Instruction Manual for Pixel-to-Pixel Correspondence Program

1. Purpose

The main purpose of this software is to realize the pixel-to-pixel correspondence of mass spectrometry imaging (MSI) data obtained by MSI.

2. Principles

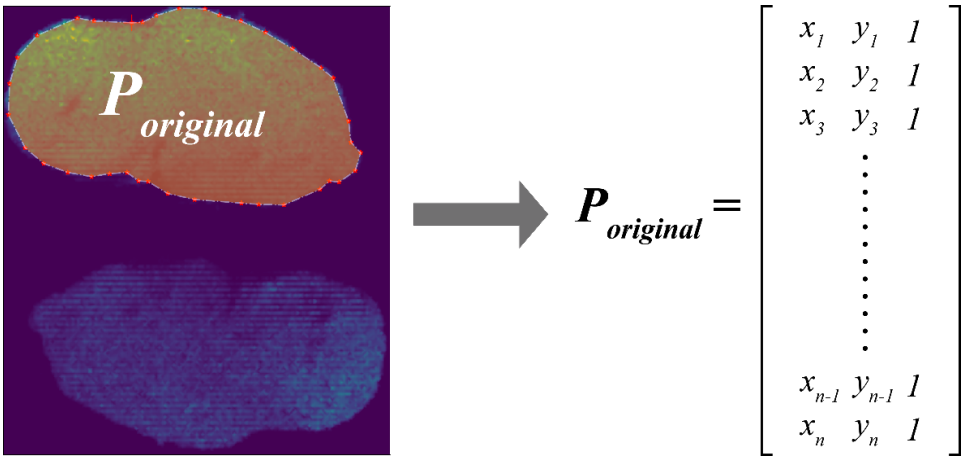


Figure 1

The matrix consists the coordinates of the image data points in the original selection is defined here as $\boldsymbol{P}_{origninal}$, then the matrix $\boldsymbol{P}_{origninal}$ can be represented as shown in Figure 1.

The overlap between the selection area and the reflection selection area was through the following transformation methods. Meanwhile, this process was recorded in real time. Figure 2 briefly shows the six basic transformation diagrams we use.

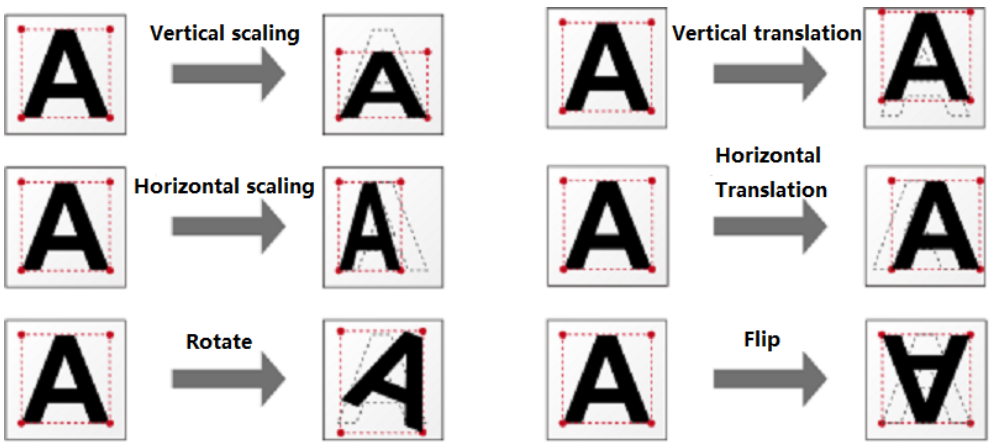
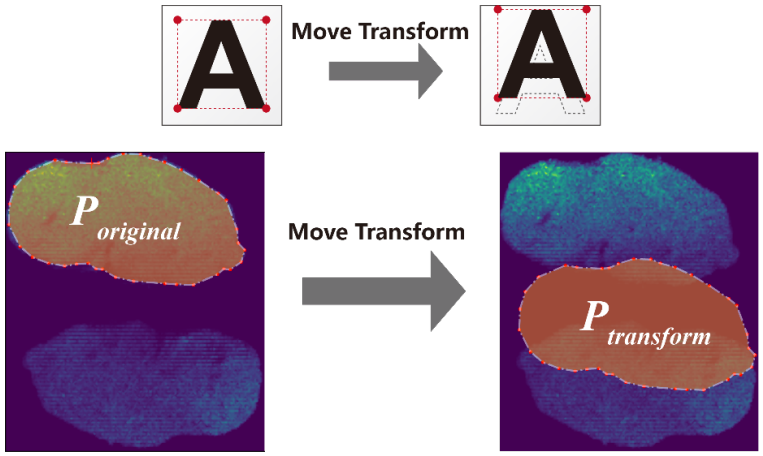


