Image acquisition, managing and processing software

ISCapture

Instruction Manual

- Key to the Instruction Manual
 - IS is shortened name used for ISCapture
 - Square brackets are used to indicate items such as menu
 names, button names and window names that appear on the computer screen.
 - >> indicates the selection procedure from the menu. Example:[Capture] >> [Main Control] >> [FlatFielding]
 - * : Marks tips for using the software.
 - ! Marks information that should be read before use.

Help

- Refer to [Help] >> [About ISCapture] menu for software information and technical support.
- Provide the following information when it is required to obtain the technical support:
 - Camera model and S/N (serial number);
 - 2 Software version number;
- 3 Description of the problem. Screenshots of the problem would be useful.

MAIN FEATURES OF IS	3
SYSTEM REQUIREMENTS	3
CHAPTER1: GETTING STARTED	4
WHAT YOU NEED	4
STARTING ISCAPTURE	5
CHAPTER2: IMAGE ACQUISITION	8
Basic Control	9
TAKING STILL IMAGES AND VIDEOS	11
Capturing and Saving Individual Images	12
Capturing and Saving a set of Images	13
Video recording	13
File Save Destination	15
Exposure Control	15
Auto Exposure	16
Manual Exposure	17
Gain, Frame Speed & Data Width	18
COLOR CONTROL	19
Flat Fielding Function	19
Fluorescence Settings	21
Black Level	21
Levels	22
Capture Mode	23
Parameter Group	24
LIVE IMAGE MEASUREMENT	24
Edit Scale Line	27
Create Calibration File	29
Calibration Table	32
Measurement List	33
Measurement	34
Annotate	35
Layer	37
LIVE IMAGE SHORTCUT	40
CHAPTER3: IMAGE MANAGEMENT	41
CHAPTER4: IMAGE PROCESSING	43
IMAGE PROCESSING	44
Extend depth of focus	
Image stitching	
Image stitching	
LABEL	
HDR IMAGE	
FLUORESCENCE	
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Main Features of IS

- Advanced camera control
- Acquisition of still images and video. Available image types: JPEG,
 BMP, TIFF and RAW.
- Fluorescence imaging settings
- Live image measurements
- Image management
- Extend depth of focus (Focus Stacking)
- Image Stitching
- Fluorescence combination function for still images
- High dynamic range (HDR) function
- Still image measurements

System requirements

os	Windows XP/ Vista/ 7/ 8 (32 & 64bit)	
СРИ	Intel processor (Core2 Duo or higher is recommended)	
Memory	2GB or More is recommended	
USB ports	USB2.0 Hi-Speed port	

Chapter1: Getting Started

This chapter explains preparatory steps and basic ISCapture (hereinafter, "IS") operations.

What You Need

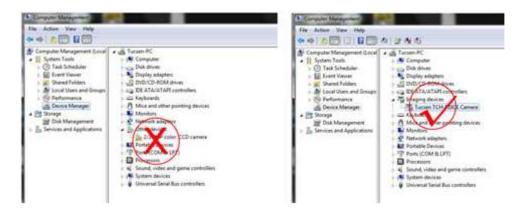
To use the camera, you need to install the camera driver and ISCapture application software:

- 1. Find the ISCapture Setup.exe file from the CD, double-click on it to start the installation and hit "Next".
- 2. The installer package selects the "C:\Program Files\" as the default file destination.
- 3. After the installation is finished, the IS shortcut will be created on the desktop.

Driver installation:

- 1. Double-click on the driver "Camera Driver Setup.exe" to start the installation.
- 2. Follow the steps to finish the installation.
- 4. After the installation is finished, please go to the Device Manager to check if the driver was installed properly. When the driver is installed correctly, there is no yellow mark with the camera under the Imaging

Device in Device Manager. Please see the picture below:

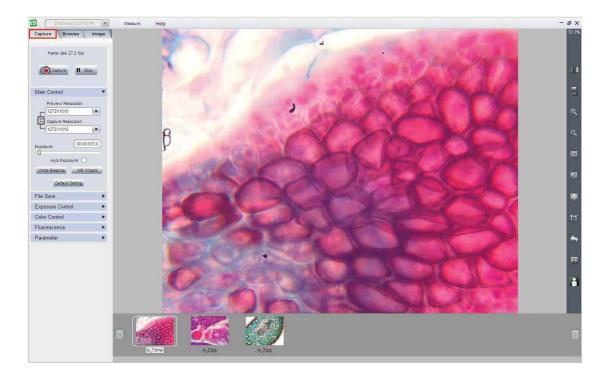


Starting ISCapture

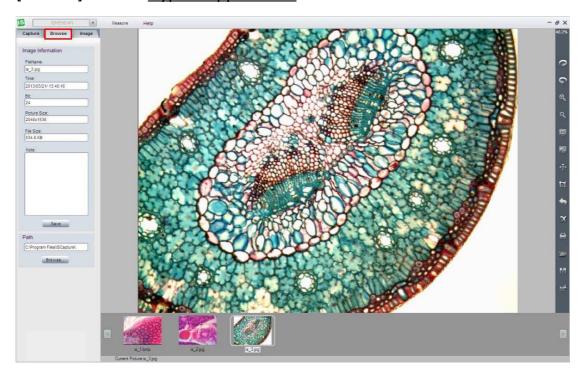
After installation, a software shortcut will be created on the desktop. Double-click on it to start ISCapture.

When IS starts up, a live image window appears. Parameters to get proper images can be set up, save still pictures or videos. The [Capture tab] window provides image acquisition settings. [Browse tab] window allows management of all the images. The [Image tab] window provides advanced image processing functions.

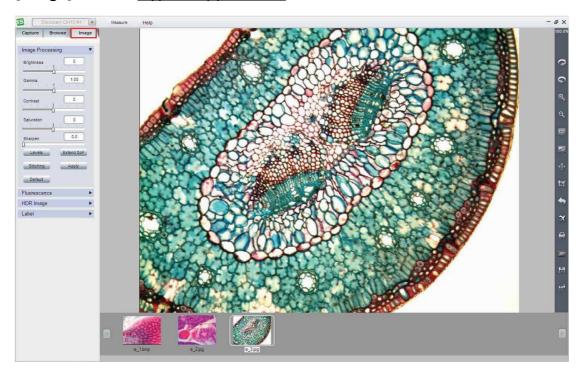
[Capture] window-typical appearance



[Browse] window-typical appearance

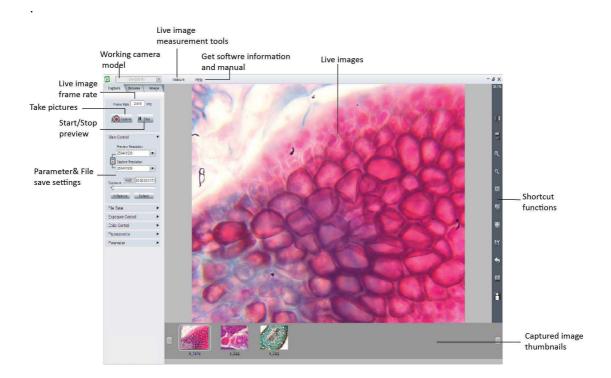


[Image] window-typical appearance



Chapter2: Image Acquisition

Adjust camera parameter settings to get proper live image; live image measurement and save still pictures and videos.



