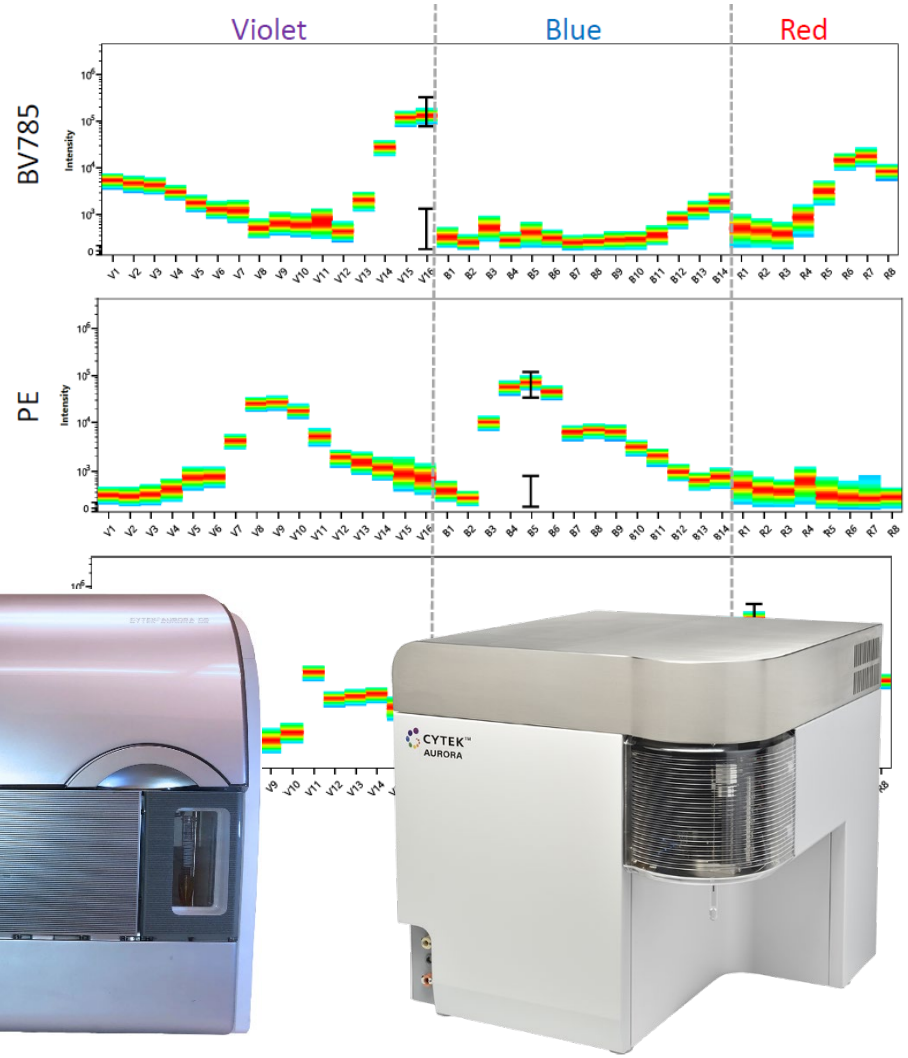
Cytek Spectral technology

full spectrum flow cytometry and sorting



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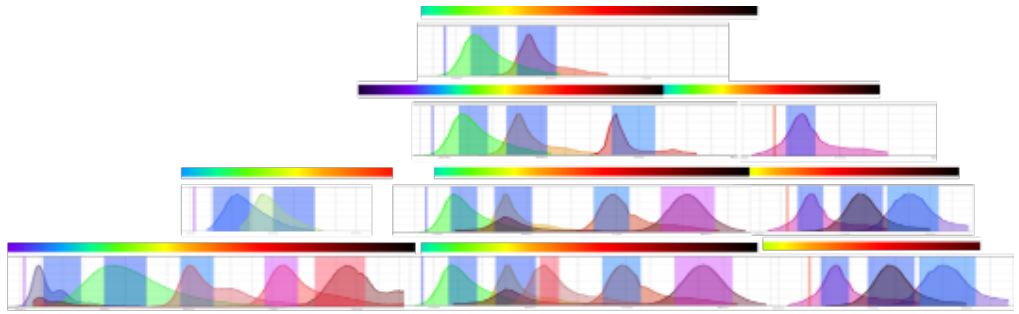
Trends in cytometry

