Instruction Manual for Image Fusion Program

1. Purpose

The main purpose of this software is to enhance the resolution of tissue slice mass spectrometry imaging(MSI), which needs to integrate MSI images with morphological images based on neural network algorithms. Furthermore, a higher resolution image of the distribution of various metabolites on the tissue surface can be obtained.

2. Principles

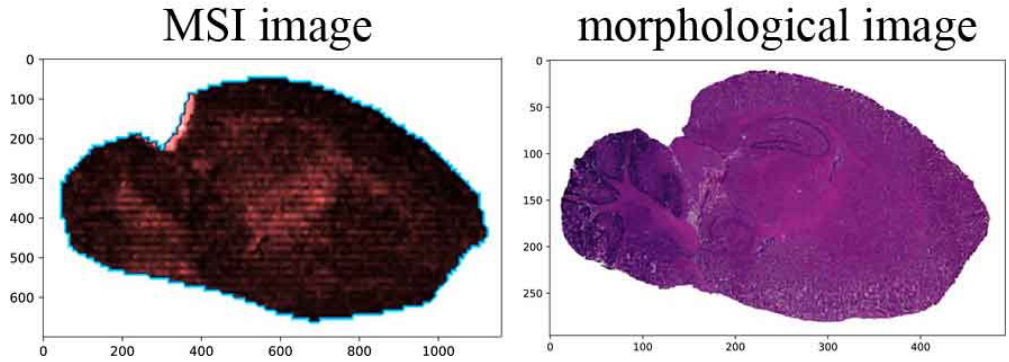


Figure 1

As shown in Figure 1, the left image is the image obtained from MSI of tissue slices. This image can be directly exported from the MSI file or generated through various processing methods. The image on the right is a morphological image.

The acquisition methods of MSI images and morphological images are completely different, and their resolutions also differ greatly. The integration of MSI images and morphological images needs to realize pixel-to-pixel correspondence between these two kinds of images.

The principle of realizing pixel correspondence is to select regions on morphological images, which correspond to MSI image selections through a series of reversible transformations. This transformation process is recorded to until pixel-to-pixel correspond between these two kinds of images.

Figure 2 briefly illustrates this process.

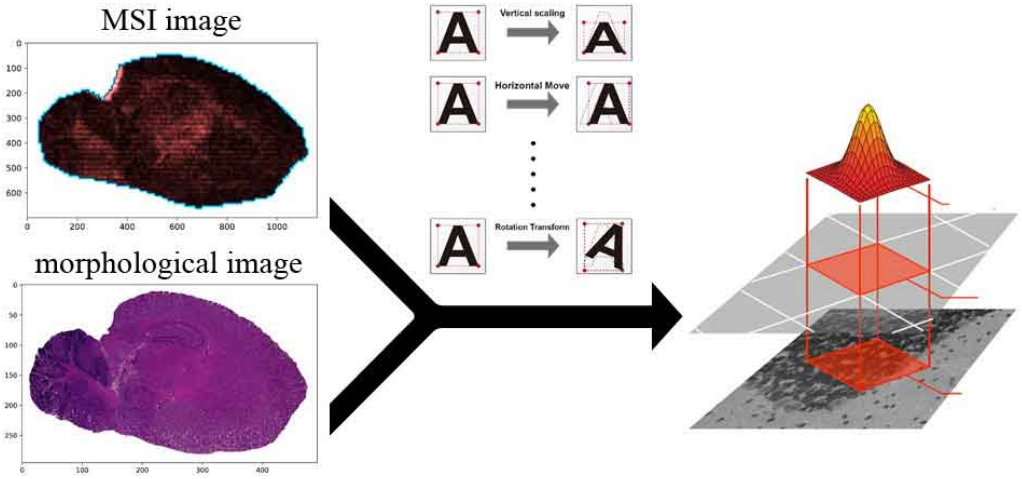


Figure 2

After pixel correspondence between MSI images and morphological images is completed, a list of pixels corresponding information to each pixel can be obtained.

Here, we set some constants. (Table 1)

Table 1

Abbr.	Exp.
n_sample	The number of pixels extracted from HE image for neural network training
n_feature	The number of features extracted from HE image
n_metabolites	The number of metabolites used for training neural network
Input Value	The matrix used as the input value when training the neural network
Target Value	The matrix used as the output value when training the neural network

Each morphological image pixel serves as a ***sample***, and the RGB values on each pixel serve as ***feature***. The intensity of metabolites contained in pixels on the corresponding MSI image is used as a ***Target Value*** to generate a neural network training dataset.

