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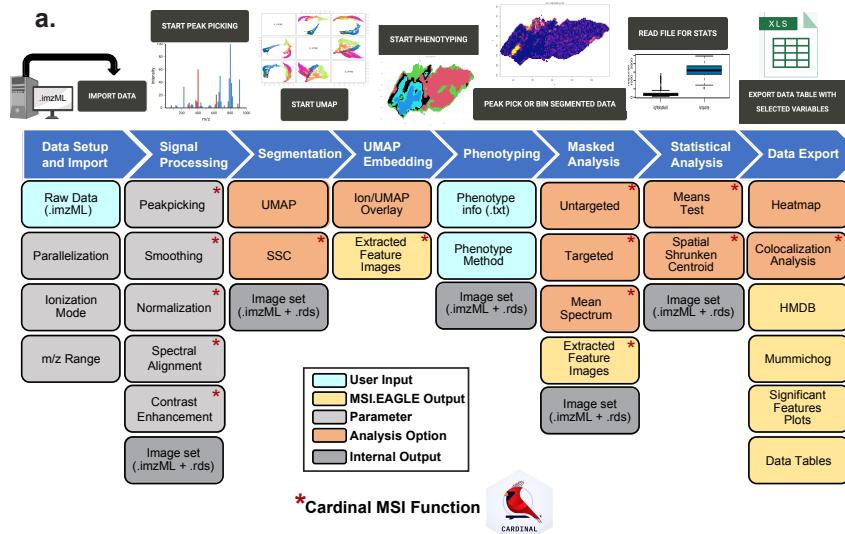
# Introduction

MSI.EAGLE is a modular R Shiny app designed to provide a user-friendly interface for processing of mass spectrometry imaging data. It relies heavily on the *Cardinal* R package for underlying spectra processing and certain data analysis functions, providing a number of other options for data analysis and visualization. A major advantage of the workflow and structure is processing of larger datasets which include multiple runs and the ability to easily manipulate whole datasets or subsets. The underlying architecture of the app is setup as individual modules accessed via tabs in the user interface, and the high-level major functions of each module are described in the diagram below. The modular layout also encourages further development of algorithms and methods which can be easily implemented. Within the modules there are a few workflow related dependencies to keep in mind:

- Data setup and import, Signal Processing / reading in files, Segmentation, and Phenotyping share data internally, and the data must be read in through the "File Restore and Overview Analysis" tab (corresponding the Signal processing arrow in the diagram below) or imported and processed as imzML data.
- The Depth Analysis tab relies on re-analysis of input raw data, and thus .imzML files must be available (and read into memory) from the Data Setup tab along with a set of coordinates from the same dataset to guide detailed analysis.
- The Heatmap and Colocalization tabs currently rely on having already performed a statistical analysis in the Statistics Tab.

Considerations for each tab will be outlined in subsequent further pages of the guide.

While running the app it is recommended that the user have access to the R console as there may be important information regarding dataset sizes, errors and other info.



# Installation

You can install the development version of MSI.EAGLE as follows:

**Step 1:** Install Bioconductor packages

```
install.packages("remotes")

if (!require("BiocManager", quietly = TRUE))
  install.packages("BiocManager")

BiocManager::install(c("BiocParallel", "Cardinal"))
```

**Step 2:** Install Git  
<https://git-scm.com/book/en/v2/Getting-Started-Installing-Git>

**Step 3:** Install MSI.EAGLE

```
remotes::install_github("http://weljie.myds.me:30003/aalim/DESI_Shiny_Processing_script.git",
  , branch="modules")
```

Running the app:

To run the app, the user can either specify directory and parallelization parameters, or MSI.EAGLE will use the current R working directory for both raw files and data output, as well as use the number of available cpu cores – 2 for parallel processes. These parameters can also be specified in the app; however, it may be more convenient to set defaults if working from startup scripts specific to each project. In the following example, the app is started based on defaults:

Here we define directory and parallelization parameters before running the app. The directory containing .imzML files is 'rawd' and the directory where the app is going to look for processed data is 'wd'. The number of cores to use is specified by ncores.

```
> wd = ('path/to/workingdirectory')
> rawd = ('path/to/rawfilesdirectory') # rawd = wd
> ncores = as.integer(parallel::detectCores()/2) # use 1/2 of the available processors
> ncores = as.integer(parallel::detectCores())-2 # us all but 2 available processors
> library(MSI.EAGLE)
> MSI.EAGLE()
```

## Data Setup

The purpose of this tab is to import initial imaging data for processing. The interface provides a set of parameters for processing files in .imzML format. Currently only data in centroid mode is fully supported, although continuous data can be read in (not fully tested). Files in imzML format are read from the Raw files directory and a selectable table presented to the user. Once one or more files are selected, import parameters are selected to be used by [Cardinal 3](#) and the data imported via the "Import Data" button. After Import, a small subset of the data can be optionally processed directly in order to quickly check spectral quality via the "Extract Sample and MS Sample Plot". Full processing is available in the next tab.

