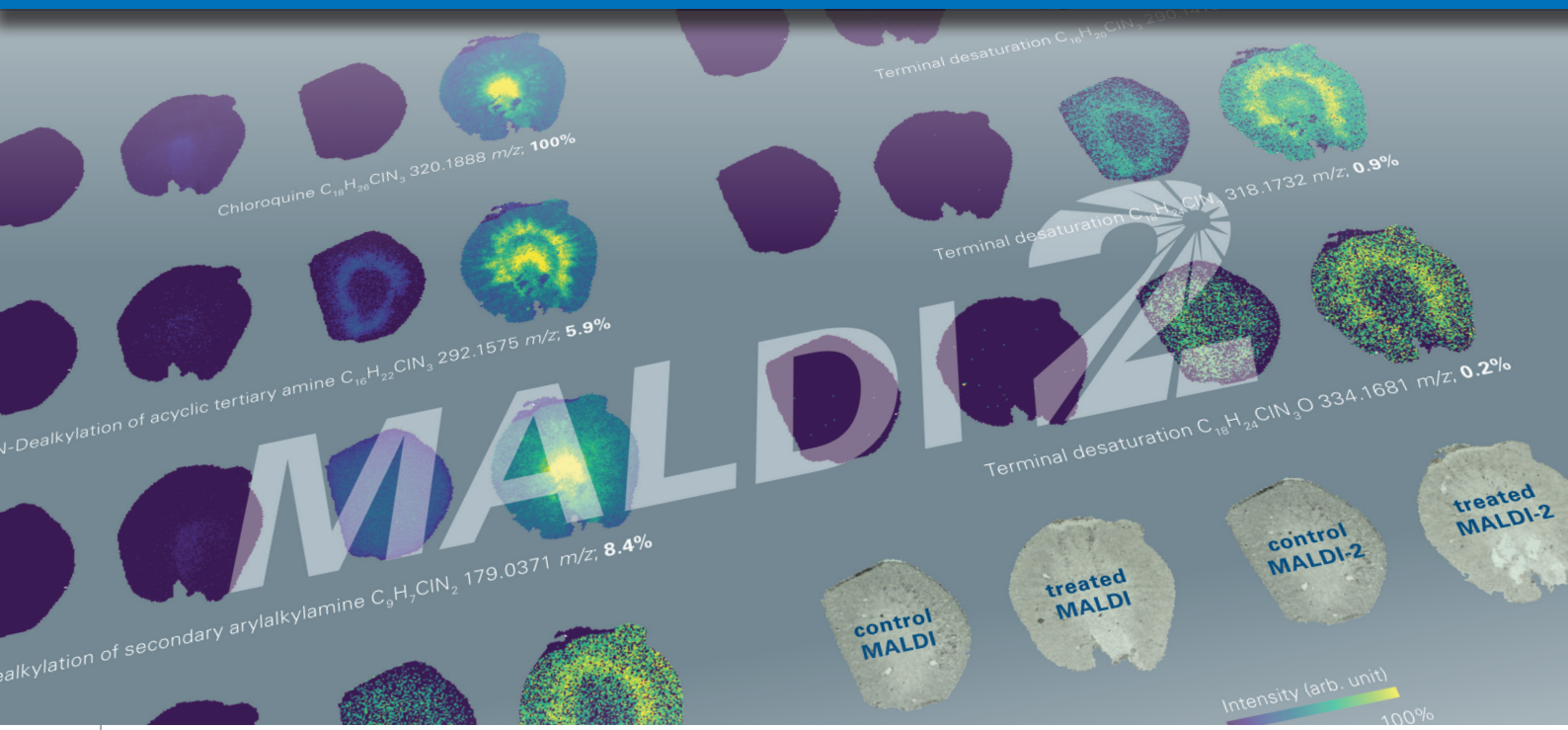

The combination of MALDI-2 and timsTOF fleX brings targeted drug imaging to the next level

Matrix-assisted laser desorption/ionization (MALDI) Imaging is a powerful tool for DMPK studies, providing untargeted and targeted measurements that can be deployed early in the discovery pipeline.