Figure 2

The six basic transformations mentioned above can all be represented by corresponding matrices. Here, we use $\boldsymbol{M}_{transform}$ to provide a general definition of all transformation matrices. Additionally, $\boldsymbol{P}_{tranform}$ is defined to represent the matrix composed of the coordinates of the image data points generated after transformation.

Next, a detailed introduction to the $M_{transform}$ is described corresponding to various basic transformations.

2.1 Move transform

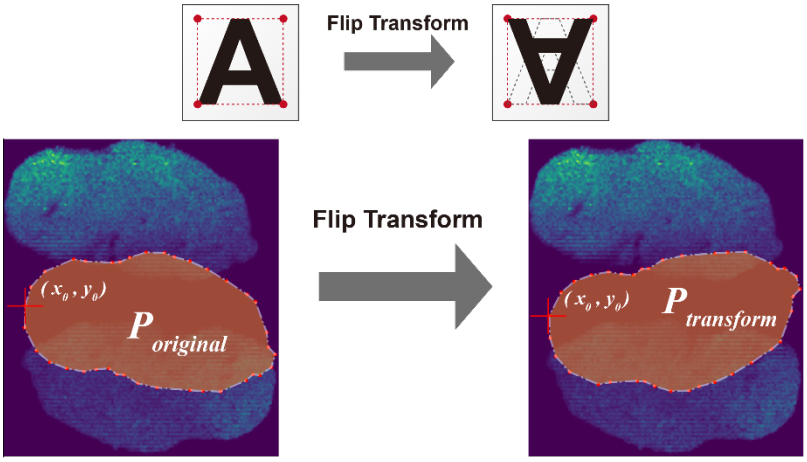


Move d_x *in x axis, Move* d_y *in y axis*

$$P_{transform} = P_{original} \cdot \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ d_x & d_y & 1 \end{bmatrix}$$

Figure 3

2.2 Flip transform

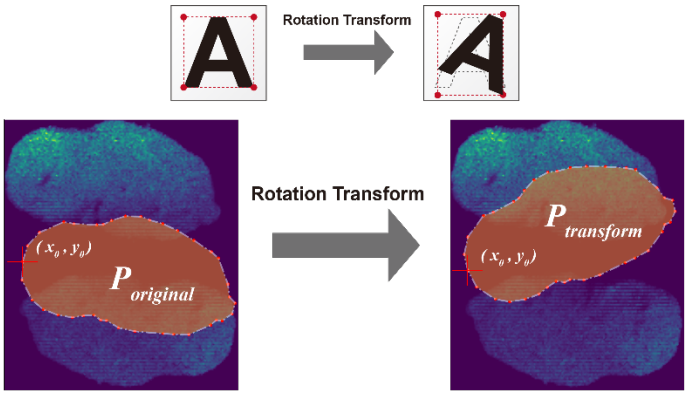


Use (x_0, y_0) *as Flip center*

$$P_{transform} = P_{original} \cdot \begin{bmatrix} 1 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 2y_0 & 1 \end{bmatrix}$$

