

Guide for Experimental Operation of Laser Speckle Imaging System

1. Connection of devices

- Firstly, the power cord is connected to the host computer of the imaging system through a power adapter;
- Connect the imaging system host with the desktop computer with the equipped three USB data cables (see the back interface in the figure below); Note: The USB3.0 interface of the host computer must be connected with the USB3.0 interface on the computer case by using USB3.0 data cable;

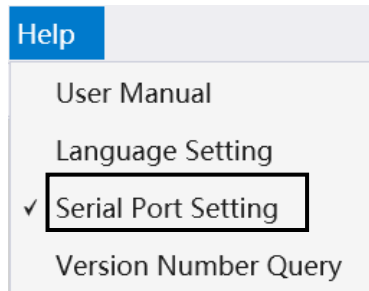


- Insert the dongle into the USB interface of the computer host;
- Make sure the scanning head is perpendicular to the desktop. Note: The bracket connecting the scanning head is rotated downward so that the scanning head is completely perpendicular to the desktop.



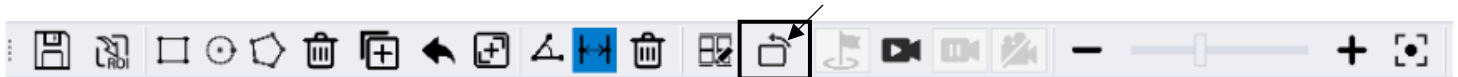
2. How to Treating with the Serial Port Connection Failure

- Check whether the connection of USB port is accurate;
- Click [Help] → [Serial Port Settings] to change the serial port, then click [OK] and restart the software.



3. Image rotation

- If the direction of the image on screen is different from the actual, it can be adjusted by the "Image rotation" button on the shortcut icon bar to ensure the consistency of the direction.



4. Background requirements

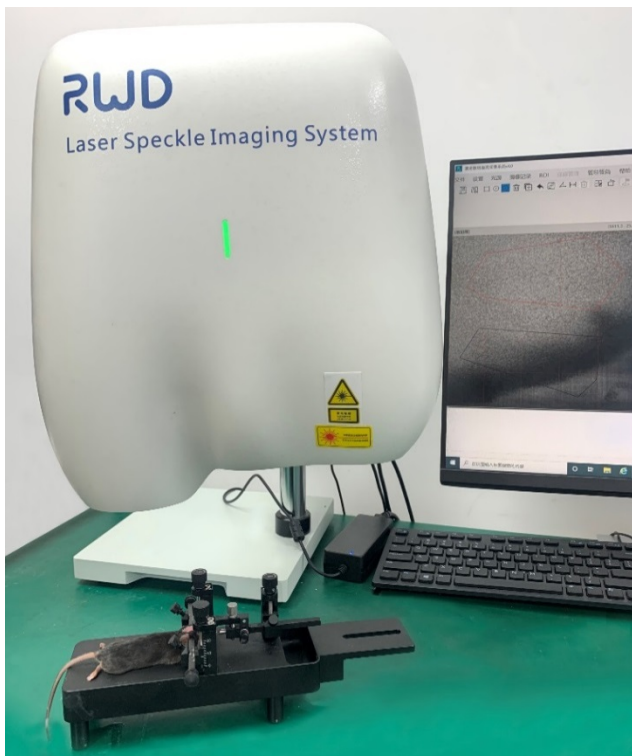
- Please choose a black non-reflective desktop for shooting background.

5. Ambient light requirements

- Normal ambient light. Please avoid the interference of oblique light source or use it by the window.

6. Suggestions on animal placement

- Cerebral blood flow-The animal is placed parallel to the scanning head, with the head facing the laser and the tail facing the bright field light source (see below).

