

# The URMCM Flow Cytometry Resource: Bonus Mass and Full Spectrum Cytometry Discussion

The logo for URMCM Shared Resource Laboratories features a cluster of approximately 15 circles of varying sizes and colors (white, grey, orange, green) arranged in a roughly circular pattern on the left side of the slide.

URMC  
Shared Resource  
Laboratories

URMC Flow Cytometry Core

Matt Cochran, Technical Director

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# Outline

## Introduction to URM C Flow Cytometry Core

- The team
- Services
- Instrumentation

## Introduction to Cytex Aurora

## Introduction to the Helios Mass Cytometer

# Our Team

## Leadership Group

- Tim Bushnell, Ph.D. Scientific Director
- Matt Cochran, Technical Director
- Wojciech Wojciechowski, Development Director
- James Java, Data Analytics

## Seven full time instrumentation/project specialists\*

- Jeffrey Capomaccio
- Justin Cobb (not pictured)
- Kate Fegan
- Meghann O'Brien
- Steven Polter
- Taylor Waldrop
- Terry Wightman



## Administration etc.

- Sharleen Slaunwhite
- Beth Laffey

# Support and Services - General

Consultation (office hrs: Zoom as requested)

- Experiment/Panel design
- Data interpretation
- Sorting strategy/setup

Instrument/Software assistance

- Slack on all computers – monitored during normal business hours
- Full remote software access/control

Data analysis

- Both Flowjo and FCS Express licenses are available
  - Information, practice data on website and FCC\_Library
- High dimensional analysis help is also available

Continuing Education

- FCC\_Library share
- Occasional seminars, lectures, and demos

# Support and Services - Data

## Analysis Computers

- PC workstation in 3-4151
  - Multiple analysis programs: ISX, Celigo, Nanosight, Flowjo
- Separate dedicated workstation for full spectrum (Aurora) analysis
  - Remote access only at this time.

## Data archiving and transfer

- FCC archives experimental data
  - Code42 automated archiving
    - Backs up every 10 minutes
    - Saved indefinitely
    - Files can be retrieved upon request (Instrument used, Exp Title, Date run)
- FCC\_Transfer provides a space for moving data from cytometers to lab
  - Not for long term storage. Space is cleared once a month.
  - Box is accessible as an alternative/backup

# Support and Services – Communication/Scheduling

Website: <http://www.urmc.rochester.edu/flow-core>

- Policies and overview – not very dynamic
- Instrument pages for all equipment
- Library contains links and useful information
- Recent updates
  - Cell sorting page overhaul
  - FAQ added to the Library page
  - Data analysis page in progress under Services.

## PPMS

- Shared between all SRLs
  - Toggle between accounts easily
- Recently updated
  - Better control/flexibility for accounting
  - New “Edit” button for existing reservations!
  - Instrument sign in page will be updated as well

## Listserve

# Instruments – Traditional Analytical

BD Accuri C6+ (Pepe)



- 2 lasers
- 4 fluorescent parameters
- Strengths: ease of use, volumetric acquisition
- Weaknesses: inflexible



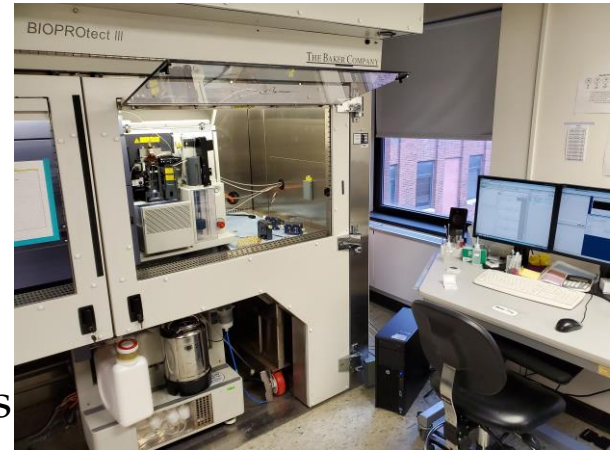
LSRII/LSRFortessa (Fozzie, Oscar, Animal, Dr. Teeth)

- 4 lasers (LSRII), 5 lasers (Fortessa)
- 18 Fluorescent parameters
- Strengths: Flexibility (fluidics and fluorescence), availability/redundancy, institutional knowledge
- Weaknesses: Aging technology (Kermit is almost 17!)



# Instruments – Cell Sorting

## BD FACSAriaII (Statler, Waldorf)



- 4 lasers
- 18 fluorescence parameters – matched to the LSRIIs
- Strengths: Flexibility\* (Fluorescence, speed, collection, setup)
- Weaknesses: Complicated, finicky



## BioRad S3e (Scooter)



- 2 lasers
- 4 fluorescent parameters
- Strengths: relative simplicity, automated control
- Weaknesses: single nozzle size, no plate sorting, automated control



# Instruments - Imaging

## Luminex Image StreamX (Sam the Eagle)

- Imaging flow cytometer
- 4 lasers
- 9-10 fluorescent parameter
- Strengths: Best of both worlds, multiple magnifications, sub-micron resolution
- Weaknesses: throughput, aging technology, EOL



## Nexcelom Celigo S (Stinky the Stinkweed)

- Plate based high throughput imaging
- Brightfield plus 3 fluorescent parameters
- Strengths: speed, ease of use, kits and established assays
- Weaknesses: somewhat inflexible, moderate resolution



