**Guide: From MSI data to publishable figures**

This guide explains the workflow of going from Mass Spectrometry Imaging (MSI) data to having figures that fit with journal publishing standard (i.e. overlaid images are not flat and can be edited as vector elements - <https://www.nature.com/documents/nprot-guide-to-preparing-final-artwork.pdf> )

You will start with the MSI data (imzML and ibd), extract intensity-containing tif-images for your m/z-values of interest with an R-script, and then convert the tif-images to .svg-files with a viridis look-up table.

The guide assumes that you have installed:

* R 4.2.2 (or newer) - <https://cran.r-project.org/>
  + Tidyverse 2.0.0 (or newer) – Can be installed in R with install.packages(“tidyverse”)
  + Cardinal 3.0.1 (or newer) – <https://www.bioconductor.org/packages/release/bioc/html/Cardinal.html>
  + ijtiff 2.3.0 (or newer) - Can be installed in R with install.packages(“ijtiff”)
* FIJI 2.11.0 <https://imagej.net/software/fiji/downloads>
  + BioVoxxel 2.5.7 <https://imagej.net/plugins/biovoxxel-toolbox> - Can be installed in FIJI by going to "Help" --> "Update..." --> "Manage update sites" and tick the "BioVoxxel" update site. Click "close", "apply changes" and restart ImageJ.

It also assumes that you have normalized MSI data in imzML and ibd format. Data is normalized by Total Ion Current (TIC) or Root mean square (RMS) before being uploaded to METASPACE.

**Download MSI data**

On METASPACE you can download the imzML and ibd data for you sample like so:

1. Find your datasets of interest
2. Click “dataset overview”
3. Click “actions” and then “download”
4. Download both files (imzML and ibd)
5. Place the files in a fitting directory on your PC.

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A picture containing graphical user interface

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**Prepare a feature list with m/z-values**

Our goal with the R-script is to extract intensity-containing tif-images, for different m/z-values. However, an ibd-file has tens of thousands of mass spectra. Thus, we need to limit the number of m/z-values that we create images for.

The main function in R requires a feature list, which is really just a data frame with a column named “m/z” containing m/z values that are known to exist in the data. You can create a table yourself, or – especially if you are interested in many m/z values – you can extract all the annotated masses of your data from METASPACE.

1. Go to the “annotations” tab in metaspace
2. Change the Database to the relevant database for your study
3. Under “add filter” add “select dataset”
4. Select all of your relevant datasets
5. Change the FDR to 50% to include molecules identified at 50% False Discovery Rate confidence
6. Click Export to CSV and select “annotations table”

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The downloaded table can be used as your Features list.

**Extract tifs with R**

Open the R-script extract\_imzml\_tifs.R. The script contains a main function extract\_peak\_imgs() which takes a Cardinal::MSImagingExperiment, a dataframe of mz-values, and an export directory and returns 32-bit tiff-images.

There is example code for data import with Cardinal, for import of feature metadata and for filtering of the feature metadata.

Once you have your files ready, you can extract the tifs using

extract\_peak\_imgs(msi\_data, features, out\_dir, overwrite = T, bits\_per\_sample = 32)

You can choose to never overwrite images by setting overwrite = F, and change the bit depth (to e.g. 8, 12, 16). 32 bit is recommended to include as much information as possible.

Once you have run the function, the output directory should be populated with directories named after your m/z-values, and each of these directories should contain one image per sample.

**Convert tifs to viridis svg**

The final step is to use FIJI to convert your tif-images to publishable figures.

Open the imageJ macro imzmltif\_to\_viridis.ijm.

You can either open the macro file itself to run it or open the macro editor in FIJI with ctrl+shift+n and then open the macro itself. Run the macro from the macro editor with ctrl+r.

When you run the macro, it will ask for an input directory (the one you populated with R), a file name suffix (tif), and a pixel length number and pixel length unit. You can find the latter in METASPACE under Annotations by scrolling down underneath the image and expanding the metadata section. The number on METASPACE is µm / px (i.e. the length of 1 pixel in µm).

The macro will create a new directory called “input”\_viridis with directories for each m/z value, which should be populated with .svg files. These files can be imported into Inkscape or Illustrator and exported as .pdf for publication.