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Accelerating Pharma Research with Sensitive Spatial Analysis of Challenging Molecules

Abstract

To reduce attrition rates in pharmaceutical research and development (R&D), powerful quantitative analytical methods are needed to monitor therapeutic compounds, their metabolites, and target engagement. The ability of matrix-assisted laser desorption/ionisation (MALDI) imaging to perform quantitative spatial analysis of drugs and their metabolites, as well as pharmacodynamic (PD) biomarkers in tissues, is accelerating its use in pharmaceutical research. Advances in technology and methodology are opening MALDI imaging up to analysing a wider range of small molecules without compromising image resolution.

The discovery and development of safe and efficacious pharmaceutical products is a lengthy and expensive process that, today, still suffers from frequent failure to progress a drug through to clinical trial. Overall failure rate in drug development has been reported at over 96%, including a 90% failure rate during clinical development[1]. Attrition rates are particularly high for drugs targeting previously 'undrugged' proteins and for diseases with poorly understood pathogenesis. Researchers must establish early in the development pipeline the exact distribution of the drug in the appropriate tissues, understand and quantify *in situ* drug metabolism, and establish any off-target activity and toxicity that could pose a safety risk. Drugs that fail late in the development or clinical phase not only incur huge costs for pharma companies, but inflate the price of drugs that do succeed through research and development (R&D). There is also a risk that high attrition rates could deter companies from pursuing drugs with innovative mechanisms of action.

One challenge that both academic and industrial researchers face is the translatability of preclinical research into clinical applications. This reinforces the need for a better understanding of the molecular defects leading to complex diseases, which can provide new insights into fundamental biology and translational opportunities[2]. Powerful quantitative analytical methods are needed to monitor therapeutic compounds, their metabolites, and target engagement, but traditional analytical techniques such as liquid chromatography mass spectrometry (LC-MS), although widely used, face limitations such as a lack of spatial information which is required for establishing efficacy and toxicity within different tissues. The emergence of advanced mass spectrometry (MS) techniques, such as matrix-assisted laser desorption/ionisation (MALDI) imaging, has enabled the non-radioactive, label-free, and non-destructive localisation of targeted therapeutics as well as endogenous biomolecules – from metabolites to proteins – in an untargeted manner[3].

Adding a Spatial Dimension to Molecular Analyses

MALDI imaging is a label-free omics technique that enables spatially resolved molecular analysis of single cells with high-throughput and high spatial resolution, enabling the monitoring of both drug metabolism and pharmacokinetics (DMPK), with absolute quantification. Relatively new techniques such as trapped ion mobility spectrometry (TIMS) are now being utilised in MALDI imaging workflows to separate structural isomers.

TIMS is a gas-phase MS technique that enables ions to be separated according to their collisional cross-section (CCS) – a measure of how likely they are to be deflected by a collision with other gas molecules as they drift through an ion tube. This provides another way of separating ions and adds an additional dimension to the analysis.

For pharma R&D, this allows researchers to image a wider range of small molecules and their metabolites in tissues and establish spatially resolved evidence of target engagement, PK/PD, and drug-induced toxicity. The greater depth of molecular information in a spatial context available during R&D improves the chance of a drug candidate's success in clinical trials.

However, research centred around small molecules typically tests the limits of MALDI sensitivity and molecular coverage. Ion suppression and quantification limit molecular characterisation. The recent introduction of a novel laser-induced post-ionisation (PI) method has addressed the trade-off between sensitivity and resolution and, together with TIMS, increases the sensitivity of MALDI imaging by up to two or three orders of magnitude depending on sample, matrix, and analyte, compared with traditional MALDI methods[4]. Importantly, this opens the technology to a wider range of small molecules, including classes of phospho- and glycolipids, liposoluble vitamins, glycans, and steroids, without compromising image resolution.

Laser-induced PI and TIMS are an essential combination that add significant value to MALDI imaging, enabling stronger signals, fast data acquisition speeds, high molecular separation, and CCS alignment to further improve the number of possible molecular identifications[5].

Boosting Sensitivity in Targeted Drug Imaging

Drug compound imaging experiments have demonstrated the significant sensitivity enhancement of MALDI imaging when target compounds and metabolites are imaged with laser-induced PI methods. In one study, a dilution series of standard compounds was used to define the ionisation efficiency of the drug of interest, called BI-YYY[6]. Solutions of caffeine, chloroquine, rosuvastatin, reserpine and BI-YYY were spotted onto control liver tissue and analysed under traditional MALDI and laser-induced PI conditions.