Abstract

The combination of MALDI with laser-induced post-ionization (MALDI-2) is a recently introduced method that has demonstrated significant enhancement of sensitivity in mass spectrometry imaging. In this Application Note, we determine the practical benefit that MALDI-2 provides for imaging drug compounds. The MALDI-2 ionization efficiency of the investigated drugs is established by using MALDI Imaging results on a standard dilution series. Results show that MALDI-2 increases the

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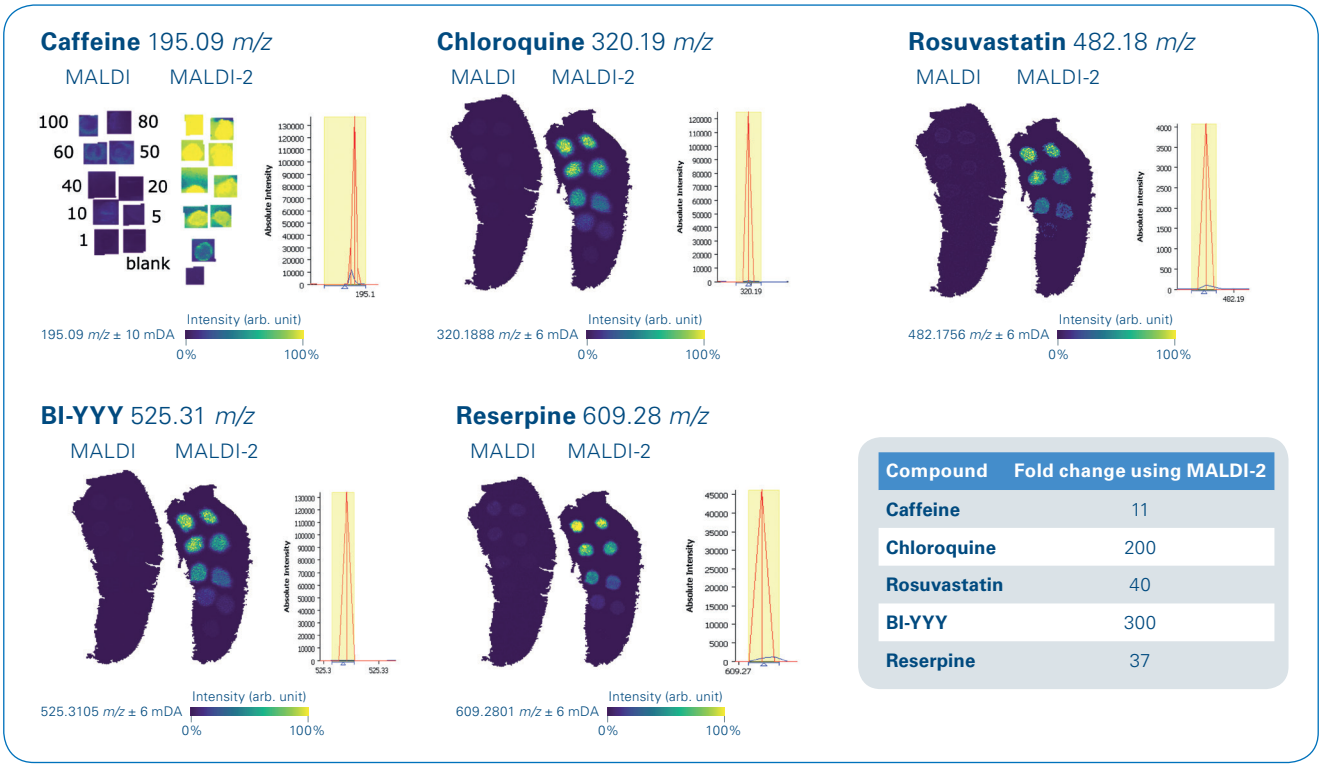

