Next Generation Infrared Matrix-Assisted Laser Desorption Electrospray Ionization Source for Mass Spectrometry Imaging and High-Throughput Screening

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Introduction

Infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) is an effective platform for mass spectrometry imaging (MSI) of biological tissues and high-throuput screening (HTS) of biochemical samples without tedious sample preparation[1]. To improve IR-MALDESI-MS analyses and overall source versatility, an entirely new architecture was designed and constructed. Here, we present the characterization of the Next Generation (NextGen) IR-MALDESI source as an improved platform for MSI and HTS featuring a vertically mounted IR-laser, computerized three-dimensional translation sample stage, aluminum enclosure, and novel source-to-mass spectrometer interface plate. The NextGen source component geometries were optimized using design of experiments (DOE) to efficiently maximize target ion abundances in a minimized number of experiments[2]. Also, the NextGen IR-MALDESI source can be coupled to numerous Orbitrap mass spectrometers to accommodate a greater number of research labs. Documentation of all components utilized in the NextGen source will be provided in the future publication.

NextGen IR-MALDESI 3D Model

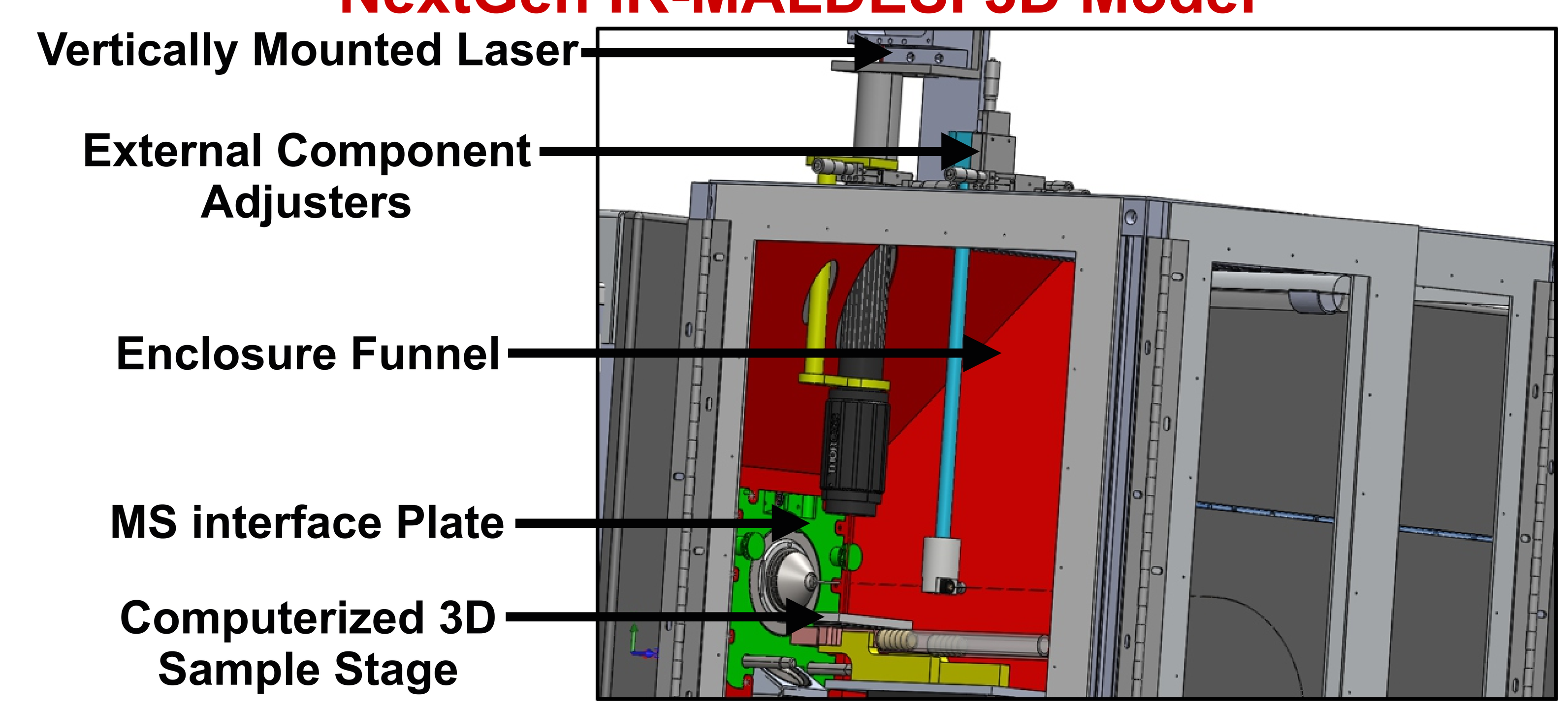


Figure 1. Three-dimensional soildworks model of the Next Generation IR-MALDESI source with new components labeled.

