DVS Sciences

Introductory CyTOF®2 Operator Training Course

July 2013

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Introductory CyTOF® 2 Operator Training Course

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CyTOF®2 Technical Overview

Utilizing the Power of the Atomic Spectrum

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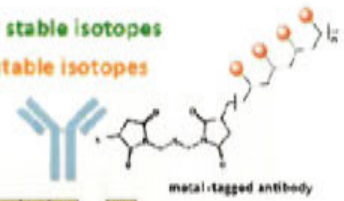
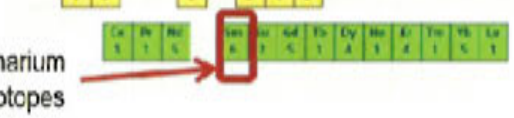
13 lanthanides: 37 stable isotopes

24 elements: 67 stable isotopes





Samarium
6 isotopes

The detector has ninety three mass channels.
DVS sells 32 MAXPAR metal kits plus Rhodium and Iridium intercalator reagents.

Utilizing the Atomic Mass Spectrum

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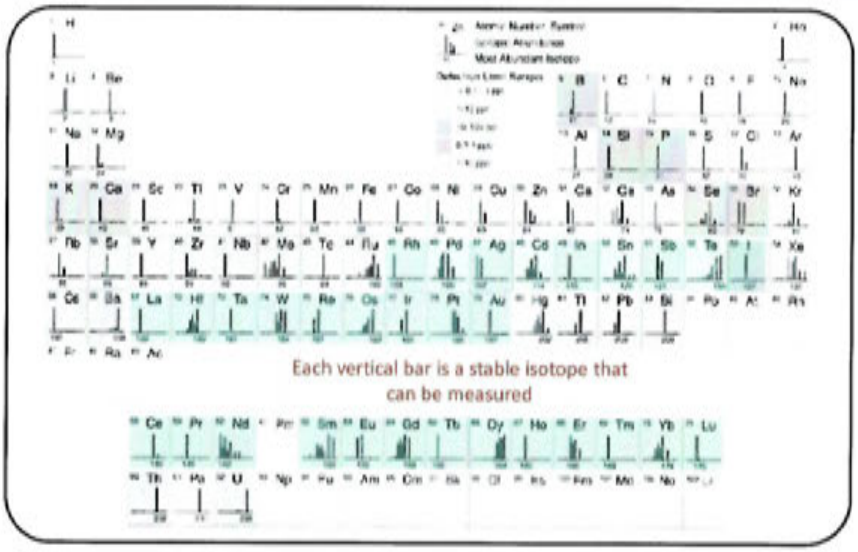

©Erin Simonds

Atomic Mass Spectrum

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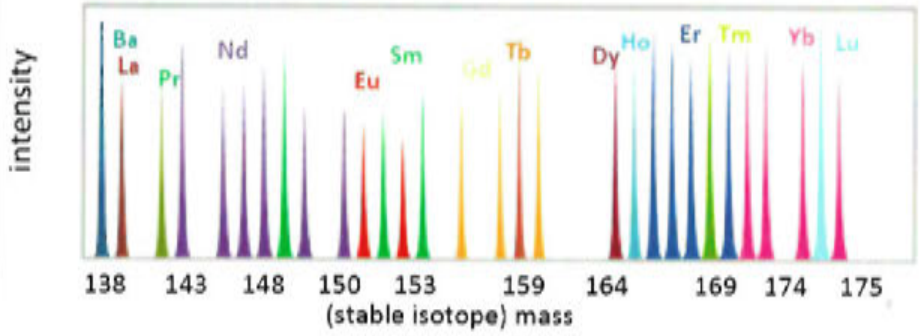

- Abundant tags of similar intensity
- Discrete signals: minimal overlap
- Single metal controls not required
- Background cellular signal: zero

Mass Cytometry

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CyTOF® 2 Mass Cytometer

- Inductively Coupled Plasma (ICP) Time-of-Flight (TOF) mass spectrometry discriminates metal-conjugated probes on a per-cell basis
- 103-193 Dalton atomic analytical mass range
- Single cell suspension input
- .fcs and .txt data output
- Optional Auto-sampler (96 well plates)

