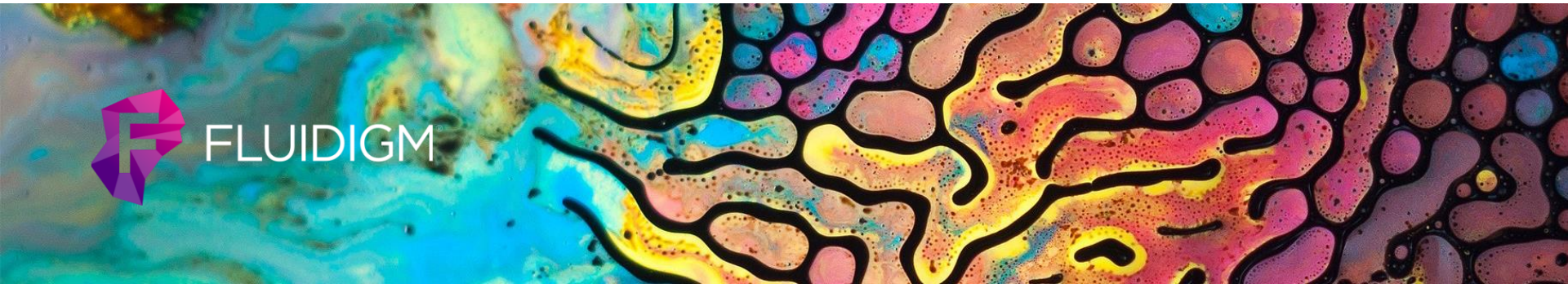
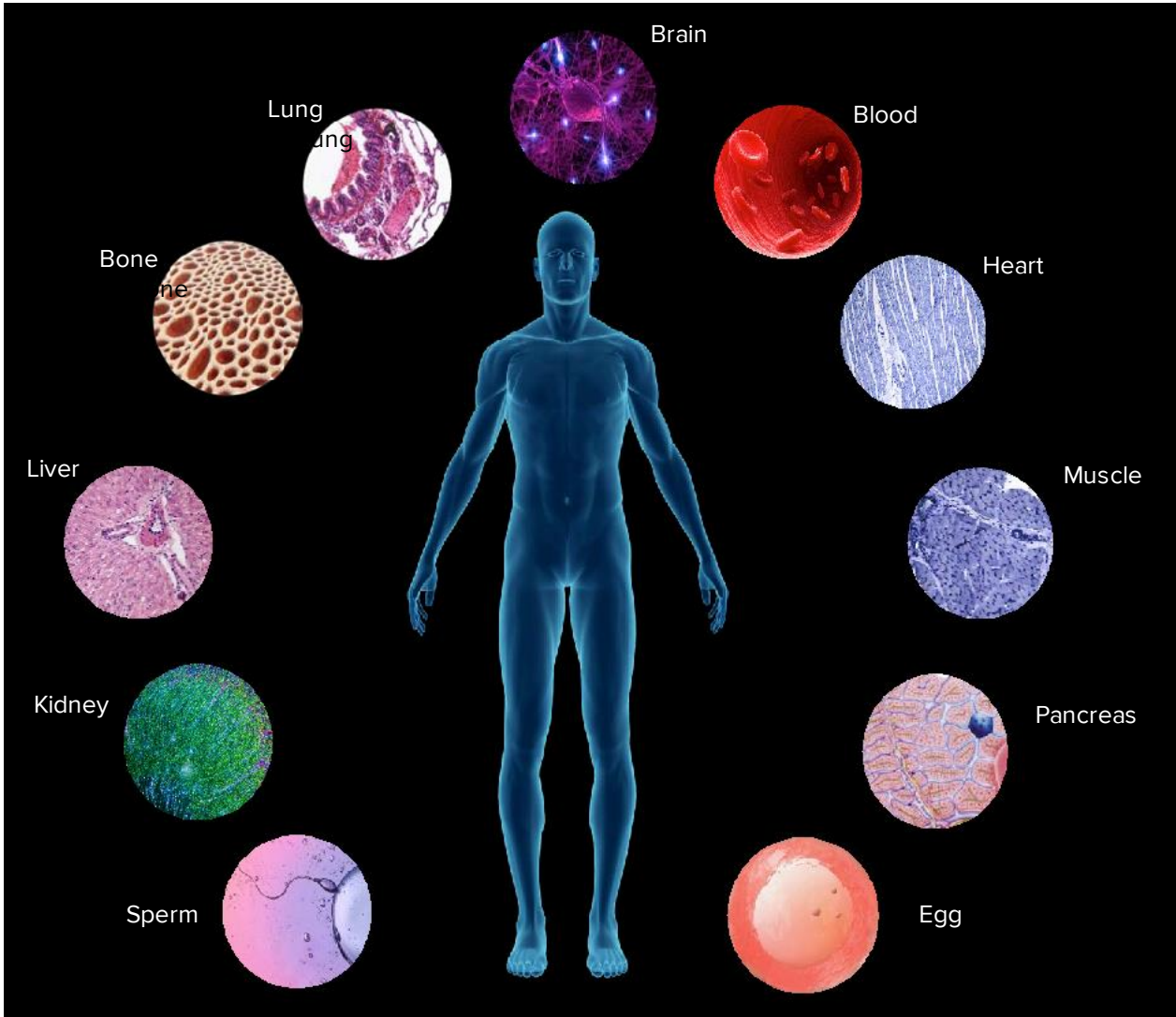
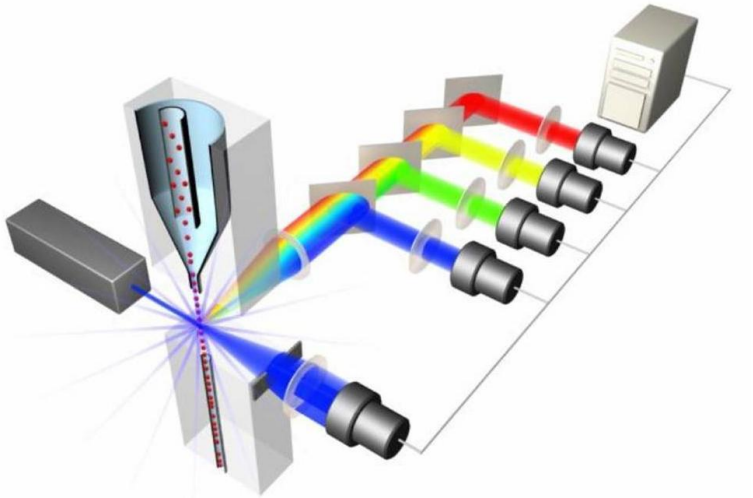
Helios Technical Overview



Every tissue is heterogeneous





流式技术

——最经典的单细胞技术

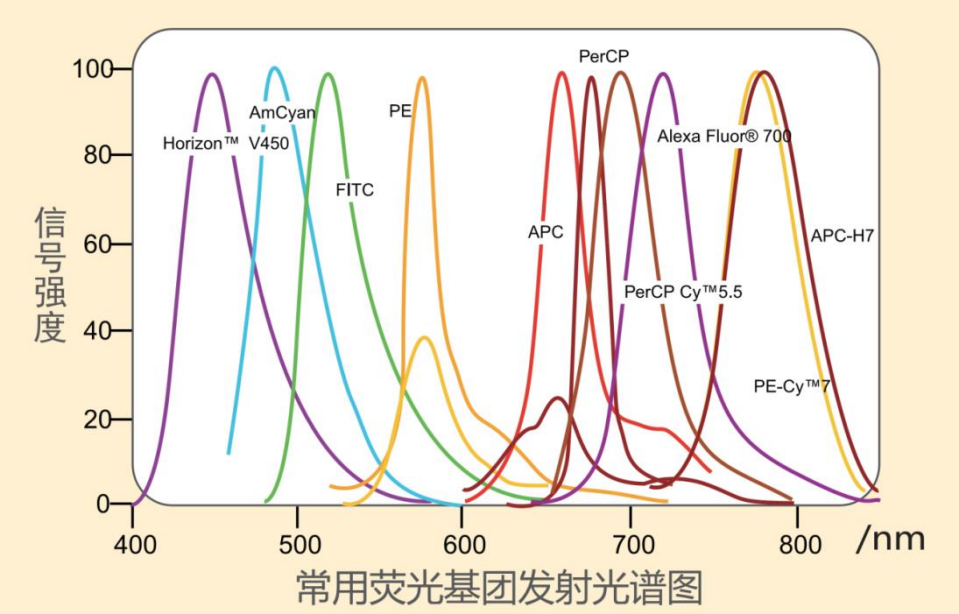
对单细胞的多参数检测

↓

对样本进行亚群和功能分析

流式技术的瓶颈：

——荧光发射光谱的重叠



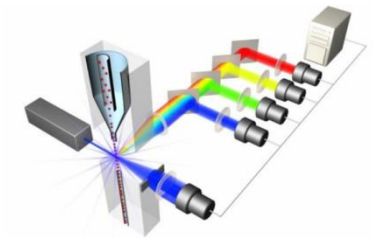
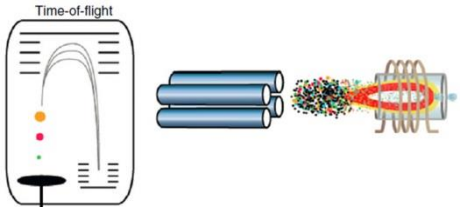
常用荧光基团发射光谱图

造成的问题：

- 通道间的相互干扰
- 通道数量很难进一步提升

需要寻求更好的标签和检测系统

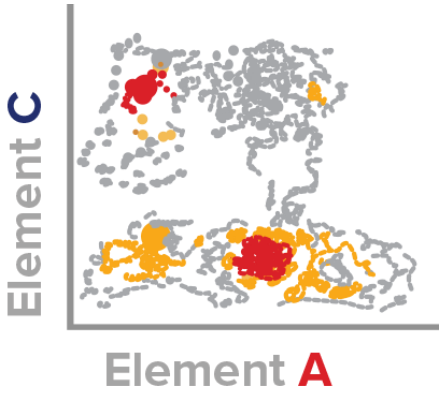
质谱流式技术的诞生：

	传统流式技术	质谱流式技术
标签系统	 荧光基团偶联抗体	 金属元素偶联抗体
检测手段	 荧光光路	 ICP质谱

Mass Cytometry: 50+ Parameters on Millions of Cells

Discovery of new biology
Comprehensive functional profiling

Basic research
Drug discovery
Clinical research



1	2	3	4	5
Metals	**Panels**	**Resolution**	**Channels**	**Cells**
>50 tags	42+ markers	1 Da	135	Millions

研究者可以全面获得样本表型和功能的信息

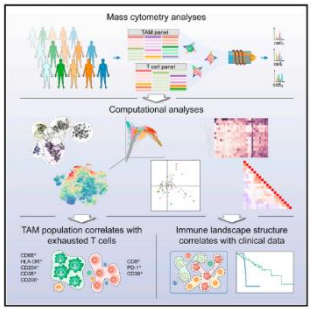
FLUIDIGM

Resource

Cell

An Immune Atlas of Clear Cell Renal Cell Carcinoma

Graphical Abstract



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Vito Riccardo Tomaso Zanotelli, ...,
Bernhard Reis, Dana Pe'er,
Bernd Bodenmiller

Correspondence

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In Brief

Applying mass cytometry for high-dimensional single-cell analysis depicts an in-depth atlas of the immune microenvironment in clear cell renal cell carcinoma patients, thereby linking immune compositions with clinical features.

Highlights

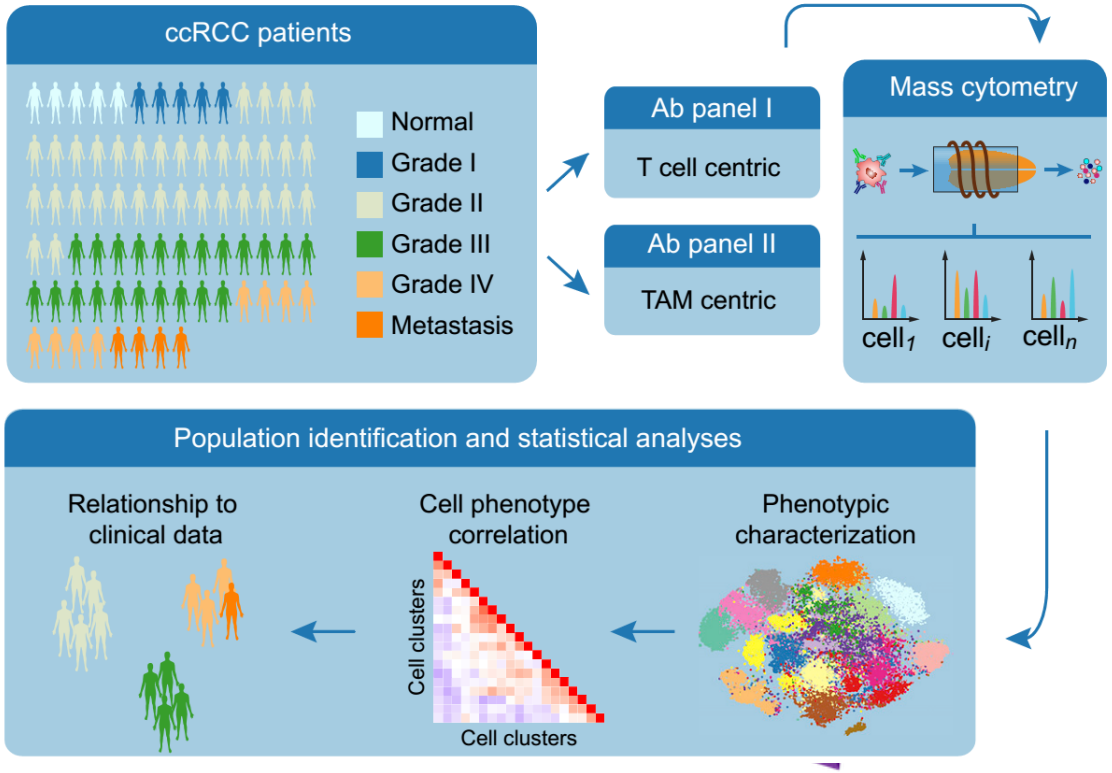
- Mass cytometry reveals the immune cell diversity of the ccRCC tumor ecosystem
- PD-1$^+$ cells display heterogeneous combinations of inhibitory receptors
- CD38$^+$CD204$^+$CD206$^-$ tumor-associated macrophages correlate with immunosuppression
- A specific immune signature is linked to shorter progression-free survival

Chevrier et al., 2017, Cell 169, 736–749
May 4, 2017 © 2017 The Author(s). Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.cell.2017.04.016

CellPress

36

32



Cell

Resource

Single-Cell Trajectory Detection Uncovers Progression and Regulatory Coordination in Human B Cell Development

