The CyTOF

Practical considerations for successful data acquisition

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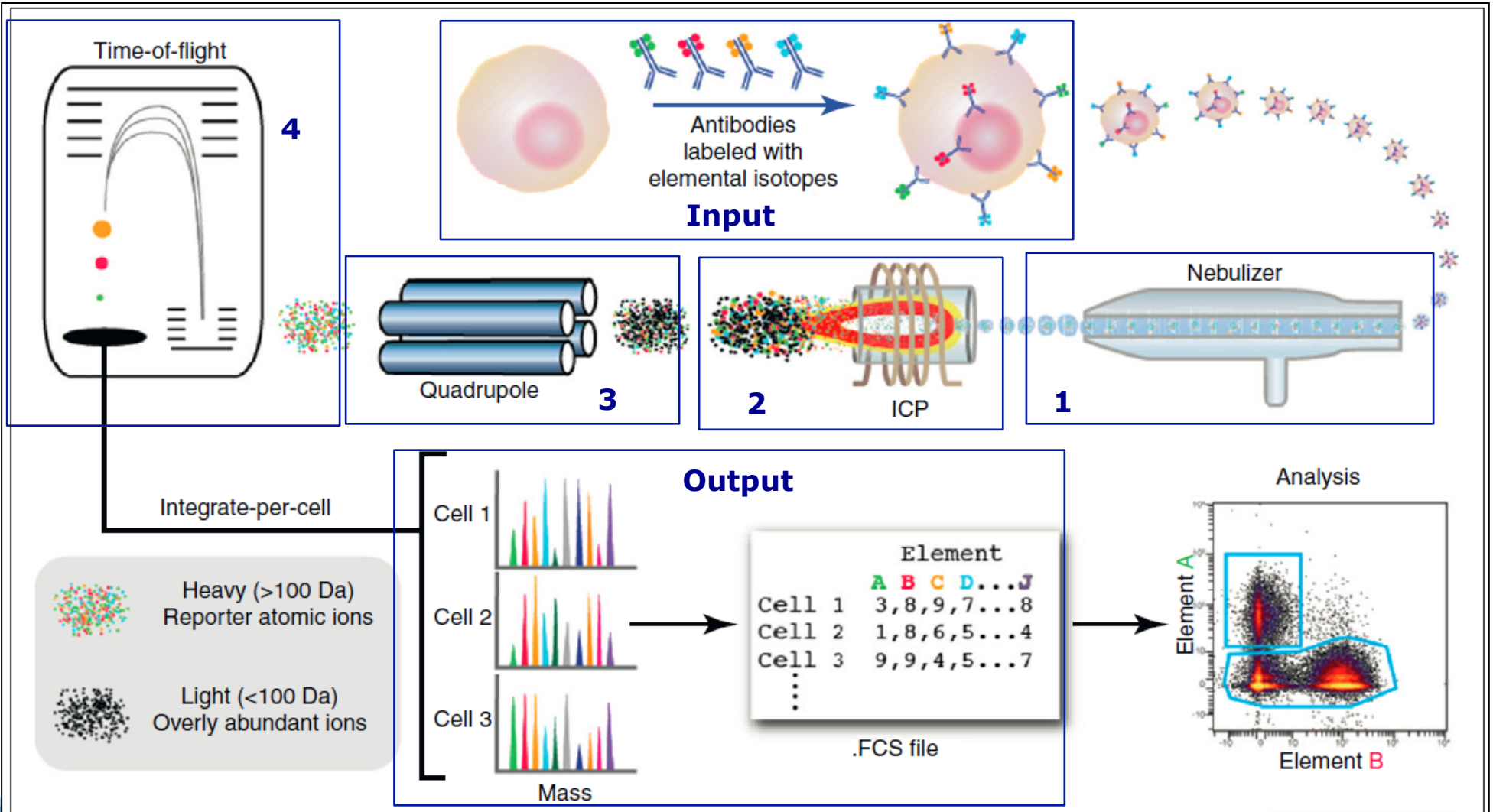
MEDICINE *of* THE HIGHEST ORDER

UNIVERSITY *of* ROCHESTER MEDICAL CENTER

**Some slides adapted directly from Fluidigm/DVS Biosciences

Summarizing the System



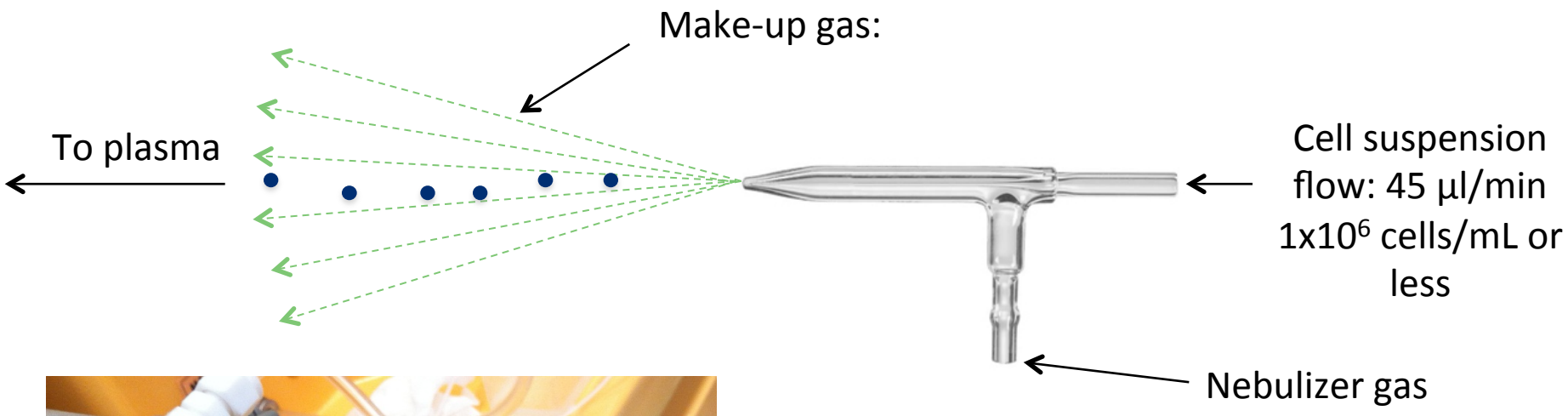
Bendall S. *et al*. (2012). Trends in Immunology, **33** (7), 323-332

