RWD

Dual Color Multi-Channel Fiber Photometry (410&470)

User Manual **B**

RWD Life Science Co.,Ltd

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Contents

1. Product Introduction

1.1. Overview

Thank you for your choosing Dual Color Multi-Channel Fiber Photometry (410&470) manufactured by RWD.

Before you assemble and use the product for the first time, read all the documents shipped with it carefully, which can help you use the product in a more favourable way.

RWD Life Science Co., Ltd. is committed to continuously improving product functions and service quality. RWD reserves the right to make changes to any product described in this manual and the content of this manual without prior notice.

If you require the latest product information, please call or write to us, or visit our website (http://www.rwdstco.com/). If there is any inconsistency between the actual use of the product and this manual, or if you have any questions or suggestions, please contact us.

The device is to be used only for animal experiments and pre-clinical scientific research. It should never be used on humans.

1.2. Safety

To avoid injuries to laboratory animals, operators and damage to the system during operation, please read section **2 "System Safety"** carefully.

If you have any questions or suggestions about product safety, please contact our customer service support.

The device should be operated and managed by trained professionals.

1.3. Description

This Dual Color Multi-Channel Fiber Photometry (410&470) is designed, developed and produced by RWD Life Science Co., Ltd. It is a low fluorescence signal detection and the system based on multi-mode fibers, can excite fluorescence stably for a long time and detect the weak change of fluorescence signal. The system is a device to record the changes of neuronal activity in animal population by sCMOS camera after the LED light source is used to excite fluorescence, which has a wide range of applications in the study of brain loop function.

The purpose of optical fiber recording is to reflect the changes of nerve cell activity in real time based on the fluorescence of detection probe. Gene-encoded neural probes are widely used in the field of neuroscience to characterize neuronal activity. The system can be applied to the fluorescence detection of various GFP-based neural probes, such as GCaMP, dLight, iGluSnFR, etc. The neural probe was expressed in the neurons of the target brain region, and the excitation light was introduced into the relevant brain region through the optical fiber to excite the generation of fluorescence, for measuring fluorescence intensity.

1.4. Product Features

- Two LED excitation light sources: 470 nm is used for the fluorescence excitation of green calcium indicator, and 410 nm is used to eliminate the influence of motion noise.
- Up to 9 channels of fluorescence signal recording can be supported for simultaneous experiments.
- Support 4 TTL signal input and 4 TTL signal input.
- **Sequential /Continuous** excitation output mode.
- User-friendly UI for easy operation.
- Up to 10,000 hours of service life.

1.5. Environment Requirements

Prepare the operating environment according to the table below to ensure operability and safety of the system.

	Description
Operating environment	Temperature range: 15 ℃~35 ℃
	Humidity range: 15%~80% (non-condensing)
	Air pressure range: 57 kPa~106 kPa
Storage environment	Temperature: range -20 ℃ ~60 ℃
	Humidity range: 10%~80% (non-condensing)
	Air pressure range: 50 kPa~106 kPa
Working power supply	AC power range: 90 V~264 V, 50/60 Hz, 2 A
	DC power range: 12 Vdc, 6 A, 72 w Voltage fluctuation of the power supply cannot exceed 10% of the working voltage range.

1.6. Product Parameters

Parameter	Description
Dimension	315mm×290mm×111mm
Weight	3.9 kg
Frame rate of the fiber photometry camera	10~300 fps
Exposure time range	Depends on the frame rate of the fiber photometry camera. If the frame rate is 10 fps, the exposure time range can be set to 1~30 ms; if the frame rate is 300 fps, the exposure time range can be set to 1~2 ms.
Frame rate of the behavior camera	30 fps
LED power regulation percentage	0~100%
Gain	1~100

1.7. Product List

Configuration	Items	Qty	Description
Standard	Host	1	Fiber photometry.
Standard	BNC cable	2	TTL input/output.
Standard	Power cord of the host	1	Power supply.
Standard	Power adapter	1	Power supply.
Standard	USB 3.0 cable	1	Transmit the signals of the fiber photometry camera.
Standard	USB 2.0 cable	1	Connect the device and the computer, downloads programs, controls the main board, and transmits marker signals.
Standard	Dongle	1	Used for software.
Standard	Behavior camera set	1	Shoot behavior videos.
Standard	Optical fiber	1	Connect to the experimental subject's brain.
Standard	Computer	1	Used to install and operate software.
Standard	USB hub	1	USB expansion interface.
Standard	USB Disk	1	Backup software.

2- System Safety

⚠ Read safety instructions carefully. For safety purposes, pay attention to the following items.

- **Connect cables correctly.**
  Ensure that all cables are securely and firmly connected to the device.
- **Avoid having exposed electrical cables.**
  Do not touch any internal electronic devices or circuits.
- **Turn off the device in the case of suspected faults.**
  If the device has potential safety problems or cannot operate normally, contact authorized technical support personnel.
- **Connect the device properly.**
  Connect the device properly to prevent operation difficulties and disconnection of the device.

⚠ 2.1. Precautions

- Insert the power cord correctly to prevent poor power contact.
- Avoid shaking, water, moisture, compression and fire.
- Move and carry the device carefully to avoid damage to and dropping of the device.
- Untrained personnel are not allowed to operate the device. Use reliable grounding devices.
- Keep the optical fiber interface clean and cover it using a dust cap when the device is not in use.
- RWD is not responsible for any device failure caused by improper cleaning, maintenance and operation.
- If the equipment is not in use for a long time, it is recommended to turn off the host and computer.
- If you disassemble and assemble the device host without authorization from RWD, the company will no longer provide any quality assurance or technical maintenance service commitments for the device. If you have any technical problems, contact authorized personnel or RWD for support.

3- Product Structure and System Connection

Figure 3-1

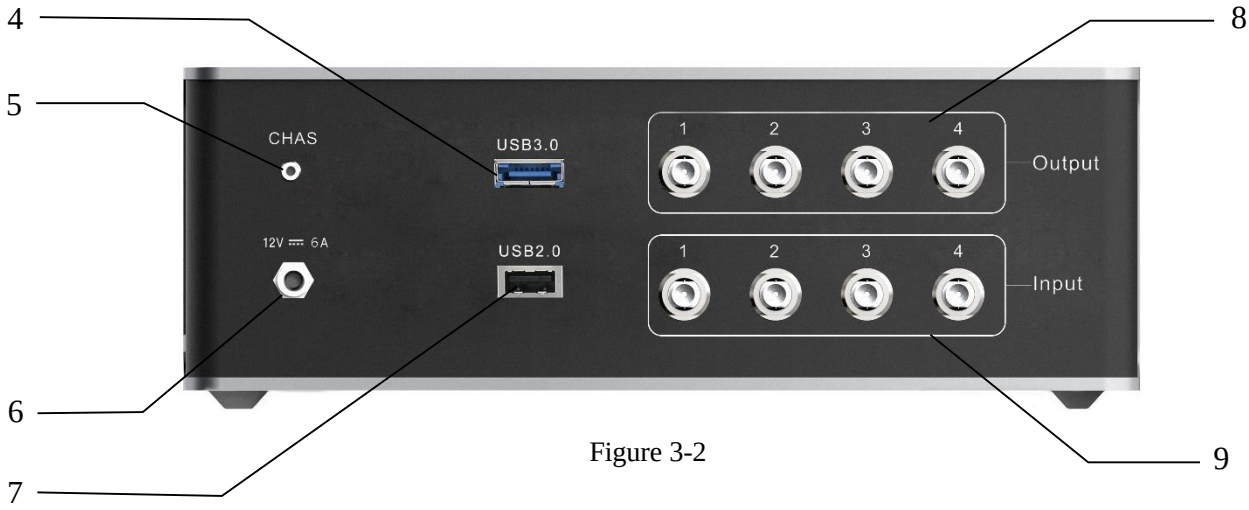


Figure 3-2

No.	Interface Name	Qty	Description
1	Power switch	1	Turns on/off the device.
2	Optical fiber interface	1	Connects the optical box and multi-mode fiber bundles.
3	Focusing knob	1	Focus on fiber end faces.
4	USB 3.0 interface	1	1) Downloads software, controls the main board, marks external enhancement. 2) Transmits camera videos.
5	Grounding interface	1	Reserved for electrophysiology device grounding.
6	12V DC power interface	1	Powers the entire device.
7	USB 2.0 interface	1	Controls hardware communication.
8	Output BNC connectors	4	Outputs 1-4 are signal channels.
9	Input BNC connectors	4	Inputs 1-4 are channels of external signals.

- System connection

  1） First, connect one end of the power adapter to the power supply and the other end to the 12V power interface of the host.

  2） Connect the USB cables to your computer

  Note: be sure to use a USB 3.0 cable to connect the USB 3.0 interface on the host to the USB 3.0 interface with power supply mark on the computer. The other USB cable is used to connect the USB 2.0 interface on the host and any USB interface on the computer (or 3.0 USB Hub). The behavior camera must also be connected to the USB3.0 interface on the computer (or 3.0 USB Hub).

  3） The fiber optics bundle is inserted into the optical interface for collection.

Figure 3-3

4- Software Introduction

4.1. Environmental requirements of the software

A 64-bit operating system of Windows 10 Home

4.2. Summary of main functions of the software

4.2.1. Data collection

1) Continuous collection and sequential collection
2) Synchronous collection of behavior video
3) Automatic marking, manual marking, ROI marking
4) Triggered collection, delayed collection and collection duration setting

4.2.2. Data analysis

1) Output ΔF/F, and Z-Score
2) Output the behavior track heat map of the experimental subject
3) Output data smoothing, baseline correction and motion correction results
4) Analyze the data event, generate the fluorescence heat map corresponding to the trail and mean±sem

4.2.3. Overview of shortcut keys

Icon	Meaning
	Reconnect (Refresh)
	Minimize
	Normal
	Maximize
	Close
	Start/Stop Setting
	Open Save Dir
	Save/Change Save Dir
	Auto Scale
	Zoom In
	Zoom Out
	New Files
	Save
	✓Check the first 50 markers at once

5- Operation Example

1) Ensure that behavior testing of an experimental animal is carried out in a dark room or a laboratory with a stable environmental brightness. Otherwise, the signal recording results can be affected.
2) Prepare an animal that has been injected with a virus and implanted with an optical fiber needle. The virus must carry a GFP-based neural probe (such as GCaMP6), and animal virus expression must last for at least two weeks.
3) Connect the system. For operation details, see section ***3-*** "***Product* Structure and System Connection**".
4) Press the power switch to turn on the device. Double-click the icon  to start the software on the desktop. Click **Photometry** and confirm that devices are properly connected. The software automatically displays the main data collection tab.
5) Connect the fiber bundle. Shake the fiber end faces towards the light source, and turn the focusing knob until the fiber end faces are clearly visible in the software.
6) Click **Preview**. Select 470 nm as the light source, use the optical power meter to measure the output power, and adjust the optical power to an expected value (for example, 20 uw) and measure. Change the light source to 410 nm and perform the same operations. After the measurement is completed, set the light source mode to 410 nm & 470 nm.
7) Access the Channel Setting window. Set the sampling frame rate and select channels (CH1 to CH9 for chosing). Shake the fiber end faces towards the light source or increase the gain. Confirm the position of the fiber end faces, adjust the ROI size and drag the ROI to a proper fiber end face. Set the gain to 1 and save the settings.
8) Point the behavior camera at the area where the animal resides, and adjust the behavior camera until the video is clearly visible.
9) Set the channel of hardware marker as Input1 and set the signal output of the external device to high level 3.3 to 5 V.
10) Before starting a formal experiment, keep the preview state for 5 to 10 minutes. The warm-up process can stabilize the baseline.
11) Connect the optical fiber to the optical fiber needle on the animal head through a sleeve.
12) Adjust the power of the 410 nm light source to make the fluorescence value of 410 nm close to that of 470 nm.
13) Click [Record] to collect data. After completing the experiment, click [Stop] to stop data collection.
14) Switch to the Analysis tab to analyze the raw data collected. You can also view the △F/F and Z-score data analysis results, fluorescence heat map, and behavior trajectory.
15) You can change other parameters, modes and settings according to specific experimental needs.

6- Data Collection

6.1. Initialization

Install the multi-channel fiber photometry software, and double-click the desktop software icon to enter the operation interface.

Figure 6-1

Click [Photometry] or [Analysis] to enter the data collection or analysis interface.

When [Photometry] is selected, the software will automatically detect whether the Host, the fiber photometry camera, and the behavior camera are connected normally. If the connection is normal, it will automatically enter [Photometry]. If the interface in Figure 6-2 shows that the connection status of the corresponding device is "disconnected", please click the refresh button or conduct a self-check, and reconnect the device to ensure that the connection status is "connected".

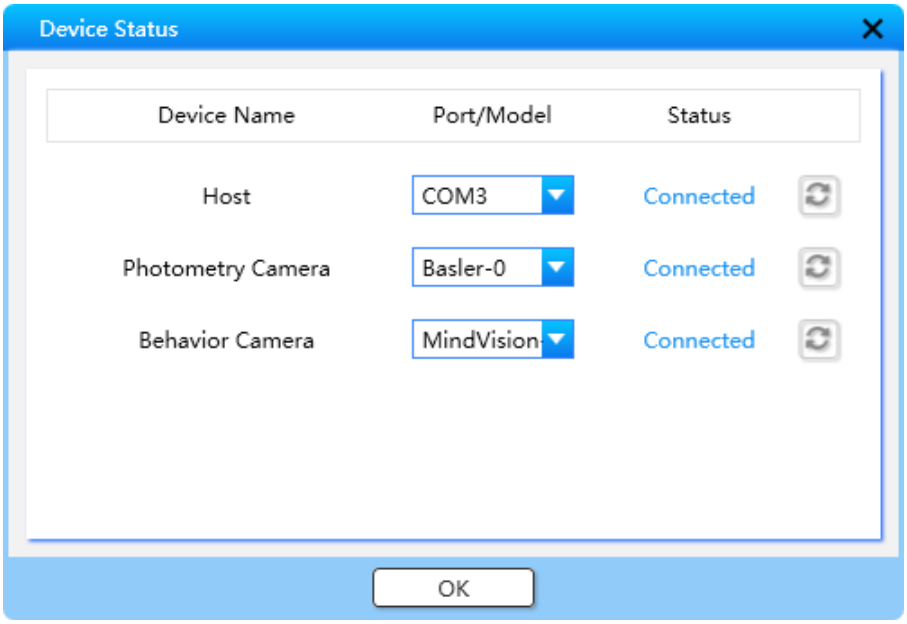


Figure 6-2

If the connection of the Host, the fiber photometry camera, and the behavior video is identified as normal, the system will automatically go to the main interface.