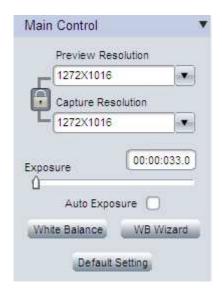
Start IS with the camera attached to a PC, the live image begins automatically. If IS is run first before the camera is connected, click to start the preview.

IS supports multiple camera functions (Only for H series or later).

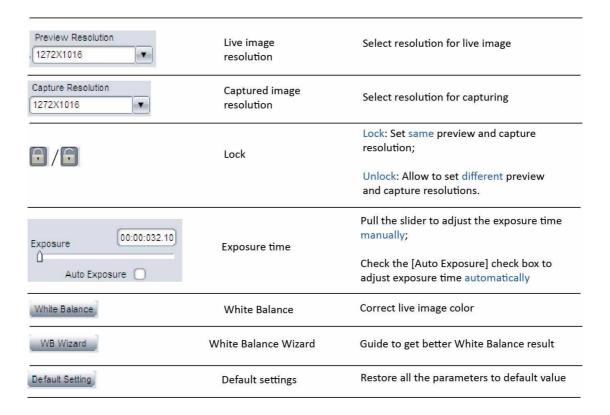
Stopping the current preview enables the selection of cameras connected to the system via the dropdown menu.



Basic Control



Provide basic camera settings:

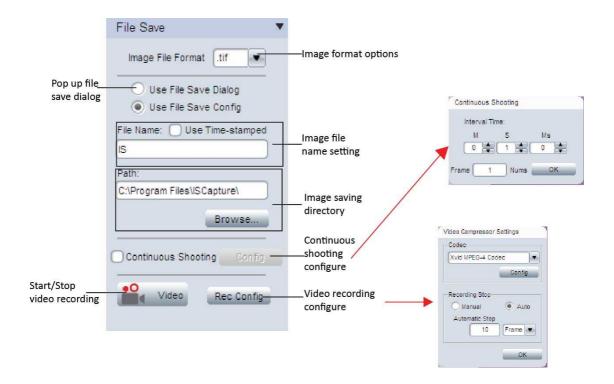


After setting the brightness live images, it is recommended to apply White Balance to correct the live image color. To get better white balance effects, please follow the following steps:

- 1. Move the sample to the blank area;
- Unselect [Color Enhancement] (It is unselected by default in [Color Control] panel);
 - 3. Push [White Balance];
 - 4. Move the sample back.

Or you can follow the [WB Wizard] to get better white balance result.

Taking Still Images and Videos



In the [File format] dropdown menu , 4 file formats are available:
 JPEG, BMP, TIFF and RAW.

Raw image file contains minimally processed data from the camera. It needs to be read in imaging software, for example, Photoshop, ImagJ etc. If the image is in the color camera raw file format, color information only can be seen after decoding the Bayer matrix

• In [Use File Save Dialog] mode

• Use File Save Dialog , a pop up file save configure window will appear when the [Capture] or [Video] button is pushed. Enter the desired file name and directory path at this pop-up window.

File save directory camera capture files - ← 🗎 💣 📰 -Save in: Desktop Libraries System Folder H Tucsen System Folder Image file name Computer Save Save as type: TIFF Files (*.tif) Cancel • Image format options

• [Use File Save Config] • Use File Save Config , allows the file save name to be pre-set, format, image quantity, capturing interval time and the file save directory. Upon executing [Capture] or [Video] , IS will save files as set.

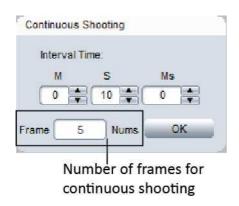
Capturing and Saving Individual Images

- Enter preferred name in the [File Name] field

 If do not key in anything, "IS" is used by default.
- Select [Use Time-stamped]
 Use Time-stamped to name the image by the capture time automatically. The time-stamp file name will be in the form of "MMDDHHmmSS". Here "MM" indicates the month; "DD" indicates the day; "HH" indicates the hour; "mm" indicates the minutes; and "SS" indicates the seconds.
- Click [Capture] to take one image with the pre-set file name.

Capturing and Saving a set of Images

- Click [Config] to set continuous capturing image numbers and the interval time.

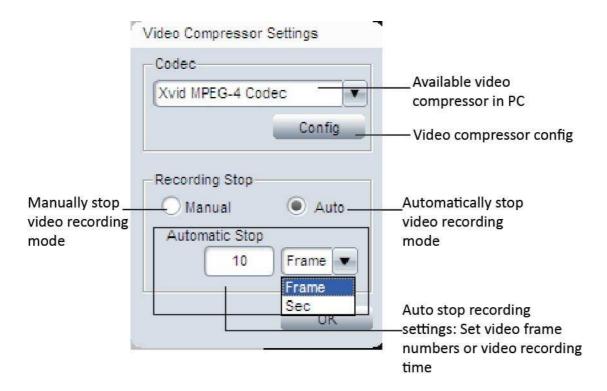


 This set of image file names follows the same format as set for individual image capturing. If [File Name] is used, image names will be in the form "X", "X-1", "X-2" (where X is the character/s you entered or "IS" by default).

Video recording

Click [Video] (Stop video recording.

Click [Rec Config] to get video recording configure window.



It provides [Manual] and [Auto] modes to stop the recording.

- In [Manual] mode, you need to click on [Video] button to start and stop the recording.
- In [Auto] mode, pre-set the number of frames or the time for videos and click on [Video], IS will stop the recording automatically after pre-set number of frames are saved or pre-set time is up.
- [Rec Config]>>[Codec] will also list all the available video compressors in PC.

The video taken without compression will be very large size. IS will automatically search the installed video compressors on the PC.

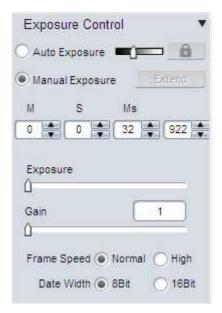
File Save Destination



Click [Browse...] to change file save destination. The default path is the software installation folder. Usually it is "C:\Program Files\ISCapture\".

This default destination might NOT be allowed storing files if the user is not logged in as Administrator in Windows Vista, 7 or later. Image may not save if the default path is used. We recommend either to change the file saving path or release this folder "Write" authority for other user accounts.