1980 (1 Laser, 2 colors) →

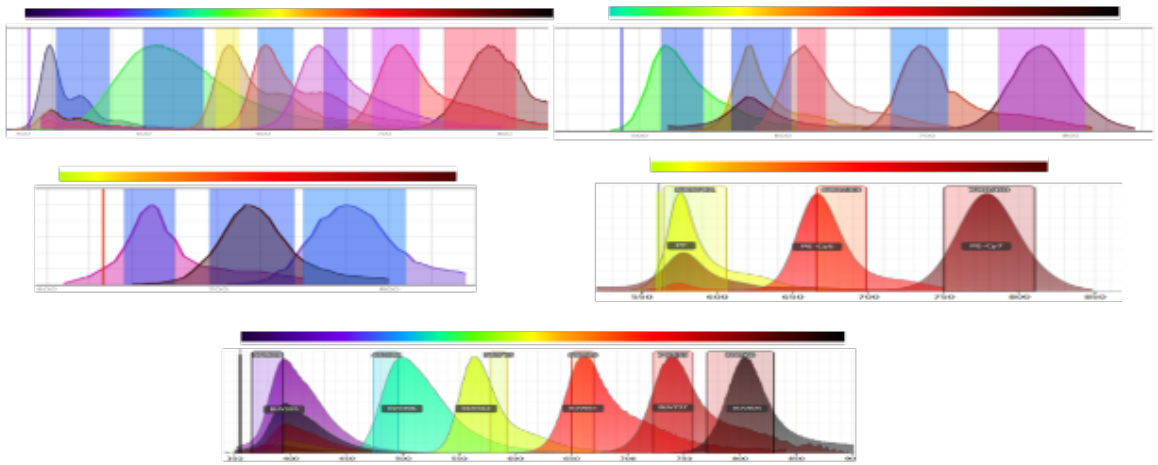
1990 (2 Laser, 4 colors) →

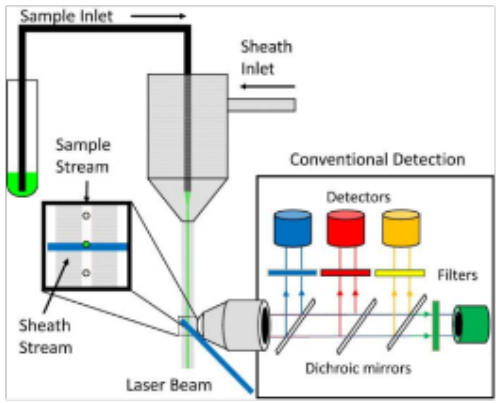
2000 (3 Laser, 8 colors) →

2010 (3 Laser, 13 colors) →



2018 (5+ Lasers, 20-50 channels)



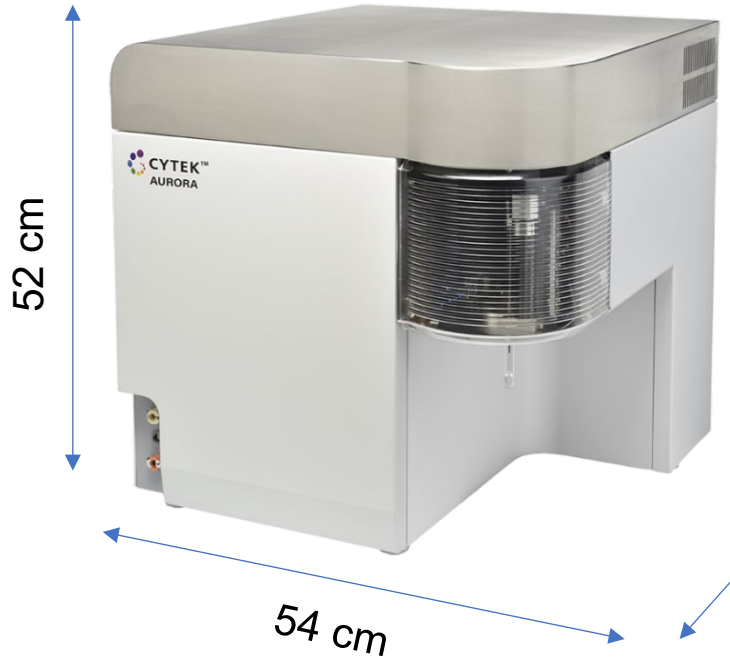


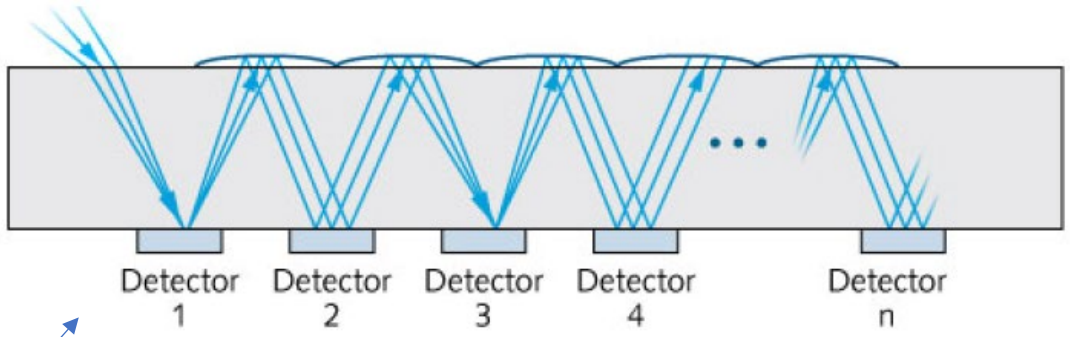
Nolan and Condello (2013) Current Protocols in Cytometry

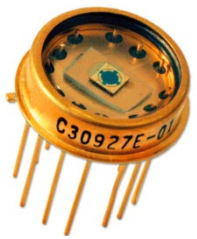
It is very hard to develop filters and dyes that allow people to increase the number of parameters, and maintain sufficient sensitivity after compensation…

