Supporting Information

Determination of Optimal Electrospray Parameters for Lipidomics in Infrared Matrix Assisted Laser Desorption Electrospray Ionization (IR-MALDESI) Mass Spectrometry Imaging

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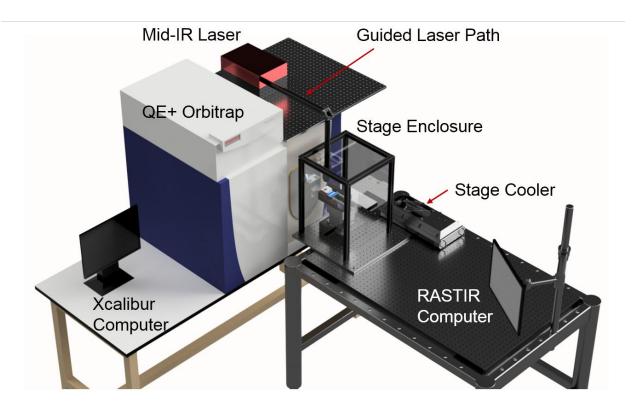
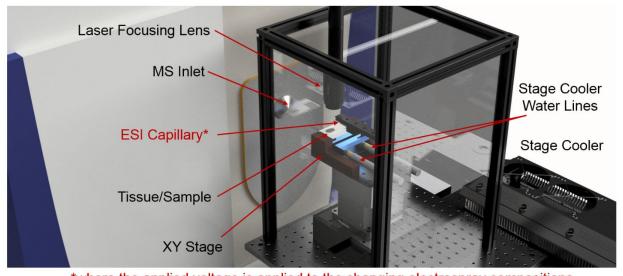


Figure 1. Schematic of IR-MALDESI experimental setup.



*where the applied voltage is applied to the changing electrospray compositions

Figure 2. Close-up of IR-MALDESI sampling, with the components changed for this experiment highlighted in red.

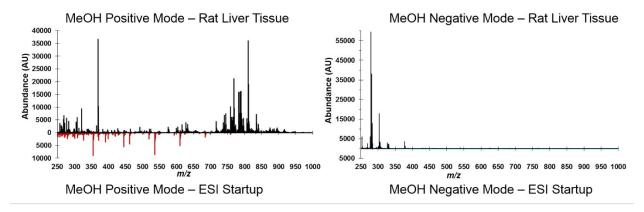


Figure 3. Representative spectra showing the differences in on tissue analysis in both polarities compared to the ESI background spectra collected before daily experiments.

| ACN Gradient | | | MeOH Gradient | | | |
|--------------|-------|----------------|---------------|--------|----------------|--|
| Time | % ACN | Voltages | Time | % MeOH | Voltages | |
| 0 | 35 | 2.5, 2.75, | 0 | 45 | 2.25, 2.5, | |
| 2.5 | 35 | 3.0, 3.2, 3.4, | 2.5 | 45 | 2.75, 3.0, | |
| 32.5 | 75 | 3.6, 3.8, 4.0, | 32.5 | 75 | 3.2, 3.4, 3.6, | |
| 35 | 75 | 4.2, 4.5 | 35 | 75 | 3.8, 4.0, 4.2 | |

Table 1. 30-minutes long gradients were run over shorter solvent gradient ranges for both ACN and MeOH to determine which solvent compositions and voltages that are best for tissue analysis in positive mode.

| ACN Gradient | | | MeOH Gradient | | |
|--------------|-------|----------------|---------------|--------|----------------------------|
| Time | % ACN | Voltages | Time | % MeOH | Voltages |
| 0 | 40 | 2.25, 2.5, | 0 | 20 | 20 22 24 |
| 2.5 | 40 | 2.75, 3.0, | 2.5 | 20 | 3.0, 3.2, 3.4, |
| 27.5 | 75 | 3.2, 3.4, 3.6, | 27.5 | 55 | 3.6, 3.8, 4.0, 4.2, 4.5 |
| 30 | 75 | 3.8 | 30 | 55 | 4.2, 4.3 |

Table 2. 25-minutes long gradients were run over shorter solvent gradient ranges for both ACN and MeOH to determine which solvent compositions and voltages that are best for tissue analysis in negative mode.

Excel Spreadsheet (.xlsx file): Details of Lipids Used for Characterization in both the positive and negative ion mode. **Note:** This is a separate file.