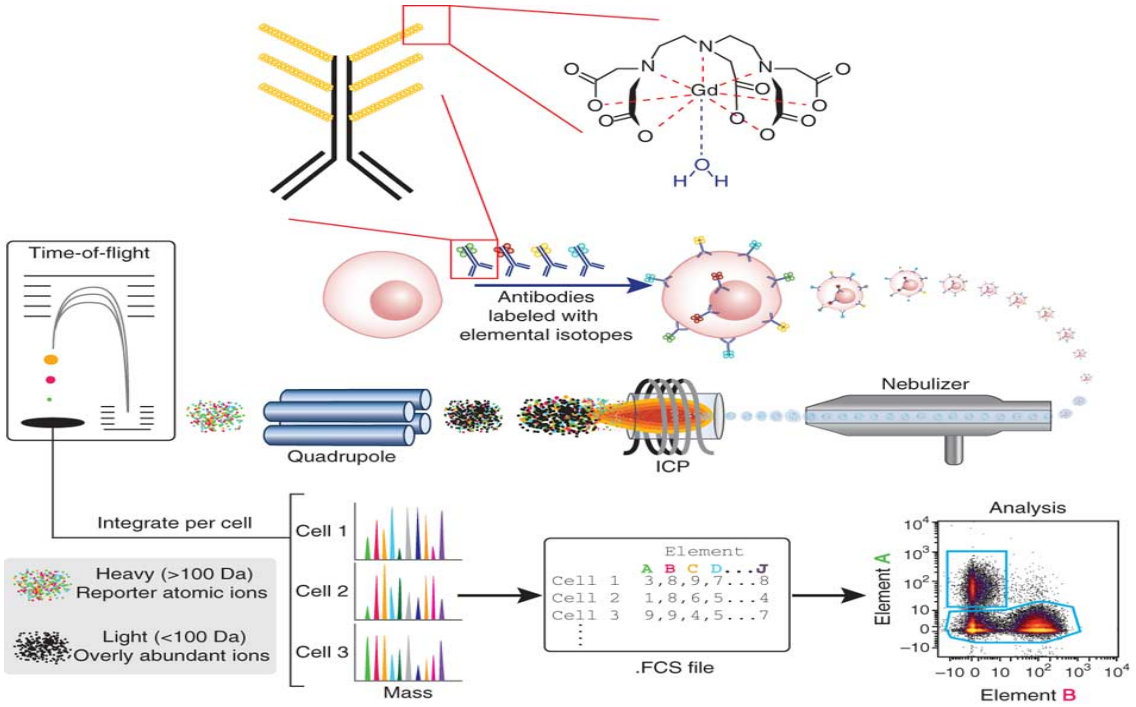
Mass Cytometry

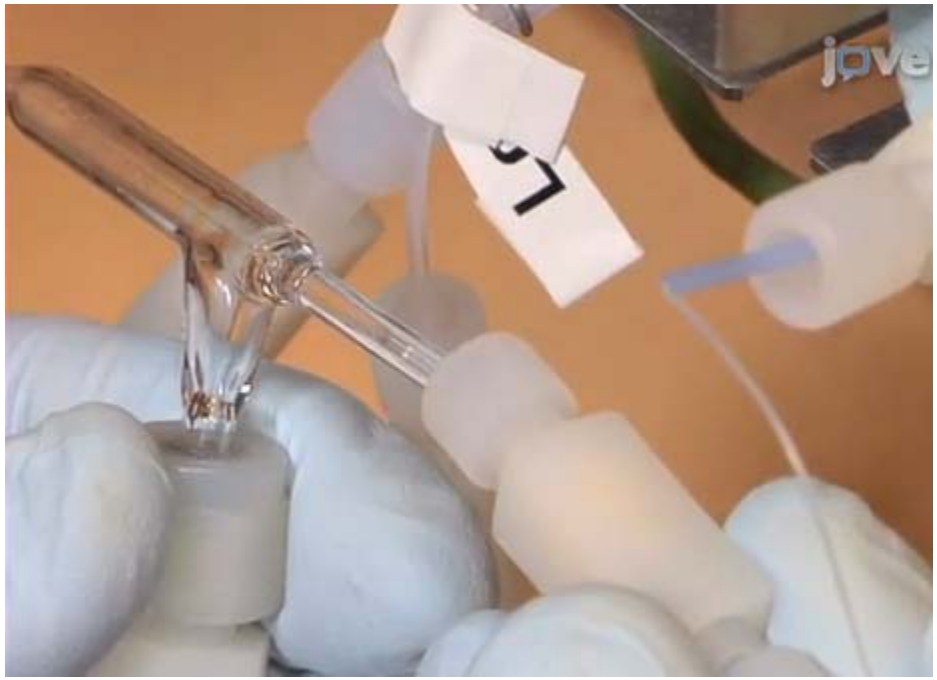
Principle of the CyTOF



Components of a Mass Cytometer

- CyTOF: Cytometer with a Time of Flight Mass Spectrometer
  - TOF and quadrupoles covered in Week 2 lectures on mass spectrometry
- What makes an aqueous flow of single cells compatible with mass spectrometry?
  - Nebulizer
  - Inductively-coupled plasma (ICP)

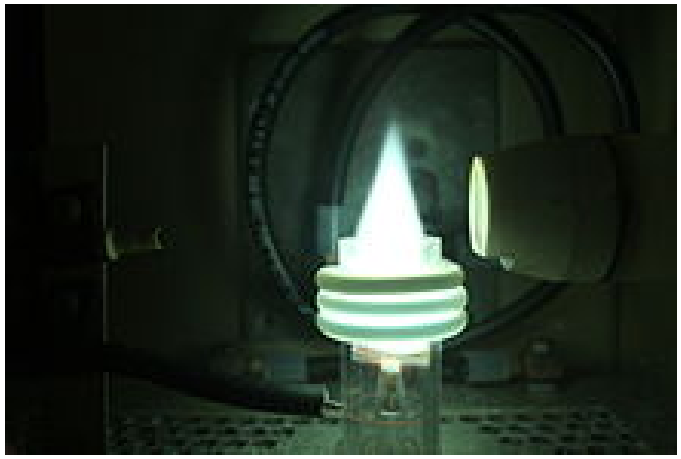
Nebulizer



http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3499083/

- Argon gas is mixed at finely tuned pressures and flows with the aqueous sample with (mostly) monodisperse cells
