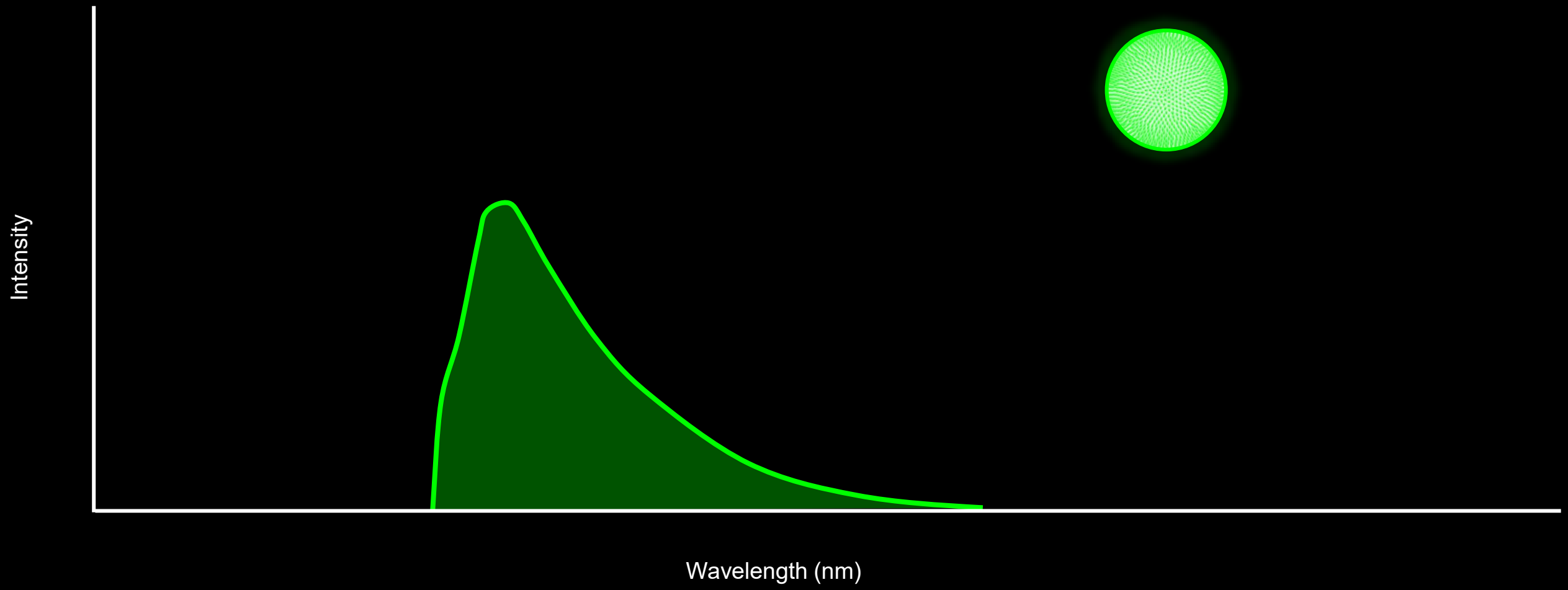
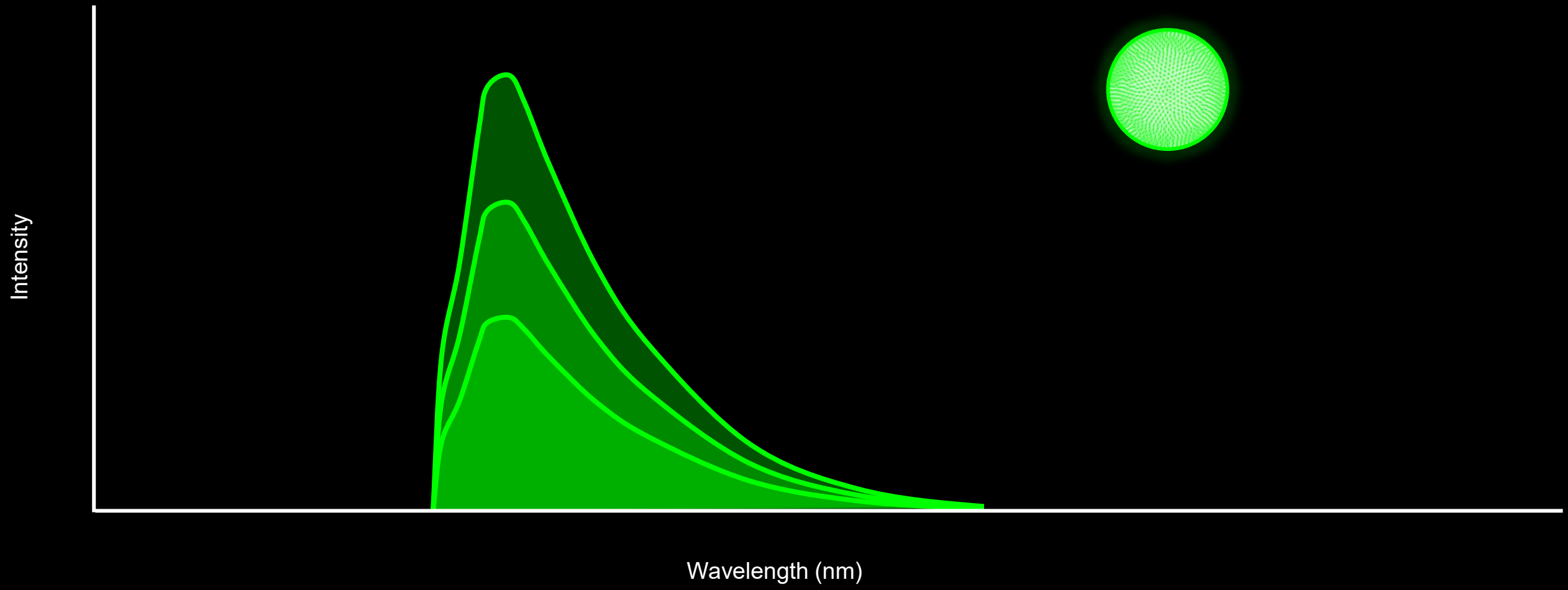
TRAINING:
Sony analyzer

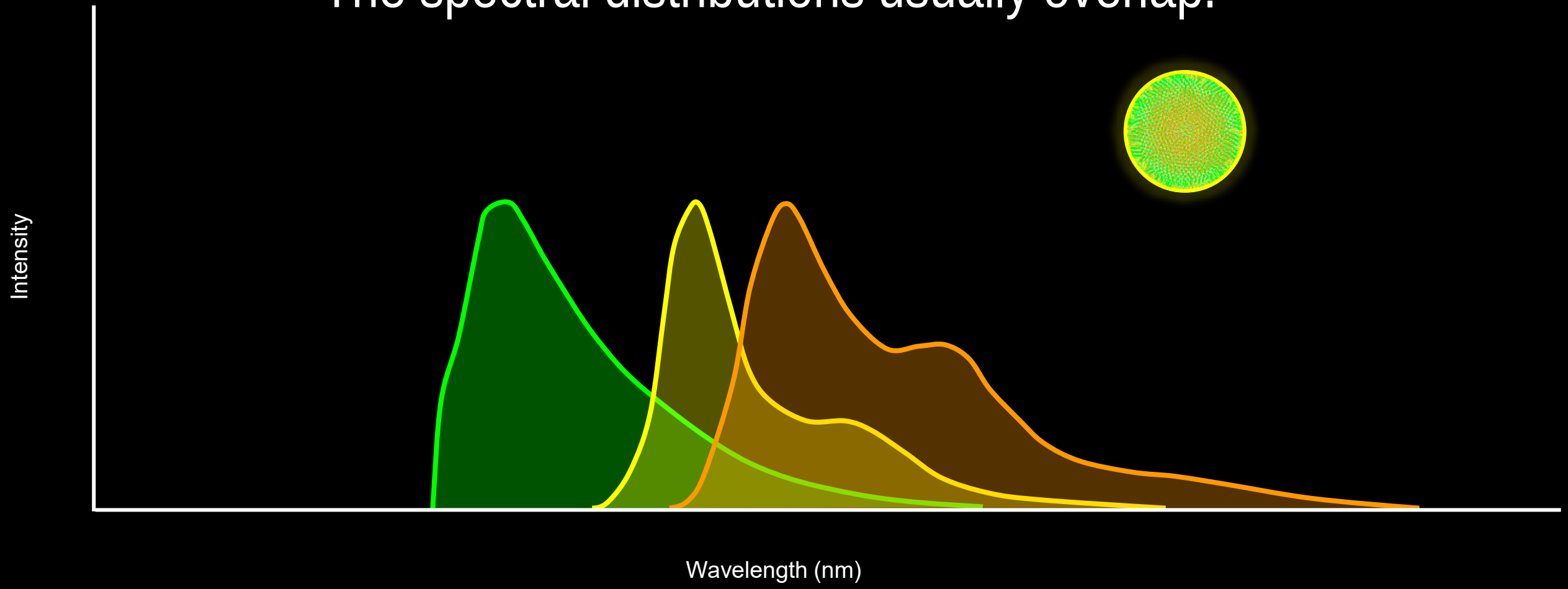
The fluorescence intensity may vary from cell to cell…



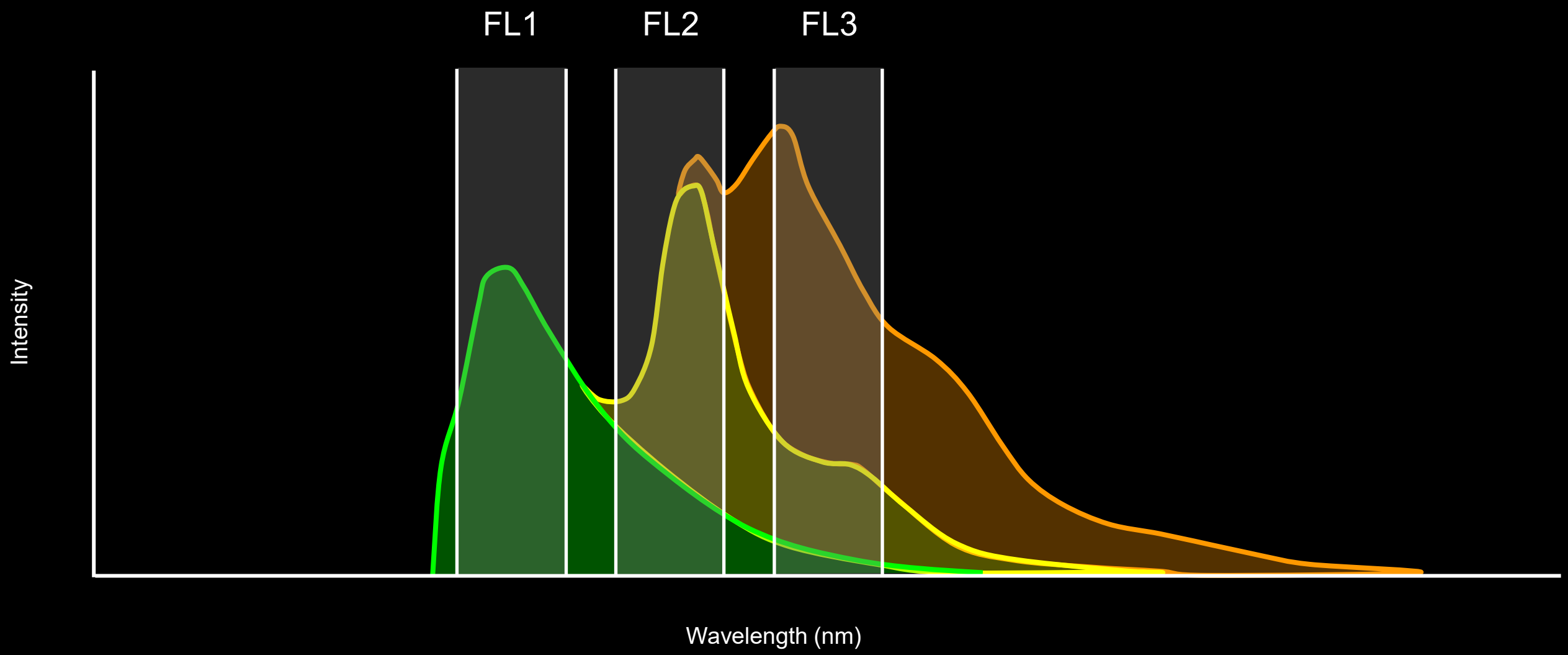
…but the proportional spectral distribution does not.



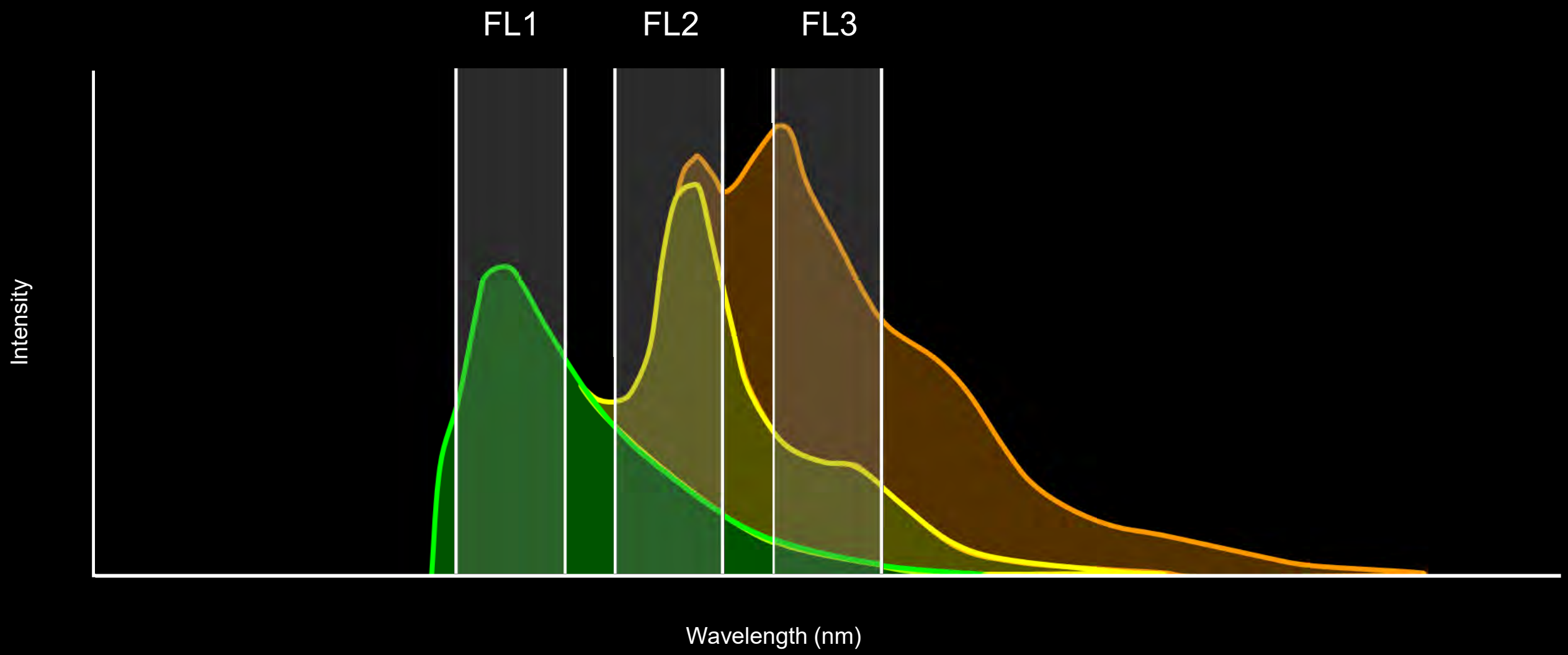
Cells are often stained with more than one fluorochrome,
each having its own spectral distribution.
The spectral distributions usually overlap.



