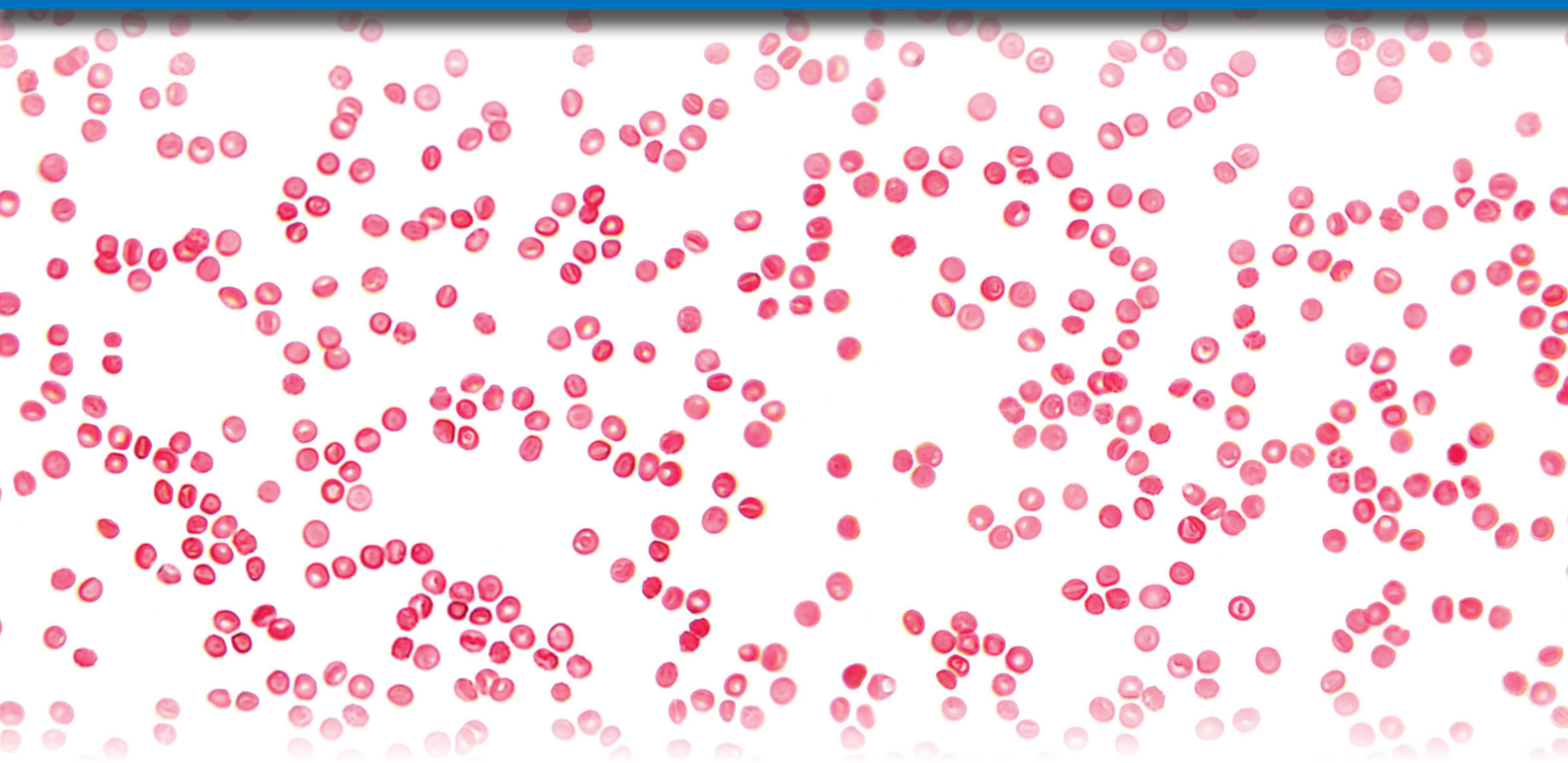

# Single Cell Lipid Analysis using the Bruker ultrafleXtreme TOF/TOF and the 7T solariX MRMS Mass Spectrometers

Matrix assisted laser desorption ionization (MALDI) mass spectrometry imaging (MSI) visualizes the distribution of a variety of molecules in biological systems, such as small metabolites, lipids, peptides, and proteins.

