

## ● Caution! MALDI-2 detects analyte classes never seen before

**MALDI-2 Technology empowers your MALDI Imaging studies with unsurpassed sensitivity and ability to map more compounds. The laser based post-ionization technology enhances the detection of numerous classes of phospho- and glycolipids as well as liposoluble vitamins, glycans and steroids without compromising image resolution. Generate more images and extract more information from your samples with MALDI-2.**

### Challenge

Steroids are a biologically important class of compounds and there is great interest to study steroid distributions using MALDI Imaging. As important components of cell membranes, steroids affect membrane fluidity and cell signaling. Hundreds of steroids can be found in plants, animals and fungi. Due to their nonpolar core structure, steroids do not ionize well by traditional MALDI without specialized on-tissue derivatization protocols. Therefore, mapping of these key molecules using traditional MALDI Imaging systems is not possible.

### Solution

MALDI-2 is a novel two-laser technology that will significantly boost the sensitivity of many compounds and is ideally suited for integration into Bruker's timsTOF fleX platform. In MALDI-2, the first laser generates the ejection plume. Immediately after this event, a second laser is passed through the expanding plume to ionize neutral matrix molecules through a 2-photon process. Within the plume, charge is transferred from the photo-ionized matrix to neutral analyte molecules. This charge transfer considerably boosts total analyte ion yields, especially for ions prone to ion suppression. Steroids are one such analyte class that benefits strongly from MALDI-2.

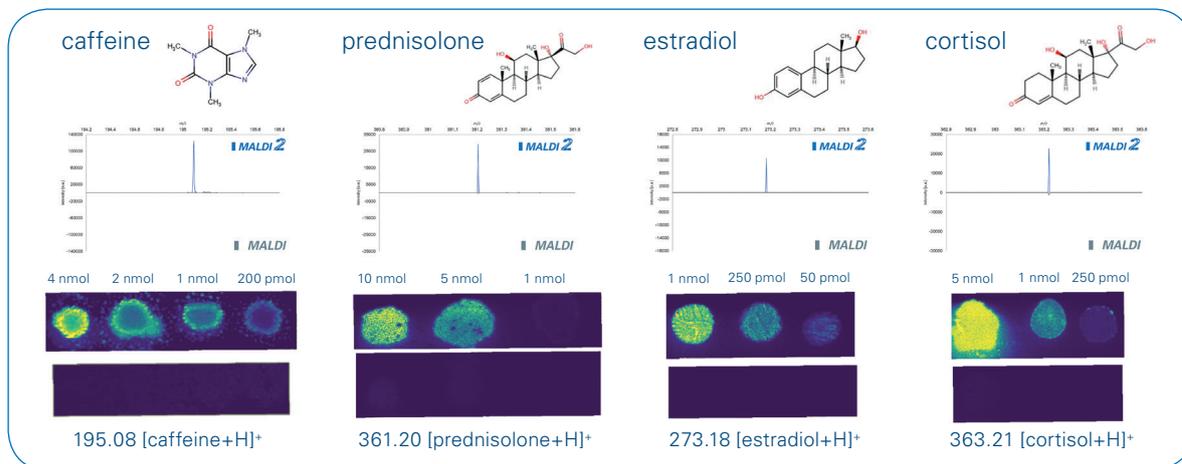


Figure 1: Comparing detection sensitivity from MALDI and MALDI-2. Dilution series of caffeine, prednisolone, estradiol and cortisol were independently spotted onto liver homogenate and imaged with MALDI-2 on and off. Use of MALDI-2 yields significantly higher sensitivity for all four test compounds.

# Caution!

timsTOF fleX with MALDI-2 Technology will revolutionize your imaging experiments by delivering more images at higher sensitivity.

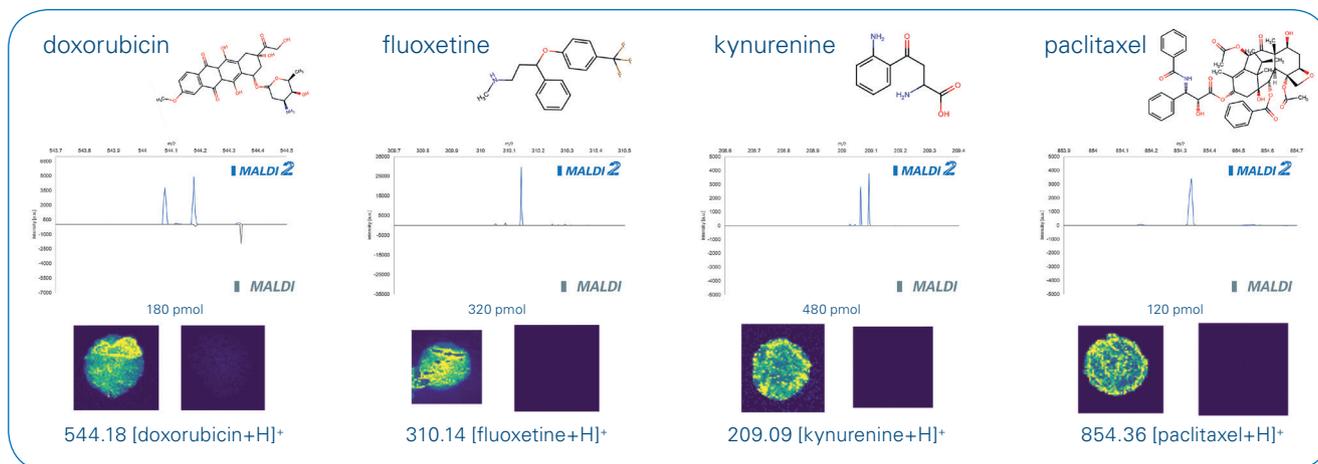


Figure 2: MALDI-2 increases sensitivity of steroids, doxorubicin, fluoxetine, kynurenine and paclitaxel. A single dilution of each of the four test compounds were spotted onto liver homogenate and imaged with MALDI-2 on and off. Use of MALDI-2 clearly provides higher sensitivity for the test compounds.

Many different compounds including steroids have been analyzed on our timsTOF fleX with MALDI-2. A dilution series (or a fixed concentration in case of proof of concept measurements) of each compound has been spotted on liver homogenate to mimic a natural tissue background. The tissue was then coated with 2,5-diacetophenone (DHAP) matrix using the HTX sprayer. All investigated compounds show a much higher sensitivity with MALDI-2 compared to MALDI alone (Figure 1 and Figure 2). We observed a sensitivity boost by up to 2-3 orders of magnitude depending on analyte and concentration. See Figure 3 for details.

Compound	Fold increase on tissue MALDI-2 vs MALDI
cholesterol	200
caffeine	100
prednisolone	100
estradiol	Not detectable by MALDI
cortisol	150
17- $\alpha$ -hydroxyprogesterone	60
diclofenac	>500
doxorubicin	25
fluoxetine	>500
kynurenine	Not detectable by MALDI
paclitaxel	>500
haloperidol	1.6

Figure 3: Selection of test candidates that benefit from MALDI-2 and the respective sensitivity gain realized with use of MALDI-2.

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