



# Mass Spectrometry Imaging Using Desorption Electrospray Ionization

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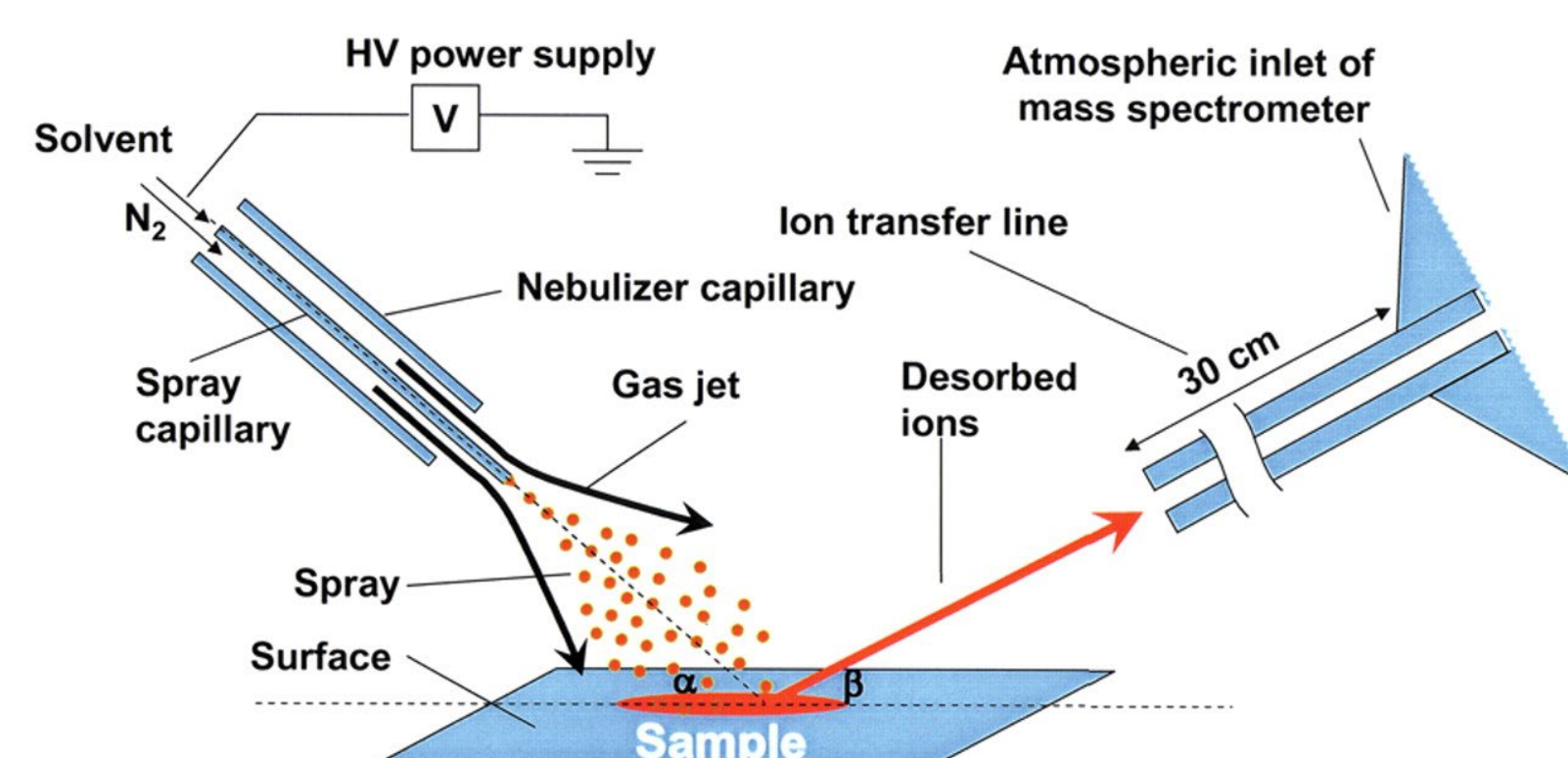
## Background & Significance

Poison dart frogs are small, brightly colored amphibians that secrete toxic, bitter-tasting chemicals from their skin. These compounds are alkaloids, naturally occurring nitrogenous compounds, and they are obtained from their diet.<sup>1</sup> To not be sickened or killed by these alkaloids, the dart frogs must sequester them away from important organs or develop immunity to their effects, and for the frogs' predators to be sickened or killed, they need to be sufficiently noxious and able to reach necessary structures. Locating toxins in the frogs' bodies using mass spectrometry imaging<sup>2</sup> is the main goal of this work.

