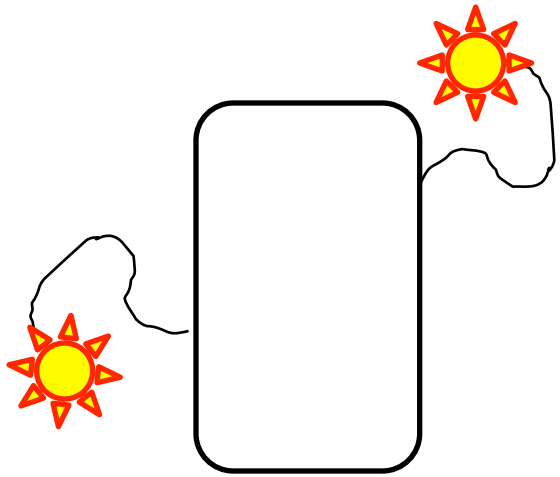
Adaptation and Design of Panels for the CyTOF

December 5, 2014
CyTOF User Meeting – University of Virginia
Mike Leipold

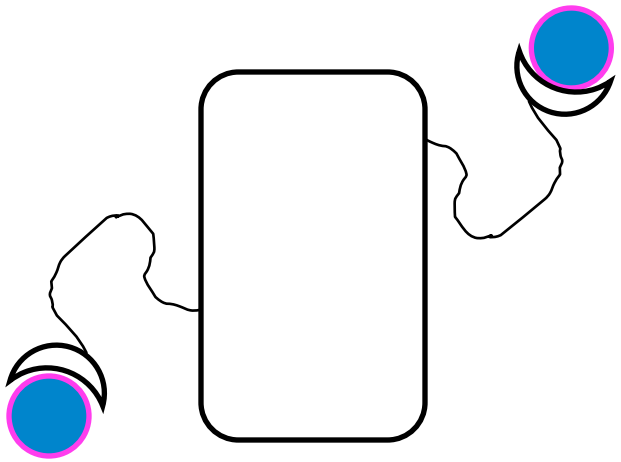
Two Different Approaches to Simultaneous Detection

Fluorophores



Fluorometer

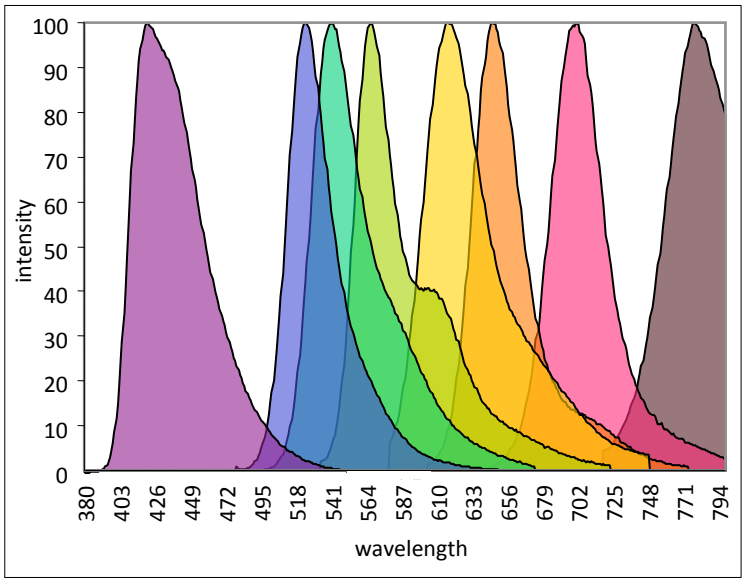
Elemental Tagging



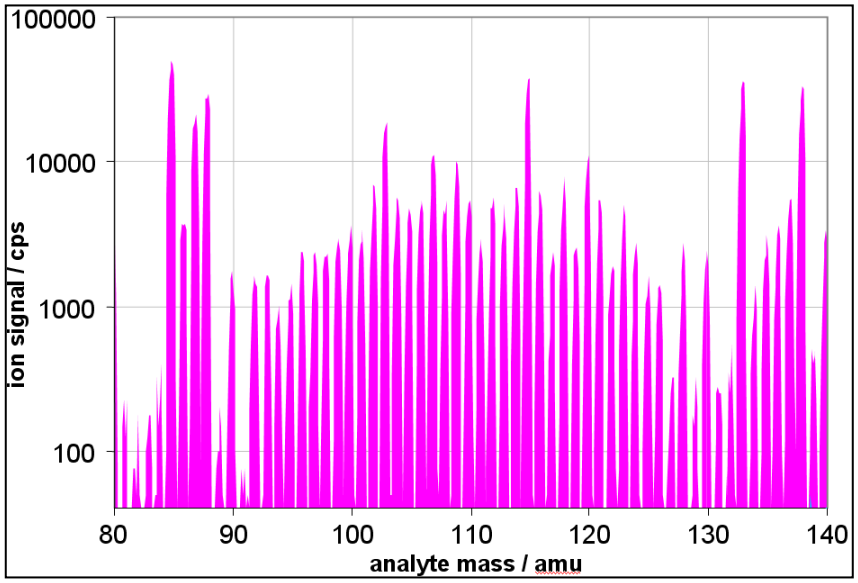
Inductively Coupled Plasma
Mass Spectrometry
(ICP-MS)

Two Different Approaches to Simultaneous Detection

8 Alexa Fluorophores



Elemental Tagging



LSR II

CyTOF

Overview

1. CyTOF – the machine, pros and cons

2. Panel development – background, desired signals

3. Experimental procedures – antibodies, reagents

4. Running samples – example data
   - LSRII vs CyTOF
   - Flu 2010, 2011

Part 1 – The CyTOF

Single Cell Analysis - CyTOF™ Machine

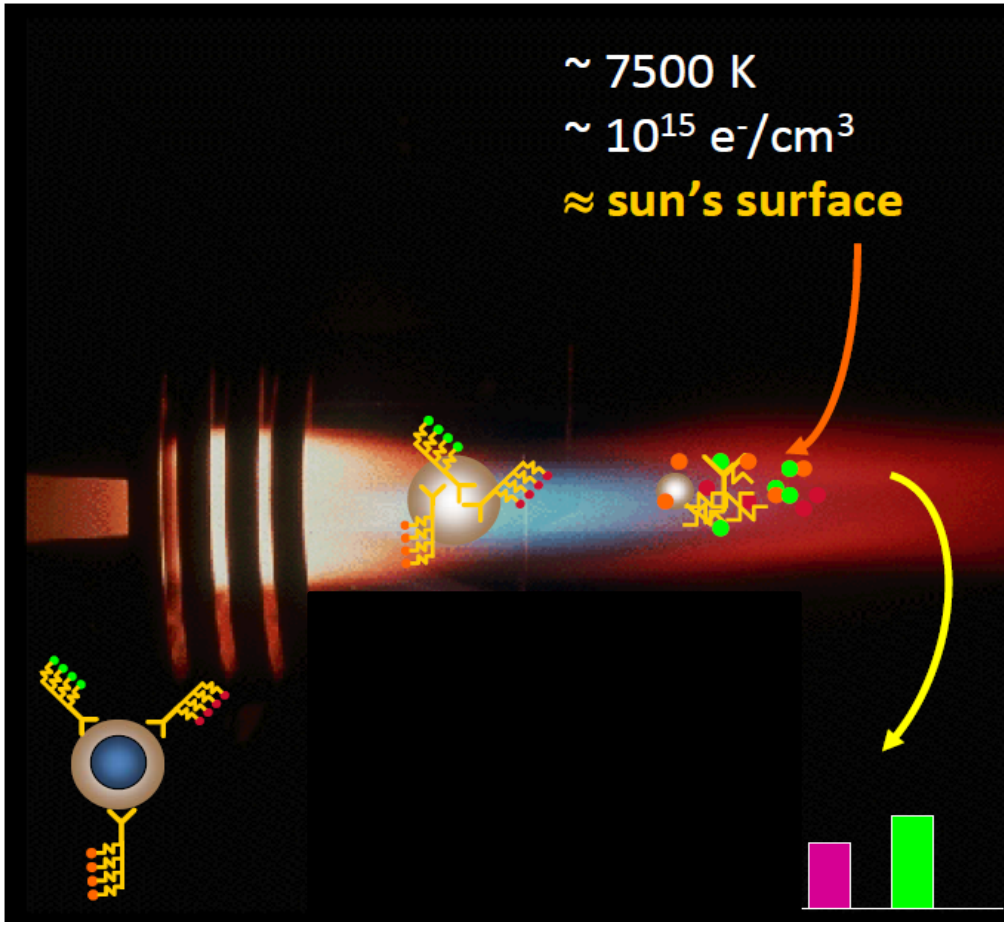


Figure courtesy O. Ornatsky

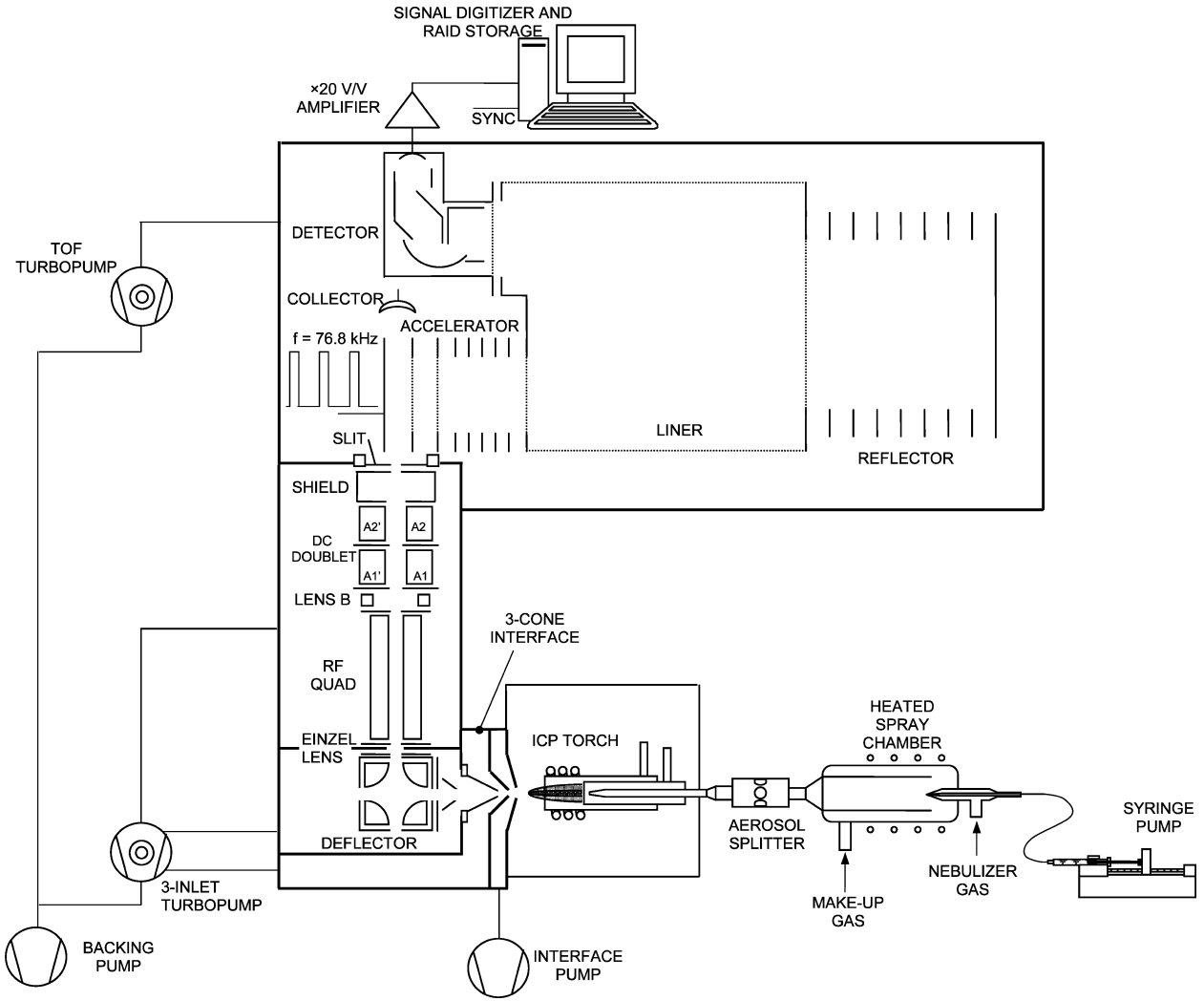
Flow Interface to ICP-MS

- Nebulizer – vaporizes

- Argon Plasma – atomizes and ionizes

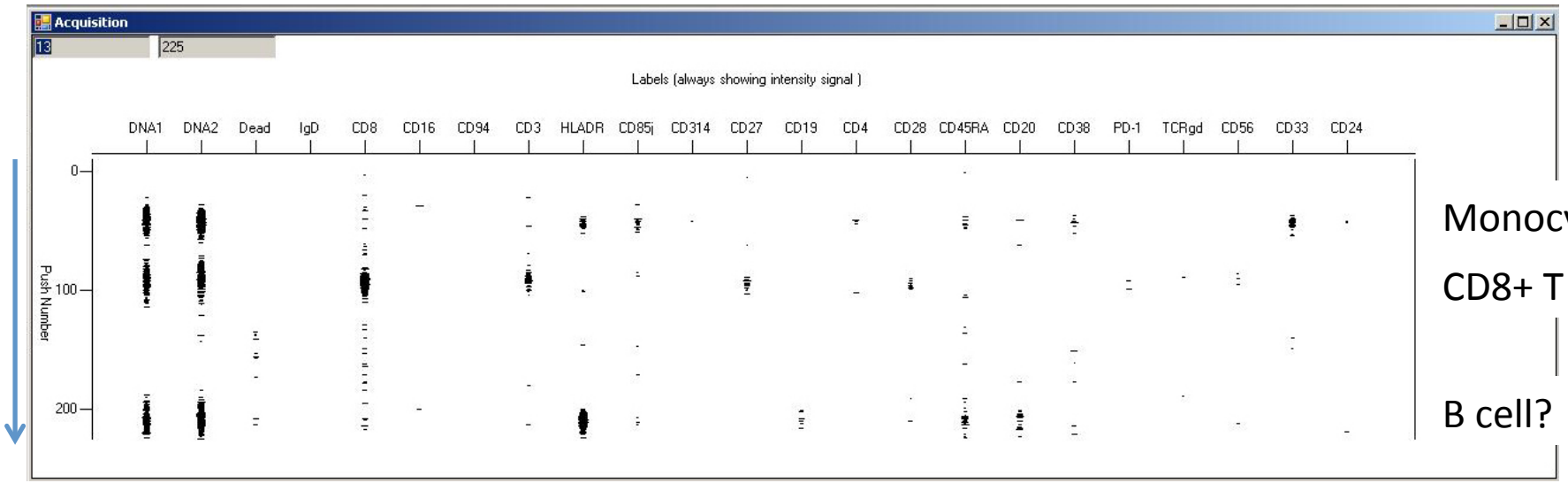
- Mass analyzer

Single Cell Analysis - CyTOF™ Machine



Published in: Dmitry R. Bandura; Vladimir I. Baranov; Olga I. Ornatsky; Alexei Antonov; Robert Kinach; Xudong Lou; Serguei Pavlov; Sergey Vorobiev; John E. Dick; Scott D. Tanner; *Anal. Chem.* **2009,** 81, 6813-6822.
DOI: 10.1021/ac901049w

Display of Cell Events



-"Push" analogous to time: 76,800 pushes/sec: 220 displayed here (1/400 of data)

Pros of CyTOF

- Advantages:
  - Minimal "spectral" overlap – higher dimensionality (more probes at once)
  - Quantitative – broad dynamic range
  - Not light- or time-sensitive
  - Minimal background
    - no analogy to autofluorescence: low/nil biological background for lanthanides

Cons of CyTOF

- Disadvantages:
  - Destructive: (currently) no way to recover interesting cells
  - Slower: limit of 1000 cells/sec; practical limit for best resolution often in ~400 cells/sec range
  - Cell transmission efficiency: only ~20-30% of cells that enter machine get counted
  - Ion transmission efficiency: only ~1 in 10,000 ions that enter machine get counted

Cons of CyTOF

- Disadvantages:
  - Postprocessing: cannot set “on fly” gates to get only “live intact singlets”
    - Events registered by CyTOF contains debris, doublets, etc
    - Might only get ~50% of total events as “live intact singlets”
  - No analogy to FSC or SSC: MUST have marker (M^{n+}) for any gating

Single Cell Analysis - CyTOF™ Machine

- CyTOFv1 – ~2010-May 2013
    - Mass window ~93 units: eg, AW 103-195

- CyTOFv2 – May 2013-current
    - Mass window ~135 units: eg, AW 78-212
    - More automated tuning, including recordkeeping
    - Improvements in ion optics
        - tighter peaks, less "spillover"

Part 2 – Panel Design

Relevant Issues to Panel Design

1. Background – any non-desired signal
   - contaminating signals
   - antibody titer (not discussed)

2. Desired Signal
   - antibody clone
   - metal label – analogous to fluorophore choice

Relevant Issues to Panel Design – Background Signals

* Desired signal only at Mass "M"

1. M+16 – oxide – cannot eliminate, can only limit
2. Metal salt impurities – M+1, M-1, etc; Ln
3. Environmental contamination – sample
4. Instrument

* All potential sources of background, even spillover!

