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IS ISCapture

Image acquisition, managing and processing software

INSTRUCTION MANUAL

INSTRUCTION MANUAL KEYS

IS is short for ISCapture

[ ] Square brackets are used to indicate items that appear on the computer screen Such as menu, button and window names.

>> indicates the selection procedure from the menu.

Example: [Capture] >> [Main Control] >> [FlatFielding]

Indicates useful tips for using the software.

Indicates important information that should be read before use.

Indicates additional useful information.

HELP

Refer to [Help] >> [About ISCapture] menu for software information and technical support.

Have the following information ready for technical support:

1. Camera model and S/N (serial number);
2. Software version number;
3. Description of the problem. Screenshots of the problem would be useful

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MAIN FEATURES OF ISCAPTURE

- 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)
CPU	Intel processor (Core2 Duo or higher is recommended)
Memory	2GB or More is recommended
USB ports	USB2.0 Hi-Speed port

CHAPTER 1: GETTING STARTED

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 click "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 "Camera Driver Setup.exe" to start the installation.

2. Follow the steps to finish the installation.

3. 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 as reference:
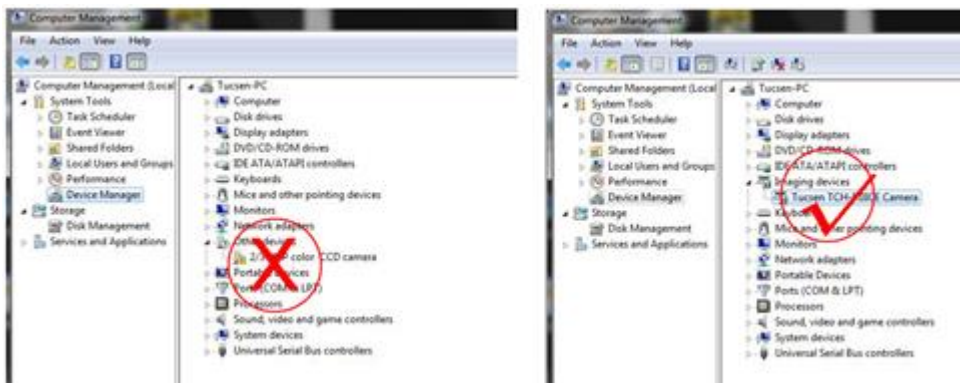
Starting ISCapture

After installation, a shortcut ![IS] will be created on the desktop. Double-click 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
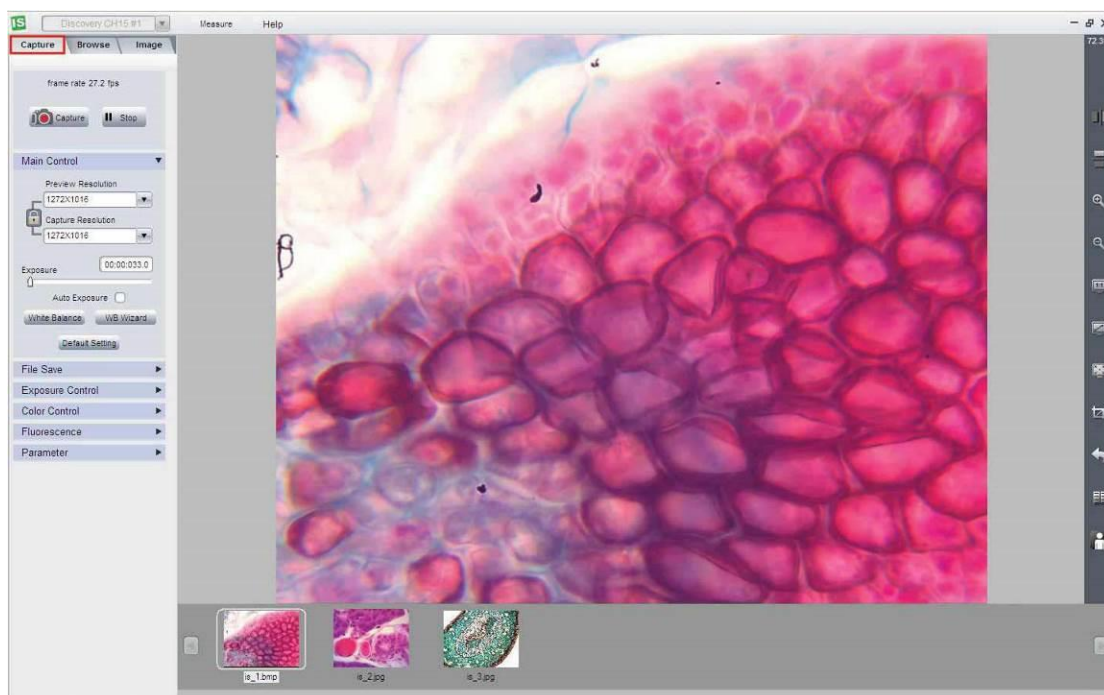
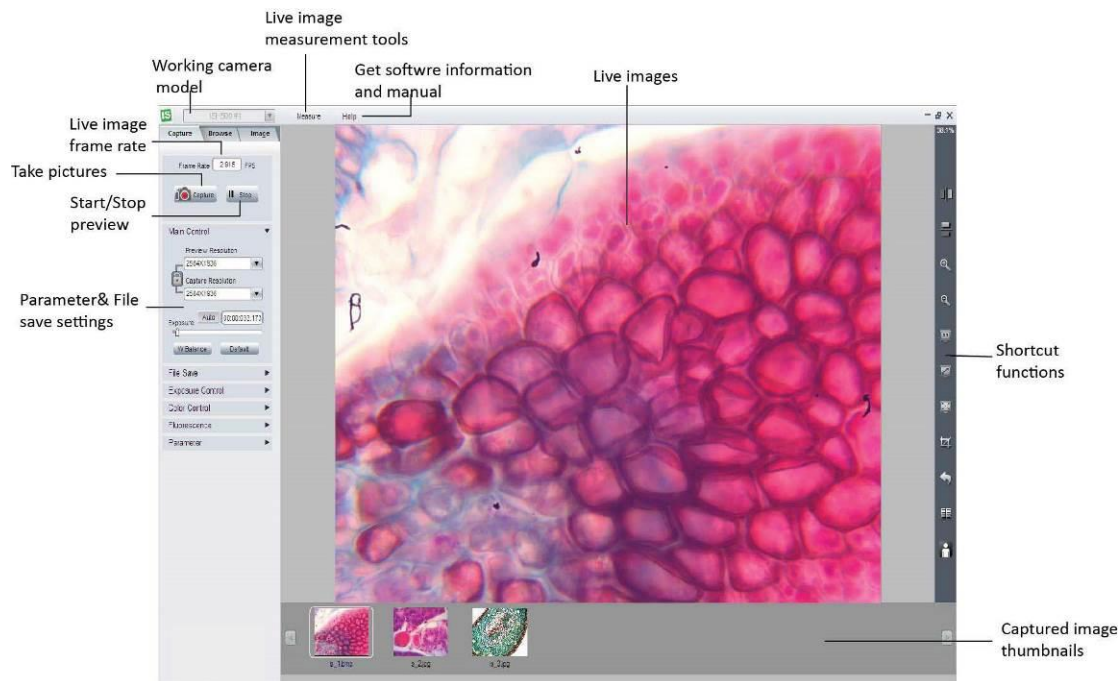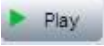## [Browse] window-typical appearance
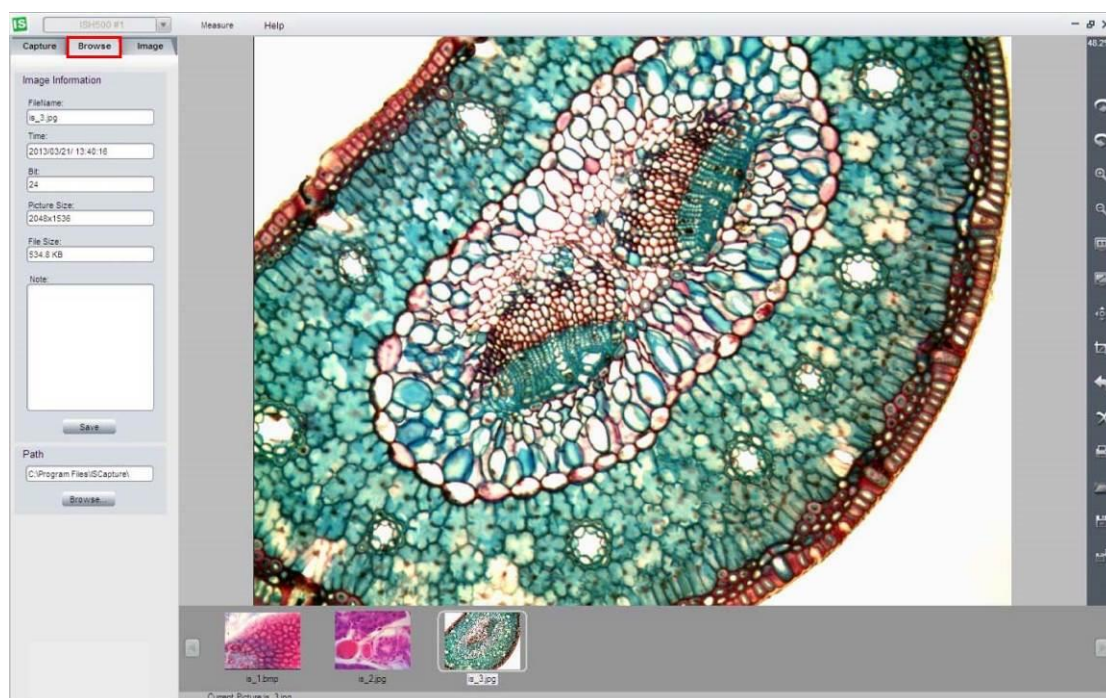
[Image] window-typical appearance

CHAPTER 2: IMAGE ACQUISITION

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

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When you start IS with the camera attached to a PC, the live image begins automatically. If you IS run before the camera is connected, click [▶ Play] to start the preview.
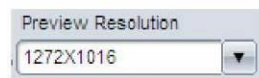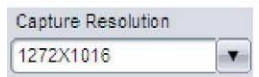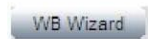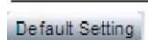
IS supports multiple cameras (Only for H series or later). Stopping the current live preview will enable you to select another camera connected to the system via the dropdown menu.

