

NEWS

Glass Expansion Newsletter | October 2016 | Issue 41

APPLICATION SPOTLIGHT

MicroMist Nebulizer for Single Cell Mass Cytometry

Noel Casey^a, Adeeb Rahman^b, Ryan Brennan^c and Jerry Dulude^c

- a. Atomic Spectroscopy Consultant, Watertown, MA 02472. noelcasey@gmail.com
- b. Director of Technology Development, Human Immune Monitoring Core, Icahn School of Medicine at Mt. Sinai, New York, NY 10029. adeeb.rahman@mssm.edu
- c. Glass Expansion Inc. Pocasset, MA 02559. geusa@geicp.com

Introduction

Mass cytometry is the current state-of-the-art technology in single cell-analysis that is transforming the fields of immunology, cancer biology, vaccine development, drug discovery, and translational medicine⁽¹⁻⁶⁾. This new tool is providing scientists with the most detailed overview of normal and diseased cells and how they respond to various drug treatment options. The technology was developed at the University of Toronto and commercialized by DVS Sciences Inc. in 2009 before being acquired by Fluidigm Corp in 2014. Since its introduction, three generations of instruments have been released (CyTOF, CyTOF2 and Helios) and the number of publications utilizing mass cytometry has nearly doubled every 2 years.

Mass Cytometry combines the elemental quantitation, specificity and dynamic range of Inductively Coupled Plasma Time of Flight Mass Spectrometry (ICP-TOF-MS) with the single cell analysis capabilities of traditional fluorescence-based flow cytometry to study, diagnose and develop treatments for diseases. The number of parameters that can be simultaneously measured on single cells by traditional flow cytometry is restricted by a limited range of available fluorochromes, spectral overlap between fluorochromes and intrinsic cellular autofluorescence. Mass cytometry uses enriched stable isotope metals attached to antibodies using metal-chelating reagents to label specific protein biomarkers in cells from blood, tissues, or cell cultures, with minimal mass spectral overlap between each metal tag. The mass cytometer measures the expression of these biomarkers in each individual cell according to the type and concentration of each metal detected. The latest generation of instrument provides more than 120 detection channels at typical acquisition rates of up to 500 cells/second⁽⁷⁾.

The primary sample introduction challenge facing Mass Cytometry is that its acquisition rate is ~10 times slower than conventional flow cytometers. So using a robust and reliable sample introduction system that maximizes the numbers of intact single cells entering the ICP while maintaining instrument detection limits is key. Glass Expansion's latest research addresses factors that can affect cell integrity prior to introduction to the ICP and ways to improve the efficiency and reliability of sample transport in Mass Cytometry. A newly designed MicroMist™ concentric nebulizer with a zero dead volume capillary connection is evaluated in addition to a tool to accurately monitor sample delivery.

Glass Expansion News

New Sales & Marketing Manager

Glyn Russell has taken up the position of Sales & Marketing Manager at Glass Expansion's Australian head office. Glyn has extensive experience in the analytical equipment industry and is particularly knowledgeable regarding ICP instrumentation. He will continue to enhance Glass Expansion's reputation for highest quality products and customer support. He replaces Peter Liddell who has retired.

In this issue:

- **Application Spotlight**.....1 – 5
- **GE News**.....1
- **New Products**.....6
 - WindTunnel Mobile Cart
- **Instrument News**.....7
 - From Agilent
 - From Analytik Jena
 - From Horiba Jobin Yvon

Mass Cytometry Instrument Overview

The mass cytometer consists of five components including sample introduction, the inductively coupled plasma (ICP) ion source, ion optics, time-of-flight (TOF) analyzer, and detector. The liquid cell suspension sample (1×10^3 cells/mL) is introduced to the nebulizer at a flow rate of 30-45 $\mu\text{L}/\text{min}$ and aerosolized into fine single-cell droplets using a nebulizer gas flow of 0.15 to 0.25 L/min argon. A makeup gas of 0.7 to 0.9 L/min argon is used to optimize the ICP conditions independently of the nebulizer gas to ensure appropriate aerosol flow through the plasma and maintain the integrity of individual cells. The single-cell droplets then pass through a single-pass spray chamber which is heated to a temperature of 200°C , resulting in the evaporation of the remaining water within the cells before entering the ICP.