Raw files directory (ie. imzML file location)  
/Volumes/User/Woolfork/Desktop/DESI

Working directory (ie. where results are stored)  
/Volumes/User/Woolfork/Desktop/DESI

**SELECT DIRECTORY**

Regex syntax to extract files if needed.  
(.\*).imzML\$

Parallelization mode  
 None  
 MulticoreParam  
 SnowParam

Number of cores to use  
6

Peak picking params  
qTof!

resolution for peakpicking (ppm)  
15

tolerance for spectral alignment (ppm)  
50

m/z min for import  
50

m/z max for import  
1700

**IMPORT DATA**

Limits for plotting spectrum to ensure MS looks reasonable  
% of pixels to randomly sample for plot  
1

**EXTRACT SAMPLE AND MS SPECTRUM PLOT**

Choose sample dataset  
Human Renal Cell Carcinoma (RCC)

**LOAD DEMO DATA**

See [Intro to BiocParallel](#) for Parallelization mode options

Select Number of Cores to Use  
Number of cores to use is typically  $\frac{1}{2}$  the available processors

Select Peak picking parameters  
peak picking parameters is a way to set default parameters for peak picking resolution and spectral alignment tolerance

Select resolution for peak picking (ppm)

Select tolerance for spectral alignment (ppm)

Select m/z min for import

Select m/z max for import

Select % of pixels to randomly sample for initial visualization plot  
For large datasets, its best to randomly sample only 1% of pixels

Choose Sample Dataset  
This is an option to load demo data for following the workflow of the MSI.EAGLE app. The data from this option will be used in the remainder of the app.  
Suggested parameters are located in images  
To load this package:  
> BiocManager::install("CardinalWorkflows")

Setup data directories, some options, and import .imzML files

folder= /Volumes/MacTork/MALDI/02-18-2023\_Files/  
wd= /Volumes/MacTork/MALDI/02-18-2023\_Files/

Show 10 rows

Select files for further processing

**files**

1	03012023_DESI vs MALDI Fly 100um_Slide_A_Analyte 2AFAMM
2	

Showing 1 to 2 of 2 entries

**Image visualization ions**

- All
- Custom

**Contrast enhancement**

**Smoothing options**

Draw color key?

Image plot width (px)

Image plot height (px)

Extended font options?

**Select files for further processing and Import Data**  
R Console will give object summary containing feature, pixel, and spectral information

**Extract sample and MS Spectrum plot to confirm proper data import**

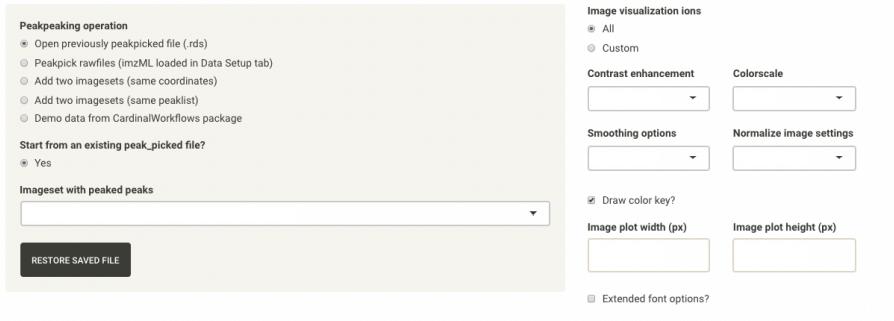
The plotting speed will depend on the size of the dataset and will initially need to be calculated and decompressed as a MSProcessedImagingExperiment object

**Other Features**

Image visualization ions: all ions or a custom m/z can be visualized for all selected files  
 Contrast enhancement, Colorscale, Smoothing options and Normalization image settings:  
 Draw color key places the key within the visualization plot  
 Image plot width and height: change the size of the plot.

## File Restore and Overview Analysis

The purpose of this tab is to manipulate datasets either by de novo peak picking, restore previously processed files, adding or subsetting existing datasets. 10-100 files / runs can be manipulated at once .



**Open previously peakpicked file (.rds)** opens a previously processed dataset using a peakpicked .rds file from the directory.

**Peakpicked raw files (imzML loaded in Data Setup tab)** opens a new dataset using raw data imzML files that must be already available in memory from the previous tab.

**Add two image sets (same coordinates)** combines two imagesets containing the coordinates. The coordinates must have been peak picked already, but usually with different peak lists (i.e., same x,y coordinates, but different m/z lists). The features selected option/filtered allows for uses of specific features to create subsets.

**Add two image sets (same peaklist)** combines two image sets containing the same peak list. The two sets must come from the same original peakpicked file (i.e., adding two datasets with different x,y coordinates but the same peak list). The features selected option/filtered allows for uses of specific features to create subsets.

## Segmentation – UMAP

The purpose of this tab is to segment a dataset to summarize the MS imaging experiment define regions of interest and assign phenotypes to the pixels. MSI.EAGLE implements two methods for this, UMAP in the first tab, and shrunken spatial centroids in the neighboring tab.

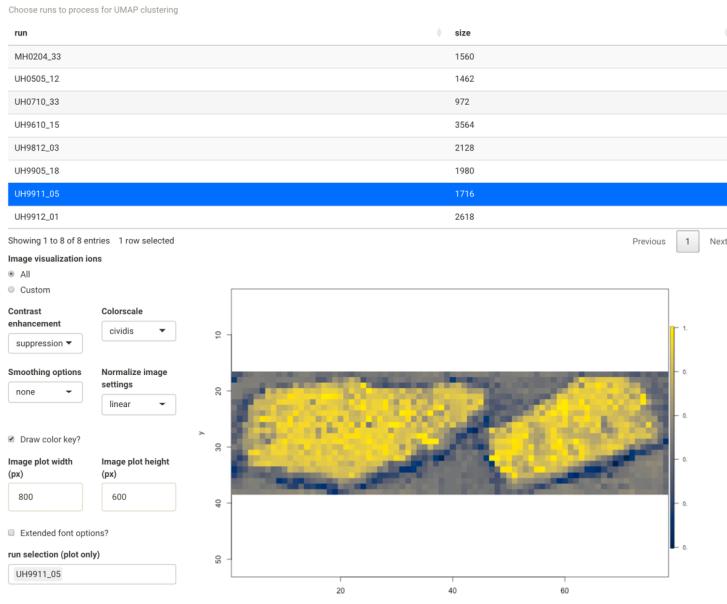
There are different goals for segmentation in this tab, both based on the Uniform Manifold Approximation and Projection for Dimension Reduction (UMAP) technique. This technique scales a dataset in size and dimension in a way that preserves the structure of the data, giving a visual representation of distinct data clusters that allows for assumptions to be made about the data. The implementation here relies on the '[uwot](#)' library in R and further information about specific parameters can be found in the package documentation.