Figure 4

2.3 Rotation transform



Use (x_0, y_0) *as Rotation center, Rotation Angle (clockwise) is α*

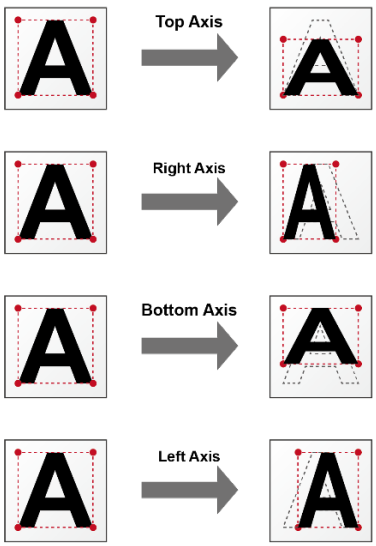
$$P_{transform} = P_{original} \cdot \begin{bmatrix} cos\alpha & sin\alpha & 0 \\ -sin\alpha & cos\alpha & 0 \\ (1-cos\alpha)x_0+y_0 sin\alpha & (1-cos\alpha)y_0+x_0 sin\alpha & 1 \end{bmatrix}$$

Figure 5

2.4 Scaling transformation

The scaling transformation is relatively complex, which can be achieved by dragging the mouse in the program. As shown in Figure 6, a rectangular control area appears around the selection area. The transformation is completed by dragging the four axes of the control area (left, right, top, and bottom).

Hence, $M_{transform}$ generated by dragging these four axes for scaling is different from each other. Here, we define the minimum x coordinate in the $P_{orginal}$ coordinate matrix of all points in the selection area as x_{min} and the maximum x coordinate as x_{max}. The minimum y-coordinate is y_{min}, and the maximum y-coordinate is y_{max}. The scaling factor is defined as r.



$$P_{transform} = P_{original} \cdot \begin{bmatrix} 0 & r & 0 \\ 1 & 0 & 0 \\ 0 & y_{min}(1-r) & 1 \end{bmatrix}$$

$$P_{transform} = P_{original} \cdot \begin{bmatrix} r & 0 & 0 \\ 0 & 1 & 0 \\ x_{min}(1-r) & 0 & 1 \end{bmatrix}$$

$$P_{transform} = P_{original} \cdot \begin{bmatrix} 0 & r & 0 \\ 1 & 0 & 0 \\ 0 & y_{max}(1-r) & 1 \end{bmatrix}$$

$$P_{transform} = P_{original} \cdot \begin{bmatrix} r & 0 & 0 \\ 0 & 1 & 0 \\ x_{max}(1-r) & 0 & 1 \end{bmatrix}$$

Figure 6

Table 1. The list of abbreviations corresponding to each transformation

Abbr.	Exp.
$M_{move}(dx,dy)$	Move Transform: Move d_x in x axis, Move d_y in y axis
$M_{reverse}(x_0,y_0)$	Reverse Transform: Use (x_0,y_0) as reverse center
$M_{rotation}(x_0,y_0,\alpha)$	Rotation Transform: Use (x_0,y_0) as rotation center, rotation angle(clockwise) is α.
$M_{scale}(d,r)$	Scaling Transform: Use d as the scaling axis, the scaling ratio is r.

After defining these basic transformations, n transformations are performed on the original selection to align with the reflection selection (Figure 7). The matrix after n transformations is defined as $P_{tranform}\{n\}$, the formular is shown as:

$$P_{transform}\{n\} = P_{original} \cdot \underbrace{\{ M_{move}(dx,dy) \cdot M_{rotation}(x_0, y_0, \alpha) \cdot \cdot \cdot \cdot M_{scale}(d, r) \}}_{n \text{ times } M_{transform}}$$

