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Vitesse Note **NT07**

Using Vitesse time of flight ICP-MS to measure metal tagged markers and endogenous elements in single cells with the reduction of data using Nu Quant and common flow cytometry software packages

- Analysis of individual cells
- Full elemental coverage from Na to U
- Accurate cell identification through fast transient signals
- Export of cell data to the flow cytometry standard file format (FCS)

Introduction

Flow cytometry has proven to be a valuable tool for the analysis of single cells in the field life sciences. Rather than examining cells in bulk, a stream of cells is examined individually for certain analytical parameters, giving much greater insight into a population of cells and allowing robust statistical analysis. The determinants measured vary depending on the detector used. The “mass cytometry” technique is now well established where metal labelled antibodies have proven to be a highly efficient tool for the identification of certain proteins in a cell using a time of flight mass spectrometer as a detector. While the identification of cells is possible by these means, the endogenous elements like Fe, Ca, P, are not within the measurable range of the instruments used, so much of the information required to understand the metallomics and metabolomics on a per cell level is lost. With the introduction of the Vitesse, a technique is now available that can provide all of the information available with the traditional mass cytometry technique whilst simultaneously providing the information from the endogenous elements to broaden the understanding of the cell systems studied.

Methodology

The robustness of any analysis relies on getting a statistically relevant number of measurements to provide reliable information, preferably in a low amount of time. Especially when examining individual cells, the number of examined cells needs to be high to obtain reliable data in order to overcome the biological variability. To minimize the total analysis time each cell needs to be examined quickly and in rapid succession. In single cell ICP-MS a liquid suspension of cells is aspirated through a specialised nebuliser and spray chamber system so they reach the plasma and can be detected individually. Various commercially available single cell introduction systems are easily connected to the Vitesse. Figure 1 shows the setup using a single cell spray chamber from Glass Expansion.

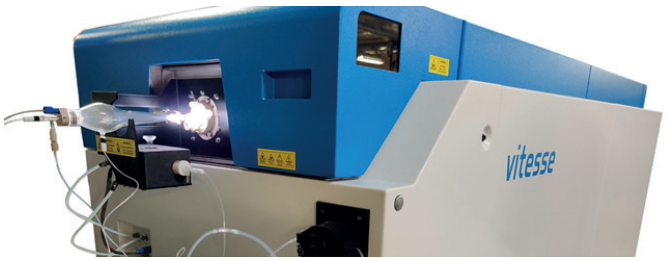
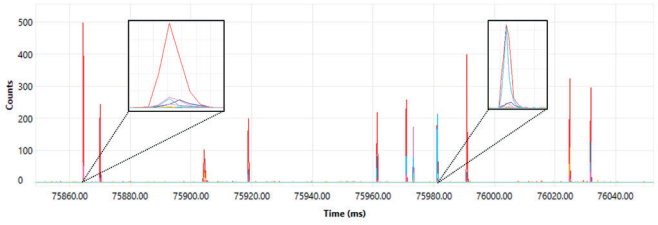


Figure 1: A typical setup for single cell analysis showing a specialized introduction system connected to the Vitesse.

The measurable signals generated from elements within the cell last between 0.5 to 2 milliseconds so the cell suspension is diluted to a level where there is negligible risk of signals from more than one cell overlapping. To achieve a high number of cells per time, the concentration in the suspension and transport efficiency of the nebuliser and spray chamber needs to be as high as possible while still being able to differentiate individual cells. To then detect the signals above the background levels of dissolved elements in solution, a high time resolution for the transient signal is needed. By using a



continuous uninterrupted storage time of 80 µs per spectrum, each cell event can be fully profiled to achieve a high certainty in cell identification as

shown in figure 2.
In the Nu Quant software each cell event is identified, using the same established principles as used for nanoparticle analysis (explained in application note NT04), and each isotope integrated resulting in a list of cell events and their elemental contents. Using this approach, hundreds of cells can be examined per second allowing for good statistical analyses in a reasonable time.

Data Reduction

The fast dwell time and high number of isotopes observed results in very large datafiles reaching multiple gigabytes in size within tens of seconds. The analysis of this data is therefore a challenging task. As previously mentioned, the Nu Quant software reduces the transient signals to single events making the analysis a much more manageable task. This data can then be further examined and visualized within Nu Quant using user editable python scripts to examine important properties of the cells analysed. For instance, a differentiation between cell events and internal standards can be made based on the ^{153}Eu and ^{140}Ce content. Since the beads used for internal standardisation in this case (Fluidigm EQ beads) are high in these two elements and these elements concentrations are comparatively low in the cell events, plotting the intensities on log scale against each other allows the identification of two distinctly different clusters (figure 3). After an automatic grouping into these clusters, we can now identify the internal standards and further examine the other events for their elemental contents.

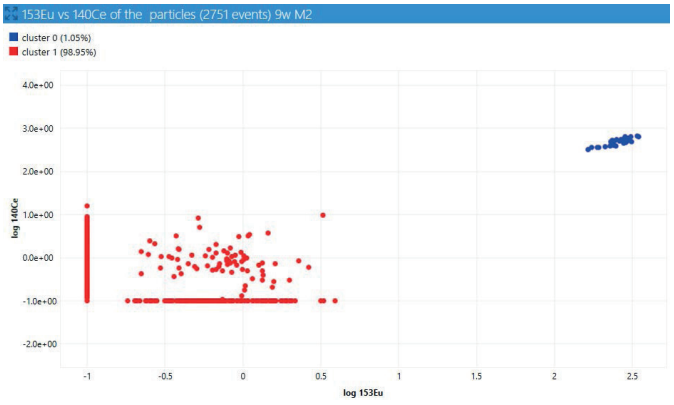


Figure 3: Differentiating cell events from calibration beads using ^{153}Eu and ^{140}Ce as plots of the log of signal. The blue cluster shows the calibration beads (high in both isotopes) and the red cluster shows the remaining events (low in both isotopes).

