

MSI Analyte Browser: A web-based MicroApp for visualization and annotation of MS imaging data

INTRODUCTION

Mass spectrometry imaging (MSI) is a rapidly developing molecular imaging modality that can directly map the spatial distribution of thousands of analytes across a sample, enabling exploratory discovery in biological and materials science.

MSI produces large amounts of multi-dimensional data, leading to the need for more powerful statistical techniques and software packages in order to guide and streamline data interpretation.

A new web-based micro-app called MSI Analyte Browser is presented which provides tools for image annotation and isotope identification as well as image and spectral visualization and automatic identification of analytes of interest. The MSI Analyte Browser delivers 2D visualization of user-selected m/z images and analyte spectra at user-selected pixels resulting in greater selectivity, more information and accuracy than other currently available options.

With the introduction of the SELECT SERIES™ MRT and the High resolution (>200,000 FWHM) and sub ppm mass accuracy (<200ppb) offered by the multi reflecting time of flight mass analyzer, a higher confidence can now be attributed to identifications made by mass analysis alone.

In addition, the putative identifications generated in the app can help guide the user with future target analysis, from the same (DESI) or consecutive (DESI and MALDI) tissue sections.

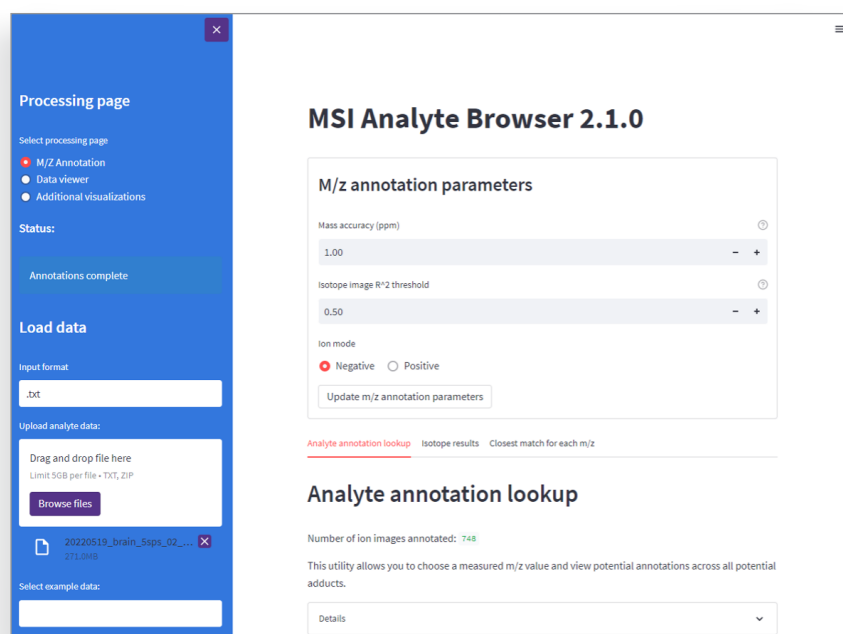


Figure 1. Overview of app layout showing m/z annotation parameters.

EXPERIMENTAL CONDITIONS

Fresh frozen mouse (*Mus Musculus*) brain whole organ was frozen at -80 °C, the organ was sectioned at -20 °C with a section thickness of 18 µm using a cryostat (Leica). The sections were stored at -80 °C until analysis.

Tissue analysis was performed by DESI™ XS on a SELECT SERIES™ MRT multi reflecting Q-ToF mass spectrometer, tissue sections were defrosted and dried under ambient conditions for 15 mins prior to analysis.

MS conditions were as follows:

Source type: DESI
 Cone voltage: 40 v
 Capillary voltage: 0.8 kV
 Nebulizing gas: Nitrogen
 Nebulizing gas pressure: 0.8 bar
 Ionization mode: Negative
 Solvent: 95% MeoH + 200 pg/µL Leu- Enkephalin

Data were acquired at 5 pixels per second with a 50 µm pixel size. The data were lock mass corrected by continuous lock mass correction against Leu-Enkephalin (m/z 554.2620). After processing the data via HDI™ the generated peak picked analyte file was loaded into the MSI analyte browser.