The system will enter the [Photometry] page by default, as shown in Figure 6-3. From left to right are the parameter setting area, the trace area, and the behavior video area.

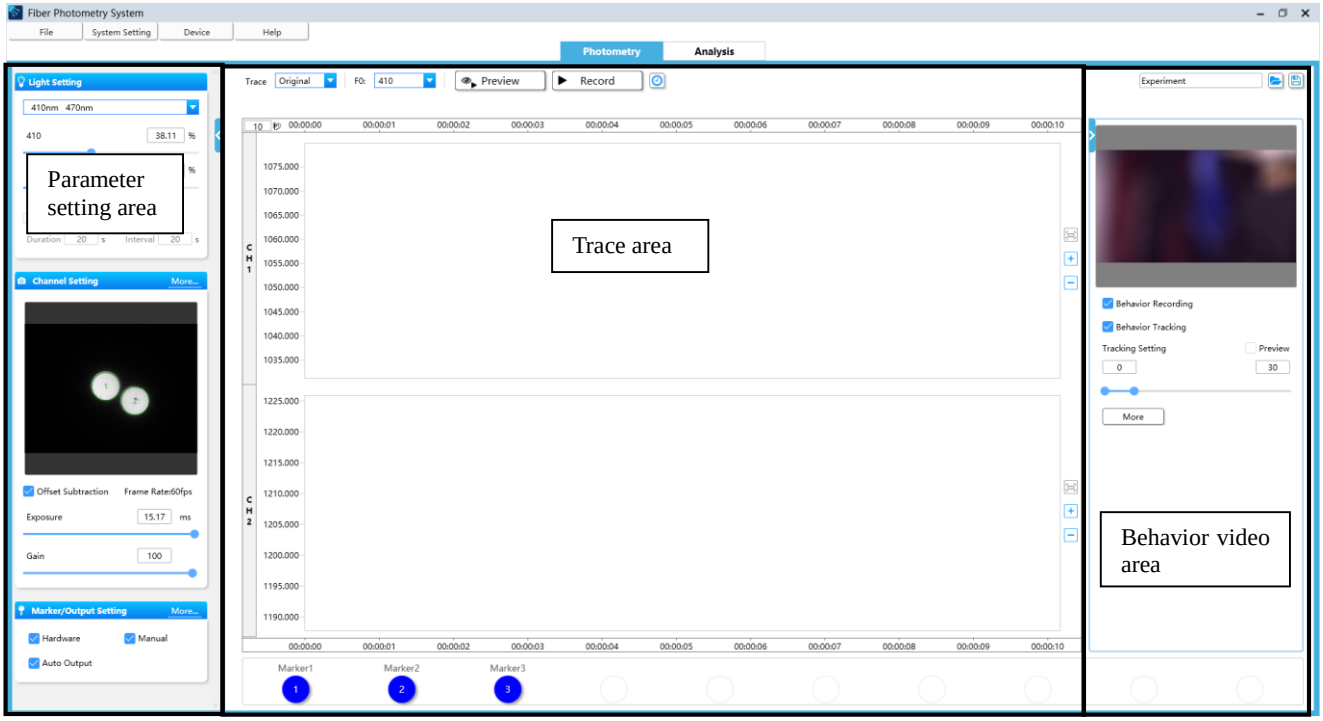


Figure 6-3

6.2. Light setting

Click  to select the Light mode. The Excitation modes include 410nm, 470nm or 410nm & 470nm. Drag the slider to set the light power proportion of the corresponding light.

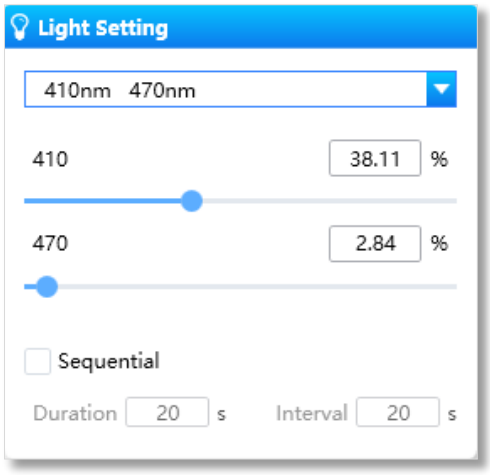


Figure 6-4

When [Sequential] is checked, the sequential excitation mode will be used to output excitation light in preview and collection. The output of excitation light will continue within the duration, and stop at intervals. The output cycle will go according to the set **Duration** and **Interval**. As shown in Figure 6-4, the duration is set to be 20 seconds, and the interval is set to be 20 seconds. When the collection starts, the excitation light will be sent continuously for 20 seconds. After that there will be a 20-second interval, during which the software is still running,

but the excitation light will be turned off. At the end of the interval, the excitation light will be sent for the next 20 seconds. Such a cycle repeats itself.

If it is not checked, the excitation light will be output continuously according to the selected light mode.

6.3. Channel/Camera parameter setting

This interface displays the current camera frame rate/exposure duration/gain.

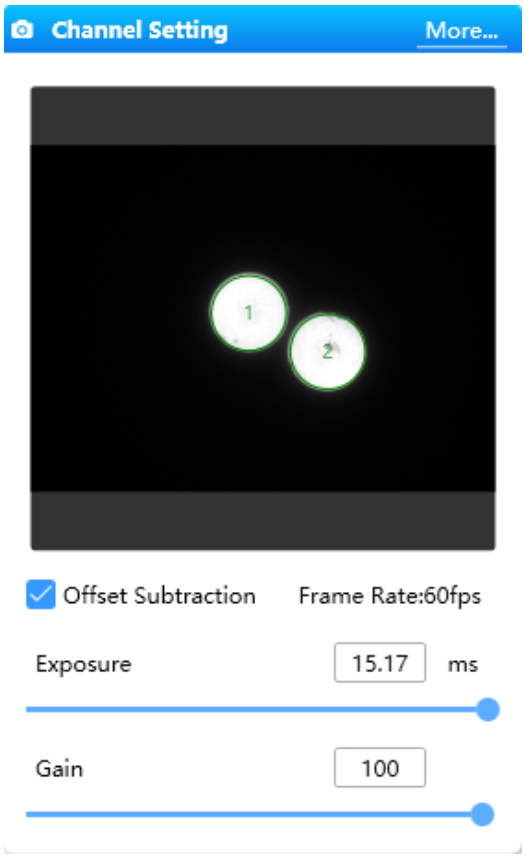


Figure 6-5

Offset Subtraction: If it is checked, the offset subtraction algorithm can be used in the process of fluorescence raw data collection to reduce the background value of the camera and the influence of the fiber spontaneous fluorescence on the data. When using low fluorescence fiber, it is not recommended to turn on offset subtraction.

Click [More] in the upper right corner of Figure 6-5 to enter the channel setting window, as shown in Figure 6-6. Click to select the camera frame rate. The higher the frame rate, the larger the amount of data collected. 60-100fps is usually recommended.

Drag the slider to adjust the exposure duration and gain. It is recommended to use the corresponding maximum exposure at the selected frame rate. In data collection, gain value of 1 is recommended.

The software supports up to nine channels of signal collection. In [Channel Setting], desired channel such as CH1/CH2 can be chosed. For each channel checked, a green ROI will be generated in the camera view. By adjusting the gain and changing the brightness of the fiber end face, or by shaking the fiber toward the light, the position of the fiber end face can be found in the view. Drag the ROI to cover the corresponding fiber end face, and adjust the gain to the lowest value after adjusting the ROI.

[ROI Diameter]: change the green ROI diameter to fit different fibers by adjusting the value in

the box or clicking and selecting the green ROI to adjust.

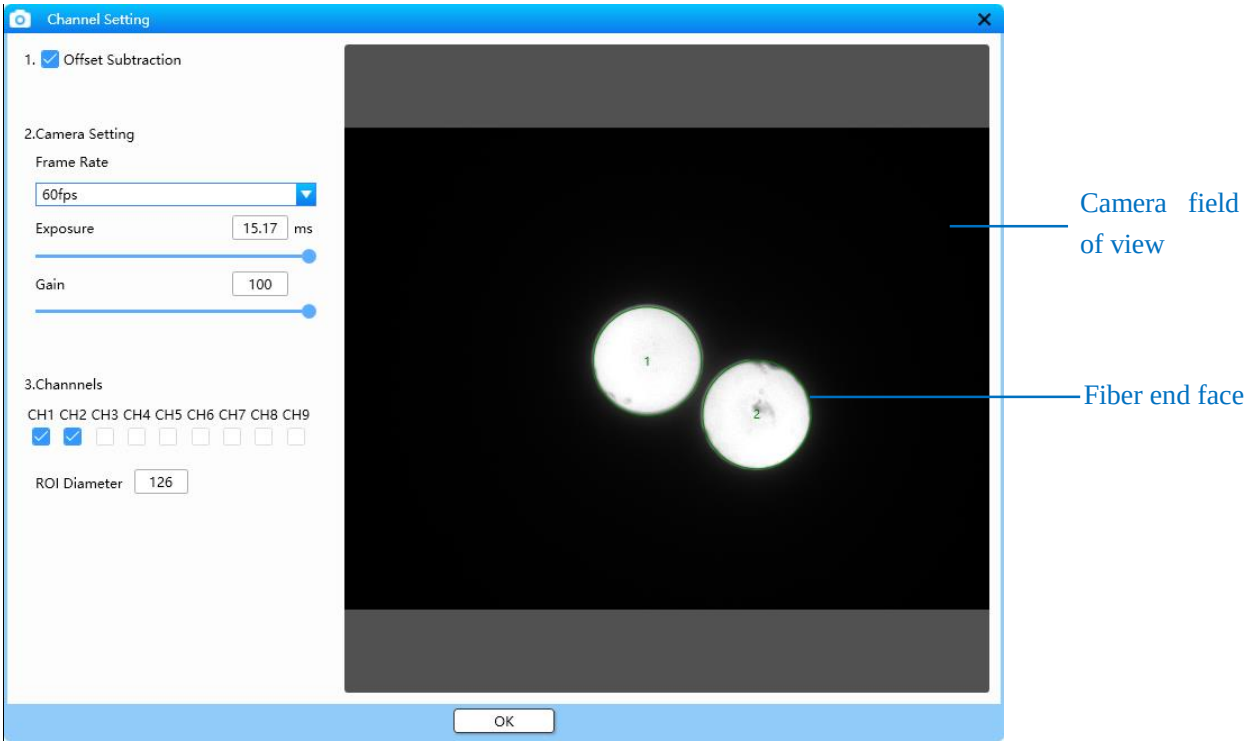


Figure 6-6

6.3.1. Adjusting the fiber end face

The fiber end face shall be clearly visible and located in the center of the view. If the fiber end face is not visible, not clear, or deviated, please select the following methods to adjust.

1) Make sure that the device and the fiber are connected well. At this time, if the fiber end face cannot be displayed normally, please aim the fiber end face at a strong light, such as lamplight, flashlight, etc., after which the fiber end face will be visible.
2) If the fiber end face is not clear, the gain in Figure 6-6 can be increased.
3) Adjust the focusing knob until the fiber end face is clearly visible in the view.

6.4. Behavior video

Adjust the behavior camera and aim it at the observed animal. The video parameters can be set and the behavior trace can be tracked in real time, as shown in Figure 6-7.

If "Behavior Recording" is checked, the behavior video file can be obtained synchronously in the process of fluorescence data collection. If it is not checked, the behavior video will not be saved.

If "Behavior Tracking" is checked, the behavior-related file can be output synchronously during behavior recording. If user has drawn the ROI and made the marking action, the automatic marking data will be output synchronously.

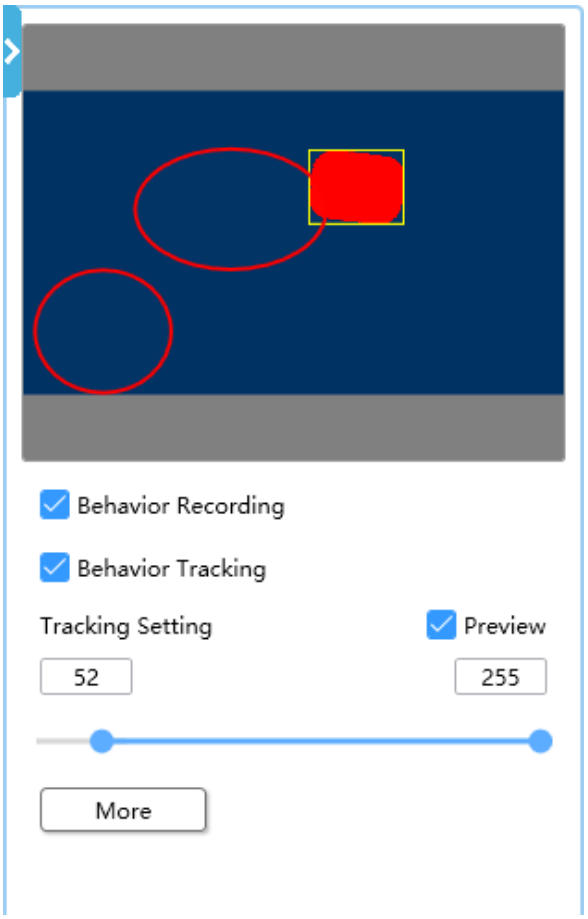


Figure 6-7

Click [More] to enter the behavior camera setting area.
Click ROI to draw circle, rectangle or polygon. Up to nine ROIs can be drawn.

