ImageFocus 4.0

USER GUIDE

Version 329271

System requirement

- 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

Getting started   -   Mount the camera on a microscope

All Euromex CMEX-1 (article dc.1300c), CMEX-3 (article dc.3000c), CMEX-5 (article dc.5000c), CMEX-10 (article dc.10c), sCMOS-1, sCMOS-3 and 5 Mpix cooled CCD cameras (dc.5000i) are delivered with a C-mount projection objective except the 5 Mpix cooled CCD cameras that comes without a projection objective

When the 5 mm ring is screwed to the camera, the camera has a C-mount. Without this 5 mm ring, the camera is a CS-mount camera

The cameras with mounted objective can be inserted into a standard 23.2 mm tube of a microscope or with 30 or 30.5 mm to 23.2 mm adapters into the eyepiece of a stereomicroscope

Cameras without mounted projection objective can be directly screwed to a C-mount (with 5 mm ring attached) adapter or CS mount (without 5 mm ring attached)
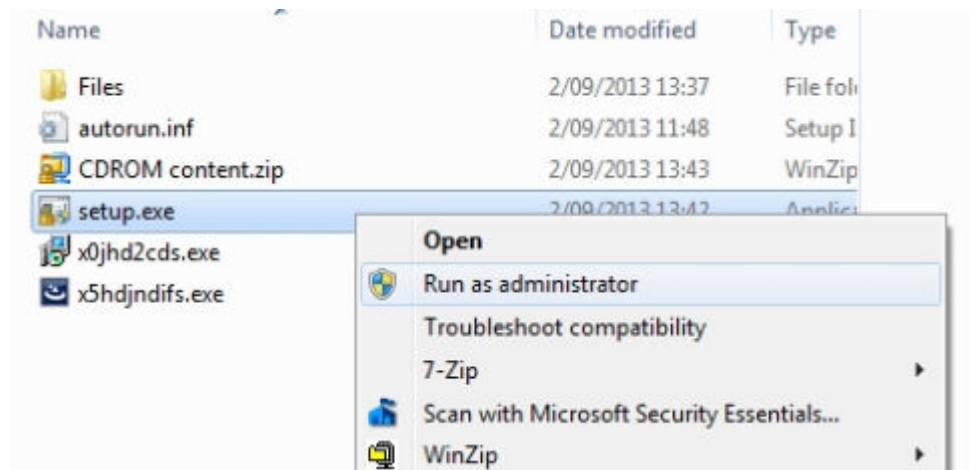
e.g.

C-mount photo adapter

Getting started   -   Install the software

In order to use the Euromex CMEX-1, CMEX-3, CMEX-5, CMEX-10, sCMOS-1, sCMOS-3 and 5 Mpix cooled CCD cameras, you need to install the camera drivers and the application software ImageFocus 4.0

 Therefore, select setup.exe, right mouse click and run setup.exe with "Run as administrator"

After finishing the installation, you can go to the Device Manager of your computer to check if the driver was installed properly

Starting up ImageFocus 4.0

Double-click on one of the shortcuts to start ImageFocus 4.0

If you need the software in one language only, you can delete the other shortcuts

When IMAGEFOCUS 4.0 starts up, the live image window appears. You can set the parameters to get correct images, save still pictures or videos. The [Capture] window provides image acquisition settings. The [Browse] window allows you to manage all your images. The [Image] window offers advanced image processing functions