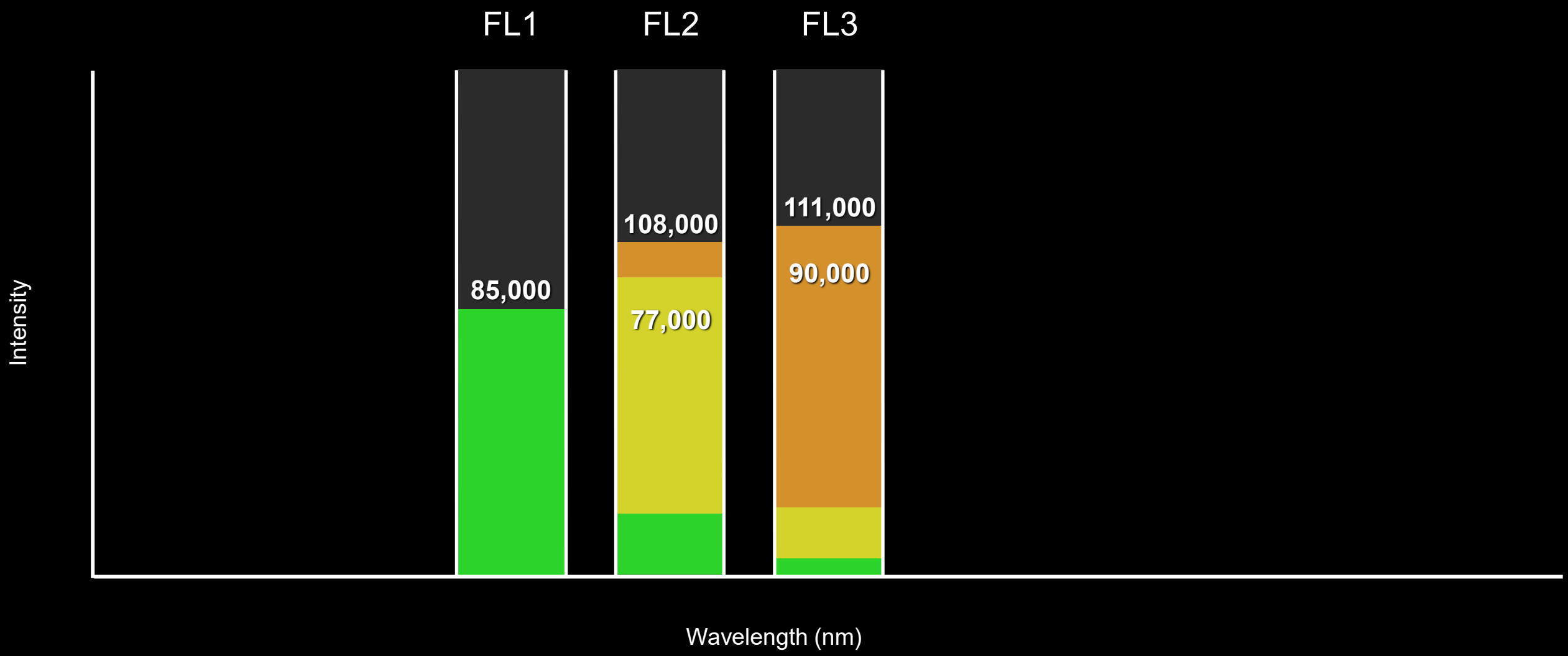
Conventional Flow Cytometry



Conventional Flow Cytometry

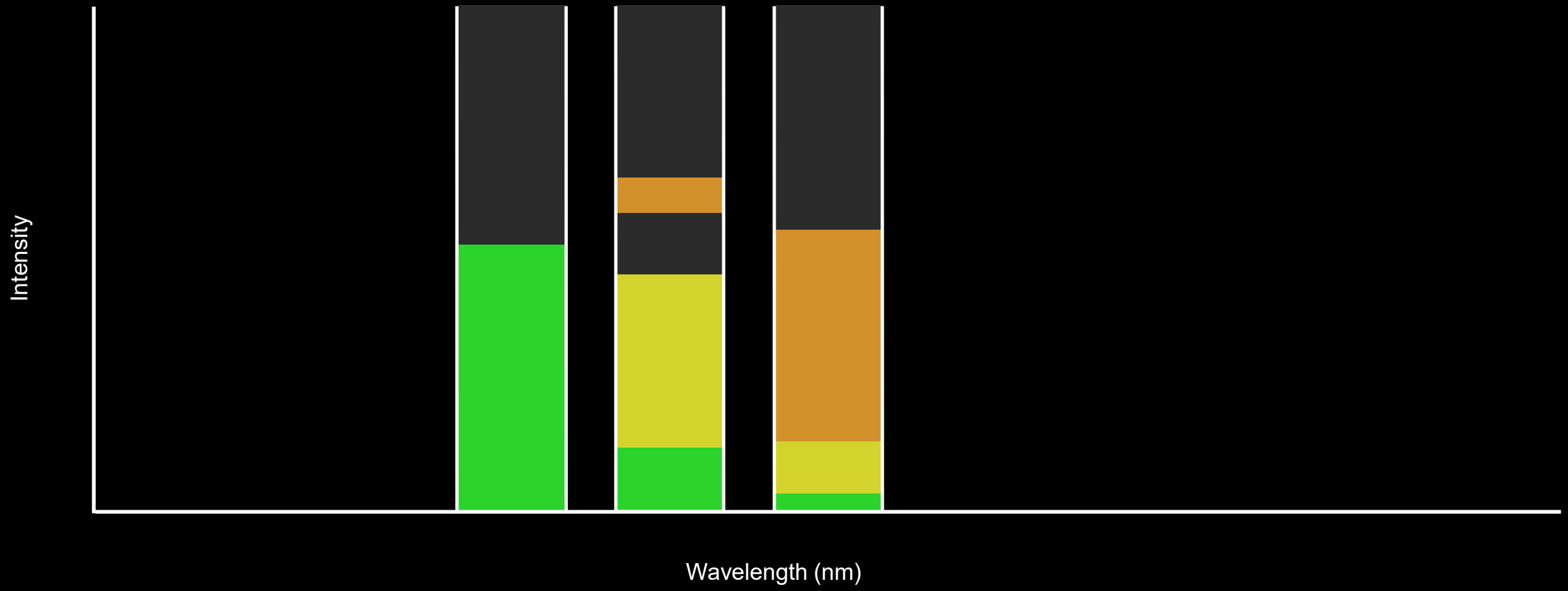


Conventional Flow Cytometry



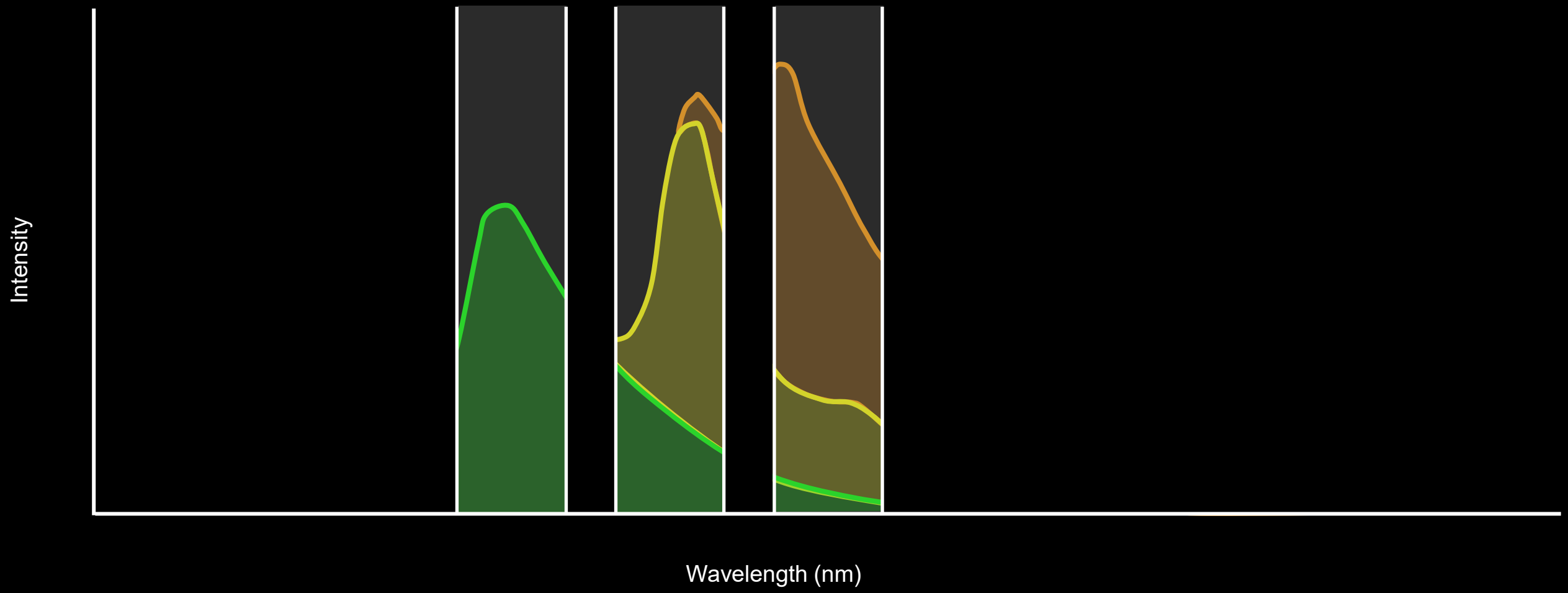
Spectral Flow Cytometry

No subtraction.



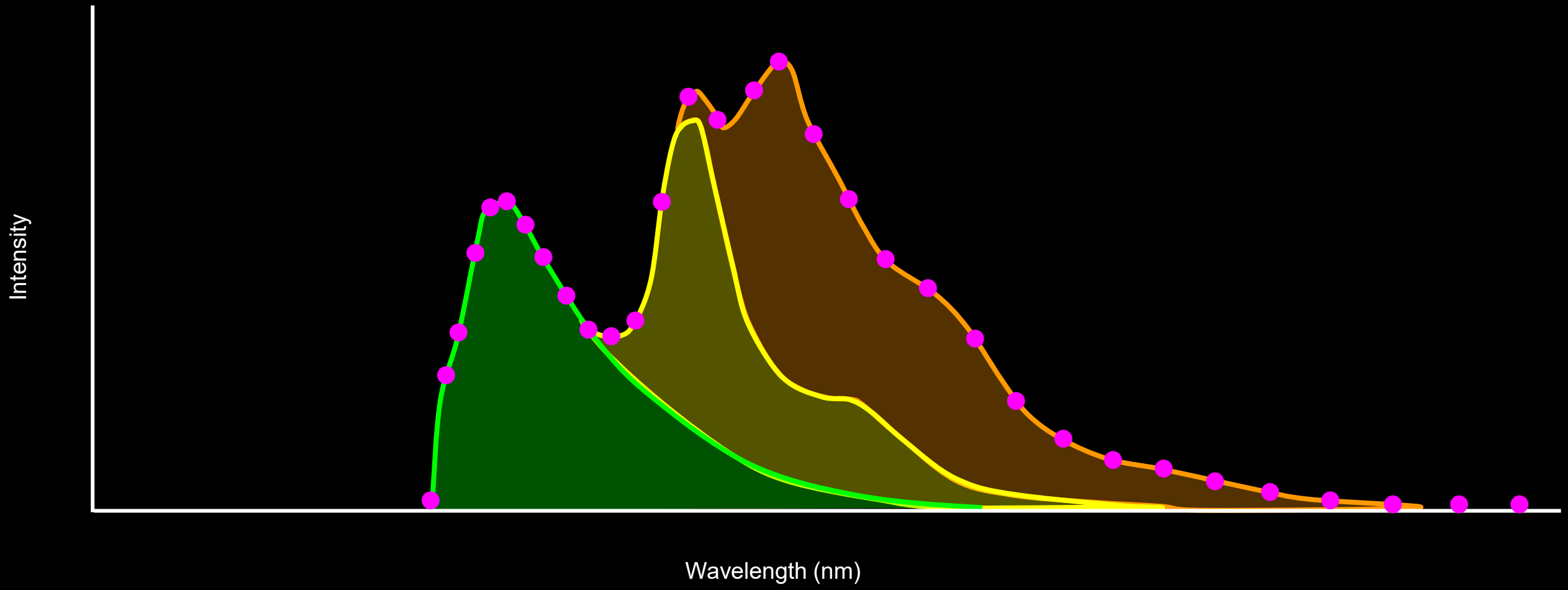
Spectral Flow Cytometry

Measure all of the light...



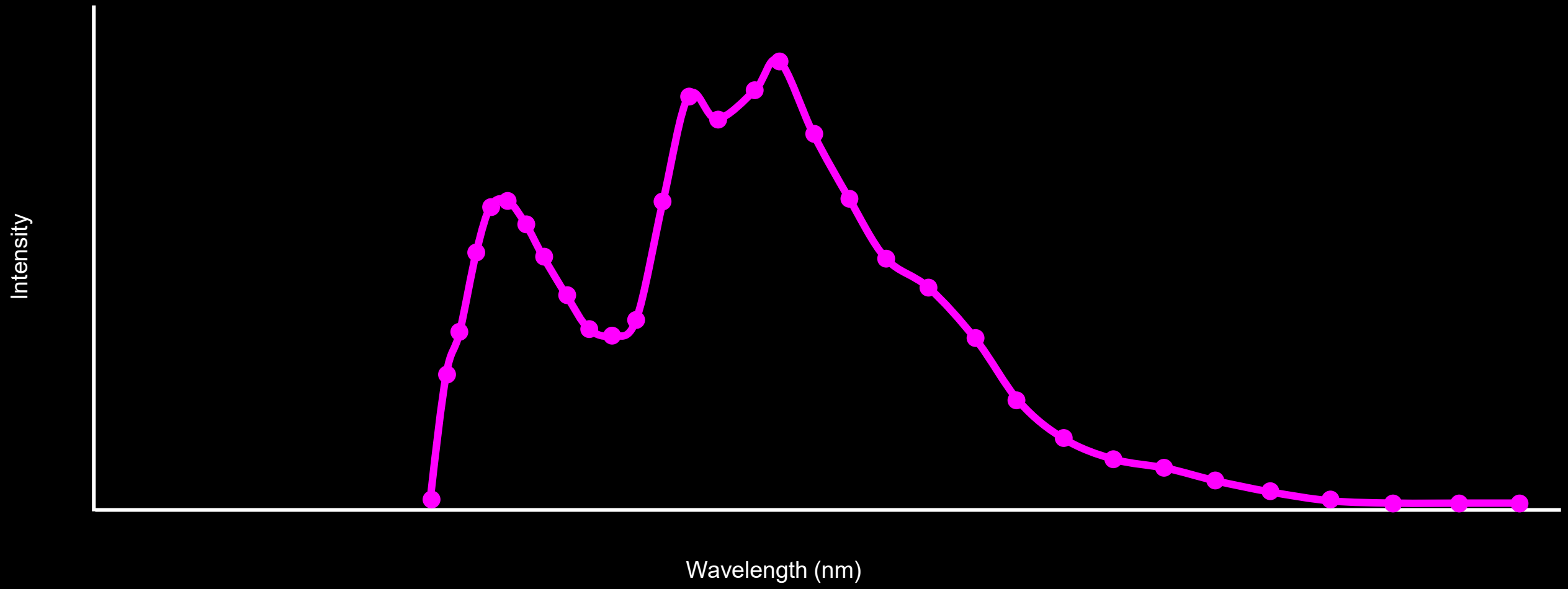
Spectral Flow Cytometry

by using many more detectors…



Spectral Flow Cytometry

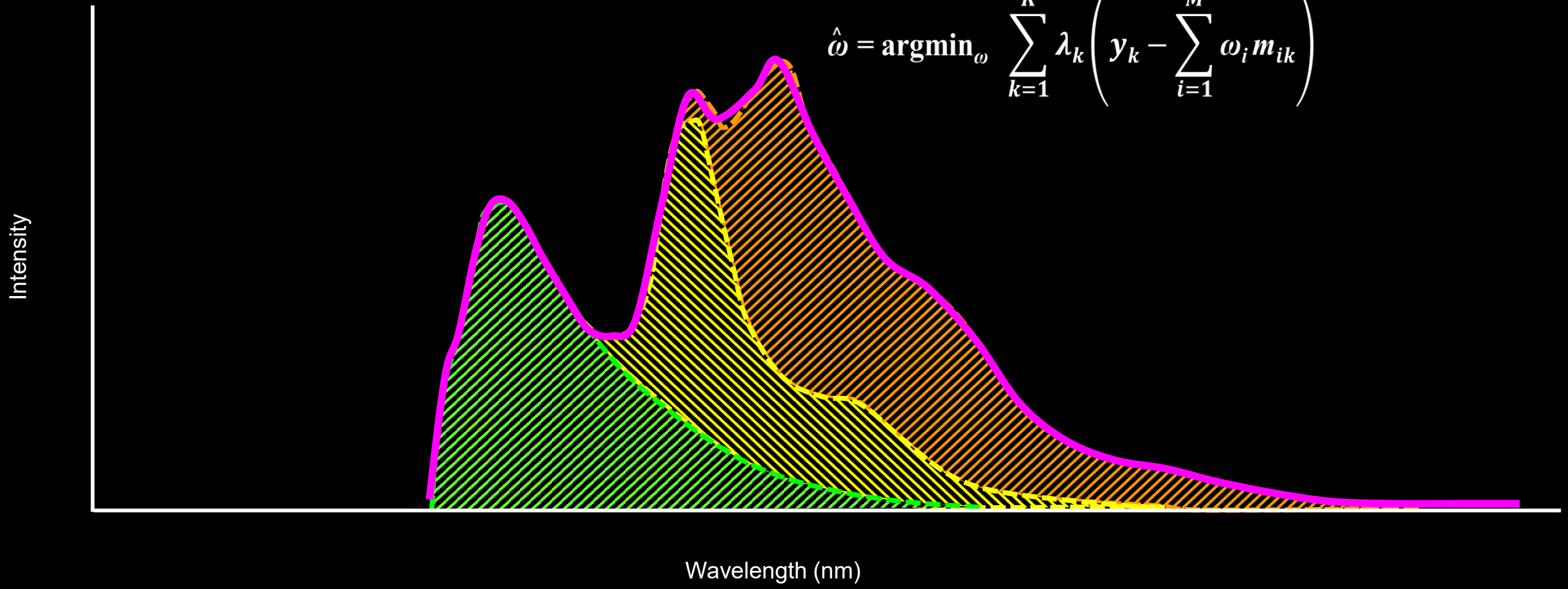
…to define the sum of all of the fluorescence from each cell.



