Mass cytometry

Dr. Henrik Mei
Deutsches Rheumaforschungszentrum Berlin (DRFZ)
mei@drfz.de
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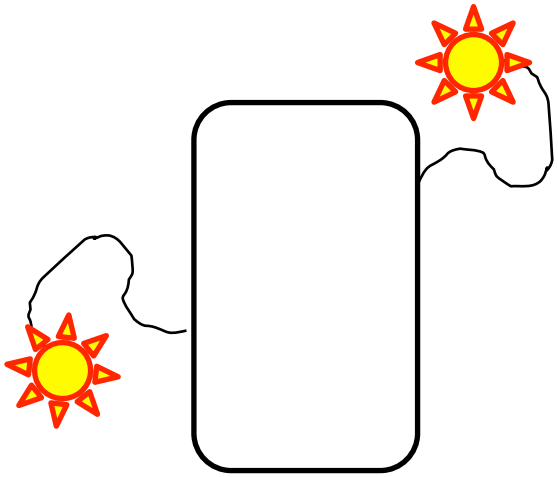
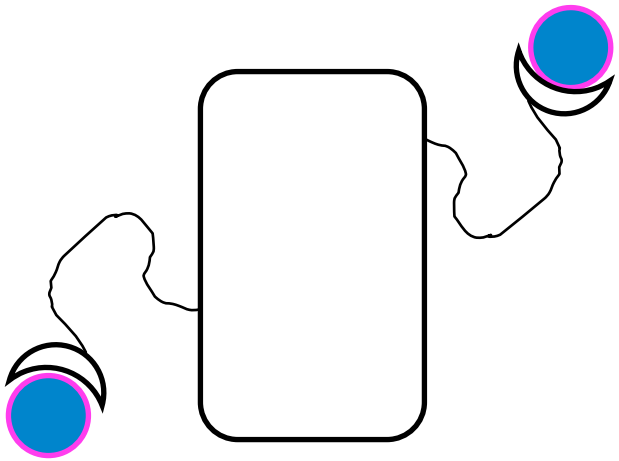
Two Different Approaches to Simultaneous Detection

Fluorophores



Fluorometer, Photodetector

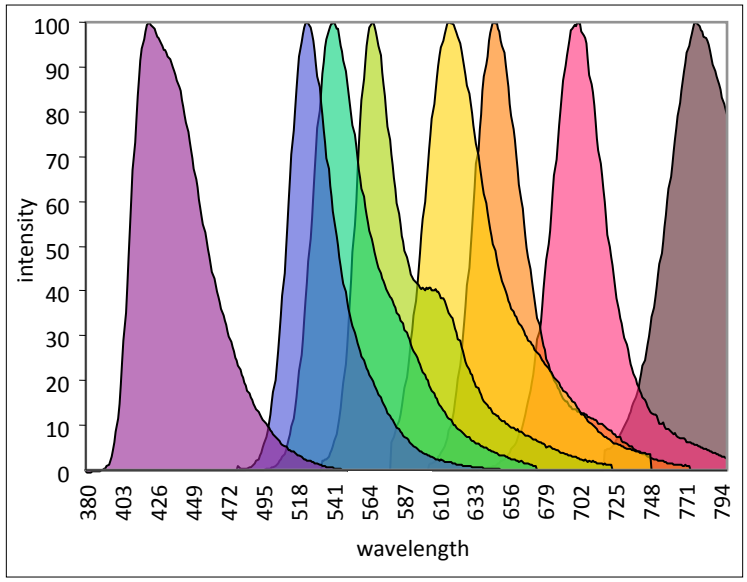
Elemental Tagging



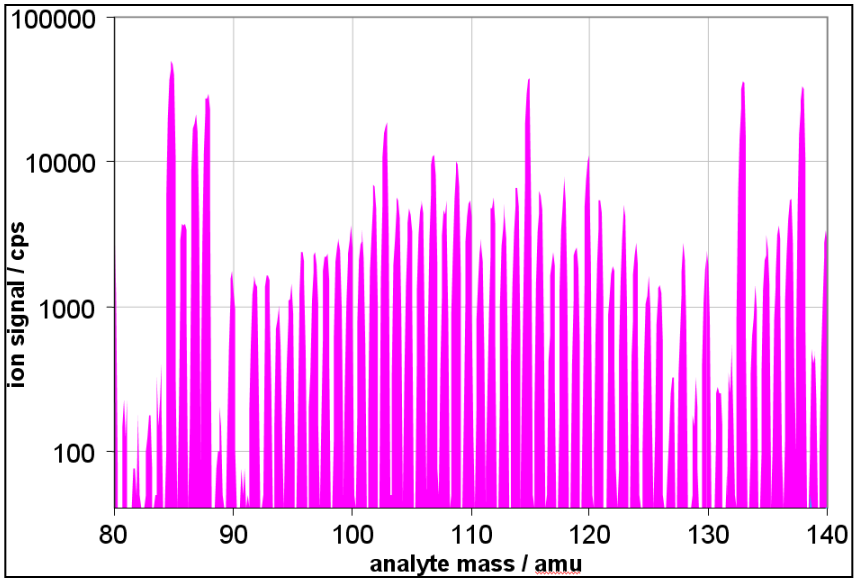
Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Two Different Approaches to Simultaneous Detection