MAXPAR® Kits and Metal-conjugated antibodies

- Metal-conjugated antibodies (as of April 2013)
  - ~106 anti-human, 54 anti-mouse, 9 secondary antibodies
  - Reactive with cell surface, cytokine and signaling epitopes
- MAXPAR® Element labeling kits for antibody conjugations

Mass Cytometry Workflow

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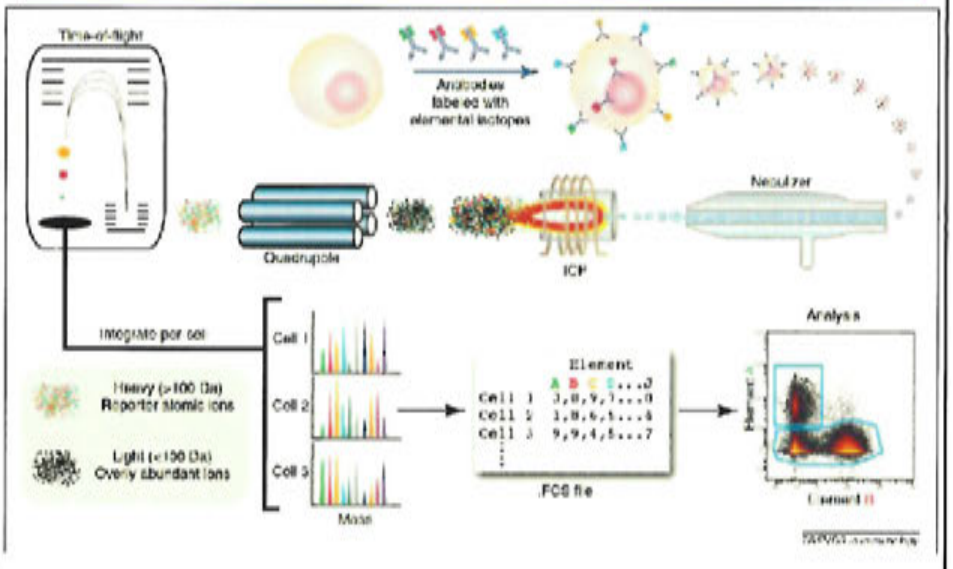

Bendall S. *et al.* (2012). Trends in Immunology. **33** (7), 323-332

Journey of the Cell: Outline

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INPUT = Cells in liquid suspension stained with metal-conjugated probes
OUTPUT = Individual metal ions separated on the basis of mass

The CyTOF® 2 achieves this through the following steps:

1. Sample introduction and ionization
   PURPOSE - to strip water from the cells followed by vaporization, atomization and ionization within the plasma
2. Ion filtering
   PURPOSE - to filter out unwanted endogenous low mass ions and argon
3. Time of flight ion separation and detection of metal probes
   PURPOSE - to separate the smallest from the highest mass ions; the time taken to reach the detector being proportional to mass

CyTOF® 2 Overview

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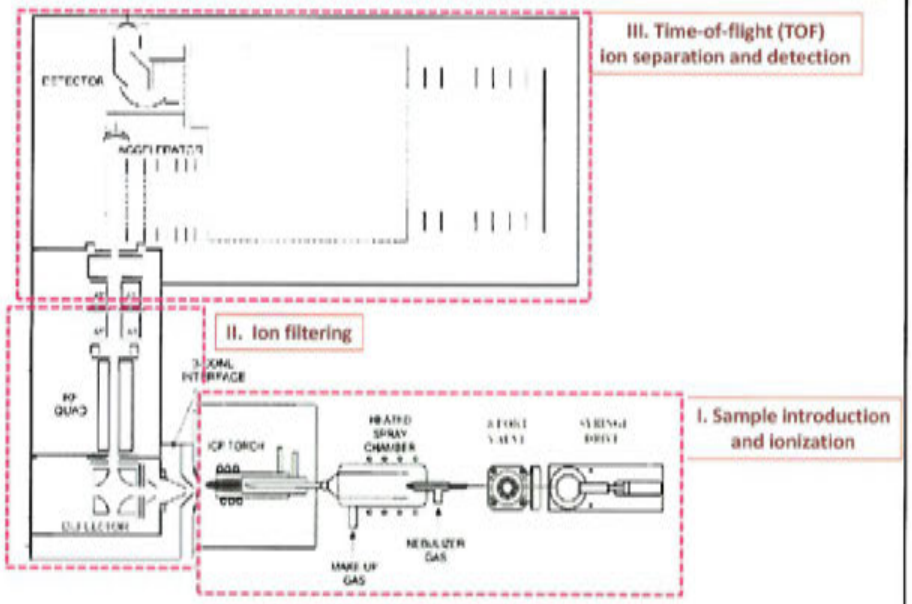