Sean C. Bendall,[1,2,7] Kara L. Davis,[1,3,7] El-ad David Amir,[4,7] Michelle D. Tadmor,[4] Erin F. Simonds,[1] Tiffany J. Chen,[1,5,6] Daniel K. Shenfeld,[4] Garry P. Nolan,[1,2,*] and Dana Pe'er[4,6,*]
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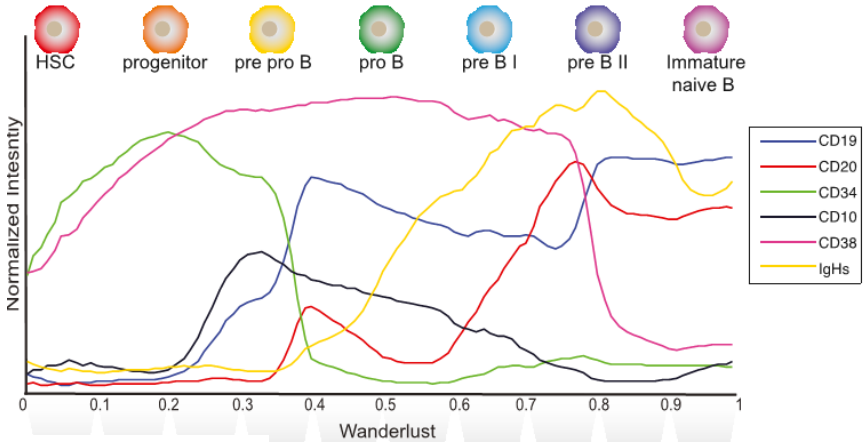
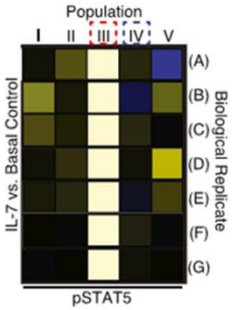
http://dx.doi.org/10.1016/j.cell.2014.04.005

Big Panel

21 细胞表面 Marker + 6 B发育相关蛋白 + 15 信号通路蛋白 = 42 抗体的 Panel

Discovery





Cell Stem Cell
Resource

CellPress

A Continuous Molecular Roadmap to iPSC Reprogramming through Progression Analysis of Single-Cell Mass Cytometry

Eli R. Zunder,[1,4] Ernesto Lujan,[2,3,4] Yury Goltsev,[1] Marius Wernig,[2] and Garry P. Nolan[1,*]
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Stanford University School of Medicine, Stanford, CA 94305, USA
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http://dx.doi.org/10.1016/j.stem.2015.01.015

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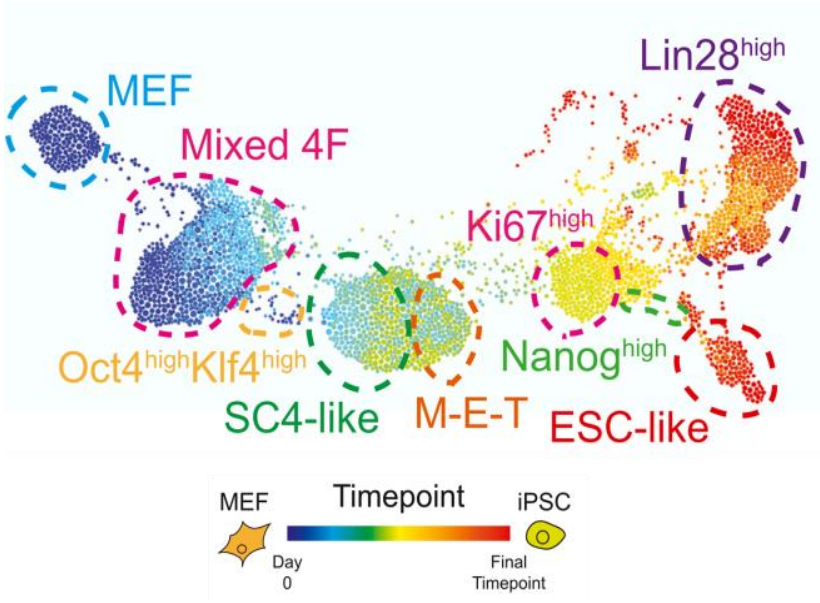
示例

利用质谱流式检测了诱导不同时间的MEF细胞中35个胞内外蛋白，展示了MEF细胞被诱导为iPSC过程的“路线图”



Cell Stem Cell 16，323-337，2015

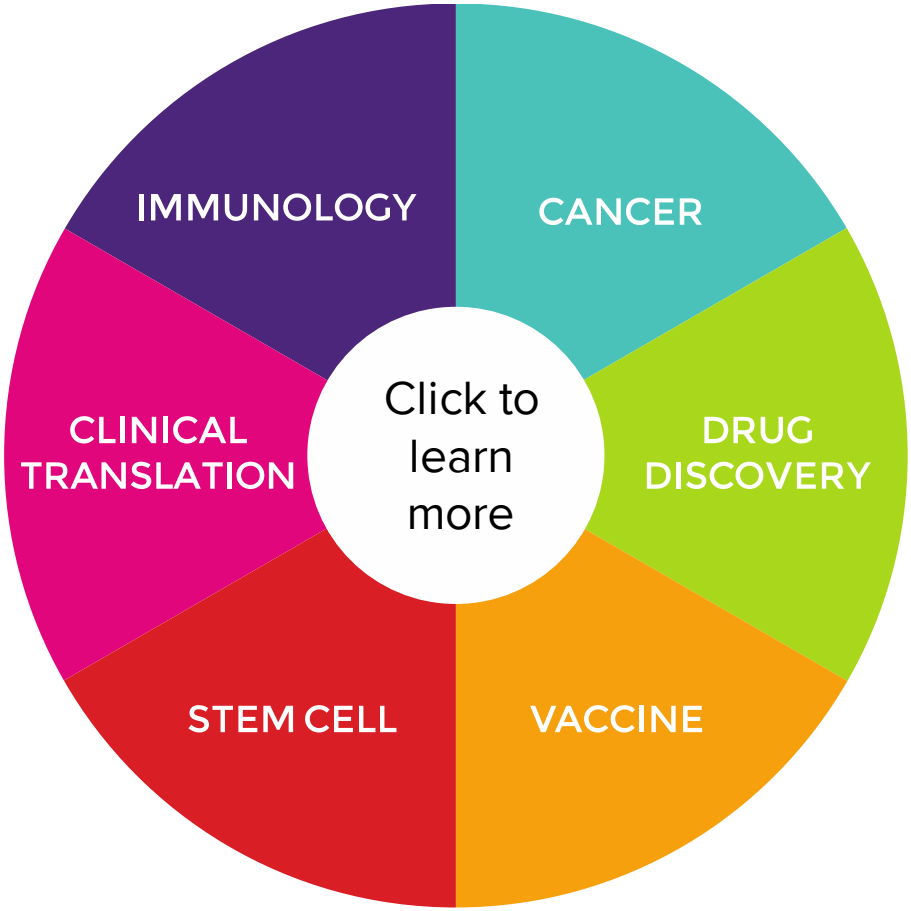
FLUIDIGM

Mass Cytometry Research

Applications

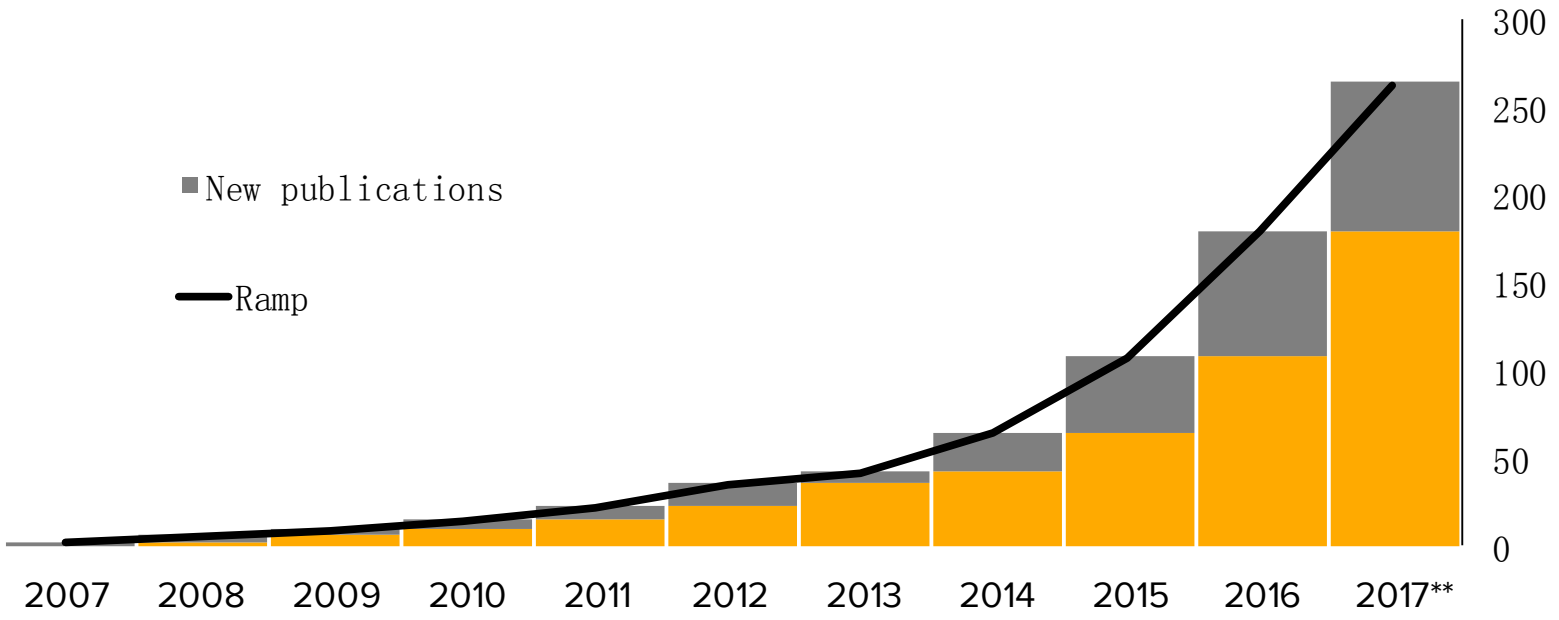
- Phenotyping
- Signaling and transcription
- Cytokines and growth factors
- Cell death and apoptosis
- Cell cycle and proliferation

Research areas



Mass cytometry publication ramp

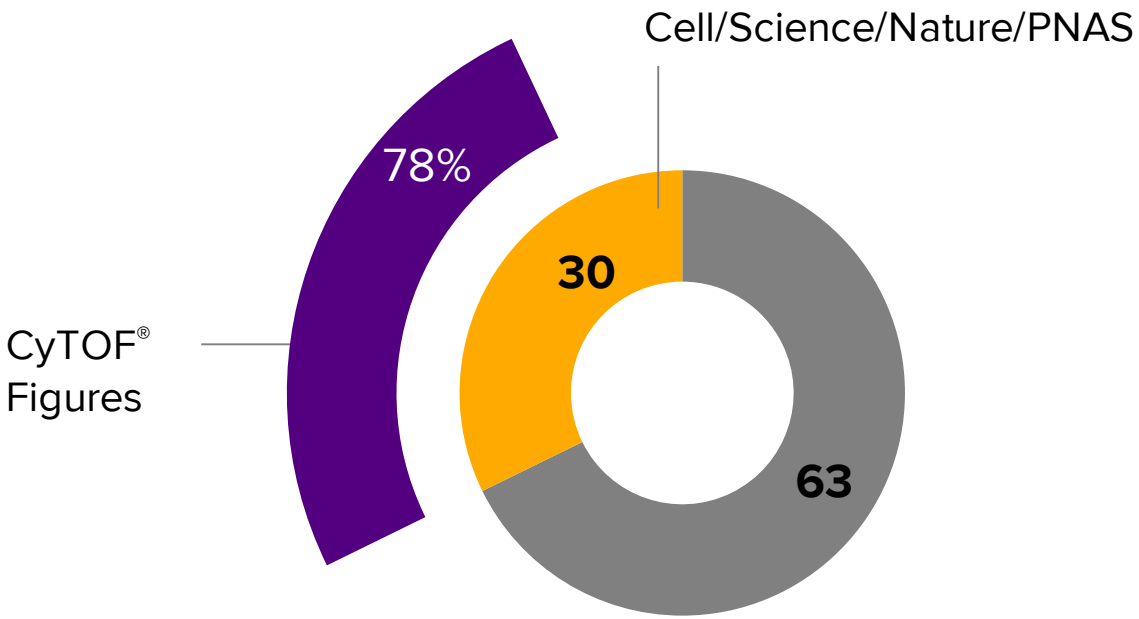
Peer-reviewed publications*



*Does not include commentaries or reviews

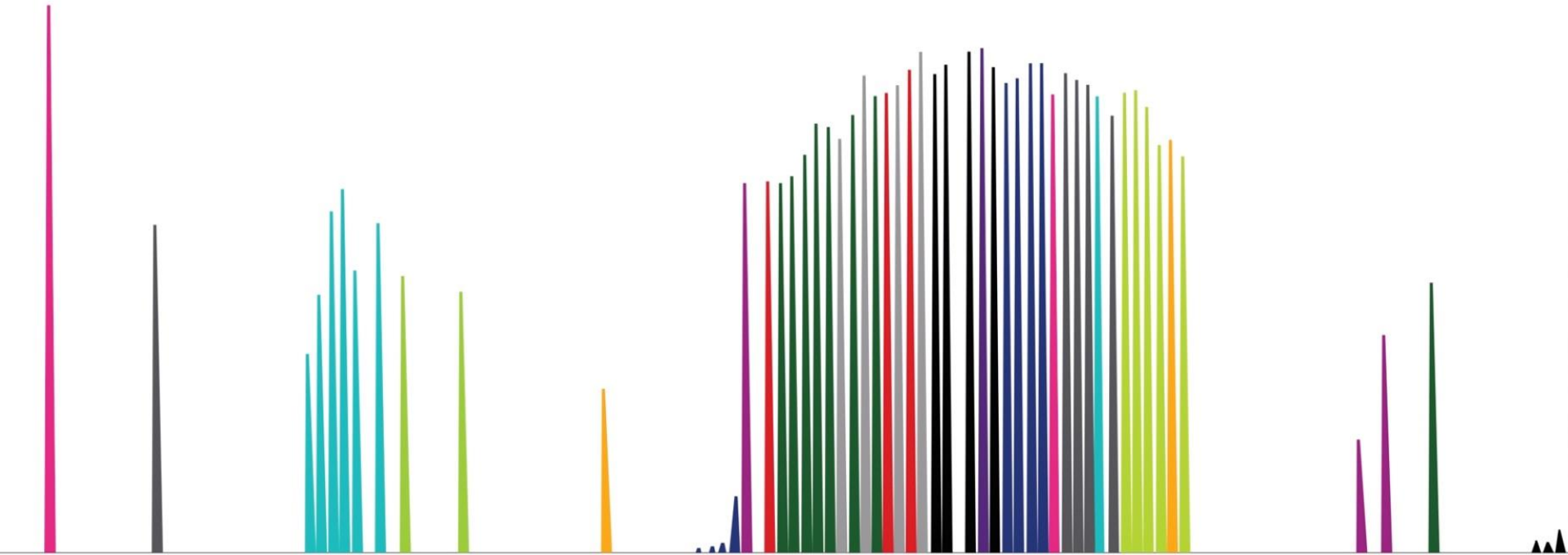
**As of July 15, 2017

Impact



Mass cytometry is responsible for breakthrough discoveries, published in top tier journals.

