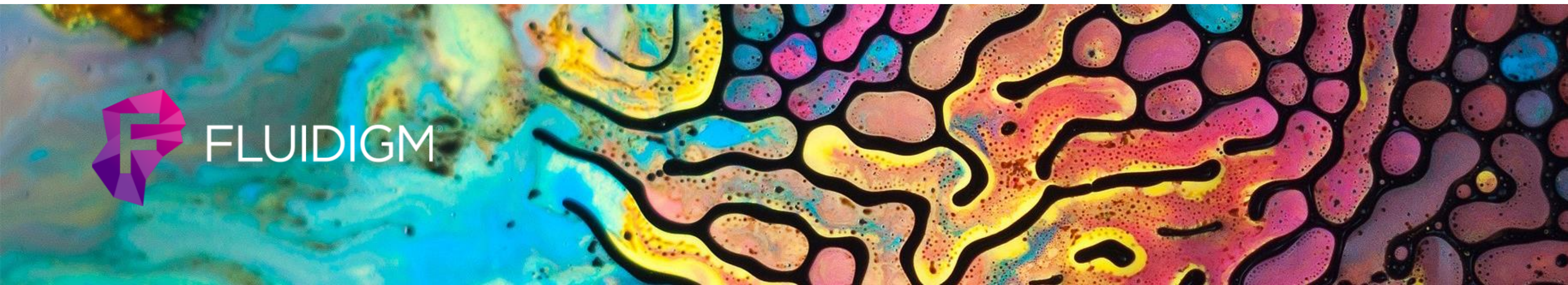
Basics of Sample Acquisition and Analysis



Outline

In this module, we will discuss:

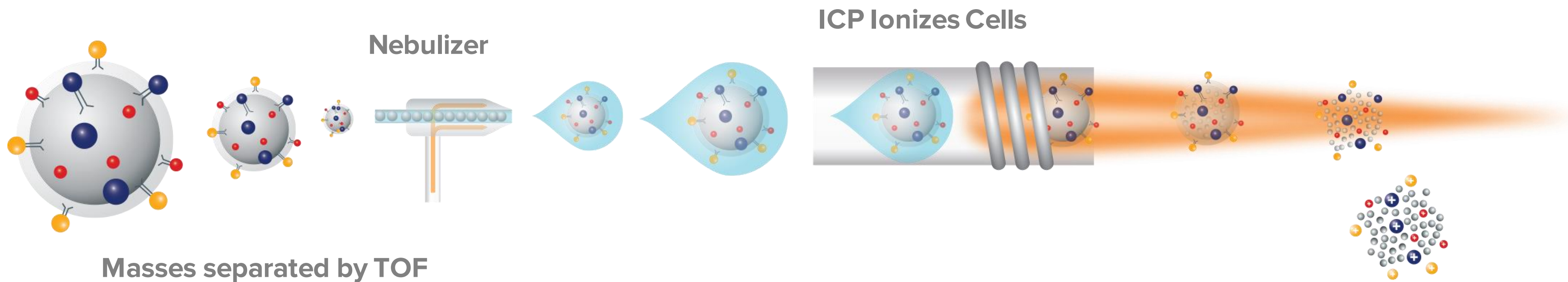
Basics of mass cytometry signal processing and how it is impacted by sample quality and delivery.

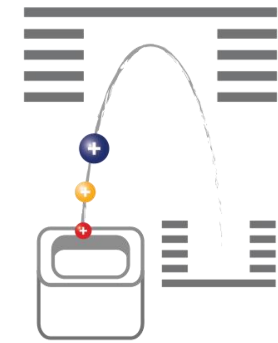
Best practices

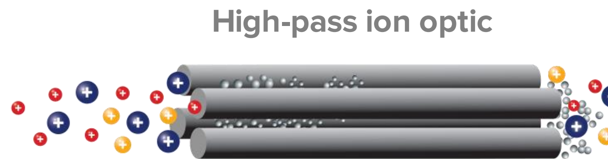
- Sample preparation
- Instrument QC
- Sample acquisition

