

You're in the right place.

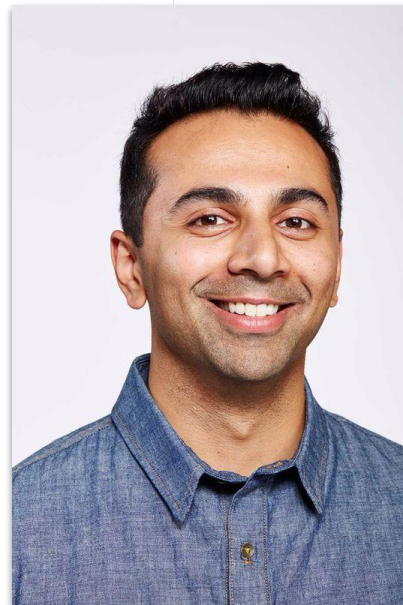
Read more about Teiko's spectral flow service @
[**teiko.bio/spectral-flow-cytometry**](https://teiko.bio/spectral-flow-cytometry)

Teiko.bio

Spectral vs conventional flow basics series

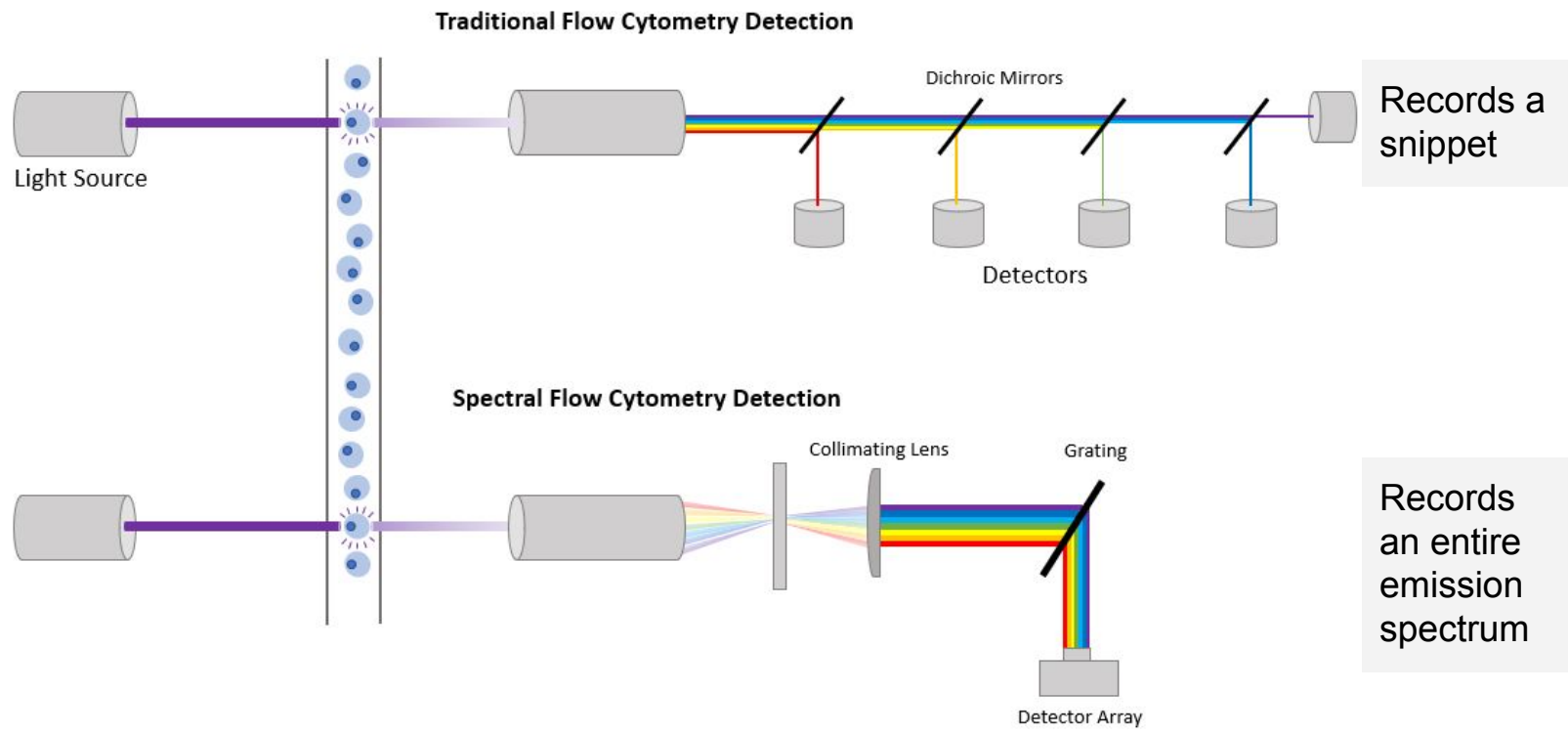
How spectral allows higher-parameter analysis