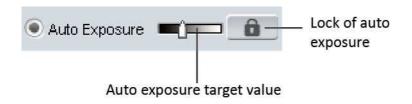
Exposure Control



Change the Exposure time and Gain to adjust the image brightness.

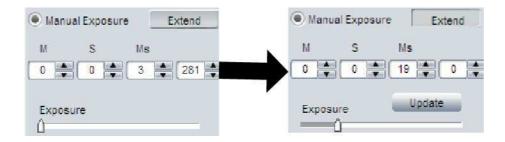
Select frame speed to get different live image frame rate. Set 8-bit or 16-bit data width for captured images.

Auto Exposure



- Check [Auto Exposure] checkbox, software start to adjust the exposure time automatically to get proper brightness of live images.
- Auto exposure target value: Set the reference exposure time for auto exposure adjustment. It helps the auto exposure to find the proper exposure time faster. For example, if the imaging target is quite bright, a lower target value is set it will indicate a shorter exposure time to the software.
- Lock: will stop the auto exposure calculation. While auto exposure is working, it will keep on calculating the image brightness to get proper exposure time. During this, if you already see a good live image for you, you can push to lock it.

Manual Exposure



Adjust the exposure time manually. Two ways to change the exposure time:

- Key in the exposure time in the edit box directly 20 , then click
 to confirm it.
- Pull the slide bar to change the exposure time.

[Extend] Extend is used to get longer exposure time. This function is ONLY available for CCD cameras. For other cameras especially the CMOS camera, the maximum exposure time is shorter than 1 second, then [Extend] will be gray out.

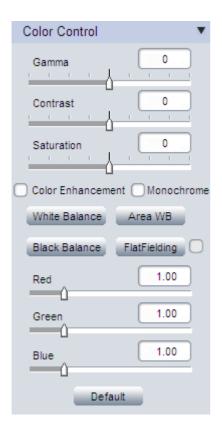
[Update] Update appears after [Extend] is selected. Click on to stop the previous exposure time and restart the new one immediately. For long exposure applications, we strongly recommend that [Update] is used to start a new setting. This will help to get the new exposed image earlier. If the exposure time is less than 2-3 seconds, it is

not necessary to use it.

Gain, Frame Speed & Data Width

Gain		Increase the power of the image data. Higher gain gives brighter images, but also makes the noise signal more obvious.
Frame Speed	High Speed	Corresponding to high pixel clock. Gives faster frame rate.
	Normal Speed	Offer lower frame rate than High Speed, but gives longer maximum exposure time.
Data Width	8-bit	8-bit images use 2^8 = 256 gray levels to represent image details.
	16-bit	16-bit images use 2^16 gray levels to represent image details. ONLY available for CCD & Discovery series cameras in .Tiff and .Raw formats.

Color Control



Is used for adjusting image color, gamma, contrast and saturations.

Flat Fielding Function

Flat fielding function is used to correct the uneven background brightness.

- Click on [FlatFielding] FlatFielding oto start the flat fielding parameter
 calculation and apply it to the live images.
- When the check box FlatFielding is unchecked, the calculated flat fielding parameter is NOT applied to the live images.



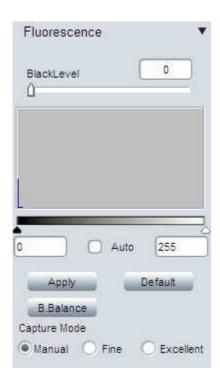
To get better flat fielding result, Move the sample to a blank area

first, apply the flat fielding, then move back the sample.

When the lighting is changed, re-do the [FlatFielding] to correct the uneven brightness or when using a different objective.

Gamma is used to obtain correct reproduction of intensity. Default value (Gamma = 0) is recommended in most of cases.	
Contrast is the difference between the brightness brights and the darkest darks in an image. Higher contrast will make the shadows become darker and the highlights brighter. High contrast will lost more image details. Default value (Contrast = 0) is recommended.	
Adjust image saturation. Saturation is the intensity of color in the image.	
Used to make the image color more vivid. Before doing White Balance, it recommends to uncheck this function, then apply WB	
Check the checkbox to get a grayscale image	
White balance. Give reference to true white for the cameras. Correct image color	
Manually select the white color area in the image as the white balance reference	
Black Balance. Correct black color. Usually use in fluorescence application.	
Correct image uneven brightness. Uncheck the check box: cancle background brightness correction.	
Adjust the intensity of red in the image. [Red] = 1 means the original intensity of red in the image.	
Adjust the intensity of green in the image. [Green] = 1 means the original intensity of green in the image.	
Adjust the intensity of Blue in the image. [Blue] = 1 means the original intensity of blu in the image.	
Restore the parameter settings to the initial value and apply white balance.	

Fluorescence Settings



Included in our software are useful parameter settings for fluorescence or low light imaging. It helps to get better images easier and faster.

Black Level

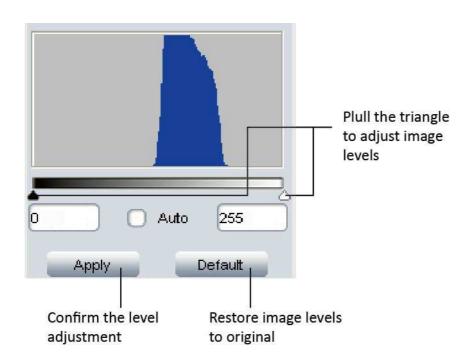


Black level function defines the brightness level at the darkest part of the image. In low light imaging, it can help to see more details in the dark area.

In low light application, it usually needs quite a long exposure time to get proper images. If you set a long exposure time at the beginning,

you might need quite a long time to find your target and get a proper image (wait for finishing a long exposure to get a new frame image, adjust, wait...). When searching for the imaging target at the beginning, we recommend to set a shorter exposure time, but make larger Gain and Black level first. After you find the target, then reduce the Gain and Black level, and increase the exposure time. This will aid in a better image acquisition.

Levels



Live image histogram.

To adjust the live image levels automatically: select [Auto] check box.

Adjust image levels manually:

Pull the little triangles to adjust the live image levels. Move the white
 triangle towards left, it is able to reveal some information in dark

area. If move the black triangle towards right, it will reveal bright area information.

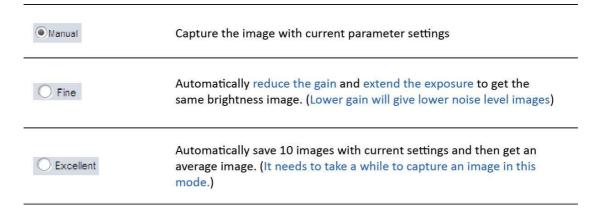
You can also key in the image levels directly

After adjusting the levels, click Apply to confirm the setting. If you need to go back to the original image, click Default to restore the image.