Spectral Flow Cytometry

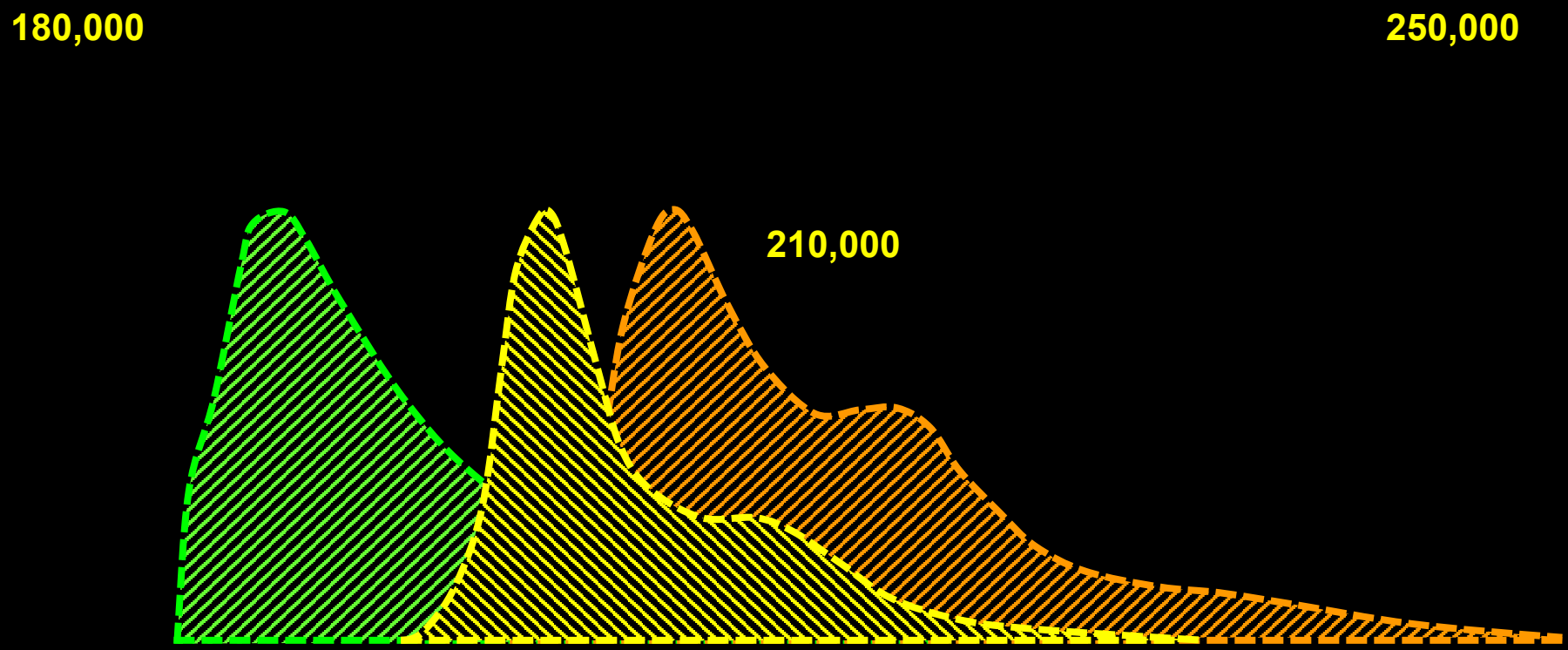
Then apply a different kind of math...

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



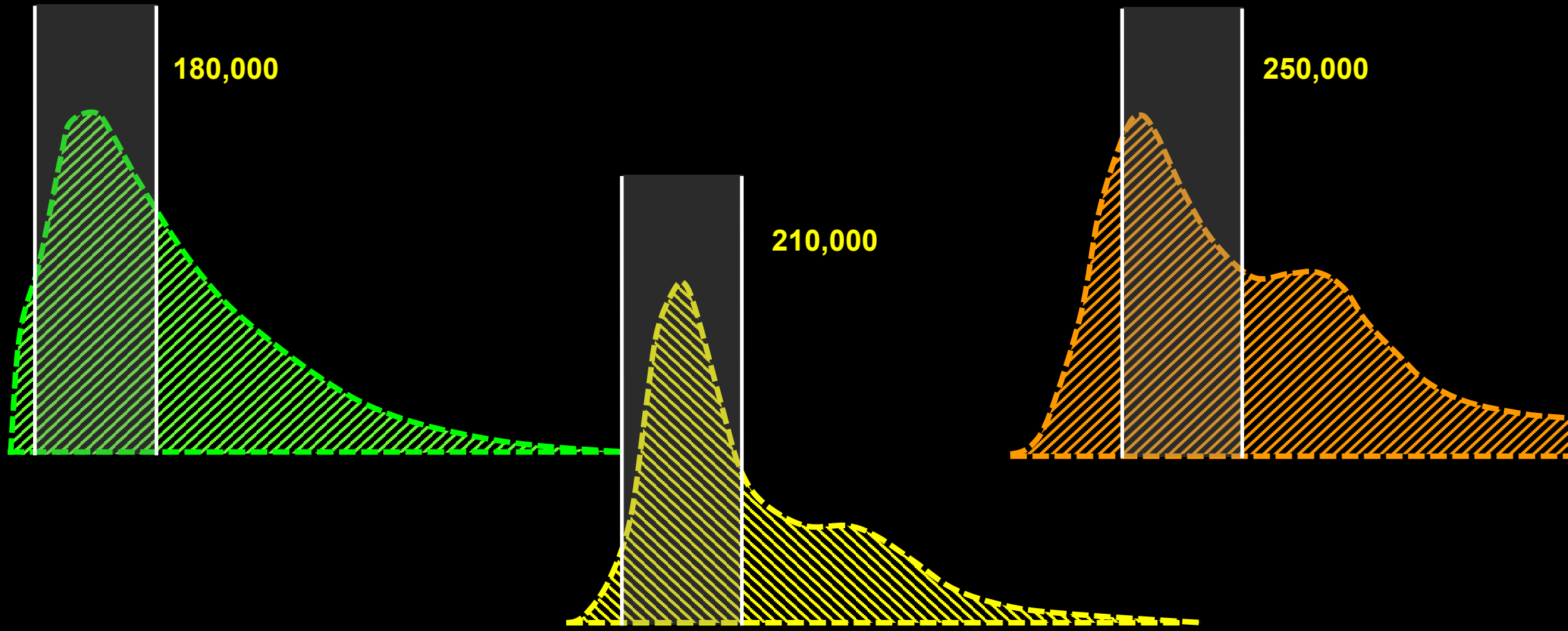
Spectral Flow Cytometry

…to determine the intensity of each fluorochrome on each cell.



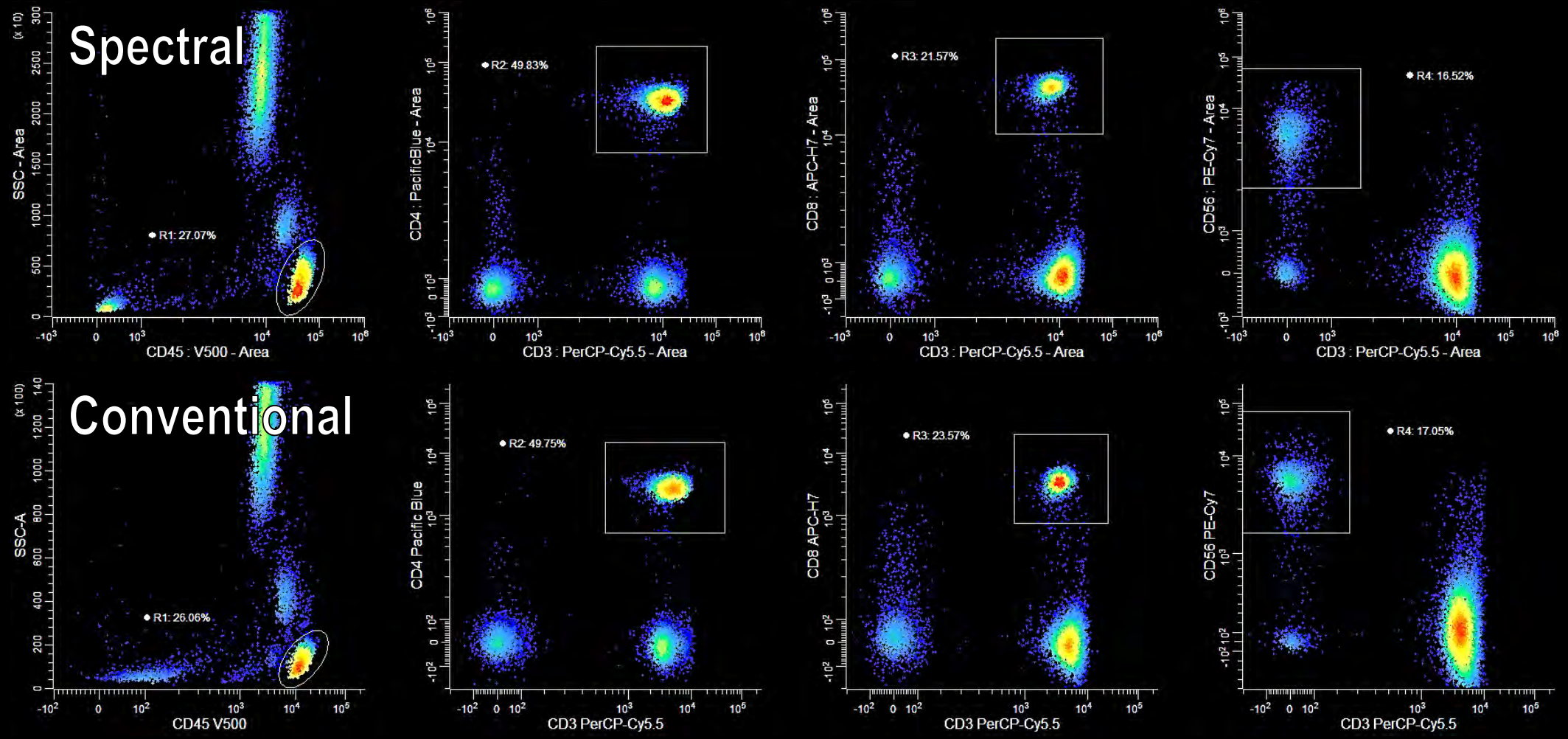
Spectral Flow Cytometry

The intensity measurements are derived from the entire range of each fluorochrome's emission, rather than just a small fraction.

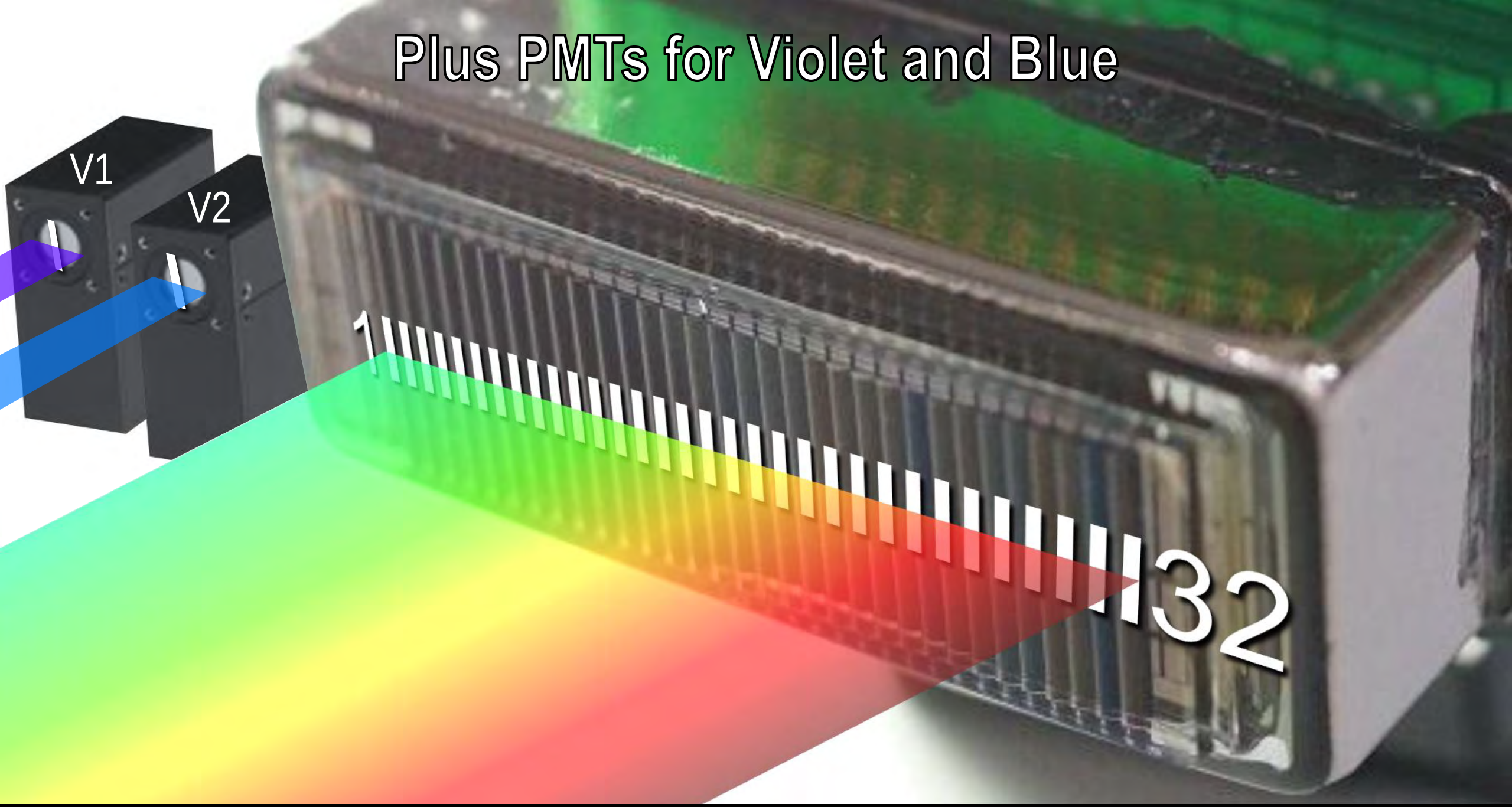


Parametric Data

The intensity of each fluorochrome on each cell, derived from the spectral data, is plotted just like conventional flow cytometry data.