IR-MALDESI for MSI and Direct Analysis

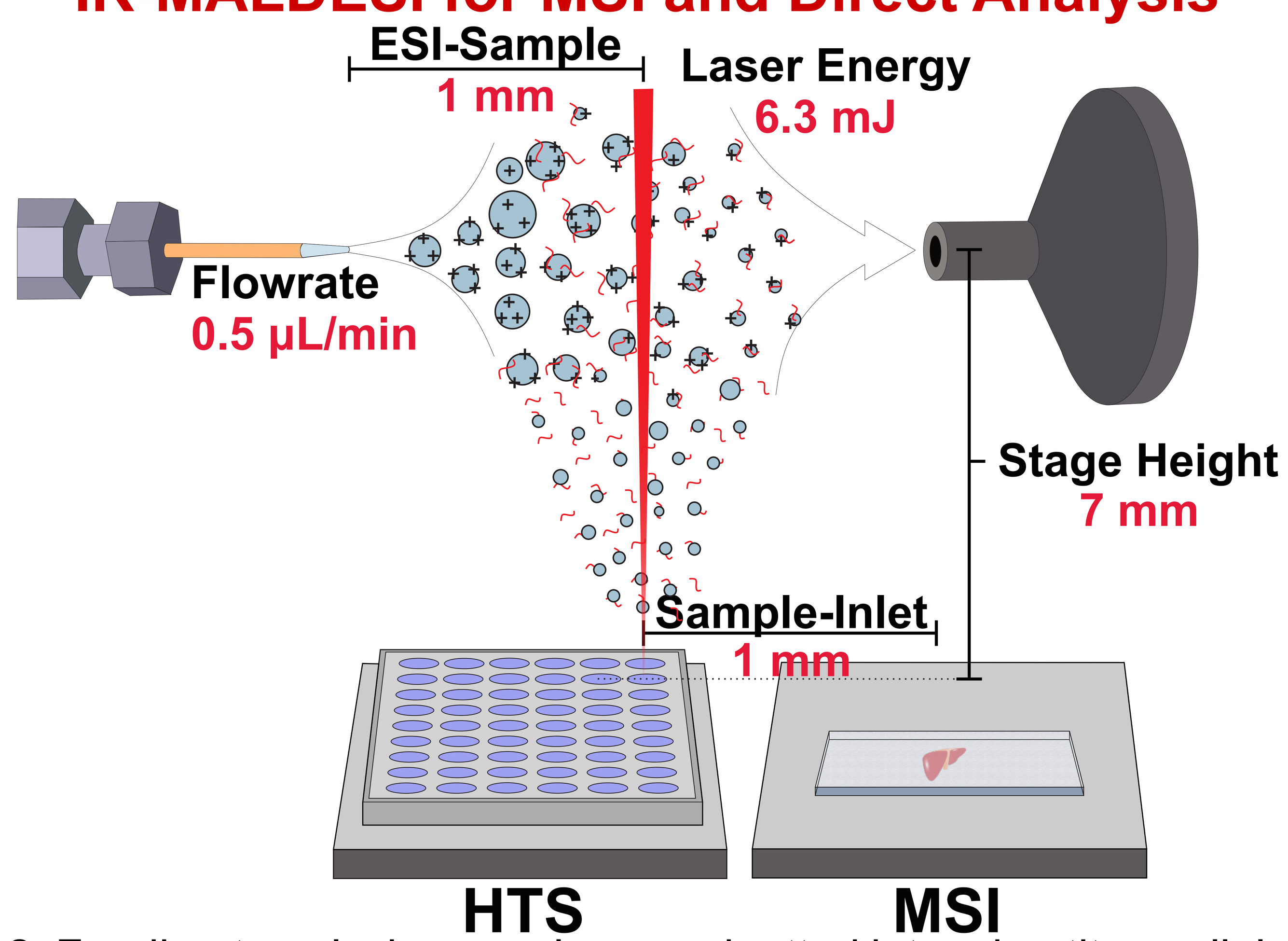


Figure 2. For direct analysis, samples are pipetted into microtiter wellplates and the 2970 nm laser is focused at the top of the sample volume. For MSI, tissues are thaw mounted onto a glass slide, placed on the translation stage, cooled to -8°C, and exposed to controlled ambient humidity to form an ice matrix on the surface of the tissue. The mechanism for ionization is the same across both analysis types. IR-MALDESI is coupled to a high resolution, accurate mass Orbitrap Exploris 240 mass spectrometer (Thermo Scientific, Bremen, Germany) for mass analysis. Flexmix was used as a model HTS sample for the optimization of NextGen IR-MALDESI component geometries. The optimized factors resulting from the DOE experiments are labelled with its respective optimal value in red.