Compat footprinth

71 kg
84 kg with plate
loader







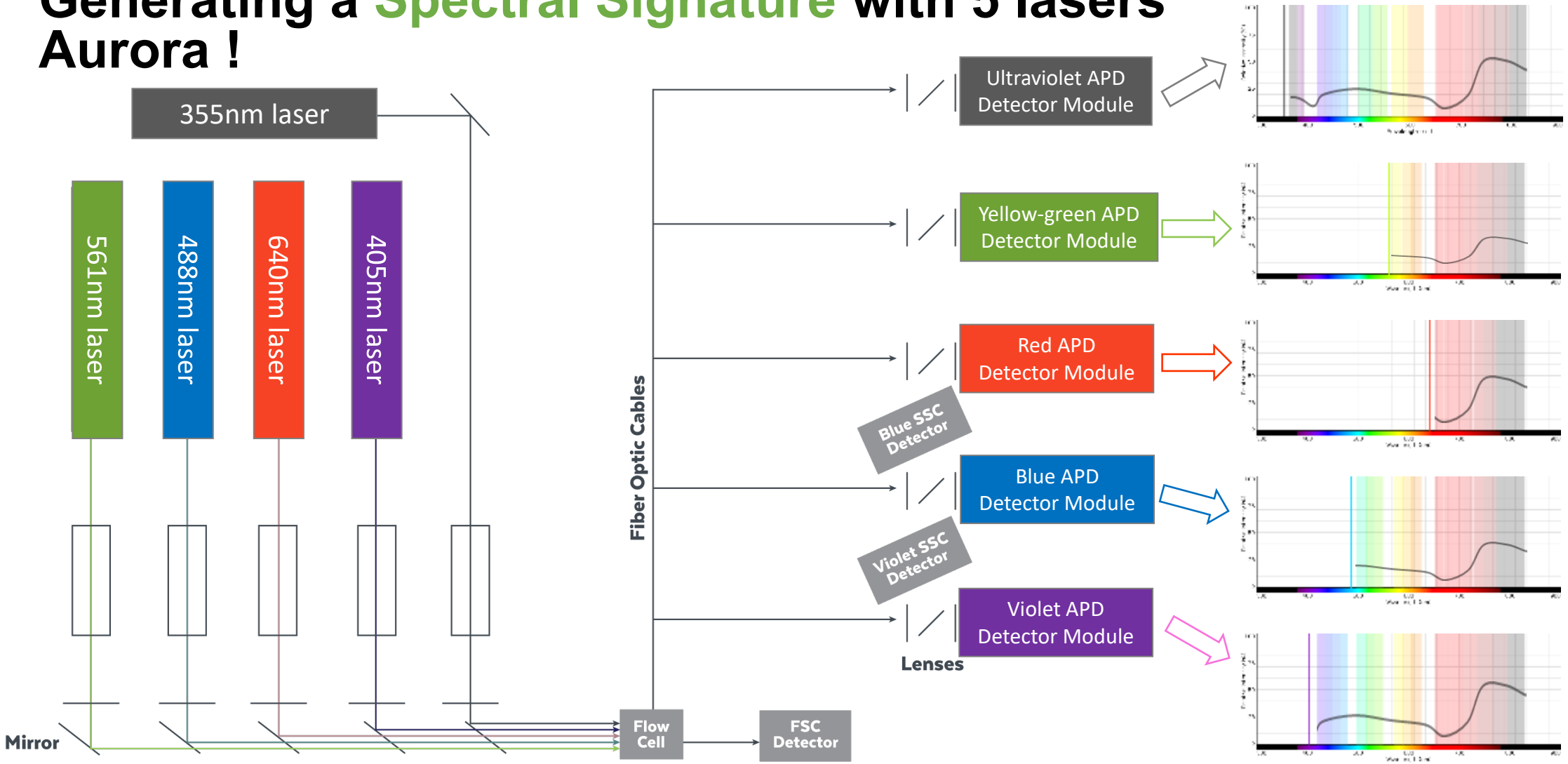
Northern Light and Aurora : Spectral flowcytometers

From 1 Laser to 3 Lasers - up to 41 parameters :
Blue, Blue Red,
Blue Red Violet

From 3 Lasers to 5 Lasers - up to 67 parameters :
Blue Red Violet, Blue Red Violet Yellow, Blue Red Violet UV, Blue Red Violet Yellow UV

<up to 70 nm detection in size

Generating a Spectral Signature with 5 lasers Aurora !



The 5 laser Aurora!

Laser	Channel	Center Wavelength (nm)	Bandwidth (nm)	Wavelength Start (nm)	Wavelength End (nm)
Ultraviolet	UV1	372	15	365	380
	UV2	387	15	380	395
	UV3	427	15	420	435
	UV4	443	15	435	450
	UV5	458	15	450	480
	UV6	473	15	465	480
	UV7	514	28	500	528
	UV8	542	28	528	556
	UV9	581	31	566	597
	UV10	612	31	597	628
	UV11	664	27	650	677
	UV12	691	28	677	705
	UV13	720	29	705	734
	UV14	750	30	735	765
	UV15	780	30	765	795
	UV16	812	34	795	829
Violet	V1	428	15	420	435
	V2	443	15	436	451
	V3	458	15	451	466
	V4	473	15	466	481
	V5	508	20	498	518
	V6	525	17	516	533
	V7	542	17	533	550
	V8	581	19	571	590
	V9	598	20	588	608
	V10	615	20	605	625
	V11	664	27	651	678
	V12	692	28	678	706
	V13	720	29	706	735
	V14	750	30	735	765
	V15	780	30	765	795
	V16	812	34	795	829

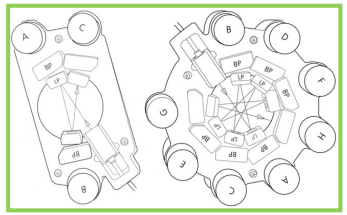
Laser	Channel	Center Wavelength (nm)	Bandwidth (nm)	Wavelength Start (nm)	Wavelength End (nm)
Blue	B1	508	20	498	518
	B2	525	17	516	533
	B3	542	17	533	550
	B4	581	19	571	590
	B5	598	20	588	608
	B6	615	20	605	625
	B7	660	17	652	669
	B8	678	18	669	687
	B9	697	19	688	707
	B10	717	20	707	727
	B11	738	21	728	749
	B12	760	23	749	772
	B13	783	23	772	795
	B14	812	34	795	829
Yellow Green	YG1	577	20	567	587
	YG2	598	20	588	608
	YG3	615	20	605	625
	YG4	660	17	652	669
	YG5	678	18	669	687
	YG6	697	19	688	707
	YG7	720	29	706	735
	YG8	750	30	735	765
	YG9	780	30	765	795
	YG10	812	34	795	829
Red	R1	660	17	652	669
	R2	678	18	669	687
	R3	697	19	688	707
	R4	717	20	707	727
	R5	738	21	728	749
	R6	760	23	749	772
	R7	783	23	772	795
	R8	812	34	795	829