- Outlet is a fine mist of droplets, each containing zero or one cell (mostly)

ICP

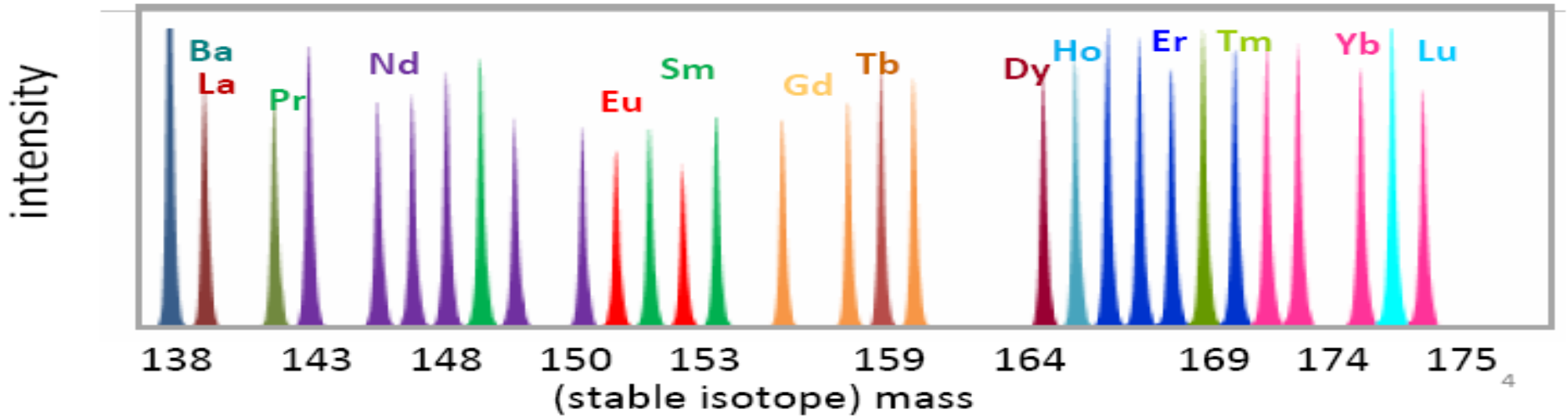


http://en.wikipedia.org/wiki/Inductively_coupled_plasma

- Electric field is rapidly oscillated through coils (inductive field), which accelerates a small packets of electrons injected into an argon gas stream.
- This creates a plasma of positive ions and electrons—the plasma torch—which approximates conditions on the surface of the sun
- As the nebulizer stream flows into this plasma, the contents of each cell are “burned” and converted into a cloud on ions for subsequent MS analysis

What Can Be Measured by a Mass Cytometer?