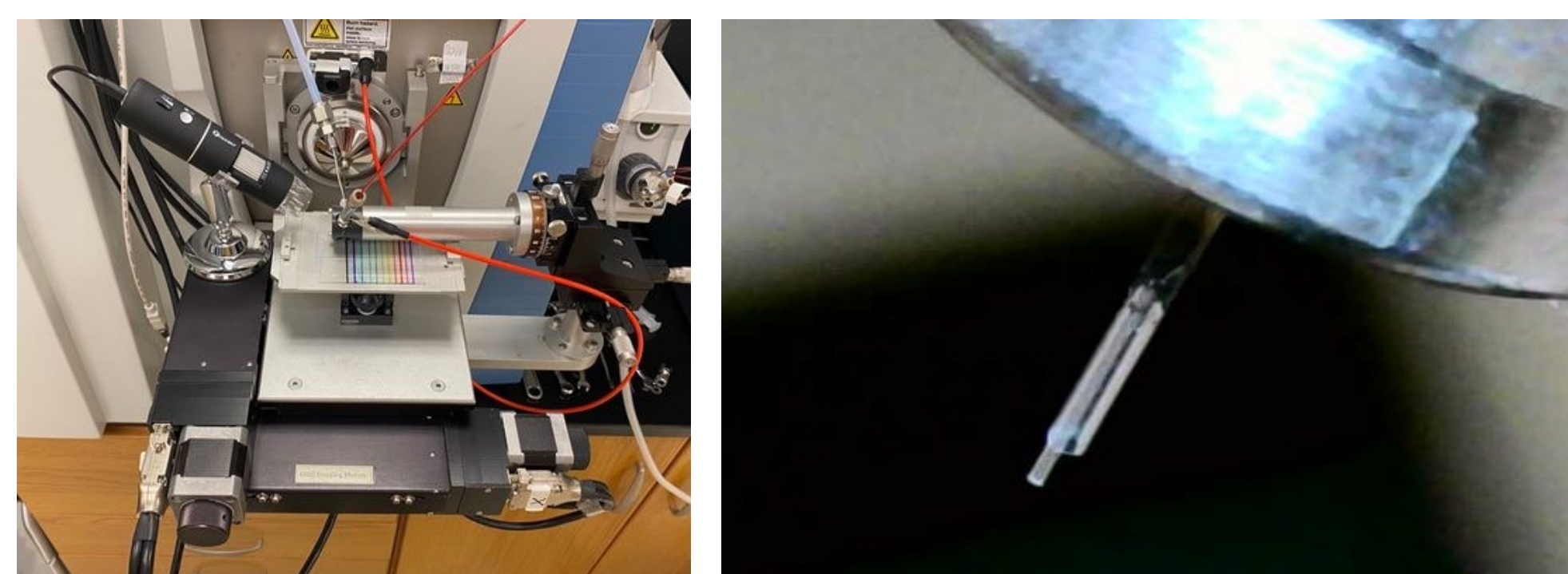


## Desorption Electrospray Ionization

In order to perform mass spectrometry, molecules first need to be ionized. Desorption Electrospray Ionization (DESI) is a form of ionization in which a charged spray of solvent picks up, ionizes, and transfers molecules from a surface into the mass spectrometer (below).<sup>3</sup>

