

# Mapping Nano-Electrospray Ionization Plumes on an Orbitrap Fusion Lumos Tribrid Mass Spectrometer Equipped with FAIMS Pro

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## ABSTRACT

Owing to weaker pneumatic suction, the emitter is commonly positioned within  $\leq 1$  mm of the FAIMS Pro orifice for adequate ion transmission at nano or capillary flow rates. Here, we demonstrate that with the addition of a sheath gas, the region of optimal performance can be significantly extended several millimeters away from the orifice without loss in performance. Proteome coverage was generally found to follow the positional pattern observed from solvent ions wherein heatmaps contain a diagonal region at the emitter angle.

## INTRODUCTION

Electrospray ionization (ESI) generates gas phase ions when a sufficiently high voltage is applied to a liquid exiting an emitter with micron dimensions. When the emitter is positioned near a mass spectrometer inlet, which also serves as a counter electrode, gas phase ions are drawn into the instrument. At nano and capillary flow rates, the optimal emitter-to-inlet distance is typically  $< 5$  mm. Because the pneumatic suction can be substantially reduced or eliminated when FAIMS Pro is installed, the emitter is commonly positioned within  $\leq 1$  mm of the FAIMS Pro orifice when performing analyses at nano or capillary flow rates to ensure high transmission.

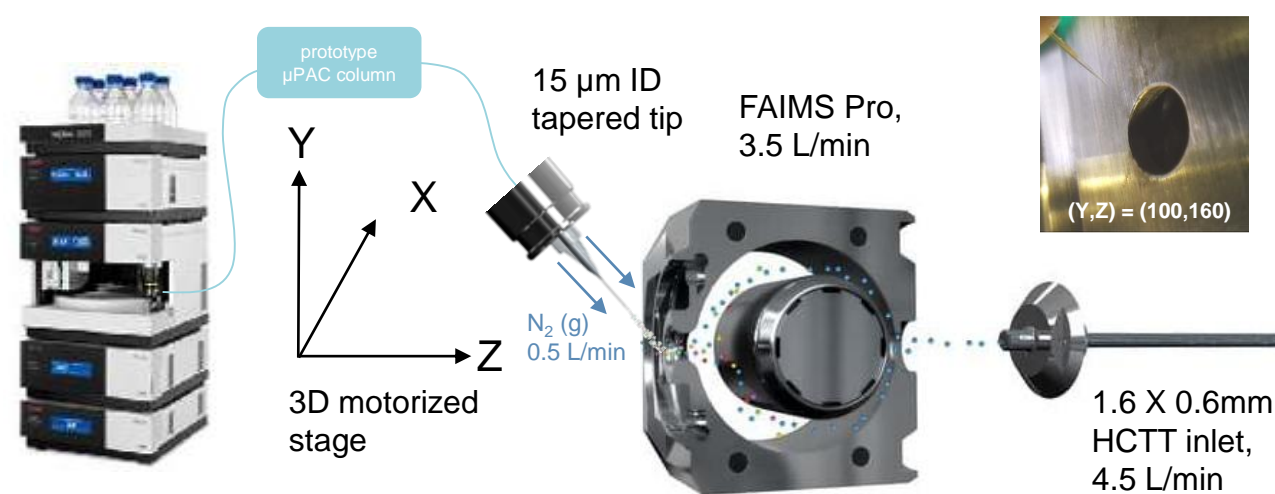
Often, the user positions the emitter at a predefined arbitrary fixed distance from the inlet during initial experimental setup; however, under these conditions, many subtle factors influence the optimal position, including: 1) electrospray mode (i.e.: spindle, multi-spindle, cone jet, multi-jet, etc.), 2) electric field, 3) fluid dynamics, and 4) solvent composition. Here, we explore the interplay between these dynamics by mapping the ESI plume in two-dimensional coordinate space.