Compound	Fold change using MALDI-2
Caffeine	11
Chloroquine	200
Rosuvastatin	40
BI-YYY	300
Reserpine	37

Figure 1: Spotted dilution series from 100 µM down to 1 µM concentration and blank, the complete series is shown in the first example caffeine. Additionally chloroquine, rosuvastatin, BI-YYY and reserpine are shown in MALDI (left side) and MALDI-2 (right side) mode. A table with the intensity change of the mean peak intensity in MALDI and MALDI-2 for the whole dilution series is shown.

sensitivity for all compounds by orders of magnitude. Two specific drugs of interest were further investigated in dosed tissue. For both target compounds as well as metabolites, MALDI-2 demonstrated significant enhancement of sensitivity. This substantial improvement over traditional MALDI Imaging is particularly significant for DMPK studies.

Introduction

The timsTOF fleX is the newest MALDI Imaging instrument in the Bruker portfolio. With its dual ESI/MALDI capability, timsTOF fleX is a versatile analytical tool, ranging from targeted label-free molecular imaging to untargeted SpatialOMx studies that integrate very sensitive LC-MS/MS PASEF measurements with high-resolution MALDI Imaging. The newest generation of timsTOF fleX is now available with innovative MALDI-2 technology, which results in higher sensitivity for MALDI Imaging studies.

MALDI-2 uses a post-ionization laser to improve the MALDI experiment. This provides access to compounds typically not accessible by MALDI, with very high sensitivity [1,2]. The post-ionization leads to both a boost in ion yields and a reduction of ion suppression effects, resulting in a sensitivity boost of up to 2-3 orders of magnitude compared to traditional MALDI (depending on sample, matrix and analyte). For a MALDI Imaging experiment, this increased efficiency translates to more than double the number of molecules detected per pixel – resulting in much improved physiological context. In addition to boosting detection sensitivity, MALDI-2 also makes it possible to detect and image chemical classes typically not ionized by traditional MALDI. The post-ionization event significantly boosts the sensitivity of analytes like sterols, statins, hormones, vitamins, glycans and other notable biomolecules. This results in more confident identifications of compounds, especially in targeted approaches.

Methods

Fresh-frozen liver and kidney tissue sections were cut in 10 µm thickness and mounted on Bruker IntelliSlides. After drying, a 0.5 µl mixture of the following substances were spotted on top of the tissue: caffeine, chloroquine, rosuvastatin, BI-YYY, and reserpine. Each mixture was spotted in a dilution range from 1 µM to 100 µM (9 spots). The slides were scanned using a Bruker TissueScout to obtain a grayscale reference image for automated teaching of the IntelliSlides. The slides were then

coated with 2,5-Dihydroxyacetophenone (DHAP) matrix (15 mg/mL in ACN:H_2O:MeOH, 8:1:1, v:v) using a TM-sprayer (HTX Technologies). In detail, 20 matrix layers were applied with a flow rate of 0.125 mL/min, a velocity of 1200, and a nozzle temperature of 60°C.

MALDI Imaging data was collected on a timsTOF fleX with MALDI-2 technology. Mass spectra were acquired in positive ion mode, with a *m/z* range of 100-2000, a 50 µm pixel size and 1kHz laser repetition rate. For comparative measurements, the MALDI-2 laser was turned on/off as needed. 30 laser shots per pixel were used for all measurements. The same parameters were used for dosed tissue imaging of liver and kidney slices. Animals were dosed orally once with either 100 mg/kg chloroquine or 120 mg/kg of BI-YYY and sacrificed 24h or 2h post-dosing, respectively. Data was visualized using SCiLS Lab. Tentative annotation of drug metabolites, was based on the metabolism prediction of the drugs, using the BioTransformer (3) module integrated in a workflow with a preliminary version of Metaboscape 2021. The Superbio parameter was selected to compute CYP450, EC-based, phase II and possible gut microbial transformations resulting in 35 chemical structures having 19 distinctive molecular formulae for which target masses were then computed. In case of isomeric metabolites a randomly selected example is shown for a given *m/z*-value. As the color intensities in figure 4 are metabolite specific, the background of the corresponding blank was subtracted, and the percentage of parent ion intensity is shown.

