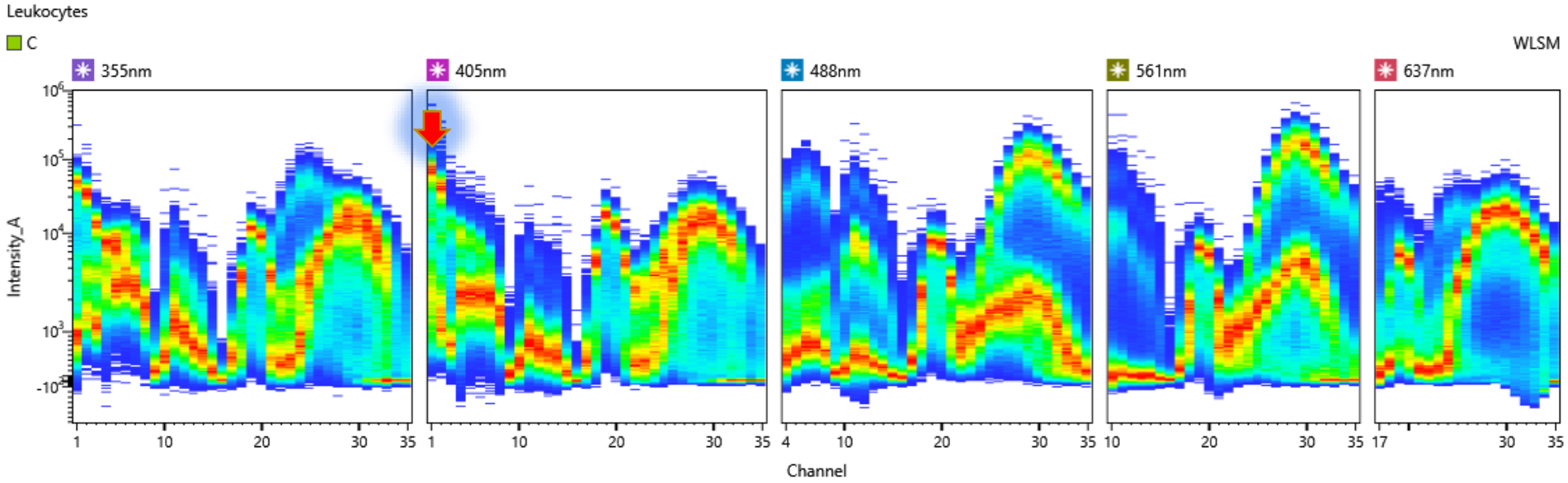


BUV737 - beads

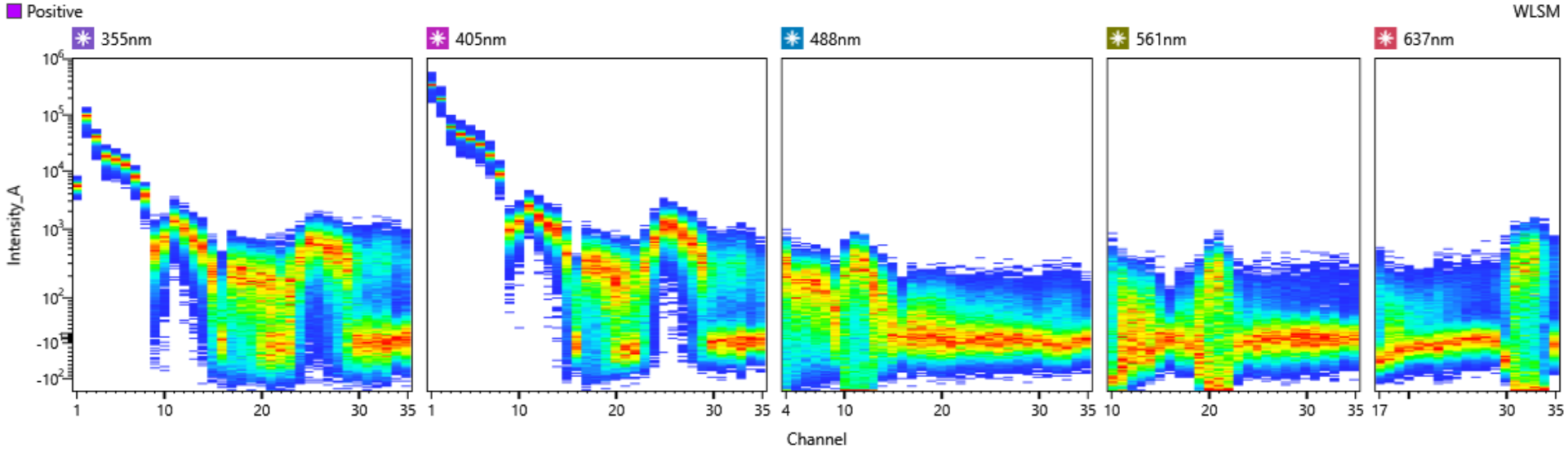


SONY

10c PBMCs: Raw Data

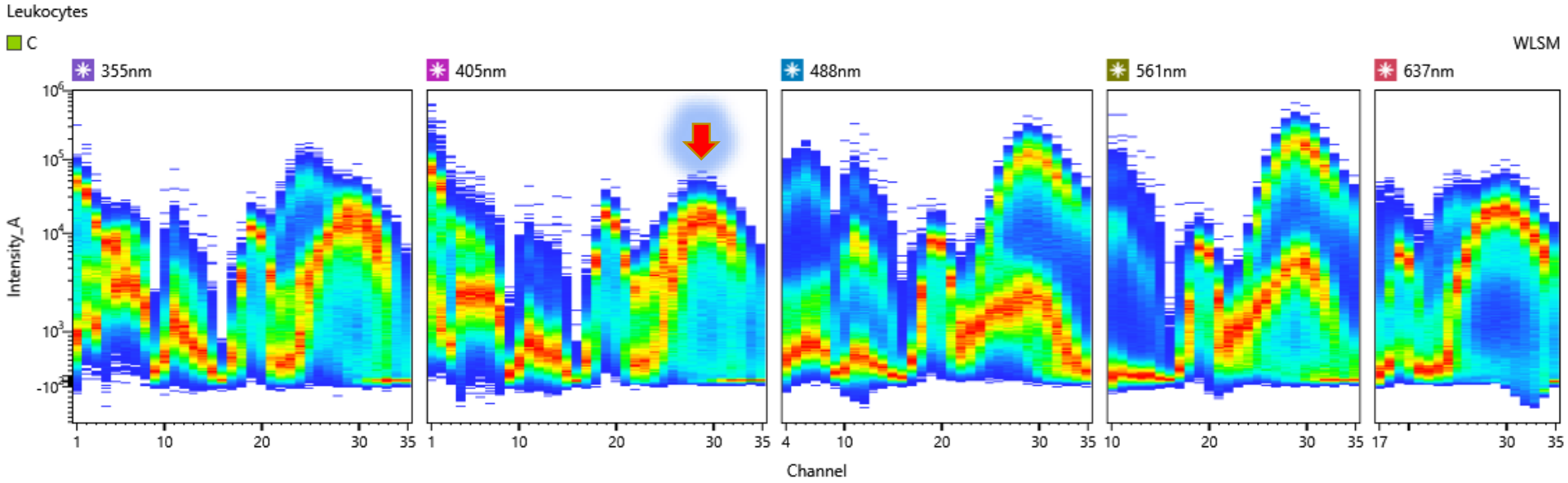


BV421 - beads

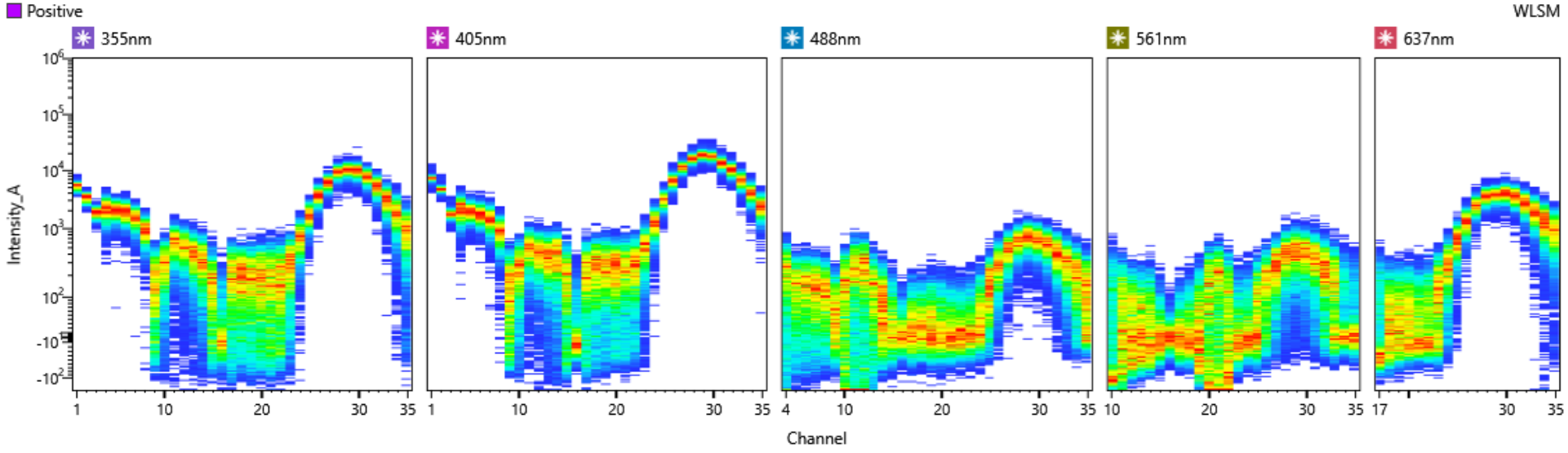


SONY

10c PBMCs: Raw Data

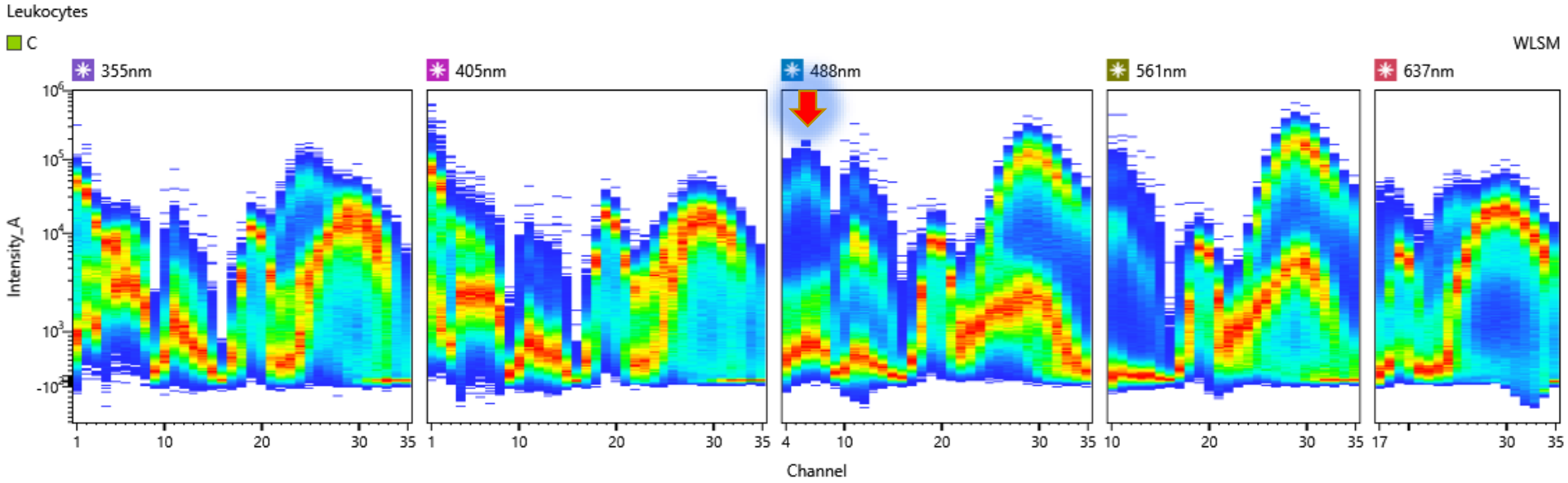


BV785 - beads

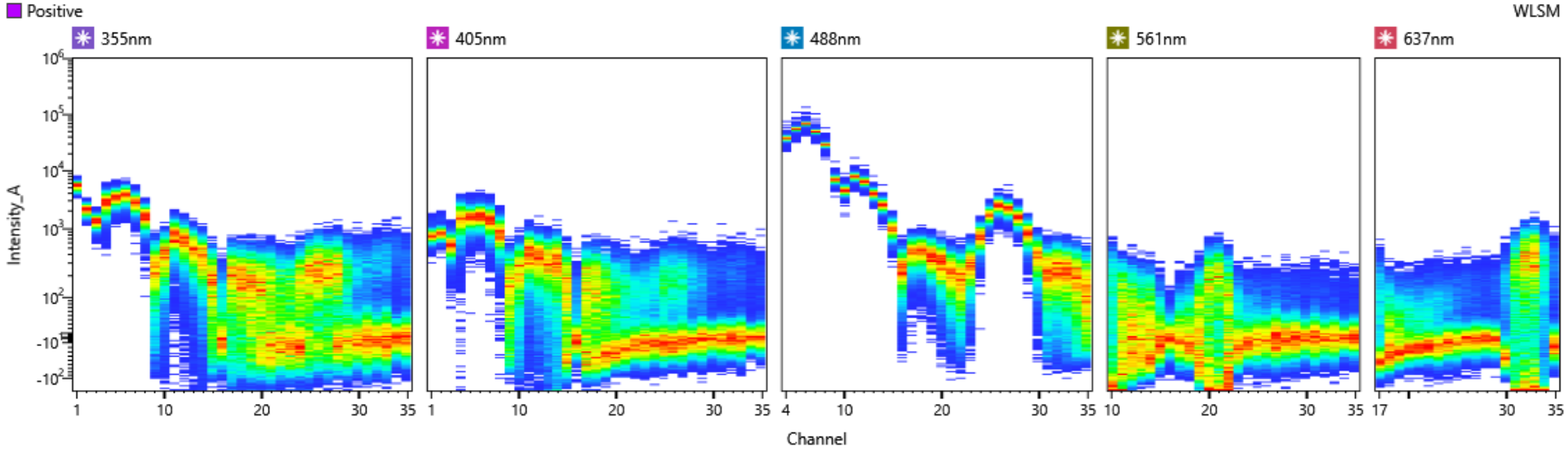


SONY

10c PBMCs: Raw Data

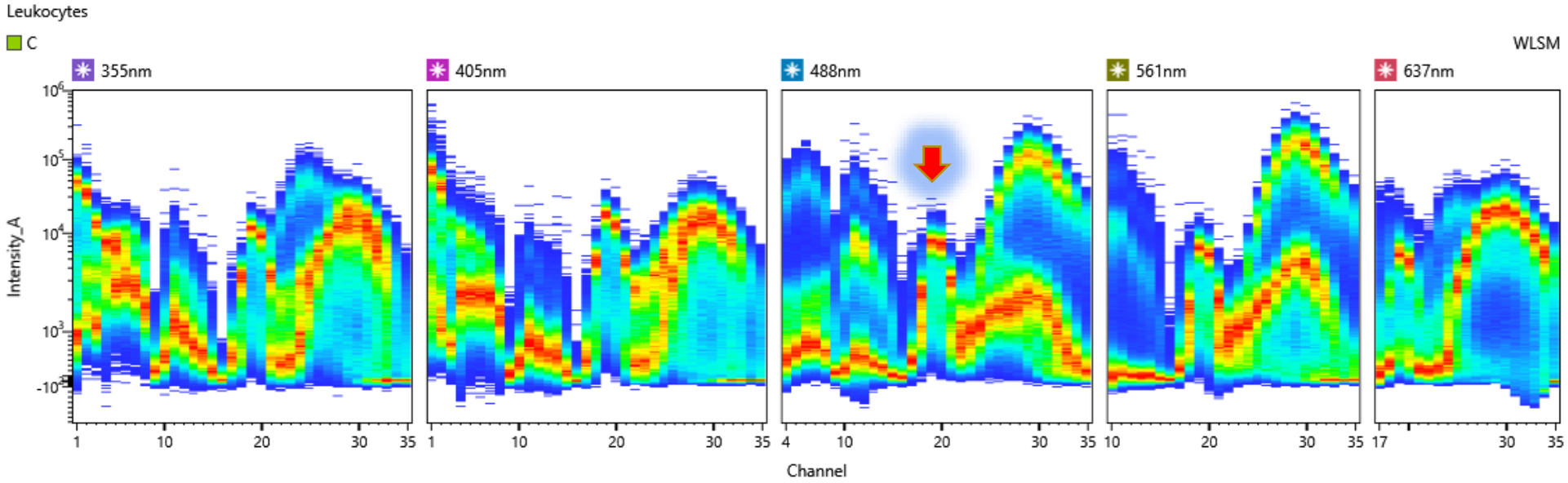


AF488 - beads

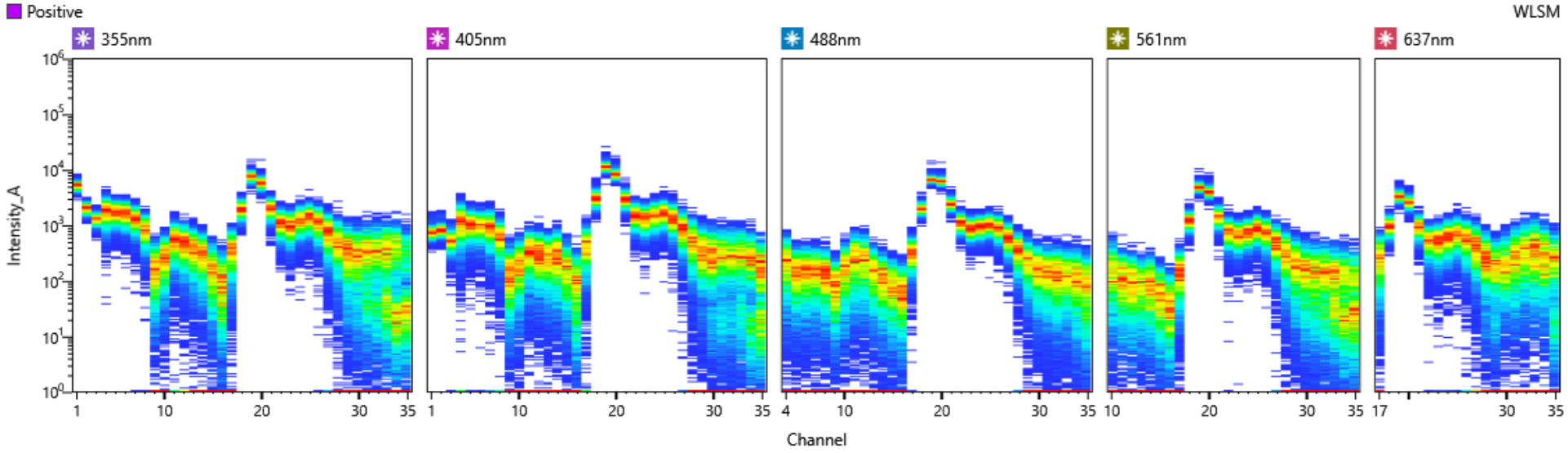


SONY

10c PBMCs: Raw Data

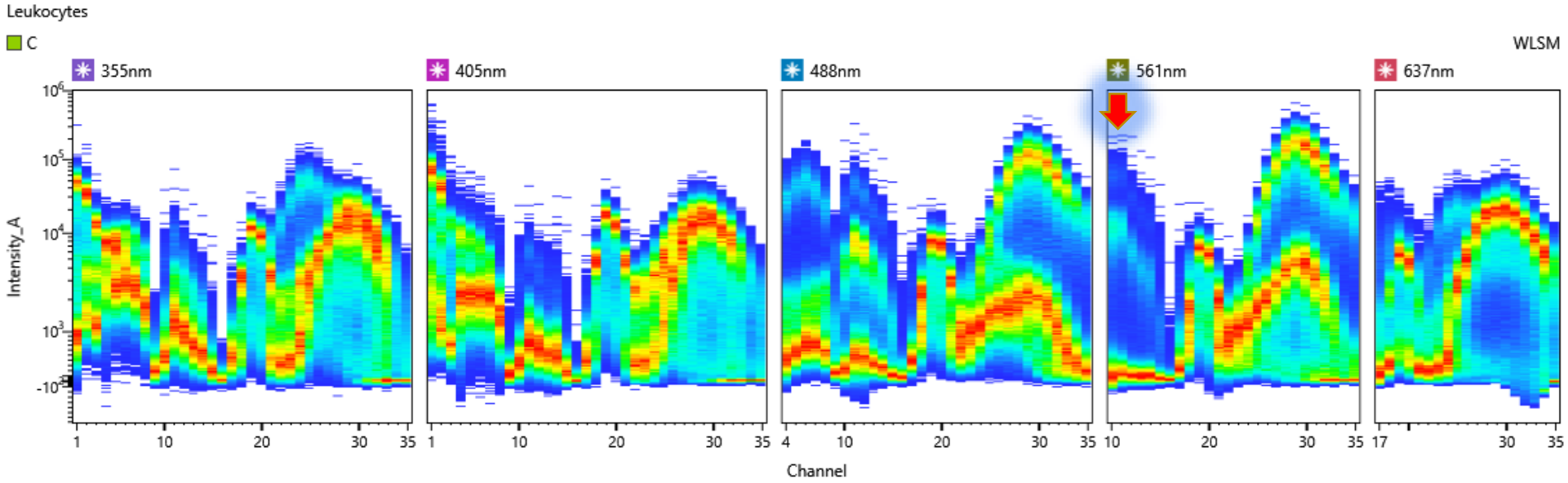


PerCP - beads

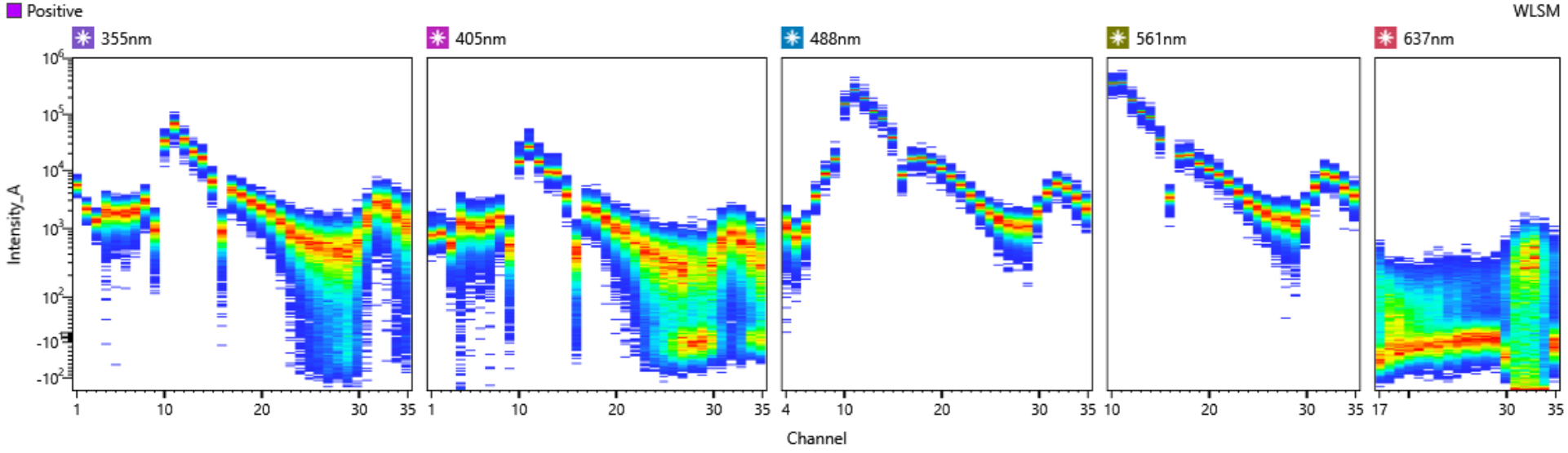


SONY

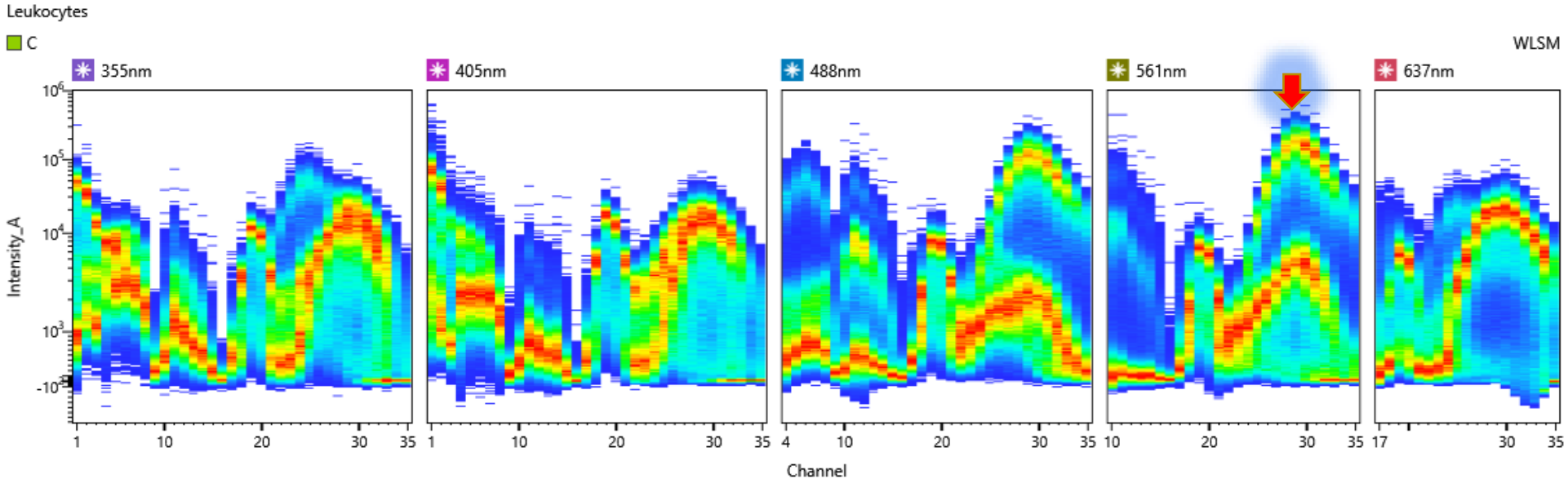
10c PBMCs: Raw Data



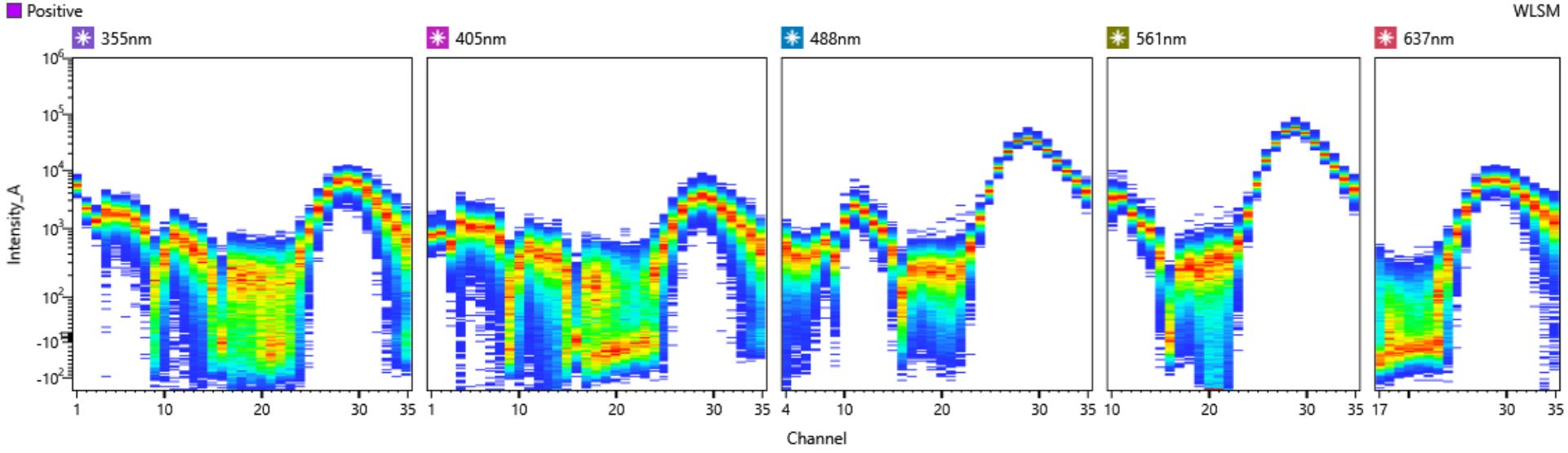
PE - beads



10c PBMCs: Raw Data

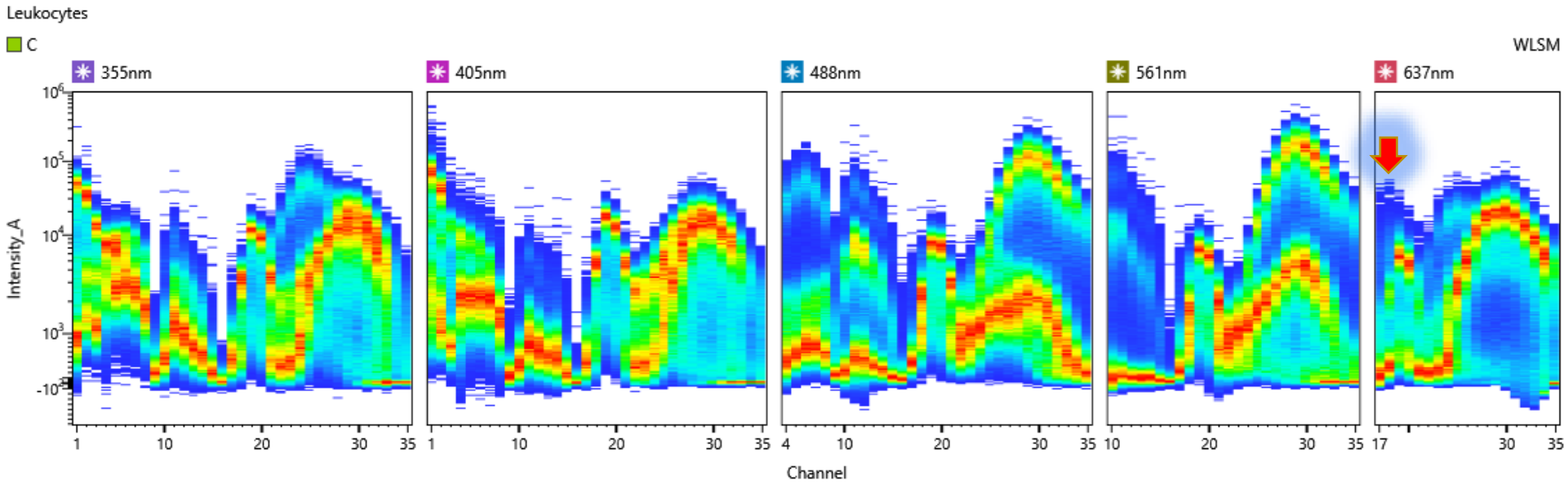


PE-Cy7 - beads

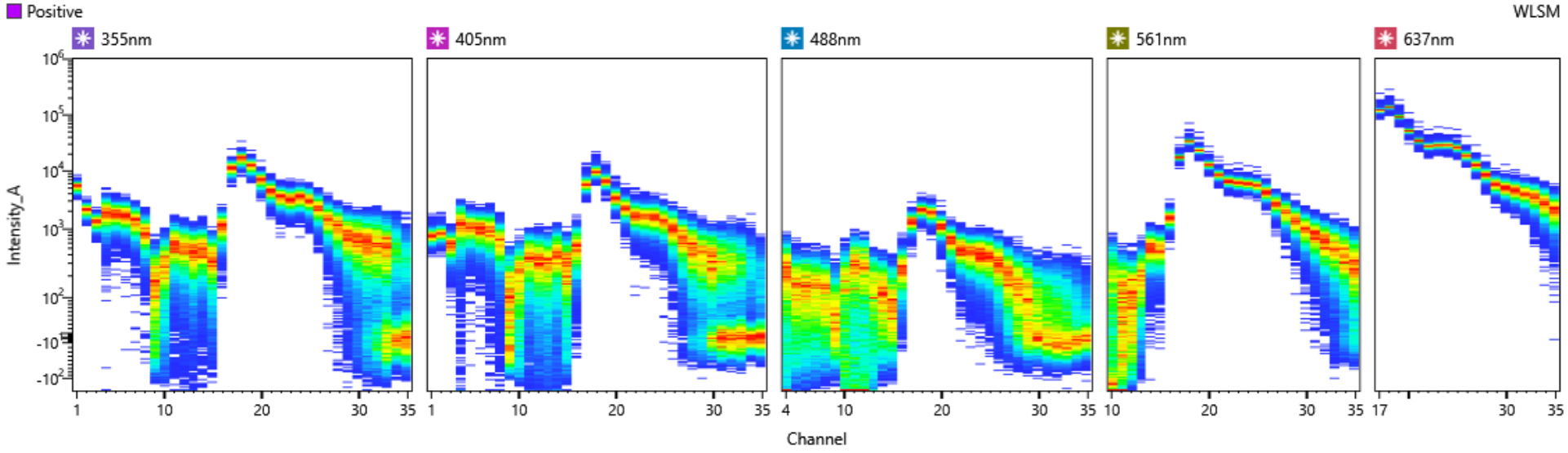


SONY

10c PBMCs: Raw Data

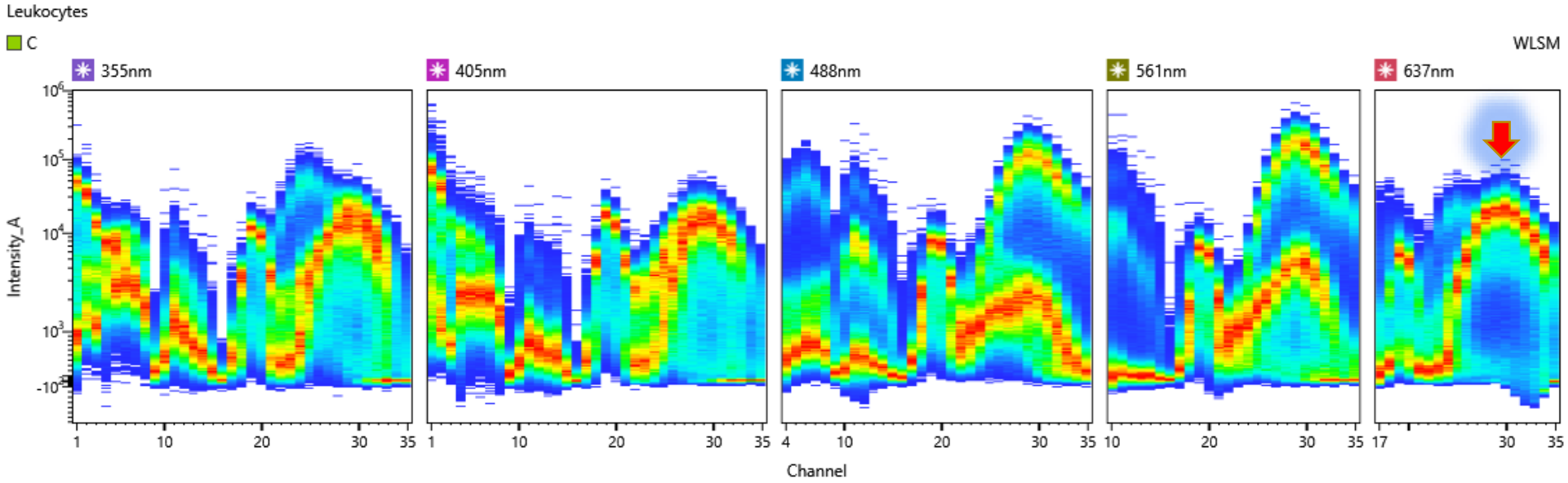


APC - beads

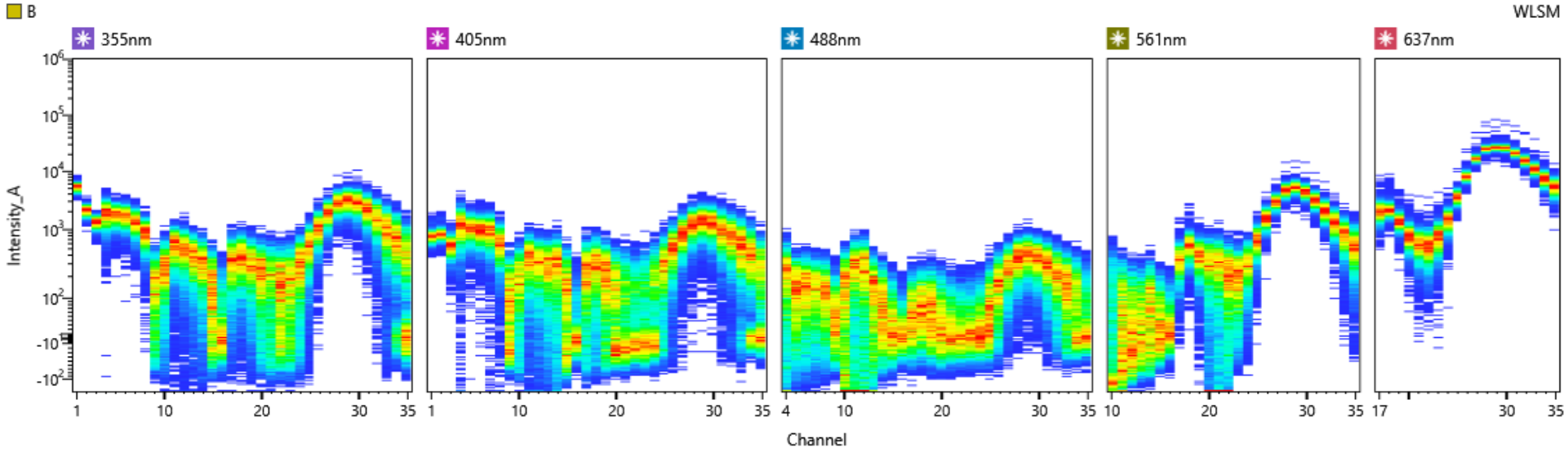


SONY

10c PBMCs: Raw Data

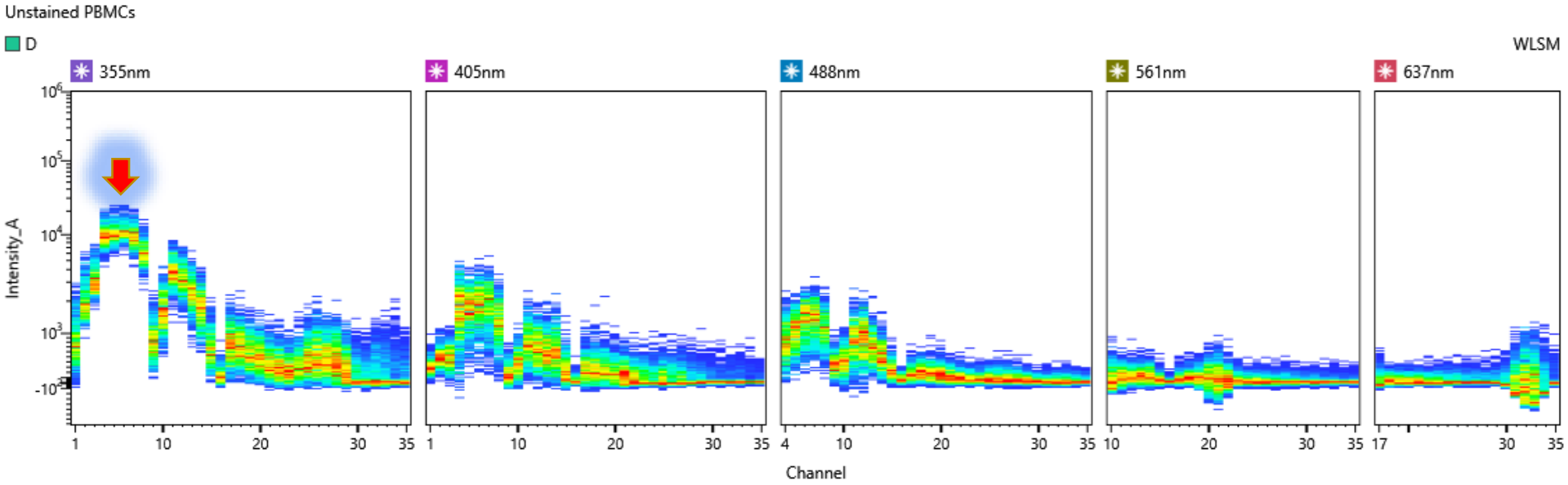


APC-Cy7 - beads

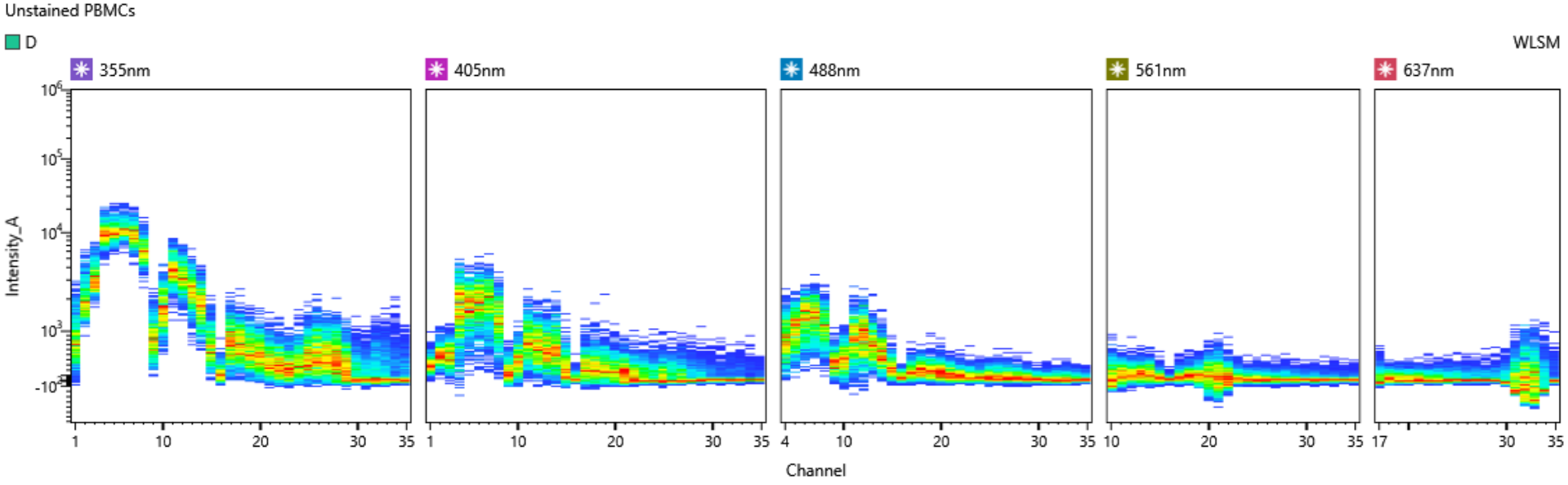


SONY

10c PBMCs: Raw Data



Unstained PBMCs



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

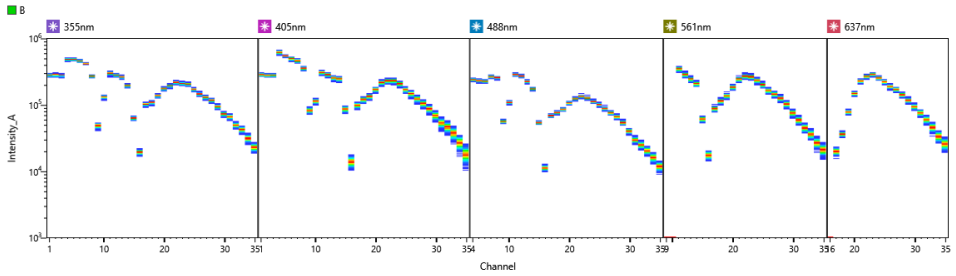
1. QC
2. Experiment
3. Colour Panel (colour + marker)

   or

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

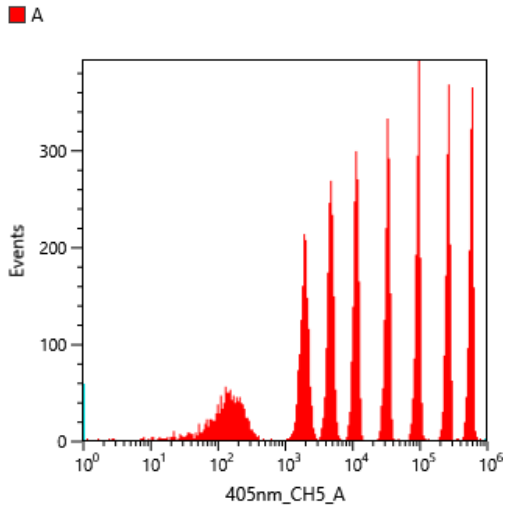
Daily:

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



Performance:

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



TRAINING - [administrator] - ID7000 Software

File | QC | Cytometer

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

QC

QC | Experiment | Acquisition | Analysis | Library

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	⊿rCV	PMT V	⊿PMT 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.

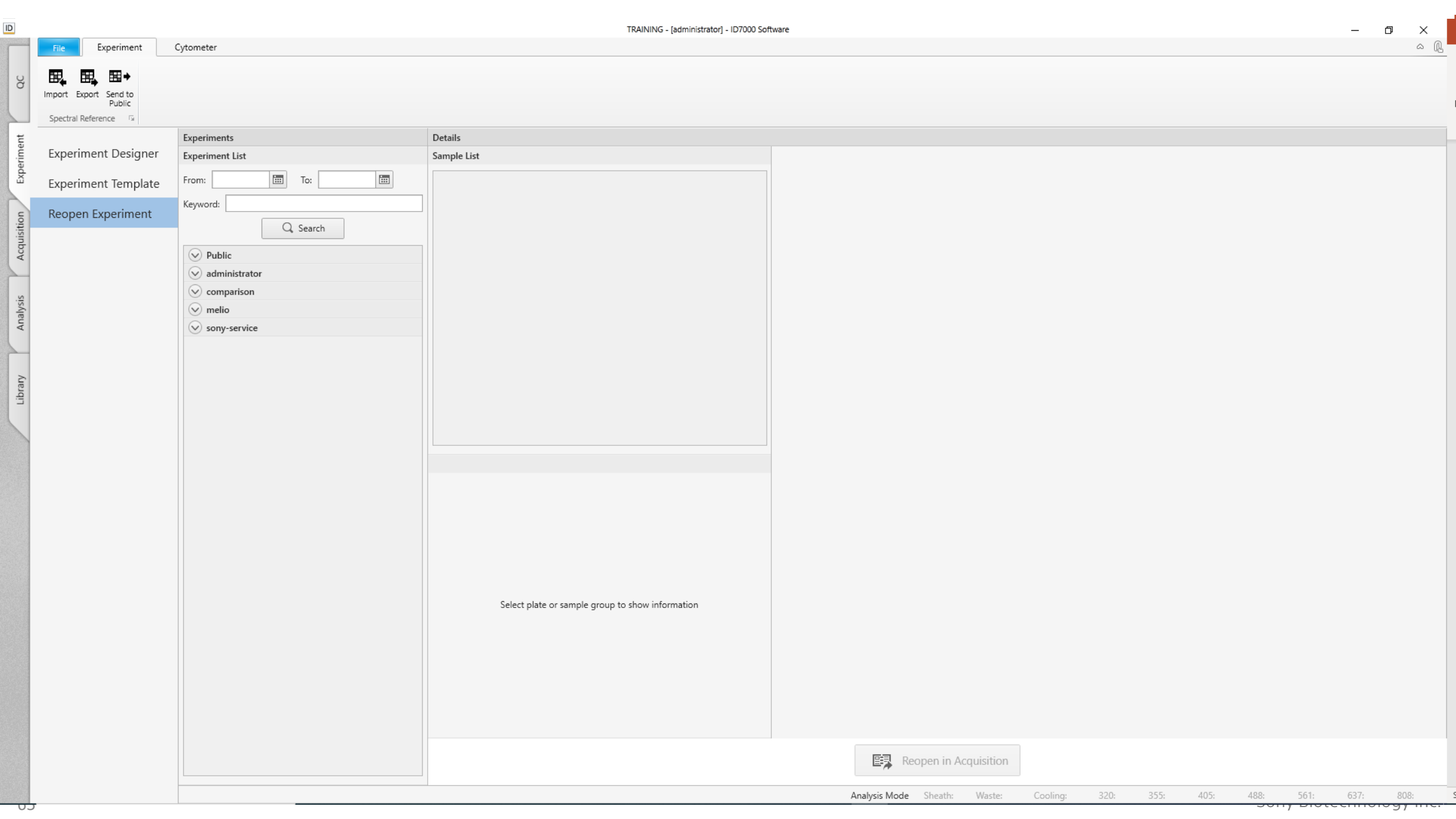
Analysis Mode | Sheath: | Waste: | Cooling: | 320: | 355: | 405: | 488: | 561: | 637: | 808:

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


TRAINING - [administrator] - ID7000 Software
File
Experiment
Cytometer
Import
Export
Send to Public
Spectral Reference
QC
Experiment
Acquisition
Analysis
Library
Experiment Designer
Experiment Template
Reopen Experiment
Experiments
Experiment List
From:
To:
Keyword:
Search
Public
administrator
comparison
melio
sony-service
Details
Sample List
Select plate or sample group to show information
Reopen in Acquisition
Analysis Mode
Sheath:
Waste:
Cooling:
320:
355:
405:
488:
561:
637:
808:

ID7000 Workflow

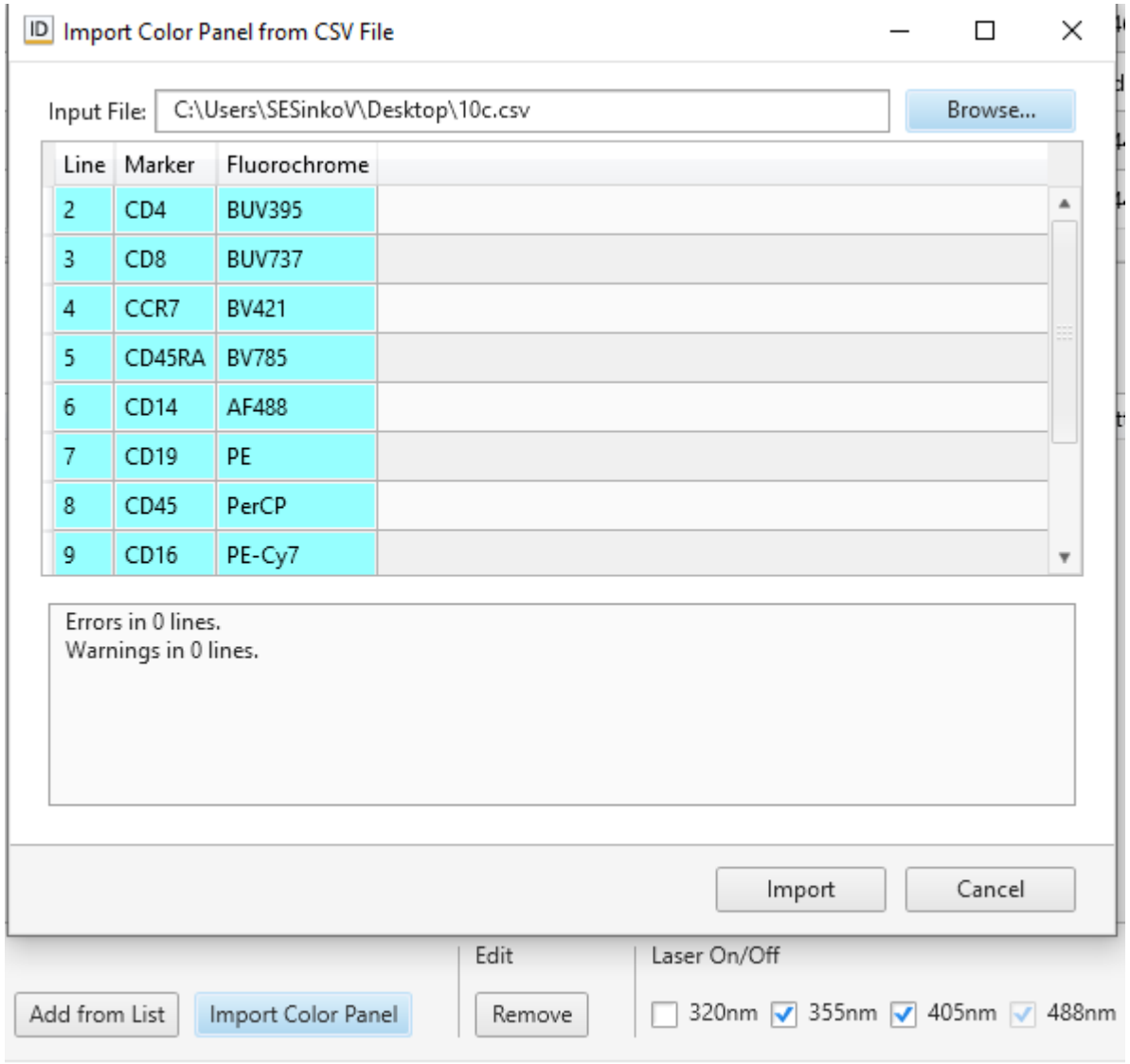