NextGen Laser Energy Measurments

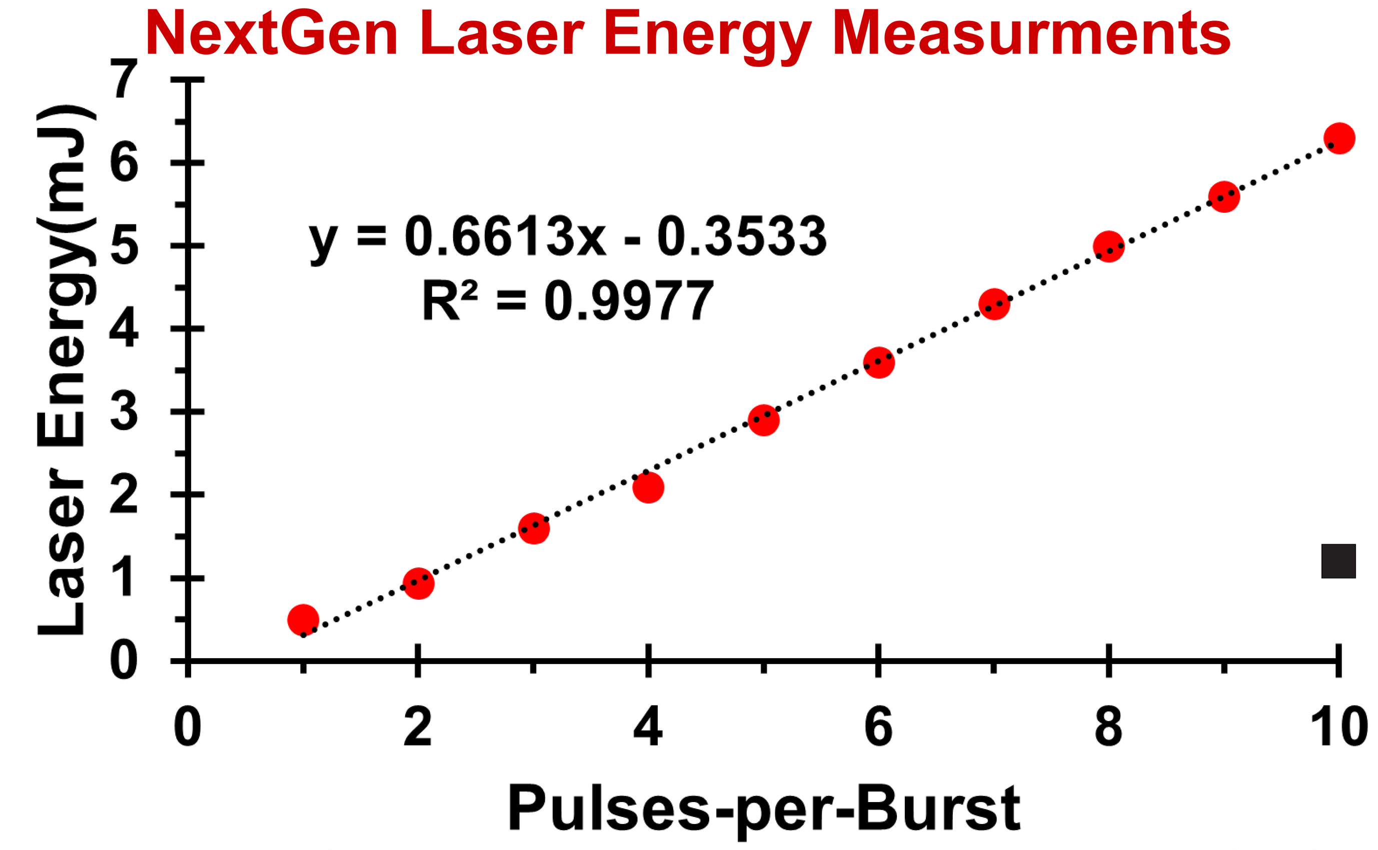