8 Alexa Fluorophores



Elemental Tagging



Usually Lanthanides

LSR II

CyTOF

Single Cell Analysis - CyTOF™ Instrument

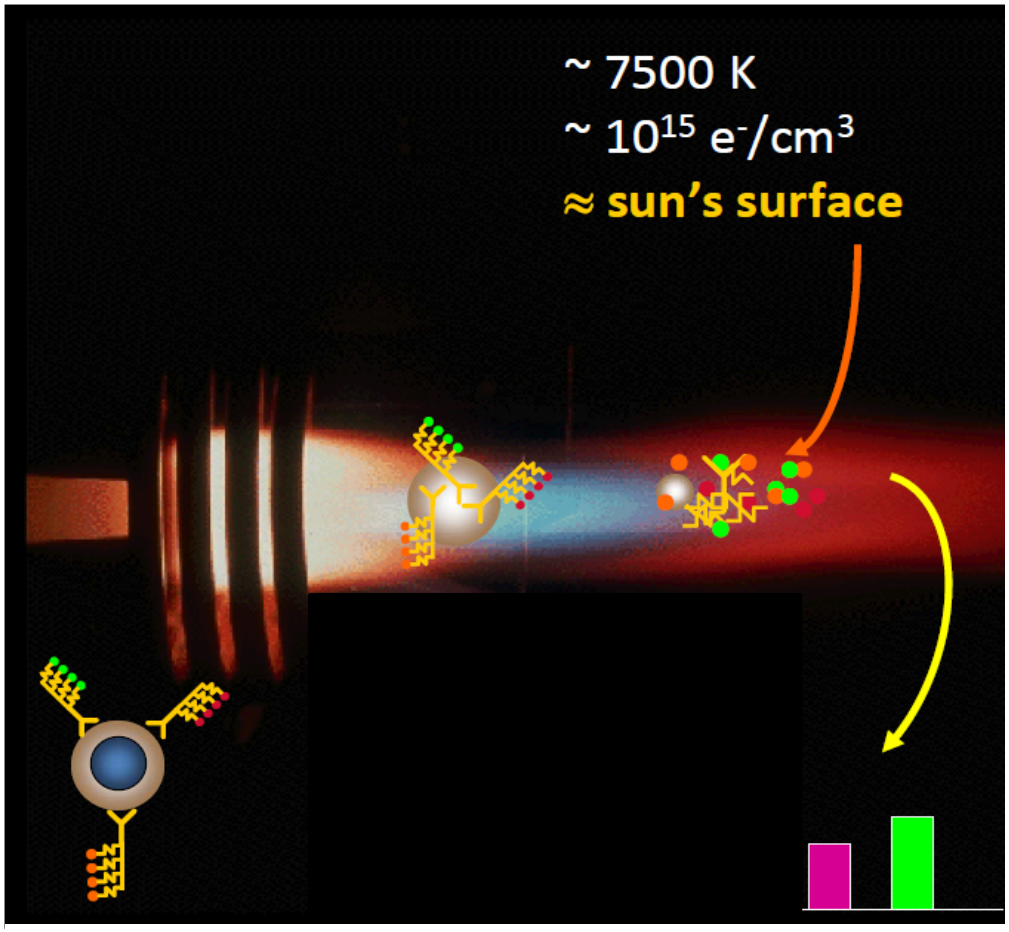


Figure courtesy O. Ornatsky

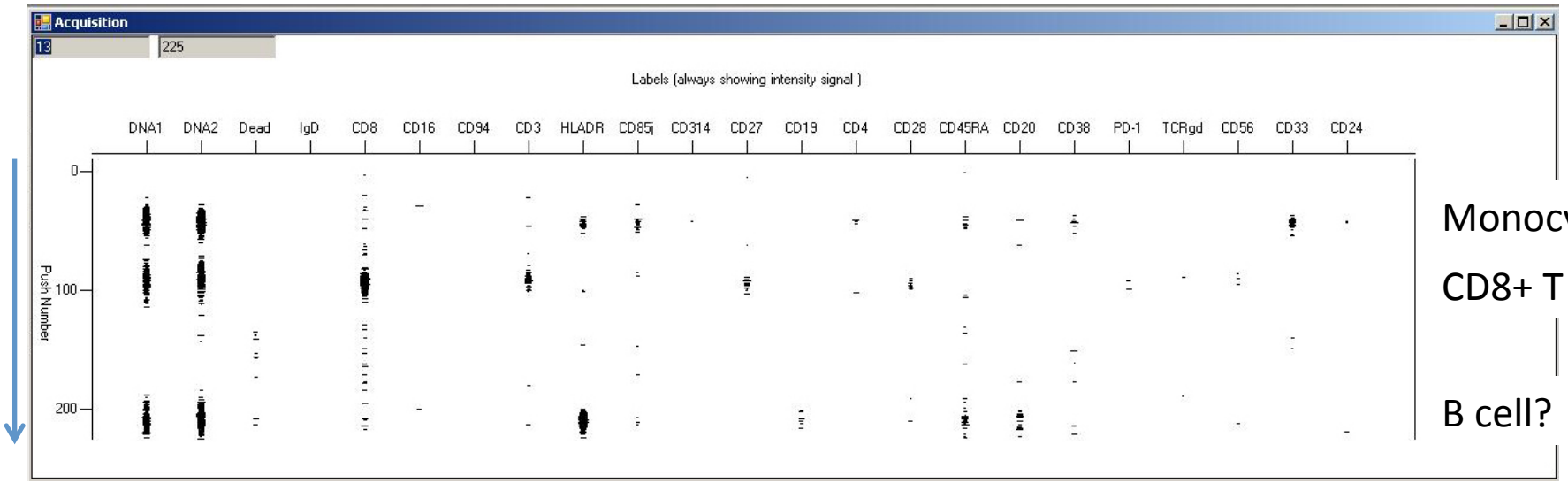
Flow Interface to ICP-MS

- Nebulizer – vaporizes

- Argon Plasma – atomizes and ionizes

- Mass analyzer

Display of Cell Events during Measurement



-"Push" analogous to time: 76,800 pushes/second: 220 displayed here (1/350 of data)