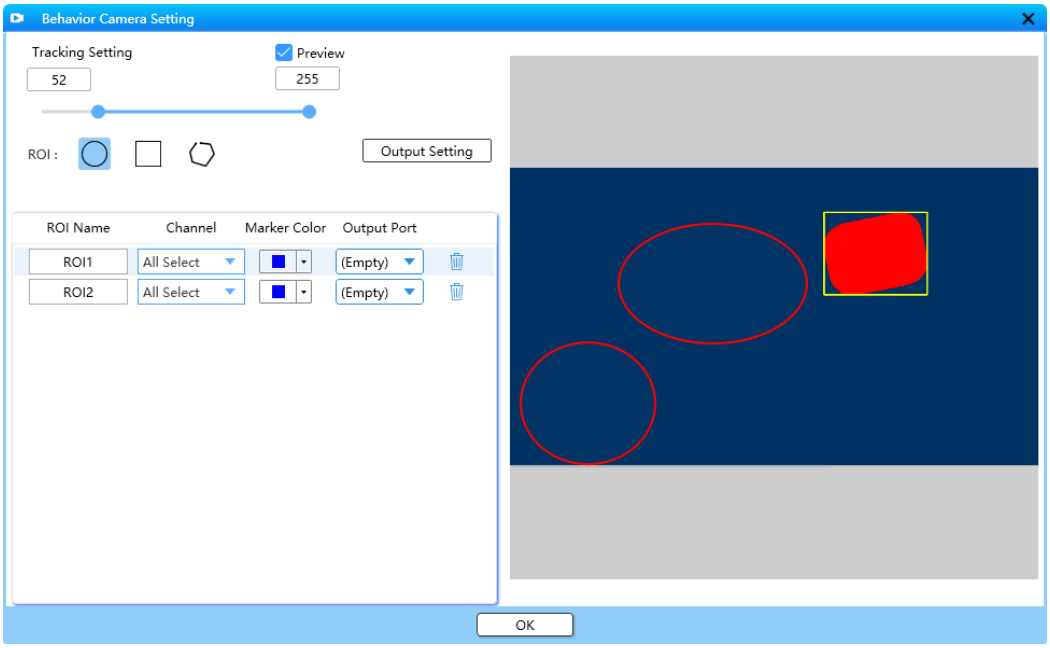


Figure 6-8

Adjust the corresponding value of [Tracking Setting] to adjust the grayscale threshold of target recognition, with a setting range: 0-255.

Check [Preview] to view the tracking status in real time.

6.4.1. Output setting

Click [Output Setting] to enter the output setting interface, where you can set the frequency and pulse width of TTL signal at the corresponding output terminal, and apply the setting of TTL signal to the output port selected by the corresponding fluorescence signal "automatic output" and behavior video ROI "output". The same output port can be set for multiple ROIs.

Note: When the frequency is set to be "0", the signal will stay at a high output level according to the set duration.

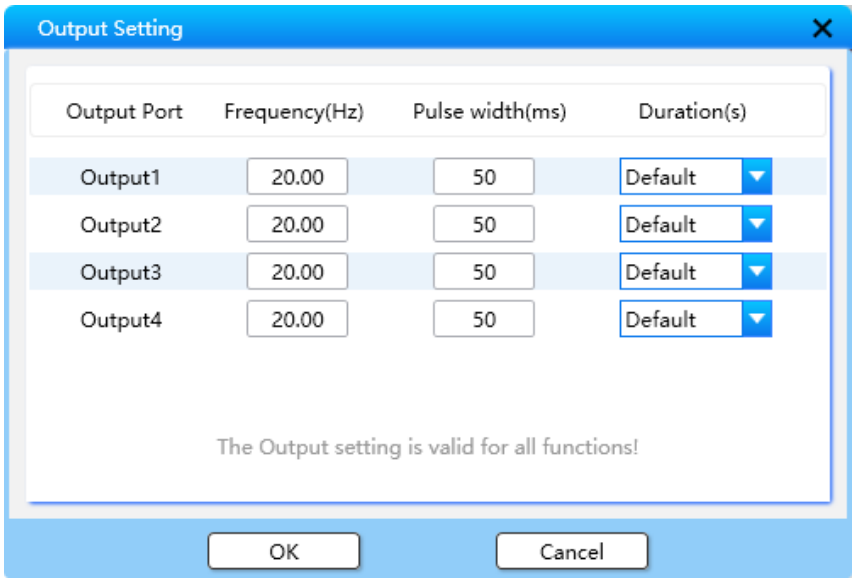


Figure 6-9

When the animal enters the ROI area, the automatic marker will be triggered and take effect in the corresponding set [Channel], and marker information will be displayed and recorded on the fluorescence trace.

6.5. Marker parameter setting

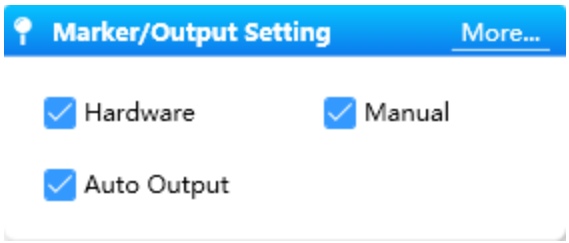


Figure 6-10

Check [Hardware]: turn on the external signal marker. During signal collection, if the system recognizes that there is a signal input at the input port, it will identify the automatic marker signal function on the fluorescent signal. It is necessary to connect the interface of the external device with the input port of the fiber photometry system through the BNC line.

Check [Manual]: turn on the manual marker. During signal collection, if the system recognizes that a manual marker action is triggered, the manual marker signal function will be identified on the fluorescent signal. You can manually mark different events by tapping the keyboard or clicking any marker icon 

below the trace.

Check [Auto Output]: turn on the automatic output marker. In signal collection, if the system recognizes that the fluorescence signal of a certain channel meets the set ΔF/F trigger threshold, or the collection has been started, it will output TTL signal to the external device.

Click [More] to open the [Marker/Output Setting] window. All the **Hardware, Manual and Auto Output** parameters shall be set respectively.

[Hardware]

When the input port recognizes the external signal input with a high-level signal in the range of 3.3-5V, the software will automatically mark the event with the set marker color on the collection trace at this time point. The event will take effect in the corresponding [Channel] (multiple channels can be selected), and the marker information will be displayed and recorded on the data.

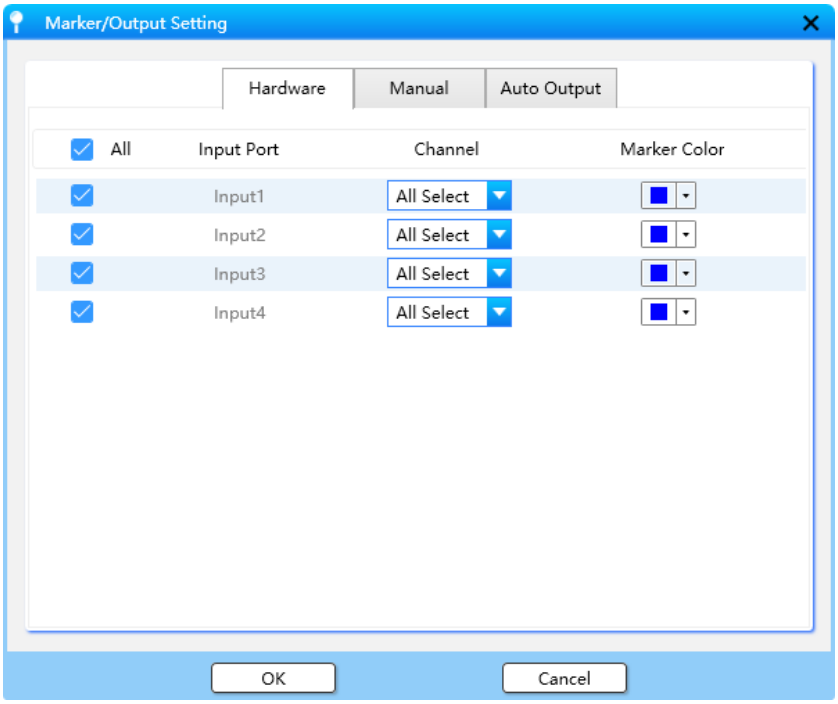


Figure 6-11

[Manual]

Marker name and marker hotkeys can be customized. When the manual marker is triggered, it will take effect in the corresponding [Channel].

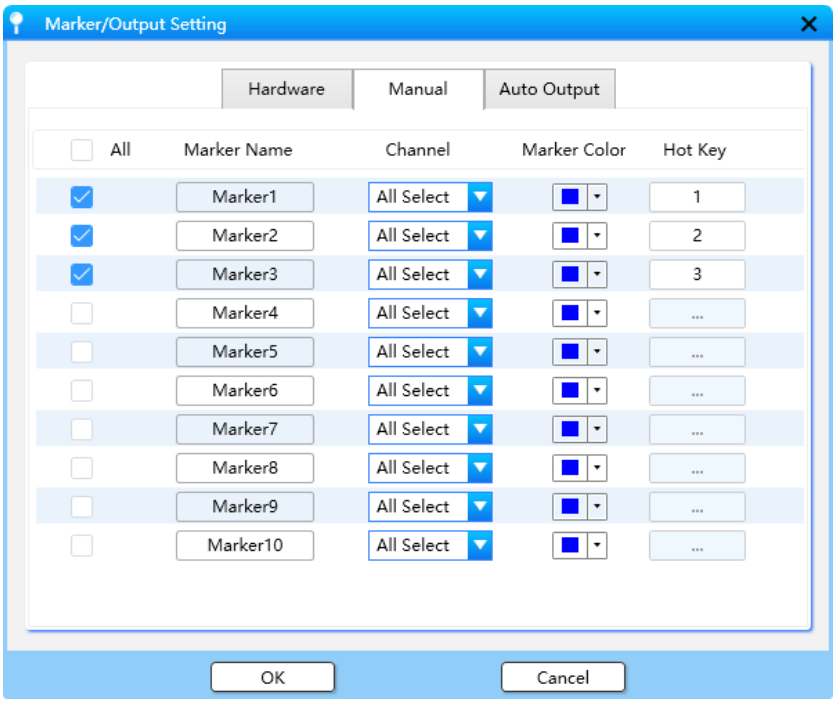


Figure 6-12

When the marking event is triggered, the diagram of the event on the fluorescence signal trace is as follows:

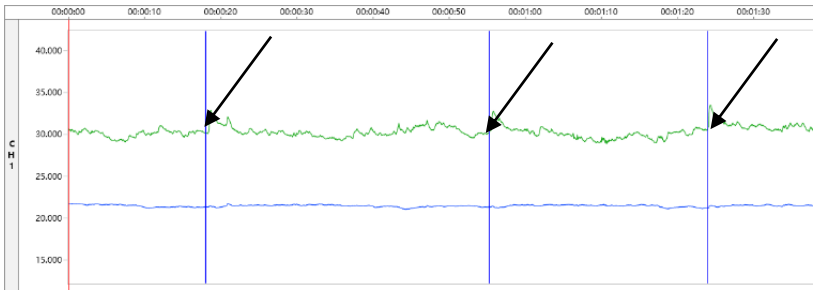


Figure 6-13

[Auto Output]

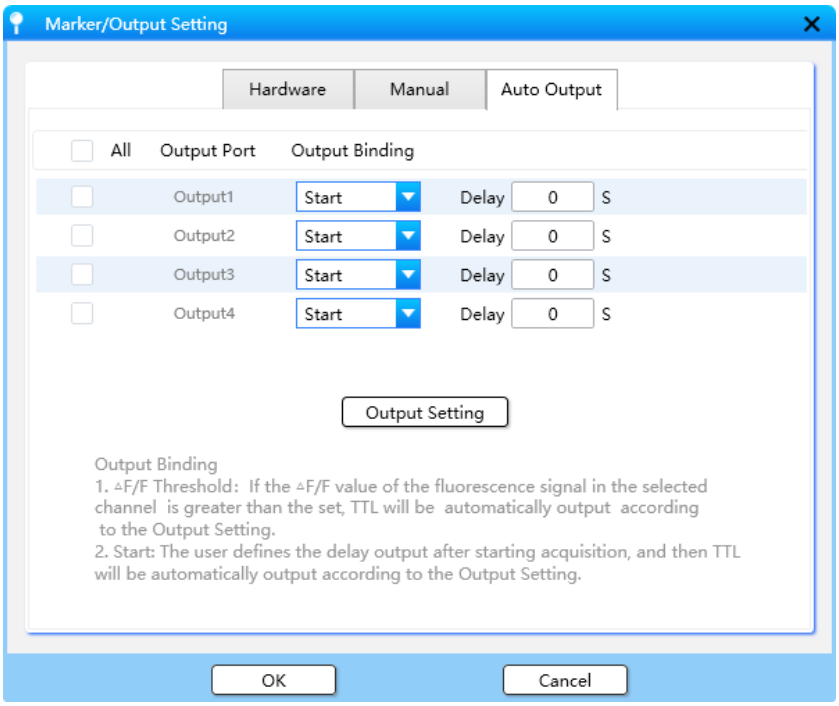


Figure 6-14

Select [Start]: There will be signal output when fluorescence data are being collected, also can customize the delay after the start of collection, that is, the TTL signal will be output when the set delay time is reached. The frequency, pulse width and duration of the output signal can be adjusted through the [Output Setting] below.

Select [ΔF/F Threshold]: If the ΔF/F value of the fluorescence signal in the selected channel is greater than the set, TTL will be automatically output according to the Output setting. Refer to 6.4.1 - Chapter description for [Output Setting].

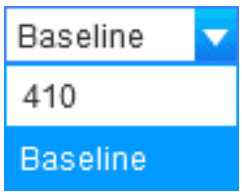
6.6. Preview

After parameter setting, click [Preview] to preview the fluorescence data trace, and click [End] to end the trace preview.

