

RWD

RWD Life Science Co.,Ltd

Laser Speckle Imaging System

User Manual

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1-Introduction

1.1 Overview

Thank you first for choosing the Laser Speckle Imaging System manufactured by the RWD Life Science Co., Ltd (hereinafter referred to as the RWD)!

For better use of this product, please read the supplied instructions carefully before the initial installation and use of this product.

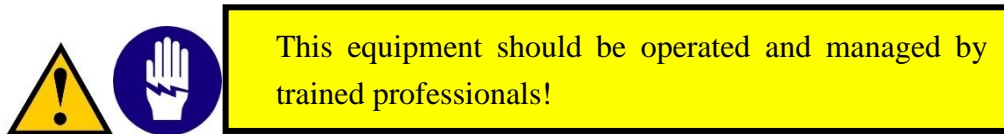
RWD have endeavored to improve the product function and service quality. RWD reserves the right to change or alter the contents of user manual without prior notice.

If you would like to acquire the latest product information, welcome to call us or visit our website (<http://www.rwdstco.com/>). If you find any discrepancy between the actual situation and this manual during the practical use of our product or if you have any question or suggestion, welcome to contact RWD.

1.2 Safety

Please read the “2-System Safety” section carefully before use to avoid causing some damages to the operator and the imaging system during application.

If you have any question or suggestion regarding safety, please contact RWD for after-sales support.



1.3 Features

- Non-contact and no contrast agent required;
- Autofocus;
- Motors Zoom Lens;
- Output of multiple indicators: video, image, blood perfusion data, and diameter analysis;
- High resolution of 2048×2048;
- High speed imaging up with frame rate up to 120fps;
- Micron-sized spatial resolution;
- Optional imaging modes: Full Speed Mode, High Speed Mode, HD Mode and High SNR Mode;

- Fast and real-time imaging, and continuously real-time monitoring;
- New all-in-one design makes operations more convenient;
- Easy to install, regulate and move.

1.4 Application

This product provides a real-time dynamic blood flow monitoring and video imaging recording method for basic research of life science, which is one of the most important bases for understanding pathological or physiological indicators of tissues and organs.

1.5 Environmental Requirements

The equipment operating environment is prepared according to the conditions listed below to ensure the operability and safety of the laser speckle imaging system.

	Description
Working environment	Temperature: 15 °C-35 °C
	Humidity: 20%-80% (non-condensing)
	Air pressure: 70kPa-100kPa
Storage environment	Temperature: 10 °C-40 °C
	Humidity: Relative humidity not more than 80%
	Air pressure: 70kPa-100kPa
Working power supply	AC: 100-240V AC, 50/60Hz 3A DC: 24.0V

1.6 Parameters

Parameters	Description
Blood monitoring perfusion range	0-5000PU
Image acquisition speed	The blood flow imaging speed is not less than 100 frames per second
Image monitoring area	1.57mm×1.57mm-90mm×90mm
Camera pixel/resolution	2048×2048
Spatial resolution of image	Higher than 3um/pixel
Effective pixels per unit area	>5,000,000 pixels/cm ²
Optical multiplication ratio	12:1

1.7 Product List

Accessory methods	Items	Quantity	Description
Standard	Host	1	For blood flow imaging
Standard	Adapter	1	For power supply of equipment
Standard	Power cord	1	For connection with power supply
Standard	USB extension cord	3	For connection with camera and communication of main control board
Standard	HP desktop computer	1	For installation of analysis software
Optional	Gimbal bracket	1	For adjustment of product angle and distance in all directions
Optional	Cross bracket	1	For fixing the product and adjusting its distance
Optional	Mouse adapter	1	For fixing the mouse during the experiment

2-System Safety



Please read the safety instructions carefully. For safety's sake, please be aware of the following:

- **Correct connection cables**
Make sure all connection cables are safely and firmly connected to the equipment.
- **Avoidance of all exposed electronic cables**
Do not touch any electronics and wiring inside the equipment!
- **Shutdown due to suspected failure**
In the case of equipment safety hazard or failure of normal operation, please contact the authorized technical support personnel.
- **Correct connection of equipment**
Connect the equipment correctly to avoid operation difficulty or even disconnection of equipment due to incorrect connection.

2.1 Notes

- Plug in the power cord to prevent improper power contact;
- Pay attention to shockproof, waterproof, moisture proof, pressure proof and fireproof measures;
- When not in use for a long time, please disconnect the power connector; prohibit sharing the power plug with non-native equipment; and place the instrument in a dust-free or less dust environment as much as possible to avoid contamination of optical devices;
- It is forbidden to hang other items on the bracket;
- During moving and handling equipment, pay attention to handling strength to prevent damages to instrument or falling of instrument;
- It is strictly forbidden for untrained personnel to operate the instrument. Please use a reliable grounding device.
- The responsibility for equipment failure arising from any improper cleaning, maintenance and operation of the equipment should be borne by the user.
- If the equipment host is disassembled without the authorization of RWD, RWD will no longer fulfill its commitments for quality assurance and technical maintenance services to the equipment. For any technical problems please contact the authorized personnel or RWD for supports.

2.2 Safety Instructions



Note: Do not look directly into the lighting hole during the operation of equipment.

- The instrument is equipped with a laser indicator light. When the indicator light is on, it indicates that any laser is emitted from the lighting hole! The emitted laser is either visible or invisible laser. The invisible laser is invisible to naked eyes, but it will also cause damages to eyes;
- The equipment should be placed away from the water source, and in a well-ventilated place with constant temperature and humidity, to avoid direct sunlight, and corrosive, flammable and explosive gases and chemicals;
- Avoid dangerous conditions such as tilting, vibrating and crushing of equipment during placement or handling operations;
- Pay attention to the voltage, current and frequency of power supply and check whether ground terminals are normal. Avoid running the equipment under overload conditions.

3-System Connection and Operation

The laser speckle imaging system consists of a host and a software system.

- Host

It includes an optical microscopic imaging part, and an image capture part. The optical microscopic imaging part is an optional configuration that according to the resolution, field of view and other requirements. The image capture part digitizes the captured images and sends them to the computer for analysis and calculation.

- Software system

It includes the software, one desktop computer, one keyboard and one mouse.




Figure 3-1

- 1) Connect the power cord to the host of imaging system by a power adapter firstly.
- 2) Connect the host of imaging system and desktop computer with 3 USB cables (as shown figure 3-2).



Figure 3-2

Note: the USB3.0 port of data cables must be connected to the USB3.0 port of the computer.

- 3) Start the software.
- 4) Adjust the position of observation object or equipment so that indicator laser at the center of the observed area, click the autofocus icon  on the auto/manual focus zone or click +/- to manually fine focus as shown in figure 5-3, to get a sharp image.
- 5) Carry out real-time analysis and monitoring of flow rate or pipe diameter/pipe angle.
- 6) When the flow rate map should be saved, the functions of continuous recording or interval recording can be selected as required for settings and saving.
- 7) After completed, turn off the software and computer, close the power switch of imaging system, unplug the power cord, and put instrument away.