BASIC CONTROL

Provide basic camera settings:

|		
	Live image resolution	Select resolution for live image
	Captured image resolution	Select resolution for capturing
	Lock	Lock: Set same preview and capture resolution; Unlock: Allow to set different preview and capture resolutions.
	Exposure time	Pull the slider to adjust the exposure time manually; Check the [Auto Exposure] check box to adjust exposure time automatically
White Balance	White Balance	Correct live image color
WB Wizard	White Balance Wizard	Guide to get better White Balance result
Default Setting	Default settings	Restore all the parameters to default value

☀ After setting the brightness of the 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;

2. 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
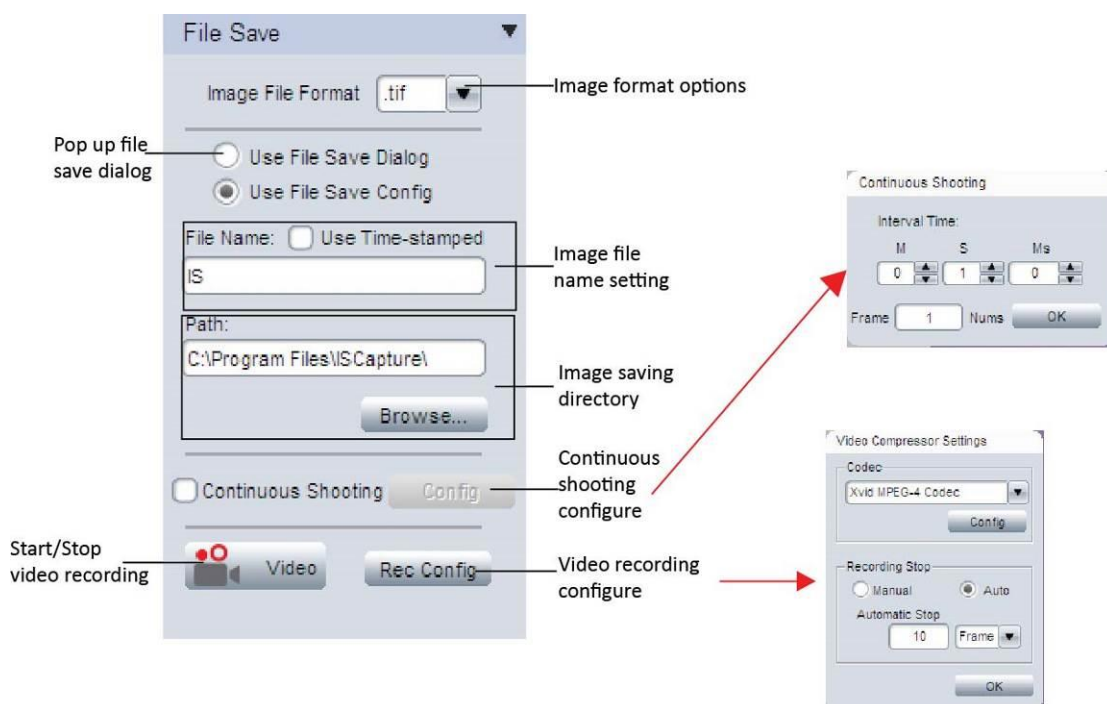
1. In the [File format] dropdown menu, 4 file formats are available: JPEG, BMP, TIFF and RAW.

> 📝 Raw image files contain minimally processed data from the camera. It needs to be read in imaging software, like Photoshop or ImagJ. If the image is in camera raw file format, color information can only be seen after decoding the Bayer matrix

2. In [Use File Save Dialog] mode ⦿ Use File Save Dialog, a pop up window will appear when the [Capture] or [Video] button is pushed. Enter the desired file name and directory path.

File save directory

Image file name

Image format options
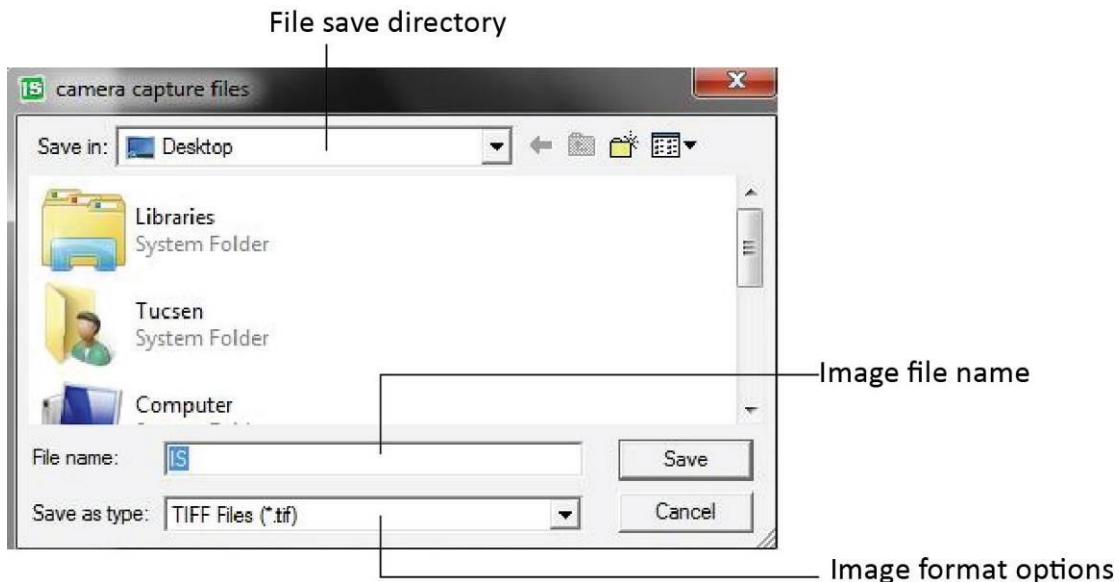
3. [Use File Save Config] ⦿ Use File Save Config, allows the file name, format, image quantity, capturing interval time and the file save directory to be pre-set. Upon executing [Capture] or [Video], IS will save files automatically according to the settings.
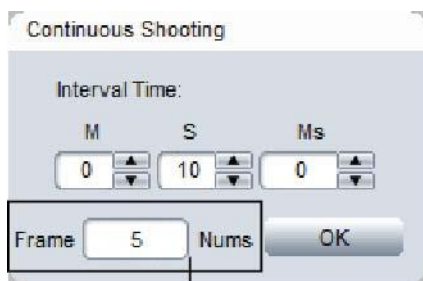
Capturing and saving individual images

1. Enter preferred name in the [File Name] field [IS   ]. If you don't type anything, "IS" is used by default.

2. Select [Use Time-stamped] ☑ Use Time-stamped to name the image using 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.

3. Click [Capture] to take one image with the pre-set file name.

Capturing and saving a set of images

1. Click the [Continuous Shooting] checkbox ☑ Continuous Shooting, the software will automatically save a set of images after a single [Capture] is executed.

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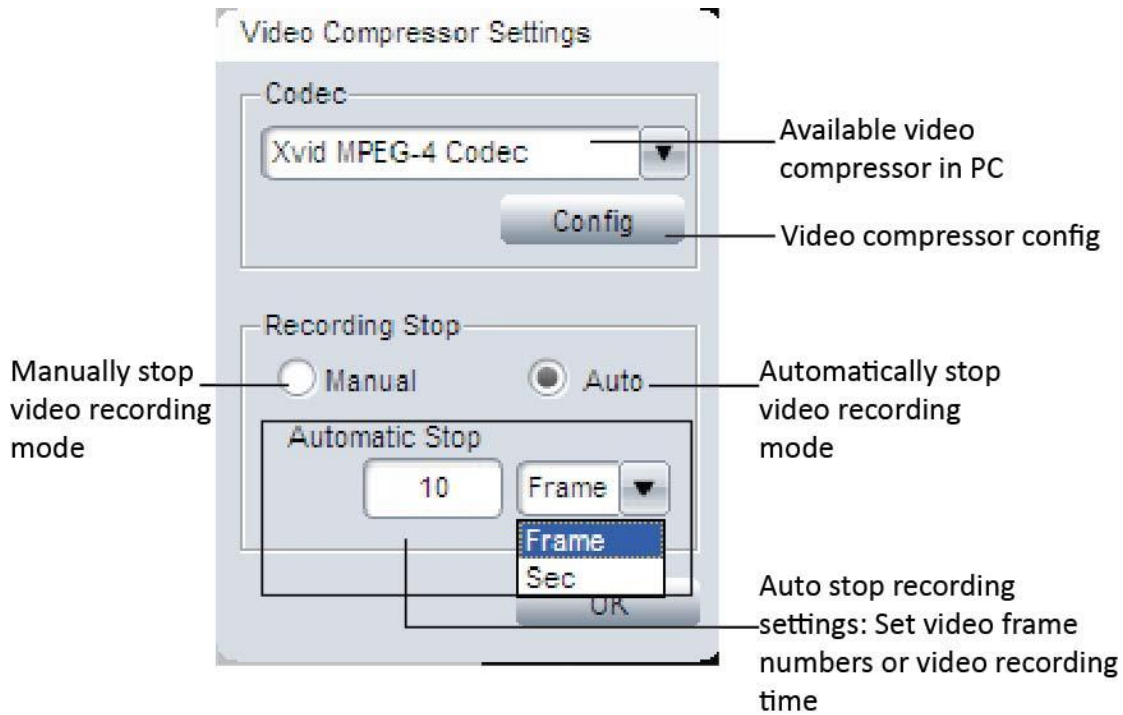

Number of frames for continuous shooting

3. 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

1. Click [Video] / , start/ stop video recording.

2. 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 on the PC.

> Without compressing the video, it will be very large. IS will automatically search the PC for installed video compressors to make the video file size smaller.

![llumins logo]

File Save Destination

Click [Browse...] to change the file destination for saving images. The default

path is the software installation folder, usually "C:\Program Files\ISCapture\".

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

EXPOSURE CONTROL
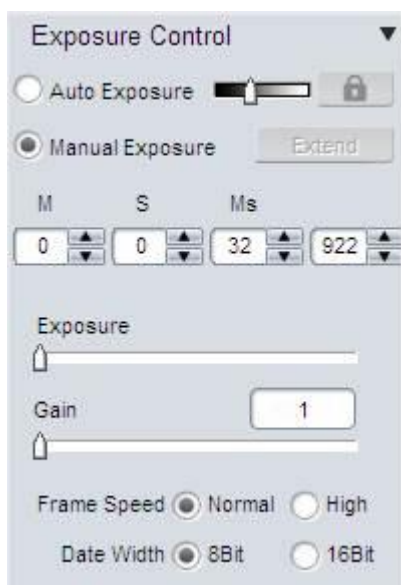
Change the Exposure time and Gain to adjust the image brightness. Select

frame speed to get different live image frame rates. Set 8-bit or 16-bit data

width for captured images.

Auto Exposure
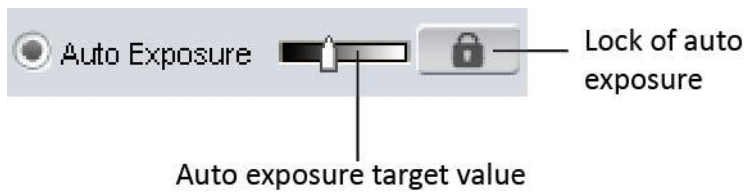
- Check [Auto Exposure] checkbox for the software to adjust the exposure time automatically to achieve proper brightness of the live images.

- Auto exposure target value: Set the reference exposure time for auto exposure adjustment, as it helps the auto exposure function to find the proper exposure time faster. For example, if the imaging target is quite bright, a lower target value is set; it will tell the software a shorter exposure time is needed.

- 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.
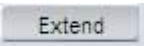
Manual Exposure
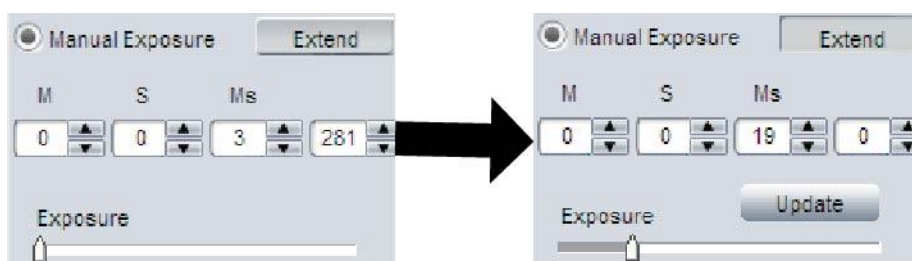
Adjust the exposure time manually. Two ways to change the exposure time:

- Key in the exposure time in the edit box directly <img> 20 </img>, then click <img></img> to confirm it.

- Pull the slide bar to change the exposure time.