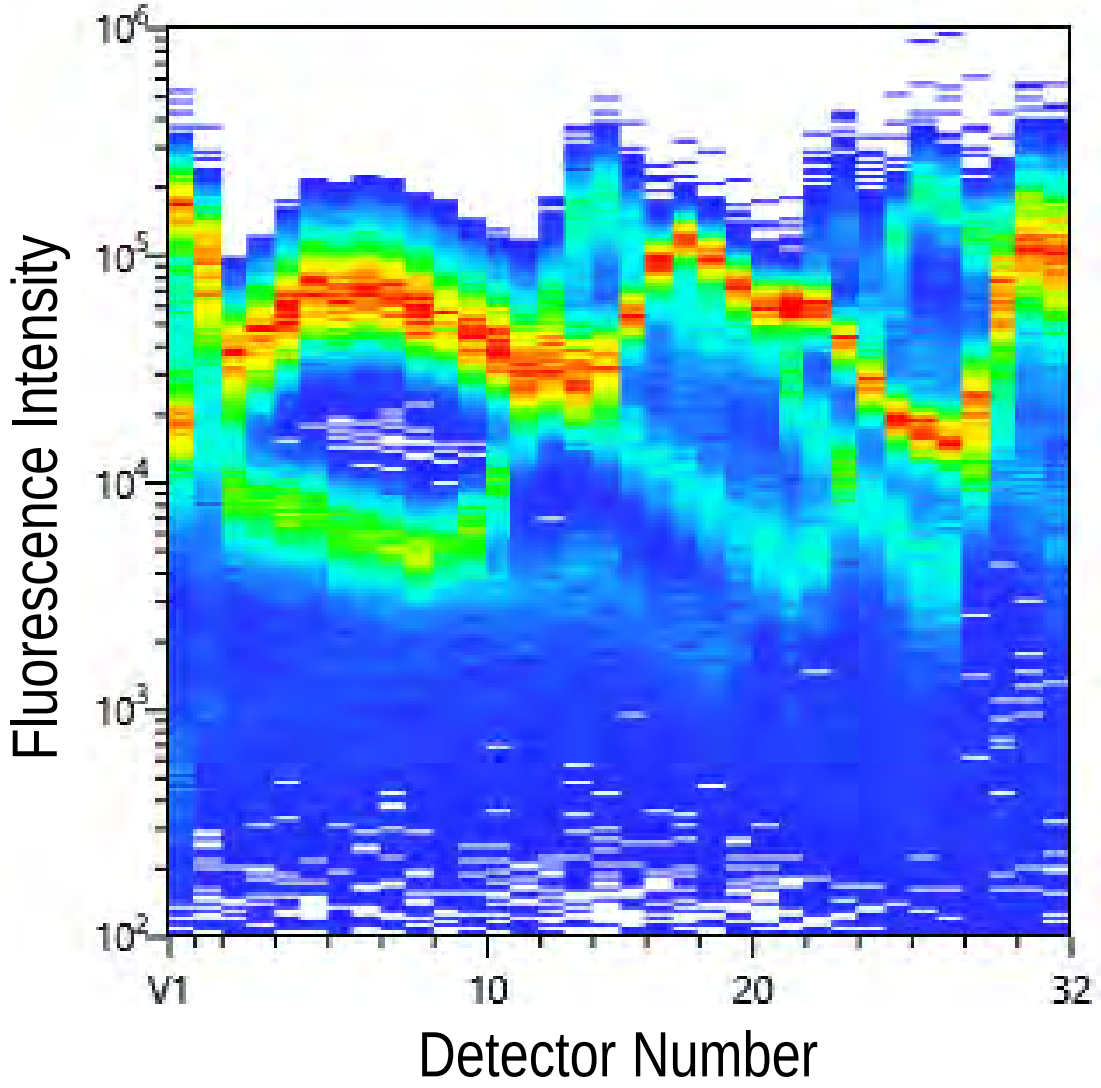


Plus PMTs for Violet and Blue




SPECTRAL
VISION

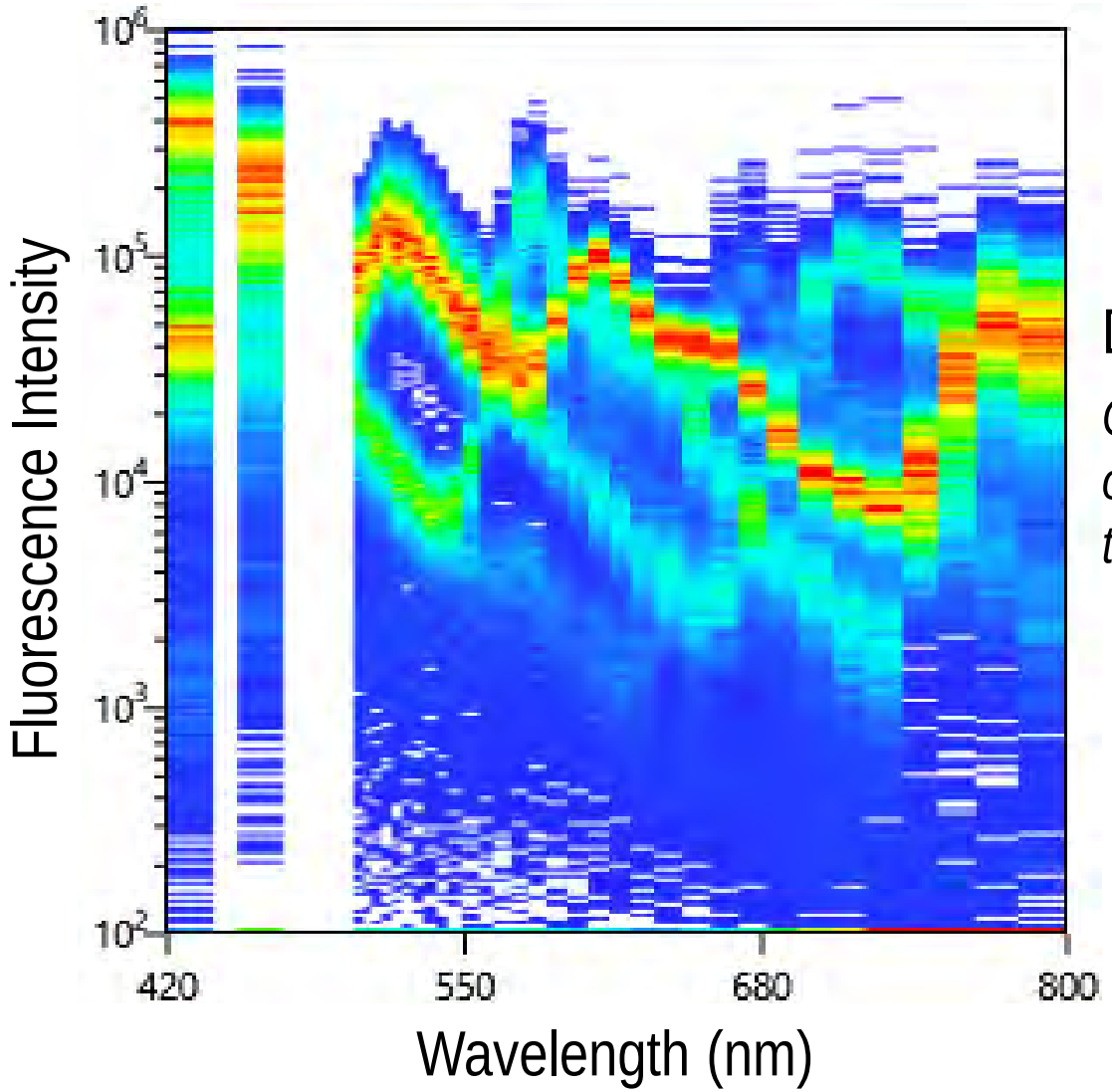
Spectral Data Plot



Density Plot

Color indicates the number of cells at each intensity throughout the spectrum.

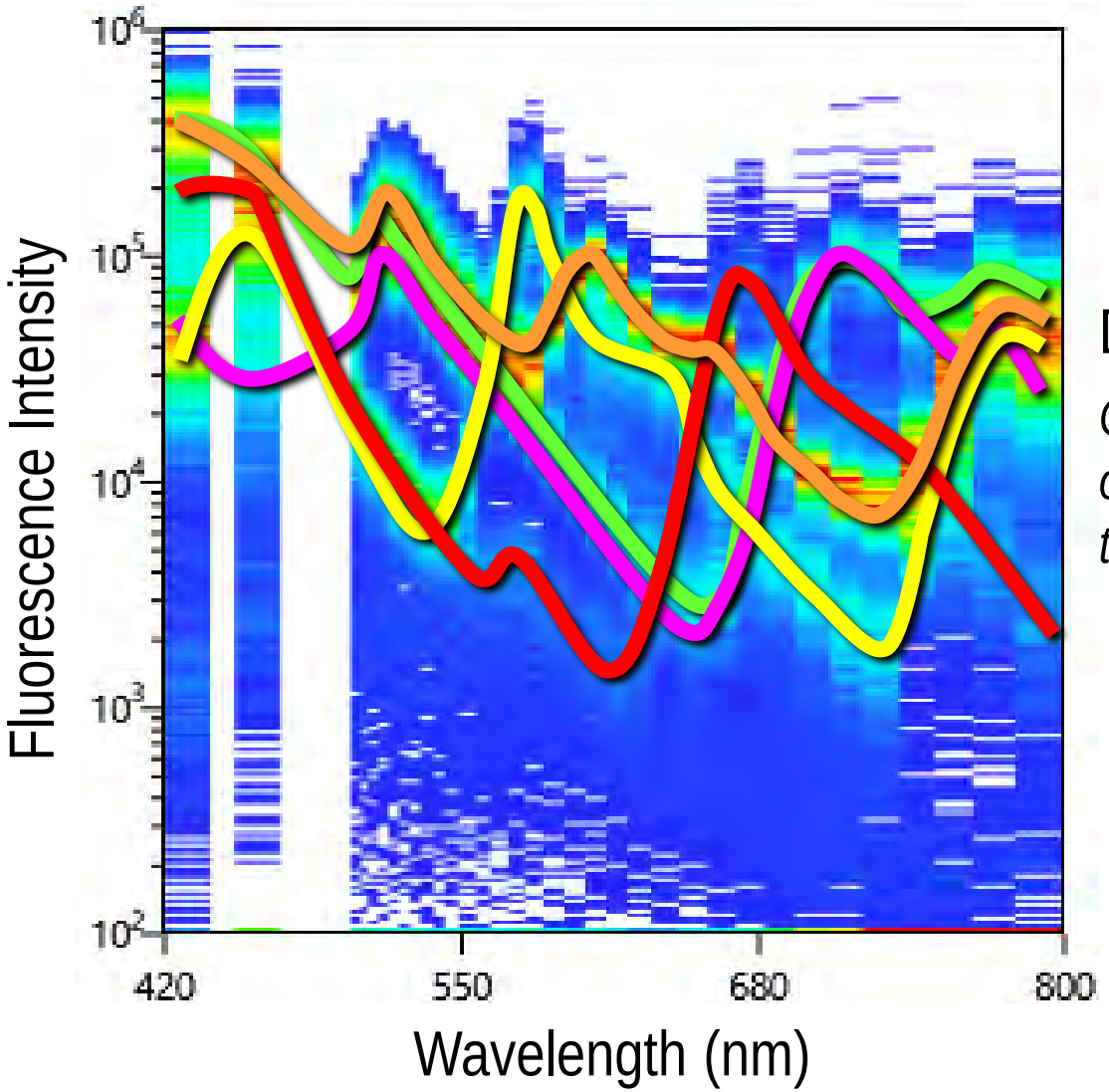
Spectral Data Plot



Density Plot

Color indicates the number of cells at each intensity throughout the spectrum.

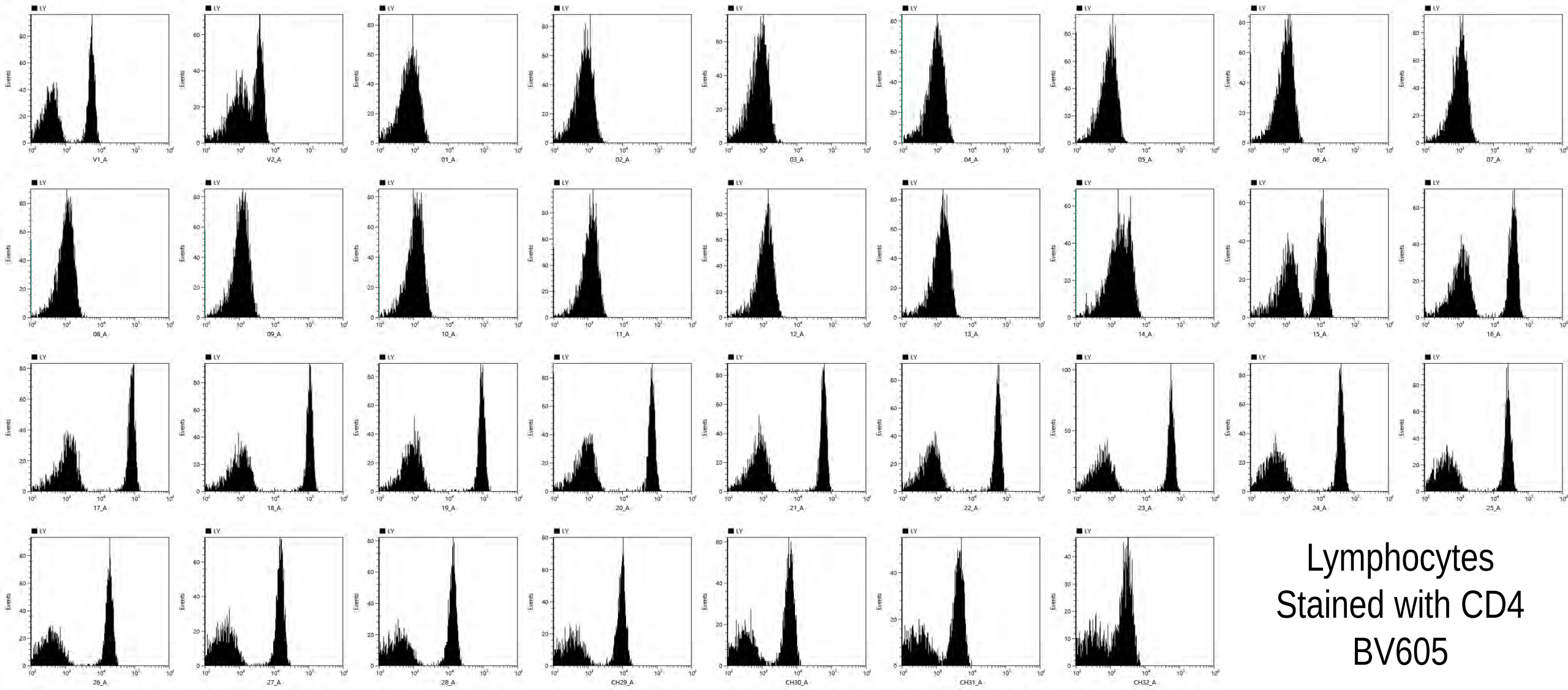
Spectral Data Plot



Density Plot

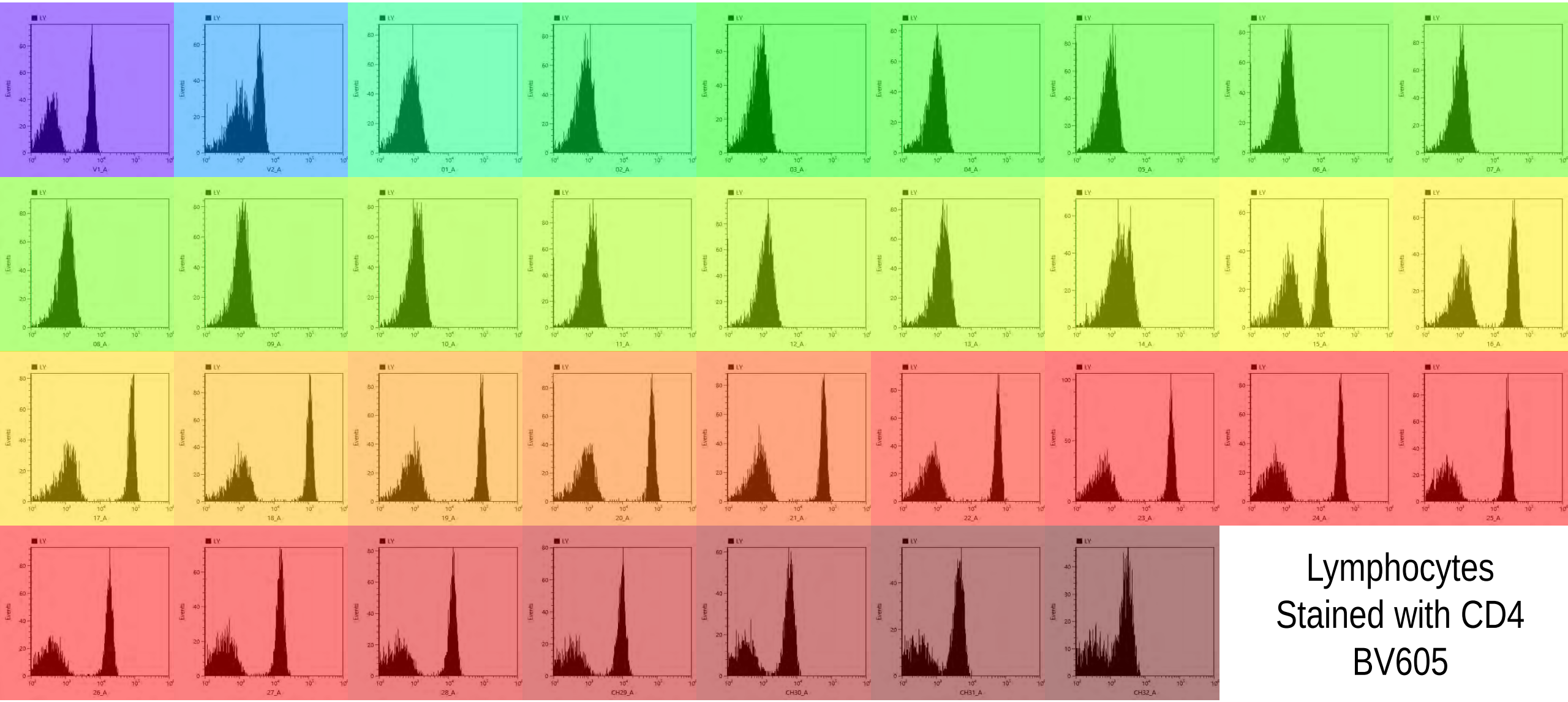
Color indicates the number of cells at each intensity throughout the spectrum.