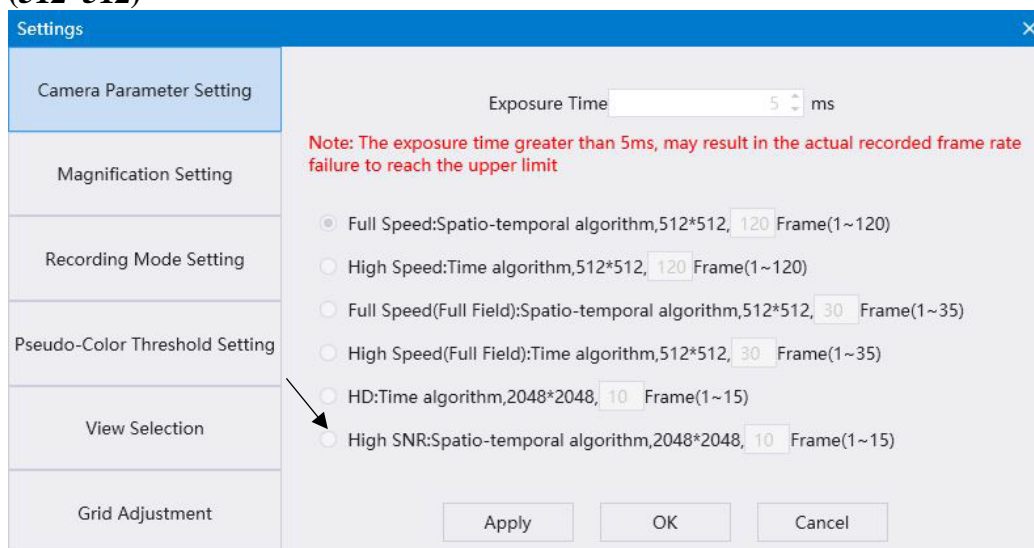


- It is recommended to use with mouse/rat holder. Some objects (such as cotton balls) can be placed under the mouth of animals to make the skull as horizontal as possible.

Note: The monitoring of cerebral blood flow needs split the scalp of mice; to monitor the blood flow of lower limbs, the lower limbs of mice should be placed in supine position after depilation, and the lower limbs of mice should be fixed with double-sided tape; Depilation is required for skin flap

shooting.

7. Suggestions on the selection of imaging mode and exposure time (note that the setting conditions before and after recording baseline should be consistent)
 - Click [Settings] → [Camera Parameter Setting] and select **High SNR: Spatio-temporal algorithm, 2048*2048**. To reduce the data size, you can also select **Full Speed: Spatio-temporal algorithm, (512*512)**



When the device is connected to the camera, this interface can set the exposure time and imaging mode of the camera.

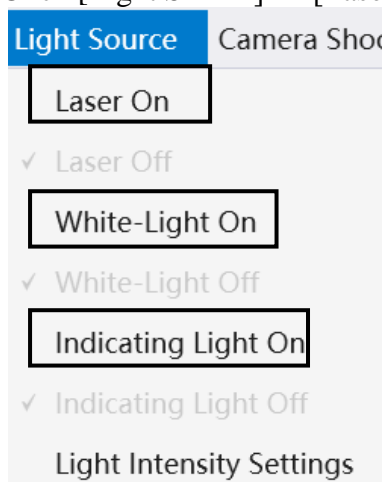
Optional exposure time: 1ms/2ms/5ms/10ms

(Note: If the exposure time is greater than 5ms, the actual recording frame rate may not reach the upper limit.)

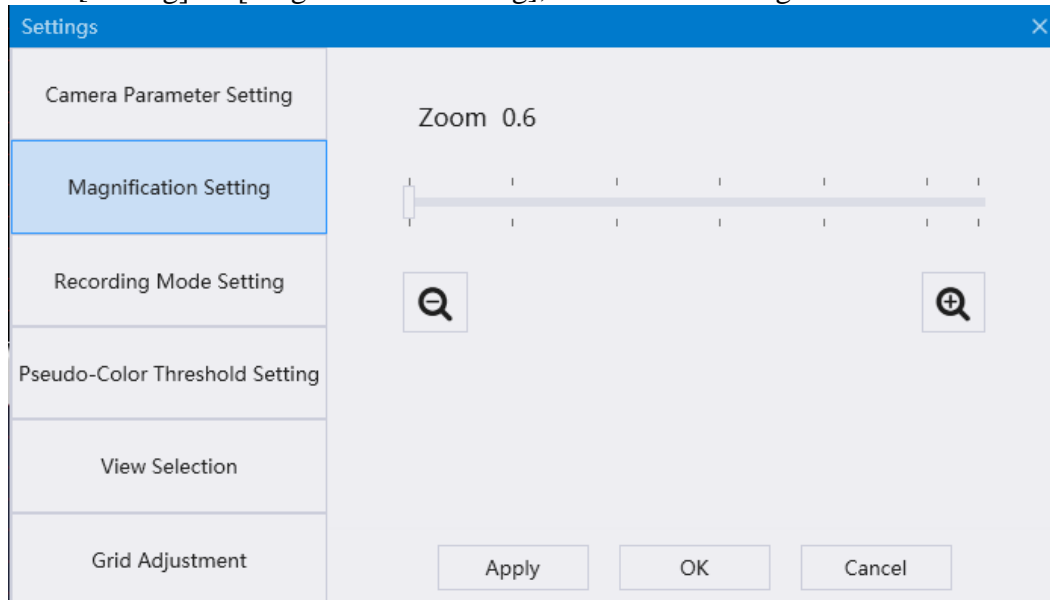
- The exposure time is adjusted according to the brightness of speckle pattern, which is generally recommended to be about 5ms in rats and 5-10ms in mice;
- Make sure to use the same camera parameters for the same experimental sample, that is, the same exposure time and imaging mode.

