Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary information

Mapping metabolites from rough terrain: laser ablation electrospray ionization on non-flat samples

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Experimental Part

Assembly of LAESI source

The LAESI source was assembled on a custom-built table made of a 70 mm by 70 mm solid aluminium breadboard (Thorlabs, Newton, NJ, USA) and aluminium framework. The table was positioned in front of a LTQ Orbitrap mass spectrometer (ThermoScientific, La Jolla CA, USA).

A four-dimensional sample manipulation system was assembled from a DDS220/M DC servo stage (220 mm travel distance), two MTS50/M-Z8 (50 mm travel), and one MTS25/M-Z8 (25 mm travel) compact translation stages (all from Thorlabs) and designated as the B-, X-, Y- and Z-axis, respectively.
The X, Y and Z-axes are orthogonal to each other; the X and Y-axes are oriented within the plane of the breadboard, and the Z-axis is oriented perpendicular to the X and Y-axes in the third dimension. The whole XYZ system was mounted on the B-axis which is parallel to the Y-axis. A holder for microscope slides was custom-made from a PEEK block and mounted onto the Z-axis. An OPO-pumped, infrared pulse laser operating at 2940 nm wavelength (Opotek, Carlsbad CA, USA) was mounted on a 45 mm x 60 mm x 60 mm NEXUS breadboard (Thorlabs) and operated at 2940 nm wavelength. The beam path was oriented parallel to the plane of the table and widened with CaF2 lenses (Thorlabs) in a 20 / 100 telescope optic. Laser energy attenuation took place inside the telescope optic and was facilitated by adding absorptive neutral density filters of various optical density (Thorlabs). The widened beam was redirected downwards, orthogonal to the table plane, with a Sapphire turning mirror (Layertec, Mellingen, Germany). An aspheric ZnSe lens (Thorlabs) was used to focus the widened beam with a theoretical focal length of 25 mm. In Figure 1B, a schematic drawing of the optical system is shown. As an electrospray emitter, a PicoTip (New Objective, Woburn, MA, USA) with a distal conductive coating, 360 µm outer diameter and 10 µm inner diameter was used.

The electrospray emitter was clamped down onto a custom-made PEEK plate opposite to the equally clamped down steel capillary, bridging the gap to the orifice of the mass spectrometer. A round hole of 25 mm allowed the laser beam to pass through the PEEK plate between the electrospray emitter and the orifice of the capillary. In negative ion mode, a solution of 33.3 % propan-2-ol in deionized water with 0.4 % ammonium acetate as additive was delivered to the electrospray emitter at 0.4 µl/min with a PEEK capillary (VICI Jour, Schenkon, Switzerland) of 100 µm inner diameter and a Fusion 100T syringe pump (Chemyx, Stafford, TX, USA). The basic operating principle is shown in Figure 1A.

The whole system is housed in a Plexiglas cover opaque to IR-light of wavelengths longer than 2800 nm. The laser is interlocked to prevent the cover opening during the experiments, and the laboratory is equipped with warning signs if the laser is firing.

Acquisition of height profiles

A confocal distance sensor (CDS) (μ-Epsilon, Ortenburg, Germany) was mounted with its optical axis parallel to the vertical part of the previously described beam path and offset along the B-axis by 108 mm.

This way, the X, Y and Z-axes of the sample manipulation system were shared by the LAESI-source and the CDS. Four positions on the sample slide were marked with white correction liquid (Stanger, Schwöllbogen, Germany) and subsequently ablated. The actual positions of the ablation marks were compared to the expected positions within the relative coordinate system of the CDS. Offset-values for the X and Y-axis were applied to correct for small deviations between the focal point of the laser and the measurement spot of the CDS in the XY-plane.

Height profiles of sample surfaces were recorded by moving the sample below the CDS with the same line-by-line raster to be applied in the ablation experiment in the LAESI source. At every step, a Z-value was recorded and saved for the corresponding XY coordinates. A fixed offset value was added to the measured Z-values to account for the different position of the laser's focal plane along the Z-axis, compared to the CDS' measurement spot. For the purpose of visualization, false colour representations of the recorded height profiles were corrected for trends in consecutive Z-values that originated from an imperfectly orientated sample holder and will here be henceforth referred to as topographic maps. These maps are represented using a standard 'viridis' color gradient scale13 where navy blue represents low intensity and the movement from green to yellow represents an increase in intensity for individual pixels on the imaged sample.

Missing values encountered while recording the height profiles of very rough sample surfaces were corrected using an in-house script written in R (version 3.2.3, 2015-12-10, R Foundation for Statistical Computing, www.r-project.org). The script performed missing value imputation using iterative mean substitution based on height profiles of the neighbouring pixels.