[Capture] window
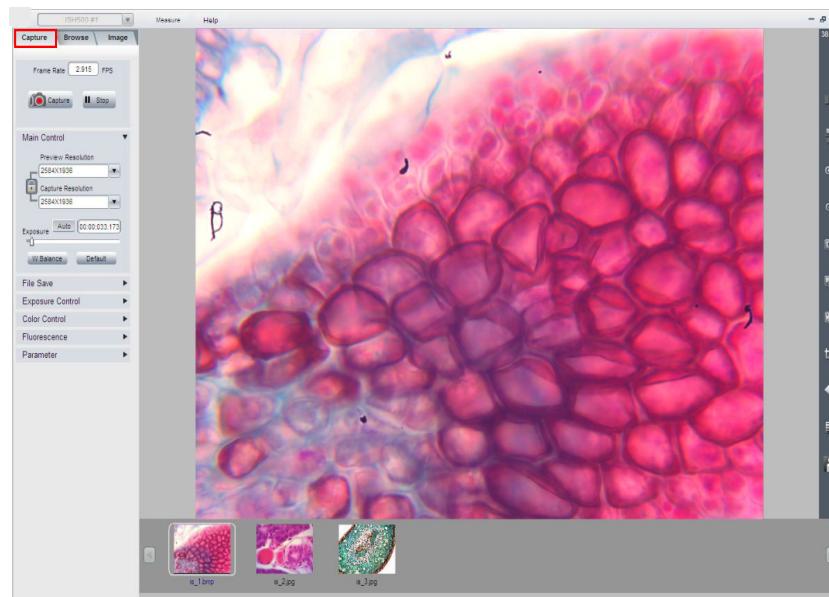
[Browse] window
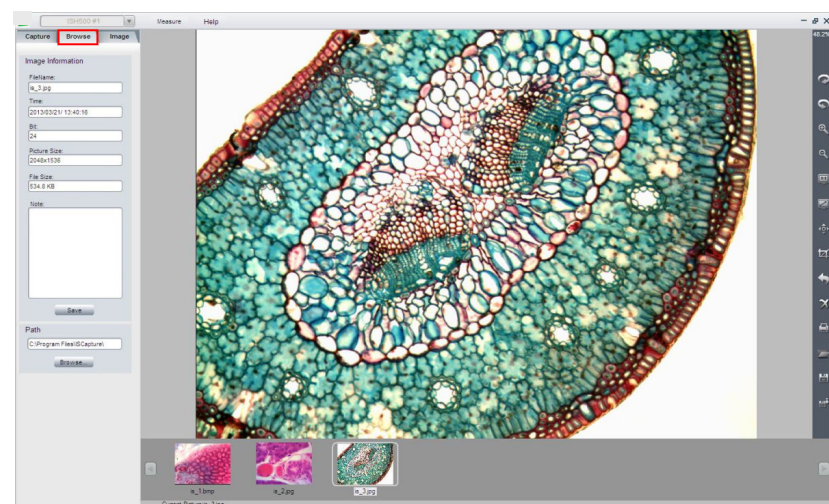
[Image] window
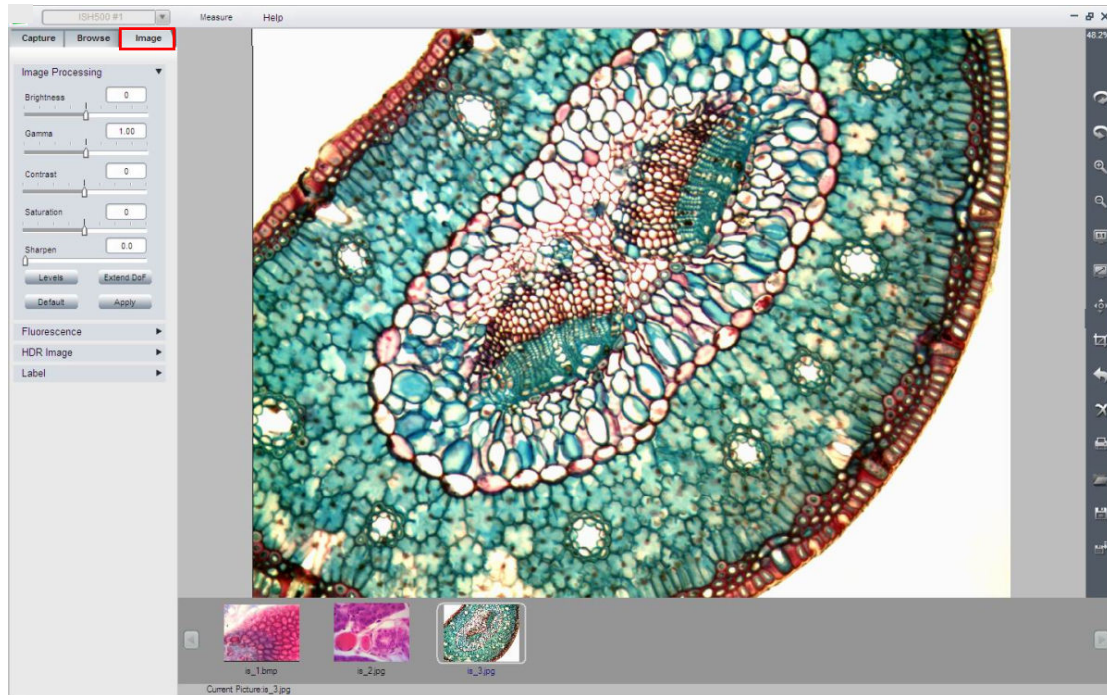
Image acquisition

Adjust the camera settings to get correct live images, do live image measurements and save still pictures and videos
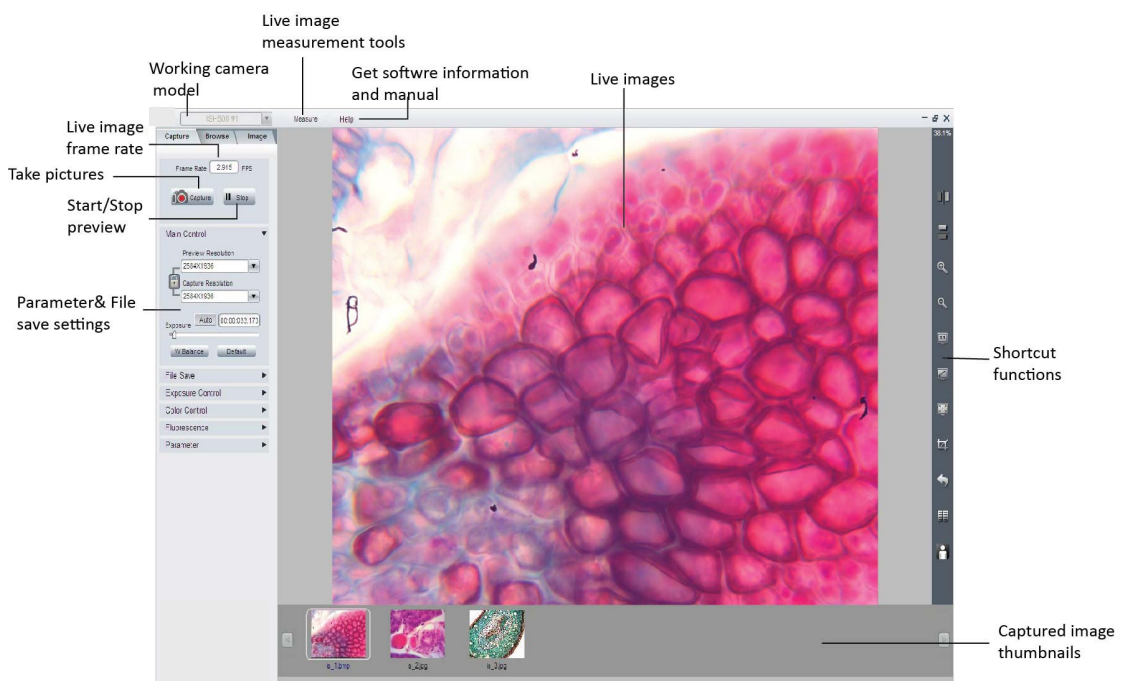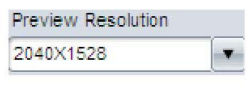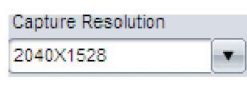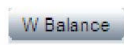
Start IMAGEFOCUS 4.0 with a camera attached to a free USB 2.0 port of the computer. The live image will be displayed automatically. If IMAGEFOCUS 4.0 was already running, connect camera, click to start a preview session
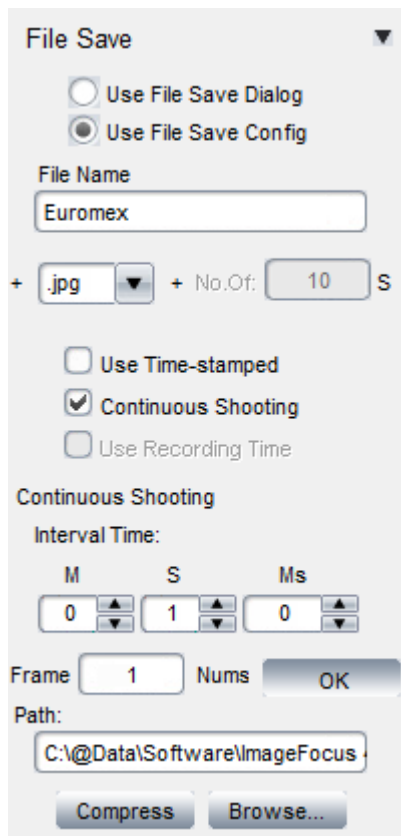
Basic controls
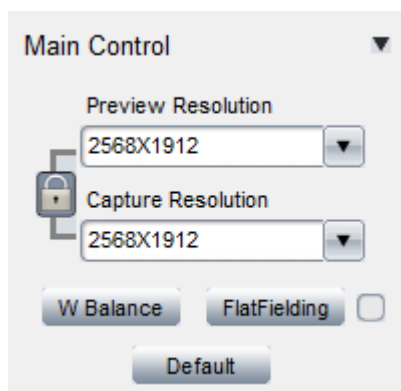
Provide basic camera settings:

	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.
	White Balance	Correct live image color

To perform a correct white balance, please follow below steps:

1. Move the sample out of the field of view
2. Unselect [Color Enhancement]
   (It is unselected by default in [Color Control] panel)
3. Don't use to much brightness to do the white balance
4. Click the [W Balance] button
5. Move back the sample
6. Check the color rendering of the sample

Take still images and videos

Under the File Save section, select if a File Save dialog must be used or a specific configuration must be executed

Enter a default file name

Select which format must be used:
.bmp, .jpg, .tif, .raw ou .avi (video)

If one select "Continuous shooting", click "Change" in order to set the interval time of the continuous shooting and the number of frames to capture. Click OK to confirm the settings

For video recording (.avi) one can set recording time or the number of frames to record

Enter the path to save the pictures / video

If a compression has to be applied to the file, select it from "Compress" and select one of the available compression modes (codec) on you computer
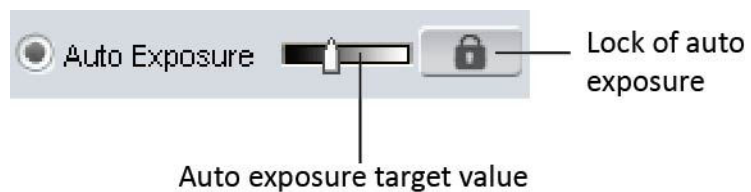
Exposure Control
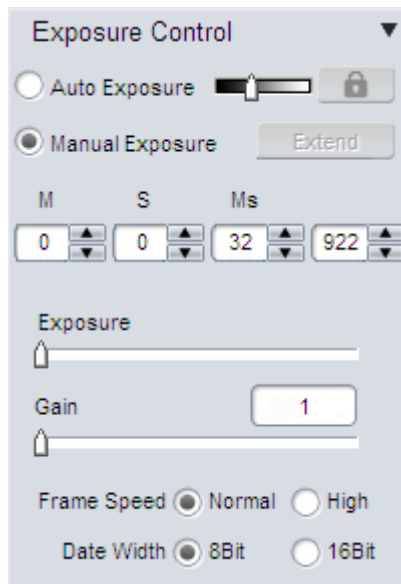
Here the user can change the Exposure time and the Gain of the internal operational amplifiers to adjust the image brightness
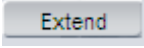
You can also select the frame speed mode 'High' to get higher live image frame rate

You can also set a 8-bit or 16-bit data format for the captured images (only available for CCD and sCMEX camera's)

Auto Exposure

- Check the [Auto Exposure] checkbox if you want the software to adjust the exposure time automatically to get correct – not overexposed - brightness of live images. This can be useful when used with stereo microscopes as brightness change continuously with magnification

- Auto exposure target value: You can pre-set a specific reference exposure time for the auto exposure adjustment. It helps the auto exposure function to find more rapidly a correct exposure time. If the imaging target is quite bright, set a lower value to tell the software that it not necessary to set long exposure times

- Lock: This will freeze the auto exposure calculation. While auto exposure is working, it will keep on calculation of the image brightness in order to get correct exposure time. If you got good live images, you can click to lock it

[Extend] **Extend** is used to enable longer exposure times. This function is ONLY available for CCD cameras.

A [Update] **Update** button appears after you selected [Extend]

Click on it to stop the current exposure time and start with the new specified exposure time immediately. With long time exposure applications, we strongly recommend to click [Update] to start the new setting. It will generate faster the new image. If the exposure time is less than 2-3 seconds, it is not necessary

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.
	16bit	16-bit images uses 2^16 = 65.536 gray levels to represent image details. ONLY available for CCD & sCMEX eries camera in .tiff and .Raw formats

## Color Control - Adjust image color, gamma, contrast and saturations
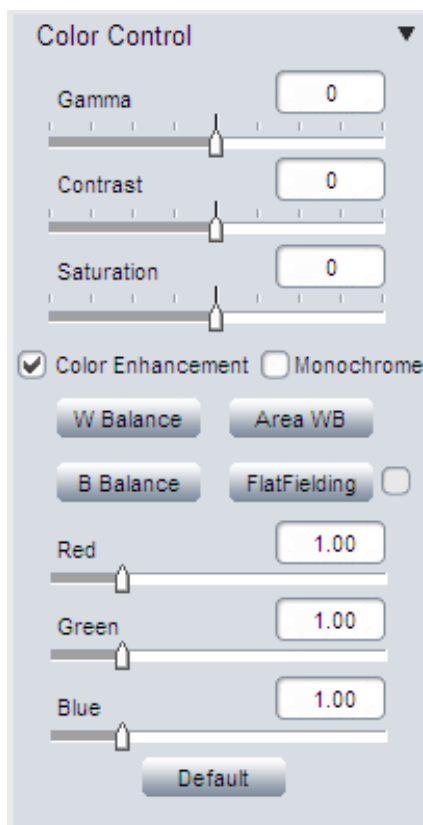
Gamma	Gamma is used to obtain correct reproduction of intensity. Default value (Gamma = 0) is recommended in most of cases.
Contrast	Contrast is the difference between the brightness brights and the darkest darks in an image. Higher contrast will make the shadows become darker and the highlights brighter. 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
W 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
B 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 red in the image.
Default	Restore the parameter settings to the initial value and apply white balance.