Next, various transformations on morphological images were performed to achieve diversity in training data. (Figure 3)

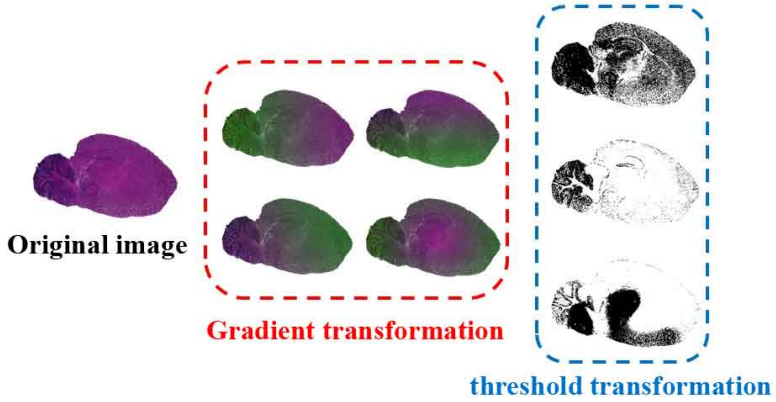


Figure 3

Next, a ***n_sample*n_feature*** data matrix based on morphological image was obtained, which is used as the ***Input Value***. A ***n_sample*n_metabolites*** data matrix was obtained for ***Output Value***. Users can use this data for neural network training.

After training the neural network, users can reselect the slice regions of interest and extract each pixel in the selected region to form a dataset. By inputting this dataset into a trained neural network, the predicted intensity of each metabolite at each pixel can be obtained.

By reconstructing the metabolite intensity predicted by the neural network according to the coordinates of the original pixel points, the image generated by fusing MSI and morphological images can be obtained.

3. Instruction

This software is written based on Python 3.2. The Python libraries required in the software include PyQt5，Matplotlib，Numpy，Scipy，xlrd，xlwt，progressbar，pyimzml，os and PIL。

This software needs to be used in conjunction with '**Pixel-to-Pixel Correspondence Program**'.

3.1 Obtaining MSI Data

Users need to refer to the '**Pixel-to-Pixel Correspondence Program**' to perform MSI selection and corresponding selection data export operations. Then, an Excel file was generated after export. (Figure 4)

	A	B	C	D	E	F	G	H
1	Metabolites Names	Selection x	Selection y	Reflect x	Reflect y	104.10699Selection intensity	104.10699Reflect intensity	104.10699Intensity difference
2	胆碱	44	3	72	144	20365	14587	-5778
3	乙酰胆碱	45	3	71	144	12742	11529	-1213
4	乙酰胆碱碎片	46	3	70	144	24026	29103	5077
5	GABA	47	3	69	144	30568	16832	-13736
6	GABA碎片	59	4	57	143	22665	30548	7883
7	DA	58	4	58	143	9232	24733	15501
8	去甲肾上腺素	57	4	59	143	20039	30997	10958
9	谷氨酸	56	4	60	143	22229	22876	647
10	谷氨酰胺	55	4	61	143	32067	23942	-8125
11	二羟基苯乙酸	54	4	62	143	21855	35437	13582
12	3-甲氧基酪氨酸	53	4	63	143	20734	26271	5537
13	天门冬氨酸	52	4	64	143	31064	31407	343
14	天门冬酰胺	51	4	65	143	28459	36327	7868
15	腺苷	50	4	66	143	20149	37378	17229
16	AMP	49	4	67	143	31775	35114	3339
17	ADP	48	4	68	143	34153	33143	-1010
18	肌酸	47	4	69	143	31277	31506	229
19		46	4	70	143	26395	42360	15965
20		45	4	71	143	32523	25106	-7417
21		44	4	72	143	33756	39857	6101
22		43	4	73	143	26663	26865	202
23		42	4	74	143	24675	26447	1772
24		39	5	77	142	25573	28536	2963
25		40	5	76	142	25250	18383	-6867
26		41	5	75	142	23548	23746	198
27		42	5	74	142	26190	33650	7460
28		43	5	73	142	30811	33382	2571
29		44	5	72	142	32418	26753	-5665
30		45	5	71	142	35193	31286	-3907
31		46	5	70	142	27044	42727	15683