> ⚠ [Extend] <img>Extend</img> 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] <img>Update</img> appears after [Extend] is selected. Click on <img>Update</img> 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

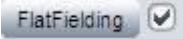| Gain		Increase the power of the image data. Higher gain gives brighter images, but also makes the noise signal more obvious.
Frame Speed	High Speed	Corresponding to high pixel clock. Gives faster frame rate.
	Normal Speed	Offer lower frame rate than High Speed, but gives longer maximum exposure time.
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

The color control settings are used for adjusting image color, gamma, contrast and saturations.
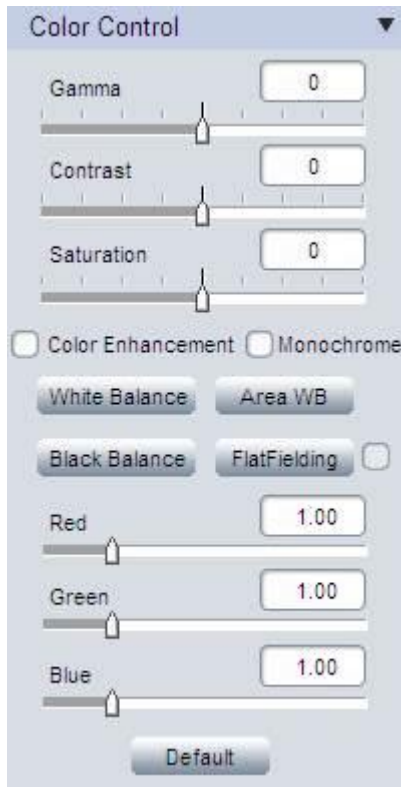
Flat Fielding Function

The flat fielding function is used to correct the uneven background brightness.

- Click on [FlatFielding] FlatFielding ☑ to 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.

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

Other Color Control Settings

Gamma	Gamma is used to obtain correct reproduction of intensity. Default value (Gamma = 0) is recommended in most of cases.
Contrast	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.
Saturation	Adjust image saturation. Saturation is the intensity of color in the image.
Color Enhancement	Used to make the image color more vivid. Before doing White Balance, it recommends to uncheck this function, then apply WB
Monochrome	Check the checkbox to get a grayscale image
White Balance	White balance. Give reference to true white for the cameras. Correct image color
Area WB	Manually select the white color area in the image as the white balance reference
Black Balance	Black Balance. Correct black color. Usually use in fluorescence application.
FlatFielding	Correct image uneven brightness. Uncheck the check box: cancle background brightness correction.
Red	Adjust the intensity of red in the image. [Red] = 1 means the original intensity of red in the image.
Green	Adjust the intensity of green in the image. [Green] = 1 means the original intensity of green in the image.
Blue	Adjust the intensity of Blue in the image. [Blue] = 1 means the original intensity of blue in the image.
Default	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
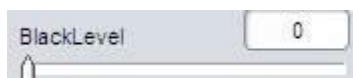
Black level function defines the brightness level at the darkest part of the image.

In low light imaging, it can help to see more details in the dark area.

> 💡 In low light conditions, you usually need a long exposure time to get proper images. We recommend setting a shorter exposure time, but increasing the Gain and Black Level when searching for the image. After you find the target image, reduce the Gain and Black Level and increase the exposure time as this will help in better image acquisition.

Levels
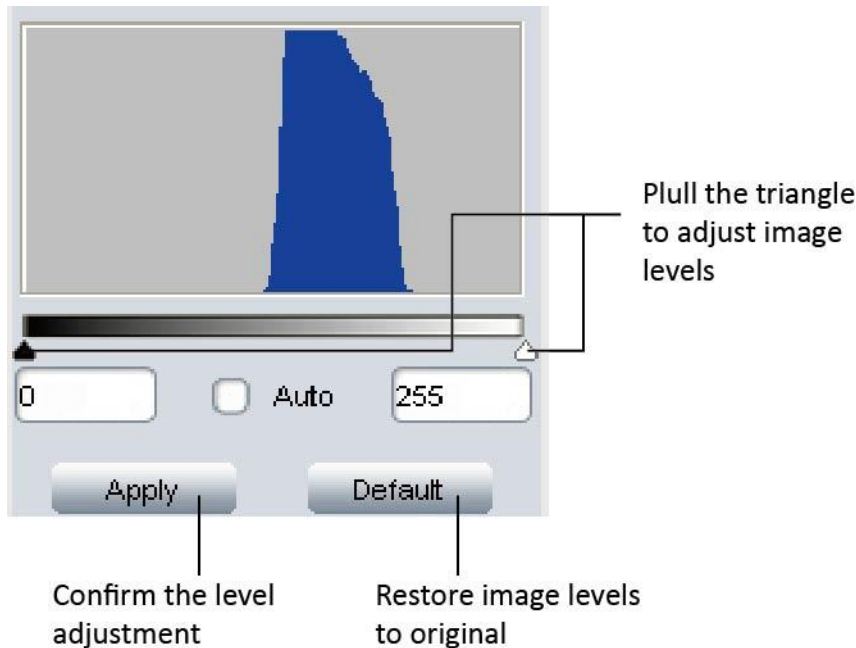
The live image histogram can help you make adjustments.

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 left, to reveal more information in the dark areas. If you move the black triangle right, it will reveal more information in the bright areas.

- You can also key in the image levels directly

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

Capture Mode
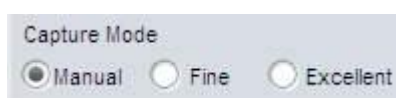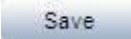
Three capture modes are specially developed for fluorescence imaging.

|	
⊙ Manual	Capture the image with current parameter settings
○ Fine	Automatically reduce the gain and extend the exposure to get the same brightness image. (Lower gain will give lower noise level images)
○ Excellent	Automatically save 10 images with current settings and then get an average image. (It needs to take a while to capture an image in this mode.)

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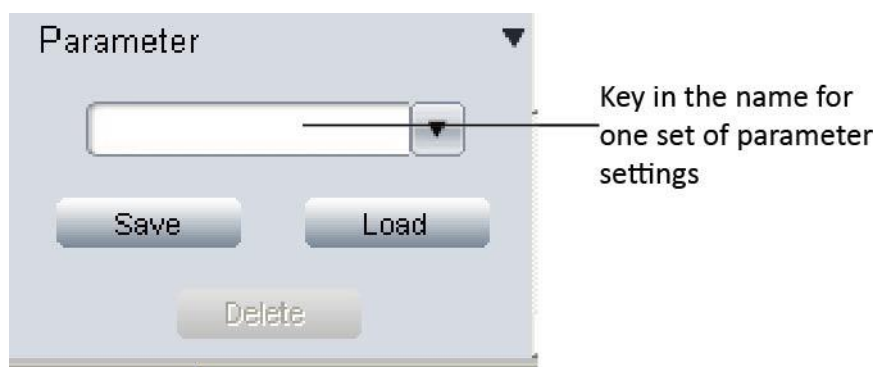
Save parameter sets for different applications. The saved parameters include exposure time, gain, frame speed, data width, gamma, contrast, saturation, color enhancement status, monochrome, RGB gain and black level. It allows users to save 20 set parameters (available in Ver 3.6 or later).

- 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 Load 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
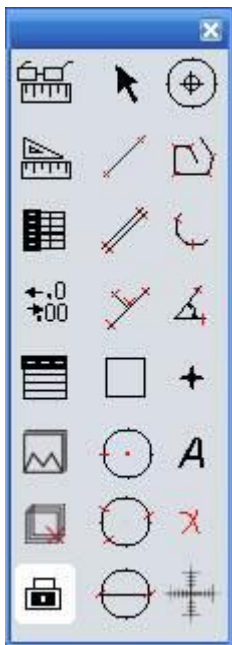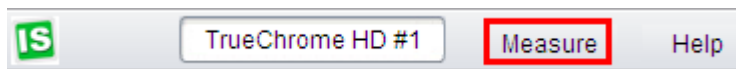
	Show Scale Line	On/off the scale line on the picture
	Calibrate	Create Calibration file
	Calibrate Table	Available calibration file list. Allow to add, edit and delete calibration file.
	Decimal	Set measurement precision. Allowed decimal range is from 0 to 7
	Measurement List	List all the measurement 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.
	Select	Select to change measurement or the measurement data position
	Line	Measure the length

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	Parallel	Measure the distance of parallel. Allow to do multiple parallels' distance measurement. Double clicking to end parallel measurement.	
	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.	
	2-points Circle	Use center point and point on the circle to draw a circle. Give the radius, area and perimeter of circle	
	3-points Circle	Use 3 points on the circle to draw a circle. Give the radius, area and perimeter of circle	
	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	
	Polygon	Measure polygon area and perimeter.	
	Arc	Measure a curve angle, radius and length.	
	Angle	Measure the angle	
	Point	Counter. Count the quantity.	
	Annotate	Add remarks on the images.	
	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

Scale Property

Character
Text ☐ BKColor ☐ ☐ Transparent BG
Line ☐ ☐ 2 ▼

Change scale text, background, line color and line thickness

Shape
Color ☐ ☐ 2 ▼

Change scale frame color and thickness

Info
Length: 100.00 pixel
ScaleName: Scale ABC

Edit displayed scale line length, name and font

Calibration Property
name=default;
length=1.00;totalPixel=1.00;
unit=pixel;unit\Pixel=1.0000000

Scale information

Goto CalibrationTable — Link to calibration table

Default   OK   Cancel

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

- Edit scale character

Scale background color. Default is WHITE

Change text color Default is RED

Check to get transparent background

Character
Text ☐ BKColor ☐ ☐ Transparent BG
Line ☐ ☐ 2 ▼

Scale line color Default is RED

Set scale line thickness
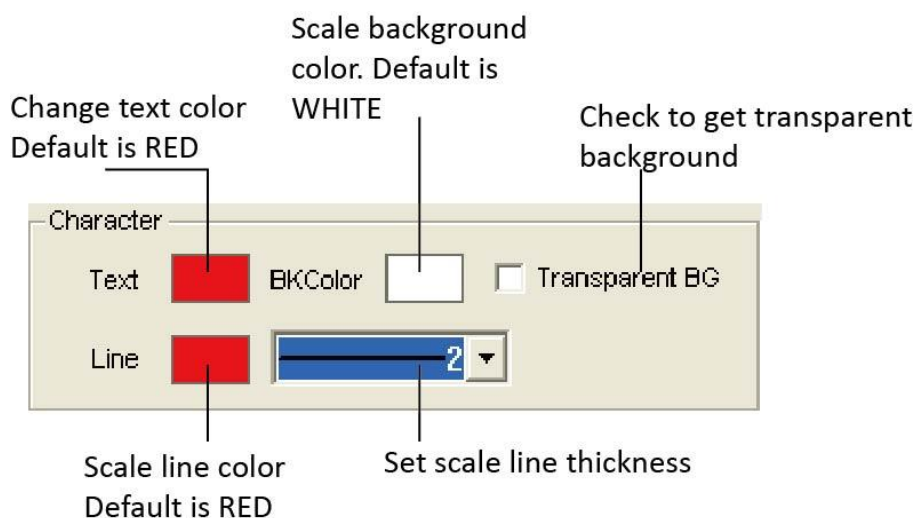
- Edit the frame of the scale

Change scale frame color
Default is RED

Frame thickness

Shape

Color [red] ───2 ▼
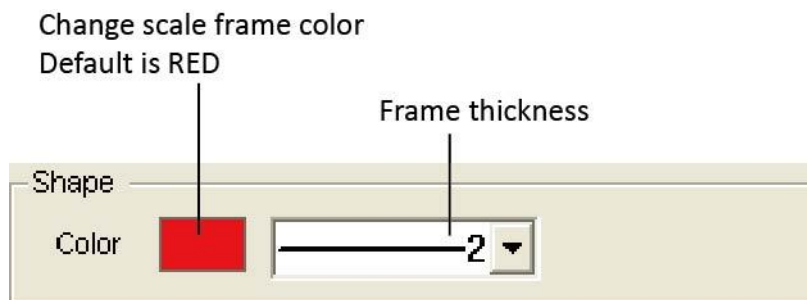
- Edit scale line length and name

Edit scale name

Change displayed
scale line length

Change scale name
font and text size

Info

Length: 100.00 pixel

ScaleName: Scale ABC
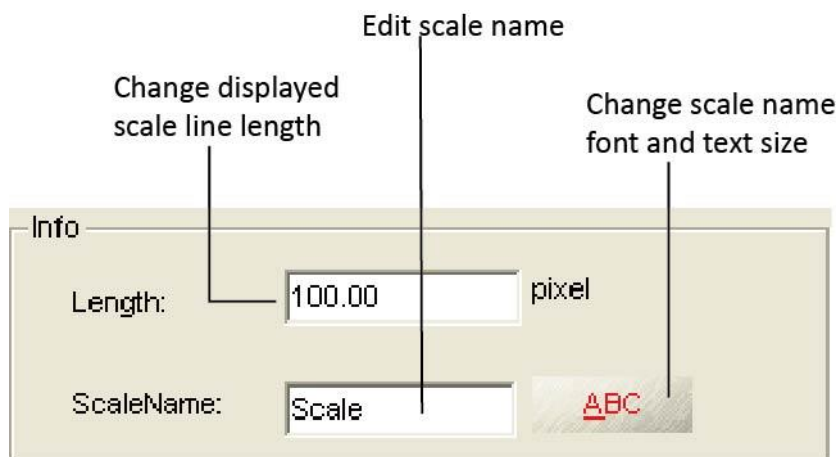
Create Calibration File

To measure the sample's 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 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.