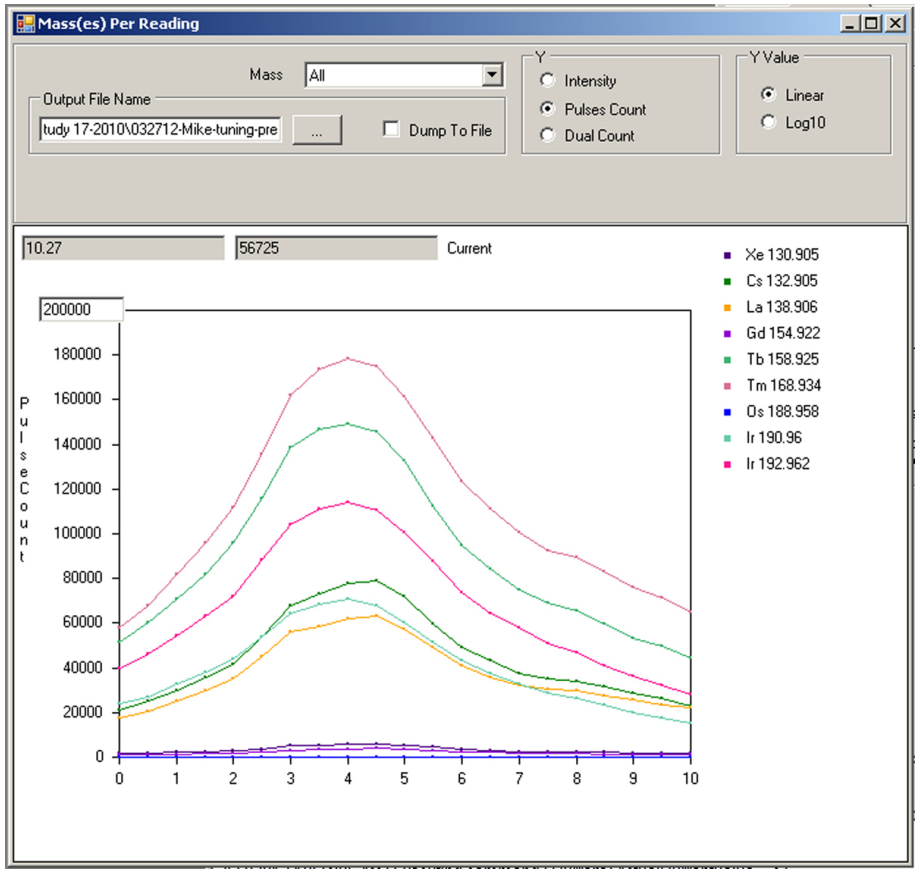
Relevant Issues to Panel Design – Background Signals

* Desired signal only at Mass "M"

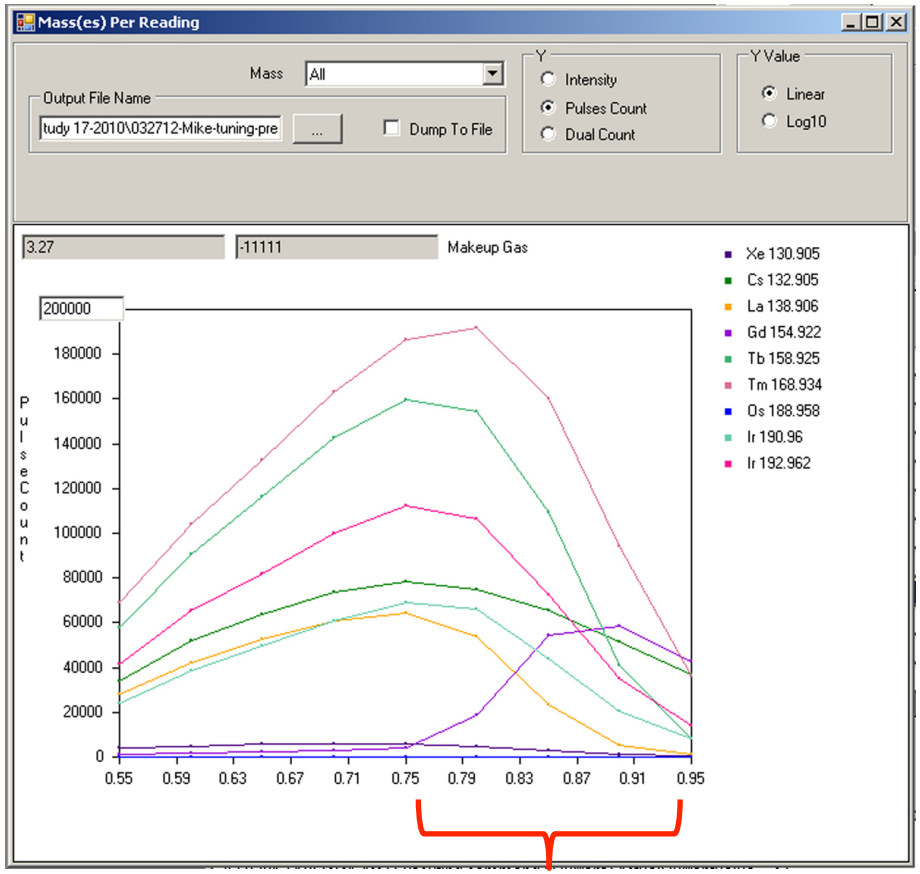
1. M+16 – oxide – cannot eliminate, can only limit
   - Proper daily machine tuning
   - Lower AW Ln = worse

Daily Tuning – Liquid Tuning Solution

Current profile

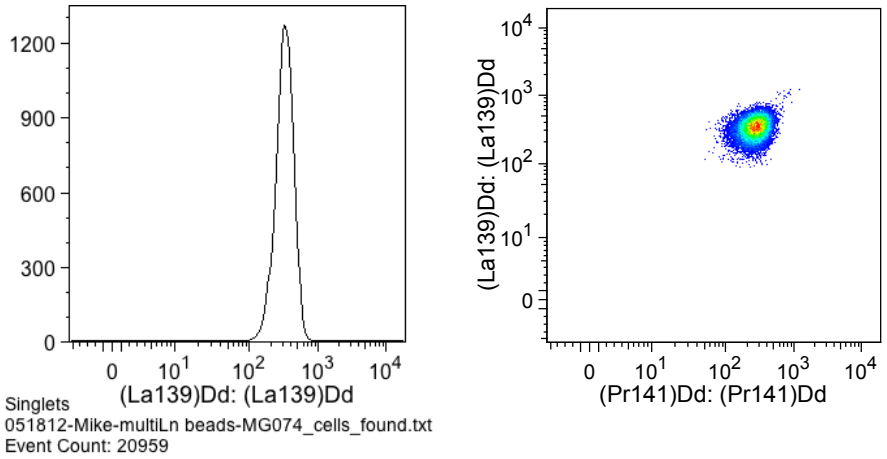


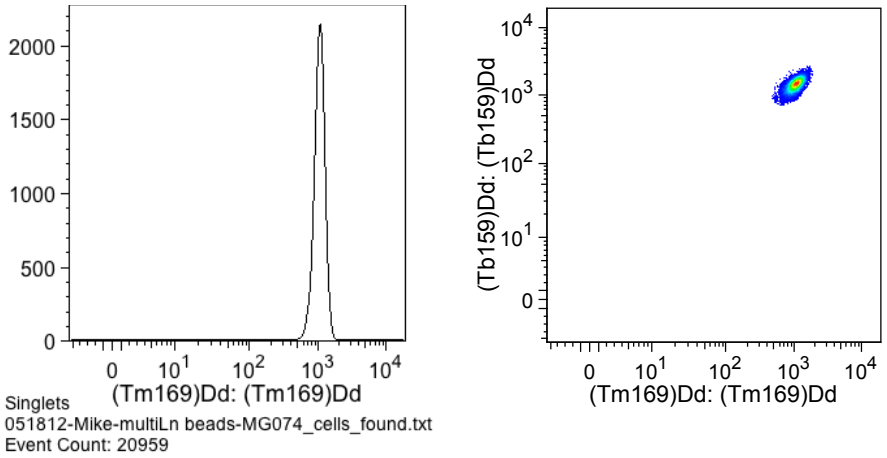
Make-up Gas profile

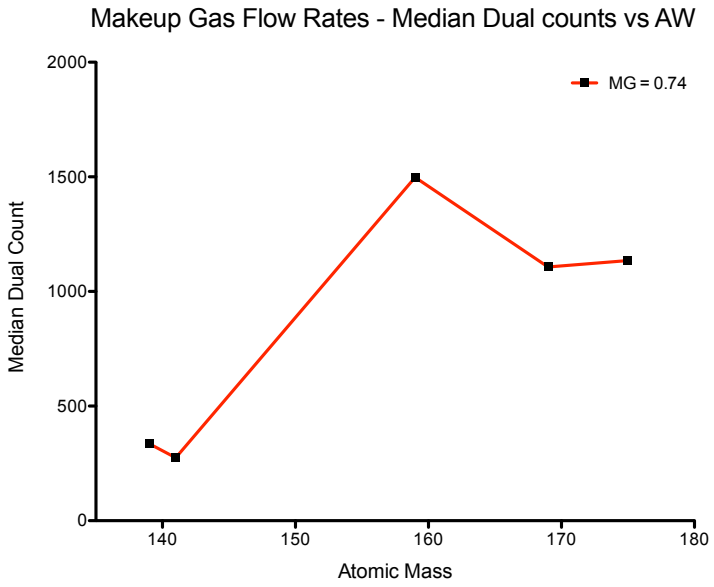


DVS Elemental Beads – "Cells"

- Polystyrene beads: contain nat. abund. Eu; or La, Pr, Tb, Tm, Lu
- Act as "cells" run in acquisition mode (vs. tuning/solution mode)

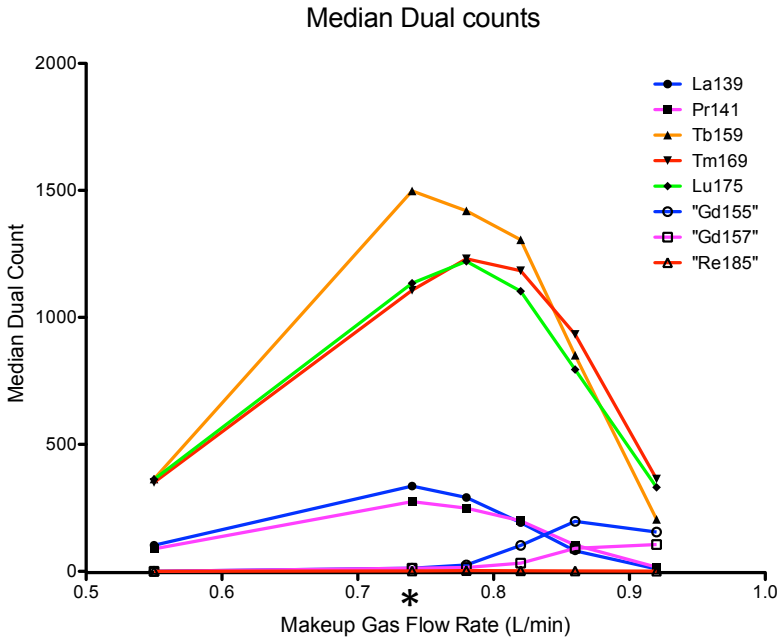




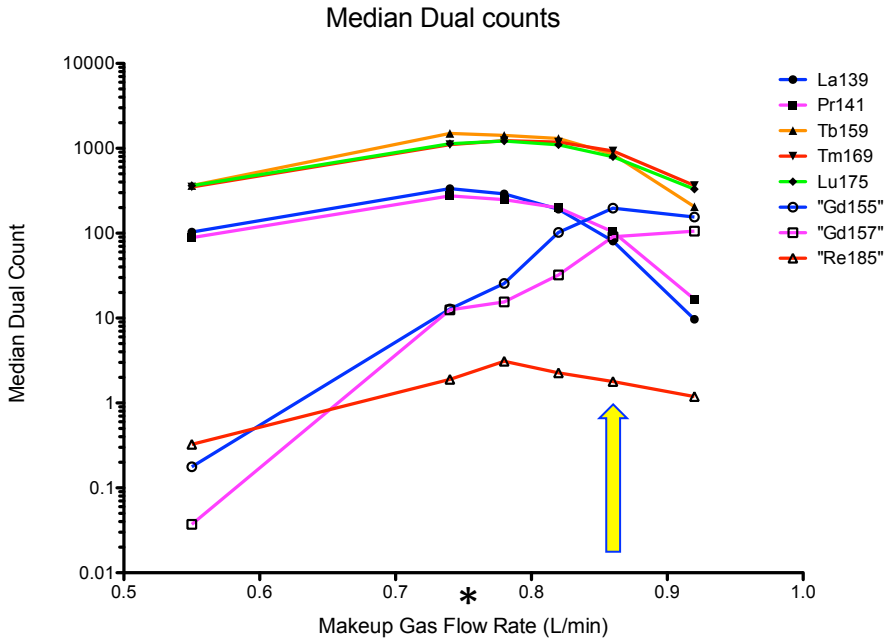


Effect of Tuning – Beads - Oxidation

Linear Scale



Log Scale



- At high MG flow rate, oxidation can be significant (decreases M, increases M+16 spillover)
- Even a few percent can be significant for high-abundance markers (eg, CD57, histone proteins, CD45, etc)

Relevant Issues to Panel Design – Background Signals

* Desired signal only at Mass "M"