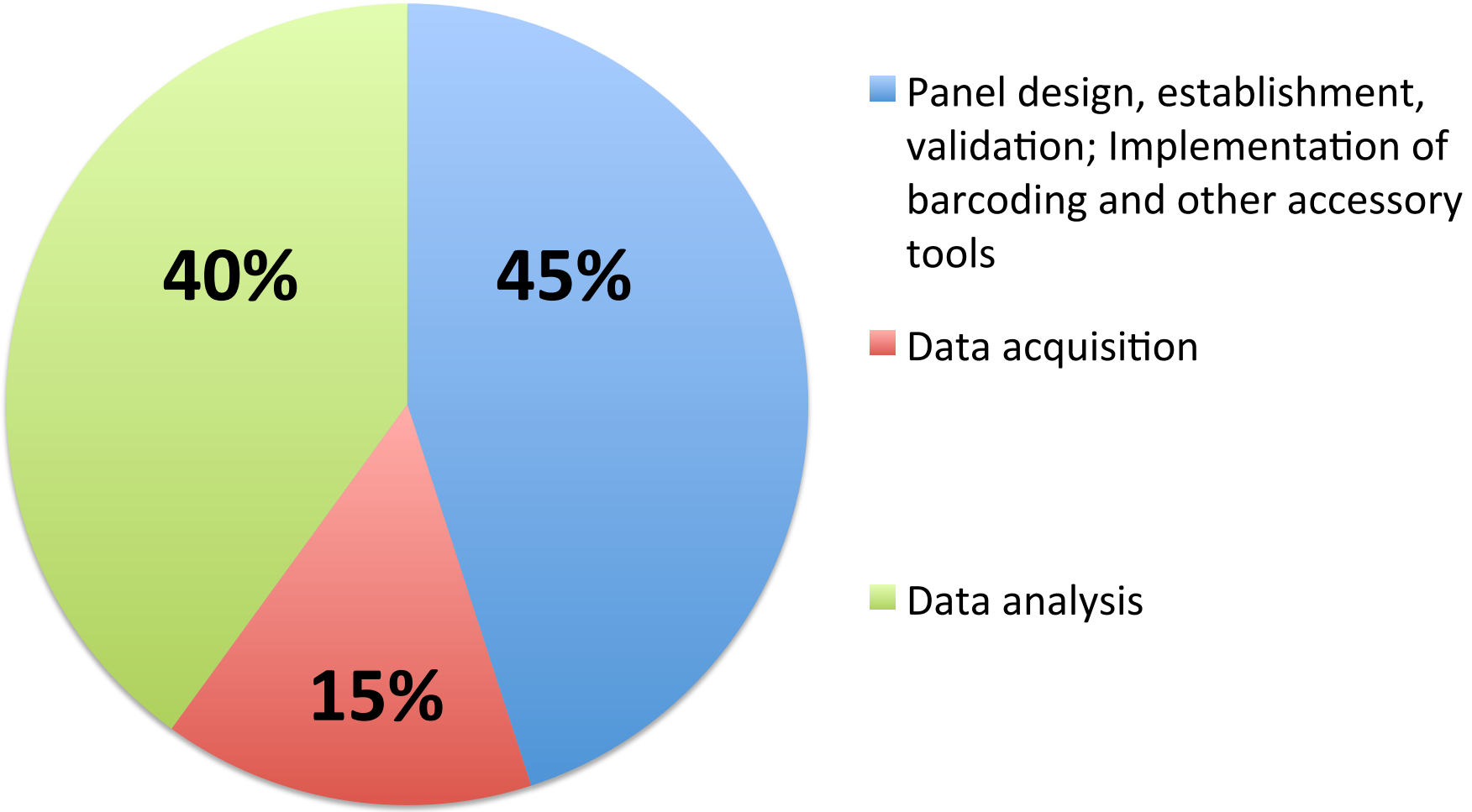
Pros of mass cytometry

- Advantages:
  - Minimal "spectral" overlap – higher dimensionality (~50 out of 135 channels currently used)
  - Quantitative – broad dynamic range
  - Not light- or time-sensitive
  - Minimal background
    no analogy to autofluorescence: low if any biological background for lanthanides

Cons of mass cytometry

- Disadvantages:
  - Destructive: (currently) no way to recover interesting cells
  - Slower data acquisition: 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 instrument get counted
  - Ion transmission efficiency: only ~4-5 in 10,000 ions that enter machine get counted.
  - No "Live gating"; therefore, events registered by CyTOF contains debris, doublets, et c; might only get ~50% of total events as "live single cells"
  - No analogy to FSC or SSC: MUST have marker (M^{n+}) for any gating

Three phases of mass cytometry projects



FACS vs. CyTOF

Technology		Fluorescence flow cytometry	Mass cytometry
Measurement basis		Fluorescent probes	Stable mass isotope probes
Experimental design			
Max no. of measurements		20 (18 fluorescence)	37 (including DNA)
Theoretical no. of subsets[a]		2.6×10^5	1.4×10^{11}
Panel design complexity (no. of probes)	Easy	<8	37
	Moderate	8–12	
	Hard	12–18	
Sensitivity range for different probes[b]		0.1–10	1–2
Sample throughput			
Sampling efficiency		> 95%	< 30%
Measured cells/s		25 000	500–1000

Live cell measurement possible

All samples fixed and permeabilized and washed in MilliQ water

Bendall et al., Trends Immunology 2012

Algorithmic data analysis tools are very helpful but require a high degree of data consistency

Algorithms for flow cytometry (Aghaeepour Nat. Methods 2013)

Dimensionality reduction

- Principal component analysis (PCA)
- t-SNE (stochastic neighbor embedding) → viSNE & Accense (Amir Nat Biotech. 2013, Shekhar et al., PNAS 2014)

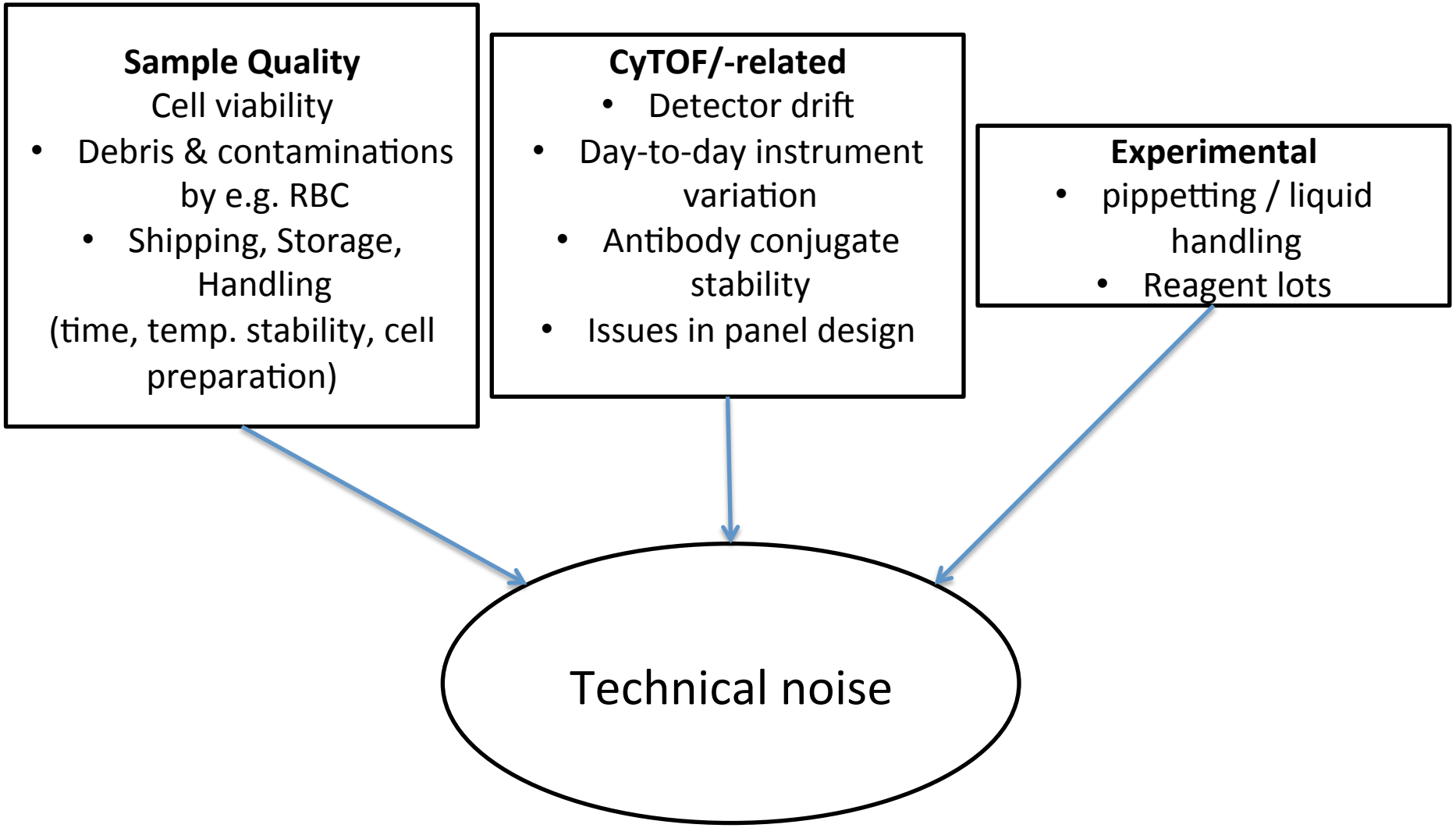
Clustering

- SPADE (Qiu et al., Nat. Biotech. 2011, Bendall et al., Science 2011)
- Citrus (enables group comparisons, statistical equipment (Bruggner et la., PNAS 2014)
- ImmunoCLust (Soerensen et al., Cytometry A 2015)
- Swift (Mosmann et al., Cytometry 2014)
- developmental / time dimension: Wanderlust (Bendall Cell 2014), Zunder et al., Cell Stem Cell 2015)

Cytobank.org (Kotecha et al.)

