

MALDI Imaging Image Analysis Flow User Guide

Table of contents

1. Introduction
2. User Interface
 - 2.1 Load Files
 - 2.2 Preprocess
 - 2.3 Binning
 - 2.4 Peak Picking
 - 2.5 Kmeans

1. Presentation.

This is an analysis flow for MALDI Imaging images developed by Ismael Luna Alvarez, junior MS Data Analyst and Eduardo Chicano Galvez Coordinator of the mass unit of IMIBIC.

The flow has been developed based on the R programming language and the Cardinal package for image analysis. The interface has been developed with R's interface creation package, Shiny.

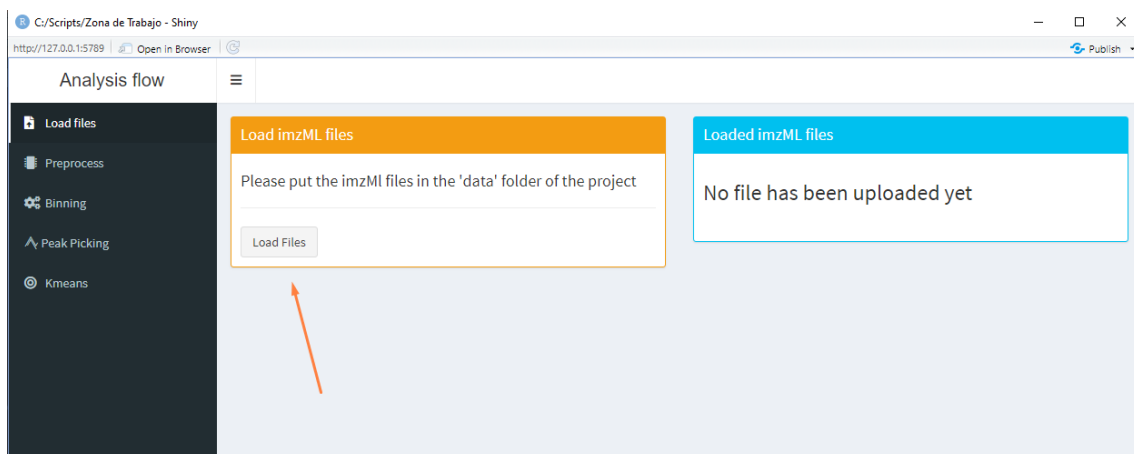
2. User Interface.

2.1 Load Files

To upload our files all we have to do is include our .IMZml and .ibd files in the data folder that was created when we unzipped the initial file.

Nombre	Fecha de modificación	Tipo	Tamaño
Imagenes	23/09/2021 13:18	Carpeta de archivos	
tejidoA2cristalA2.ibd	25/02/2021 8:41	Archivo IBD	391.501 KB
tejidoA2cristalA2.imzML	25/02/2021 8:41	Archivo IMZML	9.576 KB
TejidoBcristalA2.ibd	25/02/2021 12:18	Archivo IBD	674.688 KB
TejidoBcristalA2.imzML	25/02/2021 12:18	Archivo IMZML	16.505 KB

Once we have them inside the folder in our interface, we select the upload files section and click on the upload button.



And the files will be uploaded

Loaded imzML files

The uploaded files are:

Show **10** entries Search:

	imzMLFiles	
1	tejidoA2cristalA2	
2	TejidoBcristalA2	

Showing 1 to 2 of 2 entries Previous **1** Next

2.2 Preprocess

In the preprocess tab we will have a series of parameters that we can change when carrying out the preprocessing.

Preprocess

The files will be pre-processed with the methods you select below

Normalize method:

tic

Do you want to apply the Smooth method to pre-processing?

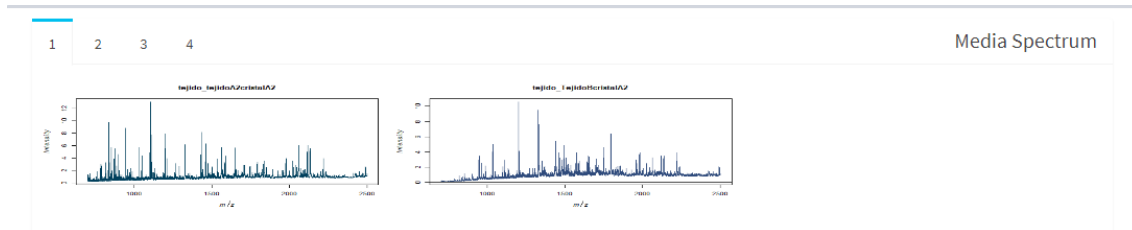
☒ No
☐ Yes

Do you want to apply the Reduce baseline method to pre-processing?

☒ No
☐ Yes

Preprocess Files

Once we have adjusted our parameters we will continue with the preprocessing and automatically in the next window we will see the average spectra of each fabric.



Once we have the preprocessing done, in the tab below we can calculate the 3 maximum intensities for each tissue and generate an image with each one of them, which will be automatically saved in the data folder.