Background removal can be used to remove the background of the slide used for imaging and anatomical segmentation is used to further segment tissue images for anatomical annotation. Both make use of UMAP parameters based on the nearest neighbors, the number of nearest neighbors used to construct the cluster and minimum distance between the points in the space. As the number of nearest neighbors increase, more neighboring points are connected and constructs a representation of the data that closely aligns with the global structure of the data. As the minimum distance increases, the UMAP spreads out the projected points and decreases the clustering of the data and reduces the global structure. The difference between the two implementations (background vs anatomical) is that the anatomical goal allows for access to a greater set of features for assigning and visualizing phenotype information assigned to the pixels, along with some slight differences in default parameters.

The UMAP PCA components runs a PCA on the dataset prior to UMAP, which is useful when the dataset is very large. The NULL feature runs on the entire dataset and gives more depth in colors and clustering. Tissue images uses a reduced dataset due to image size.

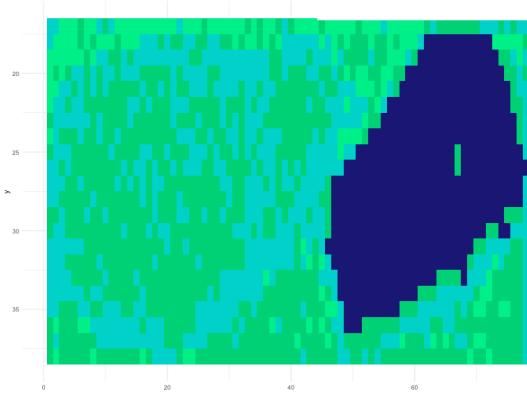
Color visualization of the UMAP embeddings can occur using R DBSCAN (via the 'dbscan' package or via method to color the UMAP space via mapping of x, y and z coordinates to rgb color space [1]. The R color reduction quantile allows for control of the color distribution of the clusters. A higher value should be used initially to accurately remove the background of the slide. With each successive UMAP, the value can be decreased to give more defined colors of background regions to be removed. The background can be segmented by removing colors one at a time while observing the original image as well as visualizing the UMAP space. The store processed data function should be used before beginning another UMAP of the image.

## Background Removal

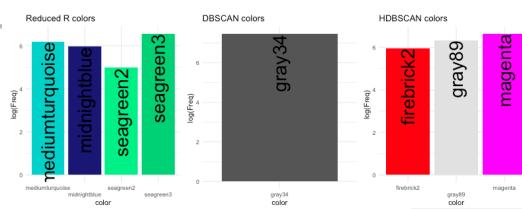
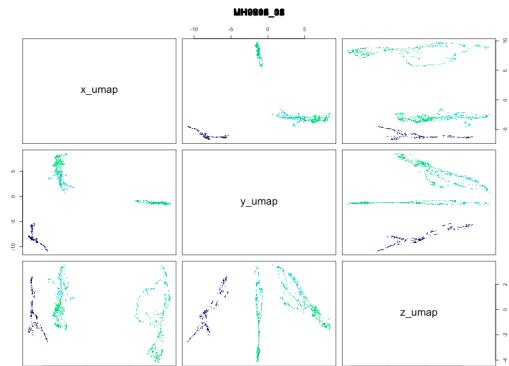


Example from demo data derived from the 'CardinalWorkflows' package. Select the data from the table to analyze via UMAP

UMAP may be performed as a group of data files or as individual data files. If performed as individual data files, the data must be restored and saved before unselecting the current file and selecting a new file to UMAP. Once all the data files are individually processed, then, the overall UMAP data can be saved as one file.



Original UMAP above, UMAP embedding (top right) and frequencies of pixels assigned by color (right)