## MATERIALS AND METHODS

Spatial mapping of nano-ESI plumes was carried out on a Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ Mass Spectrometer equipped with Thermo Scientific™ FAIMS Pro™, by mounting the emitter on a three-dimensional XYZ stage controlled by stepper motors. Note that 1 step = 48  $\mu$ m unless otherwise noted. Heatmaps for select solvent and abundant background ions were acquired as a function of the emitter position in the YZ plane at a predefined X optimum.

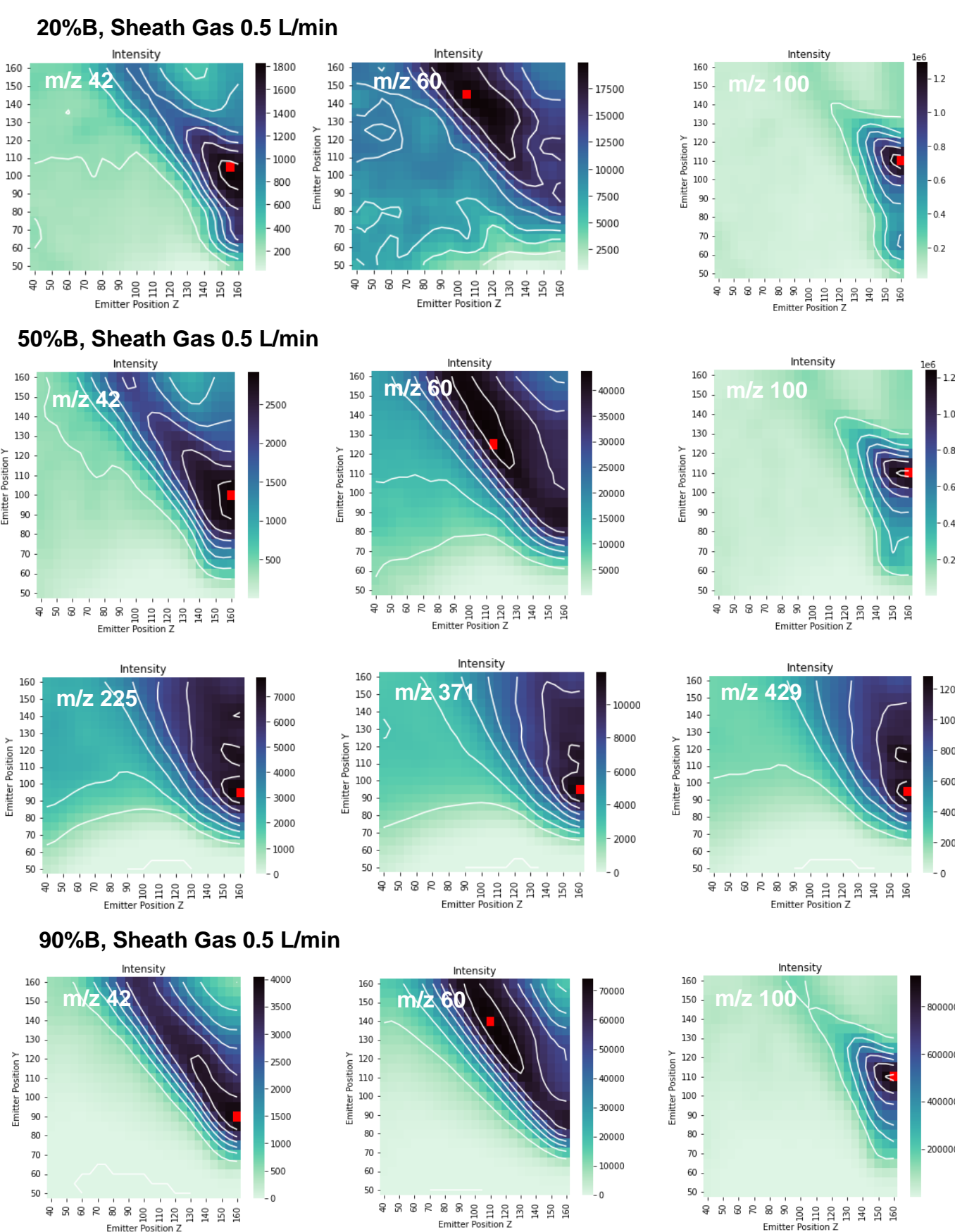
In all experiments a tapered emitter with 15  $\mu$ m ID was used. The voltage drop from the liquid junction upstream of the emitter to the FAIMS Pro front electrode was 1.75 kV. Unless specified, a coaxial sheath gas of nitrogen was used at a flow rate of 0.5 L/min. FAIMS Pro voltages remained off during acquisition of solvent and background ion heatmaps but remained on during LC-MS experiments.