High-parameter cytometry for clinical trials

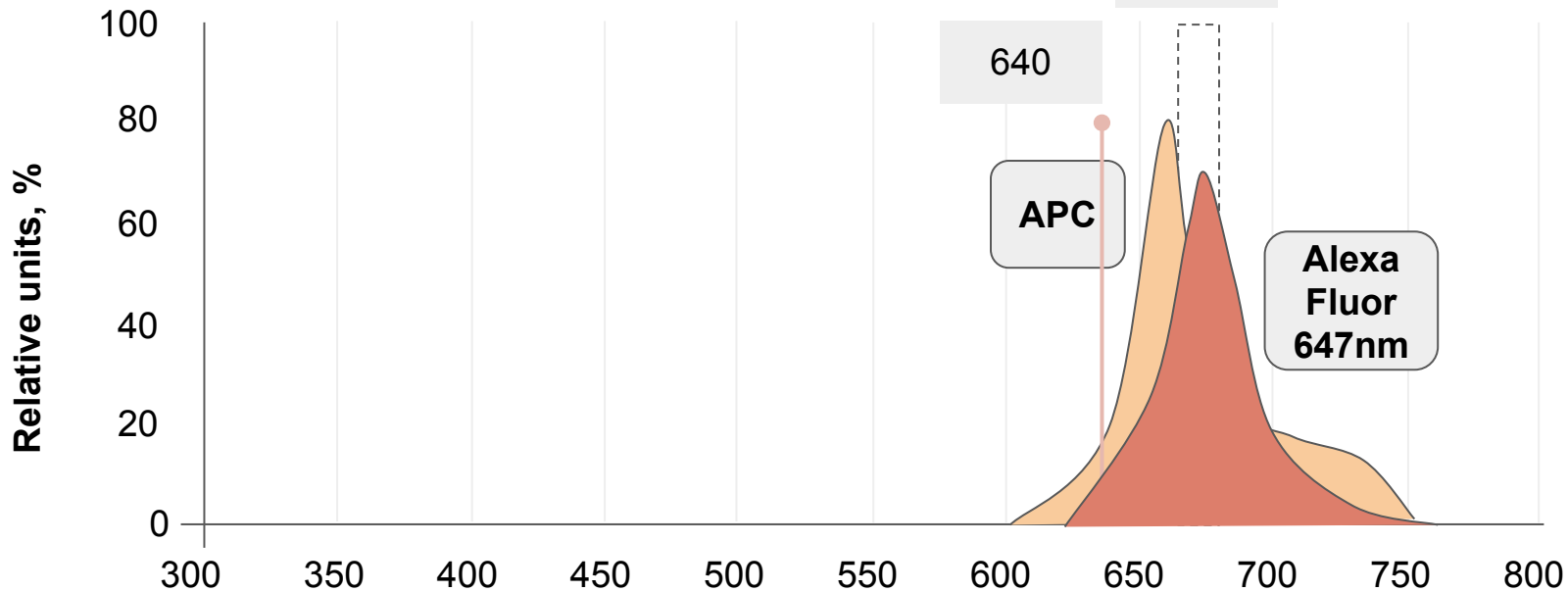


Ramji Srinivasan
Teiko CEO

Problem



Adapted from [FluoroFinder](#)