2. Metal salt impurities

a) Few lanthanides are naturally 100% monoisotopic

- e.g., Nd144 signal in Nd145 salt
- M+1, M-1, etc

b) Other lanthanide contaminations – all Ln chemistry is similar

- La139 most common contaminant

Purity of Isotopes – Liquid Stock Solutions

CyTOF Channel

Single-Isotope Sample

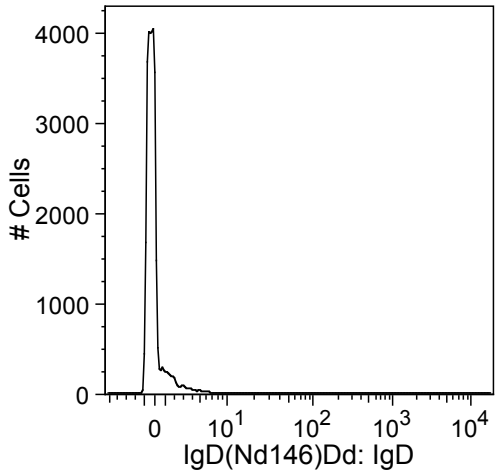
		La 139	Ce 140	Pr 141	Ce 142	Nd 143	Nd 144	Nd 145	Nd 146	Pm 147	Nd 148	Sm 149	Nd 150	Eu 151	Sm 152	Eu 153	Sm 154	Gd 155	Gd 156	Gd 157	Gd 158	Tb 159	Gd 160	Dy 161	Dy 162	Dy 163	Dy 164	Ho 165	Er 166	Er 167	Er 168	Tm 169	Er 170	Yb 171	Yb 172	Yb 173	Yb 174	Lu 175	Yb 176	Hf 177	Hf 178
La	139	75	2	0.2	0.1	0.1	0.1	0	0	0	0	0	0	0	0	0	0	3.4	0.2	0	0	0.1	0	0	0	0	0	0	0	0	0	8	0.3	0.1	0	0	0	0	0	0.1	0.1
Pr	141	0.3	0.2	77	2.3	0.2	0.1	0.1	0.1	0	0	0	0	0	0	0	0	0	0	4.8	0.2	0.1	0	0	0.2	0	0	0	0.1	0.1	0.1	0.2	7.5	0.3	0	0	0	0	0	0	0
Ce	142	0.6	0.1	0.3	81	2.9	0.6	0.1	0.2	0.1	0.1	0	0.1	0	0	0	0	0	0	0	4.6	0.3	0.1	0	0.1	0	0	0	0	0	0	0.1	0.4	0	0	0	0	0	0	0
Nd	143	0.9	0.1	0.1	1	80	4.3	0.5	0.3	0.1	0.1	0.1	0.1	0	0	0	0	0	0	0	0.1	4.7	0.3	0.1	0.7	0.1	0	0	0	0	0	0.1	0.1	0	0	0	0	0	0	0
Nd	144	1.3	0.1	0.1	0.3	0.5	75	2.8	0.5	0.1	0.1	0.1	0.1	0	0	0	0	0.1	0	0	0	0.1	4.1	0.2	0.5	0.1	1.2	0.1	0	0	0	0.1	0.1	0	0	0	0	0	0	0	0
Nd	145	1.9	0.1	0	0.3	0.3	1.4	77	6	0.4	0.2	0.1	0.1	0.1	0	0	0	0.1	0.5	0.1	0	0.1	0.1	4.4	0.5	0.1	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nd	146	3.7	0.2	0	0.2	0.2	0.4	0.5	79	2.7	0.5	0.1	0.1	0.1	0.1	0	0.3	0.2	0.1	0	0	0.2	0	0	4.1	0.2	0.1	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0
Pm	147	5.9	0.2	0	0	0	0.1	0.1	0.4	72	6	1.3	0.4	0.1	0.6	0.1	0.5	0.3	0.1	0	0	0.2	0.1	0	0.1	1.1	0.2	0	0	0	0.5	0.1	0	0	0	0	0	0	0	0	0
Nd	148	2.4	0.1	0	0.4	0.2	0.4	0.2	0.7	0.4	80	2.9	0.8	0.1	0.1	0.1	0.1	0.1	0.1	0	0	0.1	0	0	0.1	0	3.5	0.2	0.1	0	0.1	0	0	0	0.4	0	0	0	0	0	0
Sm	149	3.4	0.2	0	0	0	0	0	0.1	0.3	1.3	76	4.8	0.4	0.8	0.1	0.4	0.2	0.1	0	0	0.1	0	0	0	0	0	1	0.1	0	0	0	0	0	0.1	0	0.5	0.1	0	0	0
Nd	150	0.7	0.1	0	0.3	0.2	0.5	0.2	0.4	0.1	0.3	0.3	84	3.4	0.4	0.1	0.1	0.1	0.1	0	0	0.1	0.6	0	0	0	0	0	3	0.2	0	0	0	0	0	0	0.1	0	0	0	0
Eu	151	0.5	0	0	0	0	0	0	0	0	0	0.1	0.3	88	3.7	1.3	0.1	0.1	0.1	0	0	0	0.2	0	0.1	0	0	0	0	0.1	0	0	0	0.2	0	0	0	0	0	0	0
Sm	152	0.5	0.1	0	0	0	0	0	0	0.1	0.1	0.2	0.3	0.5	86	3.9	1.4	0.1	0.1	0.1	0	0.1	0.1	0	0	0	0	0	0	0	0.9	0.1	0	0	0	0	0	0.3	0	0	0
Eu	153	1.4	0.1	0	0	0	0	0	0	0	0	0	0.1	0.4	0.4	87	4	0.5	0.1	0	0	0.3	0.1	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0.1	0	0	0
Sm	154	1.2	0.1	0	0	0	0.1	3.2	0.2	0.1	0	0.1	0.1	0	0.9	0.2	82	3.4	0.8	0.1	0.1	0.1	0.1	0.2	0.1	0	0.1	0	0	0	0	0	0.8	0.1	0	0	0	0	0	0	0
Gd	156	0.2	0	0	0	0.1	2	0.1	0	0	0	0	0	0	0	0	0.1	1.1	81	7.8	1.7	0.1	0.5	0.1	0.2	0.1	0.3	0	0	0	0	0	0	0	1.4	0.2	0	0	0	0	0
Gd	158	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0.2	0.1	0.2	0.5	1	84	4.3	2	0.1	0.1	0	0	0.4	0	0	0	0	0	0	0	0	1.3	0.1	0	0	0
Tb	159	3.2	0.1	0	0	0	0	0	0	0	0	0	0	0.2	3.1	0.1	0.1	0.2	0	0.1	0.2	83	4.4	0.5	0.1	0	0	0	0	0	0	0	0	0	0	0	0	1.4	0.1	0	0
Gd	160	1.6	0.1	0	0	0	0	0	0	0.1	0.7	0	0	0	0	0	0	0.3	0.4	0.3	1.2	0.3	85	4.3	0.5	0.1	0	0	0	0	0	0	0	0	0	0	0.1	0	1.4	0.1	0
Dy	162	0.1	0	0	0.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	3.4	0.2	2	80	7.2	1.4	0.1	0	0	0.1	0.1	0.1	0	0	0	0	0.1	0	0	1
Dy	164	0.1	0	0	0	0.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0.2	0.5	1.6	87	4	0.5	0.1	0	0.1	0.1	0	0	0	0	0	0	0	0
Ho	165	2.7	0.1	0	0	0	0	0	0	0	0	0	0	0.1	0.2	2.4	0.1	0.1	0	0	0	0.2	0.1	0	0	0.1	0.3	83	5.1	0.7	0.1	0	0	0.1	0	0	0	0	0	0	0
Er	166	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0.1	0.3	85	6.6	1.1	0.1	0.1	0.1	0	0	0.1	0	1.5	0.1	0
Er	167	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0.6	0.1	0	0	0	0	0.1	0.2	1.5	84	7.6	0.8	0.3	0.1	0	0	0	0	0	0	0
Er	168	1.8	0.1	0	0	0	0	0.1	2.1	0.1	0	0	0	0	0	0	0.1	0.1	0	0	0	0	0	0	0.1	0	0.1	0	0.4	1.9	83	4.1	0.9	0.1	0	0	0	0	0	0	0
Tm	169	1.5	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0.2	0	0	0	0	0	0	0	0.1	0.5	85	3.5	0.5	0.1	0.1	0.1	0.1	0.1	0	0
Er	170	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0.8	0.7	1.2	0.5	86	4	0.6	0.1	0.1	0.1	0.1	0	0
Yb	171	0.6	0	0	0	0	0	0	0	0	0	0.1	3.3	0.1	0	0	0	0	0	0	0	0	0.4	0	0	0	0	0	0.1	0	0.1	0.1	0.5	80	8.8	1.3	0.6	0.1	0.2	0.1	0
Yb	172	1.7	0.1	0	0	0	0	0	0.1	1.4	0.1	0	0	0	0	0	0	0.1	0	0	0	0.1	0	0	0	0	0	0	0	0	0.2	0.1	0.1	0.6	82	6.7	1.3	0.1	0.2	0.1	0.1
Yb	174	0.4	0	0	0	0	0	0	0	0.1	1.5	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0	0.1	0.3	0.7	87	5.1	1.1	0.1	0.1
Lu	175	1.2	0.1	0	0	0	0	0	0	0	0	0	0.1	1.9	0.1	0	0	0.1	0	0	0	1.2	0.1	0	0	0	0	0	0	0	0	0.1	0	0.1	0.1	0.1	0.4	79	8.2	1	0.1
Yb	176	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0	0	0.1	0	0	0	0.1	0.3	0.2	2.1	0.5	89	5.3	0.7

- M+1 impurities – some isotopes worse than others
- M+16 oxidation impurities – affects all isotopes; lower AW worst

BD Ig κ Capture Beads and MAXPAR Antibodies

M-1

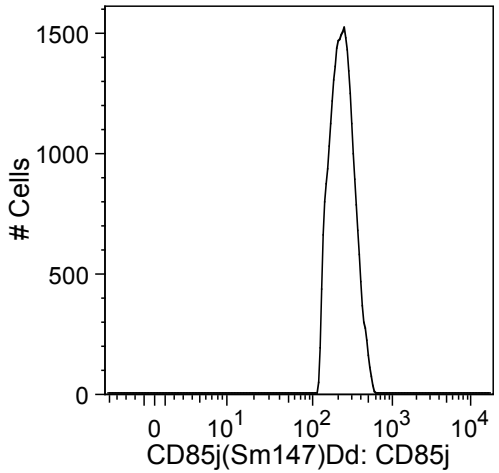
146



No "Sm146"
- 146 is Nd

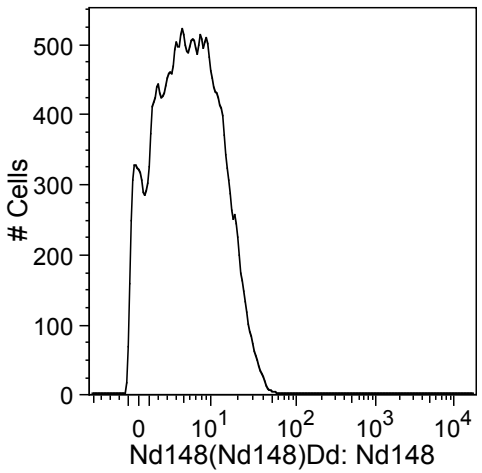
M

Sm147



M+1

148



* Spillover due to elemental and/or isotopic impurities, not strictly mass proximity

Major Contaminants/Spillovers: Percentage of M

	M-2	M-1	M	M+1	M+2	M+16
In113	0	-0.3	100	-0.1	1	0
Nd142	-0.2	-0.2	100	0.53	-0.1	3.39
Nd143	-0.4	-0.2	100	1.32	-0.3	3.06
Nd144	-0.4	-0.3	100	0.2	-0.3	2.68
Nd146	-0.1	-0.1	100	0.42	-0.1	2.99
Sm147	-0.2	-0.2	100	2.58	0.09	0.44
Sm149	-0.2	-0	100	1.71	-0.3	-0.08
Nd150	-0.2	-0.2	100	0.43	-0.2	1.67
Eu151	-0.1	-0.1	100	0.52	0.11	-0.12
Sm152	-0.2	-0.2	100	0.43	-0.1	-0.08
Sm154	0.25	-0	100	0.5	-0.1	0.3
Gd156	-0.1	0.31	100	2.46	0.24	0.72
Dy164	0.05	0.77	100	0.37	-0	0.72
Ho165	-0.1	-0.1	100	0.71	-0.1	0.53
Er166	-0.1	-0	100	1.16	-0	0.67
Er167	-0.2	0.27	100	2.14	-0.1	0.52
Er168	-0	0.6	100	0.72	0.01	0.99
Er170	0.3	-0.1	100	0.86	-0.1	0.97
Yb171	-0.2	-0.1	100	3.15	-0.1	-0.47
Yb174	-0	0.24	100	0.66	0.06	-0.03
Lu175	-0.2	-0.1	100	2.33	-0.1	63.8
Yb176	0.74	-0.1	100	1.31	-0.1	-0.22

* Ir191 contam?

- M+1 and M+16 are usually the major spillovers

- Only Fluidigm metals shown; non-Fluidigm metals can be worse

Major Contaminants/Spillovers: Cell Data

* Formerly, Fluidigm sold Dy162 and Dy164, but not Dy163
  – Dy purity issues

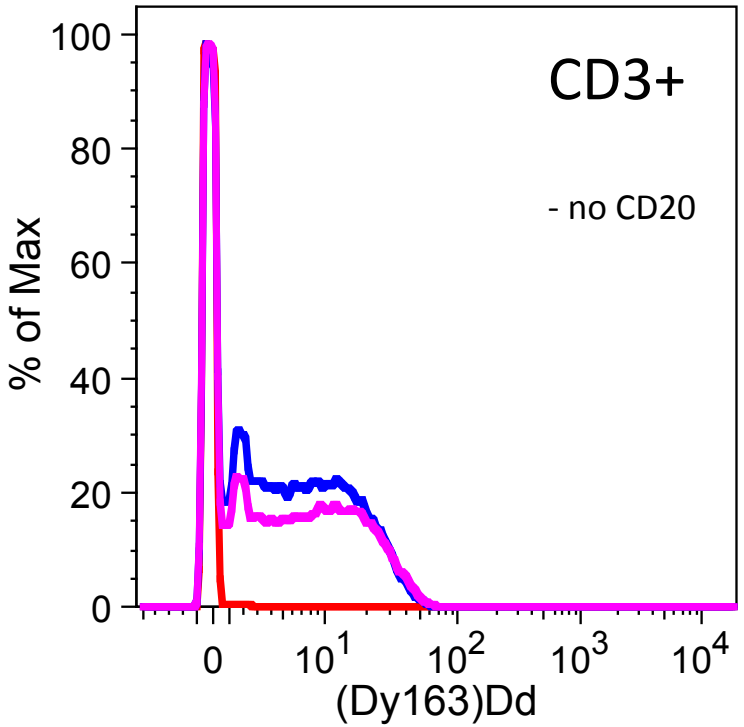
Regular Phenotyping panel:
CD45RA-Dy162
CD20-Dy164

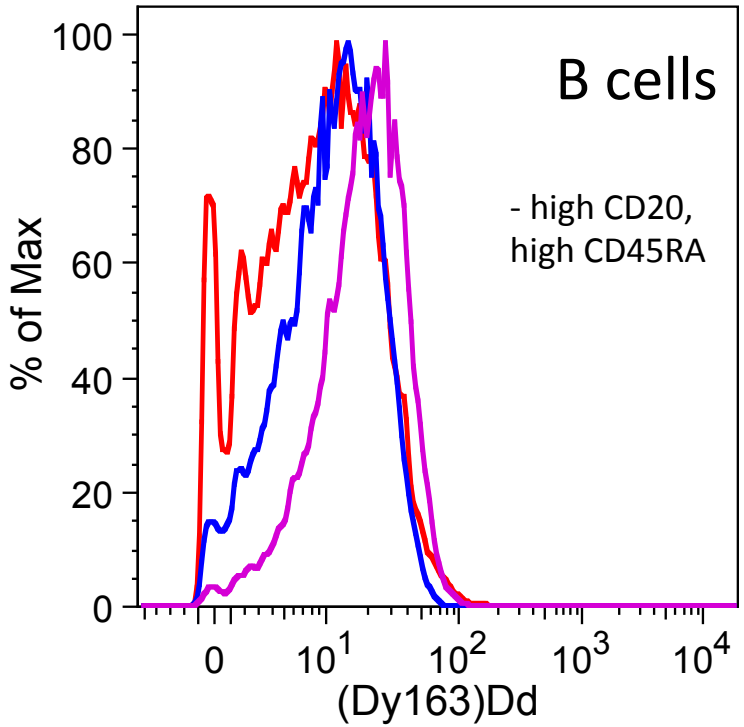
* no Dy163 antibody in experiment, so not usually monitored

Major Contaminants/Spillovers: Cell Data

CD45RA-Dy162
CD20-Dy164
* no Dy163 antibody in experiment!

Magenta: both Dy162, Dy164
Blue: Dy162 only
Red: Dy164 only





Relevant Issues to Panel Design – Background Signals

3. Environmental contamination

- Reagents – PFA lots with microparticles, MeOH with free metal, Ar gas with Sn

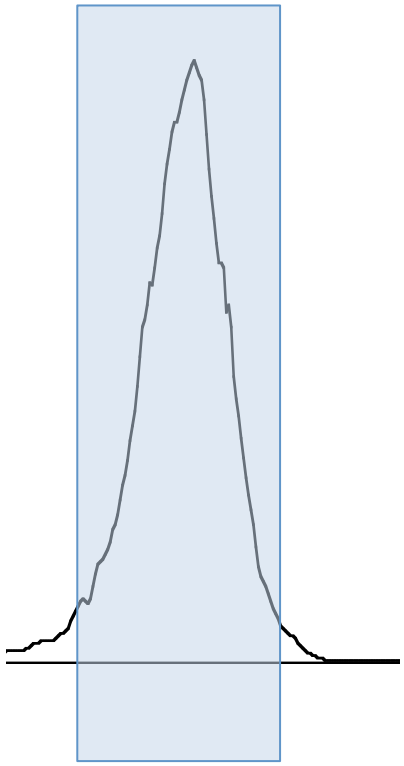
- Biological sourcing – Iodine (free I vs IdU), Pt in cisplatin-treated donor samples

- Lab dust

- Barium, lead, etc – dish soap, syringes, reagent sourcing, striker flint, etc

Relevant Issues to Panel Design – Background Signals

4. Instrument Abundance Sensitivity
   – left (M-1) and right (M+1) leg of ion peak
      - CyTOFv1: 0-1%
      - CyTOFv2: <0.3%



- Worse with high AW masses: peak less Gaussian, M+1 leg wider

Major Contaminants/Spillovers: Percentage of M

* CyTOF spillover - still much less than Fluorescence spillover!
* All proportional to signal at Mass "M".....

1. Match marker abundance with metal brightness
   - 1% of 300 is 3 (background)
   - 1% of 3000 is 30 (probably not background)

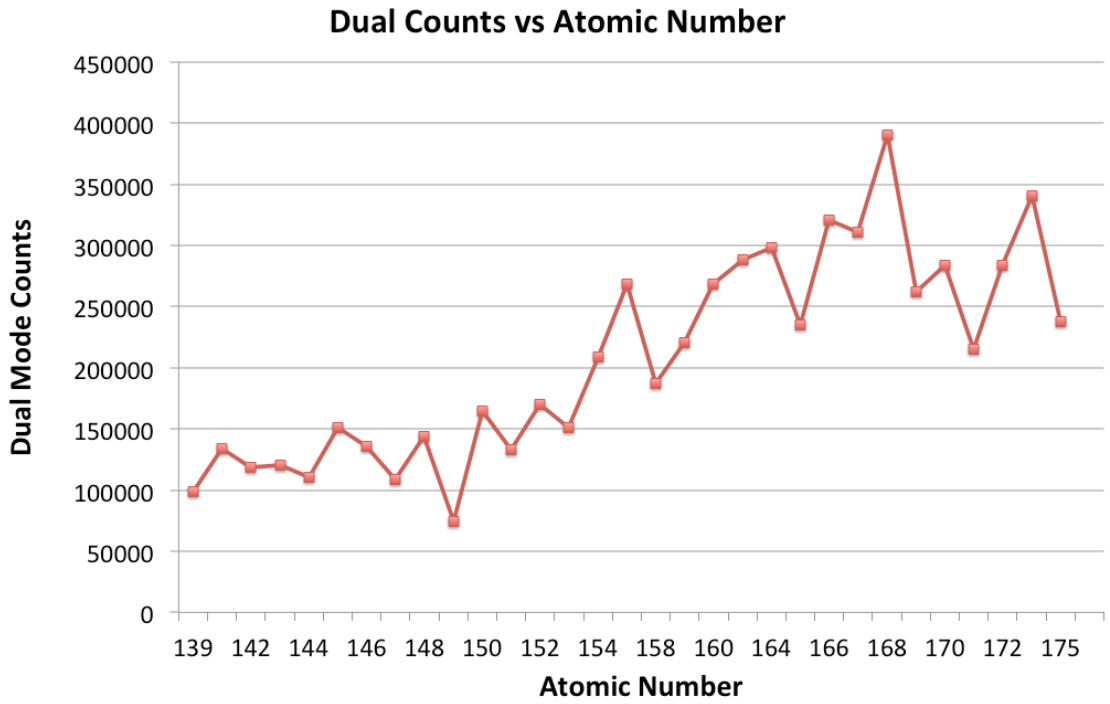
2. Mutually exclusive lineages
   - T cell marker followed by B cell marker