## Introduction

Generally for MSI, mass spectra are acquired in a raster pattern, pixel by pixel, to build a chemical image of detected biomolecules. While this technique works well for many samples and tissues, there are other approaches that are more efficient when probing dispersed cells on a slide. By performing an acquisition via a uniformly spaced grid, cells may be split into multiple pixels, dividing the chemical information and complicating data analysis. Moreover, while imaging populations of dispersed or cultured cells,

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most of the analysis time is spent acquiring spectra from empty spaces between cells rather than the cells themselves. This can lead to hours of imaging, with only a small fraction of the spectra containing cellular data. Accordingly, we have developed alternative approaches that are more efficient for examining thousands of individual cells dispersed on a slide (Figure 1).

To increase acquisition efficiency, we created the freely available microMS software [1], which automatically locates the spatial positions of fluorescently labelled cells or objects. Using these positions, microMS creates a geometry file that is compatible with Bruker MALDI mass spectrometers, allowing fast and efficient acquisition of individual cells randomly seeded on a sample substrate. Though developed for single cell analysis, this software can be used for many sample types that may require creation of non-standard geometry files with high precision and accuracy, such as bacterial colonies [2] and other objects. Further, microMS enables multimodal analysis of the same individual cells (or samples) across multiple Bruker mass spectrometry (MS) platforms and stages, including orthogonal approaches such as immunochemistry [3] and capillary electrophoresis [4]. Here, we describe the use of microMS to study lipids within single rodent brain cells on the Bruker ultrafleXtreme and 7T solariX XR MALDI MS systems.

## How to perform single cell measurements

Full experimental details have been described in detail [3], but are briefly outlined here. Dissected rodent cerebellar tissues were enzymatically and physically dissociated before being transferred onto indium-tin oxide glass slides with etched fiducials. Brightfield and fluorescence images were acquired on a Zeiss Axio M2 microscope. Cell locations were found using the high fluorescence from a nuclear dye and filtered by size, shape, and distance using the microMS software [1]. Slides were then coated with 0.1 to 0.2 mg/cm$^2$ of dihydroxybenzoic acid using an automatic sprayer described previously [5]. Single cell analysis was performed on a Bruker ultrafleXtreme TOF/TOF mass spectrometer with a mass window of 500–3000. The "Ultra" (~100 µm footprint) laser setting was used and 300 laser shots were accumulated at 1000 Hz and 60% laser energy for each cell. Additional experiments were performed on a Bruker 7T solariX XR Magnetic Resonance Mass Spectrometry (MRMS) system with a mass window of 150–3000, yielding a transient length of 2.94 s, and each MALDI spectrum was acquired with 20 laser shots at 1000 Hz and 60% laser energy with ~100 µm footprint. Cell coordinates were obtained using microMS and filtered with a 100 µm distance filter to prevent mixing of information from too closely located cells. Select slides were then immunostained with primary antibodies against glial fibrillary acidic protein and neurofilament-light chain and fluorescently labelled secondary antibodies.