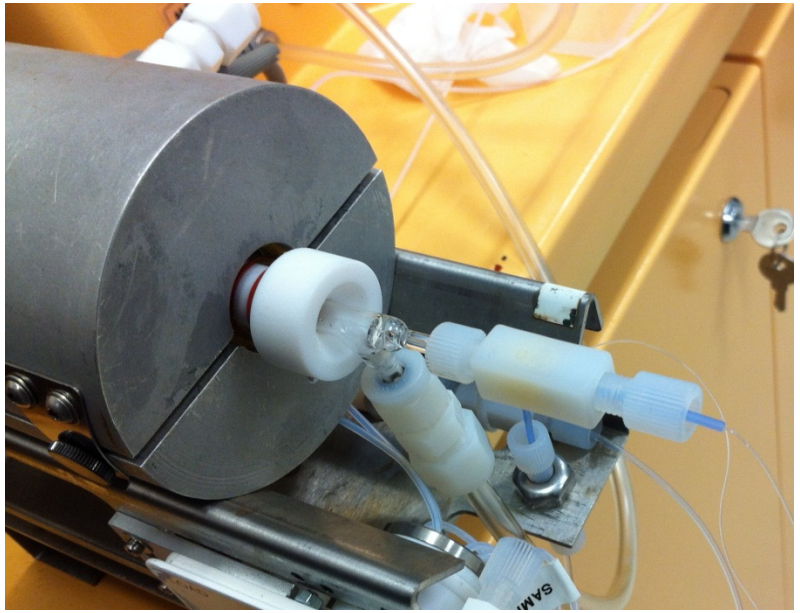
Input: Sample Introduction

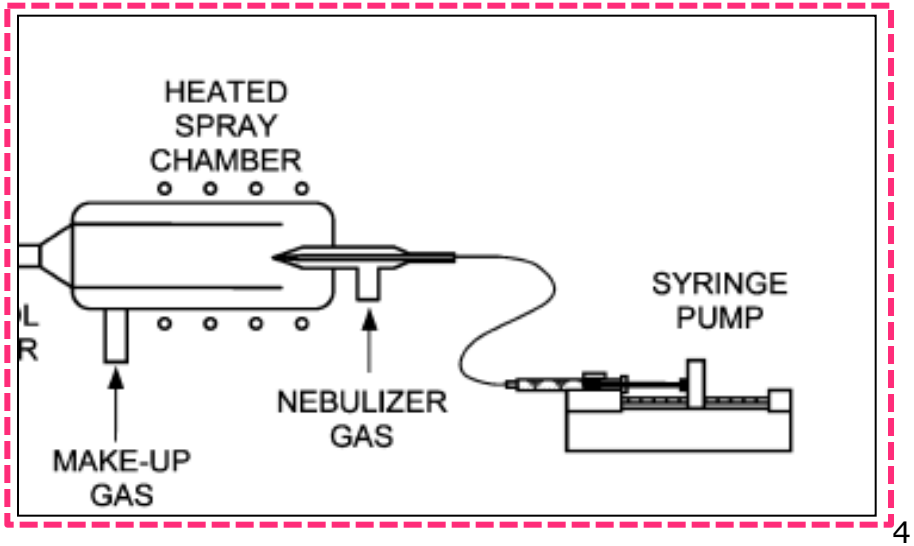
Sample concentration can effect data quality

- Maximum ~500 events per second during run (CyTOF 1-2).
  - The rate that event ion clouds can be collected.
    - Sample introduction at .045 mL/min (used to be .06 mL/min)
  - Not all events are cells.
- Maximum 5 pushes/hr – each push = 10 minutes + washing
  - 4-5 samples with one push each
  - 2-3 samples with 2 pushes
- Maximum ~2.5x10^6 cells run per hour