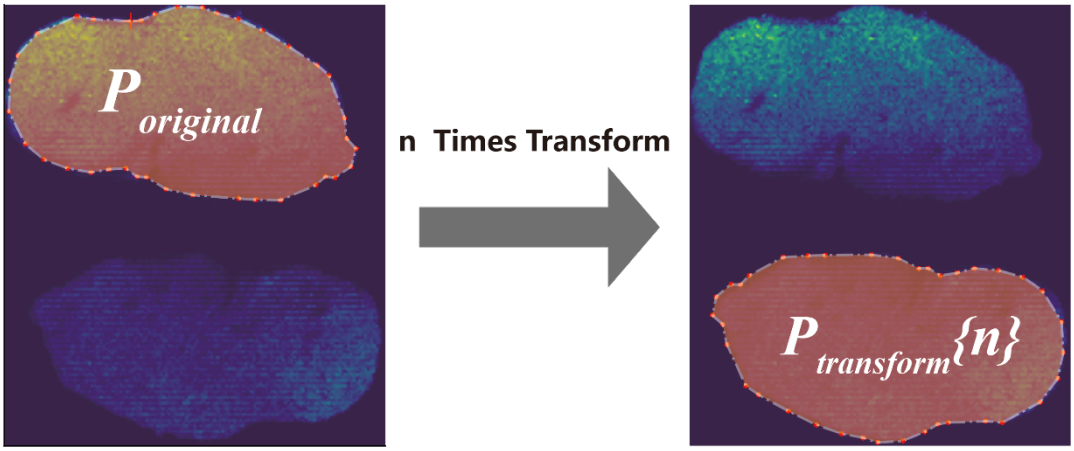


Figure 7

In matrix $P_{tranform}\{n\}$, $P_{tranform}\{n\}(i)$ is defined as all the data in the i-th row, the formular is shown as:

$$P_{transform}\{n\}(i) = [\, x_i \;\; y_i \;\; 1 \,]$$

Here, x_i and y_i are the values after n transformations, and there is a high probability that they are not integers. However, the coordinates corresponding to each pixel in the image are integers realitily. Next, we determine the final pixel point in the mapping area by searching for the nearest integer point to x_i and y_i. S_i is defined as the search matrix corresponding to x_i and y_i. $P_{reflect}$ is defined as the matrix composed of the coordinates of all points in the final mapping area. $P_{reflect}(i)$ is defined as all data in the i-th row of $P_{reflect}$. The formular is shown as:

$$P_{reflect}(i) = P_{transform}\{n\}(i) \cdot S_i$$

After obtaining $P_{origninal}$ and $P_{origninal}$, the signal intensity corresponding to each data point based on these data are obtained. $I_{original}$l is defined as the signal intensity composition matrix for

each data point in $P_{orginal}$. $I_{reflect}$ is defined as the signal intensity composition matrix for each data point in $P_{reflect}$. $I_{subtract}$ is defined as the difference matrix between the data point signals of the selection area and the corresponding selection area. The formular is shown as:

$$I_{subtract} = I_{reflect} - I_{original}$$

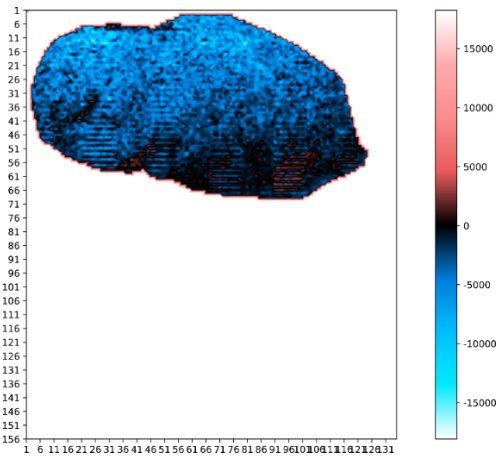


Figure 8

With the generated $I_{subtract}$ and $P_{orginal}$, we finally plotted the difference image (Figure 8).

3. Instruction

This software is written based on Python 3.2. The Python libraries required in the software includePyQt5，Matplotlib，Numpy，Scipy，xlrd，xlwt，progressbar，pyimzml，os and PIL。

At present, the software only supports mass spectrometry imaging data in imzml format.

3.1 Set up metabolite data table

As shown in Figure 9, the metabolite data that needs to be extracted from the imzml file should be saved into an Excel file.

	A	B	C	D
1	Compound	Exact mass	strat *m/z*	end *m/z*
2	胆碱	104.10699	104.14658	104.218
3	乙酰胆碱	146.11756	146.0481	146.16965
4	乙酰胆碱碎片	87.04406	87.19679	87.23909
5	GABA	104.06278	104.22038	104.61737
6	GABA碎片	58.06513	58.2621	58.50777
7	DA	154.07843	154.00744	154.0725
8	去甲肾上腺素	170.07334	169.88857	170.08331
9	谷氨酸	148.05261	147.93086	148.06332
10	谷氨酰胺	147.06859	147.0343	147.08462
11	二羟基苯乙酸	169.04171	169.00217	169.05297
12	3-甲氧基酪氨酸	212.08391	212.81019	212.89877
13	天门冬氨酸	134.03696	134.0534	134.2328
14	天门冬酰胺	133.05294	133.0265	133.12127
15	腺苷	268.09621	268.14136	268.33546
16	AMP	348.06254	349.36598	349.4744
17	ADP	428.02887	425.07901	425.17428
18	肌酸	132.06893	132.01327	132.13571