Journey of the Cell: Sample Introduction and Ionization

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1. **Sample introduction and ionization**
   PURPOSE - to strip water from the cells followed by vaporization, atomization and ionization within the plasma
   Input: Single cell suspension
   Output: Ions derived from metal-conjugated probes, endogenous cell components and argon

Sample Introduction

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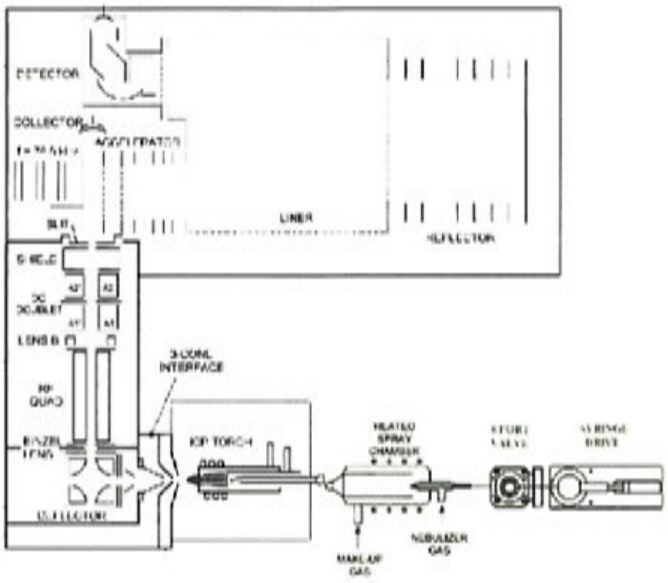

Comparison of Obtained Cell Frequencies

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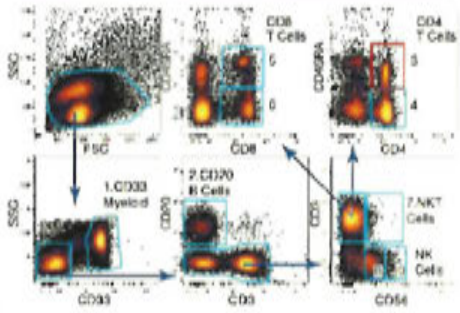
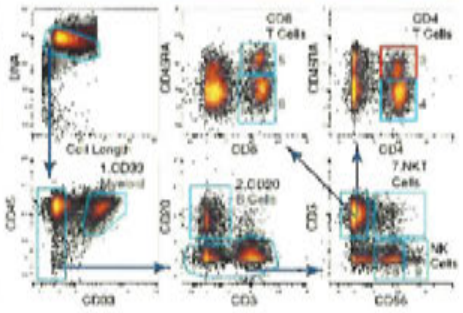
Conventional flow cytometry



Mass cytometry



Inductively Coupled Plasma (ICP)