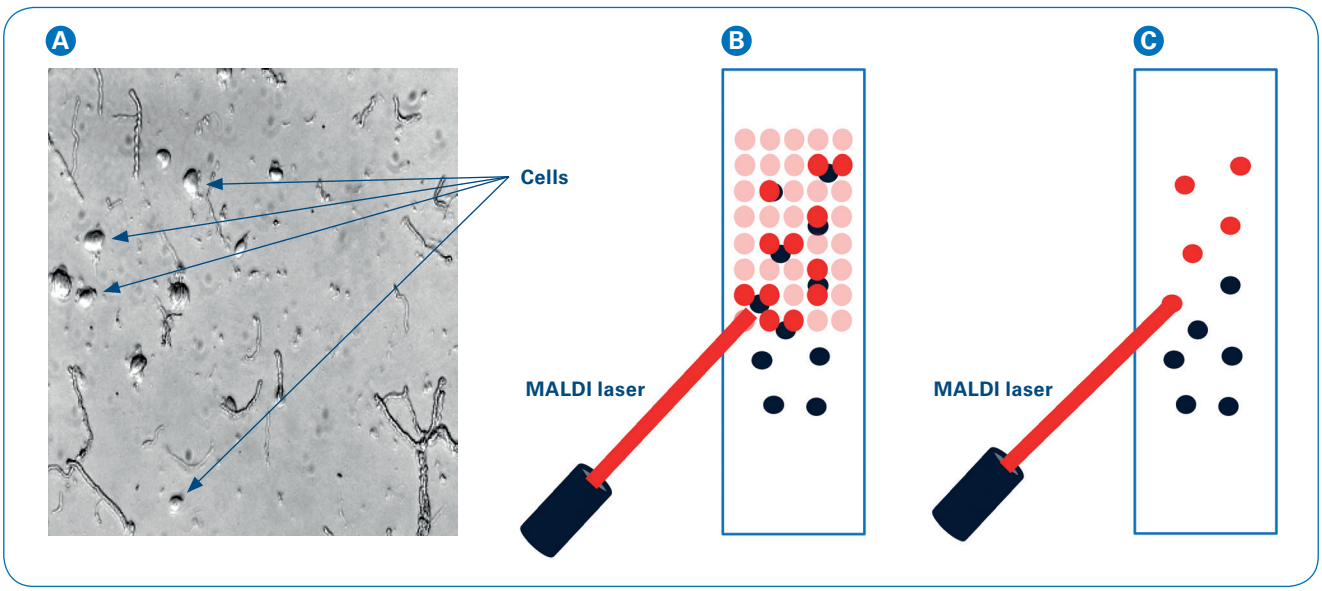


*Figure 1: Characterizing a slide with dispersed cells. (A) A slide has a collection of cells mixed with non cellular objects where we only want to characterize the cells with MS. We optically image the slide, determine the locations of the cells we want to measure, and feed these target locations into the mass spectrometer. (B) Mass spectrometry imaging acquires data in a regularly spaced raster pattern and so spends valuable time on the empty spaces and does not center the laser onto the cells. (C) Instead of mass spectrometry imaging, here we acquire spectra from the positions of the cells of interest. Because MS data is only acquired from the cells, because the empty space on the slide is not interrogated and because the acquisitions are centered on the cells of interest, the approach is faster and produces more reproducible data. Using microMS, we expand this approach to slides with tens of thousands of cells.*

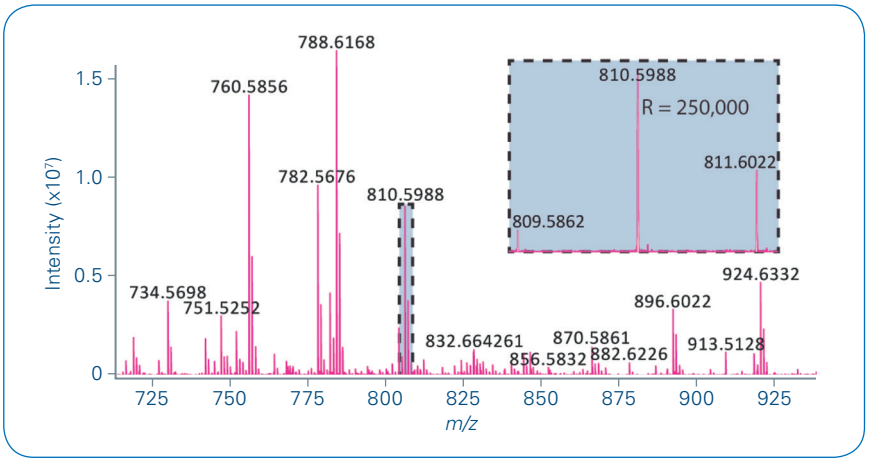


*Figure 2: Single rodent cell spectrum taken on the Bruker 7T solariX XR system. Several lipids with a resolution of ~250,000 are labelled with high signal-to-noise ratios.*