Figure 9

3.2 Select imzml file and target metabolite data

As shown in Figure 10, users can select the imzml file path for mass spectrometry imaging in the red frame, and select the Excel file containing metabolite information generated in Step 3.1 in blue frame. After selection, the target metabolite name will appear in dropdown green frame. Select the target metabolite and click the "Start Drawing" button to start imaging.

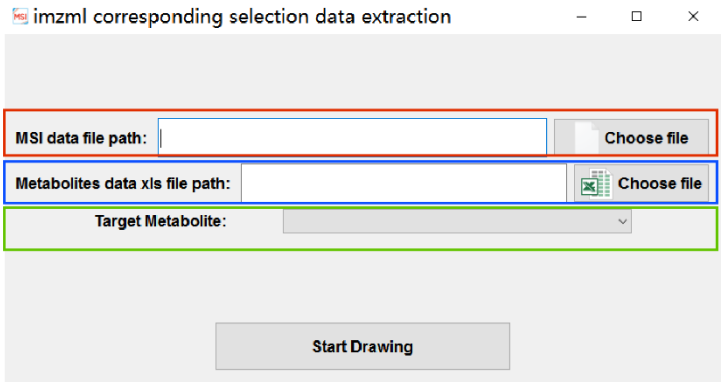


Figure 10

3.3 Introduction to MSI Interface

The MSI interface is shown in Figure 11. This interface is mainly divided into image area, trajectory learning area, and data function area.

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

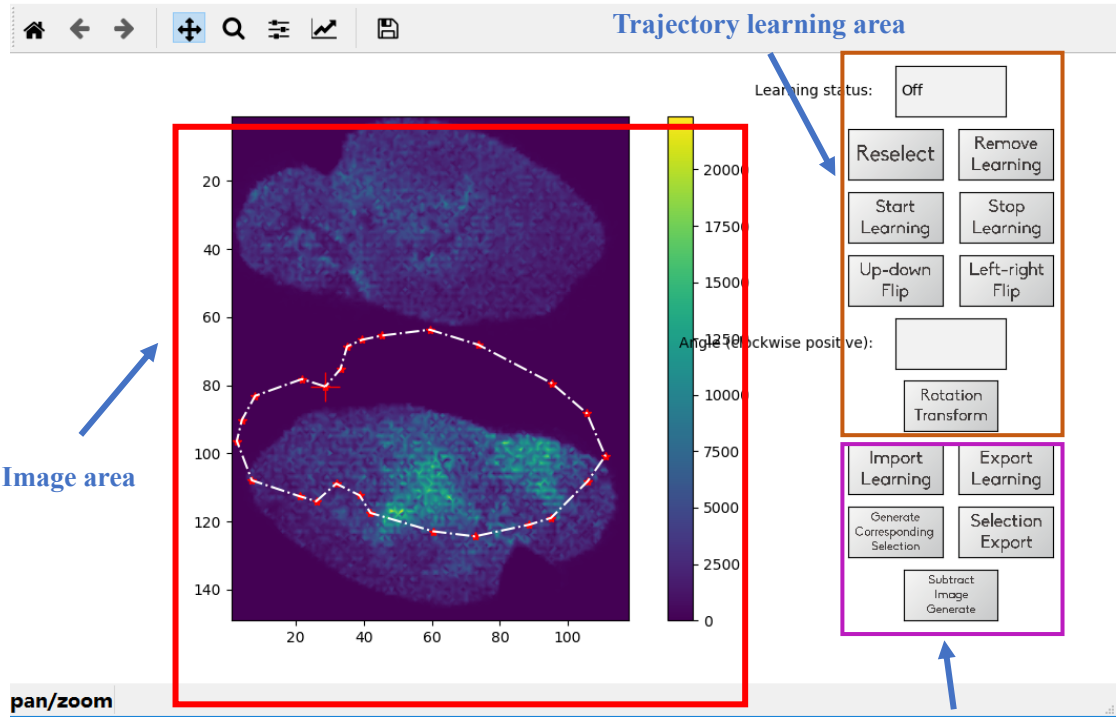
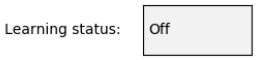