LC-MS proteomics experiments were carried out using 200 ng loads of HeLa cell protein digest. Samples were analyzed using a prototype  $\mu$ PAC column at 1500 nL/min. In all cases, a 1  $\mu$ L injection volume was used. The gradient was ramped from 1% to 45%B (where [A]: 0.1% formic acid in water, [B]: 0.1% formic acid in 80:20 acetonitrile:water) over 5 minutes. A single FAIMS Pro compensation voltage of -50 V was applied. Raw files were searched in Thermo Scientific™ Proteome Discoverer 3.0™ using Chimerys and Percolator to filter results to a 1% FDR.



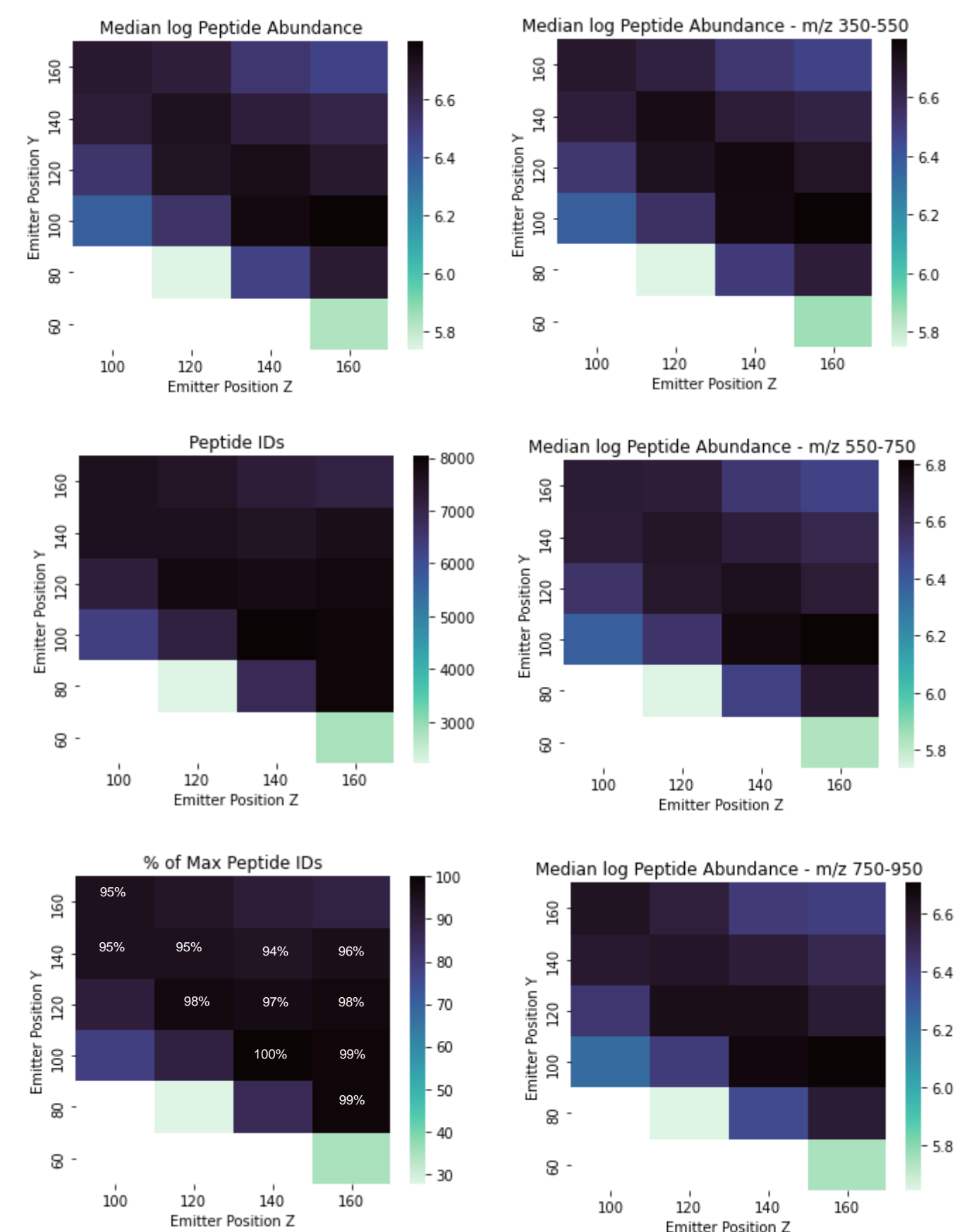