# Instruments – Flow Adjacent

## Agilent Seahorse XFe96 (Lew Zealand)

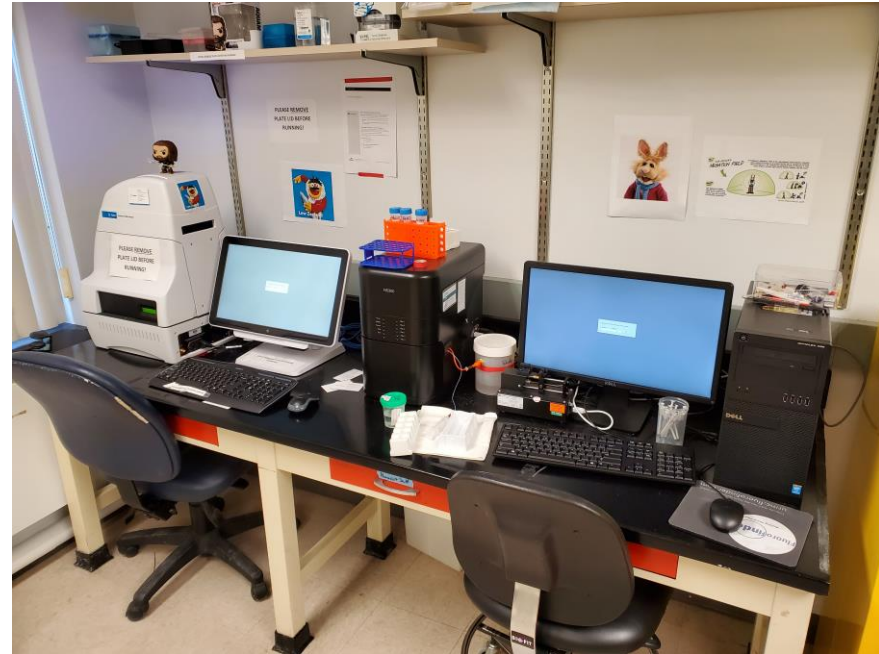


- Cellular metabolomics
  - Measures changes in O<sub>2</sub> and pH
- Strengths: ease of use, high sensitivity
- Weaknesses: cell numbers

## Malvern Nanosight NS300 (Bean Bunny)



- Small particle analysis
  - ~500nM down to ~20nM
  - Sizing and counting
  - Limited fluorescence capabilities
- Strengths: ease of use, broad size and concentration ranges, sample input flexibility
- Weaknesses: fluorescence limitations, “black box”



# FULL SPECTRUM CYTOMETRY CYTEK AURORA

Introduced in January 2020 just in time for everything to fall apart

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# What is Full Spectrum Cytometry

- Introduced by Cytex in 2017 at the annual Cyto meeting and took off
- Commercialized by Sony 2013 (SP6800) but didn't catch on.
- Original work stretches back to 1979 (Wade et. al.)

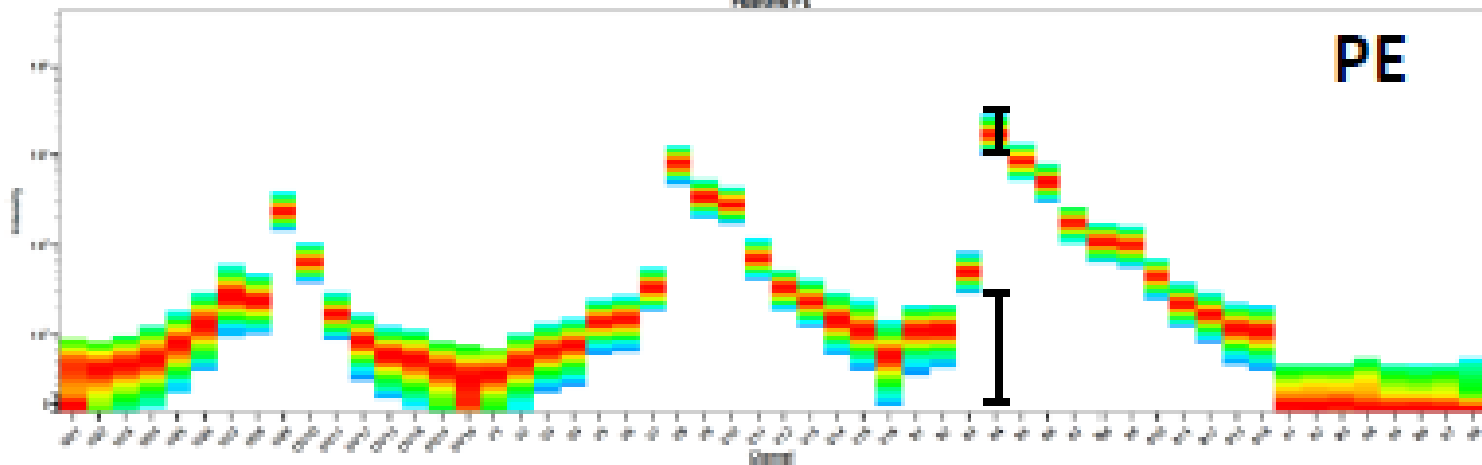
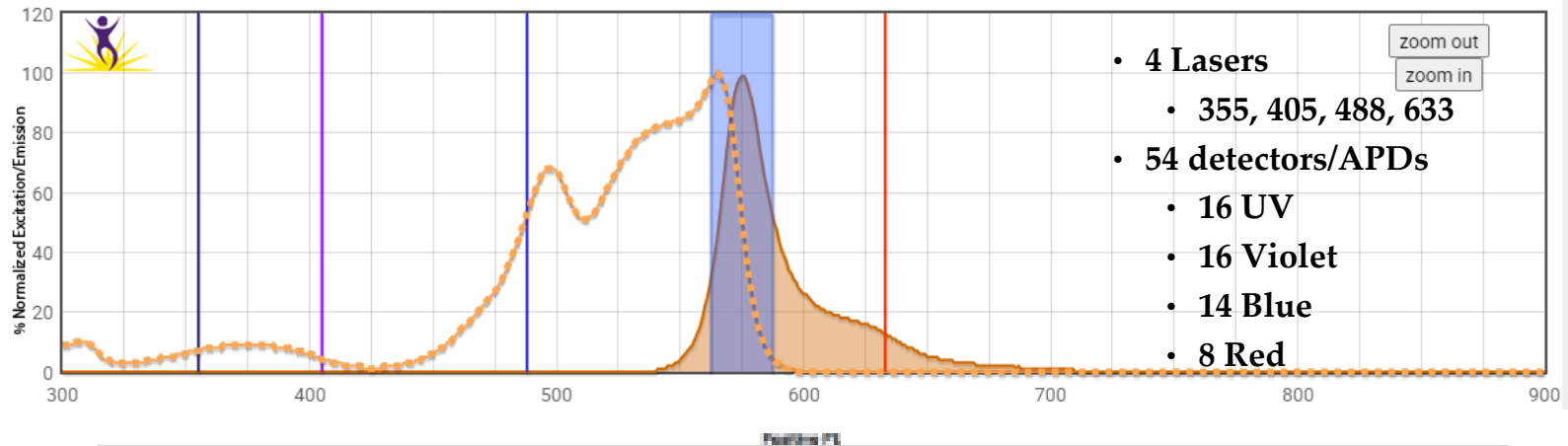