4-Examples of Experimental Scheme

4.1 MCAO

- 1) Turn on the system power switch, and start up the software, switch the working mode to online mode;
- 2) Setting: the operating distance is recommended at 15cm, the exposure time is 5ms, and the laser power is 110mW, the camera mode is selected as High SNR Mode or HD mode, the recording mode is selected as continuous, the recording time is set as 60-120 seconds, and the file saving path is set;
- 3) Cut the scalp of the mouse, remove the fascia on the skull, place the target area in the center of the visual field, perform auto focus or manually fine focus to get a sharp image, and adjust the magnification according to the size of the target imaging visual field;
- 4) Set the region of interest (ROI), select the ischemic area and the corresponding area on the normal side;
- 5) Start recording, first record 30-60 seconds, then click pause to complete the blood flow detection before MCAO operation;
- 6) After MCAO operation, click record and continue to record for 30-60 seconds;
- 7) After completion of recording, the software was switched to offline mode, ROI blood flow reduction percentage = (preoperative ROI perfusion - postoperative ROI perfusion)/preoperative ROI perfusion, cerebral blood flow reduction percentage after modeling was more than 70%, indicating the success of the model.
- 8) Other recording modes and other functions (measurement of pipe angle and pipe diameter) can be used at your need according to their needs.

5-Software Intro

Double click the software icon to start up, the software will enter the main interface of offline working mode by default after initialization completed. Please switch to online mode by yourself (see Section 5.1.1).

If there is a prompt of “serial port open failed”, please click [help] → [serial port setting] (see section 5.9.2) to select the serial port connection.

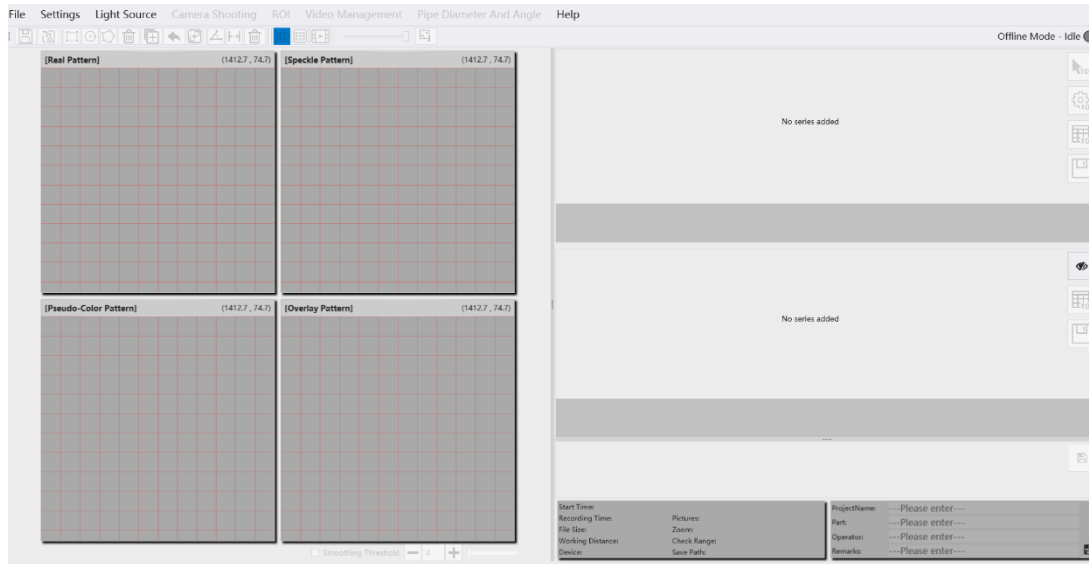


Figure 5-1

Menu bar

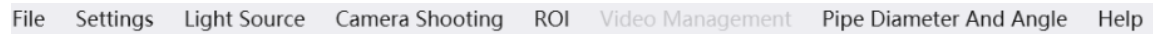


Figure 5-2

Shortcut icon bar and auto/manual focus zone



Figure 5-3

Smoothing threshold

When [smoothing threshold] is checked, the smoothness of image edges can be adjusted by clicking +/- or dragging the slider left or right.



Figure 5-4

5.1 File

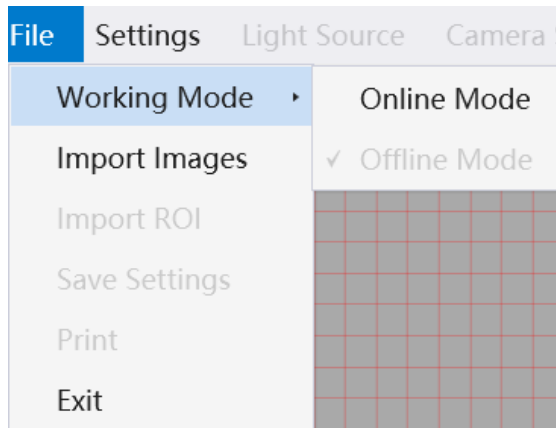


Figure 5-5

5.1.1 Working Mode

Click [File] → [Working Mode]

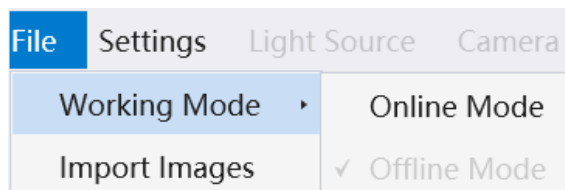


Figure 5-6

The software starts with offline mode by default. In the offline mode, some functions are invalid, and software will carry out data analysis for recorded and saved images, but camera and laser will not run automatically in this mode.

Select the online mode, the camera and laser automatically run, and the gray distribution of the original image and the change of flow rate in the ROI can be displayed in real time in the main interface during operation.

5.1.2 Import Images

Click [Import Images] to pop up a dialog box for the path selection, please select and import the file of .RDT format.

5.1.3 Import ROI

Click [Import ROI] to pop up a dialog box for the path selection, select the .xml file with ROI record and then import. The shortcut icon corresponding to this function is:



5.1.4 Save Settings

Used to set the save path, file folder name, picture and data format of the camera shooting, and so on.

Click [Save Settings] to pop up the dialog box below, enter the file folder name, selected picture and data format and save path as prompted, and click [OK]. The

shortcut icon is .

The default setting [Confirm in the pop-up window before starting recording] could be cancel selected.

Note: It is recommended to choose a save path other than C disk.