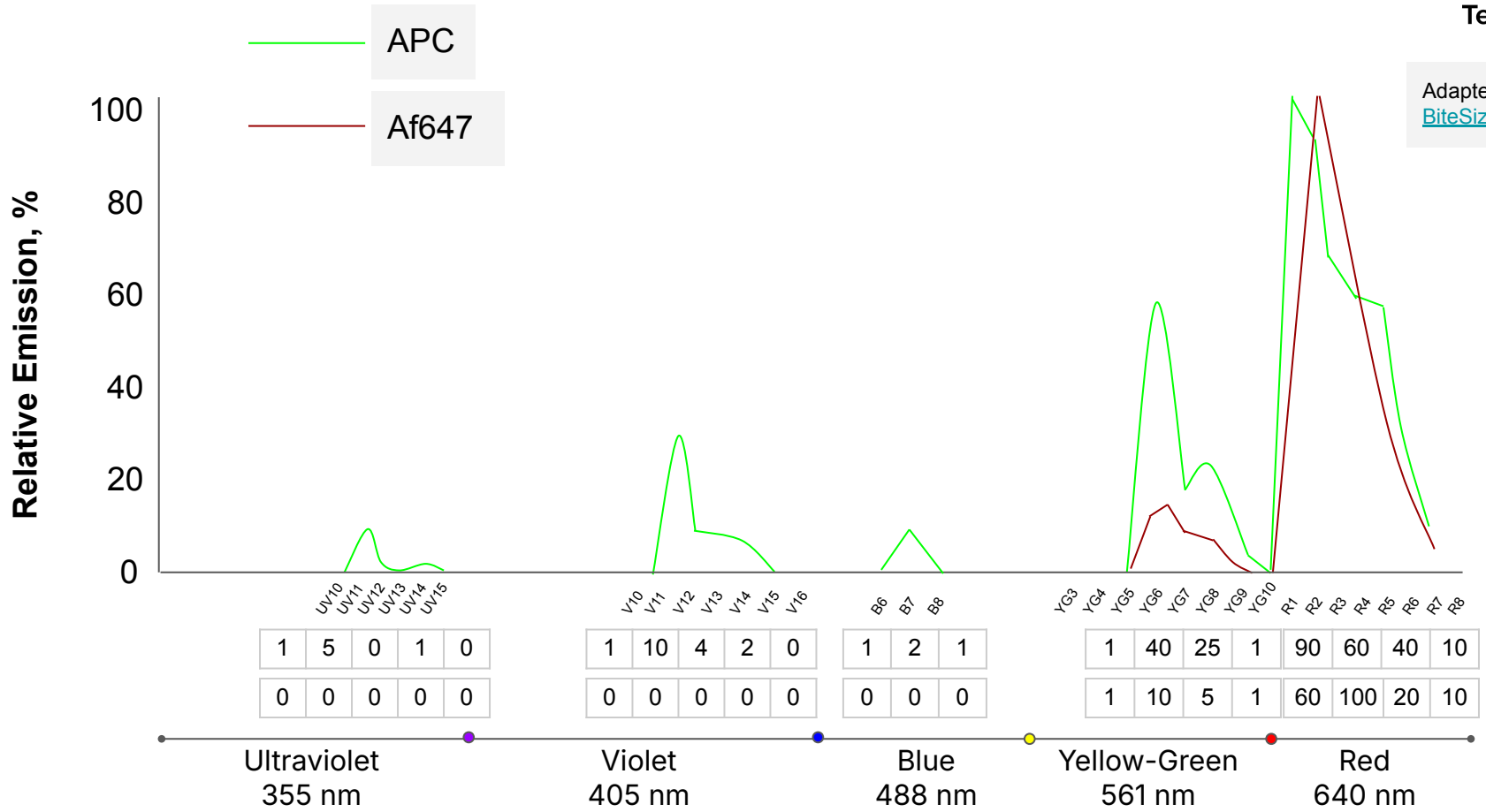


Adapted from [BiteSizeBio](https://www.bitesizebio.com)



Wavelength, nanometers (nm)

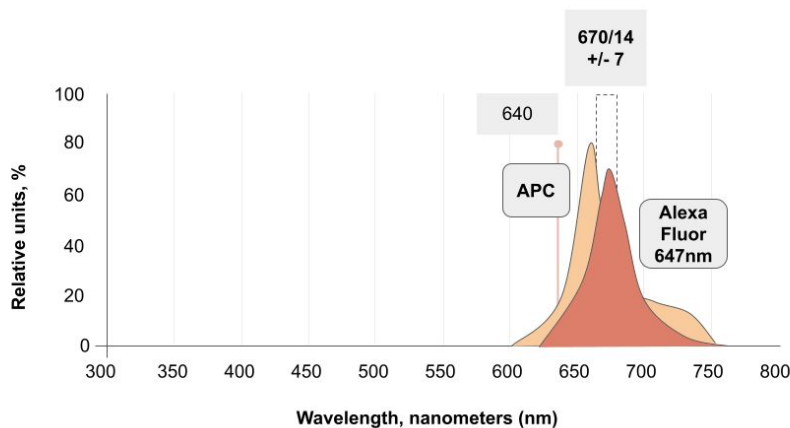
Adapted from [BiteSizeBio](#)



