User Instructions

for Mosaic 1.6

Contents

1 Window

Status Bar

The status bar displays software version, camera model and real-time frame rate.

Menu Bar
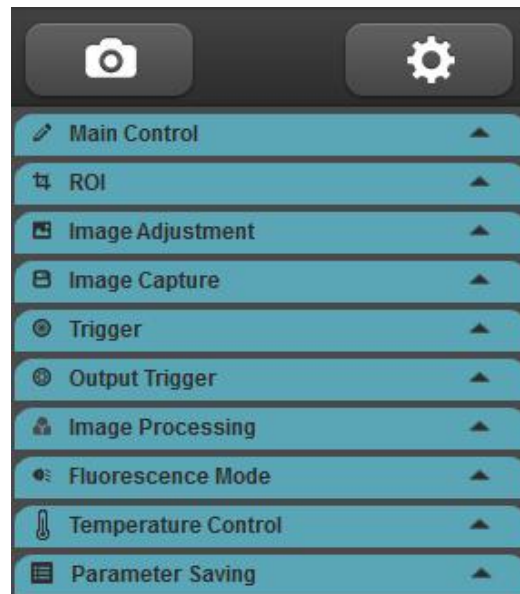
All the functional modules supported by camera are listed on menu bar and the modules can be expanded or retracted.

In Setting module, you can choose to present or hide the corresponding module.
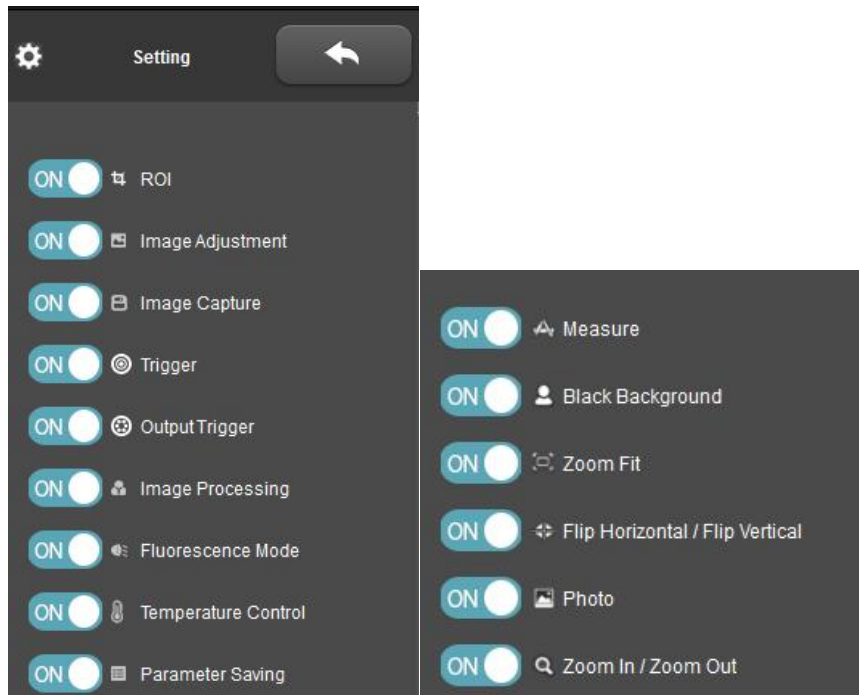
Preview Window

Preview window will display preview image, gray level/RGB value, scale, coordinates and current resolution.
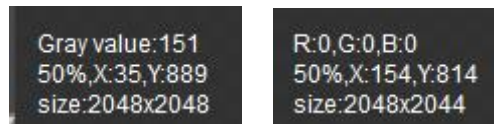
Mono cameras   Color cameras

Shortcut Menu

To satisfy various applications, the software is designed with multiple shortcut keys, so user can acquire an image quickly and conveniently. Specific functions are shown in the following table.

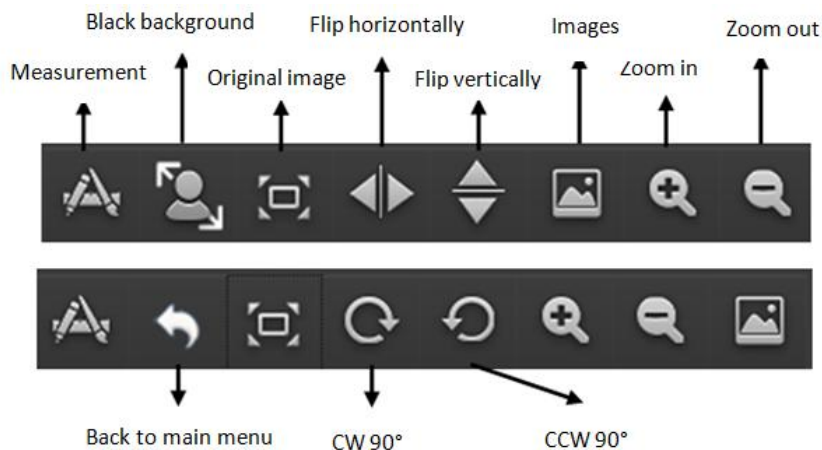| Shortcut function	Shortcut key	Remarks
Move image	Long-press mouse wheel for movement	Both preview interface and image processing interface are effective
Black background	Ctrl+B	Full screen display of preview window
Mirror horizontally	Ctrl+H	Only effective when preview interface is enabled
Mirror vertically	Ctrl+V	Only effective when preview interface is enabled
Zoom in	Ctrl+=\+,mouse wheel forward	Both preview interface and image processing interface are enabled
Zoom out	Ctr+-, mouse wheel backward	Both preview interface and image processing interface are enabled
Images	Ctrl+P	Both preview interface and image processing interface are enabled
Self-adaptive/original image switching	Ctrl+F	Both preview interface and image processing interface are enabled
CW	Ctrl+L	Only effective when image processing interface is enabled
CCW	Ctrl+R	Only effective when image processing interface is enabled
Images selection path	Ctrl+O	Only effective when gallery interface is active
Back to main interface	Ctrl+Q	Only effective when images interface and image processing interface are active
Switch of LED	F2	Click software interface and press F2 to turn on or off the LED (D400BSI V2/V3 &D95 V2&D4040&D4040BSI).
Switch of PI heating film	F3	Click software interface and press F3 to turn on or off the LED (D4040&D4040BSI).

2 Image Capture

2.1 Main Control

Configure the basic working status of camera to ensure that the camera works normally.
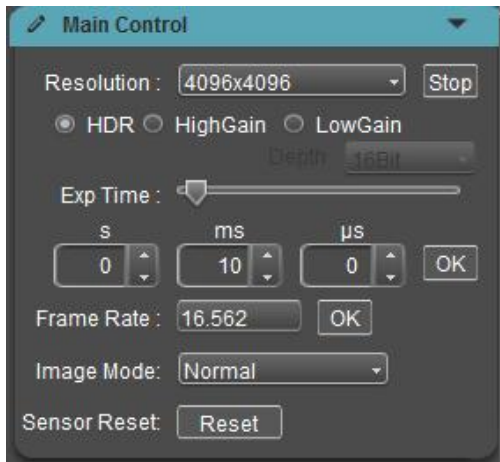
Dhyana 4040V2 / 4040BSI
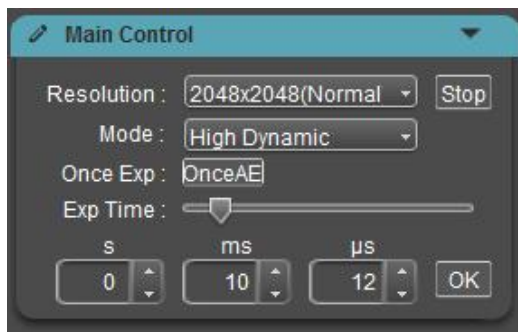
Dhyana 400BSI V3
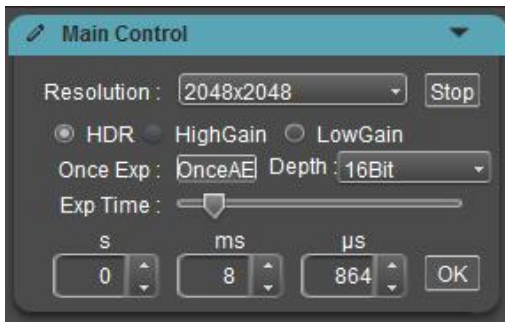
Dhyana 401D

Dhyana 201D
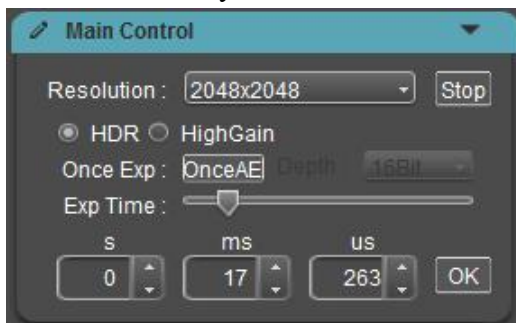
Dhyana 95V2

Dhyana 400D
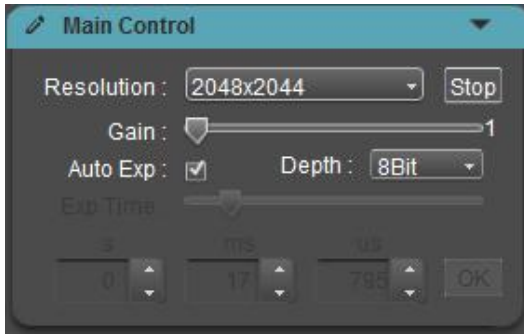
Dhyana 400DC

Dhyana 400BSIV2
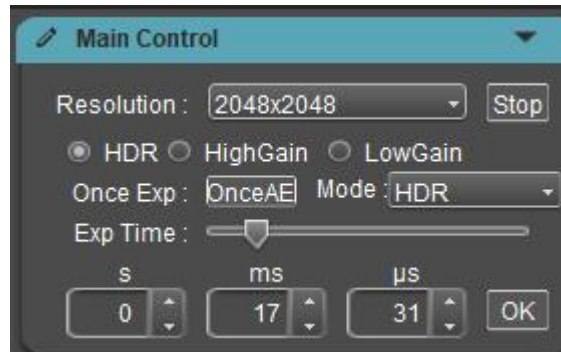
Dhyana XF/XV95

● **Preview (Live/Stop):** Start/stop preview. After preview is disabled, the image will be frozen.

● **Resolution:** Select the camera resolution.

● **Gain Mode**: Only Dhyana 400BSIV3 supports different gain modes, including High Dynamic mode, High Speed mode, High Sensitivity mode, and Global Reset mode. The default is High Dynamic.

● **Gain:** User can choose the most appropriate gain mode to transmit and preview image data depending on specific application requirements. (The gain mode supported by each camera is different and the Dhyana400DC camera is designed with HDR gain mode.)

● **Bit Depth:** Only Dhyana 400DC/401D/201D support. The displayed images are 8bit data images. Mosaic allows users to select 8bit or 16bit data for display preview depending on actual applications.

● **Exposure Control:** Set the camera exposure time.

Auto Exposure: The color camera supports auto exposure. If **[Auto Exp]** is checked, the software will acquire images of appropriate brightness by automatic adjustment of exposure time depending on image brightness. The auto exposure time range is 0ms~300ms. Dhyana 400DC supports only.

OnceExp: The monochrome camera supports OnceAE. Click **[OnceAE]**, the software automatically adjusts the exposure time according to the brightness of the specimen to obtain a suitable intensity image and locks the exposure time in the range of 1ms ~ 300ms. The range of Dhyana 400BSI V2.0 is 500μs-300ms. The range of Dhyana 401D/201D is 100μs-10s.

Manual Exposure: Depending on actual applications, user can set the camera's exposure time manually. General maximum exposure time 10s, D4040/4040BSI maximum support up to 300s.

- **Frame Rate:** The camera output frame rate can be customized according to requirements. Only Dhyana 4040 and 4040BSI support.

- **Image Mode:** Provides several groups of fixed images to detect whether the chip drawing is normal, including horizontal slope, vertical slope, static diagonal slope and rolling diagonal slope.

- **Sensor Reset:** Enables chip data to be reset and CameraLink is removed. Image is drawn automatically during the reset.

2.2 ROI

ROI (Region of interest) is used to define an area within the field of view and the camera only transmit the image information within the window. Smaller ROI helps to lower the image information quantity to be transmitted by the camera and processed by the computer. This will improve the frame rate of the camera. The ROI size for Dhyana cameras can be set by user.The row is an integer multiple of 4, and the column is an integer multiple of 8.
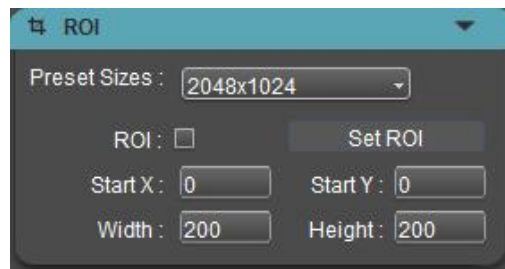
The methods of setting ROI:

1) **Quick ROI:** Click and check **[ROI]**, and drag the mouse to draw a window zone on preview image; the interface will display coordinate of the ROI start point and the current resolution. Then, click **[√]** below or double click mouse or press"ENTER" to apply ROI.

2) **ROI setting:** Manually input coordinate values of start point and ROI resolution. Input the offset, width and height of rectangle; then click **[Set ROI]** to apply ROI.
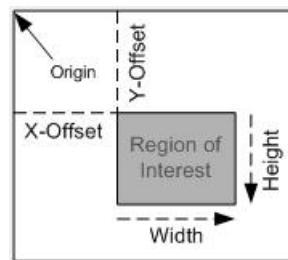
3) **Preset ROI:** The software is pre-configured with six central ROI of common size. Click the drop-down menu to quickly set the desired central ROI.

Note:

The set width of ROI must be integer multiples of 8 and height of ROI must be integer multiples of 4 and must not exceed the full resolution of the sensor. If the values entered are not compliant, the software will adjust them to the nearest compliant values.

ROI size supports a minimum of 48 (column) x40 (row) pixels and Dhyana 400BSI V2/V3 can support 48 (column) x 8 (row).

