CyTOF® Mass Cytometer

Massively Multi-Parametric Cytometry

Discover what you have been missing

Cellular analysis on an unprecedented scale

The CyTOF® Advantage

- Simple, highly multiplexed single cell analysis
- Robust and reproducible performance
- Absolute quantitation of target molecules
- Easy, convenient workflow

The CyTOF® Mass Cytometer introduces the next generation of highly multiplexed single cell analyzers which will afford new insights into the biology of complex cellular networks[1].

Unlike traditional flow cytometry where detection of multiple probes is hindered by the physical limitations of fluorescence emission spectral overlap, the CyTOF instrument uses atomic mass spectrometry to allow simple, independent detection of up to 33 metal labeled antibodies (and two DNA intercalators) based on currently available reagents. The instrument has the capacity to measure 100 independent signals and new reagents will be introduced to take advantage of its full capability in the future.

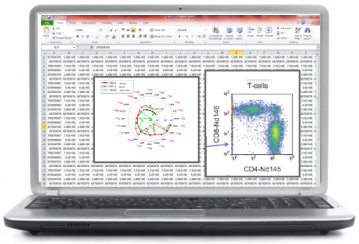
The CyTOF system is the first inorganic mass spectrometer specifically built for biologists and single cell applications. The inductively coupled plasma mass spectrometer at its heart has been used extensively by analytical chemists and is proven in the quantification of elements and stable isotopes in a variety of complex matrices. The application of this technology to multi-parametric single cell analysis brings robust and reliable analytical performance to a powerful, enabling new application.

Familiar workflow, more data

Sample Preparation

Sample Processing

Data Analysis



1 Cell Surface Immunostaining
Incubate cells with metal tagged antibodies
▼
Wash
▼
Fix Cells
▼
Incubate with a metallointercalator[2]
▼
Wash

2 Intracellular Immunostaining
Fix cells
▼
Block non-specific binding groups
▼
Stain with metal tagged antibodies
▼
Wash
▼
Fix Cells
▼
Incubate with a metallointercalator

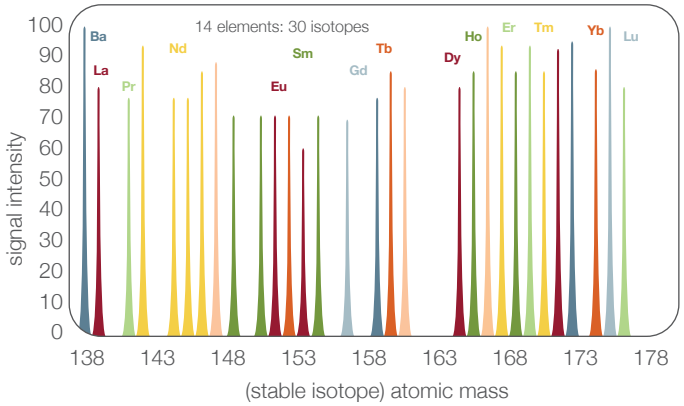
Introduce individual samples into the CyTOF® Mass Cytometer
(similar to a flow cytometer)
▼
Collect data.
(Analyzes 10^5 cells in four minutes, regardless of the number of tag elements.)

Raw data is processed to give a signal strength for each analyte represented by both an integrated analog intensity value and an integrated number of counted ions. Data output is similar to a typical flow cytometry experiment and can be exported as text and FCS3.0 formats to be fully compatible with flow cytometry data processing software.