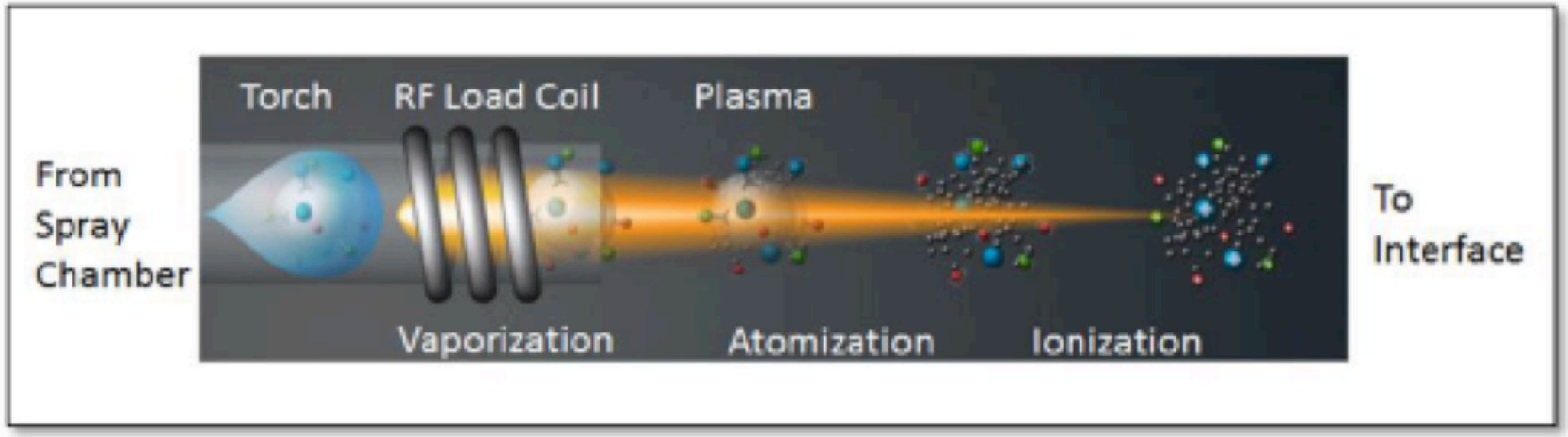
1. Sample Introduction



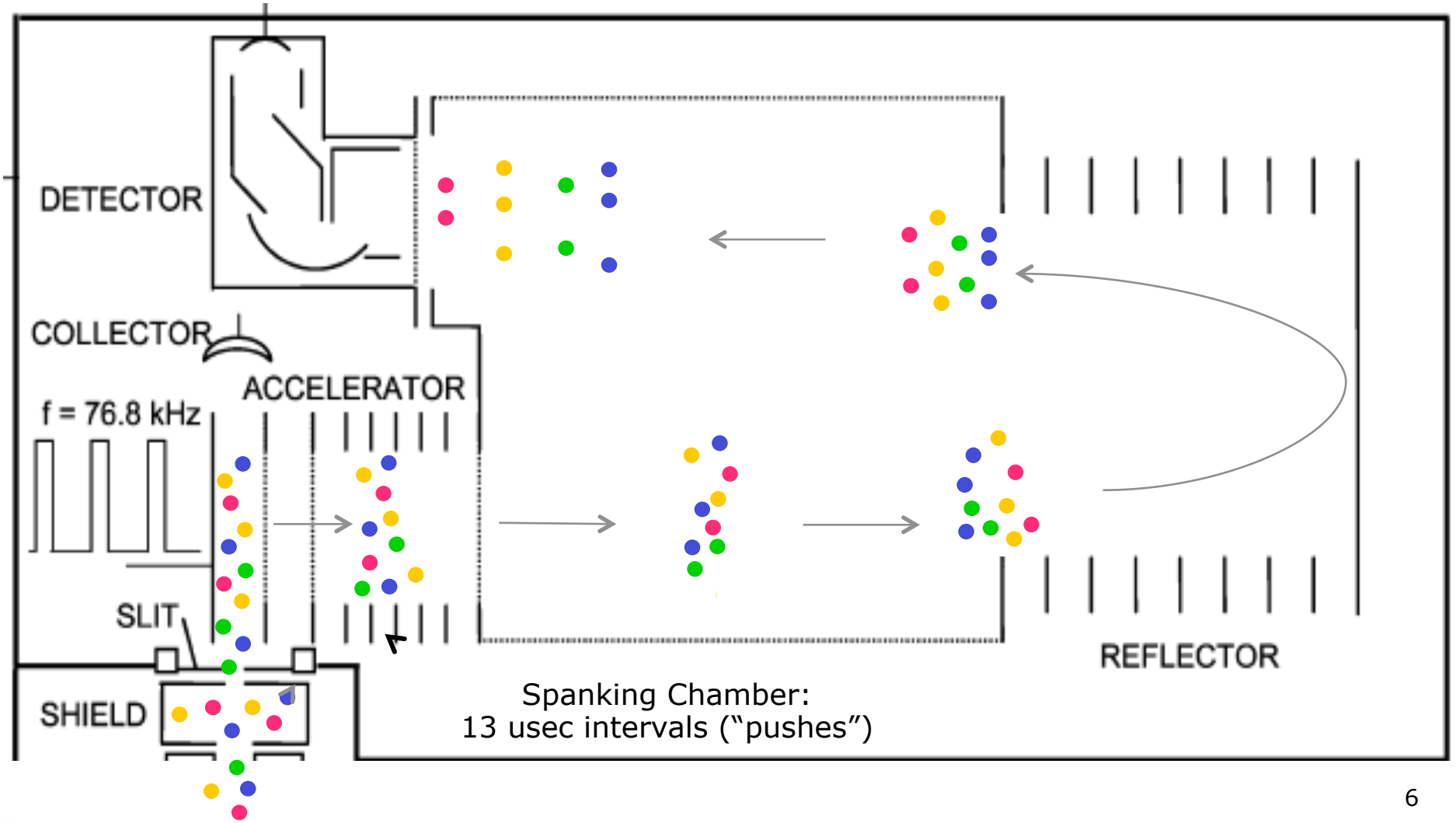




2. Ionization

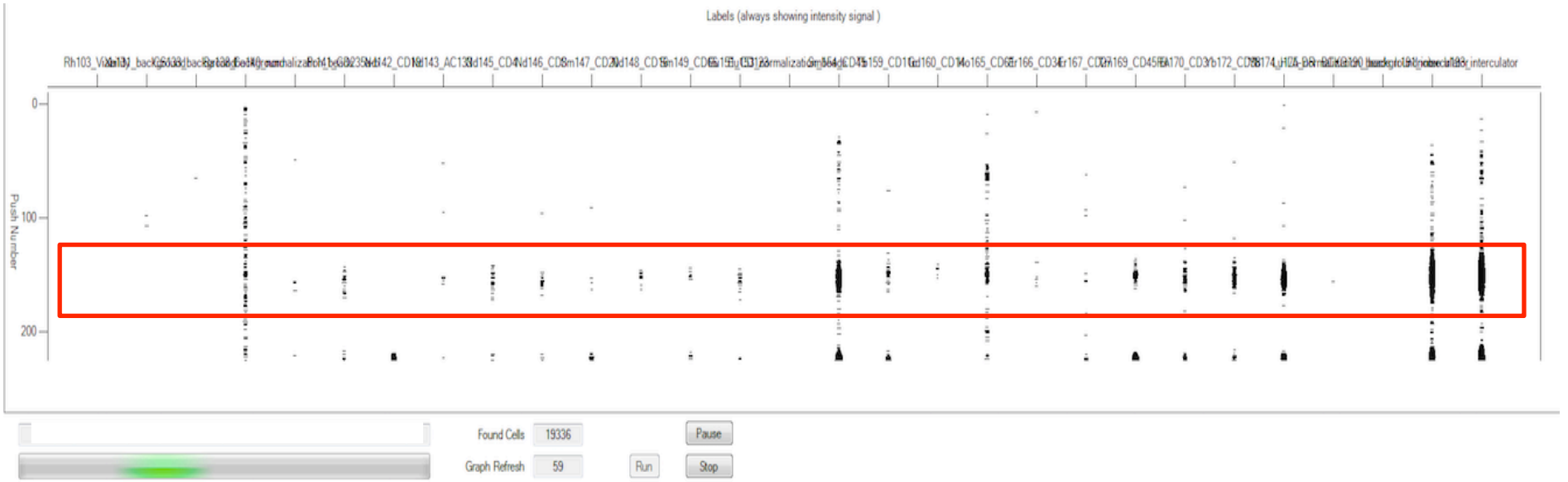


4. The TOF Chamber



Spanking Chamber:
13 usec intervals ("pushes")

The Raindrop Display