- Whats the big idea?
  - What if, instead of trying to pinpoint the best fluor for each filter/detector set, we measure “everything” and use all that information?

# Enter the Spectral Signature

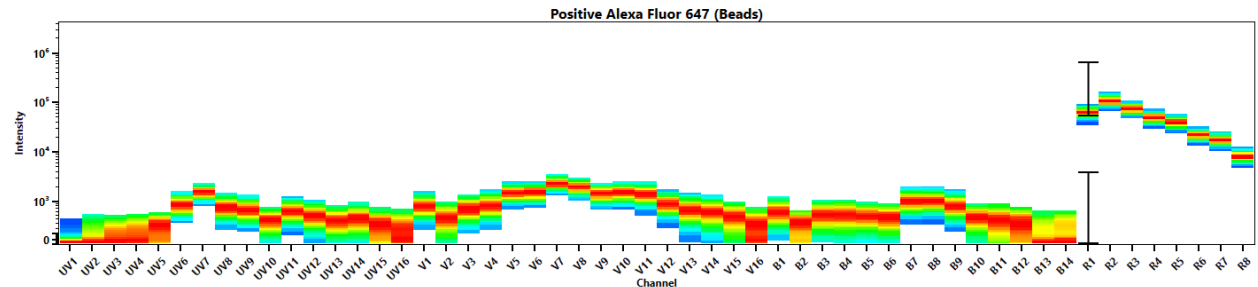
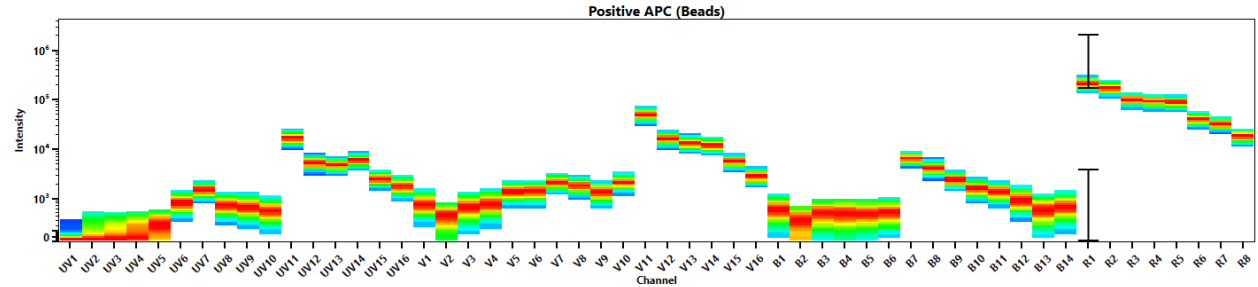
➤ Measure from ~370nm – 810nm for everything.

## Fluorescence Spectra Analyzer

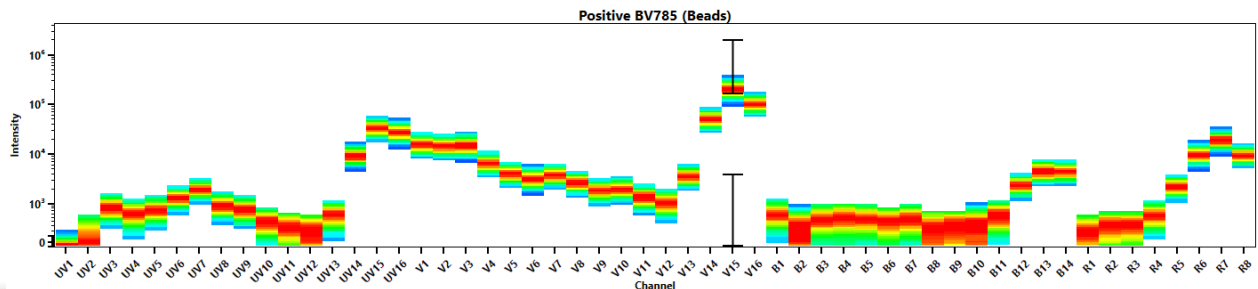
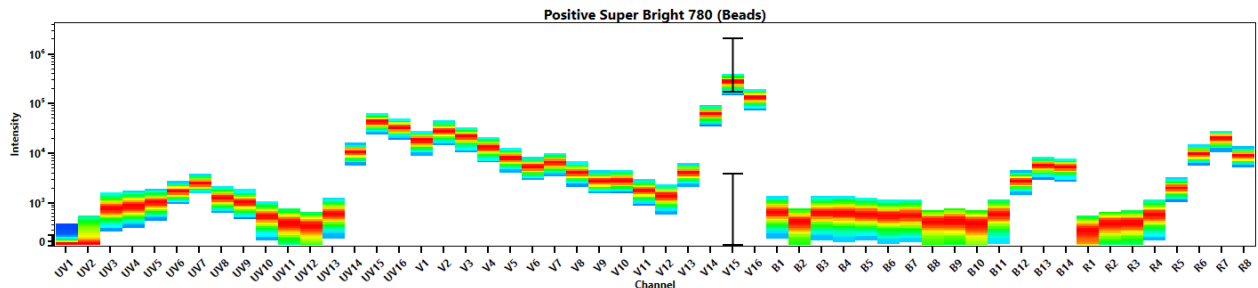