Simple, highly multiplexed single cell analysis

Up to 100 independent detection channels

The unique multiplex analysis capability of the CyTOF® system is already enabling new insights into the functional complexity of biological systems at the single cell level. A recent example published in Science[3] describes how the technology was used with an immunophenotyping panel 31 labeled antibodies to provide a uniquely detailed view of cell differentiation in the human hematopoietic system. The CyTOF technology currently enables analysis up to 100 stable isotope labels in a single sample. Currently, DVS Sciences Inc. provides labeling kits for 33 different metal tags and will increase the range in the future to fully utilize the multiplex detection capabilities of the instrument. Because the mass spectrometer provides at least 3 orders of magnitude resolution between adjacent detection channels, compensation is not required. Additionally, the sensitivity is practically the same across all channels measured and as a consequence, the combination of labels used in an experiment can be essentially arbitrary. Designing experiments is simple whether you are using as little as a single labeled antibody or as many as 33. In addition, with effectively no overlap between atomic mass signals, inherent compensation problems are completely eliminated. For the first time, complex multi-parametric cytometry is within easy reach of any life science laboratory.



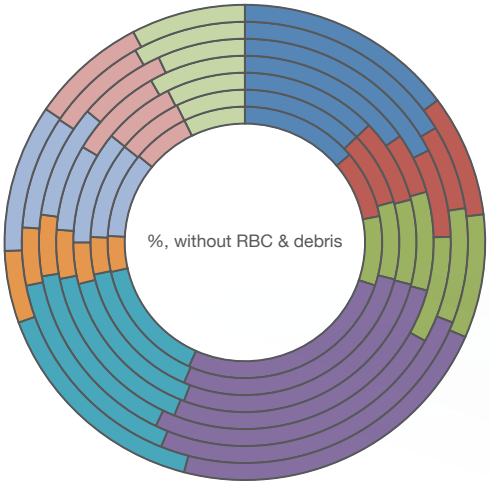
138-178 segment of mass spectrum for a homogeneous sample of several enriched isotopes of lanthanides. The complete separation of detection signals allows selection of any combination of labels to be used in multiplexed single cell analysis.

In contrast, the limitations of fluorescence signal overlap significantly restricts the number of simultaneous probes that can be used, especially when proteins are present at very different concentrations.

Robust and reproducible performance

Reproducibility between samples, between systems

The use of atomic mass spectroscopy is well established in the field of elemental analytical chemistry and the CyTOF® instrument will deliver robust and reproducible performance from the first day of installation in your laboratory. Data generated is highly reproducible with a low coefficient of variation between technical replicates, between systems and between users. This characteristic makes its use particularly well suited to multi-site or longitudinal studies.



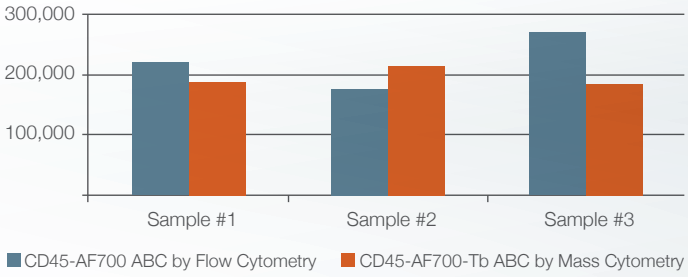
CD14	Tm169
CD45	Tb159
CD66	Dy164
CD4	Nd142
CD16	Nd144
CD8a	Nd146
CD20	Gd156
CD11b	Gd158
HLA-DR	Er166
CD13	Er170
CD15	Sm147
CD45RO	Sm152
CD3	Pr141
CD33	Ho165
CD38	Yb171
CD19	Yb176
CD56	Eu151
CD44	Eu153
CD45RA	
Ir 191	
Ir 193	

Seven aliquots of CB were analyzed on seven consecutive days. Ten gated populations were established based on the markers listed.

Absolute quantification of target molecules

The CyTOF instrument offers the specificity, dynamic range and quantitative capability of atomic mass spectrometry in a format that is familiar to flow cytometry practitioners. The quantitative aspect of flow cytometry is currently underutilized primarily due to absence of independent quantitative analytical methods and lack of standard materials. Mass cytometry offers a unique opportunity to simultaneously analyze more subtle quantitative changes in intracellular and membrane bound proteins within complex cell populations.