8. Adjustment of laser position for effective monitoring

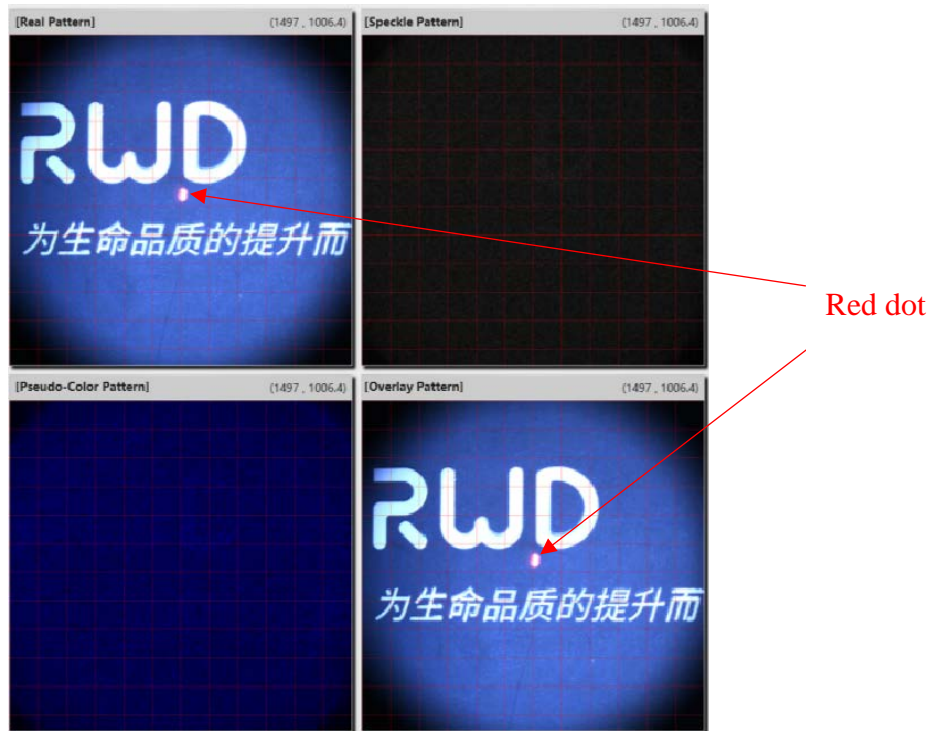
- Click [Light Source] → [Laser On / White-light On /Indicating Light On]



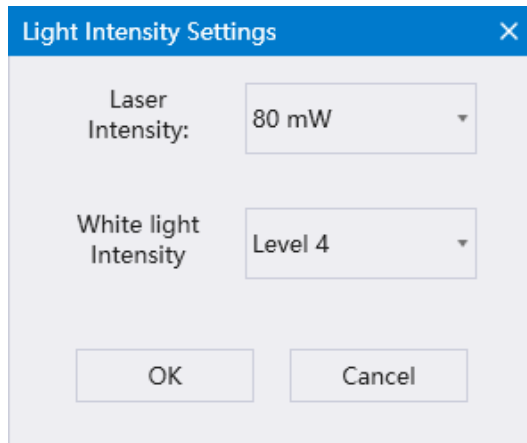
- Click [Setting] → [Magnification Setting], and select the magnification to be zoom 0.6



- Make sure that the sample is in the center of the image on screen, the red dot of indicating light is on the sample and approximately in the center of the screen.



- Turn off the indicator light. Select [Light Source]-[Light Intensity Settings], the laser intensity is 80mW by default, and it is recommended to set 80mW or 100mW. Pay attention to the consistent laser power conditions of the same sample. The white light intensity has 1~4 levels, and the default is level 4, adjust the intensity per your need.