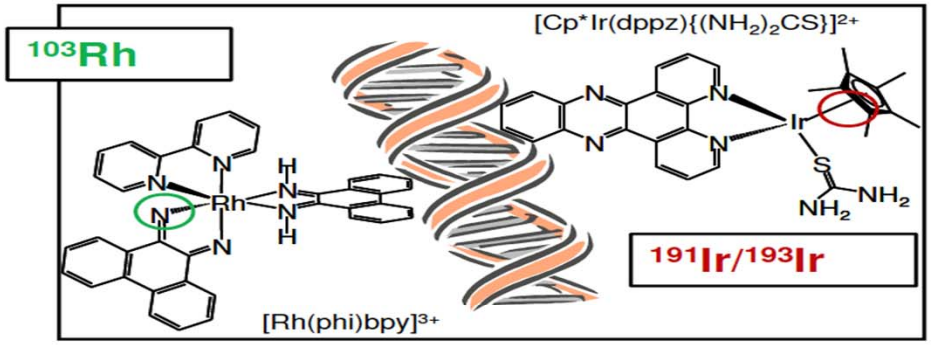
Elements for Unique Mass Tagging of Antibodies



How are cells identified?

DVS Sciences

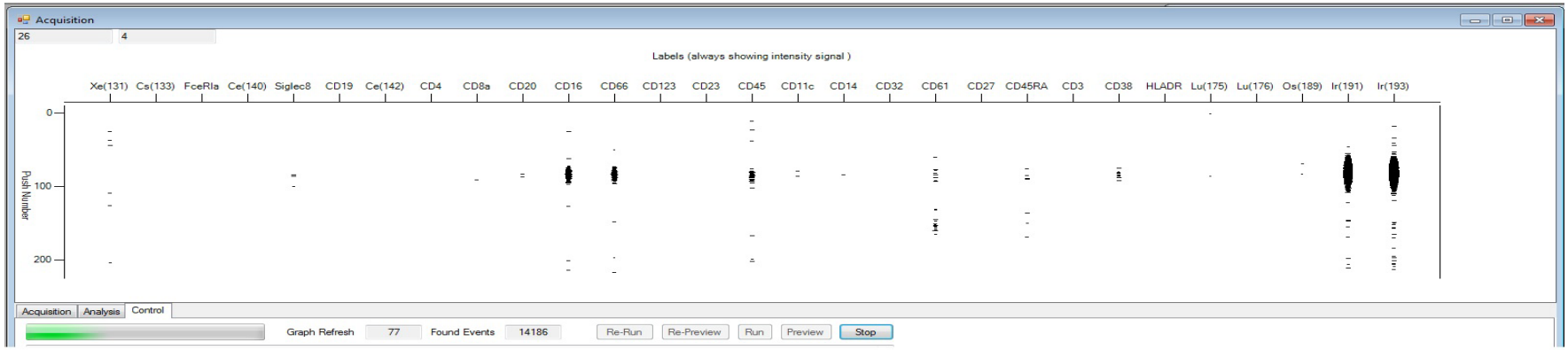
- A Rh- or Ir-based nucleic acid metalointercalator is added to all samples

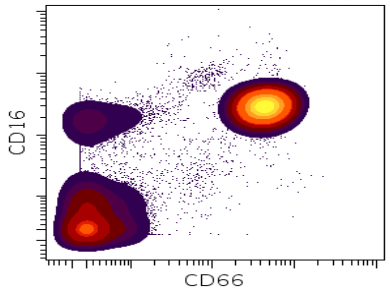


- A live/dead analysis can be done by adding one intercalator BEFORE cell staining, and the other AFTER staining and fixation.