CD45 ABC Summary	Sample #1	Sample #2	Sample #3	Average	Std Dev
CD45-AF700 ABC by Flow Cytometry	215,447	177,569	269,865	220,960	37,881
CD45-AF700-Tb ABC by Mass Cytometry	189,050	209,615	187,245	195,303	10,147



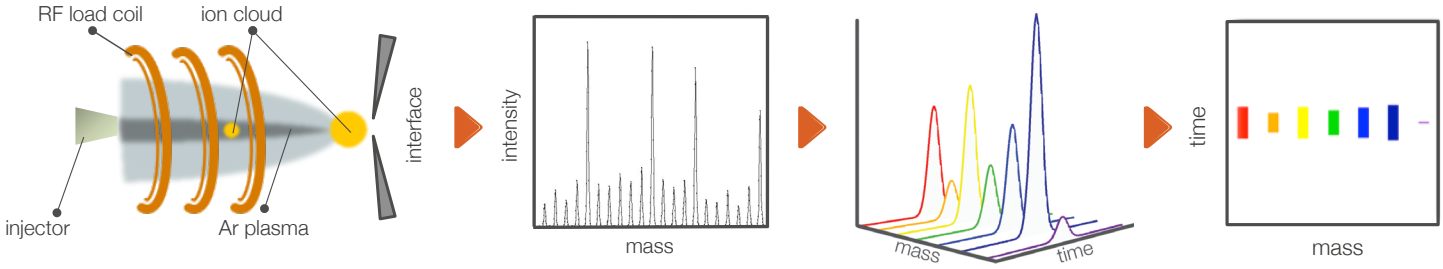
In the example above, flow cytometry and mass cytometry were compared using the same affinity probes on the same cell samples. The data generated on each platform generally agrees with published literature for CD45 expression values on normal lymphocytes. A key component in mass cytometry quantification system is the accurate determination of the number of metal atoms attached to each antibody molecule. Utilization of multiple lanthanide labeled antibodies is robust because no compensation matrix is required.

Proven technology, novel application

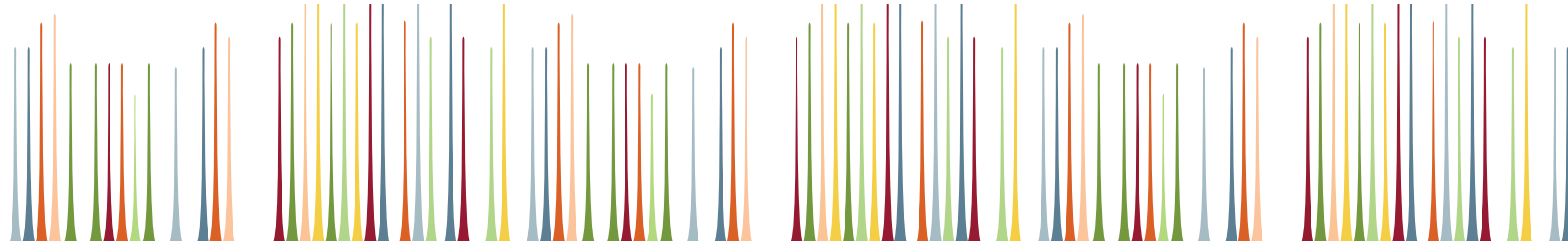
CyTOF® Mass Cytometry

The CyTOF instrument is based on the application of robust and proven atomic mass spectrometry uniquely applied to single cell analysis. Samples containing metal-labeled cells are introduced individually into an Inductively Coupled Plasma, were the cells are atomized and ionized. The atomic ions are extracted into the ion optics and time-of-flight regions where they are separated by mass and counted. The elemental signature of the cell includes the element tags introduced with the antibodies and metallointercalators. The presence of the tag element indicates that the antibody found and bound the target biomarker, and the intensity of the signal is directly proportional to the number of antibodies bound per cell (ABC). The elemental composition of each cell is separately analyzed. In a typical cell analysis experiment, four minutes of raw data collection is sufficient for analysis of 10^5 cells independent of the number of tag elements.