Figure 4

3.2 Select image files

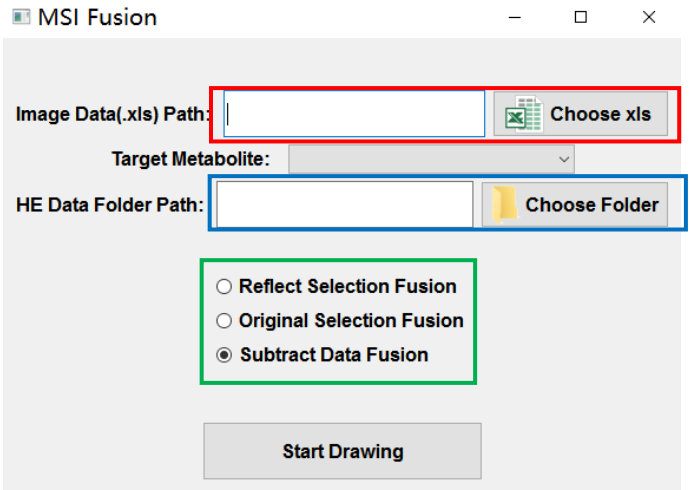


Figure 5

As shown in Figure 5, users should select the Excel file generated in 3.1 in the red frame. After selection, the target metabolite name will appear in the drop-down list frame. Then, users can select the target metabolite to be imaged. The folder containing morphological images should be selected in the blue frame. In this folder, multiple morphological images are supported, including images generated by various transformations on the original morphological images. The images contained in this folder will be used as raw data for neural network training.

There are three options in the green frame. Users can choose to fuse the original selection image, reflect selection image or difference image as needed.

Finally, click the 'Start Drawing' button to start imaging.

3.3 Image fusion interface

The image fusion interface is shown in Figure 6. This interface is mainly divided into MSI image area (red frame), morphological image area (green frame), and functional area (blue frame).

Table 2 provides an overview of the functions of each button in the interface.