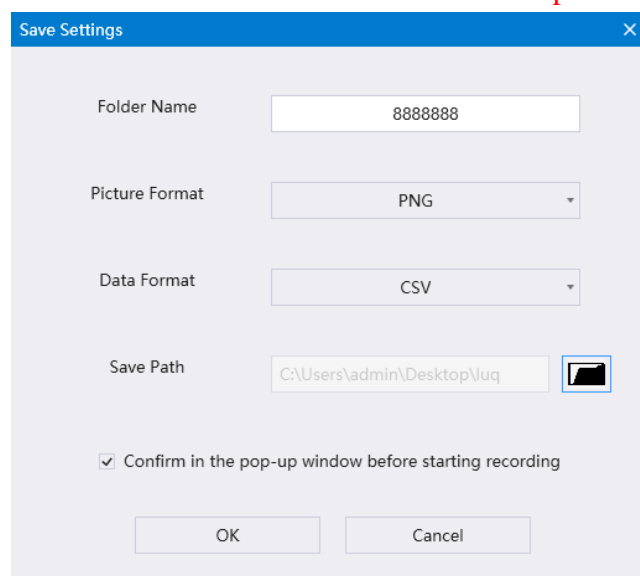


Figure 5-7

5.2 Setting

This interface allows setting of camera parameter, magnification, recording mode, pseudo-color threshold, view selection, grid adjustment.

In the following setting operations, click [Apply] or [OK] to save current parameters, and the current window will be closed after clicking [OK].

5.2.1 Camera Parameter Setting

The exposure time and imaging mode of the camera can be set when the camera well connected.

Exposure time is optional: 1ms/2ms/5ms/10ms

Note: The exposure time greater than 5ms, may result in the actual recorded frame rate failure to reach the upper limit.

It provides four imaging modes for selection: full-speed mode, high-speed mode, HD

Mode, and high SNR mode.

Full Speed Mode: resolution 512*512, 1~120FPS, and image is processed by stLASCA.

High Speed Mode: image resolution ratio 512*512, 1~120FPS, and image is processed by tLASCA.

HD Mode: image resolution ratio 2048*2048, 1~10FPS, and image is processed by tLASCA.

High SNR Mode: image resolution ratio 2048*2048, 1~10FPS, and image is processed by stLASCA.

Explanation:

stLASCA (spatial-temporal based laser speckle contrast analysis) : By calculating the standard deviation and mean of the $N_s*N_s*N_t$ pixels gray values to obtain the spatiotemporal contrast image. The stLASCA gives consideration to both temporal and spatial resolution of the image, and the image SNR can be obtained without time or space average and the high image SNR can be obtained without temporal or spatial average. Even there is a highly reflective object in the background (such as desktop, etc.), the imaging will not have a background redness, avoid interfering with the imaging of organisms.

tLASCA (temporal based laser speckle contrast analysis) : The standard deviation and mean value of the gray value at the same position in the time series image are used to obtain the time contrast image, and then one N_s*N_s space window is used to smooth the image. tLASCA is characterized by high spatial resolution, high imaging resolution of small blood vessels, but low temporal resolution, and slow response to blood flow changes.

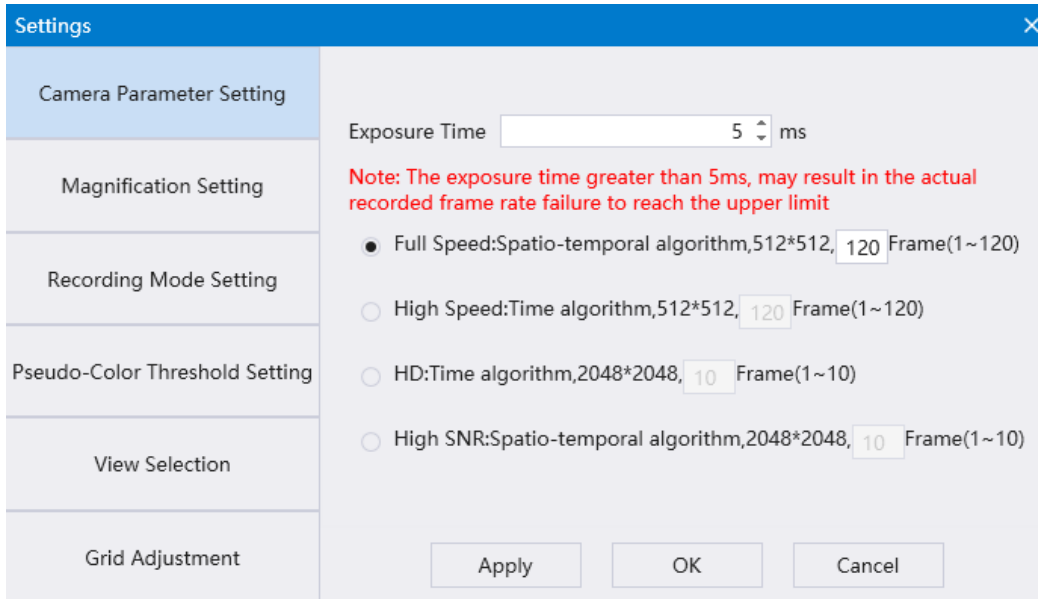


Figure 5-8

5.2.2 Magnification Setting

In the online mode, click the key   or drag the slider to adjust the magnification.

Adjustment range: 0.6~7.2

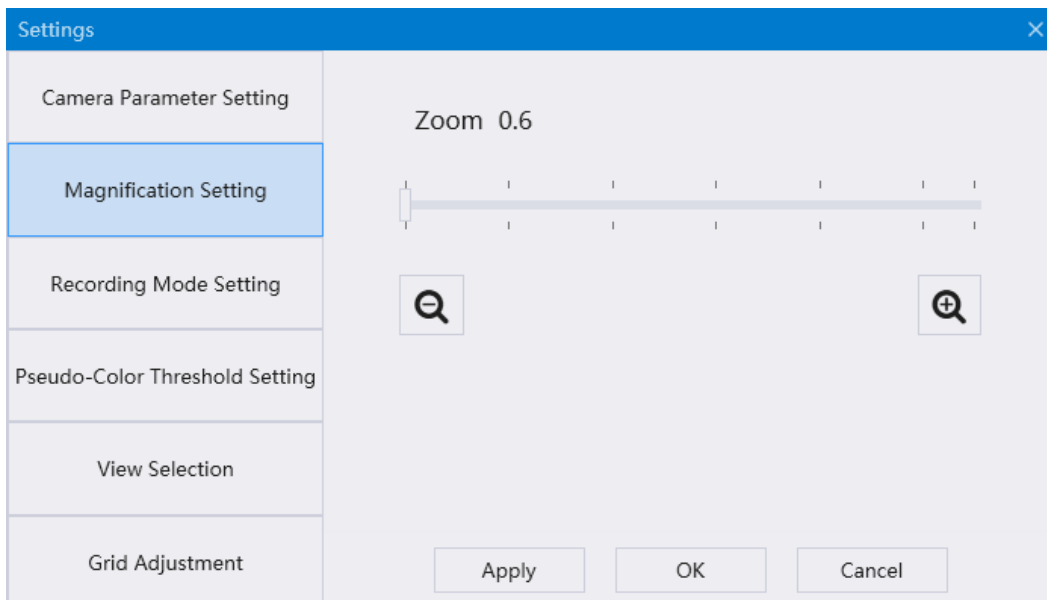


Figure 5-9

5.2.3 Recording Mode Setting

The recording mode includes [Continuous Recording] and [Interval Recording]. There are two recording method in the [Interval Recording] mode. The one is to set only the total duration and the interval time. The set duration and interval time range are 0~18,000s and 0~1,200s respectively. The other one is to set the record time and the interval time, and take both of them as a cycle, and then set the cycle times. The setting range of recording time and interval time is 0~1,200s and 0~1,200s.