Once the single-cells with the specific isotope-tagged antibodies enter the ICP they are atomized, excited and ionized to form an ion cloud. The ion cloud is extracted through a three-aperture plasma-vacuum interface to produce a transient signal with a typical half width of $200\mu\text{s}$. After the ions enter the interface, they pass through a high pass optic that transmits ions >80 amu which are then directed toward the time of flight (TOF) analyzer, where they are separated according to their mass-to-charge ratio. The signal from the detector is digitized by an analog-to-digital converter (ADC) and the data is converted to mass and integrated signal intensity (cps). The mass spectra are recorded in $13\mu\text{s}$ intervals, and the data is exported to a standard flow cytometry data format. Because of the size and complexity of the data collected, several data mining techniques have been applied to mass cytometry datasets.

As with traditional ICP-MS instruments, signal drift occurs over time because of several different factors including a buildup of sample material on the interface cones such that daily maintenance is critical. Signal drift compensation in Mass Cytometry uses a single particle-based analog of internal standardization. Samples are spiked with metal-encoded polymer beads that contain a known concentration of cerium, europium, holmium, and lutetium. The beads allow normalization within a run, between different runs on the same instrument, and provides a direct comparison between different instruments and different laboratories.

Mass Cytometry Sample Introduction

Introducing cells into a Mass cytometer without compromising cell integrity continues to be a major challenge and an active research goal of the ICP atomic spectrometry community⁽⁸⁻¹¹⁾. However, knowledge gained from understanding the fundamentals of aerosol generation and transport into the inductively coupled plasma has led to significant advances in sample introduction which make single-cell analysis a more reliable and routine measurement technique⁽¹²⁻¹⁴⁾. These improvements include the combination of high efficiency low flow rate nebulizers with heated single pass spray chambers.

Prototype MicroMist Nebulizer

Glass Expansion is well known for high quality nebulizer designs and precision manufacturing. Unique to all Glass Expansion glass concentric nebulizers is the trademark VitriCone™ sample channel. The VitriCone sample channel (Figure 1) is created by machining constant bore heavy stock glass tubing (Figure 1A) to create the desired aerodynamic exterior (Figure 2A) while maintaining a consistent internal diameter. Other nebulizer manufacturers heat and draw a thin fragile capillary from glass tubing to create the

internal capillary. This process often produces a sample capillary with varying inner diameter, an increase in the porosity of the glass, and harmonic vibrations from the flow of argon, all of which degrade performance and lifetime of the nebulizer. A diagram comparing a Glass Expansion nebulizer (A) to other manufacturers (B) is shown in Figure 2.