Sensitivity for all five test compounds was significantly increased by laser-induced PI. In particular, the peak intensity of BI-YYY was enhanced by a factor of 300 using the laser-induced PI method.

After determining the laser-induced PI enhancement of BI-YYY signal under controlled conditions, rats (n=2) were dosed with BI-YYY or chloroquine and analysed by MALDI imaging to determine whether the sensitivity advantage translates to better

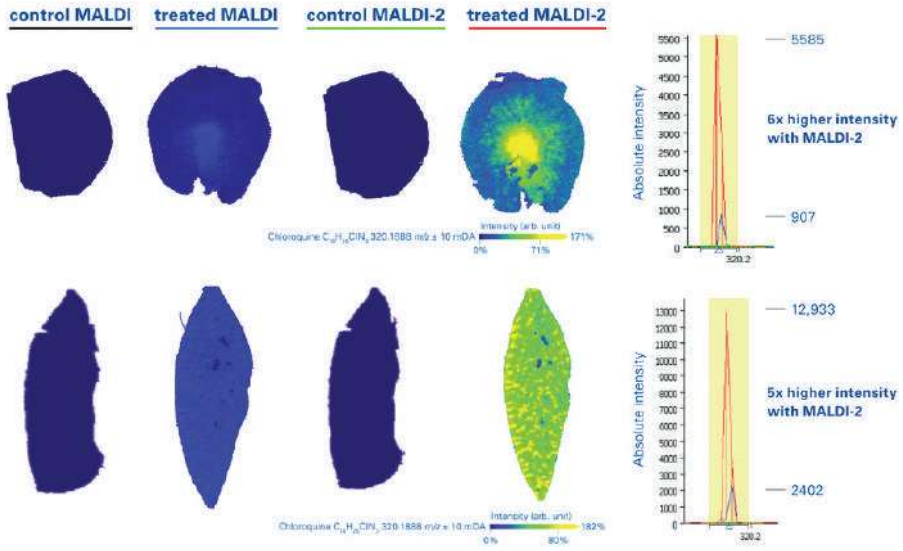

Figure 1: Rat control and dosed organs were compared between MALDI and laser-induced post-ionisation (MALDI-2, timsTOF fleX, Bruker Daltonics). BI-YYY intensity is shown in SCiLS Lab software where yellow colours indicate higher intensities than blue colours as shown in the intensity gradient. Kidney (top) and liver (bottom) from control and BI-YYY dosed rats were used to compare the ionisation efficacy in MALDI and laser-induced post-ionisation (MALDI-2). The intensity difference between the two treated samples is shown in the extracted mean spectra.

drug localisation information. Images from BI-YYY dosing exhibited 8.5-fold higher peak intensity with laser-induced PI in kidney and a six-fold higher intensity in liver, based on mean peak intensity (Figure 1).

Additionally, metabolites of chloroquine were investigated to explore the detection limit of laser-induced PI in comparison to MALDI. As with findings from BI-YYY dosed tissue, images reveal that the signal intensity for chloroquine in kidney was six-fold greater using laser-induced PI (Figure 2). For chloroquine in liver, results were similar where mean peak intensity reveal a five-fold higher intensity boost from laser-induced PI.

Laser-induced PI methods substantially enhanced the sensitivity for many compounds, delivering new distribution information from previously undetected metabolites as well as providing lower limits of detection for target compounds, both vitally important to DMPK studies.

Elevating Biomarker Discovery

The direct analysis of tissue sections by MALDI imaging has facilitated significant advances in biomarker discovery[7]. Traditional biomarker studies using genomics, tran-scriptomics, metabolomics, and proteomics techniques often require extraction of potential biomarkers from samples, resulting in the destruction of important histological information[8]. But untargeted MALDI imaging methods make it possible to identify target molecules that may be locally concentrated, providing spatial information about potential new biomarkers.

Glycoprotein biomarkers are a group of glycoproteins involved in pathological processes, which can be used as indicators of certain diseases in the clinic. Given that most of the US Food and Drug Administration (FDA)-approved biomarkers for different types of cancer are glycoproteins[9], these proteins are often targeted for biomarker discovery. *N*-glycans are important players in a variety of disease pathways, including different types of cancer, (auto)immune diseases, and viral infections, and MALDI imaging is an important tool for obtaining spatial information on glycans in tissue.