3. Dump channels for most contaminated isotopes
   - analyze the "negative" cells

Desired Signal Intensity- Choice of Metal

Reminder: you cannot detect anything that doesn't have bound metal

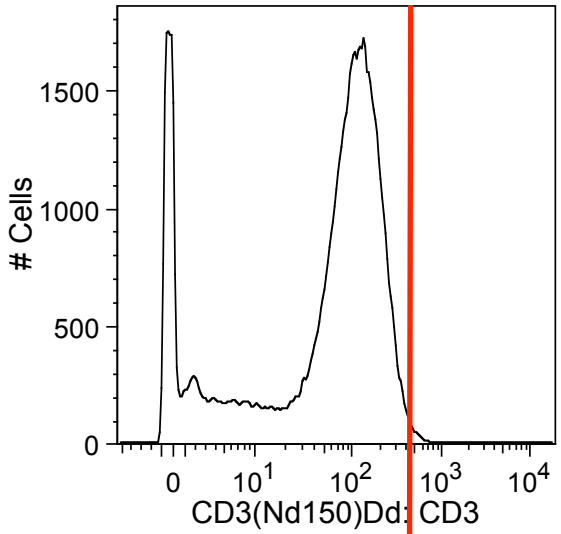
- No scatter to gate monocytes from lymphocytes, etc

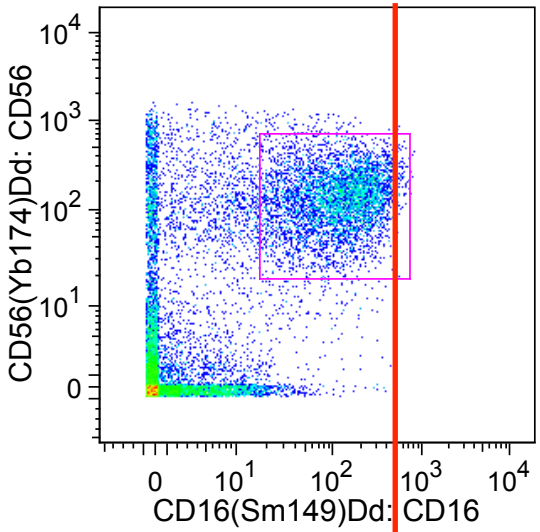


- Only about 3-fold difference in signal across lanthanides
- Switching lanthanides might help only in borderline cases

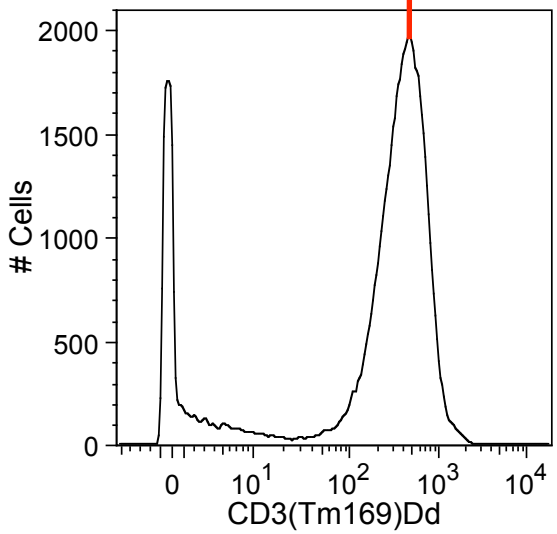
Desired Signal Intensity- Choice of Metal

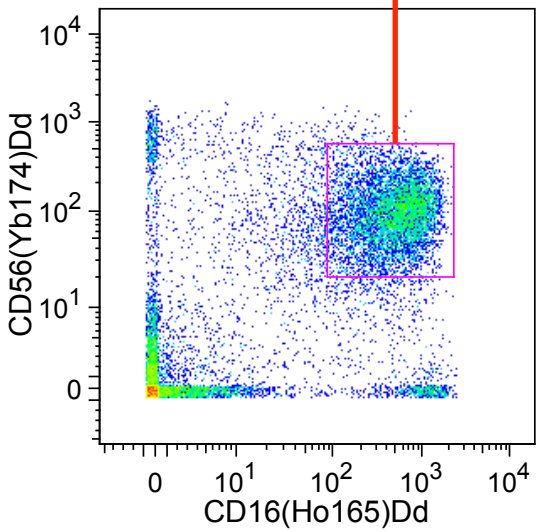
Lower sensitivity
(139-150)





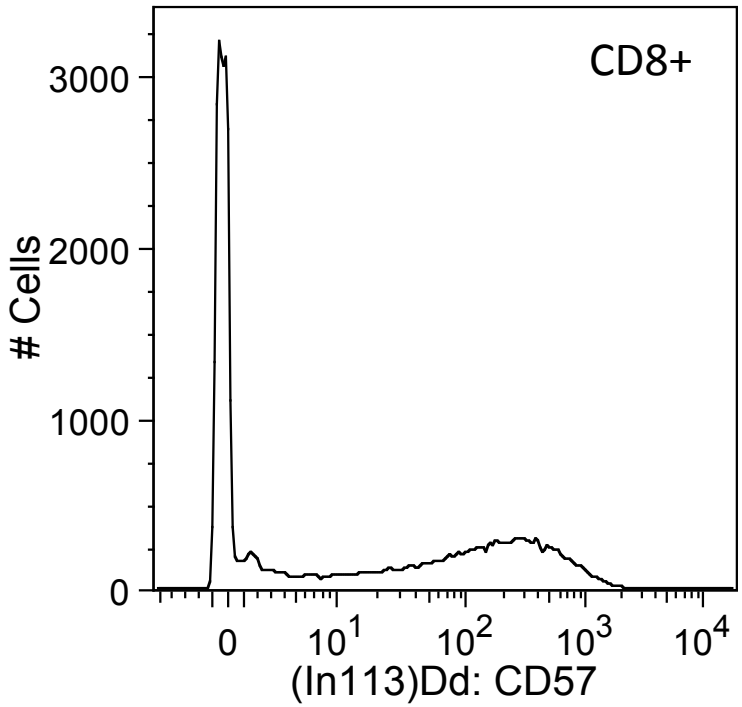
Higher sensitivity
(165-170)

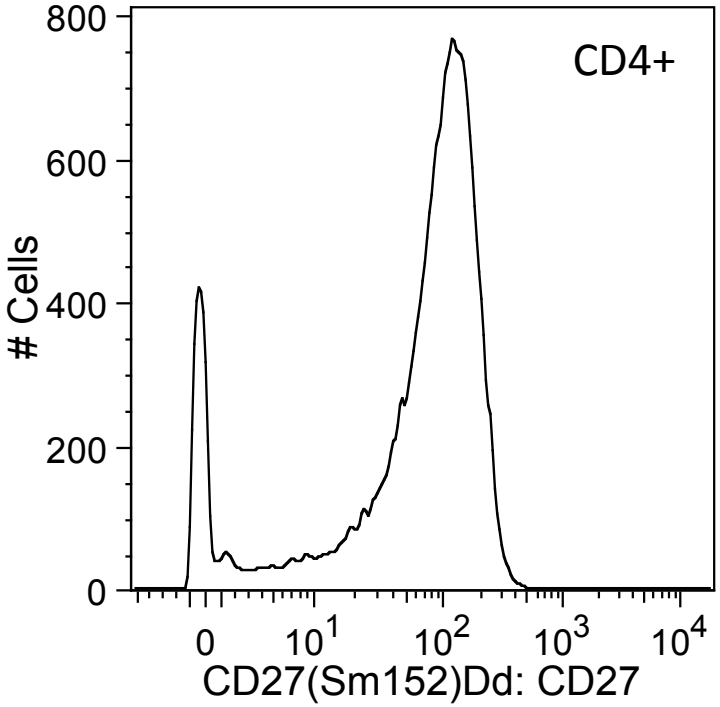




Desired Signal Intensity- Choice of Metal

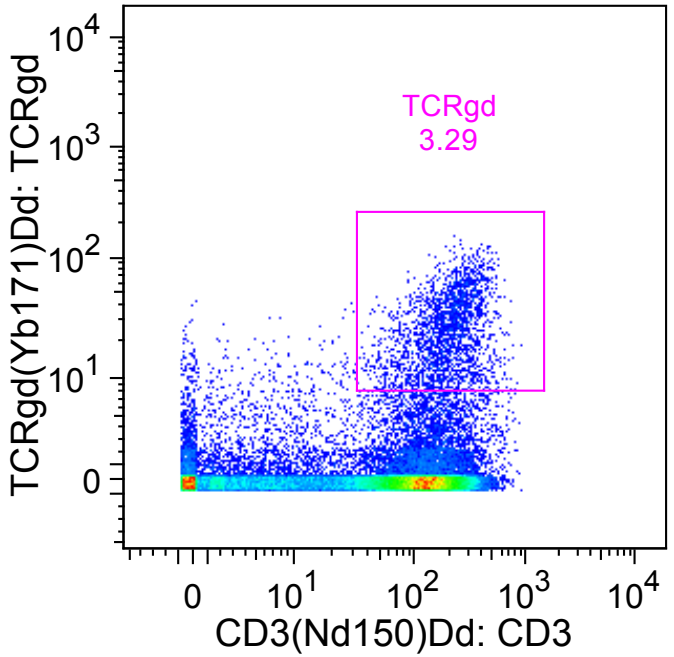
1. "Dim" metals – In, La, Pr, Nd, Sm
   - Bright/abundant markers (eg, CD57)
   - True bimodal (eg, CD27)

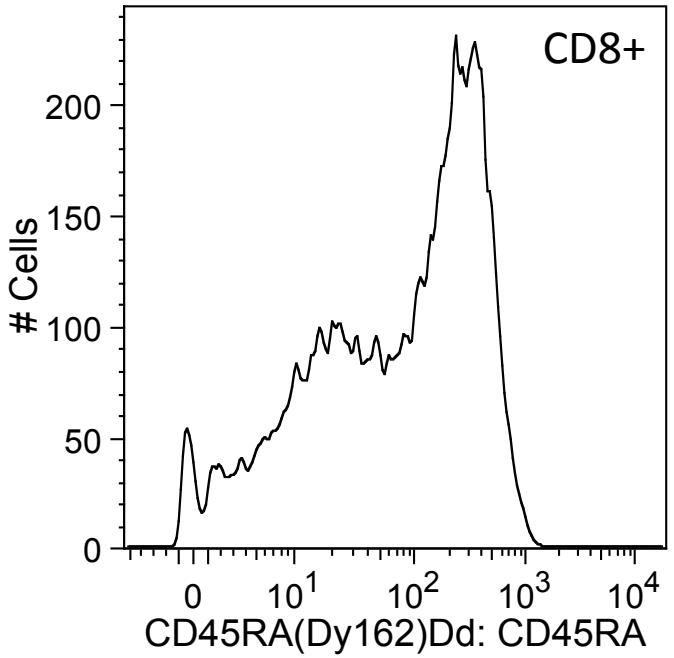




Desired Signal Intensity- Choice of Metal

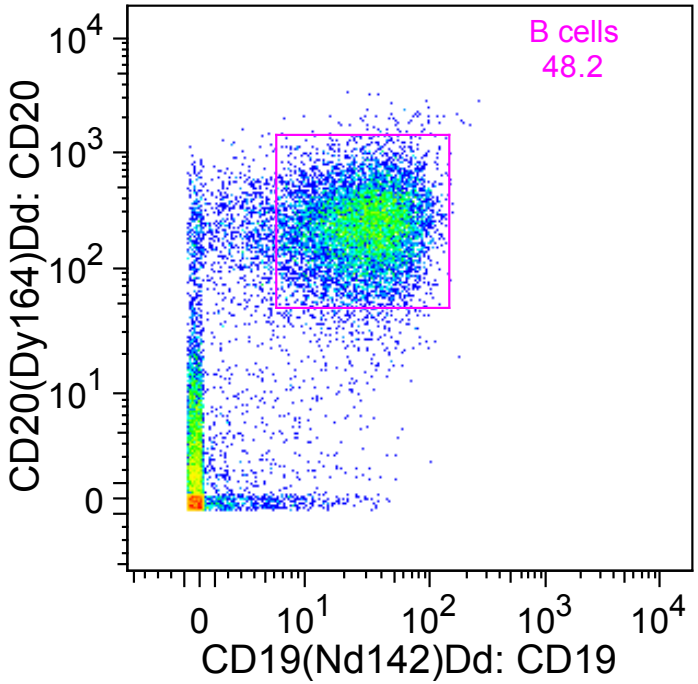
2. "Bright" metals – Tb, Dy, Tm, Yb
   - Dim/rare markers
   - "Smear" distribution (eg, CD45RA, CCR7)
   - Small fold-change

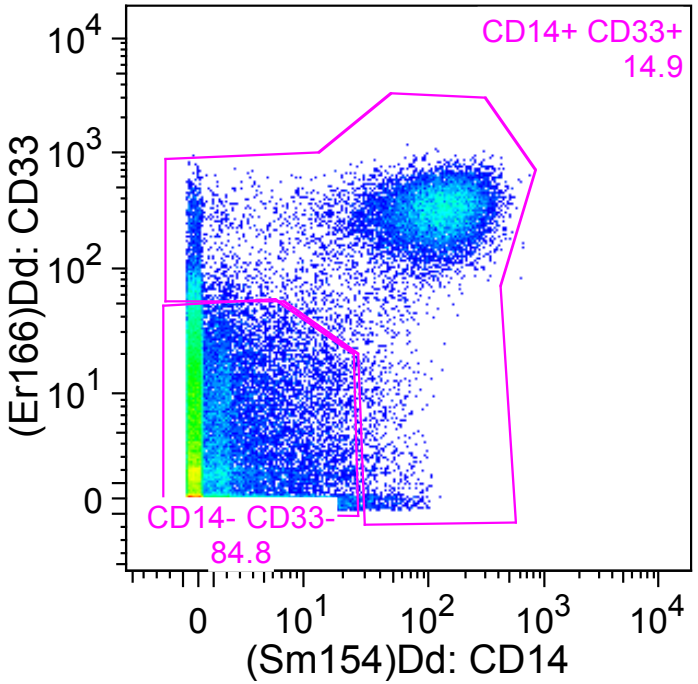




Desired Signal Intensity- Choice of Metal

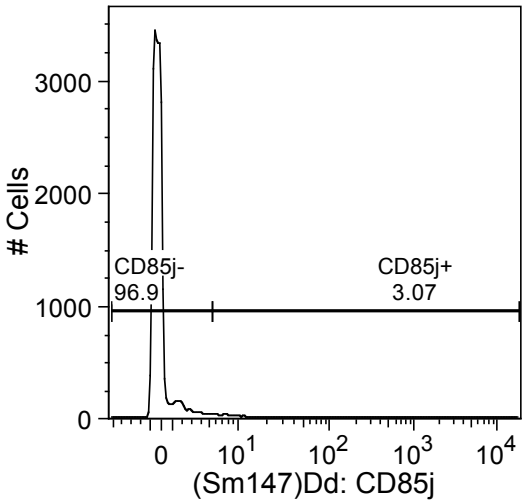
3. Recommend bivariates
   - one bright, one dim
   - two mediums – "smear", or subpopulations





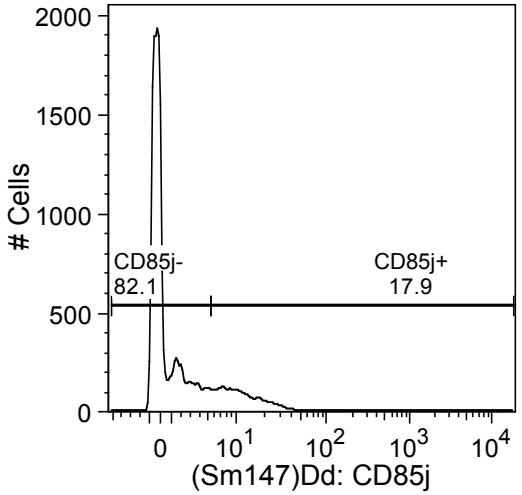
Desired Signals – CD85j as a Borderline Case

CD4+



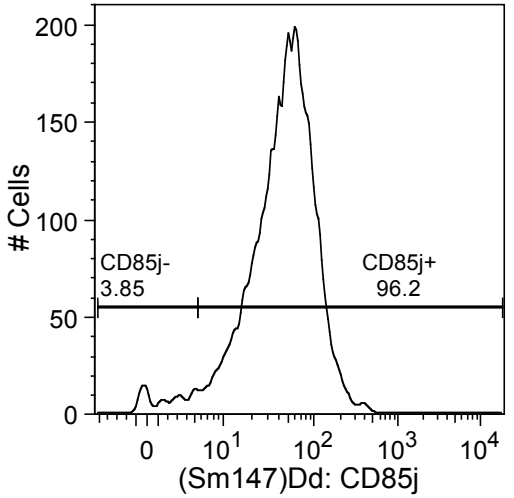
"negative"

CD8+



subpopulation

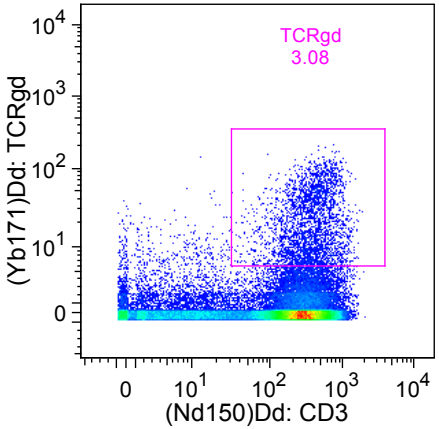
Monocytes

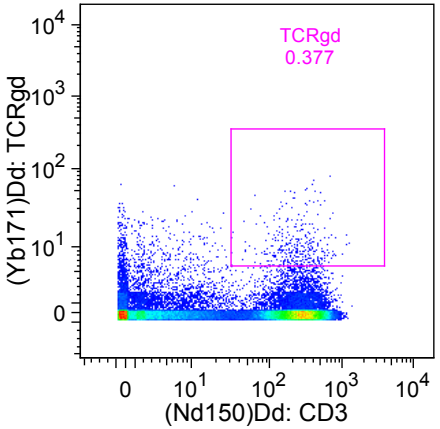


"positive"