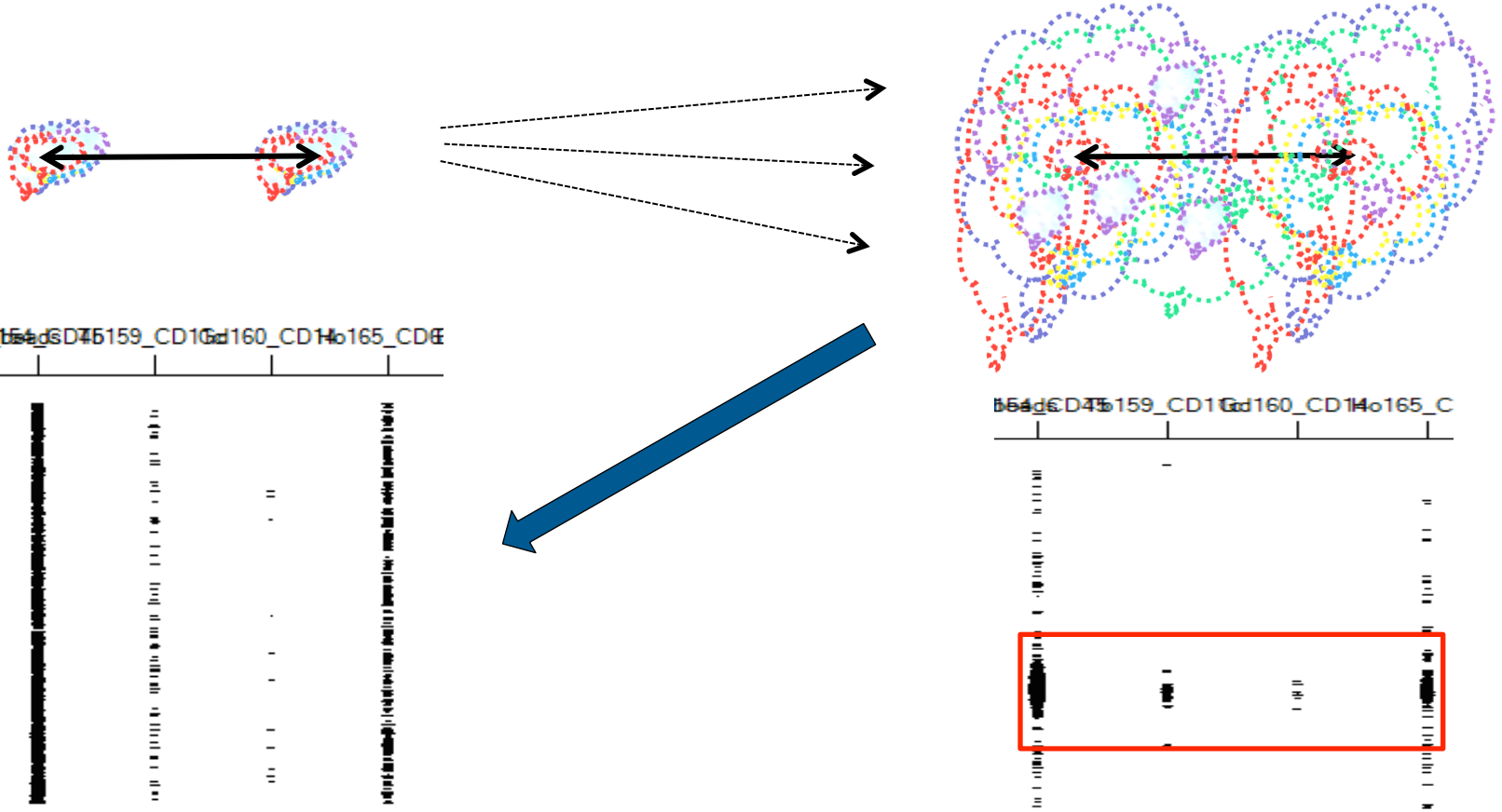
- 29 Parameters Total
- 200 pushes shown
- Some background in the Ba channel
- Good event rate ~300 evt/sec
- Software processes the raw data to generate the FCS file.
  - System also generates a “raw” IMD file