2.3 Binning
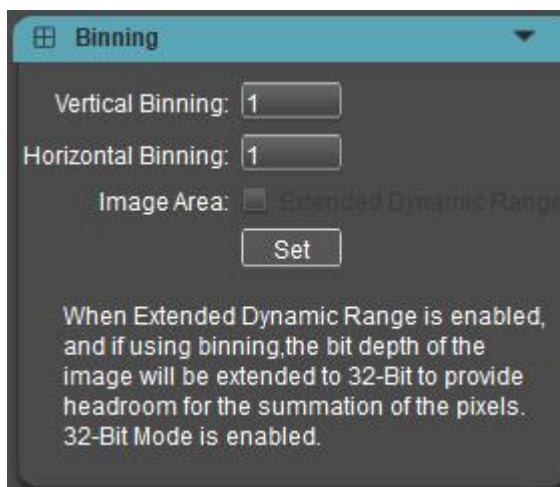
The software under the Binning module can realize any combination of Binning, customers can set any Binning according to their needs and click Set to take effect, at this time the preview image is the image after Binning, but the image data width will not change; If you need to expand the image data after Binning, you need to check Extended Dynamic Range to expand to 32-bit data, because the image data is too large to preview, the preview image is a full-resolution image, at this time you need to shoot and save the camera, 32-bit images only support saving Raw images, do not support preview.

Note:

Currently the Binning module is only available for Dhyana XF/XV95

2.4 Image Adjustment

1. Histogram

During image acquisition, more authentic and vivid images are often needed for observation and analysis; the levels adjustment feature helps users to get better image effects.

Mono camera   Color camera (Dhyana 400DC)

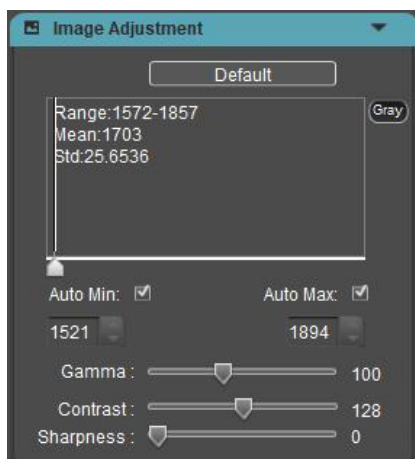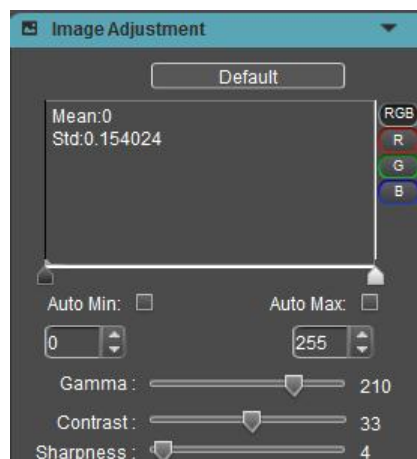Adjusting levels redefines gray value or R\G\B of each channel and redistributes their pixels proportionally. Adjusting images' level zone and increasing the range of highlight zone in the image can brighten the image; of course, it is also possible to darken a bright image; the user can also adjust the three channels RGB and enable image color of the corresponding channel to change.

With the histogram, the user can manually adjust the shade of image, Gamma and brightness to calibrate image tone, including contrast, also brightness and layer of color images.

Auto level: By checking **[Auto Min]** or **[Auto Max]**, the software will automatically define maximum and minimum pixels of each channel as white and black, and redistribute the pixels proportionally between the two limits.

The Mean (average gray value), Std (Standard Deviation) value, and Range (gray value range) of the current preview image can be displayed in real time in the histogram. When the auto level is off and the histogram module is shrunk, camera frame rate can increase.

Note:

Auto level is not supported for individual RGB channels.

Gamma: The brightness of a corrected image on the monitor; the default value is 210 for color cameras and 100 for black-and-white cameras.

Contrast: The difference between the brightest white and darkest black in an image, the default value of color cameras is 33, and the default value of black and white cameras is 128.

Sharpness: Reflects the sharpness of the image plane and the sharpness of the image edges, default value 4 for color cameras, default value 0 for black and white cameras.

Offset: Adjustable gray value of dark field background from 0 to 1023, only CameraLink Dhyana 4040 and 4040BSI support.

2. Color adjustment (Only Dhyana 400DC)

Mosaic allows the user to adjust the display parameters of the image depending on actual applications to obtain the image effects that better meet user needs. Default parameters are recommended reference values.
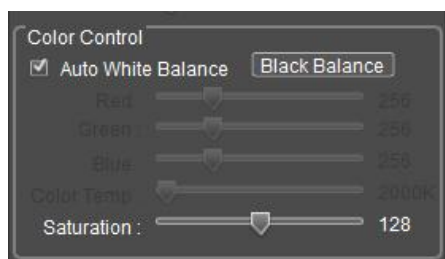
- **White balance:** By adjusting the proportion among three components R, G and B, camera can reveal the most authentic image color under varied light conditions. As default, the camera adopts automatic white balance and if there is stable lighting, the user does not need to check **[Auto White Balance]** to lock RGB gain at current white balance.

- **Black balance:** Lock black balance and set a group of defined R/G/B gain values, so that camera can properly restore image colors in weak light applications. It is generally used for imaging under a bioluminescence microscope.

- **RGB gain:** Manually adjust gain values of R/G/B channels.

- **Color temperature:** Obtain current proximity color temperature depending on R/G/B values or make manual adjustment and matching depending on actual ambient color temperature; as a result, white balance can be calibrated in a more accurate way.

● **Saturation:** Refers to the purity of color. The higher the purity is, the brighter the color will be; the lower the purity is, the darker the color will be.

● **Default:** Reset levels, Gamma, white balance, contrast, saturation, sharpness and DPC to factory defaults.

2.5 Image Capture

Capturing currently required data from real-time video data stream and record them in the form of image or video for later-stage development and analysis.
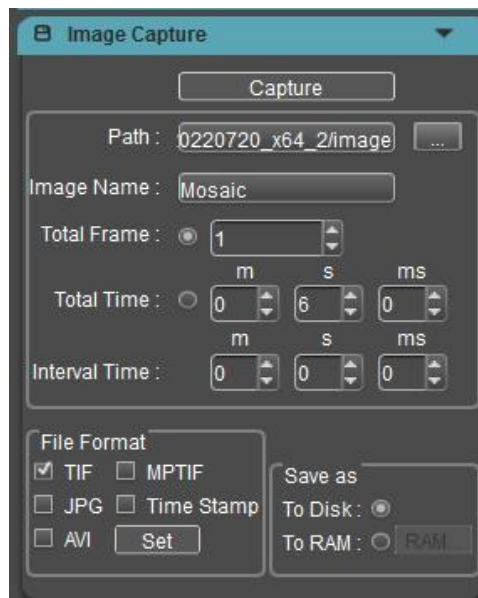
1. Save path

Under default status, the image file shall be saved to a filefolder specified in defined **[Path]**, user could modify save path through **[…]**.

2. Image name

A file will be saved with the name "Mosaic" as default, but the name can be customized. The image name can contain up to 64 bytes (exclusive of special characters \/:*?"<>| under half width; special characters cannot be input and there will be a popup window for prompt).

The image is named as follows:

Mosaic_1_1.tif
Mosaic_1_2.tif
Mosaic_1_3.tif
Mosaic_2_1.tif
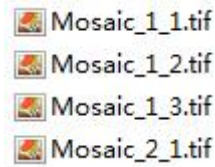
3. Total Frame

Capture image as per set frames: Up to 99999999 frames can be set.

4. Total Time

Total length of image captured: Capture images as per set time length.

5. Interval Time

Interval time between two images.

6. File Format

SupportingTIF\JPG\MPTIF\Time Stamp\AVI and TIF format is the default format. Only one of the four formats can be checked, and the image format and video format can be checked at the same time.

TIF: Lossless image format, which can save all data transmitted by the camera without loss, and is suitable for applications with high image quality requirements. It is recommended to use ImageJ professional image processing software to view TIF images. Windows Photo viewer and other software may cause the colors presented in the images to be inconsistent with the actual.

JPG: Lossy compressed image format, the image size is small, but the quality of the original image data is degraded.

MPTIF: Save multiple TIF format images as a MPTIF format image.

Time Stamp: Save TIF original image, image information, stack image. This function is to meet the needs of high-precision observation of dynamic changes in the process and the time accuracy is 1us. The image information records the start and last time of each frame, the start location of the frame ID, the resolution, the bit depth, camera model, SN（only for USB3.0 camera）, gain mode, exposure time, DPC and frame loss statistics.

AVI: The image data is saved in video format for playback of the dynamic process of the sample. Clicking the Settings button next to AVI will bring up a dialog box to select the compression format and video playback frame rate.

The video mode supports two formats: consistent record and play time or frame rate playback.

1) Video playback rate: Frames per second, can be customized by the user. In the video setting, the Play Speed function is checked to indicate playback at the frame rate. If the Play Speed is not checked, the recording time is the same as the playing time.

2) The default mode of the software is unchecked, and the recording duration is consistent.

Video playback frame rate: the number of frames played per second during playback, which is customized by the user;

Compression: Software provides 3 types of compression formats, FullFrame (NoCompression) / Microsoft_Video1 / Intel_IYUV. Videos of different compression formats result in different file sizes and user may select one depending on actual demands.

Note:

1) JPG and AVI format can support gamma, contrast, color gradation, 3D noise reduction, integration effects, not support fluorescent staining;

2) The image in TIF\MPTIF\Time Stamp format is the original image, without staining, gamma, contrast, color scale effect. When the image is saved To Disk, it

has 3D noise reduction, integration effects, flat field correction and dark field correction. When the image is saved To RAM, it does not have 3D noise reduction, integration effect, flat field correction and dark field correction because it is suitable for high-speed mode.

3) After the monochrome camera adjusts the level, the preview interface displays the effect after the adjustment, but the pixel gray value of the preview interface does not change; the captured image is the original image, that is the gray value of the image does not change.

4) Color camera is same as before. After the color gradation is adjusted, the preview interface displays the effect after the adjustment, and the pixel gradation value changes; the captured image is the image after the gradation is adjusted, that is, the gradation value of the pixel also changes. To facilitate customer preview and qualitative analysis.

5) Except for the HDR gain, the TIF taked with the other gains is inconsistent with the Mosaic preview. The image is 12 bits of data, with a high 4-bit complement of 0.

7. Save mode

The camera supports two saving modes:

[To RAM]: Save the data into computer memory; after image capturing, write the data into a disc file. By clicking the setting button, a prompt box will pop up, prompting the memory size and maximum image quantity. Also, user can customize the memory allocation.
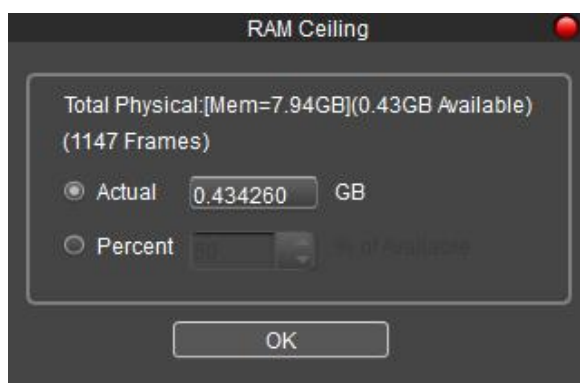
In this way, camera can maintain high frame rate transmission during image capture, which can avoid data loss due to decreased frame rate. Limited by the size of memory space, this mode is not suitable for capturing images for a long time.

[To Disk]: Instant reading and writing. In the process of capturing images, the function will automatically write the data into disk. When capturing images this way, the camera frame rate will be reduced due to data file reading and writing. This is good for long-term shooting, but not recommended for quickly-changing scenes.

8. Image capture mode

Single images: When the Total Frame is set as 1, click bottom Capture once to capture one frame of image data and save one image.

Continuous capture: Capture multiple frames of image data at preset conditions, save multiple images and record the captured images into a video (AVI checked) simultaneously. Before continuous capture ends, the user can click **[Stop]** to stop it.

Video recording: Capture multi-frame image data at preset conditions and record them into a video.

2.6 Trigger & Rollingshutter Control
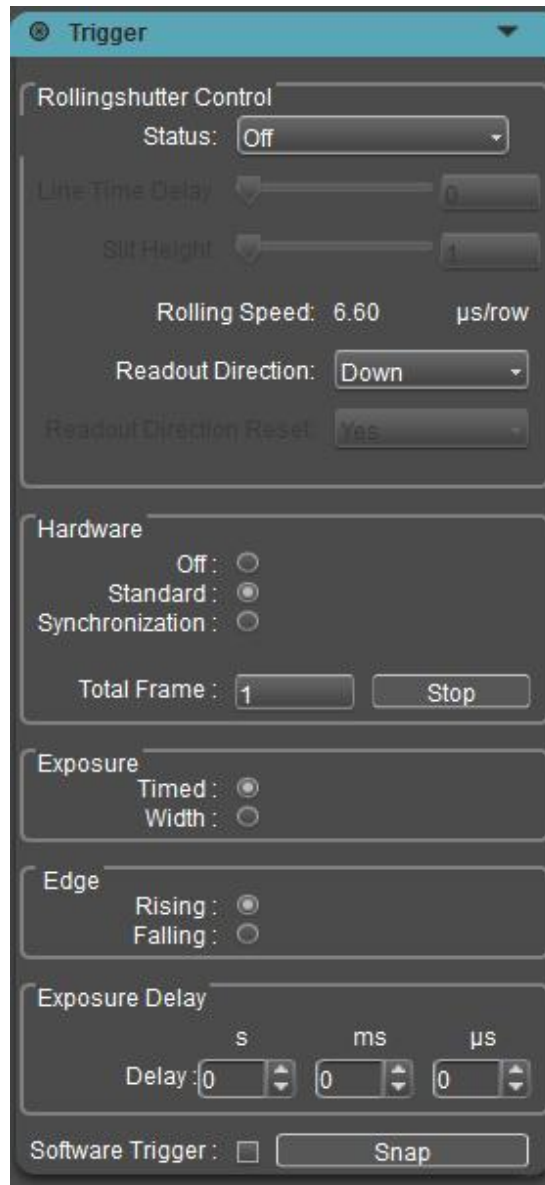
There are two modes of image output: frame mode and streaming mode.