Analyte annotation lookup

Number of ion images annotated: 748

This utility allows you to choose a measured m/z value and view potential annotations across all potential adducts.

Details ▼

Search results by:

Measured m/z
 Formula
 Name

Select a measured m/z :

885.5497 ▼

Number of matches in HMDB: 25

	Measured m/z	Adduct	Error (ppm)	Formula	Name
16306	885.5497	[M-H]-	0.1782	C47H83O13P	PI(16:0/22:4(10Z,13Z,16Z,19Z))
16307	885.5497	[M-H]-	0.1782	C47H83O13P	PI(16:0/22:4(7Z,10Z,13Z,16Z))
16308	885.5497	[M-H]-	0.1788	C47H83O13P	PI(18:0/20:4(5Z,8Z,11Z,14Z))
16309	885.5497	[M-H]-	0.1782	C47H83O13P	PI(18:0/20:4(8Z,11Z,14Z,17Z))
16310	885.5497	[M-H]-	0.1782	C47H83O13P	PI(18:1(11Z)/20:3(5Z,8Z,11Z))
16311	885.5497	[M-H]-	0.1782	C47H83O13P	PI(18:1(11Z)/20:3(8Z,11Z,14Z))
16312	885.5497	[M-H]-	0.1782	C47H83O13P	PI(18:1(9Z)/20:3(5Z,8Z,11Z))
16313	885.5497	[M-H]-	0.1782	C47H83O13P	PI(18:1(9Z)/20:3(8Z,11Z,14Z))
16314	885.5497	[M-H]-	0.1782	C47H83O13P	PI(18:2(9Z,12Z)/20:2(11Z,14Z))
16315	885.5497	[M-H]-	0.1782	C47H83O13P	PI(20:2(11Z,14Z)/18:2(9Z,12Z))

Figure 2. m/z annotation page showing results for query of m/z 885.54968.

RESULTS

Peak picked data were loaded into the MSI analyte browser by selecting the peak picked analyte file created by HDI in the raw data folder. The data set used was generated using a high resolution multi reflecting ToF Mass spectrometer and as such an appropriately tight search tolerance was used for the peak annotation. A mass accuracy cutoff of 1ppm was specified. Figure 2 shows the returned results for m/z 885.54968 which was putatively identified as the [M-H]- adduct of the phosphatidylinositol lipid PI(38:4) with the elemental composition $C_{47}H_{83}O_{13}P$. Twenty five matches were returned from the HMDB database, all structural isomers of PI(38:4) with a PPM error of 0.1782.

The *m/z* annotation page:

- Provides tools for users to explore tentative chemical annotations of individual ion images through matching measured peak masses to entries in the public human metabolome database
- The user inputs a mass tolerance in ppm and selects positive or negative ion mode
- The algorithm then estimates a neutral mass under the assumption of several common adducts depending on the ion mode and returns all entries in the HMDB with monoisotopic masses within the mass tolerance
- Results can be searched by measured mass, chemical formula, or chemical name
- The algorithm also identifies potential isotope images by comparing mass differences along with a user-defined m/z correlation metric.

In addition to the results for a selected mass, a list of the top match for each peak can be generated (figure 3) in both cases the results can be saved as a .csv file.