Note: ROI data generated during interval recording cannot be used as raw data.

The continuous recording time can be set the range from 0 to 1,800s, record up to 1,000 frames.

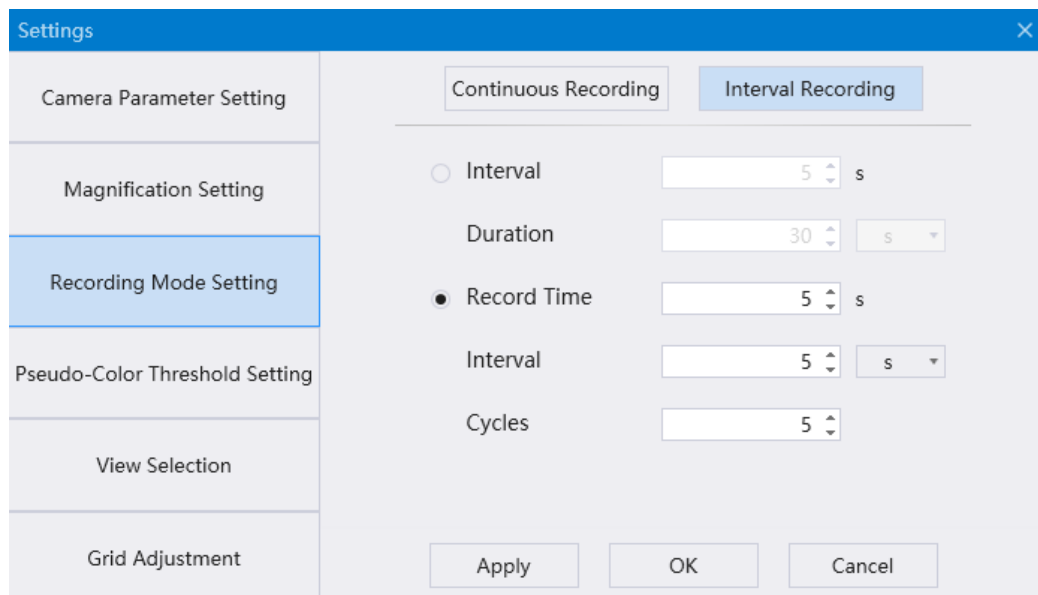


Figure 5-10

5.2.4 Pseudo-Color Threshold Setting

Drag the slider in the figure 5-11 to adjust the pseudo-color threshold. Users can preview the real-time image on the main interface and adjust the settings accordingly.

High threshold setting range: 100-200

Low threshold setting range: 0-100

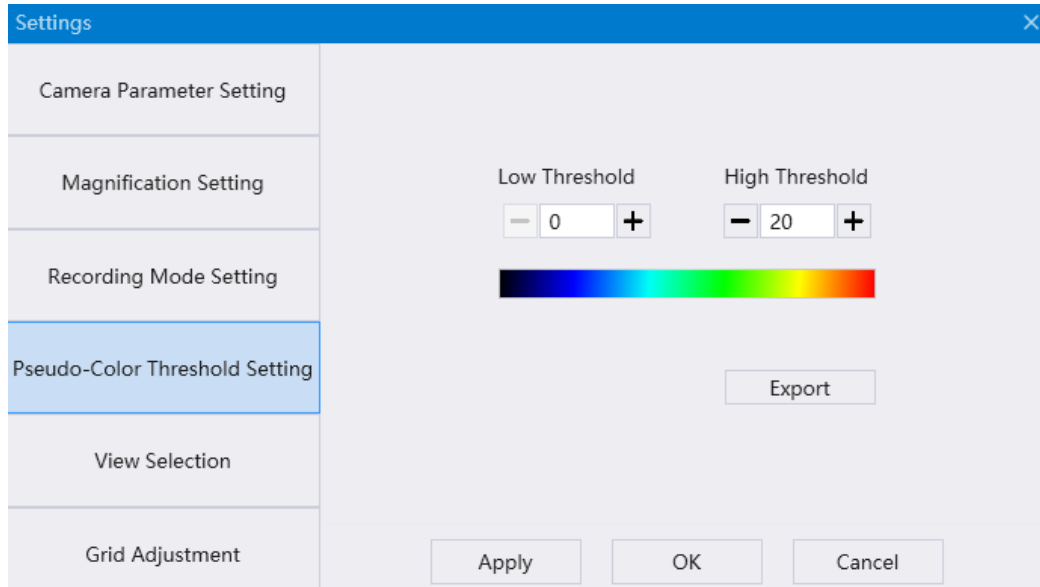



Figure 5-11

5.2.5 View Selection

Here you can select the patterns displayed on the main interface: Real Pattern, Speckle Pattern, Laser Pattern, Pseudo-Color Pattern, Overlay Pattern. Up to four of them can be selected for display. The shortcut icon is . Laser pattern can only be displayed in online mode.

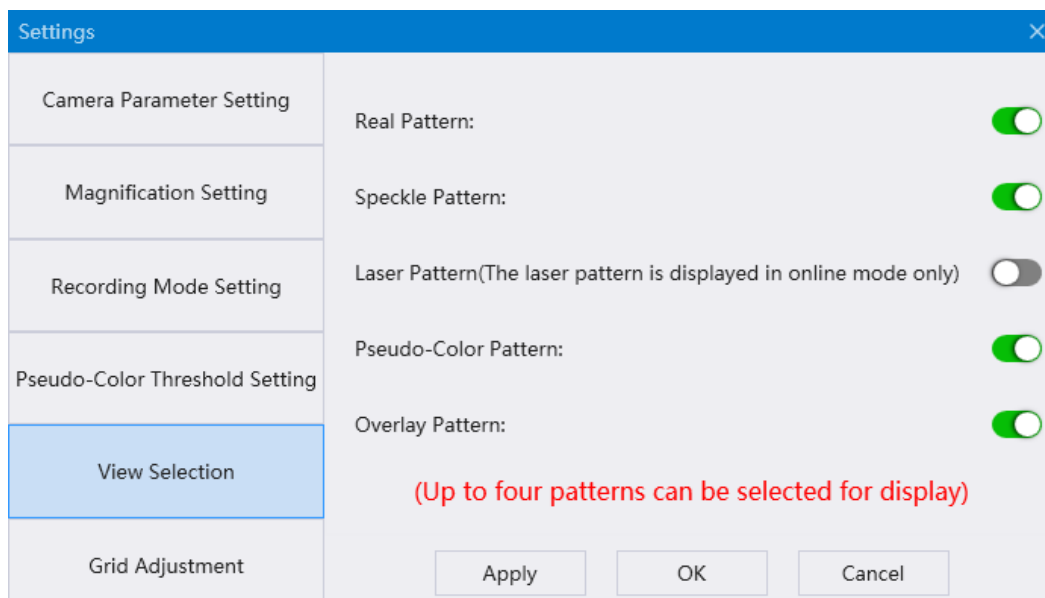


Figure 5-12

5.2.6 Grid Adjustment

There are 40 variety of grid sizes optional for users, such as 1*1, 2*2, 3*3, 4*4 ...40*40 and color can be selected. When adjusting the grid, the preview image of the main interface shows the real-time changes, and the user can observe and set it at the same time.