Sample Concentration and Cleanliness

Too close

Diffusion

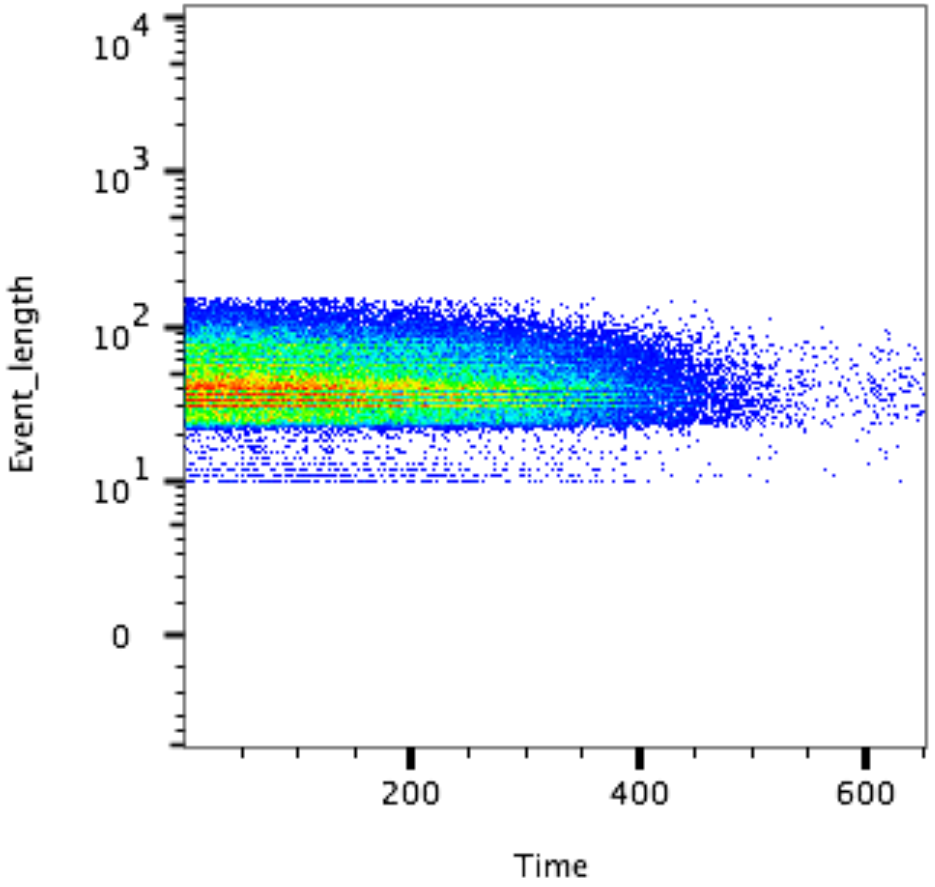
Cloud Fusion



Not so good

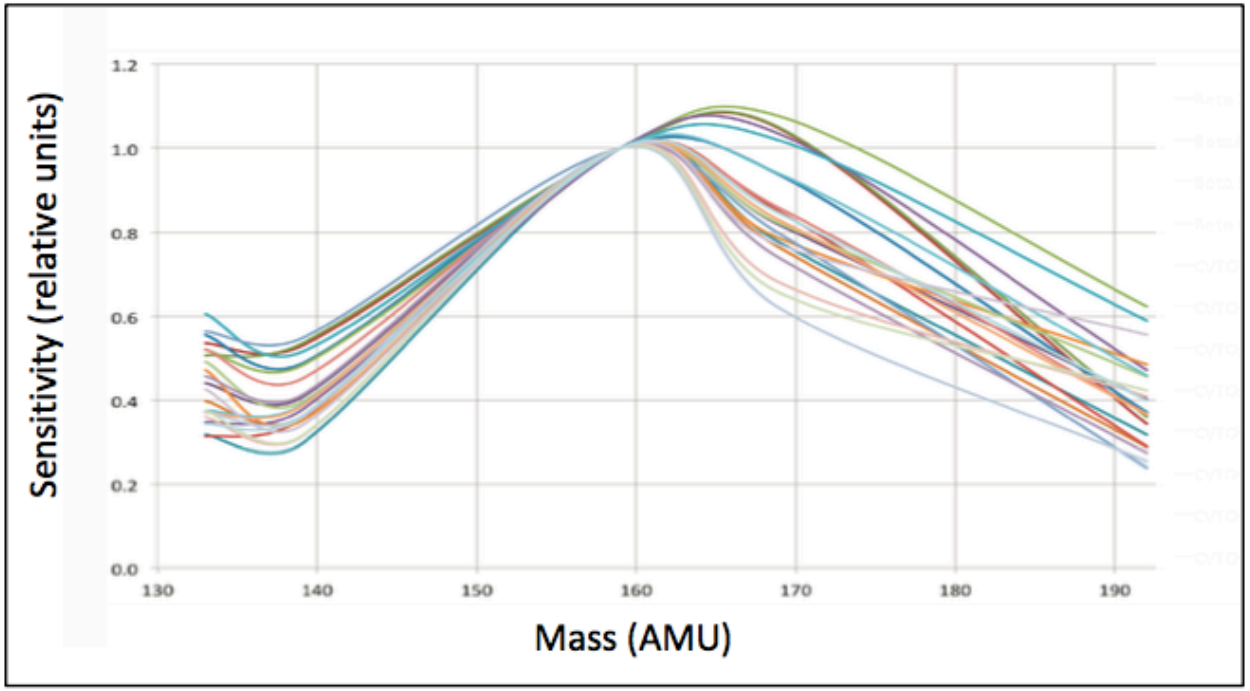
Good

Sample Flow



Metal Sensitivity – Panel Design

- Maxpar Panel Designer – web based tool with guides
- An introduction to mass cytometry: fundamentals and applications - Cancer Immunol Immunother (2013) 62:955–965



- We often recommend starting with a Fluidigm pre-set panel as a base/scaffold
  - Adding additional markers is fairly straightforward

Input: Sample Prep for the CyTOF

Single cell suspensions

- Sample preparation is critical.
  - A little more than "just" flow staining
- As free of debris and clumps as possible.
  - No scatter parameters for gating***
  - Every particle contained within the sample can contribute to signal