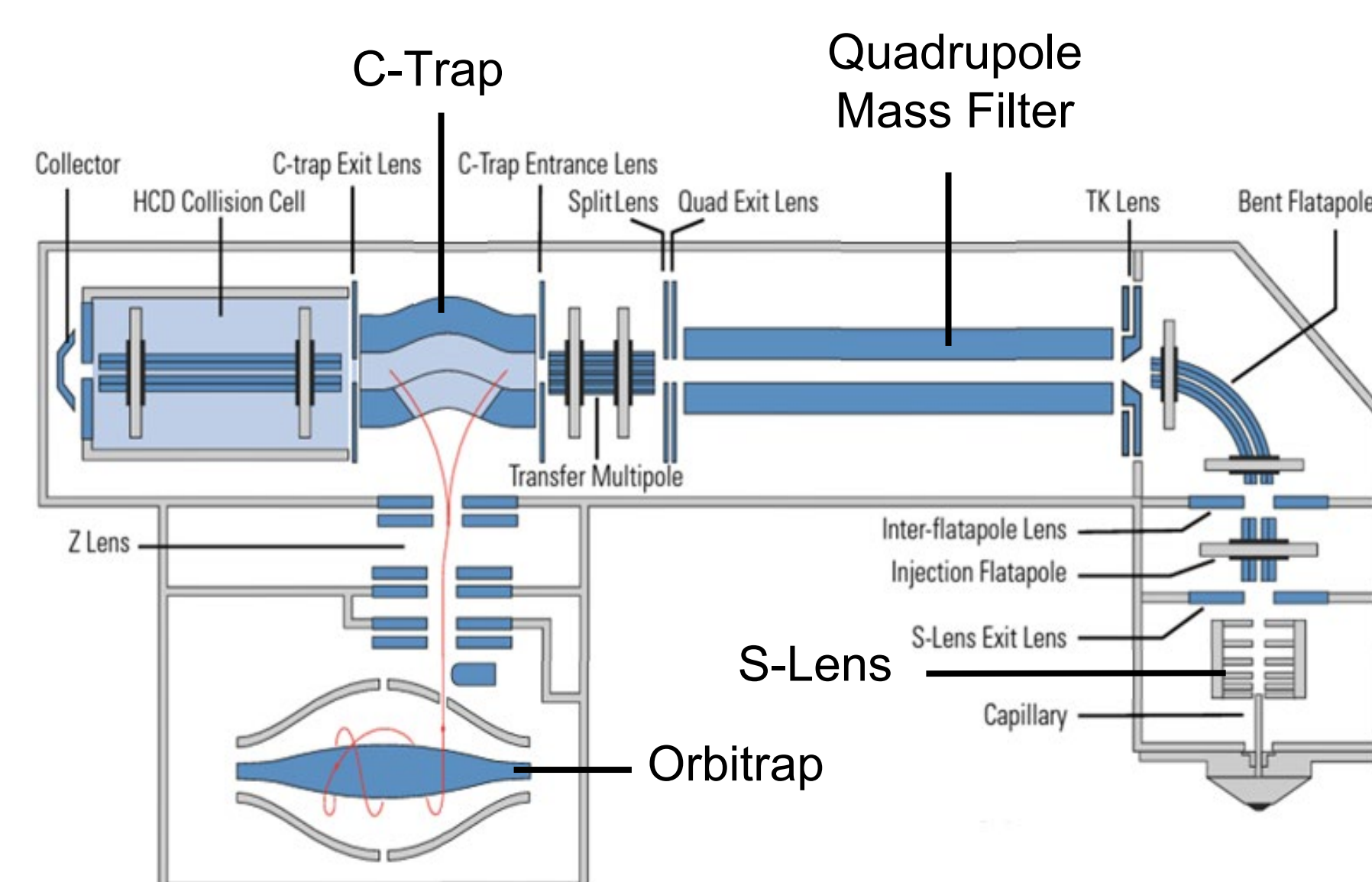