Frame mode: external trigger mode. The camera outputs individual images when hardware trigger occurs. There are four modes of triggering; hardware trigger (standard, synchronization and global) and software trigger.

Streaming mode: real-time preview mode, images are continuously captured by the camera and output in a stream.

1. Off

This means that the trigger mode is in the Off status; the camera is always capturing images.

When standard, synchronization or global mode is selected, the camera will switch to trigger and wait mode. Camera can take an image only after trigger signal is received.

Note:

When Off is selected, the trigger mode is closed, but if there is a signal input, the software can still capture and save images. This function is specially designed and similar to shutter function of a cellphone.

2. Standard

The Standard mode comprises several configuration modules.

Exposure

Timed: If enabled, exposure time will be set by the software.

Width: If enabled, exposure time will be set by the width of input pulse.

Edge

Rising: Trigger by the rising edge.

Falling: Trigger by the falling edge.

Delay: User can set a specific delay time before camera is triggered for exposure after a trigger signal is received.

Dhyana 400BSIV3 adds support for the configurable Total Frame in Standard/Time mode. Software/ Hardware trigger supports both Standard mode and Rollingshutter control mode.

Standard/Time: This mode can control the number of frames to be triggered, and the number of frames will be taken continuously according to the setting after receiving the trigger signal. This mode includes **[Total Frame]** option and **[Stop]** button.

[Total Frame] Set the total number of pictures to be taken automatically and continuously, the default is 1 picture, the range is 1~65535. After setting the parameter, you need to press the **Enter** key to take effect.

[Stop] End the picture acquisition when the frame acquisition is finished.

Note:

[Total Frame] = 1, send a trigger signal, one picture will be captured.

[Total Frame] = 65535, send a trigger signal, the camera will produce 65535

pictures continuously. You can click **[Stop]** to stop the process.

3. CC1(Cameralink standard trigger mode)

This mode is the same as Standard mode, but the difference is that only Dnyana

4040 & 4040BSI cameras support CameraLink. It can be triggered directly through

the acquisition card CC1, and the trigger signal is transmitted to the camera

through the CameraLink line without SMA interface.

4. Synchronization

This mode can be configured with one method; that is to say, exposure time will be

determined by pulse width.

Edge

Rising: Trigger by the rising edge.

Falling: Trigger by the falling edge.

5. Global

Global mode is emulation of a global shutter in rolling shutter mode by

simultaneous exposure of all rows through pre-triggering and external light source

coordination. The Dhyana 400BSIV3 no longer supports Global mode under

CameraLink and USB3.0.

Two configurations are available:

Exposure

Timed: If enabled, exposure time will be set by the software.

Width: If enabled, exposure time will be set by the width of input pulse.

Edge

Rising: Trigger by the rising edge.

Falling: Trigger by the falling edge.

6. Software Trigger

Under software trigger mode, by ticking Software Trigger and clicking [**Capture**] entering the trigger waiting status, and click [**Snap**], an image acquisition command will be issued to the camera. One image can be caqptured each time when [**Snap**] is clicked.

Note:

1) When the external trigger mode and the real-time preview mode are switched to each other, cancel the Apply button and check the different modes to take effect directly.

2) When trigger mode selection, exposure mode selection, boundary selection, and delay setting are performed, cancel the process of clicking Apply button after selection and take effectdirectly.

3) Shielding resolution, ROI, AVI format when entering external trigger mode.

4) Support saving parameters.When the software is turned on next time, it is still in the external trigger mode.

5) Trigger mode can support MPTIF and Time Stamp format.

6) External trigger can supportcontrol start and end, so MPTIF and Time Stamp formats are supported. The steps are as follows:First select the trigger mode, then configure the capturing parameters, then click Capture, and finally input the pulse signal.

7) Trigger mode supports real-time preview, frame rate update, parameter modification (same as stream mode), but does not save the picture, save the picture need to set the number of sheets, names and other parameters in the **[Image Capture]** module and click **[Capture]** to shoot the picture.

8) After choosing the trigger mode, the interface has stopped the picture, but the trigger output still has the stream mode signal, only after clicking Capture, it really enters the trigger frame mode and the output signal stops.

7. Rollingshutter Control

Supported only by the Dhyana 400BSI V3, the Rollingshutter feature allows the camera to seamlessly synchronize with the movements of the LightSheet imaging system, exposing the sample from top to bottom or bottom to top repeatedly, line by line, thus significantly improving the signal-to-noise ratio. The main applications are in life LightSheet imaging is used in the life sciences, particularly in applications requiring LightSheet imaging, such as neurodevelopmental microscopy.

Status: Three modes of operation are supported:

Off (**Default**): When "Rollingshutter Control" is set to **[Off]** mode, the line time of Sensor is 1 line time by default. Line time is the inherent (minimum) line cycle time of the Sensor. For example, the Line time in High Sensitivity mode is 11.2us, and the Rollingshutter Control function is off.

Line Time Delay: When "Rollingshutter Control" is set to "Line Time Delay" mode, add the number of "Line Time Delay" between "Reset" and "Readout" signal (range: 1 to 100ms/Line time (unit: number of Line times) to increase the value of Rolling Speed (range: 2 * Line time to 100ms, unit: us/row). The software automatically calculates and displays the value of Rolling Speed.

$$Rolling\ Speed = Line\ time_{(sensor)} + （Line\ time_{(sensor)} × Line\ Time\ Delay）$$

The frame rate of imaging in this mode depends on the number of lines to be imaged and the line cycle time, which can be calculated by the following formula:

$$Readout\ time_{(image)} = Rolling\ Speed × N_{rows}$$

$$Frame\ rate = 1/ (Readout\ time_{(image)} + Exposure\ time)$$

N_{rows} is the total number of rows of pixels to image.

Note:

1) When the exposure time is less than frame period (Line time *2048), camera has the highest frame rate;

2) The calculation formula of Readout time is slightly inaccurate, and the actual Readout time of rolling shutter is = Rolling Speed * (N-1) +Line Time (because Rolling Speed is not added to the last Line).

3) When the scanning direction is down-cycle, the line scanning order is 1->2048, 2048->1. "Rolling Speed" in the last line can be added or not.

Slit Height: When Status is set to **[Slit Height]** (line width) mode, the scan line width can be set, that is, the scan line width can be directly changed to set the size of the active pixel area. Slit Height = Number of pixel rows between "reset" signal and "readout" signal. The range of **[Slit Height]** is 1~2048.

In addition: the HighSpeed mode is two-line read-out, so the range of HighSpeed mode **[Slit Height]** is from 2 to 2048, and can only be set to even number.

The software will automatically calculate the required [**Line Time Delay**] and Rolling Speed, the formula is as follows:

- HighSpeed mode:

$$\textit{Line Time Delay} = \textit{Exposure time}_{\textit{(Lines)}} \div \left(\textit{Slit Height}_{\textit{(Lines)}} \div 2\right)$$

- Modes other than Highspeed mode:

$$\textit{Line Time Delay} = \textit{Exposure time}_{\textit{(Lines)}} \div \textit{Slit Height}_{\textit{(Lines)}}$$

$$\textit{Rolling Speed} = \textit{Line time}_{\textit{(sensor)}} + \left(\textit{Line time}_{\textit{(sensor)}} \times \textit{Line Time Delay}\right)$$

Note：

1) When Status is set to **[Off]**, **[Line Time Delay]** and **[Slit Height]** cannot be set; **[Rolling Speed]** is not gray, **[Readout Direction]** is optional;

2) When Status is set to **[Line Time Delay]**, the value of Rolling Speed is increased or decreased by changing the value of this parameter. **[Slit Height]** is read-only and cannot be set. The display value is the value obtained by the following formula：

- HighSpeed mode:

$$\textit{Slit Height} = \left(\textit{Exposure time}_{\textit{(Lines)}} \div \textit{Line Time Delay}\right) \times 2$$

- Modes other than Highspeed mode:

$$\textit{Slit Height} = \textit{Exposure time}_{\textit{(Lines)}} \div \textit{Line Time Delay}$$

3) When Status is set to **[Slit Height]** and **[Line Time Delay]** is read-only, it cannot be set. The displayed value is the value obtained by the following formula:

- HighSpeed mode:

$$\textit{Line Time Delay} = \textit{Exposure time}_{\textit{(Lines)}} \div \left(\textit{Slit Height}_{\textit{(Lines)}} \div 2\right)$$

- Modes other than Highspeed mode:

$$\textit{Line Time Delay} = \textit{Exposure time}_{\textit{(Lines)}} \div \textit{Slit Height}_{\textit{(Lines)}}$$

4) *Line Time Delay , Exposure time* $_{\textit{(Lines)}}$ and *Slit Height* $_{\textit{(Lines)}}$ must maintain the

correlation of the above formula. When two parameters are determined, the value of the third parameter should be automatically re-given.

Readout Direction: Scanning Direction. It supports the control of the Readout Direction of the rolling shutter. When Status = Off, it also supports the control of the scanning Direction, and the frame rate in the Down-Up cycle Direction decreases accordingly.

● Down: The downward scan direction is the default scan direction for sCMOS cameras. The shutter starts from the first row at the top of the Sensor and scans down to the last row at the bottom. Each subsequent frame capture starts with the first row at the top.
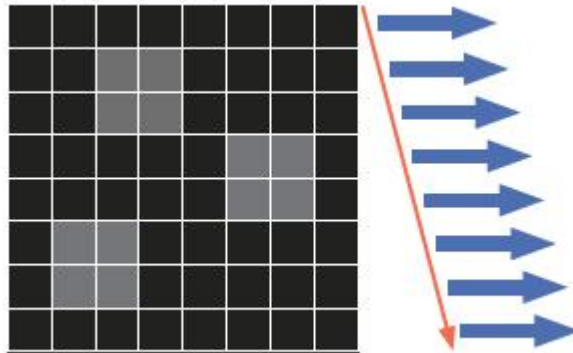

● Up: The upward scanning direction. The shutter starts to scan from the bottom row to the top row. Each subsequent frame is captured starting from the bottom row. The direction of the image acquired in this mode will not be reversed, which is consistent with the image in the downward scan mode.
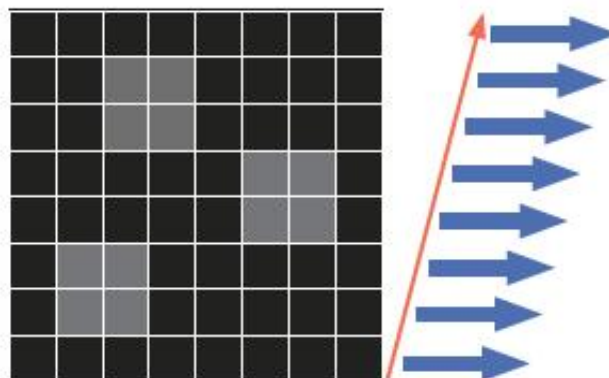
● Down-up Cycle: When scanning Up and Down alternately, the shutter starts from the first row at the top and goes Down to the last row at the bottom. For the next frame, the shutter will start at the bottom row and scan up to the top

row, and so on. The direction of the image acquired in this mode is consistent with the downward scanning direction.
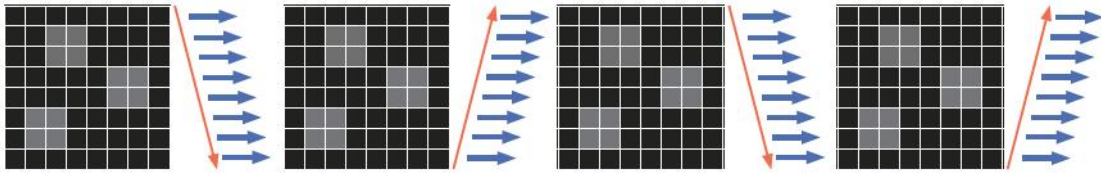
Readout Direction Reset: This function is only available in Down-up Cycle mode and affects the orientation of the first graph in each set. When **[Readout Direction]** is Down or UP, **[Readout Direction Reset]** is unavailable.

The default setting is Yes, which ensures that the first frame of each new collection sequence starts at the top line and scans down.

When set to No, the first frame of Each New Collection Sequence starts at the position of the last frame in the Previous Sequence. If the last frame ends at the bottom row, the first frame of the subsequent collection starts at the bottom row and propagates upward.

The example validates the following: sequence mechanism for each set of graphs:

1) **It's triggered one signal at a time and one graph at a time**

     Readout Direction Reset = Yes, the scanning Direction of each picture is from top to bottom: Down-Down-Down-Down;

     Readout Direction Reset = NO, the scanning Direction of each picture is: Down-Up-Down-Up;

     (One graph at a time is a set of independent sequences)

2) **Trigger each time to send multiple signals or send a signal Standard mode**
     Total Frame set multiple graphs, can be a group of sequence of graphs :

      Readout Direction Reset = Yes

      The first sequence is Down-Up-Down

      The second series is Down-Up-Down

      The third sequence is Down-Up-Down

      Readout Direction Reset = NO, only when the number of outgoing images is odd, the Direction difference is available

      The first sequence is Down-Up-Down

      The second series is UP-Down-Up

      The third sequence is Down-Up-Down

      When the number of images is even, the scanning direction of each image

is the same:

  The first sequence is Down-Up

 The second series is Down-Up

  The third sequence is Down-Up

Note:

1) Rollingshutter Control：Both Cameralink and USB3.0 are supported, and both stream and trigger modes are supported. The Global Reset mode does not support the function.

2) When the Rollingshutter Control is turned on **[Live]**, the parameters can be adjusted and real-time preview can be achieved.

3) Support Rollingshutter Control in ROI;

4) The parameters under **[Line Time Delay]**, **[Slit Height]** and "Image Ajustment" all support manual input in text box.

5) In Standard/Width mode, Total Frame is not supported, because the second image cannot copy the first one. When the second image is generated, the software or camera cannot remember the pulse width of external signals, so the exposure time is equivalent to no high level signals. In Time mode, the exposure time is taken from the software every time.

6) In the continuous saving images process of Standard/Time Total Frame, setting exposure, setting gain mode, setting ROI, switching horizontal mirroring, setting trigger output parameters, setting rollingshutter parameters, these operations (involving live/stop operations) will lead to the drawing directly stop; Switching on and off the DSNU, PRNU, LED, fan adjustment, vertical mirroring, color levels, and capturing does not affect the images of saving.