# Spectral Signature Interpretation

- Comparing APC and Alexa647
  - Similarity 0.93



- Comparing SB780 and BV785
  - Similarity 1.0



# Spectral Signature Interpretation

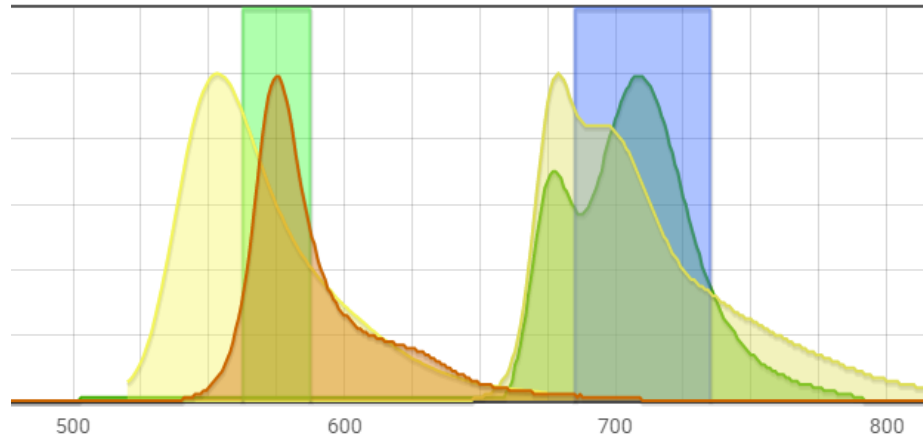
	Color/Format
1	<b>BUV395</b>
2	<b>BUV496</b>
3	<b>BUV563</b>
4	<b>BUV661</b>
5	<b>BUV737</b>
6	<b>Brilliant Violet 421</b>
7	<b>VioBlue</b>
8	<b>Brilliant Violet 480</b>
9	<b>Brilliant Violet 605</b>
10	<b>Live/Dead Fix Red</b>
11	<b>Brilliant Violet 650</b>
12	<b>Brilliant Violet 711</b>
13	<b>Brilliant Violet 750</b>
14	<b>SuperBright 780</b>
15	<b>BB515</b>
16	<b>Alexa Fluor™ 532</b>
17	<b>PE</b>
18	<b>PE-Dazzle 594</b>
19	<b>PE-Cy5</b>
20	<b>PerCP-eFluor 710</b>
21	<b>PE-Cy7</b>
22	<b>APC</b>
23	<b>Alexa647</b>
24	<b>APC-R700</b>
25	<b>APC-eFluor 780</b>

	BUV395	BUV496	BUV563	BUV661	BUV737	BV421	Vio Blue	BV480	BV605	BV650	BV711	BV750	Super Bright 780	BB515	Alexa 532	PE	PE-Dazzle594	LIVE DEAD Red	PE-Cy5	PerCP-eFluor 710	PE-Cy7	APC	Alexa Fluor 647	APC-R700	APC-eFluor 780
BV421	0.07	0.09	0.02	0.01	0.01	1	0.8	0.28	0.08	0.12	0.11	0.08	0.12	0	0.01	0.01	0.01	0.01	0	0	0	0	0	0	0
Vio Blue	0.04	0.14	0.03	0.01	0.01	0.8	1	0.59	0.1	0.11	0.1	0.07	0.12	0.01	0.02	0.03	0.02	0.04	0.01	0.01	0	0.01	0.01	0.01	0.01
BV480	0.08	0.41	0.11	0.02	0.01	0.28	0.59	1	0.17	0.08	0.05	0.04	0.06	0.07	0.06	0.11	0.06	0.11	0.01	0.01	0.01	0.01	0.02	0.01	0.01
BV605	0.02	0.08	0.17	0.15	0.06	0.08	0.1	0.17	1	0.57	0.19	0.13	0.09	0.01	0.11	0.23	0.39	0.84	0.1	0.12	0.03	0.08	0.02	0.03	0.02
BV650	0.02	0.03	0.04	0.44	0.15	0.12	0.11	0.08	0.57	1	0.46	0.25	0.17	0	0.03	0.06	0.18	0.43	0.26	0.3	0.04	0.36	0.21	0.18	0.08
BV711	0.01	0.02	0.01	0.3	0.42	0.11	0.1	0.05	0.19	0.46	1	0.69	0.48	0	0.01	0.02	0.06	0.13	0.21	0.69	0.15	0.26	0.23	0.46	0.23
BV750	0.01	0.02	0.01	0.12	0.37	0.08	0.07	0.04	0.13	0.25	0.69	1	0.82	0	0.01	0.02	0.04	0.08	0.08	0.42	0.23	0.08	0.04	0.13	0.2
Super Bright 780	0.01	0.02	0.01	0.06	0.2	0.12	0.12	0.06	0.09	0.17	0.48	0.82	1	0	0.01	0.01	0.02	0.05	0.04	0.27	0.25	0.04	0.02	0.07	0.2
BB515	0.01	0.08	0.05	0	0	0	0.01	0.07	0.01	0	0	0	0	1	0.25	0.1	0.05	0.02	0.01	0	0	0	0	0	0
Alexa 532	0.01	0.08	0.33	0.02	0.01	0.01	0.02	0.06	0.11	0.03	0.01	0.01	0.01	0.25	1	0.88	0.57	0.32	0.16	0.06	0.03	0.01	0.01	0	0.01
PE	0.02	0.06	0.29	0.02	0.01	0.01	0.03	0.11	0.23	0.06	0.02	0.02	0.01	0.1	0.88	1	0.48	0.33	0.11	0.04	0.02	0.01	0.01	0.01	0.01
PE-Dazzle594	0.01	0.03	0.14	0.05	0.03	0.01	0.02	0.06	0.39	0.18	0.06	0.04	0.02	0.05	0.57	0.48	1	0.77	0.41	0.19	0.06	0.03	0.01	0.01	0.01
LIVE DEAD Red	0.02	0.06	0.17	0.17	0.07	0.01	0.04	0.11	0.84	0.43	0.13	0.08	0.05	0.02	0.32	0.33	0.77	1	0.27	0.16	0.04	0.12	0.09	0.06	0.03
PE-Cy5	0	0.01	0.03	0.36	0.16	0	0.01	0.01	0.1	0.26	0.21	0.08	0.04	0.01	0.16	0.11	0.41	0.27	1	0.53	0.14	0.41	0.4	0.27	0.1
PerCP-eFluor 710	0.01	0.01	0.02	0.26	0.38	0	0.01	0.01	0.12	0.3	0.69	0.42	0.27	0	0.06	0.04	0.19	0.16	0.53	1	0.36	0.25	0.24	0.45	0.2
PE-Cy7	0	0	0.01	0.03	0.2	0	0	0.01	0.03	0.04	0.15	0.23	0.25	0	0.03	0.02	0.06	0.04	0.14	0.36	1	0.03	0.03	0.07	0.19
APC	0	0.01	0.01	0.77	0.21	0	0.01	0.01	0.08	0.36	0.26	0.08	0.04	0	0.01	0.01	0.03	0.12	0.41	0.25	0.03	1	0.93	0.56	0.23
Alexa Fluor 647	0	0.01	0.01	0.71	0.19	0	0.01	0.02	0.02	0.21	0.23	0.04	0.02	0	0.01	0.01	0.01	0.09	0.4	0.24	0.03	0.93	1	0.64	0.23
APC-R700	0	0	0	0.49	0.4	0	0.01	0.01	0.03	0.18	0.46	0.13	0.07	0	0	0.01	0.01	0.06	0.27	0.45	0.07	0.56	0.64	1	0.36
APC-eFluor 780	0	0.01	0.01	0.17	0.29	0	0.01	0.01	0.02	0.08	0.23	0.2	0.2	0	0.01	0.01	0.01	0.03	0.1	0.2	0.19	0.23	0.23	0.36	1