<i>m/z</i>	Measured <i>m/z</i>	Total number of matches	Formula [closest match]	Name [closest match]
872	890.6304	23	C48H91NO11S	3-O-Sulfogalactosylceramide
873	890.6395	23	C48H91NO11S	3-O-Sulfogalactosylceramide
874	890.6845	7	C50H98NO7P	PC(24:0/P-18:1(11Z))
875	891.4754	9	C42H80O13P2	PGP(18:1(11Z)/18:1(11Z))
876	891.5585	78	C46H85O14P	PG(i-20:0/6 keto-PGF1alpha)
877	891.5964	6	C47H89O13P	PG(a-21:0)/PGF1alpha)
878	891.6235	29	C49H90NO8P	PE(20:3(5Z,8Z,11Z)/24:1(15Z)
879	891.6341	29	C49H90NO8P	PE(20:3(5Z,8Z,11Z)/24:1(15Z)
880	891.6428	23	C48H91NO11S	3-O-Sulfogalactosylceramide
881	892.6190	29	C49H90NO8P	PE(20:3(5Z,8Z,11Z)/24:1(15Z)
882	892.6281	29	C49H90NO8P	PE(20:3(5Z,8Z,11Z)/24:1(15Z)
883	892.6347	29	C49H90NO8P	PE(20:3(5Z,8Z,11Z)/24:1(15Z)
884	892.6458	23	C48H91NO11S	3-O-Sulfogalactosylceramide
885	892.7761	0		
886	893.6245	0		
887	893.6490	3	C56H90O6	TG(15:0/18:4(6Z,9Z,12Z,15Z)/
888	893.7792	3	C56H106O5	TG(15:0/20:2n6/O-18:0)
889	894.6251	25	C50H90NO10P	PS(20:3(5Z,8Z,11Z)/24:1(15Z)
890	898.5085	31	C46H78NO14P	PS(20:3(5Z,8Z,11Z)/6 keto-PG
891	898.6987	0		
892	899.5120	31	C46H78NO14P	PS(20:3(5Z,8Z,11Z)/6 keto-PG
893	899.7022	0		
894	900.5148	18	C47H80NO11P	PE(22:2(13Z,16Z)/20:5(7Z,9Z,
895	900.7053	0		
896	900.7139	0		
897	901.5443	57	C47H83O14P	PI(18:0/20:3(5Z,8Z,11Z)-O(14)
898	901.7176	0		
899	902.5034	62	C50H78NO9P	PC(20:5(5Z,8Z,11Z,14Z,17Z)/i
900	902.5484	48	C51H82NO8P	PE-NMe2(22:5(4Z,7Z,10Z,13Z,
901	902.6030	30	C49H90NO9P	PE(24:0/20:3(5Z,8Z,11Z)-O(14)
902	902.6393	22	C50H94NO8P	PC(18:2(9Z,12Z)/24:1(15Z))

Figure 3. generated list showing the closet match for peaks present within the set ppm tolerance.

The MSI analyte browser application also allows rapid visualization of selected peak images via the data viewer page;

The data viewer page enables:

- Individual ion images to be viewed (Figure 4 A) in an interactive manner, with controls for colormap and scale, panning, zooming (figure 4 B) etc.
- Clicking on a pixel in the ion image window will open its spectrum in the interactive pixel spectrum window (Figure 4 C), where hovering over a peak reveals the annotation with the lowest mass error.
- An average interactive spectrum for the annotated dataset can also be generated.