2.7 Output Trigger
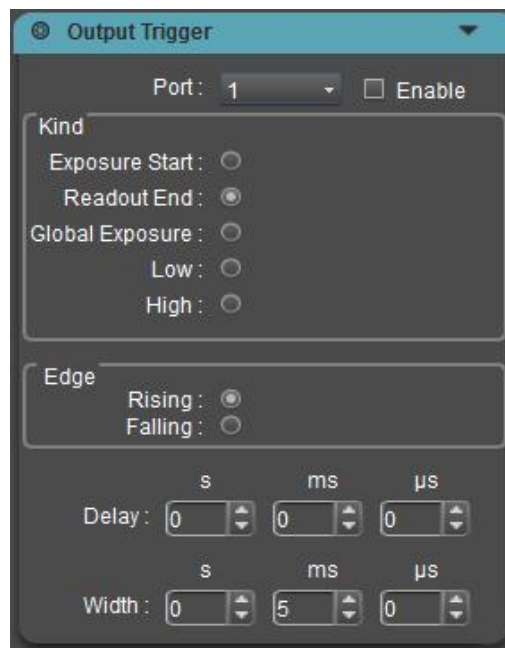
The camera has three trigger output ports TRIG.OUT1, TRIG.OUT2, and TRIG.OUT3, which correspond to Port1, Port2, and Port3 in the software interface. The three output signals are on by default and are controlled by software.

Dhyana 400BSIV3 adds a **[Enable]** button, which allows off / on to trigger the output signal in stream mode and trigger mode. At the same time, the signal is output only when preview Live, and not output signal when Stop.

When the software is turned on, the signal will output. The camera outputs the level signal to a third-party device as its input signal. The three signals can work independently and can be output to different devices at the same time.

1. Port

The hardware interface of trigger output has three pins, which can be configured for three ports. The trigger output is always on, and the signals of the three ports do not interfere with each other, and can be independently configured.

2. Kind (The configuration of the output level)

High: Output high level signal.
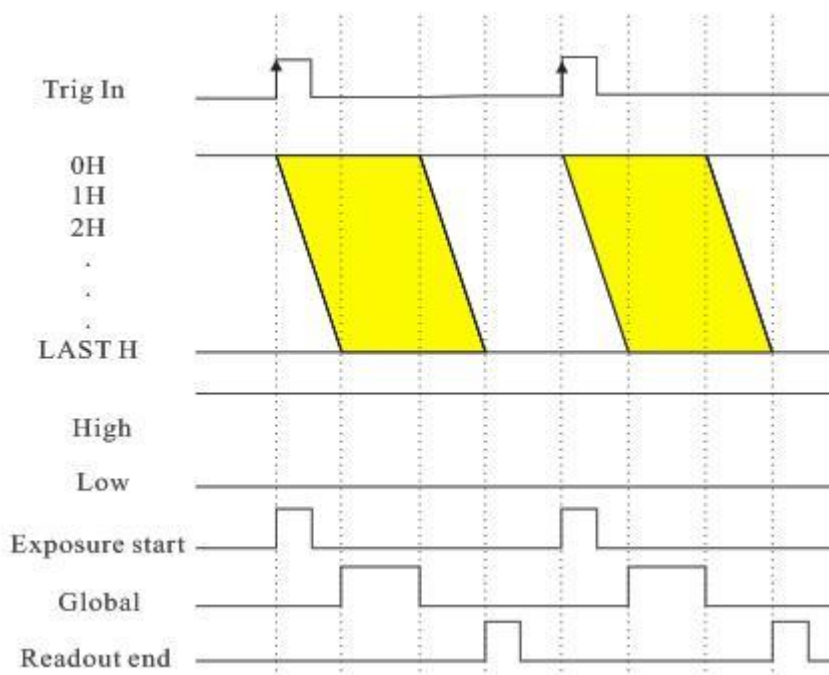
Low: Output low level signal.

Readout End: The signal output by the Readout End interface will be the level

signal from the last line starts to readout. Readout End is the default mode of Port 1.

Global Exposure: The signal output by the Global Exposure will be the level signal from the last line starts to be exposure to the end of the first line starts to readout. Global Exposure is the default mode of Port 2.

Exposure Start: The signal output by the Exposure Start will be the level signal from the first line starts to exposure. Exposure Start is the default mode of Port 3.

Dhyana 401D just support Readout End and Exposure atart signal.

3. Edge: Configuration of the output signal polarity
Rising: The rising edge of the trigger signal is valid.
Falling: The falling edge of the trigger signal is valid.

4. **Delay:** Delay time for configuring the output level signal and the default time is 0.

5. **Width:** Configure the level width of the pulse and the default width is 5ms.

Note:
1) When the Global Exposure signal output is selected, the pulse width

configuration is invalid and its output according to the actual pulse width.

2) When High or Low signal output is selected, other configurations are invalid.

3) The Delay and Width signals in the streaming mode cannot be too long, otherwise the next frame signal will be lost;

4) The configuration precision of Delay and Width signals is up to us, the setting range of Delay is 0~10s, and the setting range of Width is 1us~10s;

5) After selecting the triggering mode, the interface has stopped preview, but the triggered output still has the stream mode signal. Only after clicking Capture button, the triggering mode is really entered and the output signal stops.

Dhyana 400D / Dhyana 400BSI V1 / Dhyana 95 V1 only have three trigger output signals and they are Readout signal, Global exposure signal and Exposure start signal, High and Low signal are not supported.

Readout: The signal output by the Readout End interface will be the level signal from the end of the first line of exposure to the end of the last line of exposure, Readout End is the default mode of Port 1.

Global Exposure: The signal output by the Global Exposure will be the level signal from the last line starts to be exposure to the end of the first line to exposure. Global Exposure is the default mode of Port 2.

Exposure Start: The signal output by the Exposure Start will be the level signal from the first line starts to exposure to the last line starts to exposure. Exposure Start is the default mode of Port 3.
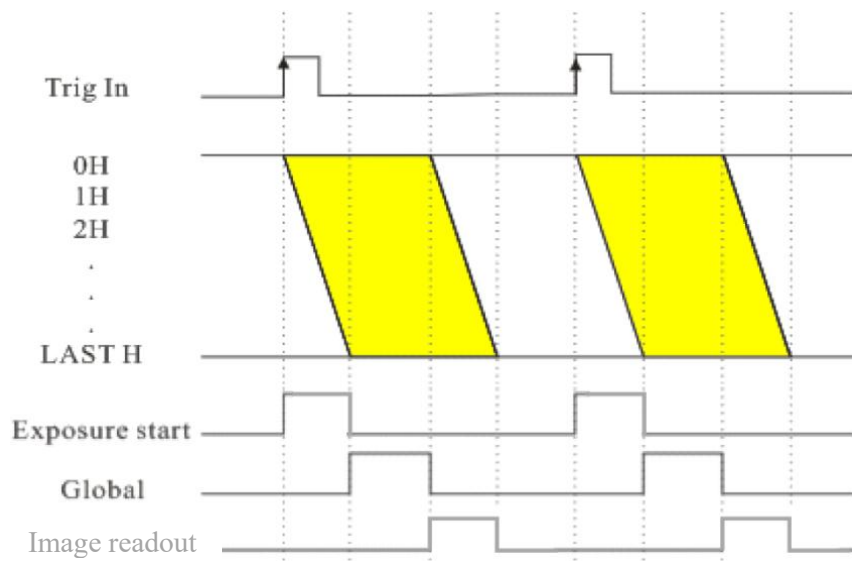
2.8 Image Processing
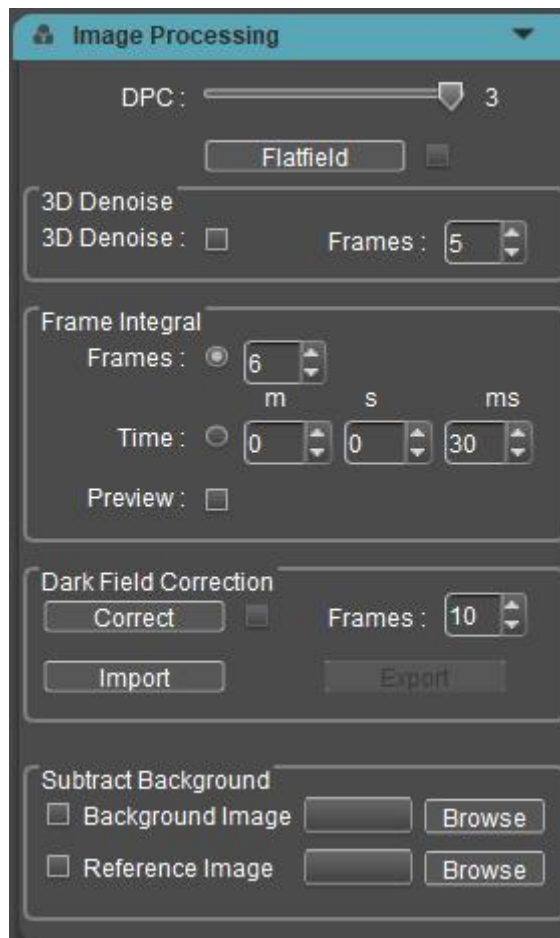
1. Defect Pixel Correction (DPC)

When it is turned on, it can remove the defect pixels on the image. The higher the grade, the stronger the correction ability. At the same time, it may cause pixel flashing in some scenes. The default grade is high.

2. Flat field correction

In actual microscopy applications, there may be phenomena such as imaging nonuniformity or color imperfections due to factors like microscope light source and optical system, or because of dirt on the objective lenses. Flat fielding can effectively reduce such defects, allowing the imaging in a more uniform way and color rendering to be smoother and more authentic.

The steps to make Flat fielding:

1) Click **[Flatfield]** to enter wizard, and move the visual field of camera to blank background zone.

2) Click **[Next]** to move the background to another blank zone and click **[FlatFielding]** to finish flat fielding.

3) Click ☑ to uncheck it and exit flat fielding; to apply flat fielding again, user just needs to check it again, without the necessity of further guide.

Note:

1) In Flat fielding, it's required to set the exposure time manually, so that the image brightness will not have any overflow; all the pixel values fall between 64DN - 254DN.

2) Except moving spots, the two background images should maintain consistent brightness in other zones; they shall not be two images that are apparently different.

3) Confirm background flat fielding target; it's recommended to use plastic, pottery or professional white balance paper.

4) During calibration, make sure that the background is a blank zone and the light is uniform. It's required to recalibrate again after lens replacement.

3. 3D noise reduction

Through several averagely concurrent images, it is possible to filter out nonoverlapping information automatically, so as to save pure and exquisite images. The user may customize the number of frames used. Take effect by ticking 3D Denoise.

Note:

To save images processed with 3D noise reduction, we need to take multiple-frame image data continuously; therefore, it takes a longer time for imaging. 3D noise reduction takes multiple-frame image data continuously to filter out noise; therefore, this is only suitable for still images. If it is used for photographing dynamic scenes, this will cause blur or smear images. In addition, 3D noise reduction is not effective for video recording. Live preview is not supported.

4. Integral imaging

Capture multiple-frame image frames continuously as per preset conditions; accumulate multiple frames of data into one image to preview integral effects in real time.
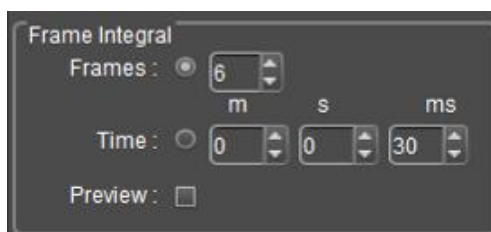
1) Integral by frame: Take images of preset continuous frames and accumulate them into one image.

2) Integral by time length: Take all the frames within the preset time period into one image; the frame data is the result of dividing integral time length by exposure time.

3) Preview: Take effect by ticking Preview. According to preset parameters, display integral effect on the image according to preset parameters, and make adjustment to optimal effects to take images. Integral photographing can brighten images to
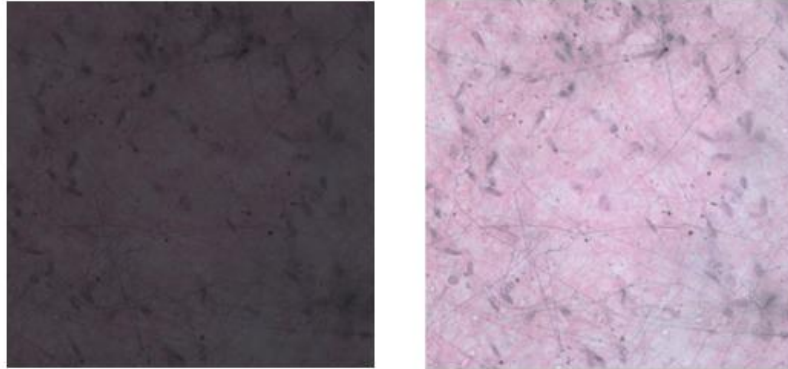
obtain better imaging effects.

Integral imaging can enhance the weak signal, improve the overall brightness of the image, and obtain better imaging effect. The integral imaging contrast effect is shown below.

Note:

1) As to integral imaging, it's required to set appropriate cumulative frames, otherwise, excessive image brightness may cause image distortion.

2) "integral frame" and "integral time length" cannot be enabled simultaneously; one mode can be selected each time.

3) Video also supports integral imaging.

5. Dark field correction

Dark field correction is used to solve the problem of chip corner brightness and background unevenness.

Note:

1) The default Correct enable cannot be checked. When the Import or Correct coefficients are turned on, the check is automatically checked and the dark field correction is turned on. At this time, the check state can be selected to be removed and the dark field correction is turned off.

2) Click on the Correct button and the box prompts"Please make the camera in dark field and choose frames", then click the "Correct" button to enable "Dark Field Correction", click on the Correct coefficient and click Cancel to cancel the operation.

3) Frames defaults to 10 frames (users can set different frames as needed, range supports 1~99).

4) Import and Export are the import and export correction factors respectively

5) Dark field correction should be done separately for different exposure times and different scenarios.

6) ResetAll can restore the Frame value and the Correct enable state, close the software and parameter group to save the Frame, close the software to clear the Correct enable and coefficient, and need to re-correct the next time the software is started.