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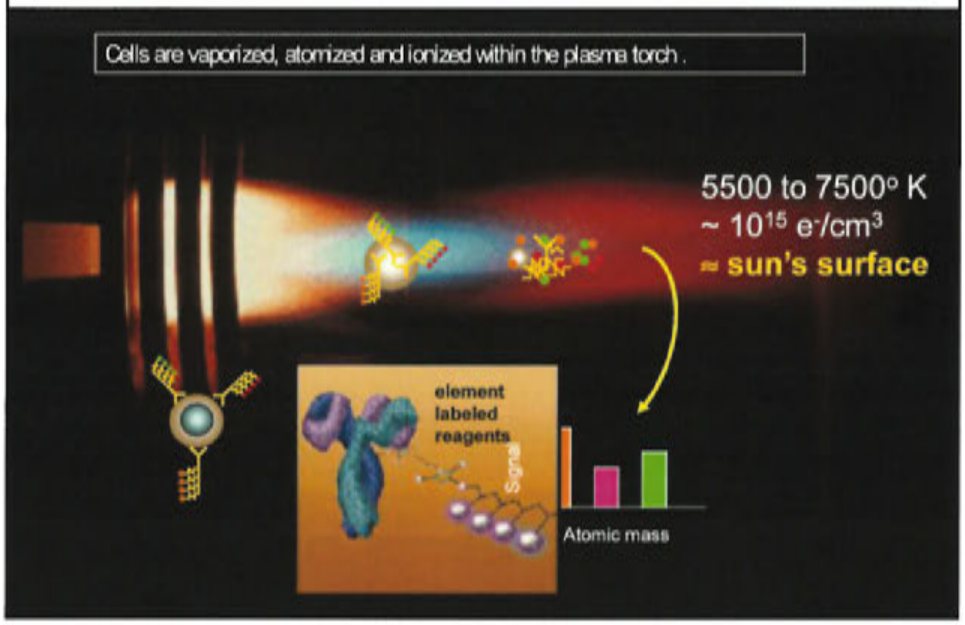

Journey of the Cell: Plasma Generation

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- Plasma is generated by inductively heating argon gas using an electrical coil.
- Argon gas flows through the torch, which itself is placed inside an induction coil supplied with a radio-frequency electric current.
- An electric spark is applied to the argon gas stream to induce free electrons, which interact with the radio-frequency magnetic field of the induction coil and are accelerated.
- The accelerated electrons collide with argon atoms, causing the release of an electron which is in turn accelerated by the magnetic field.
- This process continues, eventually converting the argon gas into high-temperature plasma consisting of argon atoms, free electrons and argon ions.

Inductively Coupled Plasma (ICP)

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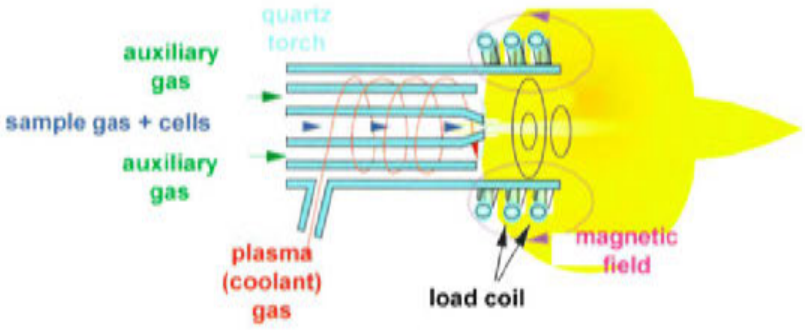
to vaporize, atomize and ionize the sample



Journey of the Cell: Ion Filtering

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2. **Ion filtering**
   PURPOSE - to filter out unwanted endogenous low mass ions and argon
   Input: Ions derived from metal-conjugated probes, endogenous cell components and argon
   Output: Highly enriched ions from the metal probes, randomly distributed in an ion cloud

Ion Filtering

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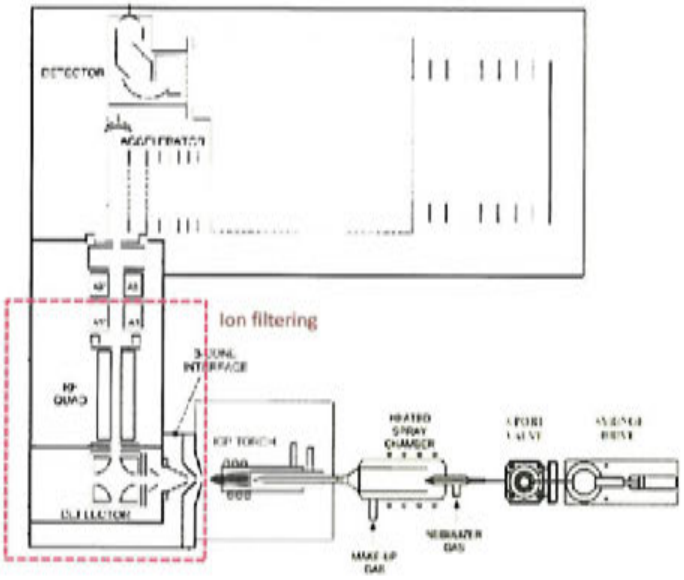