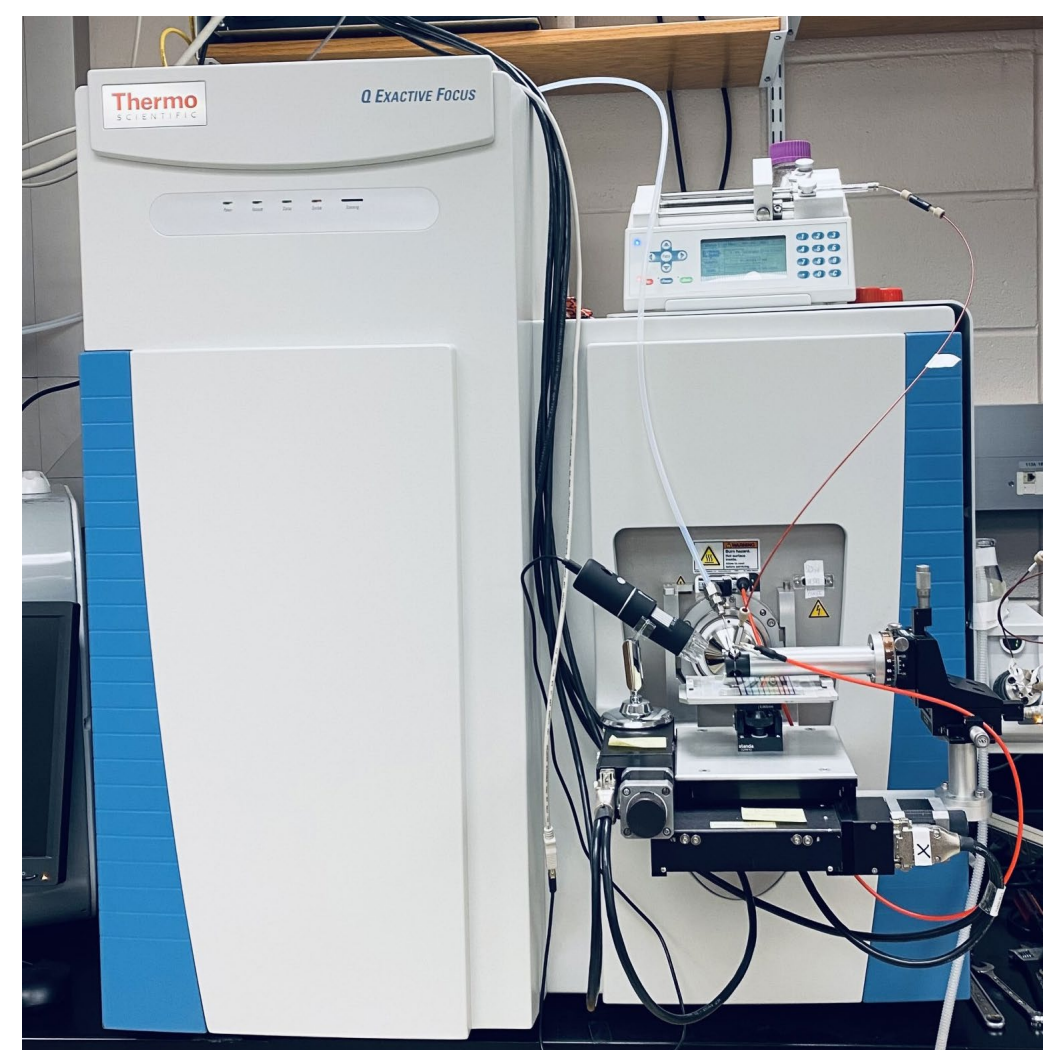