1. QC
2. Experiment
3. Colour Panel (colour + marker)

or

Reference Spectra

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

From .csv



ID7000 Workflow

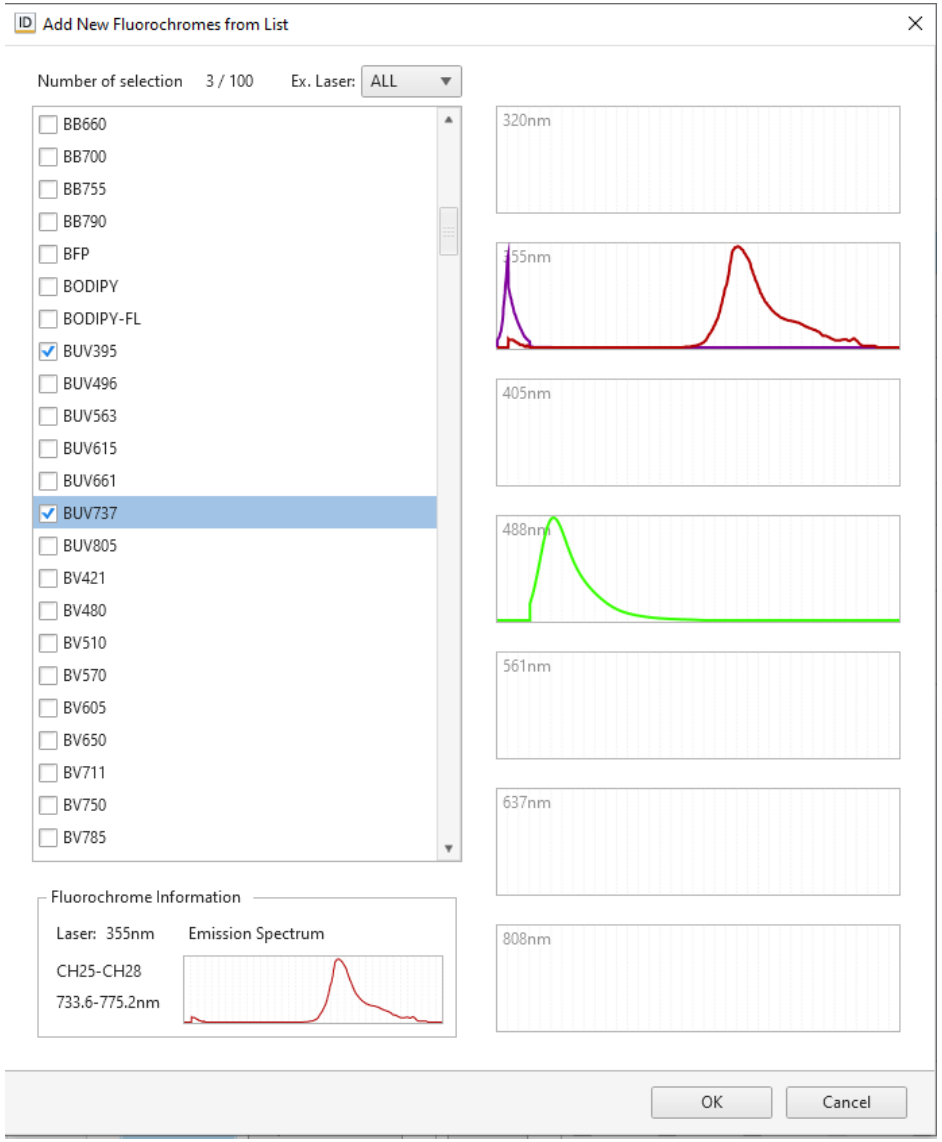
1. QC
2. Experiment
3. Colour Panel (colour + marker)

or

Reference Spectra

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

From the List



ID7000 Workflow

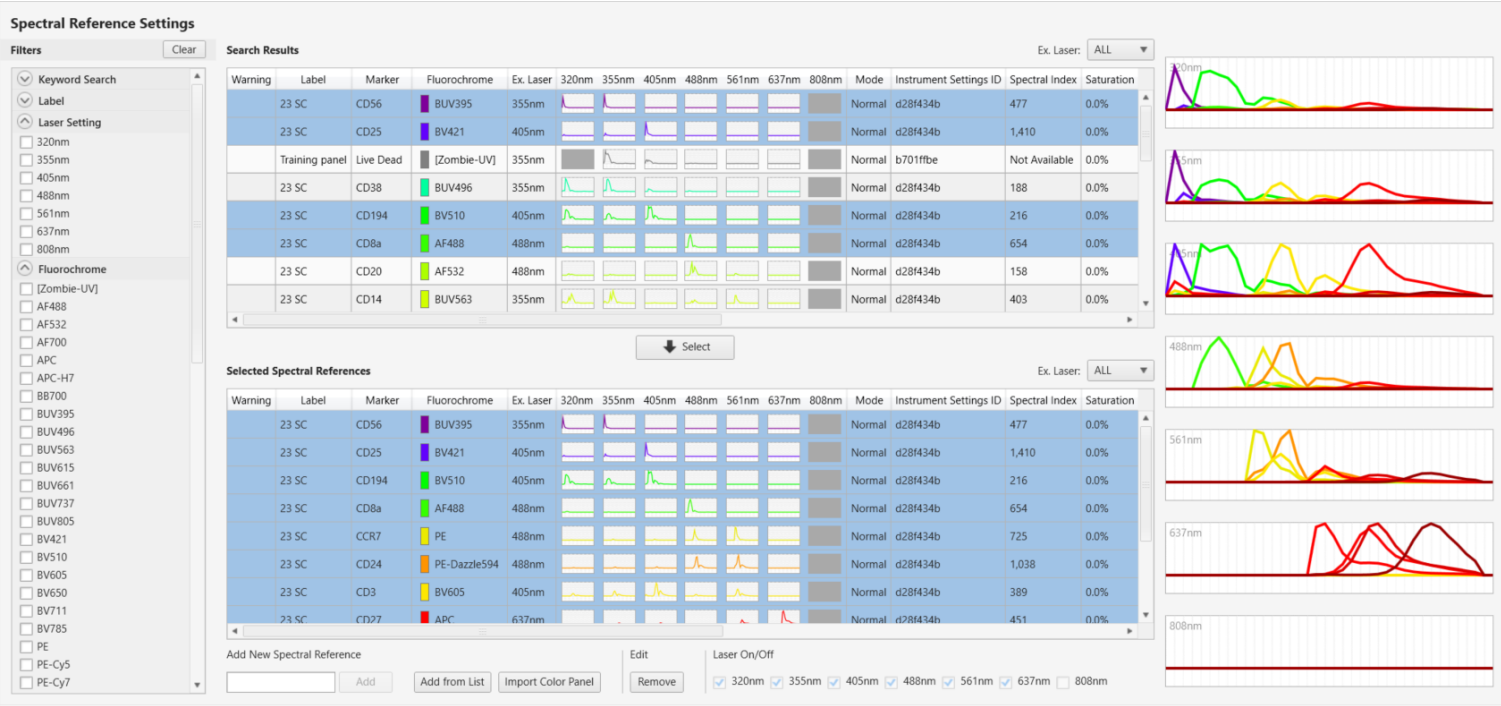
1. QC
2. Experiment
3. Colour Panel (colour + marker)

or

Reference Spectra

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

From the library

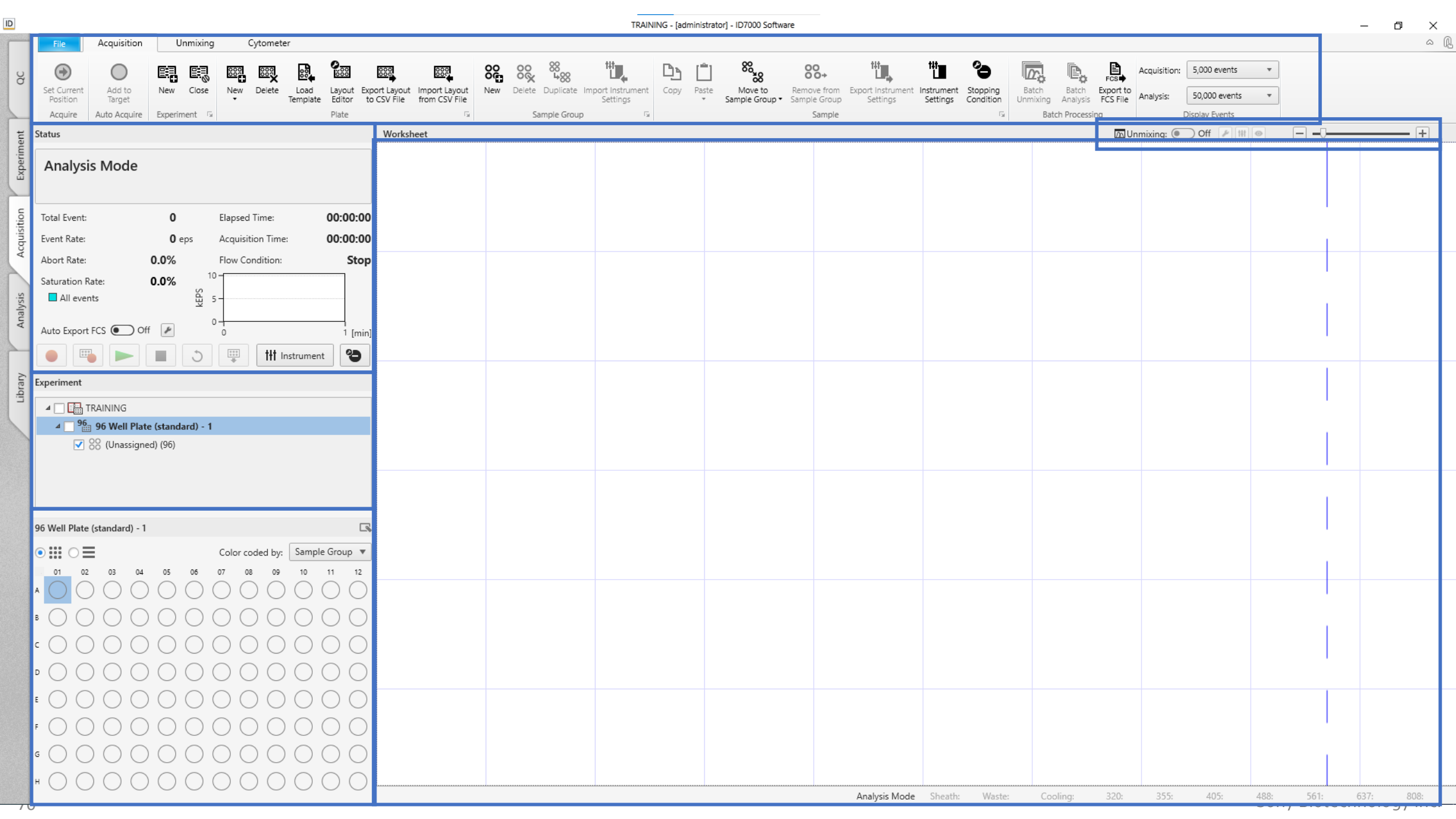


ID7000 Workflow

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

   or

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


TRAINING - [administrator] - ID7000 Software
File
Acquisition
Unmixing
Cytometer
Set Current Position
Add to Target
New
Close
New
Delete
Load Template
Layout Editor
Export Layout to CSV File
Import Layout from CSV File
New
Delete
Duplicate
Import Instrument Settings
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
Acquisition: 5,000 events
Analysis: 50,000 events
Acquire
Auto Acquire
Experiment
Plate
Sample Group
Sample
Batch Processing
Display Events
QC
Experiment
Acquisition
Analysis
Library
Status
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
kEPS
10
5
0
0
1 [min]
Auto Export FCS Off
Instrument
Experiment
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
Unmixing: Off
Analysis Mode
Sheath:
Waste:
Cooling:
320:
355:
405:
488:
561:
637:
808:

TRAINING - [administrator] - ID7000 Software

File | Acquisition | Unmixing | Cytometer

Set Current Position | Add to Target | New | Close | New | Delete | Load Template | Layout Editor | Export Layout to CSV File | Import Layout from CSV File | New | Delete | Duplicate | Import Instrument Settings | 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

Acquire | Auto Acquire | Experiment | Sample Group | Sample | Batch Processing | Display Events

Acquisition: 5,000 events
Analysis: 50,000 events

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

QC | Experiment | Acquisition | Analysis | Library

Status

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

Instrument

Experiment

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

96 Well Plate (standard) - 1

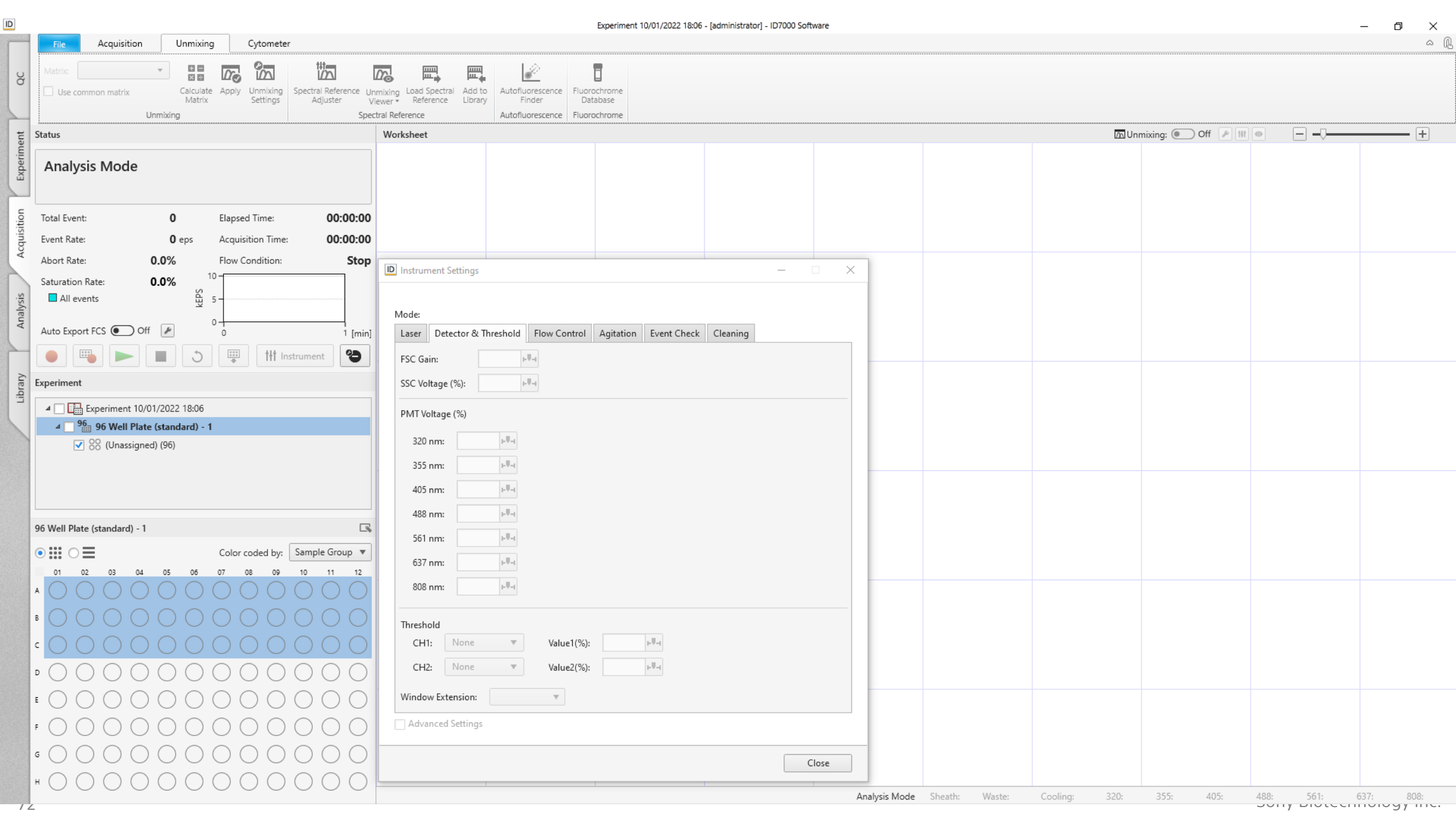
Color coded by: Sample Group

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

Worksheet

Unmixing: Off

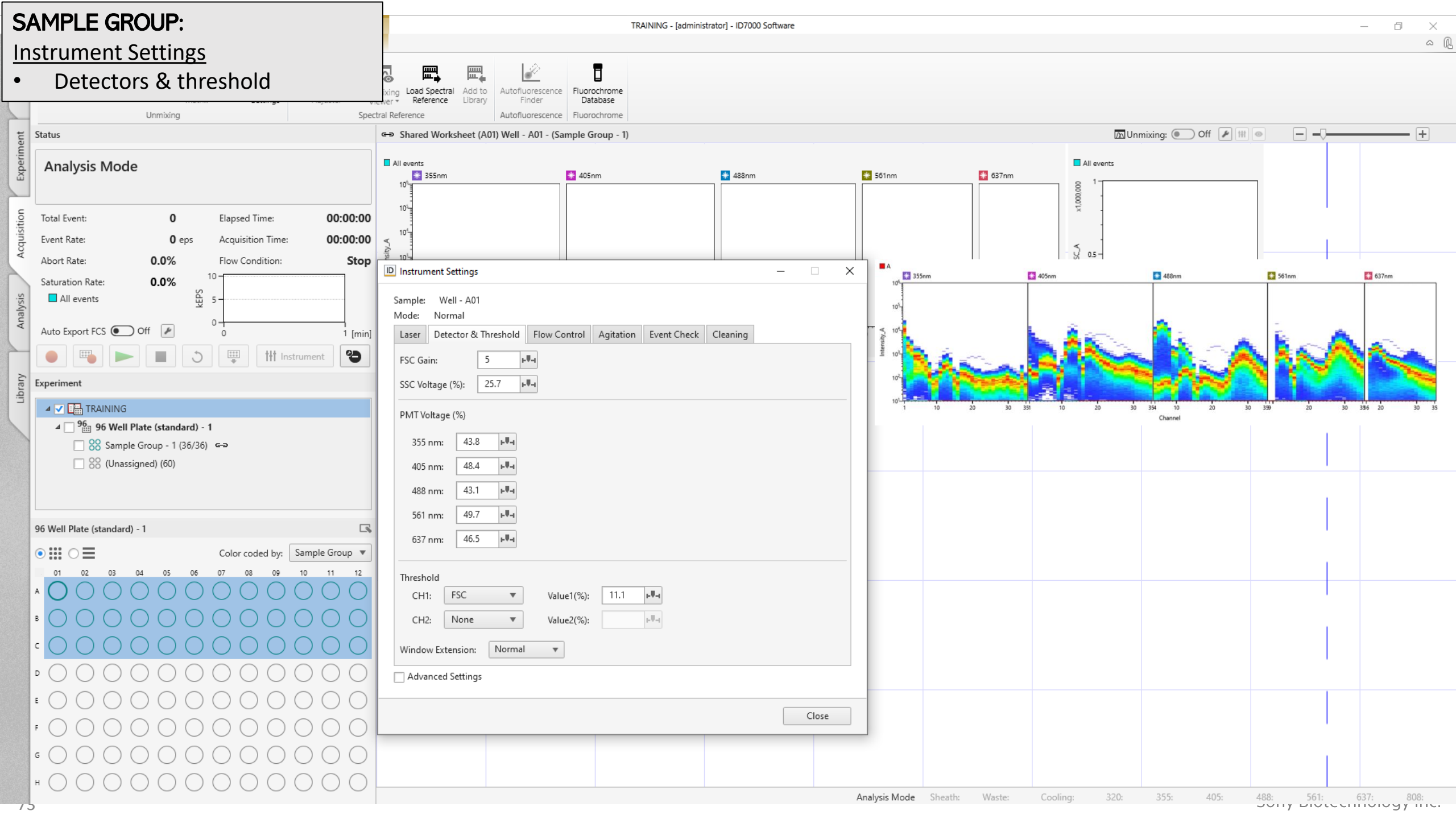
Analysis Mode | Sheath: | Waste: | Cooling: | 320: | 355: | 405: | 488: | 561: | 637: | 808:


Experiment 10/01/2022 18:06 - [administrator] - ID7000 Software
File
Acquisition
Unmixing
Cytometer
Matrix:
Use common matrix
Calculate Matrix
Apply
Unmixing Settings
Unmixing
Spectral Reference Adjuster
Unmixing Viewer
Load Spectral Reference
Add to Library
Spectral Reference
Autofluorescence Finder
Autofluorescence
Fluorochrome Database
Fluorochrome
QC
Experiment
Acquisition
Analysis
Library
Status
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
kEPS
10
5
0
0
1 [min]
Auto Export FCS Off
Instrument
Experiment
Experiment 10/01/2022 18:06
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
Unmixing: Off
Instrument Settings
Mode:
Laser
Detector & Threshold
Flow Control
Agitation
Event Check
Cleaning
FSC Gain:
SSC Voltage (%):
PMT Voltage (%)
320 nm:
355 nm:
405 nm:
488 nm:
561 nm:
637 nm:
808 nm:
Threshold
CH1: None
Value1(%):
CH2: None
Value2(%):
Window Extension:
Advanced Settings
Close
Analysis Mode
Sheath:
Waste:
Cooling:
320:
355:
405:
488:
561:
637:
808:

SAMPLE GROUP:

Instrument Settings

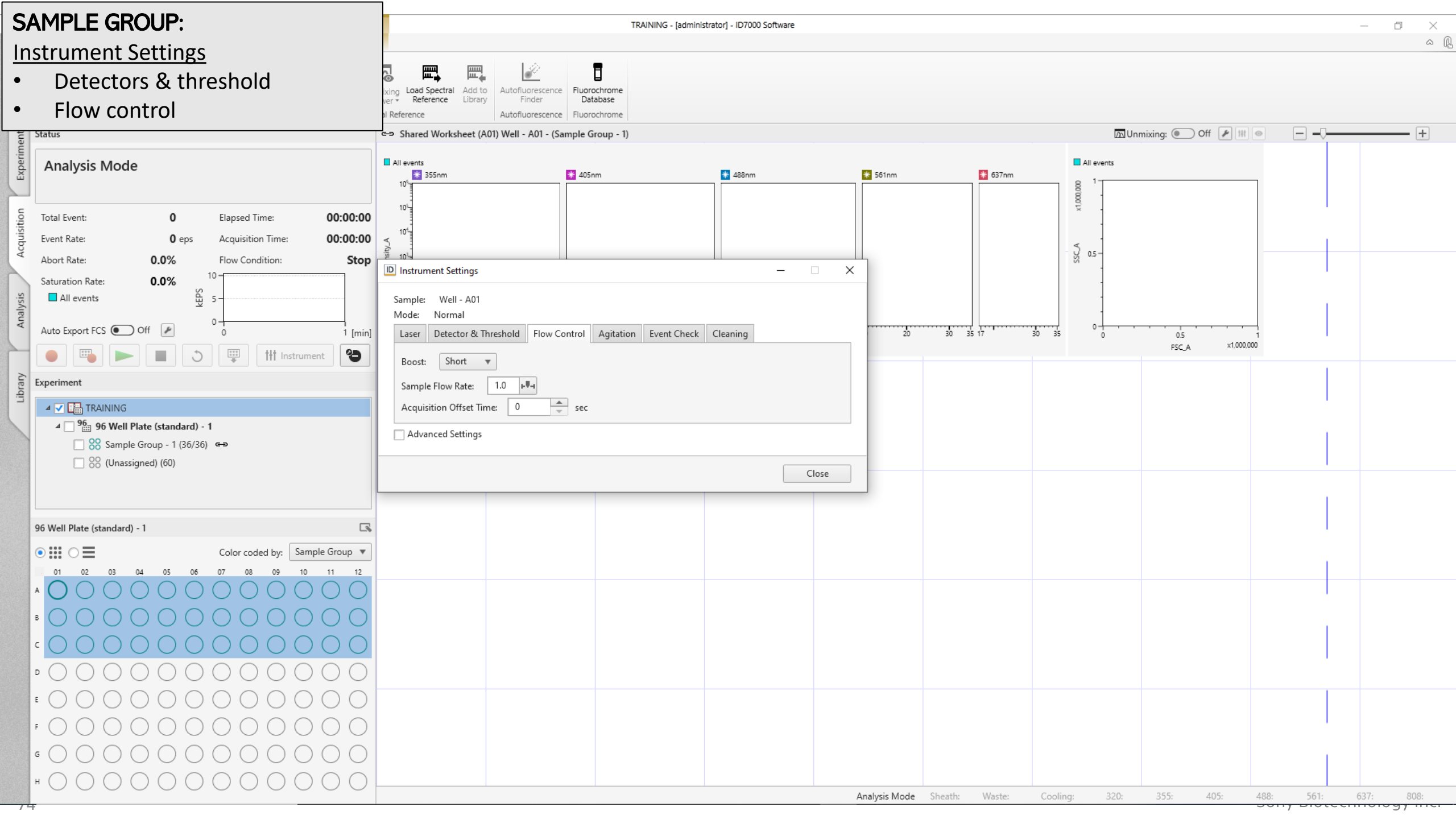
- Detectors & threshold



SAMPLE GROUP:

Instrument Settings

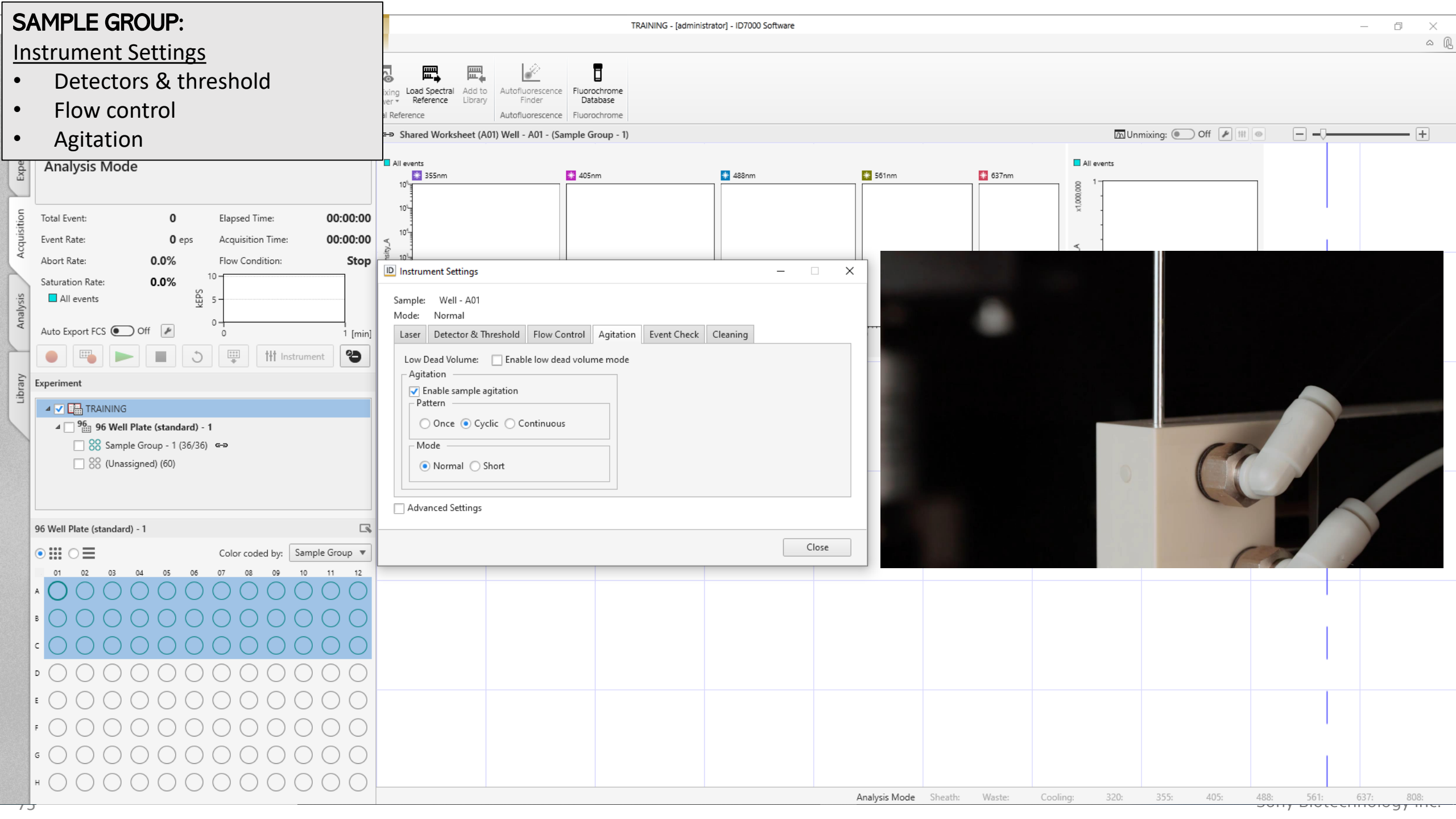
- Detectors & threshold
- Flow control



SAMPLE GROUP:

Instrument Settings

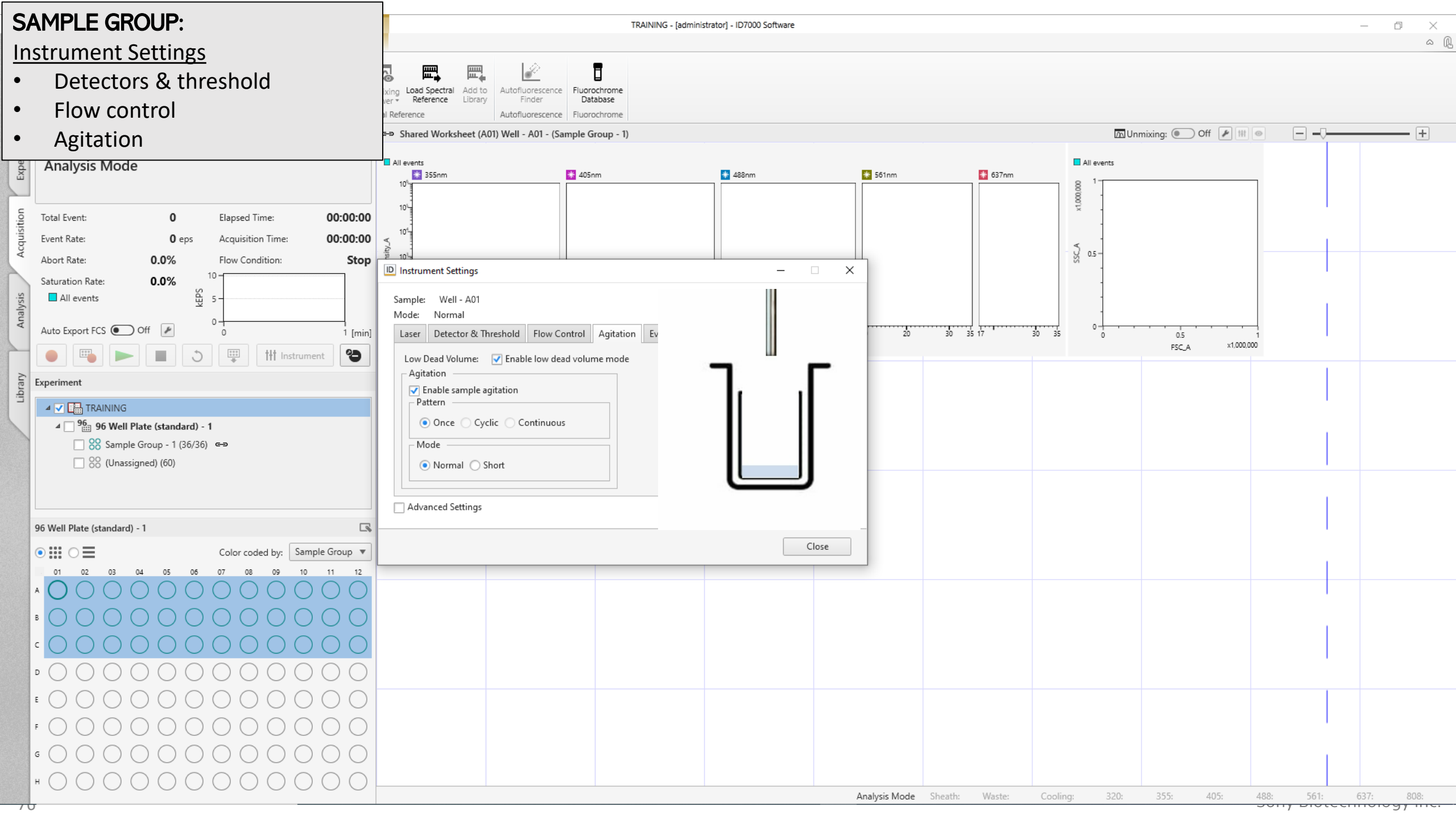
- Detectors & threshold
- Flow control
- Agitation



SAMPLE GROUP:

Instrument Settings

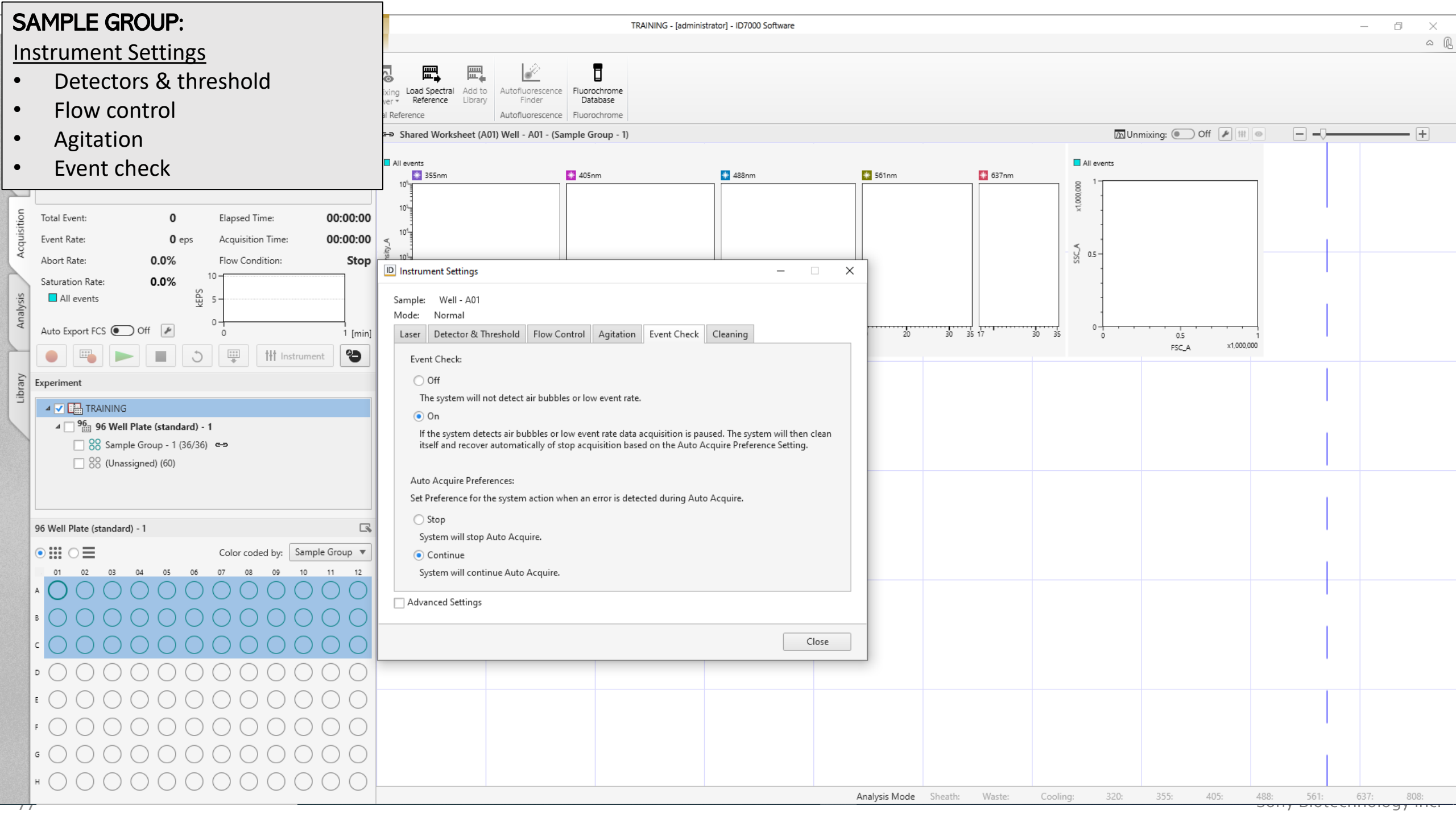
- Detectors & threshold
- Flow control
- Agitation



SAMPLE GROUP:

Instrument Settings

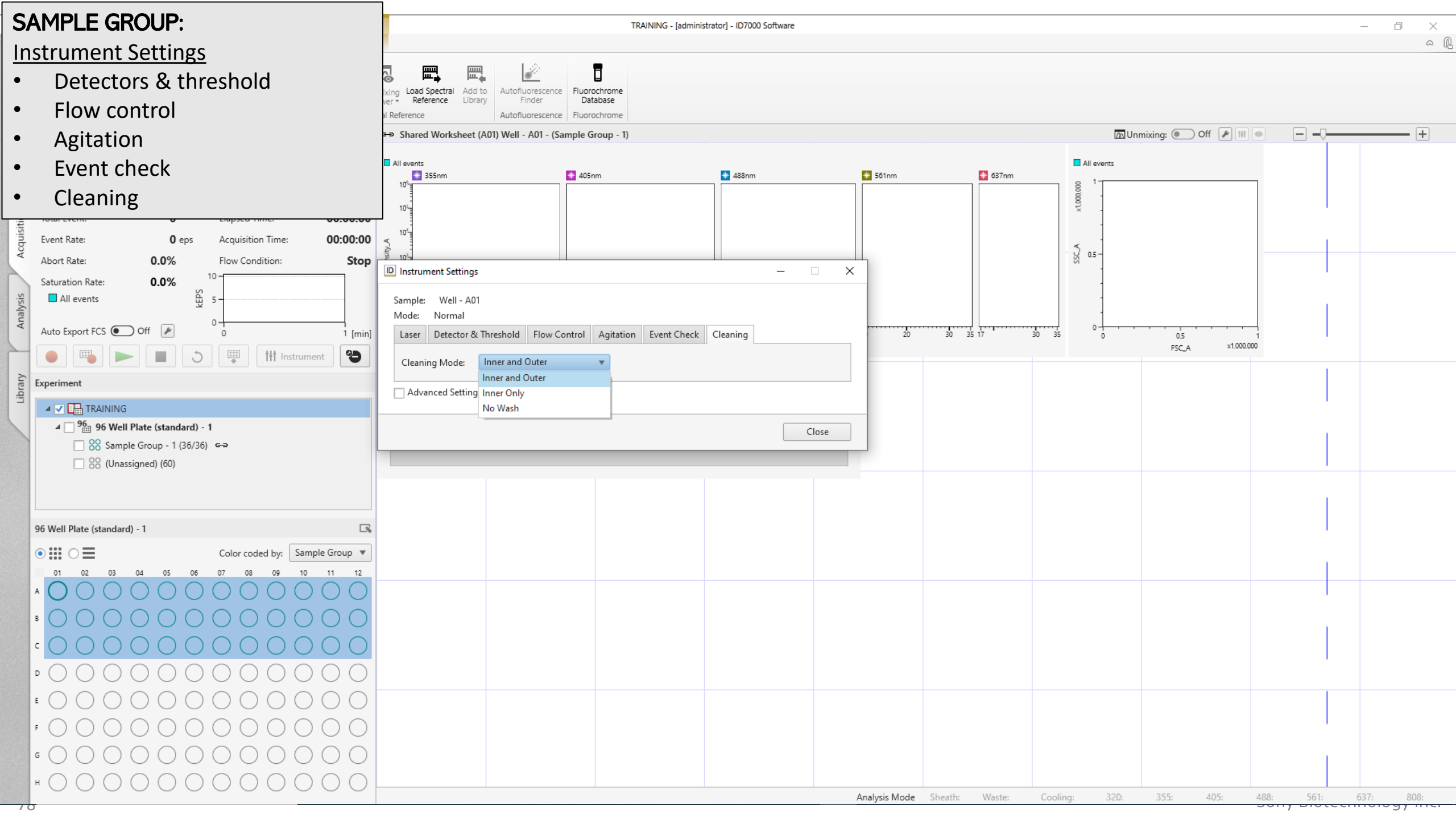
- Detectors & threshold
- Flow control
- Agitation
- Event check



SAMPLE GROUP:

Instrument Settings

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



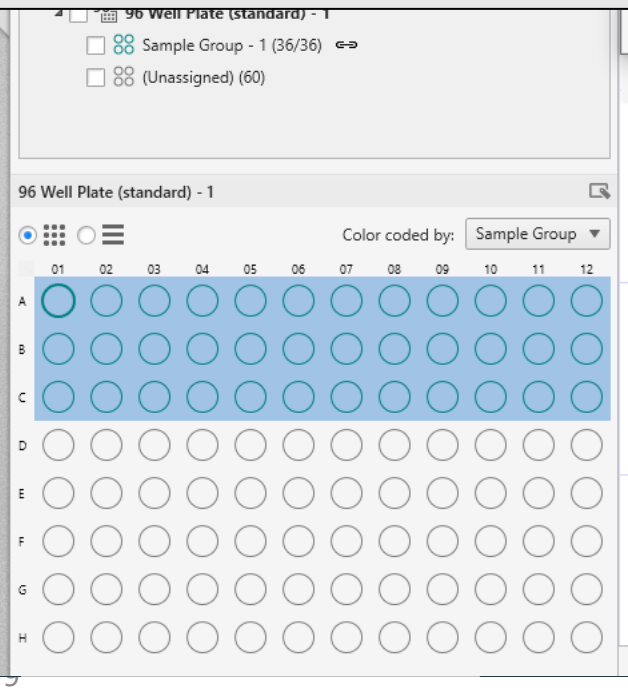
SAMPLE GROUP:

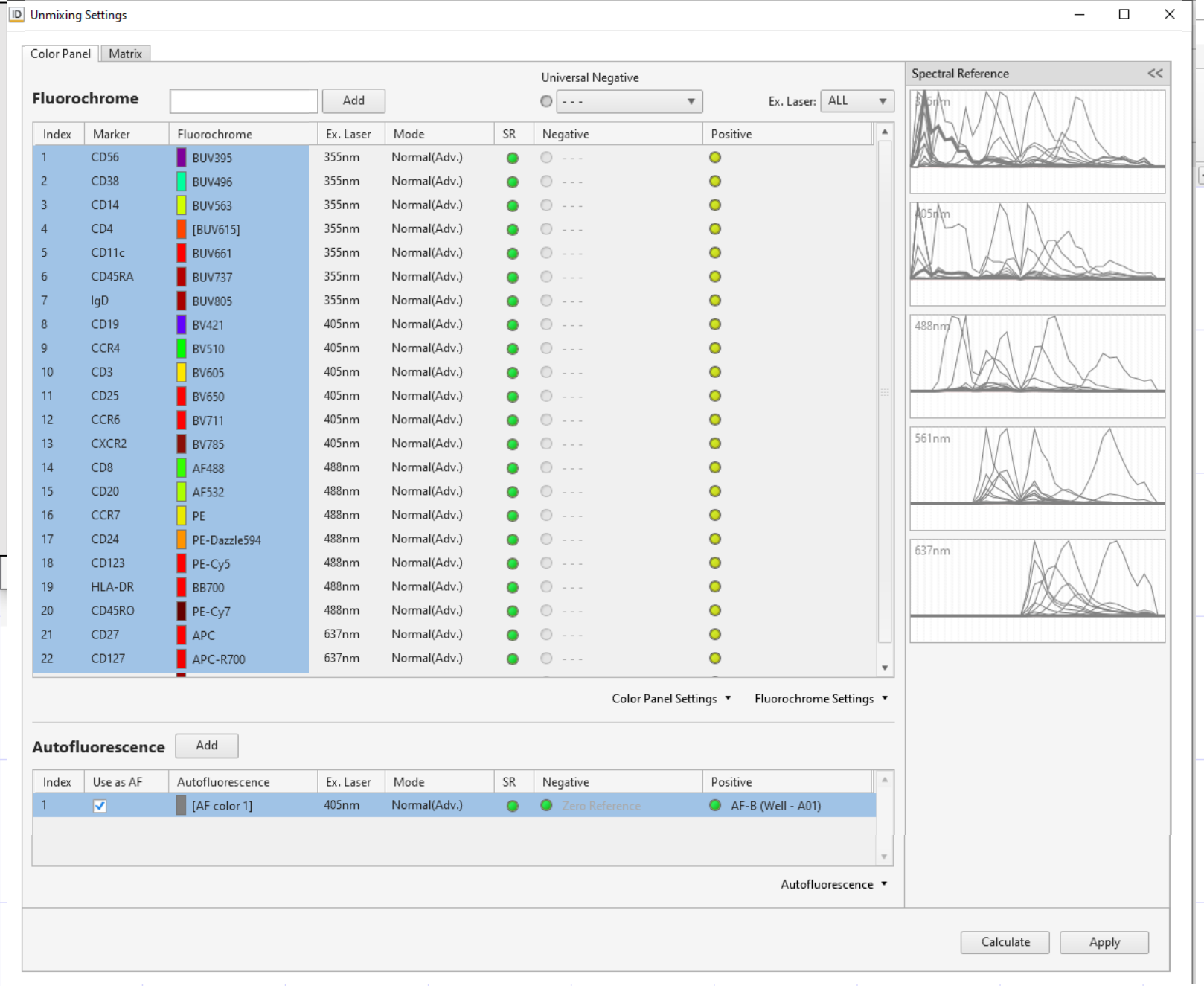
Instrument Settings

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

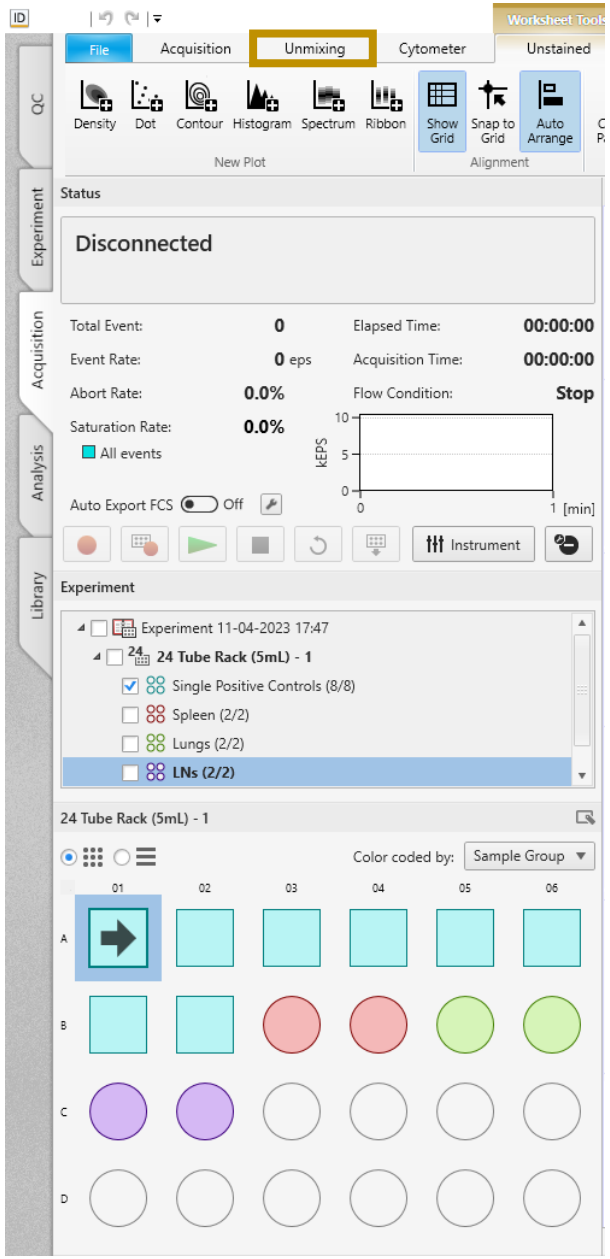
Unmixing Settings

- Colour panel
- Spectral References
- Autofluorescence



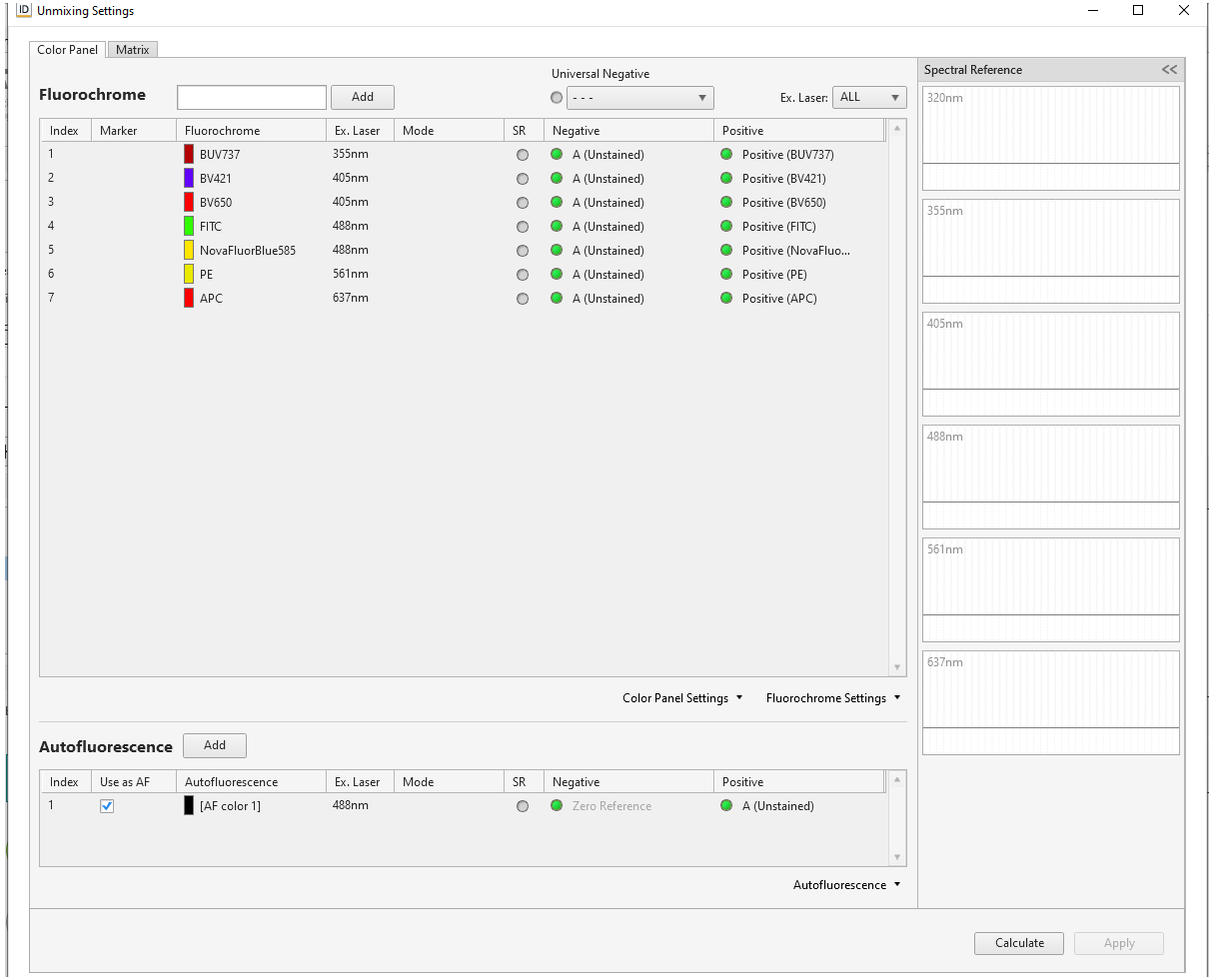


Acquisition

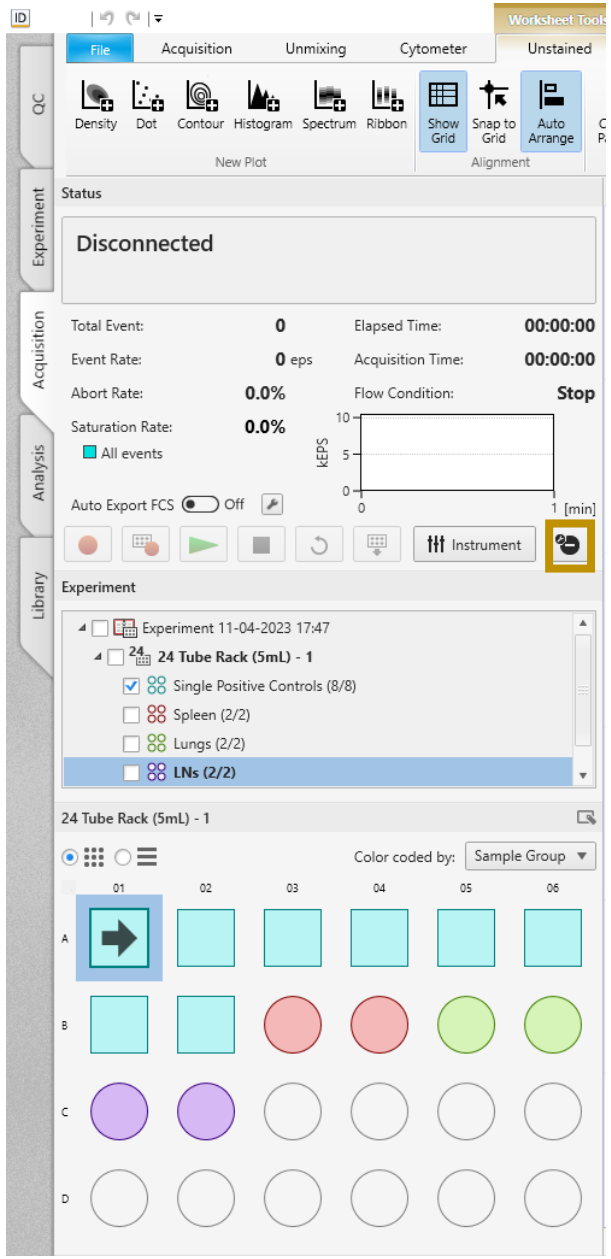


Sample groups (shared)

- Instrument settings
- Unmixing settings
- Shared worksheet



Acquisition

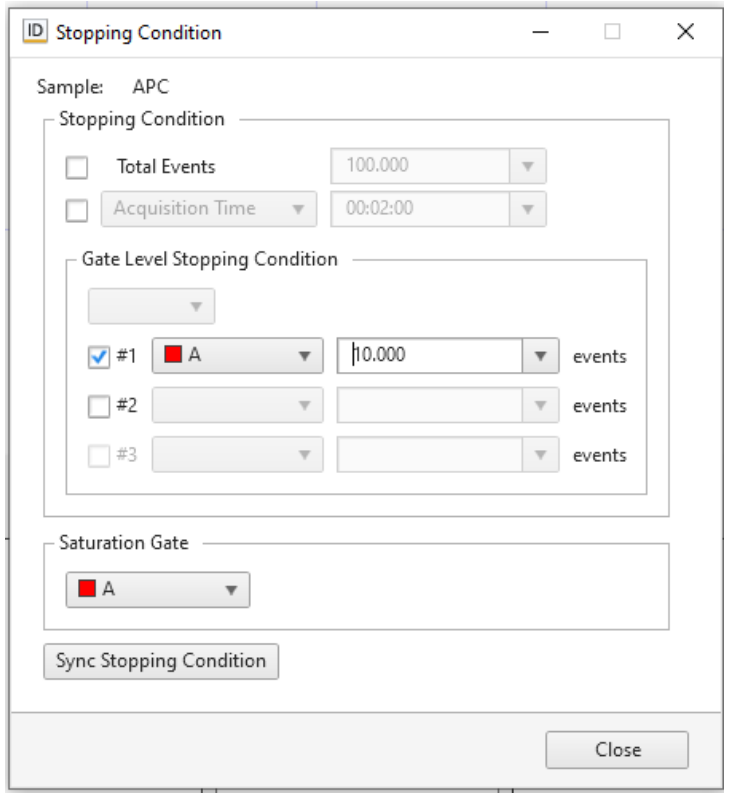


Sample groups (shared)

- Instrument settings
- Unmixing settings
- Shared worksheet

Sample groups (not shared)

- Stopping condition
- Individual worksheet

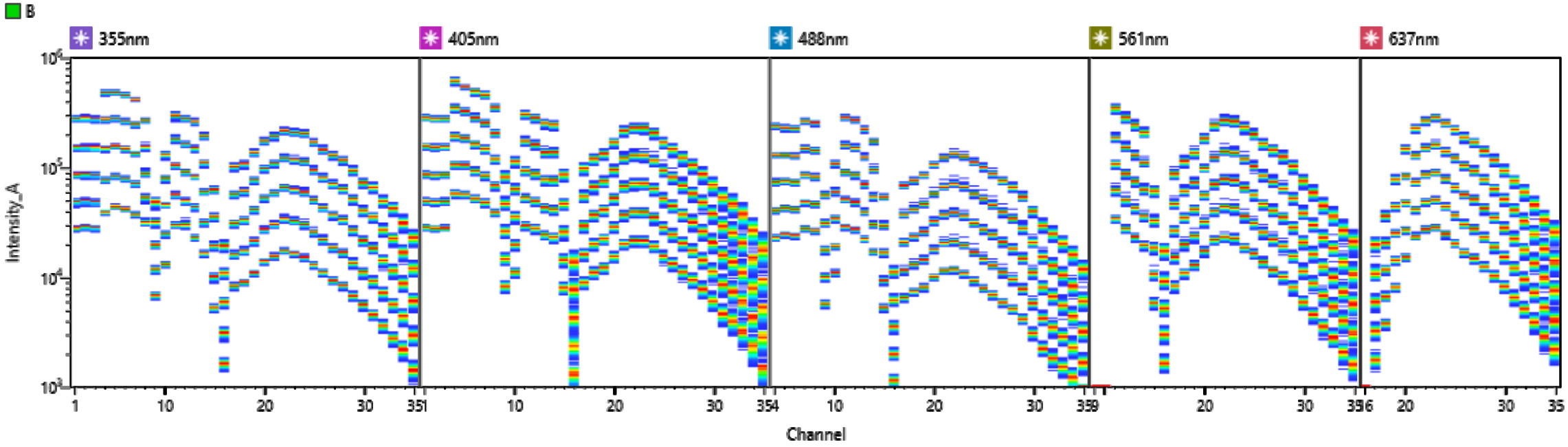


Standardization Mode

Standardization Mode (ST Mode)

Performed during daily instrument QC using Align Check beads.