Overviews: Chester & Maecker, JI 2015; Diggins et al., Methods 2015

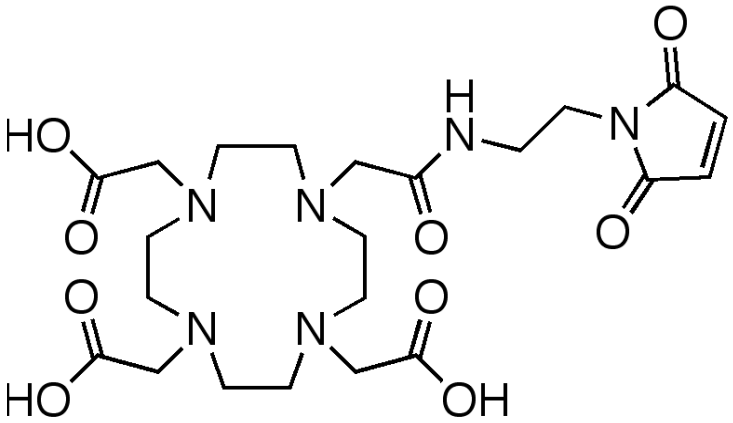
Sources of unwanted variability in data



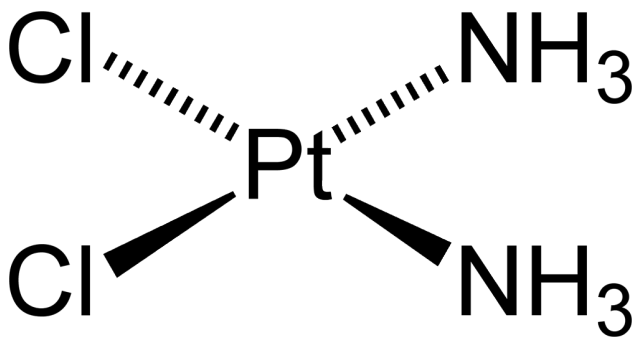
The first law of (flow) cytometry applies:

Garbage in – Garbage out!