It is important to note that cells without any tagging elements cannot be detected by mass cytometry: Only element tags can be registered with high sensitivity and specificity, and therefore there is no “auto-fluorescence”-like effects. Elemental tags are chosen from rare elements whose natural concentration in a biological sample is below the detection limit. Unstained cells are “transparent” to the mass cytometer. A complete description of the instrument can be found in Bandura et al, 2009[4].



Data acquisition process in the mass cytometer. The cell is atomized and ionized in the argon plasma forming an ion cloud, which is sampled through the vacuum interface. Mass detector (time-of-flight mass spectrometer) repeatedly registers a mass spectrum which includes a continuous range of masses with all tag elements. The intensity of each transient mass signal is tabulated separately as a function of time. The integral intensity over the cell duration (the antigen signal) is transferred into final FCS 3.0 file format.



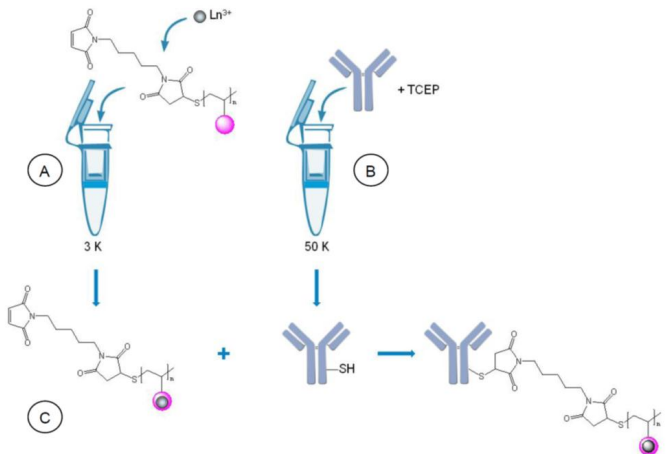
Everything you need at your fingertips

MAXPAR® Antibody Labeling Kits

Rapid, easy preparation of labeled antibodies

MAXPAR labeling reagent kits for 33 different metals are available from DVS Sciences Inc. for preparation of labeled antibodies or intracellular proteins. Kits are optimized and quality control tested for use with the CyTOF® instrument and are available in pack sizes sufficient to label 100ug or 1mg of antibody. Labeling methods are simple and convenient and require only 2.5 hours to complete. The labeling kits utilize a traditional approach of adding a metal tag using disulfide reduction (typically in the Fc region of the antibody) through a maleimide linker. Labeled antibodies are stable for approximately 6 months after preparation.

Antibody conjugation to MAXPAR metal tag



A – Lanthanide loading of metal-chelating polymer in 3K MWCO spin filter.
B – Antibody reduction with TCEP in 50K MWCO spin filter.
C – Incubation of metal-containing polymer tag with reduced antibody in the 50K MWCO spin filter.

Customer Support

DVS Sciences Inc. is fully committed to the success and satisfaction of its customers and to support the use of the CyTOF® instrument to enable new insights and discoveries in the life sciences. Each system purchase includes a site installation guide, instrument installation and testing, extensive user training, and a twelve month warranty. DVS Sciences field service is readily available to customers in the event that instrument service is required.

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4. Bandura,D.R., Baranov,V.I., Ornatsky,O.I., Antonov,A., Kinach,R., Lou,X.D., Pavlov,S., Vorobiev,S., Dick,J.E. and Tanner,S.D., 2009. **Mass Cytometry: Technique for Real Time Single Cell Multitarget Immunoassay Based on Inductively Coupled Plasma Time-of-Flight Mass Spectrometry.** Analytical Chemistry 81, 6813.