Gating basics

Mass cytometry acquisition







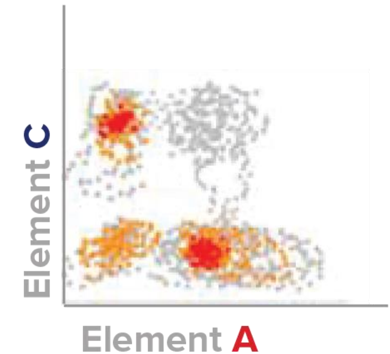
Integrate per cell

FCS file

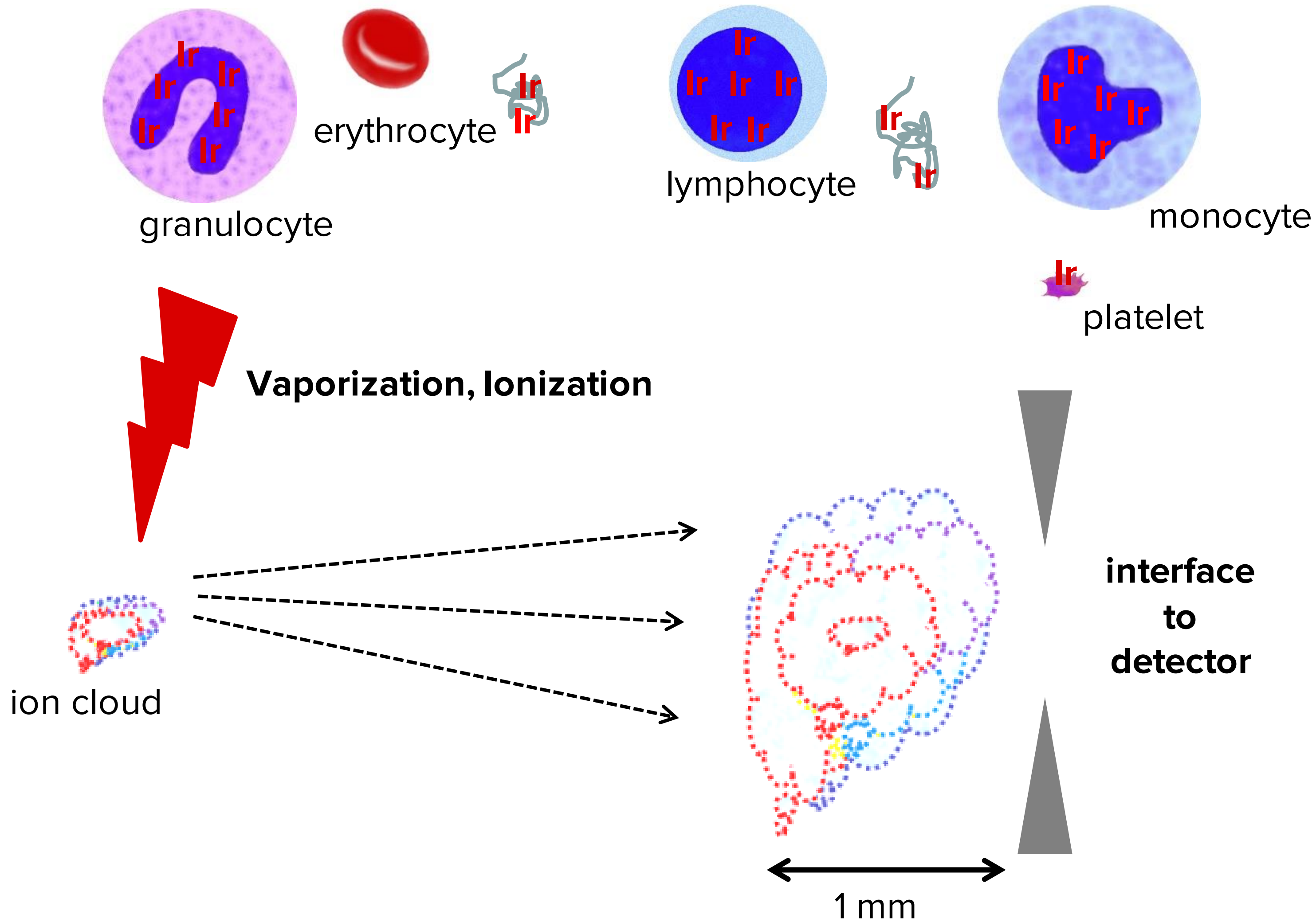
Element

	A	B	C	D	E
Cell 1	7	3	5	5	4
Cell 2	1	6	3	5	3
Cell 3	2	4	5	7	9
Cell 4	3	2	6	7	8
Cell 5	1	5	3	1	2