Click 

to select the original trace display and ΔF/F signal display.

F0 option



If Baseline is selected, you will need to set the start time and stop time

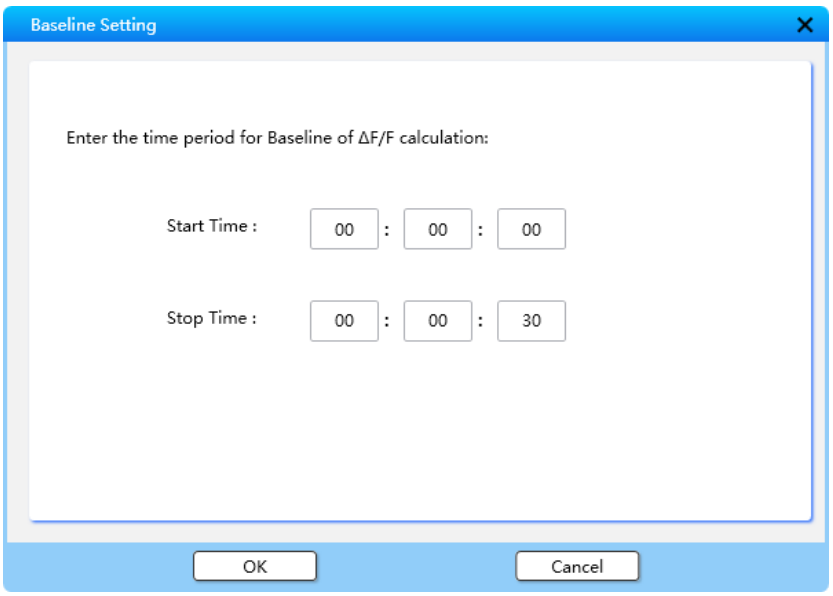


Figure 6-15

It is advisable to keep the preview for 5-10 minutes before the formal experiment, which can make the fluorescence baseline more stable.

6.7. Start data collection

After preview, click [Photometry] to start data collection, and click the icon to enter the [Start/Stop Setting] page.

Figure 6-16

[Start/Stop Setting] page

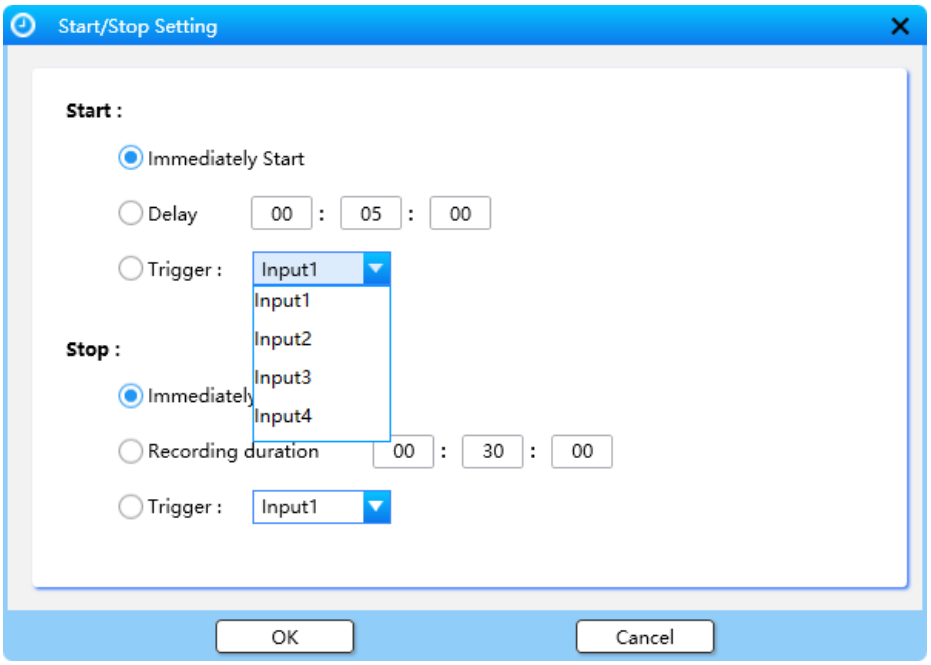


Figure 6-17

1) On the [Start/Stop Setting] page, when check [Immediately Start] and [Immediately Stop], click ▶ Record to start data collection immediately. Click ■ Stop to stop the collection immediately.

2) If ○ Delay 00 : 05 : 00 is selected in [Start] and the waiting time is set, after clicking ▶ Record, the system will automatically start data collection after the waiting time.

3) If Recording duration 00 : 30 : 00 is selected in [Stop] and the collection time is set, after clicking ▶ Record, the system will automatically collect data within the set collection time before stopping the collection.

4) [Trigger] means to start or stop data collection by triggering an external signal.
The trigger ports include Input1/Input2/Input3/Input4. In [Start], after selecting [Trigger] and any input port, the system will start data collection automatically upon the external signal that meets the triggered conditions. In [Stop], after selecting [Trigger] and any input port, the data collection will automatically stop upon the external signal that meets the triggered conditions.

Note: If [Trigger] was selected for both [Start] and [Stop], and same input port was also selected, such as input1, after clicking the ▶ Record, system will start data collection when recognizing the rising edge signal of the input1 port, and recognize the falling edge signal of the same input1 port to stop data collection. If different Input ports are selected for [Start] and [Stop], such as input1 and input2, after clicking the ▶ Record, system will start data collection when recognizing the rising edge signal of the input1 port in [Start]; and recognize the falling edge signal of the input2 port in [Stop] to stop data collection.

7- Data Analysis

After data collection, click [Analysis] on the data collection page to switch to the data analysis page, as shown in Figure 7-1. There are three subtags at the bottom of the data analysis page. Click them to switch to the [General] page, the [Fluorescent Heat Map] page, and the [Trajectory] page respectively, and the [General] analysis page will be displayed by default.

Data analysis is mainly used to analyze the collected calcium fluorescence signal data, view the video synchronously with the corresponding channel, display the fluorescence heat map and mean±SEM, trajectory and behavior heat map. You can choose to capture or export the original data and its behavior video into a fixed format picture.

If you only need to analyze the existing collected data without collection operation, you can directly click [Analysis] to enter the data analysis page when the software is started. Click [File] → [Open Files] to import the collected data file into the general analysis page. If there is video data in the collected data, the video data will be imported into the behavior video area for synchronous view.

Figure 7-1

7.1. General analysis

7.1.1. Overview

The brief of the current analysis data file are displayed: reference light, excitation light, frame rate, channel and experiment duration.

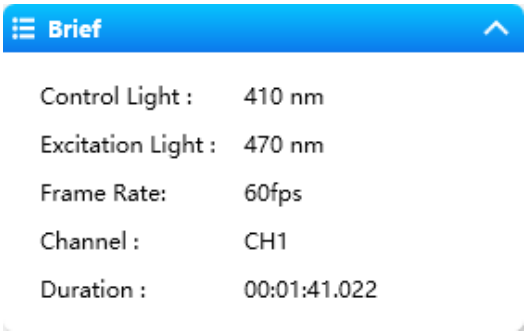


Figure 7-2

7.1.2. Data clip

Clip and select the collected data. Enter the start time on the x-axis in Figure 7-3, which corresponds to the "start time" in Figure 7-4. The "end time" is "experiment duration" by default, that is, the clipped and selected duration range.

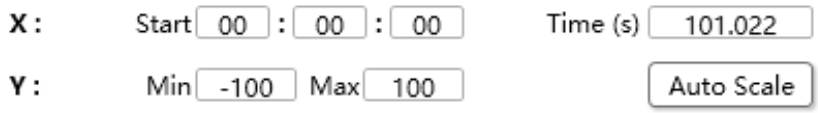


Figure 7-3

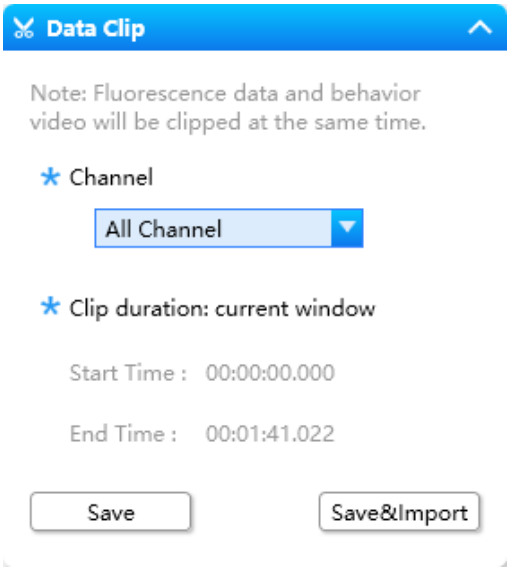


Figure 7-4

If there are multiple channels, you can click  to select which channel's data files to intercept.

Click [Save] to save the clipped .CSV data file to the selected file path. Select [Save&Import] to import the clipped data file and behavior video, and display the corresponding trace image in the interface. If there is a behavior video, the clip will also capture it synchronously.

Adjust the minimum/maximum value on the y-axis to adjust the trace display range.

Click [Auto Scale] to reset the x/y axis coordinates of all current channel traces to the original state.

Click the hotkeys  on the right side of the trace to adjust the coordinate axis, which correspond to the enlargement and reduction of the y axis of the trace and the auto scale of the x/y axis.

7.1.3. Pre-Processing

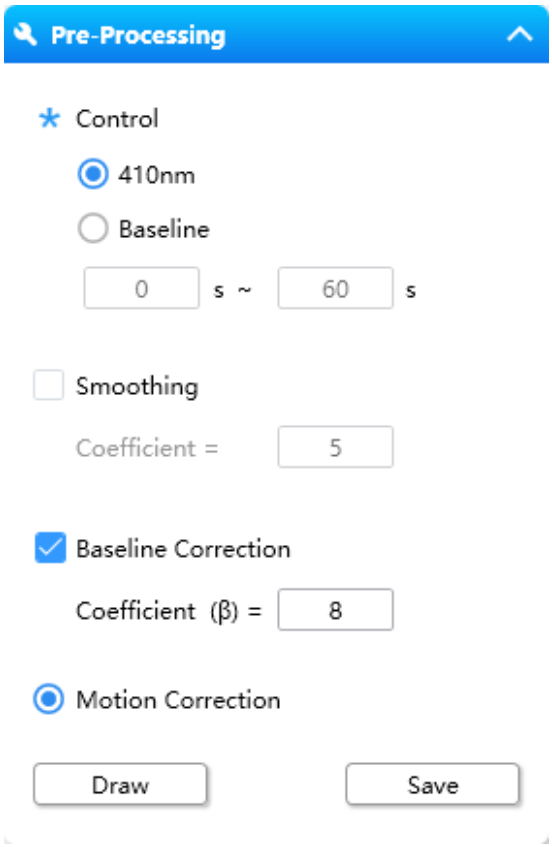


Figure 7-5

[Control]
Select the control data used to generate ΔF/F and Z-score
According to the light contained in the imported file, the options are as follows:
470: only the Baseline option is valid
410 + 470: 410nm or Baseline are optional
When Baseline is selected as the control, the Baseline time range needs to be set, and the software will calculate the median value of the set time range as the Baseline value.

[Smoothing]
You can choose whether to smooth the data. The setting range of smoothness W is 5-50. The higher the smoothness value is set, the greater the data smoothness is. The software default value is 15.

[Baseline Correction]
You can choose whether to correct the baseline data. You can correct the downward trend of fluorescence data caused by long-time data collection and LED thermal effect. Correction coefficient β setting range: 5-12.

[Motion Correction]
Only in the 410&470 mode, when select 410 as the control, the motion correction shall be used, and it will be used by default.

Click [Draw], and the trace will jump to the ΔF/F trace display by default, as shown in Figure 7-6.

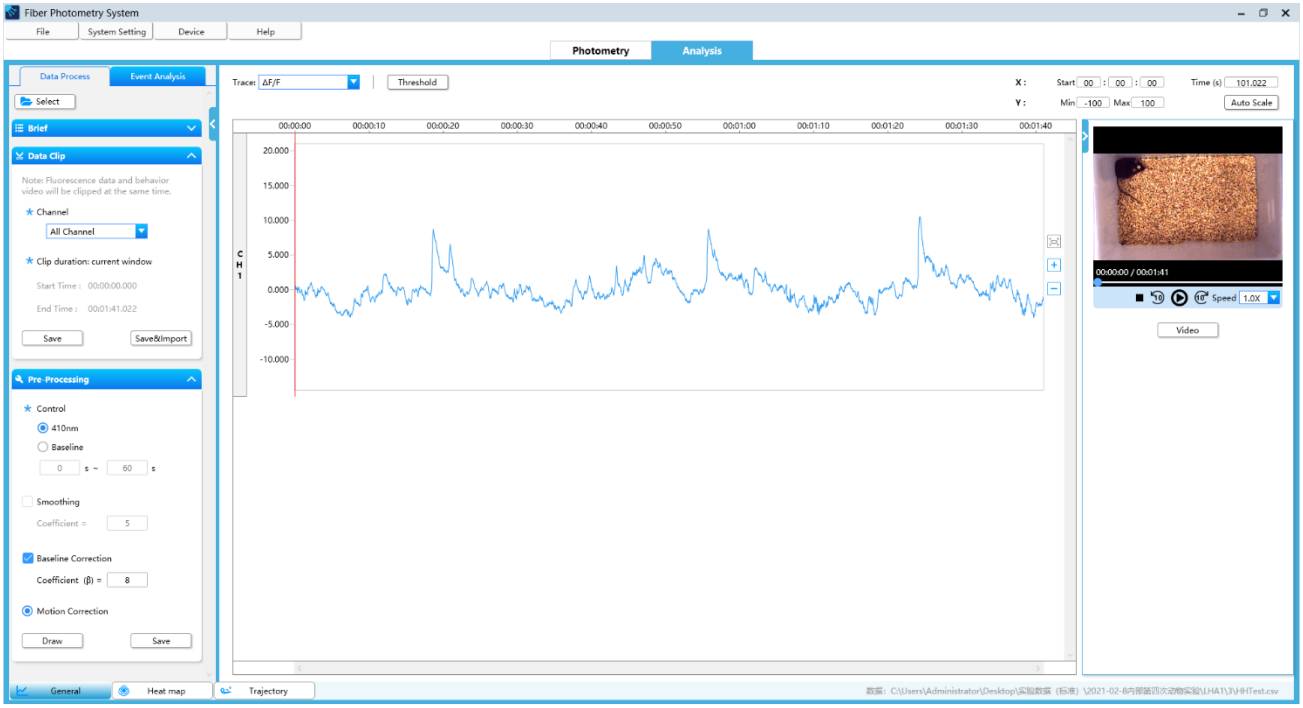
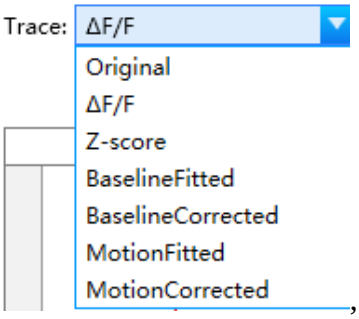


Figure 7-6

Click to open the drop-down list 

, in which there are a variety of traces to display.