Colors for UMAP visualization

R reduced

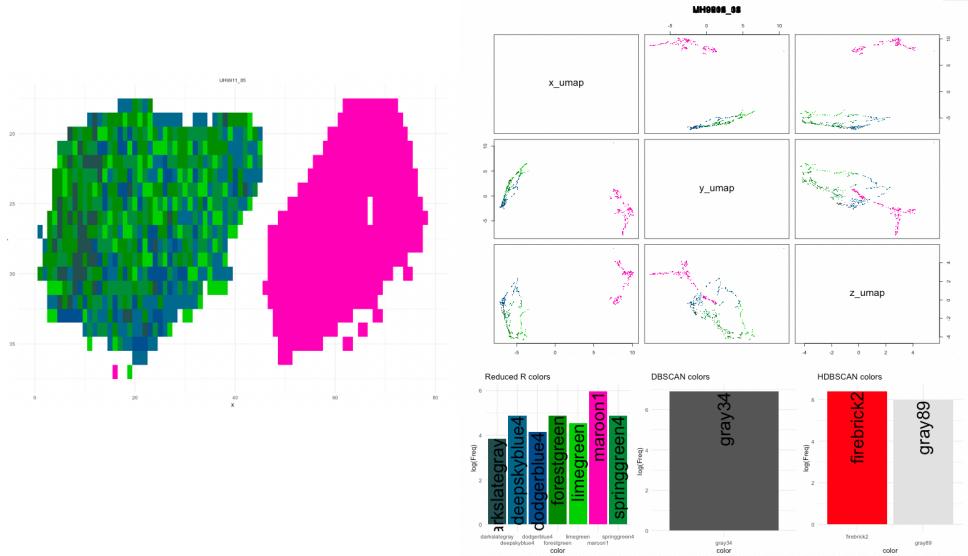
Choose colors

- mediumturquoise
- seagreen2
- seagreen3
- midnightblue

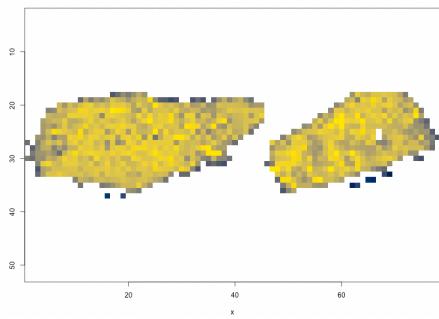
**STORE PROCESSED DATA** **SAVE PROCESSED DATA**

Uncheck the colors that corresponds to the background to be removed during the next UMAP.

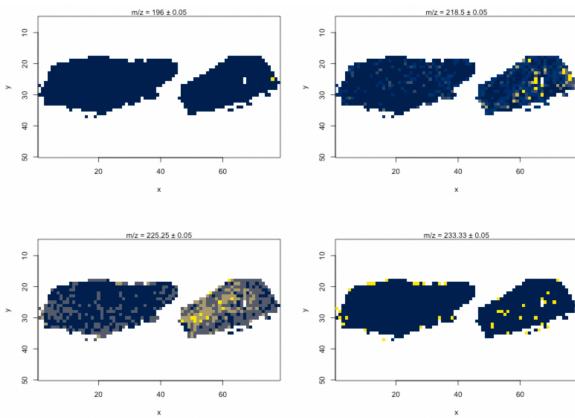
Store the processed data before reducing the R color reduction quantile and beginning the UMAP process again.



After successive UMAPs for background removal



Can check with your original image to make sure the background is being removed.



Once the background is successively removed, UMAP segmentation can also be for visualizing a specific ion using *custom image visualization of ions*.

This option can also perform *ion math* which can give the sum, min, max, standard deviation for the custom ions (current selection: none).

### Anatomical Segmentation

Under this option, the pData field for visualization must be set and Values to show must be defined or a new field must be added.

Select pData field for visualization

Values to show

Choose pData field to annotate:

Replace

Add new field

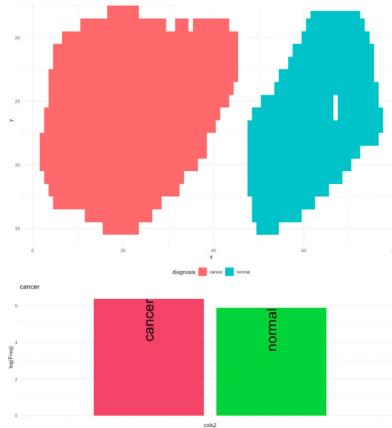
Choose field to annotate

Choose existing value

OR

Input new annotation value

**APPLY ANNOTATION**



## Segmentation – SSC

The purpose of this tab is to segment a dataset to summarize MS imaging experiment and define regions of interest.

While PCA components is a popular way for exploring a dataset, segmentation can also occur with spatial shrunken centroids (SSC) [as described by Bemis et al \(2016\)](#) [2]. This performs spatially aware nearest shrunken centroid clustering or classification a dataset. SSC is based on four parameters: method, r, s, and shrinkage (c or s). Method determines the spatial weight (Gaussian or adaptive). The r parameter is the smoothing radius, ranging from 2 and 5. The k parameter is the initial number of clusters, where a large value is higher than the expected number of clusters and the algorithm will automatically drop empty segments (ranges from 5 and 15). The c parameter default is the shrinking parameter ranging between 5 and 10, where higher values result in fewer contributing clusters to segmentation.

Because of the potential time-burden of performing SSC, a file is created which saves the analysis in the working directory. The user can re-access the file by inputting the same parameters used to generate the initial file. Each model is given as a separate option to visualize. Once a model is selected, three panels are plotted below the image plot. This is an image of the ssc clusters themselves, a weighted spectrum showing which masses are contributing to each cluster, and a plot of the statistic to visualize the highest contributing m/z values. Further analysis to extract specific m/z values can be performed by opening the saves ssc model file in R.

The SSC models may not work if there are pixels with few neighbors. As a result, the "Show fix stray pixel options?" check box is offered which will remove these stray pixels and can in some (but not all cases) allow the ssc to run smoothly. Note that at the moment one would need to perform the same stray pixel fix (same parameters) in order to restore a SSC model previously run otherwise a size mismatch will occur in the number of pixels. The SSC models are automatically saved to the working directory.