We are currently using a home-built moving stage (below, left) due to the high price of commercial instruments. The stage is controlled in the x- and y-direction by a computer (Galil Motion Control). The stage height can be manually adjusted, and there is a holder for the sprayer to be mounted on. We adjusted the parameters with the goal of getting the highest signal-to-noise ratio while keeping solvent puddling to a minimum in order to maximize resolution. The sprayer is set to be 1 mm from the sample at a 50° angle from the horizontal. The solvent flow rate is set to 2.5  $\mu\text{L}/\text{min}$  and the gas is set to 100 psi. The solvent is LC-MS & UHPLC-UV grade methanol. Since the sprayer (below, right) is a capillary inside a larger capillary, it is not supported and struggles to stay centered. We are making tapered capillary tips to strengthen the capillaries and fix this problem.



## Mass Spectrometry

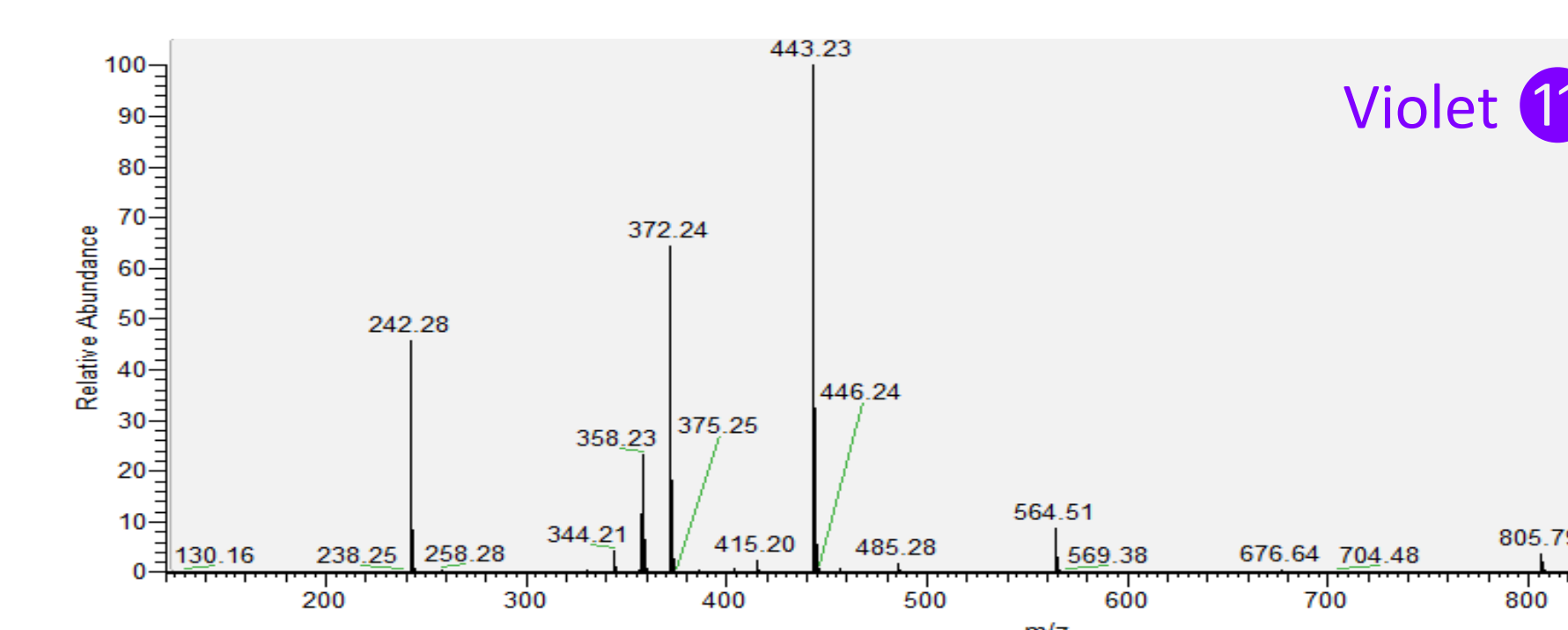
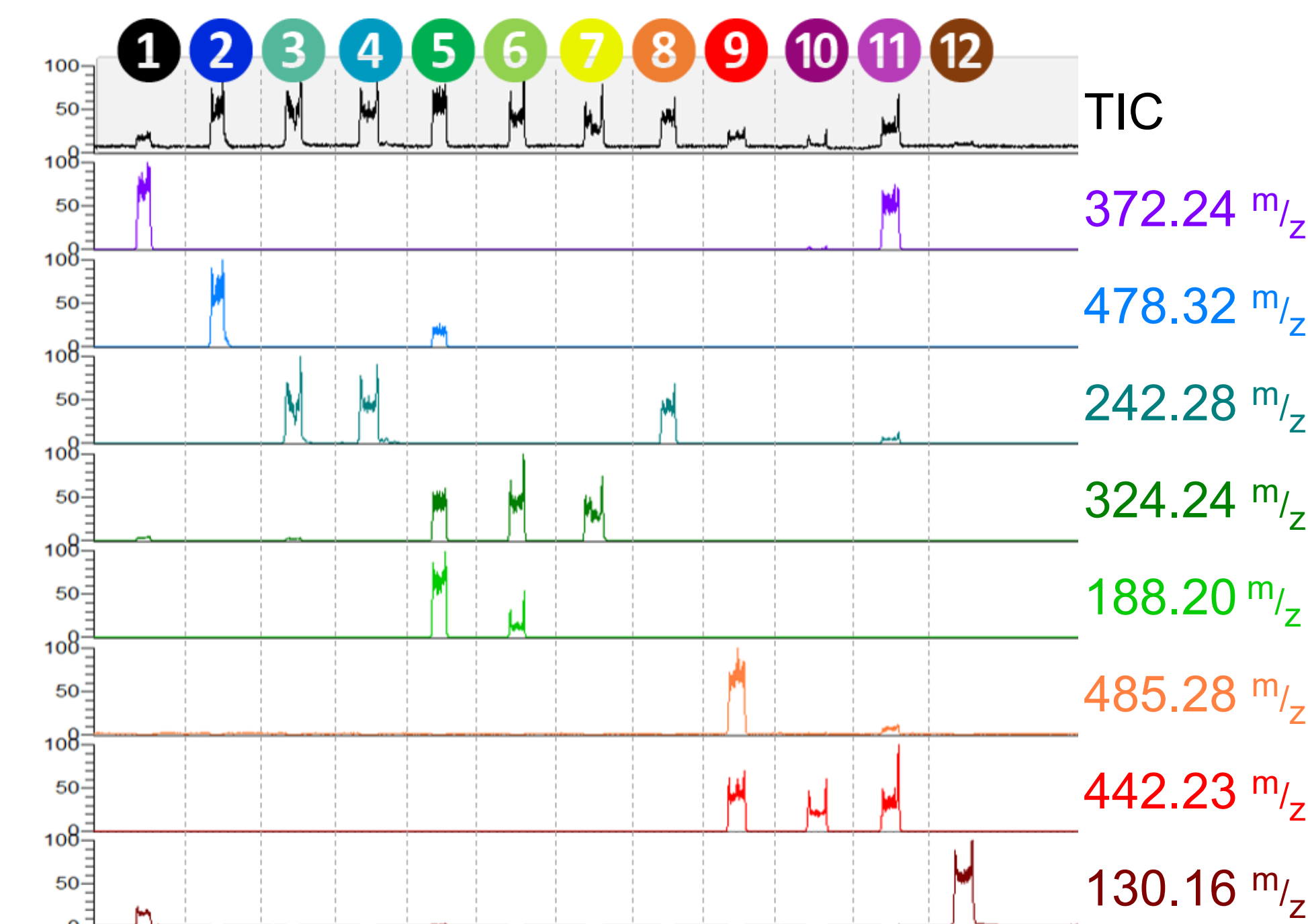
We are using a Thermo Scientific Q Exactive Focus Mass Spectrometer (inset below). It is high resolution-accurate mass quadrupole-orbitrap mass spectrometer. The set-up is comprised of the mass spectrometer and a syringe pump to provide solvent. The ions will enter the instrument. Then, an ion lens will focus and accelerate the ions. Since we are only doing full scan analyses, the quadrupole will only transmit the ions and the collision cell will not be used. The C-trap will bundle the ions and send them to the orbitrap to be detected. We are using Thermo's Xcalibur Software to view the chromatogram. After a couple of data sets, it was noticed that the number of scans for each run of an image varies. This could impact the processing of the image. We figured that a possible reason is the automatic gain control which waits until a certain threshold of ions is collected before analyzing. We plan to scan an image with automatic gain control turned off to see if the number of scans is consistent.