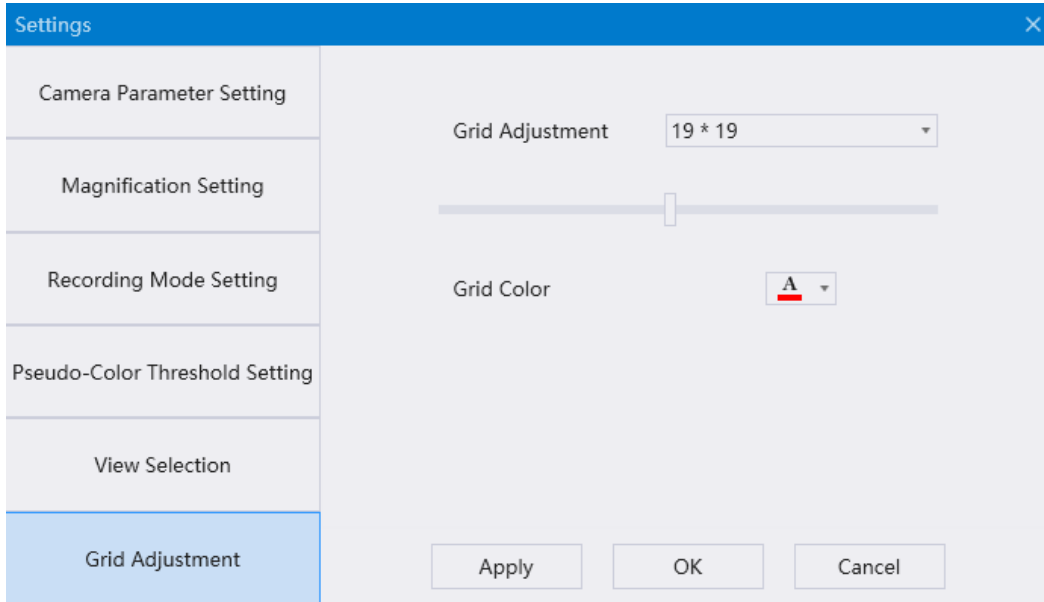


Figure 5-13

5.3 Light Source

Click to turn the laser on/off, turn white-light on/off, turn indicting light on/off and set the light intensity.

Note: the imaging system will be off if one hour without any operation in the computer, user need to click [Camera Connection] (light source will be on automatically after camera connected) to continue. The imaging system will also be off if the computer is in dormant or standby mode.

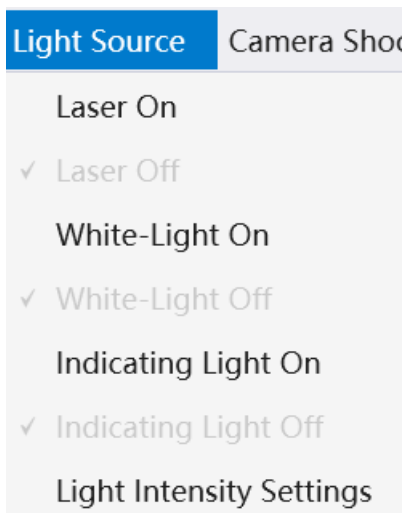


Figure 5-14

5.3.1 Light Intensity Setting

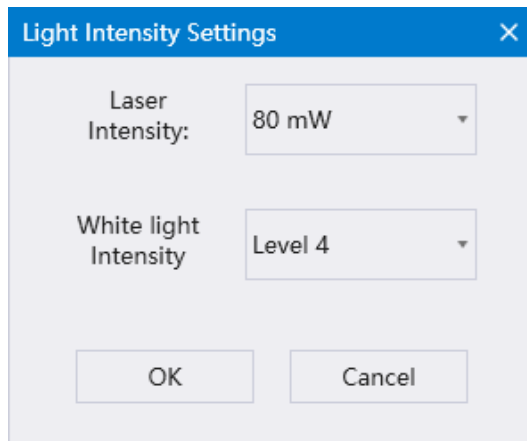


Figure 5-15

There are four options of laser intensity, 60/80/100/110MW, the default is 80mW. The white light intensity has 1~4 levels, and the default is level 4. Users can adjust the intensity per your need.

5.4 Camera Shooting

This menu only works when the camera is connected. Mainly used to control the camera and recording state.

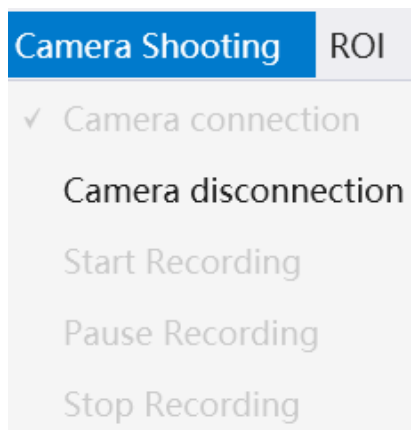


Figure 5-16

5.4.1 Camera connection/ disconnection

Click [Camera connection] to open the camera, and the main interface displays the preview image at the same time. Click [Camera disconnection] to disconnect the camera and stop camera shooting.


5.4.2 Start/Pause/Stop Recording

Click [Start Recording] to start recording according to the settings of recording mode.

The system will stop recording automatically when finished and save the data based on save settings.

Click [Pause Recording] to pause the current recording.

Click [Stop Recording] to stop the current recording and save the recorded data.

During camera shooting, user can click the shortcut icon  to mark the event.

When the event mark operation is carried out, the real-time mark record will be displayed in the information bar at the bottom right of the interface. User can also double-click [New tag] to edit the information of the event mark.

In addition, the user can enter the project name, part, operator and remarks and so on in the information bar below [event mark] bar.

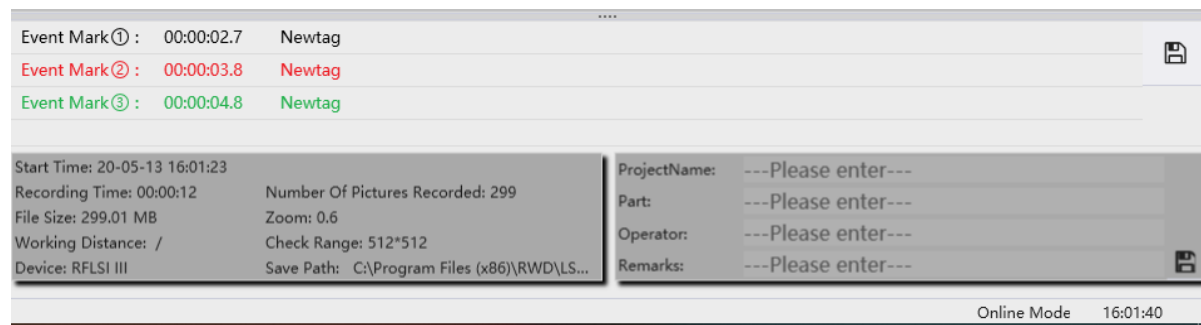


Figure 5-17

5.5 ROI

Mainly used for ROI drawing, deletion and other related operations. The image can be enlarged to draw the ROI.