质谱流式的原理介绍

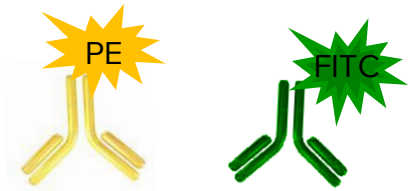


质谱流式技术的诞生：

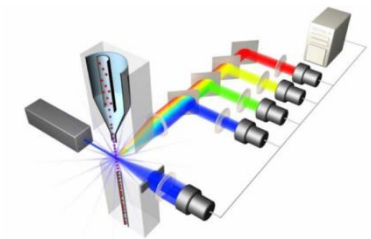
标签系统

检测手段

传统流式技术



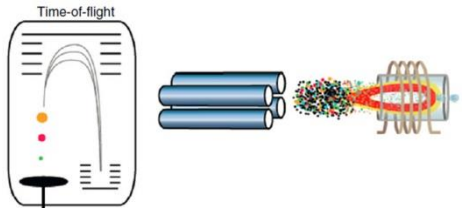
荧光基团偶联抗体



荧光光路

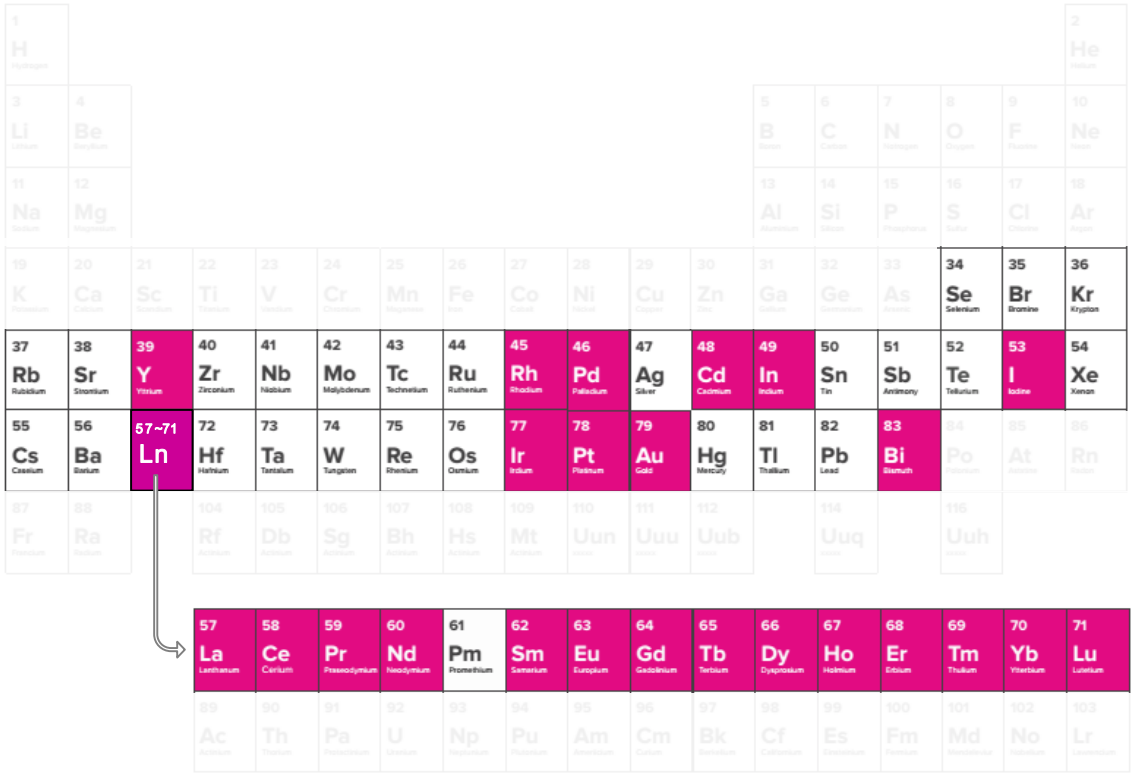
质谱流式技术

金属元素偶联抗体



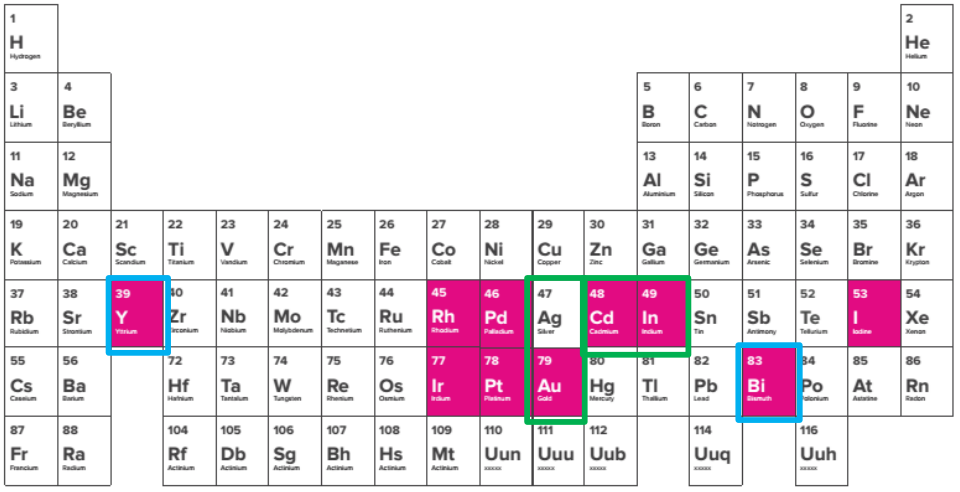
ICP质谱

丰富的标签可供选择

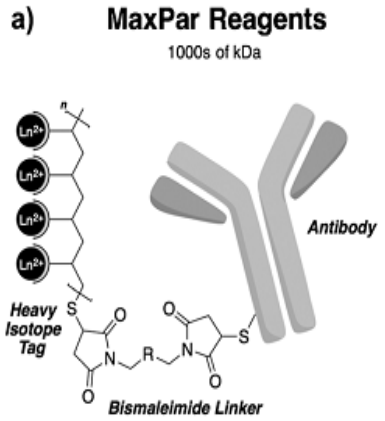


- Helios 75~209 amu
- Not rare, radioactive or biological

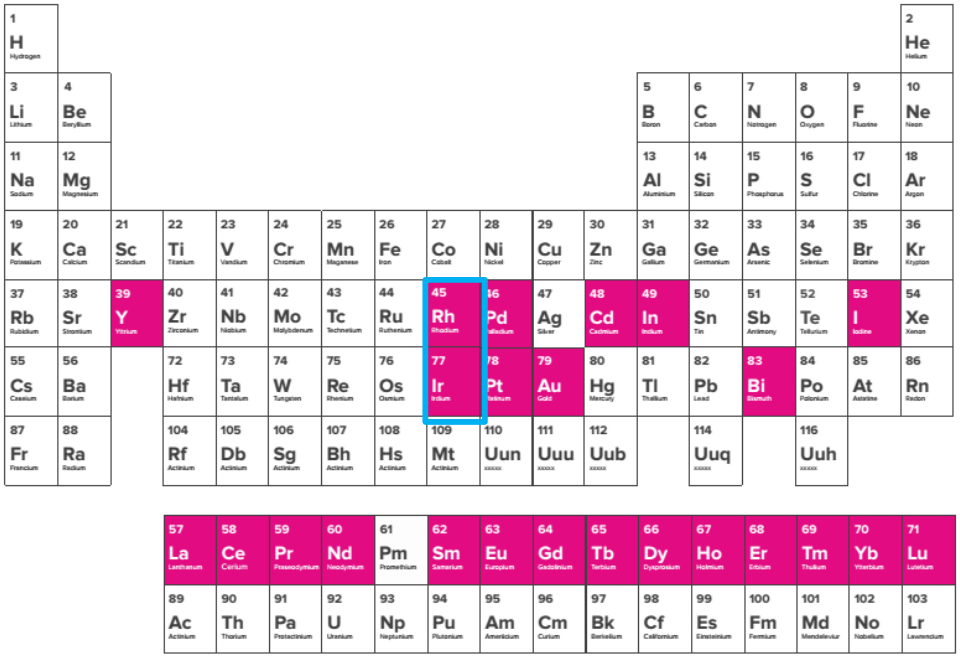
Utilizing the power of the atomic spectrum

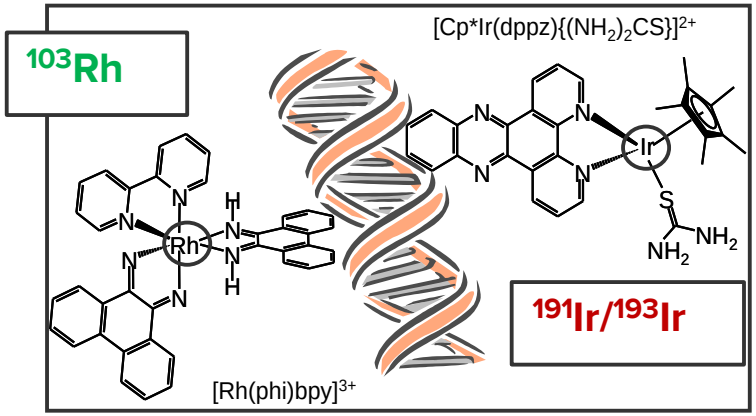


Mass cytometry elements: the stable isotopes of these 24 elements provide over 50 unique tags for use in mass cytometry experiments.

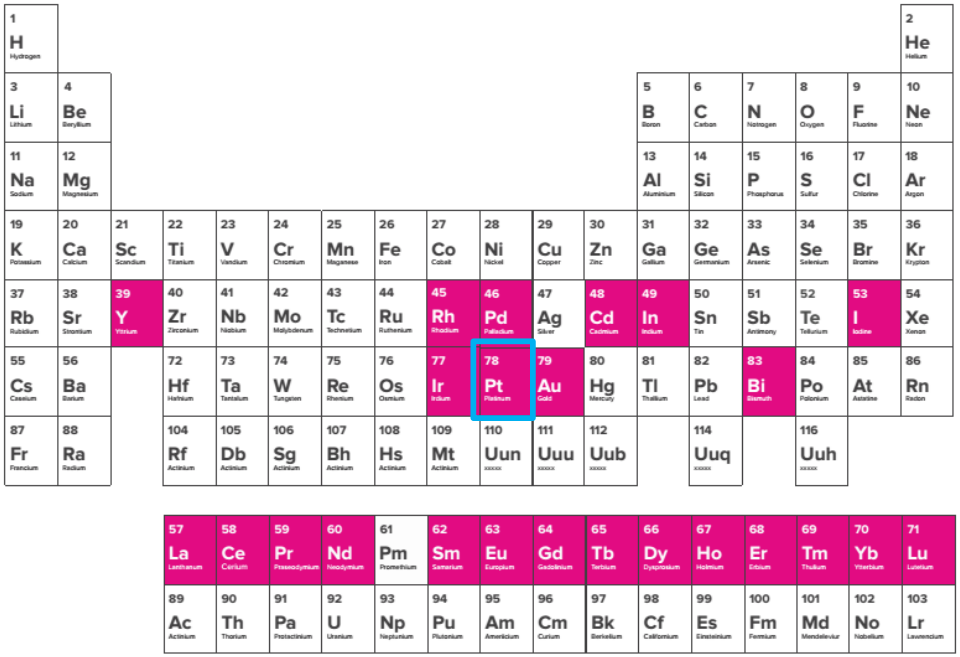


Utilizing the Atomic Mass Spectrum



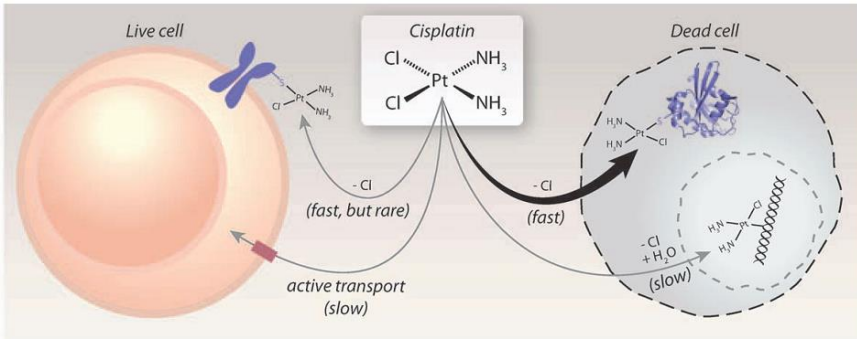


Utilizing the Atomic Mass Spectrum

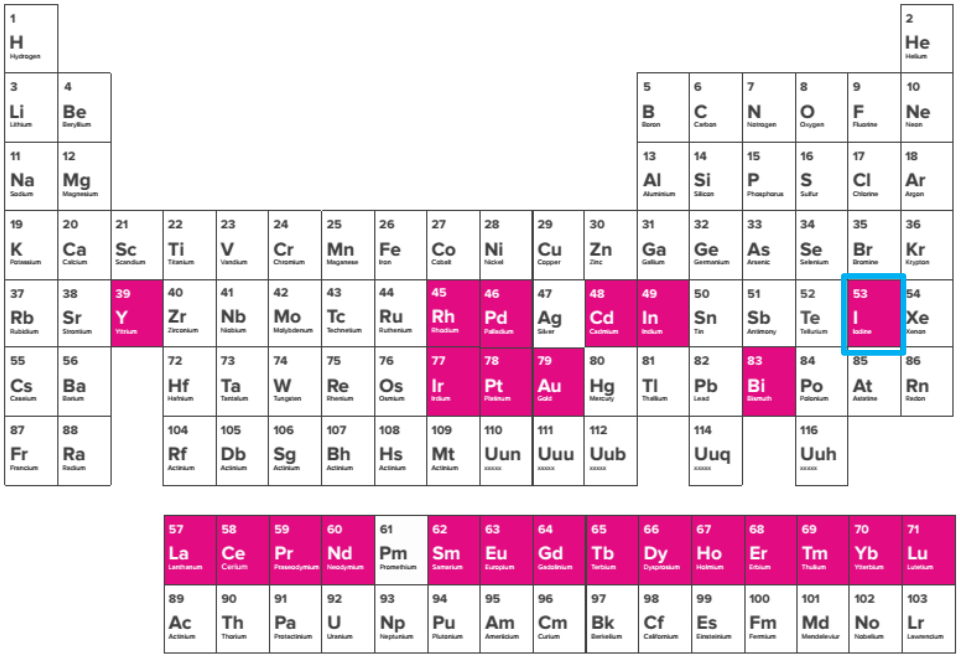


Mass cytometry elements: the stable isotopes of these 24 elements provide over 50 unique tags for use in mass cytometry experiments.