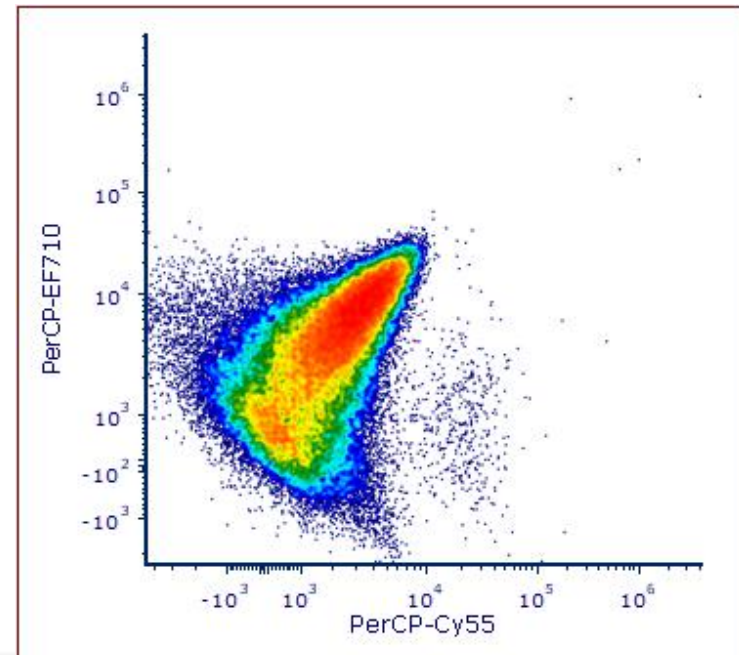
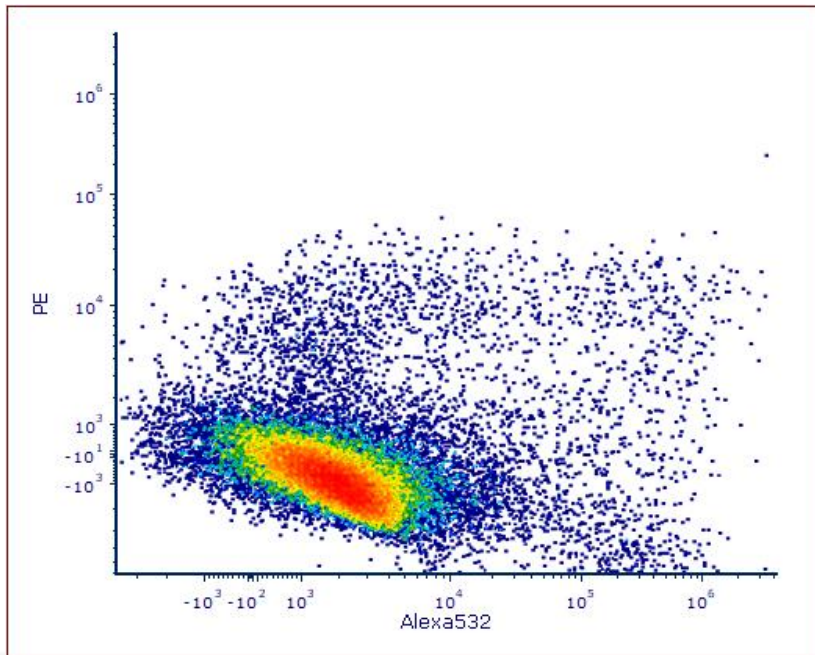
Complexity Index: 16.98

# Impossible Fluorochrome Combinations

➤ Alexa 532 and PE



➤ PerCP-Cy55 and PerCP-eFluor710





# Full Spectrum Strengths and Weaknesses

## Constantine - Strengths

- Sensitivity
- Standardization – Cytex assay settings
  - APDs with flat top lasers
- Autofluorescence as a parameter
- Small particle detection
- Plate loader and volumetric
- Resources – customer service
- Familiarity

## Weaknesses

- New
- No redundancy
- Slow warmup
- ????