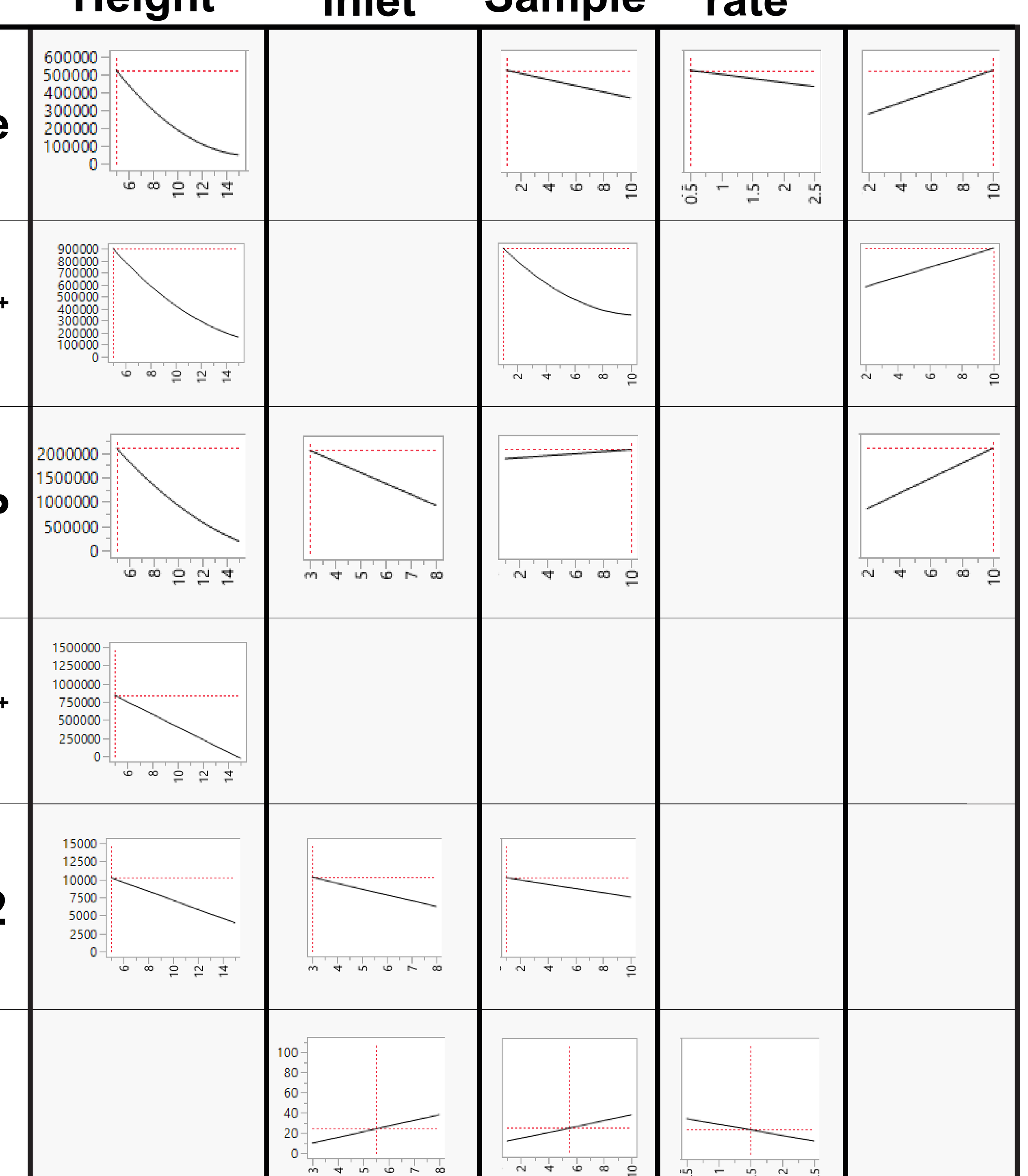