  - Filter immediately prior to running
  - Count immediately prior to running
    - Using a method that allows detection of debris
- Beware the water washes
  - Not a bad idea to seek help when attempting this.

Input: Sample Introduction

- Maximum 5 pushes/hr – each push = 10min + washing
- Maximum ~500 events per second during run.
- Maximum ~2.5x10^6 cells run per hour

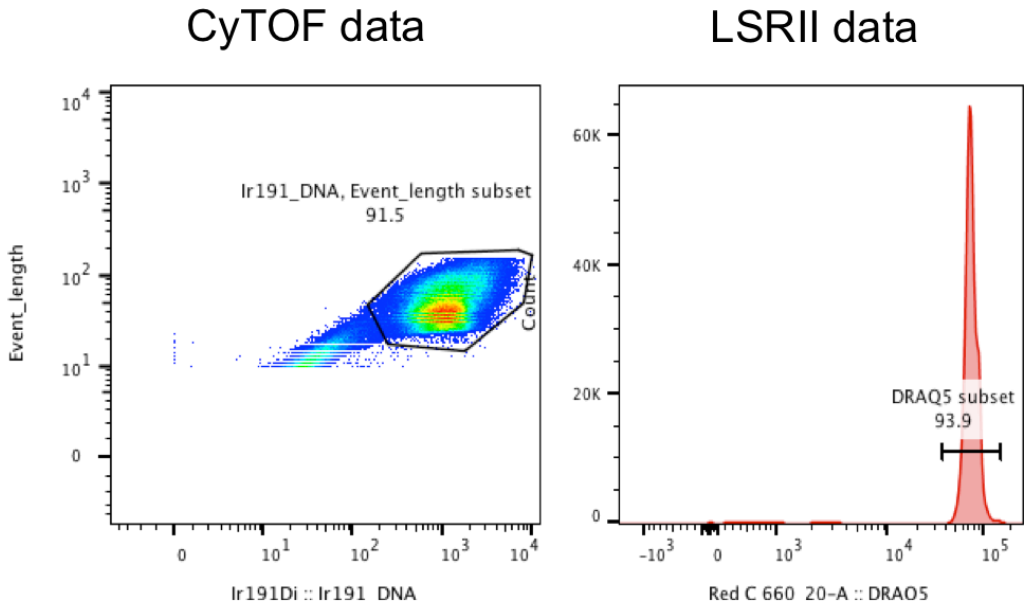
Begin with 2x10^6 cells: after staining/washing you have ~1x10^6 remaining: resuspend in 1mL and do two pushes: ~30 minutes of run time: recover ~2x10^5 cells

- Can you utilize the barcoding technologies – Palladium or CD45
  - Improves sample throughput for smaller cell numbers
  - Improves standardization of data over time
  - Easy to set up and use