6. Subtract Background

Implementation principle:

Use of Background, Reference and Signal
Example: Percentage Transmittance, Absorptance
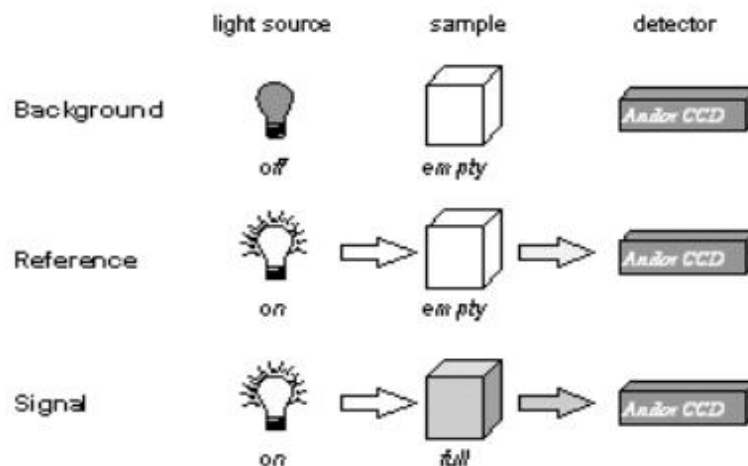
The figure above shows typical uses of background, reference and signal, commonly used to calculate things like percentage absorption or percentage light transmission:

1) Acquisition signal: uncorrected count data collected by Take Signal; If raw or background correction data related to the light source itself is required, the signal

will be data taken directly from the light source. If the reflective, transmitting, or absorption properties of a material are to be calculated, the signal will be data obtained from light that has passed through or reflected from the material under study.

2) Acquisition: Uncorrected count data, obtained in the dark, by taking the background.

3) Acquisition reference map: background correction data, acquired by reference (usually used to calculate the reflection, transmission or absorption characteristics of materials). Reference data is usually obtained from a light source, rather than the light being reflected or passed through or studied material.
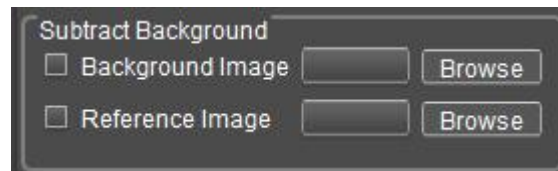
Backgroud Image and Reference Image are loaded by Browse, and when checked, the software automatically subtracts the selected image. (the captured image needs to match the resolution of the camera, if it does not match, it cannot be calculated.)

Note:

  Real-time background reduction is currently only available for Dhyana XF/XV95.

2.9 Fluorescence Mode

Florescence mode is generally used for taking fluorescent images with monochrome camera. By adding pseudo color to the image by software, user can achieve the purpose of fluorescent color.
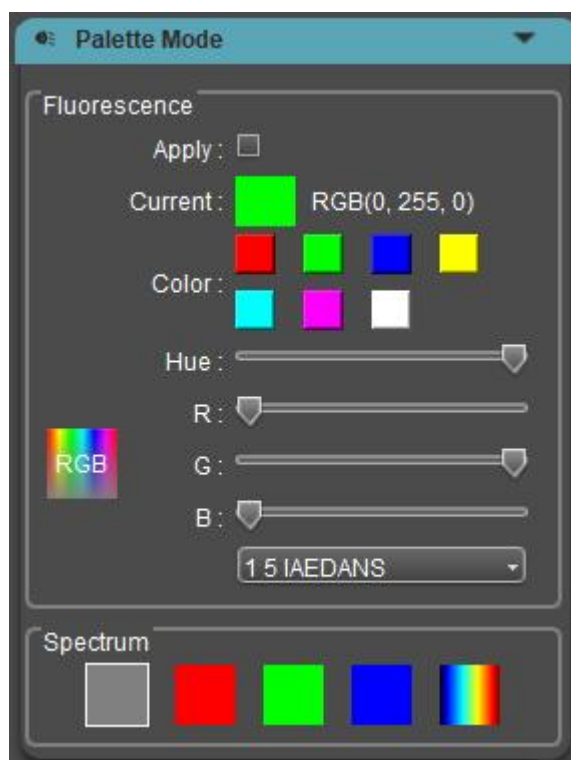
Check **[Apply]** to apply the selected fluorescence color to the image; it is permitted to obtain pseudo fluorescence image by capturing for later-stage synthesis of a complete fluorescent image. But the images taken is the original images, no florescence effect. If the image needs to be tinted, click, then right mouse click to bring up the Measurement menu, find and click **[Combined Measurement]** to apply the selected fluorescent color to the image, and shoot to save. For more features of the measurement menu, please refer to **section 4 Measurement**.
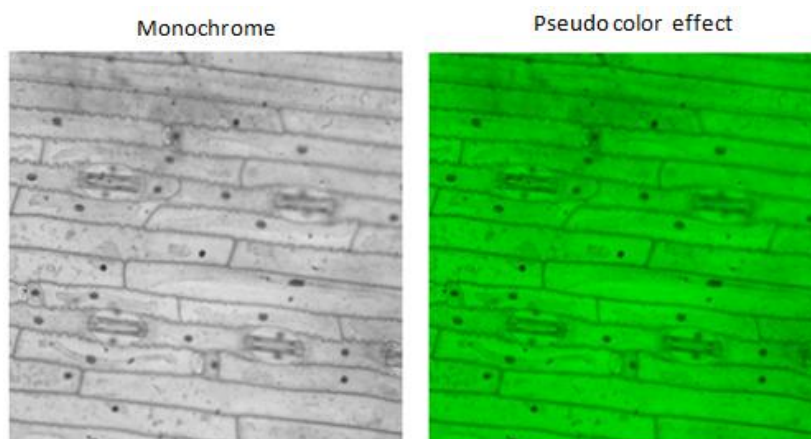
Spectrum supports black and white, red, green, blue, color, mouse selection click to take effect, like fluorescent staining, the captured image is the original image, without false color effect.

2.10 Temperature Control

There is a thermoeleectric cooler installed in the camera. The software can be used to control the cooling temperature of camera and reduce the working temperature of image sensor to reduce the noise. Dhyana 401D/201D does not support temperature control.

The heat dissipation method is divided into fan cooling and water cooling.
Fan cooling

Fixed-speed fan: The fan is always running at a fixed wind speed, and the fan speed cannot be adjusted. The temperature can support -10℃ and -20℃, and the default is -10℃.
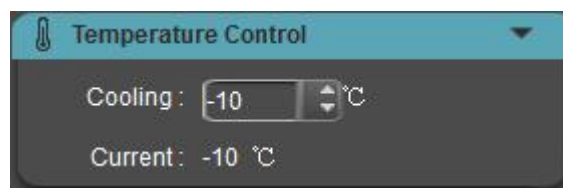
Adjustable-speed fan: After the camera is turned on, the fan keeps running at the maximum speed by default. And the fan speed can be adjusted by software. The grades are divided into High/Medium/Low/Off (Water Cooling). The temperature can support -10℃ and -20℃, and the default is -10℃. The default fan speed of Dhyana 4040 is Medium. The temperature of Dhyana 4040 is fixed -20℃.

Note:

1) The temperature of the different fan grades is fixed at -10°C. The difference between the three grades is that the speed of cooling to -10°Cis different.

2) Each time you select the Off, the following interface reminder will pop up, and you can turn off the fan after clicking OK.
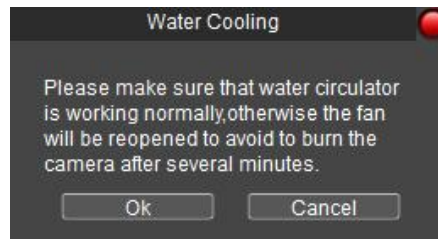
3)If the Off is selected and the water cooling is not worked, the camera will do the protection mechanism. When the current temperature reaches 0°C and above, the highest-grade speed of fan will be automatically turned on to prevent the camera from burning the chip. If the software is turned off, the protection mechanism will not take effect.

4) The fan speed will be reset after restarting the camera. You need to re-open the software to take effect the original configuration.
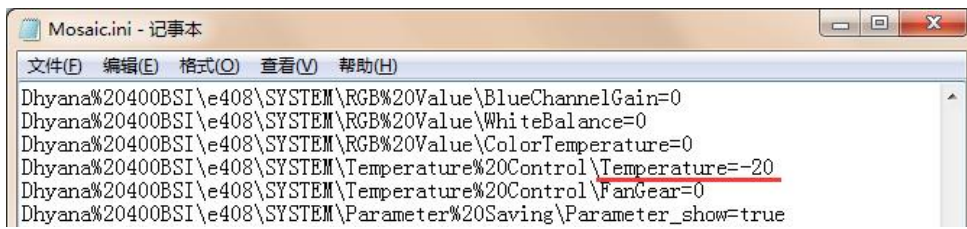
Water cooling

The camera that supports water cooling is equipped with a adjustablespeed fan. It needs to be connected with an external water cooler. See the Instructions of Water Cooling Camera.

The default temperature of water-cooled camera is fixed at -10°C (external water temperature is 20°C), but water-cooled cooling can reach -20°C or even lower. If lower temperature is required, the Temperature value in the software configuration file can be modified.

As shown below:

Dhyana%20400BSI\e408\SYSTEM\Temperature%20Control\Temperature=-20

Warning:

Water cooling and fan can be turned on at the same time, but they cannot be turned off at the same time. At least one cooling mode must be guaranteed. Otherwise, the chip may be damaged due to continuous high temperature operation.

Note:

Current temperature only shows the precision to single digits and does not display after the decimal point.

Temperature alarm

The camera supports the high temperature warning function. When the external ambient temperature is too high, the software will prompt the temperature alarm prompt message, and the camera indicator will turn red flashing (only D95V2.0 camera supports).

2.11 Parameter Saving

In actual applications, the user will switch between multiple applications the camera or use it on different platforms. To facilitate user operations, the software has provided a parameter group management function; the user may save and load the setting parameters in each different environment to save operation time. The parameter group can save the configuration parameters of the functional module mentioned in above chapters (except flat field correction and dark field correction).
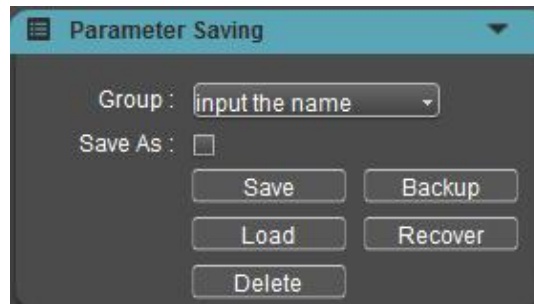
Group: Used to name a group; input a name to save and load it; you can view and load saved parameter group through drop-down menu.

Save: Save current configuration parameters into parameter group.

Load: Load a parameter group and   apply it..

Delete: Delete currently selected parameter group.

Backup: Save parameter group file to a specified filefolder.

Recover: Load the parameter file from a specified filefolder.

Save As: Save a group of parameters into an independent file.

ResetAll: Click to clear all the parameters set by the user and return to the software factory settings.

Note:

Copy parameter file to other computers for application loading. This function will enable the cross-platform transition of parameters to ensure that the user's experimental conditions remain consistent to the largest extent.

3 Browse and Edit Image

3.1 View Image

During operation, the user can click ▨ to open, view or edit images and play back or download a video. The default setting is to open an image under preset save path. In the images window, select an image thumbnail, double click to view the image; right click to **[Delete]** the image, or you can delete one or several images through **[Delete]** on the keyboard.

In the Image view window, you can switch to the previous or next picture for browsing and processing by press the "Left" or "Right" on the keyboard.
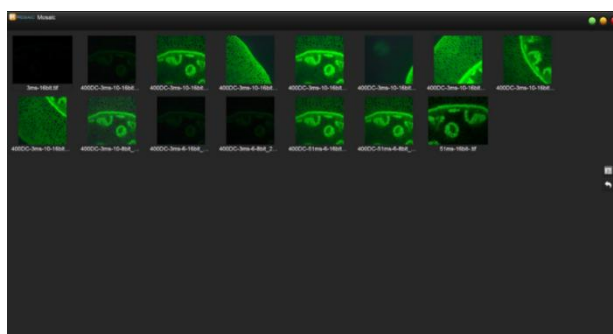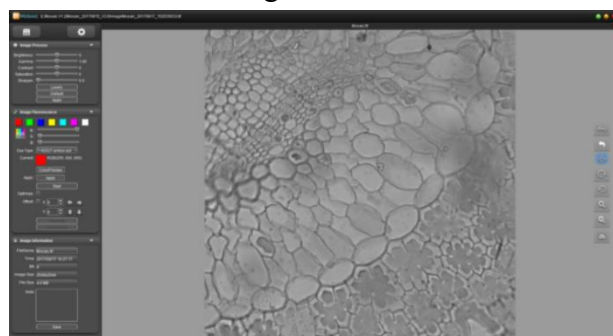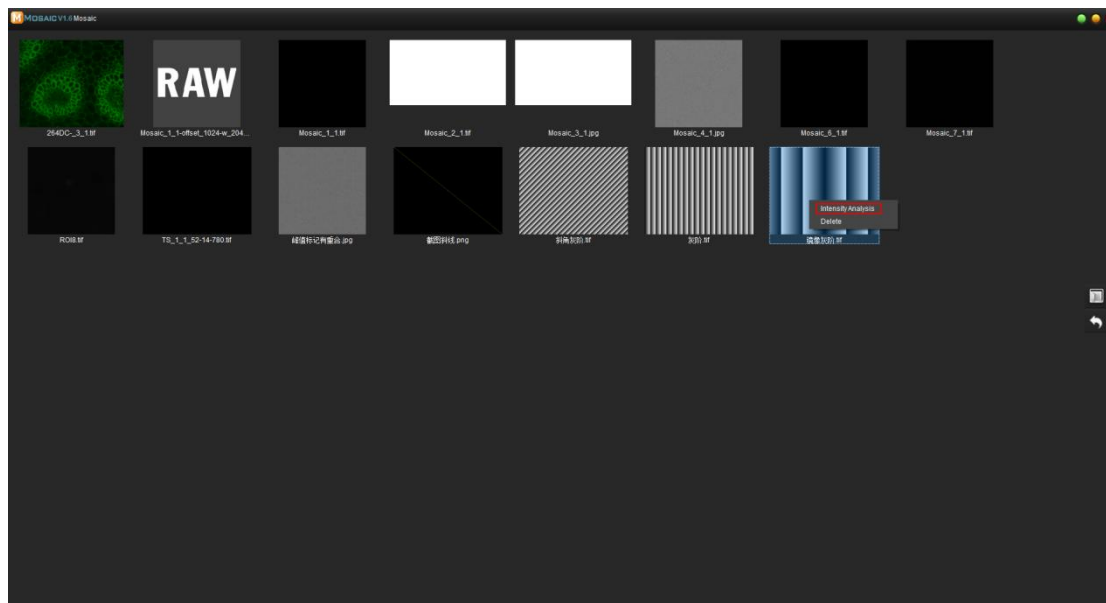

Images window

Image view window

3.2 Intensity Analysis

The intensity analysis function is applied to the field of spectral analysis, and the grayscale statistics of the images collected in specific scenarios, the marking of image positions with spectral characteristic significance, histograms, and ROI data signal-to-noise ratio analysis.