2. Click ![ruler icon] to start to create calibration file.
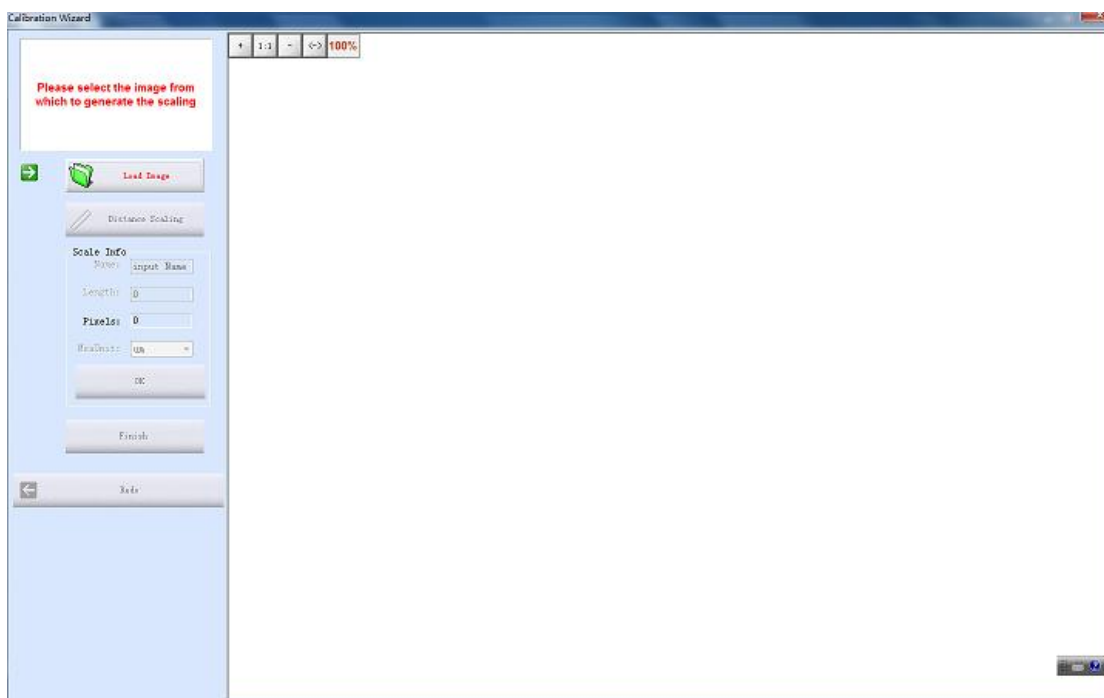
3. Click [Load Image] to load the calibration slide picture taken in Step1.
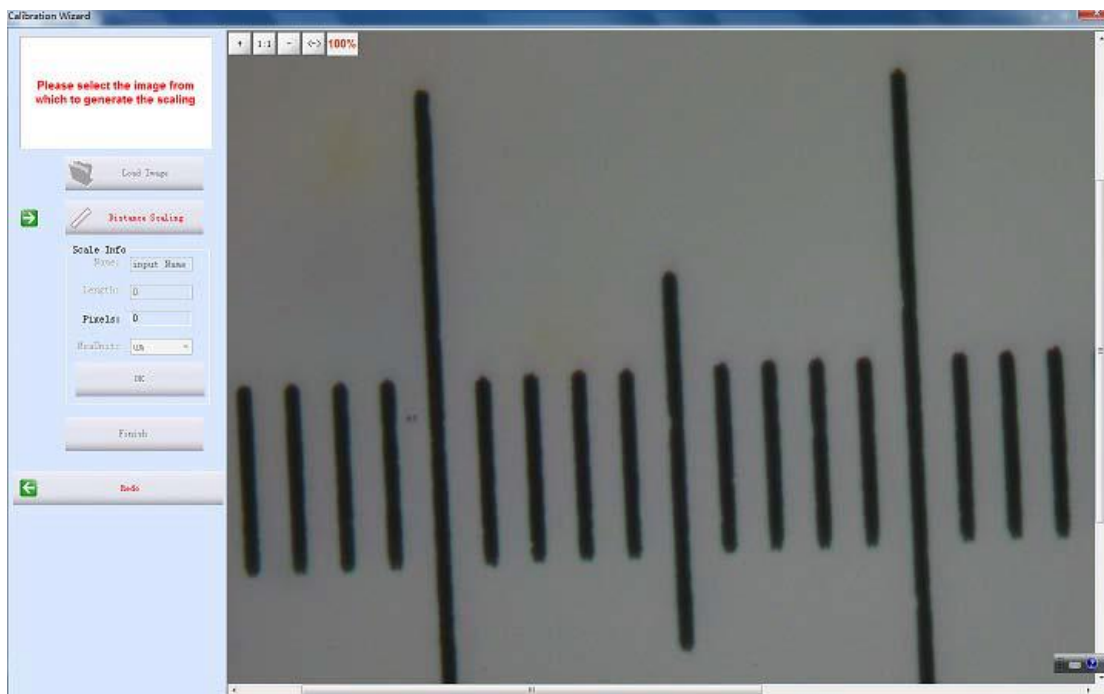
4. Click [Distance scaling] and move the cursor to the slide image, draw a line to

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.
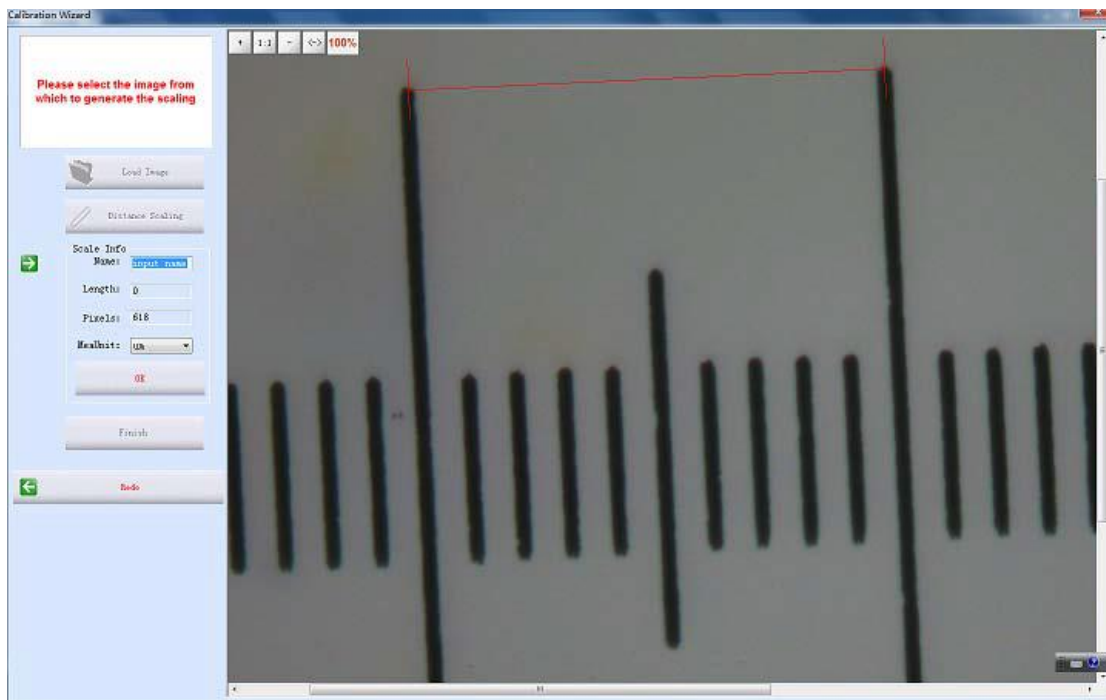

5. Enter the name for the calibration file and the length of the line you draw.

> 💡 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 files to do the calibration.

> ⚠ When keying in the length, please pay attention to the Calibration Scale Unit and the Measure Unit used. 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 0.1mm x 1000 = 1000 μm.

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 inaccurate. Please make sure the calibration file corresponds to the current image. Hence, it is useful to name the calibration file with the capturing settings or objective name.

Measurement List

Name	Length_um	Width_um	Height_um	Area_umsq	Perimeter_um	Radius_um	Angle
L1	612.73						
Parallel1	734.60						
Perpendicular1	462.92						
R1		449.58	359.67	161700.66	1618.50		
C1				420057.97	2297.52	365.66	
P1				225746.95	2283.12		
Arc1					440.31	175.46	143.79
A1							28.92
Remark1							

Save to TXT — Export the measurement data to .txt file

Save to Excel — Export the measurement data to Excel file

Copy — Copy all the measurement data to a file: txt, word or excel.

