Metabolite Imaging using DESI with Ultra-High Resolution Multi Reflecting Time-of-flight (ToF) Mass Spectrometry

Imaging

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Desorption electrospray ionization (DESI) is an established imaging mass spectrometry (MS) technique for metabolites from mammalian tissue sections. One of the biggest challenges is the confident identification of the imaged metabolites. Imaging MS with an ultrahigh mass resolution provides a high mass accuracy for more accurate identification in addition to providing cleaner discrimination of imaged ions from the neighboring chemical noise. Fourier-transform ion cyclotron resonance (FTICR) with MALDI has shown the ability to acquire ultra-high mass resolution for lipids, but requires a longer MS scan time per pixel. A longer MALDI imaging time reduces the throughput, suffers from matrix evaporation and molecular degradation tissue. MALDI imaging is generally less amenable to small metabolites, often requiring chemical derivitization. In this work, we present the utilization of multi reflecting ToF (MRT) with folded flight path geometry to image metabolites off porcine liver and mouse brain sections using desorption electrospray ionization (DESI). Multi reflecting ToF provides an ultrahigh mass resolution by increasing ToF length, such as 50 meters, using low aberration ion optics capable of producing narrow peak widths. Here, a multi reflecting ToF (Select Series MRT, Waters Corp) mass spectrometer was coupled with DESI (DESI- XS, Waters Corp) with MassLynx and High Definition Imaging (HDI) software. DESI MRT is capable of 10 pixels per sec, 200 000 FWHM mass resolving power, <500ppbmass accuracy. DESI-XS was equipped with a novel high-performance sprayer based on a cartridge and heated transfer line for more optimal transfer of ions. The temperature of around 150-200 ° C set in heated transfer line was found optimal for enhancing signal for many small metabolites, while the temperature of 400 to 450 ° C was ideal for lipid species. DESI solvent (MeOH: water, 98:2, 0.01% formic acid) running at 2 µL/min was electrospray by holding capillary at 1.0 kV with nebulizing gas pressure set at 1.5 bar. DESI images were acquired at 50-micron and 30-micron pixel sizes for liver and brain sections, respectively. Small metabolites such as lactate at m/z 89.02387 were imaged confidently with 157 ppb mass accuracy. In the brain section imaging, mass accuracies for metabolites (e.g., AMP, glutamate, taurine, adenine, aspartic acid) were between 69 and 182 ppb allowing for confident identification. The manual identifications were corroborated with the online METASPACE tool. METASPACE utilized the Human Metabolome Database (HMDB) with a maximum of 10% target–decoy false discovery rate (FDR), translating into a maximum of 10% possible false positive annotations. The manually identified metabolites matched with METASPACE with a mean metabolite-signal match (MSM) of 0.2 and 5% FDR for about half of the ions and 10% FDR for the rest. In conclusion, DESI with MRT showed the capability of imaging and confidently identifying small metabolites from liver and brain tissues.