Example of real-time data acquisition

- Whole lysed blood sample stained with a 20 parameter panel



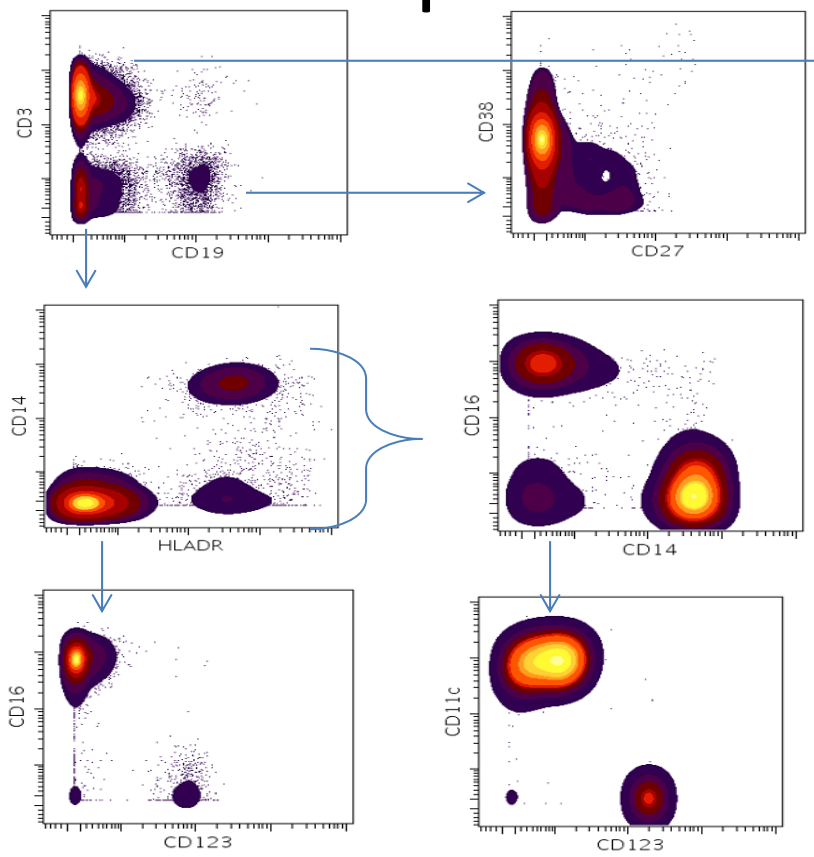


Sample pilot data

- 17 parameter human PBMC panel from DVS
- PBMCs from healthy volunteer

Target	Clone	Metal	Target	Clone	Metal
CD3	UCHT1	170Er	CD38	HIT2	172Yb
CD4	RPA-T4	145Nd	CD45	HI30	154Sm
CD8a	RPA-T8	146Nd	CD45RA	HI100	169Tm
CD11c	Bu15	159Tb	CD61	VI-PL2	165Ho
CD14	M5E2	160Gd	CD66	CD66a-B1.1	149Sm
CD16	3G8	148Nd	CD123	6H6	151Eu
CD19	HIB19	142Nd	CD235a/b	HIR2	141Pr
CD20	2H7	147Sm	HLA-DR	L243	174Yb
CD27	O323	167Er			

Sample pilot data at Mount Sinai



Gated on CD45+ singlet events

CD4 T cells (naïve/effector/memory
CD8 T cells (naïve/effector/memory
B cells (naïve/memory)
Basophils
CD16+ NK cells
CD14hiCD16low monocytes
CD14hiCD16hi monocytes
CD14lowCD16hi monocytes
Plasmacytoid DCs
Conventional DCs

Flow vs. Mass Cytometry

Mass Cytometry vs. Flow Cytometry

- Pros
  - More parameters (up to 25 fairly easily, up to 34 with some work, potential for 100+ in the future)
  - Minimal spillover between channels
  - Low variation in signal intensity across channels
  - Low background (no autofluorescence) so good signal to noise

- Cons
  - Slow (max of 500 events/s, less is better)
  - Inefficient (around 25-50% the efficiency of AriaII)
  - Weaker signal intensity than bright fluorochromes
  - Considerable instrument variability over time/environmental conditions
  - Consumables/parts are expensive and maintenance can be challenging

Ideal situations to use the CyTOF

- Rare samples with few cells (but not too few!)
- Need simultaneous information from many different populations in a heterogeneous mixture
- Need for depth in polyfunction profiling of cells in a heterogeneous mixture
- Need good signal to noise