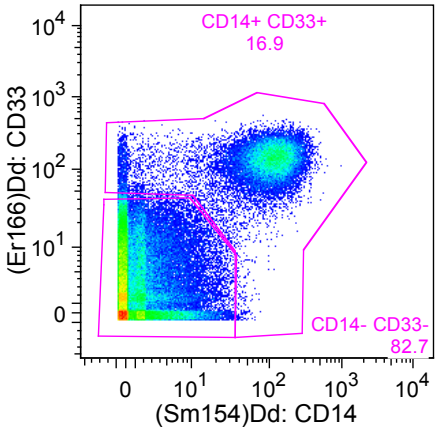
Confirm New Markers With More Than One Donor

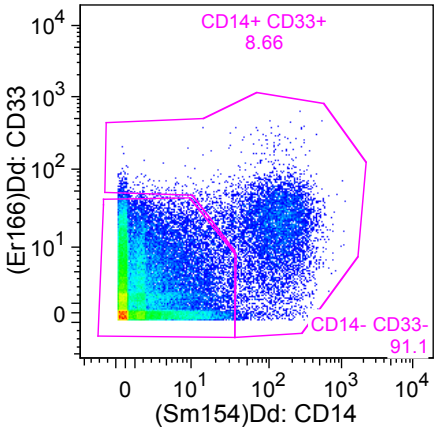
TCRgd





CD33





Part 3 – Experimental Procedure

CyTOF Sample Info

1. Reagent purity is paramount
   - MilliQ water only
   - trace metal pure salts and buffers, if possible

2. All samples - fixed and permeabilized
   - Thorough fixation - Fresh PFA!

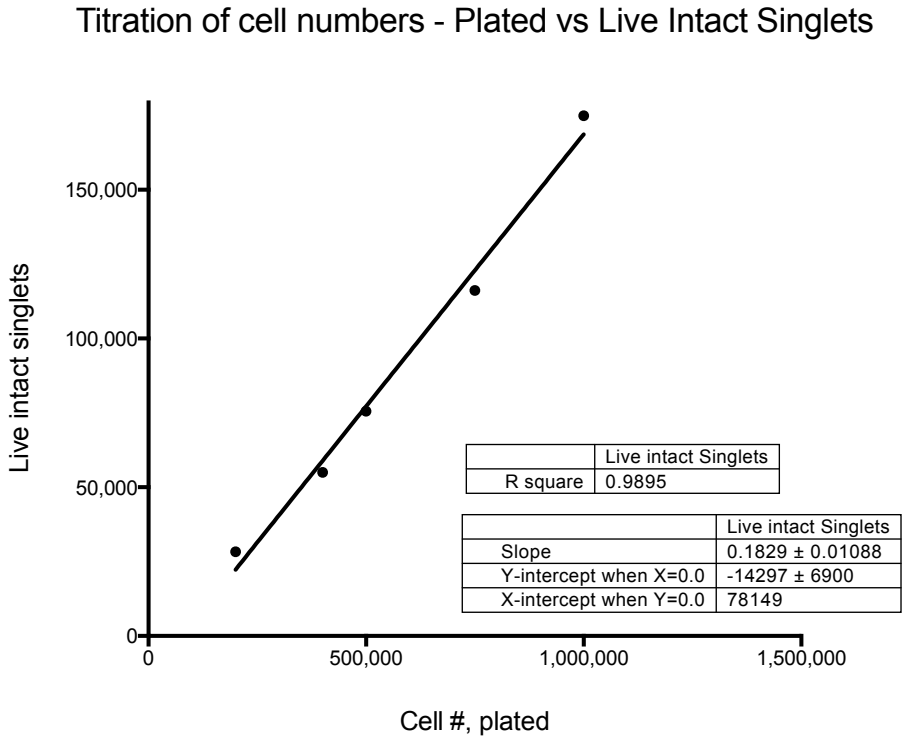
3. MilliQ water washes at the end
   - Proper fixation ensure intact cells
   - Minimizes buffer salt introduction into machine

4. Titrate all reagents for your assay conditions
   - Commercial
   - Every batch of in-house reagents – re-verify before use

CyTOF Sample Info – Starting Cell Count

* Remember: only 20-30% cell transmission efficiency
  - also, processing losses

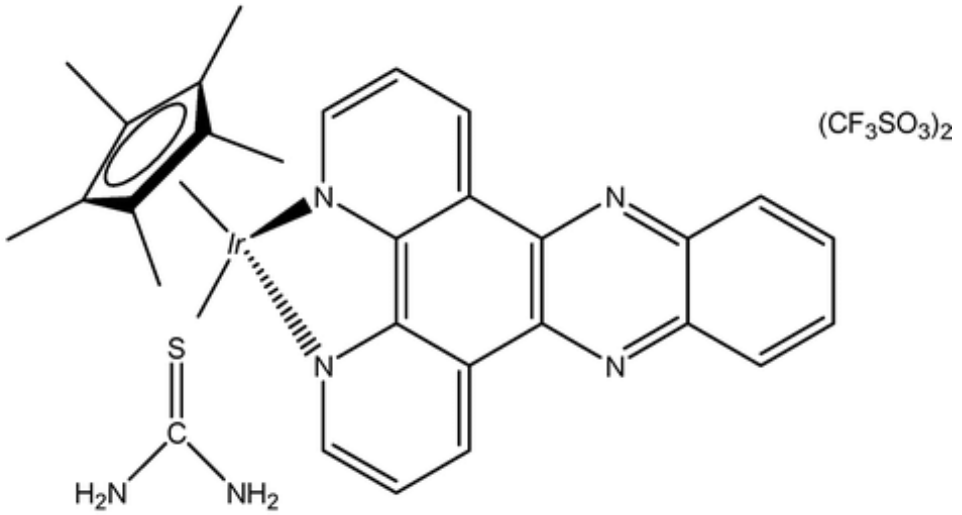
Cells Plated	Live intact singlets
200K	28K
500K	76K
1M	175K



Yao *et al*: down to 10K (major only)
HIMC: recommend at least 500K

Metal Staining of Cells - DNA Intercalator

Structure of $[Cp^*Ir(dppz)\{(NH_2)_2CS\}](CF_3SO_3)_2$



$C_{31}H_{29}F_6IrN_6O_6S_3$

Mol.Wt.:984.08 Da

- Labels <u>any</u> cell containing DNA regardless of whether any labelled antibodies bind

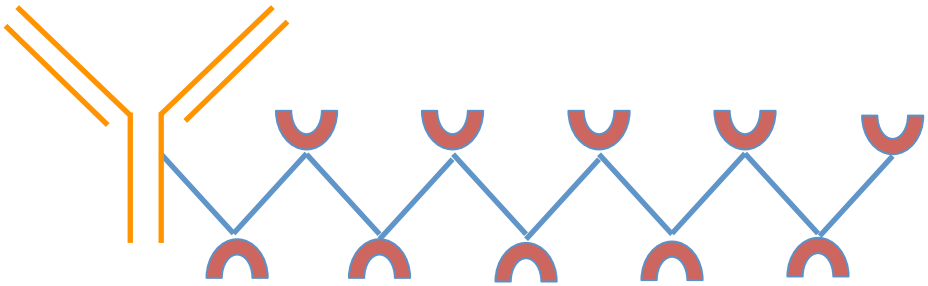
Schaferet al *J.Organomet.Chem.,* **2007**, *692*, 1300−1309

Ornatsky et al, *Anal. Chem.*, **2008**, *80*, 2539–2547

Chelating Polymer Structures

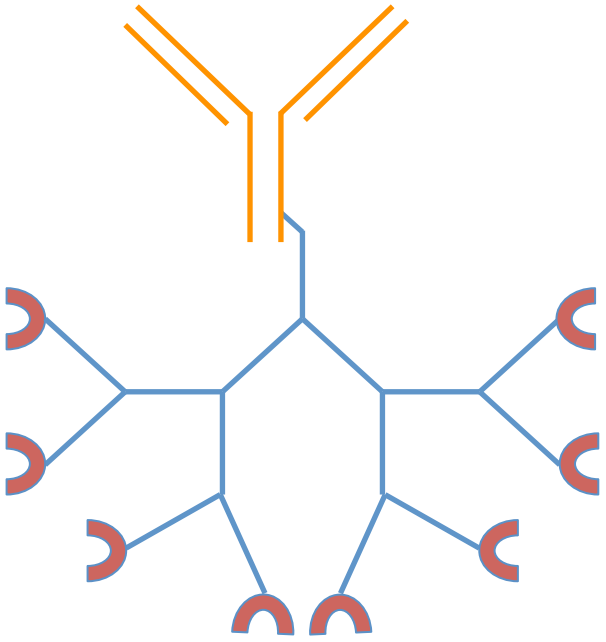
X8

linear polymer with ~22 chelation sites

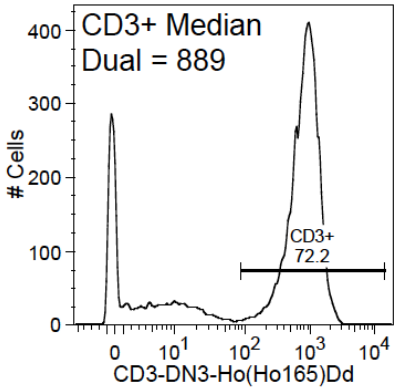


DN3

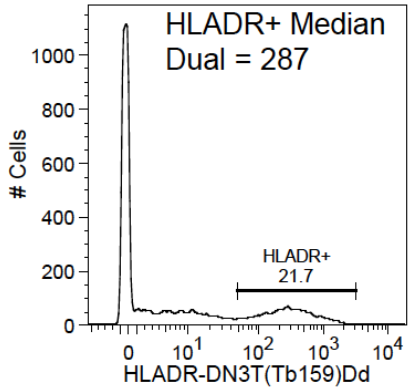
branched dendrimer,
~16 chelation sites



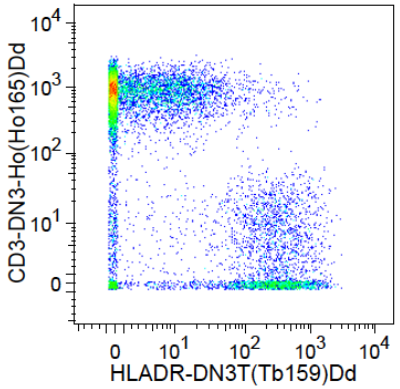
Antibodies – X8 vs DN3 Comparison



Live intact singlets
011411-PBMC-DN3-duplex-TbHo_cells_found.txt
Event Count: 14144

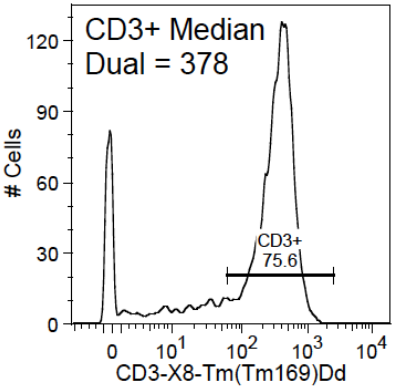


Live intact singlets
011411-PBMC-DN3-duplex-TbHo_cells_found.txt
Event Count: 14144

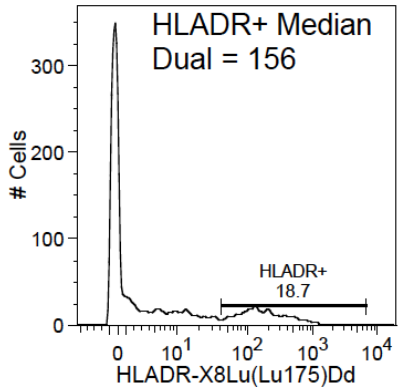


Live intact singlets
011411-PBMC-DN3-duplex-TbHo_cells_found.txt
Event Count: 14144

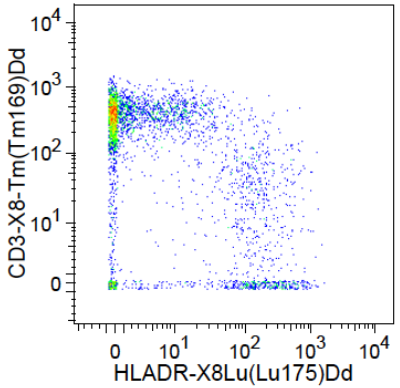
DN3



Live intact singlets
011411-PBMC-X8-duplex-TmLu_cells_found.txt
Event Count: 4519



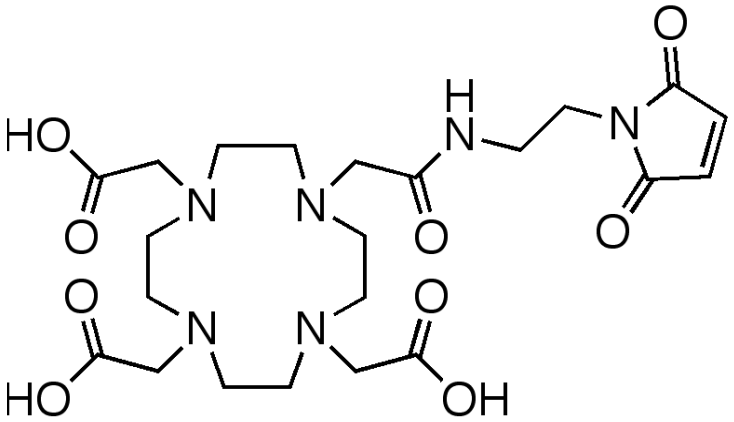
Live intact singlets
011411-PBMC-X8-duplex-TmLu_cells_found.txt
Event Count: 4519



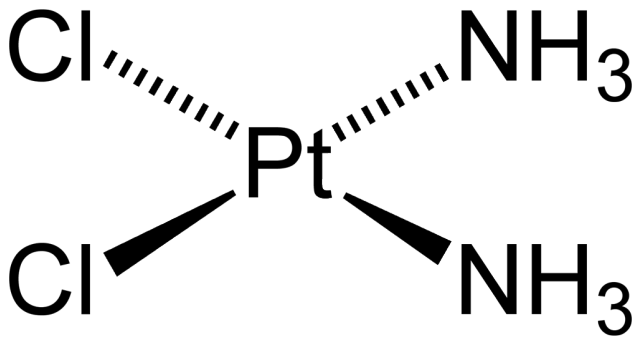
Live intact singlets
011411-PBMC-X8-duplex-TmLu_cells_found.txt
Event Count: 4519

X8

Live-Dead



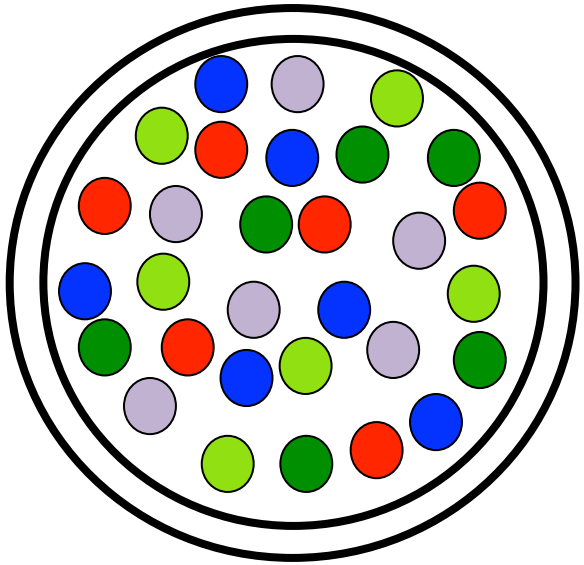
maleimide-DOTA



cisplatin

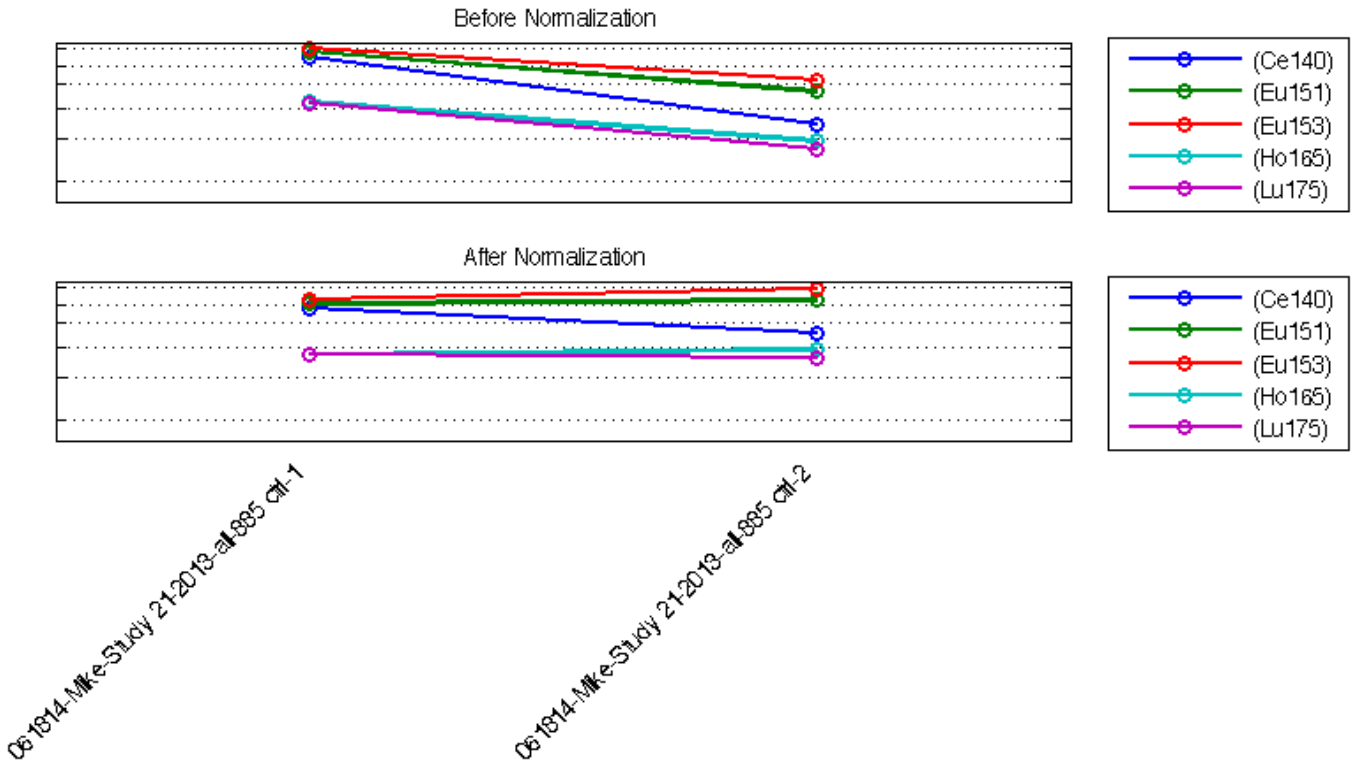
Normalization – EQ Four-Element Beads

- Polystyrene beads: contain elemental Ce, Eu, Ho, Lu
    - burn like cells, but are defined composition