Z-score

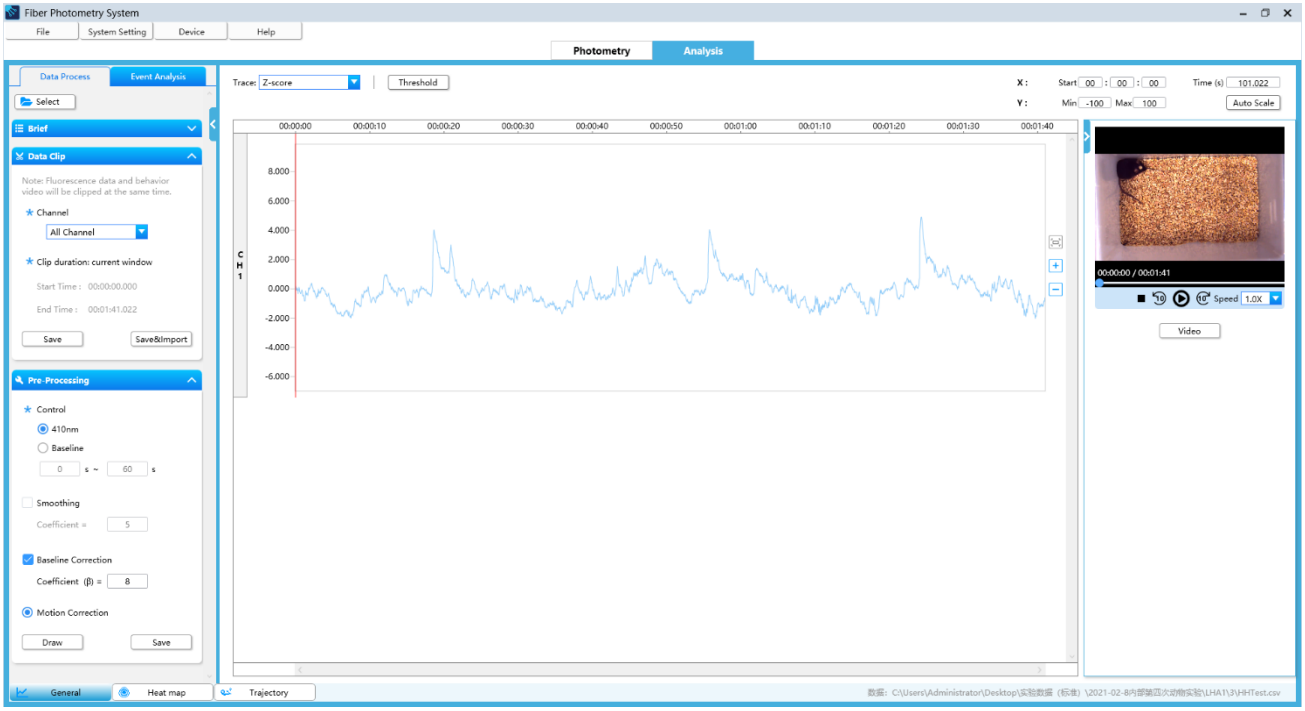


Figure 7-7

BaselineFitted (display the original data and baseline correction fitting curve)

Figure 7-8

BaselineCorrected (display the data curve after baseline correction)

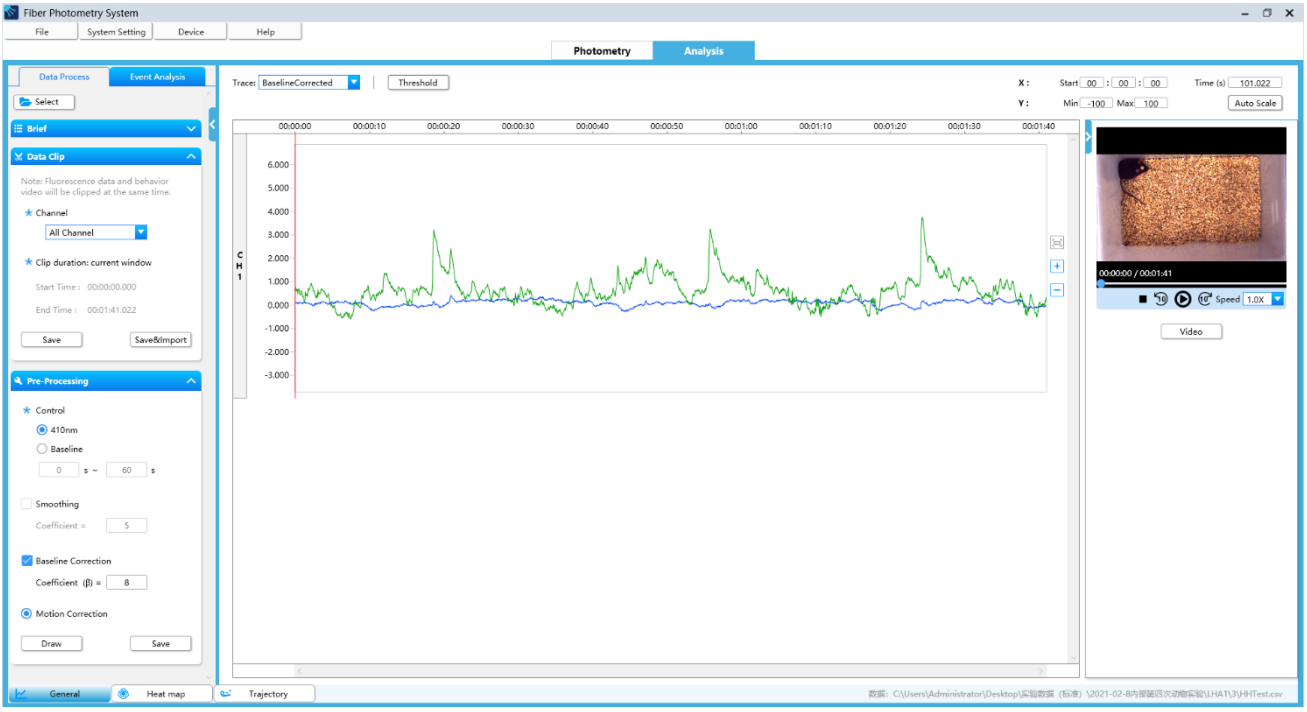


Figure 7-9

MotionFitted (display fitted410 data curve)

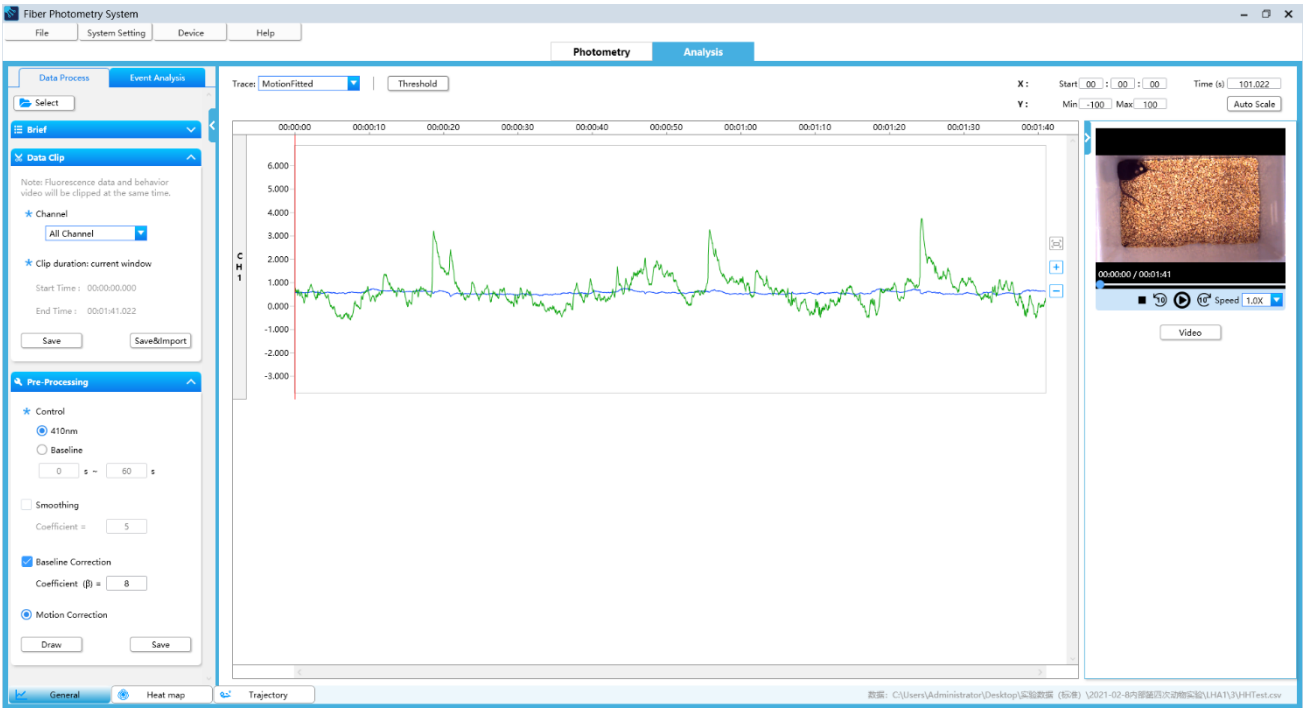


Figure 7-10

MotionCorrected (display the curve of data after motion correction (F470-fitted410))

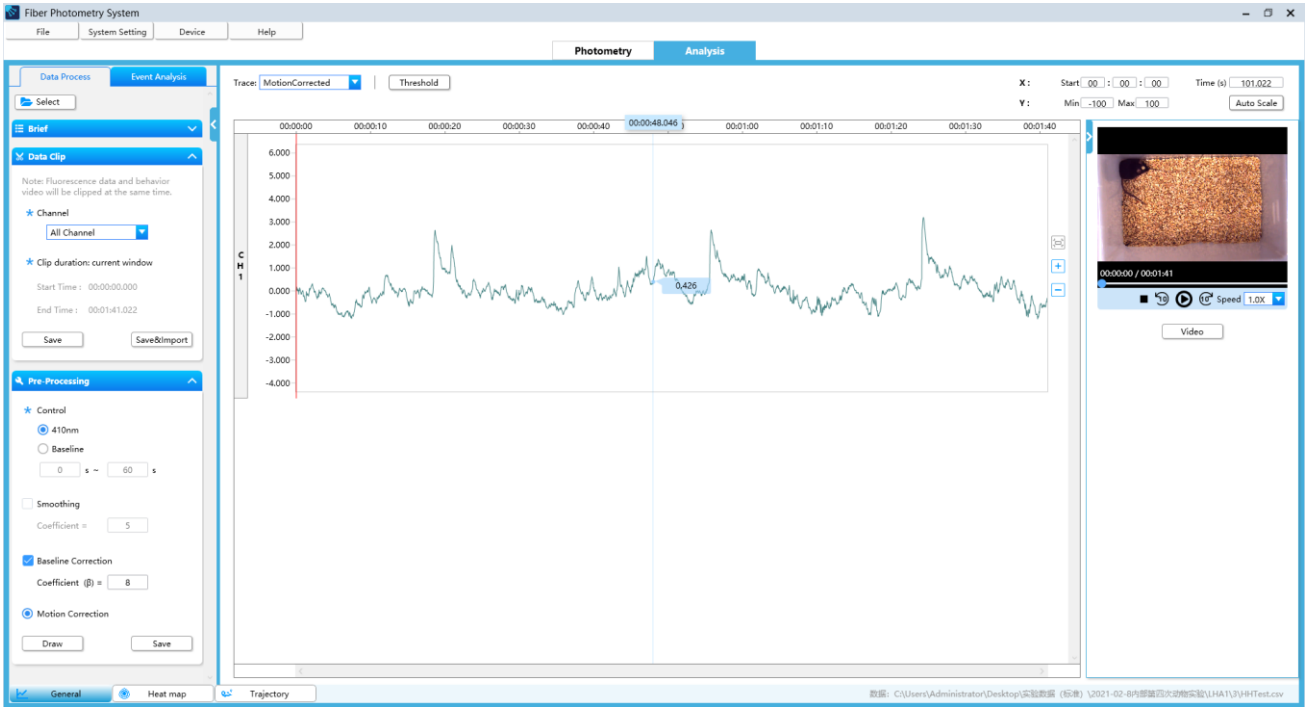


Figure 7-11

Algorithm description

1） ΔF/F

ΔF/F = (F-F1)/F0, F is the target fluorescence data (F470), in some cases F1 = F0:
If the data go through baseline correction:
If 410 is selected as the control: F1 = fitted410, F0 = median (raw data)
If 410 is not selected as the control: F1 is the baseline correction curve value, F0 = median (Baseline)

If the data does not go through baseline correction:
If 410 is selected as the control: F1=F0=fitted410,
If 410 is not selected as the control: F1=F0=median (Baseline)

2) Z-score: The standard z-score calculation method is used, that is, Z-score = (x-mean)/std, x = ΔF/F (mean is the corresponding mean value and std is the corresponding standard deviation).