Differentiating cell types

During the sample preparation, various metal label antibodies can be used to label proteins on the cell surface. When done efficiently, various cell types can be distinguished by examining the intensity of the applied metal label. This allows the differentiation of cells based on protein markers for instance figure 4 shows the deeper evaluation

Figure 4: Determining the type of cells is possible by examining the elements bound to the antibodies. In this case ^{141}Pr and ^{149}Sm were metals attached to antibodies that bind to protein expressed in B-cells. Therefore, it can be assumed that all cells in clusters 0, 2, 6, 8, 9 and 11 are B-cells.

of the red cluster from figure 3, for elements linked to antibodies which are expressed by B-cells. Therefore, it can be assumed that all these cells high in ^{141}Pr and ^{149}Sm are identified as B-cells and the rest of the population could be assessed for other labels linking them to other cell types. Furthermore, the amounts of endogenous elements in the identified B-cells can be examined and compared to other cell types.

Detailed examination in common flow cytometry software packages

Since the start of flow cytometry measurements, it has become evident that efficient tools needed to be developed to examine the large datafiles and make accurate predictions on a per cell level. To tie into existing workflows and enable the use of powerful and well-known software packages, it is possible for Nu Quant to export the found cell data to the widely used Flow Cytometry Standard file format (.fcs). This allows for easy and fast analysis in software packages like FlowJo (figure 5) or others specifically designed to deal with the types of data found in flow cytometry measurements.

After identification of the cell types it is also now possible to look at the presence of endogenous elements for each individual cell type. Due to the use of the reaction cell in the Vitesse ICP-MS, sensitive Iron measurements can be made on the most abundant but usually heavily interfered isotope of ^{56}Fe. This allows for great insight into the iron uptake of various types of cells further elucidating the role of these cells in the body. Figure 5b shows the varying distributions of iron in various different cell types.

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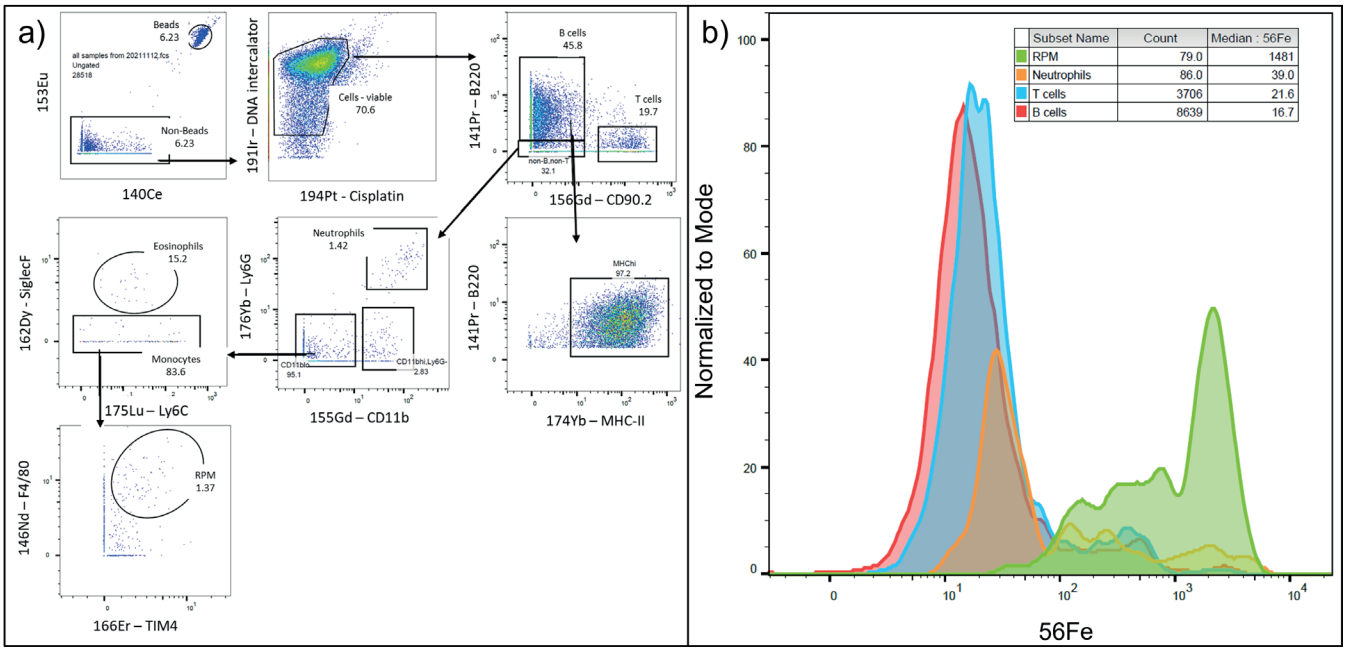

Figure 5a and 5b: Examination of single cell measurements in FlowJo after export to the .fcs file format from Nu Quant. a) shows the identification of cell types based on the used labels while b) shows the distribution of iron in the various cell types.

Conclusion

Single cell analysis is becoming established as a robust ICP-MS technique and has proven to be an important tool to examine groups of cells on an individual level. Using modern methods like SC-ICP-MS, it is possible to differentiate various cell types using metal labels. In addition to that, the Vitesse is capable of not only detecting and differentiating a high number of cells in a short time, but also measuring the endogenous elements in each cell. The information on endogenous elements in cell types is an important analytical parameter for metallomics and metabolomics to further elucidate the roll that various elements have in living organisms.

Acknowledgements

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