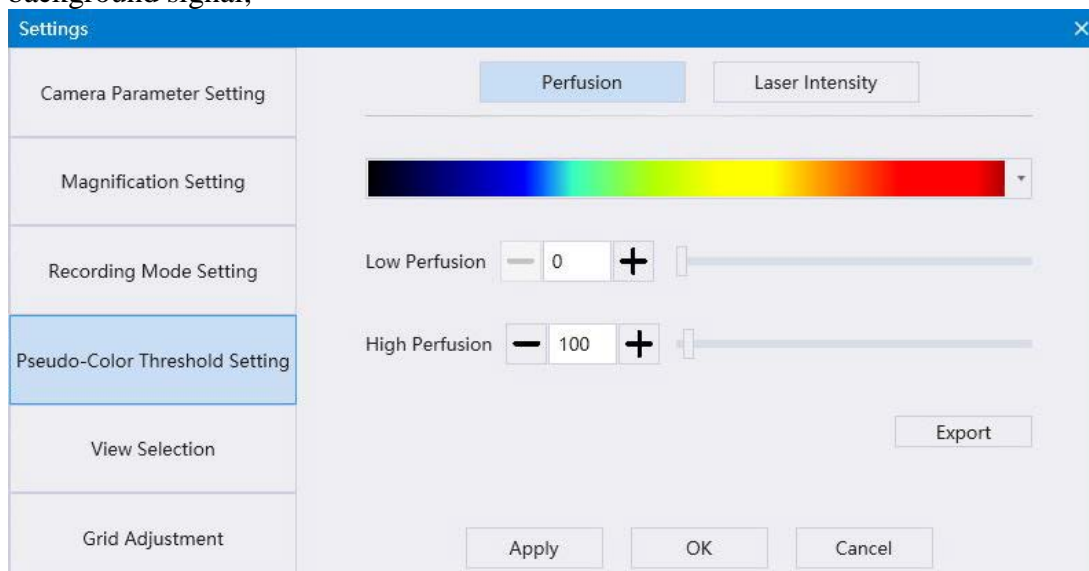


- After that, please do not adjust the height of the imaging system in this experiment.
- Click [Settings] → [Magnification Setting] again, and select the appropriate magnification to make the picture accommodate the part to be tested as much as possible.
- Adjust the manual focus slider in the shortcut icon bar or click the autofocus button, as shown below. Note: When the ambient light changes or the animal is in poor anesthesia state and shakes, it is difficult for the lens to focus automatically, so manual focusing is preferred.

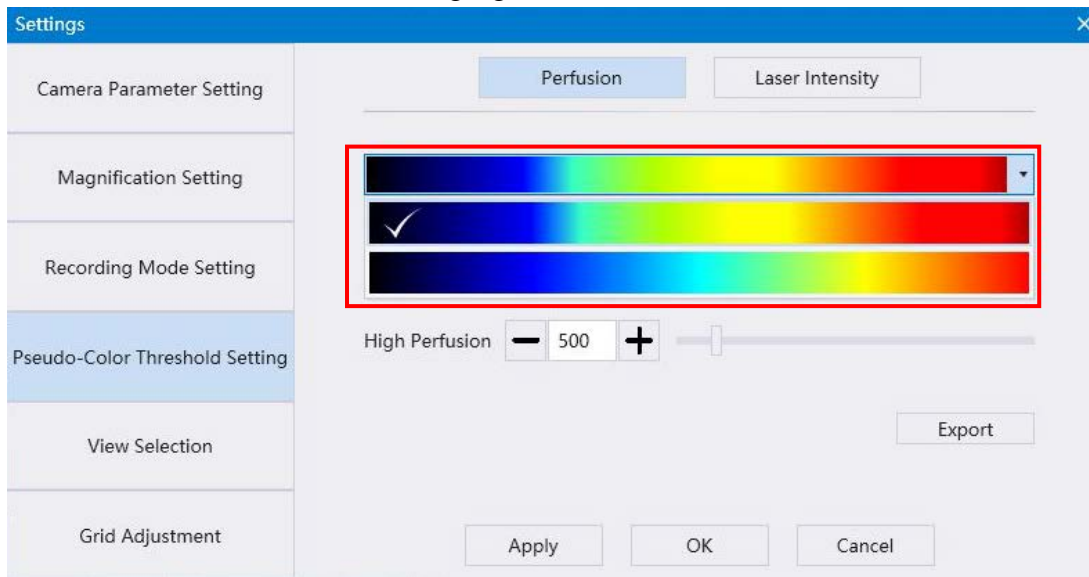


9. Selection of pseudo-color threshold

- Click [Settings] → [Pseudo-Color Threshold Setting], adjust the upper and lower limits of the threshold left and right to achieve the best visual effect of pseudo-color image to reduce the background signal;