Fluorescence Settings
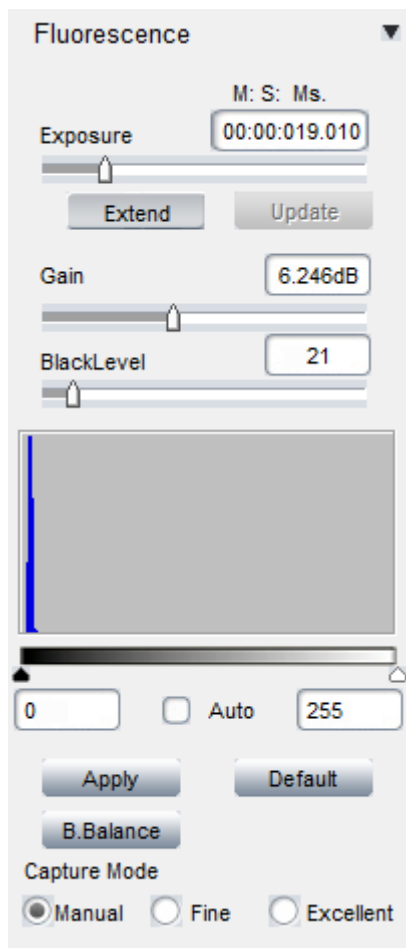
Integrate parameter settings for fluorescence or low light imaging conditions

Black level
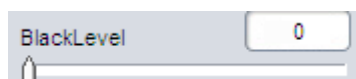
The Black level function defines the brightness level for the darkest part of the image. In low light imaging, it can help to see more details in the darker areas

In low light application, one can use long exposure times to get a correct image. However, when you start setting the camera parameters, we recommend to set short exposure times, use larger Gain levels and set the Black level first. After you find a first image you reduce the Gain and Black level, increase the exposure time
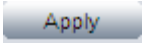
Adjust levels of histogram
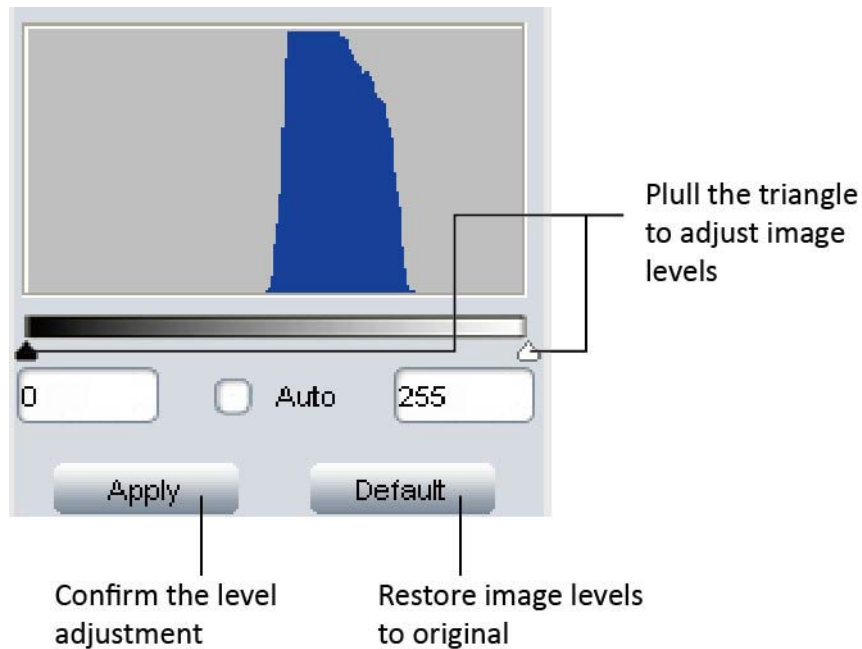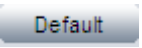
Check the [Auto] check box to adjust automatically the levels of the image histogram

Adjust manually image histogram levels:

Pull the triangles to adjust the levels. Move the white triangle to the left, it can reveal some details in darker areas. Move the black triangle to the right, it can some details in very bright areas

Click [Apply] to apply the setting. If you need reset to the original levels,

click [Default] to restore the values

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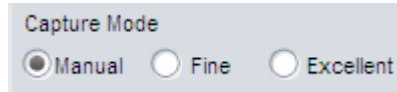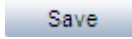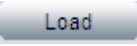
Three capture modes are specially developed for fluorescence imaging.