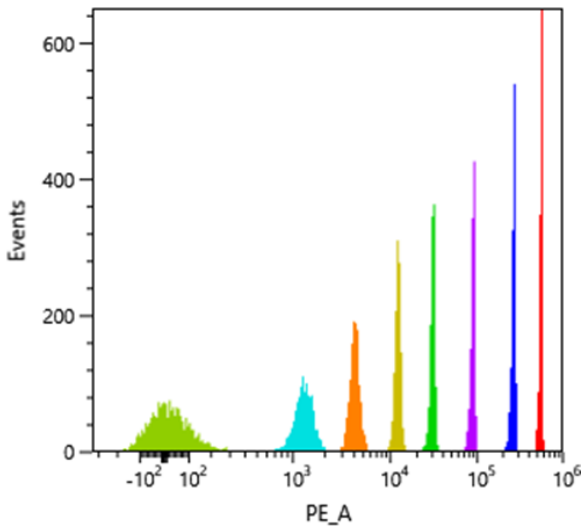
AlignCheck beads produce consistent broad spectrum fluorescence.



Std. PMTV Setting 5.00

Measurement stability over time

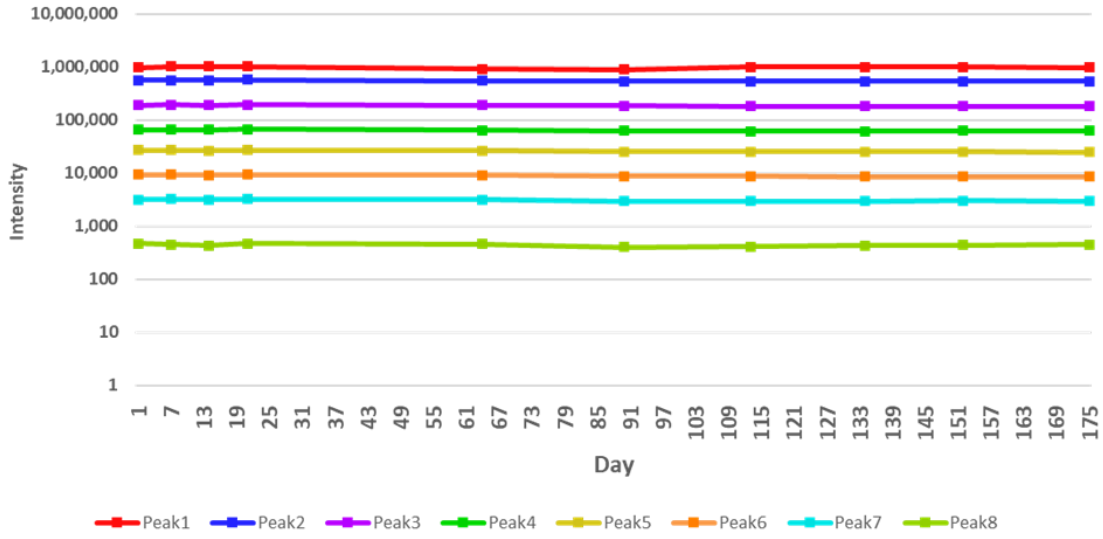
8-peak beads



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

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 %

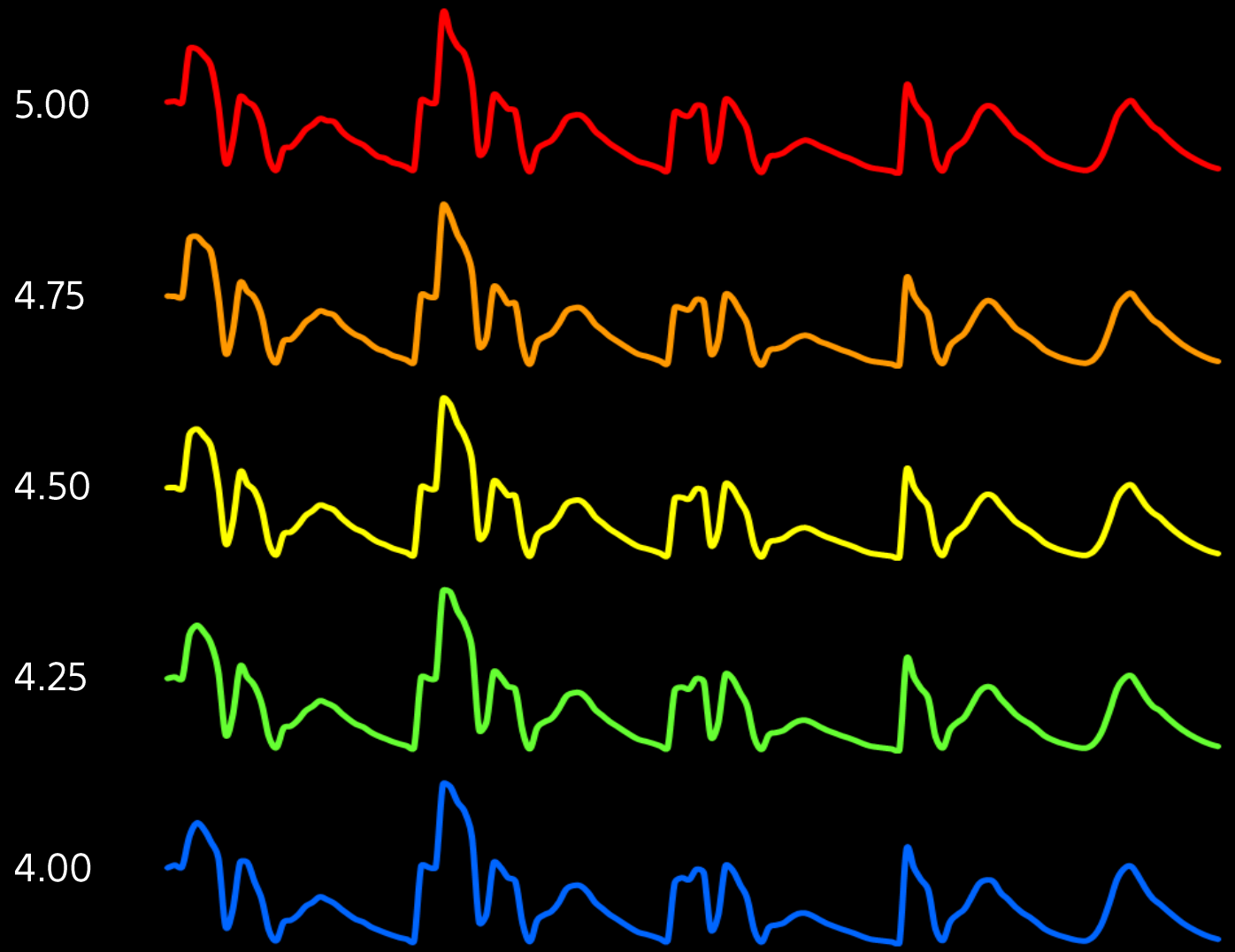
Stability over 175 days at ST value of 5



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

ST Mode - PMT voltage validation

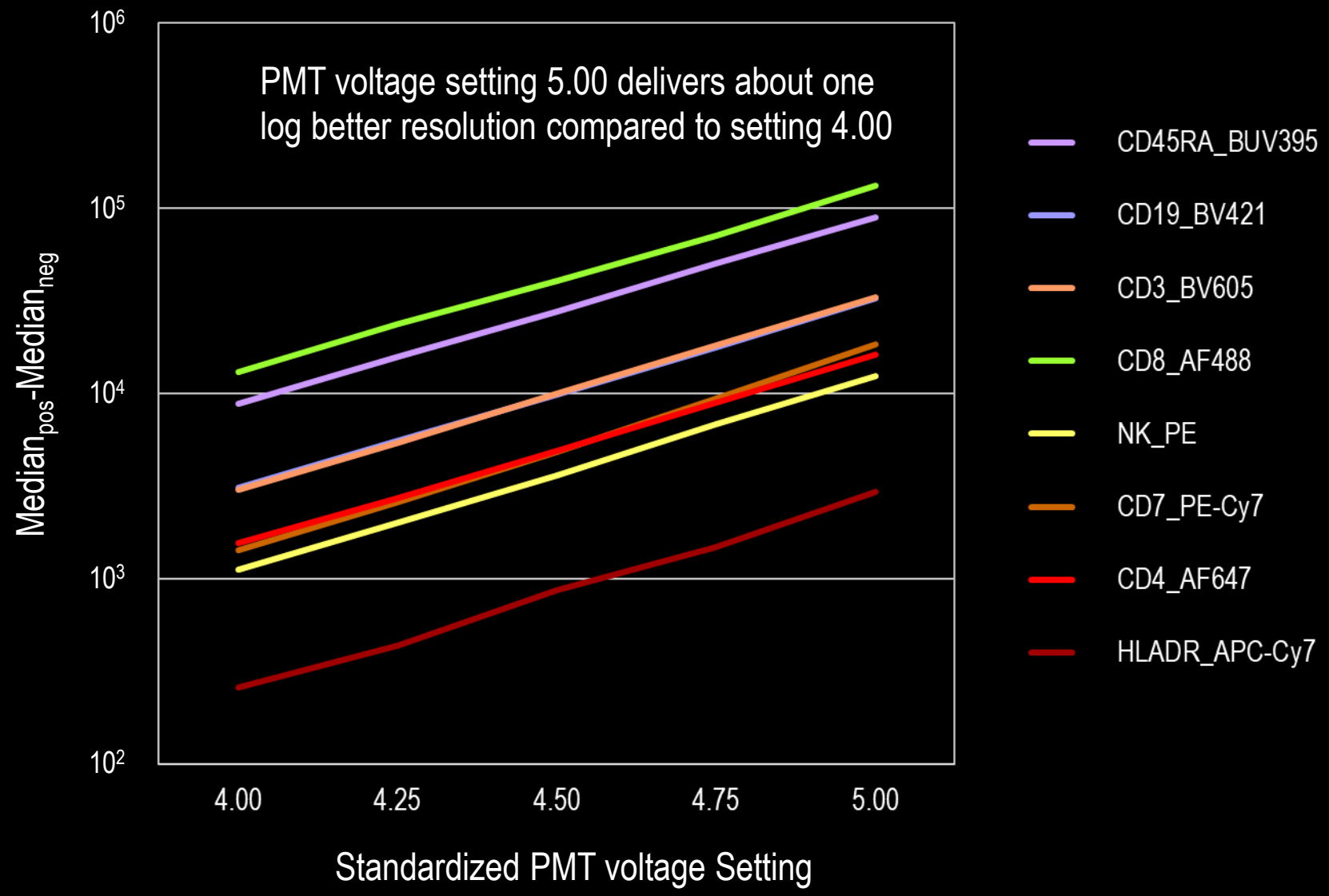
Normalized fluorescence distributions from Align Check beads acquired at five standardized PMT array voltages



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

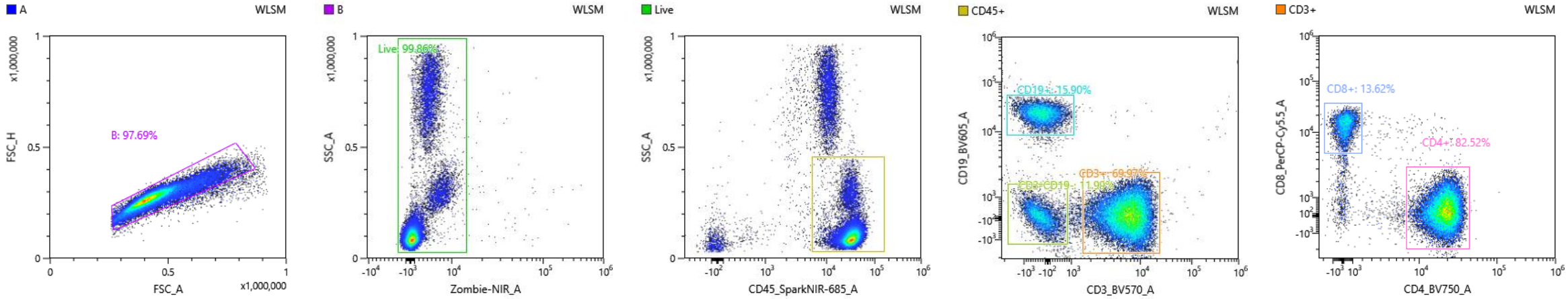
	4.00	4.25	4.50	4.75	5.00
4.00		0.996	0.987	0.980	0.981
4.25	0.996		0.997	0.992	0.991
4.50	0.987	0.997		0.999	0.996
4.75	0.980	0.992	0.999		0.997
5.00	0.981	0.991	0.996	0.997	

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

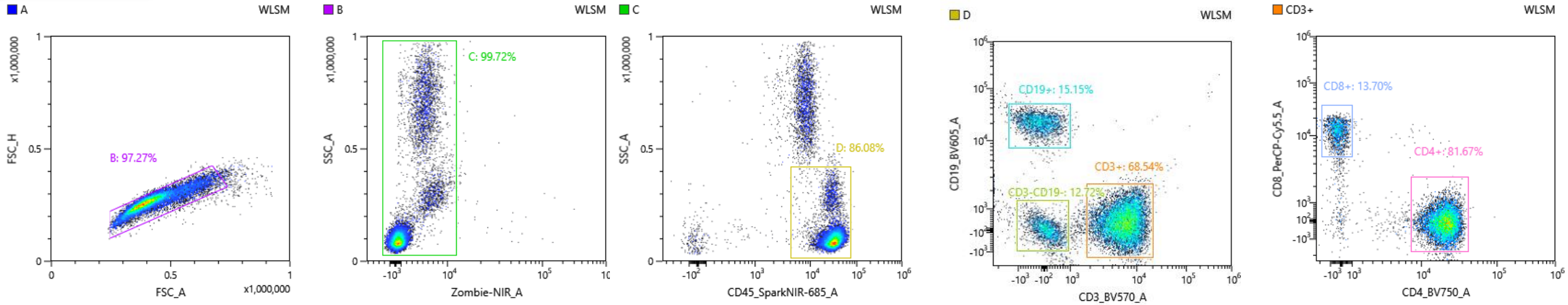


Unmixing with bead and cell-based references show comparable results

Beads

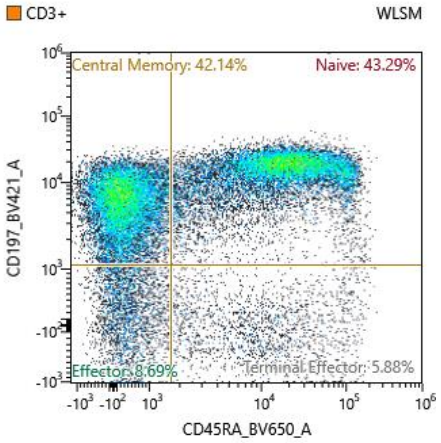


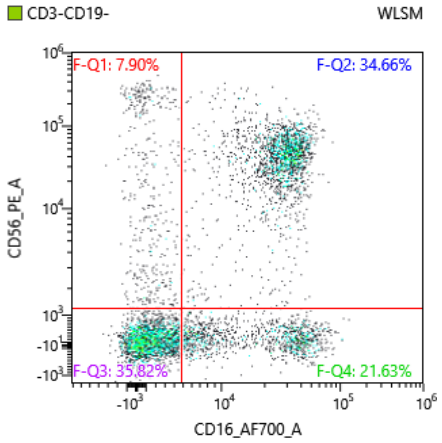
Cells

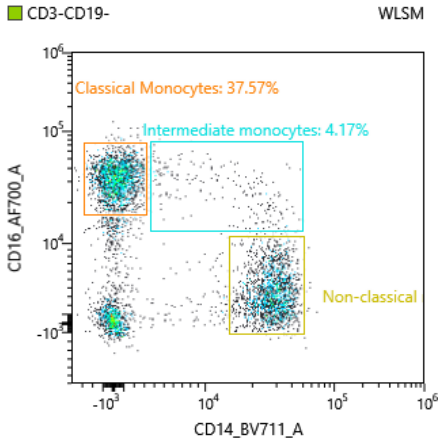


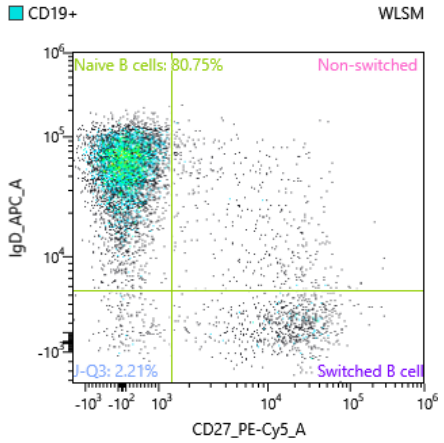
Unmixing with bead and cell-based references show comparable results

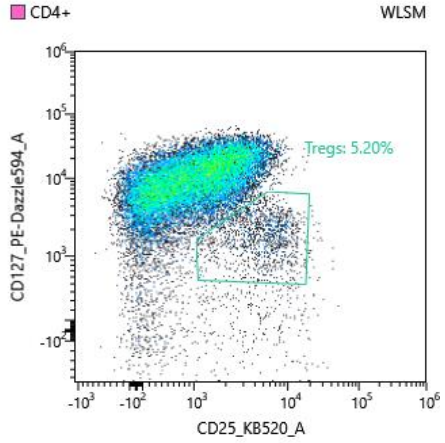
Beads



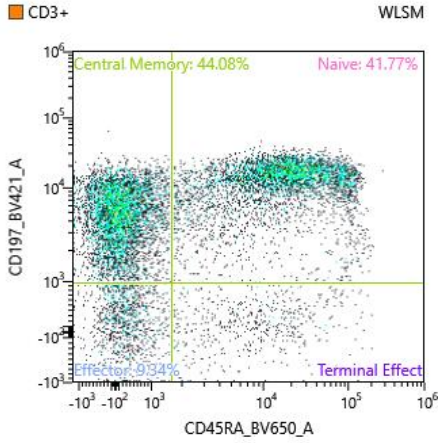


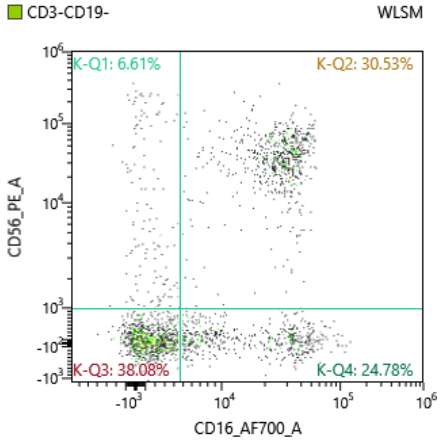


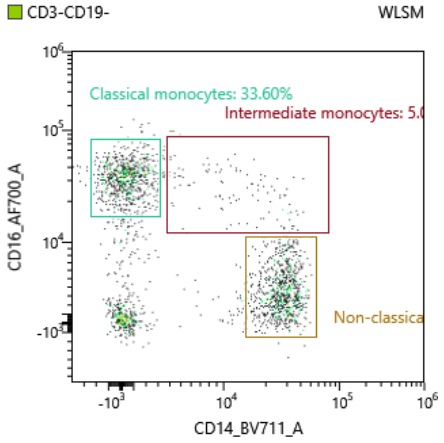


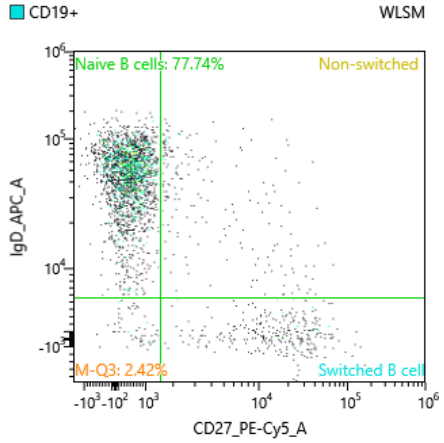


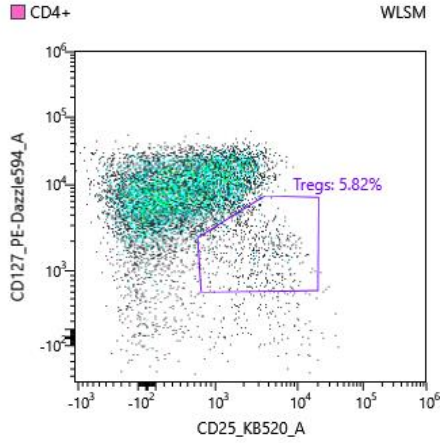
Cells



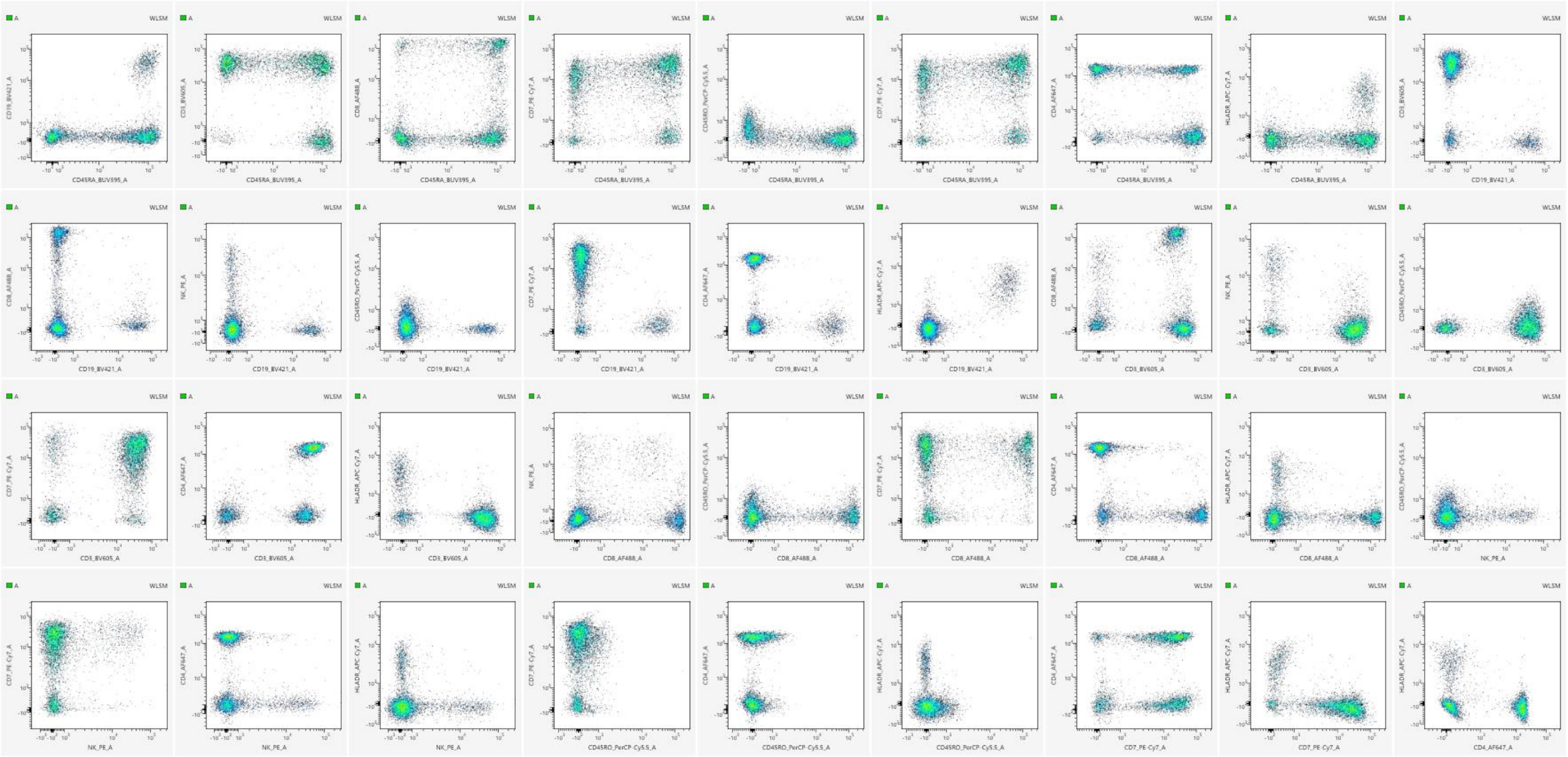






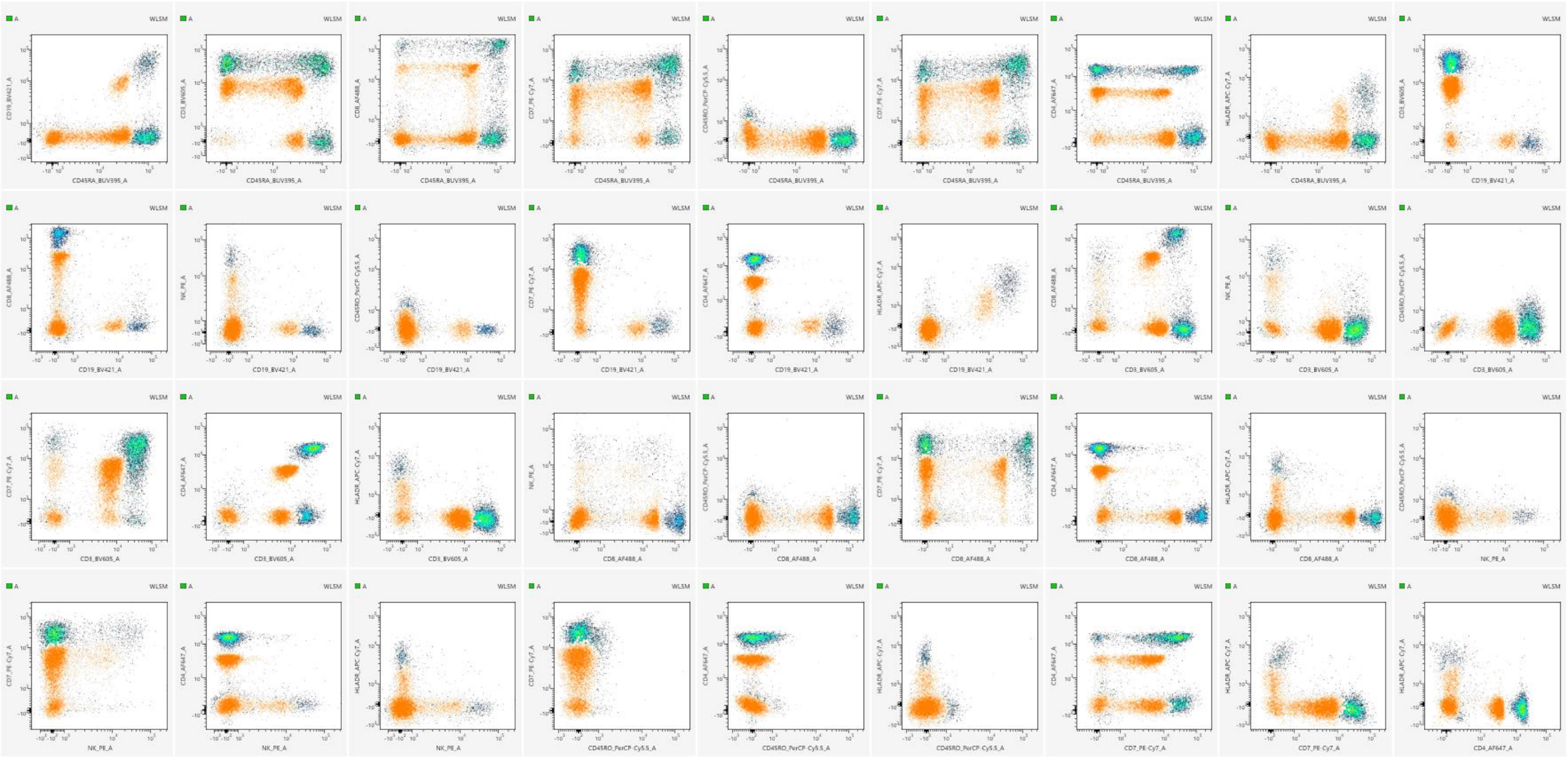


One unmixing matrix works for all standardized voltage settings

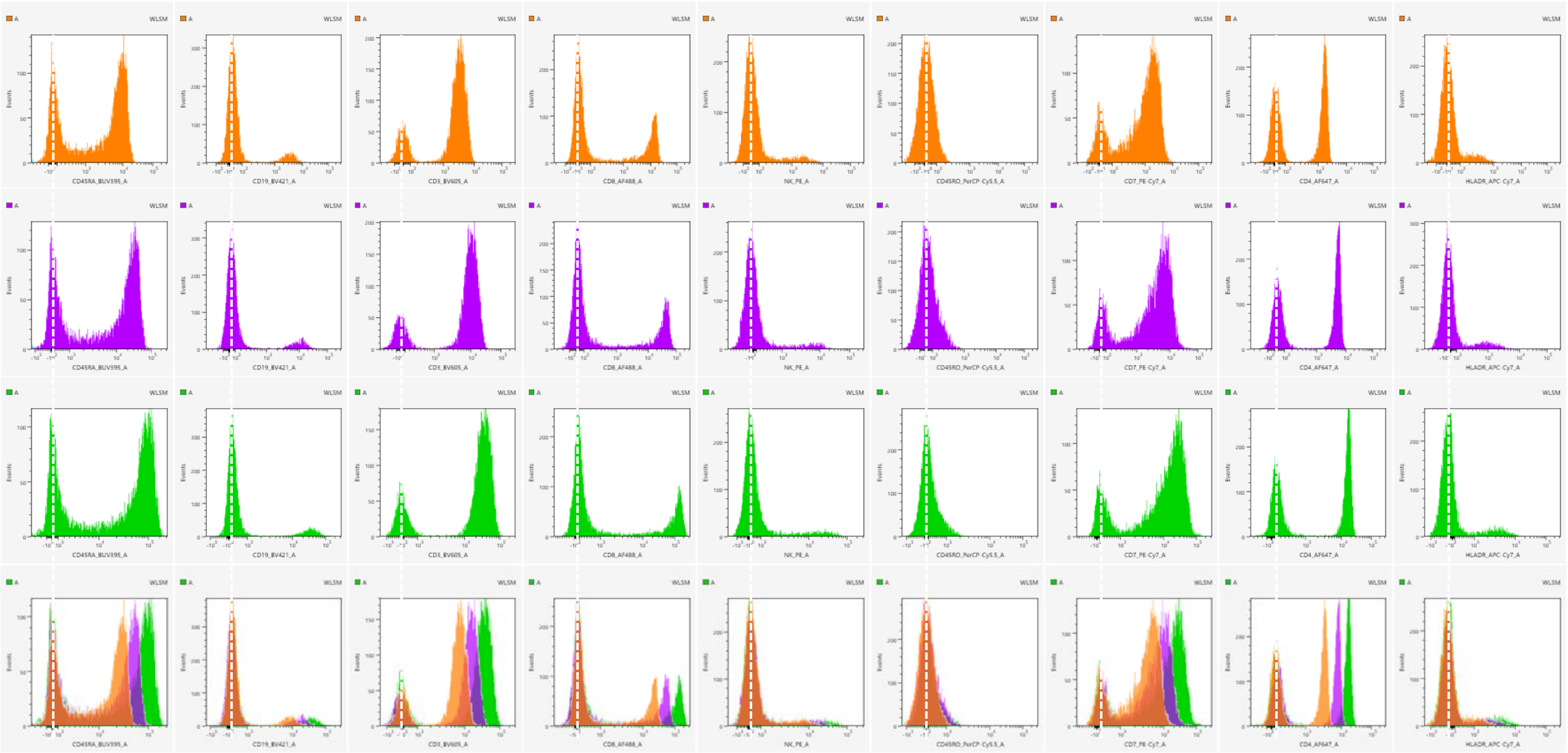


SONY

Overlay PMT Voltage 4.00 and 5.00



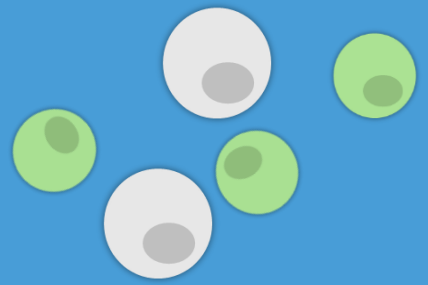
SONY 5-laser ID7000 – 9c + AF at ST PMT Settings 4.00, 4.50, and 5.00



SONY

ACQUISITION

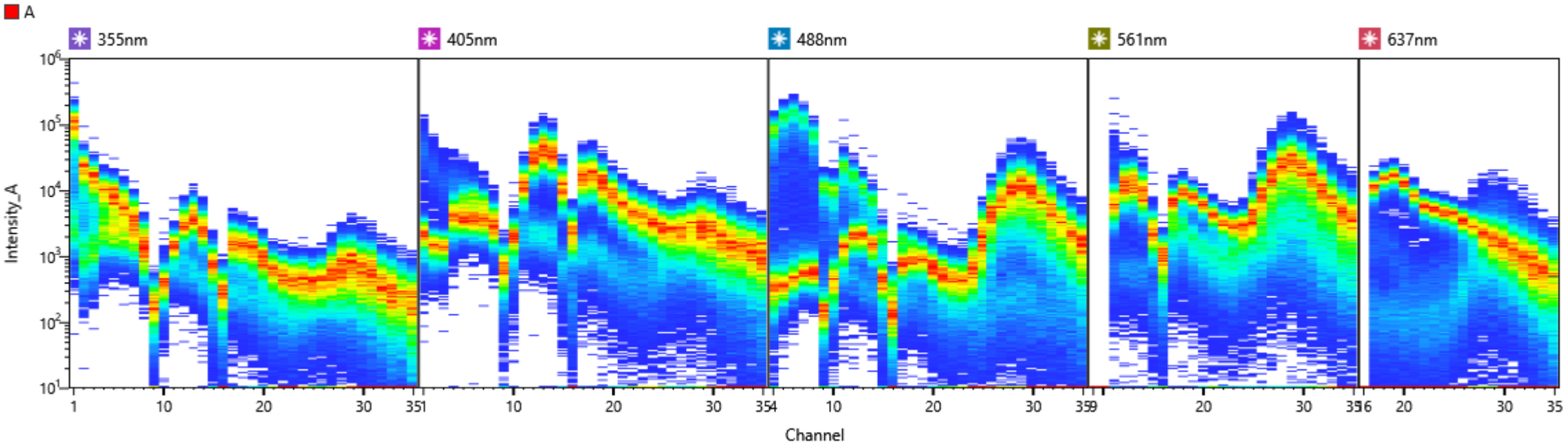
Workflow



Standardized Mode

All the detectors in the entire array with a single PMT adjustment:

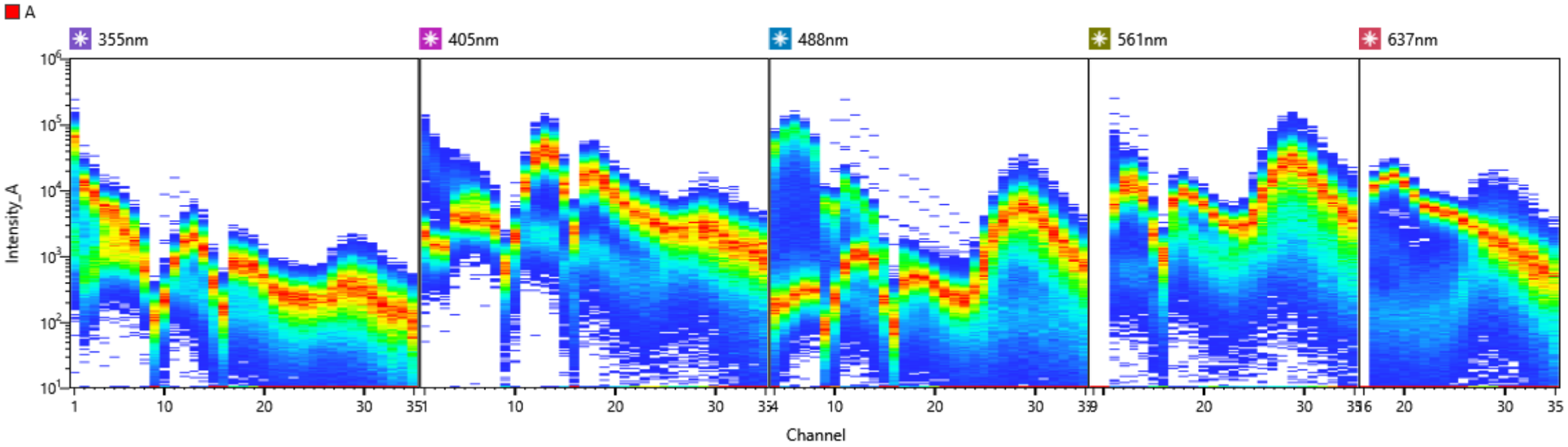
The sensitivity of all detectors increases and decreases in synchrony.