Cisplatin



Utilizing the Atomic Mass Spectrum



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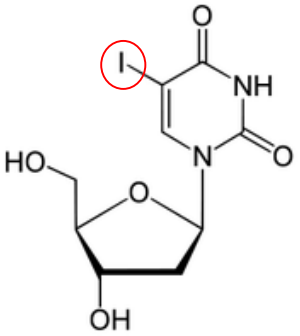
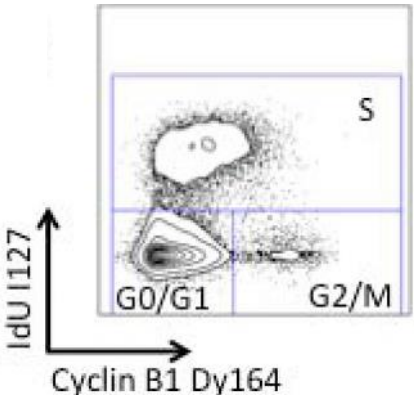
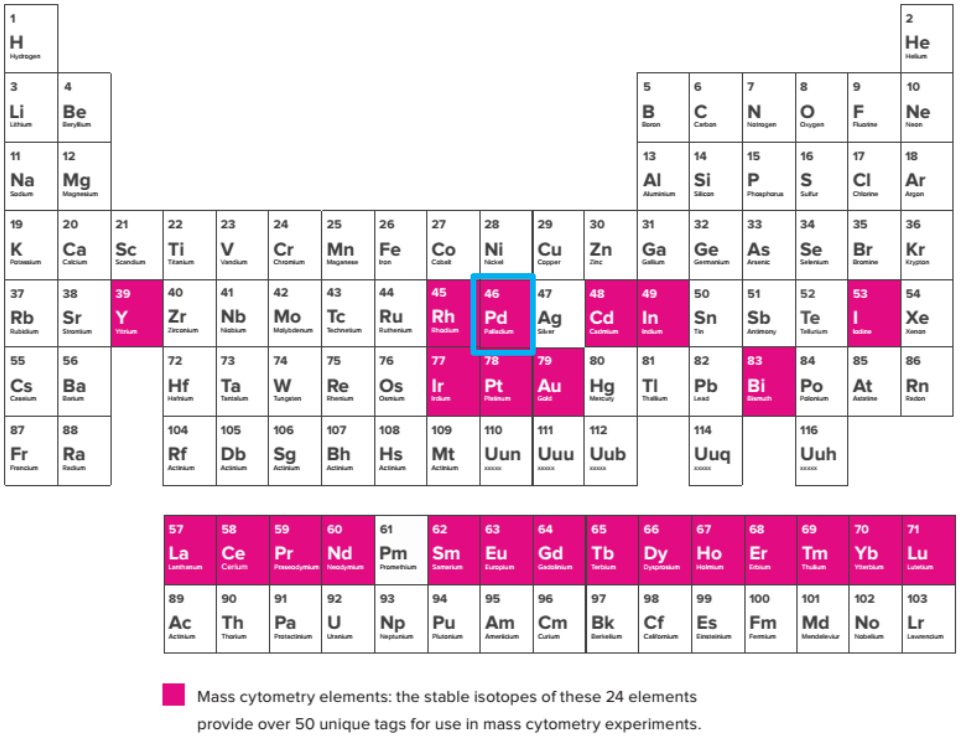


IdU

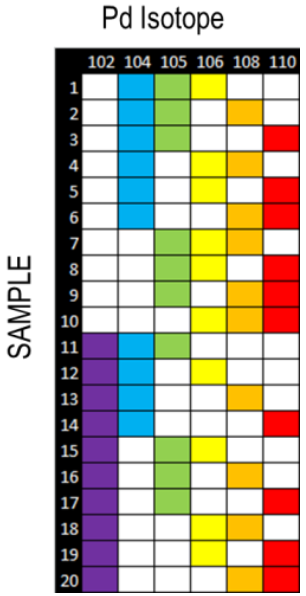


Utilizing the Atomic Mass Spectrum



Mass cytometry elements: the stable isotopes of these 24 elements provide over 50 unique tags for use in mass cytometry experiments.

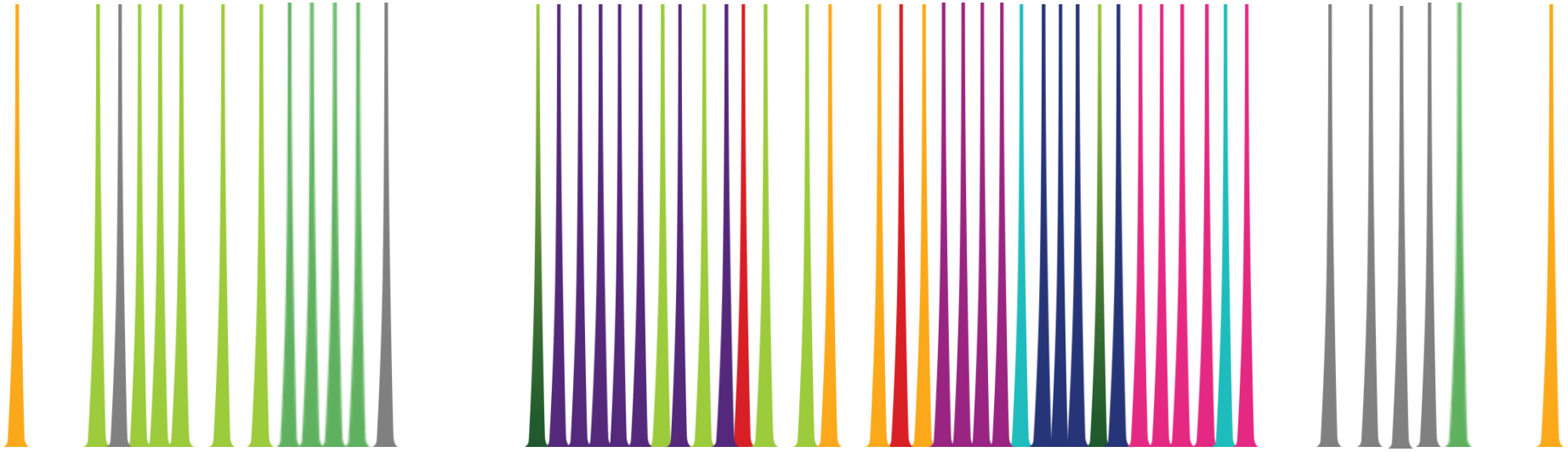
barcoding



Utilizing the Atomic Mass Spectrum

Channels

Channels Remaining 81



Panel

- PBMC Phenotyping Kit (17)
- Cytokine kit (11)
- Additional targets (9)
- 3rd Party Metal labeled Antibodies (5)

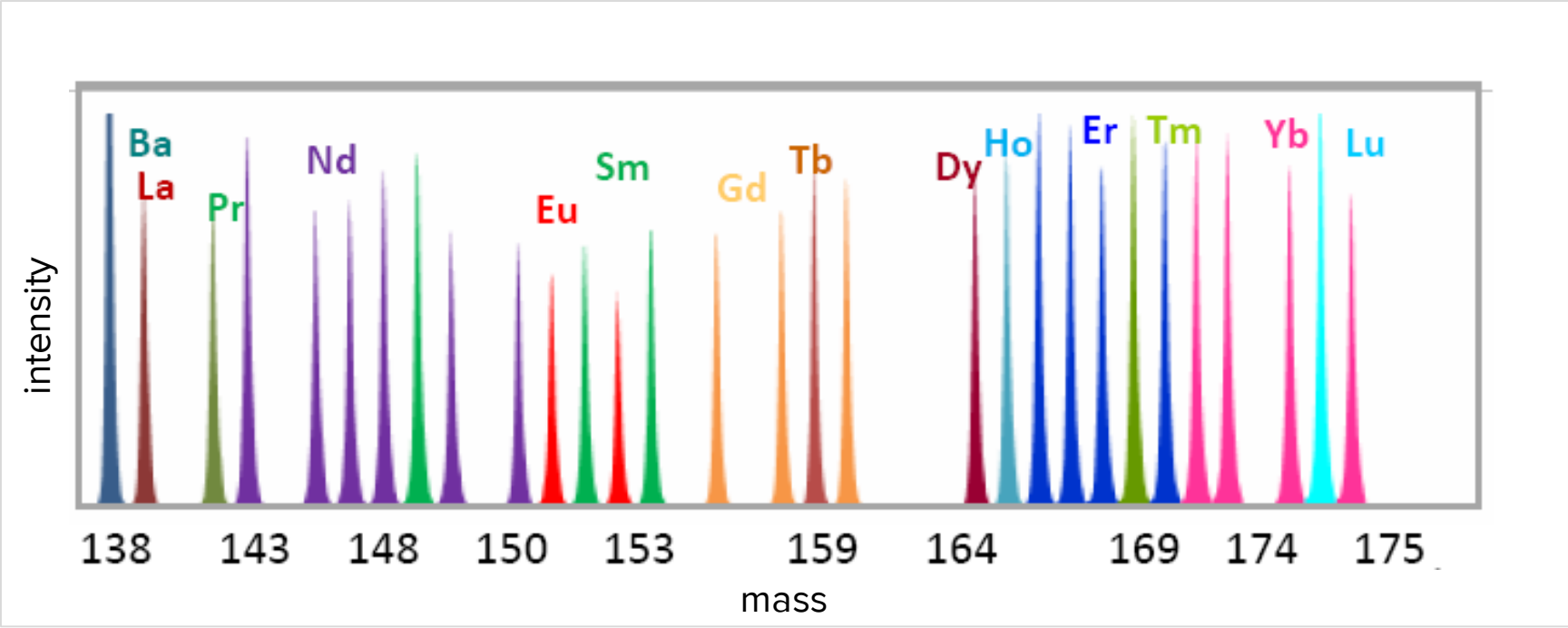
Cell-ID

- Barcoding (6)
- DNA (3)
- S-phase (1)
- Dead cell (2)

Other

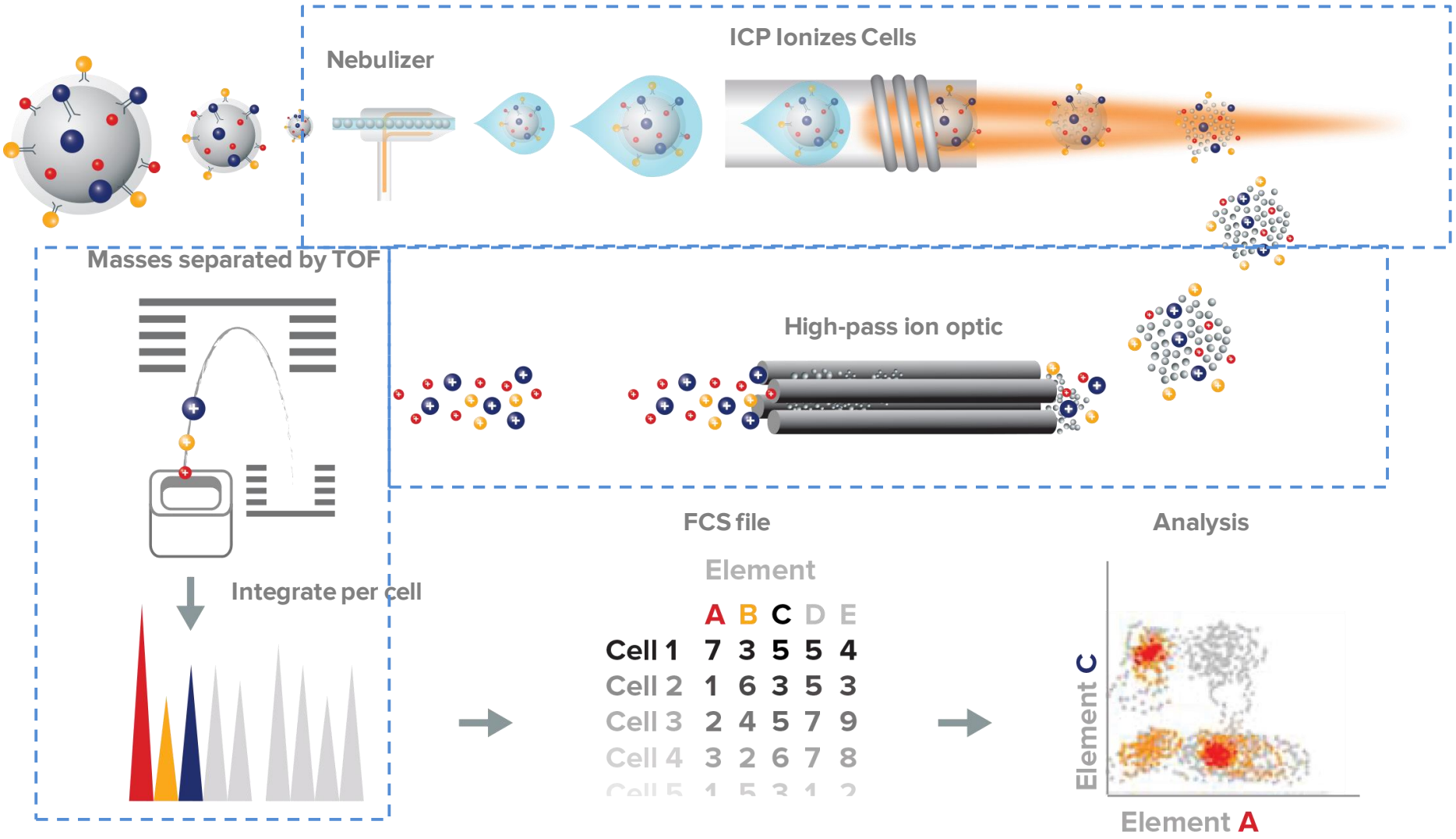
New Tags Under R&D (…)

Atomic mass spectrum

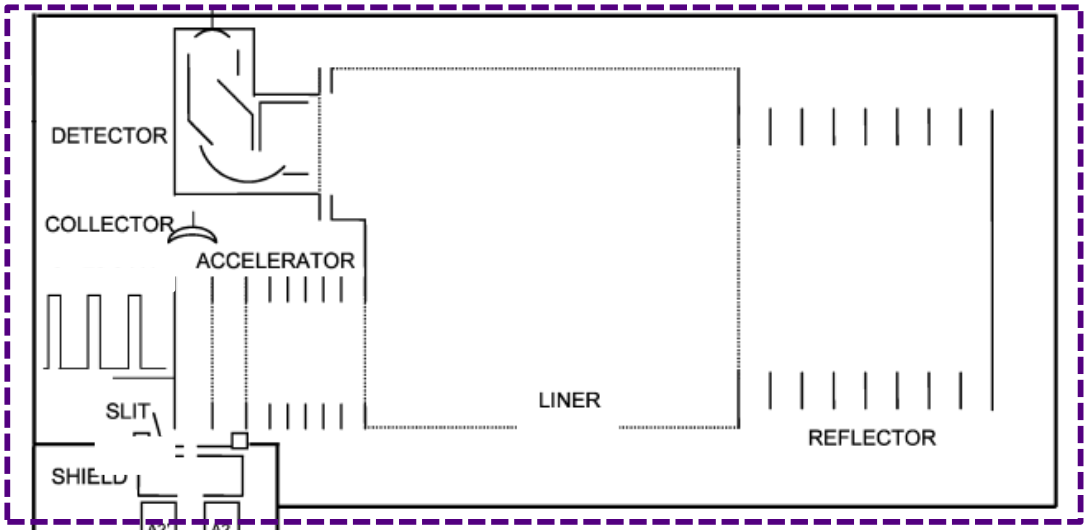


- Abundant tags of similar intensity
- Discrete signals: minimal overlap (fewer controls to run)
- Zero background cellular signal