Results

A dilution series of standard compounds was used to define the ionization efficiency of the drug of interest, called BI-YYY. Solutions of caffeine, chloroquine, rosuvastatin, reserpine and BI-YYY were spotted onto control liver tissue and analyzed under MALDI and MALDI-2 conditions. Figure 1 shows a comparison of images from each compound in the dilution series when measured with MALDI or MALDI-2. Inset spectra illustrate relative differences observed in mean peak intensity for the specific compound. This spotting experiment demonstrates that sensitivity for all five test compounds was significantly increased by MALDI-2. In particular, the peak intensity of the new drug compound, BI-YYY, was enhanced by a factor of 300 using

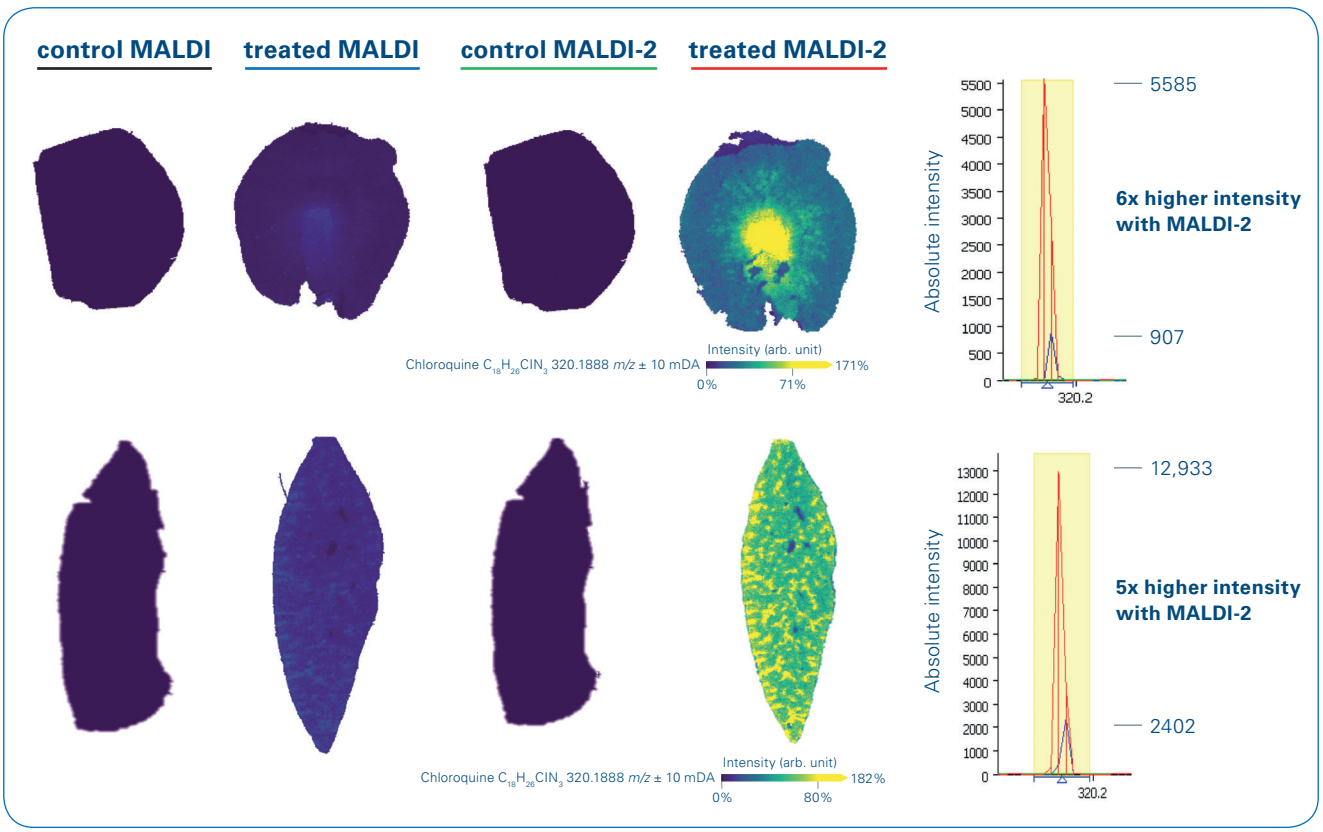

Figure 2: Rat control and dosed organs were compared between MALDI and MALDI-2. Chloroquine intensity is shown in SCiLS Lab software where yellow colors indicate higher intensities then blue colors as shown in the intensity gradient. A) kidney and b) liver from control and chloroquine dosed rats were used to compare the ionization efficiency between MALDI (left side) and MALDI-2 (right side). The intensity difference between the two treated samples is shown in the extracted mean spectra.

MALDI-2. Please note that the goal of this study was not to perform rigorous quantitation. Nevertheless, the overall intensity of each dilution spot observed for all compounds readily correlates with the concentration of the spotted solution.

After determining the MALDI-2 enhancement of BI-YYY signal under controlled conditions, the detection of ingested drugs was the next step. Rats (n=2) were dosed with BI-YYY or chloroquine and analyzed by MALDI Imaging to determine whether the sensitivity advantage translates to better drug localization information. Additionally, metabolites of chloroquine were investigated to explore the detection limit of MALDI-2 in comparison to MALDI.

The comparison of MALDI and MALDI-2 for dosed kidney or liver tissues was performed on the same slide by turning the MALDI-2 laser on/off via software control. Results for chloroquine are shown in Figure 2.

Images reveal that the signal intensity for chloroquine in kidney was 6-fold greater using MALDI-2. For chloroquine in liver, results were similar where mean peak intensity reveal a 5-fold higher intensity boost from MALDI-2.

Ion images from BI-YYY, our test drug of interest, are shown in Figure 3. As with findings from chloroquine dosed tissue, images from BI-YYY dosing exhibited 8.5-fold higher peak intensity with MALDI-2 in kidney and a 6-fold higher intensity in liver, based on mean peak intensity.

Images from selected metabolites of chloroquine are shown in Figure 4. As can be seen, most metabolites generated only weak signals using traditional MALDI. MALDI-2 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.