Figure 11

Table 2. Functions of each button in the interface

Name	Pattern	Function
Learning Status		Indicating trajectory learning status; 'On' means recording learning trajectory; 'Off': Not recording learning trajectory
Reselect		Reselect districts; click to eliminate all selection areaes in the image area
Remove Learning		Remove the learning trajectory; click to clear the current state learning trajectory
Start Learning		Start trajectory learning; click to start recording image area selection area transformation
Stop Learning		Stop trajectory learning; click to stop recording image area selection area transformation
Up-down Flip		Flip up and down transformation; click on the image area selection area to flip up and down transformation
Left-right Flip		Flip left and right transformation; click on the image area selection area to flip left and right transformation
Angle		Angle input; input rotation angle (clockwise is positive)
Rotation Transform		Rotation transformation; click on the image area selection area to rotate the angle input in the frame 'Angle''
Import Learning		Import learning trajectory; click to import learning trajectory from file to current state
Export Learning		Export the learning trajectory; click to import the current state learning trajectory into the file storage
Generate Corresponding Selection		Generate corresponding selection area; click to select the current image area and generate corresponding selection area areaes according to the learning trajectory
Selection Export		Export selected area data; click to export the MSI pixel data contained in the selection area and the corresponding reflection selection area

Subtract Image Generate	Subtract Image Generate	Generate the difference image; The difference image of the selection and reflection area will be generated based on data exported by clicking the "Selection Export" button

3.4 Software functions and usage workflow

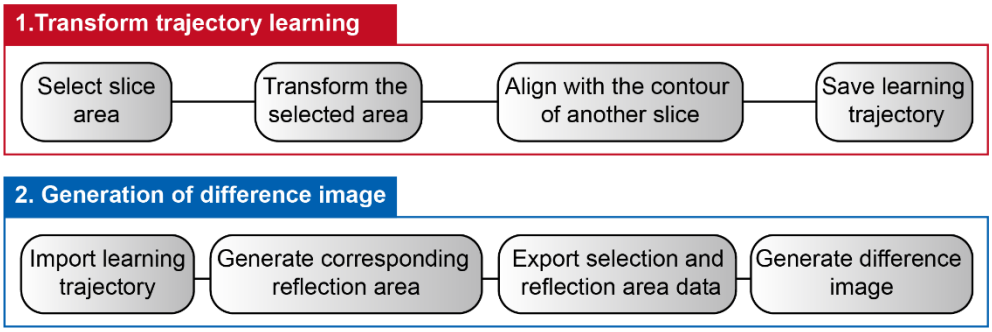


Figure 12

The main workflow is shown in Figure 12. The usage workflow mainly consists of two steps: transform trajectory learning and generation of difference images. To create a difference image, correspondence of pixel-to-pixel is needed. In the actual process, complete alignment between the two slices cannot be achieved. Therefore, a metabolite with clear image is selected. Then, alignment between the two selected regions is conducted manually, and the learning trajectory is recorded. Each pixel in the selection can be mapped to the corresponding pixel in two slices through the learning trajectory transformation. For a single imaging, only one trajectory learning process is required.

After the trajectory learning process, users can import the learning trajectory and select a new area to directly generate the corresponding selection area. Each pixel in the selection area can be matched one-to-one with the pixel points in the reflection area through learning trajectories, and the corresponding intensity can be subtracted to generate a difference image.