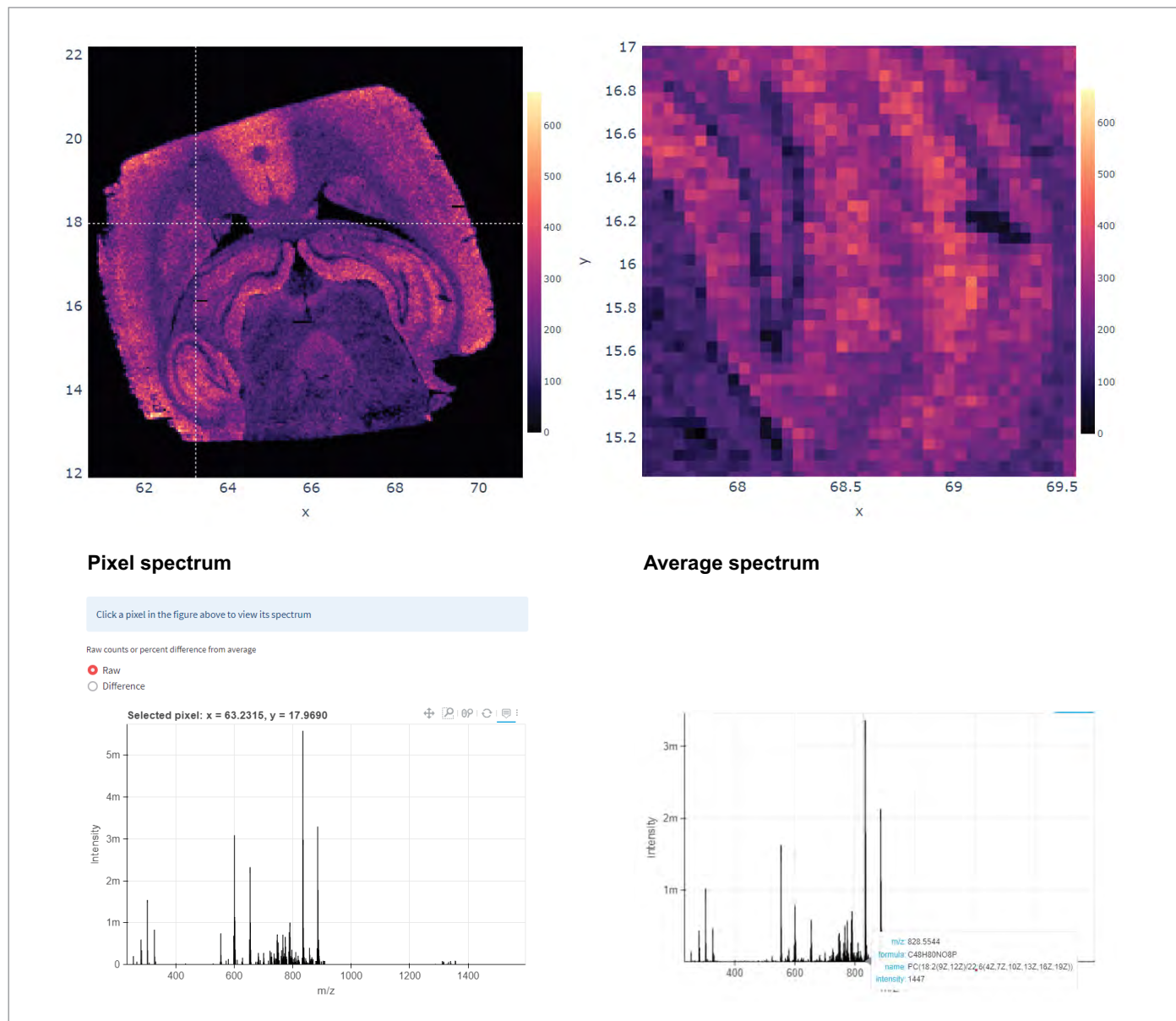


Figure 4. data viewer page visualisations – A) full image view for m/z 837.5388 ($C_{46}H_{78}NO_{10}P$), B) zoomed selection from visualisation in A, C) selected pixel spectrum from position indicated in A, D) average spectrum for data set with peak highlighted at m/z 828.5544 (top hit PC(40:8)).

Finally, the additional visualizations page allows for the display of the top 50 analytes in a grid, intended to guide selection of m/z values for further inspection in the "Ion image" tab. The top analytes are chosen by ranking m/z values by the ratio of the sum of their intensities inside and outside a custom object mask (automatically detected tissue boundary). Once chosen, the top images are ordered by m/z value and plotted as a grid in a single figure (figure 5).