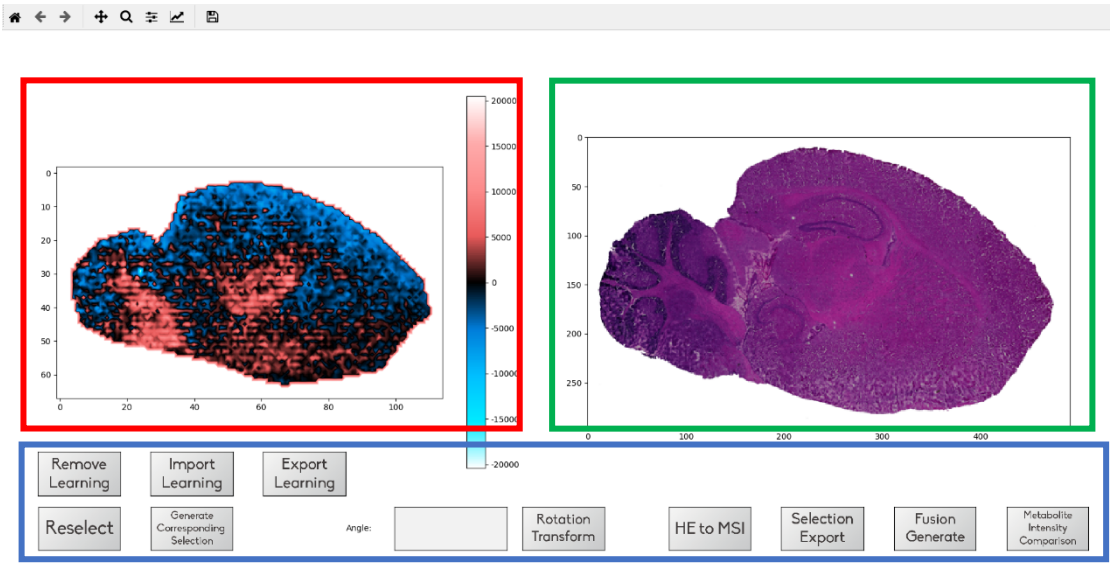


Figure 6

Table 2

Name	Pattern	Function
Remove Learning	Remove Learning	Remove the learning trajectory; click to clear the current state learning trajectory
Angle	Angle (clockwise positive):	Angle input; input rotation angle (clockwise is positive)
Rotation Transform	Rotation Transform	Rotation transformation; click on the image area selection area to rotate the angle input in the frame 'Angle'
Import Learning	Import Learning	Import learning trajectory; click to import learning trajectory from file to current state
Export Learning	Export Learning	Export the learning trajectory; click to import the current state learning trajectory into the file storage
Generate Corresponding Selection	Generate Corresponding Selection	Generate corresponding selection area; click to select the current image area and generate corresponding selection area according to the learning trajectory
HE to MSI	HE to MSI	Map the trajectory in the morphological image area to the MSI image area

Selection Export	Selection Export	Export selected area data; click to export the MSI pixel data contained in the selection area and the corresponding reflection selection area
Fusion Generate	Fusion Generate	Generate fused images using data exported from 'Selection Export'
Metabolite Intensity Comparison	Metabolite Intensity Comparison	Compare the intensities of various metabolites contained in the selected pixels of the MSI image area

3.4 **Software functions and usage workflow**

The workflow of the software is shown in Figure 7.

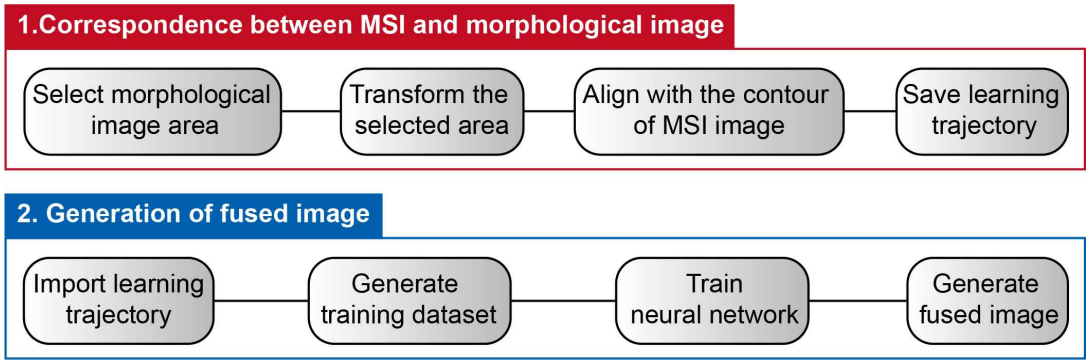


Figure 7

The usage process mainly consists of two steps: correspondence between morphological images and MSI images, and fusion image generation. Firstly, users need to correspond pixel-to-pixel of MSI images and morphological images. Since morphological images and MSI images are obtained through two completely different methods, there are significant differences in the location, resolution, and number of pixels of the slices. Therefore, the position of the slice in the morphological image and MSI image can be manually aligned by selecting a metabolite with clearer imaging. And the learning trajectory can be recorded. Each pixel in the morphological image selection area can be reflected to the corresponding pixel in the MSI image through this learning trajectory transformation. For a single imaging, only one trajectory learning process is required.

After the trajectory learning process is completed, users can simply import the learning trajectory and select a region in the morphological image to generate the corresponding area in the MSI image. Each pixel in the morphological image selection area can be corresponded with the pixels in the MSI image through learning trajectories. Users can export the features corresponding to each pixel to generate training set data.

Detailed software operation workflow:

1） MSI image area selection.

Continuously left-click the MSI image area to create a new selection control point. (Figure 8)