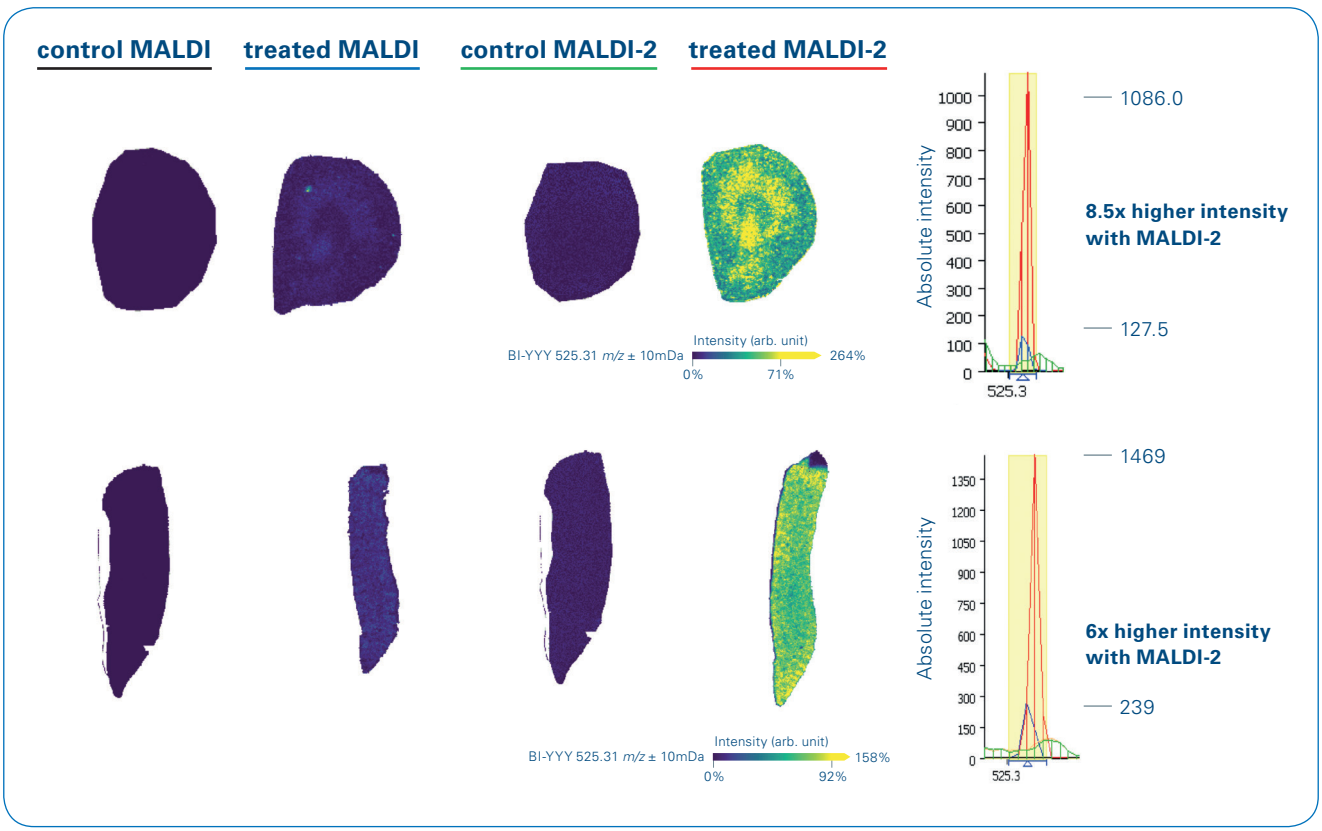

Figure 3: Rat control and dosed organs were compared between MALDI and MALDI-2. BI-YYY intensity is shown in SCiLS Lab software where yellow colors indicate higher intensities then blue colors as shown in the intensity gradient. A) kidney and b) liver from control and BI-YYY dosed rats were used to compare the ionization efficacy in MALDI (left side) and MALDI-2 (right side). The intensity difference between the two treated samples is shown in the extracted mean spectra.