Mass cytometry acquisition



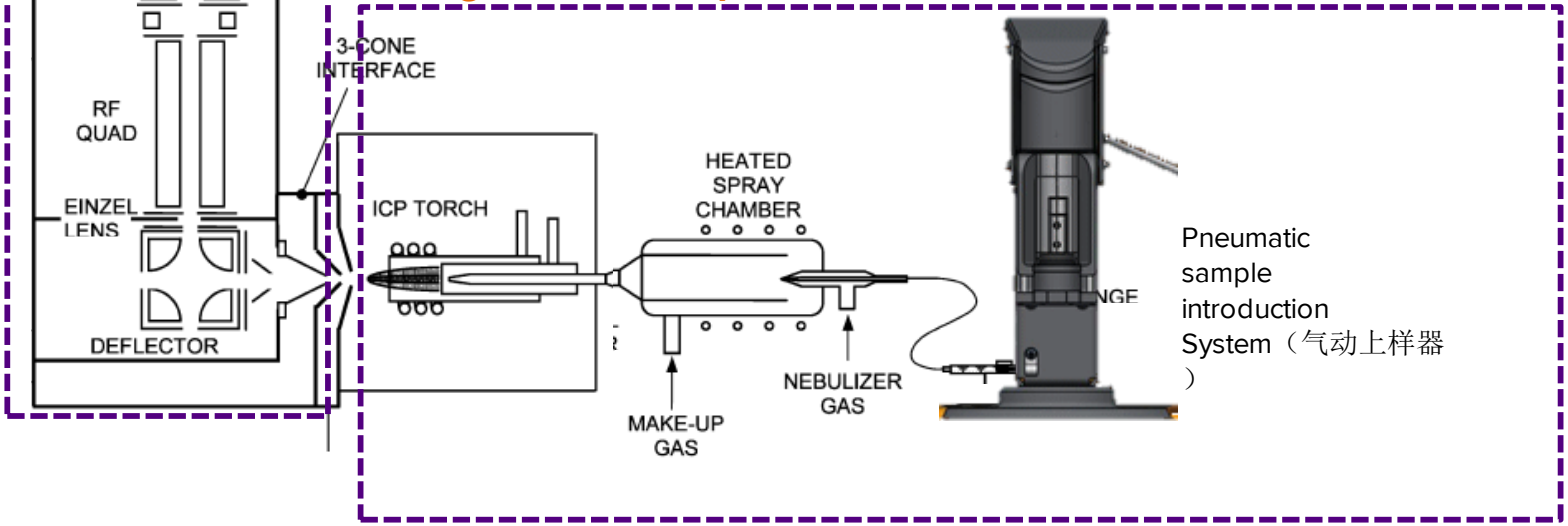
Helios overview



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

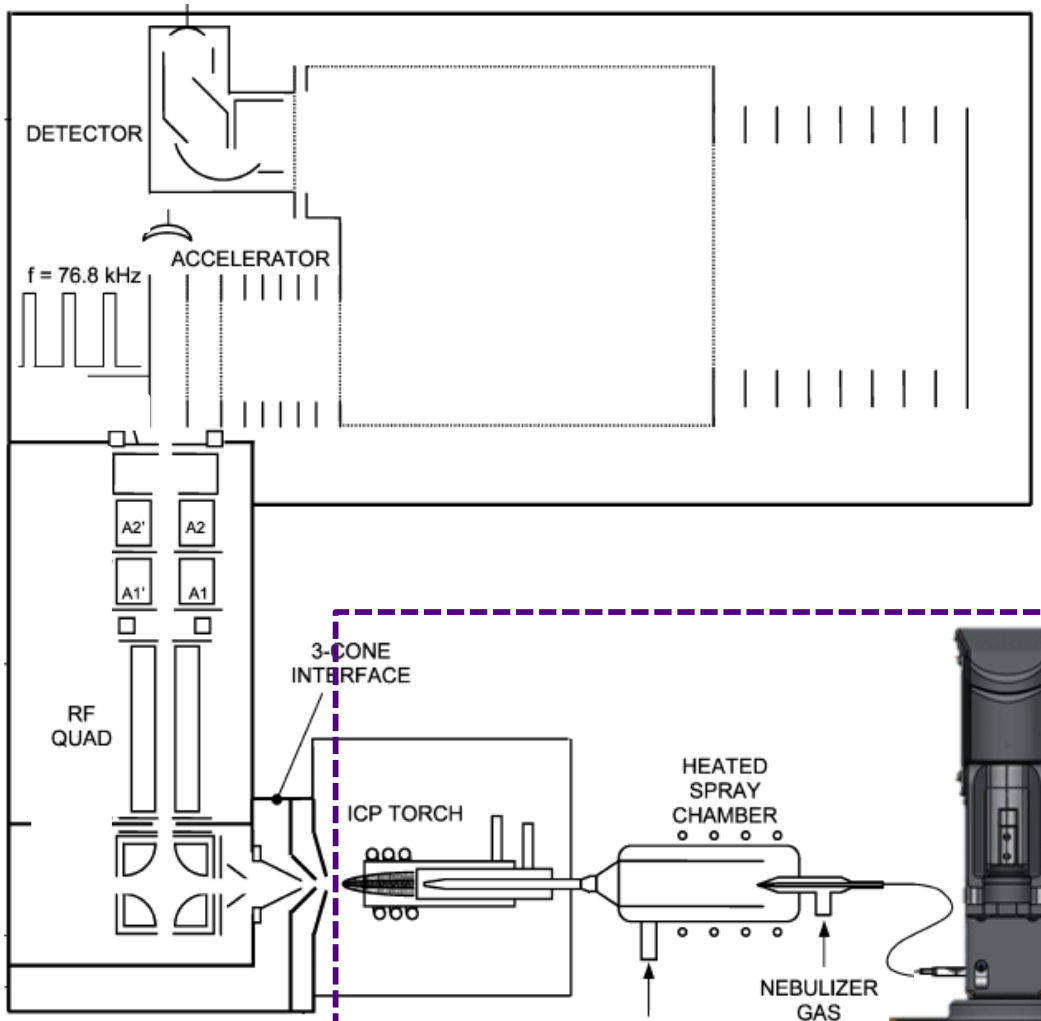
2. High Pass Ion optics

1. Sample introduction
and ionization



Pneumatic
sample
introduction
System（气动上样器
）

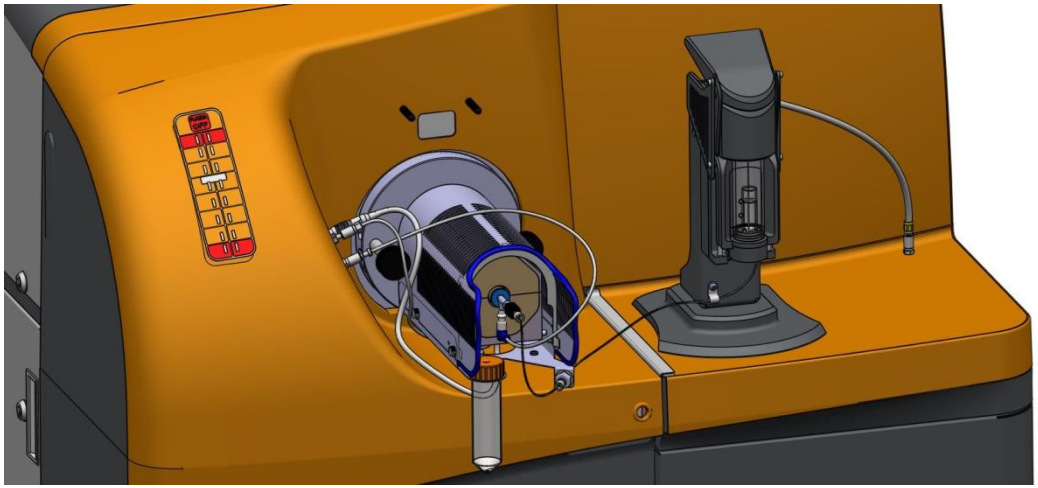
Sample Introduction

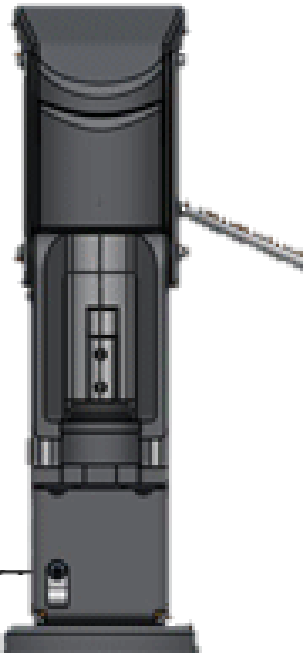


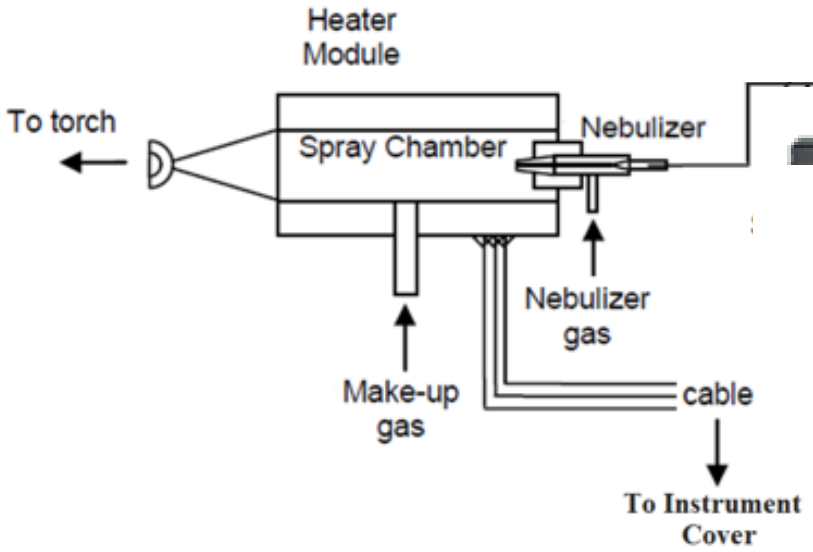
1. Sample introduction and ionization

Pneumatic sample introduction system

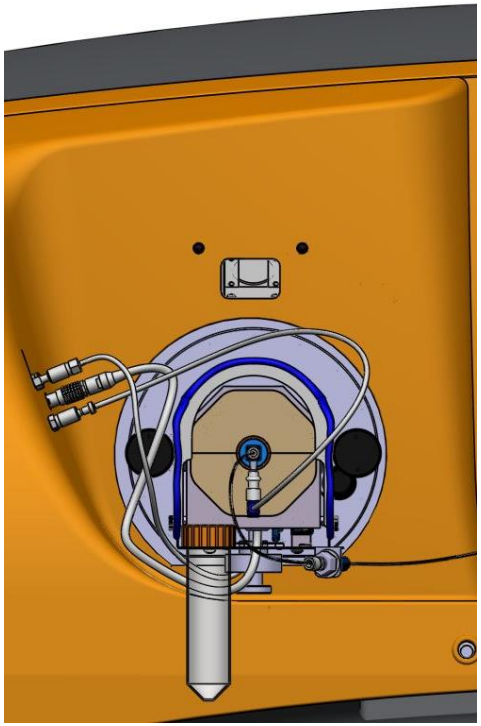
Sample introduction

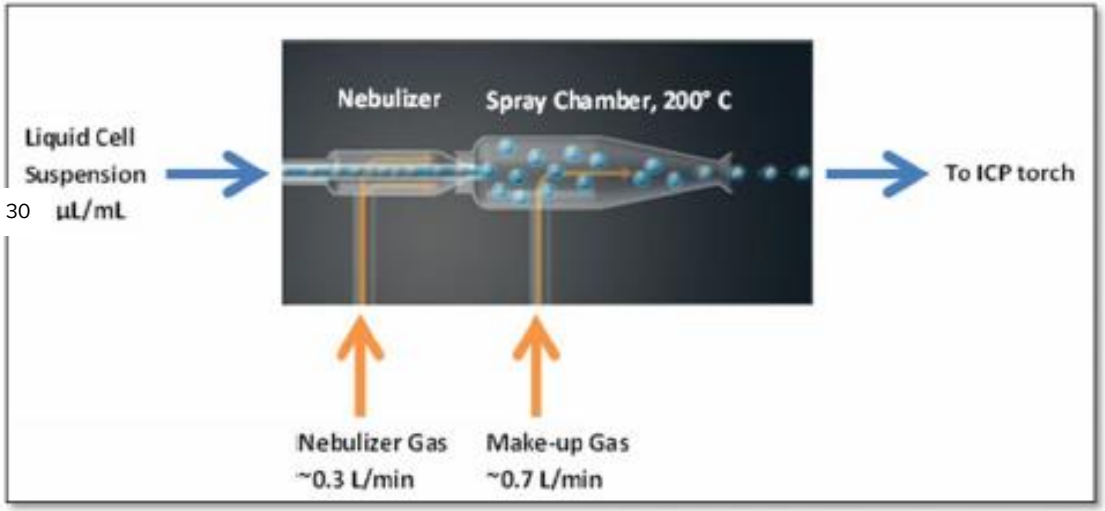


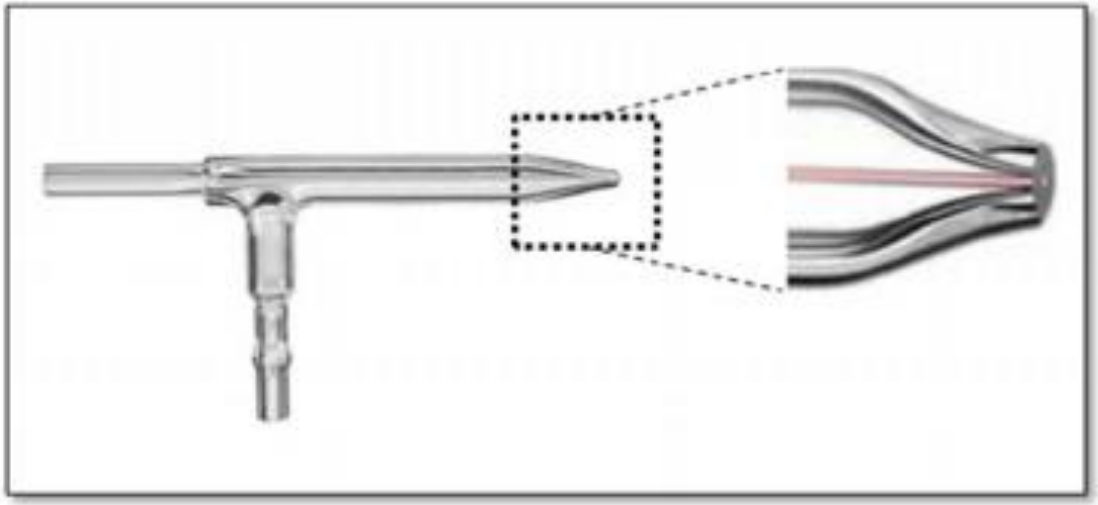




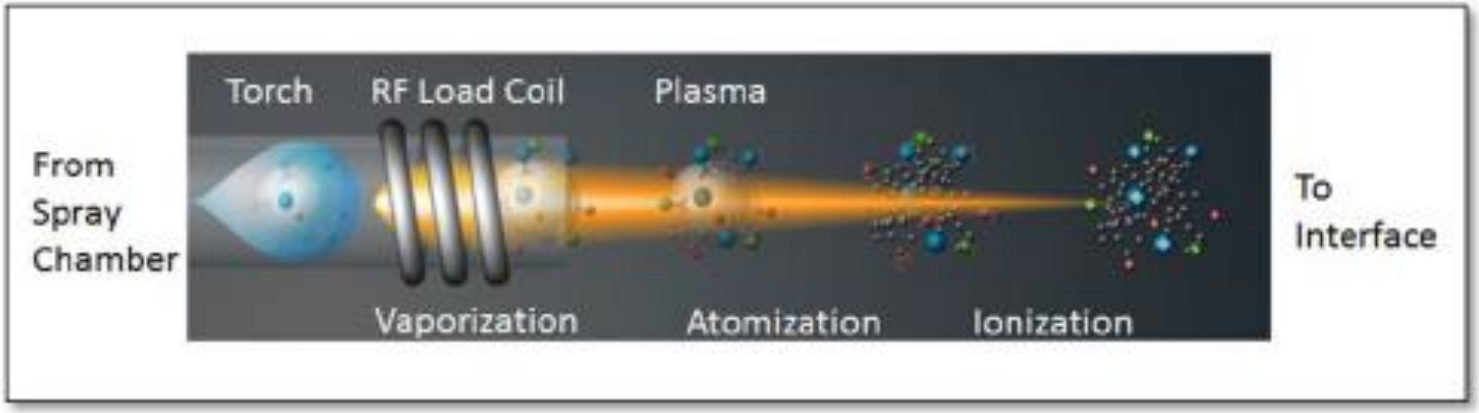
Sample introduction





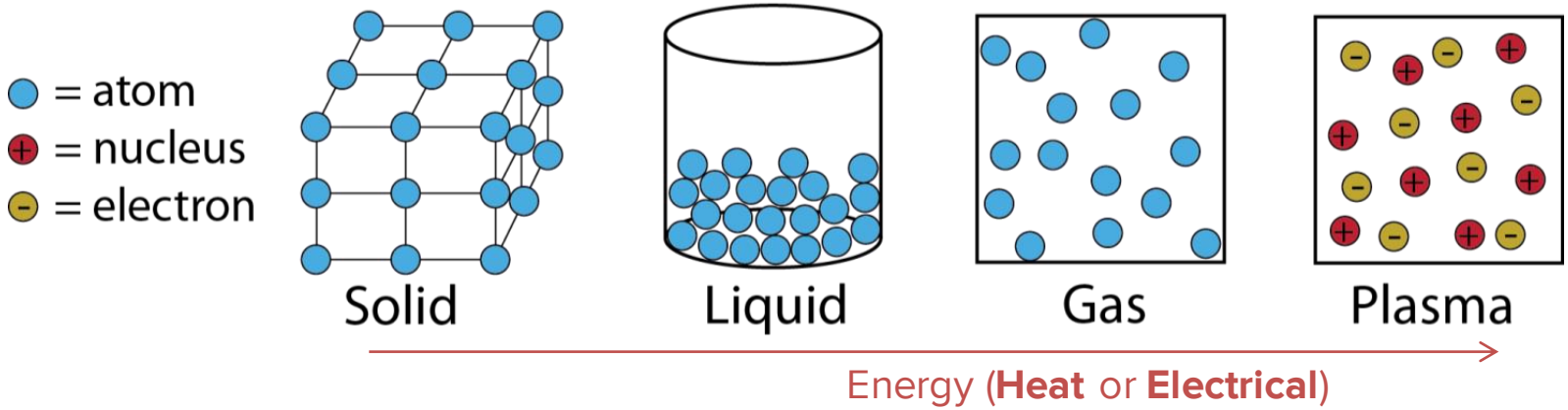


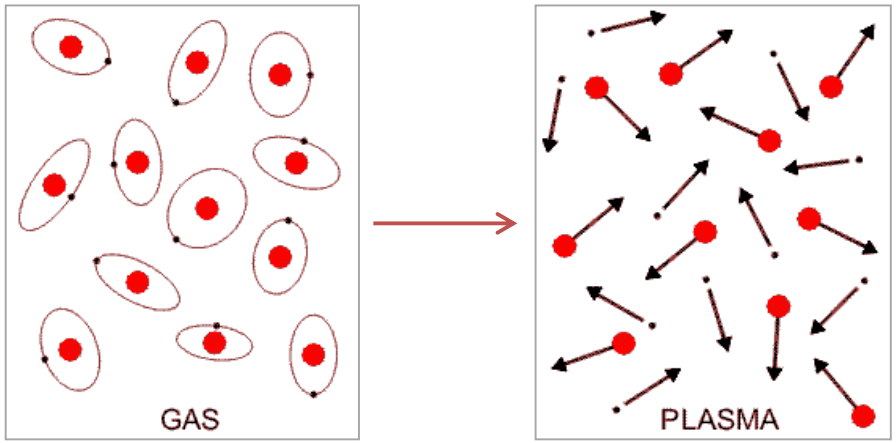
Inductively coupled plasma (ICP)
感应耦合等离子体