Figure 1. Glass Expansion glass concentric nebulizer design

Figure 1A. Constant bore heavy stock glass



Figure 1B. Machined aerodynamic exterior



Figure 1C. VitriCone machined capillary

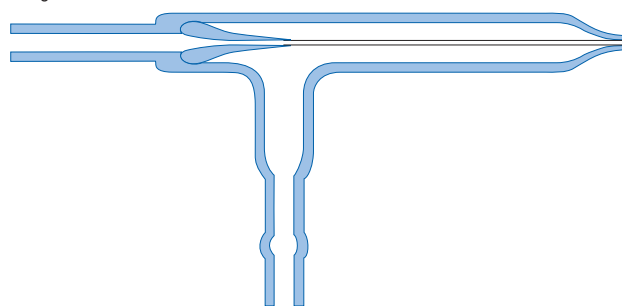


Figure 2. Comparison of nebulizer designs

Figure 2A. Glass Expansion



Figure 2B. Other brands



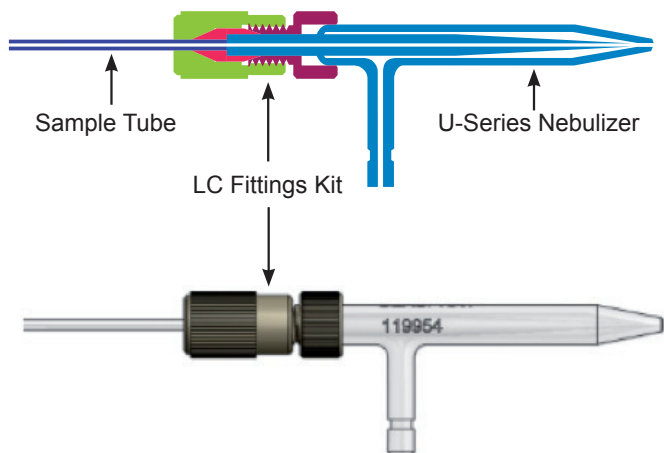
The benefits of the VitriCone construction are:

- The sample channel is guaranteed a constant internal diameter and resistant to clogging.
- The rugged precision-machined capillary resists vibration producing the best possible analytical precision.
- The industry's tightest tolerances ensure that each nebulizer will perform to the same high standards as the previous one.

Glass Expansion's U-Series nebulizer design has an extra advantage for those who are conducting single cell analysis by Mass Cytometry. Combining a U-Series nebulizer with Glass Expansion's LC Fittings Kit (P/N FT-16-8), allows for the nebulizer to be quickly and easily directly connected to the syringe or pneumatic driven sample introduction system with a zero dead volume connection.

The LC Fittings Kit is configured to connect typical eluent tubing (1.6mm or 1/16 inch OD) to the U-Series sample arm (3.2mm or 1/8 inch OD). A diagram of the kit connected to a U-Series nebulizer is shown in Figure 3.

Figure 3. LC Fittings Kit



Results

A prototype MicroMist nebulizer (P/N AR120-1-UFT02) combining all the benefits of the Glass Expansion U-Series nebulizer construction with the LC fittings Kit (P/N FT-16.8) was evaluated on a CyTOF2 instrument by Dr. Rahman. When designing the prototype MicroMist nebulizer, it was important to take into account the standard nebulizer operating conditions for single cell analysis by TOF-ICP-MS. In contrast to a typical ICP-MS concentric nebulizer, Mass Cytometry requires a very low nebulizer gas flow (≤ 0.3 L/min) and back pressure (≤ 20 psi) to keep the cells intact during nebulization. This poses a challenge, as with typical ICP nebulizers a low nebulizer gas flow and back pressure will produce a poor aerosol quality resulting in poor transport efficiency (poor sensitivity). Therefore, the prototype MicroMist nebulizer for single-cell analysis required a unique construction to achieve an optimal aerosol quality at a low gas flow to ensure an acceptable cell transmission efficiency. Cell transmission efficiency is defined as the proportion of single-cell (or single particle) events that are acquired by the instrument relative to the overall number of cells in the sample. When evaluating relative acquisition efficiency, it is therefore important to use matched samples and to keep the acquisition time, the cell (or particle) concentration and sample flow rate constant. For these experiments an HF resistant TruFlo monitor at the lowest range (P/N 70-803-0892) was utilized to continuously monitor the sample uptake rate. Single-cell analysis uses a very low uptake of 30-45 $\mu\text{L}/\text{min}$ for most methods and maintaining a constant sample flow rate is directly related to the performance of the analysis. For this reason a syringe drive or pneumatic sample loader is used to maintain a precise sample flow rate. However, like any ICP application the nebulizer can clog, leaks can occur and the syringe barrel or pneumatic sample holder can degrade, all of which can affect the sample flow rate and analytical performance. Glass Expansion's TruFlo sample monitor (Figure 4) provides a real-time digital display of the sample flow rate, so you always know the actual flow rate to the nebulizer. This enhances the day-to-day reproducibility of results and reduces the need to repeat measurements.