To reproduce the demo data as produced in this manual, below are the following parameters:

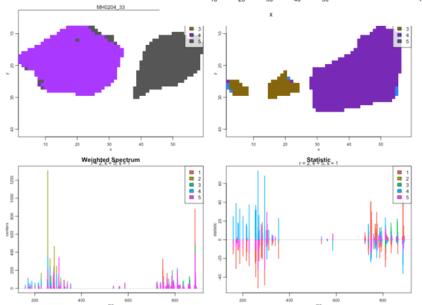
Under the Data Setup Tab:

- Load the RCC without background data
- Change the Tolerance for spectral alignment to 1200

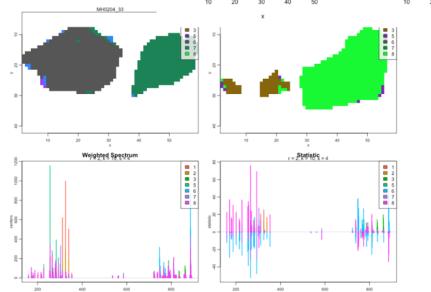
Commented [AW1]: App is now crashing

Under the File Restore and Overview Analysis

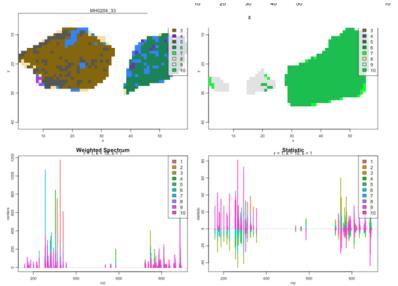
- Select Peakpick rawfiles
- Change the S/N for overview peakpicking to 3
- Change Peak picking method to mad
- Start peak picking



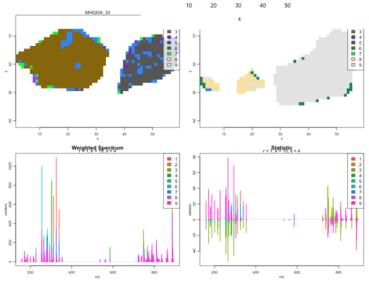
SSC segmentation for adaptive method  
 $r = 2, k = 5, s = 1$ ; shrinkage  $c(1,4)$



SSC segmentation for adaptive method  
 $r = 2, k = 10, s = 4$ ; shrinkage  $c(1,4)$



SSC segmentation for adaptive method  
 $r = 1, k = 10, s = 1$ ; shrinkage  $c(1,4)$



SSC segmentation for adaptive method  
 $r = 1, k = 10, s = 4$ ; shrinkage  $c(1,4)$

## Phenotyping

The purpose of this tab is phenotype MSI data. This is a critical step in being able to assign meaning of the data through statistics. Each pixel can have multiple phenotypes assigned. The approach used in MSI.EAGLE relies on removing the background pixels to then assign phenotypes based on areas with and without sample information. The automated method also relies on a grid-like structure of the input data.

Phenotyping can be done in two different ways. If starting from an existing peak picked file, the file must be restored from the file restore tab. If continuing data processing from segmentation, choose the 'Stored data' option. In addition, a text file with the phenotype data needs to be uploaded in the phenotyping tab and then read in. The text file must be tab-delimited with a .txt ending. The input text file for all methods must have minimally three columns, the first two being titled "Spot" and "Plate". The third and subsequent columns are the phenotype information (eg. Biological grouping, Dose, Time etc). The phenotype name will match the column header name. "Spot" must be specified at the grid position (A1, A2... B1, B2...etc.) of the sample. If there are only tissues, a single row (A1, A2, A3 etc.) can be used. When analyzing tissues, automated phenotyping in the app is dependent on collecting tissues in a grid-like pattern. The "Plate" variable defines the run to which the spot/sample is attributed. As such, some part of this variable must be contained in the run name (i.e., the name displayed on the table during import or display in the previous tabs). Furthermore, the "Plate" variable must be unique to each run. For if the runs were called 'run\_P1' and 'run\_P2', samples from the former could have plate values of 'run\_P1' or simply 'P1'.

Spot	Plate	Sample Name	Time Point
A1	P1	AGW#1	AM0800
A2	P1	AGW#3	AM400
A3	P1	AGW#4	AM2400
A4	P1	AGW#6	AM0400
A5	P1	AGW#7	AM1600
A6	P1	AGW#8	AM0800
A7	P1	AGW#13	PM2400
A8	P1	QC	NA
A9	P1	AGW#14	AM400
A10	P1	AGW#15	AM2400
A11	P1	AGW#16	AM0800
B1	P1	AGW#20	AM400
B2	P1	AGW#21	AM2400
B3	P1	AGW#24	AM400
B4	P1	AGW#25	AM400
B5	P1	AGW#27	AM0800
B6	P1	AGW#28	AM2400
B7	P1	QC	NA
B8	P1	AGW#29	AM1600
B9	P1	AGW#31	AM400
B10	P1	AGW#32	AM0800
B11	P1	AGW#33	AM0800

Sample data to insert into a .txt file for spotted samples

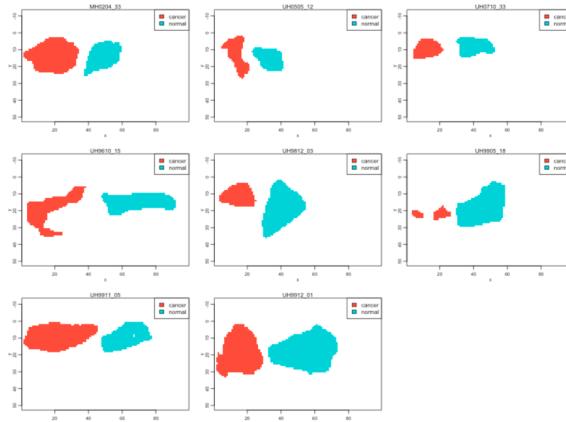
Spot	Plate	Sample Name	Phenotype
A1	P1	MH0204_33	Cancer
A2	P1	MH9294_33	Normal
A1	P2	UH0505_12	Cancer
A2	P2	UH0505_12	Normal
A1	P3	UH0710_33	Cancer
A2	P3	UH0710_33	Normal
A1	P4	UH9610_15	Cancer
A2	P4	UH9610_15	Normal
A1	P5	UH9812_03	Cancer
A2	P5	UH9812_03	Normal
A1	P6	UH9905_18	Cancer
A2	P6	UH9905_18	Normal
A1	P7	UH9911_05	Cancer
A2	P7	UH9911_05	Normal
A1	P8	UH9912_01	Cancer
A2	P8	UH9912_01	Normal

Sample data to insert into a .txt file for tissue samples

There are four phenotyping methods: spectral density, periodicity, breaks between samples, and manual.