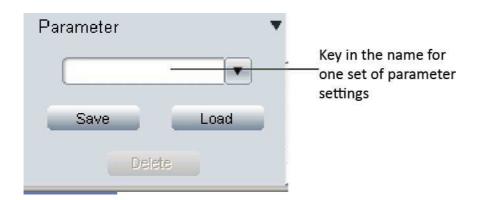
Capture Mode



Three capture modes are specially developed for fluorescence imaging.



Parameter Group

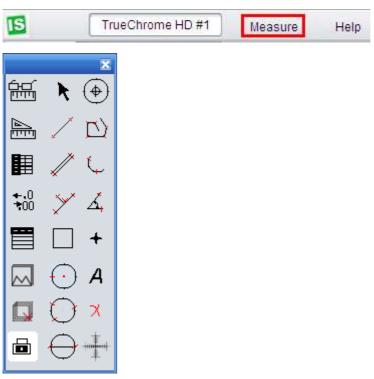


Save parameter sets for different applications. The saved parameters include exposure time, gain, frame speed, data width, gamma, contrast, saturation, color enhancement status, monochrome, RGB gain and black level. It allows users to save 20 set parameters (available in Ver 3.6 or later).

- Save parameter function: Enter a name for current parameter settings, click
 Save to save it.
- Load parameter function: Click to open drop-down menu, click on preferred parameter name and then push to make selected parameters take effect on the live image.

Live Image Measurement

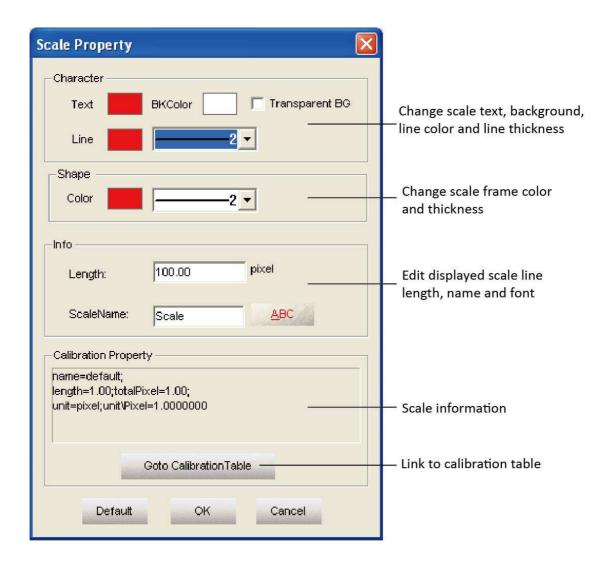
Click on [Measure] at the top of the IS to get the measurement tools



	Show Scale Line	On/off the scale line on the picture
<u>птп</u>	Calibrate	Create Calibration file
	Calibrate Table	Available calibration file list. Allow to add, edit and delete calibration file.
+. 0 † 00	Decimal	Set measurement precision. Allowed decimal range is from 0 to 7
	Measurement List	List all the measurement data
	Layer	Create multiple layers to apply measurements and save layer information
	Delete All	Delete all the measurements and layers
	UnLock/Lock	Unlock/lock the measurement operation. Allow to do same measurement continually when LOCKED. It is locked by DEFAULT.
K	Select	Select to change measurement or the measurement data position
/	Line	Measure the length
1	Parallel	Measure the distance of parallel. Allow to do multiple parallels' distance measurement. Double clicking to end parallel measurement.

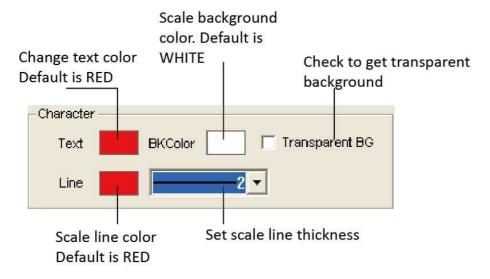
Y	Perpendicular	Measure the perpendicular length. Allow to do multiple perpendiculars' length measurement. Double clicking to end perpendicular measurement.
	Rectangle	Measure rectangle height, width, area and perimeter.
\odot	2-points Circle	Use center point and point on the circle to draw a circle. Give the radius, area and perimeter of circle
\bigcirc	3-points Circle	Use 3 points on the circle to draw a circle. Give the radius, area and perimeter of circle
\ominus	Diameter Circle	Draw a circle according to the diameter. Give the radius, area and perimeter of circle
•	Concentric Circle	Use center point and radius to draw concentric circles. Give concentric circles' radius, area and perimeter. Allow to do multiple concentric circles measurement. Double clicking to end concentric circles measurement
口	Polygon	Measure polygon area and perimeter.
L	Arc	Measure a curve angle, radius and length.
4	Angle	Measure the angle
+	Point	Counter. Count the quantity.
A	Annotate	Add remarks on the images.
Х	Delete	Delete previous measurement. Select it then click on the measurement to delete the measurement.
+	Cross-ruler	On or off cross-ruler on the images. The unit of the ruler depends on the applied calibration file.

Edit Scale Line

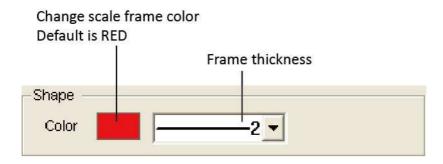


Double click on the scale to get its properties and make changes to it.

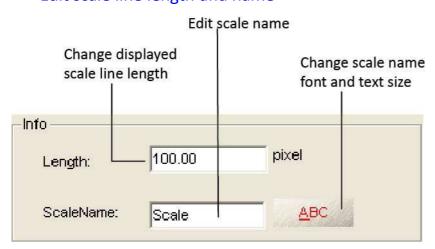
Edit scale character



• Edit the frame of the scale



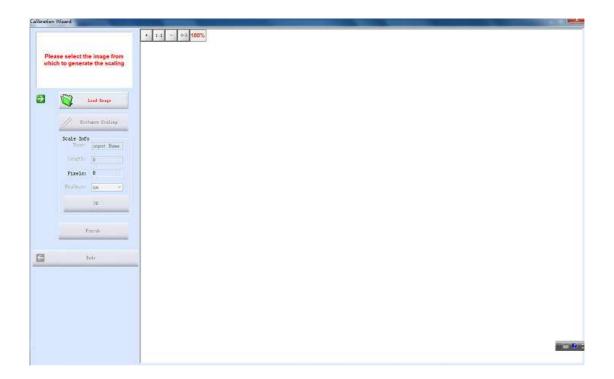
• Edit scale line length and name



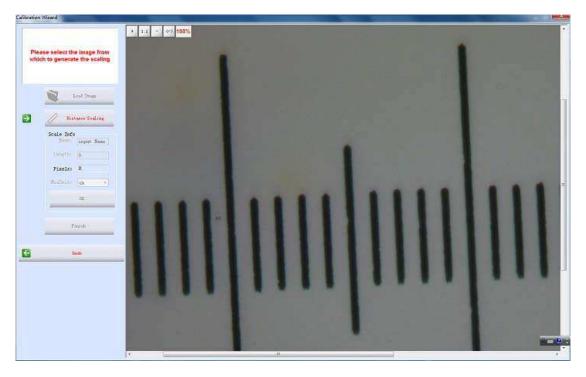
Create Calibration File

To measure the samples real size, the corresponding calibration file needs to be created first.