## RESULTS

Figure 1. YZ heatmaps acquired at the optimum X position for solvent and abundant background ions. The mobile phase composition and sheath gas settings are indicated below. The step size used for acquisition in both the Y and Z dimensions was 240  $\mu$ m (5 steps). Note that the total range was 5.3 mm and 5.8 mm for the Y and Z dimensions, respectively.



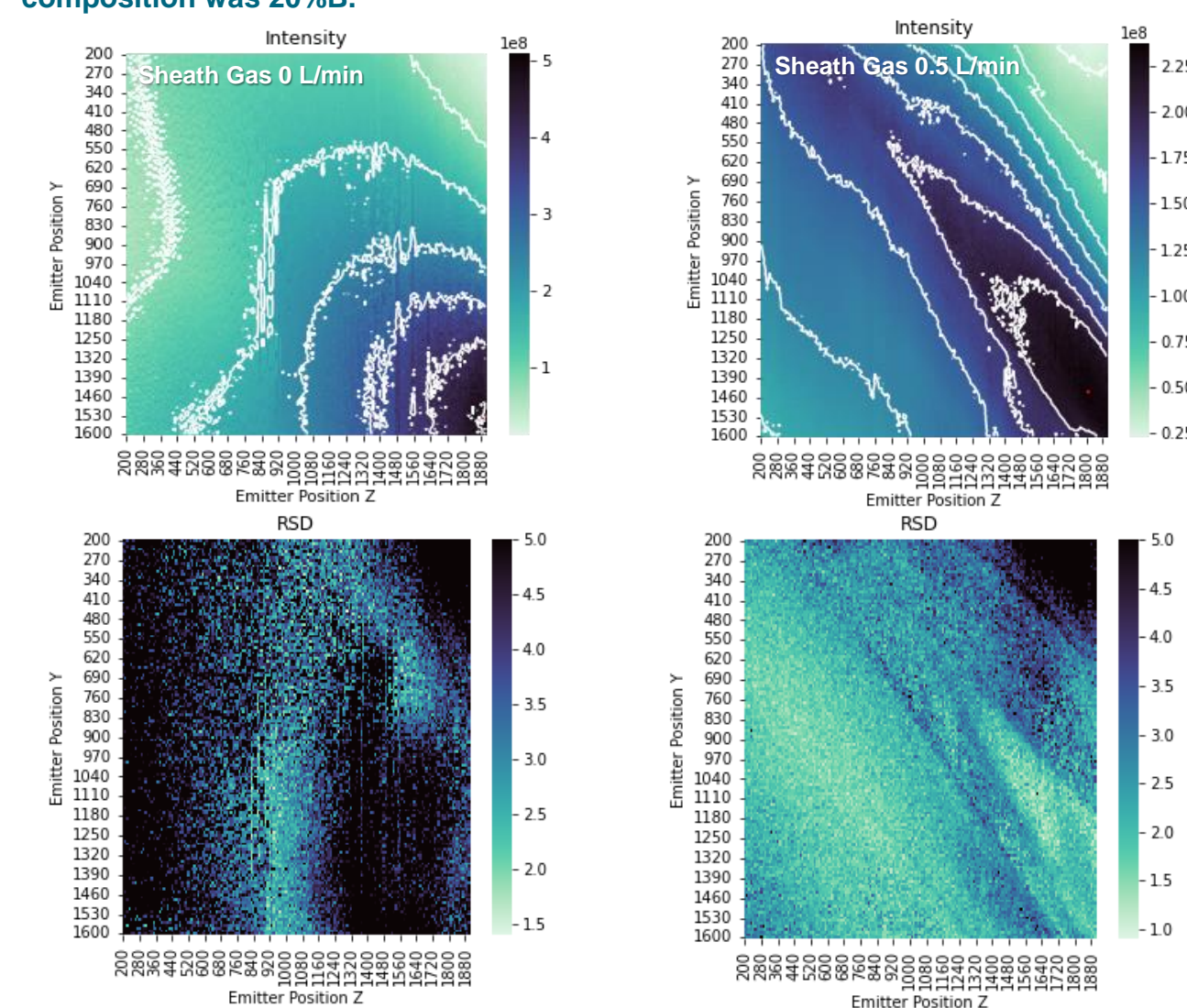
When sheath gas is used, optima tend to be located across a diagonal region approximating the angle of the emitter. Common spectral contaminant ions, including polysiloxanes, tend to also optimize near the inlet but are also dominant in regions where solvent ions are lower in abundance—in particular, above the inlet.