UV16+ V16 + B14 + YG10 + R8 = 64 Detectors

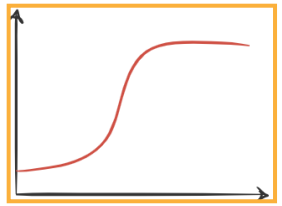
Intuitive and familiar workflow & Cytek Cloud

Conventional Cytometer

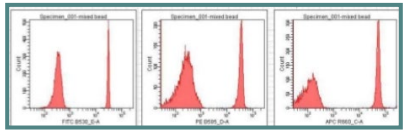
Confirm panel can be run with existing optical configuration



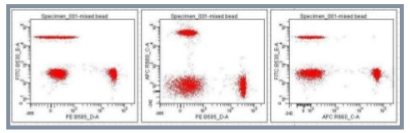
Voltage optimization



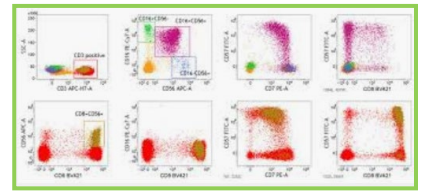
Collect unstained and **compensation controls**



Calculate **compensation**



Acquire **compensated** multicolor data

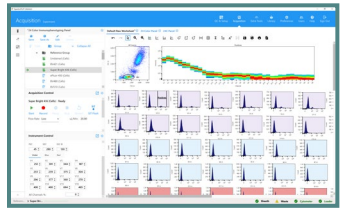


Cytek® Aurora

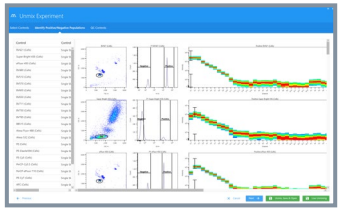
One configuration for all assays

Pre-optimized Cytek Assay Settings

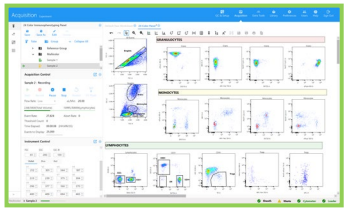
Collect unstained and **reference controls**



Calculate **unmixing** (using wizard)



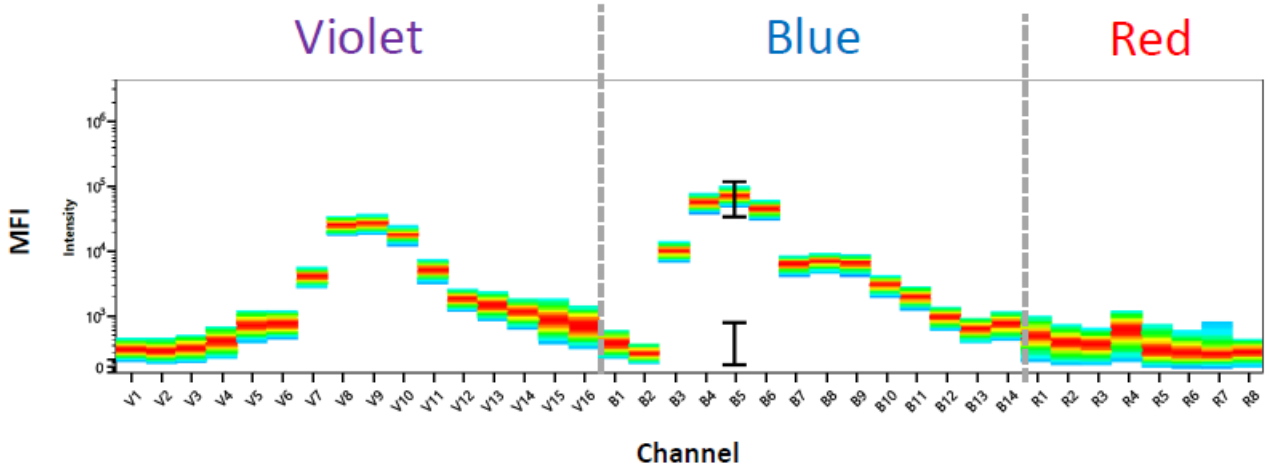
Acquire **live unmixed** multicolor data



Cytek spectral technology

Full spectrum analysis

•Entire emission spectrum is captured across the different modules and then stitched together to create a spectral signature that combines emission information from all three excitation wavelengths