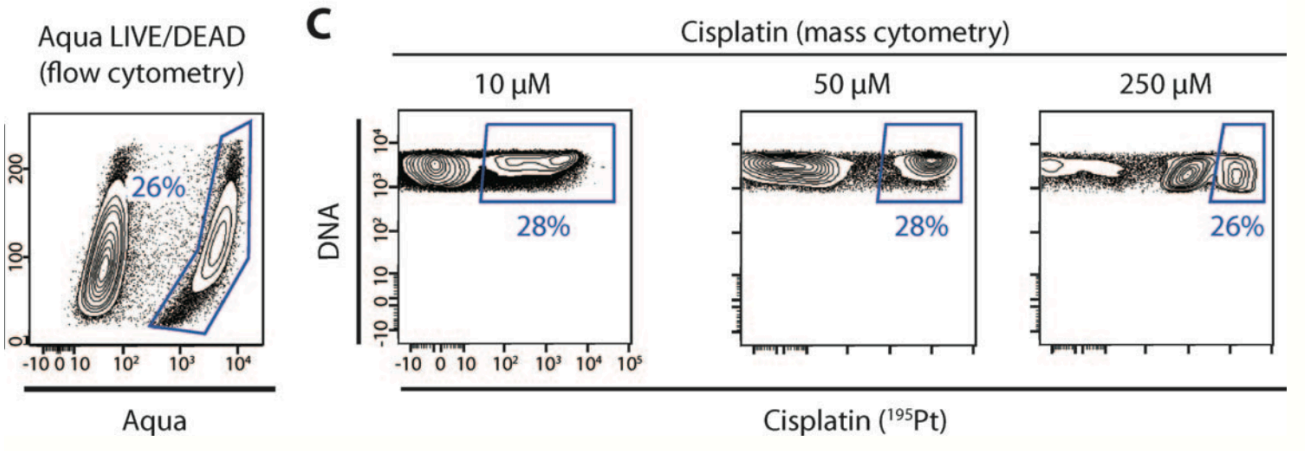
Live-Dead staining reagents



maleimide-DOTA



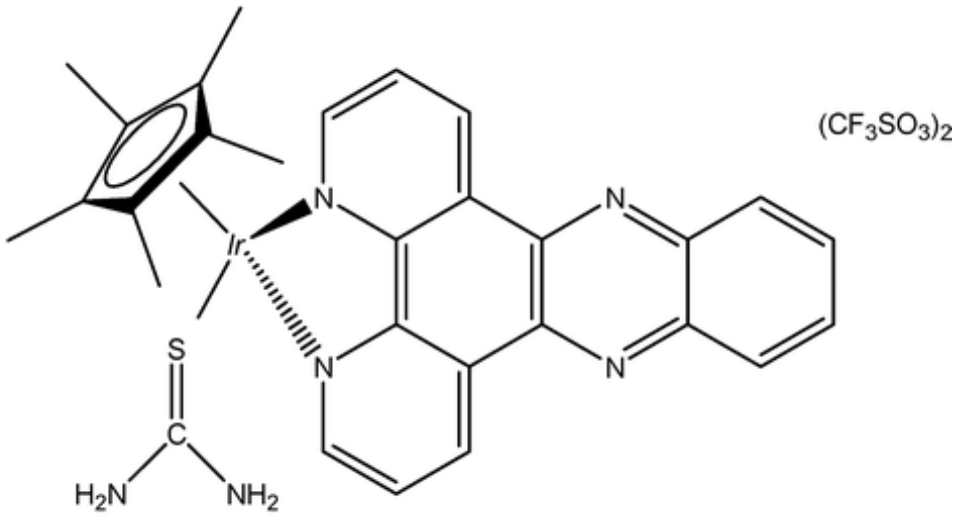
cisplatin



Fienberg et al., Cytometry A, 2012

Metal Staining of Cells - DNA Intercalator

Including cells



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

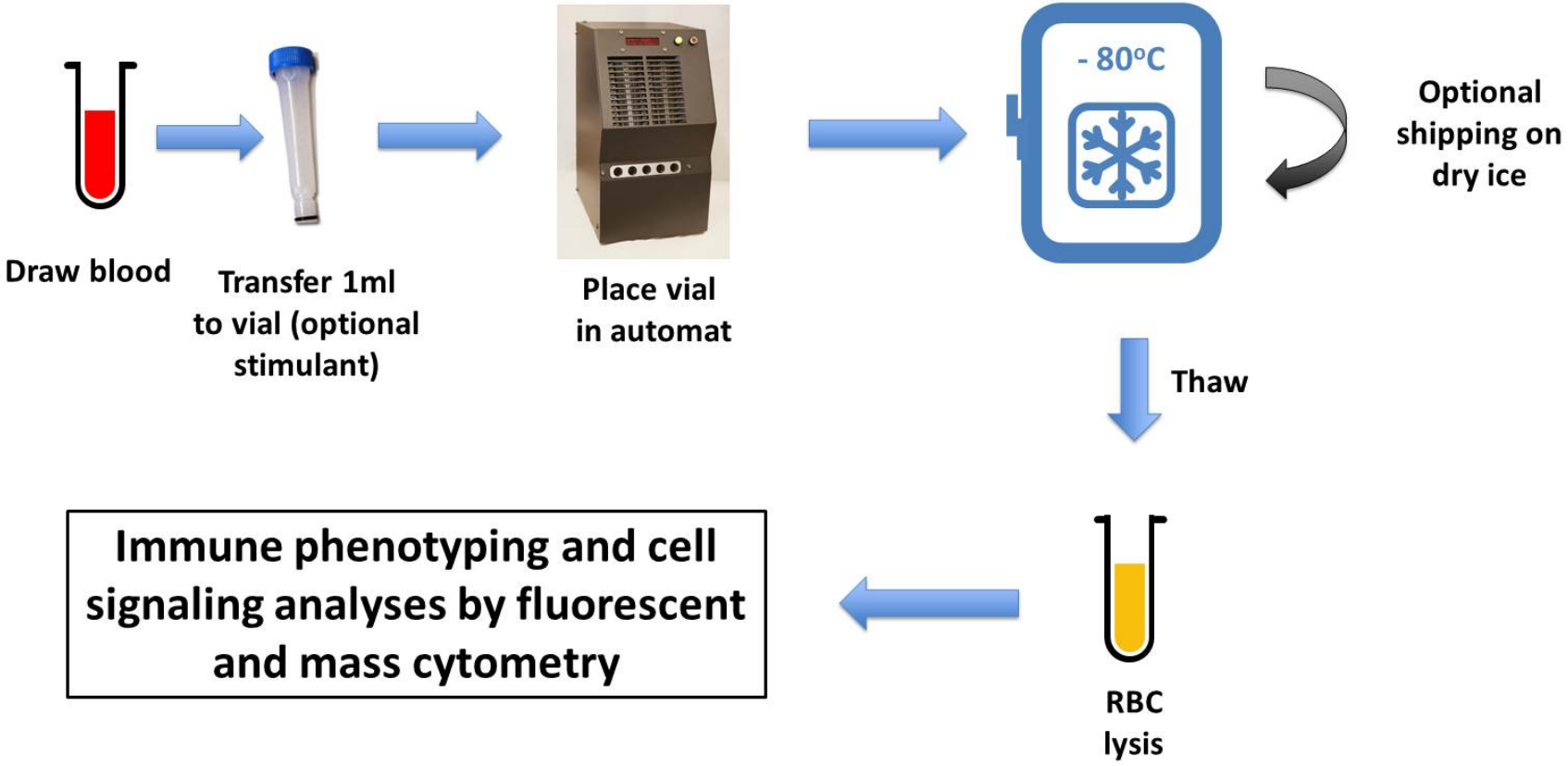
- No nucleus?
  → Specific antibody, dead cell reagent post-fix

Schaferet al *J.Organomet.Chem.,* **2007**, *692*, 1300−1309
Ornatsky et al, *Anal. Chem.*, **2008**, *80*, 2539–2547

Excluding unwanted cells / events

use a DUMP channel
carefully optimize reagent concentrations

Sample consistency
- Cryopreservation of native or *in vitro* stimulated whole blood -



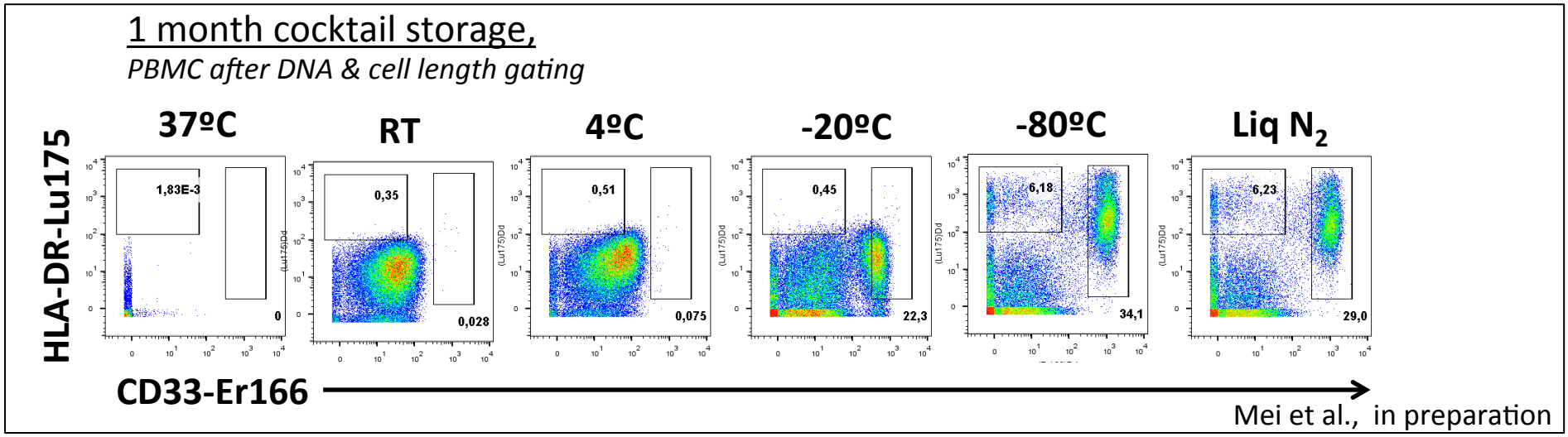
SmartTube Inc., no COI

Mei et al., manuscript in preparation

Antibody cocktail consistency

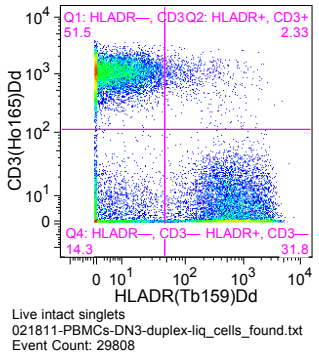
More parameters → higher chance of error in cocktail preparation

For repeat assays, e.g. longitudinal studies

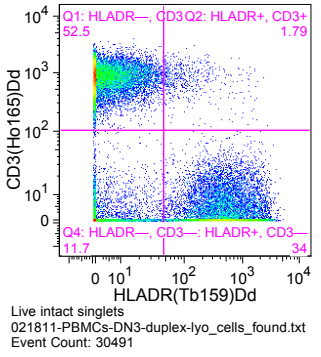


Candidate procedures

- Freezing cocktails
- Lyosphere (Biolyph LLC)
- Dry-down (Beckman Coulter)
- In-house lyophilisation