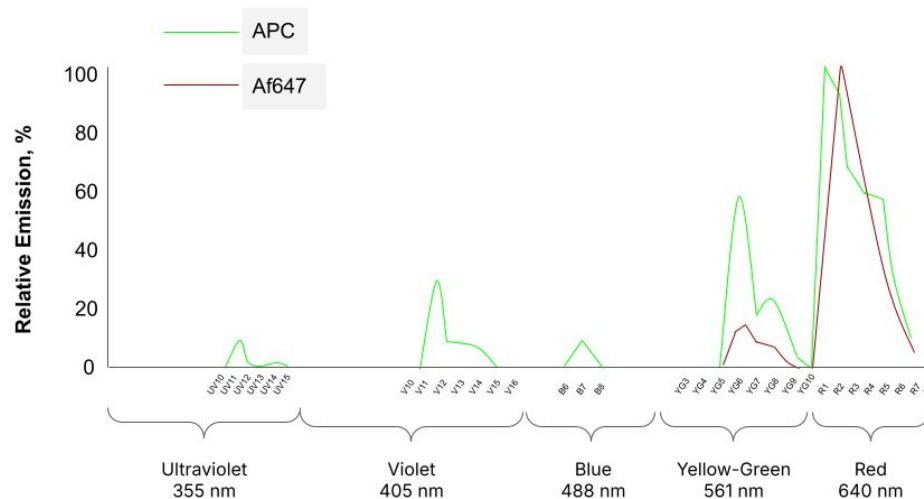
So what?

Spectral Flow can distinguish overlapping signals that would stump conventional flow

Conventional Flow



Spectral Flow



Adapted from
[BiteSizeBio](https://www.bitesizebio.com/)

That means more markers, and finer resolution

	Classical Flow	Spectral
Markers	6-8	25
Possible subtypes	~52	298
% of Cells Recovered	95%+	95%+
Days to run 300 samples at a target of 1M events per sample	~1 day	~1 day
Value	Speed, cost	Tighter variation

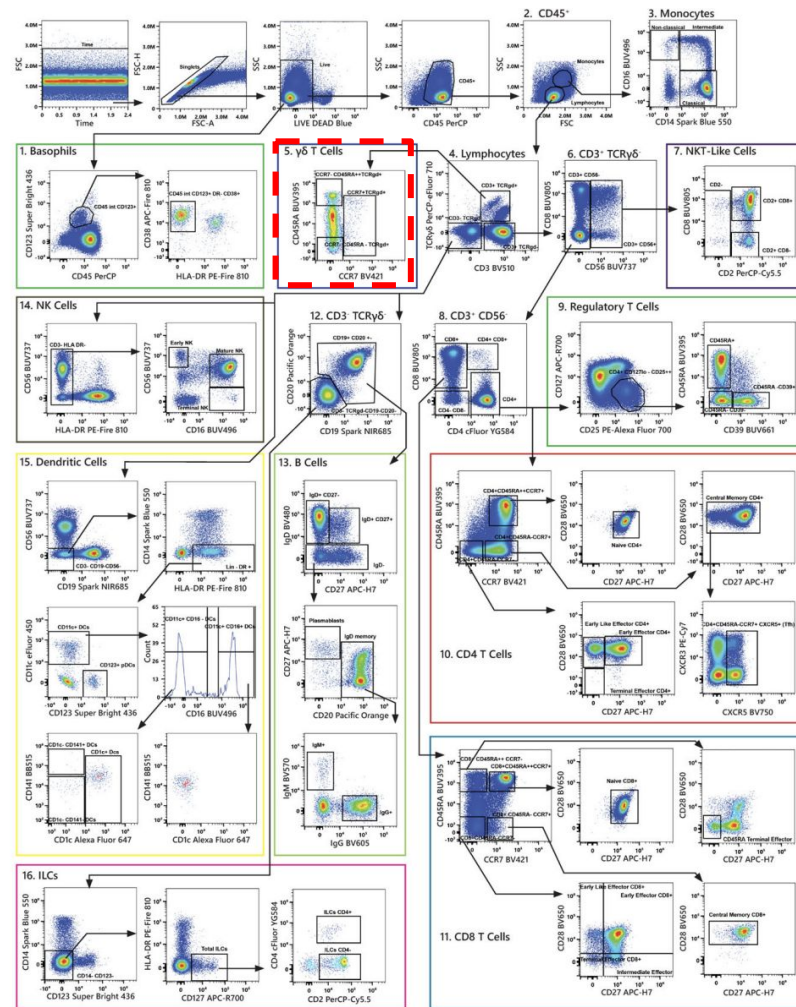
Sources: Teiko estimates, research.