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

Key in the name for one set of parameter settings
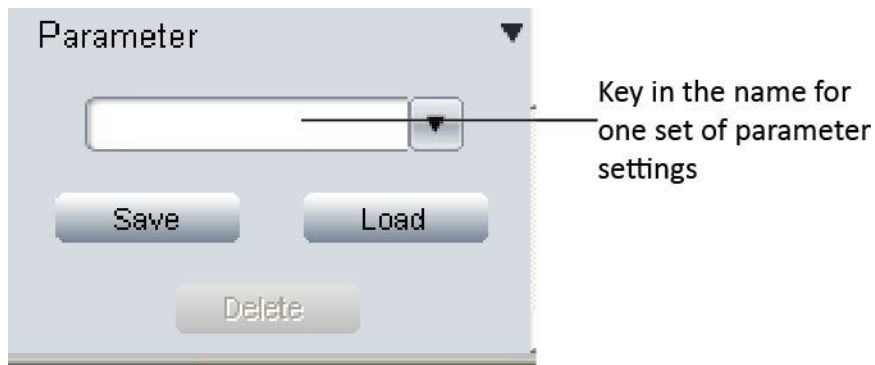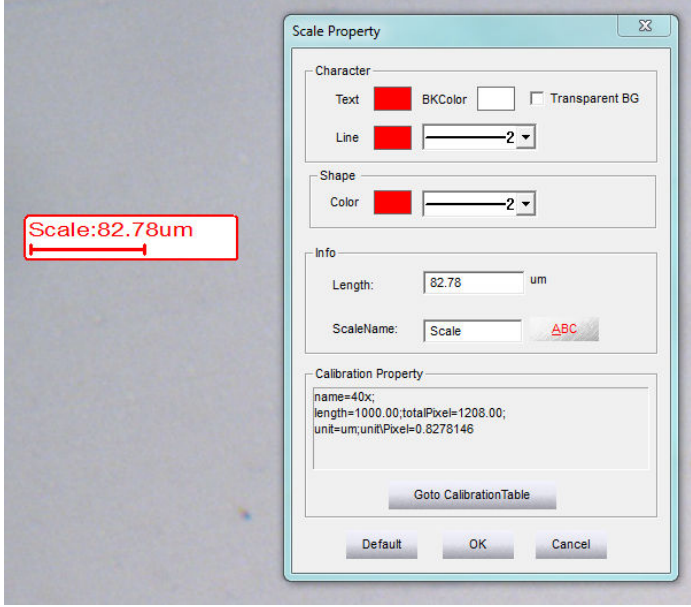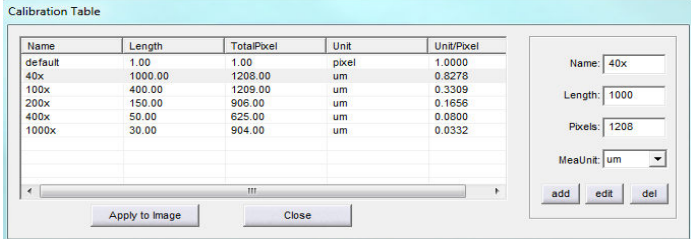
Save parameter sets for different applications. The saved parameters include exposure time, gain, frame speed, data width, gamma, contrast, saturation, color enhancement status, monochrome, RGB gain and black level
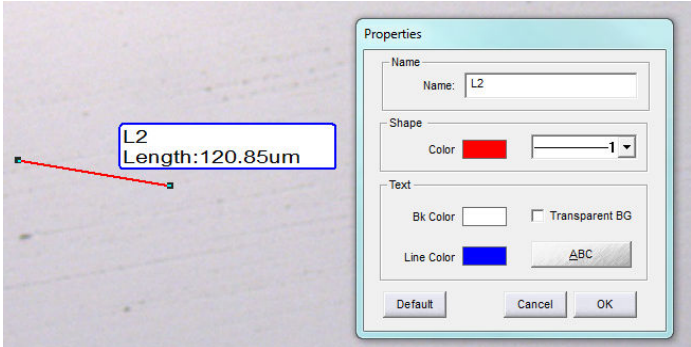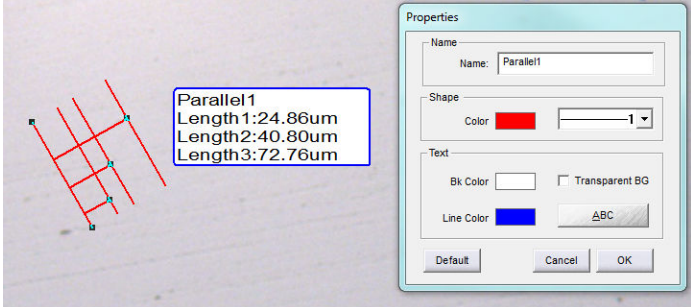
- Save parameter: Enter a name for parameter set, click [Save] to save it