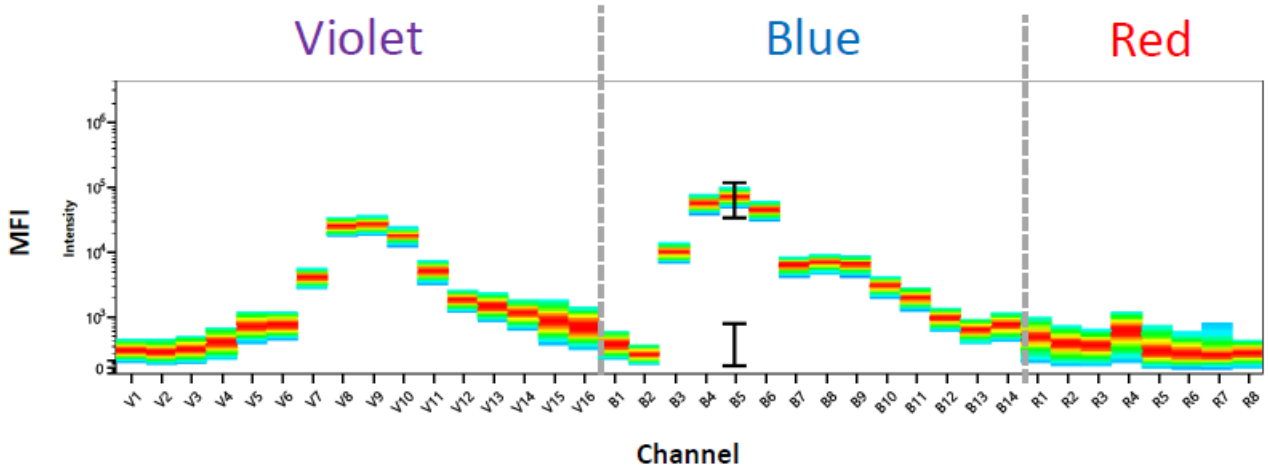
PE full spectrum

Cytek spectral technology

Spectral unmixing

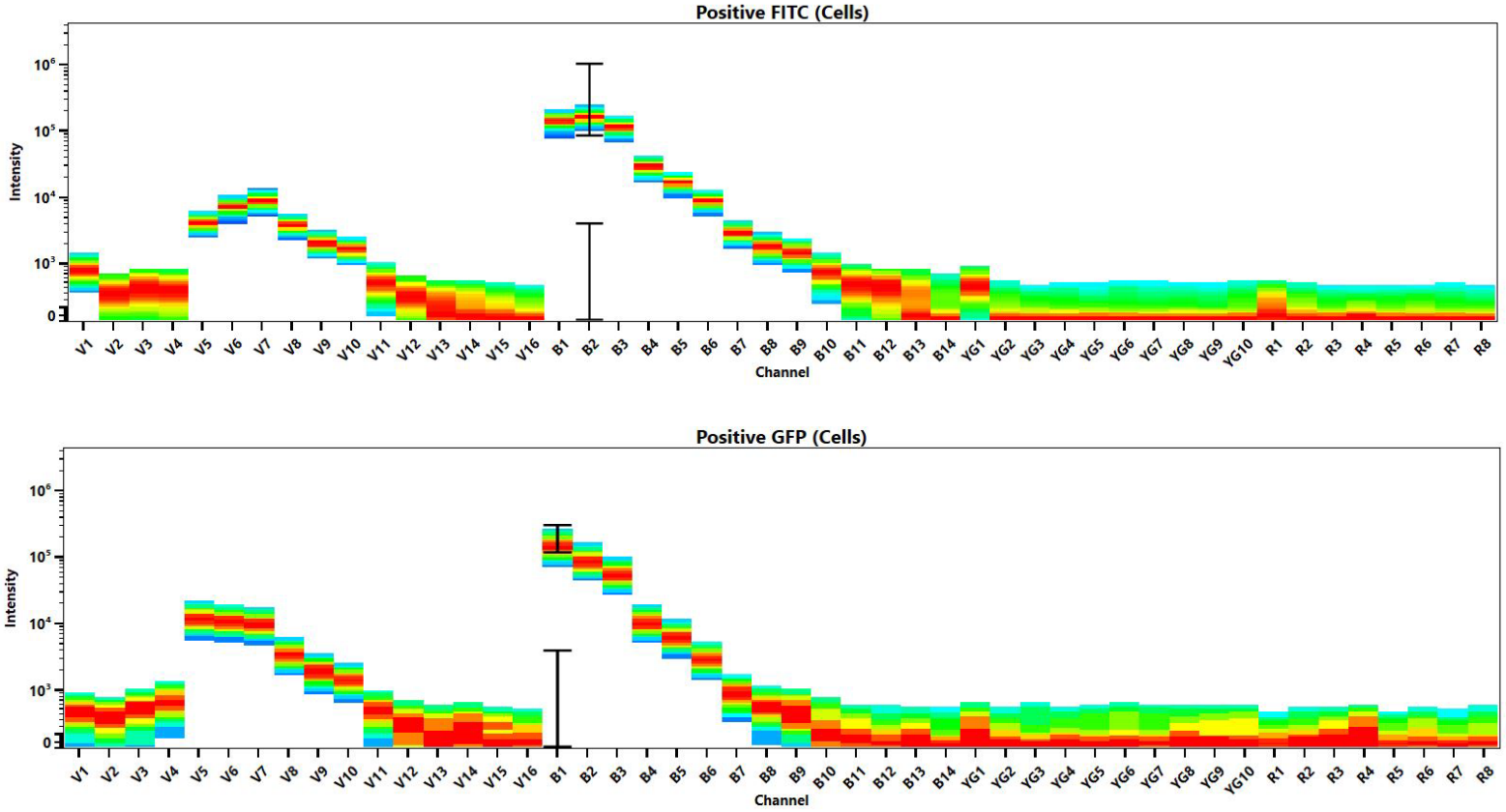
Spectral unmixing algorithms calculate the contribution of each known fluorophore's spectra to the total collected emission signal

In spectral cytometry, unmixing is used for the decomposition of fluorescent components in a multicolor sample using spectral reference controls as standards