A recent study has demonstrated the ability of laser-induced PI MALDI imaging (negative ion mode) to increase ion yields and enable the acquisition of high-quality MS/MS spectra and structural analysis of *N*-glycans from minute sample amounts[10]. To evaluate whether ion species generated from the same N-glycans in both positive ion mode MALDI and negative ion mode laser-induced PI would show the same profiles and spatial distributions in tissue, a comparison experiment was performed on human cerebellum tissues. Figure 3A shows the representative average tissue spectra obtained from the two analyses, and the recorded distributions showed the same glycans to be present in similar morphological areas upon measurement with the two ion polarities (Figure 3B-I).

By coupling laser-induced PI to a TIMS quadrupole time-of-flight (QTOF) mass spectrometer, researchers could increase detection sensitivity of molecular [M – H]- species of *N*-glycans from tissue sections of human cerebellum by approximately three orders of magnitude. Compared with traditional MALDI imaging techniques, laser-induced PI mode analysis of [M + Na]+ adducts achieved a 10-fold sensitivity increase.

An additional benefit of the analysis of glycans with laser-induced PI is the absence of peak-splitting that occurs with traditional MALDI imaging through the presence of multiple alkali-metal adducts for the same glycan (e.g., sodium and potassium adducts;

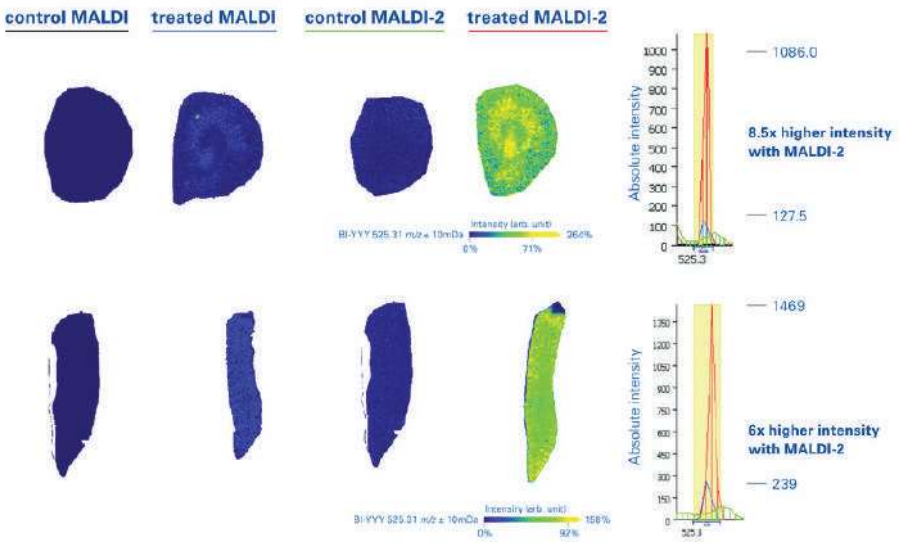

Figure 2: Rat control and dosed organs were compared between MALDI and laser-induced post-ionisation (MALDI-2, timsTOF flex, Bruker Daltonics). Chloroquine intensity is shown in SCiLS Lab software where yellow colours indicate higher intensities than blue colours as shown in the intensity gradient. Kidney (top) and liver (bottom) from control and chloroquine dosed rats were used to compare the ionisation efficiency between MALDI and laser-induced post-ionisation (MALDI-2). The intensity difference between the two treated samples is shown in the extracted mean spectra.