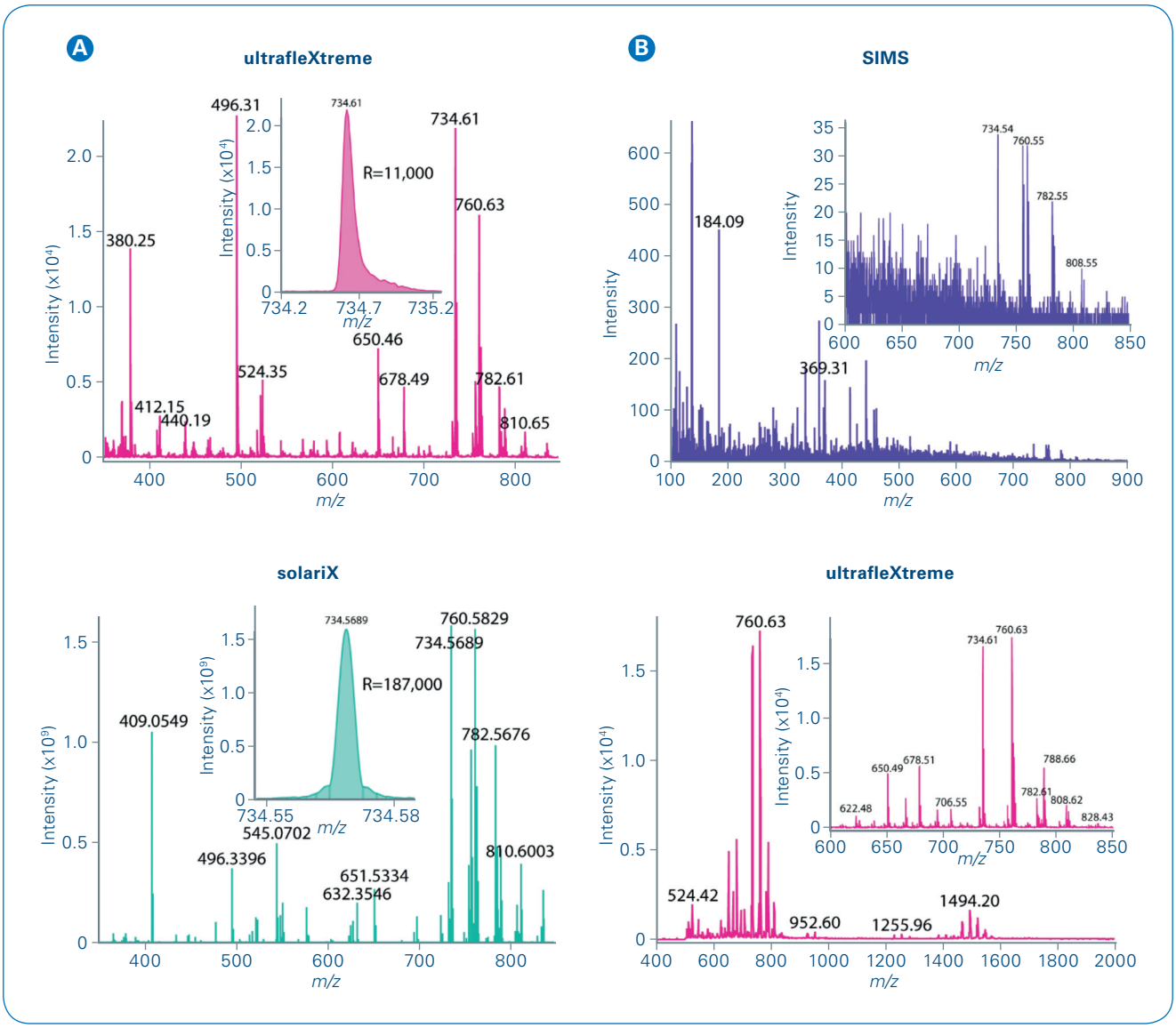


*Figure 3: Spectra from the same single cell can be serially acquired on multiple MS systems to enhance the chemical information obtained from an individual cell or sample using microMS. Ⓐ A Bruker ultrafleXtreme obtains single cell spectra at a rate of 1 cell per second and a resolution of 11,000 for the lipid mass range, and was used to screen cells for follow-up analysis by a Bruker 7T solariX XR system at a rate of 1 cell per 30 seconds and a resolution of ~180,000 for the lipid mass range. Ⓑ A custom secondary ion mass spectrometer and a Bruker ultrafleXtreme system can also be used sequentially on a single cell for obtaining both metabolic and lipid/peptide chemical information.*