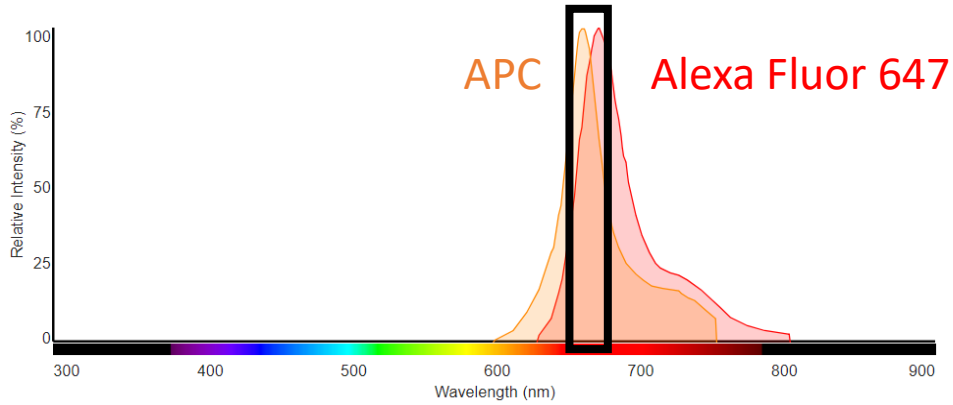
PE full spectrum

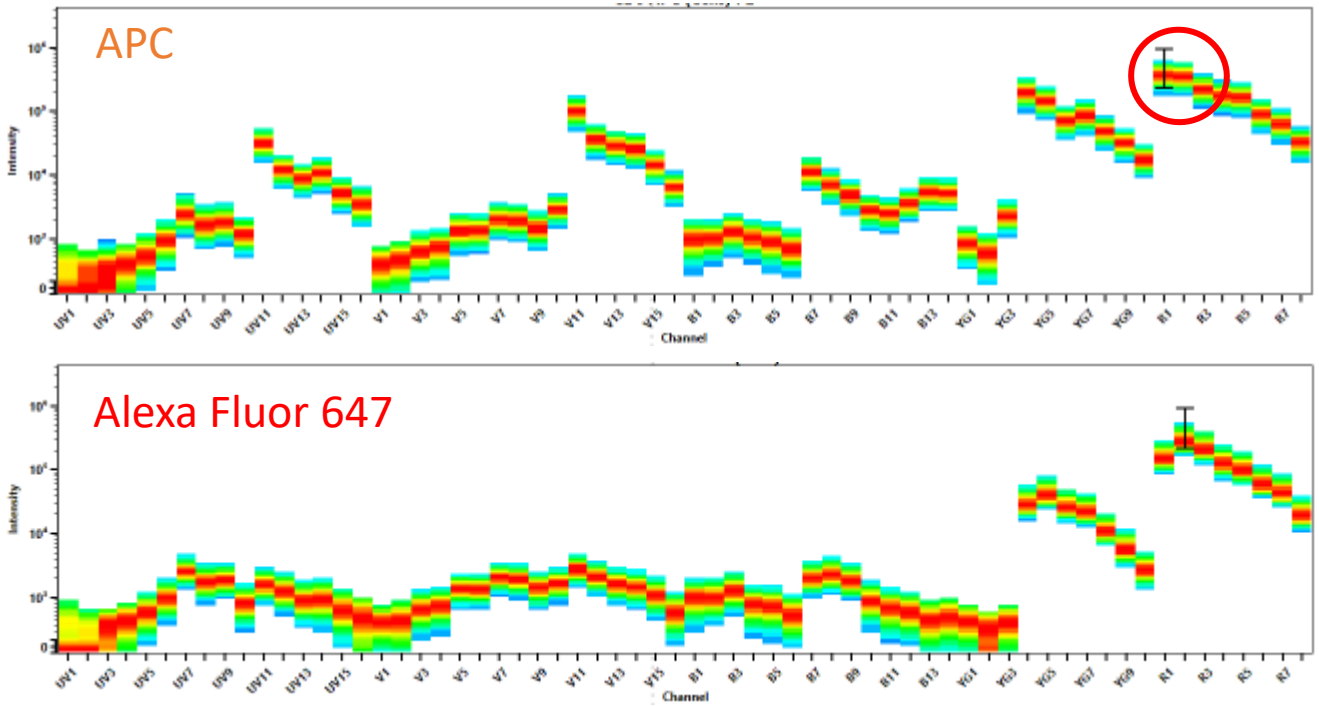
Effectively use Highly Overlapping Dyes Together, Even on Co-Expressed Markers



Figures shows the uniqueness of the spectral signatures of FITC (with emission peak on B2 channel), and GFP (with emission peak on B1 channels).

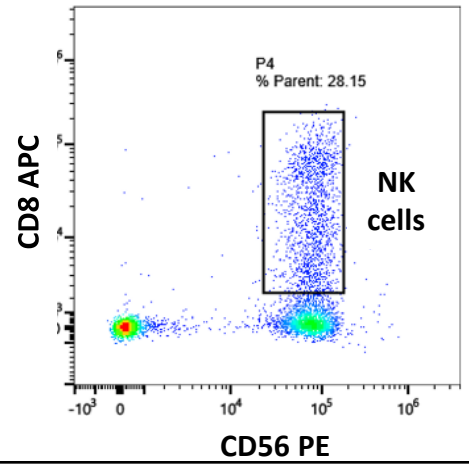
Unique spectrum allows the unmixing algorithm to assign photons to the right channel.