Spectral density and periodicity will attempt to use the frequency of the pixel density along the x and y axis to estimate the minimum to find breaks between samples. The breaks method is a simpler version which looks for areas with no pixels to assign phenotype.

The manual method will assign phenotypes based on user specified x- and y- start and end positions. These are specified in the input phenotype text file through four columns (xstart, xend, ystart, yend) added to the phenotyping .txt file for each sample.



Plotting of phenotype data by diagnosis (cancer vs normal) tissue samples

The phenotyping data (once applied) can also be plotted based on one of the columns, as well as interaction terms can be added which may be useful for downstream statistics (for example to define grouping variables). Based on these choices, various phenotyping data can be added into different or combined files by clicking the save phenotype data button.

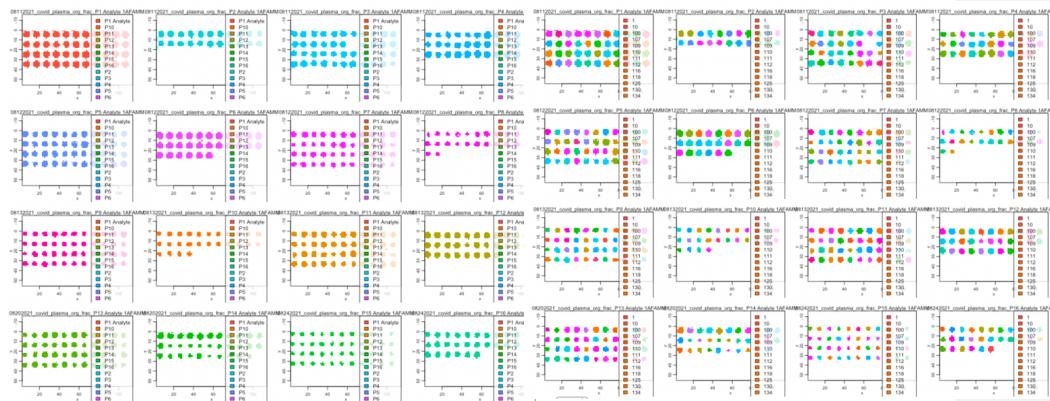


Figure 1. Interaction data added for phenotyping

## Depth Analysis

The purpose of this tab is to analyze specific pixels in the data without the burden of analyzing the complete dataset. This may be useful in a number of situations, such as analyzing only tissue pixels once background is removed or analyzing only certain tissue structures. By refining the analysis, targeted methods benefit by removing noise from pixels not relevant to the analysis which can be particularly important for low-abundance peaks which may get removed by a frequency filter (i.e., the requirement that a peak must appear in 1% of the data to not be considered noise). Another advantage of this approach is the ability to use a mean spectrum for peak picking which is reflective of tissue / pixels of interest which will also reduce spurious noise.

Because the Depth analysis relies on re-analysis of the data, the source imzML files much be available and open in memory from the 'Data Setup' tab prior to any analysis.

The 'Imageset with segmented coordinates' file is a processed file which has some subset of pixels in the data (for example, tissue only, background removed). These sample coordinates will be used to specifically extract and perform the analysis on the raw data. Note that the run names in the input coordinate file and the .imzML file must match exactly since we are assuming this is a reanalysis of the same data. This can be a bit tricky if the imzML files are being read from a sub-directory, in which case the names may not be an exact match.

The other options are analogous to those from the 'File Restore and Overview Analysis tab'

# Statistics

The purpose of this tab is to perform statistical analysis on MSI data. There are three methods from Cardinal (means test, supervised SSC, and DGMM). A description of these and specific use cases are fully described in the Cardinal manual.

## Means Test

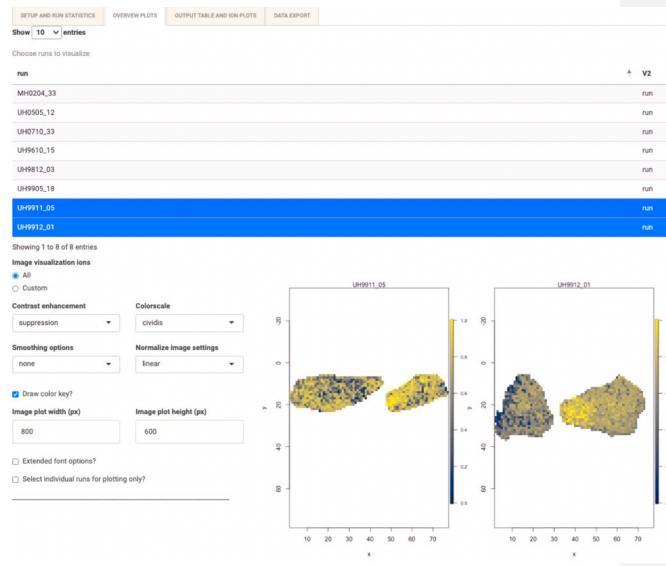
To begin statistics, the file generated from phenotyping must be read in for analysis. The simplest test is a means test from Cardinal which compares the mean of a variable in a group with the same variable in other groups across all pixels of interest. The variable for testing is then chosen and then members of this variable can be chosen along with interaction for grouping. The grouping variable should define the 'biological' replicate of the sample. As a result, all pixels and technical replicates from the same biological replicate will be considered together. After running the test, a notification will appear, and a table of the generated significant features will appear at in the 'Output table and ion plots' subtab in the main window. The FDR threshold can be adjusted based on needs and a value of 1 will give the entire list. The data table can be exported with selected variable as a CSV, excel, or PDF file.