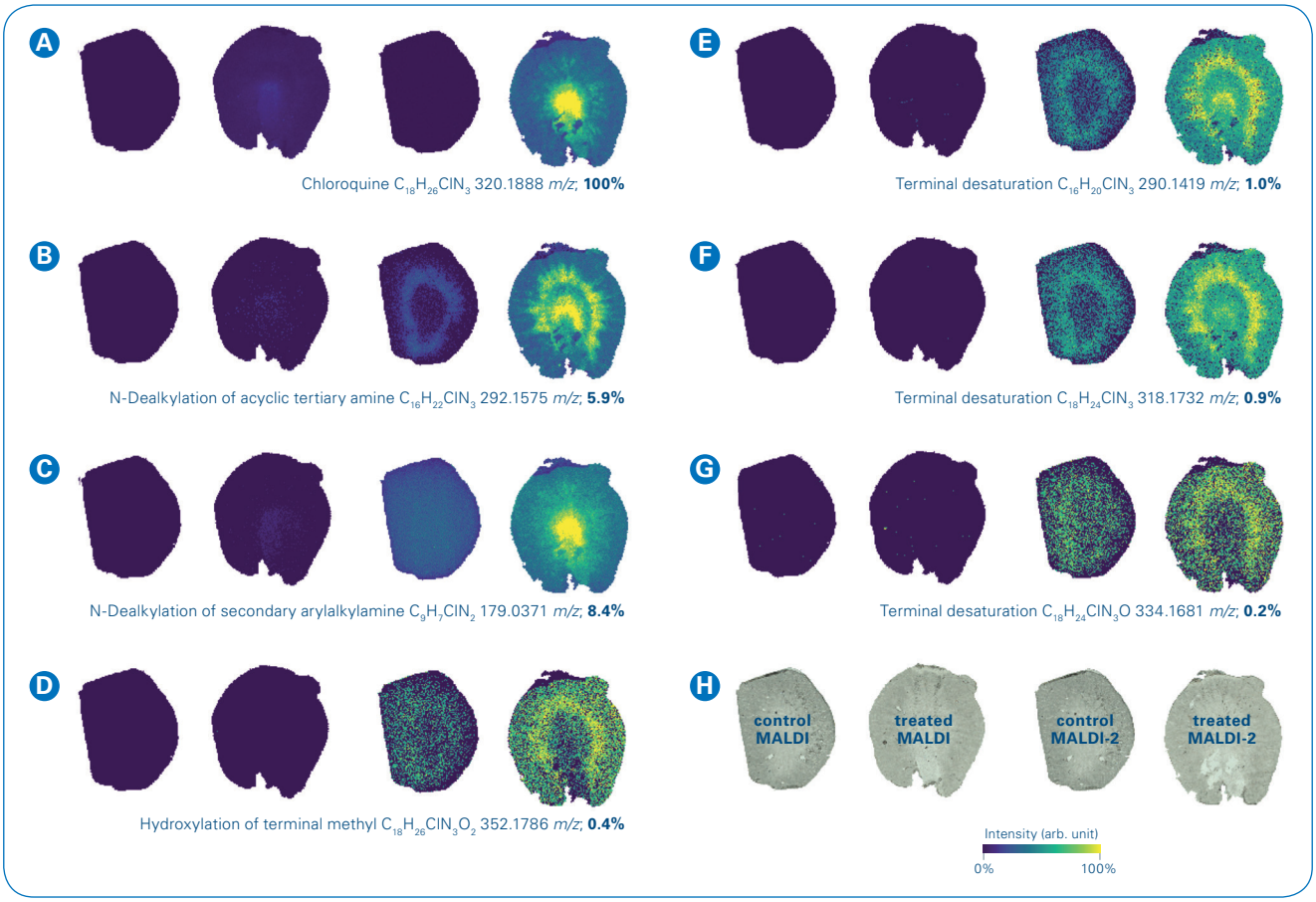

Figure 4: Metabolites of chloroquine are shown in SCiLS Lab software. MALDI (left side) and MALDI-2 (right side) kidney samples were compared. Intensity is shown in SCiLS Lab software where yellow colors indicate higher intensities then blue colors as shown in the intensity gradient. The color intensities are metabolite specific. Therefore, the percentage of parent ion intensity is shown next to the m/z *value.* (A) *shows the chloroquine intensity,* (B)-(G) *different metabolite intensities and* (H) *the measurement (MALDI or MALDI-2) and tissue annotation (control or treated).*

Conclusion

- Laser induced post-ionization is a powerful tool to boost sensitivity in targeted drug imaging approaches
- MALDI-2 Imaging provides a more sensitive analysis of tissues dosed with BI-YYY or chloroquine in comparison to standard MALDI Imaging
- Metabolites not detectable with MALDI could be identified using MALDI-2

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