Data from 34 Detectors

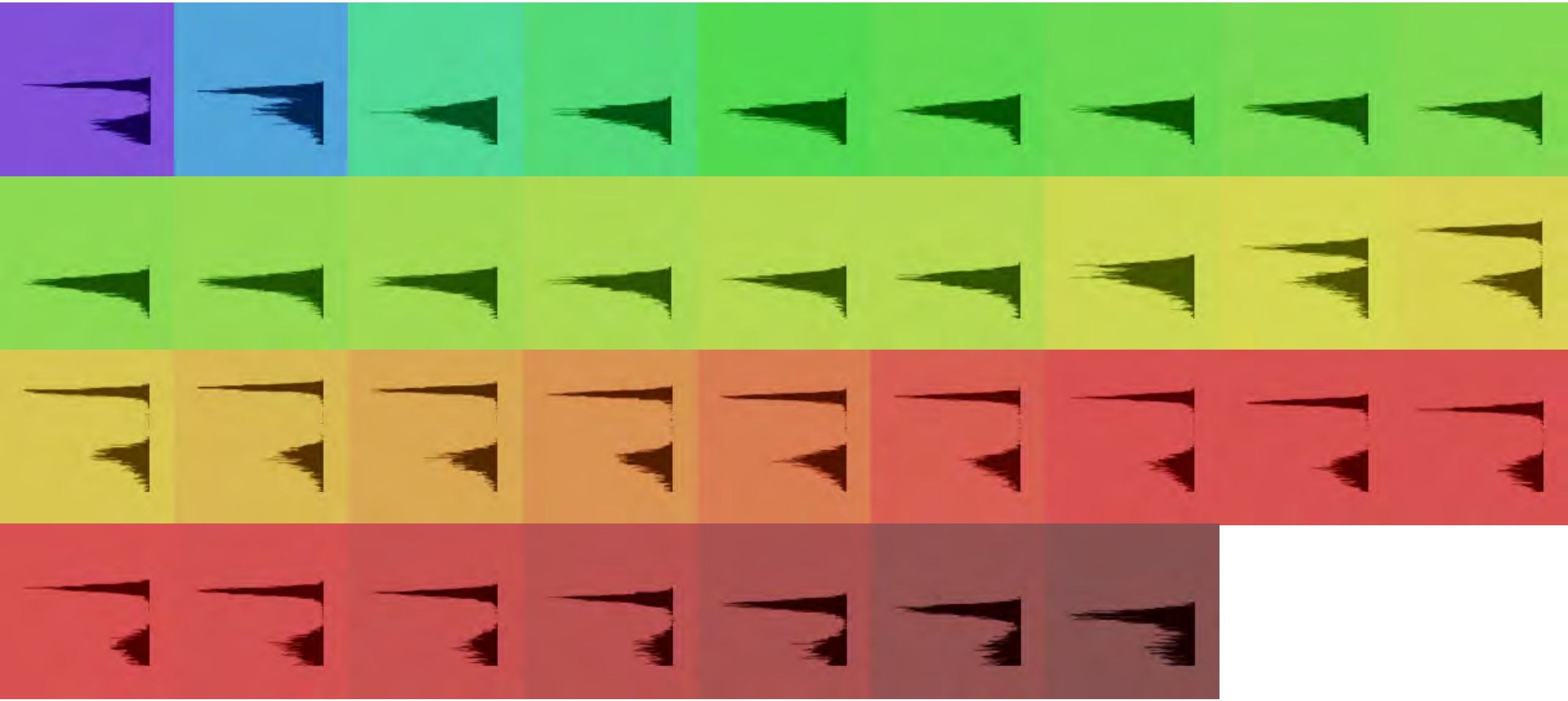


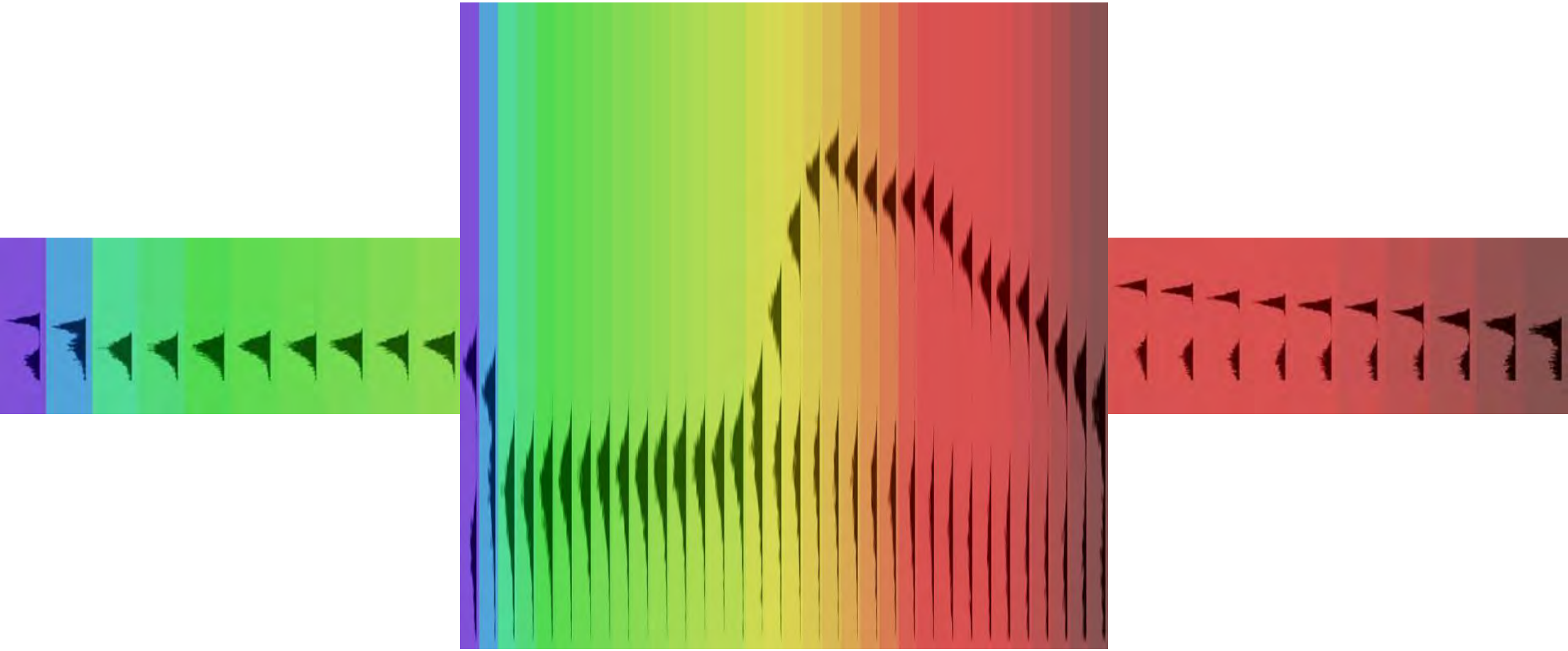
Lymphocytes
Stained with CD4
BV605

Data from 34 Detectors

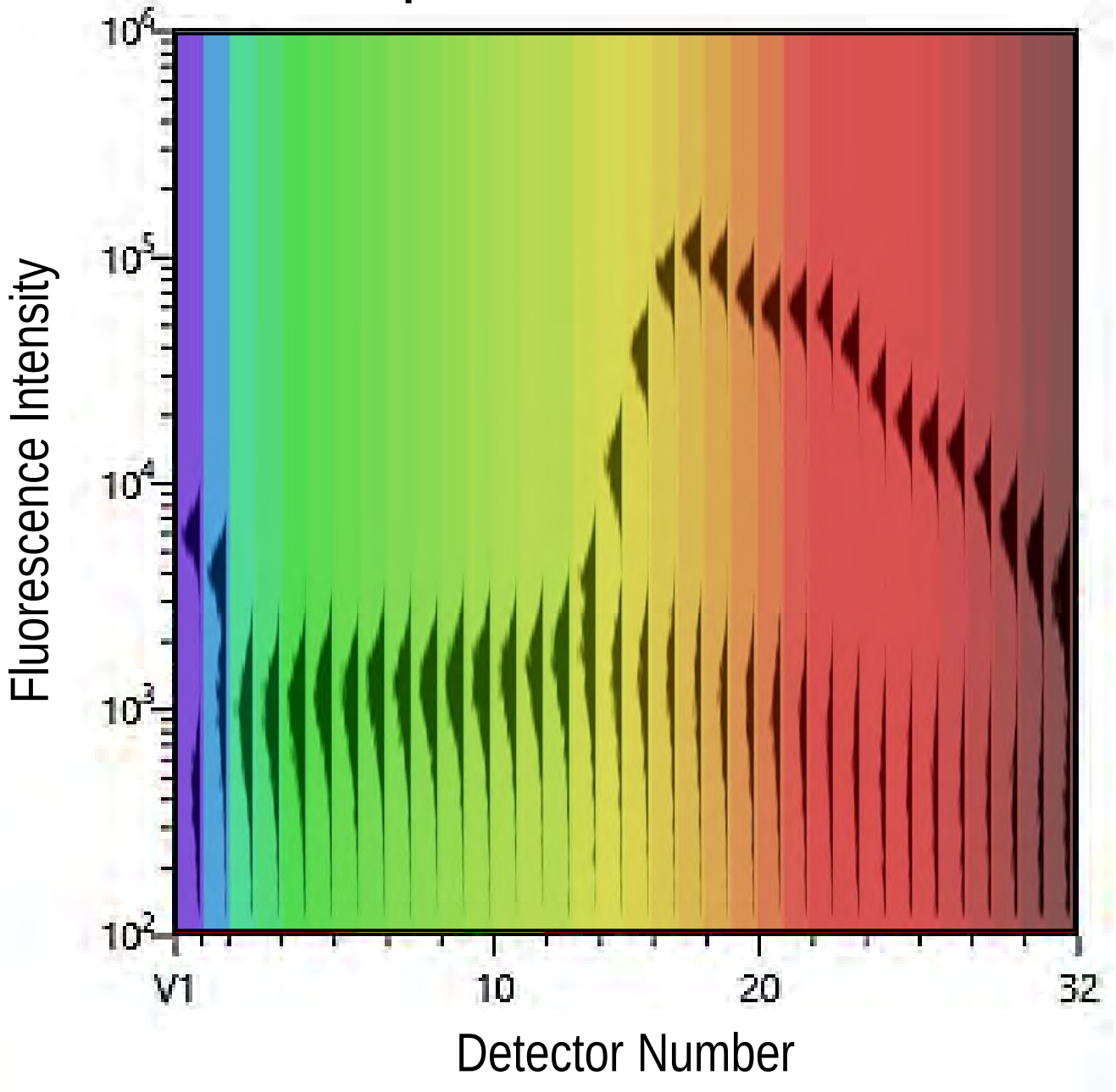


Lymphocytes
Stained with CD4
BV605

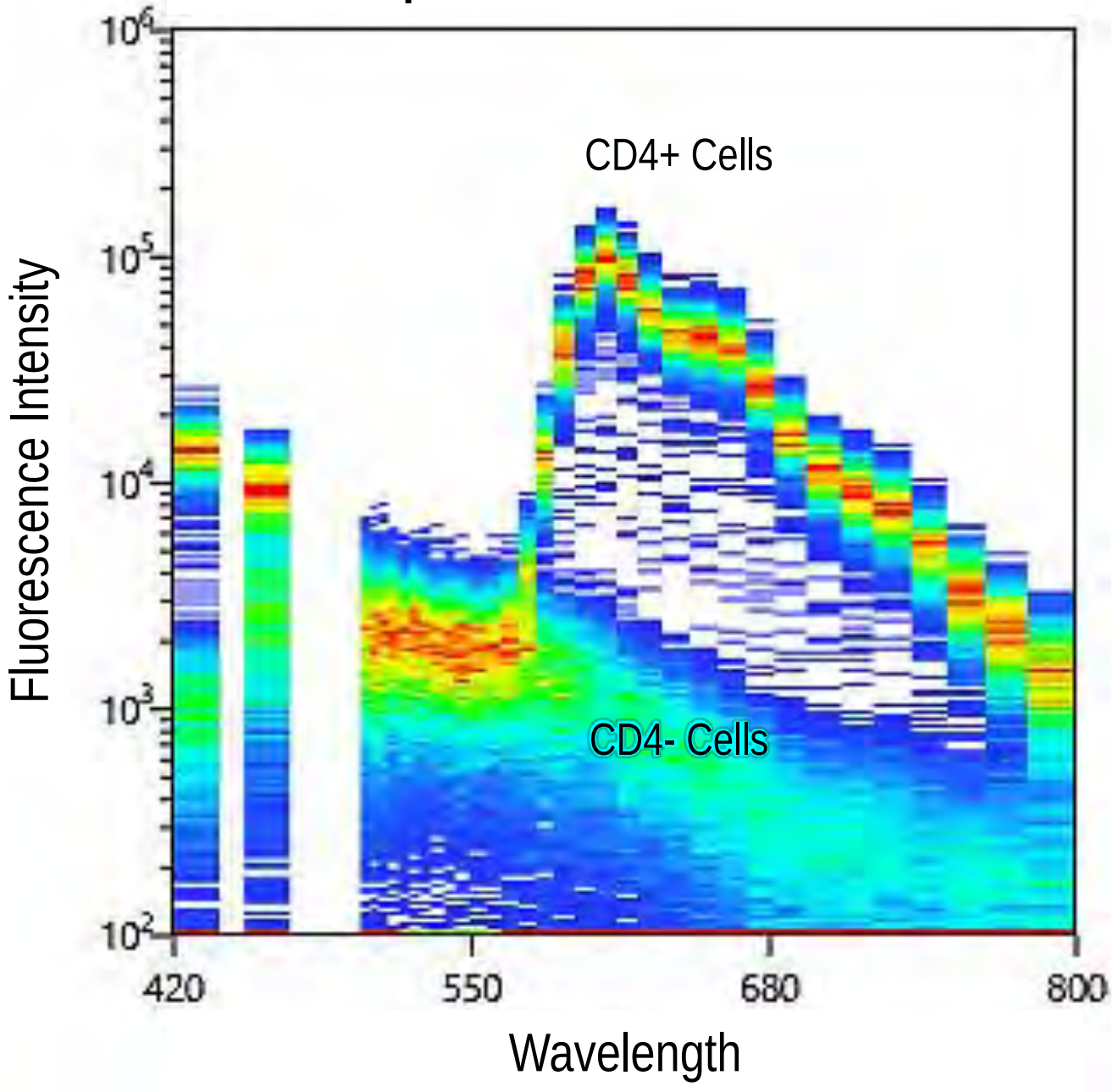