Notes:

1) Motion correction uses Robust Least Square to fit 410 data to 470 to get fitted410.
2) Baseline correction uses the iterative weighted least square algorithm to get the baseline correction curve.

Threshold Setting

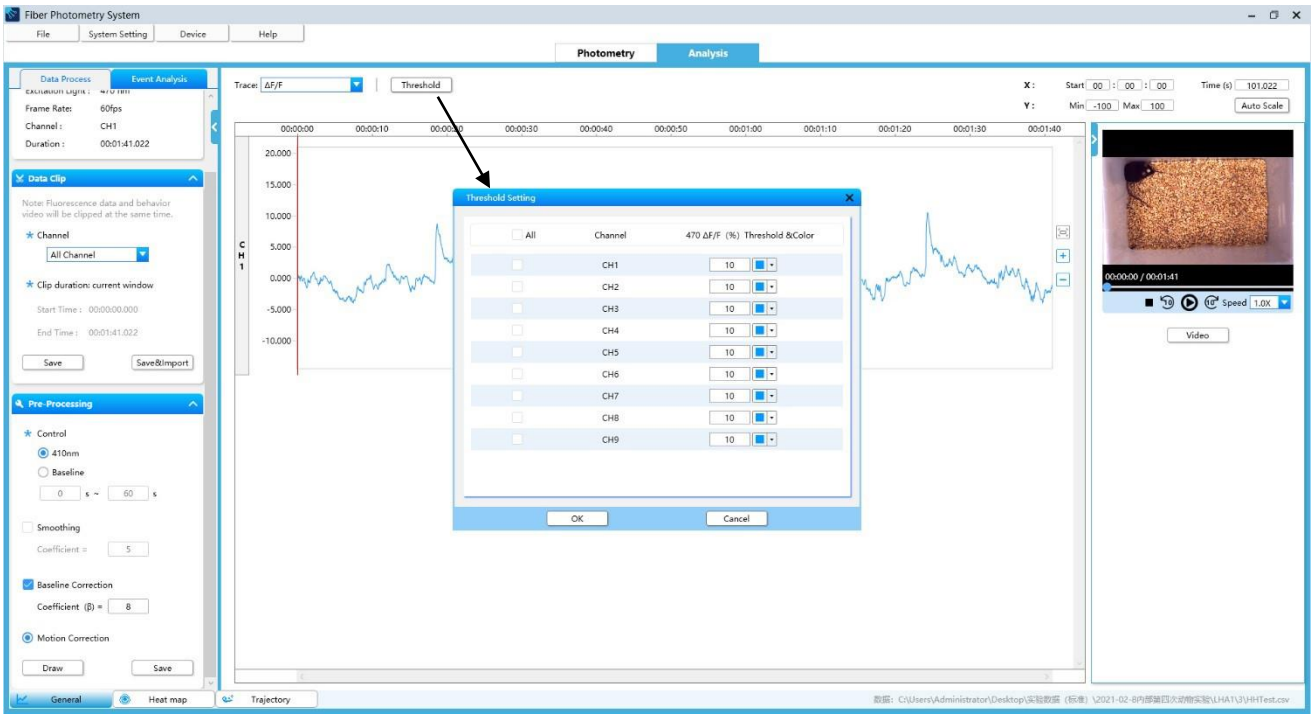


Figure 7-12

Click [Threshold Setting] and check the channel to adjust the threshold setting in the input box. The software will display the threshold dotted line of the set color in the trace display area.

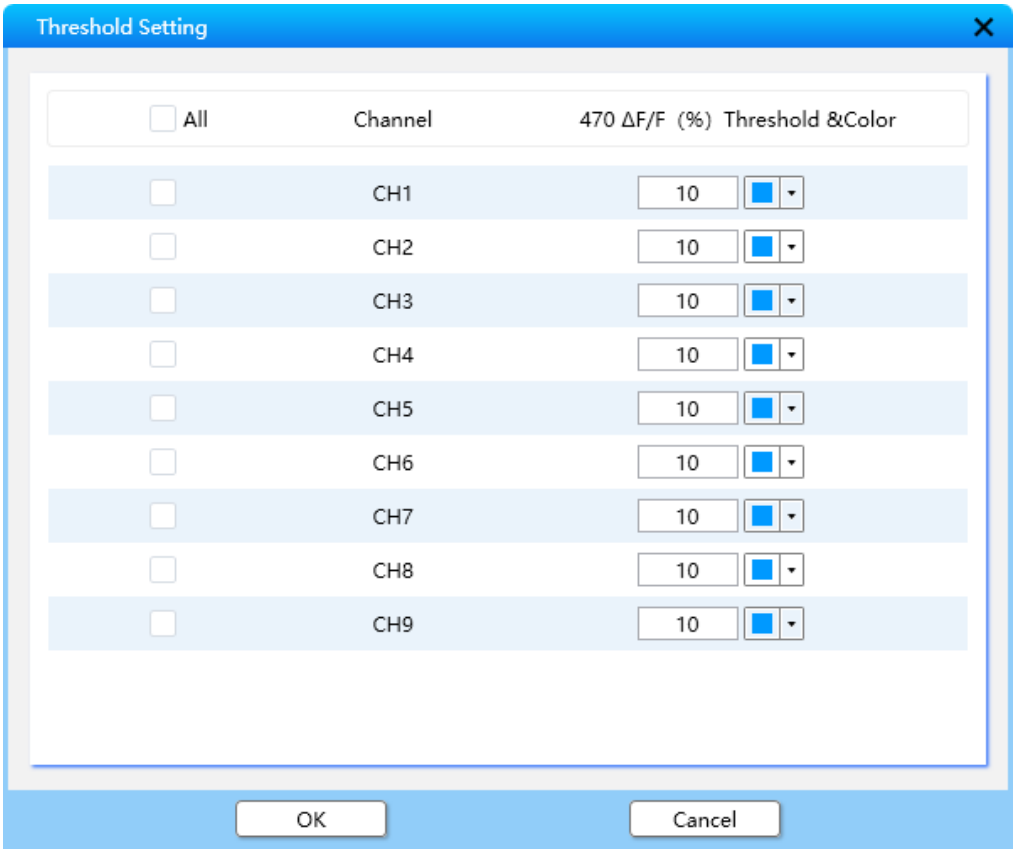


Figure 7-13

7.1.4. Event analysis

Switch to [Event Analysis] for viewing all the markers and details. The markers checked by ✓ is displayed with a small triangle marked in the trace area. Click drop-down arrow of  to change the marker color.

[New maker]

Right-click in the trace area，then click **[New Marker]→(Edit) [New Event]/(Manaul) Marker1**, if **(Edit) [New Event]** is selected, **[New Event]** will appear in the maker list, edit its name as you need.

Marker list

Figure 7-14

[Delete maker]

Click the right mouse on any marker in the trace area，[Delete marker] will appear for choosing.

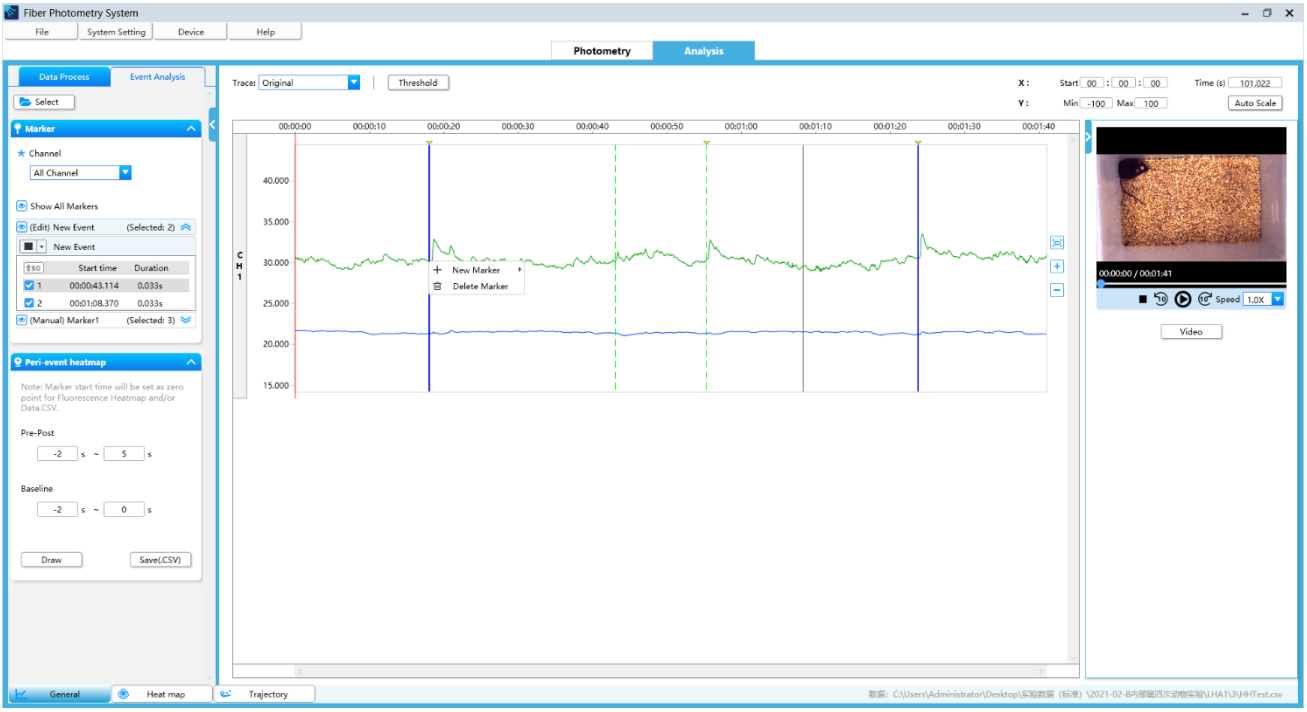


Figure 7-15

Light up the icon Show All Markers to show all markers in trace area.

[Marker]

Instruction:

1） Checked by ✓ in the maker list: Only the checked marks can be used to draw the heat map of ΔF/F&Z-score. Click ↑50 to check up to 50 markers at once.

2） Click selection: Cick on a marker in the maker list, which is hightlighted by a gray bar, meanwhile the marker is highlighted on the trace area.

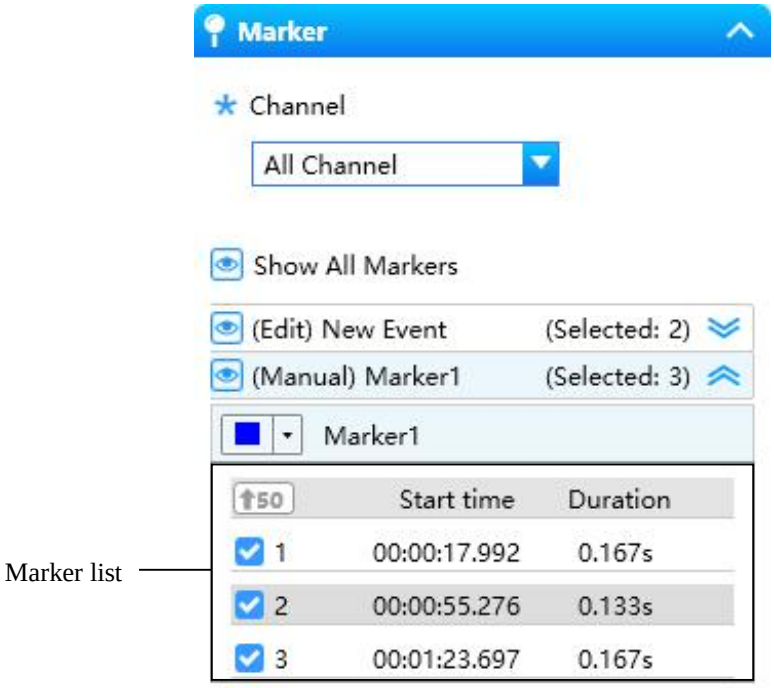


Figure 7-16

[Peri-event heatmap]

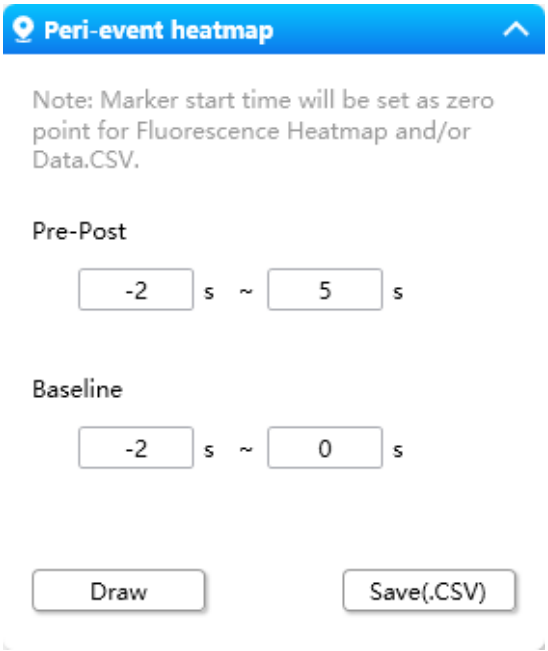


Figure 7-17

Note: Maker start time will be set as the "0" point for Fluorescence Heatmap and/or Data.CSV
Pre-Post: Maker start time was taken as "0" point to set the pre and post time. The data within this window (peri-even) is used to output the heat map and mean±sem.
Input range of Pre: -3600 ~ 0s
Input range of Post: 0 ~ 3600s

Baseline: Maker start time was taken as the "0" point to the Baseline time window for outputting heat map data, and the median value of this time window will be taken as the base value to normalize the pre-post data.