Std. PMTV Setting 5.00

Standardized Mode

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



Experimental Setup

Step 1

Single stain controls

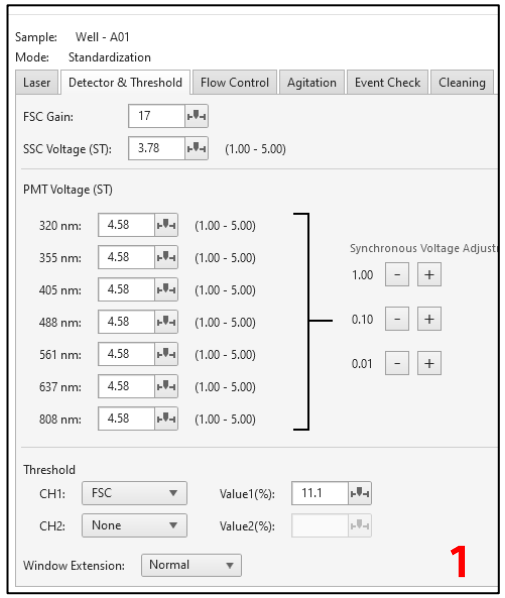
Set the gains for all decks to **4.58**

Set the gate on the beads
Select this gate for the saturation

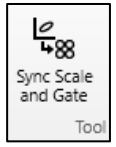
Preview the single stained controls

Record single stained controls

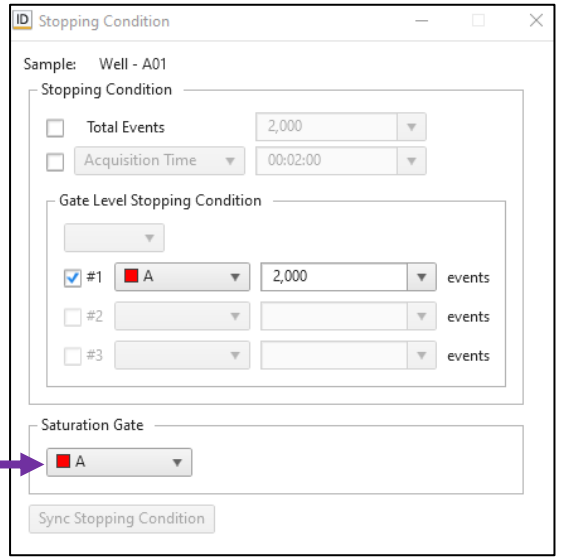
Instrument Settings



- Synchronize accross the sample group



- Sync. Stopping conditions



Exceptions:

Ensure that brightest fluorochromes are not saturated

If necessary: adjust the gains in syncronous mode and record individually

Experimental Setup

Step 2

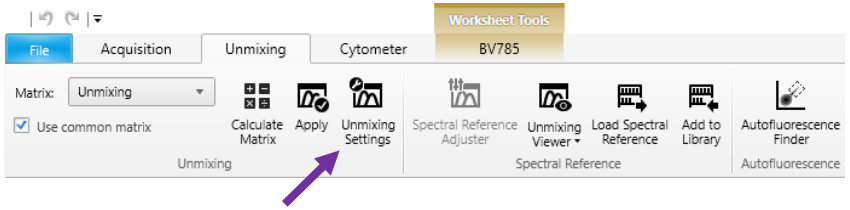
Unmixing Settings

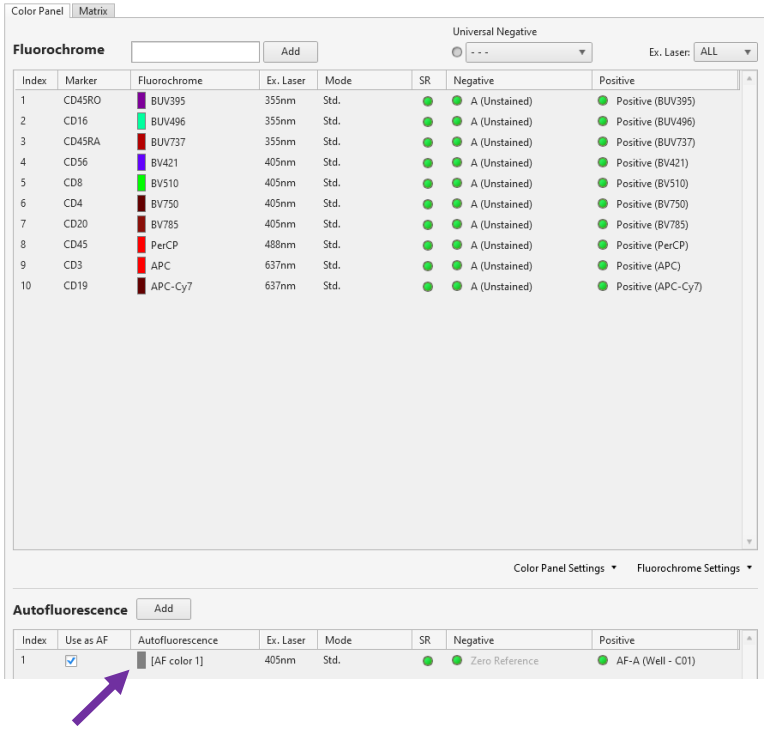
Adjust the gates for single controls (pos/neg)

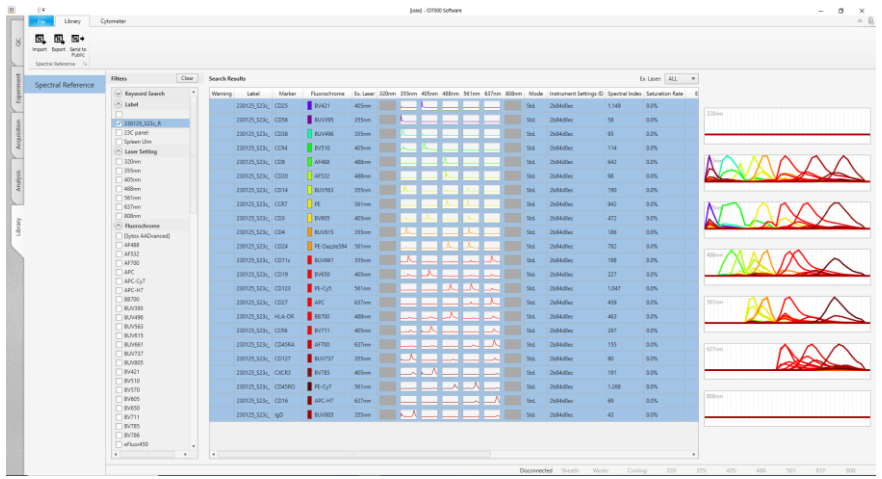
Remove AF

Calculate Unmixing and Apply

Add single stain controls to Spectral Reference Library







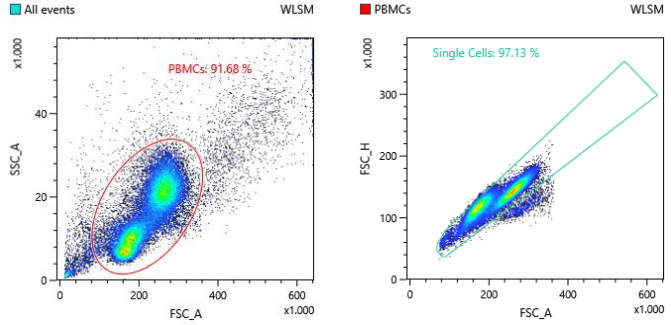
SONY

Step 3

Full stained samples

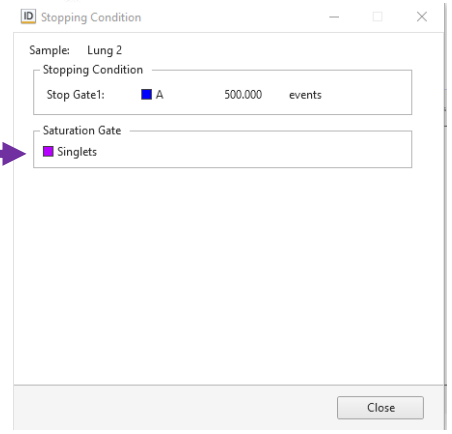
Preview sample

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



In Stopping Conditions:

Select in Saturation Gate – Singlets

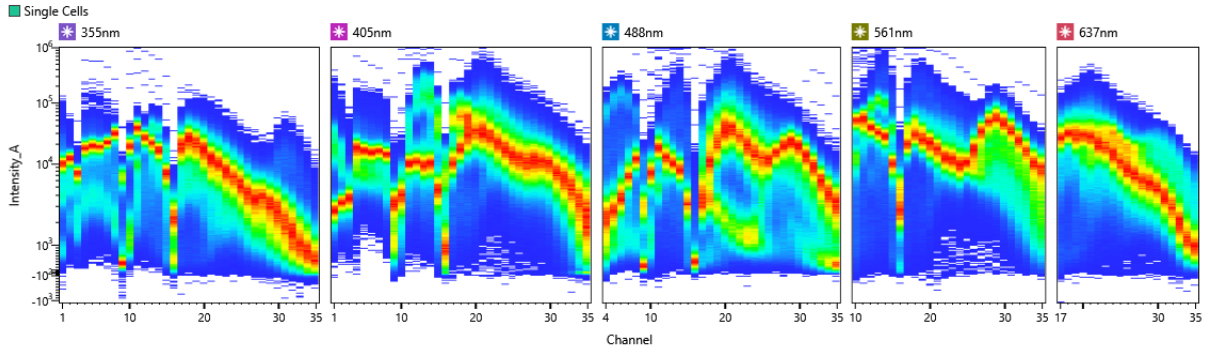


One Max

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

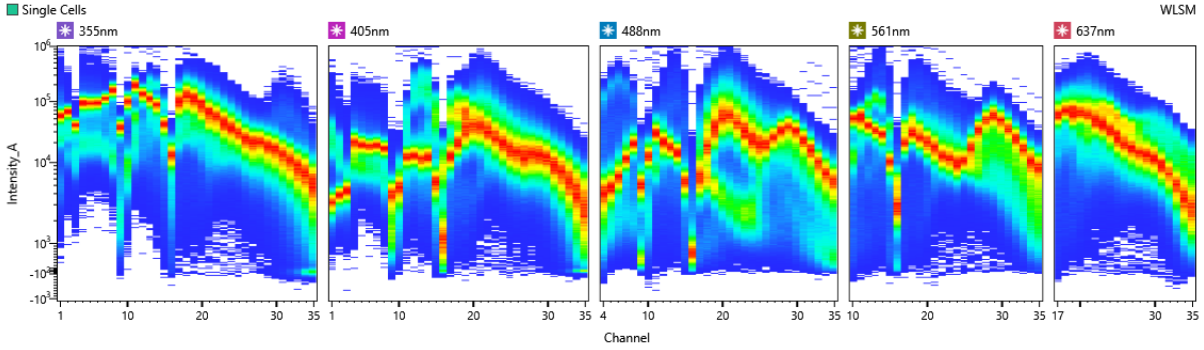


All Max

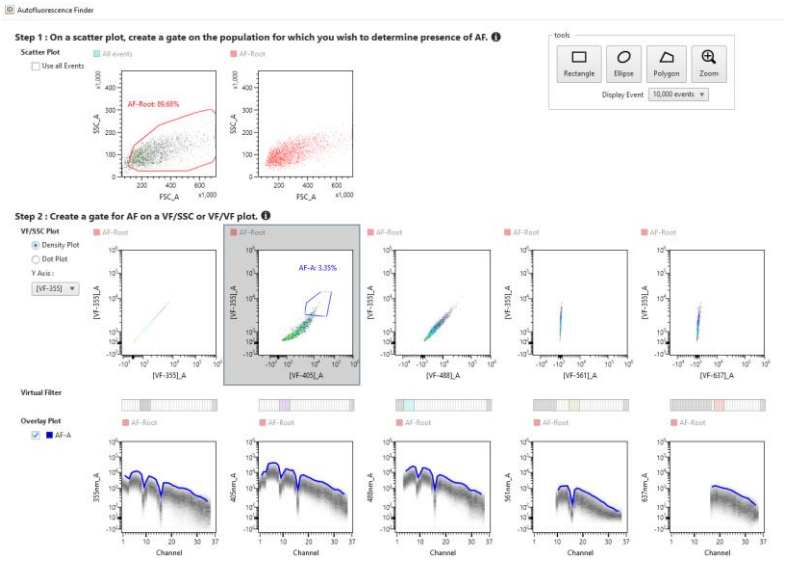
Maximize gains across the individual decks 3

PMT Voltage (ST)

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



Experimental Setup



One Max

Maximize gains in synchrony **2**

Maximize gains while viewing the Ribbon plot

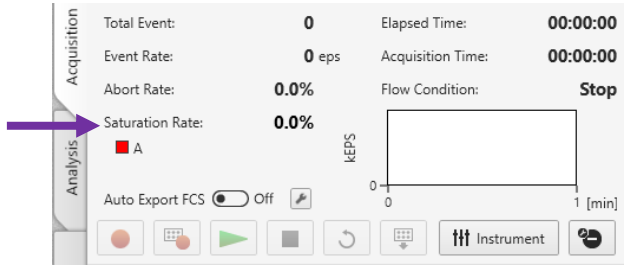
Monitor saturation rate

Acquire the unstained sample with the same settings

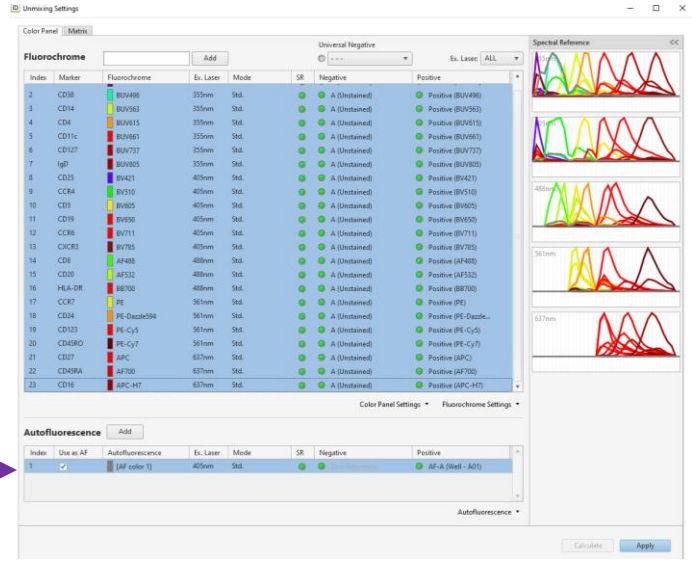
Use the unstained sample for the calculation of AF signal

All Max

Maximize gains across the individual decks **3**



Note: Keep saturation rate < 3%

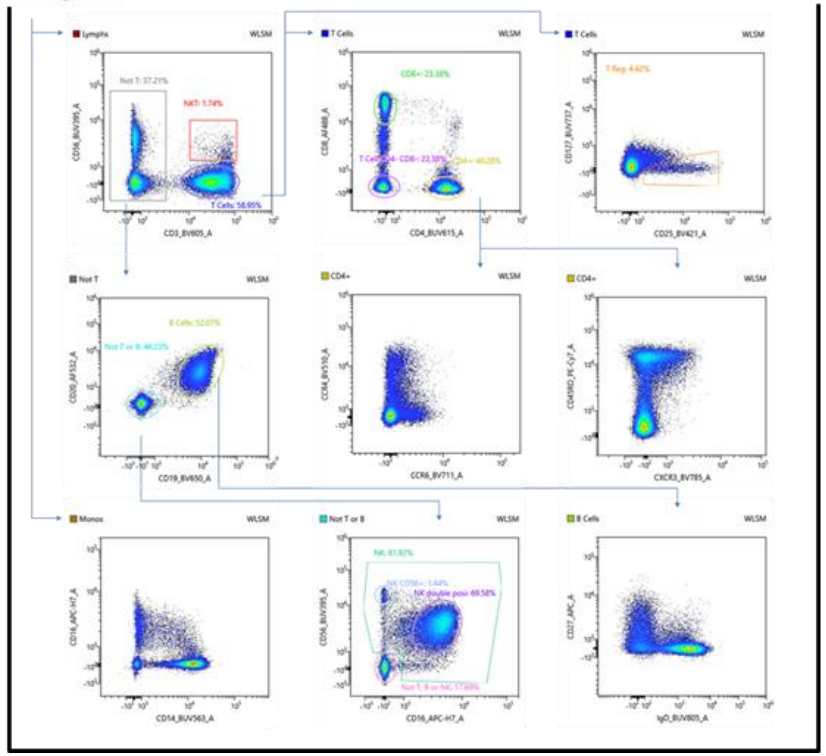


SONY

One Max

All Max

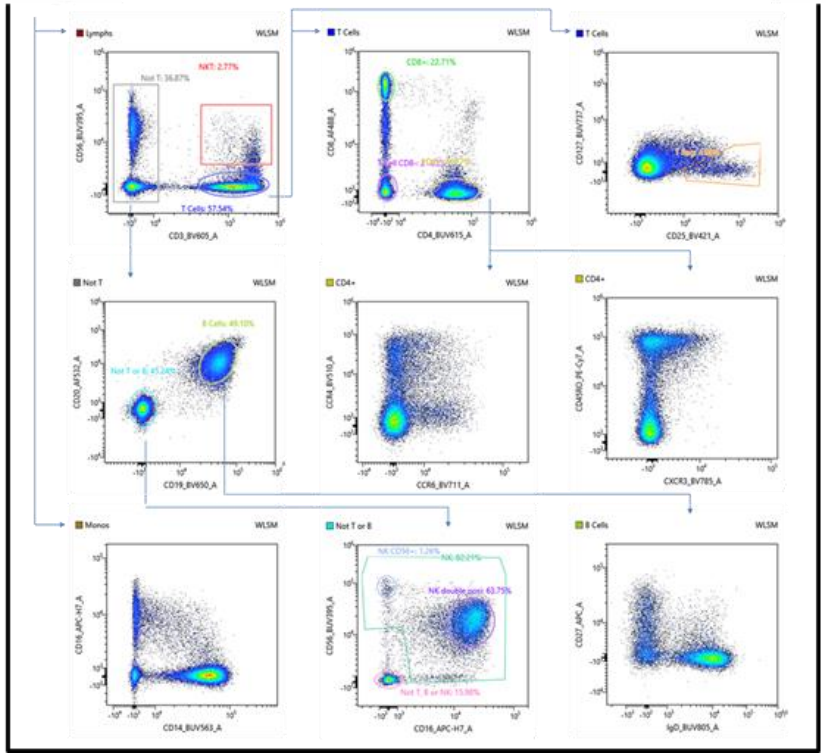
Setting 1



PMT Voltage (ST)

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

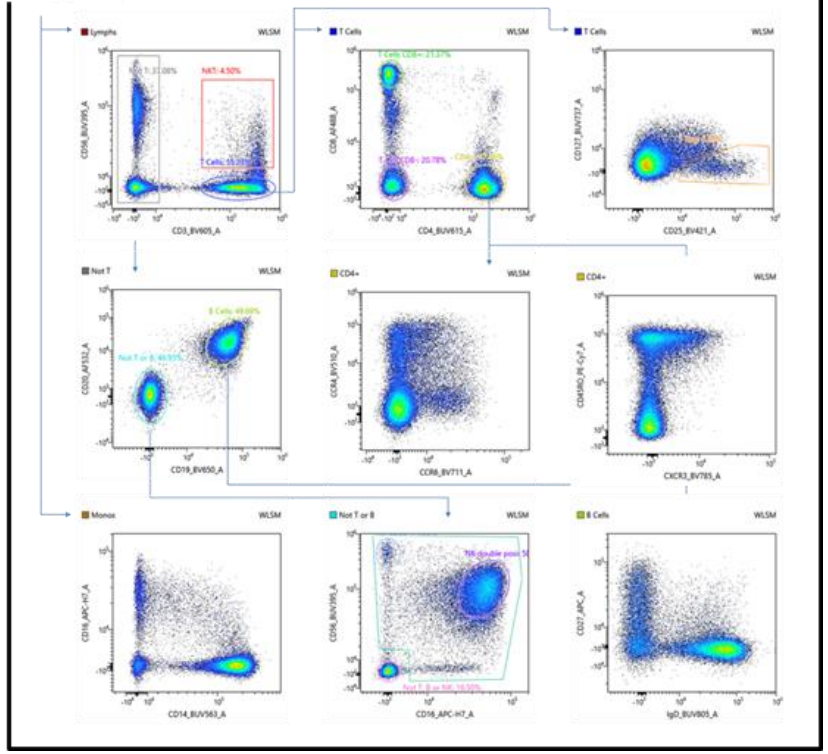
Setting 2



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

Improvement of workflow efficiency with Standardized Mode

Based on Daily QC

Minimizes subjectivity and instrument variability

Single stain controls acquired at one PMT voltage (ST)

PMT voltage (ST) for samples can me 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

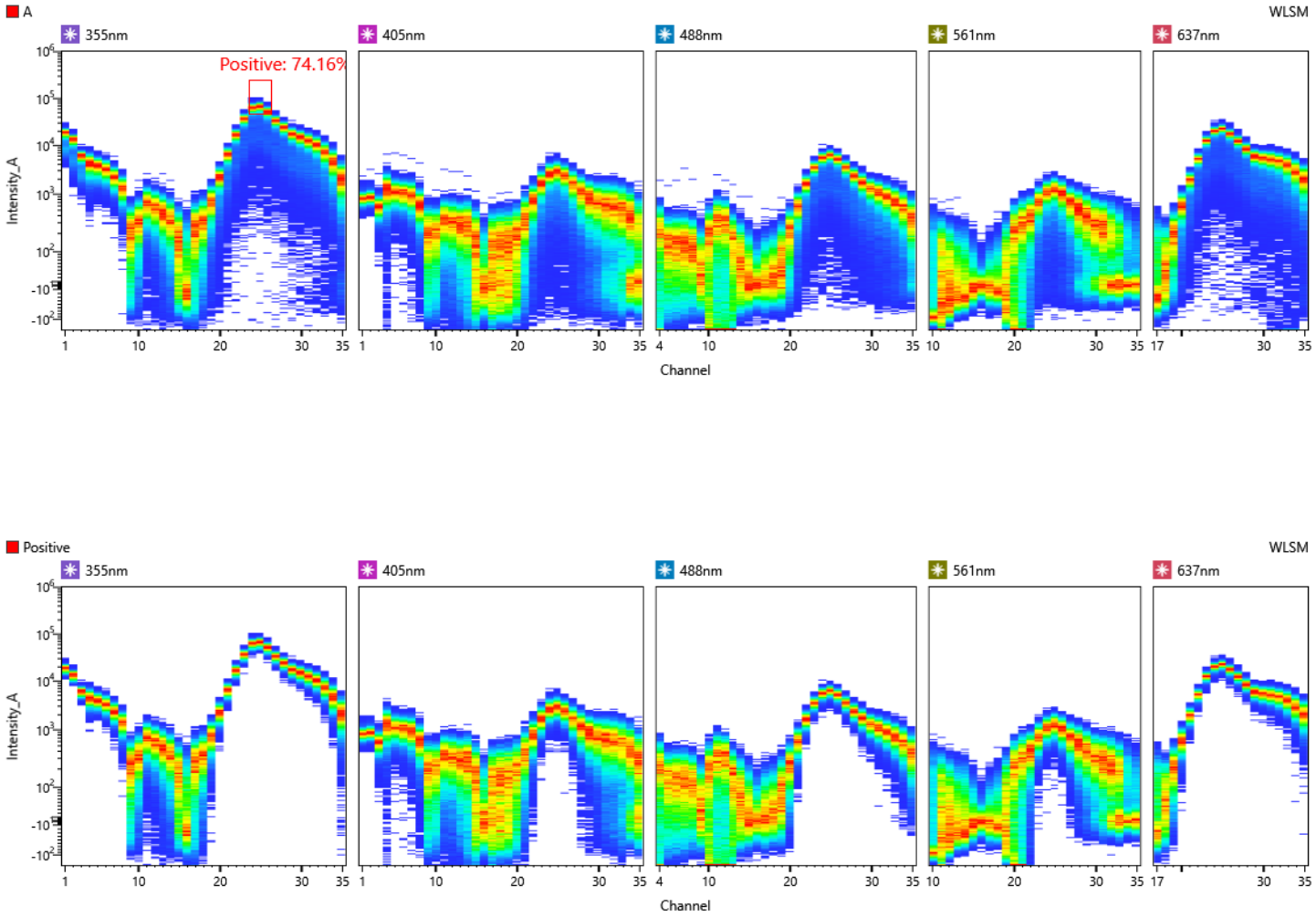
1. QC
2. Experiment
3. Colour Panel (colour + marker)

or

Reference Spectra

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

Single Colour Control



Single Colour Control

Unmixing Settings

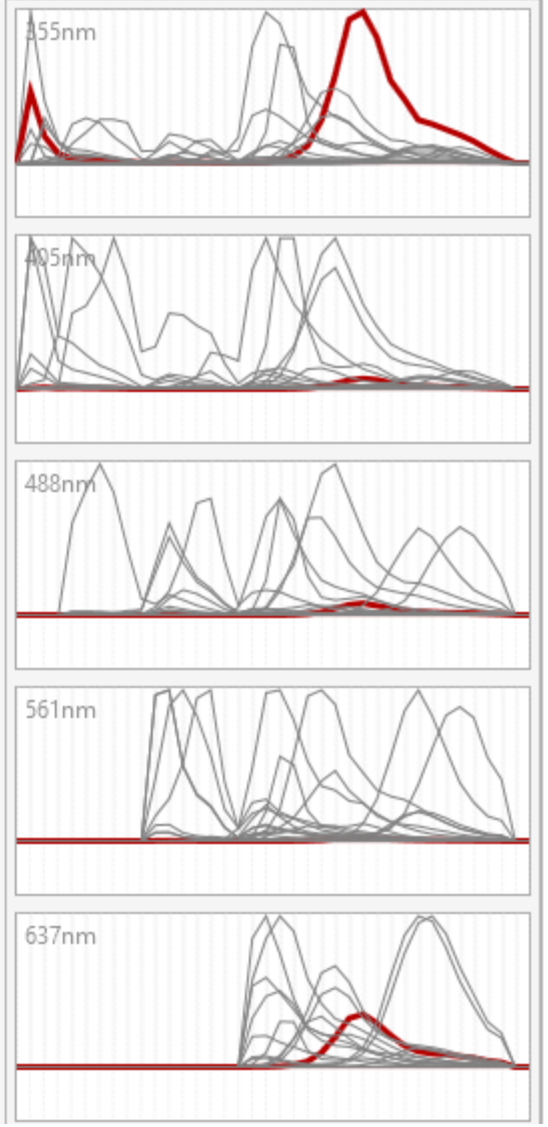
Color Panel | Matrix

Fluorochrome [ ] Add

Universal Negative: E (Unstained) | Ex. Laser: ALL

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

Spectral Reference



WLSM

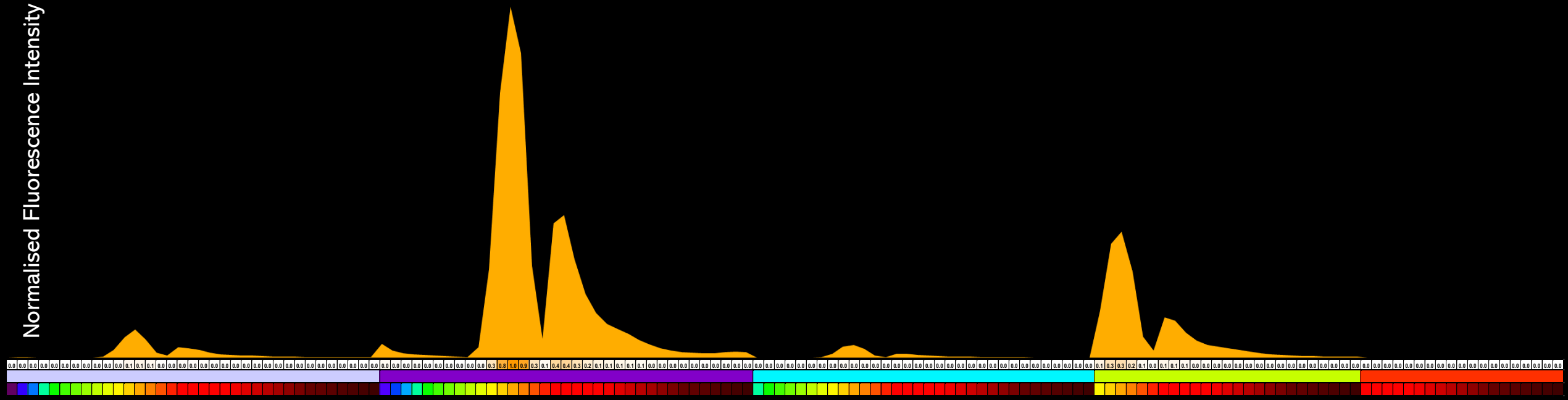
35

SONY

Reference Spectra

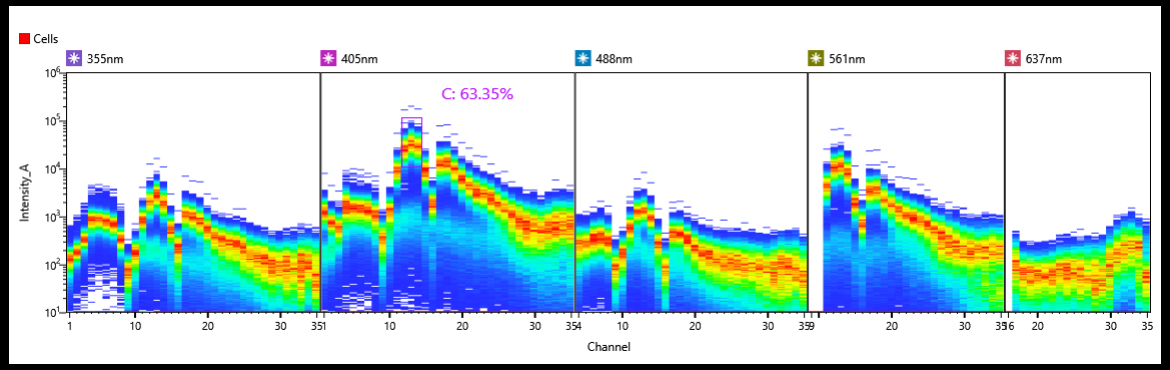
A reference spectrum is a set of values that defines the distribution of a fluorochrome's fluorescence in the detector array.

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

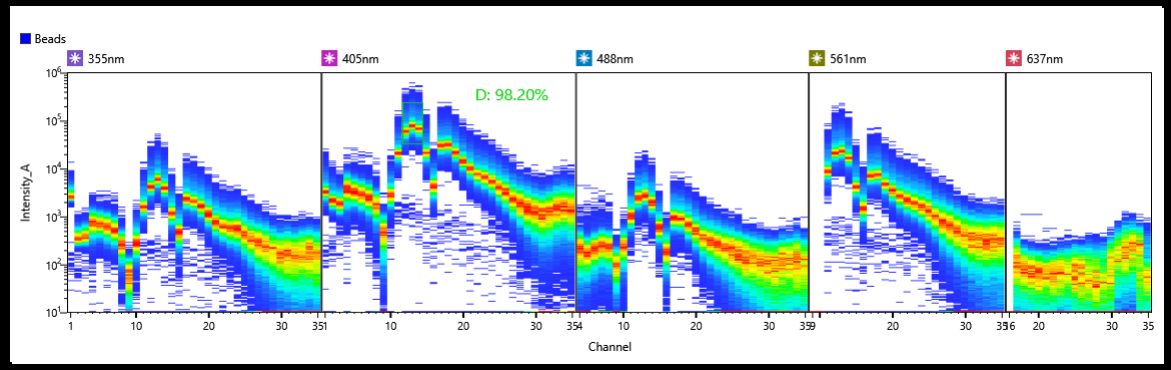


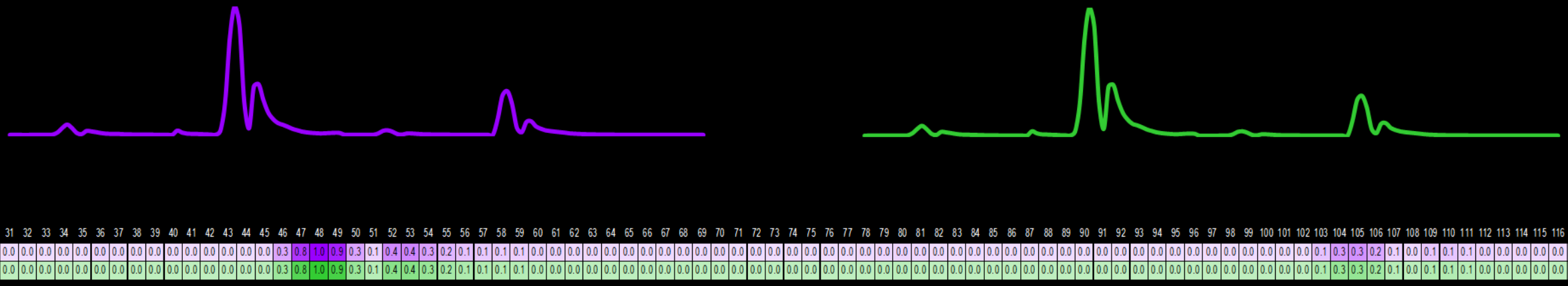
Reference Spectra are the same from single-colour cells or beads

Single-colour cells



Single-colour beads





SONY

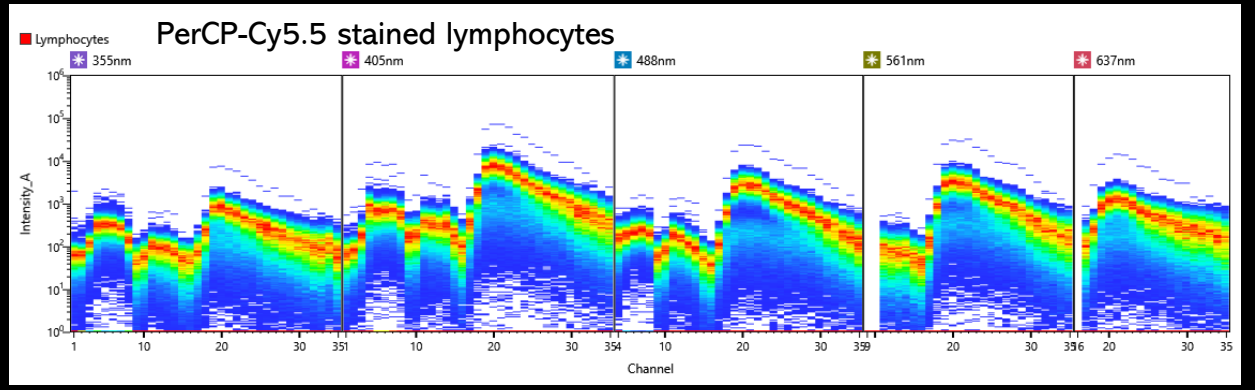
Single Colour Control Best Practices

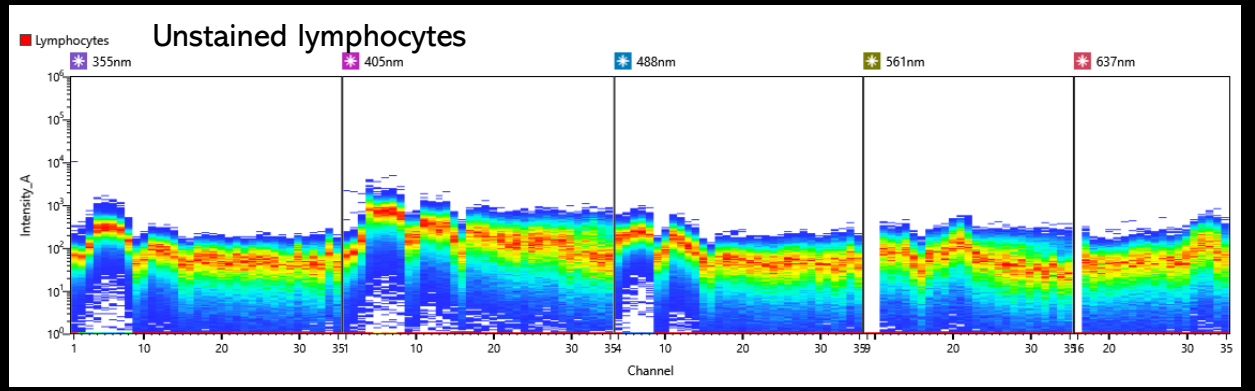
- Cells or beads will both work fine.
- Treat the controls the same way the sample will be treated.
- Antibody capture beads are preferred when possible.