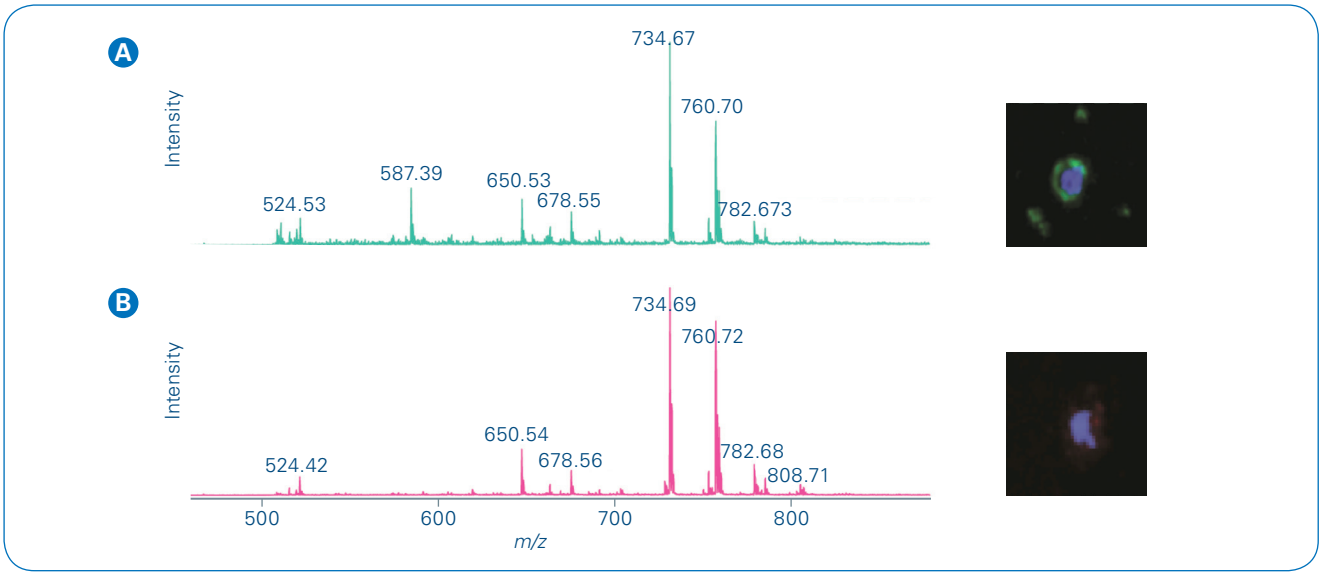


*Figure 4: Single cell mass spectrometry lipid information can be directly correlated to immunocytochemical profiles for cell identification. Spectra were acquired on a Bruker ultrafleXtreme instrument. (A) Single neuron mass spectra and immunocytochemical image. (B) An astrocyte with its corresponding mass spectra. Green and red fluorescence corresponds to neurofilament- light chain (neuronal marker) and glial fibrillary acidic protein (astrocyte marker) respectively, while blue fluorescence corresponds to a nuclear dye.*