- 1. Take pictures of the calibration slide in all the required working objectives and resolution (if a reducing lens is also used in your application, it also requires you to take the calibration slide picture with the reduce lens attached).
- If ONLY ONE objective and ONE resolution is used in the application, one calibration slide picture is enough. The calibration slide picture MUST be taken with exactly the same lens or microscope settings as the target image taken.
- 2. Click to start to create calibration file.

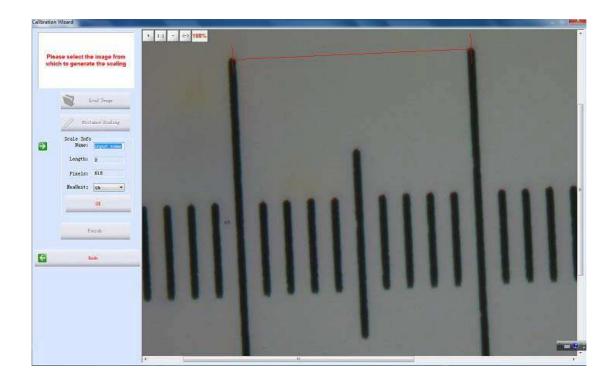


3. Click [Load Image] to load the calibration slide picture taken in Step1.



4. Click [Distance scaling] and move the cursor to the slide image, draw a line to get the reference length.

Using longer length as the reference length will give more accurate measurement results. For example, using 10 scale units as reference length will give more accurate result than using 1 scale unit.

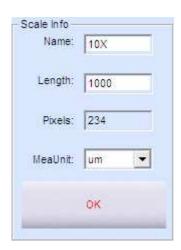


5. Enter the name for the calibration file and the length of the line you draw.

If you need more than one calibration file, using objective+reducing lens(if it is used)+resolution as the name of the calibration file is recommended. This can help to prevent using the wrong file to do the calibration.

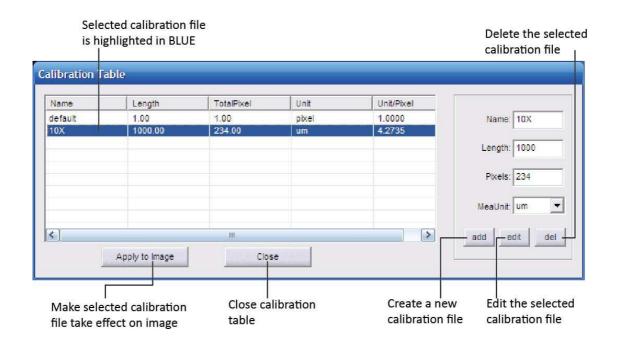
When keying in the length, please pay more attention to the calibration scale unit and the Measure Unit used here. For example, the calibration scale unit is 0.1mm; the Measure Unit is selected as µm; and

the reference length is 10 scale units, so the length should be 10 x $0.1 \text{mm} \times 1000 = 1000 \ \mu\text{m}.$



6. Click [OK] to confirm the calibration. The new calibration file named "10X" is created in the [Calibrate Table].

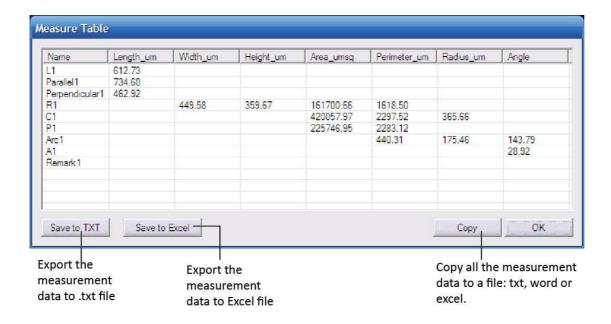
Calibration Table



- Click [Calibrate Table] to open the calibration table.
- Select the correct calibration file for current image measurement.

Using the WRONG calibration file will make the measurement result innacurate. Please make sure the calibration file is correctly corresponding to the current image. Hence, it is useful to name the calibration file with the capturing settings or objective name.

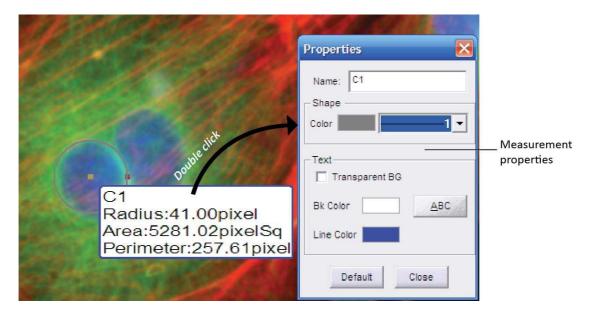
Measurement List



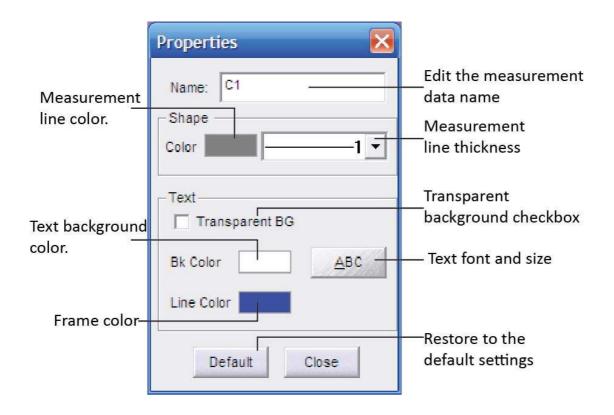
All the measurement data is listed in the [Measurement List]. The software allows you to export all the measurement data to TXT or Excel file.

Measurement

IS allows you to do line, parallel, perpendicular, rectangle, circle, polygon, arc and angle measurement. The [Count] function allows you to manually count the objects. And the [Annotate] function offers to add comments on the images.

