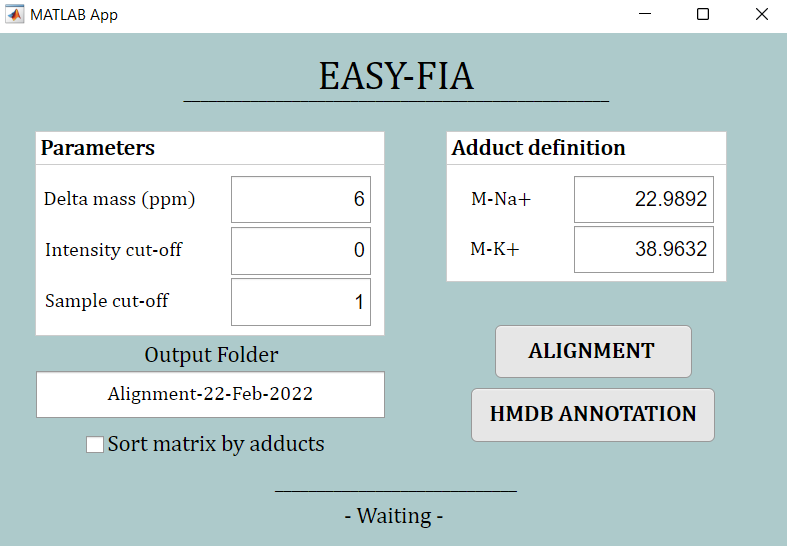
**EASY-FIA USER MANUAL**



EASY-FIA is a MATLAB application which performs blank subtraction, m/z alignment and HMDB identification of FIA-HRMS metabolomics data. It provides the user a GUI in order to set some parameters for the elaboration and to keep track of the ongoing processes.

To employ EASY-FIA, the user must download both the exe file names *EASY\_FIA.exe* and the function *import\_spectra\_csv.m* and put them in the same folder.

Before using EASY-FIA, the user must ensure that version 9.11 of MATLAB Runtime is correctly installed on the computer. To install the Runtime, the user must download from Mathworks website (<https://it.mathworks.com/products/compiler/matlab-runtime.html>) the proper version according to the computer operative system. The downloaded folder should be decompressed, then the user can proceed with the setup through the appropriate file following the instructions.

Once the Runtime is correctly installed, the user might check if metabolomics data are in the appropriate folder format. EASY-FIA requires all the sample and the blank *csv* files to be in the same folder, which must be called POS or NEG according to the ionization mode of the acquisition. Inside this folder, each blank must have the same name of the corresponding sample, with the prefix “wash” or “lav”. For instance, if the first sample is named “1”, the blank must be named “wash\_1” or “lav\_1”. The app is case sensitive and accepts the following prefixes: “wash”, “WASH”, “Wash”, “lav”, “LAV”, “Lav”. We suggest keeping the names as short and simple as possible, avoiding too much punctuation.

The accepted *csv* file must contain the *m/z* list in the first column, and the corresponding intensity in the second one. The following figure is an example of the accepted file format.

Immagine che contiene tavolo

Descrizione generata automaticamente

Before proceeding with the alignment, the user can modify some of the elaboration parameters from the *Parameters* box:

* *Delta mass (ppm)* is the tolerance interval of the instrument used for the acquisition
* *Intensity cut-off* is a threshold under which the intensities are discarded
* *Sample cut-off* is the minimum number of intensities that each *m/z* must have in order to be considered valid

The user can also decide whether to select or not the option *sort matrix by adducts*, which is available for positive ionization mode only*.* If so, the intensity matrix will be ordered in such a way that each *m/z* is listed next to its possible adducts.

When the alignment process ends, the notification “Alignment section – THE END” appears on the GUI, and the user can go on with the HMDB annotation.

The annotation section requires the user to select first the *excel* file produced by the previous alignment section and saved in the appropriate output folder, then the database *mat* file (positive or negative according to the acquisition mode). When the annotation section ends, the notification “Annotation section – THE END” appears on the GUI and the result of annotation can be found in the same output folder of the alignment section.