Figure 3. Measured laser energy as a function of the number of pulses-per-burst (PPB) applied to a sample in an IR-MALDESI-MS analysis. The previous generation IR-MALDESI source laser energy is represented as a black square.

Definitive Screening Design of Experiments (dsDOE)



	Stage Height	Sample-Inlet	ESI-Sample	Flow-rate	PPB
Caffeine					
MRFA^{2+}					
HMOP					
MRFA^{1+}					
UM 1022					
%RSD SIL-Caff					
Range	5 - 15 (mm)	3 - 8 (mm)	1 - 10 (mm)	0.5 - 2.5 (µL/min)	1 - 10
Optimal	5 mm	3 mm	1 mm	-	10

Table 1. dsDOE is an effective methodology to optimize a system where multiple factors affect the measured response[3]. Pierce Flemix calibration solution (Flexmix) was used as a model, direct analysis sample to maximize target ions over a wide *m/z* range. The %RSD of SIL-caffeine was used as a measure of ESI variability. Five IR-MALDESI factors were studied to maximize analyte abundances and minimize ESI variability. Significant factors for maximizing Flexmix ion abundances and minimizing ESI variability are represented by a graph. Optimized factor values are shown in red.

Full Factorial Design of Experiments (ffDOE)

Full Factorial Results	Tested	Previous	Updated
Stage Height (mm)	3 - 7	5	7
Sample-Inlet (mm)	1 - 5	5	1
ESI-Sample (mm)	1	1	1
Flowrate (µL/min)	0.5 - 2.5	2	0.5
Energy (mJ)	5 - 6.3	2	6.3

Table 2. The tested geometries for the full factorial design of experiments. The tested factor range was set based on the optimal factor values from the dsDOE study. The tested range of each factor, the previous factor vlaues, and the updated factor values are listed. The updated values were calulated in JMP DOE software by maximizing Flexmix ion abundances and minimizing the %RSD of SIL-caffeine abundances.