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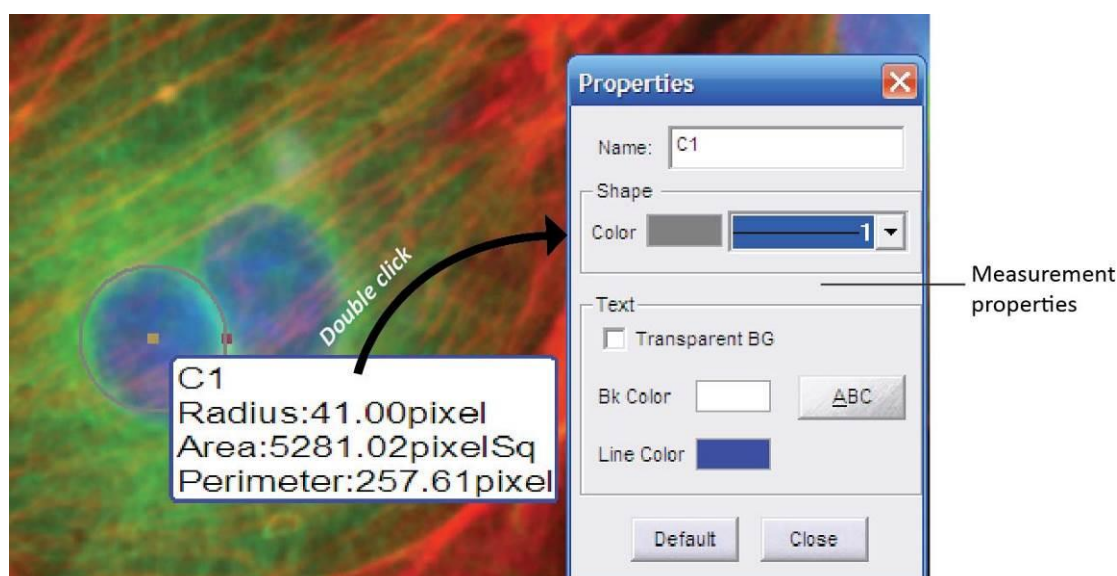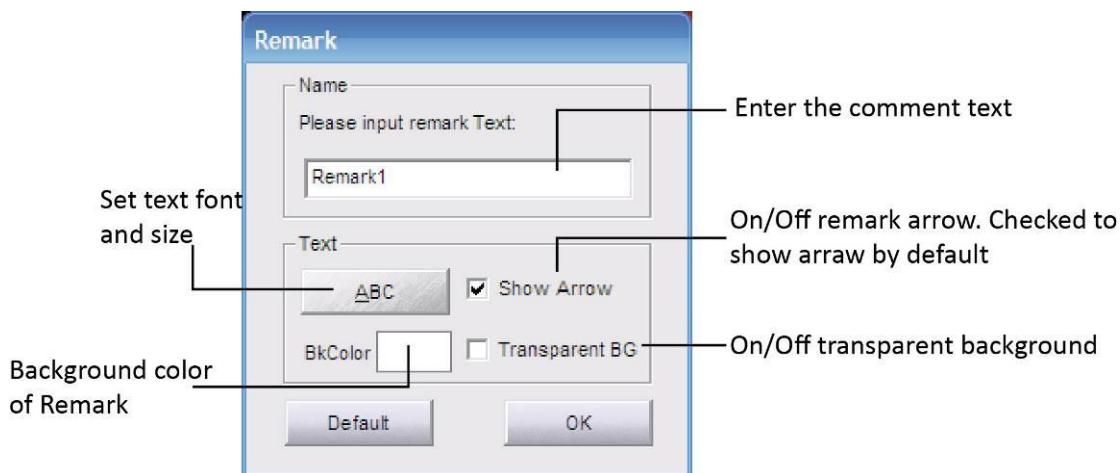
Double click on the measure data to get the measurement configure window. It allows you to change the measured data name, color, thickness, background color and the character font.
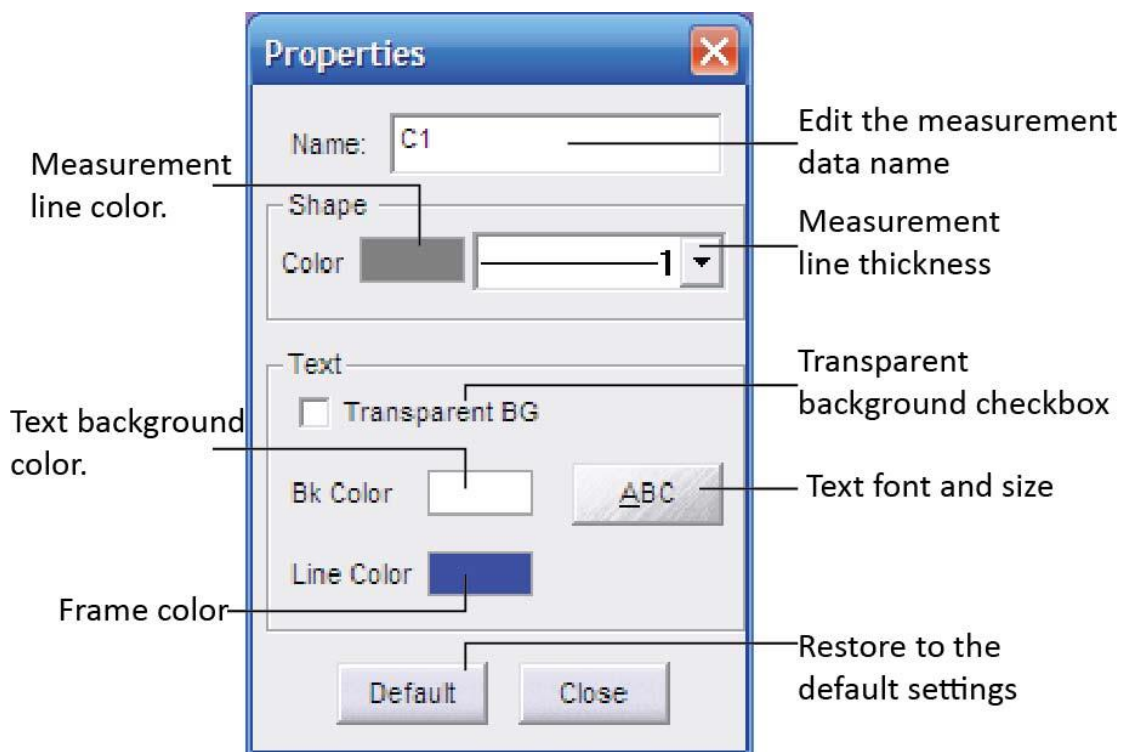

Annotate

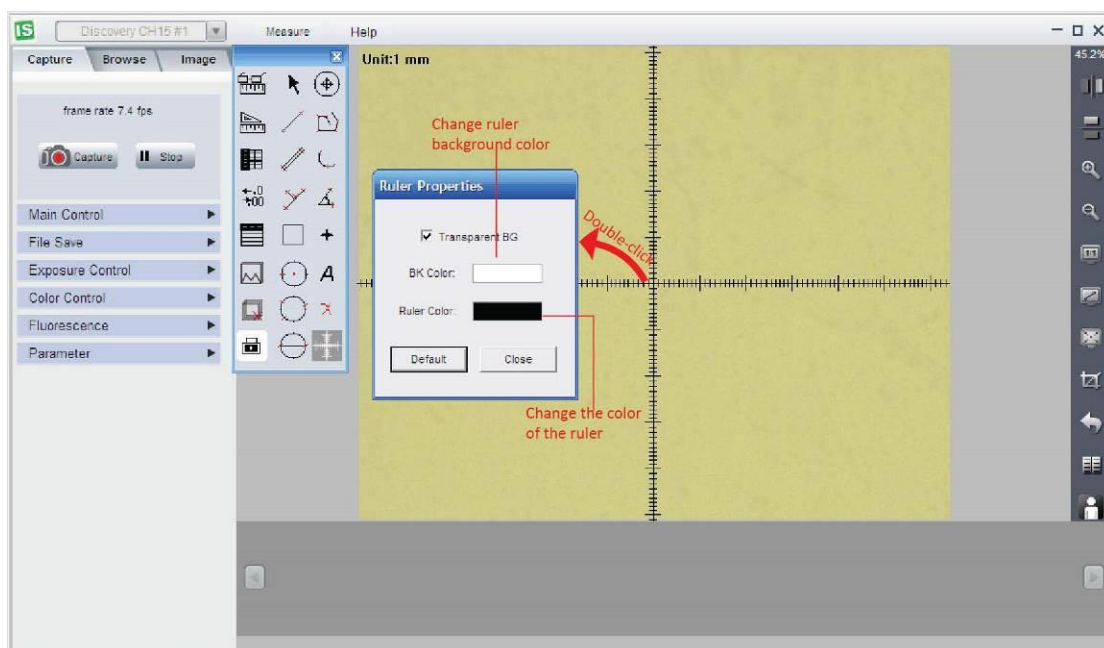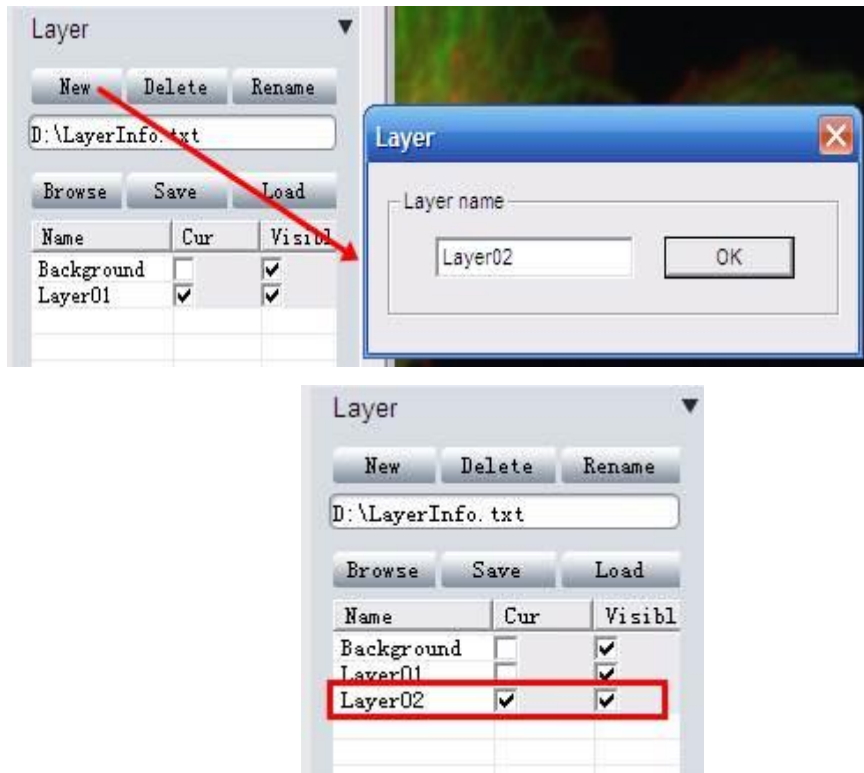Select [Annotate] and click on the image area which you prefer to add a remark. It allows you to edit the comment, change the background color and on/off the annotate arrow.