Electromagnetic energy generated by the RF load coil surrounding the quartz torch sustains argon plasma (orange) that vaporizes, atomizes, and ionizes individual cell aerosols from the spray chamber. The positive ion component of the cell-derived plasma cloud enters the ion optics and mass analyzer chambers of the Helios through the interface.

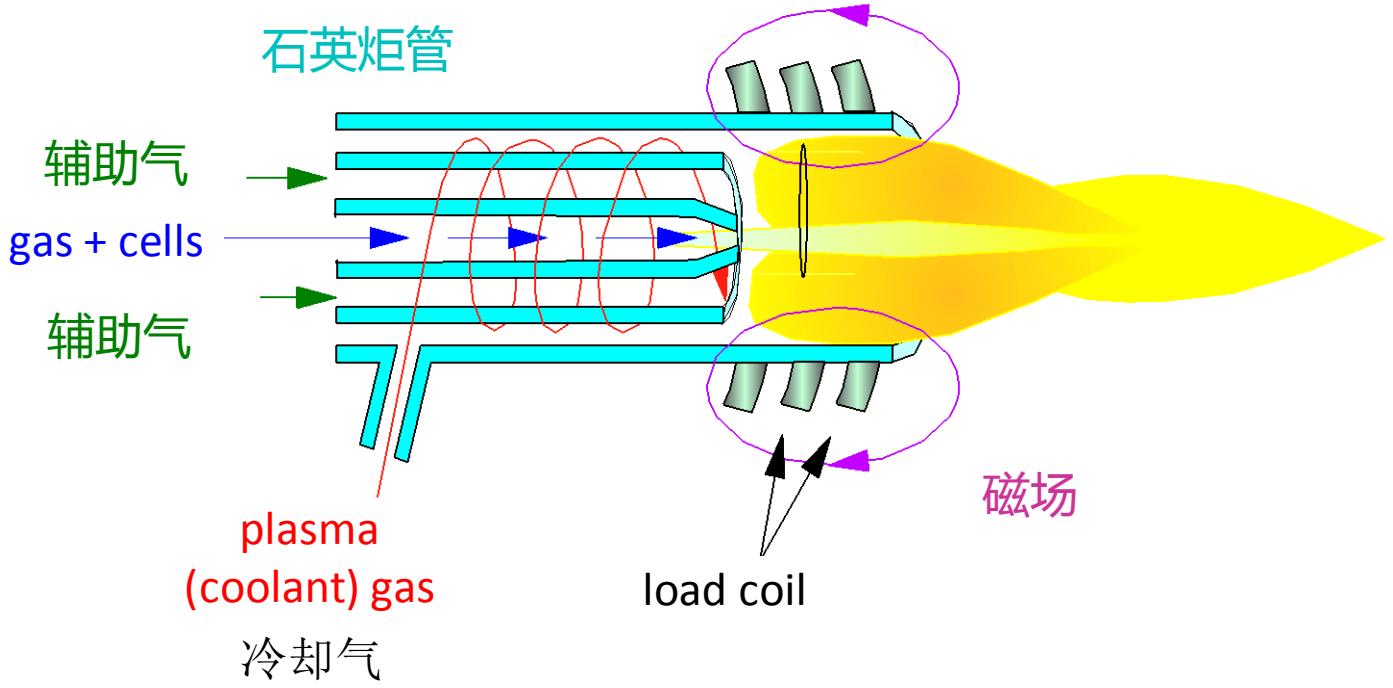
Plasma is the Fourth State of Matter





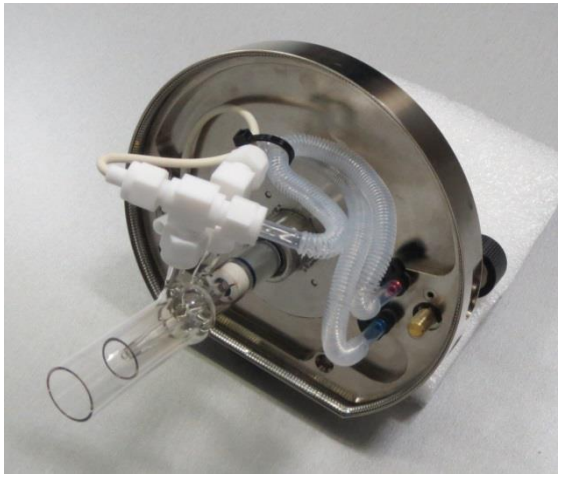
- A gas consists of single atoms
- Each atom has a positively charged nucleus surrounded by negatively charged electrons
- Applying energy causes the negative electrons to overcome the pull of the nucleus
- A plasma ‘soup’ is formed consisting of free electrons (- charge) and free nuclei (+ charge).

Inductively coupled plasma (ICP)

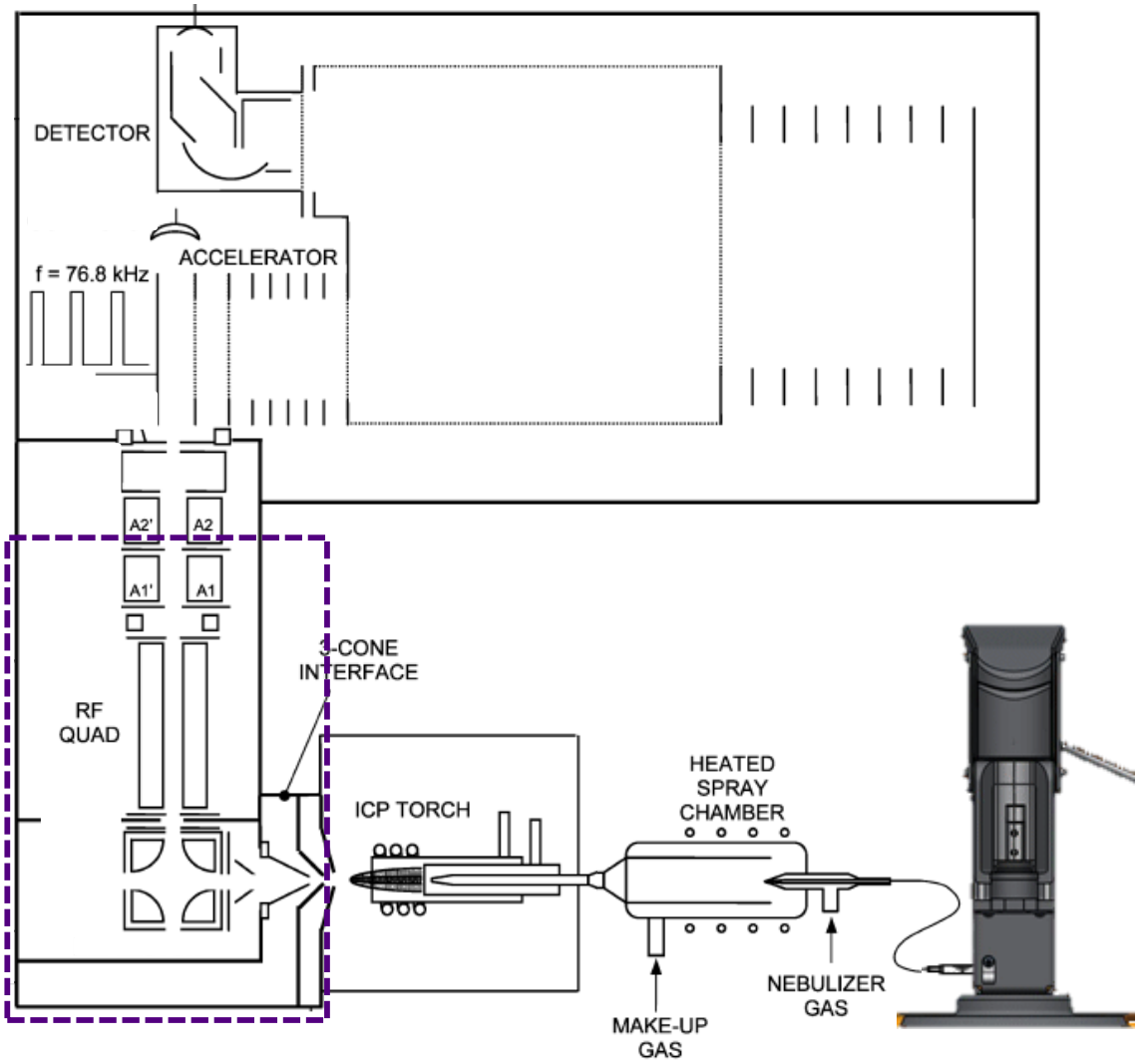


Plasma generation

- Argon gas flows through the torch
- Energy is applied to the argon gas by the ignition pin and by inductively heating the gas within the load coil
- The argon gas is converted into high-temperature plasma consisting of a mixture of argon atoms, free electrons and positively charged argon ions