Starting time input range: -3600~3599
Ending time input range: -3599~3600

Click [Draw] to get the fluorescent heat map of △F/F&Z-score generated of the peri-event data, as shown in Figure 7-18. The corresponding data of multiple markers are stored in the same file.

Click [Save image] to save the image to the selected path. By default, the name of the mean±sem data group is Group 1.

Click [Save (.CSV)] to save the fluorescence data file.

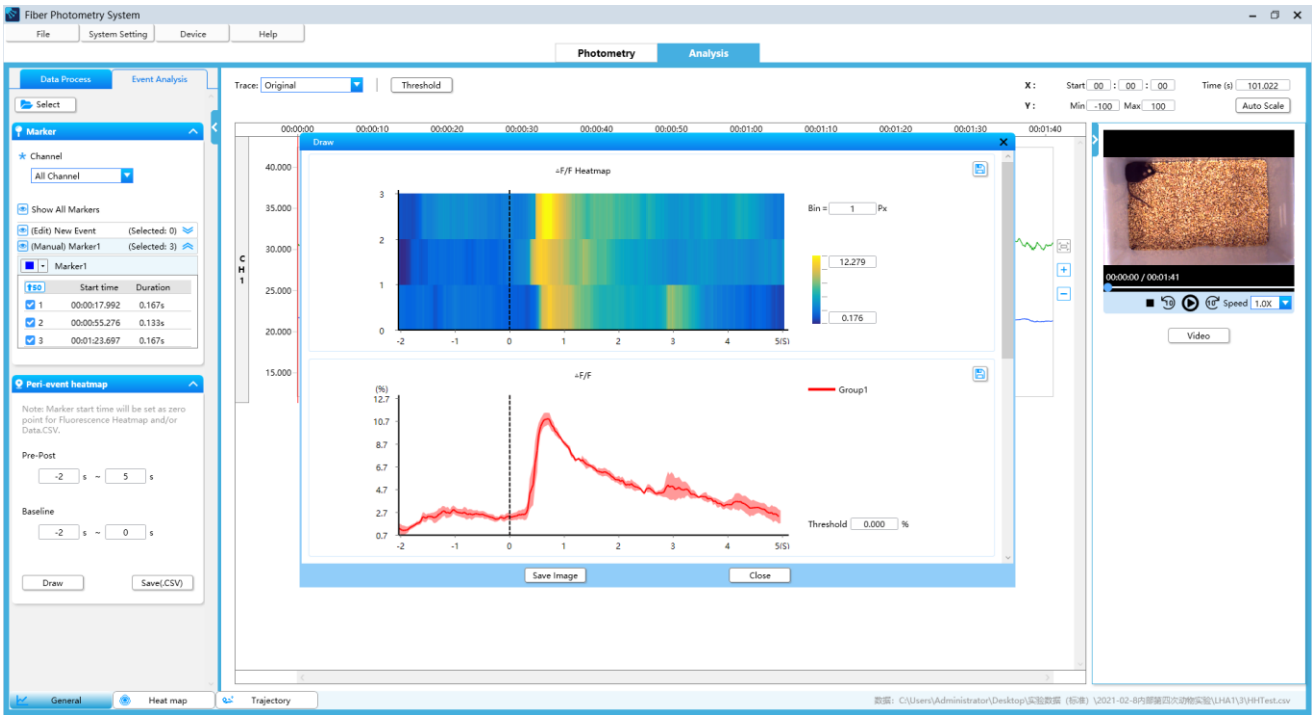


Figure 7-18

Algorithm description

The processing results in event analysis are related to the [Pre-Processing] option in [Data Process].

1） ΔF/F

ΔF/F0=（F-F1）/F0

In the Peri-event heatmap analysis interface, a time window before the event needs to be set as the baseline (different from the Baseline in data pre-processing), and F0 = median (baseline).

If the data go through baseline correction:

If 410 is selected as the control: F1=fitted410

If 410 is not selected as the control: F1 is the baseline correction curve value

If the data does not go through baseline correction:

If 410 is selected as the control: F1=fitted410

If 410 is not selected as the control: F1=F0=median (baseline)

2） Z-score：

The standard z-score calculation method is used, that is, Z-score = (x-mean)/std, in which x is the delta F/F of the peri-event, mean is the mean of the baseline time window, and std is the standard deviation of the baseline time window.

Notes:

1) Motion correction uses Robust Least Square to fit data 410 in the peri-event to 470 to get fitted410.
2) Baseline correction uses the Iterative Weighted Least Square algorithm to get the baseline correction curve.

7.1.5. Behavior video

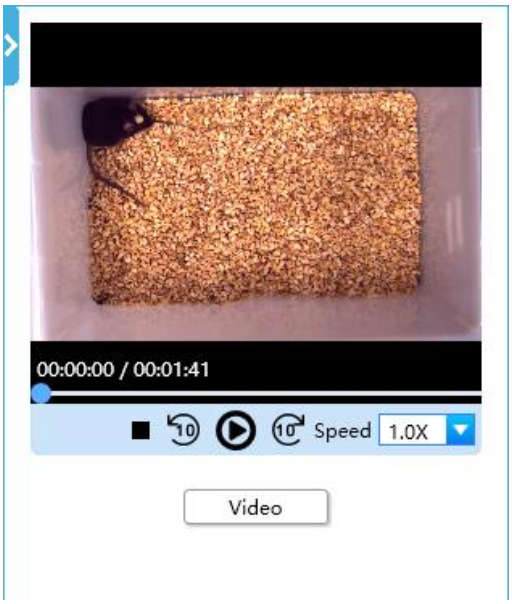


Figure 7-19

Video files in the data can be imported synchronously with the data. You can also click [Video] to import the fluorescence data file while importing the video.

Start/Pause/Stop: start pause stop

Fast-Forward/Fast-Backward: fast backward the current video and corresponding fluorescence data for 10s; fast forward the current video and fluorescence data for 10s.

Play multiple: 1.0X for setting the play multiple of video: 0.5x, 0.75x, 1.0x, 1.5x, 1.75x, and 2.0x.

Start playing behavior video: The current playing time frame is synchronized with the [Real-Time Refresh Line] of the fluorescent signal, as shown in Figure 7-20. In video play/pause, drag the horizontal slider of fluorescent data/fluorescent trace/x-axis start time to refresh the current frame and real-time refresh line to the current data position synchronously.

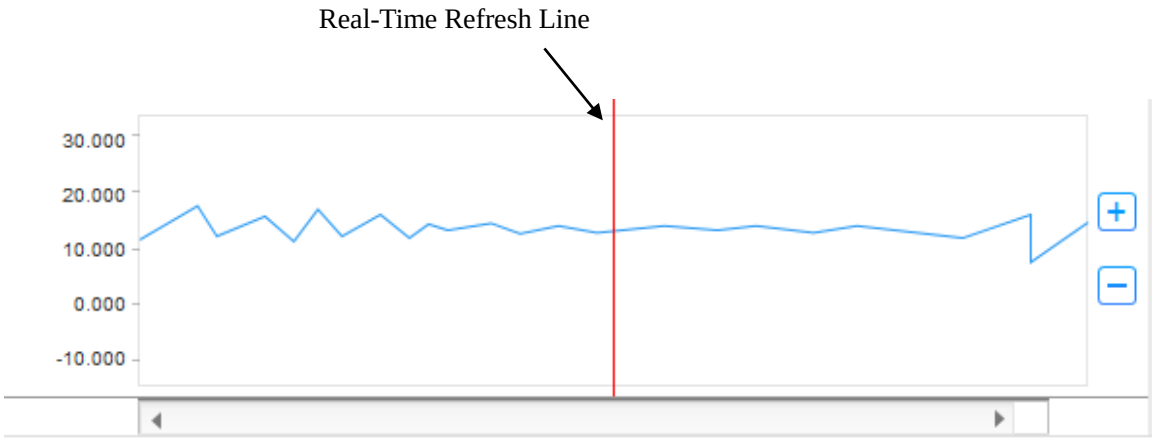
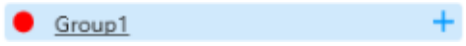


Figure 7-20

7.2. Fluorescent heat map

Click at the bottom of the interface to switch to [Fluorescent Heat Map]. Click [+] in the 

for addition, and select the fluorescence data file of XX Fluorescence_Ttrail.CSV to import.

Click [Draw], and the analysis results are shown in Figure 7-21.

Drag the mouse down to browse the right drawing area. From top to bottom: ΔF/F fluorescent heat map, ΔF/F (mean±sem), Z-score fluorescent heat map, Z-score (mean±sem).

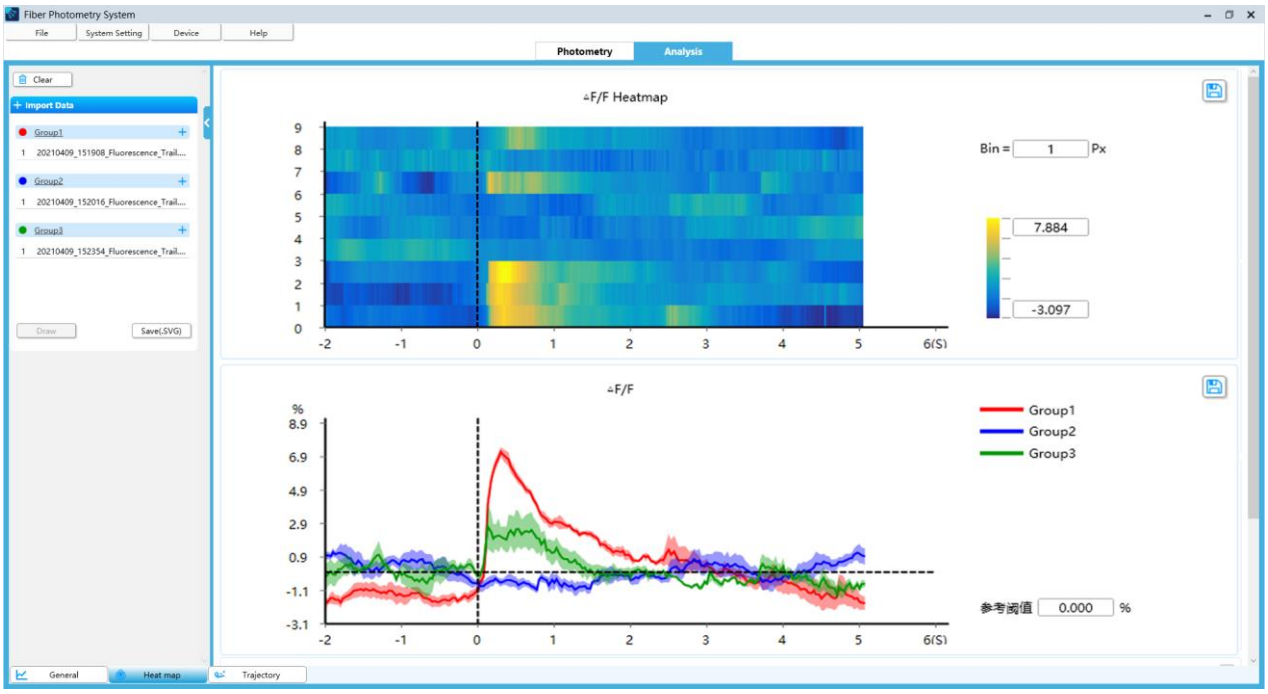


Figure 7-21

Description of ΔF/F fluorescent heat map (same as Z-score fluorescent heat map)

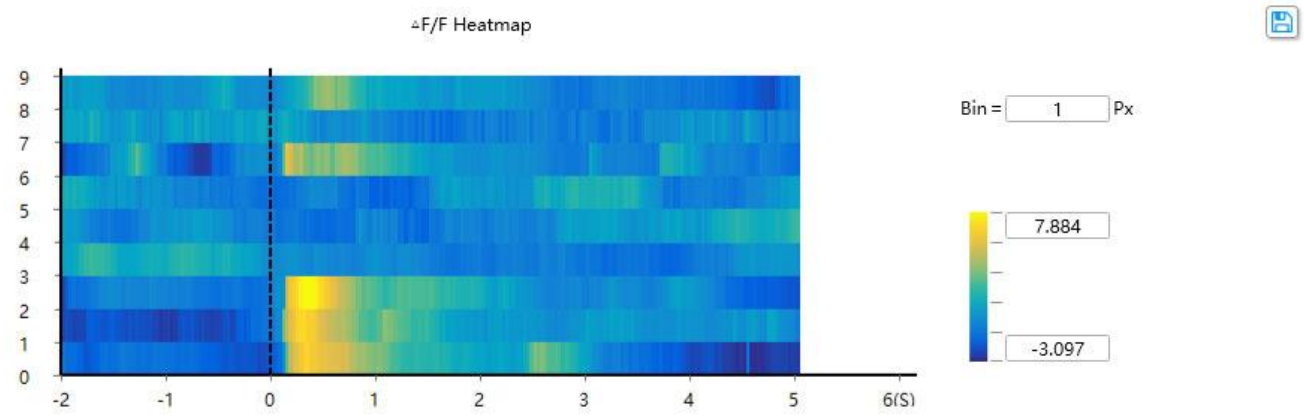
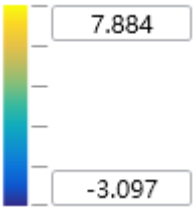


Figure 7-22

Bin value setting: change rendering fineness of the heat map by setting bin value. Bin setting range: 1px ~ 100px;

Fluorescence thermal value scale: 

Upper limit input range: -999.000 ~ 1000.000

Lower limit input range: -1000.000 ~ 999.000

Description of ΔF/F mean±sem (same as Z-score mean±sem):

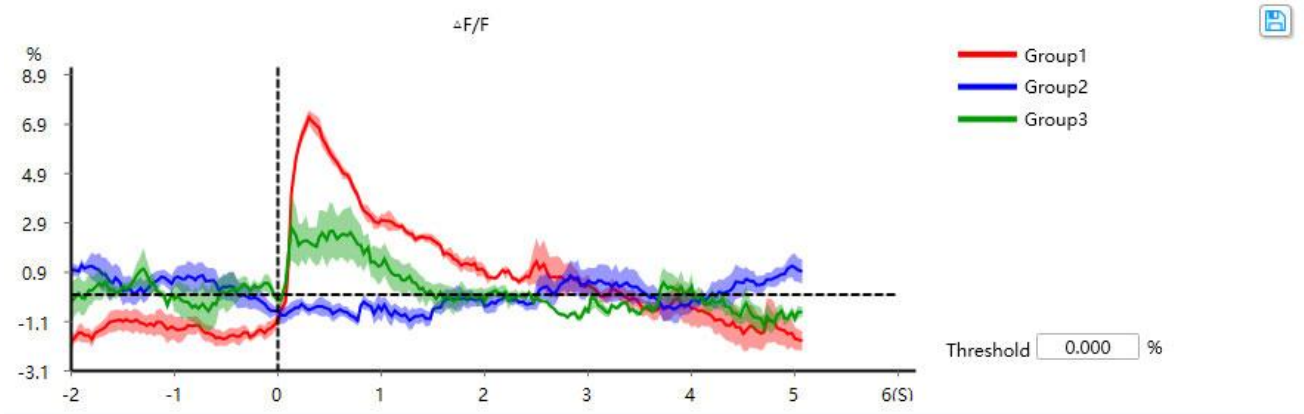


Figure 7-23

Set the Reference Threshold:

ΔF/F setting range: -1000%.000 ~ 1000.000%;

Z-score setting range: -1000.000~1000.000

7.3. Trajectory

Click to switch to the [Trajectory] interface, as shown in Figure 7-24. You can view the animal trajectory and corresponding ROI in this interface. The ROI list displays the ROI outline thumbnail and ROI name, as well as the trajectory duration of the animal in the ROI area.