# MASS CYTOMETRY FLUIDIGM HELIOS

Recently replaced the CyTOF 1.5 in January 2021

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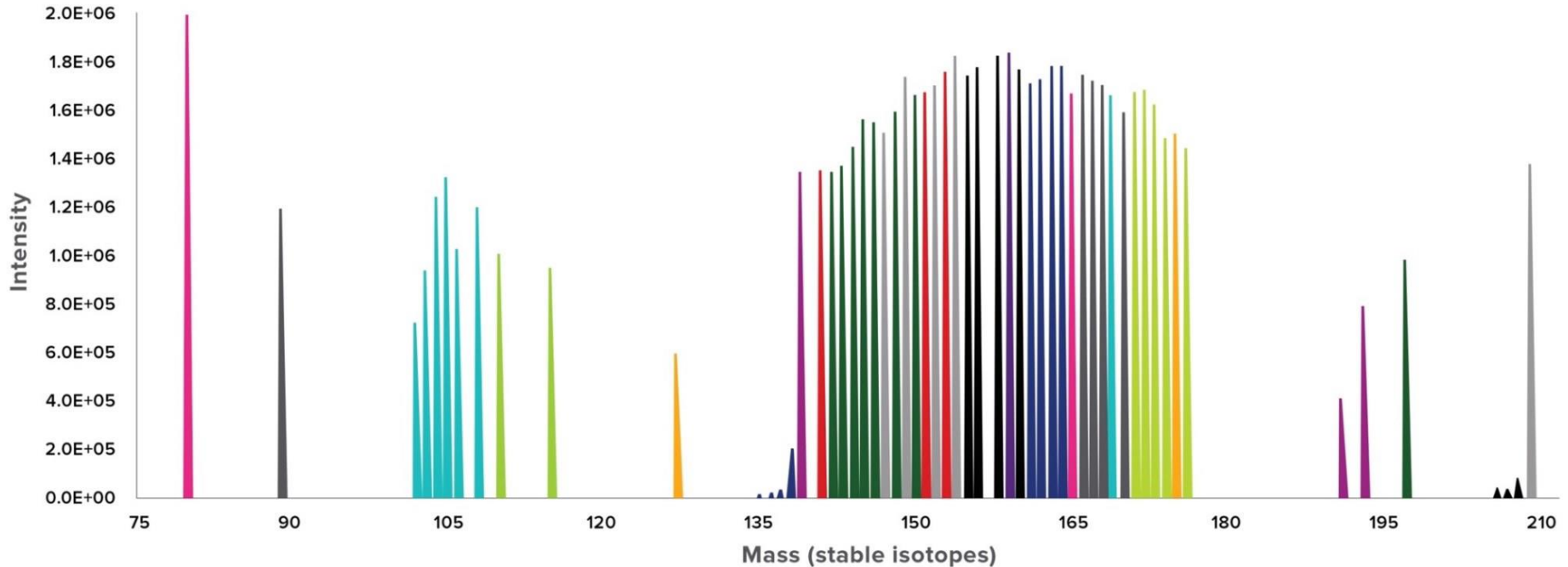


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# Evolution of the CyTOF

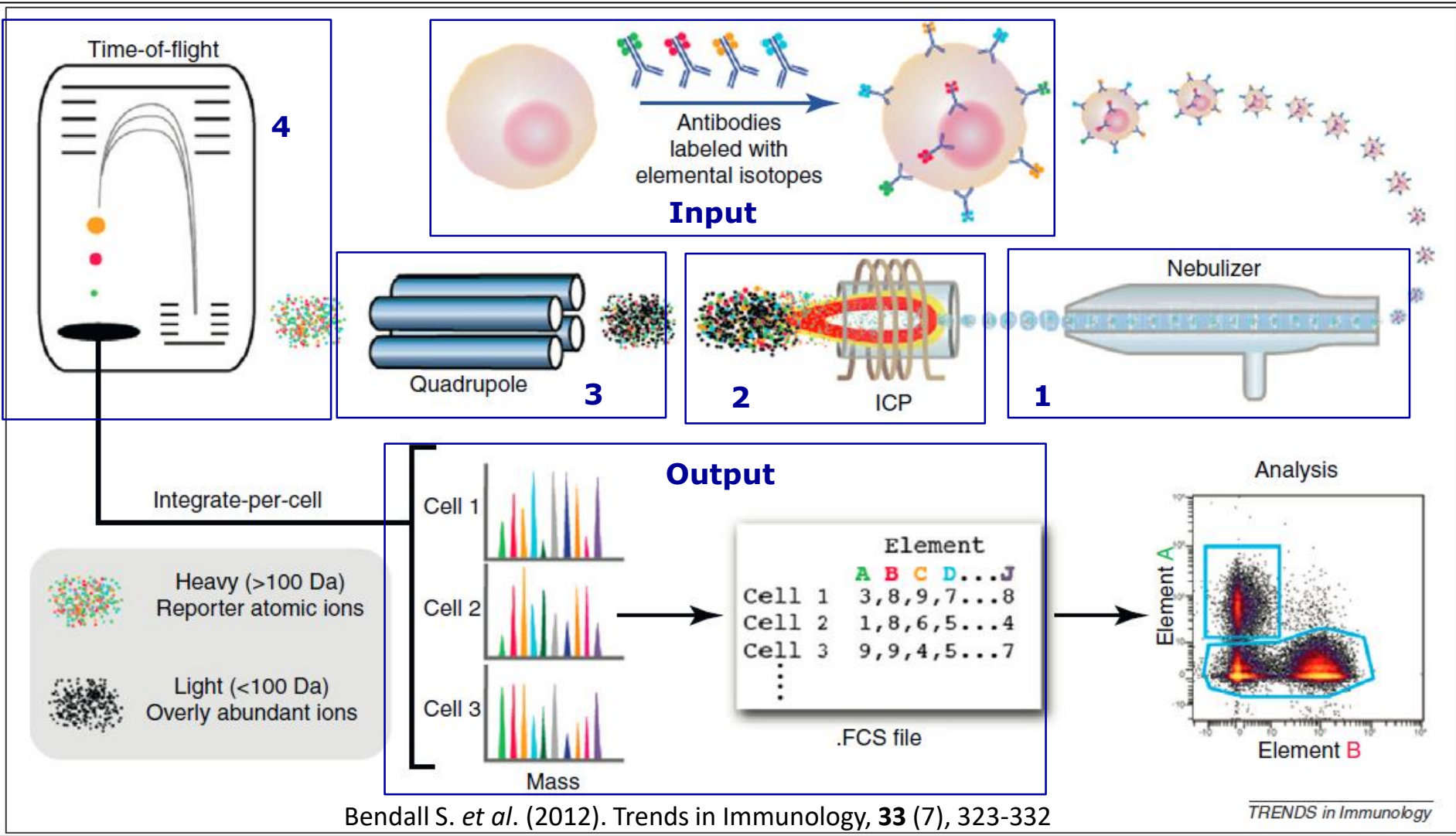
# CyTOF 'Spectrum'