SONY

Single Colour Control Best Practices

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

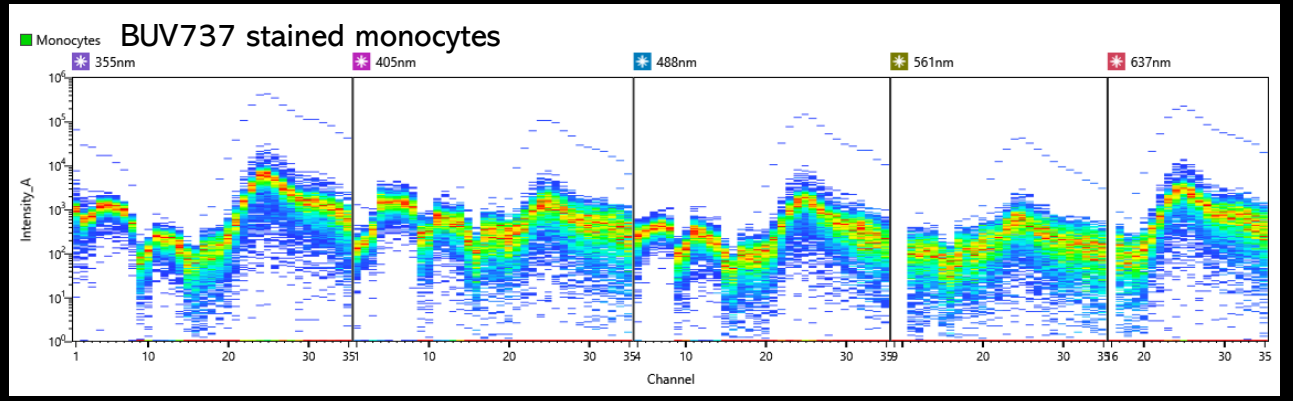


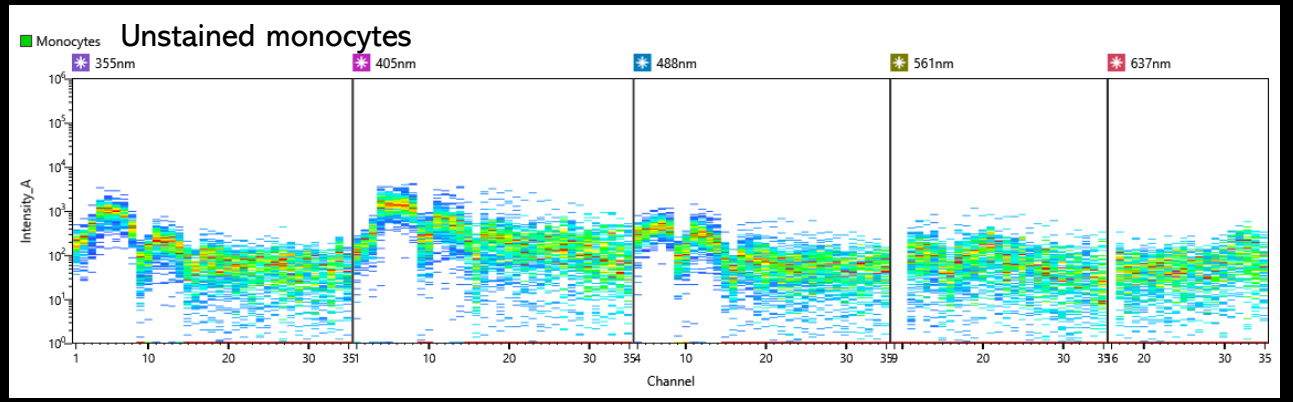


SONY

Single Colour Control Best Practices

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



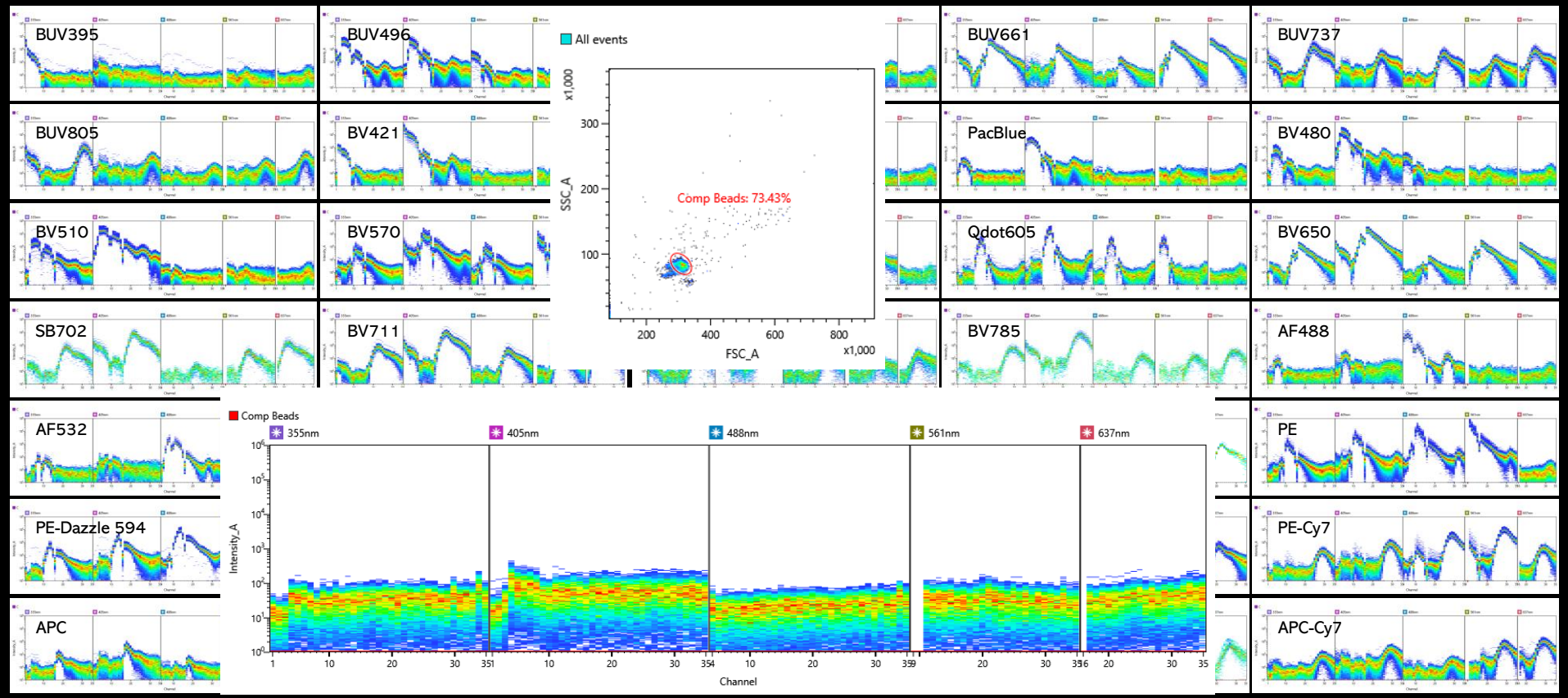


SONY

Single Colour Control Best Practices

Compensation beads

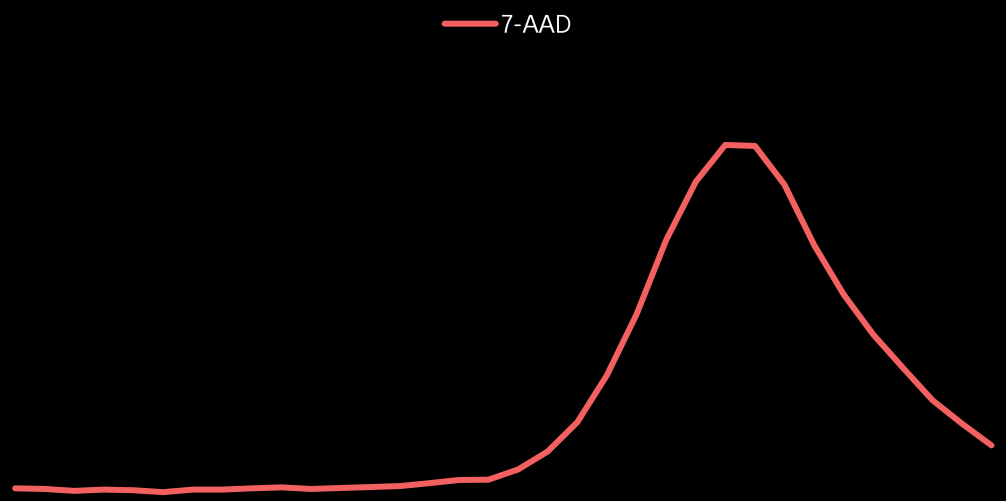
- Bright, well-defined signature
- Require only 1 negative control



SONY

Single Colour Control Best Practices

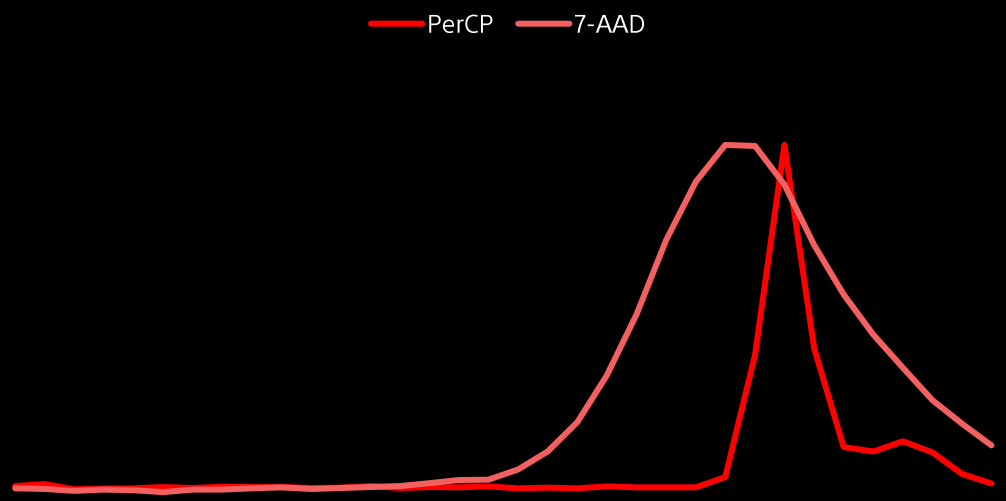
- Do not substitute similar fluorochromes for what is in your sample.

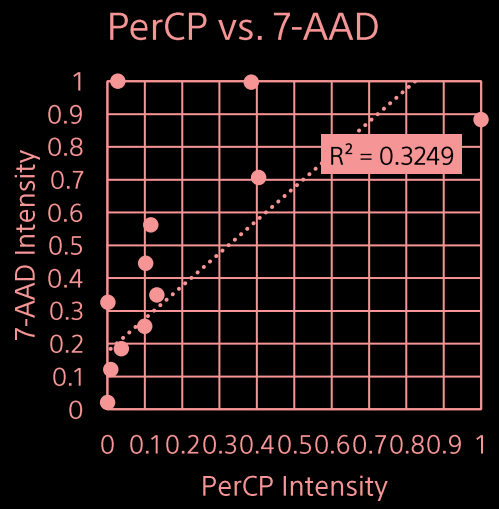


SONY

Single Colour Control Best Practices

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

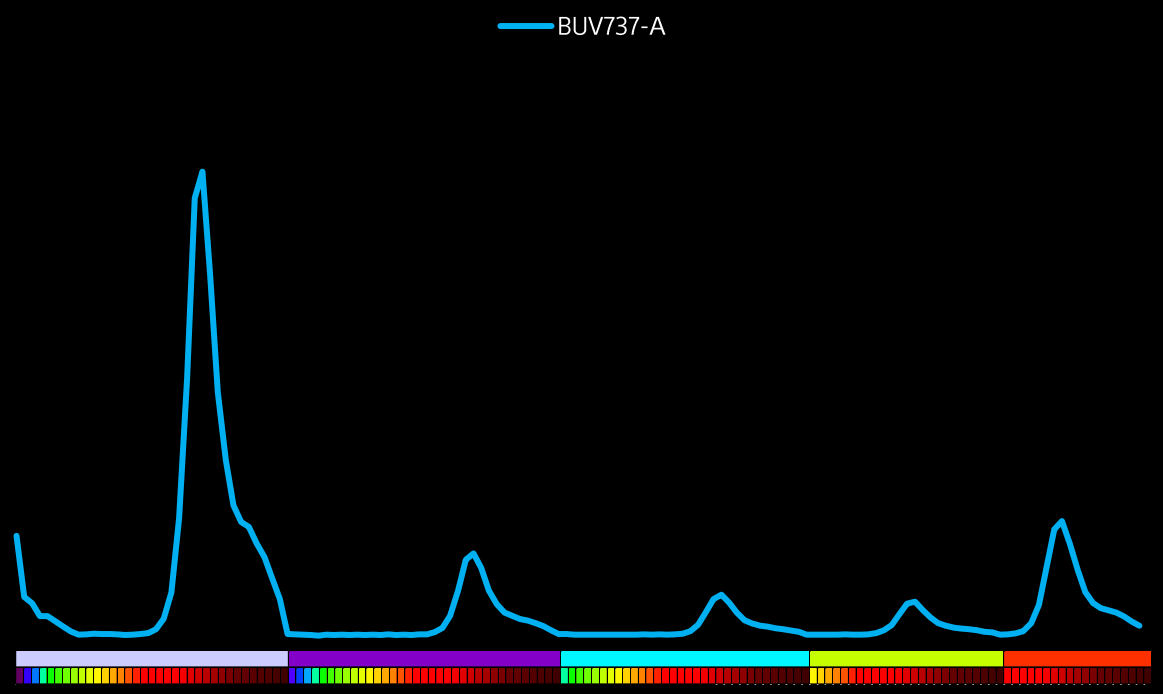




SONY

Single Colour Control Best Practices

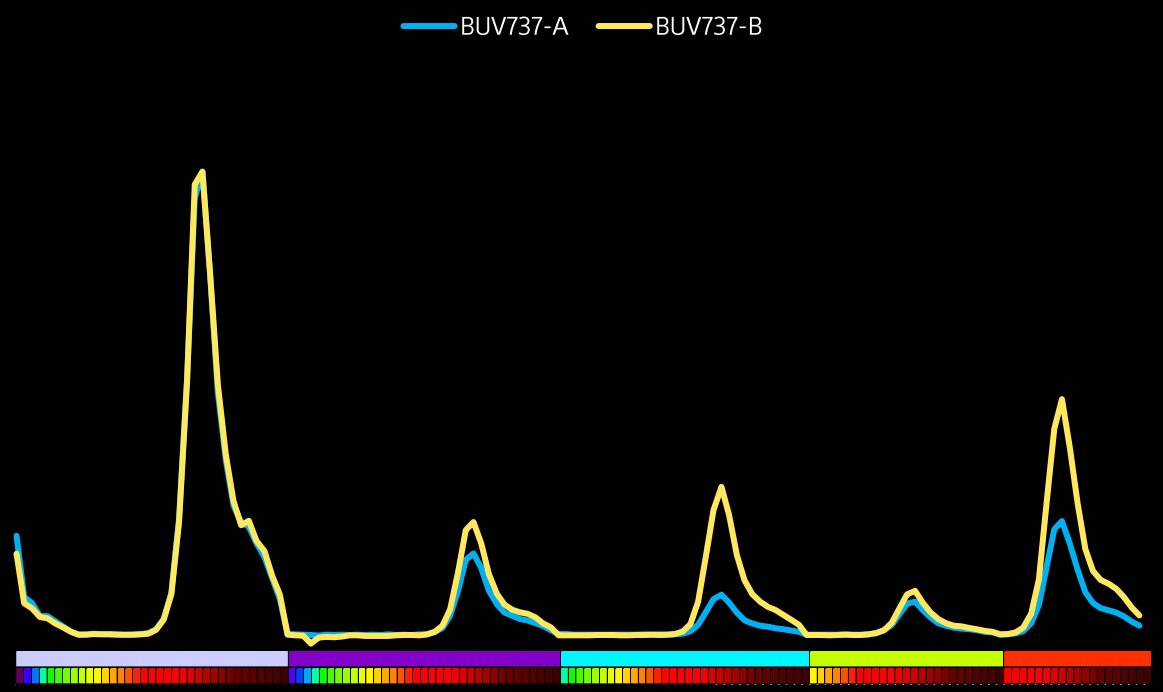
- Tandem dyes may have different degradation states.



SONY

Single Colour Control Best Practices

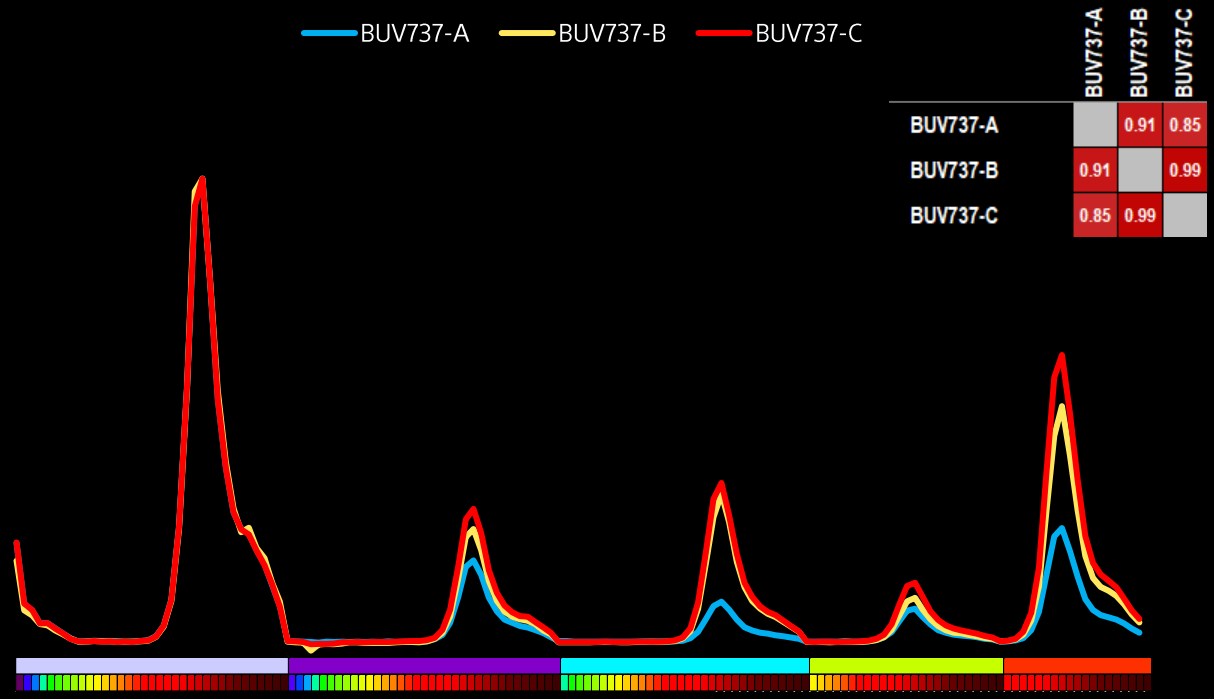
- Tandem dyes may have different degradation states.



SONY

Single Colour Control Best Practices

- Tandem dyes may have different degradation states.

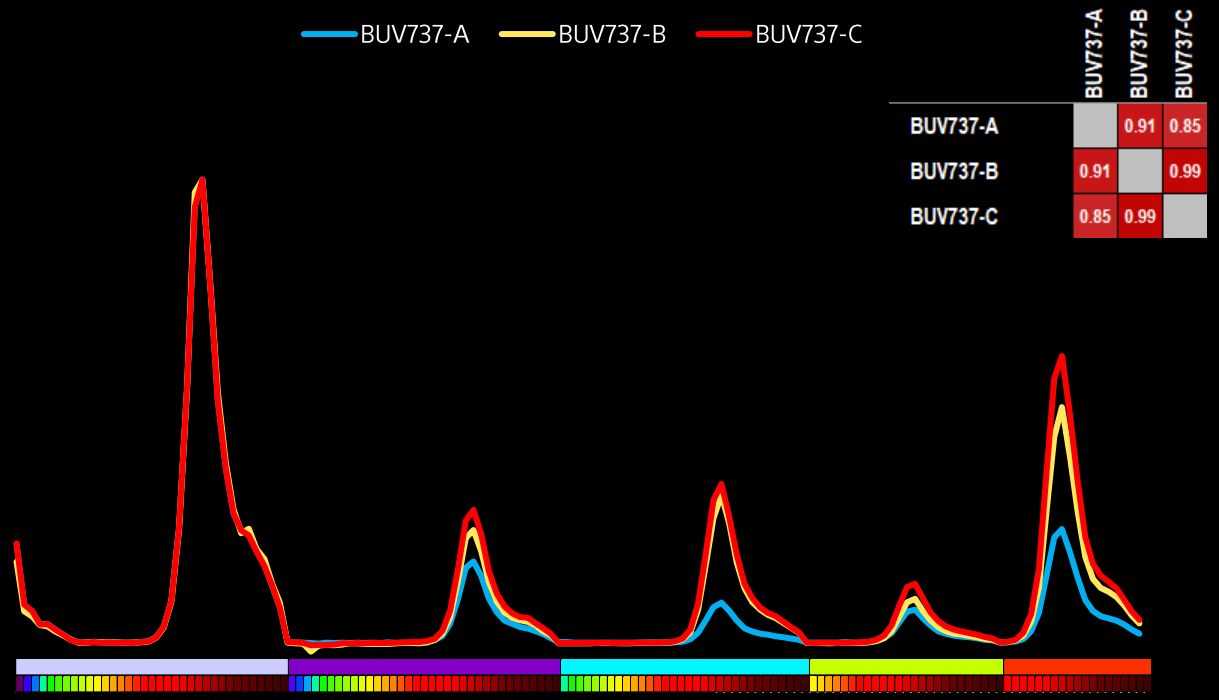


	BUV737-A	BUV737-B	BUV737-C
BUV737-A		0.91	0.85
BUV737-B	0.91		0.99
BUV737-C	0.85	0.99	

SONY

Dump Channels

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

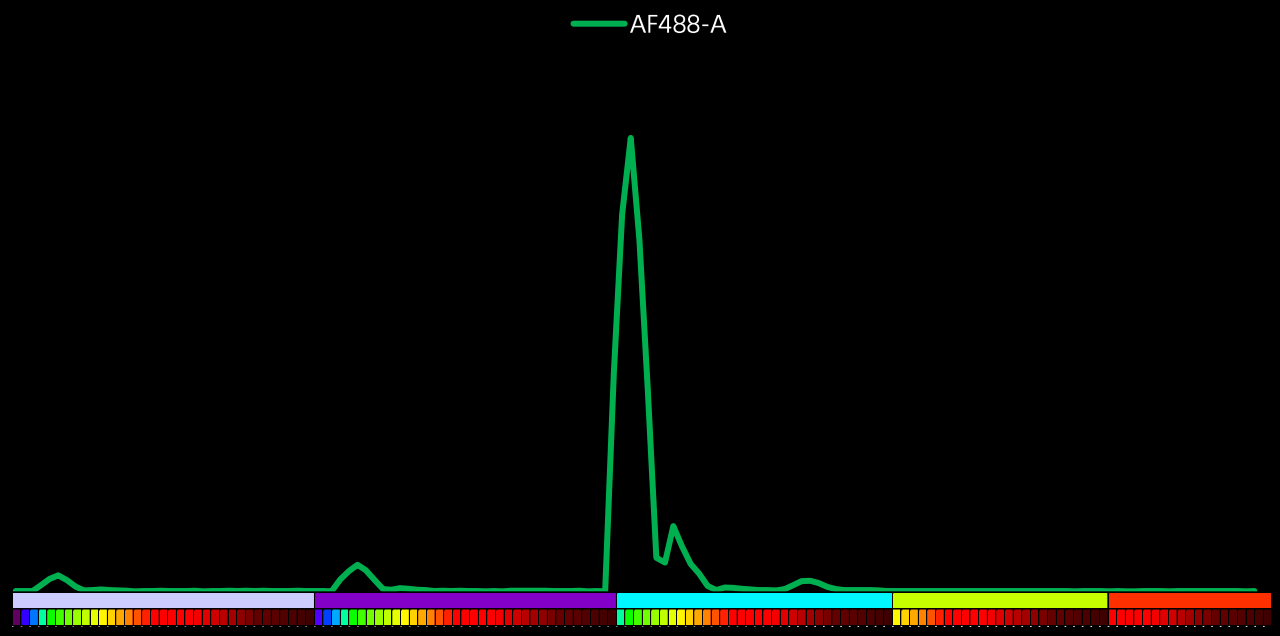


	BUV737-A	BUV737-B	BUV737-C
BUV737-A		0.91	0.85
BUV737-B	0.91		0.99
BUV737-C	0.85	0.99	

SONY

Dump Channels

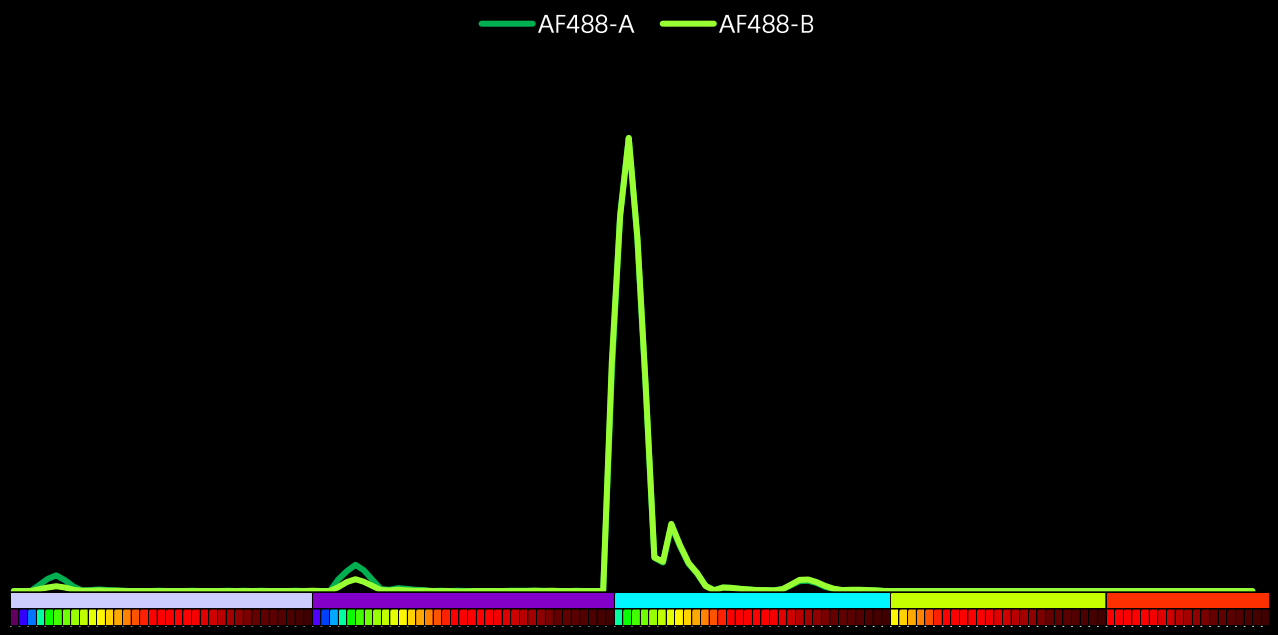
- Single molecule dyes are much more consistent with each other.



SONY

Dump Channels

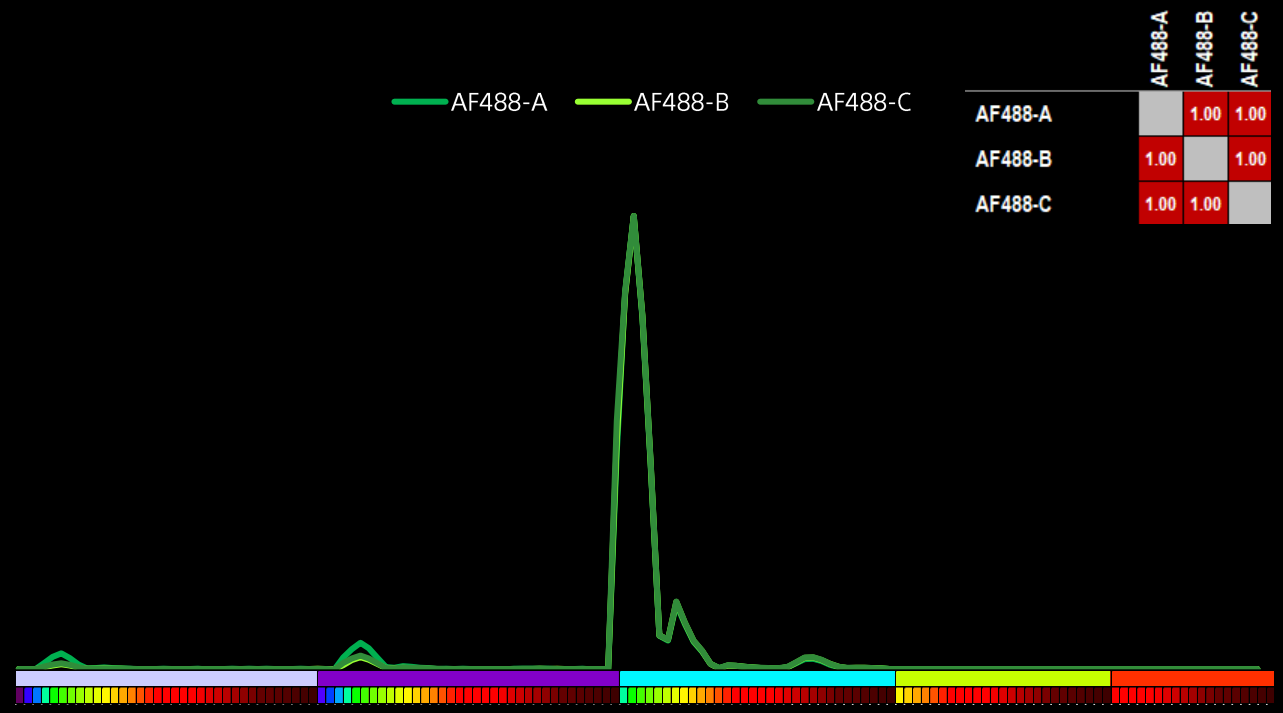
- Single molecule dyes are much more consistent with each other.