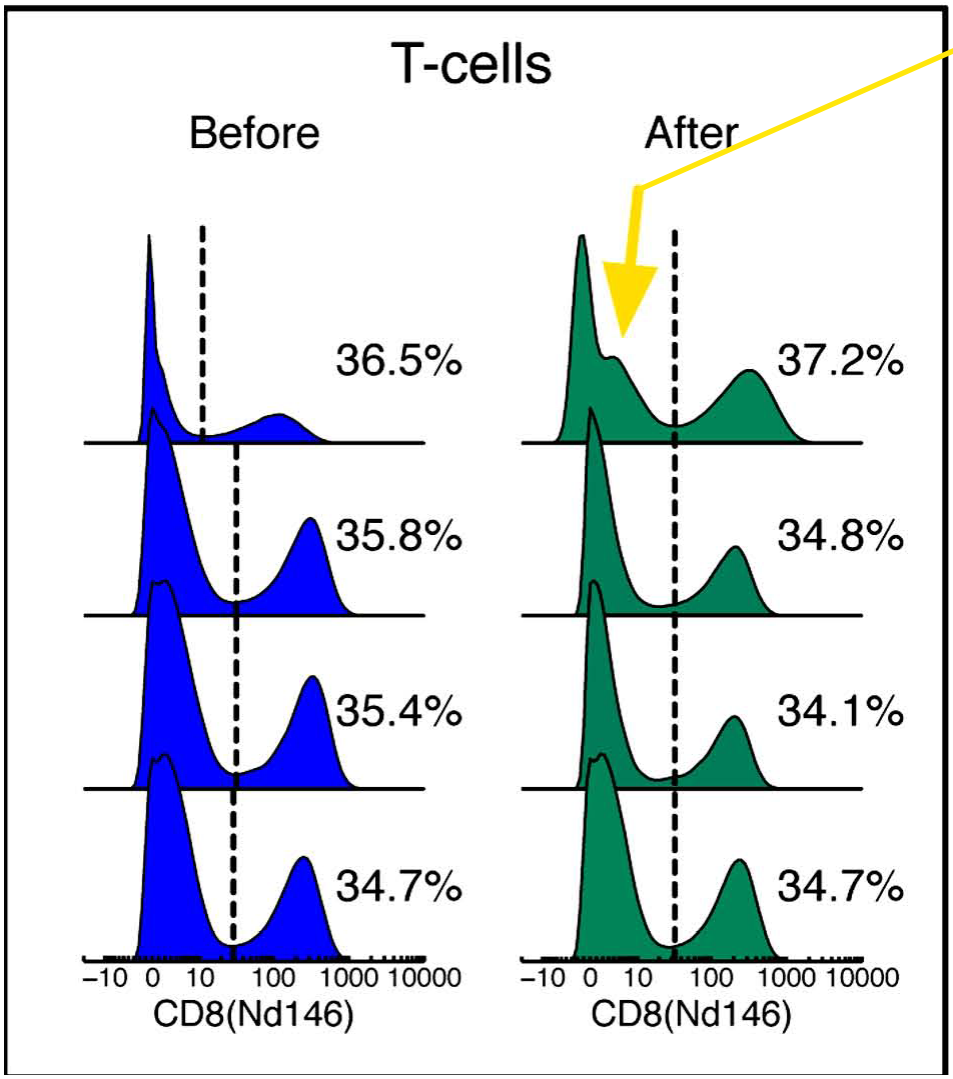


Normalization Beads - Long Runtime Decrease in Signal Intensity

10:33 AM 6:55 PM



Normalization Affects All Signals



Noise amplification!

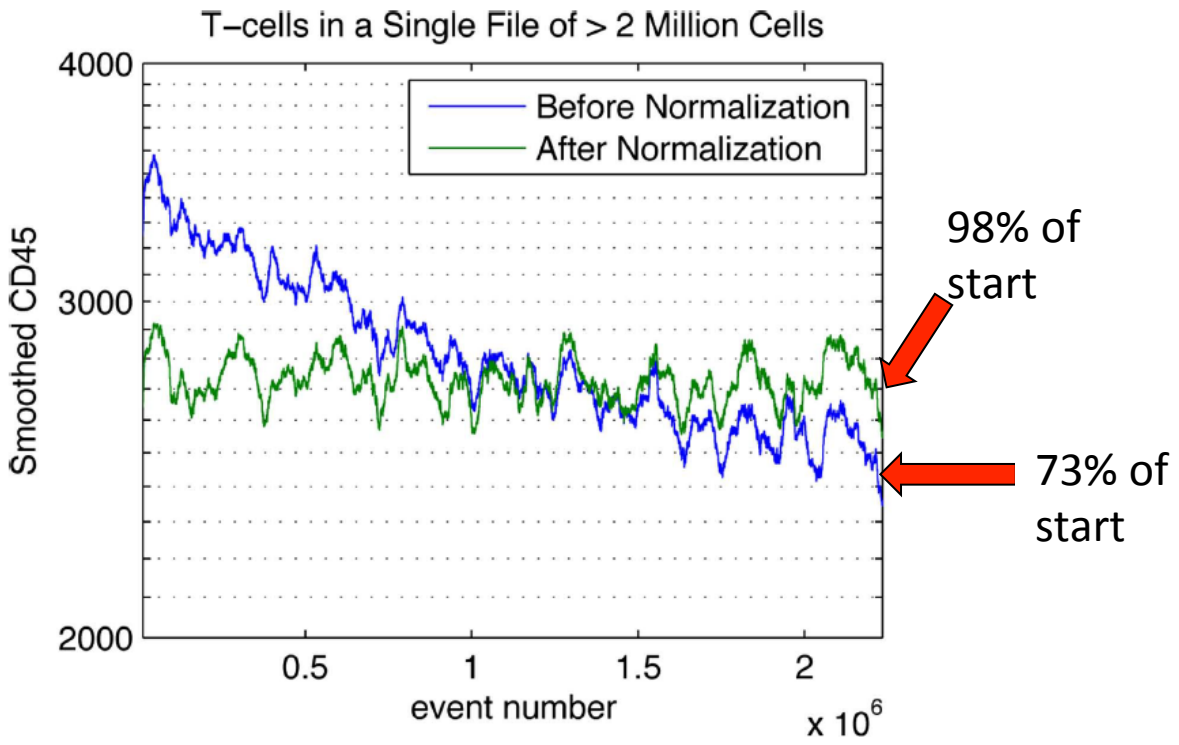
* Global Mean as baseline, rather than Minimum or Maximum
- limits noise amplification

Normalization CANNOT correct for improper machine cleaning/tuning (undetectable analyte signals).

Finck et al, *Cytometry A*, **2013**

Signal Decrease Over Very Long Run-Times

- Single file: >2 million cells = >3 hr



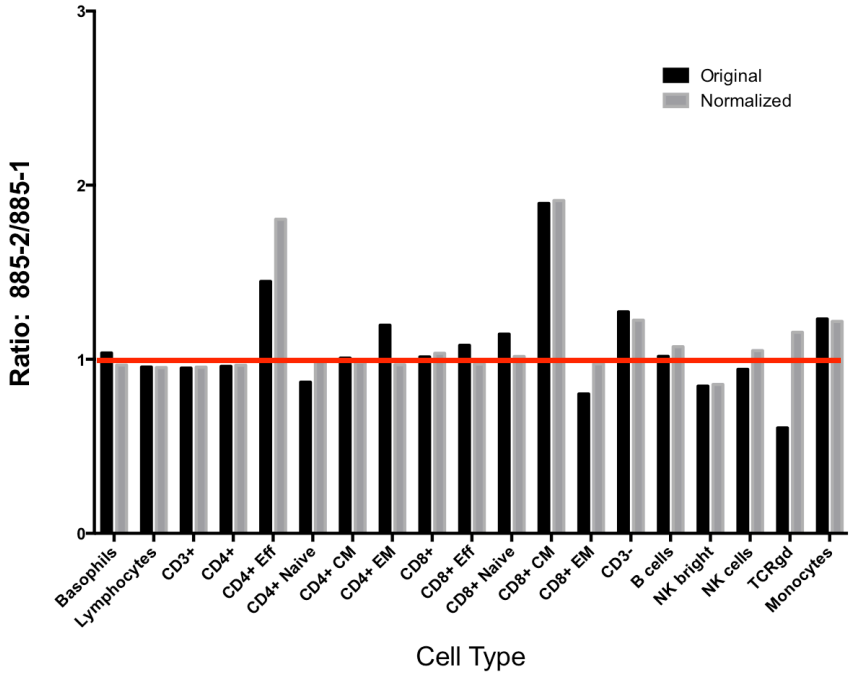
* Highlights need for moving window normalization

Finck et al, *Cytometry A*, **2013**

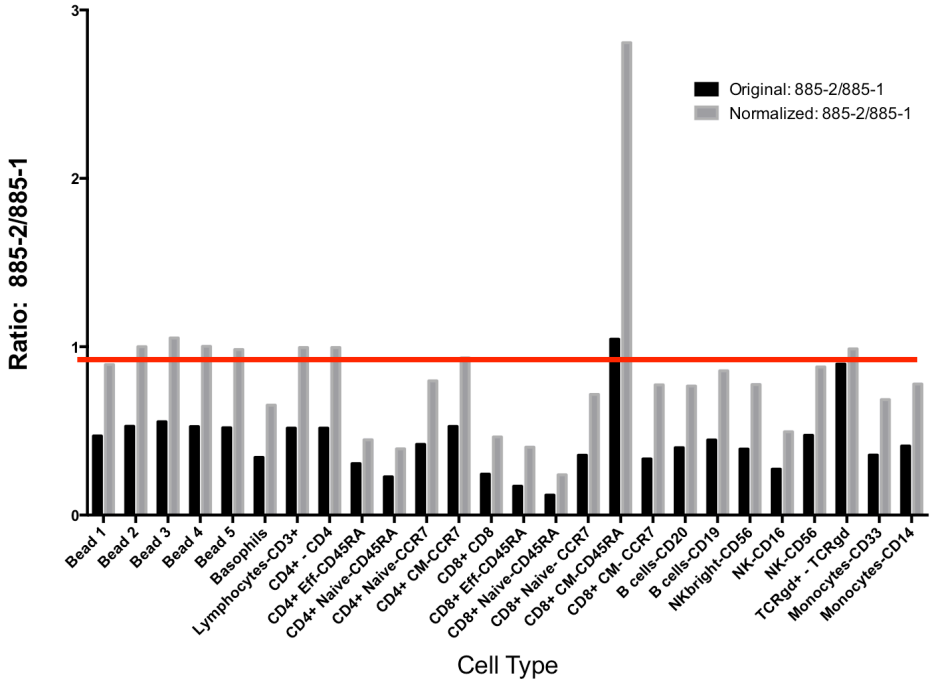
Effects of Normalization

- Control sample split in half, run 8.5hr apart; ratio after manual gating

Freq Parent



Median Intensity



* Freq Parent fairly robust;
only small gains from normalization

* Median Intensity more affected by normalization (phosphoflow...)

Part 4 – Sample Running

Running Samples

1. Warm up machine – ~15-20 min to create and maintain fully hot and stable plasma

2. Tune machine –maximize M signal while minimizing M+16 oxide formation

3. Finish washing cells – final wash in MilliQ water, then resuspension and dilution in MilliQ water.

   - residual buffer salts can affect Current value

4. Resuspend and filter immediately before injection

   - Reduces aggregates (clogs)

5. Appropriate dilution – minimize doublets

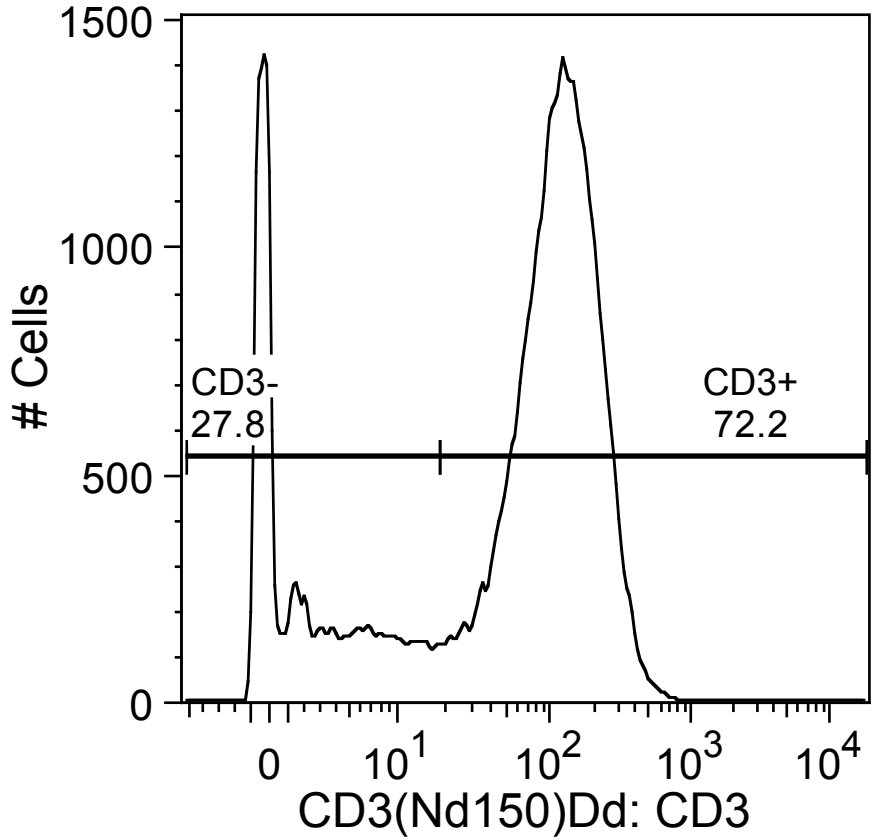
   - Around 1 cell event/screen refresh; usually ~750K/mL on cell counter