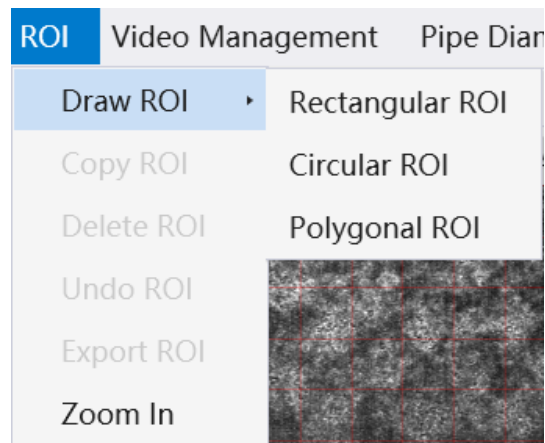





Figure 5-18

5.5.1 Draw ROI

- 1) It includes the drawing of Rectangular ROI, Circular ROI and Polygonal ROI. The corresponding shortcut icon is   .


The drawing method of Rectangular ROI/ Circular ROI: clicking the

corresponding key and then drag the mouse to select ROI.

The drawing method of Polygonal ROI: clicking the corresponding key and then click the mouse left to draw ROI, and complete by clicking mouse right.


- 2) User can draw ROI in any image. After the ROI is drawn, the corresponding positions of other images will simultaneously display the same ROI. The system supports drawing multiple ROIs and distinguishes them by different colors. Up to 50 ROIs can be drawn.
- 3) The user can also draw multiple ROIs in succession. Right click or click the corresponding draw key again to release the drawing state.

5.5.2 Copy ROI


Select ROI and click to copy the currently selected ROI. The shortcut icon is .

5.5.3 Delete ROI

Click the key could delete the currently selected ROI or depress the “Delete” key on the keyboard to delete; if no ROI is selected, click the key to delete all ROIs.

The shortcut icon is  (Delete ROI). You can also click the left mouse to drag and select multiple ROI, and click to delete.

5.5.4 Undo ROI


Click this key to undo the previous operation of Draw/Copy/Delete ROI, The shortcut icon is .

5.5.5 Export ROI

Export ROI and save it.

5.5.6 Zoom in

Click the key to make mouse cursor switch to a Zoom-In icon, user can select the zoom-in zone in the all patterns. The enlarged image of selected area is shown in the figure 5-19. The icons in the red border are in sequence: zoom in, zoom out, move (this function only be applicable to move the enlarged area of original image) , rectangular ROI, circular ROI and polygonal ROI, undo, confirm. The

shortcut icon of [Zoom in] is .

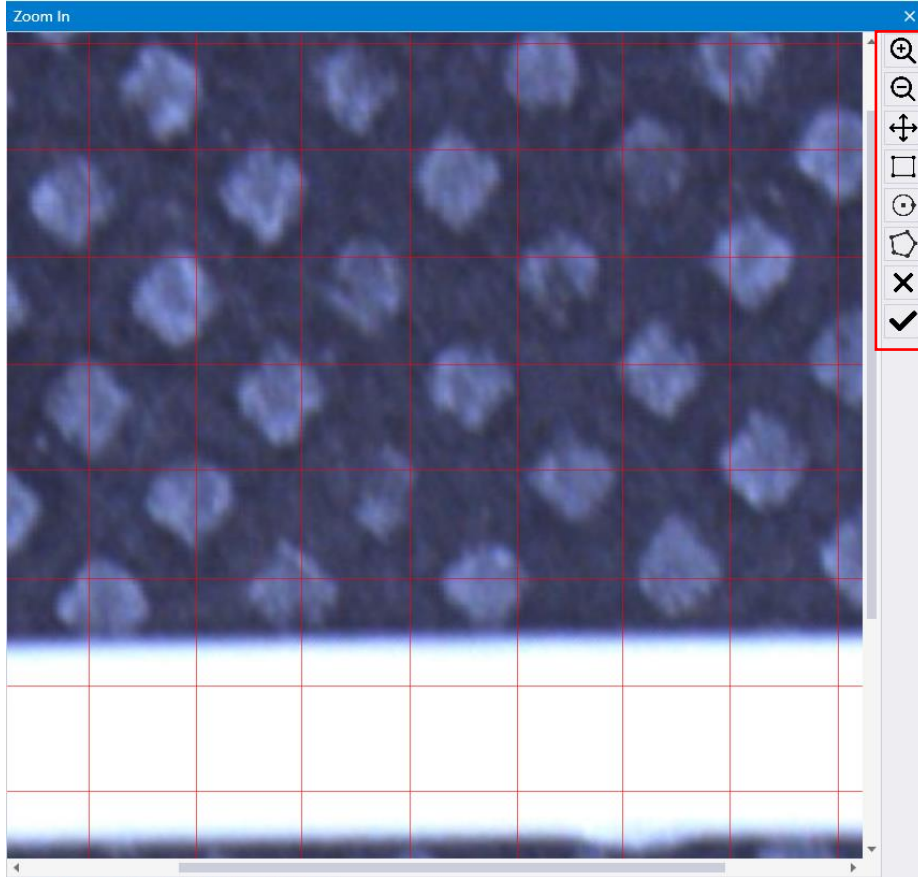


Figure 5-19

5.6 Pipe diameter and angle

Pipe Diameter And Angle	Help
Pipe Diameter Measurement ▶	Draw
Pipe Angle Measurement ▶	Delete


Figure 5-20

5.6.1 Pipe diameter measurement


Click [Pipe diameter measurement] → [Draw]. It allows to draw several pipe diameters. Up to 50 pipe diameters can be drawn and distinguished by different


colors. The shortcut icon is .

Click [Pipe diameter measurement] → [Delete]. When any pipe diameter is selected, click this key to delete the currently selected pipe diameter. If no pipe diameter is selected, click this key to delete all pipe diameters. The shortcut icon

is  (Delete diameter and angle). You can also click the left mouse to drag and select multiple diameter, and click to delete.


5.6.2 Pipe angle measurement

Click [Pipe angle measurement] → [Draw]. It allows to draw several pipe angles. Up to 50 pipe angles can be drawn and distinguished by different colors. The shortcut icon is .

Click [Pipe angle measurement] → [Delete]. When any pipe angle is selected, click this key to delete the currently selected pipe angle. If no pipe angle is selected, click this key to delete all pipe angles. The shortcut icon is  (Delete diameter and angle). You can also click the left mouse to drag and select multiple angle, and click to delete.

5.7 Curve graph



ROI and pipe diameter are drew in online mode, the real-time blood perfusion and pipe diameter will be displayed on the main interface.