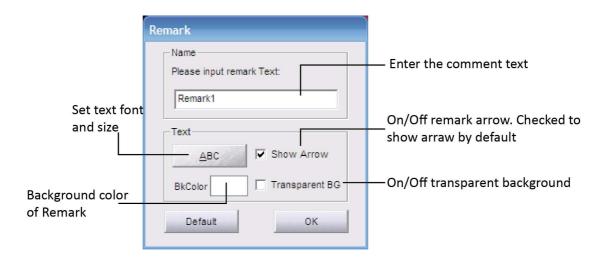


Double click on the measure data to get the measurement configure window. It allows you to change the measured data name, color, thickness, background color and the character font.



Annotate

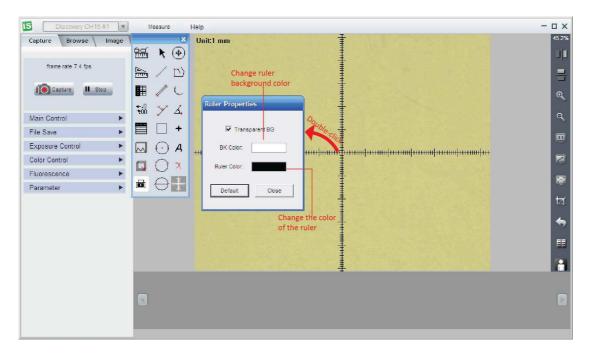
Select [Annotate] and click on the image area which you prefer to add a remark. It allows you to edit the comment, change the background color and on/off the annotate arrow.



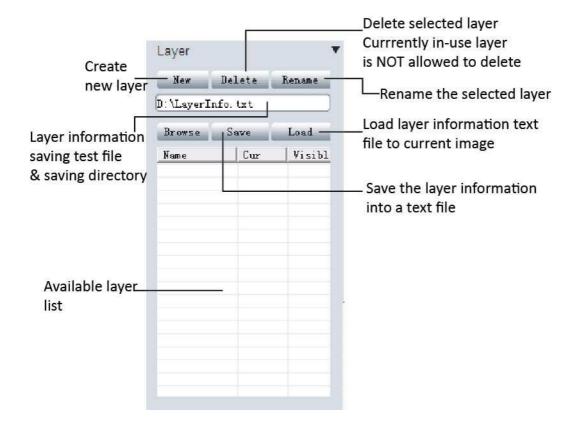
Ruler

Click on to show or cancel the cross-line on the images. The displayed ruler unit is determined by the selected calibration file.

Double-click on the ruler to get the ruler property and change the ruler color. The default color is BLACK.



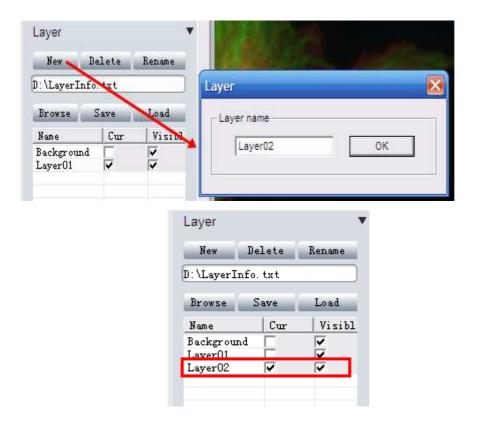
Layer



Create multiple layers for loads of measurements. The layer function makes adding a large number of measurements on the processed image review simple and easy.

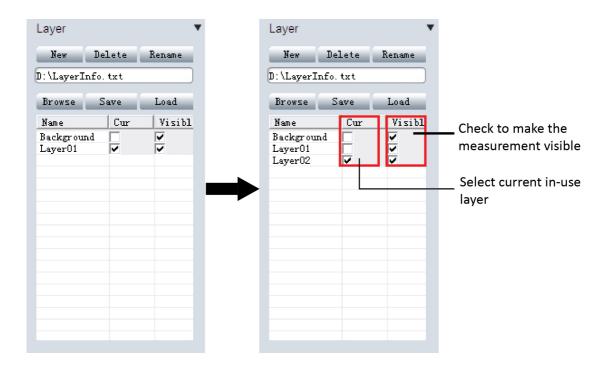
If you have already applied some measurements on the image, the [Layer] function automatically creates "Background" and "Layer01" for the current image.

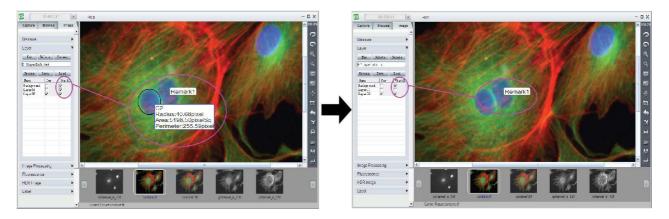
Click [New] to create a new layer. Allow to key in the preferred name for the new layer. It uses "Layer02", "Layer03"... etc as the layer name by default.



Now loads of measurements can be applied on different layers. It allows you to choose any layers to view.

Checked [Cur] means the corresponding layer is displayed currently. Select different [Cur] to switch between different layers. In the [Visible] column, the selected check box means all the measurements in the corresponding layers also display on the current layer. Uncheck the check box, and the corresponding measurement will be invisible in the current layer



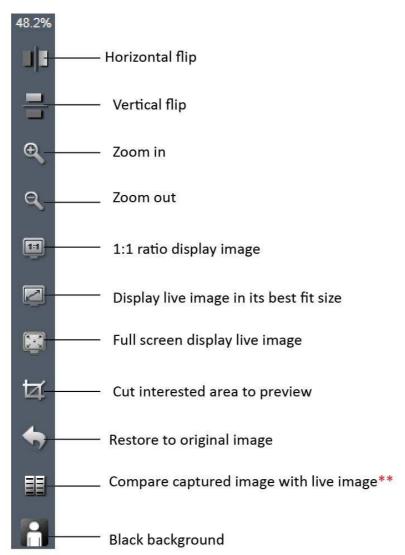


The layer information is saved in a text file.

- Click [Browse] to choose the text file saving directory and enter file name. Then click [Save] to save the current layer information in the text file. The layer information will be saved as "LayerInfo.txt" in Disk
 D by default
- Click [Browse] to find the existed layer information text file. Click
 [Load] to load the layer information to the current image.

Live image shortcut

On the right hand side of the live image window, some shortcuts are provided to process the live image quickly.



^{**} Compare function: Live image will be displayed on the left side. Click on the taken image thumbnail to select it to compare with live images (Chosen compared image will be enhanced in gray-white frame).

Chapter3: Image management

View images in [Browse] panel, it displays the image File name, capturing time, color depth (bit), picture resolution and image size. It also allows you to add comments to any individual image. When you view this image next time in the IS, it will show the image comment.