Max Intensities

Obtain the 3 maximum intensities

File:

tejidoA2cristalA2 ▼

Plusminus:

0.05

0.5

0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5

Calculate

Generate Image

Max intensity:

1106.1757719715 ▲

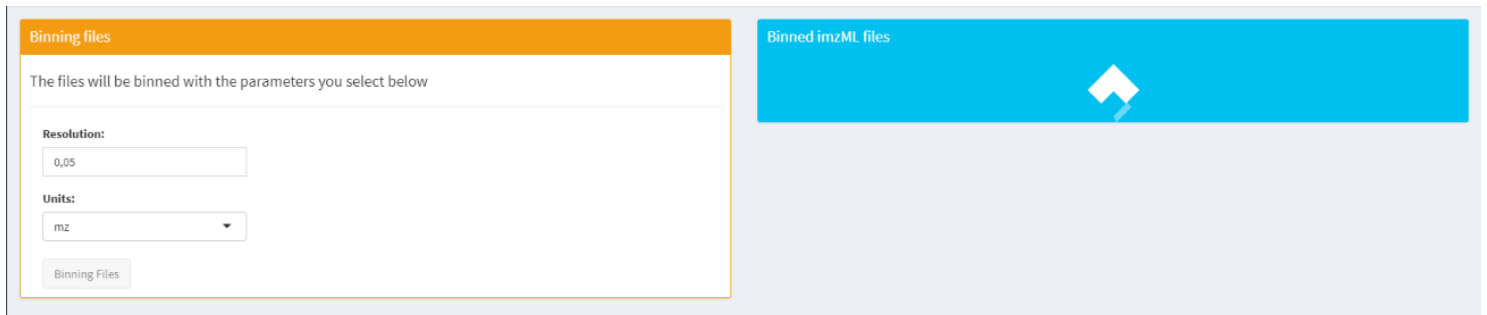
1106.1757719715

836.817102137767

1105.95074384298

2.3 Binning

In the binning tab we only have to choose the correct parameters for the process and execute it with the Binning button.

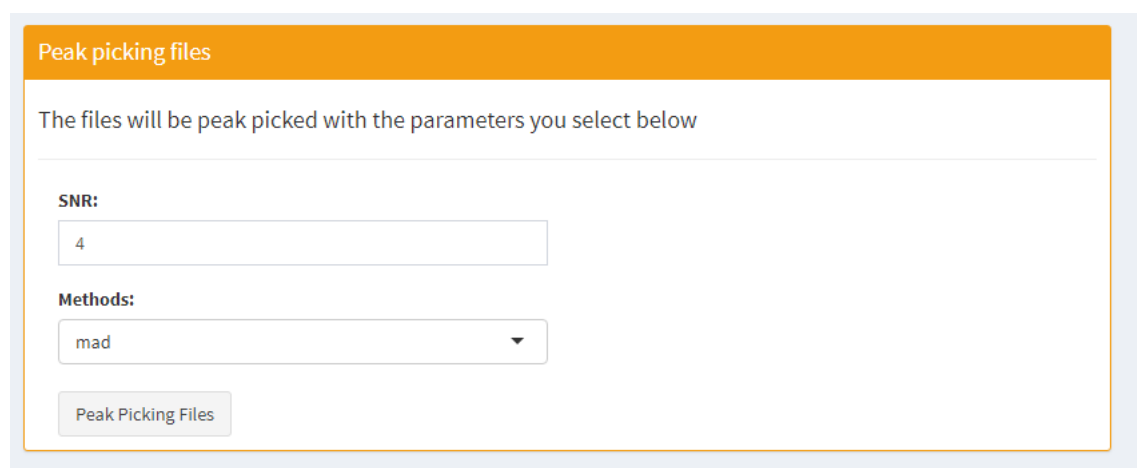


The screenshot displays the 'Binning files' interface. It features an orange header bar with the text 'Binning files'. Below the header, a message states: 'The files will be binned with the parameters you select below'. The interface includes two input fields: 'Resolution:' with a text box containing '0,05', and 'Units:' with a dropdown menu showing 'mz'. A 'Binning Files' button is located at the bottom left. On the right side, there is a blue header bar with the text 'Binned imzML files' and a white upward-pointing arrow icon.

Once the operation is complete, the files are ready for the next step.

2.4 Peak Picking

In peak picking, as in binning, we only have to choose the parameters with which we want to perform the calculation and proceed with it.



The screenshot displays the 'Peak picking files' interface. It features an orange header bar with the text 'Peak picking files'. Below the header, a message states: 'The files will be peak picked with the parameters you select below'. The interface includes two input fields: 'SNR:' with a text box containing '4', and 'Methods:' with a dropdown menu showing 'mad'. A 'Peak Picking Files' button is located at the bottom left.

2.5 Kmeans

In the Kmeans section we can create the clusters for each fabric separately with our respective parameters.

Kmeans cluster

A cluster object (Kmeans) shall be created with the following attributes

Load peaked files

File:
tejidoA2cristalA2

R:
1

K:
10

Methods:
Lloyd

Kmeans

Once the clusters have been created and the tissue has gone through the Kmeans process, we can also perform a digital dissection of the different clusters to obtain a version of the original file but dissected by clusters of our choice.

Virtual dissection

A virtual dissection will be carried out with the following parameters

Load clusters

Selects clusters for dissection
1 4 5 10 9

Digital dissection