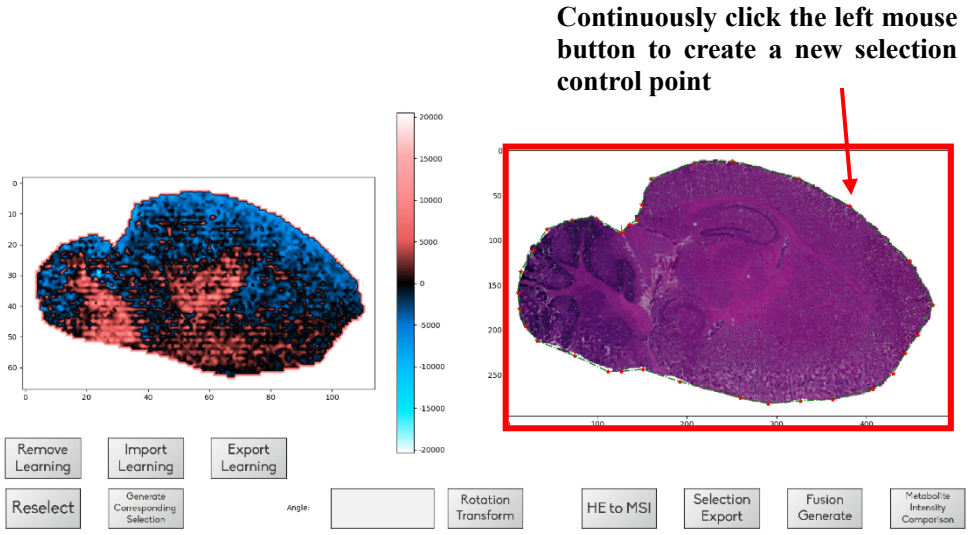


Figure 8

2） Modify the selection area.

Users can move the mouse to the vicinity of the selection control point that needs to be deleted, and right-click to delete a selection control point.

3） Copy selection to MSI image area

After selecting the area in the morphological image, click the ‘HE to MSI’ button to copy the selected area to the MSI image area. (Figure 9)

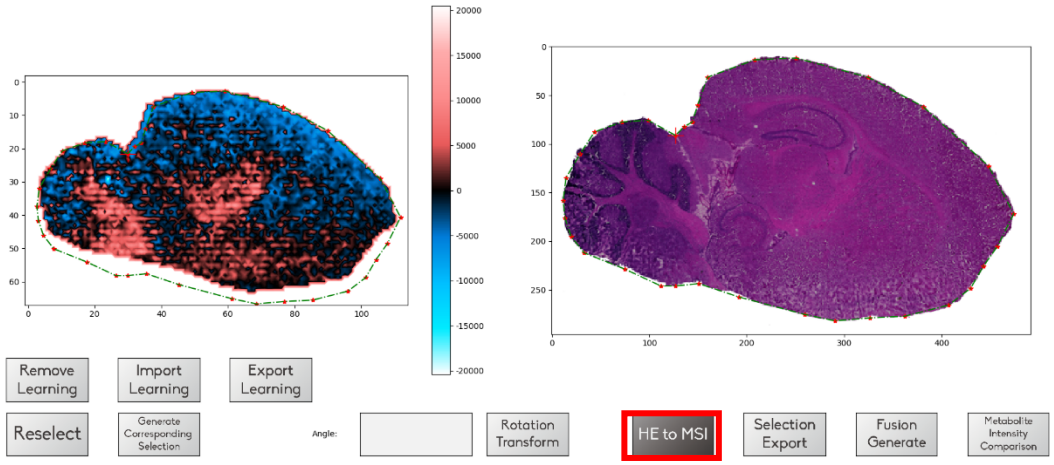


Figure 9

4） Change selection area

After the selection is completed, corresponding transformations should be made to align the selection area of the other slice. The software will automatically record the trajectory learning process.

Firstly, users should open the trajectory learning function. Opening method: Click the ‘Start Learning’ button. When the learning status frame changes to ‘On’, the learning trajectory will be recorded.

The main transformation methods include translation transformation, rotation transformation, and scaling transformation.

1º Translation transformation

There are two ways to conduct translation transformation in software: one is to hold down the 'Shift' 'and click on a new point in the image area with the left mouse button, and the entire selection area will be translated. The second way is to move the cursor to the image area (do not press) and hold down ' ↑ ↓ ← →' to perform translation. Users can hold down 'alt' and ' ↑ ↓ ← →'to perform minor translation

2º Rotational transformation

Users can enter the desired rotation angle (clockwise is positive) in the frame and click the 'Rotation Transform' button. Then the selection area will rotate by the corresponding angle.