Detailed software operation workflow:

1） Select slice area. Continuously click the left mouse button in the image area to create a new selection control point. (Figure 13)

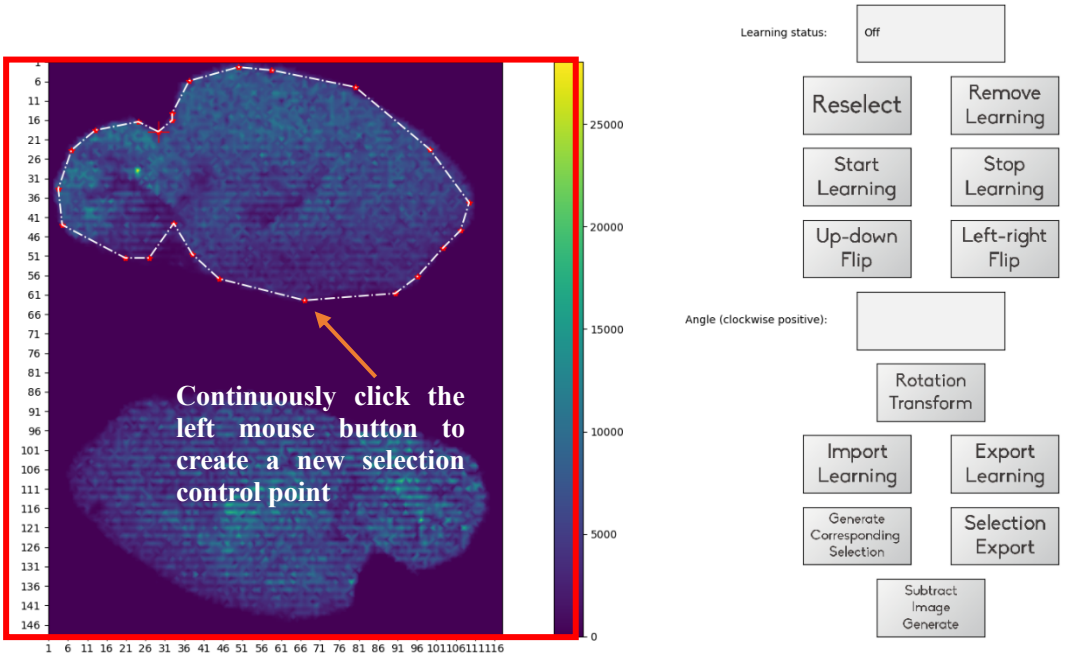


Figure 13

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. (Figure 14)

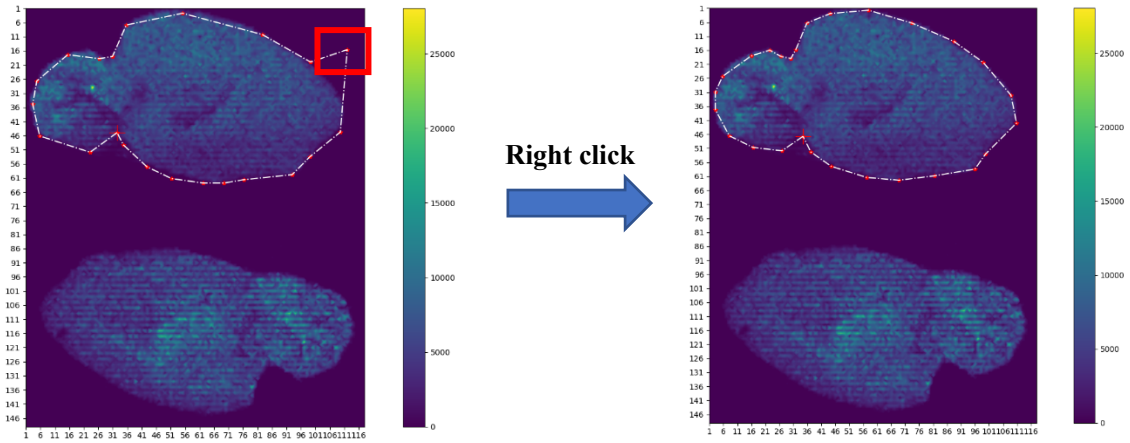


Figure 14

3） 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 flip 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 Flip transformation

Flip transformation is divided into up and down flipping and left and right flipping. Users can click the 'Up down Flip' button to achieve up and down flipping transformation; Click the 'Left right Flip' button to achieve left and right flipping transformation.

4） 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.