- Load parameter: Click [▼] to open a drop-down menu and select a set with parameter and click [Load] to load the parameters into the software
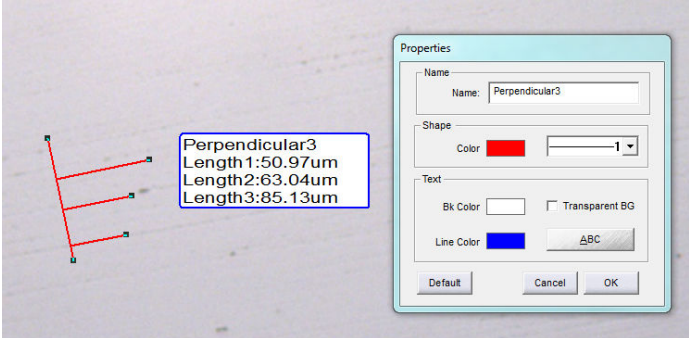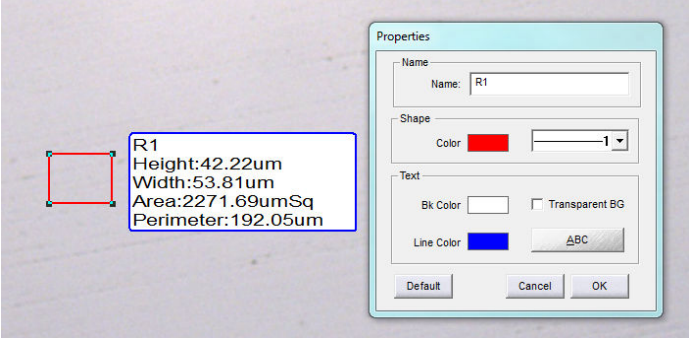
Measurements
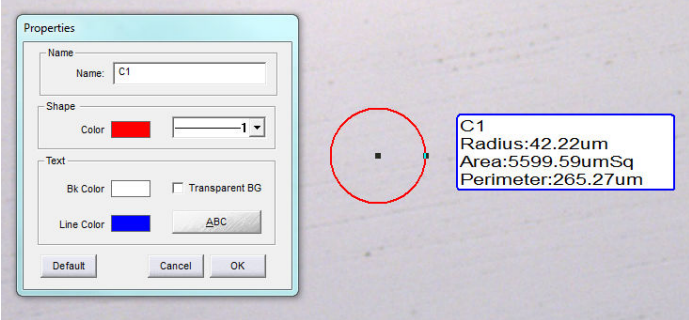
Click on the Image TAB and on [Measure]
to get the measurement icons
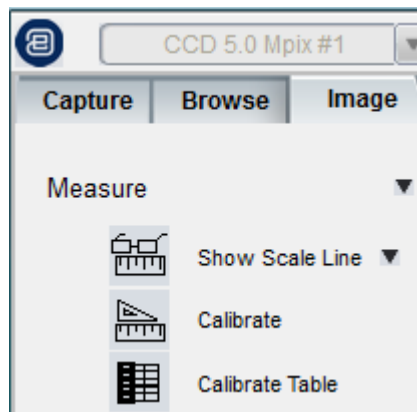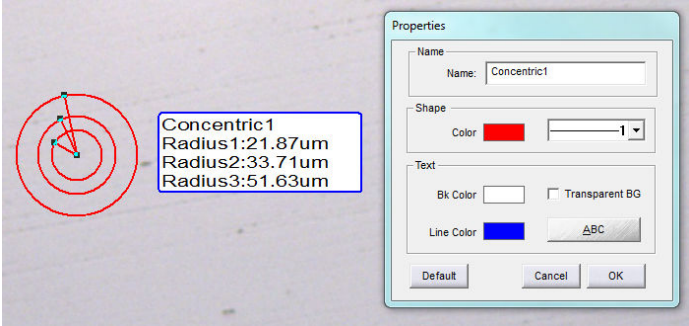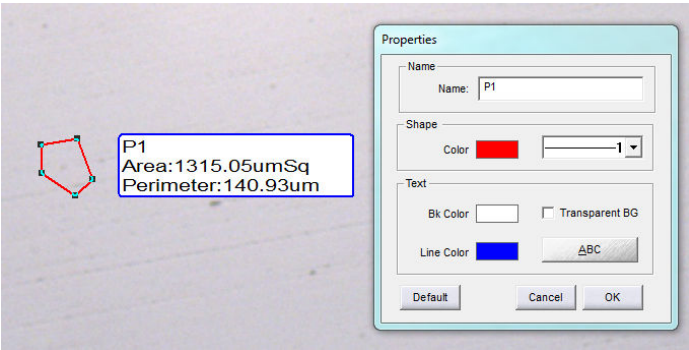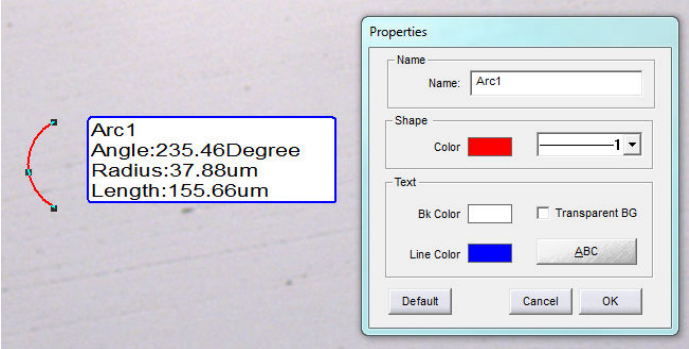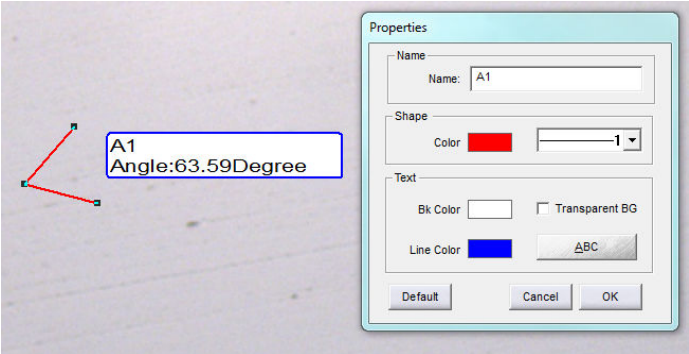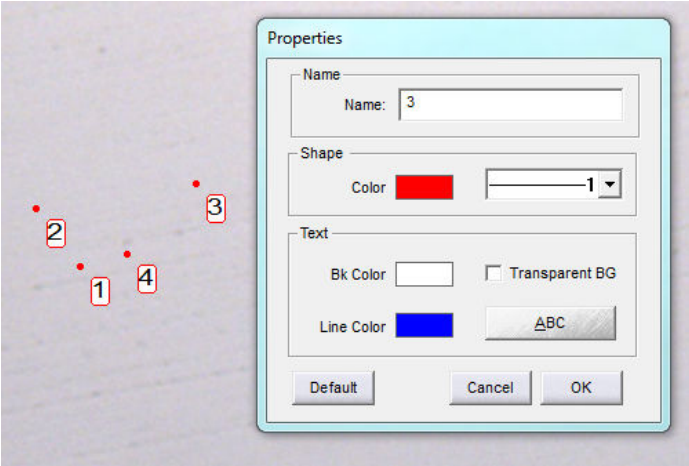
	Show Scale Line	Enable/Disable the scale line on image
	Calibrate	Perform a calibration on an image
	Calibrate table	Open/Edit the calibration table
	Decimal	Set number of decimals to be shown on image (valid values are 0 to 7)
	Measurement List	List with all measurements