Right-click Intensity Analysis through the gallery window to enter the intensity analysis.
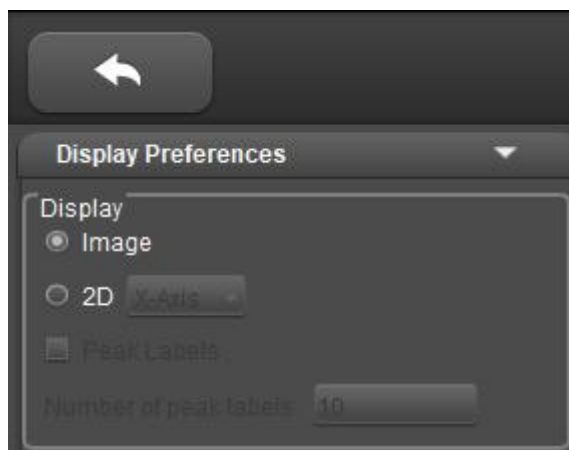
Click the arrow in the upper left corner to exit intensity analysis mode.

1. Display Preferences

1) Image

Intensity analysis of images X and Y according to mouse position, such as mouse position at [255,255] position, the gray value distribution of 255 rows and 255 columns is counted, and the mouse is located in the bottom middle position of the image by default; The scale is in Pixel Number or Wavelength, the scale is the resolution of the corresponding image, and the image scale calculation formula is as follows:

　　1) Take the lower left corner of the image as the coordinate origin and do not display the value of the origin, that is, do not display 0;

2) By default, it is divided into 5 complete large scales according to the longest side of the image, and 5 complete small scales are divided between each large scale;

Formula:

(1) After dividing the length by 5, round up;

(2) Calculate the length of the value in step (1);

(3) Calculate the corresponding length of 10 to the power of -1;

(4) Calculate the maximum scale by dividing the value of step (1) by the value of step (3), rounding up, and then multiplying by the value of step (3);

(5) Calculate the value of the small scale according to the steps of (1) ~ (4);

(6) Divide the value of step (4) by the value of step (5) and round up to calculate the actual number of small scales;

(7) Multiply the value of step (5) by the value of step (6) to calculate the final maximum scale.

Unit: pixels / scale.

For example: resolution 2048 x 1152

① 2048 / 5 = 409.6 ≈ 410；

② 410 is 3 digits;

③ Calculate 10 to the power of (3-1) to be 100;

④ 410 / 100 = 4.1 ≈ 5 ； 5 * 100 = 500；

⑤ According to the steps of (1) ~ (4), calculate the small scale value as 100;

⑥ 500 / 100 = 5；

100 * 5 = 500. That is, large scale = 500; small scale = 100.

Image supports zooming in and out, and the corresponding scale will also be enlarged and reduced;

The current intensity distribution is shown above the image, the grayscale image data is mapped to the color range of the pseudocolor bar according to the minimum and maximum values of the histogram, and the pseudocolor mapping of pixels with different grayscale values changes with color levels.

The lower-left corner of the image displays the coordinates and grayscale values of the current cursor position in real time.
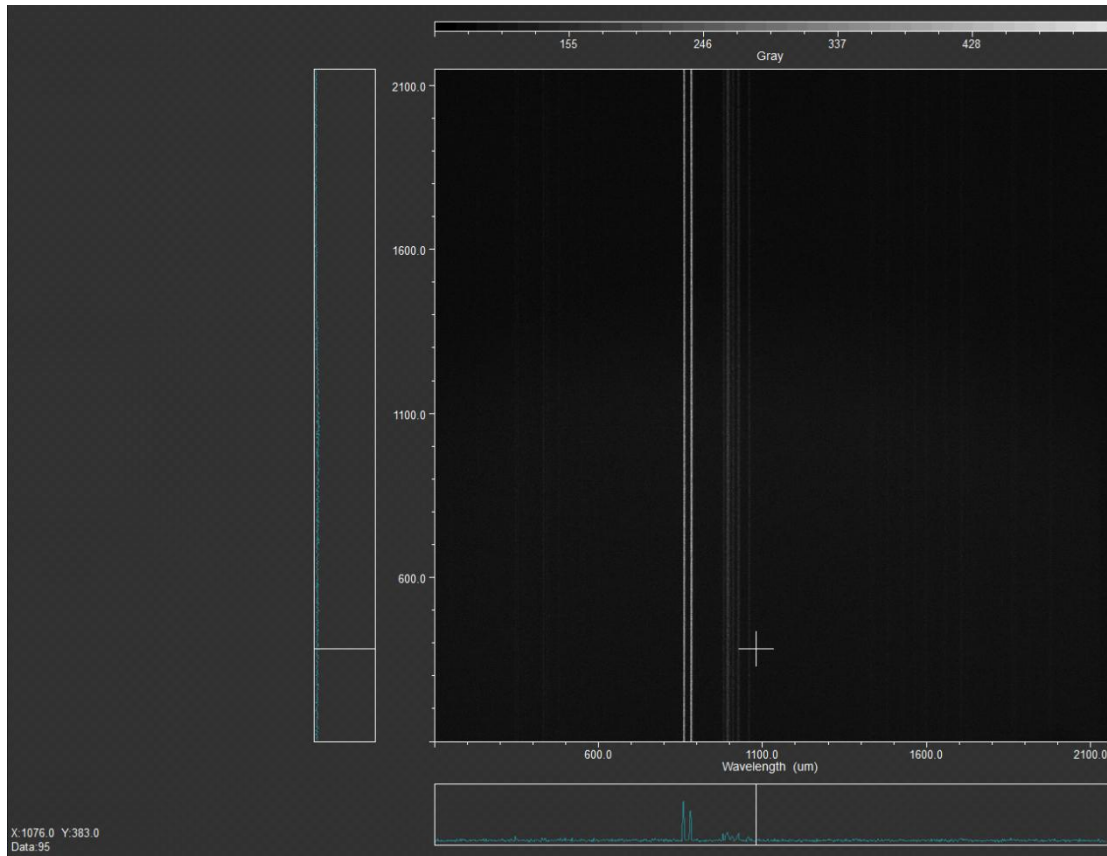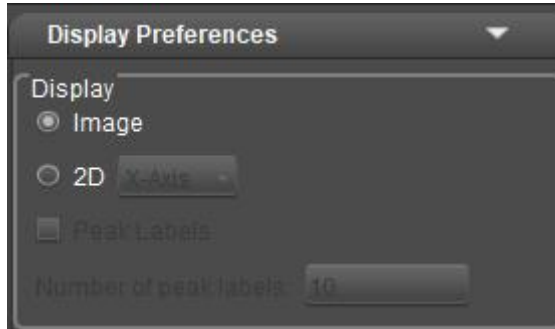
2) 2D

Analyze the single direction of the current mouse coordinate position, which can better analyze the strength of the current row or column, support X-Axis and Y-Axis switching, the abscissa is Pixel Number or Wavelength, and the ordinate is Gray.
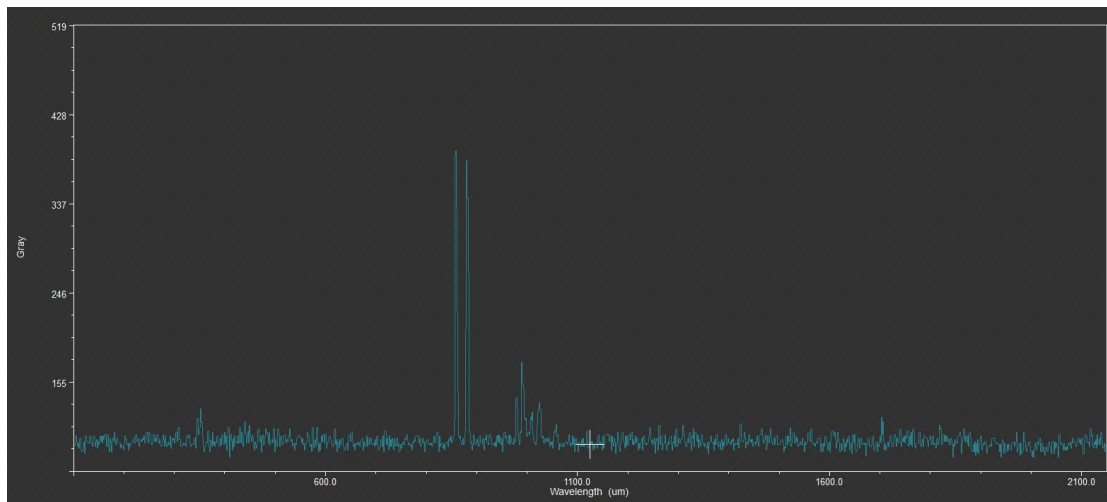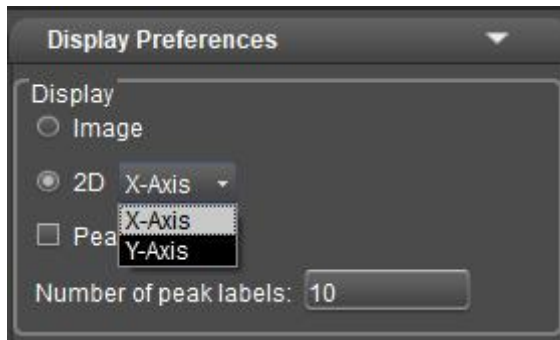
3) Peak Labels

The maximum value mark of the waveform is supported in 2D mode, and the peak labels are not supported in Image mode, and the maximum value mark is labeled based on the automatic multi-scale peak finding algorithm, and the final result will be sorted from largest to smallest according to the Gary value corresponding to the maximum value of the marker.

Note:

The automatic multi-scale peak lookup algorithm may not accurately label all scenes, but most scenes with obvious intensity contrast can be accurately labeled. Users can set the number of peak labels according to their needs, and the number can be set in the range of 1~200.
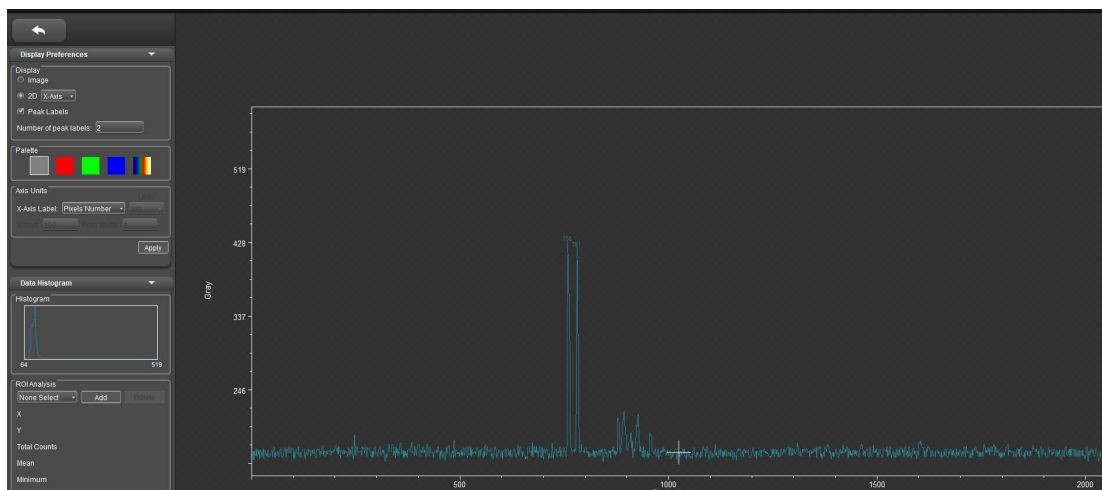
4) Palette

As with Spectrum in fluorescence mode, false color is added to the currently open image, only preview is supported in Image mode, not in 2D mode.


5) Axis Units

The abscissa of the intensity analysis supports Pixel Number and Wavelength unit switching, and click Apply to take effect after the adjustment is completed.