CyTOF®2 Plasma-Vacuum Interface

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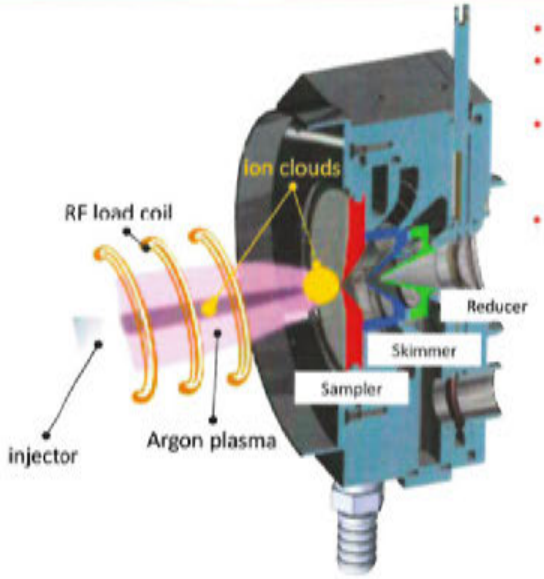
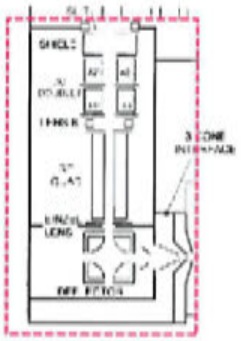


- Chiller cools the cones
- "Dirty" cones reduce sensitivity
- Cones get dirty just from having plasma on; not from running cells
- Deposits from load coil



CyTOF®2 Plasma-Vacuum Interface

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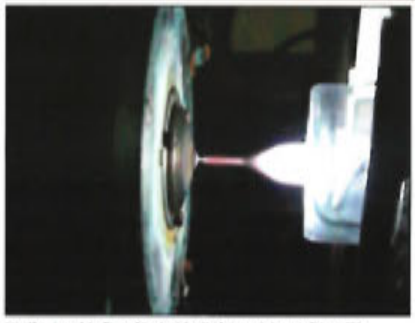

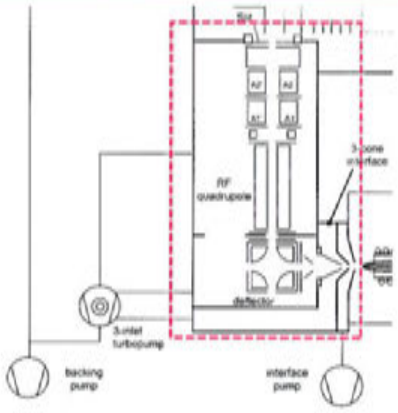

- Cones:
  Sampler, skimmer, reducer
- The ions pass through each cone into a chamber to which a sequentially lower vacuum pressure is applied
- A low vacuum pressure is required to move ions through the system and to limit random collisions during mass resolution

Journey of the Cell: Why do we Need a Vacuum?

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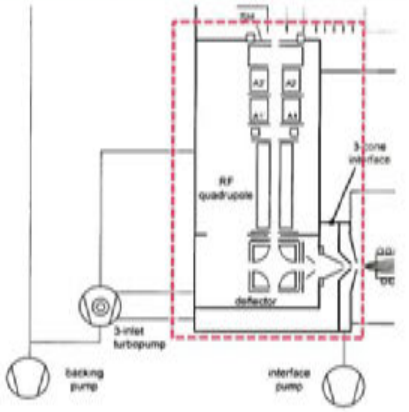
- The ions need to be in the gas-phase in order to freely pass though the system in response to the imposed electromagnetic fields
- A vacuum limits the number of random collisions and allows free movement of the ions
- A vacuum is sequentially applied as the ions travel through the cones, which removes ambient air from the system