SONY

Dump Channels

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

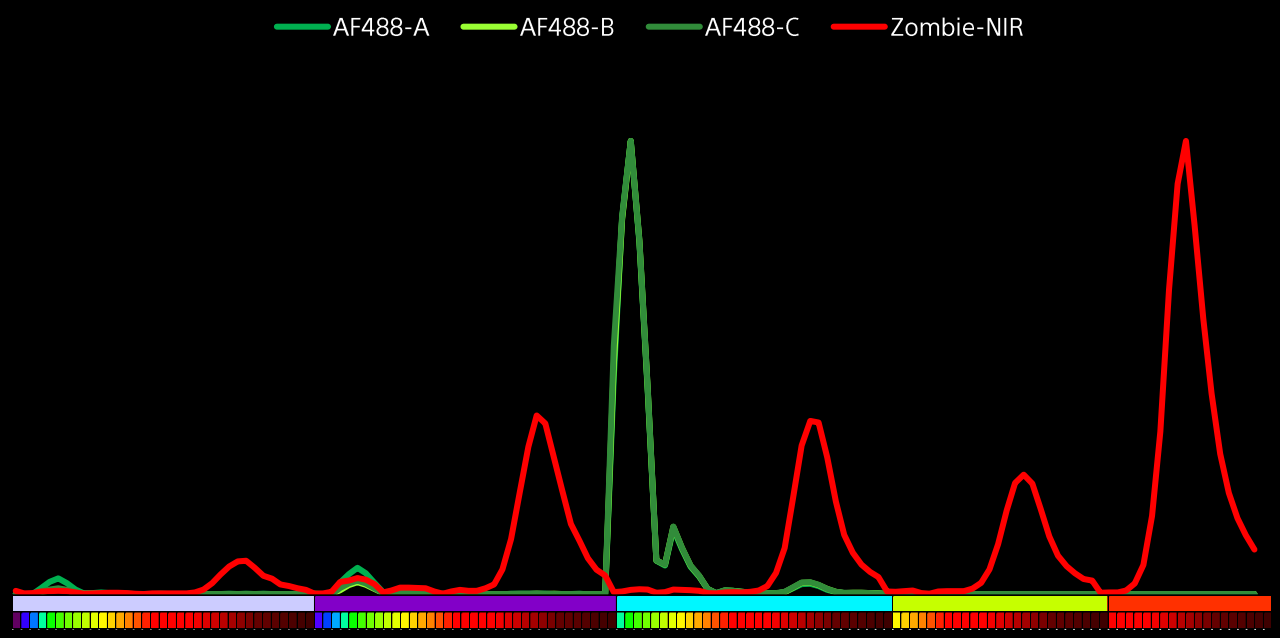


	AF488-A	AF488-B	AF488-C
AF488-A		1.00	1.00
AF488-B	1.00		1.00
AF488-C	1.00	1.00	

SONY

Dump Channels

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



Reference Spectra can be saved in the Spectral Reference Library

Single Colour Controls

Reference Spectra

Spectral Reference Library

BUV395

BV421

BV605

AF488

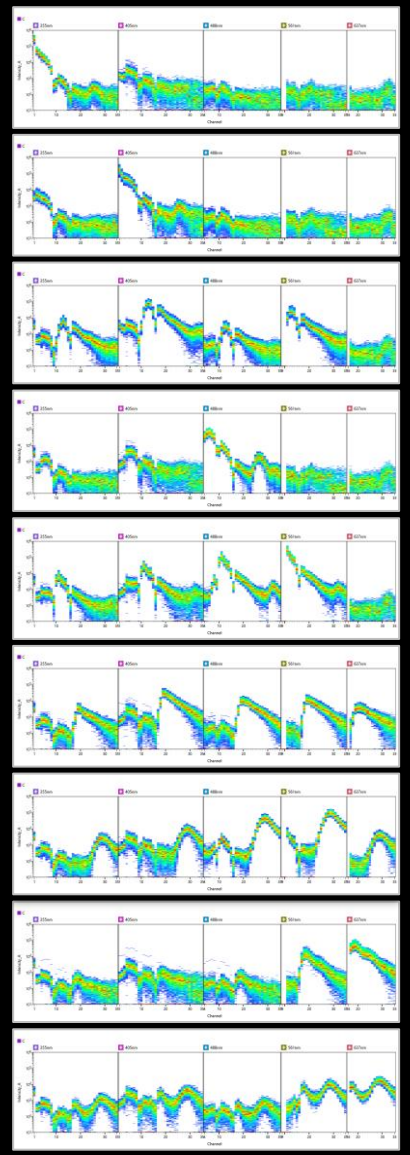
PE

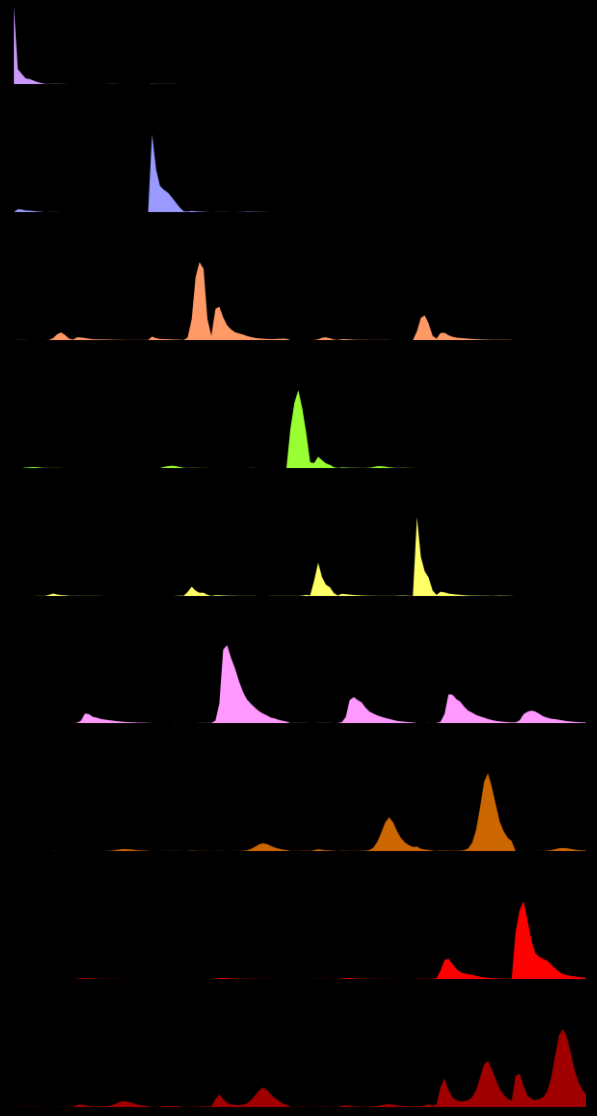
PerCP-Cy5.5

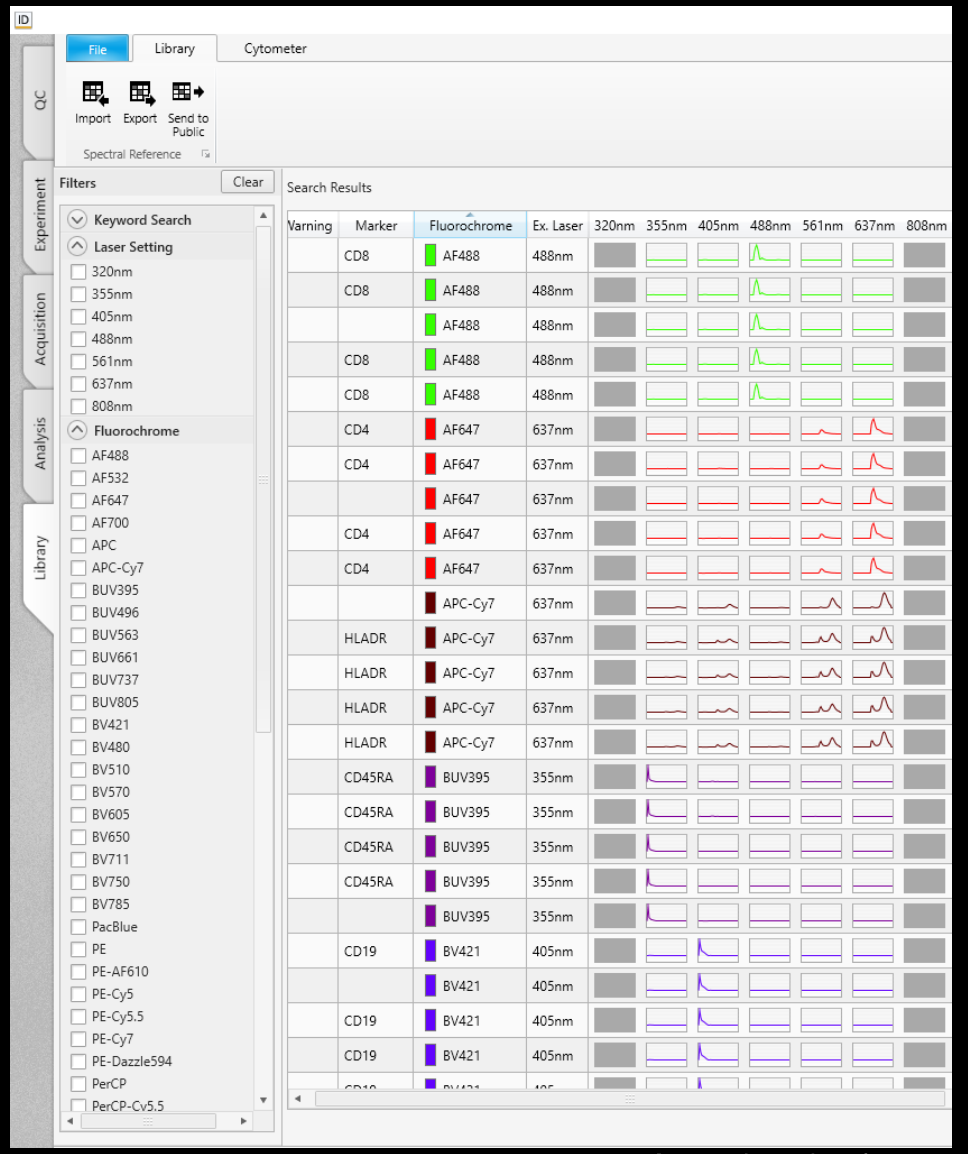
PE-Cy7

AF647

APC-Cy7







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**

Autofluorescence Finder

SONY

Autofluorescence Finder

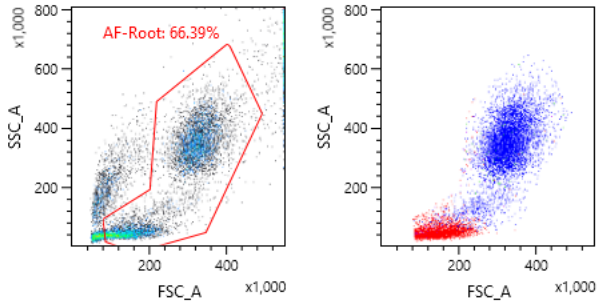
Step 1 : On a scatter plot, create a gate on the population for which you wish to determine presence of AF.

Scatter Plot

Use all Events

All events

AF-Root



tools

Rectangle | Ellipse | Polygon | Zoom

Display Event 10,000 events

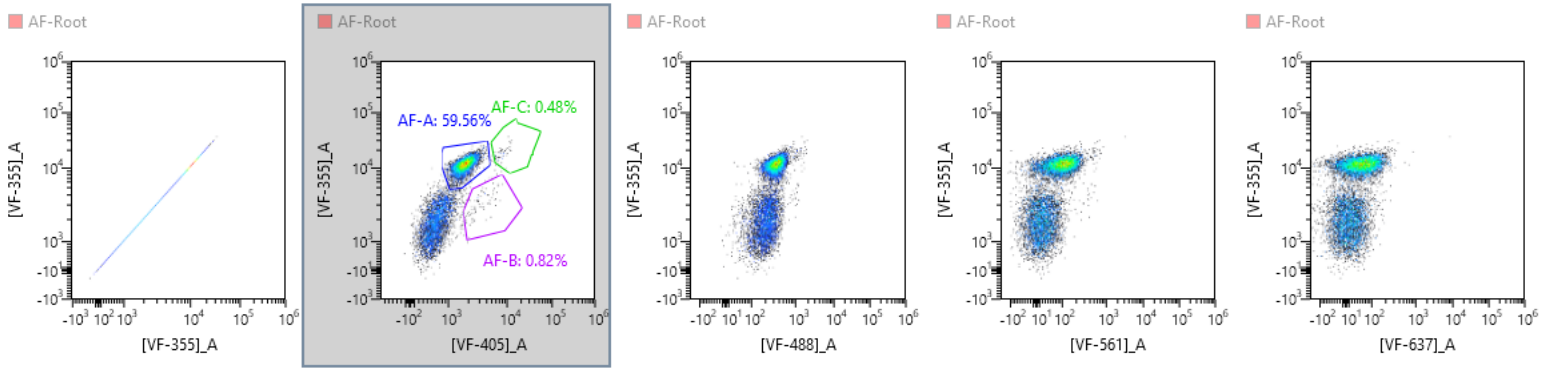
Step 2 : Create a gate for AF on a VF/SSC or VF/VF plot.

VF/SSC Plot

Density Plot

Dot Plot

Y Axis : [VF-355]



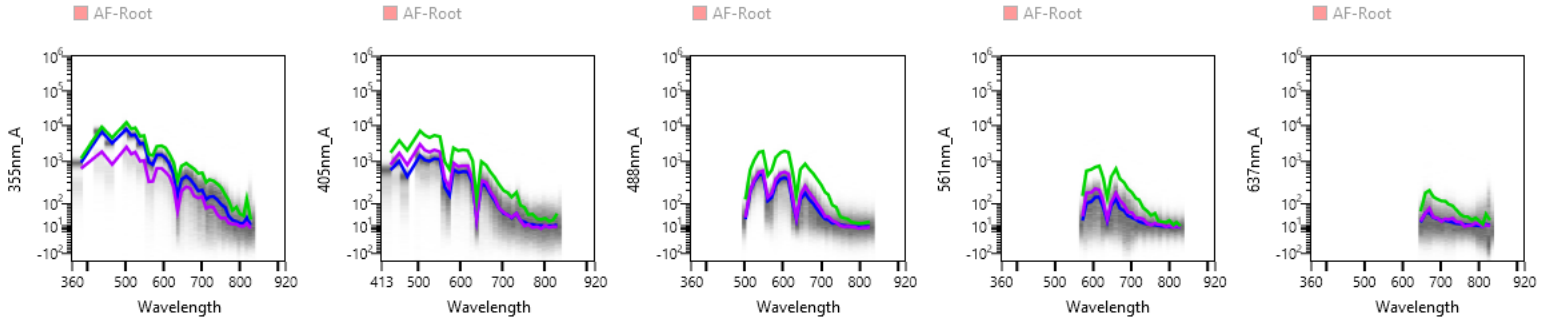
Virtual Filter

Overlay Plot

AF-A

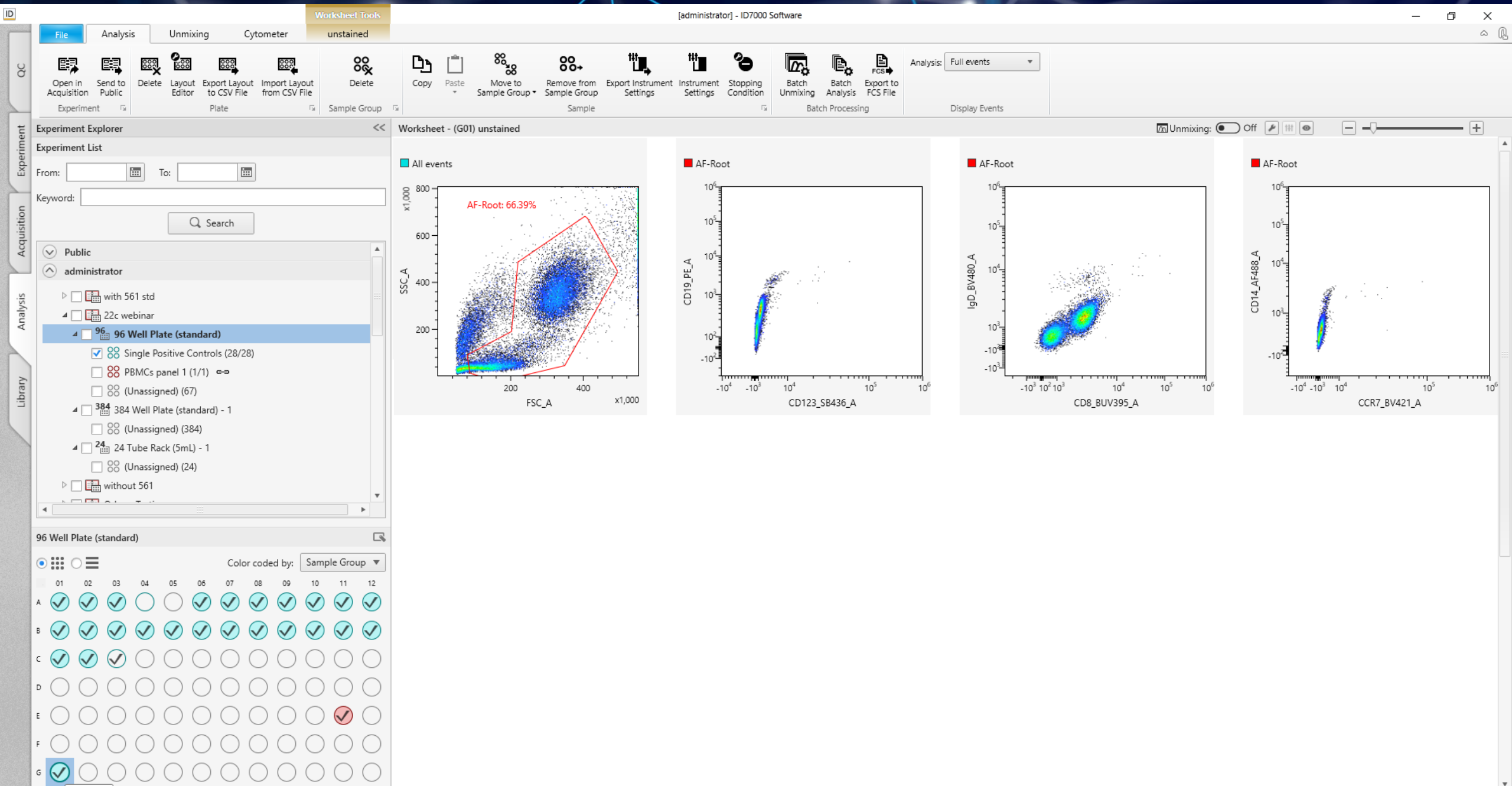
AF-B

AF-C



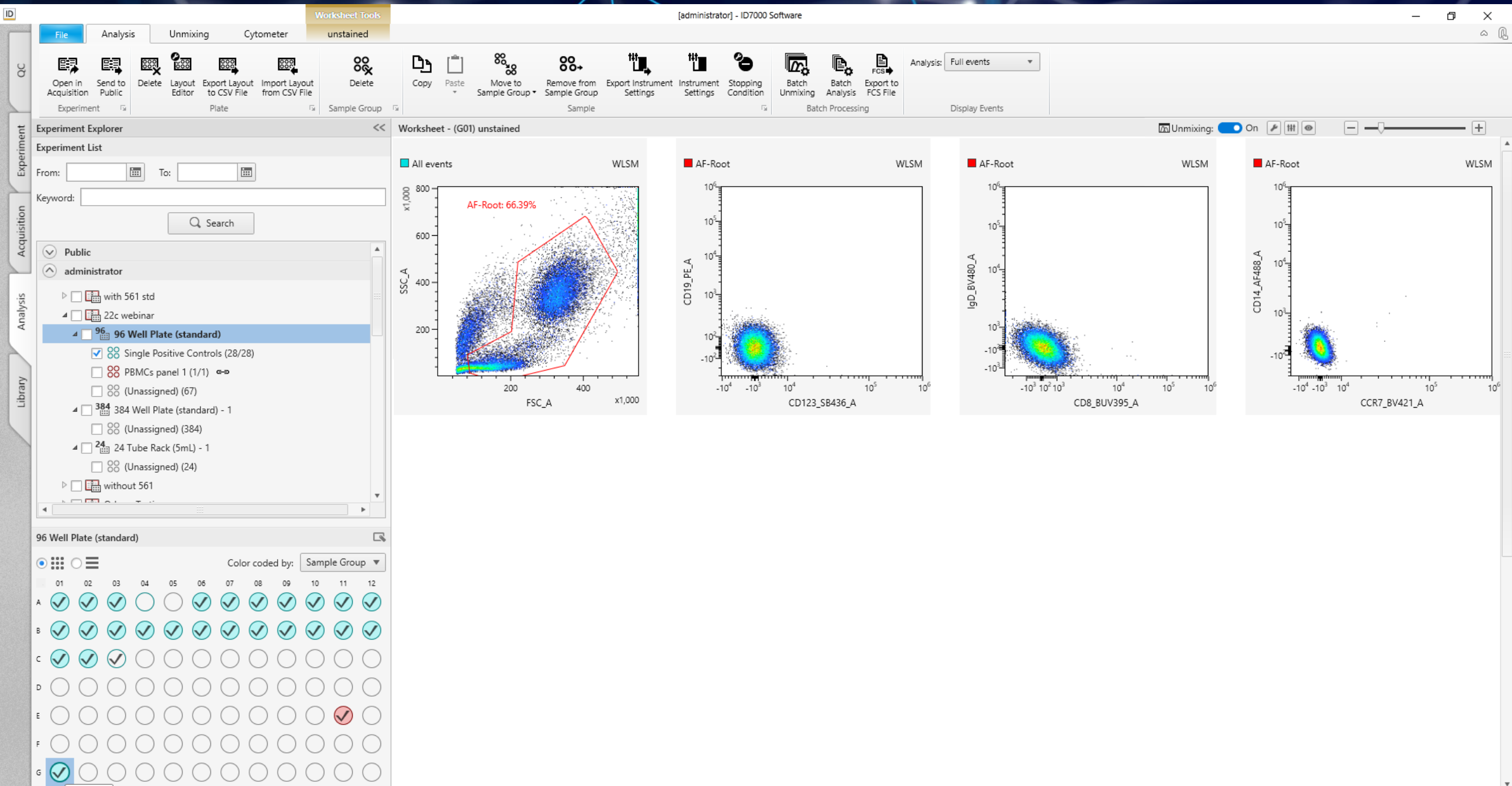
Autofluorescence Finder

SONY



Autofluorescence Unmixing

SONY



Autofluorescence

SONY

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.)		- - -	

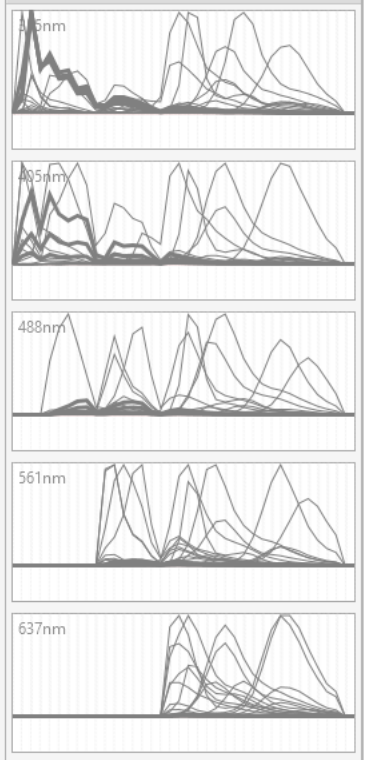
Color Panel Settings | Fluorochrome Settings

Autofluorescence Add

Index	Use as AF	Autofluorescence	Ex. Laser	Mode	SR	Negative	Positive
1	☑	[AF color 1]	405nm	Normal(Adv.)		Zero Reference	AF-A (Well - A01)
2	☑	[AF color 2]	405nm	Normal(Adv.)		Zero Reference	AF-B (Well - A01)
3	☑	[AF color 3]	405nm	Normal(Adv.)		Zero Reference	AF-C (Well - A01)

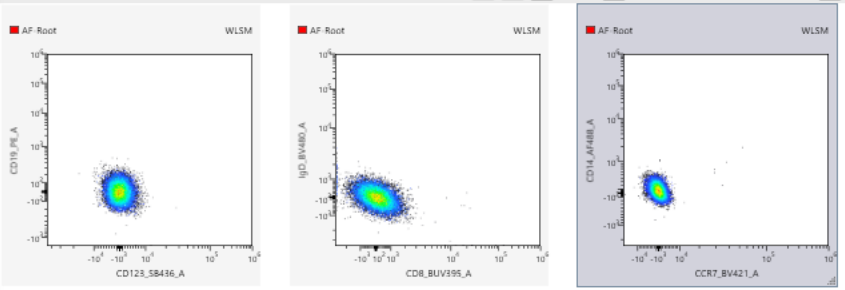
Autofluorescence

Spectral Reference



Calculate | Apply

Unmixing: On



Autofluorescence

SONY

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.)		- - -	

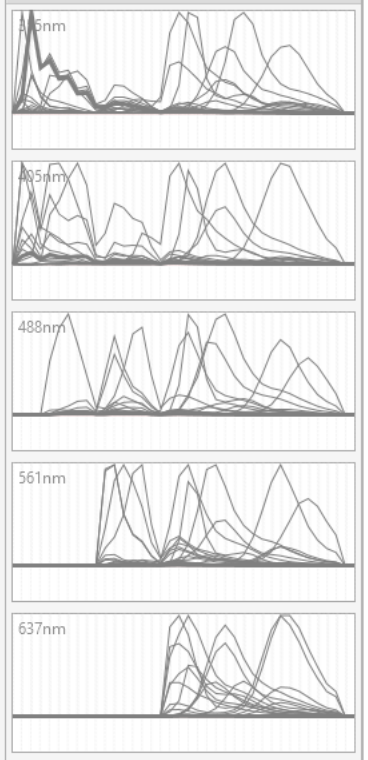
Color Panel Settings | Fluorochrome Settings

Autofluorescence Add

Index	Use as AF	Autofluorescence	Ex. Laser	Mode	SR	Negative	Positive
1	☐	[AF color 1]	405nm	Normal(Adv.)		Zero Reference	AF-A (Well - A01)
2	☐	[AF color 2]	405nm	Normal(Adv.)		Zero Reference	AF-B (Well - A01)
3	☐	[AF color 3]	405nm	Normal(Adv.)		Zero Reference	AF-C (Well - A01)

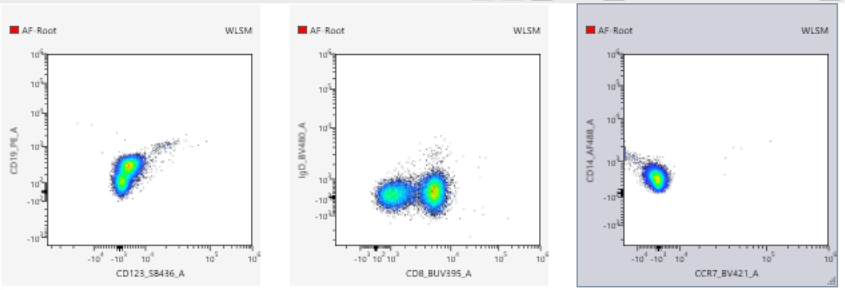
Autofluorescence

Spectral Reference



Calculate | Apply

Unmixing: On



Autofluorescence

SONY

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.)		- - -	

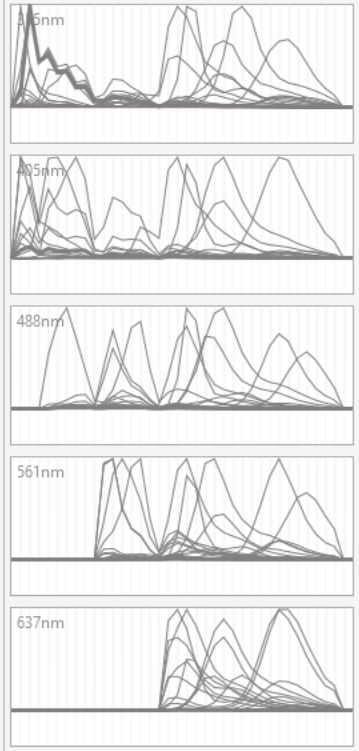
Color Panel Settings | Fluorochrome Settings

Autofluorescence Add

Index	Use as AF	Autofluorescence	Ex. Laser	Mode	SR	Negative	Positive
1	☑	[AF color 1]	405nm	Normal(Adv.)		Zero Reference	AF-A (Well - A01)
2	☐	[AF color 2]	405nm	Normal(Adv.)		Zero Reference	AF-B (Well - A01)
3	☐	[AF color 3]	405nm	Normal(Adv.)		Zero Reference	AF-C (Well - A01)

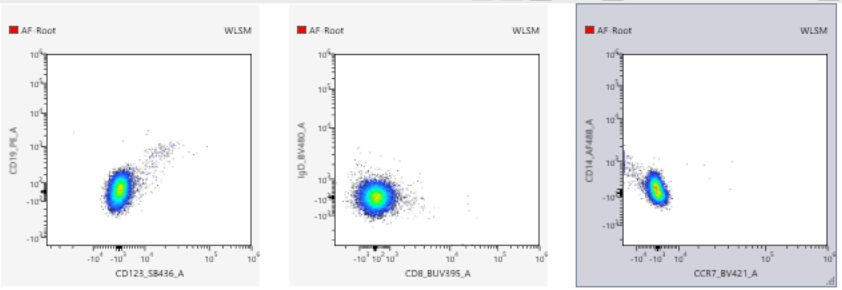
Autofluorescence

Spectral Reference



Calculate | Apply

Unmixing: On



Autofluorescence

SONY

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.)		- - -	

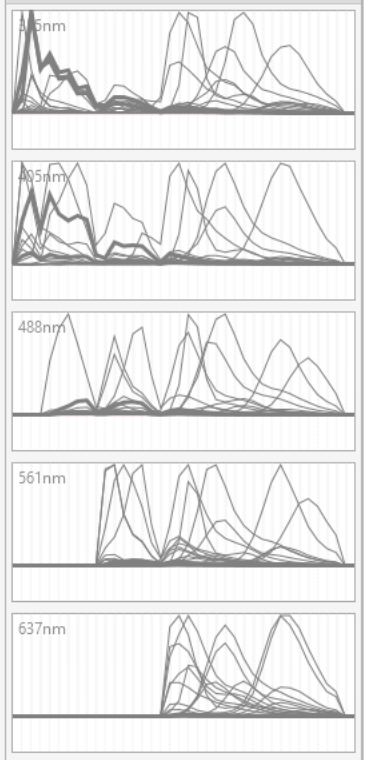
Color Panel Settings | Fluorochrome Settings

Autofluorescence Add

Index	Use as AF	Autofluorescence	Ex. Laser	Mode	SR	Negative	Positive
1	☑	[AF color 1]	405nm	Normal(Adv.)		Zero Reference	AF-A (Well - A01)
2	☑	[AF color 2]	405nm	Normal(Adv.)		Zero Reference	AF-B (Well - A01)
3	☐	[AF color 3]	405nm	Normal(Adv.)		Zero Reference	AF-C (Well - A01)

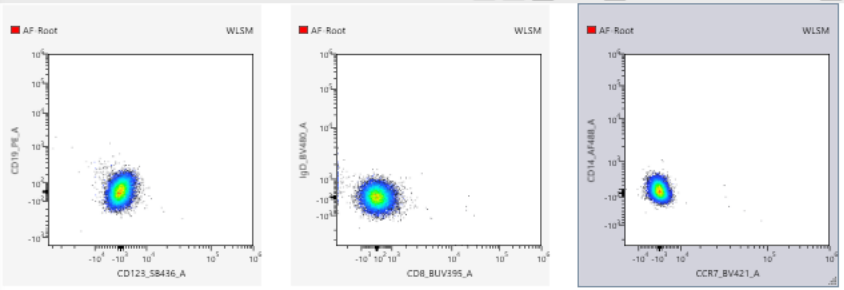
Autofluorescence

Spectral Reference



Calculate | Apply

Unmixing: On



Autofluorescence

SONY

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.)		- - -	

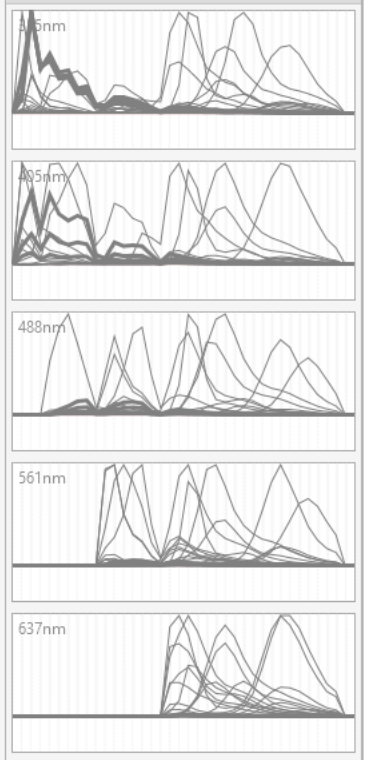
Color Panel Settings | Fluorochrome Settings

Autofluorescence Add

Index	Use as AF	Autofluorescence	Ex. Laser	Mode	SR	Negative	Positive
1	☑	[AF color 1]	405nm	Normal(Adv.)		Zero Reference	AF-A (Well - A01)
2	☑	[AF color 2]	405nm	Normal(Adv.)		Zero Reference	AF-B (Well - A01)
3	☑	[AF color 3]	405nm	Normal(Adv.)		Zero Reference	AF-C (Well - A01)

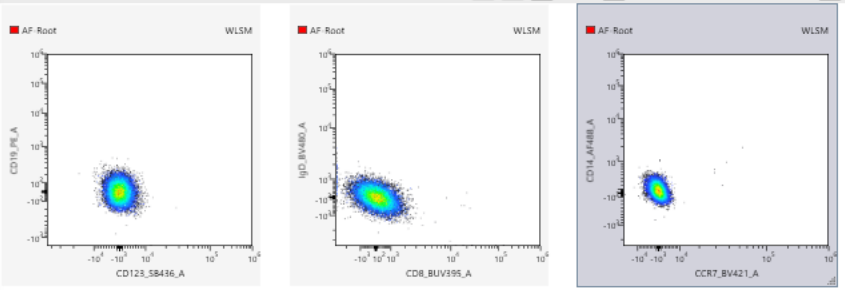
Autofluorescence

Spectral Reference



Calculate | Apply

Unmixing: On



Autofluorescence

SONY

Unmixing Settings

Color Panel | Matrix

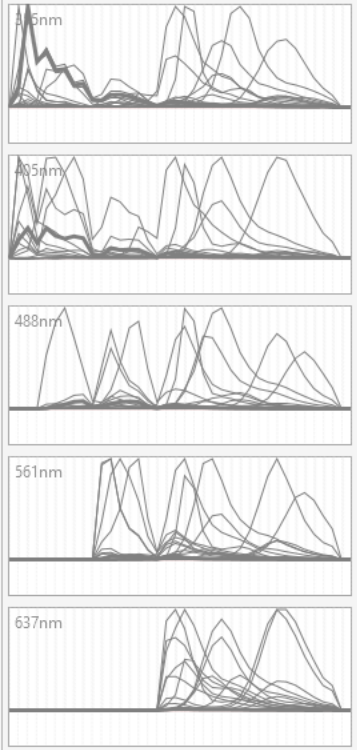
Fluorochrome Add

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

Spectral Reference



Autofluorescence Add

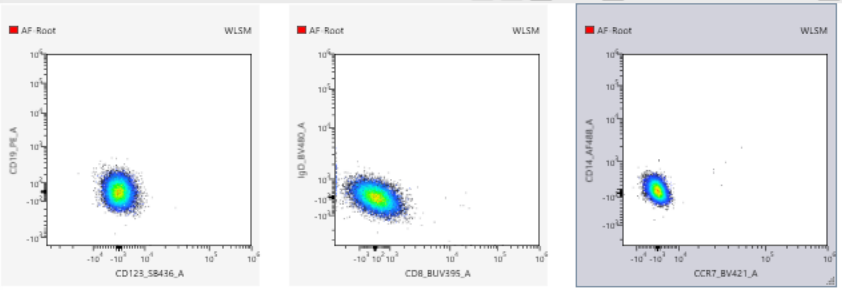
Index	Use as AF	Autofluorescence	Ex. Laser	Mode	SR	Negative	Positive
1	☑	[AF color 1]	405nm	Normal(Adv.)		Zero Reference	AF-A (Well - A01)
2	☑	[AF color 2]	405nm	Normal(Adv.)		Zero Reference	AF-B (Well - A01)
3	☑			Normal(Adv.)		Zero Reference	AF-C (Well - A01)

Autofluorescence

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

Calculate | Apply

Unmixing: On



Autofluorescence

SONY

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.)		- - -	

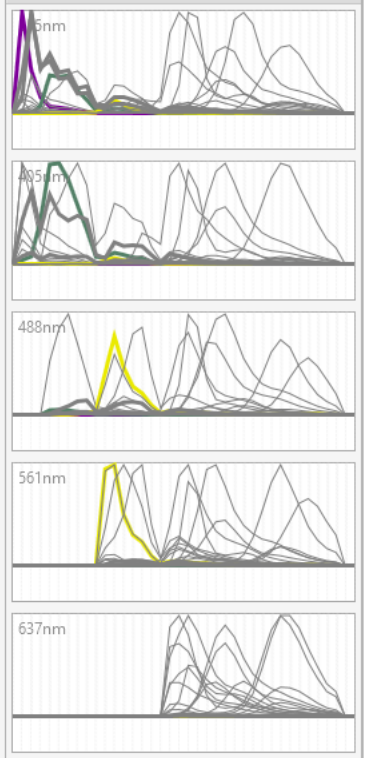
Color Panel Settings | Fluorochrome Settings

Autofluorescence Add

Index	Use as AF	Autofluorescence	Ex. Laser	Mode	SR	Negative	Positive
1	☑	[AF color 1]	405nm	Normal(Adv.)		Zero Reference	AF-A (Well - A01)
2	☑	[AF color 2]	405nm	Normal(Adv.)		Zero Reference	AF-B (Well - A01)