In action

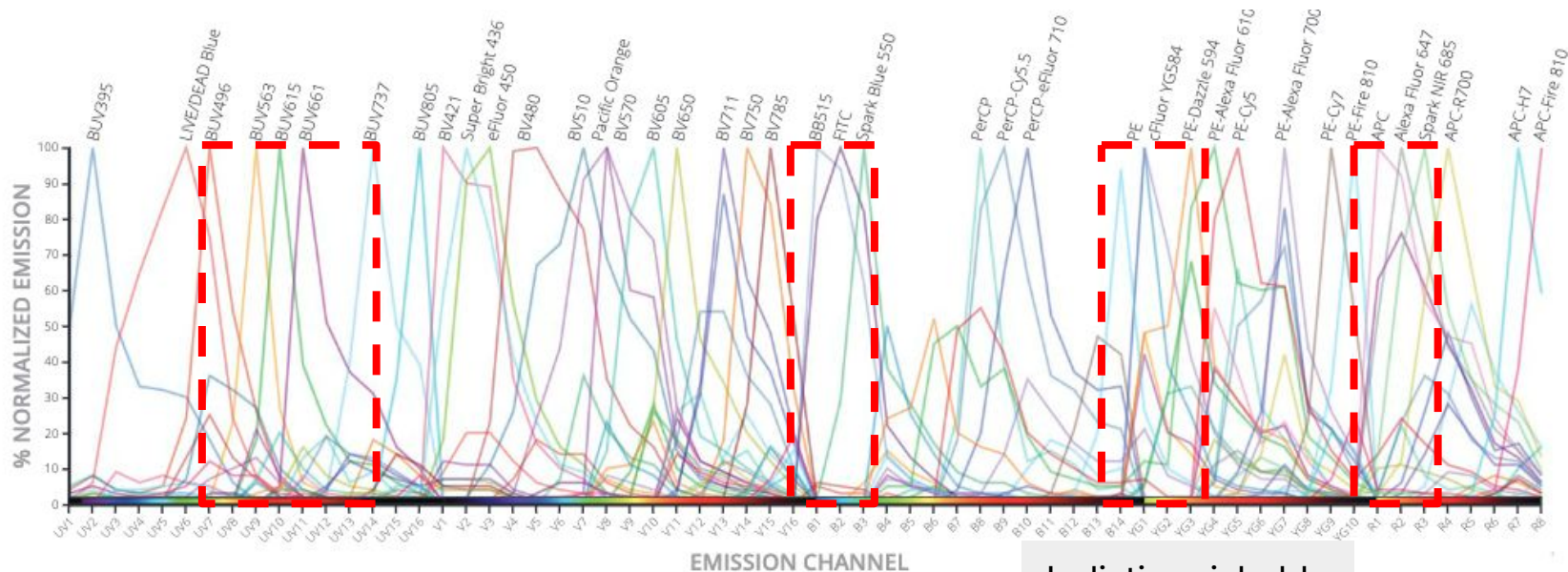
40 marker panel ([OMIP-69](#))

$\gamma\delta$ T cells also associated with “major autoimmune rheumatic diseases”... but take up a lot of channels to resolve

Markers	
Viability	CD2
CD45	CD56
CD3	CCR7
CD4	CD27
CD8	CD28
CD25	CD45RA
TCR $\gamma\delta$	CD95
CD14	CD127
CD16	CD337
CD11c	CCR6
CD19	CCR5
CD20	CXCR5
CD24	CXCR3
CD39	HLA-DR
IgD	CD38
IgG	CD57
IgM	PD-1
CD141	CD159a
CD1c	CD159c
CD123	CD314



Emission spectrum for 40 marker panel ([OMIP-69](#))