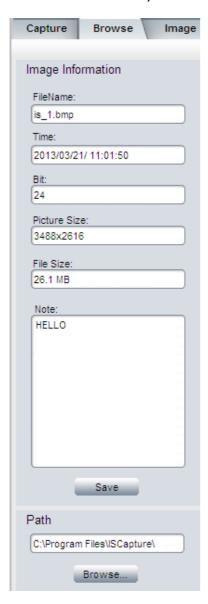
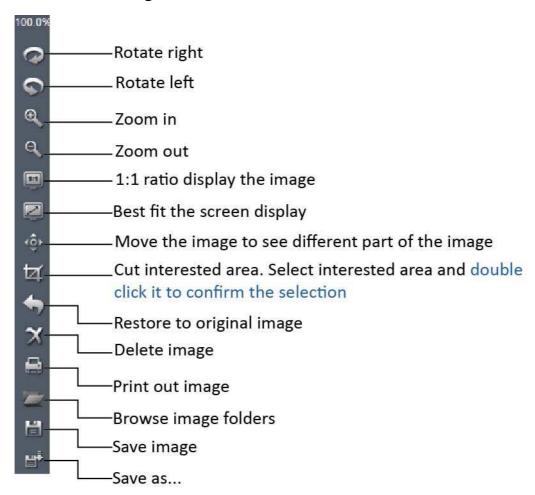


Image Management and Processing Shortcuts

IS provides some quick functions on the right hand side of the software in *Browse* or *Image* mode.



Chapter4: Image Processing



In this section, IS provides advanced image processing functions and also allows you to do the measurement on the still images.

Image Processing



Provide basic captured image processing functions and allows functions such as extended Depth of Focus and image stitching.

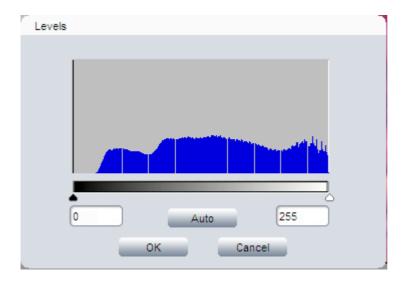
Brightness	Adjust captured image brightness. Default brightness = 0
Gamma	Adjust captured image gamma. Default gamma = 1.00
Contrast	Adjust contrast. Increase the contrast, the shadows become darker and the highlights brighter. Decrease the contrast, the highlights grow dim and the dark areas lighten up
Saturation	Adjust the color saturation. Fully-saturated colors are very bright, while low saturation are grayish.
Sharpen	Adjust the image sharpness. Sharpness is the contrast on the edges. Sharpening increases the bright and dark lines on edges.
Levels	Adjust image levels. Get more details in [Fluorescence]>>[Levels]
Extend DoF	Extend the Depth of Focus (DoF)
Stitching	Image stitching. Combine multiple images with overlapping fields of view to produce a segmented panorama or high-resolution image.
Default	Restore Brightness, Gamma, Saturation, Sharpen and levels back to the default value
Apply	Confirm to apply all the settings to the image.

After clicking [Apply], all the settings are applied to the image.

Then you can NOT revert to the original image.

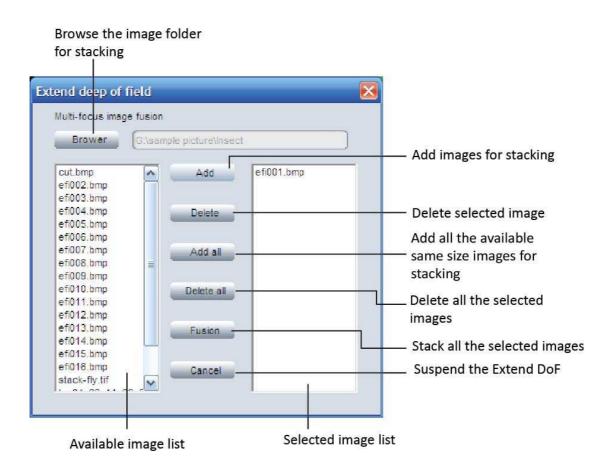
Level

Push [Levels] to get the image histogram. It allows you to adjust the image levels. The level adjustment is the same as live image level adjustment. Get more detail in [Capture]-->> [Fluorescence].



Extend depth of focus

Push [Extend DoF] Extend DoF to get below dialog. Select the corresponding images and apply the function.



- Browse the image folder which you are going to do the stacking.
- All the images in the folder will be listed on the left hand side. Click on one image, the image will be hightlighted in BLUE.
- Click [Add] to add the highlighted image to the right hand side (the selected source images for stacking).
- [Add all] button allows to add all the same size images in the left hand side to the right as stacking source images by just one click.
- Click [Fusion] to stack all the selected source images and get an image with an extended depth of field.

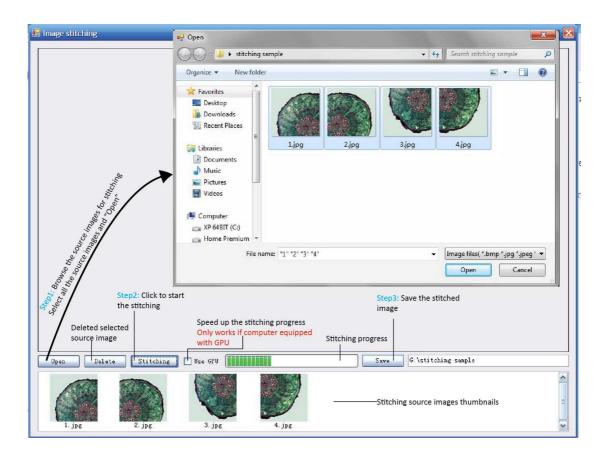
When selecting a wrong image as stacking source, just click on it and then click [Delete] to remove it. [Delete all] will remove all the selected images.

Image stitching

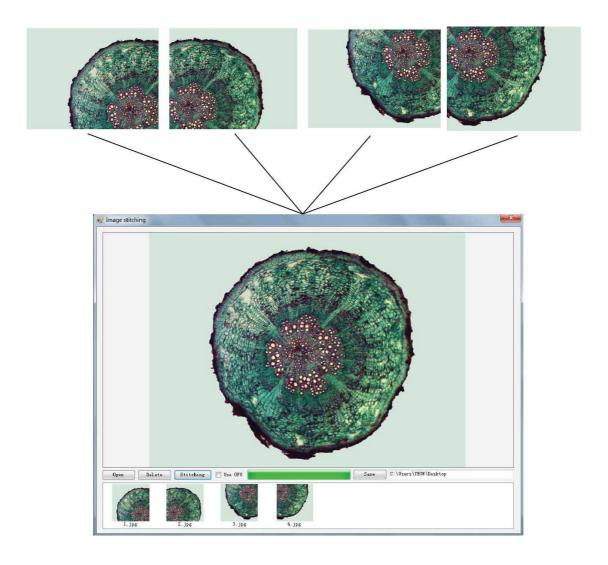
Click on Stitching to get the image stitching configuration. It combines multiple images with overlapping fields of view to produce a large panorama or high-resolution image.