- If you need to change the effect of pseudo-color coding, click on the color bar and select other color ratios, as shown in the following figure;



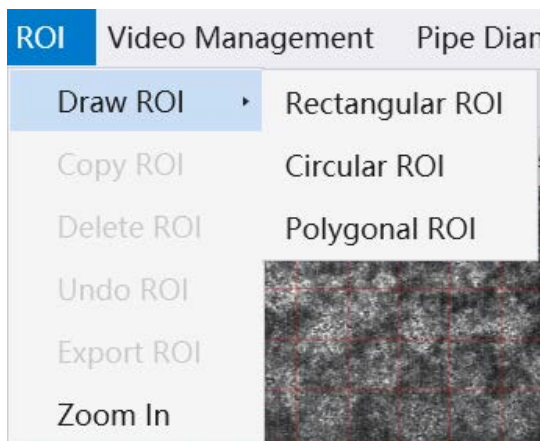
10. Noise reduction method

- The noise can be reduced by using a syringe to moisten the sample (such as the brain) with a small drop of normal saline;
- Using adapter of stereotaxic instrument can significantly reduce respiratory jitter;
- Selecting the resolution of 512*512 can improve the sampling speed to reduce the influence of respiratory jitter;

11. Drawing/Copying/Deleting ROI

- Select the appropriate ROI shape in the menu bar. ROI drawing includes Rectangular ROI, Circular

ROI and Polygonal ROI, and the corresponding shortcut icon bar is.



Drawing ROI for blood flow monitoring area of sample target

Rectangular ROI, round ROI drawing method: Click to select the corresponding graphics, drag the mouse to select ROI.

Polygonal ROI drawing method: Click to select polygonal ROI, click the left mouse button along the observation area to draw polygon, and then click the right mouse button to finish drawing.

- Select the ROI and click [ROI Delete] to delete the currently selected ROI or use the Delete key on

the keyboard to delete the current ROI. Corresponding shortcut icon .

- Select ROI, click [ROI Copy] to copy the currently selected ROI, corresponding to the shortcut icon