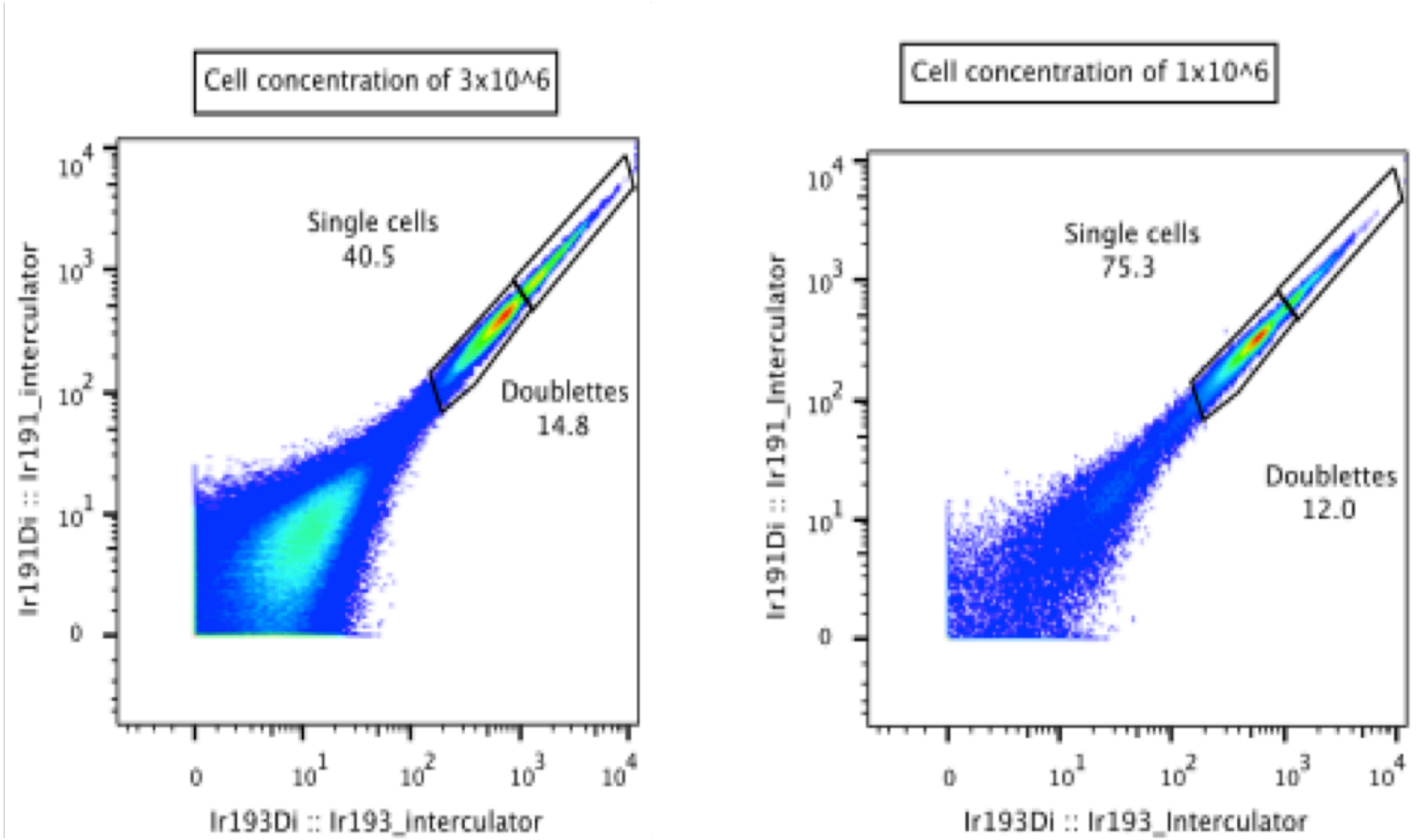
Output: Sample Recovery

- Suggested as ~30% recovery
  - Increased in the Helios
- Our numbers suggest between 20-30% in our system

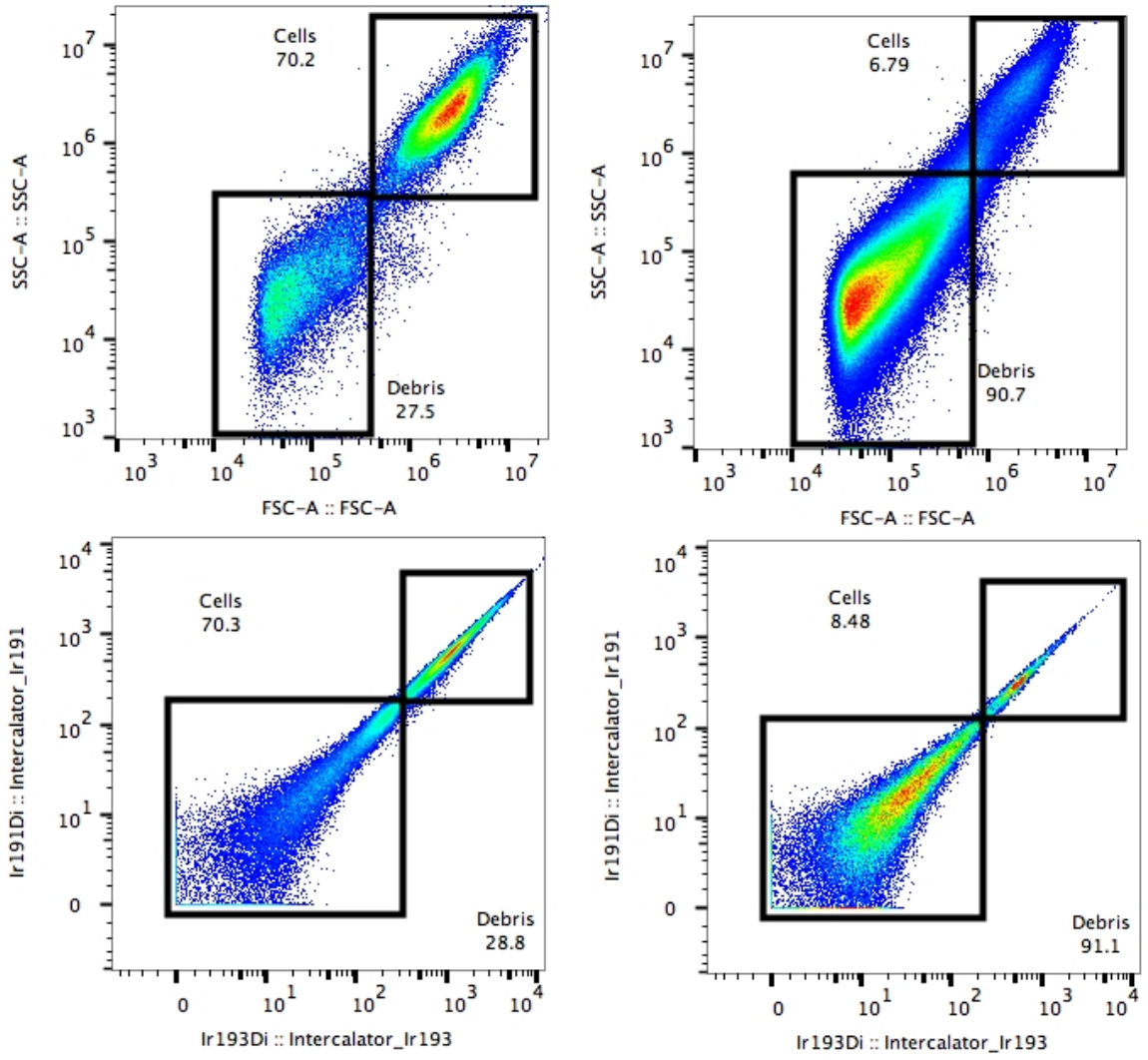
- Recovery Comparison
  - CyTOF: ~22%
  - LSR: ~31%
  - FACSCanto: ~50%