Updated IR-MALDESI Geometry Improvments

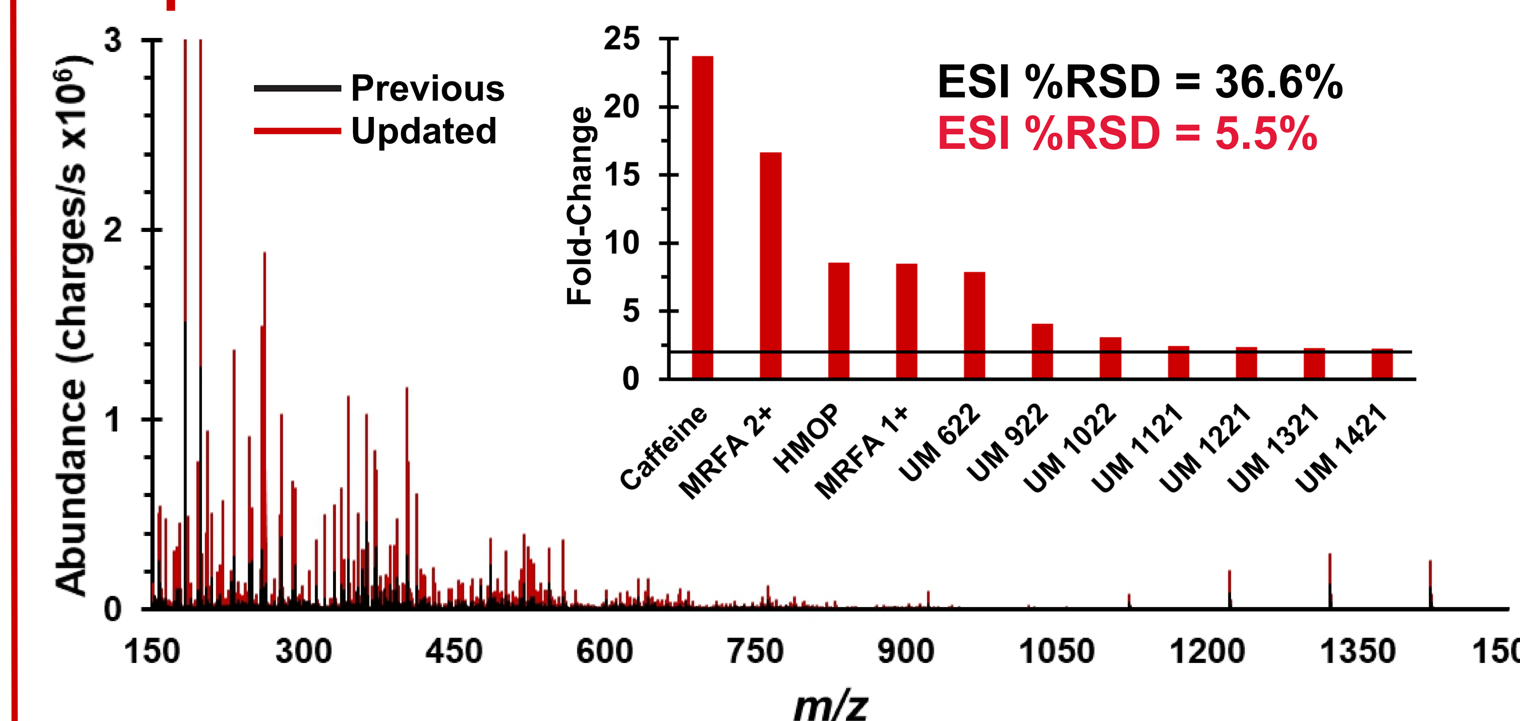


Figure 4. Overlapped mass spectra of the analysis of Flexmix by IR-MALDESI with the updated (red) and previous (black) geometries. Increases in Flexmix ion abundances are shown in the bar chart. The labelled Flexmix ions are in increasing *m/z* order. The %RSD of the SIL-caffeine abundances is a measure of the ESI variability and is shown in the top right corner of the bar chart.

Conclusions and Future Directions

- The IR-MALDESI source has been redesigned and each component has been upgraded.
- Two DOE experiments were conducted to optimize the geometries of the upgraded IR-MALDESI components for IR-MALDESI-MS.
- The updated geometries and components resulted in a 2.4 average fold increase in target ion abundances.
- Further MSI analyses with the updated geometries will be conducted.

Literature Cited

1) Bagley, et al., *Mass Spectrom. Rev.*, **2021**, 1-32.
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3) Hecht, et al., *J. Am. Soc. Mass Spectrom.*, **2016**, *27*, 767-785.

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