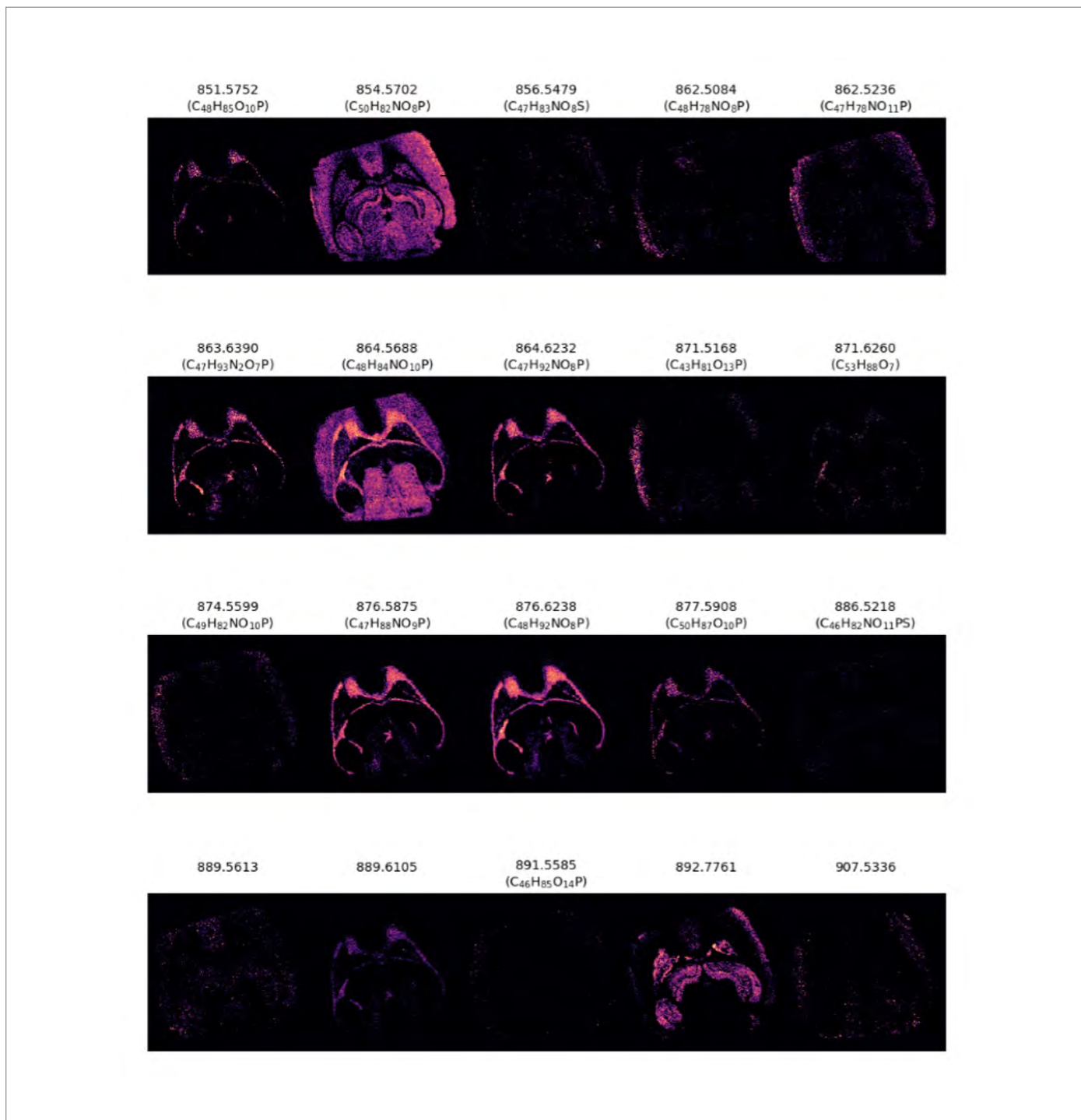


Figure 5. A subset of 20 images from the top 50 analytes showing mass and possible elemental composition.

The additional visualizations tab can also generate RGB color overlays of selected masses. Figure 6 shows the result of a 3 color overlay of m/z 888.6238 (red), 885.5497 (green), 810.5290 (blue) which have been putatively identified by accurate mass using the annotation tool as 3-O-Sulfogalactosylceramide (d18:1/24:1(15Z)) (0.1711 ppm), PI(38:4) (0.1782 ppm) and PS(38:4) (0.0083ppm).