Figure 2. Metrics related to total proteome coverage (left) and metrics related to ions binned according to m/z (right) as indicated below. In all experiments the sheath gas flow rate was 0.5 L/min. Regions shown in white are outside the area of interest and were not acquired. The total range was 4.8 mm and 2.9 mm for the Y and Z dimensions, respectively. Note that the step size of 20 corresponds to a distance of 0.96 mm.



Positions ranging across the diagonal contained in the heatmap were all found to yield  $\geq 95\%$  of the max proteome coverage. From (Y,Z) = (100,160) to (160,100) represents a 4.1 mm distance increase. Though the analytical performance generally mimics the solvent ion (m/z 42) behavior, one minor difference is that for analytical ions, the decrease in performance along the bottom of the diagonal is generally more significant than the decrease along the top.

Figure 3. High resolution scans of m/z 60 with (right) and without (left) sheath gas acquired with a step size of 40  $\mu$ m. Note that the arbitrary coordinate system used in this experiment differs from the rest of the datasets and is only shown to illustrate the fine structure of the spray plume. The flow rate was 1000 nL/min and the mobile phase composition was 20%B.



## CONCLUSIONS

The analytical performance was found to largely mirror the solvent ion (m/z 42 and 60) behavior. In some cases, select ion heatmaps show some evidence of m/z dependency within the plume as well as background ions that exist in spatial locations where analytical or solvent ions are absent. Most peculiar are background ion heatmaps that contain prominent signals above the FAIMS Pro inlet. Importantly, we find that the addition of a co-axial sheath gas used in conjunction with tapered emitters:

- substantially helps facilitate ion transport into the FAIMS Pro orifice, thereby enabling the use of significantly larger emitter-to-inlet distances at low flow rates, and,
- improves the spray stability.

Low m/z background and solvent ions exhibit position-dependent behavior, though peptide ion abundances did not show analogous trends across a range of m/z.

## ACKNOWLEDGEMENTS

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## TRADEMARKS/LICENSING

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