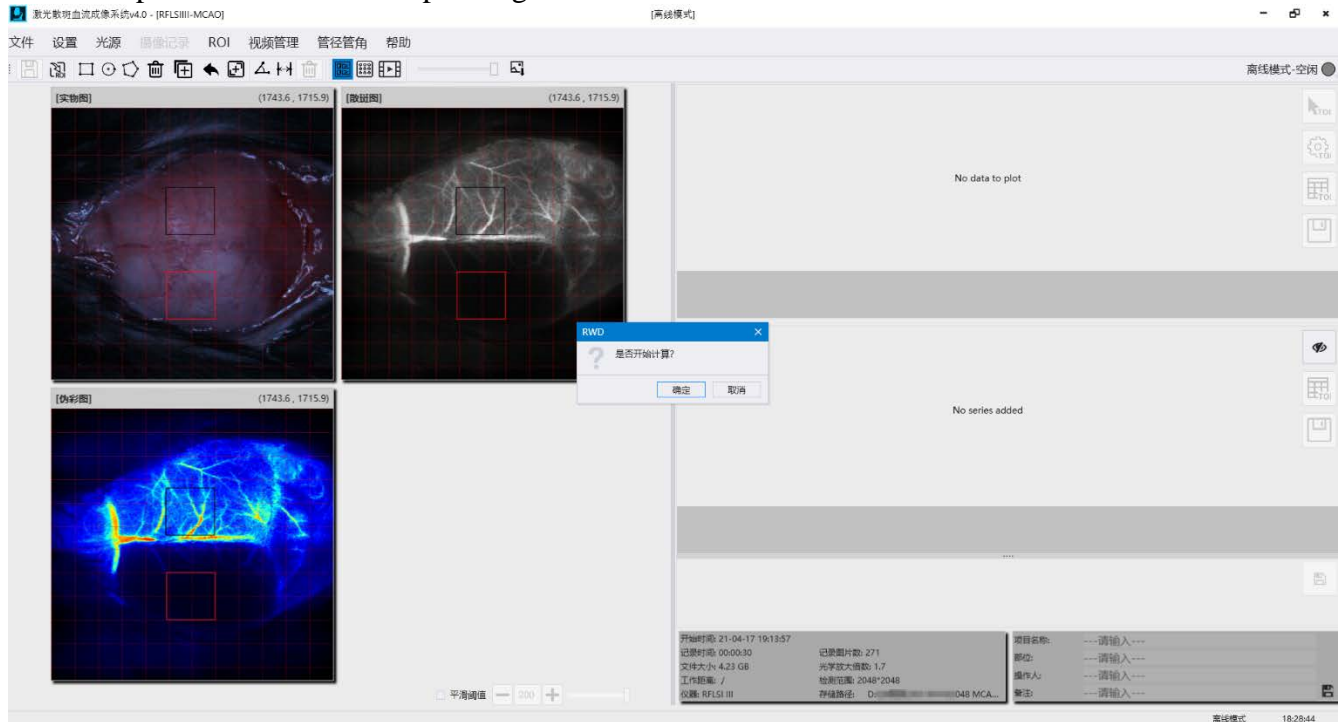


Note that if the state of "ROI Draw" is in operation and "Polygonal ROI"  as shown in the

figure is in the blue selected state, you need to click it to cancel the selected state before you can carry out other operations.

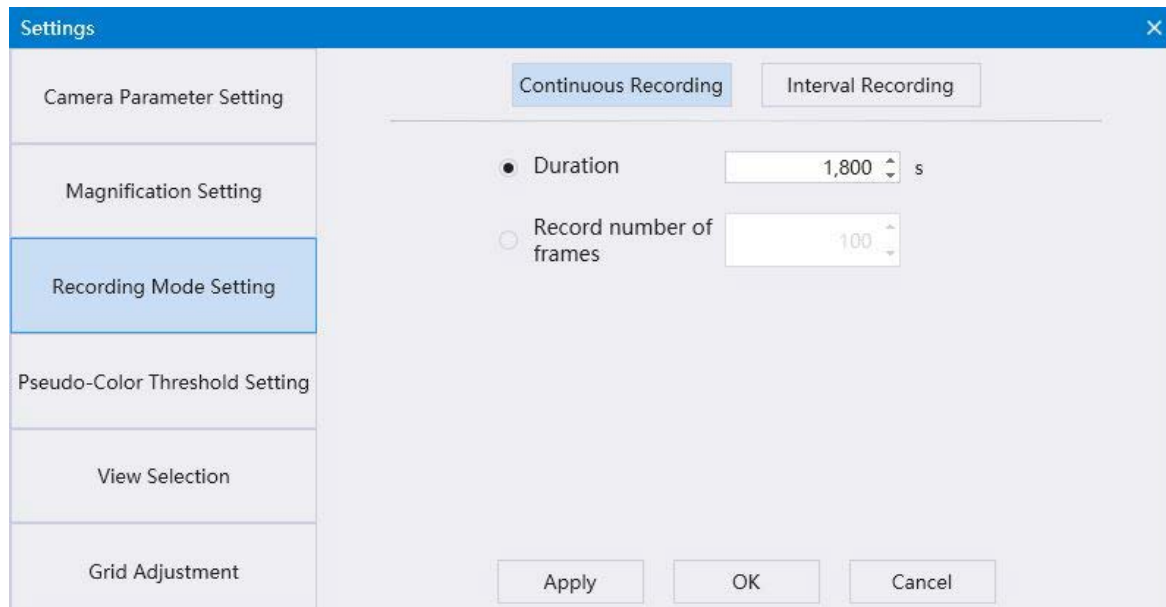
12. Collection of Perfusion of selected ROI

After selecting the appropriate ROI, double-click the right mouse button in the blank area, and the pop-up dialog box asks "Are you sure to start calculation". Click [OK], and the system will start collecting the blood perfusion in the corresponding ROI.