135 channels (75–209 Da range) to measure all existing tags, with more tags being developed

- Abundant tags of similar intensity
- Discreet signals: minimal overlap
- Single metal controls not required
- Background cellular signal: often zero

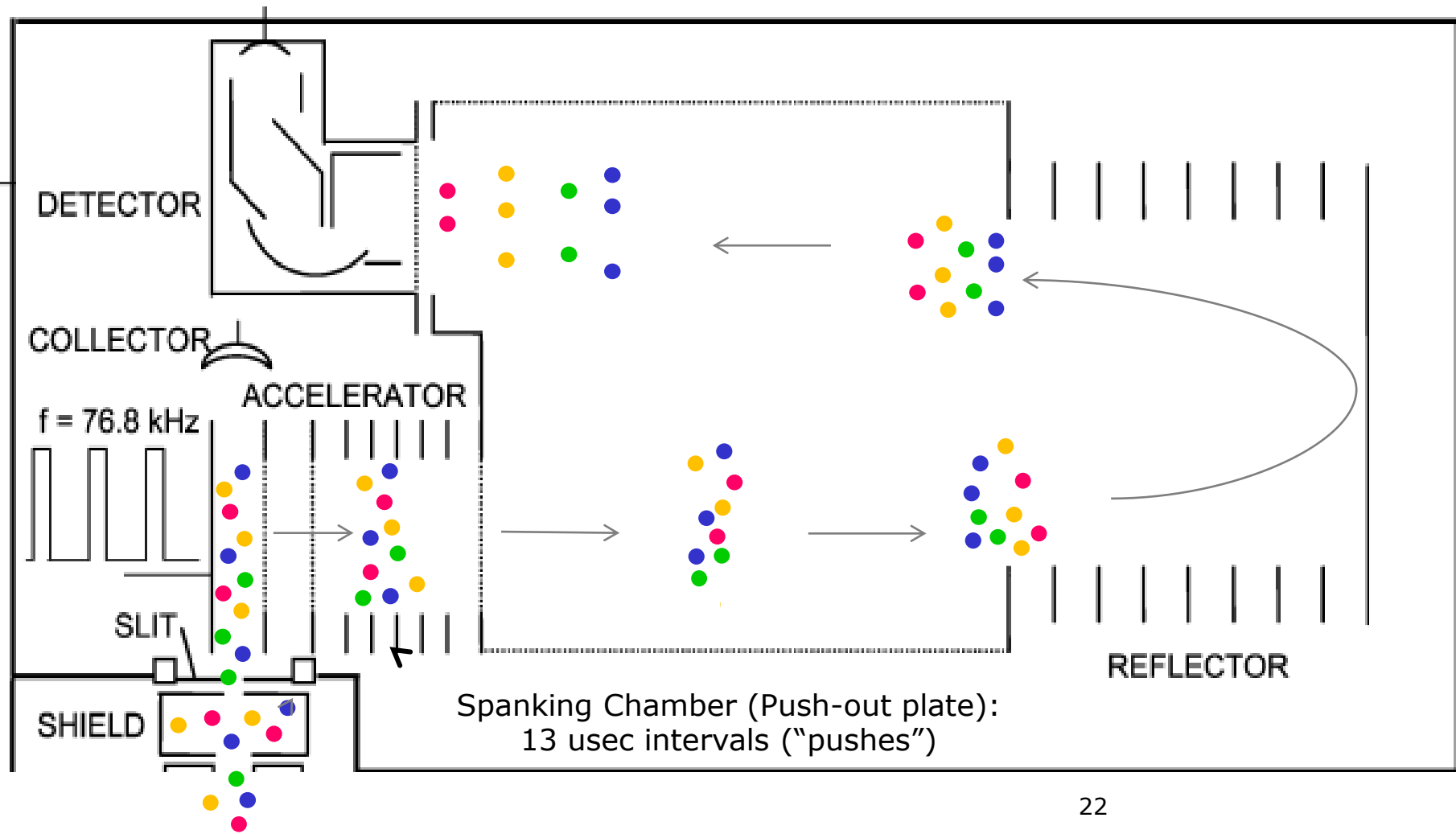
# Summarizing the System



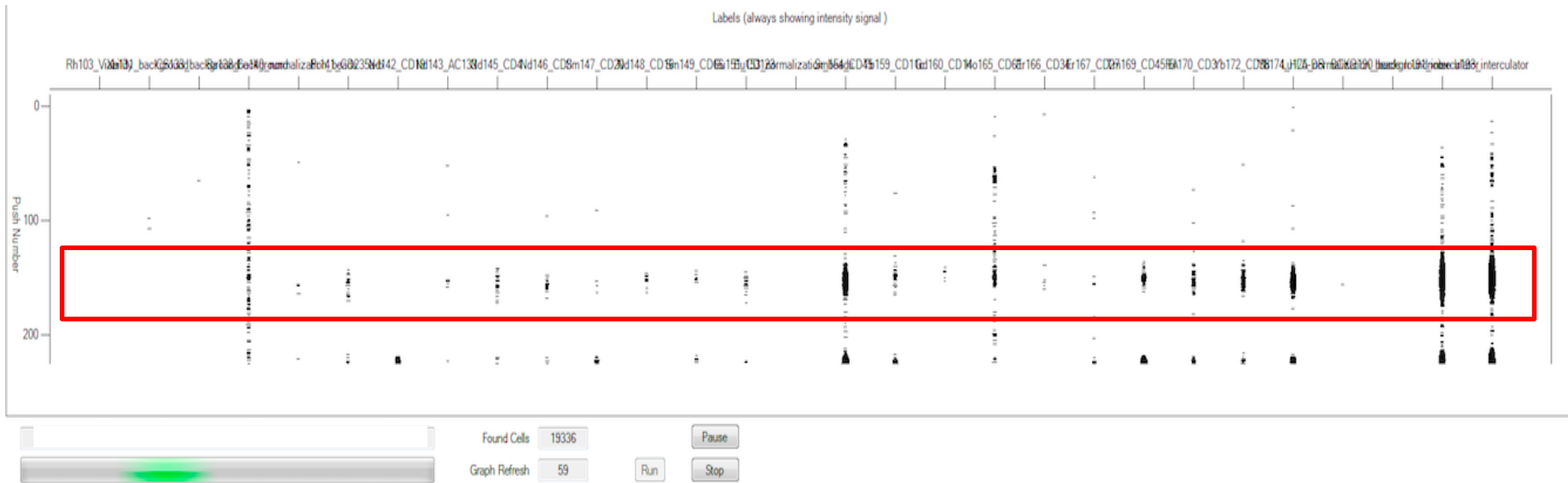
Bendall S. *et al.* (2012). *Trends in Immunology*, **33** (7), 323-332

*TRENDS in Immunology*

# 4. The TOF Chamber

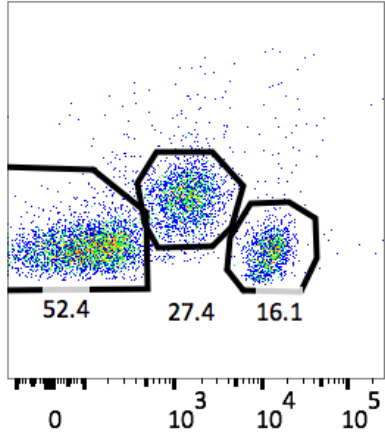
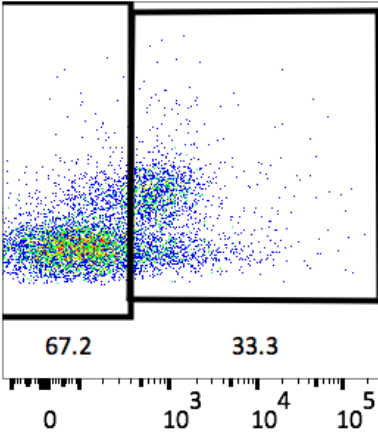
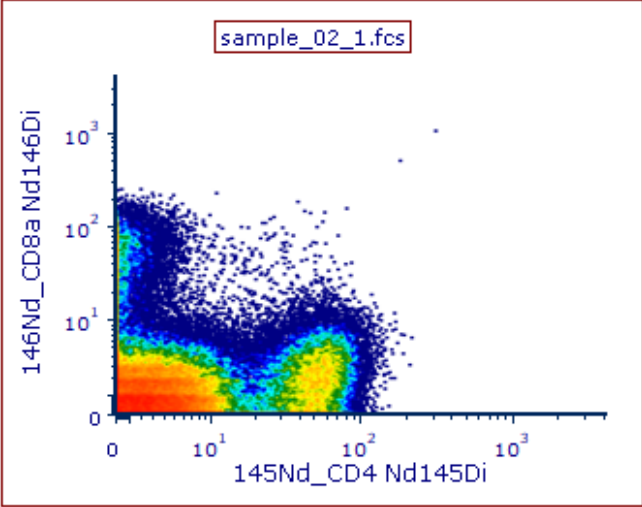


# The Raindrop Display

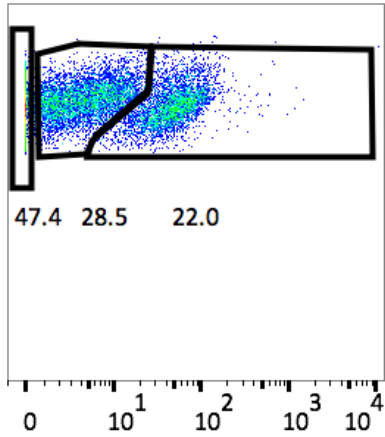
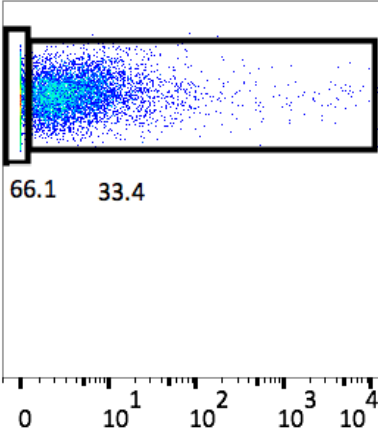


- 29 Parameters Total
- 200 pushes shown
- Some background in the Ba channel
- Good event rate  $\sim 300$  evt/sec
- Software processes the raw data to generate the FCS file.
  - System also generates a "raw" IMD file

# Output: Comparing the Signal



Flow Run



CyTOF Run

CD279

CD4



# MC Strengths and Weaknesses

## Ludo - Strengths

- Discovery and/or depth
- No autofluorescence
- Relative panel design ease
- Barcoding
- Sample storage

## Weaknesses

- All new reagents
- No redundancy
- Sample prep concerns
  - No scatter parameter
- Slow



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# THANK YOU

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