To reproduce the demo data as produced in this manual, below are the following parameters:

### Means Test (Cardinal)

The screenshot shows the 'MEAN TEST (CARDINAL)' configuration window. At the top, there are four tabs: 'SETUP AND RUN STATISTICS' (selected), 'OVERVIEW PLOTS', 'OUTPUT TABLE AND ION PLOTS', and 'DATA EXPORT'. Below the tabs, the 'Imageset for analysis' dropdown contains the path: [1] "working filename= background\_Users\_ashwoo/Desktop/\_0.Temp\_MSI-proc\_hi\_SN-peak\_picked-subset-2023-09-14.rds". Under 'Type of test', the 'Means test (Cardinal)' radio button is selected. In the 'Choose variable to test' dropdown, 'diagnosis' is chosen. In the 'Choose members to include' field, 'cancer normal' is entered. In the 'Choose interaction for grouping' field, 'run diagnosis' is entered. The 'FDR threshold to include in results' input field is set to 0.05. On the right side of the interface, a note states: 'The Imageset for analysis is a saved RDS file with background from the demo data'. At the bottom, there are buttons for 'RUN TEST', 'SAVE MEANS TEST MODEL', and 'RESTORE MEANS TEST MODEL'.

Select plots to view

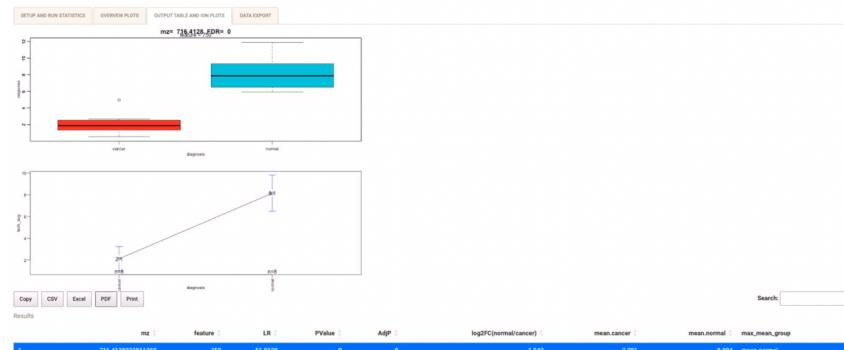


	mz	feature	LR	PValue	AdjP	log2FC(normal/cancer)	mean.cancer	mean.normal	max_mean_group
1	716.4128233811258		750	51.0128	0	0	1.942	2.291	8.804 mean.normal
2	808.31155052151934		890	48.7747	0	0	1.623	4.068	12.531 mean.normal
3	808.2567210424116		889	39.8979	0	0	1.934	4.058	15.509 mean.normal
4	740.3572055267042		794	38.5411	0	0	1.832	5.102	18.168 mean.normal
5	714.4717437515177		747	34.2426	0	0	2.337	2.281	11.524 mean.normal
6	714.941319115965		748	34.3553	0	0	1.384	2.155	5.626 mean.normal
7	742.444688428813		796	32.6884	0	0	1.757	5.298	17.901 mean.normal
8	215.2334970328965		54	31.1804	0	0	2.381	13.48	70.206 mean.normal
9	715.5781471790017		749	29.6014	0	0.00001	1.325	1.805	4.524 mean.normal
10	717.090265107317		751	29.1577	0	0.00001	1.052	2.135	4.427 mean.normal

Showing 1 to 10 of 70 entries

Previous 1 2 3 4 5 6 7 Next

Table of significant features with FDR threshold: 0.05. To generate a table with all results, change the FDR threshold to 1.



When selecting a feature, the means test generates box-and-whisker plots to visualize the differences between the conditions.

The means test model (and all other models in this tab) can be saved using the save button and reopened from a saved R.Data file in this tab.