Figure 4. TruFlo sample monitor

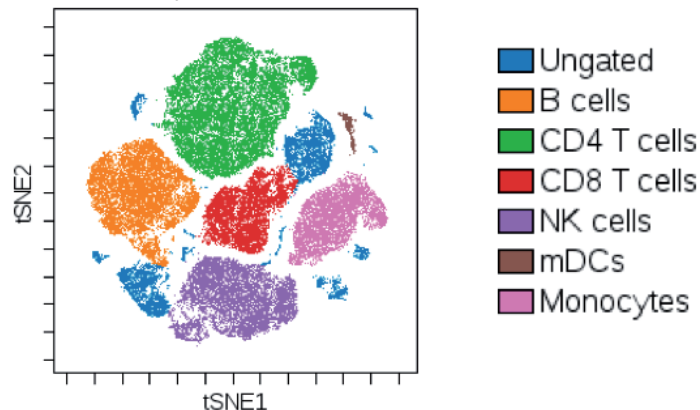


The gas flow rates for the prototype MicroMist nebulizer were optimized using Tuning Solution (Fluidigm), a high purity solution containing known quantities of defined elements. At a liquid flow rate of 45 $\mu\text{L}/\text{min}$, the nebulizer gas flow and make-up gas flow of the prototype MicroMist nebulizer optimized at 0.17 L/min and 0.76 L/min, respectively determined using both manual and routine autotuning protocols. These gas flow conditions match those utilized with the standard CyTOF nebulizer and provided an identical signal intensity. Nebulizer performance was then validated using EQ Calibration Beads (Fluidigm), which are polystyrene beads containing known concentrations of metal isotopes. EQ beads acquired using the prototype MicroMist nebulizer showed identical median signal intensity and CV to those acquired with the standard CyTOF nebulizer.

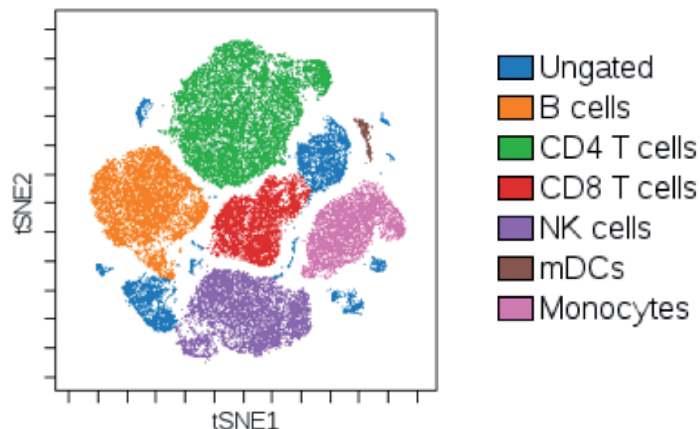
To test performance in a typical experimental application, the prototype MicroMist nebulizer was used to acquire a stained preparation of peripheral blood mononuclear cells (PBMCs) from a healthy donor. PBMCs reflect major populations of circulating immune cells, and high dimensional analyses of these samples are a powerful tool that can be used to evaluate the immune status of patients and evaluate disease progression and treatment responses. Figure 5 shows human PBMCs that were stained with a basic immunophenotyping panel and acquired using the standard CyTOF nebulizer and prototype MicroMist nebulizer. The cell concentration (1 million cells/mL), flow rate (45 $\mu\text{L}/\text{min}$) and total acquisition time (280 sec) were kept constant. The resulting data, visualized using viSNE⁽¹⁵⁾ in Cytobank, show that overall data quality, single cell staining and resolution obtained with the prototype MicroMist nebulizer were identical to the standard CyTOF nebulizer (Figure 5). The prototype MicroMist nebulizer acquired 69,827 cell events in a 280s acquisition window, providing a cell transmission efficiency of approximately 33% on the CyTOF2.

Figure 5. Stained PBMC acquired on a CyTOF2 mass cytometer using a conventional CyTOF nebulizer and the prototype MicroMist nebulizer. The cell concentration, flow rate and total acquisition time were kept constant. The resulting data were visualized using tSNE and some major immune subsets were manually annotated on the map on the basis of canonical patterns of surface marker expression.