5） Import learning trajectory

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

6） Generate Corresponding Selection

As shown in Figure 15, click the 'Generate Responding Selection' button to generate the corresponding reflective selection according to the learning trajectory.

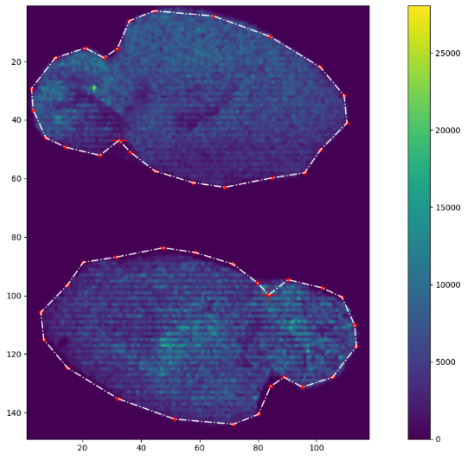


Figure 15

7） Selection and reflective selection data export

As shown in Figure 16, after generating the corresponding reflective selection area, users can click the 'Selection Export' button, and the 'Data Export' dialog box will appear.

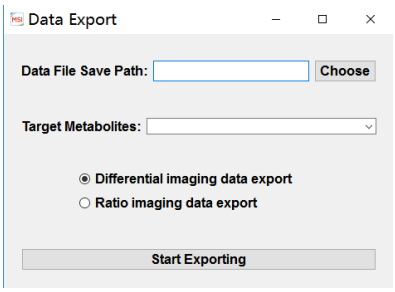


Figure 16

In this dialog box, users can choose the path to save the exported metabolite information file and which metabolites need to be exported. Click the 'Start Exporting' button to start exporting. The exported .xls file is shown in Figure 17.

	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 17

8） Generate difference image

After the data export is completed, users should click the ‘Subtract Image Generate’ button to display a dialog box. Then users should select the previously exported data file, select the metabolite that needs differential imaging, and click "Start Drawing" to generate a differential image. As shown in Figure 18, the left button is used to change the color of the image. The button below can be pulled to determine the shading range.

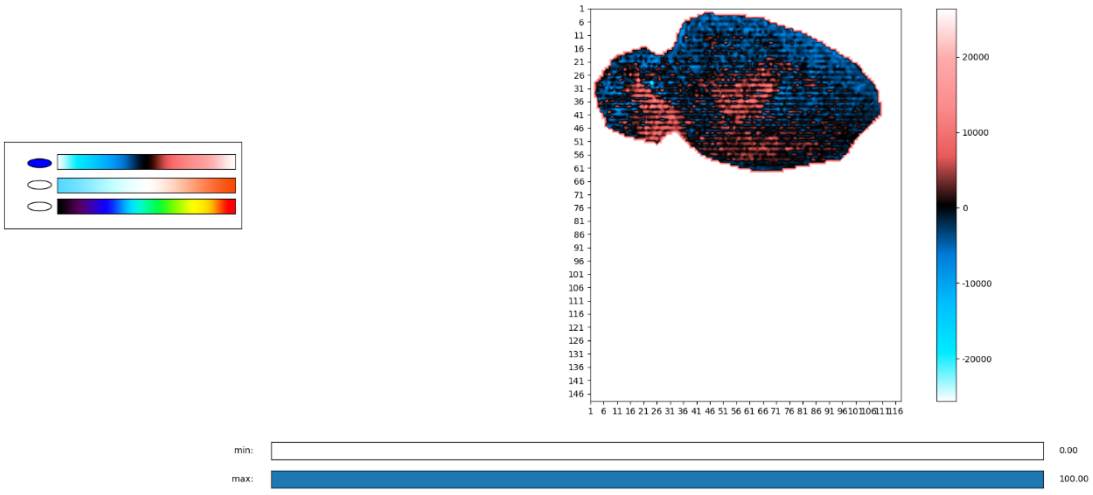

1
6
11
16
21
26
31
36
41
46
51
56
61
66
71
76
81
86
91
96
101
106
111
116
121
126
131
136
141
146
1 6 11 16 21 26 31 36 41 46 51 56 61 66 71 76 81 86 91 96 101 106 111 116
20000
10000
0
-10000
-20000
min:
0.00
max:
100.00

图 18