Ion Guide with High Pass Ion optics



Pneumatic sample introduction system

Helios plasma-vacuum interface

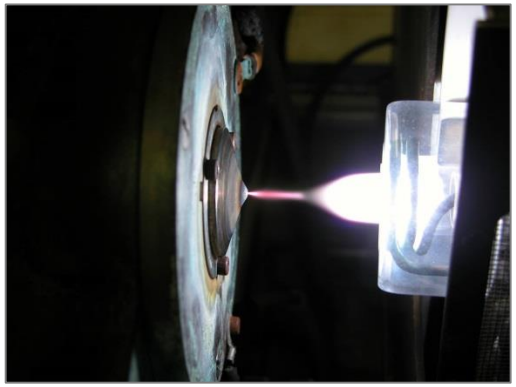
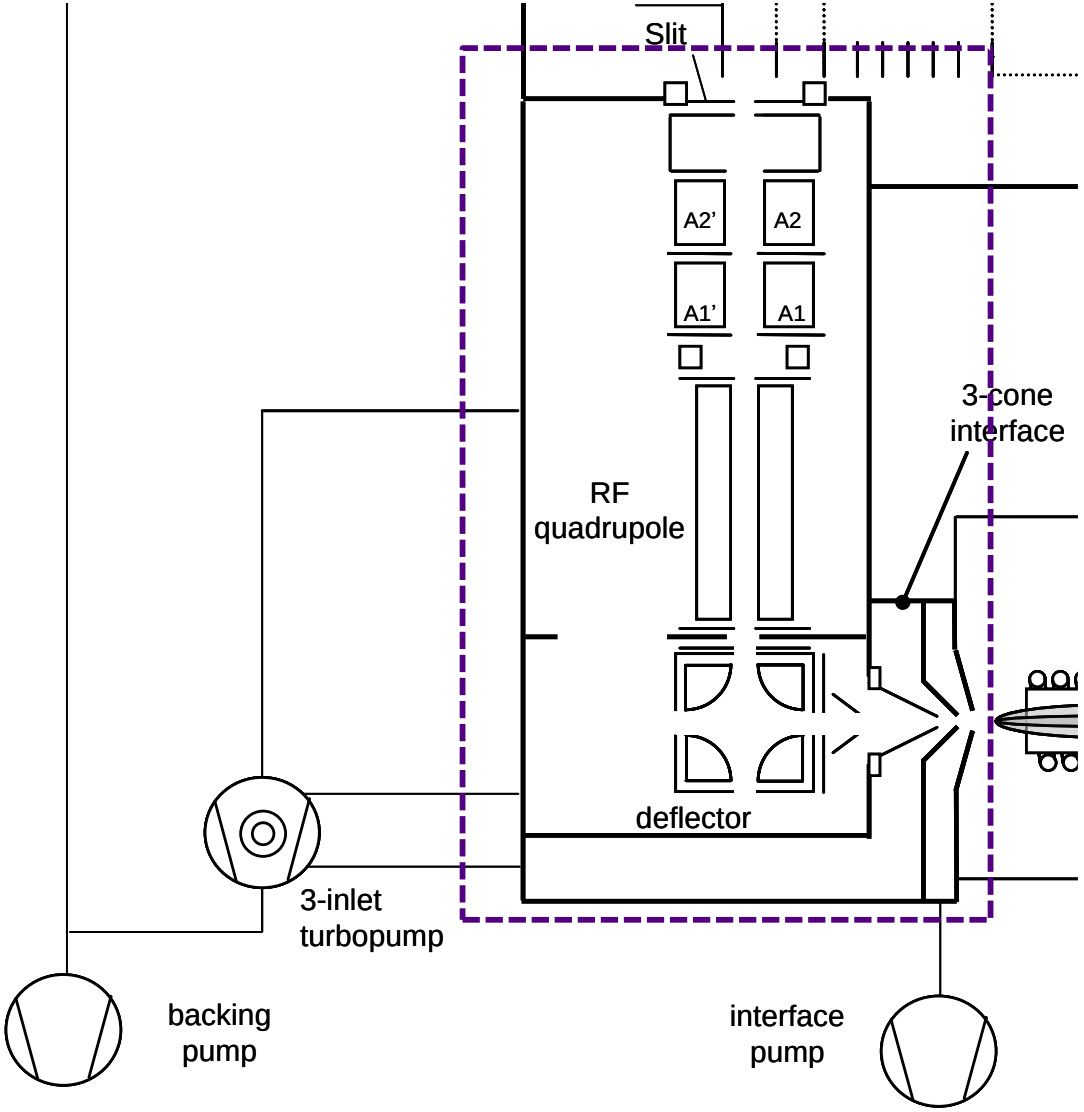
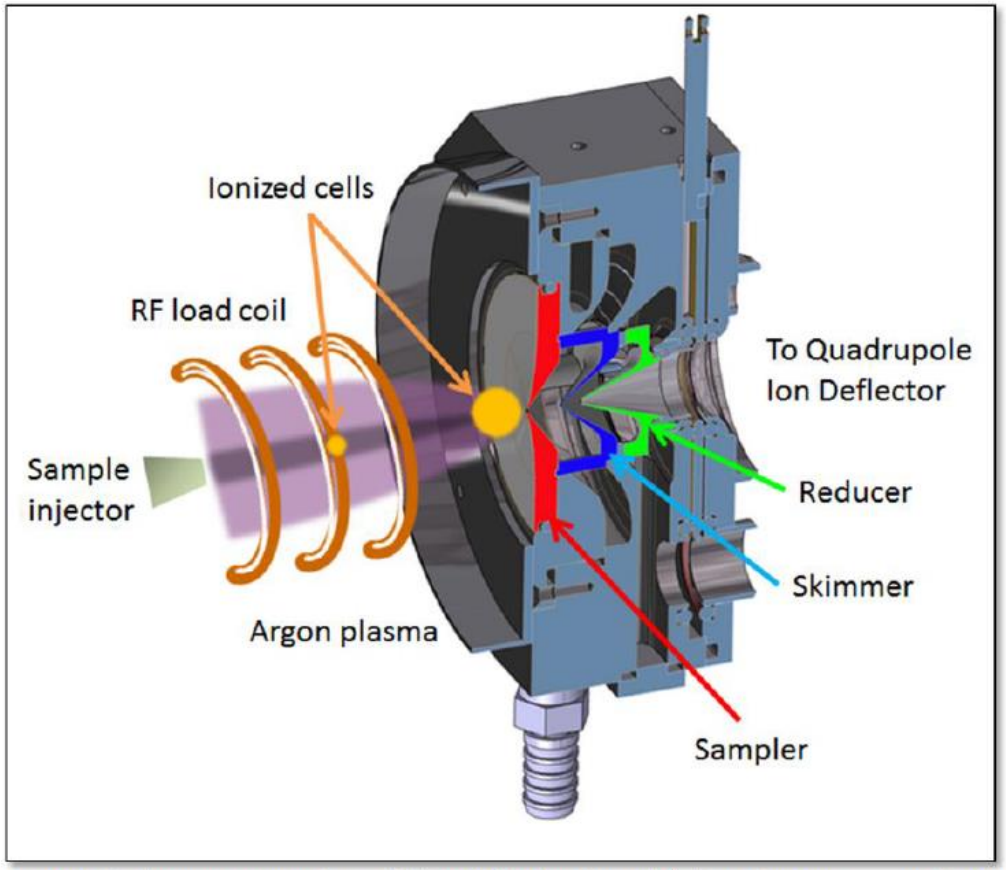


Photo credit: IRAMIS, http://bit.ly/1wbku5y

- Nickel cones:
  Sampler, skimmer, reducer
- Ions pass through each cone to a chamber with a sequentially lower vacuum pressure
- Low vacuum pressure required to control ion movement and limit random collisions

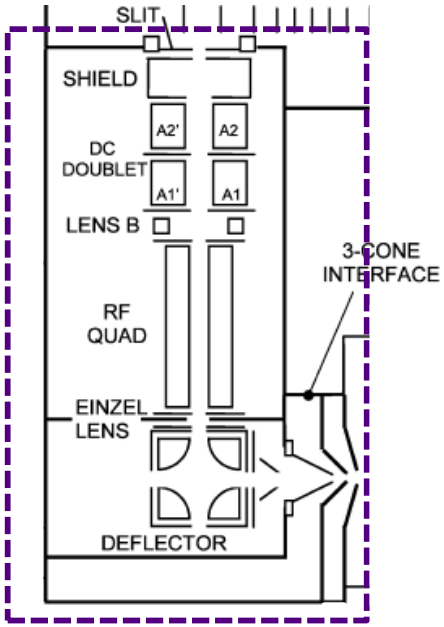


Helios plasma-vacuum interface

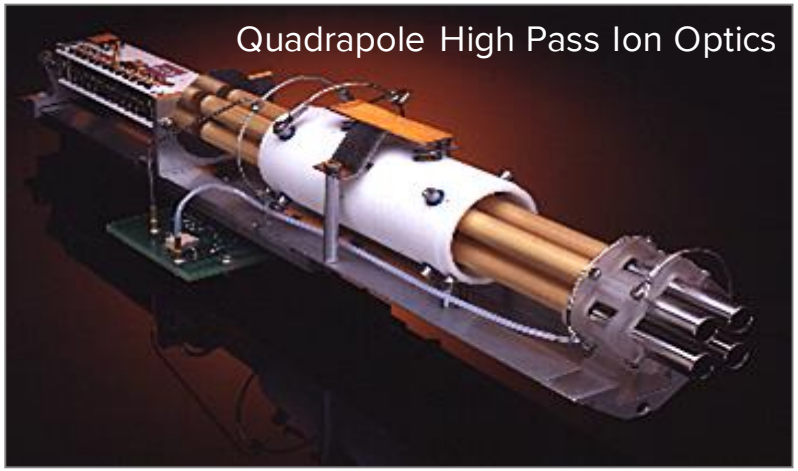


The vacuum interface which includes the three nickel interface cones: sampler (red), skimmer (blue) and reducer (green).

- Cones cooled by the chiller
- 'Dirty' cones reduce sensitivity
- Cones become dirty from plasma exposure, not only from running cells



Ion guiding of low-mass ions below 80 Da

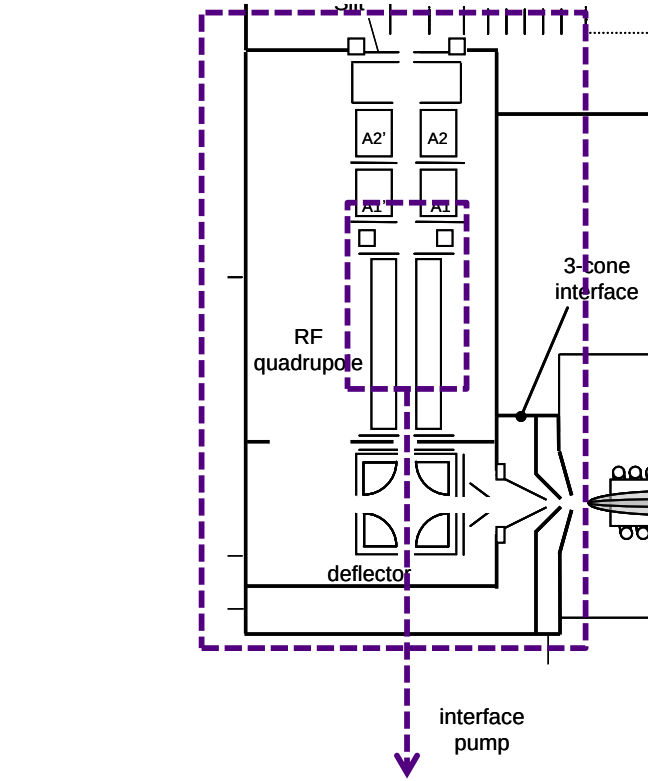


- **Quadrapole Deflector**

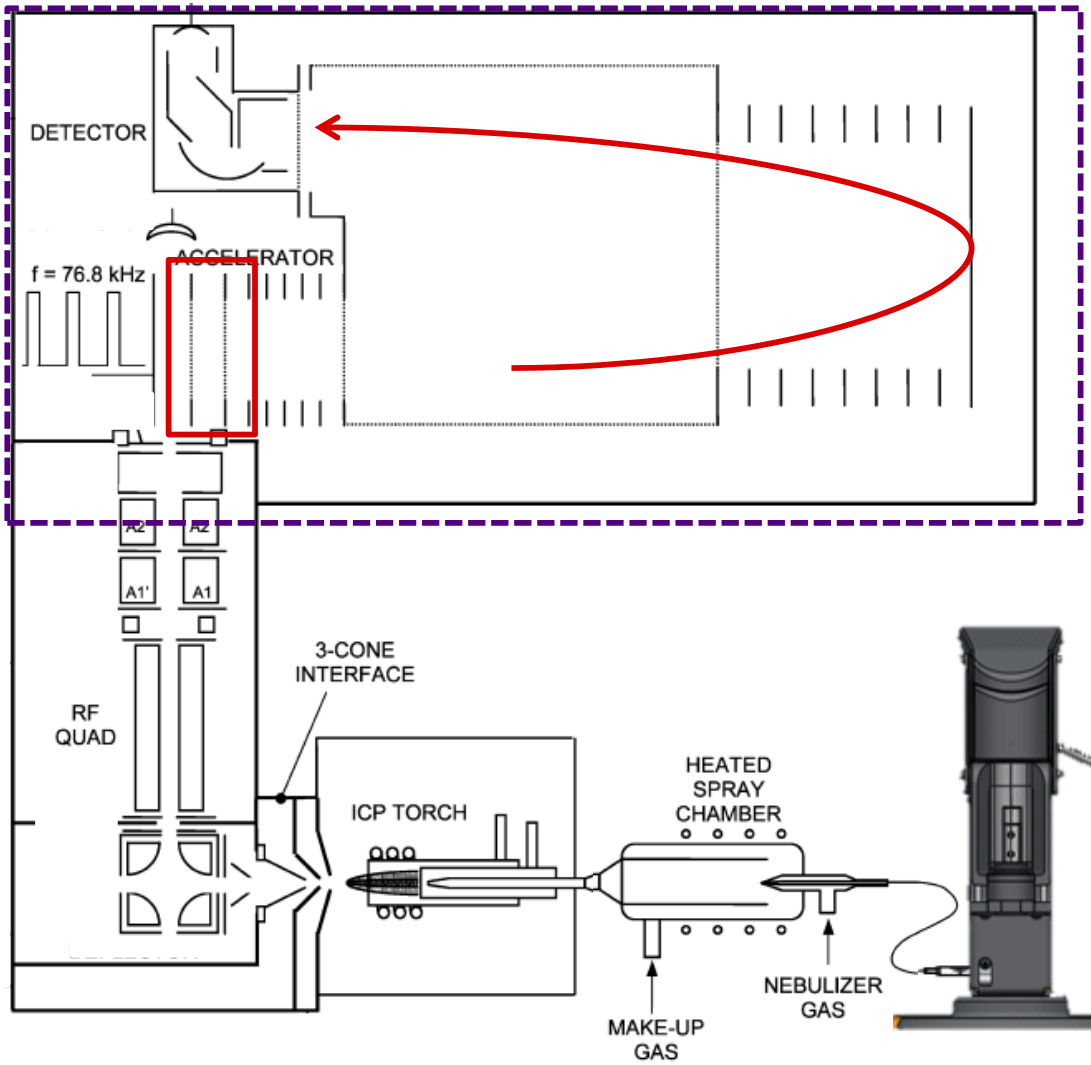
  Eliminates non-ionized particles and photons

- **Quadrapole High Pass Ion Optics**

  Eliminates ions with a mass below 75 Da, e.g. H+, C+, O+, N+, OH+, CO+, O2+, Ar+, ArH+ and ArO+