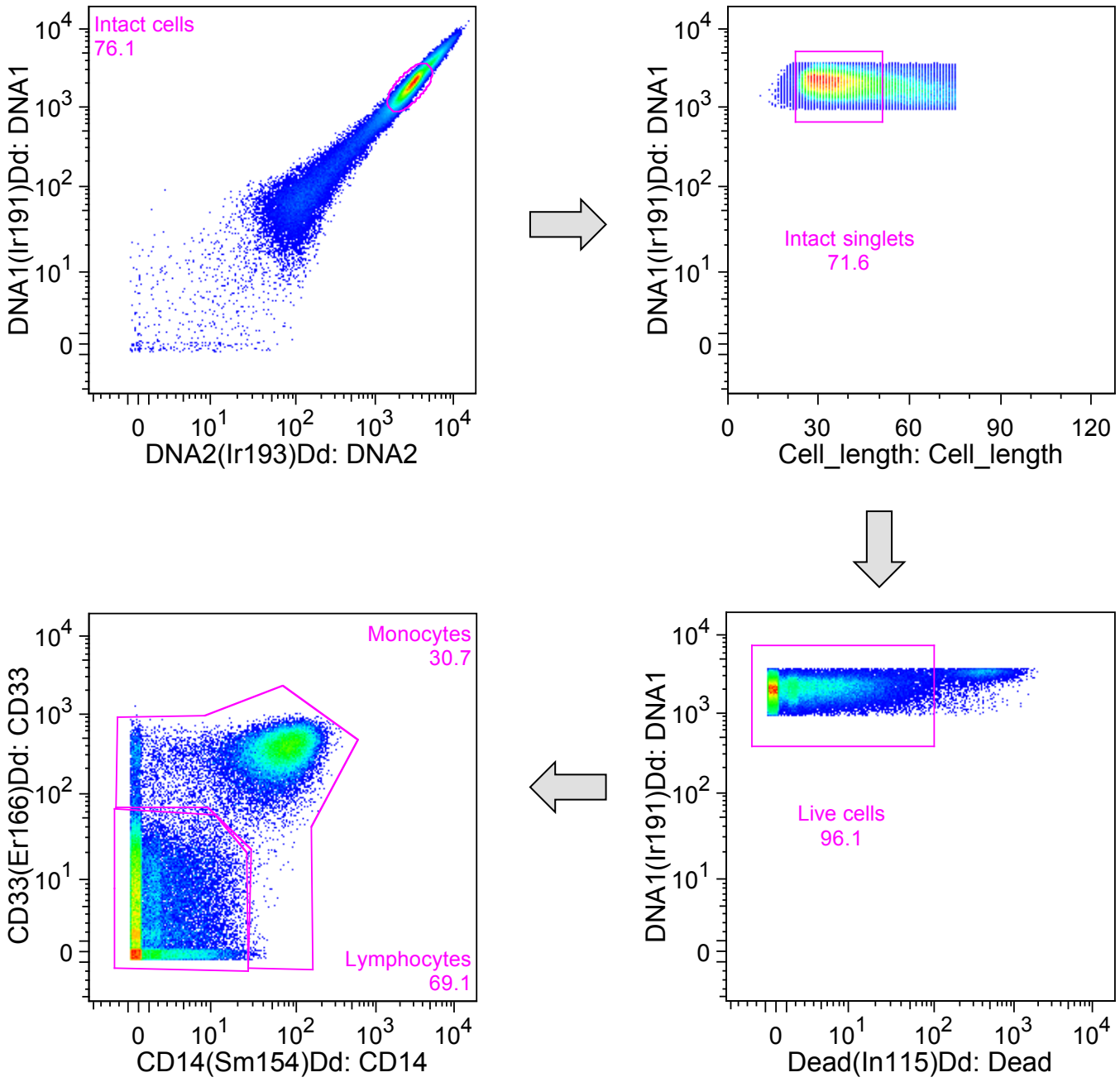
Standard CyTOF Output Appearance

- Spike at ~0 is legitimate ("true zero")

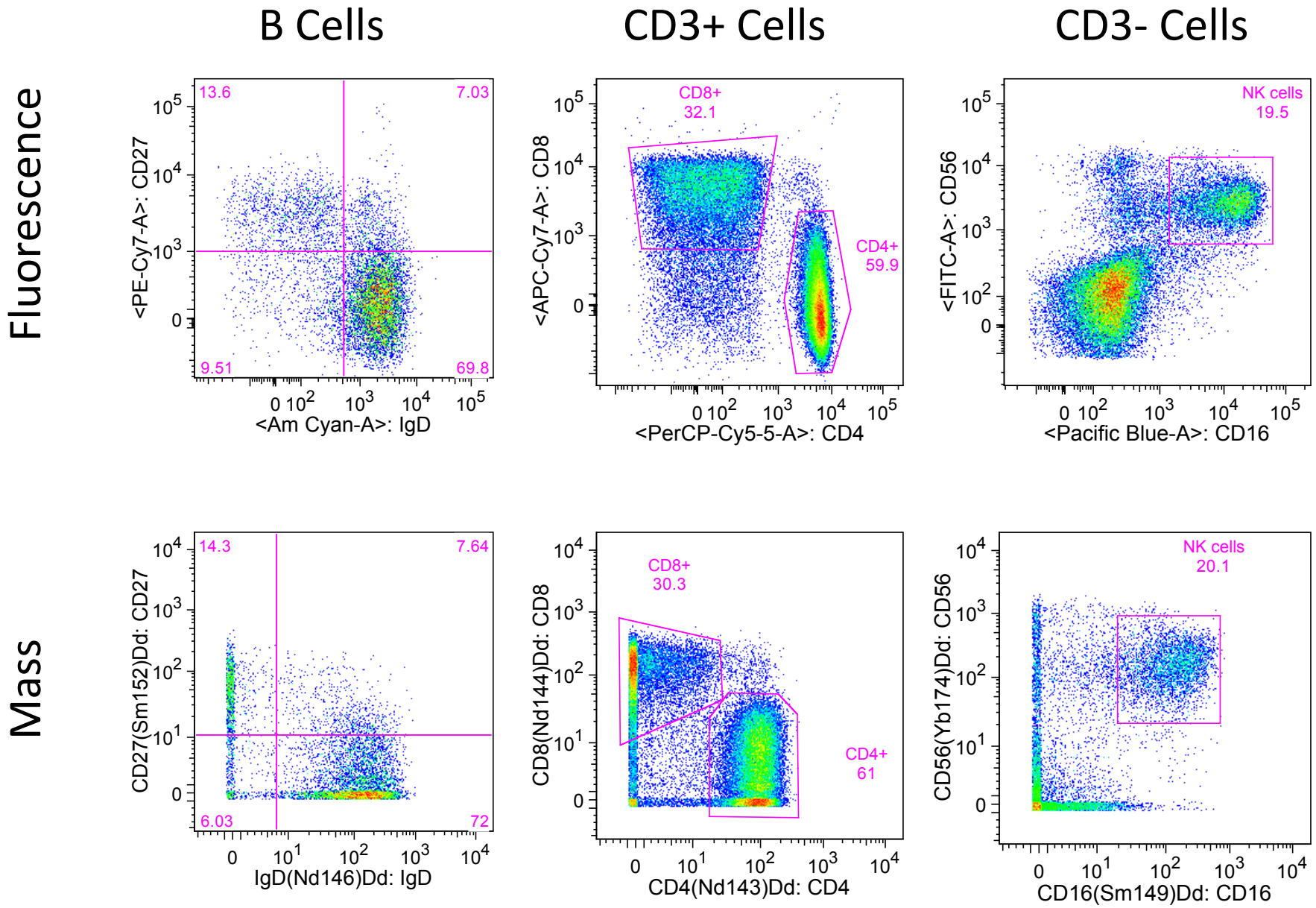
- Flow data is usually distributed above and below zero due to comps and auto-fluorescence.

* FlowJo settings must be changed for CyTOF data




Intact cells
76.1
DNA1(Ir191)Dd: DNA1
DNA2(Ir193)Dd: DNA2
Intact singlets
71.6
Cell_length: Cell_length
Live cells
96.1
Dead(In115)Dd: Dead
Monocytes
30.7
Lymphocytes
69.1
CD33(Er166)Dd: CD33
CD14(Sm154)Dd: CD14

CyTOF vs LSRII – Representative Examples



Flu 2010-2011 - CyTOF Phenotyping Panel

- Collapse 6 tube LSRII panel into 1 tube CyTOF panel – 23 markers

CD14
CD33

CD3
CD4
CD8
TCRgd

CD19
CD20

CD16
CD56

CD45RA
CCR7
CD25
CD127
CD27
CD28
CD38
CD85j

IgD
HLADR
CD24

CD94
CD161

Flu 2010, Flu 2011 Studies

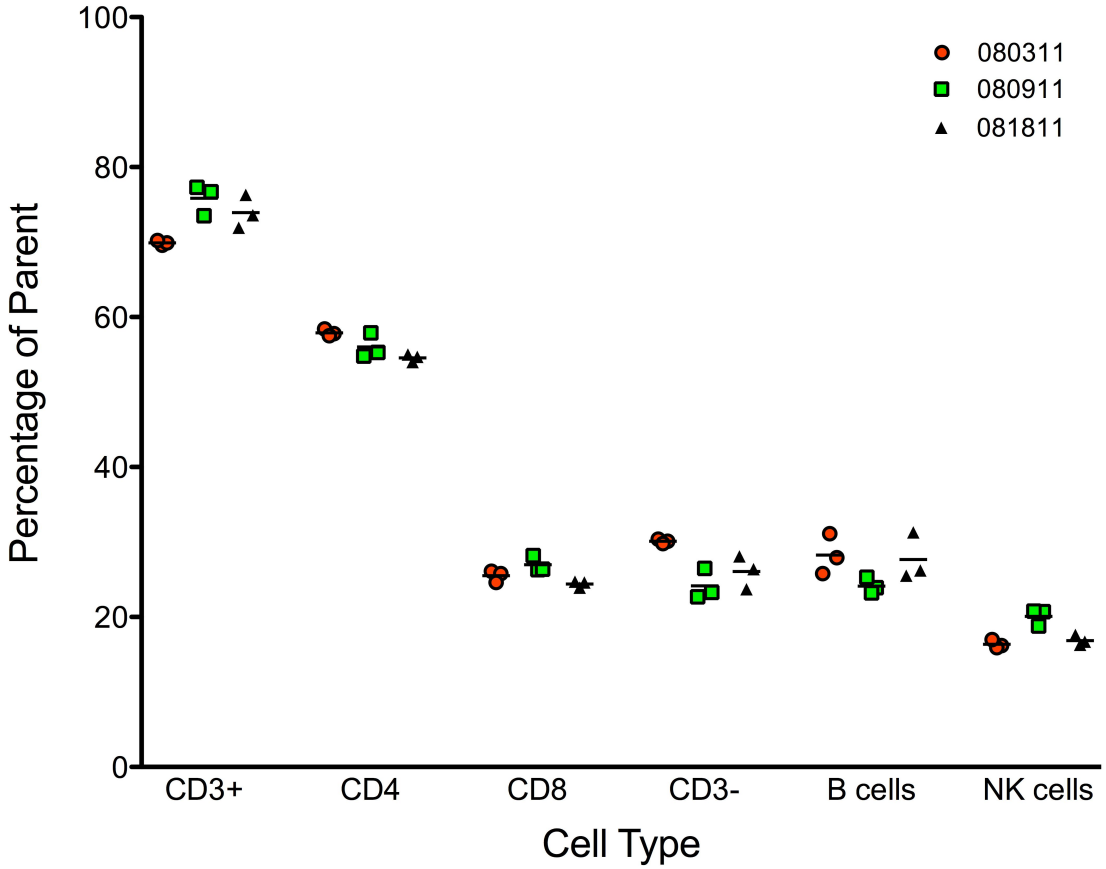
- On-going Flu vaccine studies – U19

- CyTOF Phenotyping – 2010, 2011 years
  - Day 0 (pre-vaccination) only – Study 15, 17, 18
  - Day 0, 1, 4, 7 – Study 23-2011

- 2010-2011: >300 unique donors – between-donor variability

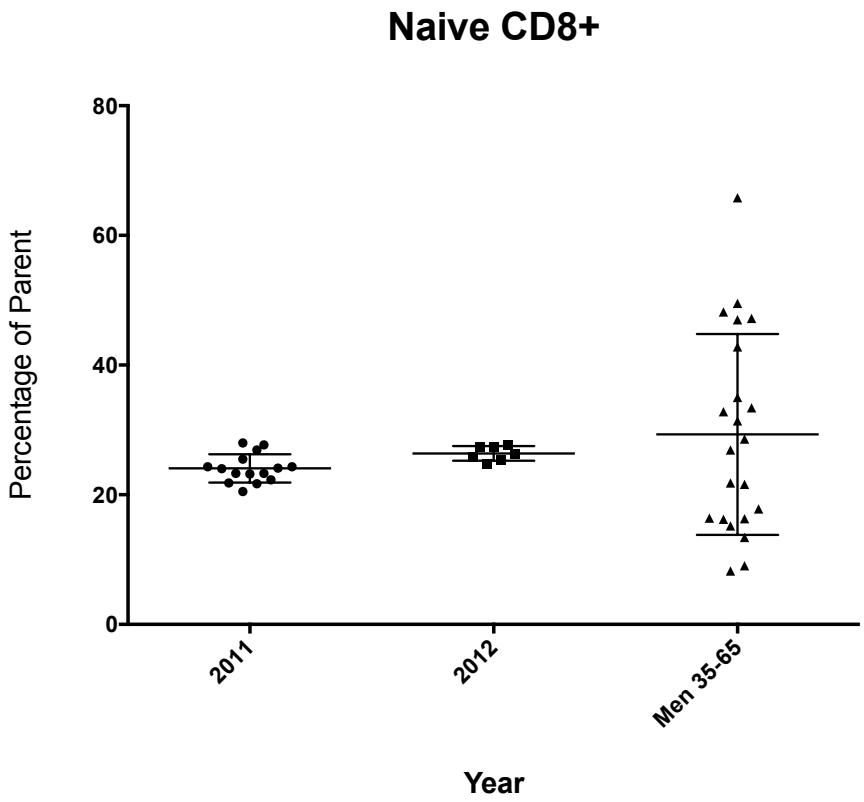
	Age Band (y/o)			
	<35	35-65	>65	Total
Male	62	22	46	130
Female	84	47	65	196
Total	146	69	111	326

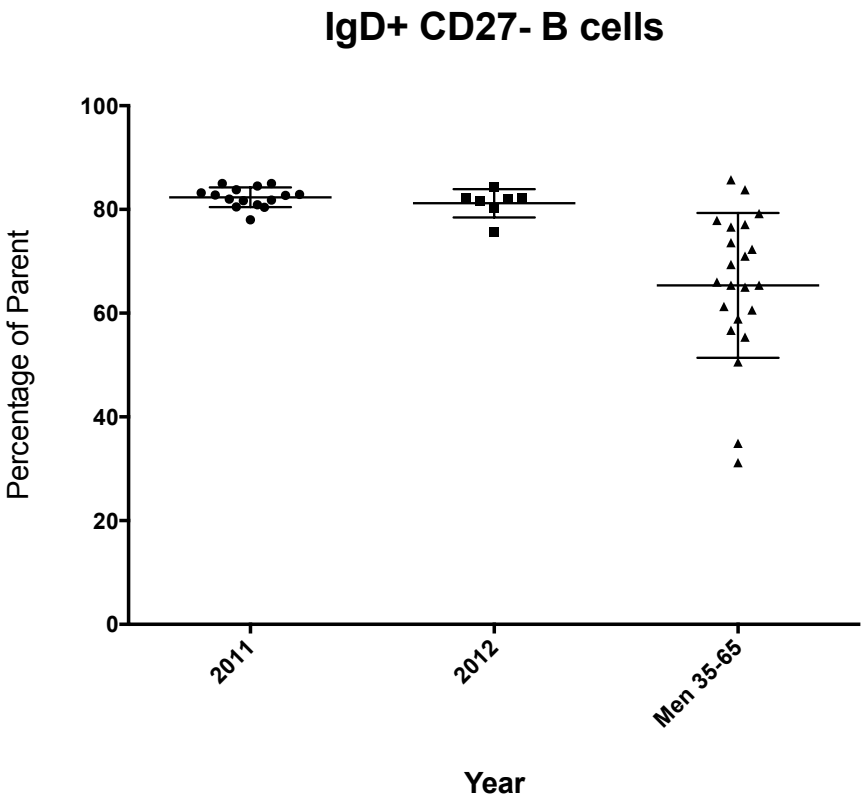
2010 Flu Studies – Consistency – 284 Control - Same Draw, Same Year Replicates



Flu 2011, 2012 Studies – Consistency – 885 Control

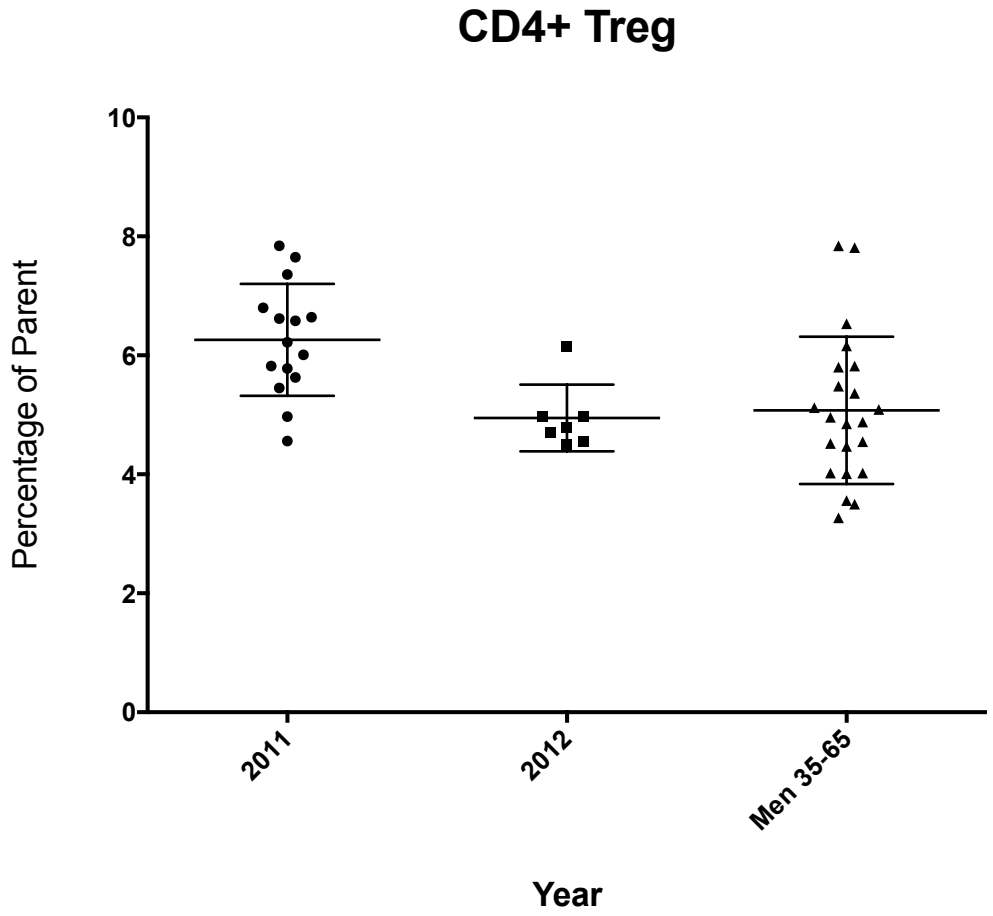
- Generally good year-to-year agreement from same 885 donor draw