Analysis

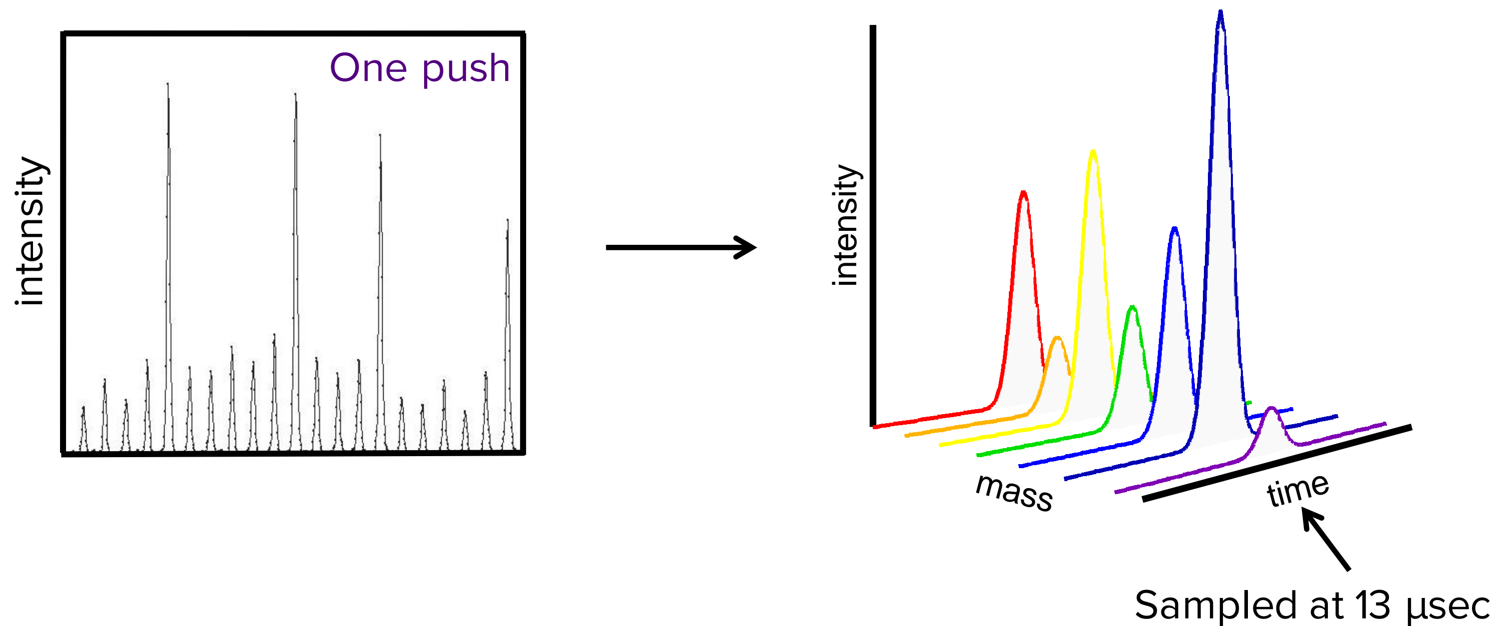


From cell to ion cloud



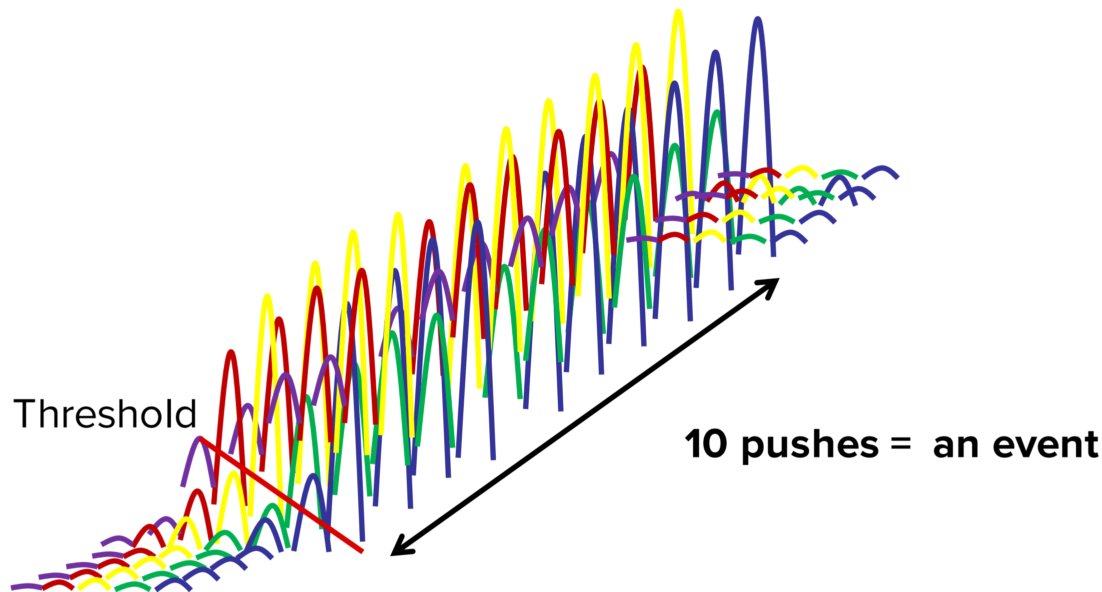
Ion cloud analysis

Each ion cloud is sampled as 13 µsec pushes. All ions within each push are measured.



The number and mass of the ions in each push are measured after being separated in the TOF chamber.

Event duration equals signal duration



Advanced

Lower Convolution Threshold	400
Signal Subtraction (per channel)	0
Min Event Duration	10
Max Event Duration	150
Sigma	3

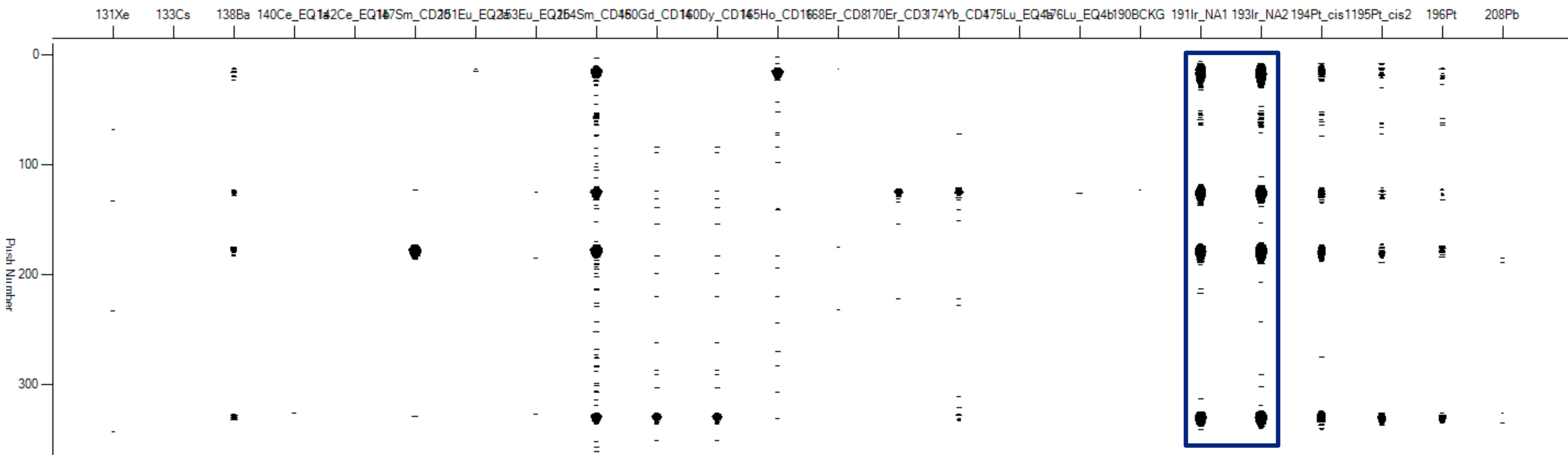
- Event duration is the period of time, measured in pushes, that the total ion signal intensity is above the threshold.
- Any events outside the range of 10 to 150 pushes are excluded from conversion to the FCS file data set.