Conventional CyTOF nebulizer



Prototype MicroMist nebulizer



Conclusions

The Glass Expansion prototype MicroMist nebulizer at optimized operating conditions for Mass Cytometry produced excellent data quality and single cell staining. Using a sample flow rate of 45 $\mu\text{L}/\text{min}$, the prototype MicroMist nebulizer produced a cell transmission efficiency of 33%. The prototype MicroMist nebulizer provides comparable performance to the standard Mass Cytometer nebulizer, but owing to the VitriCone capillary has the advantages of outstanding analytical reproducibility and improved resistance to blockages. The LC fittings kit provided a zero dead volume connection for best sensitivity and low carry-over. Further experiments are planned where the sample flow rate will be varied in the range of 30 to 40 $\mu\text{L}/\text{min}$ to improve cell transmission efficiency.

The TruFlo Sample Monitor serves as an essential component to the CyTOF & Helios instruments so that the sample flow rate is continuously and accurately monitored, ensuring the quality of cell transmission efficiency.

References

1. Dmitry R. Bardura et al., "Mass Cytometry: Technique for Real Time Single Cell Multi-target Immunoassay Based on Inductively Coupled Plasma Time-of-Flight Mass Spectrometry." *Analytical Chemistry*. 2009, 81 (16), 6813–6822.
2. Sean Bendall et al., "Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum." *Science*. 2011, 332 (6030), 687–696.
3. Scott D. Tanner et al., "An introduction to mass cytometry: fundamentals and applications." *Cancer Immunol Immunother*. 2013, 62 (5), 955-965
4. Smita Krishnaswamy et al., "Conditional density-based analysis of T cell signaling in single-cell data." *Science*. 2014, 346 (6213).
5. Kondala R. Atkuri et al., "Mass Cytometry: A Highly Multiplexed Single Cell Technology for Advancing Drug Development." *Drug Metabolism and Disposition*. 2015, 43 (2), 227-233
6. Serena Di Palma and Bernd Bodenmiller, "Unraveling cell populations in tumors by single-cell mass cytometry." *Current Opinion in Biotechnology*. 2015, 31, 122–129.
7. Farah Virani and Scott Tanner, "Mass Cytometry: An Evolution in ICP-MS Enabling Novel Insights in Single-Cell Biology." *Spectroscopy*. 2015, 30 (5), 14-22
8. Fumin Li et al., "Behavior of bacteria in the inductively coupled plasma: atomization and production of atomic ions for mass spectrometry." *Analytical Chemistry*. 2005, 77 (5), 1407-1413.
9. Koon-Sing Ho and Wing-Tat Chan, "Time-resolved ICP-MS measurement for single-cell analysis and on-line cytometry." *Journal Analytical Atomic Spectrometry*. 2010, 25 (7), 1114-1122.
10. Alexander S. Groombridge et al., "High Sensitive Elemental Analysis of Single Yeast Cells (*Saccharomyces cerevisiae*) by Time-Resolved Inductively-Coupled Plasma Mass Spectrometry Using a High Efficiency Cell Introduction System." *Analytical Science*. 2013, 29(6), 597-603
11. Shin-ichi Miyashita et al., "Highly efficient single-cell analysis of microbial cells by time-resolved inductively-coupled plasma mass spectrometry." *Journal Analytical Atomic Spectrometry*. 2014, 29, 1598-1606
12. John W. Olesik, "Investigating the Fate of Individual Sample Droplets in Inductively Coupled Plasmas." *Applied Spectroscopy*. 1997, 51, 158A-175A.
13. José-Luis Todoli and Jean-Michel Mermet, (2011) *Liquid sample introduction in ICP spectrometry: A practical guide*. Oxford, UK, Elsevier Science
14. Nicolas H. Bings et al., "Liquid sample introduction in inductively coupled plasma atomic emission and mass spectrometry—critical review." *Spectrochimica Acta Part B: Atomic Spectroscopy*. 2014, 100, 14-37.
15. El-ad David Amir et al., "viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia." *Nature Biotechnology*. 2013, 31(6), 545-552.