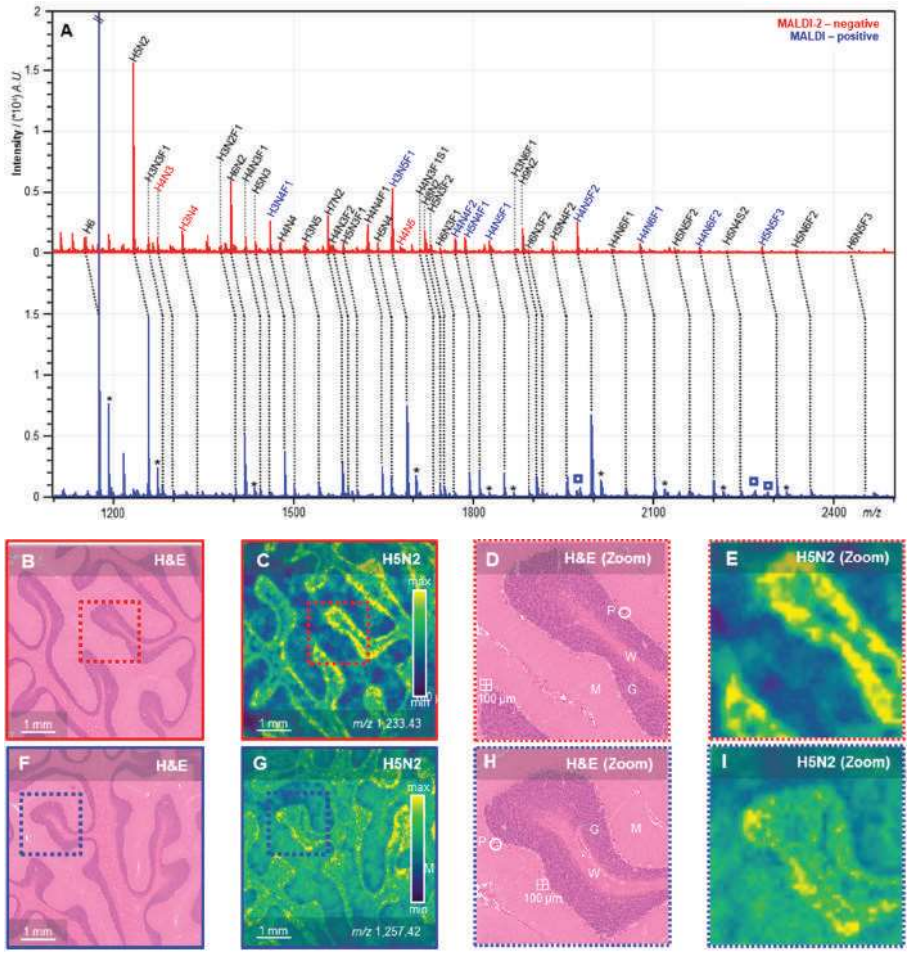

Figure 3: Spectra and images of positive ion mode MALDI imaging and negative ion-mode laser-induced post-ionisation (MALDI-2) imaging (timsTOF fleX, Bruker Daltonics). (A) Average spectra for negative ion-mode MALDI-2 imaging (red) and positive ion-mode MALDI imaging (blue) with assigned N-glycan species. Coloured N-glycan compositions represent a > ± 10% variation of intensity between positive and negative ion-mode analyses. Peaks with an asterisk (*) in the positive ion mode spectrum are potassium adducts. (B,F) H&E stained consecutive section displaying cerebellar brain morphology. (C,G) Example images for N-glycan H5N2 in human cerebellum. (D,H) Zoom in on histology with different morphological structures annotated (P, Purkinje cell, G, granular layer, M, molecular layer, W, white matter). (E,I) Zoom in on example H5N2 images. In red, negative ion-mode laser-induced post-ionisation (MALDI-2) images, and in blue, positive ion-mode MALDI imaging images. Reproduced from reference[10] in accordance with Creative Commons Attribution License.

Figure 3A). Further research is needed to assess potential differences in (post) ionisation efficiencies of various glycan classes (e.g., high-mannose vs complex-type, sialylated and/or fucosylated species), but these results show that laser-induced PI can be a useful tool for the visualisation of *N*-glycans in tissue.

Studies such as these not only facilitate developments in glycobiology research, but shed light on how evaluating *N*-linked glycosylation and other glycan classes with MALDI imaging could help provide new therapeutic and diagnostic assay leads, for example in cancer. One of the longstanding challenges of glycan imaging is the number of structural isomers. In glycobiology, how sugars are arranged on proteins dictates the impact on cancer growth and which cancer pathway has been activated. Because ion mobility separation is an MS technique that separates molecules based on their conformation, TIMS can therefore be used to detect isomers and glycoforms, and lead to more accurate cancer biomarker development.

Future Adoption in Pharma R&D

The pharmaceutical industry is in need of fast and sensitive analytical tools to visualise the spatial distribution of drug, metabolite and endogenous biomarkers directly in tissue sections. The ability of MALDI imaging to co-localise drug/metabolite distribution with histological information in a label-free manner is highly

advantageous for pharma R&D. For example, the typically heterogeneous compound distribution in tumours can be matched with tumour biomarkers in oncology drug development[11].

Recent advances in instrumentation, such as the incorporation of TIMS to QTOF MS, have led to more widespread adoption of MALDI imaging in pharma research and the development of drug discovery beyond drug disposition analysis, particularly in PD biomarker research and toxicology[3]. Laser-induced PI methods, together with TIMS, can provide enhanced measurement speeds and increased sensitivity without compromising spatial resolution, and deep molecular content when combined with QTOF MS. The combination of these technologies shows great potential as a MALDI imaging tool to support distribution, metabolism, efficacy and toxicity testing of a wide range of drug compounds in pharma research, all in the same dataset.

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