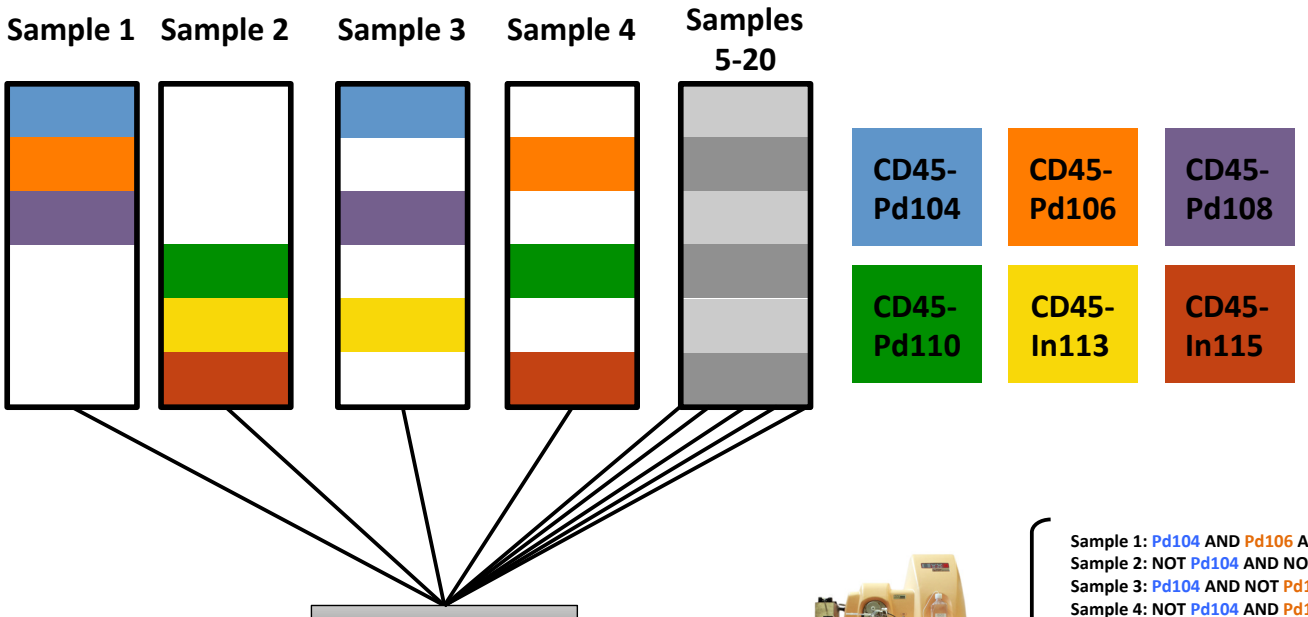


Liquid



Lyophilized

Harmonizing sample preparation and acquisition by sample barcoding and pooling



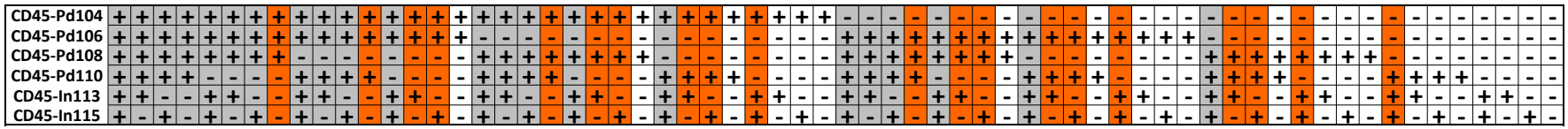
CD45-Pd104 | CD45-Pd106 | CD45-Pd108

CD45-Pd110 | CD45-In113 | CD45-In115

Combinatorial staining with six different CD45 Abs for barcoding of 20 samples

Sample 1: Pd104 AND Pd106 AND Pd108 AND NOT Pd110 AND NOT In113 AND NOT In115
Sample 2: NOT Pd104 AND NOT Pd106 AND NOT Pd108 AND Pd110 AND In113 AND In115
Sample 3: Pd104 AND NOT Pd106 AND Pd108 AND NOT Pd110 AND In113 AND NOT In115
Sample 4: NOT Pd104 AND Pd106 AND NOT Pd108 AND Pd110 AND NOT In113 AND In115
…
…
…
…
…
…
Sample 20

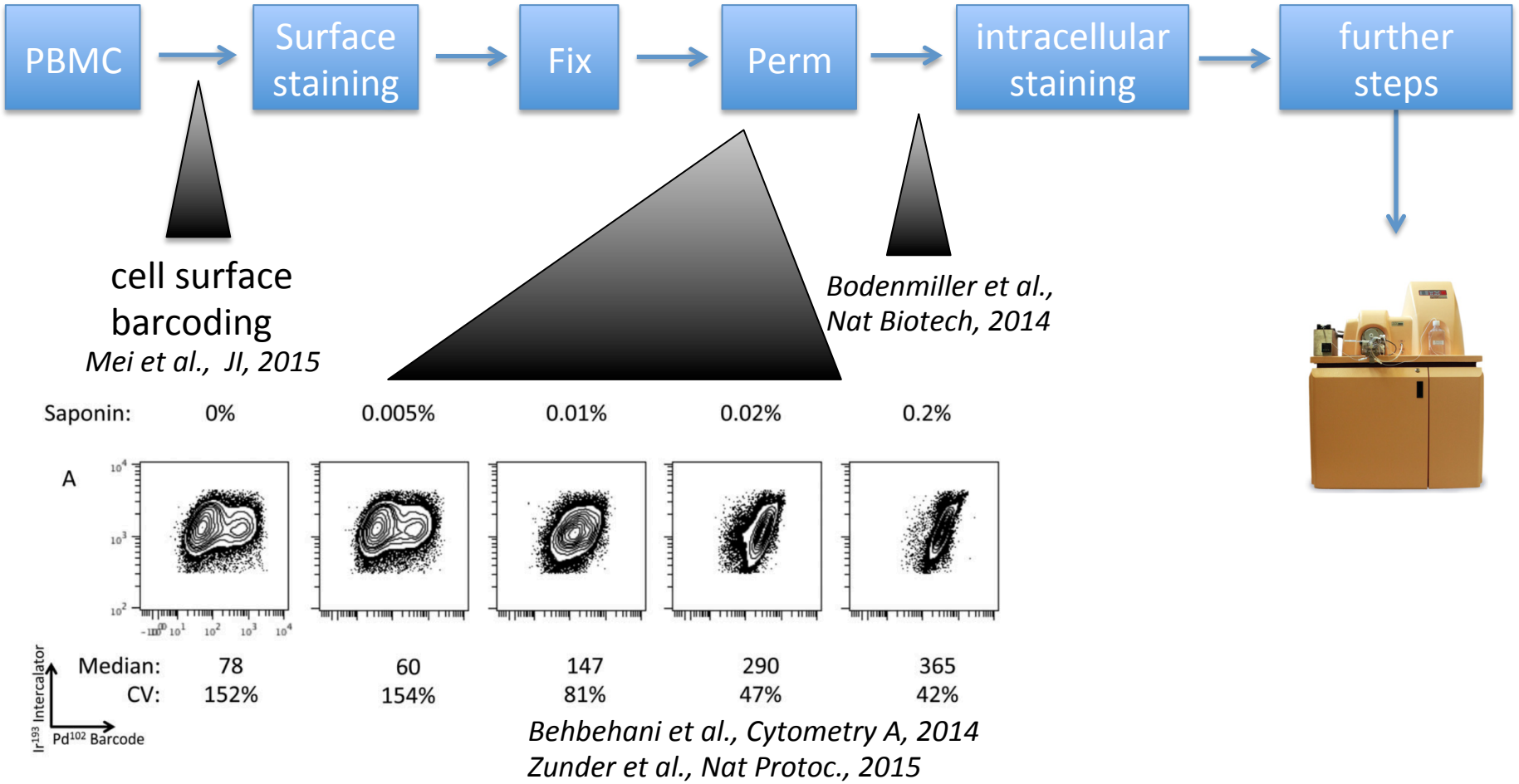
less than 3 labels
exactly 3 labels
more than 3 labels