3º Scaling transformation

Users can press 'Ctrl' and left mouse button in the MSI image area, and a yellow zoom control box will appear around the selection area. Then, users should hold down the 'Shift' key, move the cursor near the four control axes of the selection control box, and drag with the left mouse button to complete the zoom operation. (Figure 10)

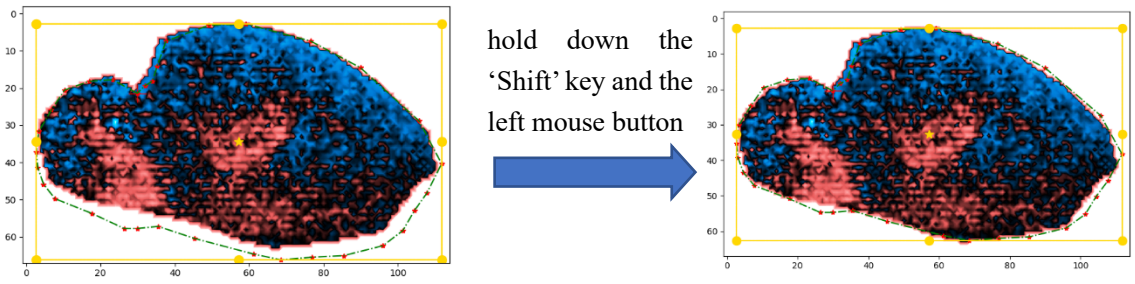


Figure 10

5） Save learning trajectory

After completing trajectory learning, click the 'Export Learning' button to export the learned trajectory. The exported learning trajectory will be stored in the Learning Recording Data.xls folder in the program running folder.

6） Import learning trajectory

After saving the learning trajectory, users can click the 'Import Learning' button to import the saved learning trajectory.

7） Generate Corresponding Selection

As shown in Figure 11, click the 'Generate Responding Selection' button to generate the corresponding reflective selection according to the learning trajectory.

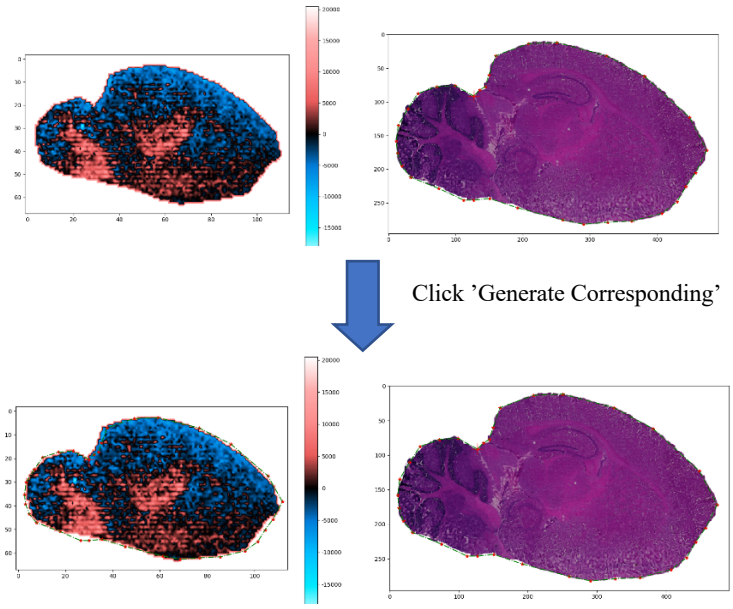


Figure 11

8） Export selected training set data

After generating the corresponding selection area, users can click the 'Selection Export' button, and the 'Data Export' dialog frame will appear. (Figure 12)

