Title (12/20)

Rapid MALDI Imaging, Sample Preparation, and Sample Analysis for High Throughput Discovery

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Introduction (XXX/120)

The long processing times associated with MALDI imaging mass spectrometry (IMS) studies are often a hindrance in large research studies. In order to improve throughput, and reduce processing times, the use of high speed sample preparation and instrumentation are necessary. An automated spray chamber system was used to reduce long preparation times; multiple samples can be sprayed at once as the coating remains uniform across all samples. A large volume and diameter spray nozzle housed in a spray chamber allows for drastically shorter spray times while creating a uniform coating. A new 5 kHz MALDI-TOF with both linear and reflecting analyzers provided rapid data collection with high spatial resolution.

Methods (XXX/120)

Tissue sections typically 10-20 μm thick were mounted on 25x 76 mm slides composed of either conductive glass or stainless steel. The MALDI mass spectrometer employed a sample plate with the standard microtiter format that accommodates up to four slides at a time. In some experiments each slide was coated separately using the automated spray chamber; in others a complete set of four slides mounted on the microtiter plate were coated simultaneously Each sample was analyzed by IMS generating complete spectra at up to 50 averaged spectra per second. The resulting data were analyzed to determine the uniformity of signal across each sample and the sensitivity and reproducibility of the approach..

Preliminary data (XXX/300)

In IMS, the adequacy of a matrix coating is determined by the amount of ion signal produced from each laser shot, the uniformity of the TIC, and the ability to identify different ions of biological significance throughout the sample. In order to achieve these parameters for an adequate coating, a matrix crystal size that is less than the laser spot diameter must be achieved. Previous studies suggested 50:50 chloroform/methanol with DHB or α-cyano produced good crystallization and a short drying time. Such volatile combinations as DHB in chloroform/methanol have been identified to produce good crystallization, and the spraying system's construction entirely of stainless steel allowed for the use of these volatile combinations. DHB or α-cyano dissolved in 50:50 chloroform/methanol was chosen for the spray chamber after numerous experiments due to superior drying time, and uniform crystallization producing crystal sizes of less than 10 μm. Performance evaluations employed rat brain sections for the initial studies. Rat brain is well-characterized, thus making it an excellent choice for testing deposition techniques. Two sodiated phospholipid ions [PC 18:0/18:1 + $Na]^+$ at *m/z* 810 and [PC

16:0/16:0 + Na]$^+$ at *m/z* 756 were chosen for comparison because of their presence in specific locations in the brain. After coating and analysis, the crystal size, TIC, ion signal, and extraction of the chosen analytes were compared.
Novel aspect (7/20)
First High-Speed Preparation/Analysis Complete System

Session
Imaging MS