Cell ID: Maxpar® intercalator-Ir product

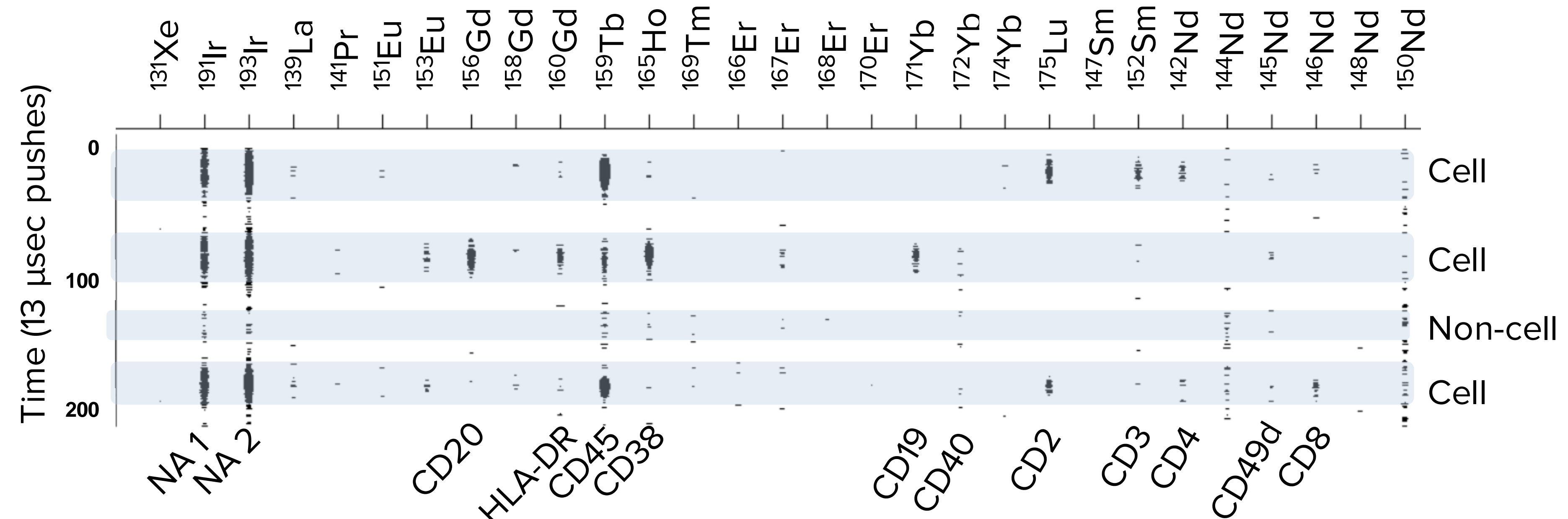
- A cationic nucleic acid intercalator containing natural abundance Iridium: ~37% ^{191}Ir and ~63% ^{193}Ir.
- Incubate cells with $^{191/193}Ir$ diluted in Maxpar Fix and Perm Buffer after antibody staining.
- All cells or particles containing DNA/RNA will bind at least some Ir intercalator.

Cell events are positive for $^{191/193}Ir$

Metals + Markers



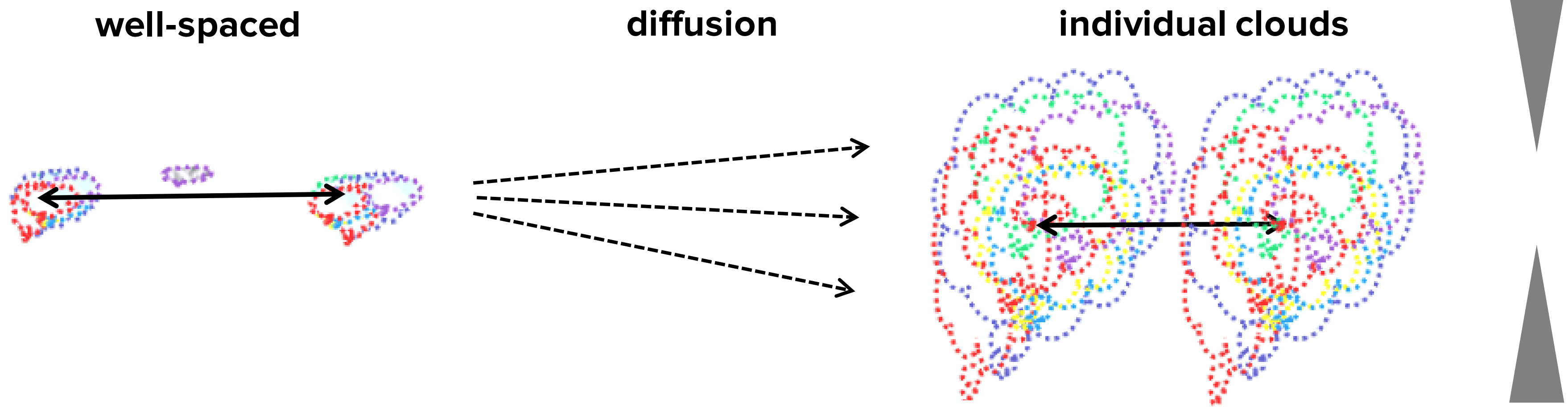
Which pushes are considered events for FCS file creation?



Ornatsky *et al., J Immunol Methods 361, 1 (2010)*

- The signal duration of 10-150 pushes determines an *event*.
- During analysis, further gating is performed to identify singlet cells based on nucleic acid signal and other markers of interest, such as CD45.