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
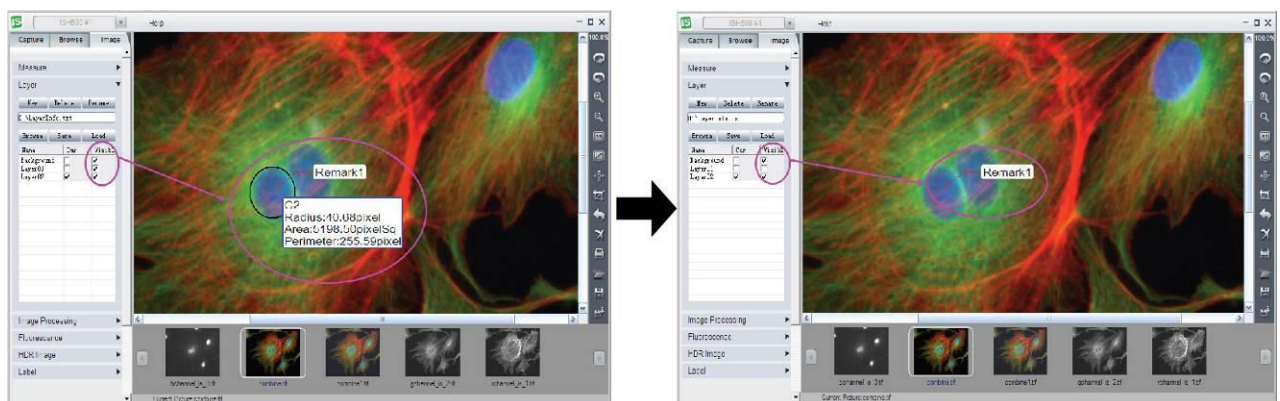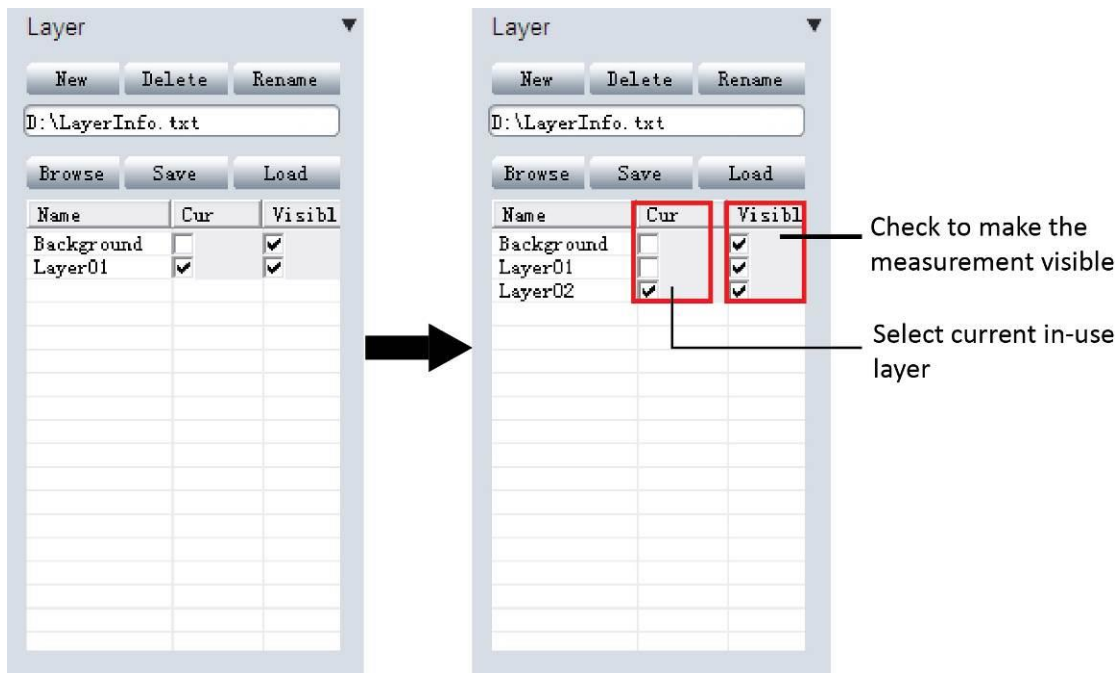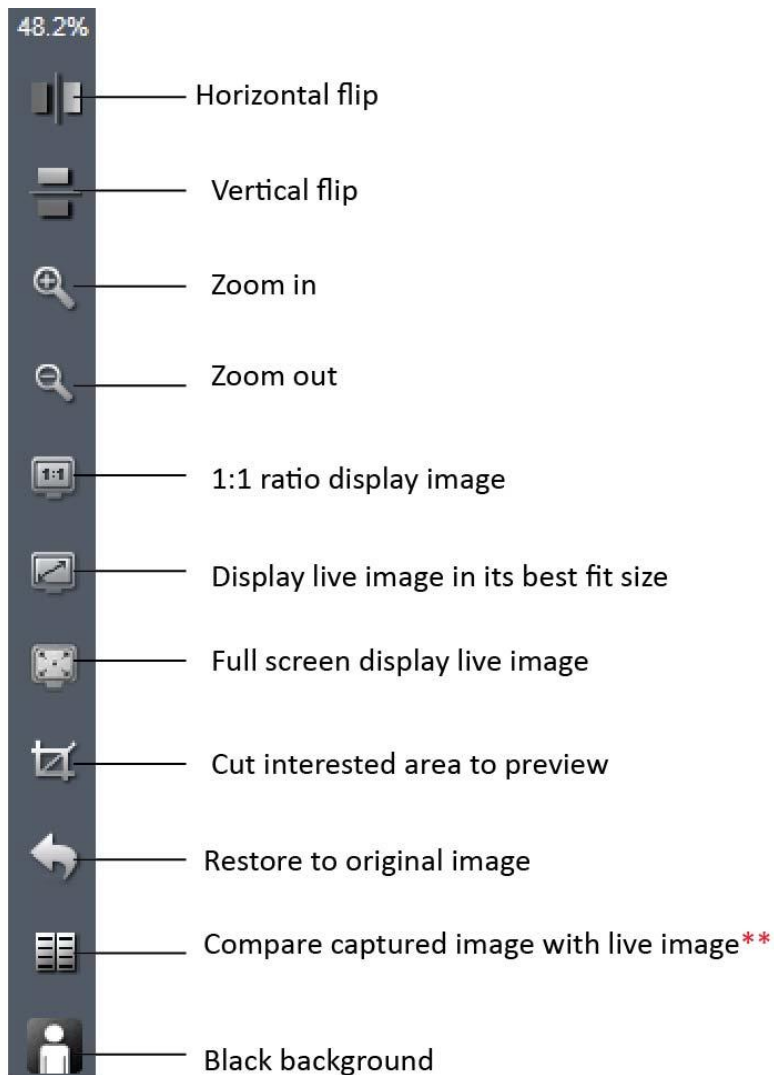
The layer information is saved in a text file.

- 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.

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LIVE IMAGE SHORTCUT

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

48.2%

— Horizontal flip

— Vertical flip

— Zoom in

— Zoom out

— 1:1 ratio display image

— Display live image in its best fit size

— Full screen display live image

— Cut interested area to preview

— Restore to original image

— Compare captured image with live image**

— Black background

** 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).

CHAPTER 3: IMAGE MANAGEMENT

View images in [Browse] panel, it displays the image File name, captured 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.
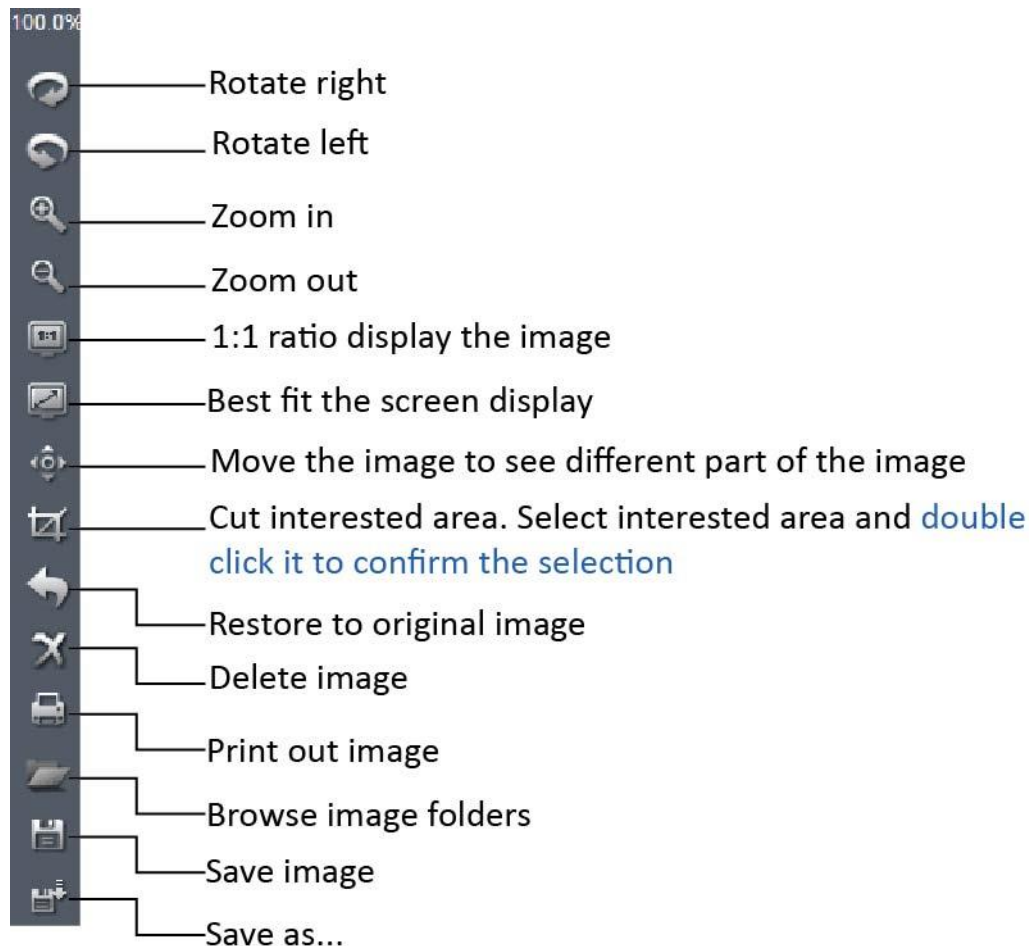
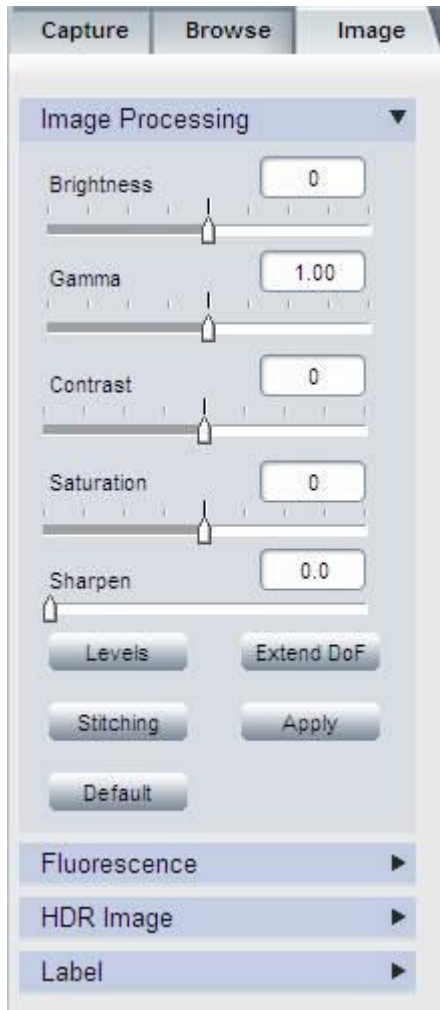|	
Image Information	
FileName:	is_1.bmp
Time:	2013/03/21/ 11:01:50
Bit:	24
Picture Size:	3488x2616
File Size:	26.1 MB
Note:	HELLO
	Save
Path	C:\Program Files\ISCapture\
	Browse...

Image Management and Processing Shortcuts

IS provides some quick functions on the right hand side of the software in

Browse or *Image* mode.

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CHAPTER 4: 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 and you can NOT revert back to the original image.
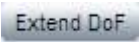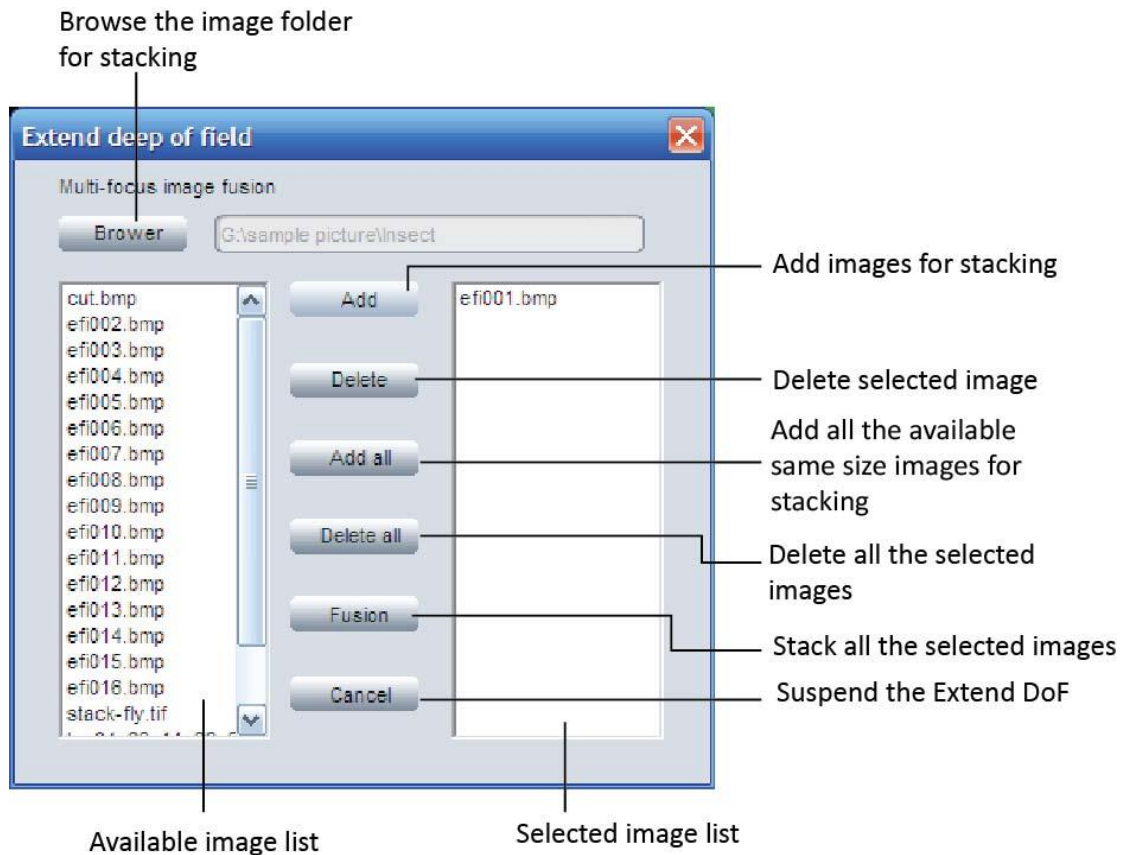
Level