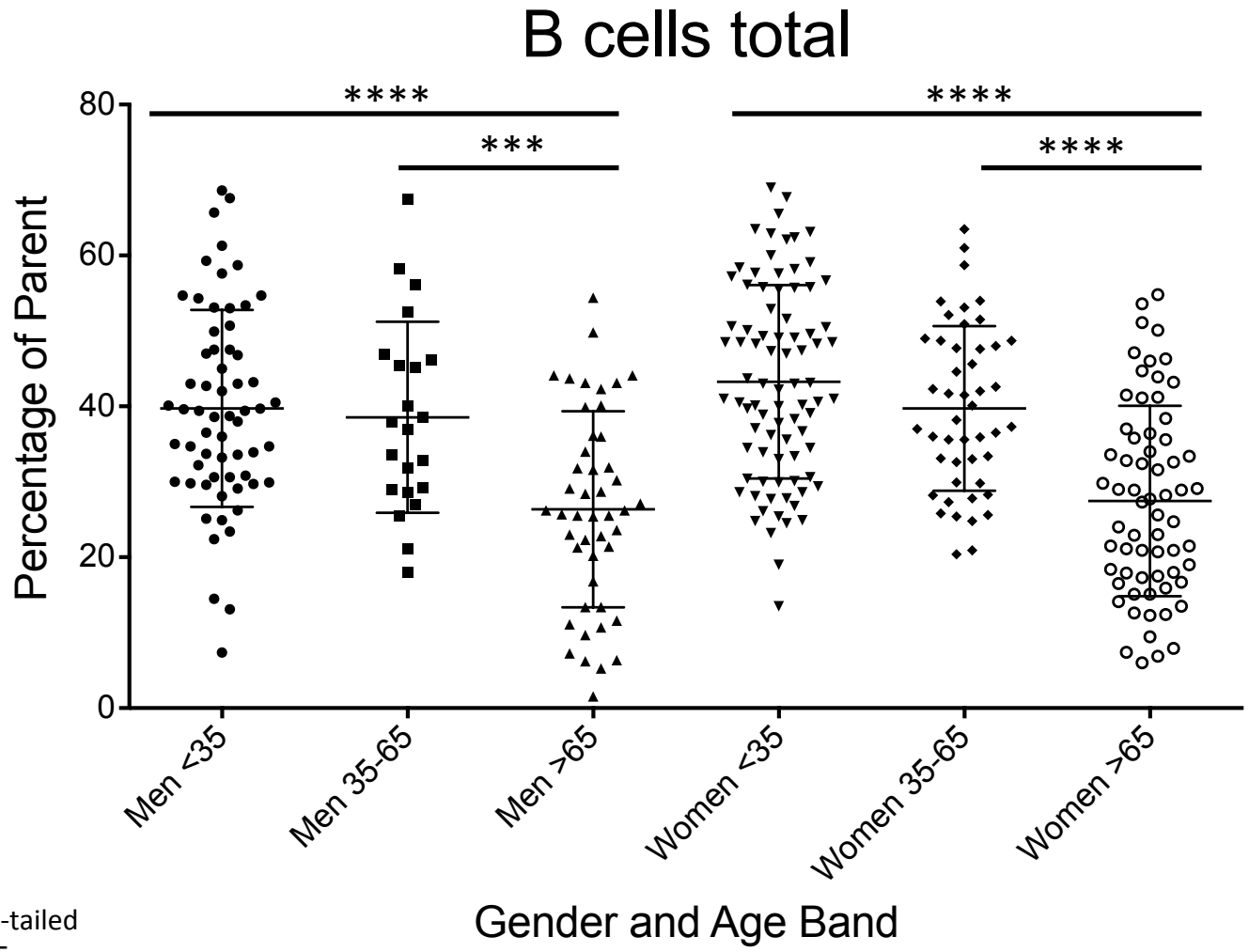


Flu 2011, 2012 Studies – Consistency – 885 Control

- Sometimes greater variability in less-frequent populations



Flu 2010, Flu 2011 Studies – Donor Variability



Mann-Whitney two-tailed

*	<0.05
**	<0.01
***	<0.001
****	<0.0001

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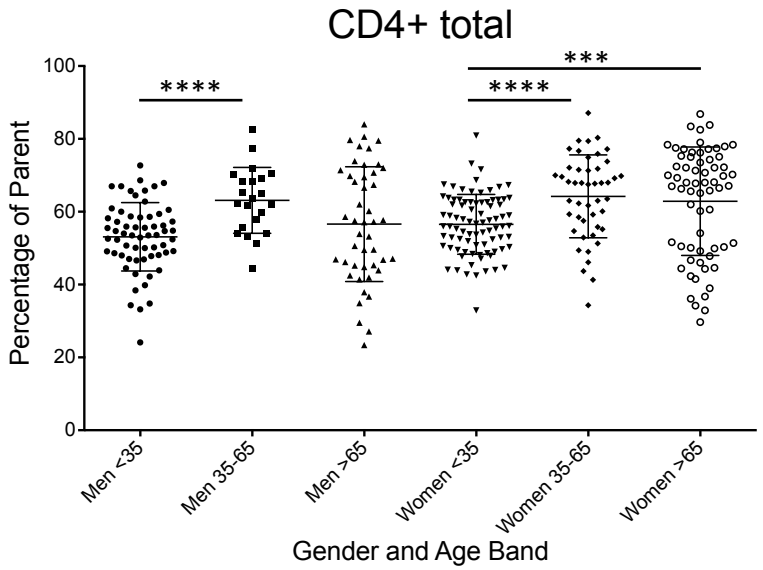

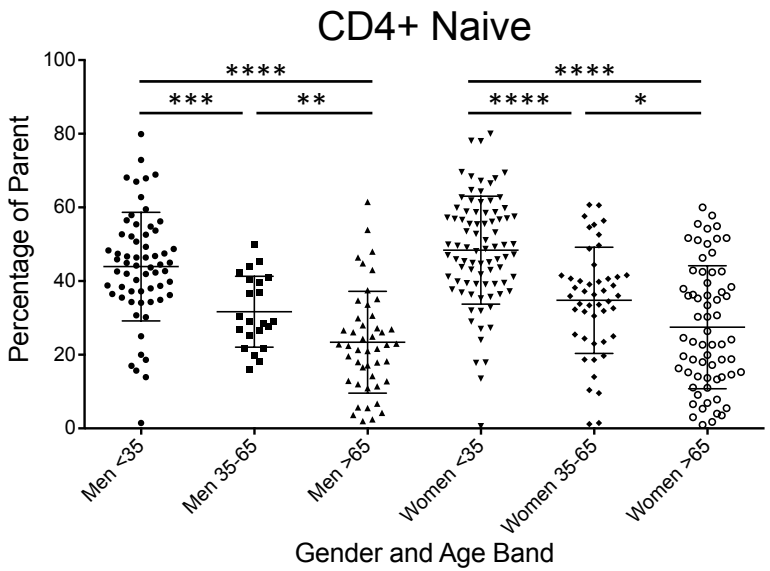

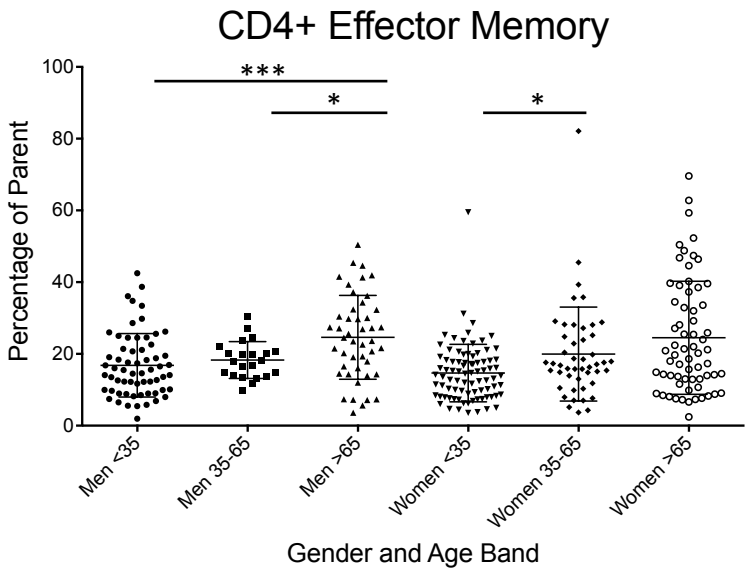

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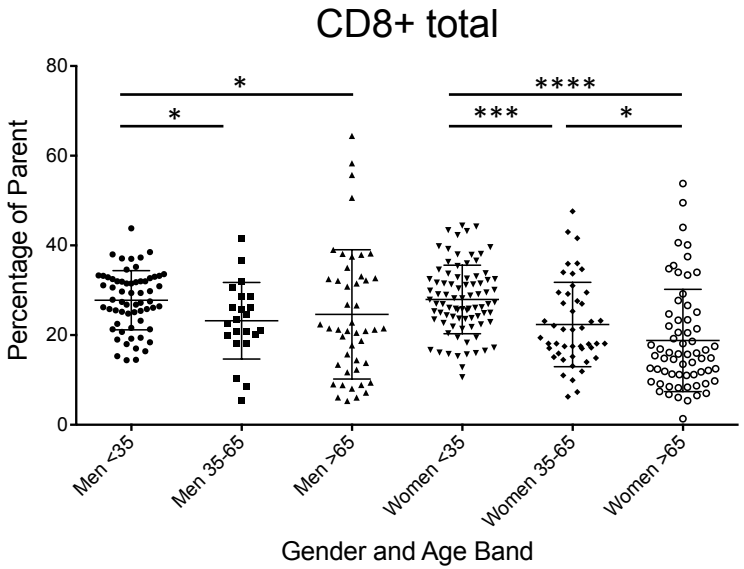
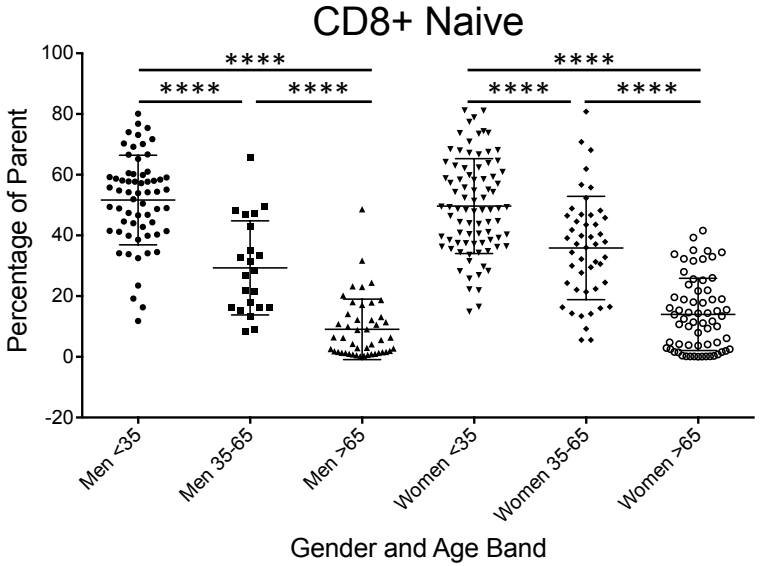
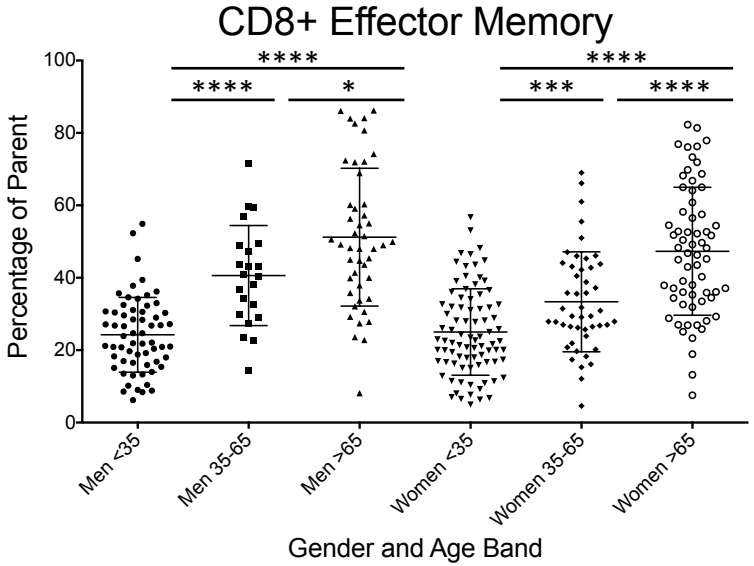
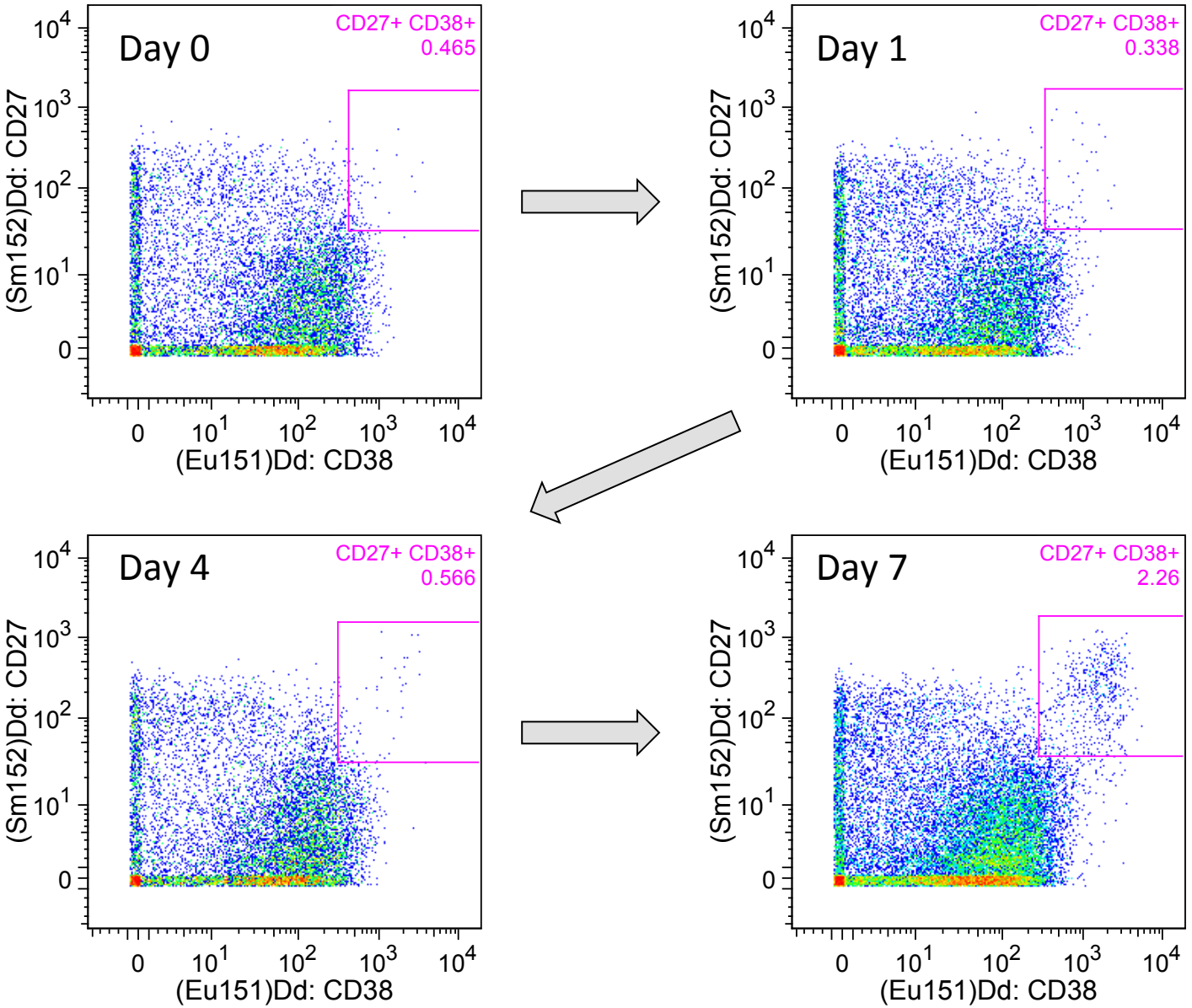






Study 23-2011



Conclusions

1. Proper tuning of the CyTOF ensures minimal sample oxidation.

2. Metal purity (both elemental and isotopic) is highly important.

3. The choice of metal label is dependent upon marker abundance, modality, and resolution needed (fold-change).

4. All samples must be stained with metals to be detected by the CyTOF. Proper fixation is important, as are MilliQ water washes.

5. Head-to-head comparisons with LSRII assays are consistent.

6. The Flu studies are an example of how a CyTOF panel can be used to monitor the surface phenotype for hundreds of donors to detect variations between gender, age-band, and time-point.

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    - Henrik Mei, Meena Malipotlalla, Holden Maecker

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    - Fluidigm/DVS Sciences

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