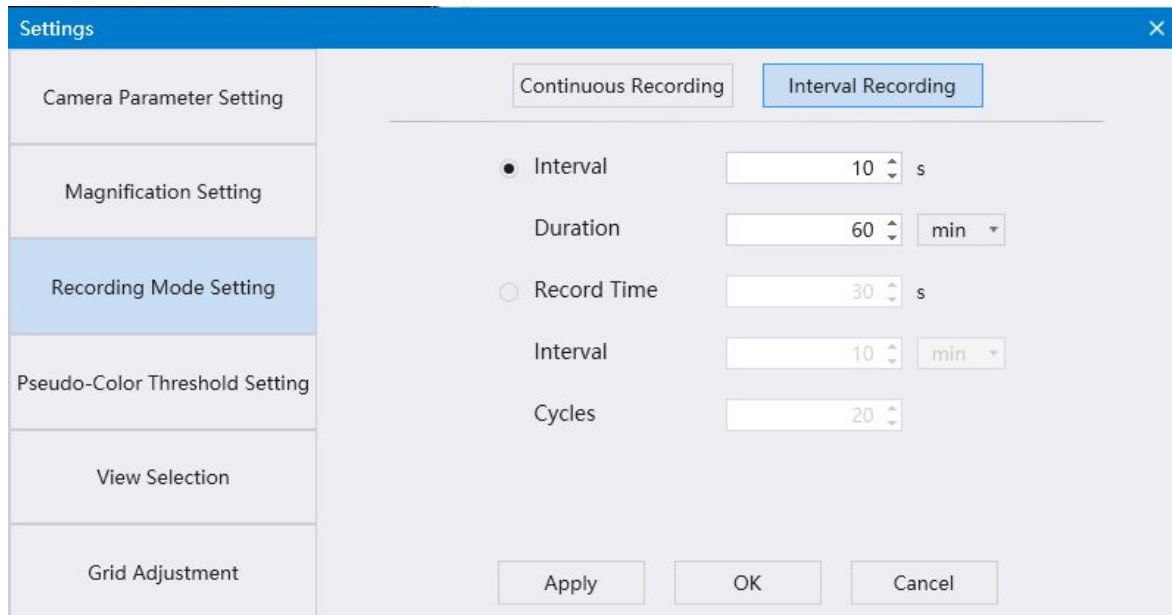



13. Selection of recording mode

- Click [Settings] → [Recording Mode Setting]. Under normal circumstances, the continuous recording mode is selected by default. The duration can be set to 8 ~ 12s.

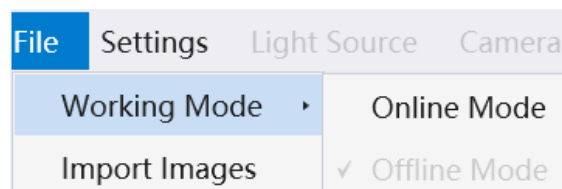


- If long intermittent recording is required, please select interval recording mode. As shown in the following figure, click to set Interval duration and Record time.

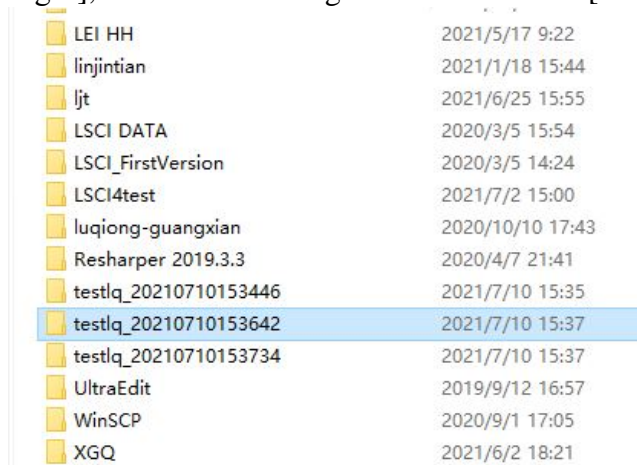


14. Click [Start Recording] in the menu bar or select the shortcut icon button , edit the file name in the pop-up window, select the storage path, and the screen will prompt "Start Recording" and "Stop Recording" after sampling is completed.

15. Import and save files in offline mode



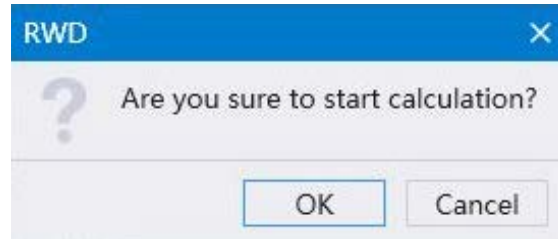
- Select [File] → [Working Mode] → [Offline Mode].
- Select [File] → [Import Images], find the saved target folder and Click [Select Folder] and import.



- No other operations can be performed during the import process.