Mei et al., J Immunol 2015

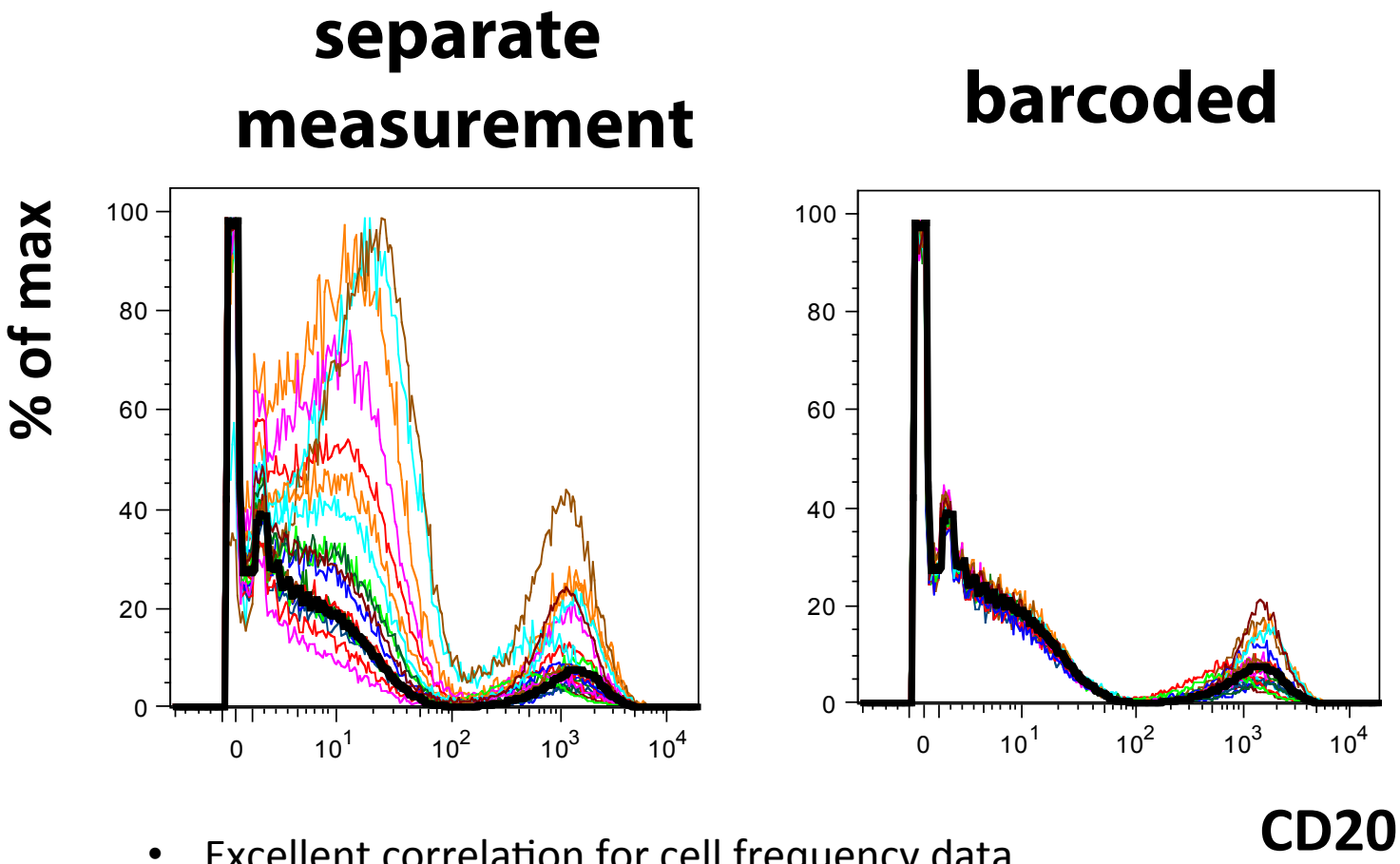
Barcoding scheme described in more details in Zunder et al., Nat. Protoc. 2015

Intracellular barcoding relies on thiol- or amine reactive reagents which require cell fixation and permeabilization prior to barcoding



Cell surface barcoding extends benefits of barcoding to additional steps of the protocol

Results from barcoded, deconvoluted data reproduce data from individually processed samples

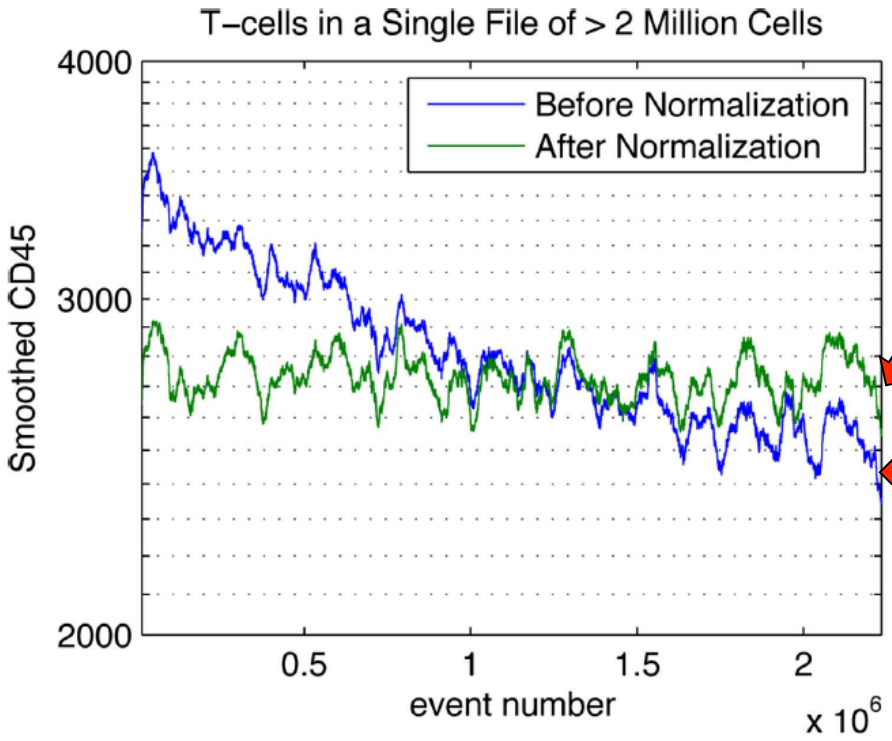


- Excellent correlation for cell frequency data
- Good to excellent correlation for signal intensity data