Click  to hide the curve graph of pipe diameter.


In offline mode, user can import the recorded images and ROI data, and then click on any ROI to start data analysis after confirmation, as shown in figure 5-21.





Figure 5-21

Click  &  in offline mode to switch **selection on pictures/TOI setting** on the graph. Place the mouse on the slider on the horizontal or vertical axis until the mouse turns into double-arrow icon, slide left/right or up/down to enlarge/shrink the curve.

5.7.1 Selection on pictures

After clicking , place the mouse on the horizontal axis and drag it to select time interval. To delete the selected area, right-click mouse twice and then click appeared "delete" word.

5.7.2 TOI setting

Click  to pop-up [TOI setting] window, click  to add more.

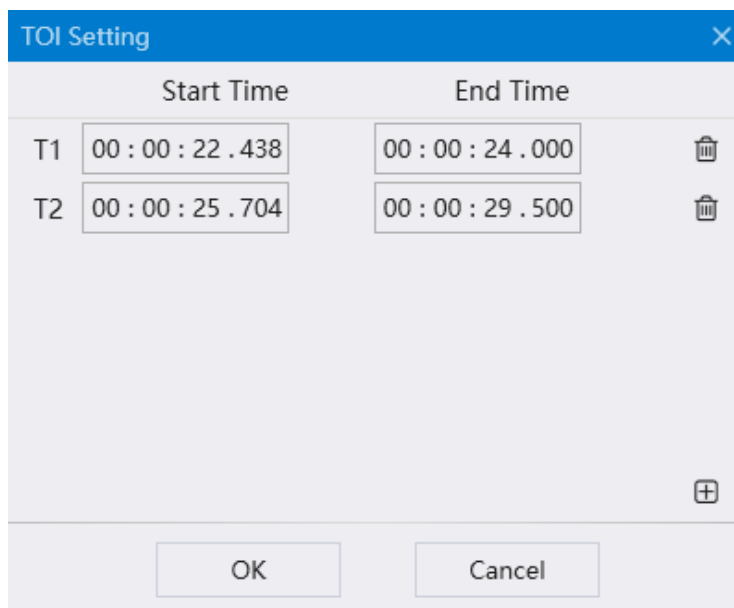




Figure 5-22

5.7.3 Blood perfusion / pipe diameter data

Click  on the graph  to view the TOI data of blood perfusion /pipe diameter. As shown in Figure 5-23 and Figure 5-24


TOI Data (Perfusion)								
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

Figure 5-23

TOI Data (Pipe Diameter)								
Pipe Diameter	TOI	Frames	Mean	Standard Deviation	Variance	Maximum	Minimum	Median
①	T1	39	6050.08	12098.51	146373928.71	9308.57	3034.91	7677.87
②	T1	39	5901.98	83.17	6917	5914.96	5888.31	5914.96
③	T1	39	6506.05	2127.87	4527827.43	6576.87	4406.51	6554.95

Figure 5-24

5.7.4 Save

Click  to save data, as shown in Figure 5-25. Users can choose three formats of .txt/.csv/.jpg for data saving.

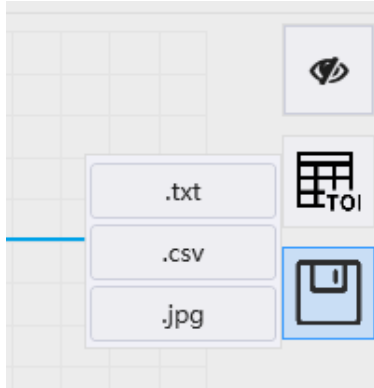


Figure 5-25

5.8 Video Management

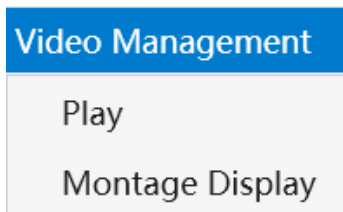








Figure 5-26

5.8.1 Play

In the offline mode, click [file] → [import images], select the recorded .RDT file and load, then click [play] to pop up the playing interface, and the corresponding shortcut icon is .

Click  to play, and play speed is optional for 0.5/1.0, click   to fast-forward, or rewind.

Click  to back to main interface, click  to enter Montage display .

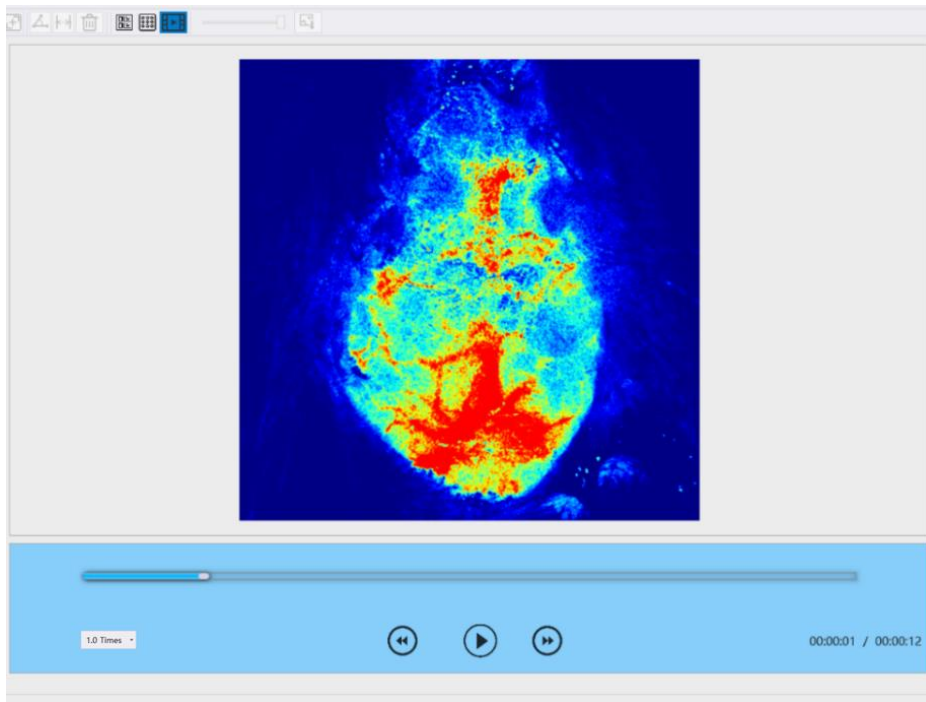


Figure 5-27

5.8.2 Montage display

Click [Video Management] → [Montage Display] to get the montage interface of pseudo-color pattern as figure 5-28.