Properly diluted cell sample with little debris

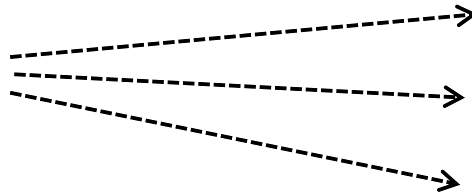


Metals + Markers

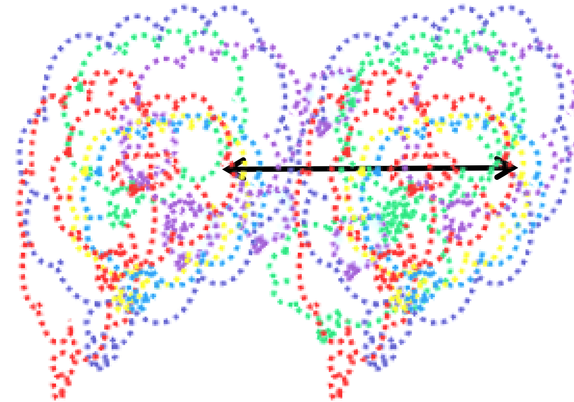
High particle concentration causes ion cloud fusion

too close

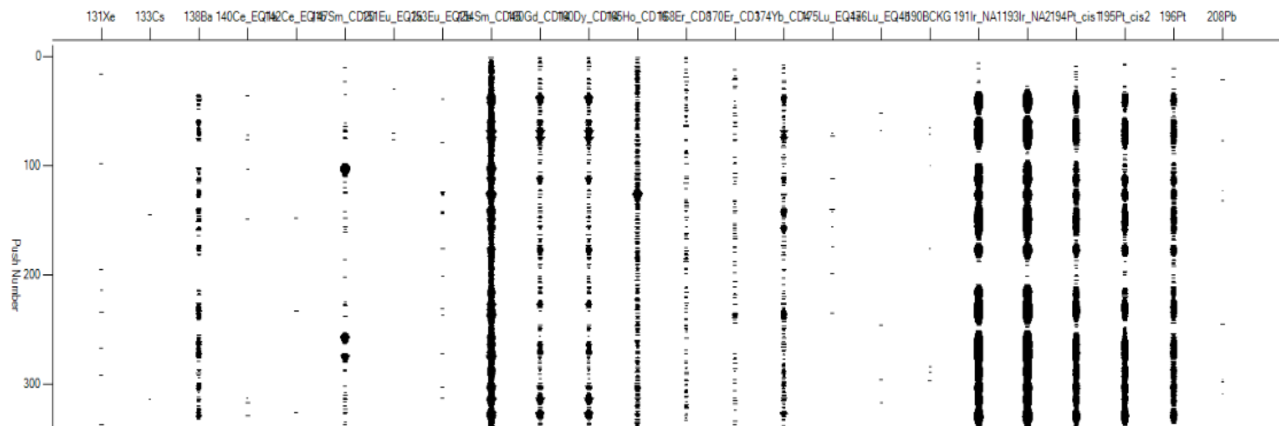
diffusion

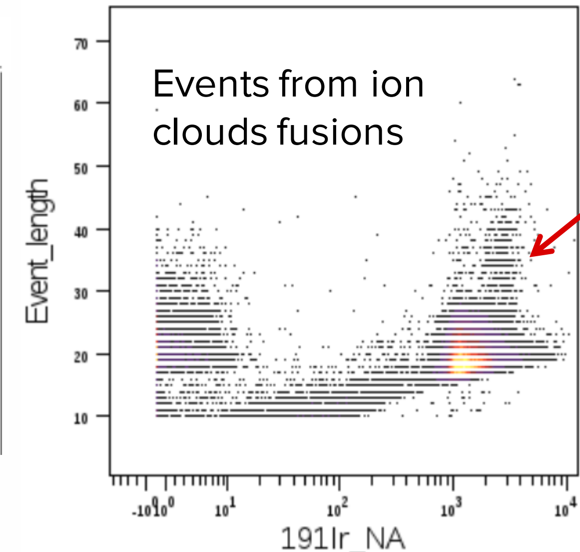


cloud fusion



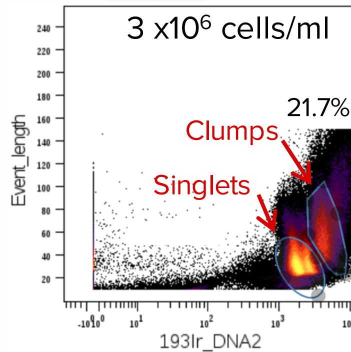
example: very concentrated cell sample (10^7 cells /mL) showing fusion events with larger event length values