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].
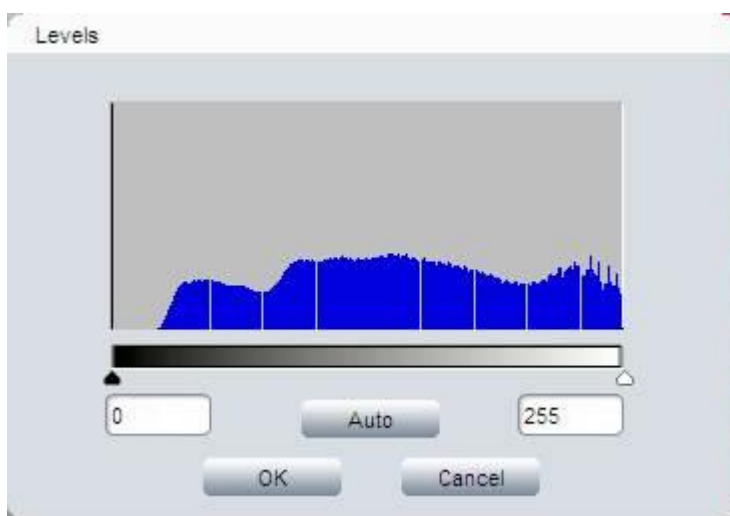
Extend depth of focus

Push [Extend DoF] to open dialog box. 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 highlighted 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.
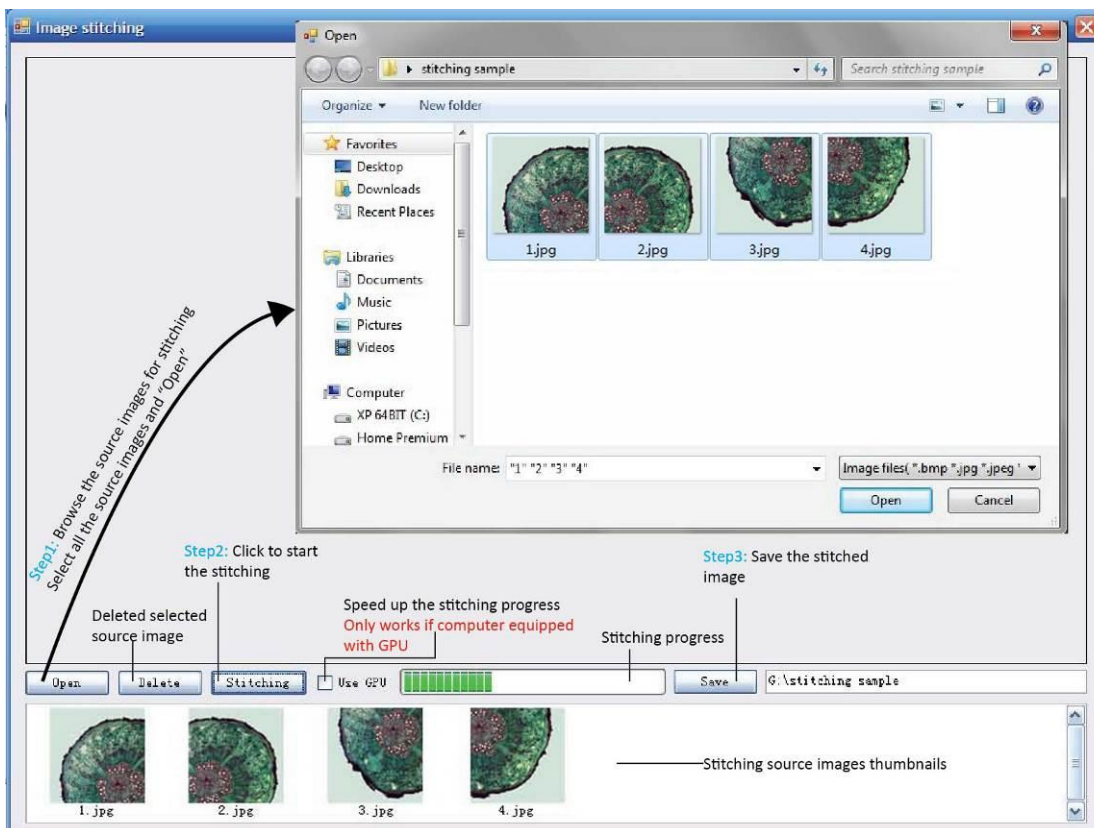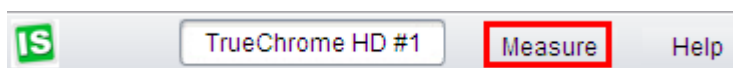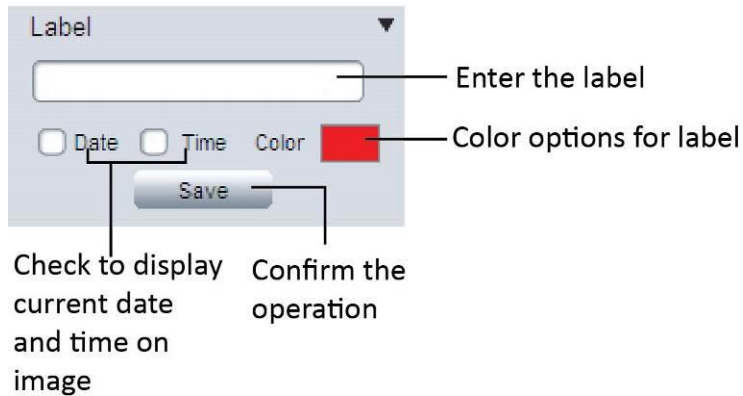
Sample images:
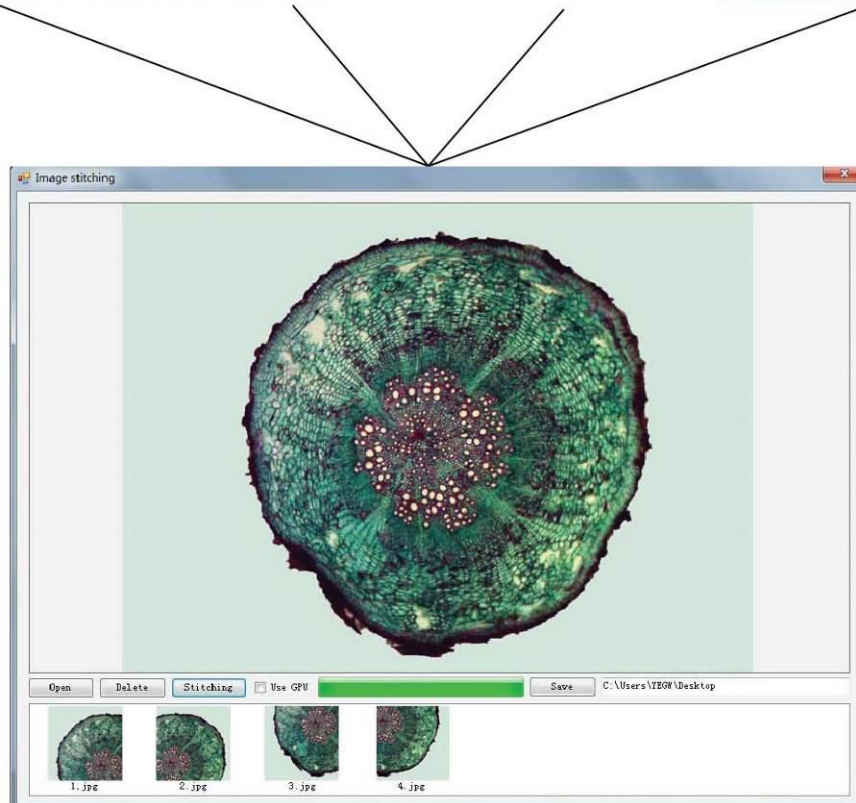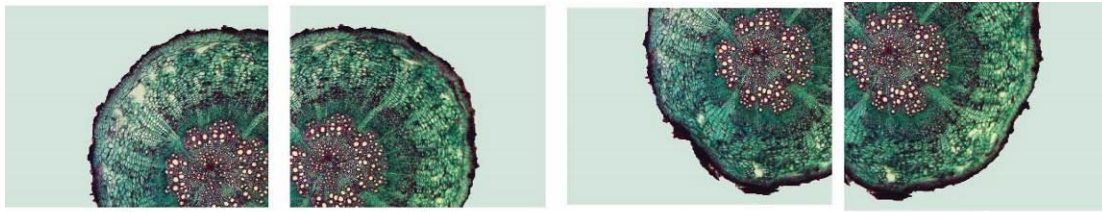
MEASURE

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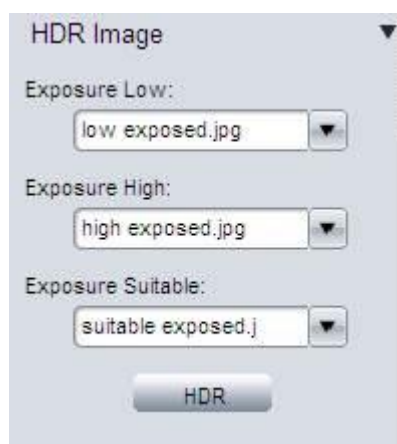
High Dynamic Range (HDR) images are used to get a greater dynamic range of an image.

- Take pictures of the same scene with different exposure times and load them into the software.

- In the drop-down menu, select the corresponding images for [Exposure Low], [Exposure High] and [Exposure Suitable].

- Push the [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 yet loaded in ISCapture, the shortcut on the right hand side allows you to browse any images.

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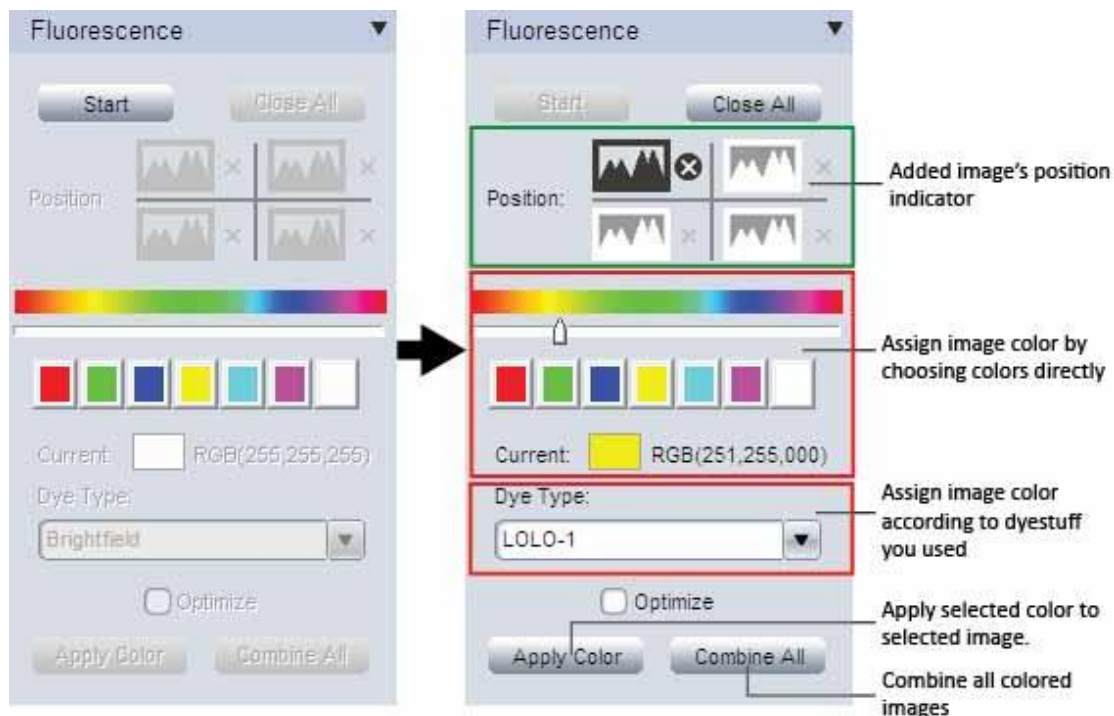
This function is used to assign fluorescence images with different colors and combine them together into one image.