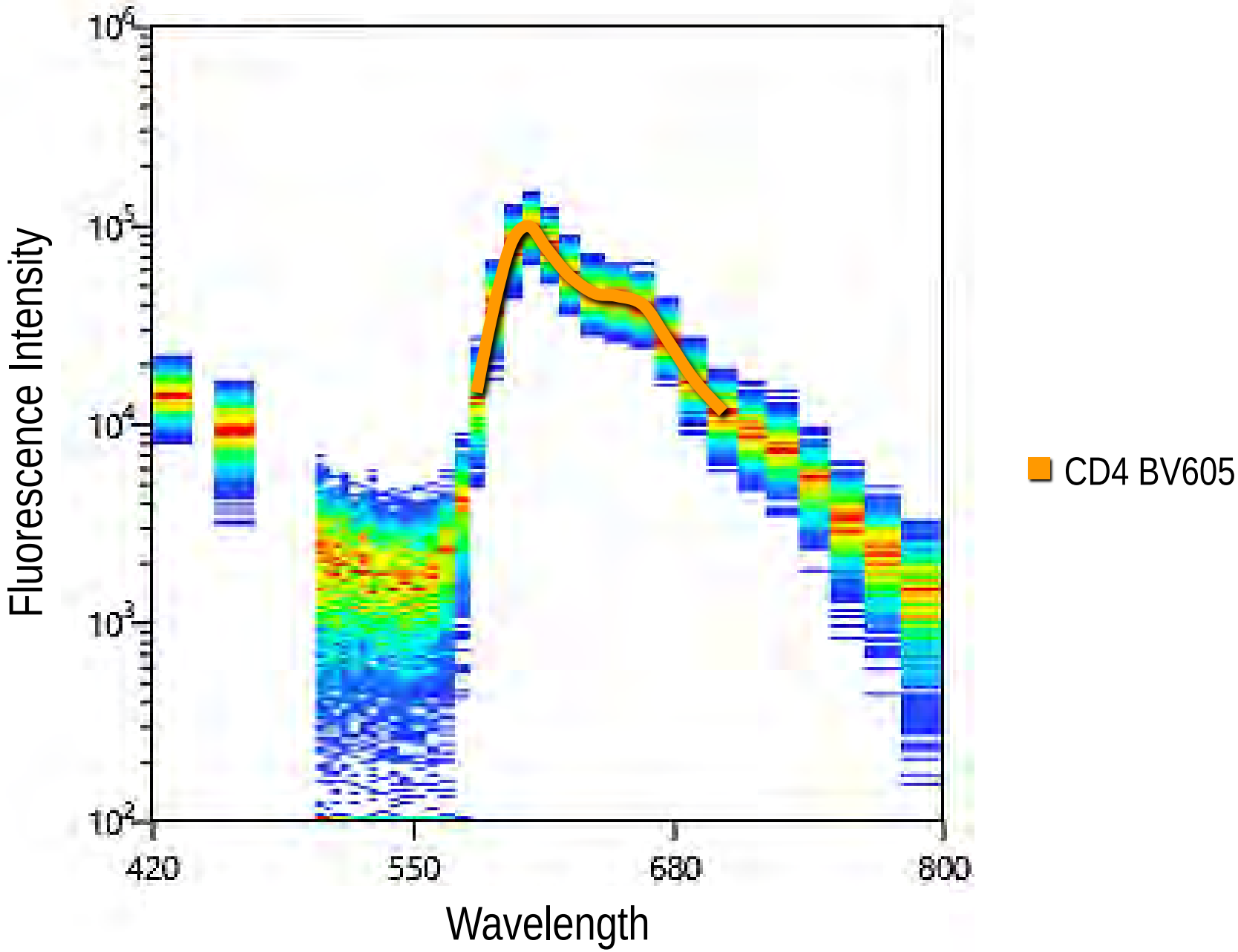
Spectral Data Plot



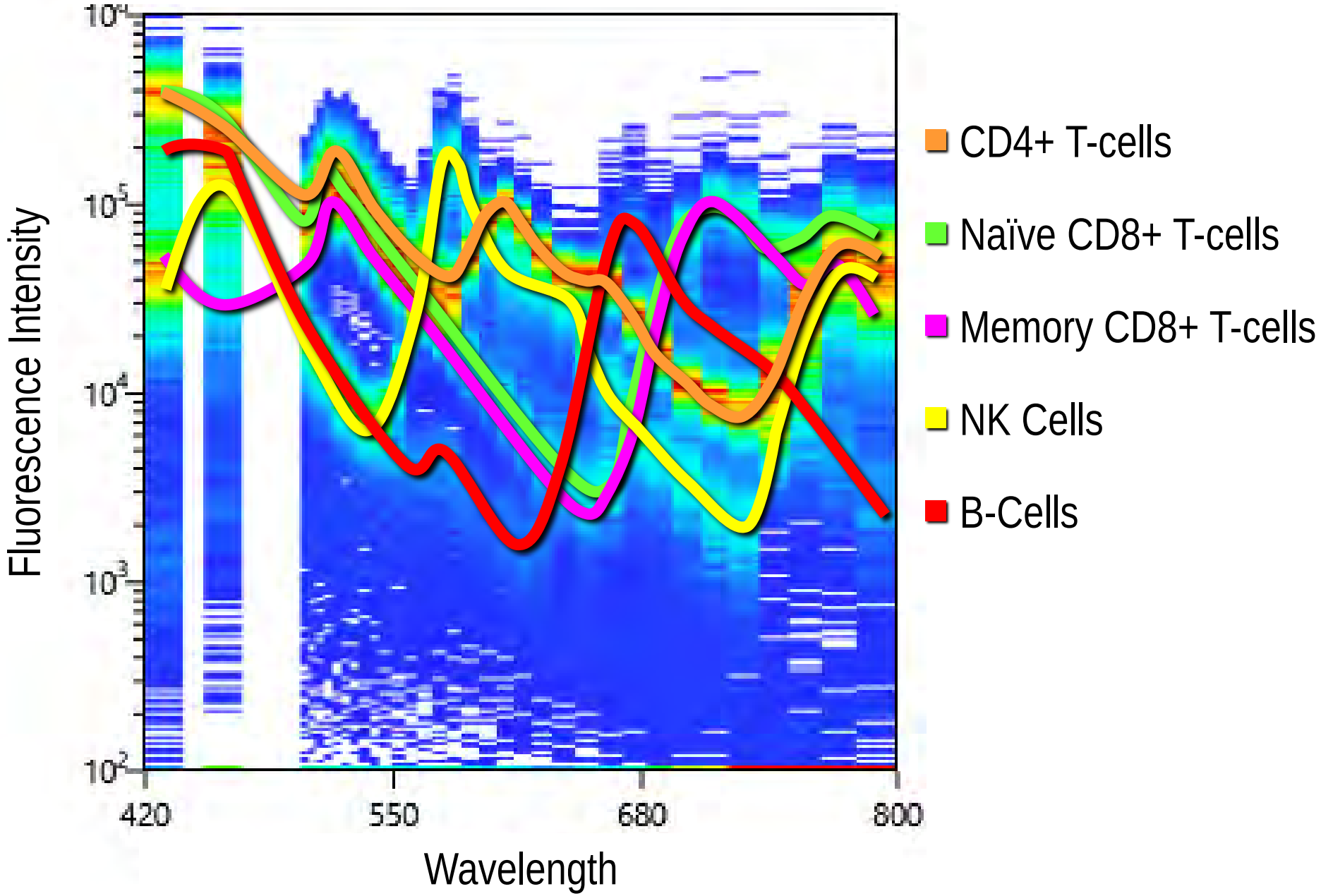
Spectral Data Plot



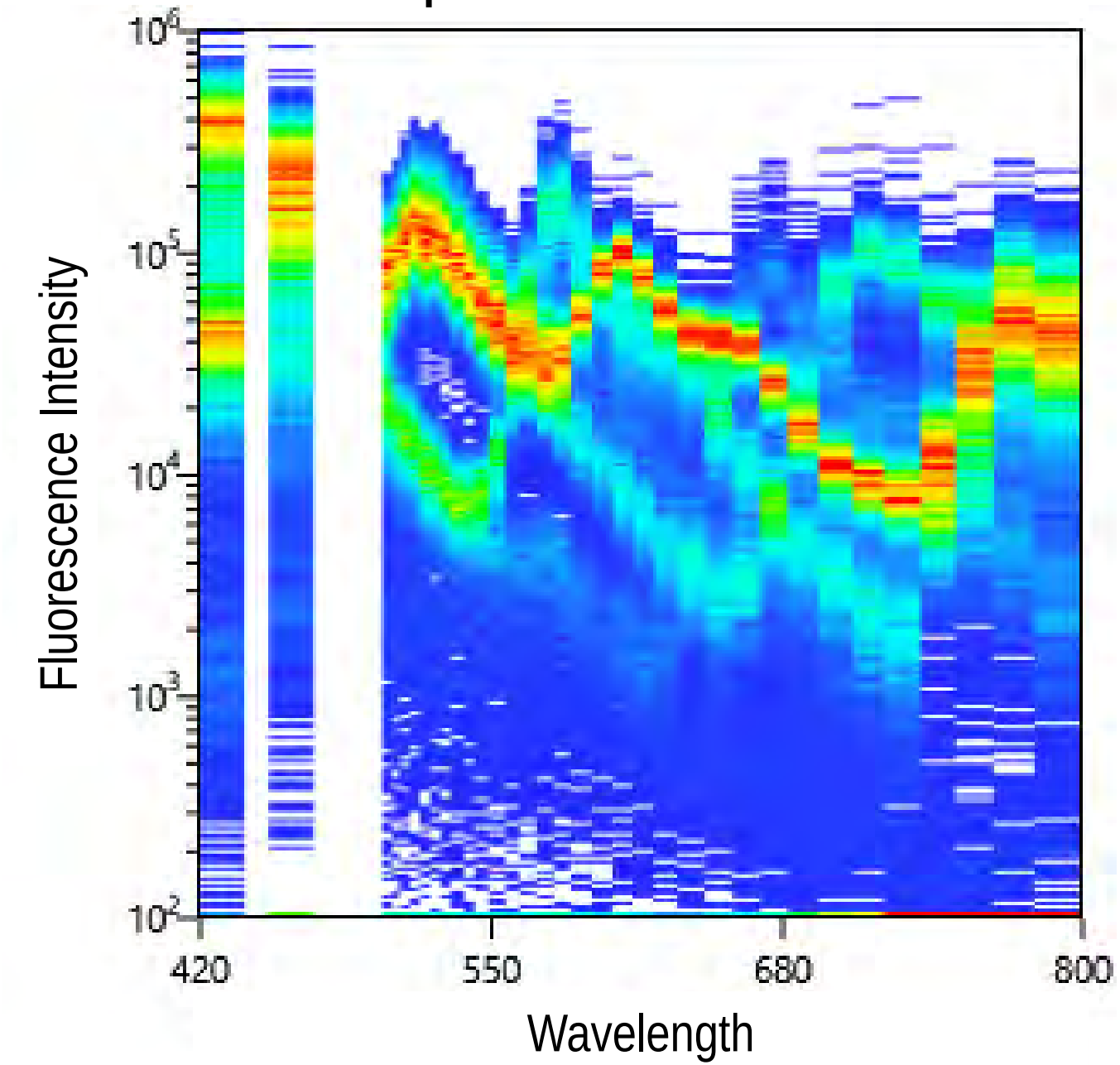
CD4+ T-cells


Fluorescence Intensity
10^6
10^5
10^4
10^3
10^2
420
550
680
800
Wavelength
CD4 BV605