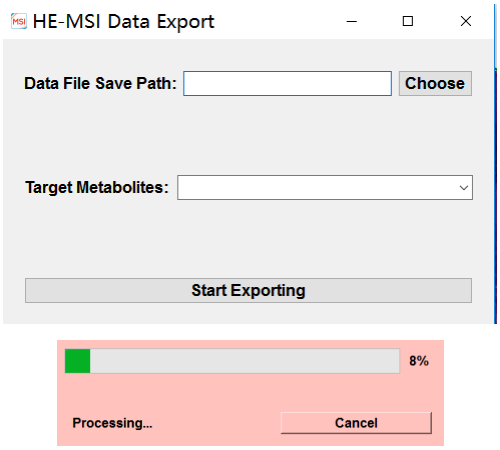
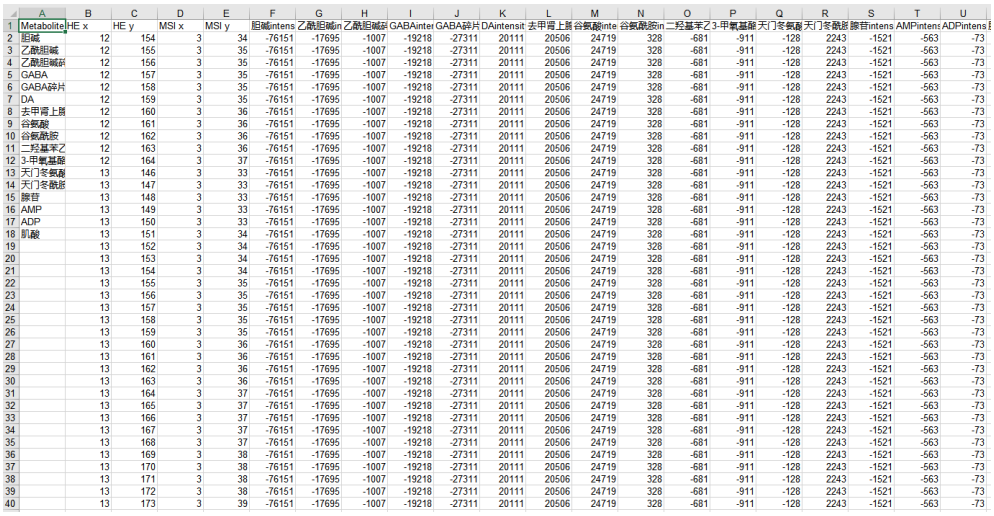


Figure 12

In this dialog box, users can choose the path to save the exported metabolite information file and the metabolites need to be exported. Click the 'Start Exporting' button to start exporting.

The exported xls file is shown in Figure 13.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	Metabolite	HE x	HE y	MSI x	MSI y	胆碱intens	乙酰胆碱in	乙酰胆碱碎	GABAinter	GABA碎片	DAintensit	去甲肾上腺	谷氨酸inte	谷氨酰胺in	二羟基苯乙	3-甲氧基酸	天门冬氨酸	天门冬酰胺	腺苷intens	AMPintens	ADPintens
2	胆碱	12	154	3	34	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
3	乙酰胆碱	12	155	3	35	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
4	乙酰胆碱碎	12	156	3	35	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
5	GABA	12	157	3	35	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
6	GABA碎片	12	158	3	35	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
7	DA	12	159	3	35	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
8	去甲肾上腺	12	160	3	36	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
9	谷氨酸	12	161	3	36	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
10	谷氨酰胺	12	162	3	36	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
11	二羟基苯乙	12	163	3	36	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
12	3-甲氧基酸	12	164	3	37	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
13	天门冬氨酸	13	146	3	33	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
14	天门冬酰胺	13	147	3	33	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
15	腺苷	13	148	3	33	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
16	AMP	13	149	3	33	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
17	ADP	13	150	3	33	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
18	肌酸	13	151	3	34	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
19		13	152	3	34	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
20		13	153	3	34	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
21		13	154	3	34	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
22		13	155	3	35	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
23		13	156	3	35	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
24		13	157	3	35	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
25		13	158	3	35	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
26		13	159	3	35	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
27		13	160	3	36	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
28		13	161	3	36	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
29		13	162	3	36	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
30		13	163	3	36	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
31		13	164	3	37	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
32		13	165	3	37	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
33		13	166	3	37	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
34		13	167	3	37	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
35		13	168	3	37	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
36		13	169	3	38	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
37		13	170	3	38	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
38		13	171	3	38	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
39		13	172	3	38	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73
40		13	173	3	39	-76151	-17695	-1007	-19218	-27311	20111	20506	24719	328	-681	-911	-128	2243	-1521	-563	-73

Figure13

9） Generate fused images

After the data export is completed, users can click the ‘Fusion Generate’ button to display a dialog box (Figure 14).

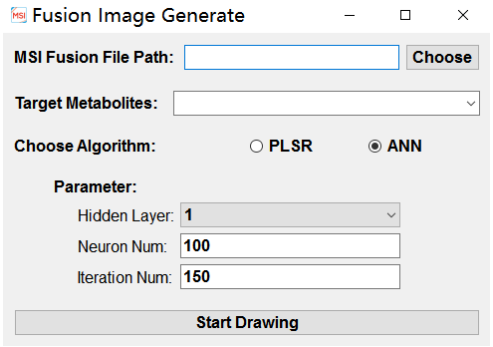


Figure 14

Users can select the previously exported data file and the metabolites that require fusion imaging (up to 9 at a time). The fusion algorithm provides two algorithms including PLSR (Partial Least Squares Regression) and ANN (Artificial Neural Network). Here, we select ANN.