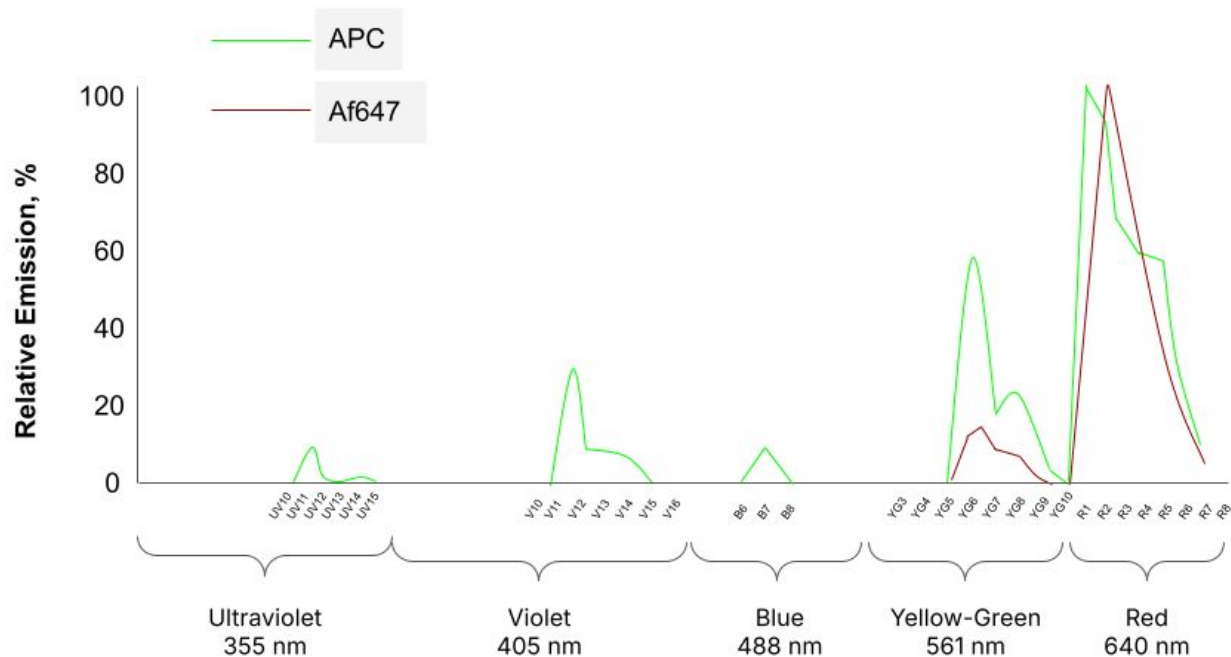


Indistinguishable
through a
bandpass filter

Unmixing preview

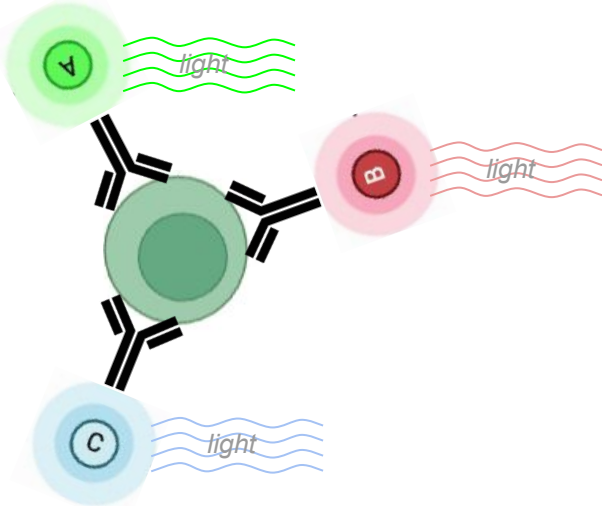
Remember this?