Ion Filtering: Masses > 100

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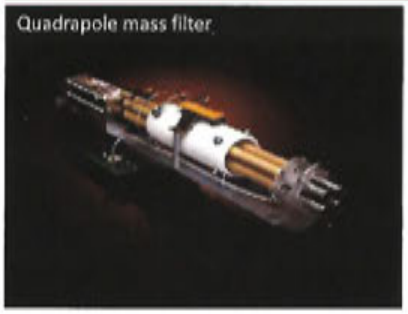
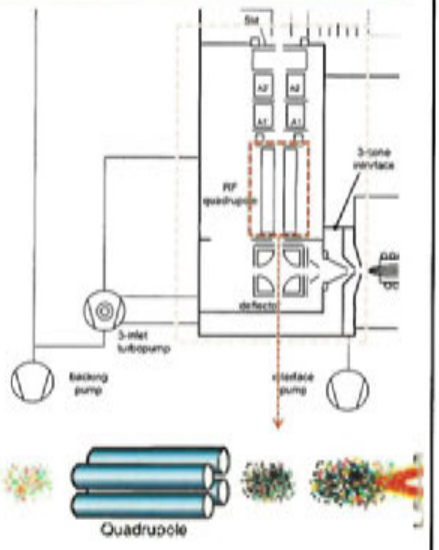


- Quadrapole Deflector:
  Eliminates non- ionized particles and photons
- Quadrapole Ion Filter:
  Eliminates ions with a mass below 100 Da
  e.g. H^+, C^+, O^+, N^+, OH^+, CO^+, $O2^+$, Ar^+, ArH^+ ArO^+ and Argon dimers



Journey of the Cell: TOF Ion Separation and Detection

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3. **Time of flight ion separation and detection of metal probes**
   PURPOSE - to separate the smallest from the highest mass ions; the time taken to reach the detector being proportional to mass
   Input: Highly enriched ions from the metal probes, randomly distributed in an ion cloud
   Output: Time-resolved ions from each individual metal probe

Time of Flight Ion Separation and Detection

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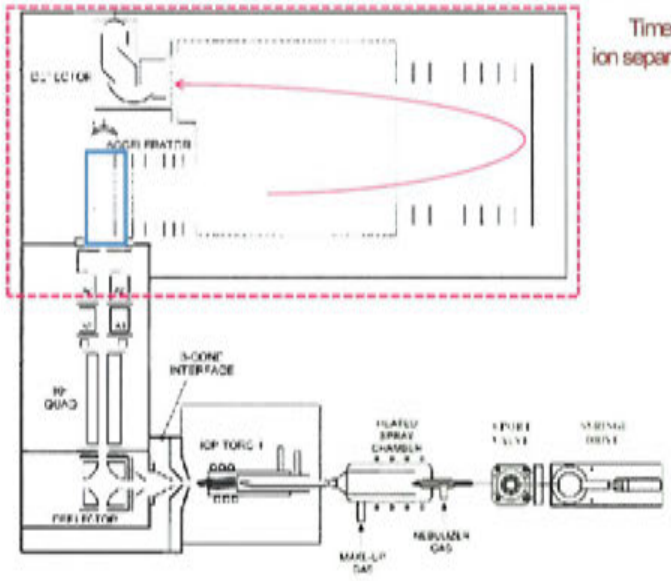

Time-of-flight (TOF) ion separation and detection

Time of Flight Separation of Ions

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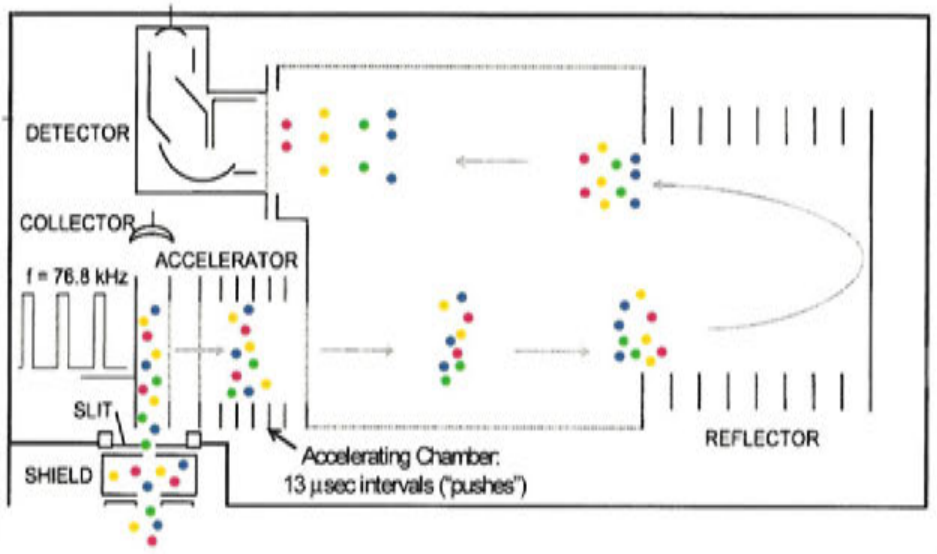