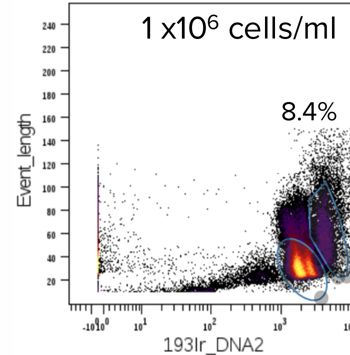


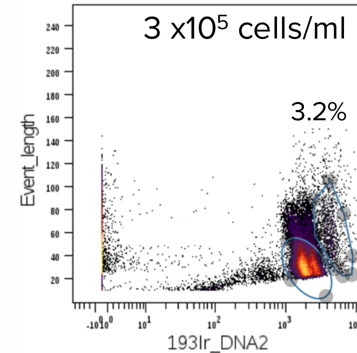


Impact of cell concentration on data quality

all events





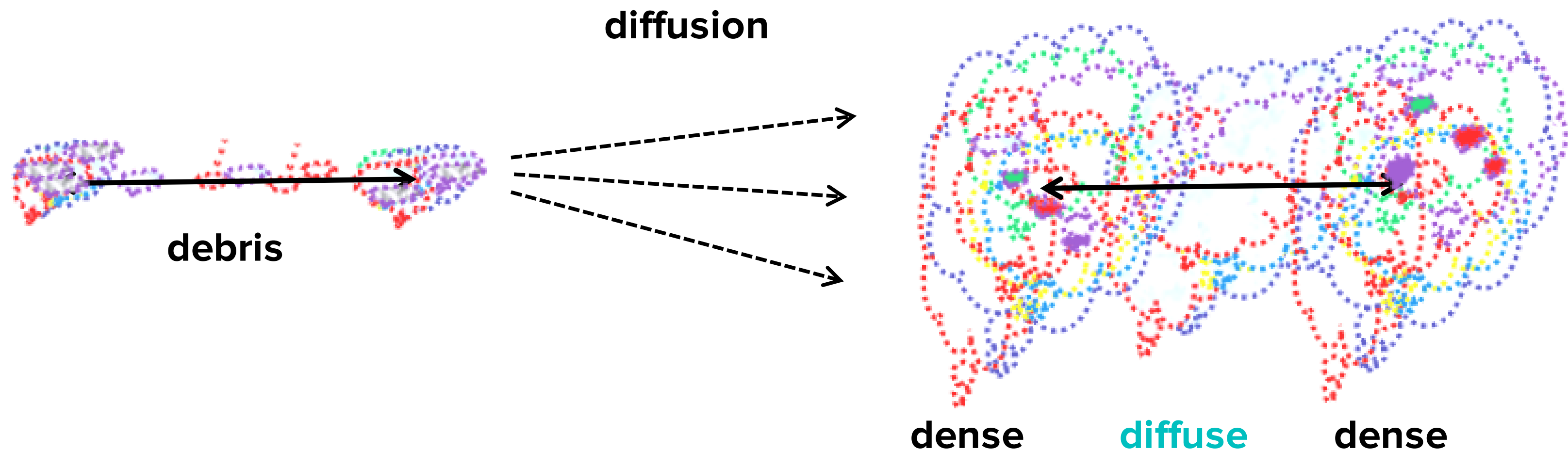


Experiment: PBMC sample, serially diluted. Acquisition time held constant.

high 193Ir (NA content) gate is more likely to contain cell clumps.

Take home Message: Run your sample below 500 events/sec

Impact of debris on data quality

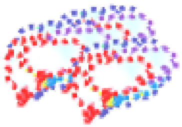


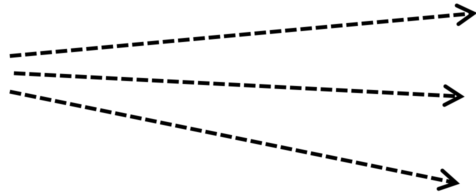
Take home message:

Reduce debris – often this means start staining process with as few dead cells as possible to minimize creation of debris during staining steps.

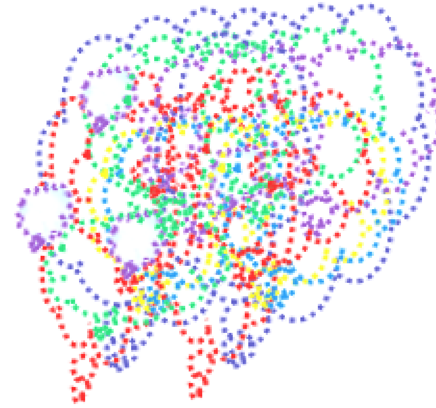
Doublet determination

cell clumps

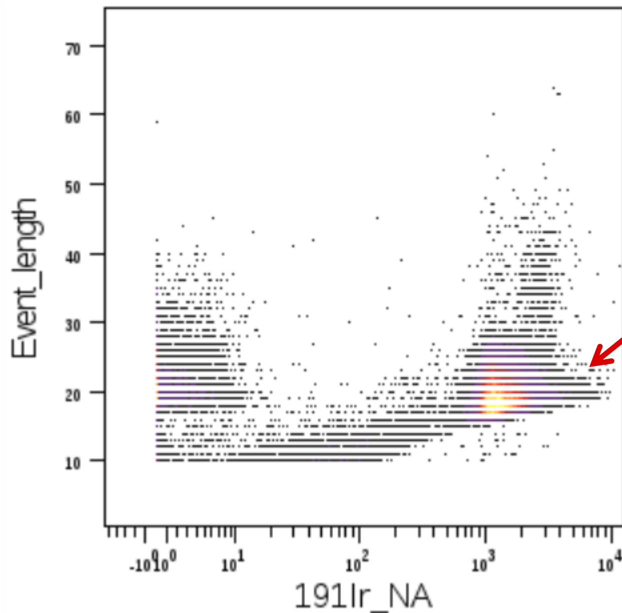




intense cloud



Larger event length value
(signal duration in 13 µsec "push" increments)



Controlling metal contamination

Source	Remedy
Glassware (Lead-^{208}Pb)	• Use plastic containers to soak parts, e.g. nebulizer • Use plastic for storage of media and reagents
Detergent used for cleaning (^{138}Ba)	• Rinse all glassware thoroughly with Type 1 ultrapure water (18.2 MΩ) (DIW)
PBS (^{138}Ba and ^{133}Ce)	• Use Ca^{++}/Mg^{++} free reagents: these will also be low for ^{138}Ba and ^{133}Cs
Water impurities	• Use only DIW for reagent preparation and cleaning of Helios® parts
Residual sample/debris in instrument	• Keep sample lines and nebulizer clean
Xenon (^{131}Xe)	• A contaminate in argon gas; monitor level in Background Template to identify a dirty tank (call gas provider)
Pipettes used with metal labels	• Clean pipettes with lab wipes and 70% ethanol after each use
Tin (^{120}Sn)	• Environmental, Igniter pin – clean with isopropanol
Worn out O-ring or some latex gloves (^{127}I)	• Change the brown sampler o-ring during maintenance, test the latex gloves before use.

Sample preparation: quality and concentration are key

Obtaining a single cell suspension:

- Sample preparation is critical: as free of debris and clumps as possible.
- Use a viability indicator such as ^{103}Rh or cisplatin (^{195}Pt).
- Filter using 35 µm cell strainers immediately prior to loading on the Helios

Cell Concentration:

- Staining: < $3x10^6$ per 100 µL staining volume (1 test)
- For injection (500 cells/sec target sampling rate is suggested, Don't be higher than 750 cells/sec)
    - $1x10^6$ /ml (0.030 ml/min introduction rate)

Best practices: instrument QC

Monitor Background

- Create template.
- Use data from install as a baseline reference.
- Collect data daily and check for changes especially after changing argon or water sources.