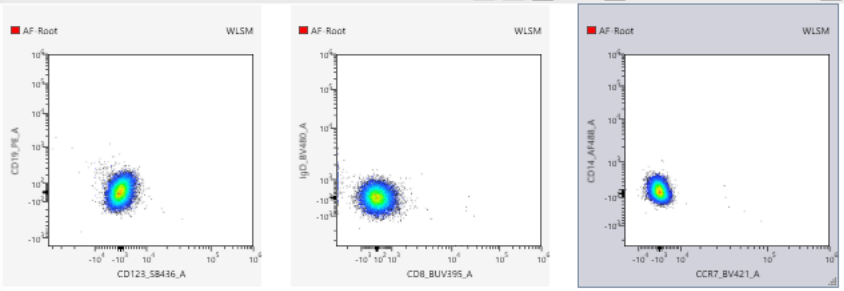
Autofluorescence

Spectral Reference



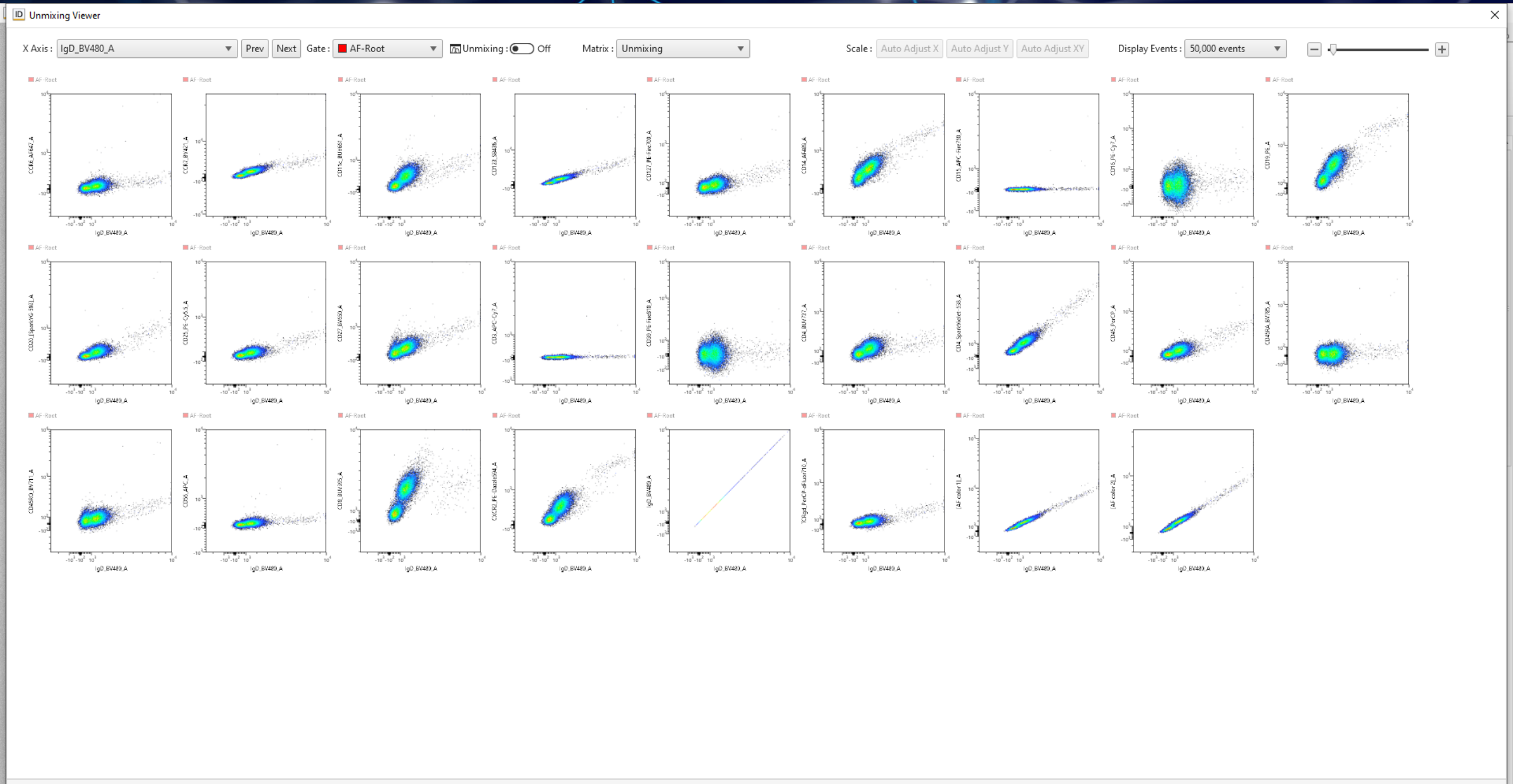
Calculate | Apply

Unmixing: On



Autofluorescence

SONY



Autofluorescence

SONY

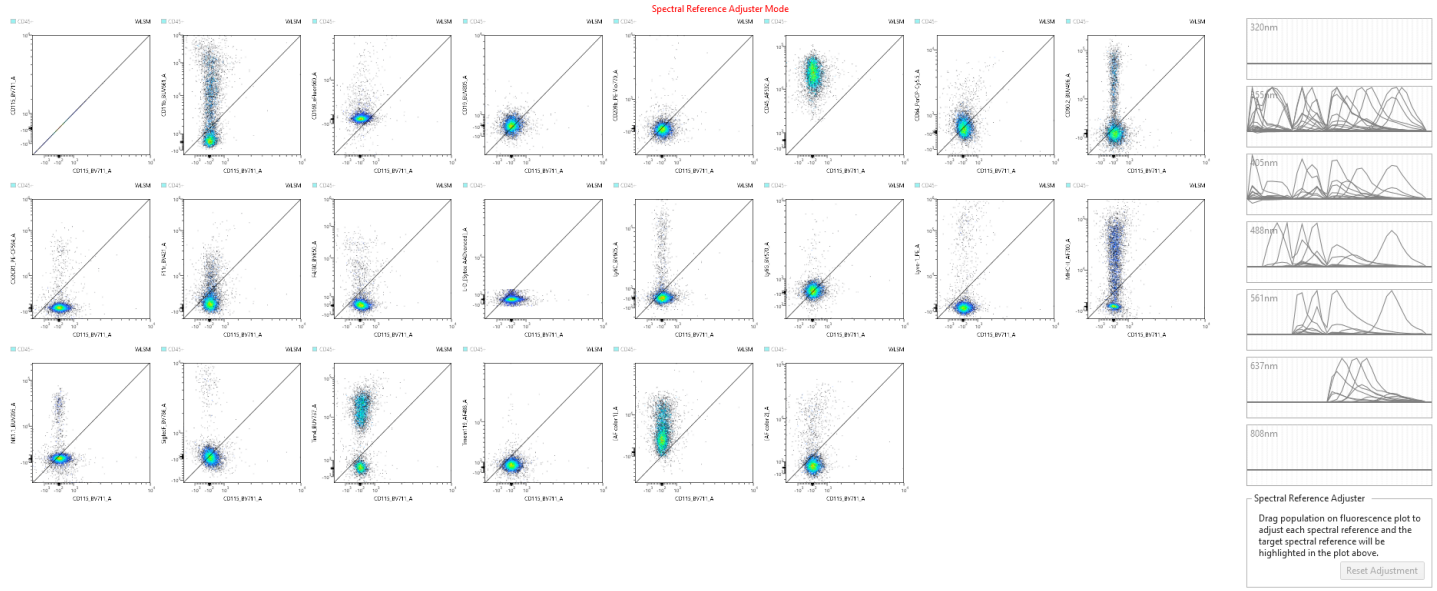
ID7000 Workflow

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

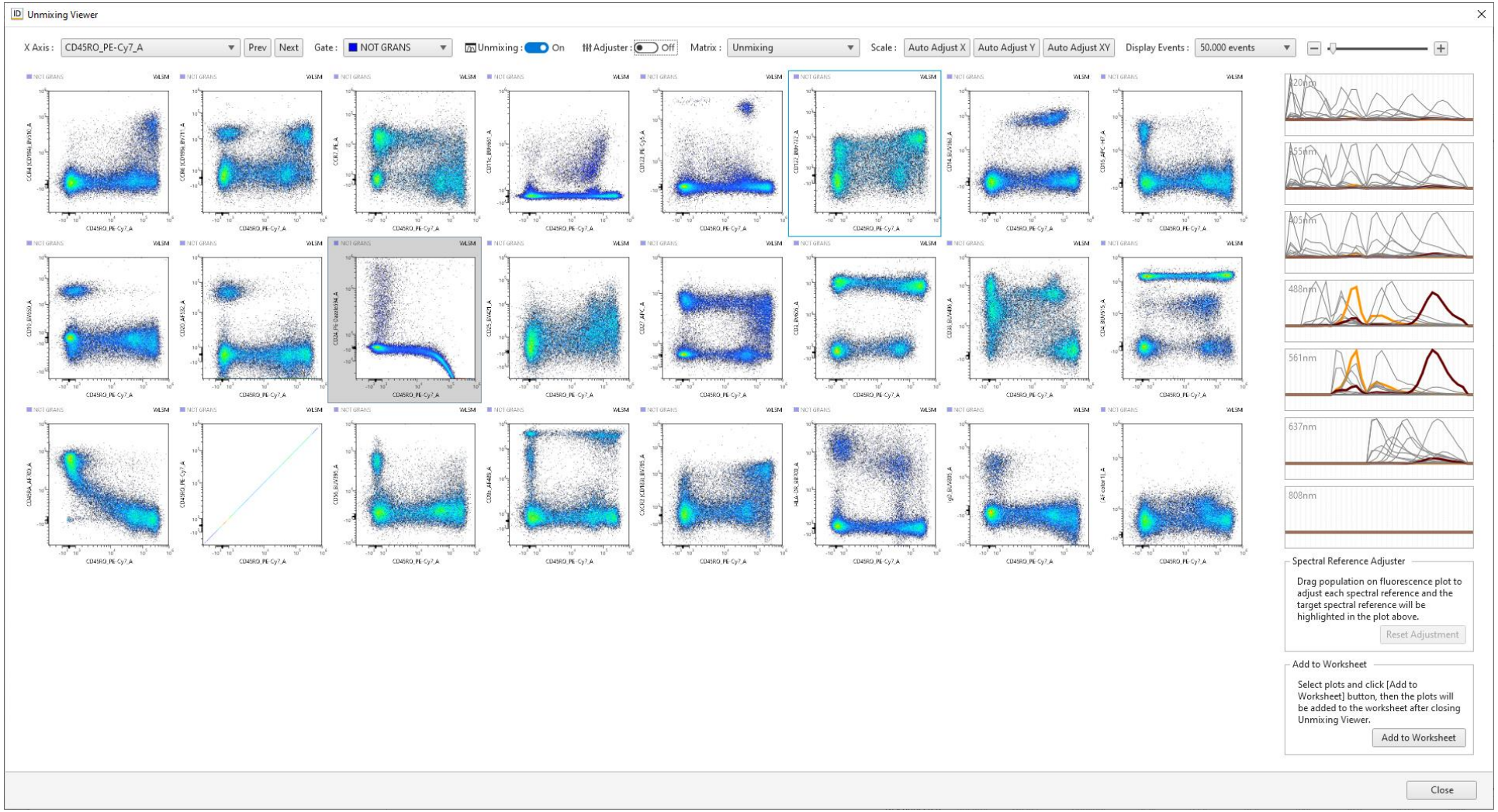


Unmixing Viewer

1. Gating strategy until singlets/LD level

2. Unmixing Viewer

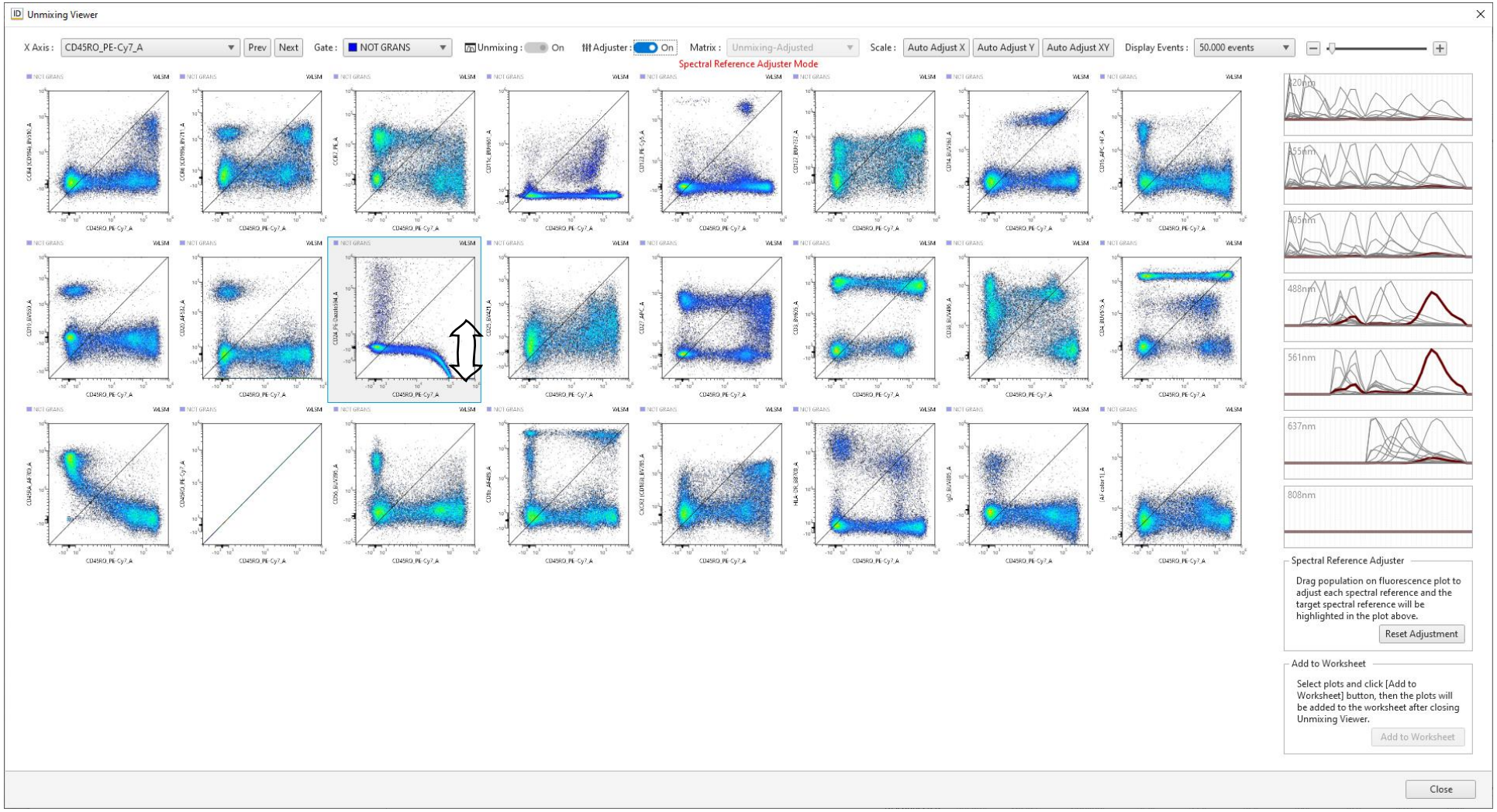
PE-Cy7



Unmixing Viewer

3. Spectral Reference Adjuster

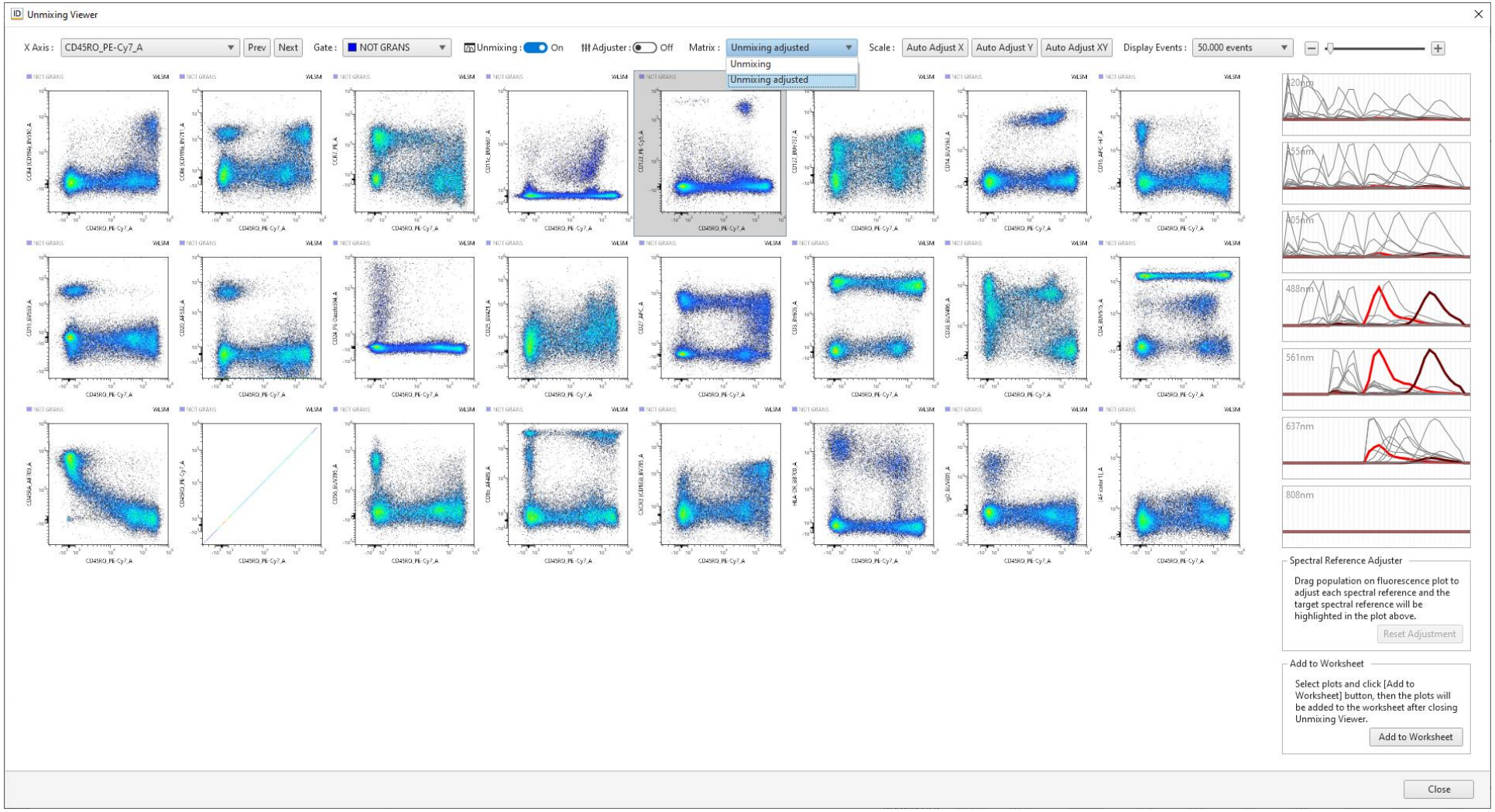
PE-Cy7



Unmixing Viewer

3. Spectral Reference Adjuster

PE-Cy7



Panel Design

Panel Design

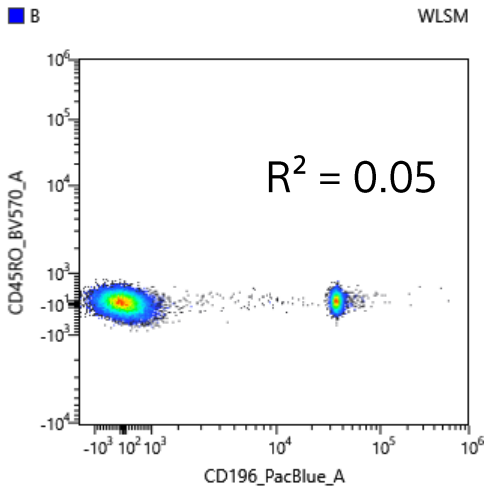
1. Know your fluorochrome
   - Similarity/R^2 value
   - Brightness
   - Availability

2. Know your sample
   - Primary: ON/OFF (lineage)
   - Secondary: continuum
   - Tertiary: low/unknown
   - Gating Strategy

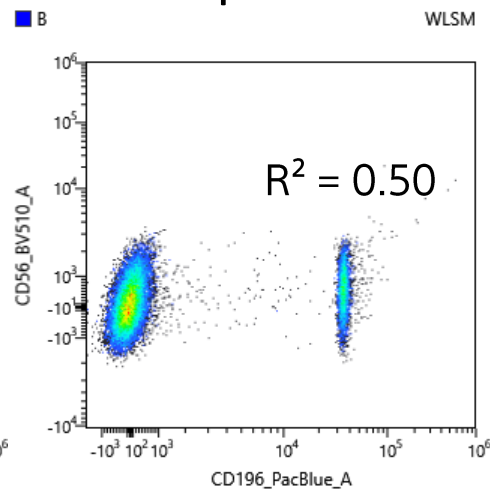
R^2 Value

	BV421	eF450	PacBlue	BV480	BV510	PacOrange	BV570
BV421		0.59	0.41	0.12	0.07	0.03	0.01
eF450	0.59		0.95	0.51	0.34	0.15	0.05
PacBlue	0.41	0.95		0.70	0.49	0.23	0.07
BV480	0.12	0.51	0.70		0.88	0.53	0.17
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	0.01	0.05	0.07	0.17	0.05	0.23	

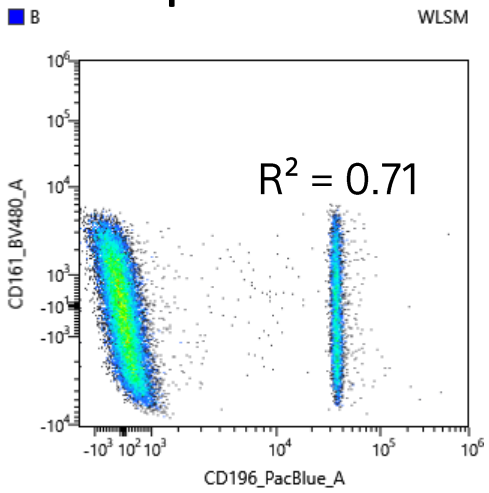
spread



spread



spread



Panel Design

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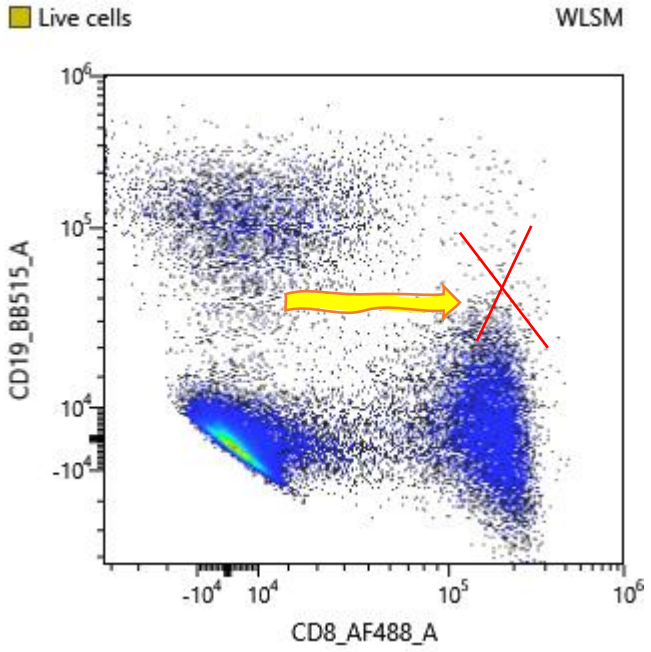
   - Similarity/R^2 value
   - Brightness
   - Availability

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

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   - Similarity/R^2 value
   - Brightness
   - Availability

2. Know your sample
   - Primary: ON/OFF (lineage)
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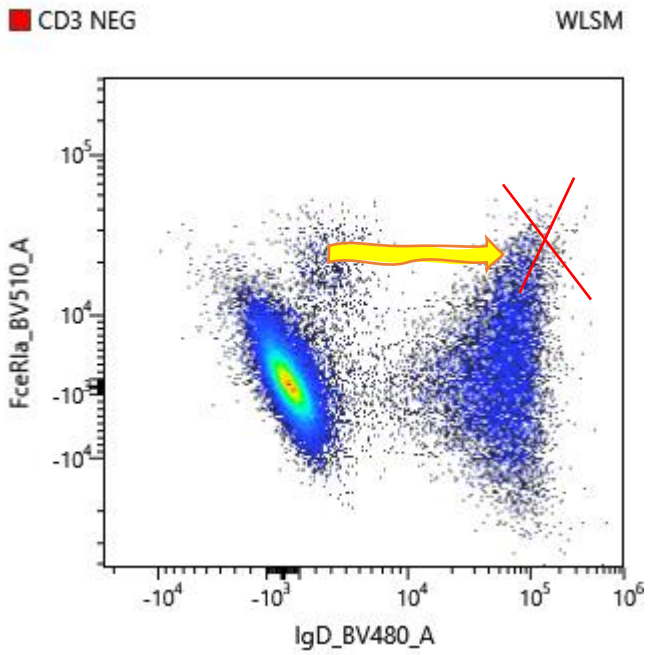
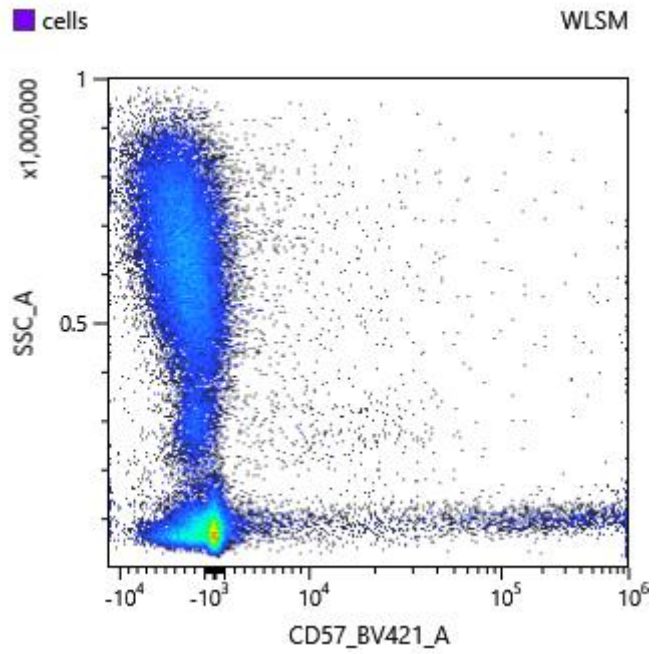
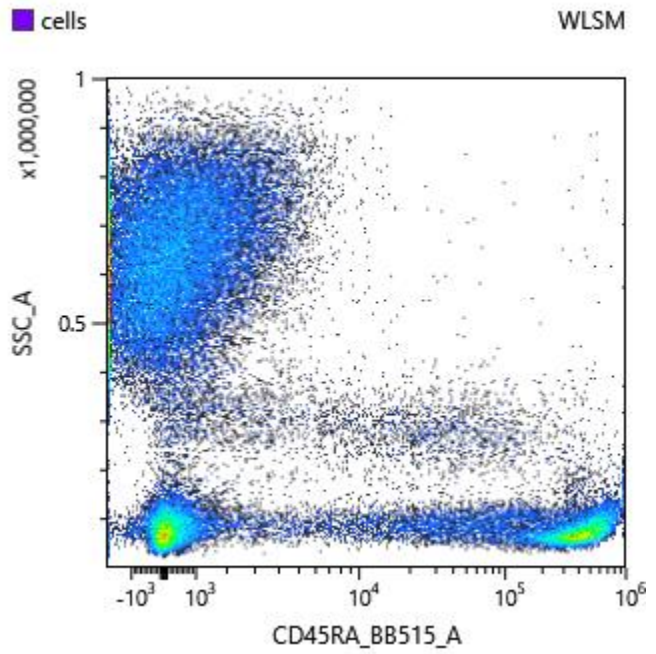


Panel Design

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





SONY

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

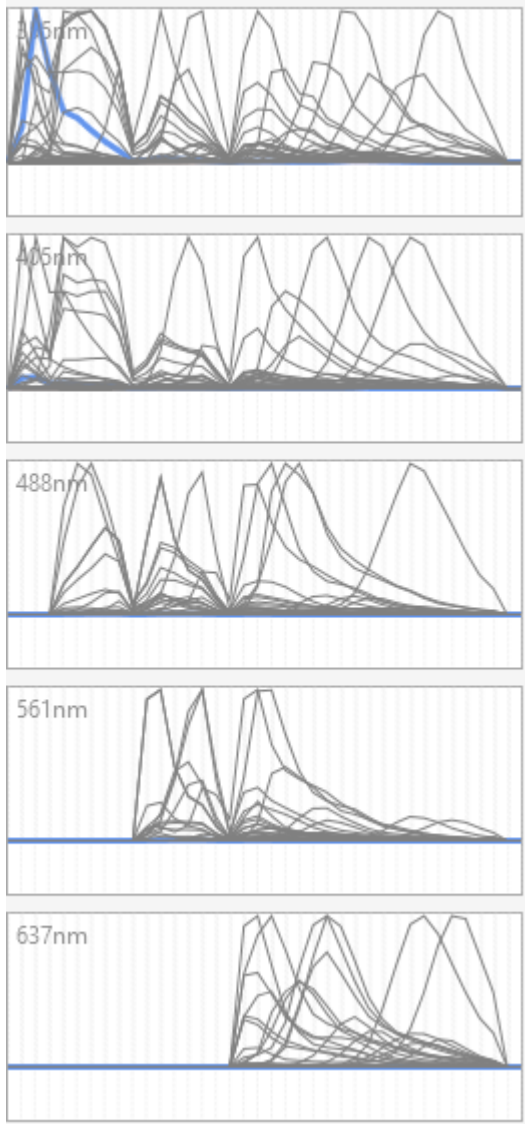
"Good" fluorochromes:
- Low overlap (e.g. BUV395)
- Bright

Panel Design

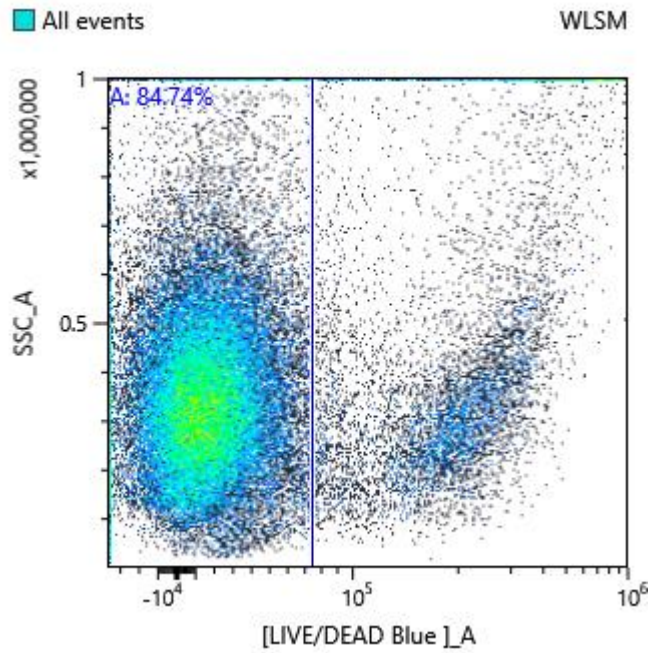
1. Know your fluorochrome
   - Similarity/R^2 value
   - Brightness
   - Availability

2. Know your sample
   - Primary: ON/OFF (lineage)
   - Secondary: continuum
   - Tertiary: low/unknown
   - Gating Strategy?

Live/Dead



Live gate = 1st gate

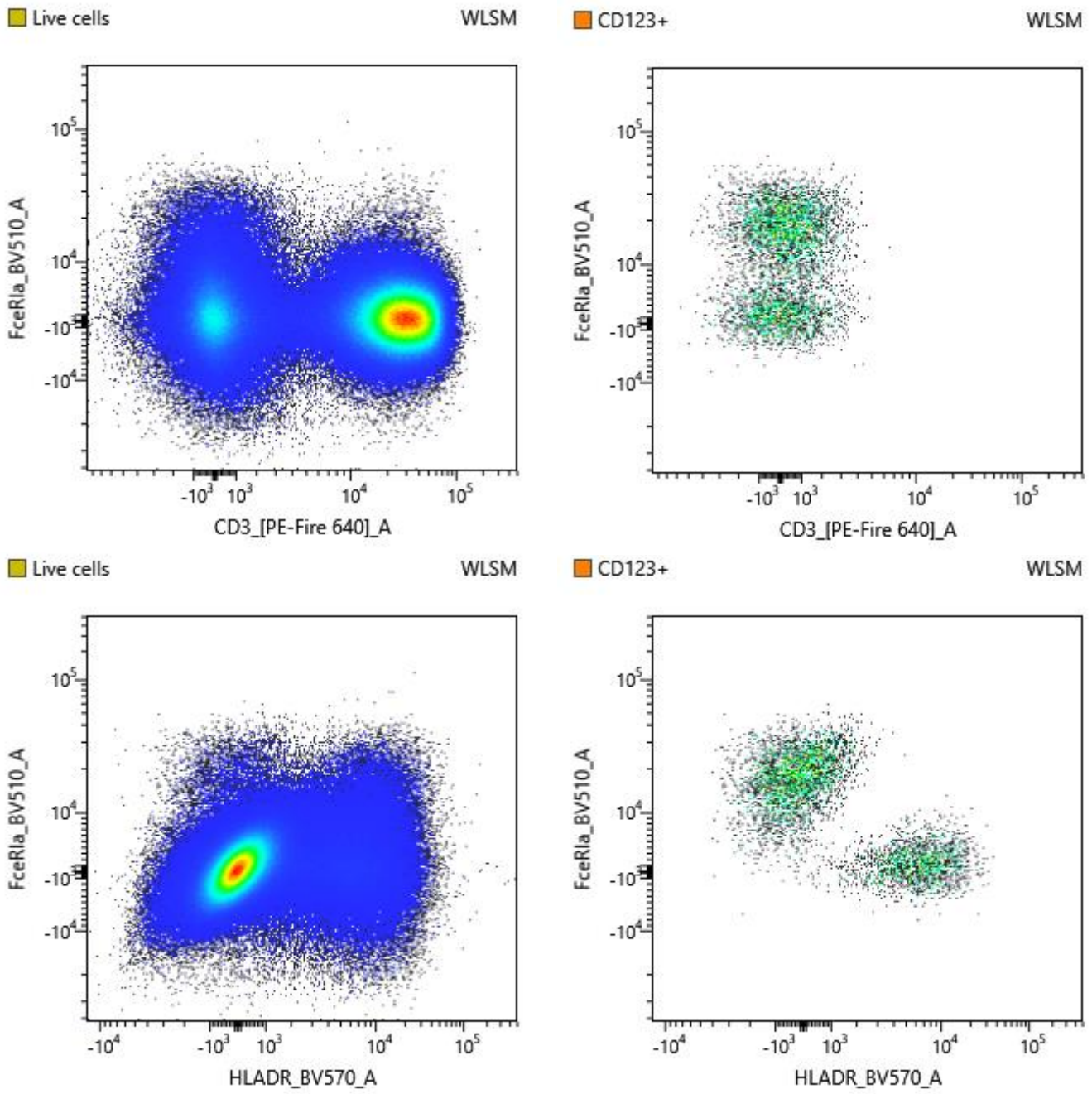


Panel Design

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



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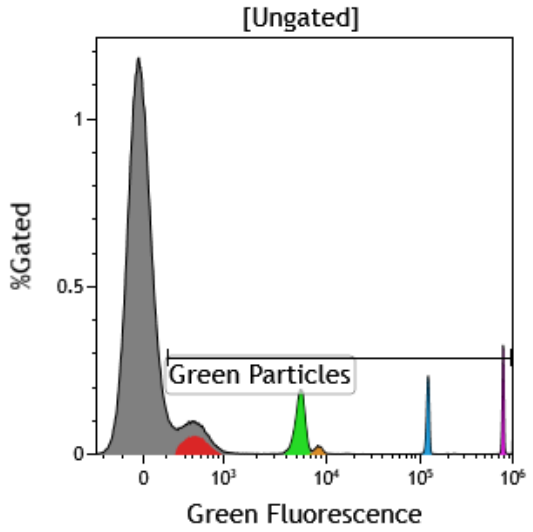
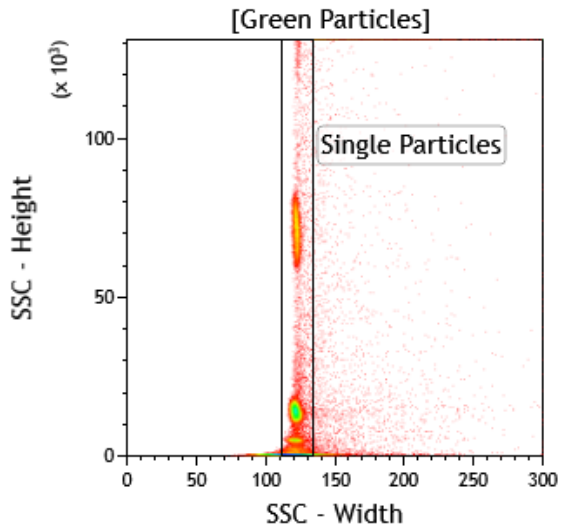
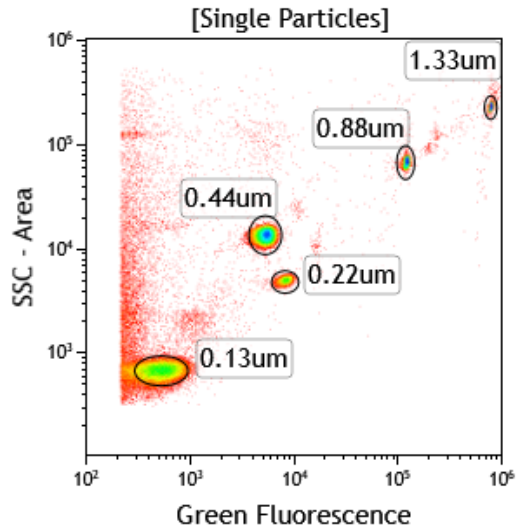
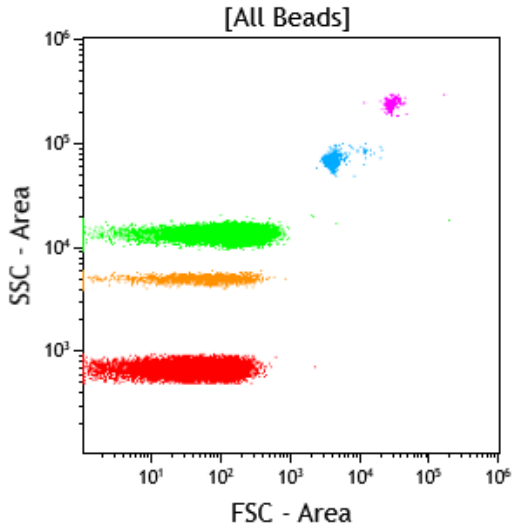
Small Particles

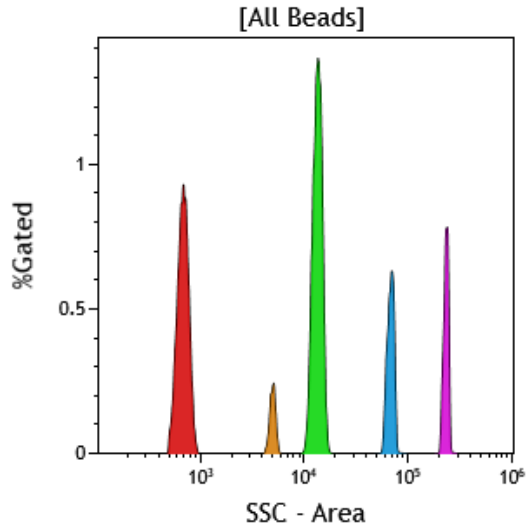
SPHERO™ Flow Cytometry Nano Fluorescent Size Standard Kit

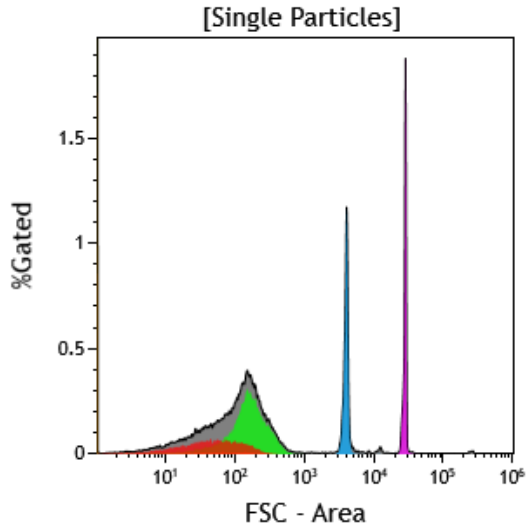












Data courtesy of: The University of Nottingham

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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?