1) When the unit is Pixel Number, X Start and Pixel Width grayed out do not support adjustment;

2) When the unit is Wavelength, X Start and Pixel Width light up can be adjusted;

3) X-Start: X-axis starting position, can enter the range 0~2000;

4) Pixel Width: X-axis step, can input range 1~100;

6) Data Histogram

● **Histogram:** Displays the histogram distribution of the current image, supports full image resolution and ROI display.
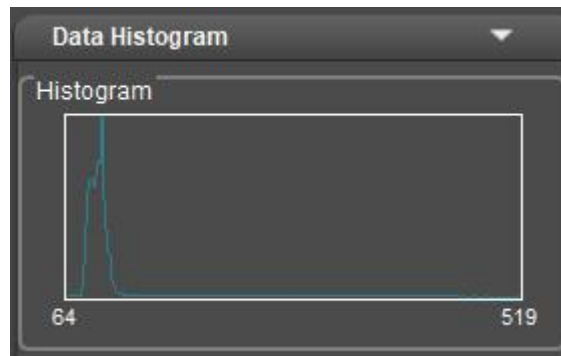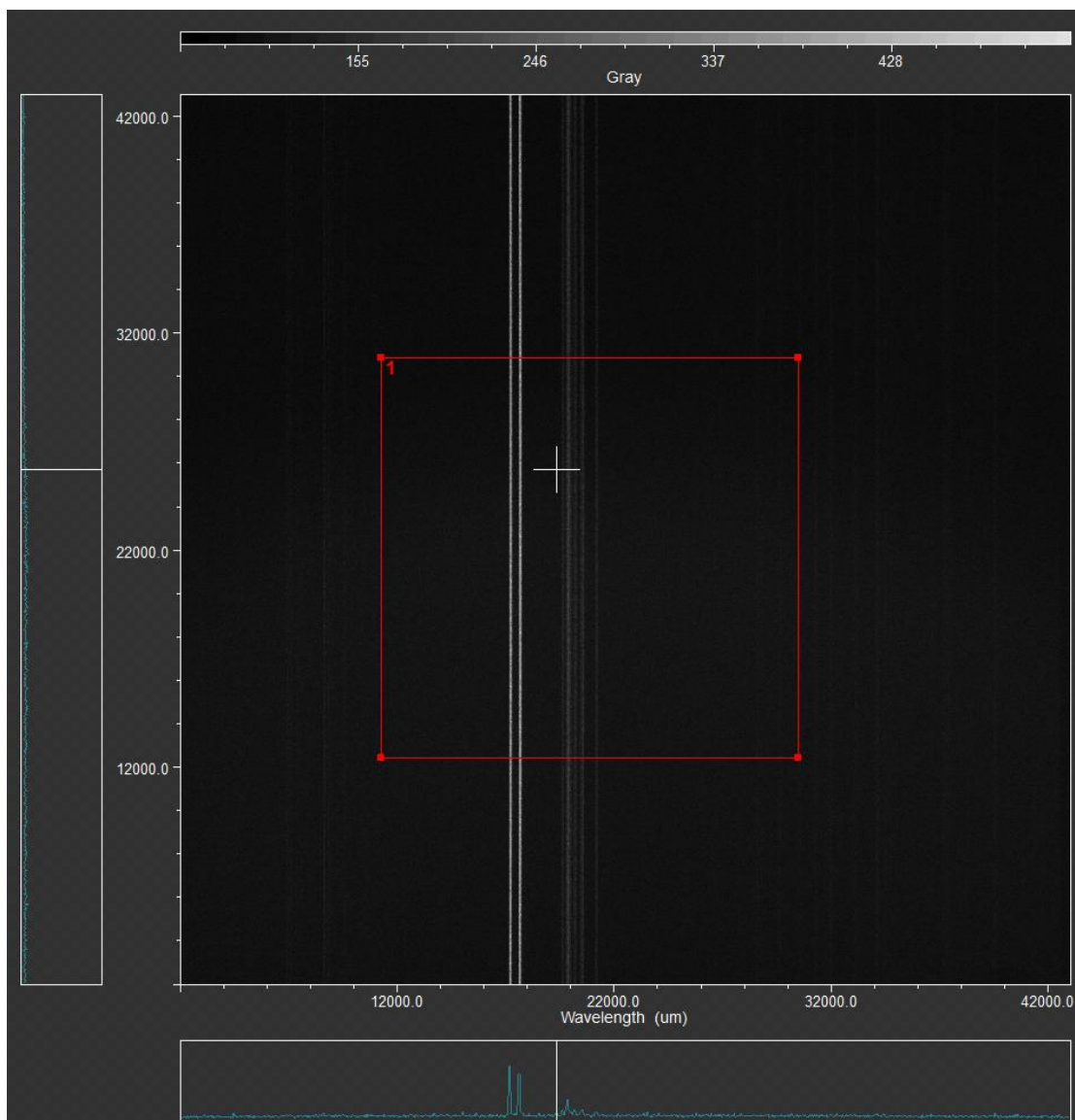
● **ROI Analysis:** Click Add to draw ROI on the image, ROI Analysis will count the selected ROI coordinates, and calculate the number of pixels in the ROI area, average gray value, maximum value, minimum value, standard deviation and signal-to-noise ratio.

   "Add ROI" button: click once, drop down the box to add the new ROI area, name ROI + serial number (such as ROI 1), and add one to each click on the serial number;

   "Delete ROI" button: Click once to delete the currently selected ROI region and the corresponding ROI region name in the drop-down box, the name after deleting the ROI will replace the deleted ROI name until all are deleted, and the ROI is selected as "None Select".
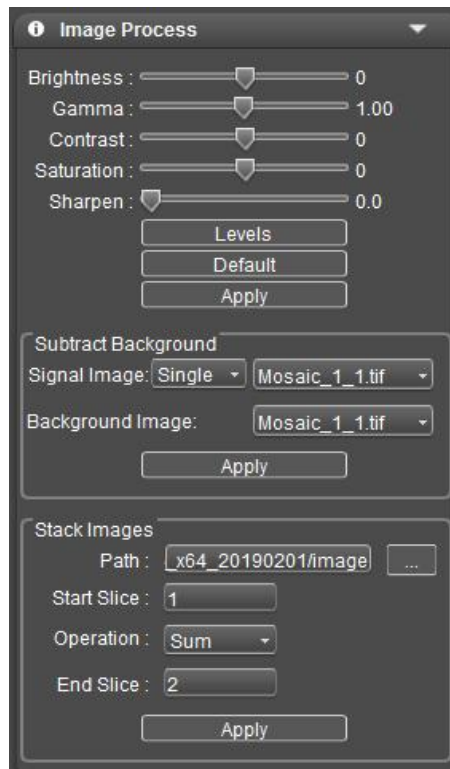
3.3 Image Process

1. Image parameter setting

Adjust image parameter to correct captured image.

Parameter setting:

Brightness: Used to set the brightness of image.

Gamma: Calibrate the display brightness of image in display.

Contrast: Ratio between maximum darkness and maximum brightness.

Saturation: The purity of color. The higher the saturation is, the bright the color will be.

Sharpen: Focus on blur edge of a part to enhance its definition or focal length degree, enabling the color of specific zone of the image to be brighter.

Levels: Used to adjust the gray level resolution of image; it's available to adjust levels manually and automatically within the range.
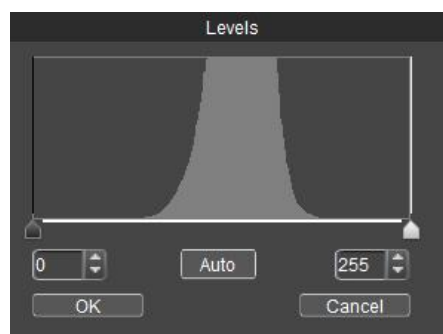
[Apply]: After parameter has been adjusted, apply parameter setting into image.

The parameters in the Image Process and Image Fluorescence modules can be saved.

[Default]: Reset current parameter to default value, including brightness, gamma, contrast, saturation, sharpen, levels, rotate and fluorescent dye.

Subtract background:

Real signal image = signal image– background image.Signal Image is set as the Signal Image, and Background Image is set as the Background Image. Click Apply to get the result image of the subtraction between the two images. The software supports single subtract background, also supports multiple subtract background, just stack select image folder,all images subtract the same background and get the new stack images after subtract background.

Signal Image has drop-down boxes Stack and Single, the default is Single;

Image sorting is only compatible with Mosaic's own chart naming rules.

The image of the signal image subtract the background can be displayed directly, and saved as the Single_Subtract_n.tif;

The image after subtracting the background from the stack images shows only the last one, and saves it as Stack_Subtract_n.tif.

Stack images

For applications such as single-molecule imaging, it is necessary to collect thousands or tens of thousands of images for each acquisition, and then perform single-molecule localization and accumulation on the acquired images, and finally obtain a superimposed result map.

Operation has a drop-down box Sum and Average optional (default Sum).

Image sorting is only compatible with Mosaic's own chart naming rules.

Start Slice and End Slice set the sequence of images to be superimposed.

The Stack overlay (Average and Sum) images can be displayed directly and saved as Average_n.tif or Sum_n.tif
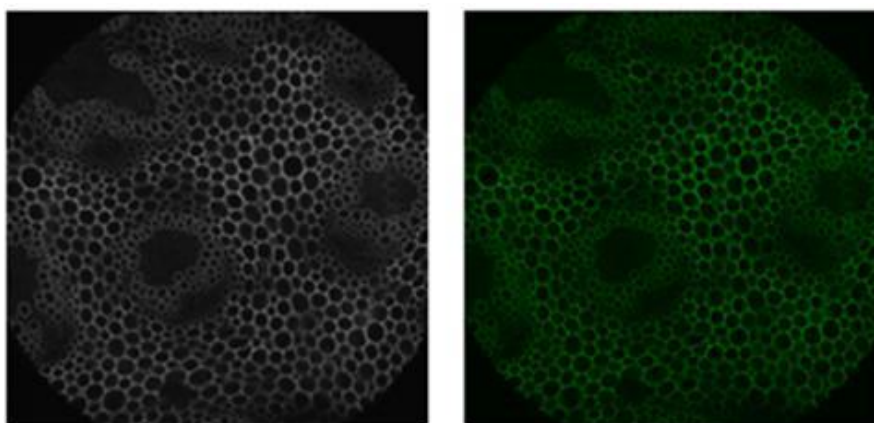
2. Fluorescence synthesis

In bioscience, different fluorescent dyes are adopted to observe different cell structures. Under a fluorescence microscope, we can only see one fluorescent image. Image fluorescence function will help users to synthesize multiple fluorescent images under the same visual field into one, thus obtaining a complete

color image.
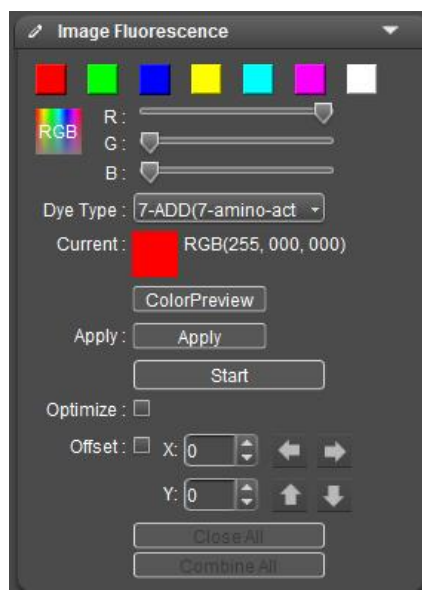
3. Staining

To find the gallery for the image to be edited, double-click to open the preview, click **[Start]** to enter the fluorescent color operation. Select the desired color, click **[Apply Color]** to see the staining effect. If other colors need to change, select the target color directly and click **[Apply Color]** again to obtain a vivid color image that is almost identical to the observation though the microscope eyepieces.


4. Optimization

Recommended to be checked to optimize a synthesized image and obtain a better effect. Unoptimized synthesis image synthesizes all the information of source image into the same image.

5. Synthesis

In fluorescence microscopy, a complete cell image is usually composed of multiple fluorescence bands. The synthesis function will integrate images under multiple fluorescence bands into a complete image. Open the gallery and select the images to be synthesized (at least two, up to four) and click **[Start]**.
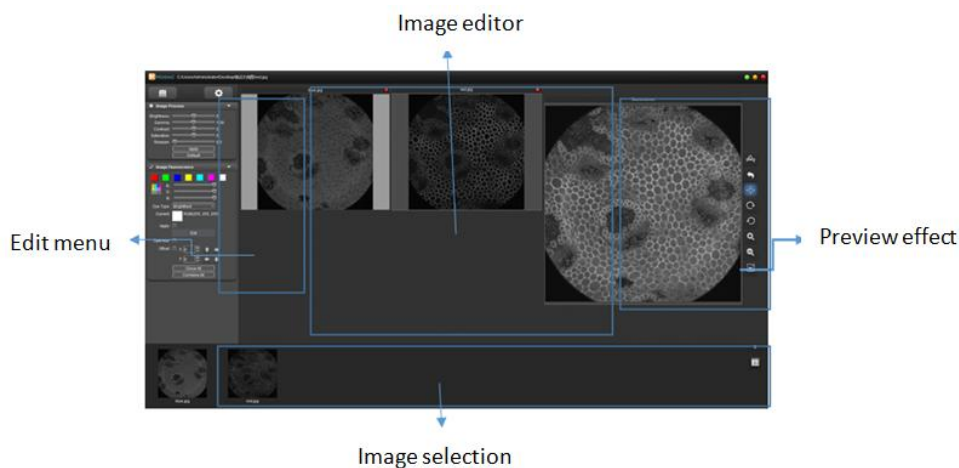
Select the desired color, click **[Apply Color]** to determine the color. Do the same for other images, the software will be a real-time display of the current color of the final rendering.The image can still be fine-tuned, the detail of the current single image and the Offset through the parameter adjustment under Image Process, to finally achieve the desired effect. Click **[Combine All]** to complete the fluorescence synthesis.

Note:

If four images of the same format are selected for synthesis, the composite image format is consistent with the original image format. If four different formats of images are selected for hybrid synthesis, the composite image format is fixed to the TIF format;

Offset: While switching filter wheels under fluorescence microscope, it may cause certain mechanical dithering, resulting in images taken by the camera to have micro pixel error. Check **[Offset]**, user may make micro movement adjustment for currently selected original image to correct pixel error. One numeric unit indicates one pixel.

**Close All:** Close the source images selected currently and select new source images for synthesis.

Combine All: Synthesize current source images into one and save it locally; the format of currently synthesized images will be the same as currently selected source image.

6. Image information

Obtain the information of captured images, including file name, capture time, bit depth, resolution and file size, to modify file name and add remarks.

In**[Note]**box, user may add corresponding remarks for current image and after content addition, click save. Next time you open the image, the remarks will show in **[Note]** edit box.
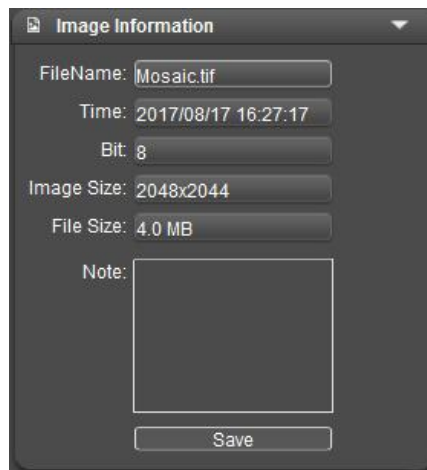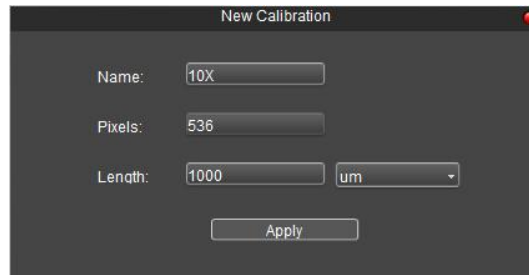
4 Measurement

Mosaic is equipped with tools for making measurements in images; for example, if you want to measure the distance between two points in an image, you may use the measurement tool to draw a straight line between the two points and get real-time measurement data. All the measurement tools can be found in the functional zone of measurement. Click "", and then right click to invoke the measurement menu. Mosaic provides users with rich measurement tools to help image analysis.