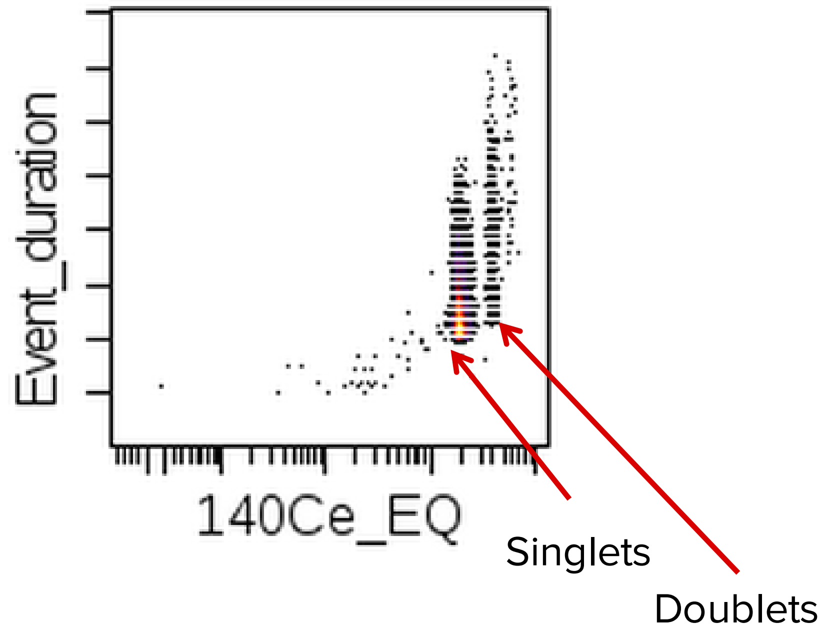
Daily Tuning

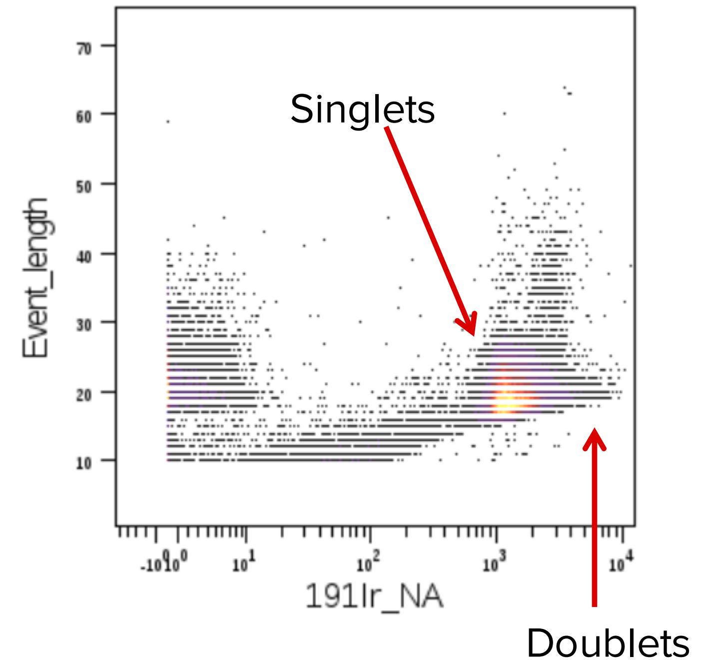
Keep Records

- QC log
- Screenshots of QC report

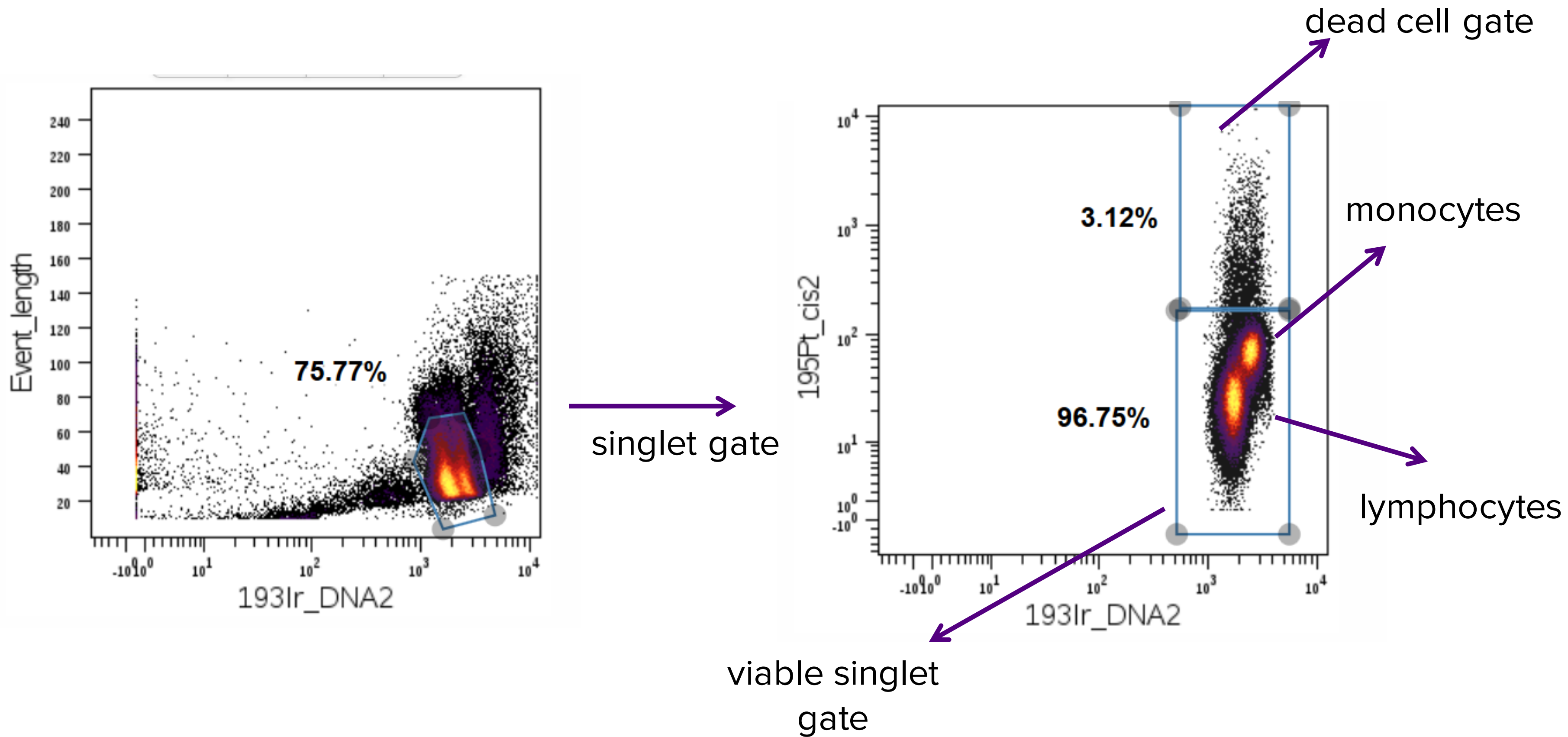
Basic gating strategy: Ir signal vs. event length