Step 1: Open the images which you want to combine 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.
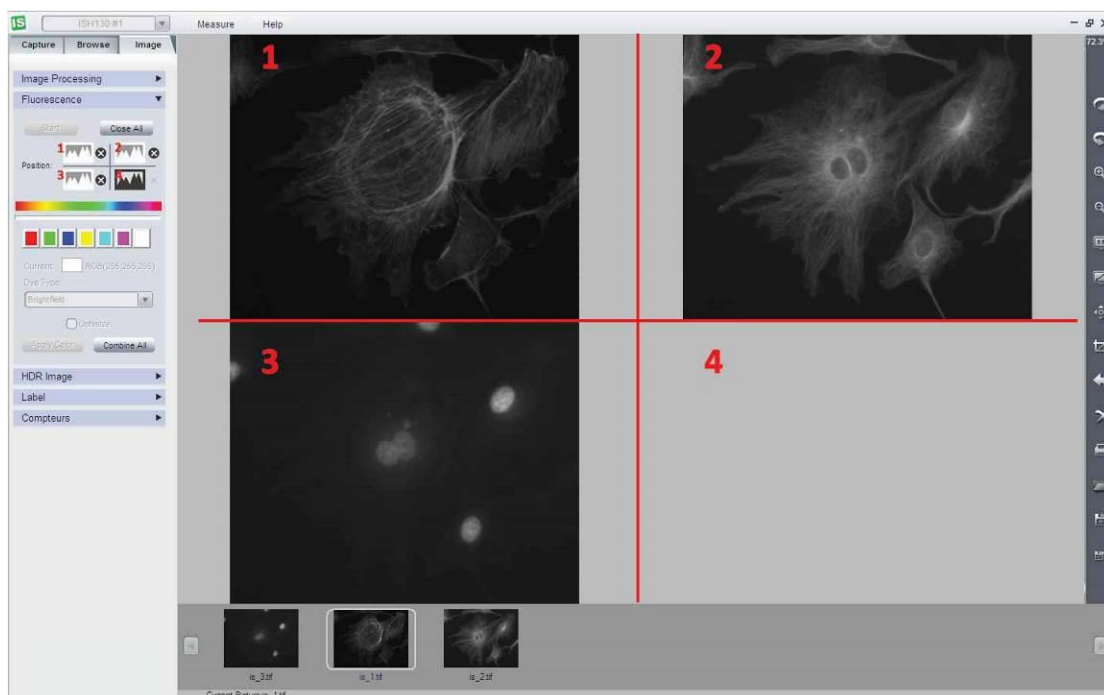

Step 3: Click on one added image indicator to start applying color for it.

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:

    1. Click on the preferred color or slide to choose it.

    2. 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.
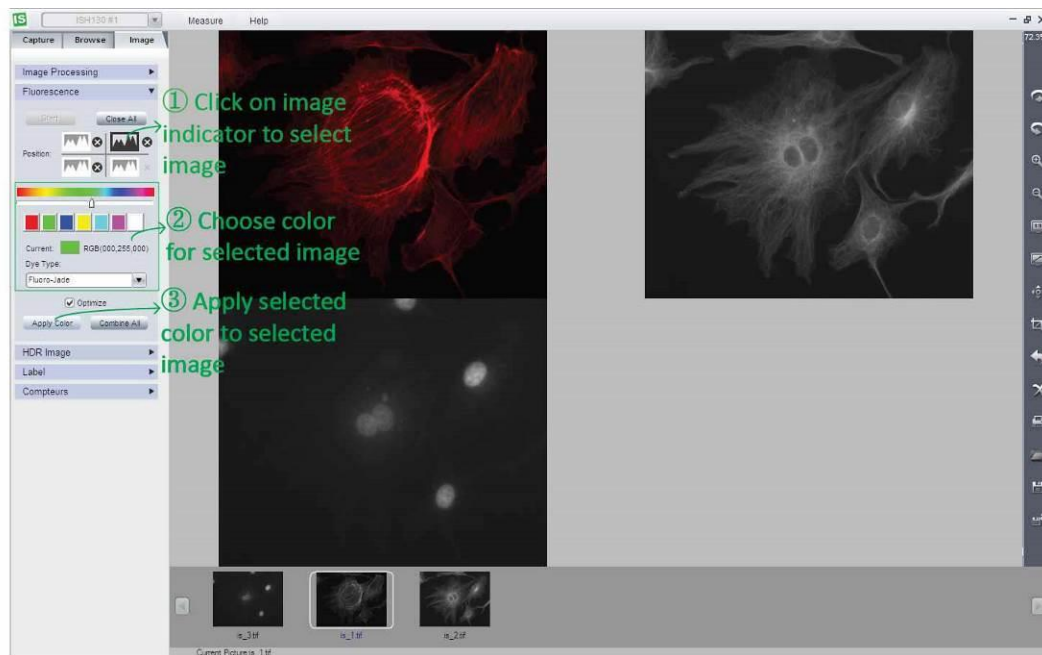
Step 4: Click on [Apply All] to combine all the colored images.
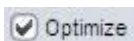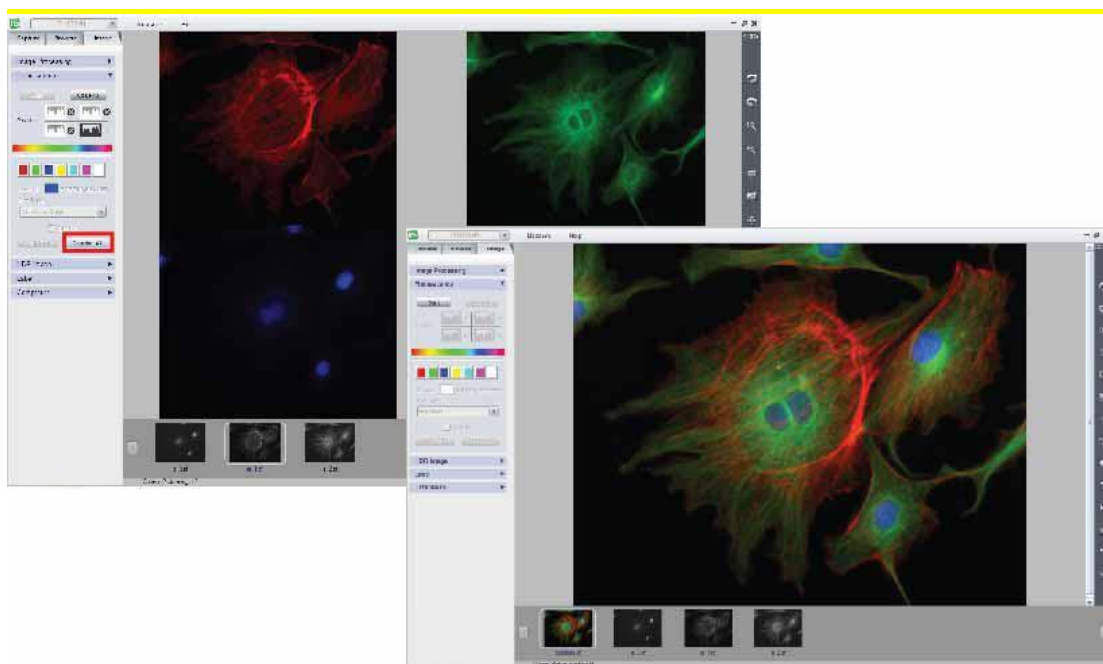
 ✓ Optimize   It is recommended to select the Optimize checkbox during combination. It will optimize image background to get a better image. But without optimization, the created image will keep all the original information. No extra processing is applied to the image data.

   After get the combined fluorescence image, Sharpen [1.6] [Sharp] function in [Image Processing] can help to get sharper images and see more image details.
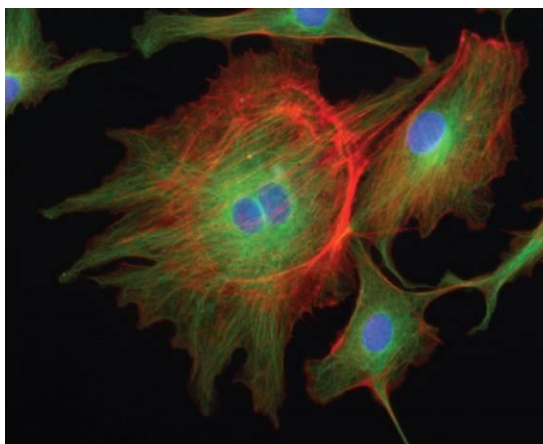
> If you added the wrong image or the wrong color to selected image, just click on the tiny cross ⊗ beside each indicator to delete it. If want to cancel current combination, just click on [Close All] to cancel the combination.
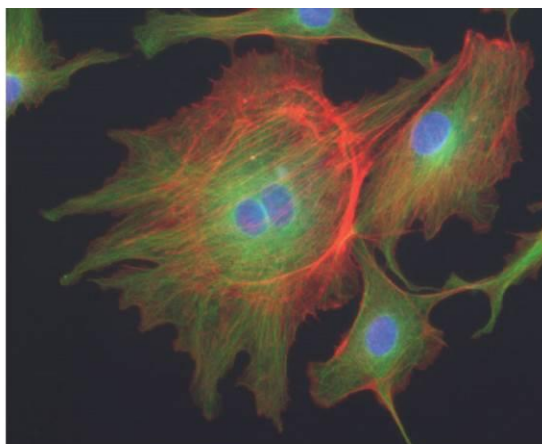
Original images:
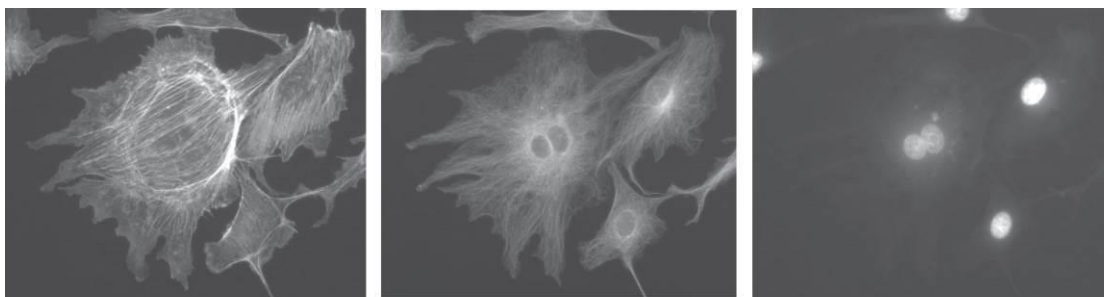
Original images

Combined image:

Combined image with optimization

Combined image without optimization