Then, users should set parameters in the ANN algorithm parameters below. Parameter range: Hidden Layer: 1-4; Neuron Num: 100-300; Iteration Num: 100-300 (Parameter settings require several attempts to achieve optimal results, incorrect settings can cause program crashes)

Lastly, user should click ‘Start Drawing’ and patiently wait to train the data set. The fusion image of the target metabolite can be automatically predicted (Figure 15). The left button is used to change the color of the image. The button below can be pulled to determine the shading range.

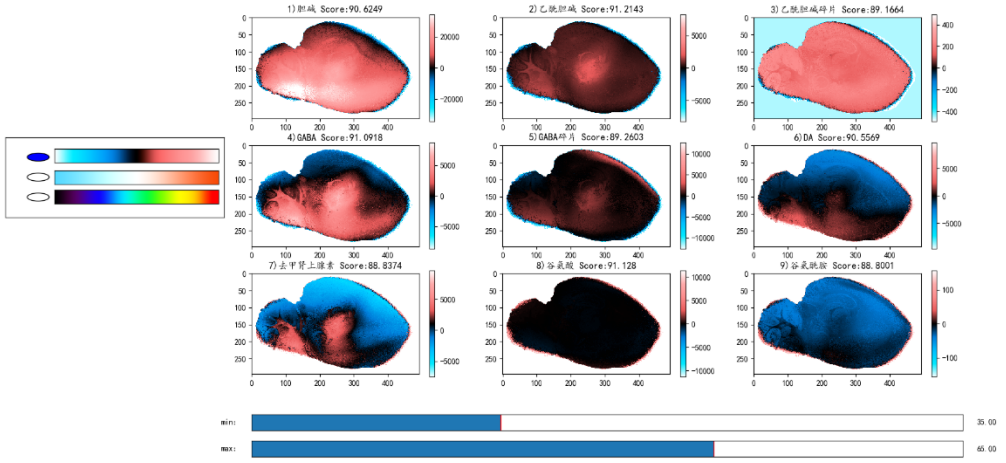


Figure 15

Tips: Fusion Image Interface Operation Guide

1º Modify shading. Click on the color bar on the left to change the coloring.

2º Modify the coloring intensity of specific metabolites. Users should move the cursor to the metabolite image frame that needs to be modified, click the left mouse button, and wait until the following prompt appears on the screen. (Figure 16) This indicates that the system has adjusted to the metabolite coloring intensity adjustment mode. At this time, users should drag ‘Scoll’ at the bottom of the screen to adjust the coloring intensity of the metabolite.

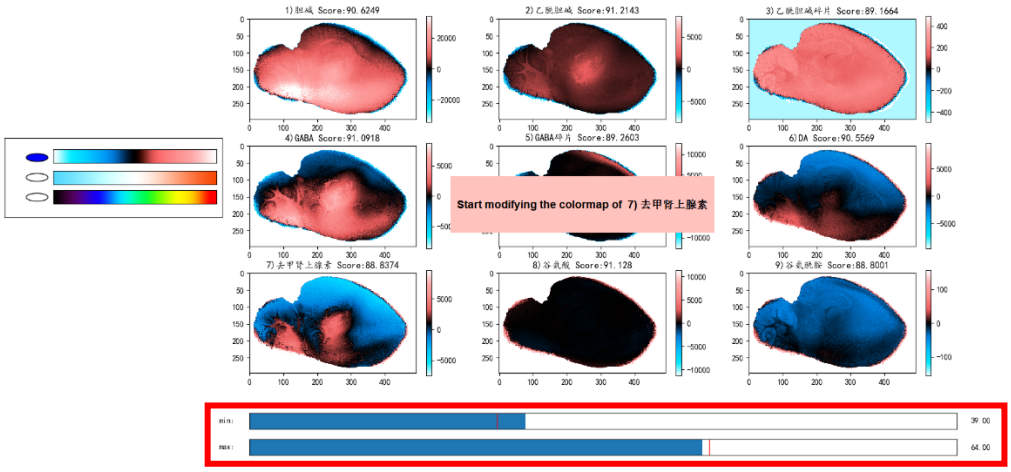


Figure 16