Beads: doublets are obvious

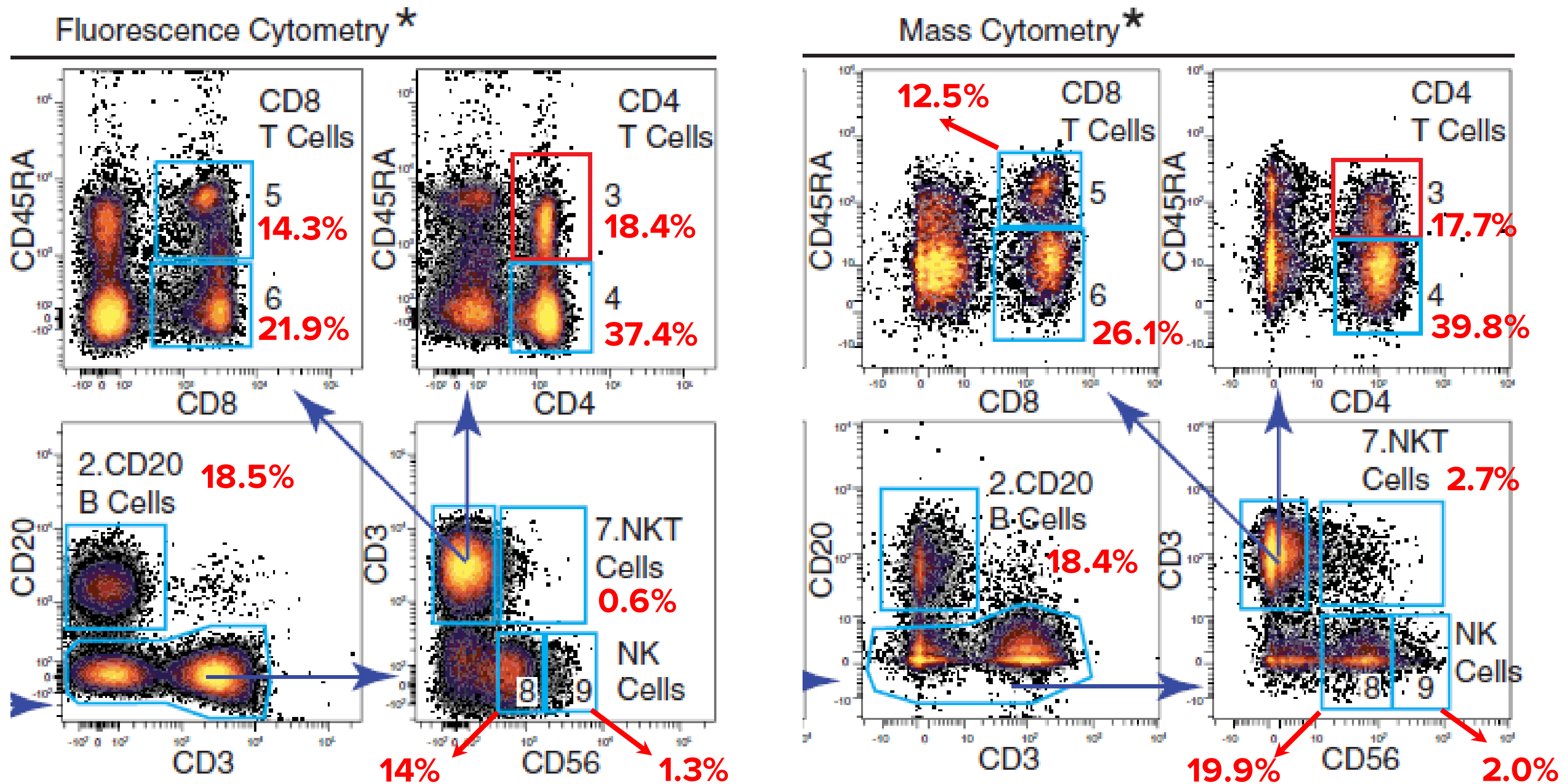




Basic gating strategy: viability



Data comparison to flow cytometry



Bendall & Simonds *et al.*, *Science* 332, 687 (2011)

Fixed PBMCs from donor labeled with 7 surface antigens:
CD3, CD4, CD8, CD45RA, CD56, CD20, CD33

* Pearson's correlation r = 0.99, $p<10^{-6}$ (2-tailed t-test) for population frequencies

Simplify the complex quest to understand and apply biology.