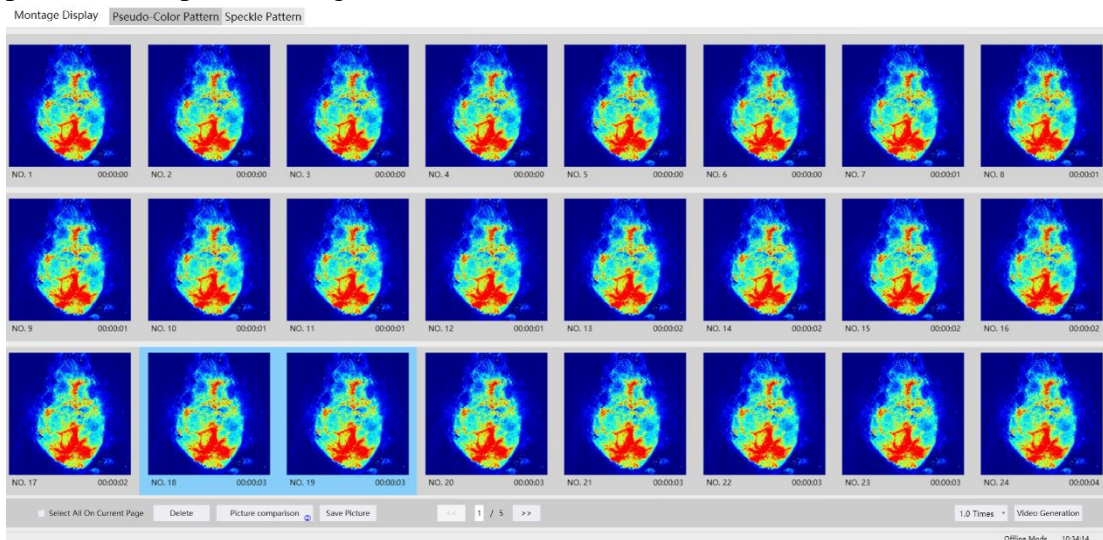


Figure 5-28

Click "Speckle Pattern" to switch to montage display of speckle pattern.

Select 1-4 pictures and click [Picture Comparison] to get the enlarged comparison pictures.

Click [Save picture] to save the picture to the required path according to the prompt.

Click [Video Generation] to regenerating new video and save the video to the required path according to the prompt.

5.9 Help

Mainly used for system related information inquiry and user manual consultation.

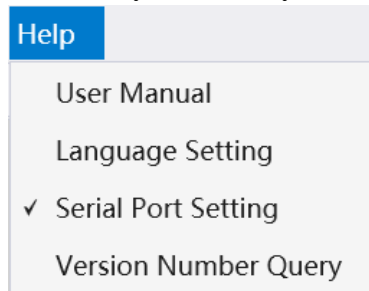


Figure 5-29

5.9.1 Language Setting

Click [Language Setting] to get the following dialog box, in which the user can click to switch the system language.

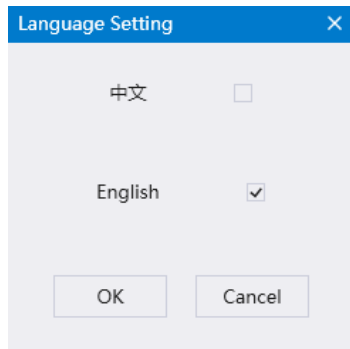


Figure 5-30

5.9.2 Serial Port setting

Click [Serial Port Setting] to get the following dialog box, in which the user can click to select serial port.

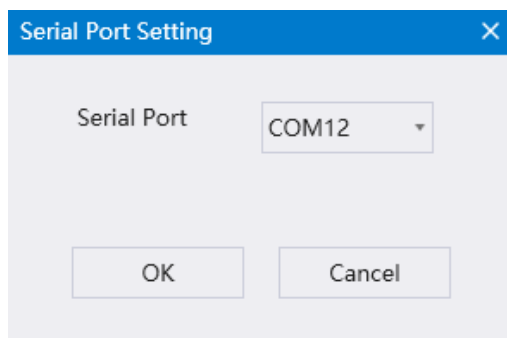


Figure 5-31

5.9.3 Version Number Query

When the system is well connected, the item of BORD Revision will show a correct version number.

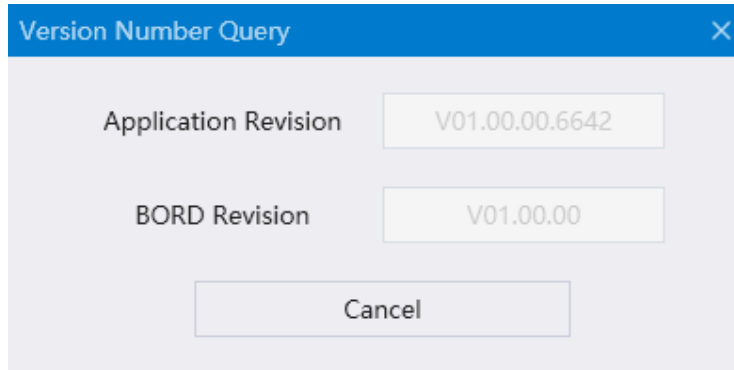


Figure 5-32

6-Maintenance

6.1 Overview

This section describes how to maintain the laser speckle imaging system to ensure that the system is in good condition.

6.2 Annual inspection

RWD recommends a comprehensive inspection of the laser speckle imaging system every year, as the annual inspection can ensure a good operating state of equipment. For details, please contact RWD.

6.3 Record keeping

It is recommended that the maintenance process should be recorded and kept after maintenance, including time, location, maintenance procedures, etc. for future reference.

6.4 Parts and materials

If the user finds that some parts or materials should be replaced during maintenance, please contact RWD for after-sales support.

6.5 Product cleaning and maintenance

- Do not scratch the instrument with hard or sharp objects;
- Do not wipe the surface of instrument with chemical reagents but water;
- Avoid vibration or collision of equipment during the handling process;
- For every two months of use, the attached tools should be used to tighten the connecting screws among parts;
- If the instrument is not used for a long time, unplug the power cord and place it in an appropriate storage environment. It is suggested to cover the optical imaging unit with cloth for dust prevention.

7-Troubleshooting

This section introduces the problems (faults) that are common to the product and their possible causes and suggested countermeasures.

Faults	Troubleshooting methods
Lighting laser cannot be turned on	1) Check whether the host power cord is loose
	2) Check whether the serial port can be found and opened
	3) Check whether the 2.0 USB cable is loose
Software startup abnormality	1) Check for original computer
	2) Check whether the 3.0 USB cable of image capture camera is loose
	3) Check whether the driver is installed correctly
Abnormal image	1) Check whether the white light of imaging system is turned on
	2) Check whether the laser of imaging system is turned on
	3) Check whether the image capture camera operates properly
Other faults or questions	1) Please contact the after-sales personnel. Do not disassemble the equipment for maintenance.

8-Warranty

The warranty of this equipment starts from the date of leaving the factory. During the warranty period, the equipment cannot be used normally due to problems such as materials and process defects. RWD undertakes after-sales service such as equipment maintenance and parts replacement.

Any damage caused by improper use or over-range use is not covered by the warranty. If repair or replacement of parts is required, the cost will be borne by the user.

If the reworked equipment was found to have been unauthorised disassembly, RWD will not provide after-sales service such as warranty, free maintenance and parts replacement.

The warranty statement (including its restrictions) is exclusively issued by RWD and covers all other warranties



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