Spectral Flow

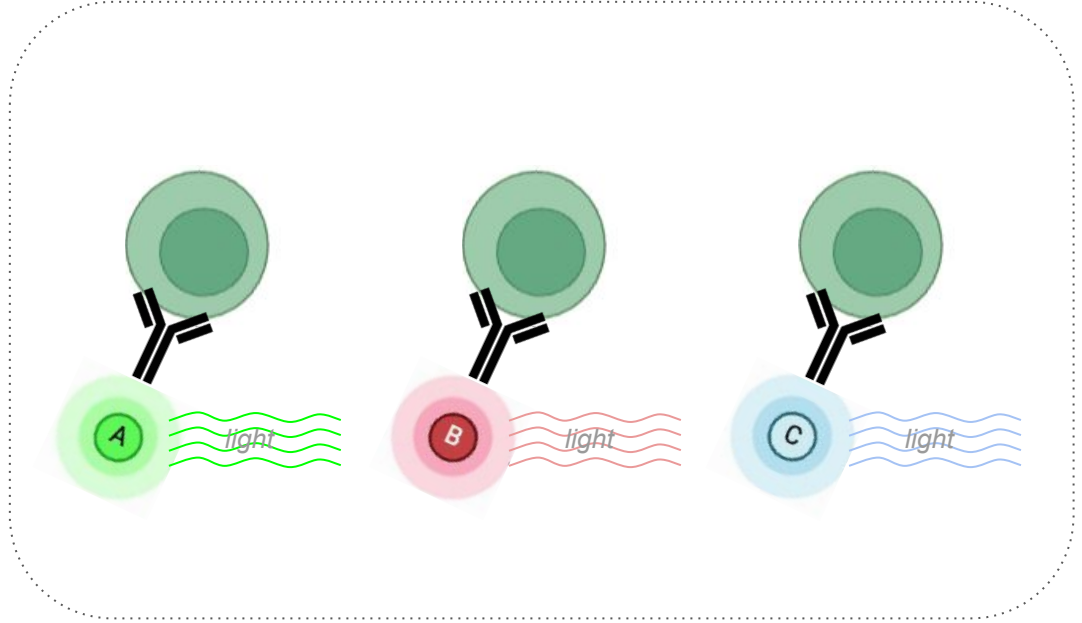


Experiment design before acquisition

Combination of the light coming from A, B and C

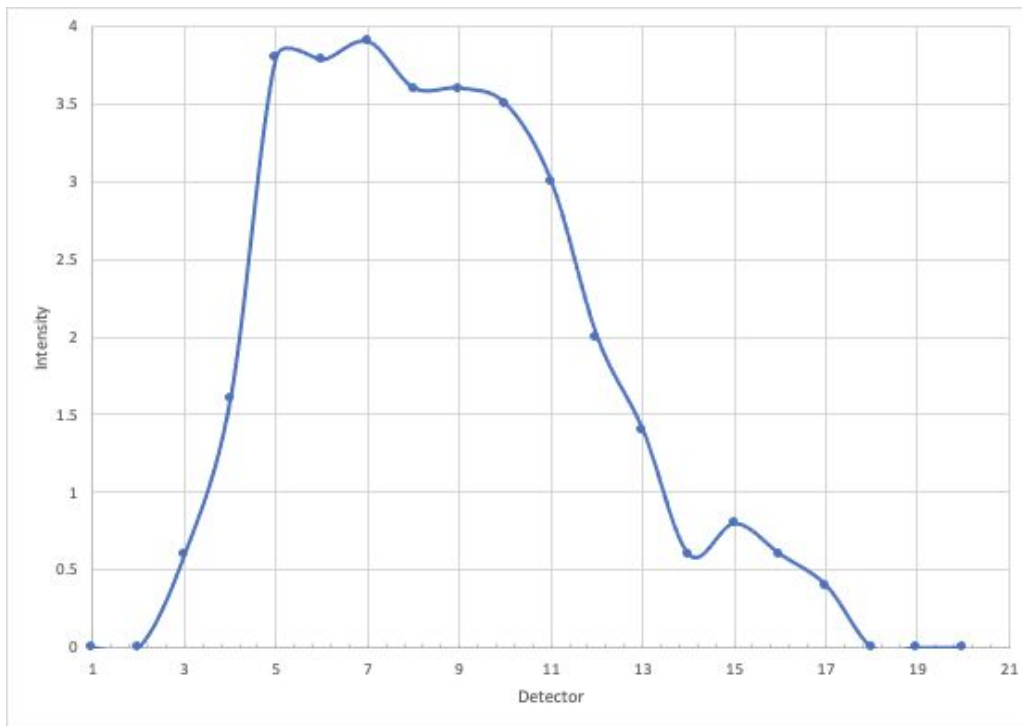


Set of single color controls (A, B and C)