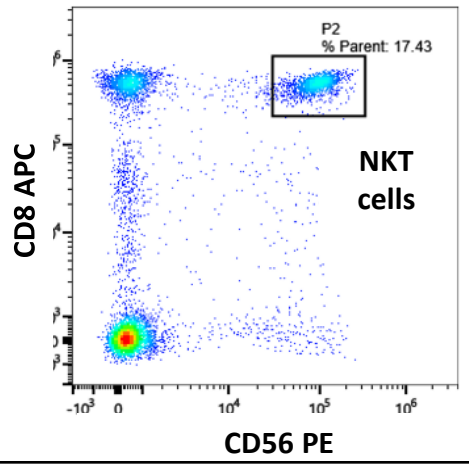




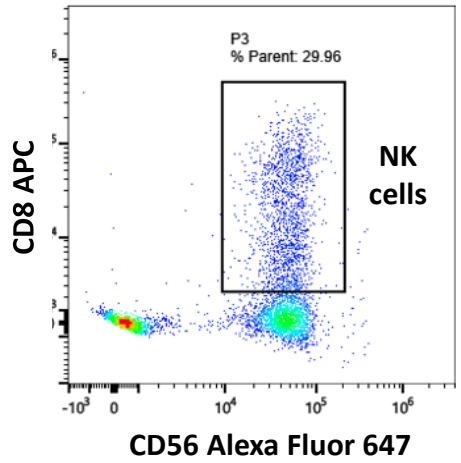
Gated on Lymphs, CD3-

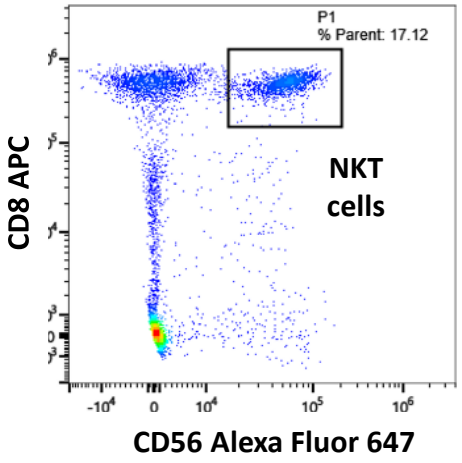
Gated on Lymphs, CD3+





Control





Test

Autofluorescence Extraction Improves Resolution for Fluorochromes in Highly Autofluorescent Regions

- Autofluorescence (AF) is light emitted by cells, depending on the cell's size, granularity, metabolic state, and even experimental conditions.
- With Cytek's Full Spectrum Profiling™ (FSP™) technology, AF is easily measured and can be treated like any other single color fluorescent control in a multicolor assay
- Extracting AF from highly autofluorescent samples can improve data clarity for large immunology panels
- Moreover, you can compare and characterize AF characteristics across cell types, microorganism types, and more using the NEW AF Explorer tool.

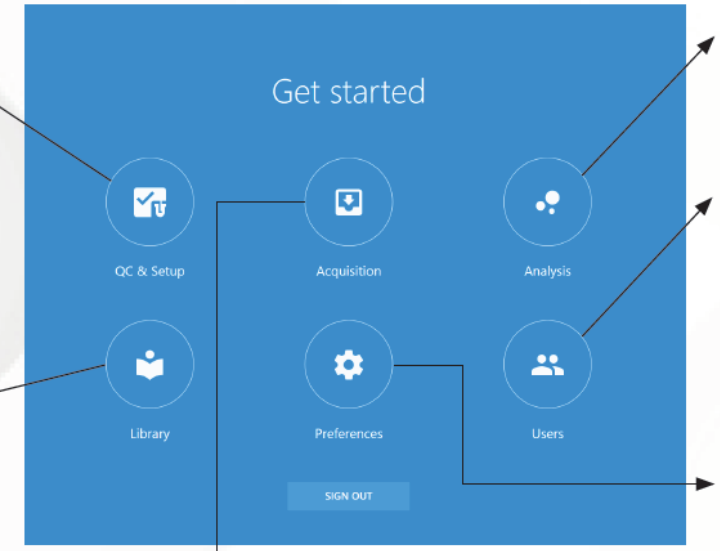
Spectroflo software guided workflows

QC and Setup:

Run Daily QC to monitor instrument performance and add reference controls. Controls are normalized during QC so you can reuse them. Minimize the number of reference controls by using the built-in reference library.

Library:

Add or remove experiment templates, worksheet templates, fluorochrome information, QC bead information, and more.



Analysis:

Unmix data and view sample spectra.

Users:

For administrative controls.

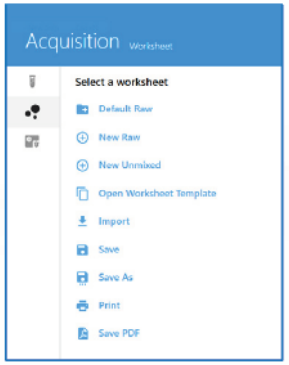
Preferences:

Customize your software appearance. Set default plot sizes, text sizes and fonts, gate colors, print layout, statistics table options, and more.

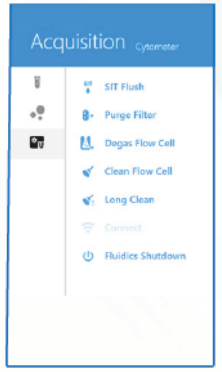
Experiment Workflow:

From the Acquisition menu, you can start a new experiment and get to your data in three simple guided steps.