Lymphocyte Subsets

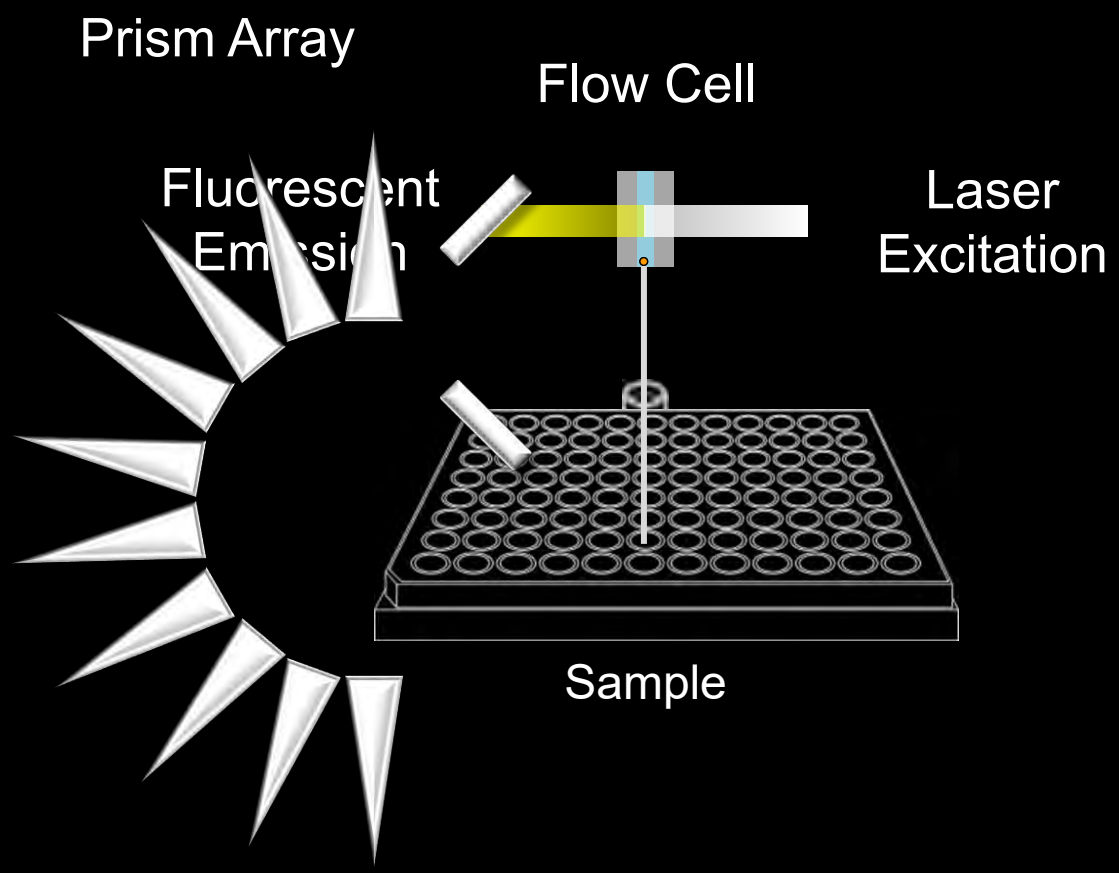


Spectral Data Plot



SONY SA3800

Spectral Technology



SONY SA3800
Spectral Technology

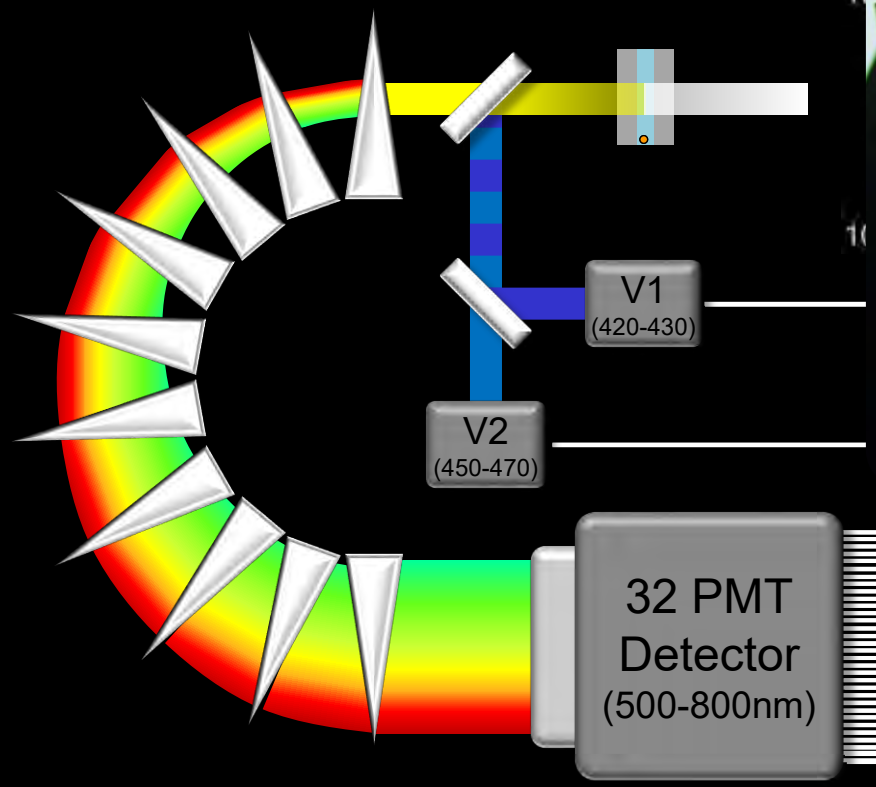
Spectral Data

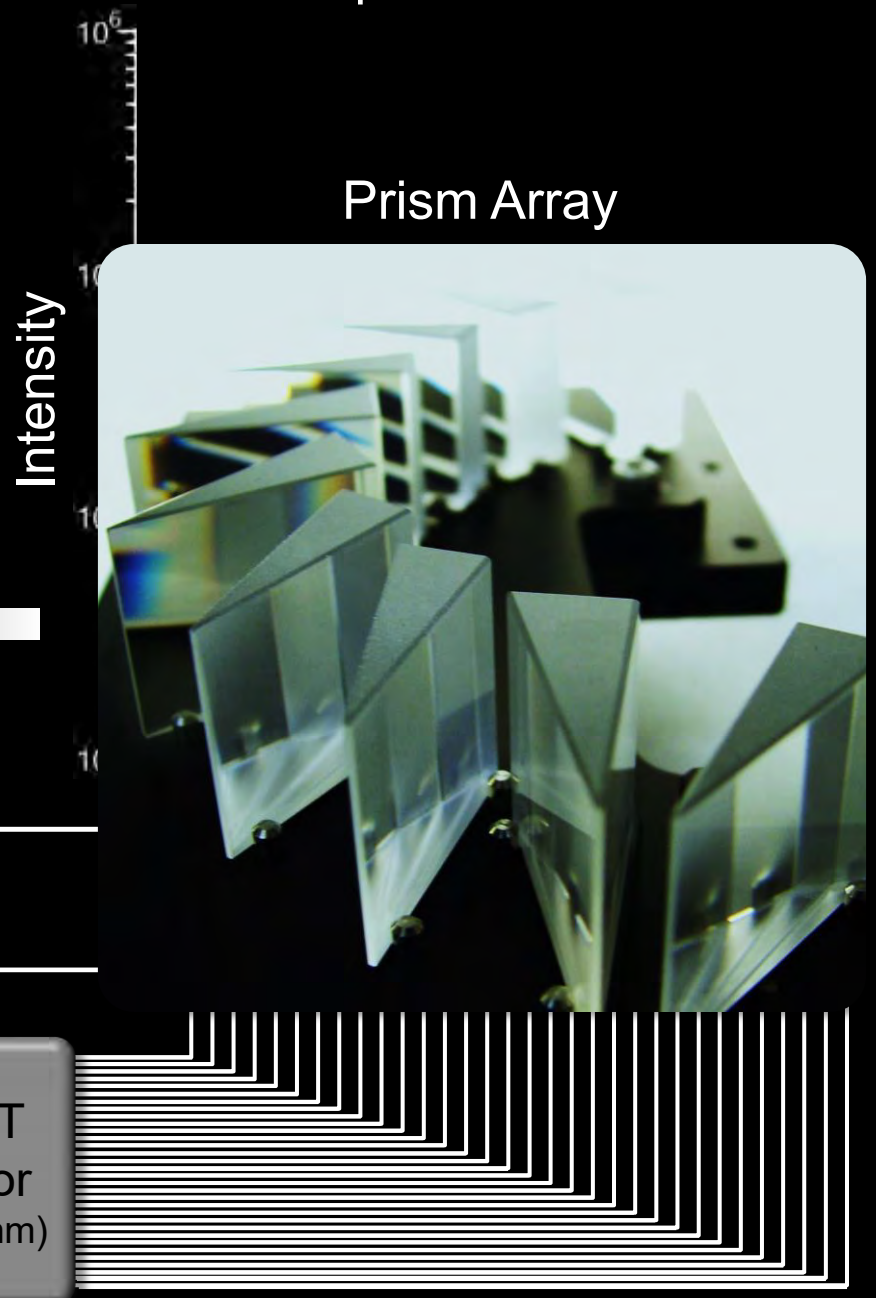
Reference Spectra

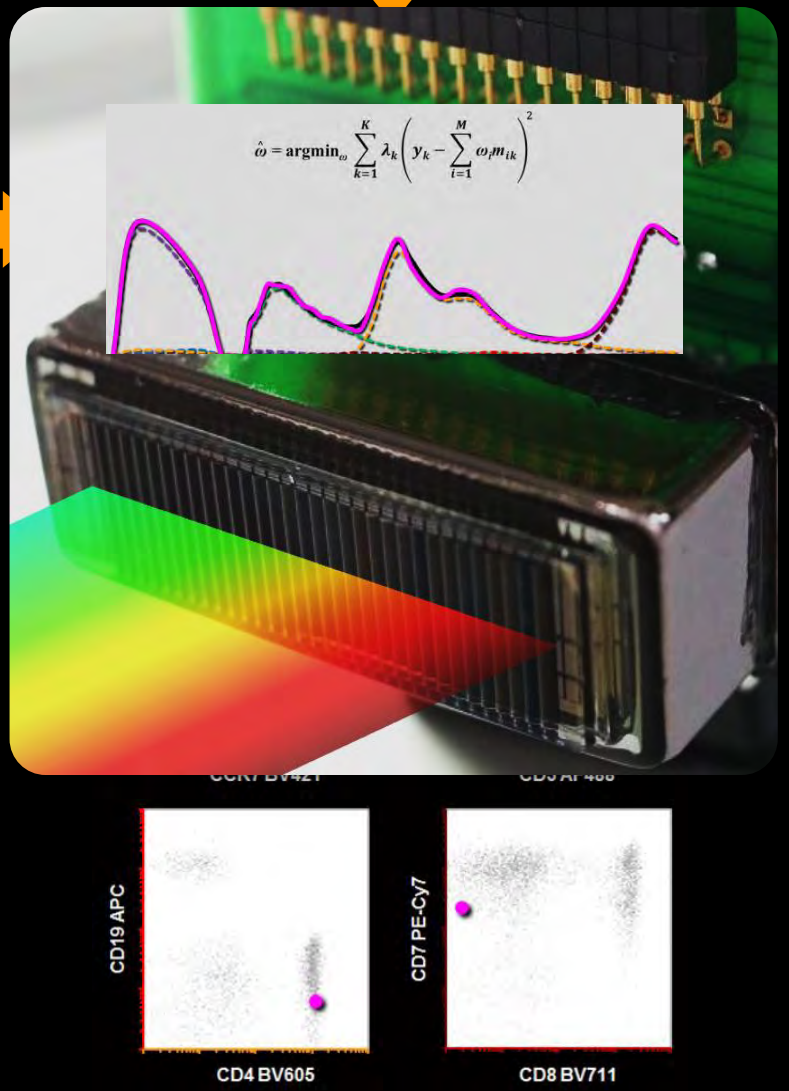
Prism Array

32 PMT Detector

Prism Array







Normalized Reference Spectras are Proportional Spectral Distributions

	V1	V2	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
BV421																																		
PacBlue																																		
AF488																																		
PE	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.9	1.0	0.7	0.4	0.3	0.3	0.3	0.3	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
BV605																																		
PE-Cy5																																		
BV711																																		
PE-Cy7																																		

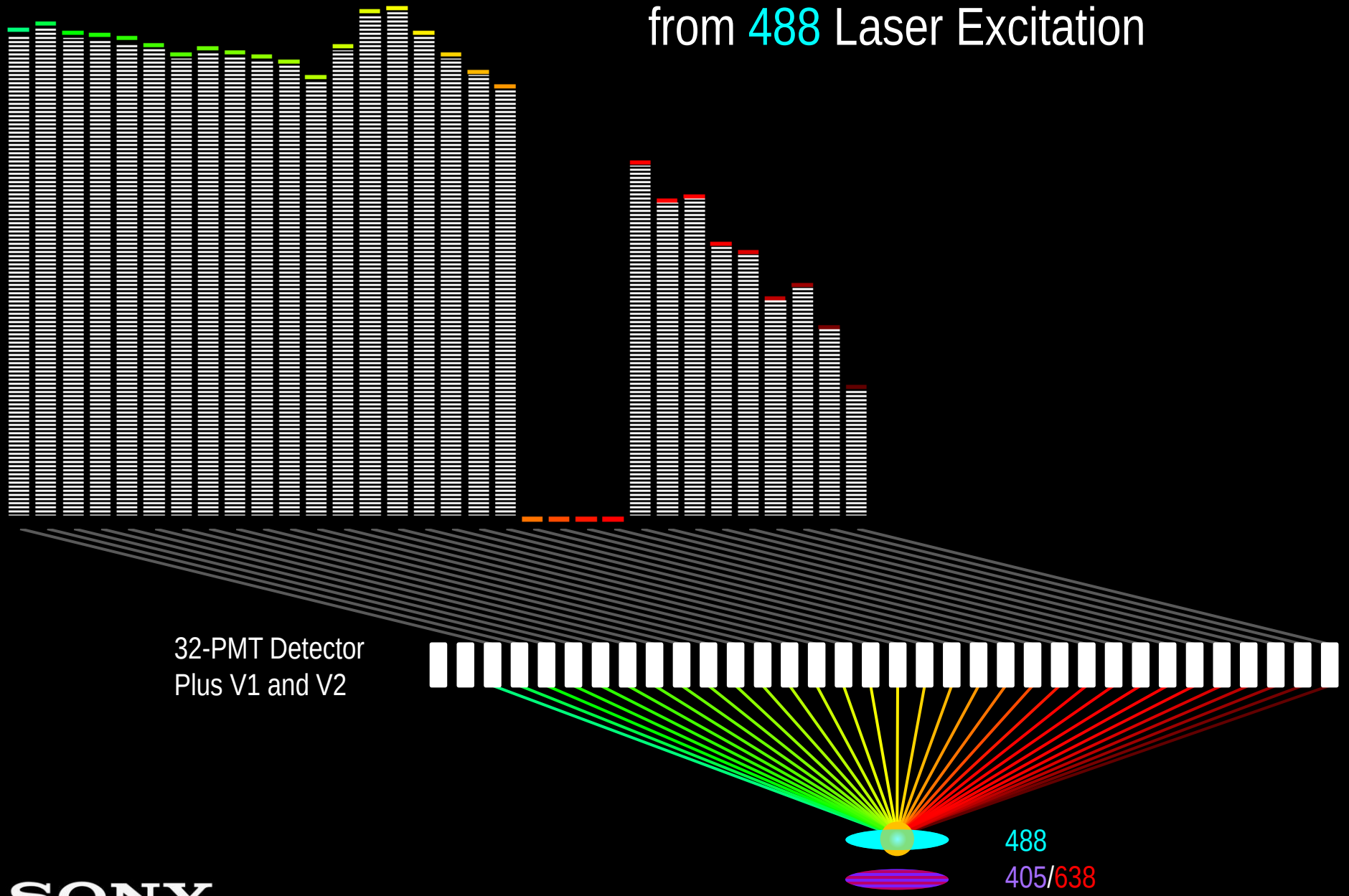
NRS are Proportional Spectral Distributions

	V1	V2	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
BV421	1.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PacBlue	0.3	1.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AF488	0.0	0.0	0.4	0.5	0.7	0.9	1.0	1.0	0.9	0.8	0.7	0.6	0.5	0.5	0.4	0.3	0.3	0.2	0.2	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PE	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.9	1.0	0.7	0.4	0.3	0.3	0.3	0.3	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
BV605	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.8	1.0	0.8	0.7	0.5	0.5	0.5	0.4	0.2	0.2	0.1	0.1	0.1	0.1	0.0	0.0
PE-Cy5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.7	1.0	0.7	0.4	0.3	0.3	0.2	0.1	0.1	0.0
BV711	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.7	1.0	0.9	0.6	0.4	0.3	0.2
PE-Cy7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.5	1.0	0.9

WHY 66 COLORS????