16. ROI addition and deletion of offline files

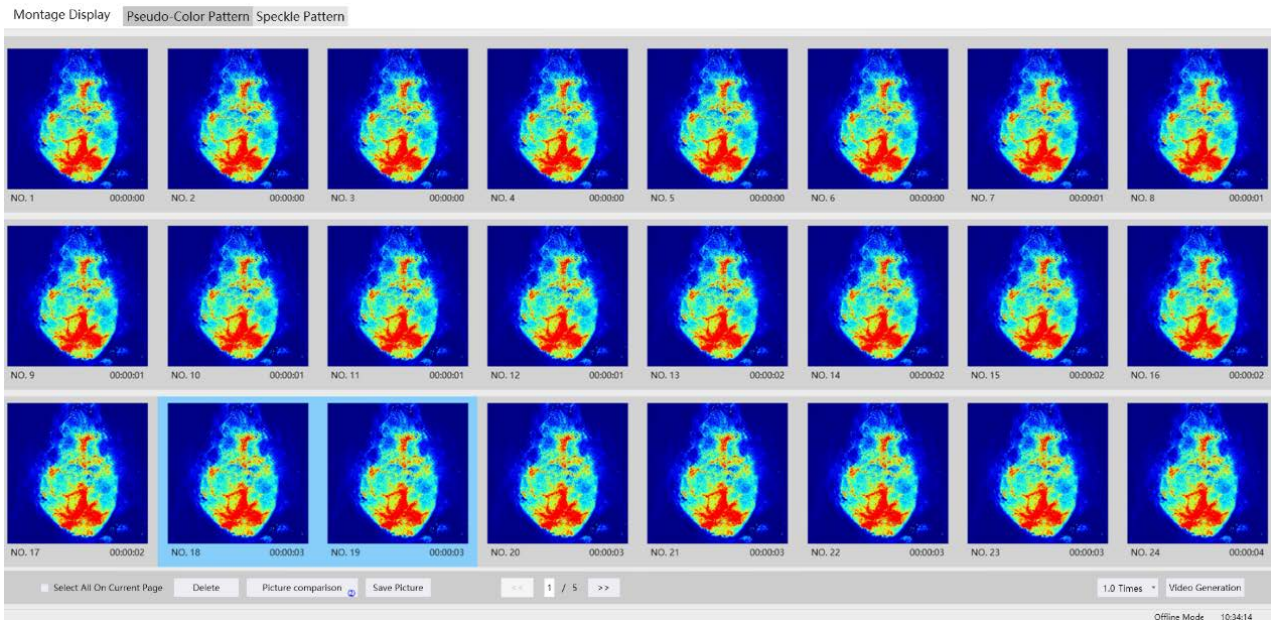
- If you need to re-add or remove ROI from the offline file, please click the ROI action option on the menu bar.
- Select any ROI on the image, right-double-click, as shown in the following figure, click OK, and the data will be recalculated.



- No other operations can be performed during calculation.

17. Export of pictures (Pseudo-Color pattern, Speckle pattern)

- Click on the menu bar, click [Video Management] → [Montage] or shortcut icon , and the user could select any 1-4 pictures in the corresponding interface.



- Click [Picture comparison] to get an enlarged contrast picture of the image. You can select any image to save or delete.
- To generate video, click "Video Generation" to generate a new video, and save the video to the required path according to the prompt.

18. Export of data

- TOI analysis can be performed on curve files at a custom time or at any time selected on images.




- When selecting on the image, hold down the left mouse button and drag on the curve file.

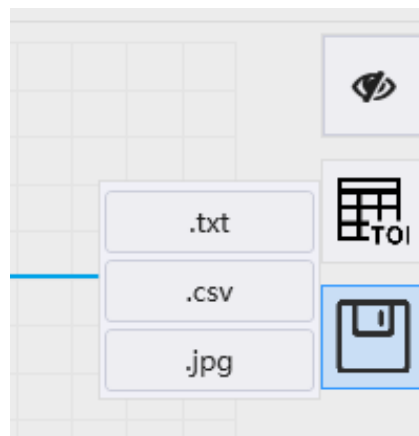


- Select and right-click to calculate the TOI data of this duration, save as Excel file as you need.

| ROI | TOI | Frames | Mean | Standard Deviation | Variance | Maximum | Minimum | Median |
|-----|-----|--------|--------|--------------------|----------|---------|---------|--------|
| ① | T1 | 39 | 360.27 | 236.28 | 55828.97 | 414.89 | 310.21 | 356.19 |
| ② | T1 | 39 | 402.34 | 143.26 | 20522.2 | 433.11 | 339.71 | 410.24 |

Cancel Export

- If you need perfusion data at all time points, click the Save icon  below the curve file to generate a CSV table.
- Click the "Save" button to choose to export the pictures of curve file.



19. Suggestions on polishing rats

- Please select a large flat drill bit.
- Vertical polishing.
- It is recommended to polish from the center seam position and gradually spread to the periphery.
- When polishing, please add normal saline to reduce the skull temperature.
- When polishing, the drill bit needs to move back and forth in the polishing area. If it stay in the same position, the skull will have different thicknesses and even hurt the dura mater.
- The speed is recommended to be about 1/4 of the maximum speed.