Measurements made by the software are all executed with an image pixel as the basic unit. For example, line length is determined by the number of pixels along the line. Pixel level measurement uses measurement calibration to convert the dimensions into a more meaningful unit, for example mm or inches.

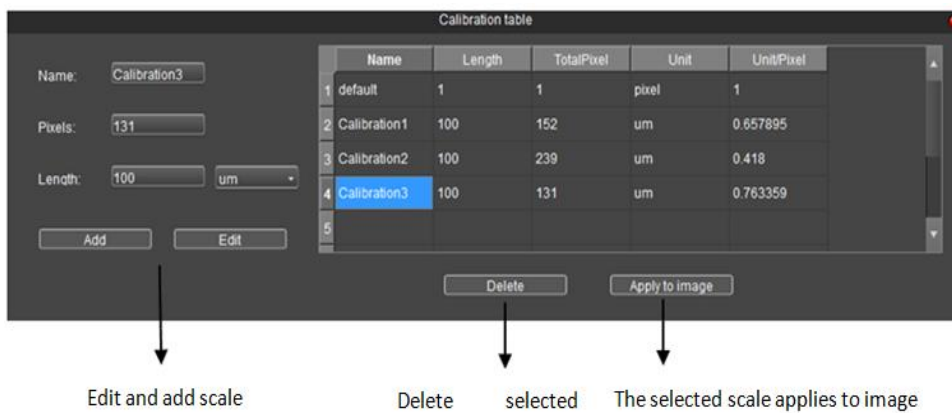## 4.1 Calibration

[Calibration] Create calibration table: Convert pixels into meaningful measurement unit. Click [Cailbration] to draw a line on the image and mark the length represented by the number of pixels of the line. Then mark the unit ruler of

all measurement items.

[Calibration Table] View or edit calibration scales: Right-click the preview screen, bring up the measurement menu bar, and click **[Calibration Table]** to view or edit the calibration ruler.

1. For example, click "Calibration 3" and click **[Apply to image]** to switch the calibration scale currently in use.

2. For example, click "Calibration 3" and select **[Delete]** below to delete the selected calibration scale, but the calibration scale in use cannot be deleted.

3. After the calibration scale is selected, the corresponding information can be modified on the left side, click **[Edit]** to save the modification to the current calibration scale, click **[Add]** to add a new calibration scale.

Note：

The calibration can be done more accurately with the aid of a micrometer. Choosing the wrong correction chart will result in the measurement result being completely wrong. When the image is measured, the correct correction table should be selected.

If a micrometer is available, the calibration can be performed more accurately with the aid of the micrometer, and the operation is as follows.

1) Place the micrometer on the microscope stage and adjust the microscope to the clearest state of the micrometer.

2) Click the measurement function ![icon], and then right-click the mouse to bring up the measurement menu.

3) Click **[Calibration]** to draw a straight line in the preview screen.

4) Enter the name of the calibration scale, the corresponding length of the line and set the correct units.

5) Click **[Apply]** to save the settings to the calibration table, and use this calibration scale to the current measurement.

Notes:

1. Under different magnification and resolution, the calibration operation should be performed separately to create the corresponding calibration scale.

2. When setting the length, selecting a longer scale length can get more accurate results. For example, selecting 10 small scale lengths will give more accuracy than selecting only 1 small scale length.

3. If you need to create multiple calibration scales, it is recommended to name them with objective + magnification + resolution. This nomenclature will avoid using the wrong calibration scale for the measurement.

4.2 Measurement Tools

Select: Select an object; select a measurement object on the image to edit its property. Double click the measurement data property, the user can set the name and item of measurement.

1) Long press left button, to move the image selected currently to other positions on the image.

2) Double click the graphics property to modify the type property of the graphics.

3) Click measurement data frame, set the name measurement content of the graphics drawn.
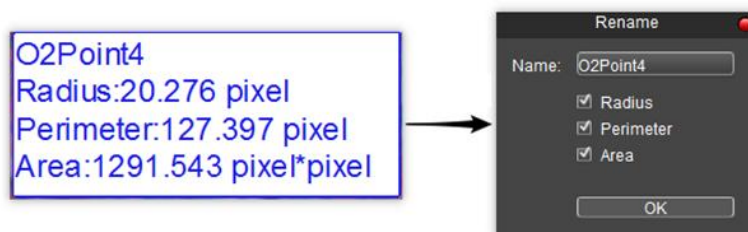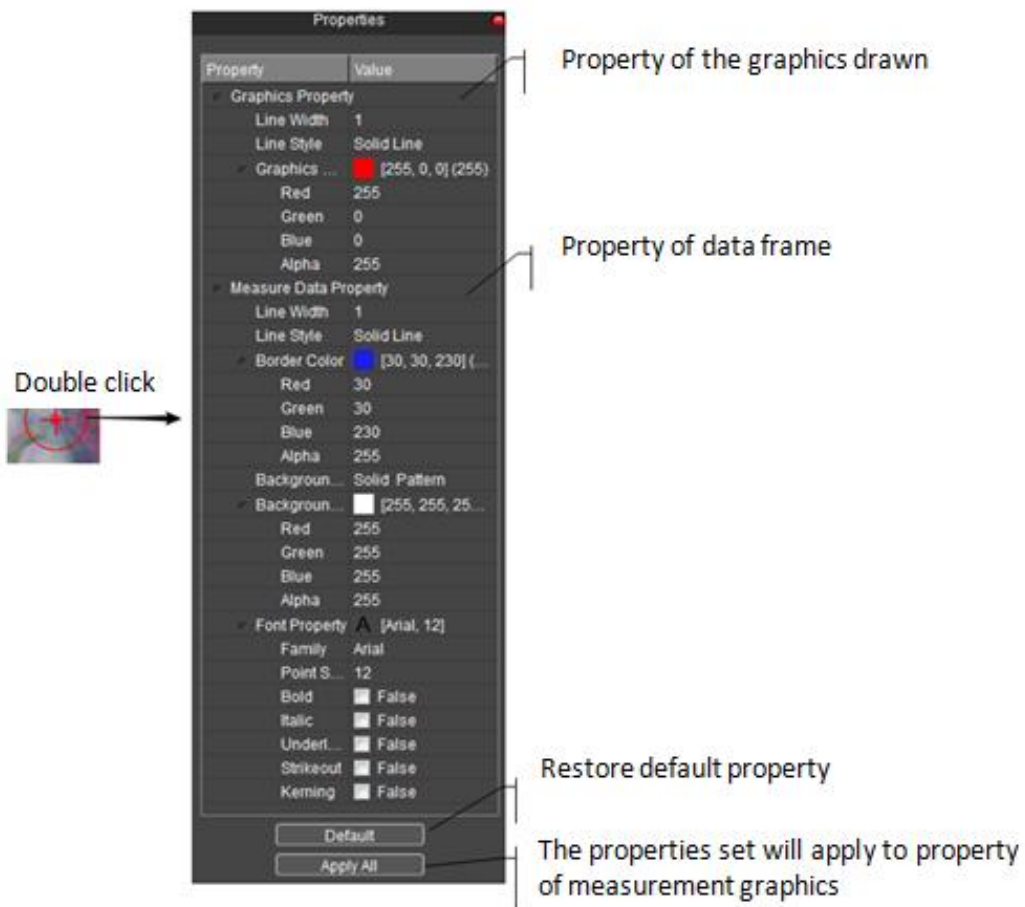
Precision: Data precision; this function is used to set data precision; 0 to 7 decimal places is allowed.

Dot: Dot count. Make manual statistic.

Line: Draw a line between points. A line will be finished at the second click.

Parallel: Draw a parallel line; draw a line first, then click again to draw another line that is parallel to the first one. Double click to finish the drawing.

Perpendicular: Draw a perpendicular line; draw a line first, then click again to draw

another line that is perpendicular to the first line; double click to finish the drawing.

Polyline: Draw a polygonal line; left click to click a new line to already existing polygonal line; double click to finish the drawing.

Rectangle: Left click to select two endpoints to draw a rectangle; it's available to measure length, width, perimeter and area.

Polygon: Draw a polygon; left click for n times to draw a n-side structure; double click to finish drawing.

O2Point: Draw circle with radius; left click to select the center of the circle; left click again to select the radius distance and finish drawing.

O3Point: Draw a circle with 3 points; left click for three times to select three points   of a circumference; click for the third time to finish drawing.

Diameter: Draw a circle with diameter; left click twice to select two endpoints of the diameter; finish drawing at the second left click.

Concentric: Concentric circle; draw a circle with radius; choose the center of a circle with the first left click and double click to finish drawing.

Arc: Draw an arc; take 3 points to draw an arc; finish drawing at the third left click.

Angle: Draw an angle, formed with two connected lines. Click to draw endpoints and finish drawing at the third left click.

DisjointAngle: Draw an angle, formed with two disconnected lines; click to draw endpoints and finish drawing at the fourth left click.

Annotate: Give comments in the graphics.

4.3 Measurement Data

Measurement Data: View measurement data of measurement graphics drawn; measurement data can be exported to Txt or Excel.

| Number		Name	Length_pixel	Width_pixel	Height_pixel	Perimeter_pixel	Area_pixelSq	Radius_pixel	Angle_°	Slope
1	1	O2Point1				2125.304	359445.041	338.253		
2	2	O2Point2				37.291	110.662	5.935		
3	3	O2Point3				1700.427	230094.624	270.632		
4	4	Line2	398.358						167.336	-0.225
5	5	Parallel1_1	75.178							
6		Parallel1_2	130.717							
7		Parallel1_3	187.791							
8	6	Polygon1				344.306	6497.333			
9	7	Rectangle1		242.667	74.000	633.333	17957.333			
10										
11										

Export data to TXT   Export data to Excel   Refresh data   Copy to clipboard

Delete: Select to delete a drawn measurement graph.

UnDelete: Undo the immediately previous Delete operation, but only one time.

Combined Measurement: With measurement photographing function for currently drawn plot and fluorescence images. Photograph will be saved into image during photographing (Enable the function; the saved picture is of 8bit bit depth).

Layer: Create multiple layers; make different measurements on different layers. This function helps to meet the measurement demands and image processing demands of different applications.
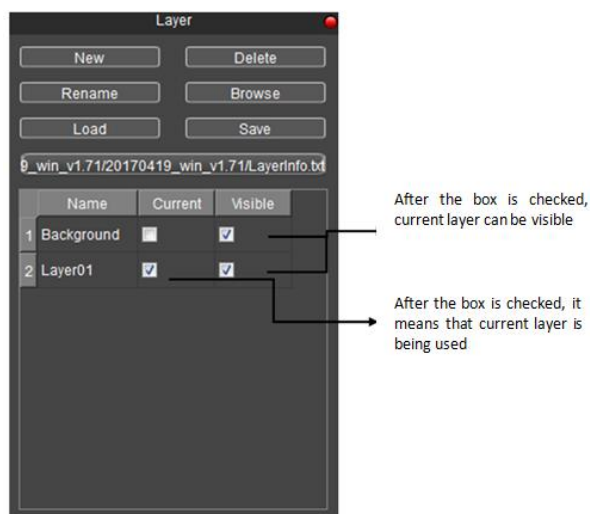
The Layer function will create "Background" and "Layer01" and existing measurements will be displayed under "Layer01" automatically.

The user may choose to display the measurement data of any layer and previewing any layer. By checking the box of a layer under **[Current]**, the user can preview the layer. By changing current layer to switch the layer displayed by the software. Check the box of a layer under **[Visible]**, the corresponding measurement information of the layer canbe displayed in current layer.

Click **[New]** to create a new layer; the user may customize the name a new layer; default layer names are "Layer01", "Layer 02", "Layer 03"...
Click **[Delete]** to delete the selected layer.
Click **[Rename]** to rename a selected layer.
Click **[Browse]** to select the path for saving the text file and file name; click **[Save]** to save layer information into the text file. The default setting is to save layer information under the root directory and name it as "LayerInfo.txt". Click **[Browse]** to find a saved image. Click **[Load]** to load corresponding layer information to the current image.

Ruler: Display/cancel cross ruler on the image. The specific unit length of ruler shall be determined by the selected calibration table. Double click any position of the ruler displayed on image to open a dialog box of ruler setting and set ruler color and background. Default settings are transparent background and black ruler.
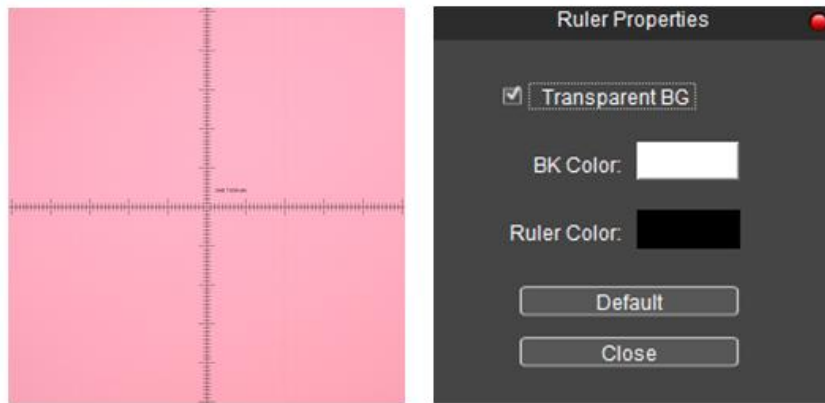
Scale: Display/cancel current scale. By selecting it with the mouse, the users may move the scale to any position on the image; double click to modify scale type and property. If users want to save the current applied scale into the captured images, locate in the Measurement menu and click Combined Measurement to save the current scale in images.

Clear All: Delete all the information of measurement graphics of current layer.