Mei et al., J Immunol 2015

Signal Decrease Over Very Long Run-Times
- Data normalization using beads -

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



98% of start

73% of start

Finck et al, Cytometry A

Metal Labeling of Antibody

Pre-conjugated Ab from Fluidigm

- chelator-decorated polymer + lanthanides, Y, Bi

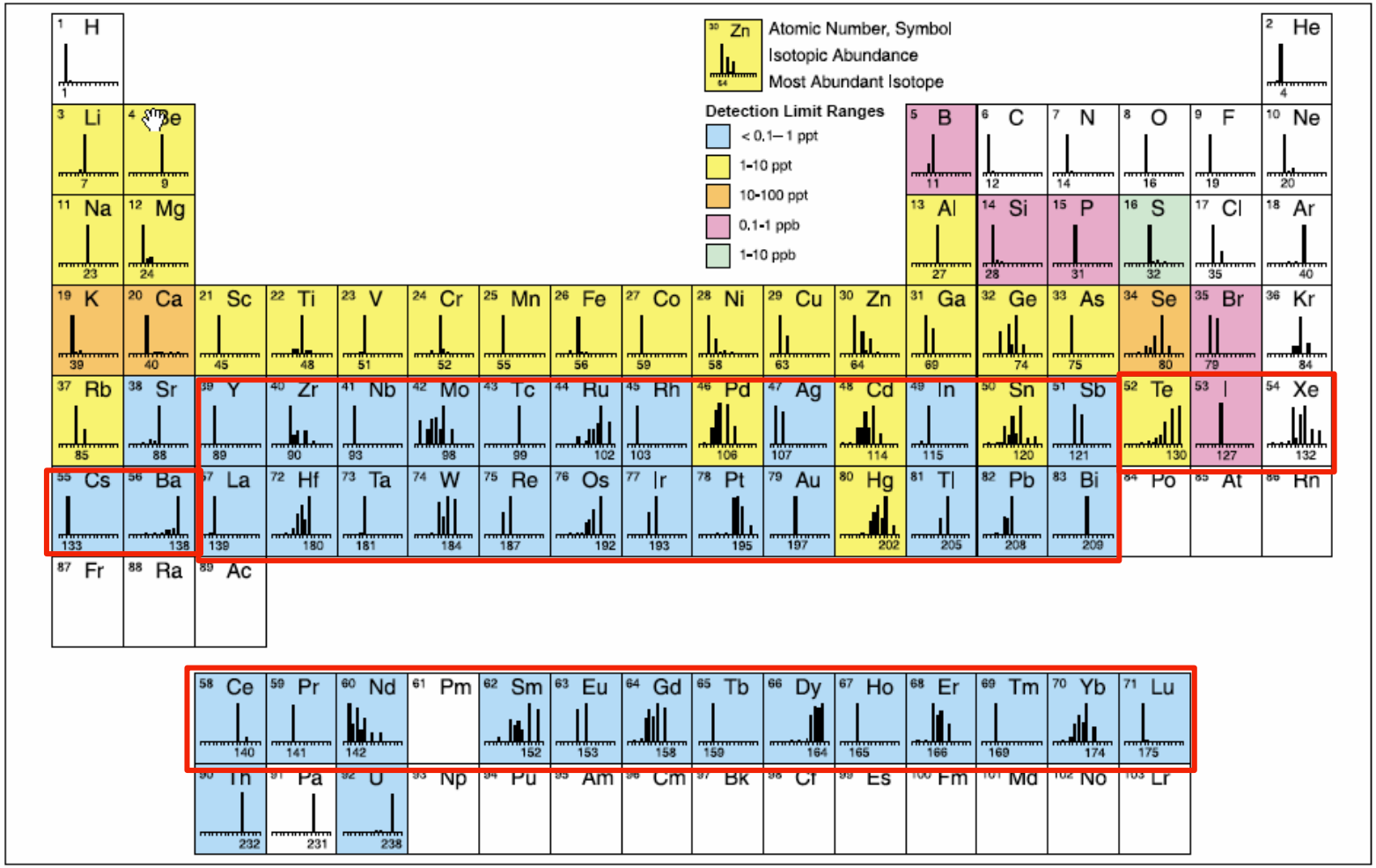
In-house conjugates with

- Lanthanides using Fluidigm kits and carrier-protein-free Abs
- Palladium (Mei et al., JI 2015)
- Platinum (Mei et al., Cytometry A 2016)

In case of conjugation failure

- secondary antibodies (anti-Ig, anti-Biotin)
- anti-fluorochrome antibodies

Potentially Suitable Elements



Lanthanide advantages:

1. Very low bioavailability
2. Enriched isotopes can be purchased and are well-resolved by ICP-MS

Applications of mass cytometry

Immune monitoring (PBMC/leukocytes)

HIMC Stanford, core facilities

- combine multi-tube FACS panels into mass cytometry panel (e.g. Nichholas et al., CytA 2015)

Tumor phenotyping

Cell signalling

Metal-labeled antibodies go well with methanol

Many parameters

TCR repertoire (Evan Newell)

NK cell inhibitory and activating receptor repertoire (Amir Horowitz)

Analysis of small & rare cell samples

Biopsies, liquor, urine, lavage

Tracking of cellular differentiation

B cells (Sean Bendall) / Stem cells (Eli Zunder)

But also: toxicology (heavy metal uptake by cells), bacteria (Leipold et al., An l. Biochem. 2011).

Mass cytometry of rare cells

Many interesting cell types are scarce and display considerable heterogeneity

- Stem cells
- Innate lymphoid cells (ILC)
- Plasma cells
- antigen-specific T and B lymphocytes

Limited throughput → intolerably long acquisition time for rare cells in their native mixture

Solution: pre-enrichment of cells

Magnetic separation

- stress for sensitive cells → consider enriching cells after staining/fixation
- metal contamination of beads cannot be excluded
- sorting according to multiple parameters is difficult

Separation by cell sorting (FACS)

- tricky to stain for markers for CyTOF previously used for sorting

Thanks!

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Christian Dose

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Mark Konrad

Tad George