32 Fluorescence Signals

from 488 Laser Excitation

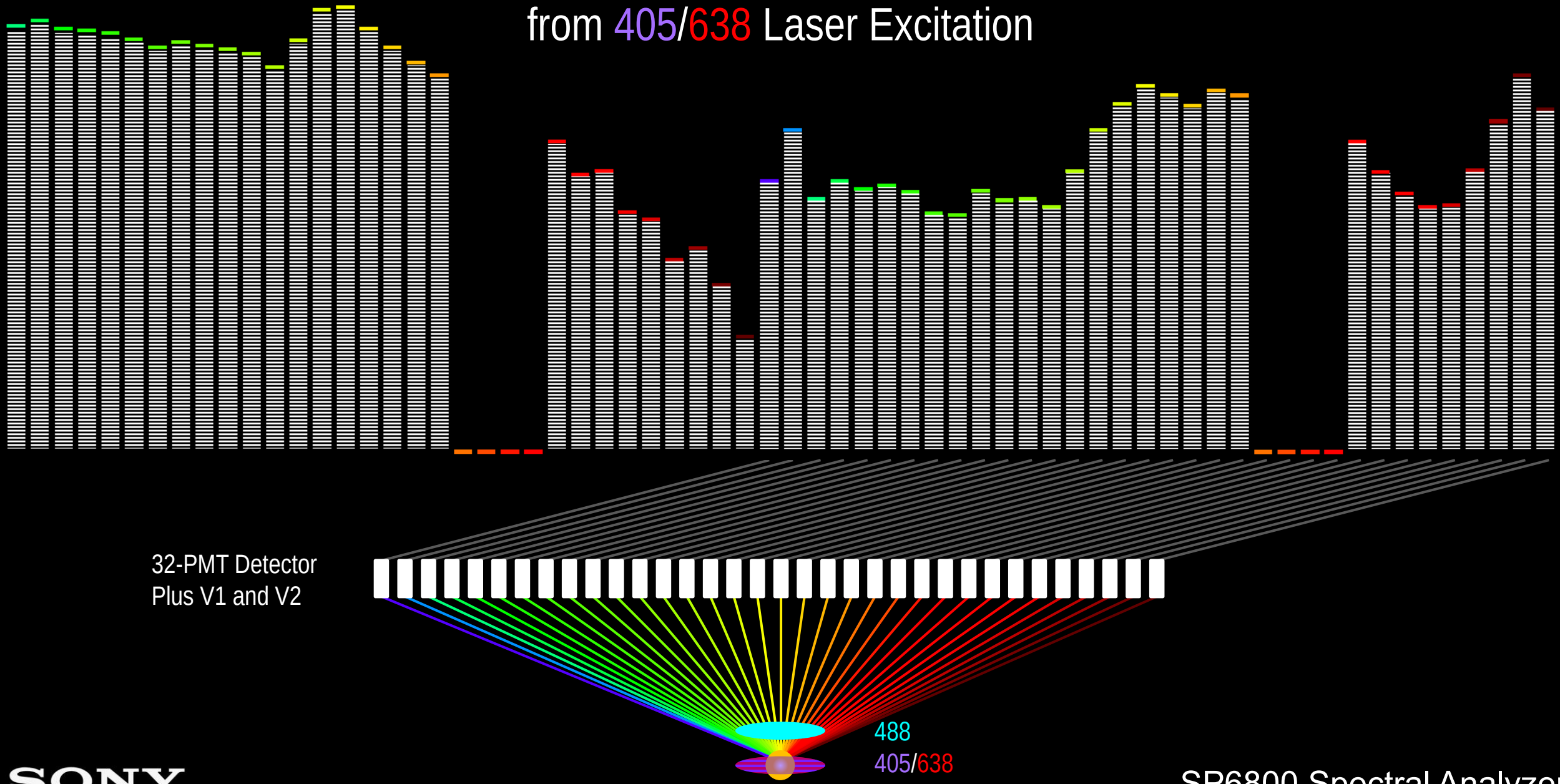


SONY

SP6800 Spectral Analyzer

34 Fluorescence Signals

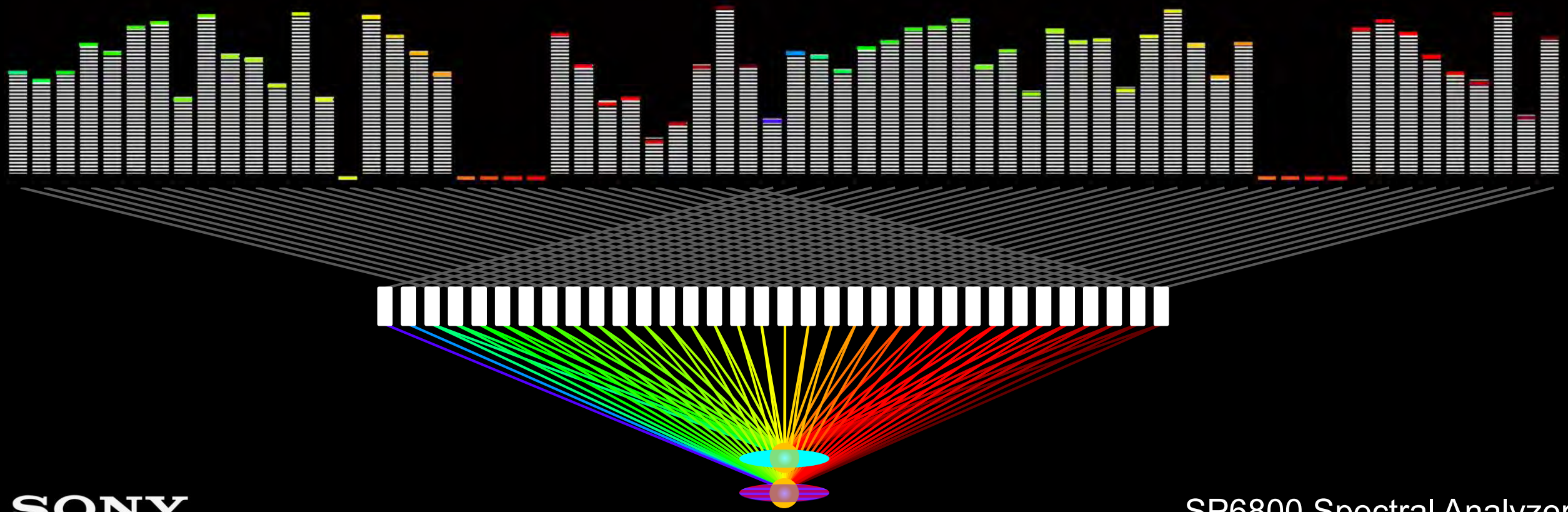
from 405/638 Laser Excitation



SONY

SP6800 Spectral Analyzer

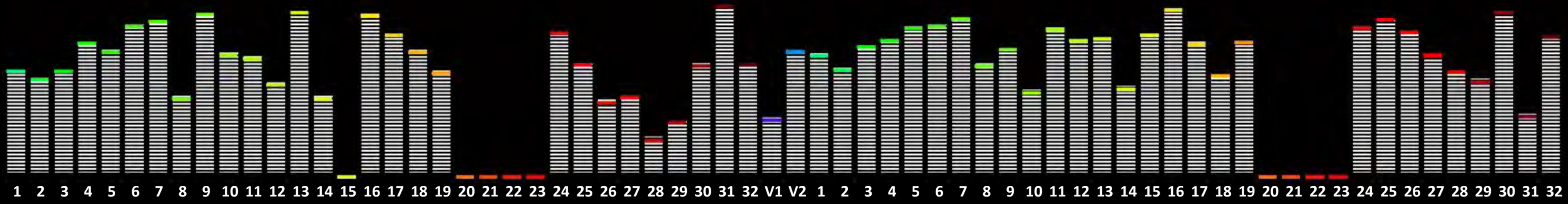
66 Fluorescence Signals from Each Cell



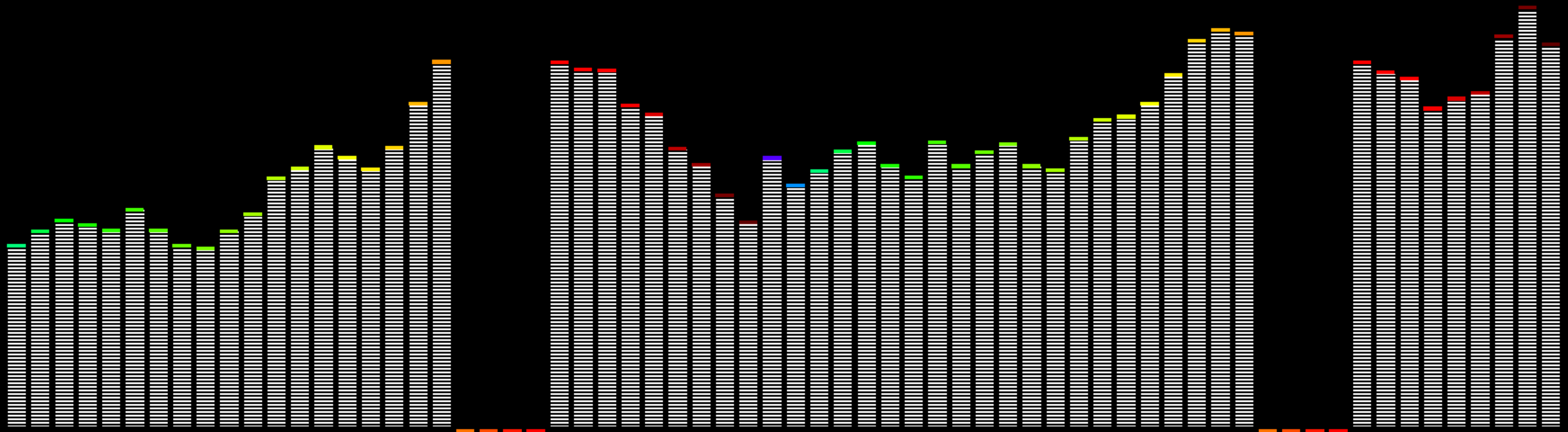
SONY

SP6800 Spectral Analyzer

66 Intensity Values are Recorded for Each Cell



66 Intensity Values are Recorded for Each Cell



1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	V1	V2	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
159	177	150	142	116	104	109	114	114	93	93	76	58	48	41	31	26	19	15	0	0	0	0	3	2	1	1	1	1	1	1	6	20	9	10	11	9	7	7	5	5	5	5	4	5	4	6	6	5	2	3	2	0	0	0	0	3	3	3	1	3	5	16	15	9
331	352	328	331	279	257	232	247	244	234	200	166	120	99	88	69	58	36	27	0	0	0	0	9	8	4	2	2	3	3	2	1	16	54	20	20	24	18	18	15	17	16	15	14	10	11	11	11	10	10	11	6	6	0	0	0	0	7	5	5	4	9	15	38	67	35
138	137	122	119	104	98	97	81	85	86	75	52	45	39	37	26	19	18	10	0	0	0	0	3	2	2	1	1	1	0	0	1	2	5	5	5	5	4	4	5	5	3	3	4	5	3	2	4	5	2	4	3	2	0	0	0	0	2	2	2	4	3	4	10	12	10
1	1	1	0	0	0	0	1	2	1	0	1	2	2	2	3	3	1	1	0	0	0	0	1	1	1	1	1	0	0	0	1	0	1	2	2	2	1	1	2	1	1	2	1	2	1	1	1	2	2	2	2	1	0	0	0	0	1	1	1	1	2	1	1	1	1
134	136	137	141	132	118	103	101	98	98	88	80	91	118	142	104	87	64	40	0	0	0	0	8	9	10	4	3	1	2	1	1	5	15	6	8	10	8	6	7	6	6	6	5	6	5	8	19	20	18	14	7	7	0	0	0	0	4	2	2	1	1	7	12	19	12
2	3	4	3	3	4	3	2	2	3	4	8	9	13	11	9	13	27	55	0	0	0	0	55	48	48	26	23	12	9	6	4	11	7	8	12	14	9	8	14	9	12	13	9	9	15	20	22	27	44	79	95	89	0	0	0	0	55	46	42	25	29	32	85	140	75
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151	165	157	146	141	124	116	116	103	110	93	79	65	41	40	33	25	20	16	0	0	0	0	5	4	3	2	1	1	1	1	0	4	13	4	7	7	5	6	4	4	4	4	5	4	4	5	3	4	5	4	3	2	0	0	0	0	2	3	2	1	1	4	8	18	12
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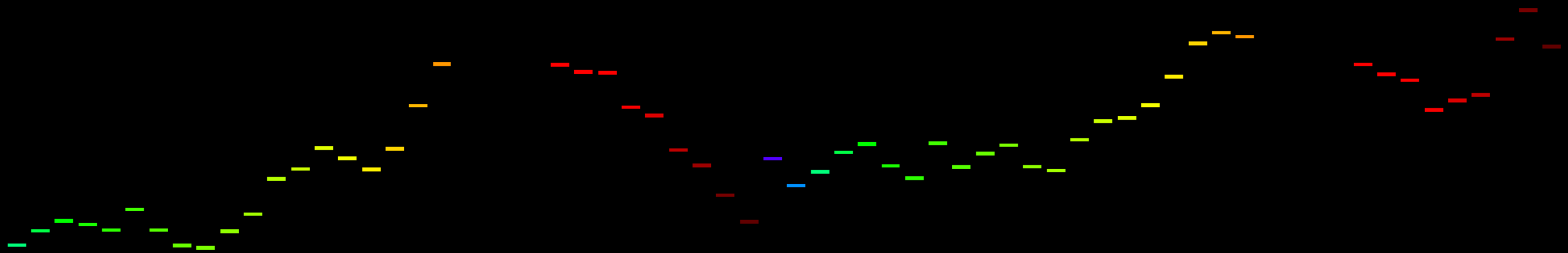
SONY

SP6800 Spectral Analyzer

These 66 Intensity Values



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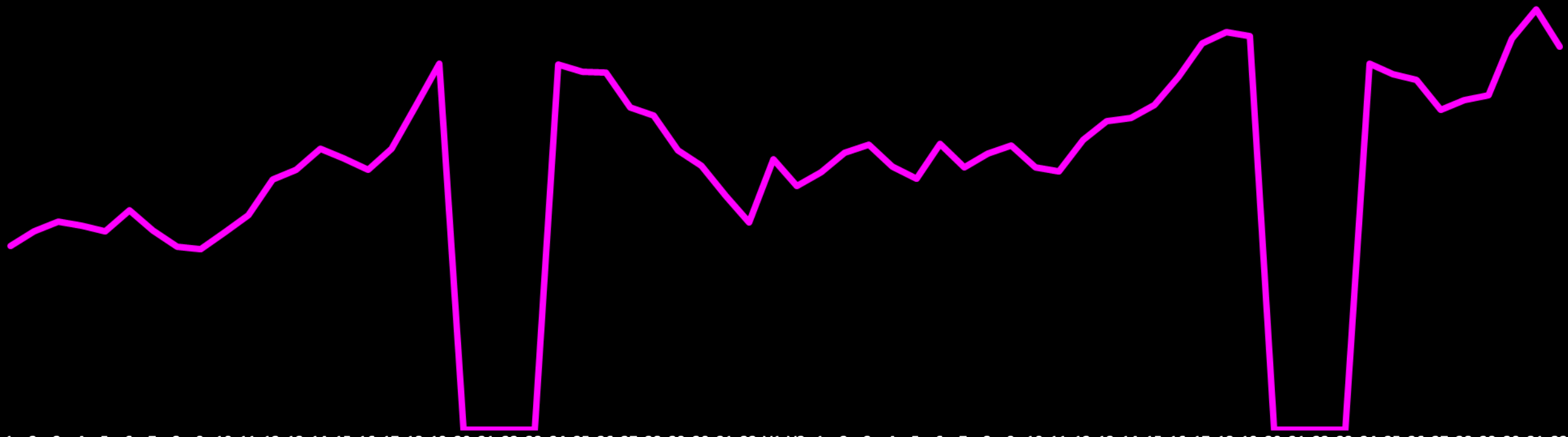
SONY

SP6800 Spectral Analyzer

Define a Single Cell Vector (y_k)



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104	99	104	107	97	70	82	75	77	73	54	58	59	67	57	40	33	30	19	0	0	0	0	4	4	3	3	2	1	1	1	2	8	24	12	11	14	12	10	8	9	6	8	7	5	6	8	15	10	10	6	4	4	0	0	0	0	6	4	4	6	5	9	19	27	19
164	178	178	170	134	130	129	121	119	124	116	98	91	94	85	59	47	39	32	0	0	0	0	7	5	6	3	2	2	1	1	1	5	19	7	10	13	10	8	8	7	6	6	6	6	6	7	10	9	9	9	7	6	0	0	0	0	5	4	3	2	3	5	12	27	18
137	141	134	119	102	101	91	79	90	96	78	62	54	47	40	28	24	20	13	0	0	0	0	3	3	3	2	1	1	1	2	1	4	11	7	7	7	5	6	7	7	6	4	5	5	3	6	8	6	4	4	5	3	0	0	0	0	3	2	2	1	2	5	12	18	15
21	23	27	29	31	34	33	35	27	25	19	17	18	12	11	9	11	11	9	0	0	0	0	6	5	6	3	3	2	5	10	6	0	1	2	2	2	1	2	2	1	2	2	1	2	2	1	2	3	3	4	4	3	0	0	0	0	15	13	10	6	8	13	47	72	45
1	3	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	2	2	1	2	1	1	2	1	2	2	1	2	2	1	1	0	0	0	0	1	1	1	1	1	1	1	0
20	26	34	39	34	35	33	32	24	23	19	18	18	13	10	9	9	10	10	0	0	0	0	10	9	14	7	5	6	10	16	10	0	1	1	1	1	2	1	2	2	1	2	2	1	2	2	1	2	3	3	2	3	0	0	0	0	16	12	9	6	10	23	57	80	42
149	151	156	145	122	116	113	103	105	105	81	77	69	64	65	45	37	22	17	0	0	0	0	8	7	5	5	9	5	3	2	1	11	32	10	11	12	12	12	12	8	8	7	7	7	7	6	9	11	7	6	4	3	0	0	0	0	7	32	94	210	287	164	128	80	37
0	0	0	1	0	0	0	0	0	0	2	1	1	1	2	1	1	0	1	0	0	0	0	1	0	1	1	1	1	0	1	0	0	1	1	0	2	1	2	1	1	1	1	1	1	2	2	2	1	1	1	0	0	0	0	1	1	1	2	1	0	1	2	1
199	205	193	186	159	166	145	145	129	128	118	94	120	178	174	111	75	70	50	0	0	0	0	20	10	12	7	7	4	3	2	2	5	13	7	9	10	7	9	10	8	8	7	6	5	9	24	31	32	19	20	21	11	0	0	0	0	8	6	7	5	6	7	18	23	13
141	129	123	117	112	99	96	86	90	80	84	86	102	152	164	122	86	55	52	0	0	0	0	9	9	8	6	4	1	1	1	1	5	16	9	6	5	9	7	8	7	8	5	5	8	8	12	22	29	17	12	8	7	0	0	0	0	5	3	3	2	3	5	14	26	14
126	118	114	117	103	111	107	115	137	157	199	269	315	316	360	407	492	589	536	0	0	0	0	429	314	300	184	181	202	485	621	214	6	17	29	33	27	27	27	31	29	30	33	36	42	58	65	76	93	106	112	121	123	0	0	0	0	225	184	173	131	207	378	1081	1535	936
122	125	119	101	102	91	74	77	78	70	65	55	45	35	33	24	24	16	16	0	0	0	0	3	3	2	2	2	1	2	2	1	4	13	6	8	6	5	5	6	5	7	4	7	6	3	4	5	4	3	2	4	3	0	0	0	0	3	2	1	1	1	7	10	17	11
25	30	36	34	35	40	40	38	29	28	24	22	21	21	18	14	19	33	66	0	0	0	0	71	62	60	35	34	15	14	9	4	1	4	16	15	14	18	19	19	22	20	18	16	16	17	15	14	17	18	16	14	35	0	0	0	0	104	101	99	94	133	87	104	124	80
13	19	23	29	26	24	25	29	22	19	15	15	14	12	15	8	9	14	23	0	0	0	0	24	22	20	9	11	4	8	11	4	1	2	3	5	5	3	3	3	3	3	3	4	3	2	3	4	4	2	4	7	11	0	0	0	0	15	15	12	6	6	11	35	59	31

Use Caution with Tandem Dyes!

SONY SA3800 Fluorochrome Guide

Select one from each group

Group	Fluorochrome	λ_{max}	Laser
1	Alexa Fluor 405	421	405
1	**Brilliant Violet 421**	421	405
2	V450	448	405
2	eFluor 450	450	405
2	**Pacific Blue**	455	405
2	CFP	475	405
3	AmCyan	489	405
3	V500	500	405
3	GFP	507	488
3	Brilliant Violet 510	510	405
3	Brilliant Blue	515	488
3	**Alexa Fluor 488**	519	488
3	FITC	520	488
3	Krome Orange	528	405
3	V550	536	405
4	YFP	528	488
4	**Alexa Fluor 514**	547	488
4	Pacific Orange	551	405
4	Alexa Fluor 532	554	488
5	Brilliant Violet 570	570	405
5	**PE**	578	488/561
6	dsRed	602	488/561
6	**Brilliant Violet 605**	605	405
6	eFluor 605NC	605	405
6	Qdot 605	605	405
6	mCherry	610	561
6	PE-Dazzle 594	610	488/561

6	PE-eFluor 610	610	488/561
6	PE-Texas Red	616	488/561
6	Alexa Fluor 594	620	561
6	eFluor 625NC	625	405
6	PE-Alexa Fluor 610	625	488/561
7	HcRed	631	561
7	mKate2	633	561
7	Brilliant Violet 650	650	405
7	eFluor 650	650	405
7	Qdot 655	655	405
7	**APC**	660	638
7	eFluor 660	660	405
7	PE-Cy5	667	488/561
7	Alexa Fluor 647	668	638
7	Tri-Color	671	488/561
7	PerCP	678	488/561
8	PE-Cy5.5	693	488/561
8	PerCP-Cy5.5	695	488/561
8	APC-Cy5.5	695	638
8	Qdot 705	705	405
8	PerCP-eFluor 710	710	488/561
8	Brilliant Violet 711	711	405
8	PE-Alexa Fluor 700	720	488/561
8	**Alexa Fluor 700**	723	638
9	APC-Alexa Fluor 750	775	638
9	APC-eFluor 780	780	638
9	**PE-Cy7**	785	488/561
9	APC-Cy7	785	638
9	APC-H7	785	638
9	Brilliant Violet 785	785	405
9	Qdot 800	800	405