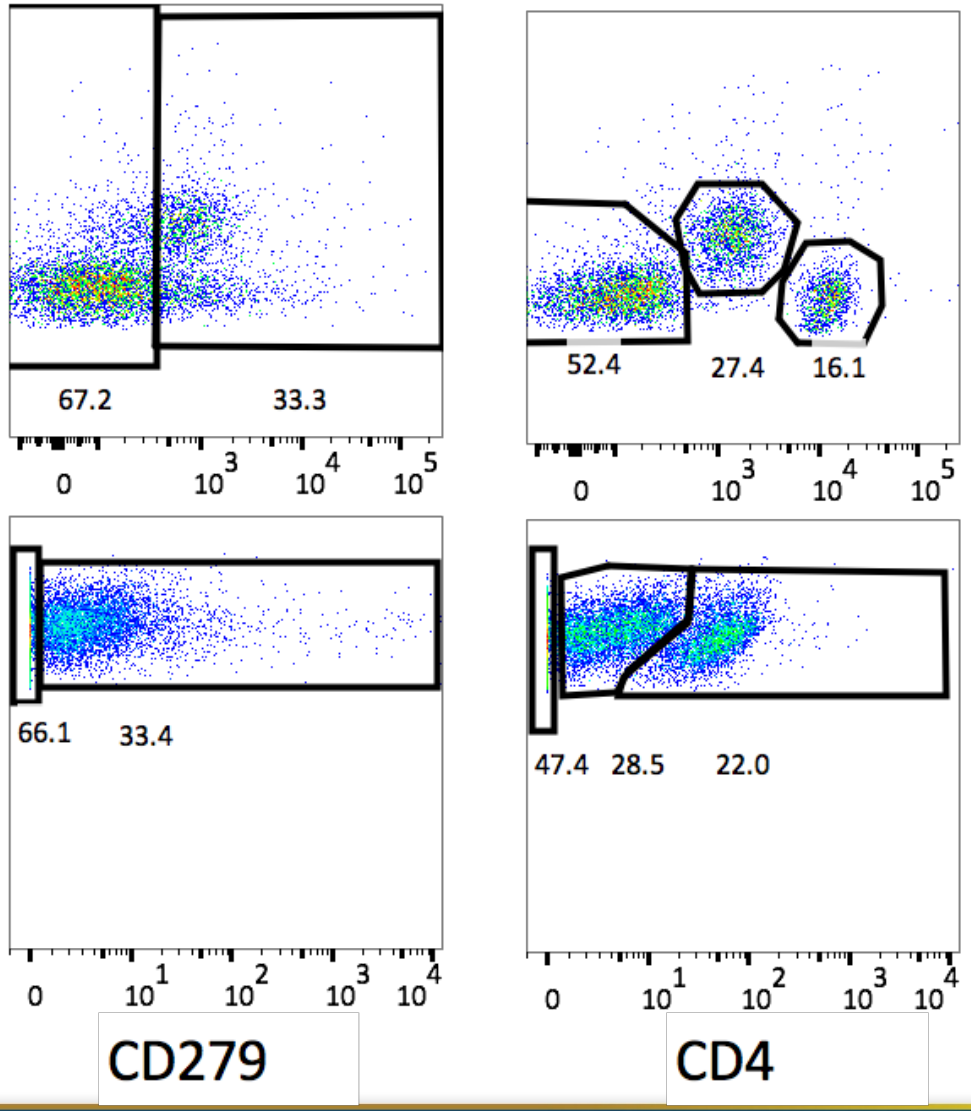
Sample Concentration



Sample Cleanliness



Output: Comparing the Signal



Flow Run

CyTOF Run

CD279

CD4