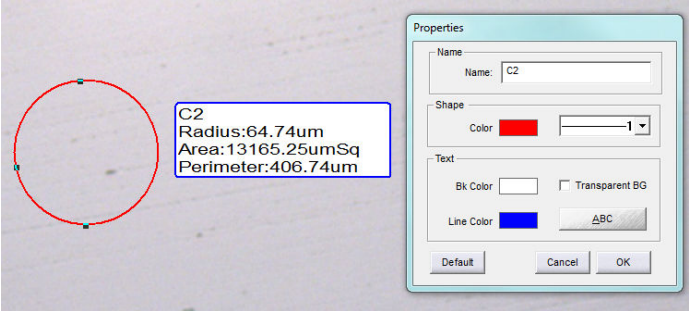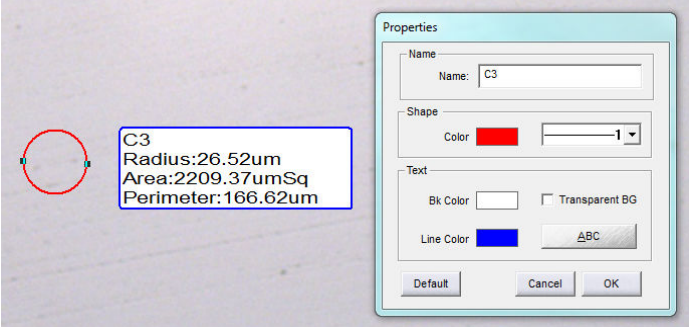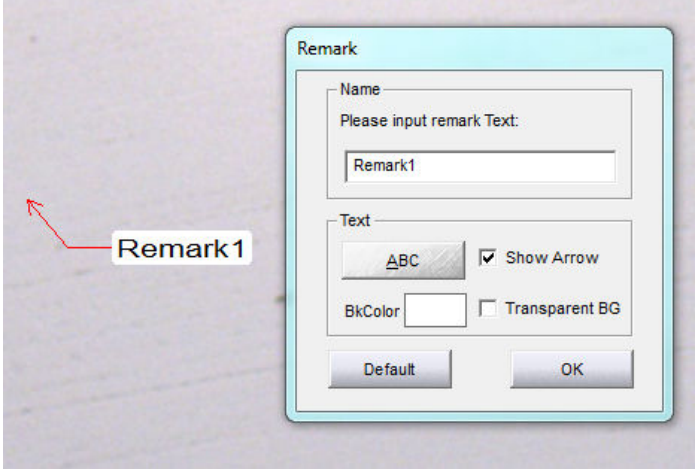|		
/	Lock	Lock and unlock
	Delete	Click on icon to enable the delete function Select the item to delete
	Select	Select tool
	Line	Perform a line measurement *Double click on text area to show and edit the properties*
	Parallel	Measure the distances of parallel lines - click a first time to start tracing the baseline - click at the end of the baseline to terminate the tracing of the base line - move the cursor to trace a second line, click on at the end of this new line to terminate the tracing of the second parallel line - double-click to terminate the function (see example below) *Double click on text area to show and edit the properties*

	Perpendicular	Measure the distances of perpendicular lines - click a first time to start tracing the baseline - click at the end of the baseline to terminate the tracing of the base line - move the cursor to trace a second line, click on at the end of this new line to terminate the tracing of the second perpendicular line - double-click to terminate the function (see example below) *Double click on text area to show and edit the properties*
	Rectangle	Measure de height, width, surface and perimeter of a rectangle *Double click on text area to show and edit the properties*

	2-points circle	Click the center of the circle to trace and move the cursor to the 2nd point of the circle Click a second time to terminate the operation *Double click on text area to show and edit the properties*
	3-points circle	Click 3 times to define the 3 points of the circle to trace *Double click on text area to show and edit the properties*
	Diameter circle	Click 2 times to define the diameter of the circle to trace *Double click on text area to show and edit the properties*

	Concentric circles	Click once to define the center of the centers of the concentric circles. Click to trace a first circle, etc … Double-click to terminate the operation *Double click on text area to show and edit the properties*
	Polygon	Measure the surface and perimeter of a polygon *Double click on text area to show and edit the properties*
	Arc	Arc measurement by 3 points *Double click on text area to show and edit the properties*

	Angle	Angle measurement Click once, move the cursor and click at the intersection point of the 2 lines of the angle. Move the cursor again and click a third time to terminate the operation *Double click on text area to show and edit the properties*
	Point	Set a point to mark something or perform a count on an image *Double click on text area to show and edit the properties*

A	Remark	Click on the location where you want to add a remark

Calibration procedure and Calibration table

Before you can perform measurements on an image, the calibration table must be updated with correct calibration values for each available magnification and camera of the microscope. This table contains a calibration value (usually µm/pixel) for each magnification of your microscope(s)

When you enter a name for a calibration value, we recommend to use the total magnification (see table below, 40x for a 4x objective together with a 10x eyepiece; if you use more than one microscope, you can use prefixes to distinguish them, e.g. M40x and S40x)

Remark: there is always a default entry that cannot be deleted

Procedure

1. Take as many pictures of a suitable calibration slide (*) as there are magnifications available on your microscope !

   Save each image with a comprehensive filename!
   We recommend to use the total magnification as filename, e.g. for an image taken with a 4x objective and 10x eyepiece: '40x'

   (*)   *Euromex cameras are delivered with 76 x 26 mm*
   calibration slides with a 1 mm / 100 micrometer reticle,
   10 µm intervals   (Order reference AE.1110)

   For stereo microscopes, we recommend to purchase an optional calibration slide with

100 µm intervals like a 50 mm / 500 micrometer slide   (Order reference AE.1112)

For zoom stereo microscopes, one can use the click-stops on the microscope in order to set the magnifications. If there are no such click-stops available, one must put some mark(s) on the microscope in order to know with which magnification a picture is taken !