<https://assets.thermofisher.com/TFS-Assets/CMD/manuals/manualbre0012255-exactive-series-manbre0012255-en.pdf>

## Mass Spectrometry Data

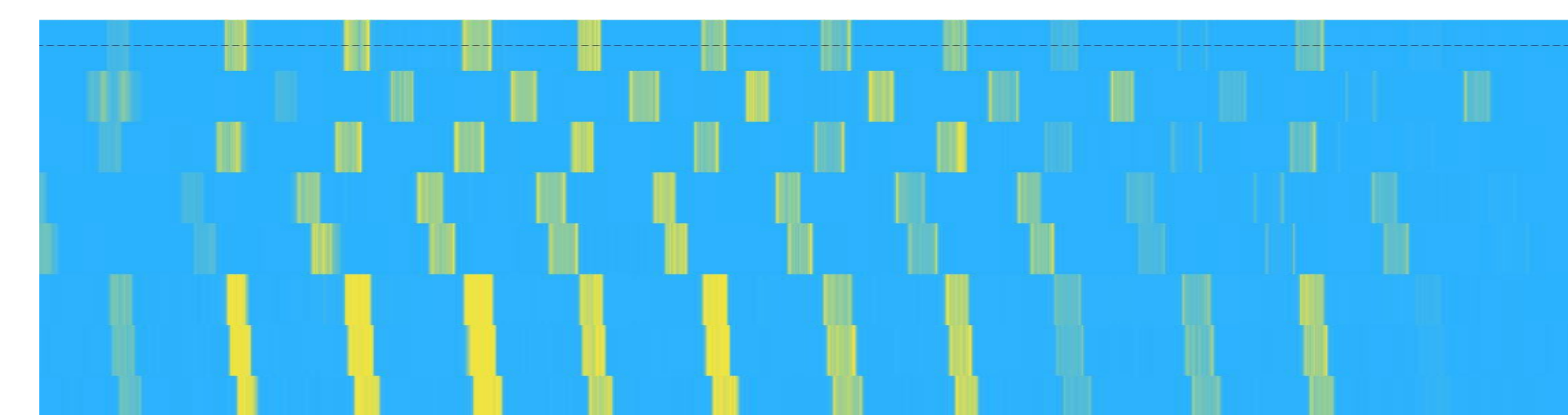
We started by scanning lines drawn in multiple colors with Sharpie® marker on a glass slide. The order of the colors can be seen at the top of the figure above in the next column. The total ion count (TIC) chromatogram is shown in the first row. This shows all the ions that the mass spectrometer detected. The y-axis is relative abundance, and the x-axis is time, which is correlated to position on the slide. The chromatogram shows obvious increases in ion abundance at the times it encounters the inks. From the data, we can see the individual dyes that are in each marker and attain a chemical formula for each from the exact mass. Each color is produced by a combination of dyes. For example, marker color #11, violet, is composed of primarily the pigments shown in rows 2, 4, 7, and 8 along with other minor dyes whose peaks can be seen in the spectrum that follows.



## Processing

After obtaining raw data, the files need to be converted into an .imzML file to view and process the 2-dimensional image. We found that most imaging literature cites conversion of raw data files into .mzML format using the MSConvert in ProteoWizard (v3.0) and then combinatoin into a single .imzML file using imzMLConverter (v1.3).<sup>4,5</sup> The newest version of imzMLConverter is 2.1, but only the older version has a graphical user interface (GUI). Unfortunately, resulting image files would not open easily in the application we are using to view and process. We also suspect some issues may be caused by each acquisition file not being the same exact number of scans, which is caused by automatic gain control, a setting which adjusts injection time for intensity of ions. With image files that could open (marker lines on a glass slide below), drifting is apparent. Further tests would need to be done to see if this is actually an issue. This is thought to be due to delays between the workstation and the mass spectrometer.

Serious problems arise when using *ad hoc* methods, which are common in the MS imaging community. Having to go between multiple processing platforms across multiple operating systems (Windows, Linux) and languages (R, Java, Perl, Python, etc...) is a common problem for the novice imaging scientist. Therefore, a single, user-friendly interface would be highly desirable in order to expand the use of this powerful technique for interrogating surfaces and tissues.



## Conclusions

For most academic institutions, it is possible to obtain the instruments for mass spectrometry imaging for a relatively affordable price. A home-built stage would cost a few thousand dollars, and many would already have a mass spectrometer. We are working to find a reliable method of scanning slides using these instruments, but processing the scans still needs improvement. For future work, we will refine our scanning and processing methods. We aim to use mass spectrometry imaging for locating compounds in tissues such as toxic alkaloids in poison dart frog samples and brain tissue samples, but DESI-MSI could prove to be extremely useful in many fields such as chemistry, biology, medicine, and forensics.

## Acknowledgments

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