1) Click [Open] browse the stitching source images. Select all the source images and open them.

- 2) Click [Stitching] to start stitching all the source images.
- 3) Click [Save] to save the stitched image in the same directory as the source images with the name of date and time stamped.
- Select [Use GPU] checkbox to speed up the stitching progress. This function only works if the computer is equipped with GPU.



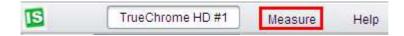
Sample images:



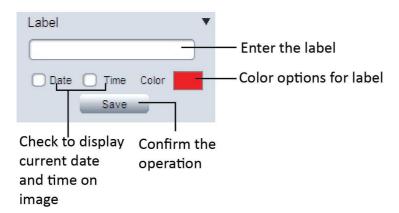
Measure

Click on [Measure] at the top of the IS to get the measurement tools.

Then select the corresponding tools to measure the still images (get more details in [Capture]>>[Live Measurement]).

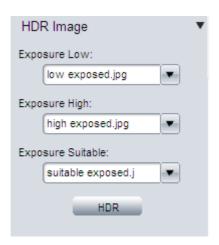


Label



- The label text will be displayed on the lower right corner of the image.
- The date and time will be displayed on the top right corner of the image.
- After clicking [Save], the image with the label will be saved as image file name+ _bak. For example, the original image file name is "IS.jpg", then this image with a label will be saved as "IS_bak.jpg". So the original image is still kept.

HDR Image

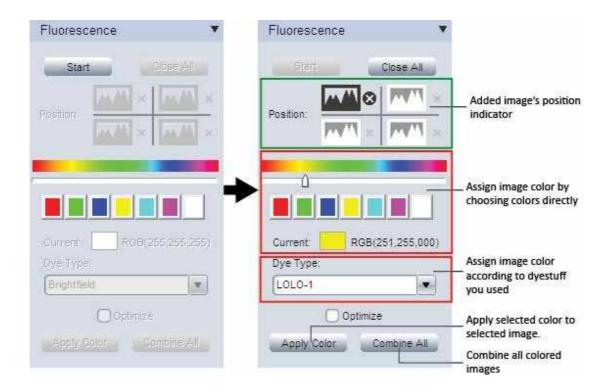


High Dynamic Range (HDR) image is used to get greater dynamic range of an image.

- Take pictures for one same scene with different exposure time and load them in the software.
- In the drop-down menu, select corresponding images for [Exposure Low], [Exposure High] and [Exposure Suitable].
- Push [HDR] button to combine different exposed images into one.
 The generated HDR image will be named as "hdr_image".

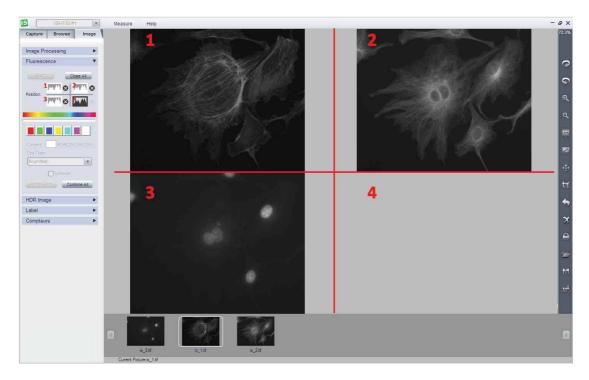
If the different exposed images are not loaded in the ISCapture yet, the shortcut on the right hand side of the ISCapture allows you to browse any image simply.

Fluorescence



This function is used to assign fluorescence images with different colors and combine them together into one image.

- **Step 1:** Open the images which are used for combination in IS, then click on [Start] to start the fluorescence combination.
- **Step 2:** Click on image thumbnails to add corresponding images. The image position indicator shows the added images' position. Maximum 4 frame images are allowed to add for fluorescence combination.

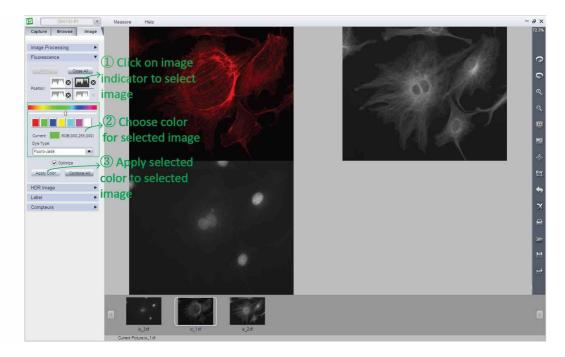


Step 3: Click on one added image indicator to start applying color for it.

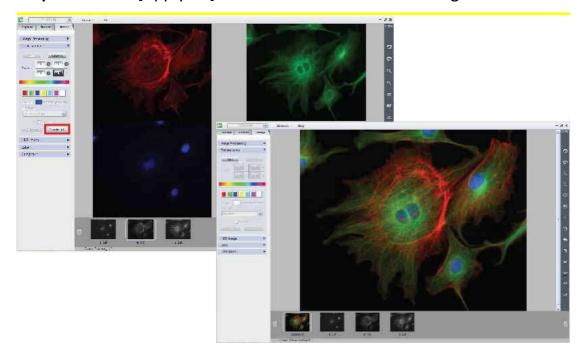
- ① Click on one image indicator to select it (The selected one will be in dark color, unselected ones will be gray white).
 - ② Assign color for selected image.

Two ways provided for color assignment:

- a. Click on the preferred color or slide to choose it.
- b. Assign the color according to the dyestuff in the drop-down menu of [Dye Type].
- ③ Click on [Apply Color] button to add selected color on the image.



Step 4: Click on [Apply All] to combine all the colored images.



Optimize checkbox is recommended to select during the combination. It will optimize image background to get a better image. But without optimization, the created image keep all the original information. No extra processing is applied to the image data.



After get the combined fluorescence image,

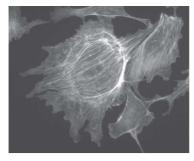


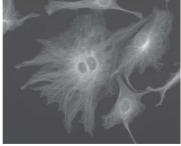
[Sharp] function in [Image Processing] can help

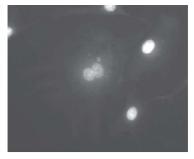
to get sharper images and see more image details.

If add wrong image or wrong color to selected image, just click on the tiny cross Solution beside each indicator to delete it. If want to cancel current combination, just click on [Close All] to cancel the combination.

Original images:



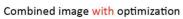


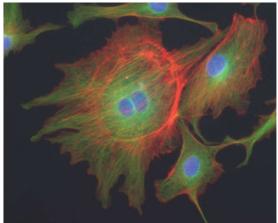


Original images

Combined image:







Combined image without optimization