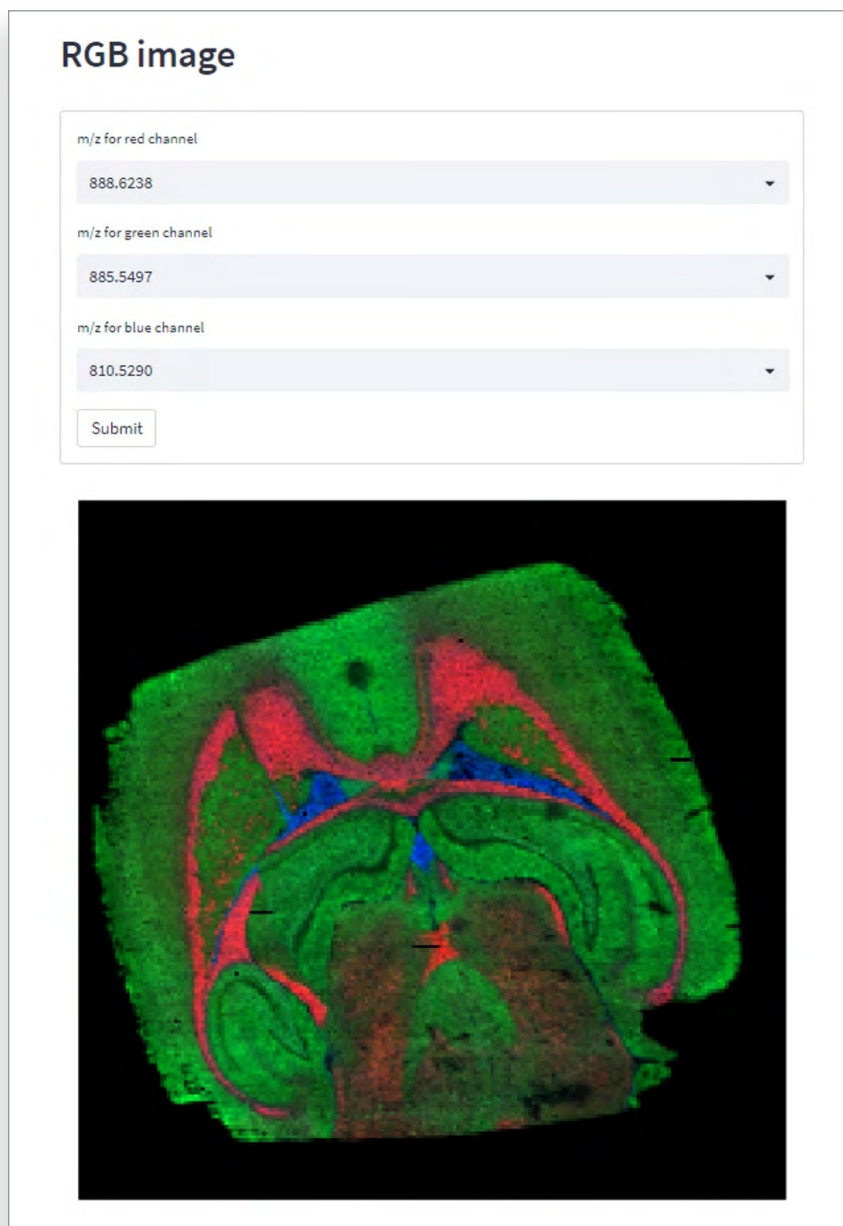


Figure 6. three colour RGB overlay m/z 888.6238 (red), 885.5497 (green), 810.5290 (blue).

SUMMARY

- A light-weight data exploration tool allows the user to explore and characterize imaging data
- Annotation tool matches spectral features across the entire dataset to tentative matches in a chemical database, allowing users to identify and search for known compounds of interest in their images
- Visualization tools allow the user to explore spatial and spectral distributions of individual peaks and scans, with additional tools to automatically suggest "top" analytes of interest and combine key features in a single RGB color figure
- This tool fosters understanding of imaging datasets by guiding chemical annotations with spatial distributions.

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