Workpackage 3: Analytical Studies

Workpackage 3 has focused on the application of the developed imaging MS methodologies, strategies and protocols to various analytically challenging problems in the life sciences. In doing so it has highlighted the advances in the European imaging Mass Spectrometry realized by the efforts of the Computis consortium. Several of the developed strategies have found their way to research professionals all over the world demonstrating the broader international impact.

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- Perform comparative bioanalytical studies

- Definition, evaluation and validation of calibration protocols
- 3D MS imaging strategies for biological tissue
- Identification and characterization of relevant biomolecules from tissues by established and new bioanalytical strategies

The concise list of analytical scientific results below is a selection of the highlight of the project. Calibration targets and comparative studies

Round robin samples and calibration targets with pre-patterned peptides, proteins and lipids have been designed and evaluated. It has resulted in standardized methodologies, protocols and sample standards between the partner laboratories. This comparison of the results of all partners demonstrated the strength and weaknesses of the imaging methods applied to determine LOD, spatial resolution and molecular sensitivity.

3D MS imaging of biological tissue

Two three-dimensional reconstructions from six slices of a MALDI-ToF analyzed human breast cancer xenograft tumor are shown in the figure below. Panel A shows m/z 616 (free heme) and panel B shows m/z 773 (PC 32:0). These masses exhibit a complementary distribution revealing some of the three-dimensional tissue morphology.

The data acquisition and analysis for this type of analysis is extremely time-consuming, as each individual section takes approximately 24 hours to prepare, analyze, process and add to the 3D volume. The protocol developed has demonstrated that for small stable molecules, it is feasible to perform 3D tissue analysis with a z-resolution of 500 micrometers. Ultimately the throughput of imaging mass spectrometry instruments will need to be improved drastically to allow the 3D imaging of less stable compounds.

Depth profiling tissue for 3D reconstruction

Ion images of 110 x 110 µm2 surface were recorded with Bi3+ primary ions. The four rows in Figure 4 show the total image (sum of all the step B measurements), the “top” images (accumulation of the step B measurements over the first part of the irradiation, i.e. during the steep σ1 slope, from the beginning to a dose density of 2.8x1015 ions.cm-2), and the “middle” and “bottom” images (sum of the step B measurements during the σ2 slope, for dose densities ranges of 2.8x1015 to 1.5x1016 ions.cm-2, and 1.5x1016 cm-2 to 2.8x1016 ions.cm-2, respectively). It can be seen that most of the intensity is concentrated in the “top” images for lipids such as cholesterol, while the intensity of the images remains readable until the end of the irradiation for sodium, potassium and phosphocholine ions.

Significant dynamic range in the spectra

Without any quantitative information in terms of amount of matter or concentration, it is possible to evaluate the ability to get significant information from a very tiny peak compared to other much more intense peaks. This is impressively highlighted when selecting a very-low-intensity peak in the mass domain produces a clear and significant image in space.

An attempt to assess the range of peaks between MALDI-Tof and Tof-SIMS spectra was carried out, from selected data processed by CEA .
- MALDI spectra can be characterized by a high-level baseline even after denoising, whereas SIMS spectra present no (or almost no) baseline.
- The ratio in amplitude between high peaks and the baseline is about 400 for MALDI spectra, and about 104 for SIMS spectra, for the data studied by CEA in the framework of the Computis project.
- The ratio between the high peaks and the small speaks with a significant physical sense is in the same order of magnitude with both imaging techniques: about 40 for MALDI, and about 50 for SIMS.
- Peaks are large in MALDI imaging and very thin in SIMS imaging. The width at mid-height of high peaks is about 5 to 6 mass units for MALDI, and only of about 0.2 mass unit for SIMS.

MALDISIMS

Amplitude of small peaks with a physical sense (in DAC units or counts)105200

Level of the baseline after denoising (in DAC units or counts)104Almost 0

Amplitude of the high peaks (in DAC units or counts)4. 106104

Width at mid-height of high peaks (in mass units)5-60.2 Table.1 Assessment of the dynamic range in spectra for MALDI and SIMS imaging (according to the data processed by CEA in the framework of the Computis project).

Characterization of human pancreas disease

Human pancreas sections were analyzed to compare lipid profiles of different disease states. Pancreatic cancer is typically diagnosed at an advanced stage, thus the survival rate compared to other types of cancers is poor. Donor pancreas, chronic pancreatitis and pancreatic cancer were sectioned to 15 µm on a cryo-microtome, washed in 70:30 ethanol:water for 1 minute and coated with 5 layers of α-cyano-4-hydroxycinnamic acid diethylamine salt (100 mg/ml, 2:1 ACN/H2O (0.2% TFA)) and 30 layers of 10 mg/mL α-cyano-4-hydroxycinnamic acid (2:1 ACN/H2O (0.2% TFA)) with an ImagePrep matrix deposition device.

The samples were analyzed on the MALDI FT-ICR MS described in deliverable 2.6 of this project. The resulting data was analyzed with statistical tools (PCA and pLSA, developed outside the Computis project) to aid in data interpretation. Accurate mass measurements from FT-ICR allowed confident identification of phosphatidylcholine and triacylglycerol lipids directly from the tissue surface without MS/MS. The three figures below show the results for healthy pancreas, chronic pancreatitis and pancreatic cancer.

Analytical studies at high spatial resolution

At JLU spatial resolution for MALDI was further increased in order to closer investigate regions of interest. The figure below shows an AP-SMALDI image of an area of the urinary bladder with two different, adjacent tissue types, acquired with a step size and ablation spot size of 5 µm. Ion distributions again correlated well with histological features indicated in the stained tissue image (Figure 21, left). The image provides pixel-sharp separation between the muscular layer (m/z = 741.5307 u, green) and the urothel tissue (m/z = 798.5410 u, red) with no blur or signal overlap (yellow pixels), demonstrating that the effective analytical resolution is indeed in the range of 5 µm.

Using dedicated imaging software, high mass accuracy and resolution were preserved when selecting mass spectral images. All images were generated using a highly discriminatory m/z bin width for image assignment of m/z = 0.01. This was necessary to distinguish compounds which were significant for structural contrast but were close in m/z.