NEW PRODUCTS

WindTunnel Mobile Cart

The WindTunnel Mobile Cart frees up bench space and allows the autosampler to be placed in the most convenient position for your analysis.

- Chemical-resistant polymer construction
- Inbuilt shelving for reagent and waste containers
- Fully mobile for easy positioning
- Locating pins to lock WindTunnel Enclosure in place
- Drain fitting to remove any accidental spills
- Lightweight and fully collapsible for easy shipping

WindTunnel Mobile Cart



Enclosure photographed with the Mobile Cart is sold separately

Part No.

Description

[70-803-1390](#)

WindTunnel Mobile Cart

[70-803-1148](#)

WindTunnel Autosampler Enclosure

[70-803-1199](#)

WindTunnel Autosampler Enclosure for Cetac XLR8

INSTRUMENT NEWS

From Agilent Technologies - New 4210 MP-AES

Eliminate the cost of expensive gases. The new Agilent 4210 microwave plasma-atomic emission spectrometer runs on air rather than flammable gas—a feature unique to Agilent MP-AES systems—making it both safer and less expensive to operate. This is especially important to laboratories in the food, agriculture, petrochemical, environmental and mining industries.

The 4210 features an array of optional accessories which extend the instrument's analytical performance, sample throughput, and ease of use. In addition, new automation software enables remote elemental analysis. This flexibility opens up new possibilities for applications such as at-site process stream monitoring and environmental monitoring. If you want more information, [please visit us here](#).



From Analytik Jena - PlasmaQuant® PQ 9000 – A New Perspective of Plasma Observation in ICP-OES

A novelty in High-Resolution (HR) atomic spectrometry, the Dual View PLUS plasma observation of Analytik Jena's [PlasmaQuant® PQ 9000](#) is now bringing state-of-the-art productivity and operator convenience to the most elaborate HR ICP-OES routines. Probing an analytically superior vertical plasma by end-on and side-on observation with complementary attenuation of both the axial and the radial view, respectively, [Dual View PLUS](#) reaches well-beyond traditional boundaries of ICP-OES and thus takes ultra-trace detection capabilities, linear dynamic working ranges as well as the overall signal stabilities, a major step ahead. Its fast adaptation of plasma views to the required sensitivities and working ranges ensures supreme operator flexibility at compatible speed and sample costs. Dual View PLUS means free selection of 2+2 plasma views in every routine and stands for instrumental flexibility instead of lengthy sample preparation. Look forward to the most sensitive axial plasma views on the market: That of [PlasmaQuant® PQ 9000 series!](#)



From Horiba Jobin Yvon - New ICP-NEO Software facilitates method development, sample measurement and results management.

HORIBA Scientific announces its newest software release for its ICP-OES spectrometers. ICP-NEO is designed to facilitate method development, sample measurement and results management. ICP-NEO includes powerful tools for sample measurement with advanced quality control protocols and retrospective analysis with respect of the integrity of raw results to match with good laboratory practice requirements. The ICP-NEO interface offers a multiple user capability with individual passwords, logoff and logon without stopping the sequence, a contextual tool bar to adapt the content with the window selected, user defined window display on the screen and diagnostics with real-time information on instrument.

The method development includes a visual display of interference free lines using S3 wavelengths database - unique database specially developed for ICP-OES by HORIBA Scientific. This database gives information not only on wavelengths but also on detection limits and it is the only one to give reliable relative intensities between emission lines.

Full automation of the analysis is possible with autosampler, the Smart Rinse feature for automatic monitoring of rinse efficiency between 2 samples, automatic control of the quality with limits on correlation coefficient and the automated Quality Control procedures fully US EPA compliant with ICV, CCV, LCS, Interference check, Paired samples, etc.

ICP Neo is available for all of HORIBA Scientific's Ultima family ICP-OES spectrometers. It is compliant with the latest Microsoft® platform, as well as Windows® 7 and 8.