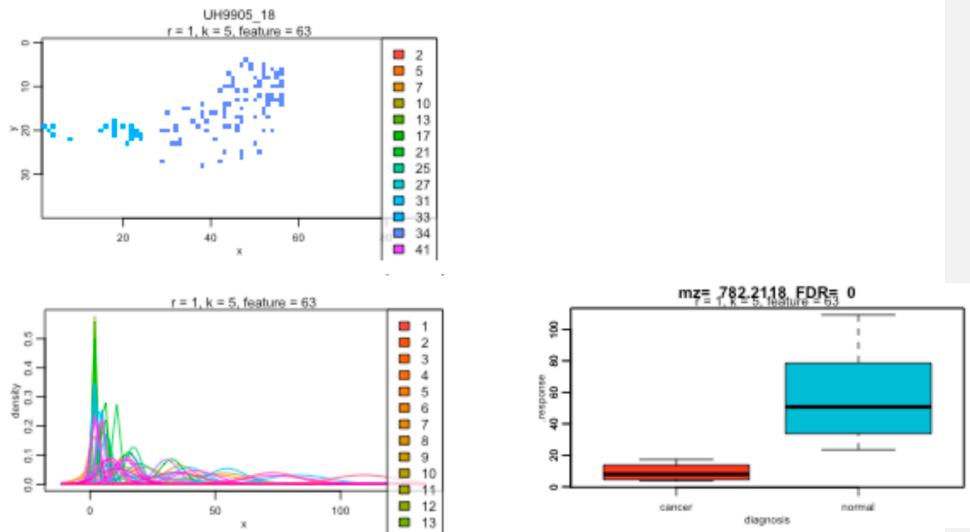
### Spatial Shrunken Centroids

**Commented [AW2]:** Visualization is a problem

### Spatial DGMM

The **Spatial DGMM** examines features individually as opposed to the entire data set. In this model, the variable to test, r value, k value, interaction groups and members are chosen (see segmentation tab) for modeling density plot and box-and-whisker plots are used to visualize the data [3].

**Commented [AW3]:** Still need to edit based on original paper



Example of density and box-and-whisker plots for DGMM model

## Heatmap

The purpose of this tab is to generate heatmaps based on statistical analysis performed on MSI data from the Statistics tab.

To reproduce the demo data as produced in this manual, below are the following parameters:

Choose variables for heatmap  
diagnosis run

Grouping variables  
 Show only significant peaks  
 Annotate columns  
 Annotate rows  
 Remove legend  
 Scale row  
 Scale col  
 Cluster rows  
 Cluster cols  
 Remove col names  
 Remove row names

Choose labels  
m/z

kmeans k-value

cutree row clusters

cutree col clusters

clustering method (R hclust)  
complete

column distance method  
euclidean

row distance method  
euclidean

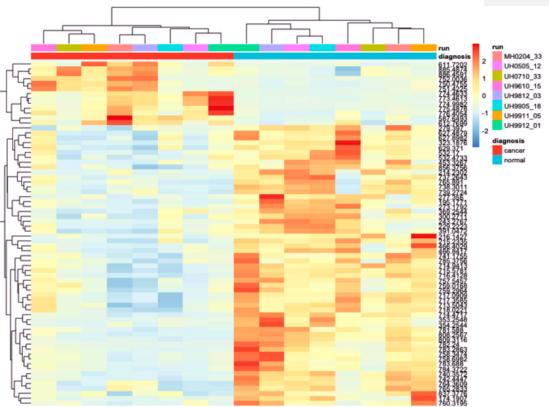
Choose filtering column for heatmap  
AdP

Filtering stat direction?  
 Ascending  
 Descending

Significance cutoff  
0.05

**GENERATE HEATMAP** **DOWNLOAD**

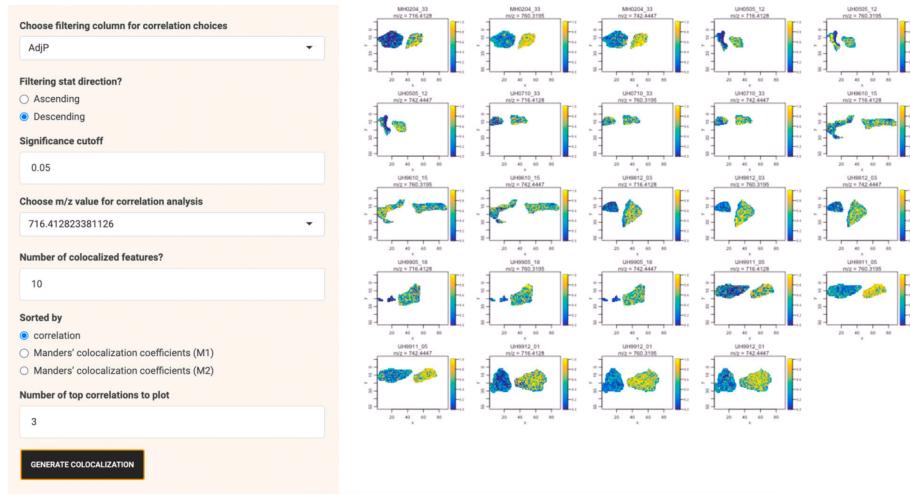
All grouping variables need to be in the field to generate individual rows on the heatmap



## Colocalization Analysis

The purpose of this tab is to visualize colocalization between features.

To reproduce the demo data as produced in this manual, below are the following parameters:



The console will generate a table of the top ten colocalized ions.

Colocalized features:					
	mz	correlation	M1	M2	
1	716.4128	1.0000000	1.0000000	1.0000000	
2	760.3195	0.4391020	0.6237656	0.6239710	
3	742.4447	0.4334985	0.6171824	0.6173856	
4	764.3609	0.4249884	0.6247531	0.6247531	
5	782.2400	0.4236315	0.6323239	0.6323239	
6	738.3011	0.4180463	0.6237656	0.6237656	
7	714.4717	0.3943332	0.6263989	0.6263989	
8	808.2567	0.3757609	0.6369322	0.6369322	
9	740.3572	0.3661958	0.6175115	0.6175115	
10	353.2548	0.3487755	0.6520737	0.6520737	

## **References**

- [1] Anal. Chem. 2019, 91, 9, 5706–5714. Publication Date: April 2019.  
<https://doi.org/10.1021/acs.analchem.8b05827>
- [2] MCP. 2016, 15, 5, 1761-1772. Publication Date: May 2016.  
<https://doi.org/10.1074/mcp.O115.053918>
- [3] Bioinform. 2019, 35, 14, 208-217. Publication Date: July 2019.  
<https://doi.org/10.1093/bioinformatics/btz345>