Click [Select] to import the stored track.csv file. There are two forms of trajectory output: trajectory heat map and trajectory map.

Click / to switch the display background of trajectory map/trajectory heat map. Click [Image] to import the customized background image.

Click to save the trajectory image to the target folder.

0 14400 It is the time scale of fluorescence thermal value. You can input the values at the upper limit "0" and the lower limit "14400" to adjust the display of the heat map.

Upper limit input range: 1s ~ 14400s

Lower limit input range: 0s ~ 14399s

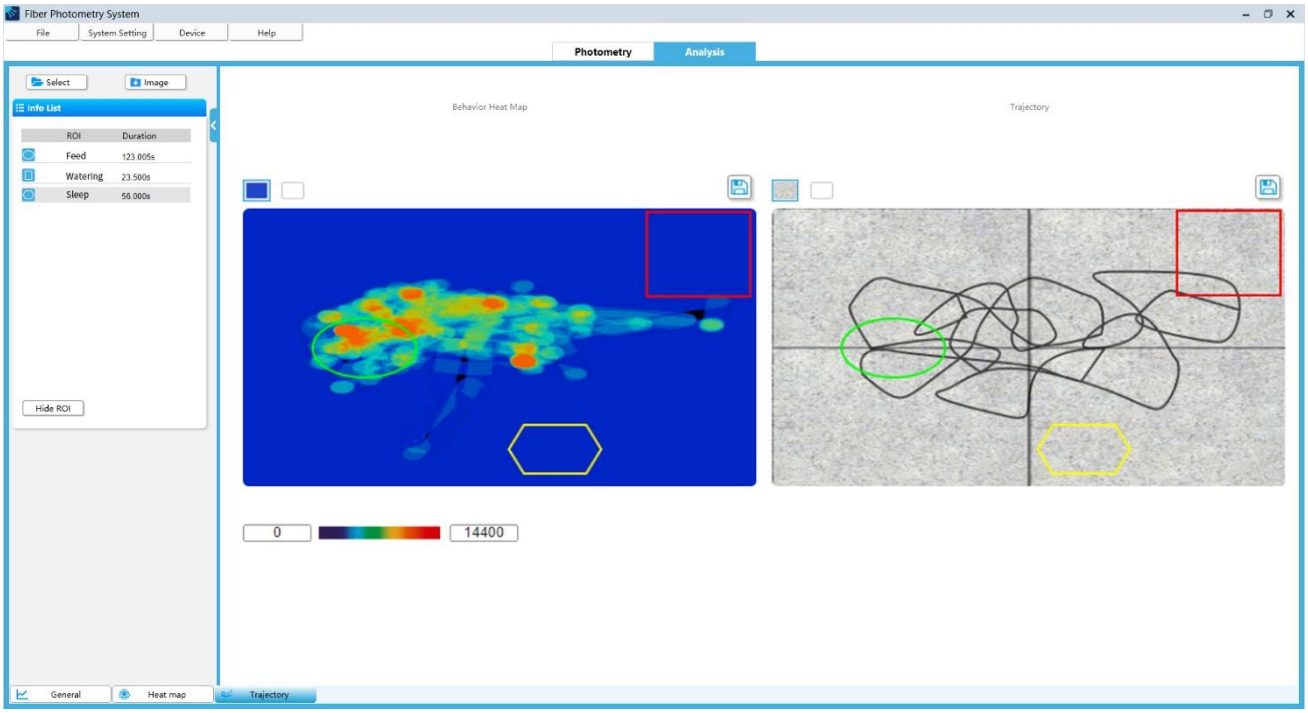


Figure 7-24

8- System Setting

Click **System Setting** on the **Photometry** or **Analysis** tab. In the window displayed, you can set the color of trace, tracking set and languages.

8.1. Trace Color

On the **Trace Color** tab, choose a color block to set the color of trace displayed on the **Photometry** or **Analysis** tab. It helps observe and distinguish trace.

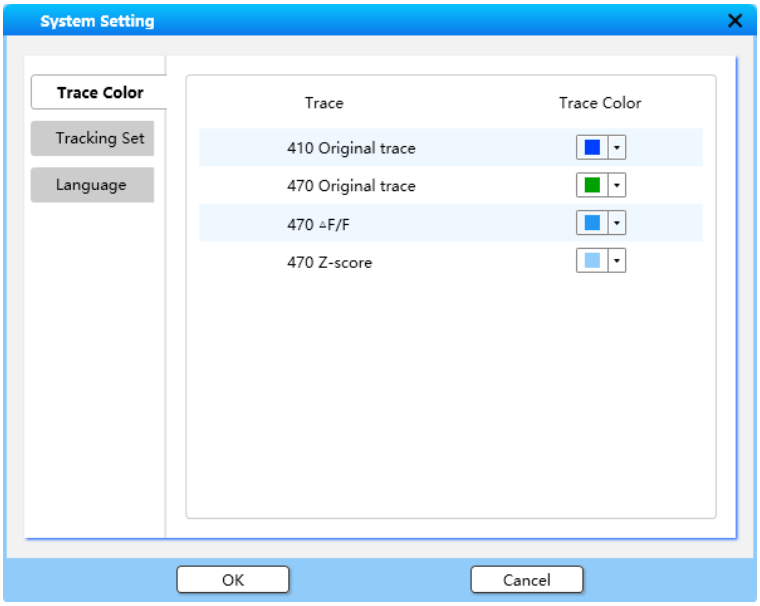


Figure 8-1

8.2. Tracking Set

The circle represents the object being observed in the behavior video, which is the target. The rectangular area represents the view of the behavior video, which is the background. On the **Tracking Set** tab, you can observe the position and size of the target in the background and customize the colors of the background and target.

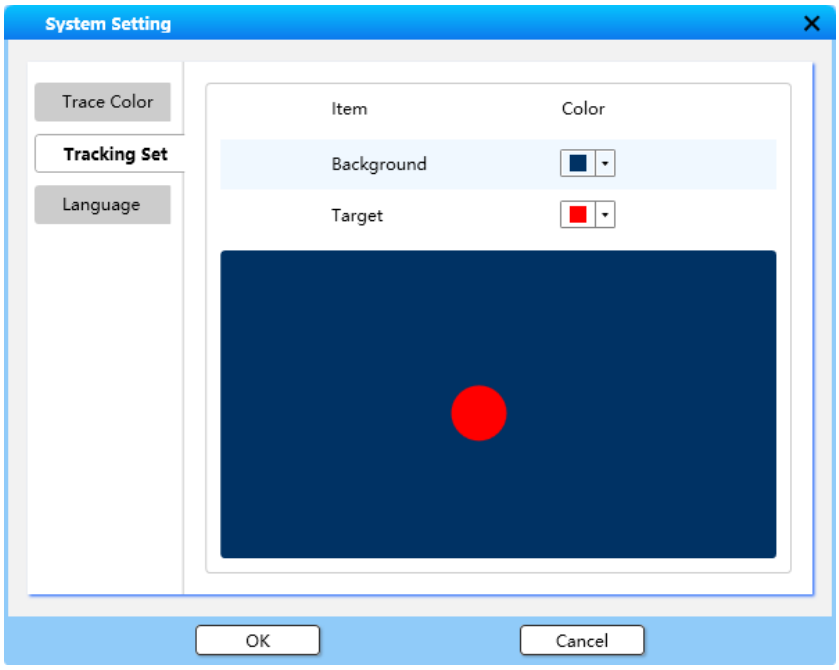
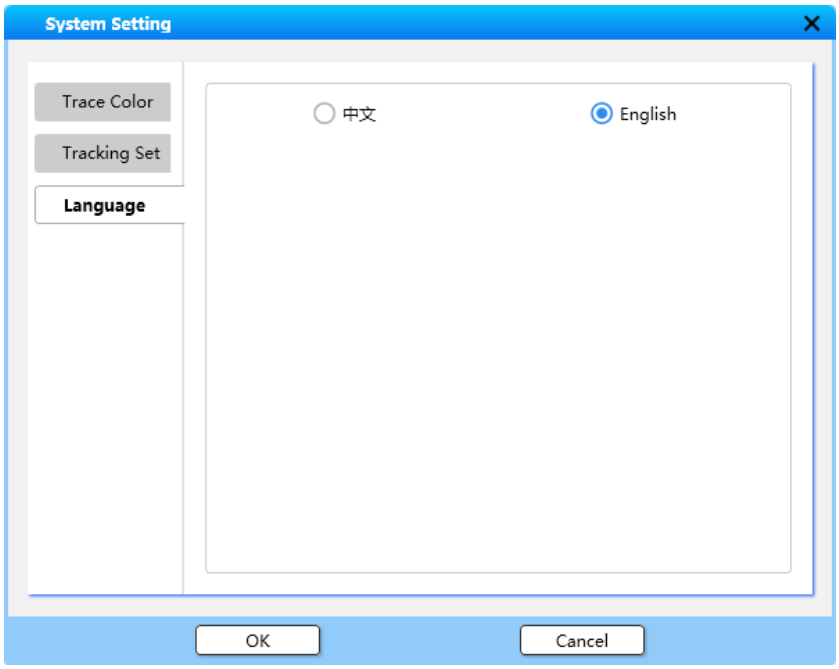


Figure 8-2

8.3. Languages

Choose Chinese(中文) or English as the system language.



9- Other Functions

9.1. File

On the **Photometry** tab, click **File** to perform the import setting / save as.

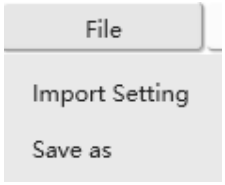


Figure 9-1

Click **Import Setting** to access the target folder and import a parameter configuration file. Click **Save Settings** to save the light source settings, channel settings, and marking/output settings.

Click **Save As** to select a new save path.

On the **Analysis** tab, click **File** to **Open Files** or **Video**.

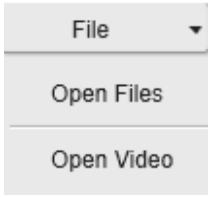


Figure 9-2

9.2. Device

Click **Device** to check the device status, as shown in Figure 6-2.

9.3. Help

You can download the instructions and visit the official website of RWD.

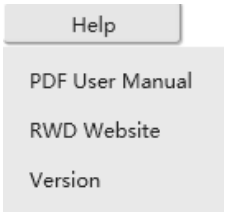


Figure 9-3

10- Maintenance

- Do not use hard or sharp objects that may scratch the device.
- Do not use chemical reagents to clean the surface of the device. Instead, use water.
- Avoid shaking and collision when you move the device.
- Use the tools shipped with the device to tighten the connecting screws between components every two months of use.
- If the device is not intended to be used for a long period of time, unplug the power cord and place the device in a proper storage environment.
- If the optical fiber is removed from the interface, please cover the optical fiber interface with red dust cap to prevent dust from affecting the use of the device.

11- Troubleshooting

This section describes the frequently encountered problems (faults) when using the device, their possible causes and countermeasures.

Problems	Solution
Abnormal data collection	1) Check that the USB 3.0 cable is connected to the correct interfaces.
Abnormal images	1) If the aperture image is blurred, turn the focusing knob to focus.
	2) If streaks appear or images are missing, the lens may be damaged. Contact customer service personnel for maintenance. Do not disassemble the device by yourself.
Abnormal light source of a fiber end face	1) If the brightness of a fiber is dimmed, the fiber may be damaged. Replace the fiber.
Other faults or questions	1）Contact customer service personnel for maintenance. Do not disassemble the device by yourself.

12- Warranty

The warranty for this device starts from the date of shipment. During the warranty period, if the device cannot be used normally due to defects in materials and workmanship, the company is responsible for customer service such as device maintenance and component replacement.

Any device damage caused by incorrect use or out-of-range use is not covered by the warranty. If you require repairs or parts replacement, the costs incurred shall be borne by yourself.

After the device to be repaired is returned to the factory and inspected, if it is found that the device has been disassembled without authorization from RWD, the company does not provide customer service such as warranty, free maintenance and parts replacement.

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

RWD Life Science

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