TOF Ion separation and detection

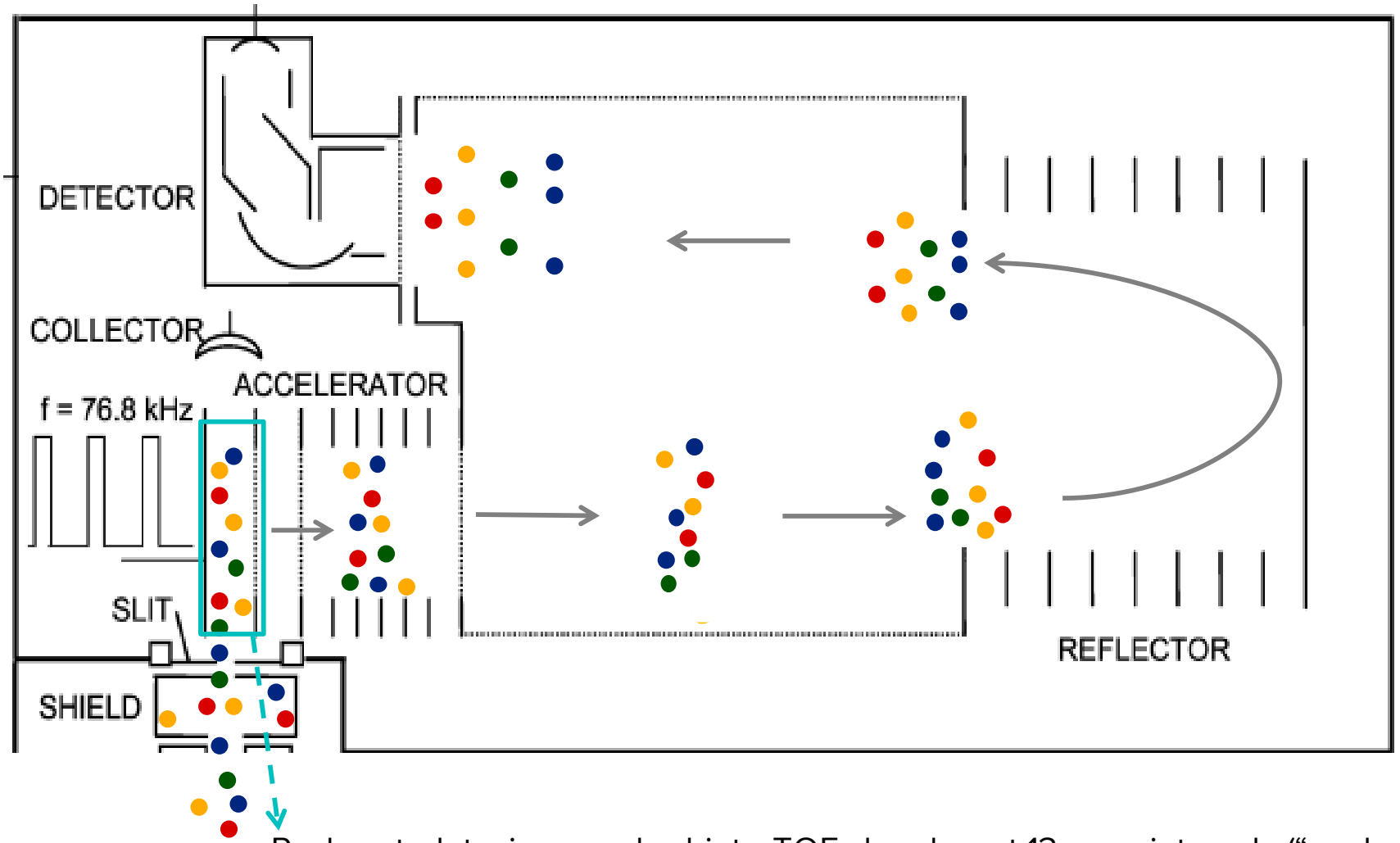


3. Time-of-flight (TOF) ion separation and detection

Pneumatic sample introduction system

TOF separation of ions

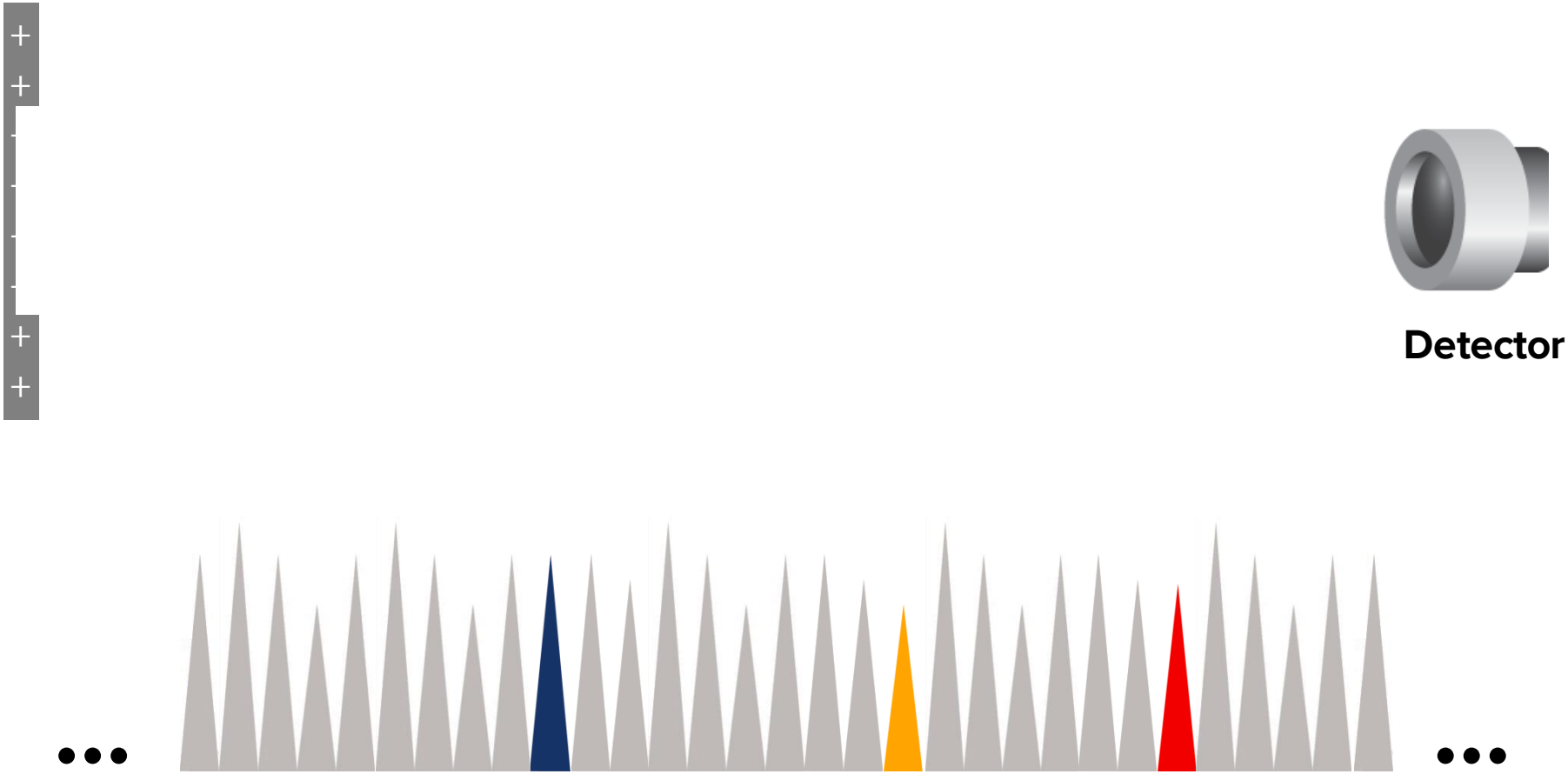
$$t = t_0 + A\sqrt{\frac{m}{z}}$$



Push-out plate: ions pushed into TOF chamber at 13 µsec intervals (“pushes”)

TOF separation of ions

Helios ionizes each cell and separates tags with 1 Da resolution.

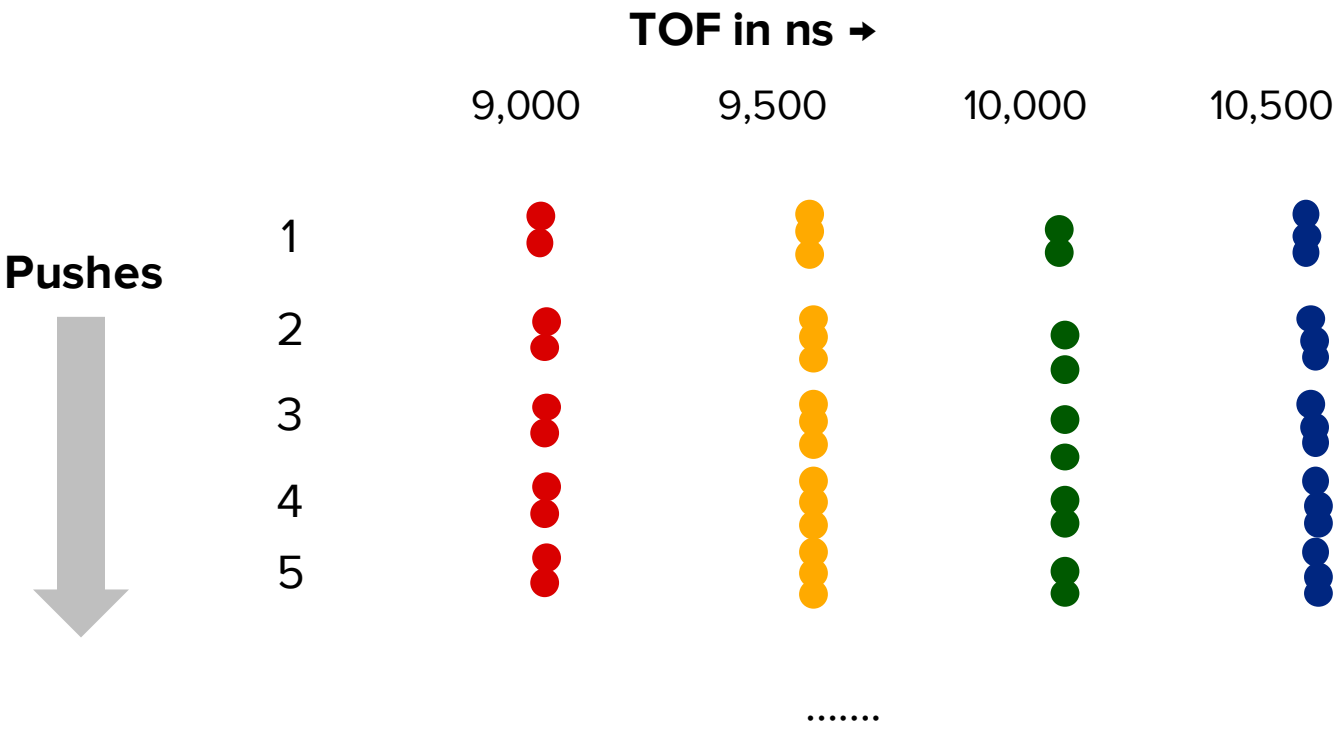


135 channels (75-209 Da range) to measure all existing tags and more to come…

Pushes vs TOF

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

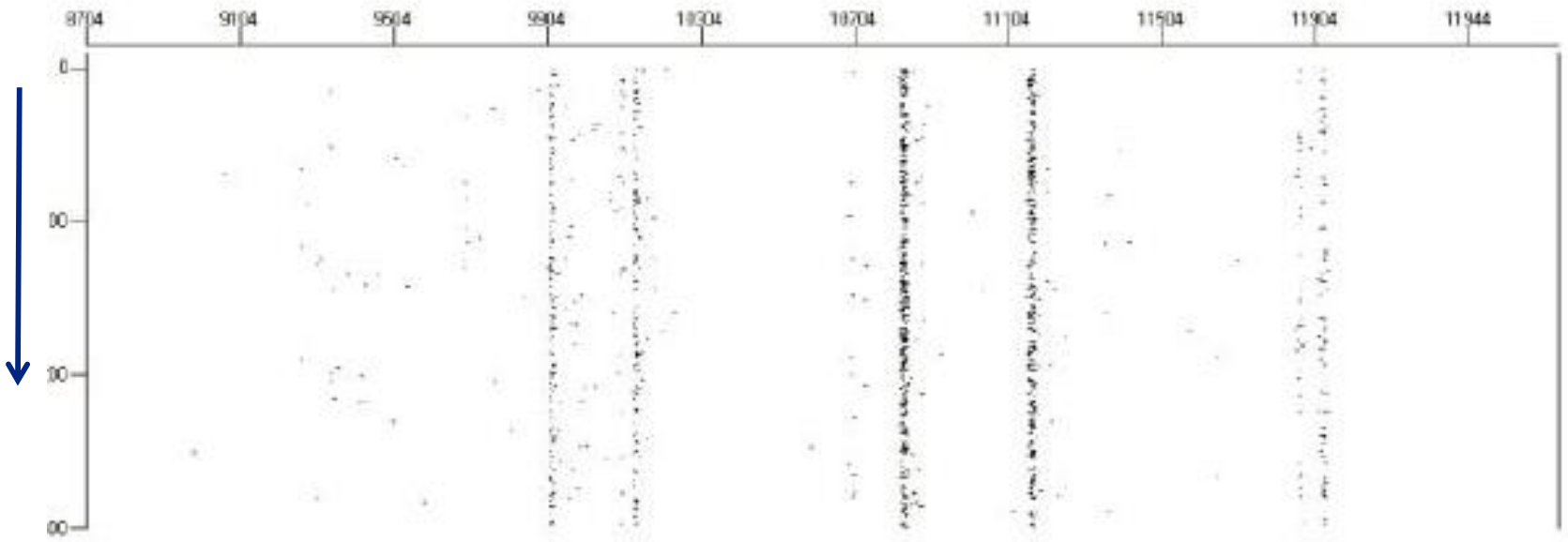
TOF: Separation of ions **within a push** by mass



Tuning solution: metals in liquid suspension

Pushes (13 µsec time slices)

Time of Flight (nsec)



Isotope:	^{133}Cs	^{139}La	^{159}Tb	^{169}Tm	^{191}Ir	^{193}Ir
ppb:	0.5	0.5	0.5	0.5	0.25	

Journey of the cell: outline

INPUT = Cells in liquid suspension stained with metal-conjugated probes

OUTPUT = Individual metal ions separated on the basis of mass

The Helios achieves this through the following steps:

1. **Sample introduction and ionization （上样和离子化）**
   PURPOSE –introduction of cells; to strip water from the cells followed by vaporization, atomization and ionization within the plasma
2. **Ion Guide with High Pass Ion optics（离子过滤）**
   PURPOSE - to filter out unwanted endogenous low mass ions and argon
3. **Time of flight ion separation and detection of metal probes（TOF检测）**
   PURPOSE - to separate the smallest from the highest mass ions; the time taken to reach the detector being proportional to mass

Simplify the complex quest to understand and apply biology.

FLUIDIGM®