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Overview and Latest Developments in Desorption Electrospray Ionization (DESI) Mass Spectrometry Imaging

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Mass spectrometry imaging (MS imaging) provides the untargeted distribution evaluation and structural characterization of a set of molecules from the surface of analysis, such as tissue sections. These molecules can be of diverse composition and weight, such as lipids, drugs or metabolites contained in sequential mass spectra (which constitute the image "pixels" after software processing). MS imaging experiment is performed by scanning the sample in its *x* and *y* axis in open environment with minimal sample preparation and this approach is named ambient MS imaging [1]. So far, there are ca. 30 relevant ionization approaches and desorption electrospray ionization (DESI) is the top ambient technique in terms of publication. DESI-MS imaging is mostly used for the analysis of lipid and other small molecules. Recent technical developments in DESI-MS imaging include (i) the use of morphologically-friendly solvent combinations, which allow tissue morphological evaluation through staining or immunohistochemistry after the chemical imaging experiment[2], and (ii) the possibility to perform higher spatial resolution (50µm lateral resolution) to pick up cell population distributions or tissue features [3].

These new technical developments allow for multimodal DESI-MS imaging in biological samples. Multimodal DESI-MS imaging will benefit from the precise alignment of diverse images, which can be, for example, to overlay ion and one optical image obtained from a tiny region of the same sample. In this work, we propose the use of a fiducial maker system, namely 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) for the precise alignment of DESI-MS ion images and optical data of tissue sections. The sodiated HEPES molecule (*m/z* 261) is readily detected in the positive ion mode and the deprotonated HEPES (*m/z* 237) in the negative ion mode, making it appropriated for both ion modes.HEPES-based fiducial marker was prepared by mixing 25mg of HEPES (H7006 Sigma-Aldrich) and 100µL of Harris hematoxylin solution (HHS32 Sigma-Aldrich) to the tissue homogenate of one mouse brain. This mixture was stored frozen in small aliquots and, as a proof-of-concept, deposited onto a mouse brain tissue section in a triangulate pattern with the aid of a steel straight pin. DESI-MS imaging has been performed using a Thermo Fischer Scientific LTQ (San Jose, CA) equipped with a linear ion trap with settings and conditions previously described. After the MS-imaging experiment, the tissue section was stained with hematoxylin & eosin (H&E) and optical images of sections of each tissue were taken using a SM-LUX Binocular brightfield microscope (Leitz, Wetzlar, Germany) and a Dino-Lite AM2011 Handheld Digital Microscope (Torrence, California). Fiducial marker position was plotted and embedded in the ion and optical images (Figure 1). Ion images and optical images were superimposed using the transparency effect in the Power Point software to precisely overlay the ion image with the tissue section. Due to its biological nature (tissue homogenate), the proposed HEPES-based fiducial marker is compatible with solvents used in histological staining and can be spotted over the tissue or outside it. In conclusion, this work introduces a system of fiducial marker for multimodal DESI-MS imaging of tissue sections. This capability can be applied for physiopathological evaluation of tissue sections in order to better map in an untargeted fashion small molecular compound location in a variety of biological and pathological conditions.

References

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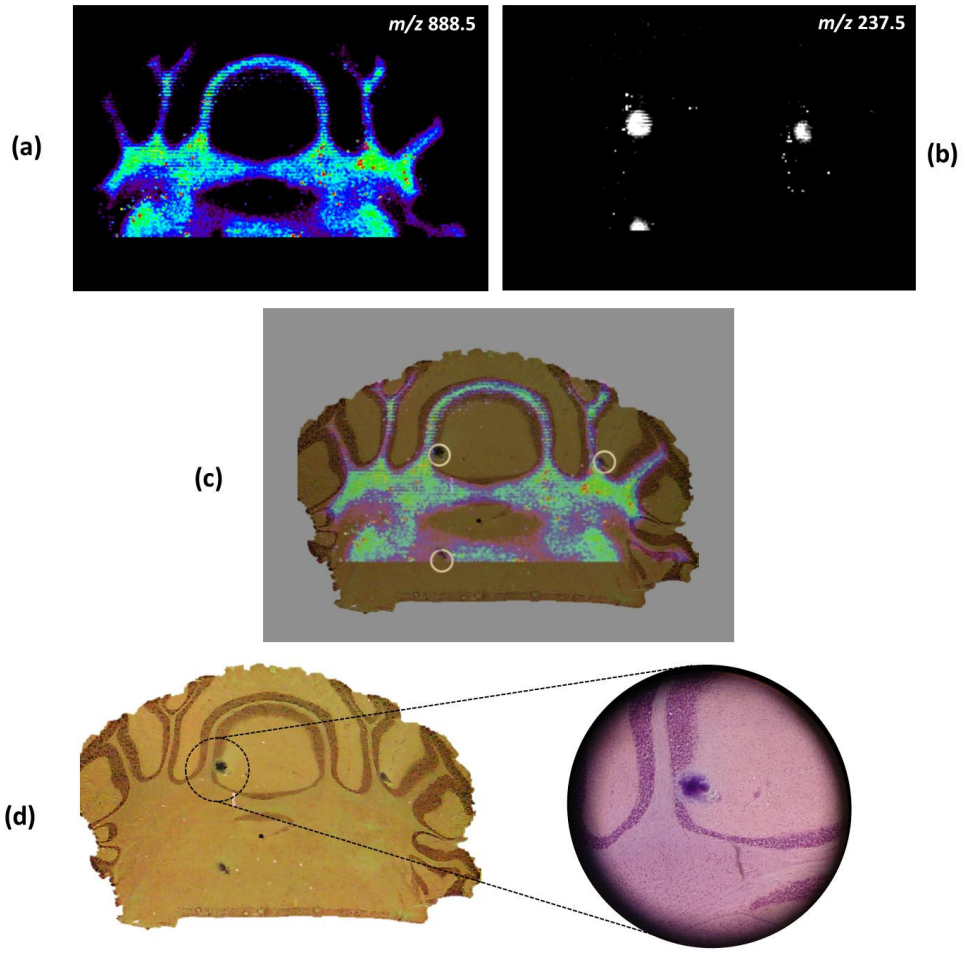

Figure 1. HEPES-based fiducial marker for DESI-MS multimodal tissue imaging. (**a-b**) Ion images of a sulfatide lipid of *m/z* 888.5 and the *m/z* 237 (deprotonated HEPES); **(c)** Ion and optical images are combined in order to align mass spectrometric and tissue morphological information. **(d)** The purple spots of the HEPES-based fiducial marker in the tissue are easily distinguishable during microscopic examination after H&E staining.

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