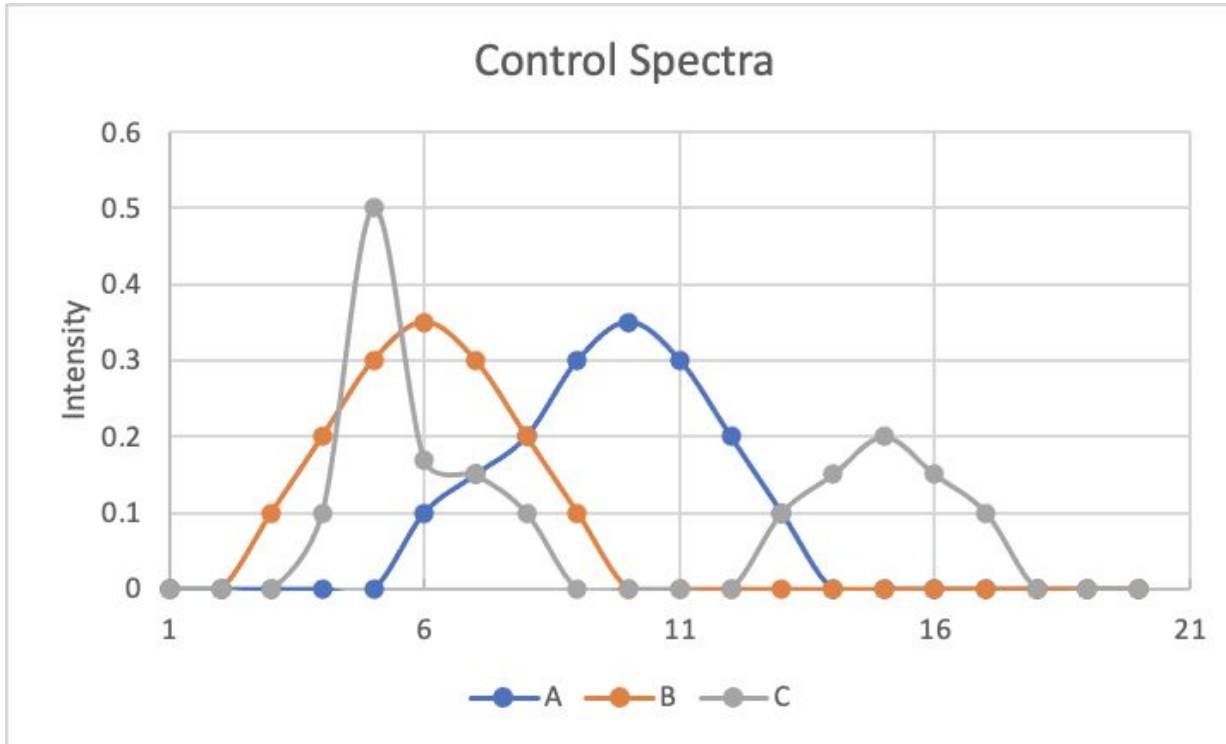


The cell with three fluorochromes: A, B and C

Observed Spectra from a cell



We need single color controls to determine intensity of each fluorochrome



Adapted from
[CheekyScientist](#)

How much of each of these fluorochromes is present on the cell?

Mixing

Detector	Individual Spectra		
	A	B	C
1	0	0	0
2	0	0	0
3	0	0.1	0
4	0	0.2	0.1
5	0	0.3	0.5
6	0.1	0.35	0.17
7	0.15	0.3	0.15
8	0.2	0.2	0.1
9	0.3	0.1	0
10	0.35	0	0
11	0.3	0	0
12	0.2	0	0
13	0.1	0	0.1
14	0	0	0.15
15	0	0	0.2
16	0	0	0.15
17	0	0	0.1
18	0	0	0
19	0	0	0
20	0	0	0

X

Channel	Attribution
A	?
B	?
C	?

=

Observed

Observed
0
0
0.6
1.6
3.8
3.78
3.9
3.6
3.6
3.5
3
2
1.4
0.6
0.8
0.6
0.4
0
0
0

Adapted from
[CheekyScientist](#)

How much of each of these fluorochromes is present on the cell?

Mixing **Attribution** = **Observed**

[20 X 3] [3 X 1] = [20 X 1]

Channel	Attribution
A	10
B	6
C	4

How much of each of these fluorochromes is present on the cell?

Detector	Individual Spectra		
	A	B	C
1	0	0	0
2	0	0	0
3	0	0.1	0
4	0	0.2	0.1
5	0	0.3	0.5
6	0.1	0.35	0.17
7	0.15	0.3	0.15
8	0.2	0.2	0.1
9	0.3	0.1	0
10	0.35	0	0
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12	0.2	0	0
13	0.1	0	0.1
14	0	0	0.15
15	0	0	0.2
16	0	0	0.15
17	0	0	0.1
18	0	0	0
19	0	0	0
20	0	0	0

X

Channel	Attribution
A	10
B	6
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=

Observed
0
0
0.6
1.6
3.8
3.78
3.9
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3
2
1.4
0.6
0.8
0.6
0.4
0
0
0

Adapted from
[CheekyScientist](#)

In sum

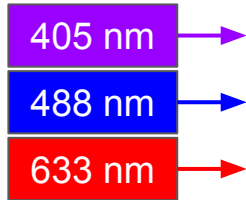
Appendix

100 Low Pass

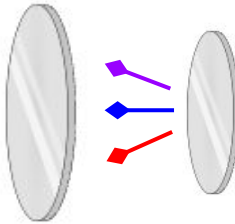


Wavelengths lower than 100 nanometers will be deflected

Excitation Lasers



Lenses and Prisms



Flow Cell

Dichroic Mirrors

735LP

640LP

550LP

Bandpass Filters

780/60

660/20

585/42

530/10

Forward Scatter

Photomultiplier Tube (PMT)

PMT

PMT

PMT

500/30

470-530 nanometer detection

Adapted from
<https://bitesizebio.com/31638/flow-cytometry-optics-system/>