Experiment Menu

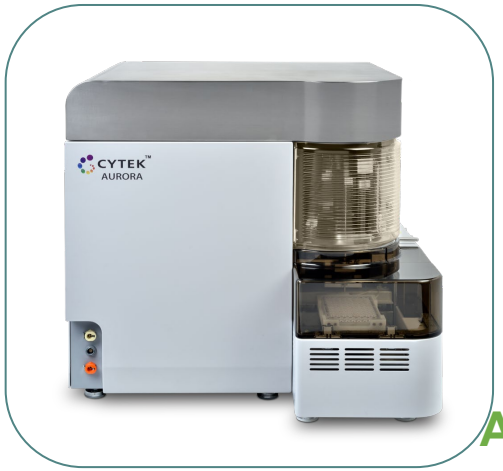


Analysis Worksheet Menu

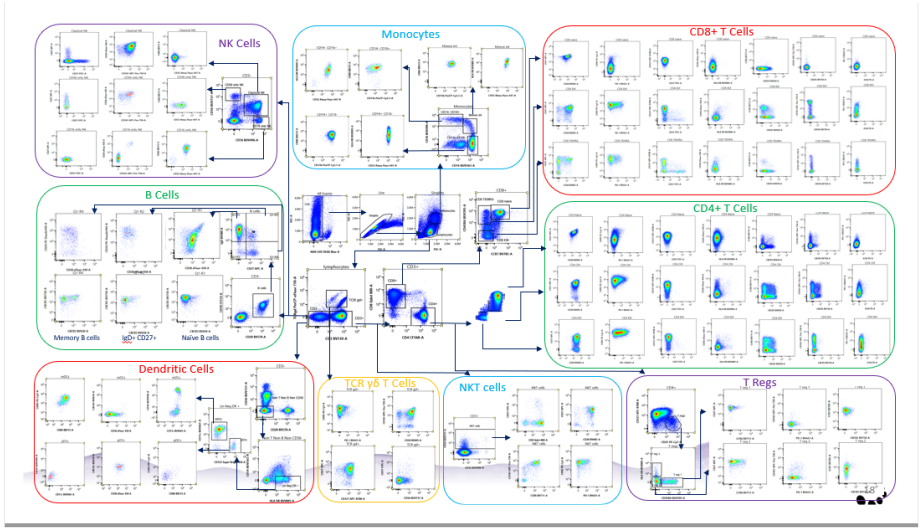


Fluidics Menu

Imagine this…A Cytek Aurora which can sort !



Assay Reproducibility – Assay Transferability



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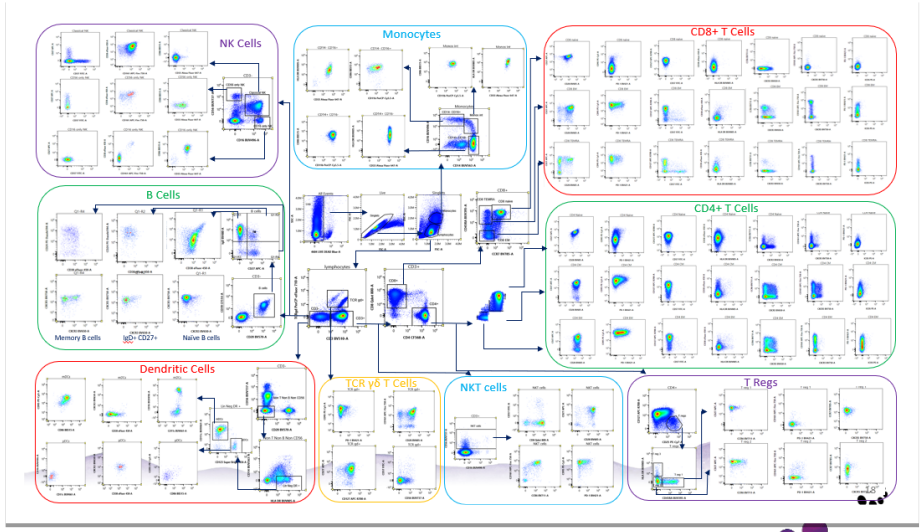

Sensitivity of detection

All Cytek instruments offer possibility of particle detection from 70nm and sensitivity to fluorescence:

FITC=< 5 MESF

PE=< 4 MESF

APC=< 3 MESF

50 Shades of Fluorescence to Follow Immune Response (Saffir) with spectral cytometry.

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CNRS UMR 7280, Inserm U1104, AMU-UM2.
** Cytek Biosciences

Introduction

Whooping cough persists as an endemic disease (40million cases and 300000 child death per year worldwide) even in vaccinated populations. This disease is caused by the gram(-) bacterium *Bordetella pertussis*. This bacteria expresses multiple virulence factors acting in concert to facilitate its adherence, survival and proliferation in human respiratory tract. More, it has been shown, in a mouse model, that $CD4^+$ Trm are generated during the time course of infection[1]. Our objectives is to decipher the cellular mechanisms underlying the "cross-talk" between antigen presenting cells and T cells that allow the differentiation, maintenance and function of Trm at the tissue level. To do this aim we developped a 48 markers panel to follow the behavior of several immune populations in the mean time in the same tube.

Methods

- Mice were infected with a wild strain of Bordetella pertussis (Tahoma I). 15 days later they were sacrificed. After intra-cardiac perfusion with PBS, collected lungs were digested with a mix of Collagenase IV/DNAse I for 30 mn at 37°C. Lung cells were seeded at $3x10^6$ cells/well and stained in PBS, 2mM EDTA, 0,5% BSA, 40% brilliant violet stain buffer(BD Biosciences) and 10% Fc block at 37°C for 45mn. Cells washed 3 times before staining in PBS + live/dead marker (Zombie NIR) for 15mn. After 3 washes cells were resuspended in PBS for analysis on Spectral Cytometer Aurora (Cytek Biosciences).
- Data analysis were performed using OMIQ.

ANTIGEN	CLONE	ANTIGEN	CLONE	ANTIGEN	CLONE	ANTIGEN	CLONE	ANTIGEN	CLONE
CD3	17A2	CD25	PC61	CD69	H1.2F3	CD196	29-2L17	Ly6G	1A8
CD4	GK1.5	CD26	H194-112	CD88	20/70	B220	RA3-6B2	MHC-II	M5/114.15.2
CD5	FAB115U	CD27	LG3A10	CD90-2	30H12	BST2	927	NK1.1	PK136
CD8a	53-6.7	CD44	IM7	CD103	2 E7	CX3CR1	SA011F11	NKp46	29A1.4
CD8b	53-5.8	CD45	30F11	CD115	afs98	EpCAM	G8.8	PD-1	29F1A12
CD11b	M1/70	CD49a	HA31/8	CD138	281-2	F4/80	BM8	Siglec F	E50-2440
CD11c	N418	CD49b	HMa2	CD154	MR1	IgM	RMM-1	XCR1	ZET
CD19	1D8	CD49d	PS/2	CD172a	P84	IgD	11-26c.2a	γδ TCR	GL3
CD23	B3B4	CD62L	MEL-14	CD183	CXCR3-183	Klrg1	2F1	Live/ Dead	Zombie NIR
CD24	M1/69	CD64	X54-5/7.1	CD192	SA203G11	Ly6C	HK1.4	AutoFluo	

THANK YOU FOR YOUR ATTENTION

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