Pushes vs ToF

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These are NOT the same thing

Pushes 13 µsec slice of the ion stream entering the ToF chamber

ToF Separation of ions **within a push** by mass

Tuning Solution: Metals in Liquid Suspension

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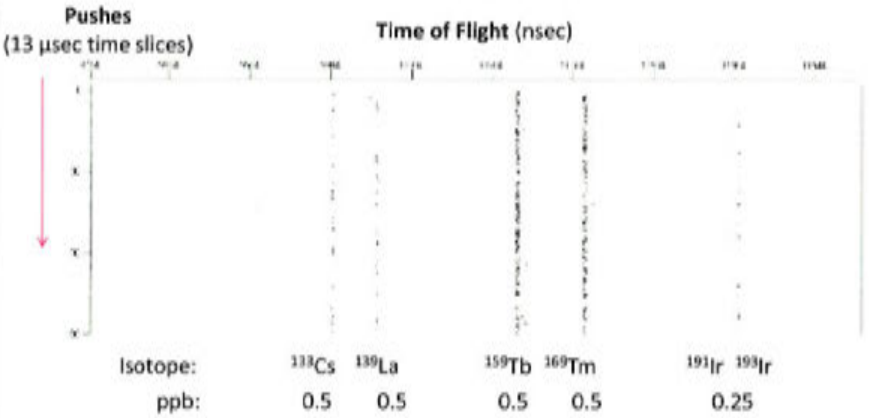
Purpose: Create a continuous stream of ions at varying density to monitor instrument performance.



Multiparameter Analysis of PBMCs: Particles

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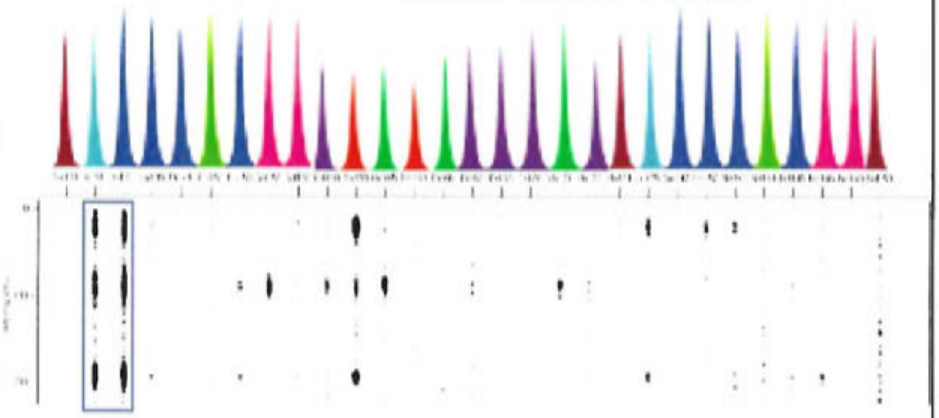
CD2 175Lu	CD13 166Er	CD36 150Nd	CD56 176Yb
CD3 152Sm	CD15 170Er	CD38 165Ho	CD64 148Nd
CD4 142Nd	CD19 171Yb	CD40 172Yb	CD71 167Er
CD7 139La	CD20 156Gd	CD44 151Eu	CD90 174Yb
CD8 146Nd	CD31 144Nd	CD45 159Tb	CD117 147Sm
CD10 168Er	CD33 141Pr	CD45RA 153Eu	HLA-DR 160Gd
CD11b 158Gd	CD34 169Tm	CD49d 145Nd	



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