## Results and Discussion

By using a custom geometry file, we acquired single cell spectra from rodent brain cells using the Bruker ultrafleXtreme TOF/TOF mass spectrometer at a rate of ~1 cell/s and ~1 cell/ 30 s on the Bruker solariX MRMS MS system. This allowed us to profile thousands of cells in a reasonable time frame, making the technique applicable for studying systems of high complexity and diversity spread over large numbers of cells, such as the brain. We can target an individual cell with a spatial accuracy of ~ 30 µm. While the ultimate spatial accuracy depends on instrument stage movement accuracy, in practice we find that it is related to the number of training fiducials and the accuracy of the training set. While the accuracy is greater than the size of most mammalian cells, we compensate for the uncertainty of position by using a laser footprint that is large enough (~100 µm) to assure that we sample the desired cells. Of course, we can improve our spatial resolution via an increase in the number of training fiducials. By only acquiring spectra for cells that are at least 100 µm apart, we limit cell-to-cell contamination within our single cell spectra.

An example single cell spectrum taken on the 7T solariX XR MRMS system is shown in Figure 2. Dozens of lipid species are detected between the *m/z* values of 700 to 925 with high mass resolution (~250,000). For most single cell mass spectra, the spectral quality is generally high, with an average of 40 lipid features detected per cell, ranging between one and over a hundred lipid features within individual cells. We mostly detected phospholipids from the outer membrane of the cell, including phosphatidylcholines, phosphatidylethanolamines, sphingomyelin, and many others. It is possible to produce similar spectra on the ultrafleXtreme at a faster acquisition rates, although at a lower mass resolution and, therefore, a fewer number of lipids that are resolved.

Because microMS can be implemented on both MALDI instruments, the same microscopy image can be used without changing the pixel coordinate of any cells, greatly simplifying multimodal MS acquisition and subsequent data analysis. For instance, initial single cell profiling experiments can be performed on the ultrafleXtreme TOF to prescreen cells for subsequent solariX MRMS MS measurements (Figure 3a) [1]. By performing the faster TOF MS measurements first, we reduce the number of targets for the subsequent, slower MRMS MS analysis, thereby increasing the efficiency of our overall experiment. Similarly, MALDI MS can be used to re-assay cells for subsequent capillary electrophoresis analysis as well [4].

MicroMS and subsequent single cell analysis on the Bruker MALDI imaging systems is applicable to other chemical classes beyond lipids, such as small metabolites, peptides, and proteins; however, each chemical class requires optimization and different sample preparation approaches.

While MS produces rich chemical details, how do we link this information to cell type? For us, this has involved the introduction of orthogonal analytical approaches for comprehensive single cell analysis. Using microMS, we have correlated ultrafleXtreme spectra with immunocytochemical (ICC) classifications on the same cells [3]. By coupling these two approaches, we obtained reproducible lipid profiles for rodent astrocytes and neurons located within the cerebellum. Because the position remains constant throughout both MALDI MS analysis and antibody staining, we directly correlate the mass spectral features and ICC-based fluorescence profiles from individual cells (Figure 4). For example, we determined that neurons (Figure 4a) have a higher abundance of phosphatidylcholine lipids compared to astrocytes (Figure 4b), which have a higher abundance of phosphatidylethanolamine lipids. Ultimately, the lipid differences between these two cell types were modest, requiring single cell MS analysis via microMS. While this example demonstrates our ability to distinguish two canonical cell types, astrocytes and neurons, using the ultrafleXtreme, the sampling procedure can be extended to other Bruker MALDI instruments, biological samples, and antibodies.

## Conclusions

- Our single cell approaches, aided by microMS, enable sparse object locations and acquisitions that are compatible with many Bruker MALDI MS instruments. By creating a custom geometry file to obtain multiplexed chemical information from each cell, the methodology provides efficient analyses of thousands of randomly-seeded cells. The workflow is easily adaptable for multiple analyses being performed on the same cellular or non-cellular targets to extend the information that can be gained from a small sample.

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