2. Click to start to perform a calibration
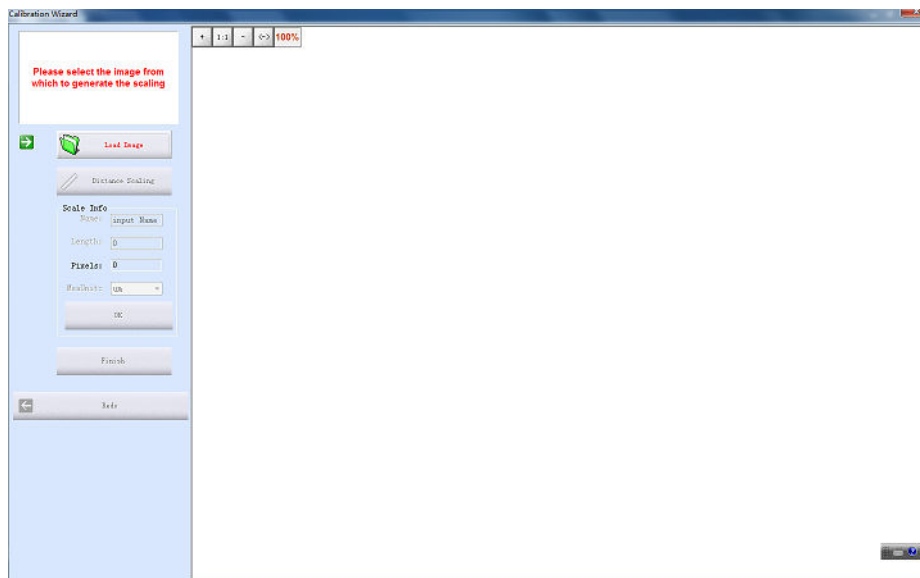
3. Click the [Load Image] button to load the first picture of the calibration slide taken in step 1

4.   Click the [Distance scaling] button and move the cursor over the image; click on mouse button to start drawing a line along the micrometer; start at the beginning of a vertical line; click the mouse button again at the end of the line; stop at the end of a vertical line

Draw a line as long as possible, as longer lines will have better precision and thus more accurate measurement results
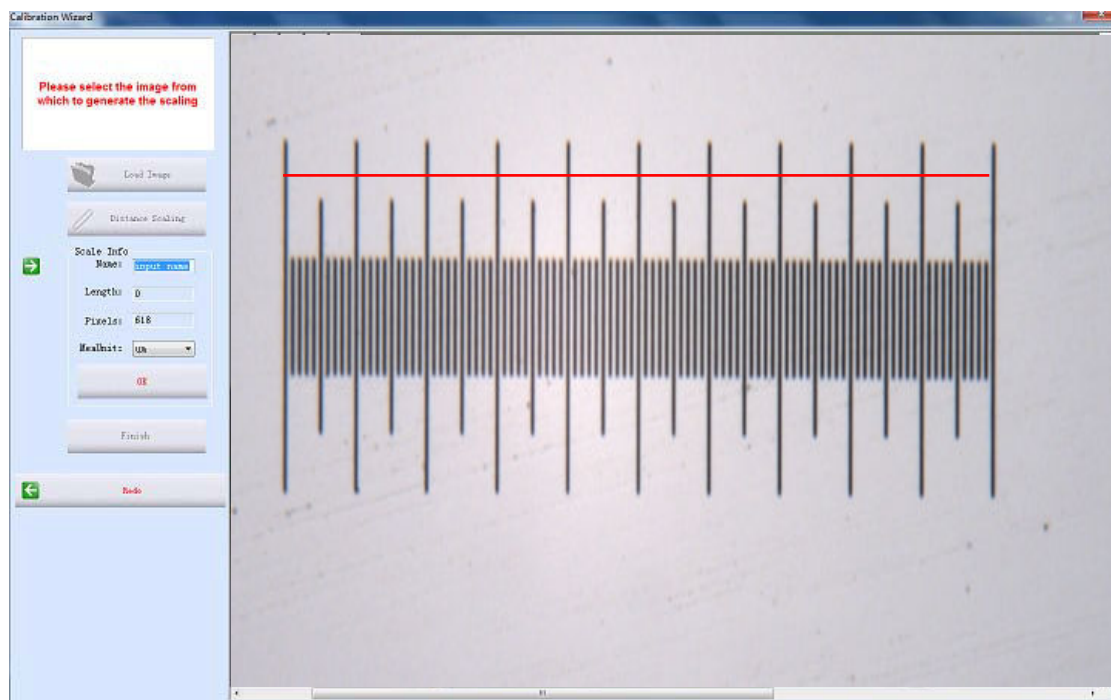

In this picture the line represents – with a 1mm/100 micrometer calibration slide with 10 µm divisions – in reality 100 divisions x 10 µm = 1000 µm long

5.
Select the measurement unit for the value "Length".
In microscopy we usually use 'µm' to specify distances

Enter in the field with the label 'Name", the total magnification e.g. '40x' (or alternatively the magnification of the objective e.g. '4x') at which the picture has been taken

Enter the real distance representing the line (in our example = 1000
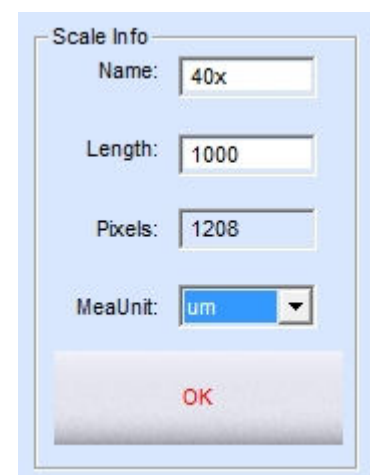
6. Click [OK] to confirm the calibration and click "Finish".
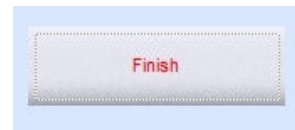
The new calibration value for "40x" will be created in the [Calibrate Table]

As you can see in table above, the calibration value is equal to 0.8278 μm/pixel

7 Repeat steps 2 to 7 as many times as there are pictures that you took during step 1

Example: with 10 objective and 10x eyepiece = 100x total magnification

Example: with 20x objective and 10x eyepiece = 100x total magnification

Example: with 40x objective and 10x eyepiece = 400x total magnification

Example: with 100x objective and 10x eyepiece = 1000x total magnification

Perform a measurement on an image

1   In the [Image] tab, select an image on which you want to realize a measurement

    Example: image taken with the 20x objective and 10x eyepiece of the microscope

2   Click [Calibrate Table] to open the calibration table

3   Select the magnification that corresponds for the selected image

Name	Length	TotalPixel	Unit	Unit/Pixel
default	1.00	1.00	pixel	1.0000
40x	1000.00	1208.00	um	0.8278
100x	400.00	1209.00	um	0.3309
200x	150.00	906.00	um	0.1656
400x	50.00	625.00	um	0.0800
1000x	30.00	904.00	um	0.0332

Calibration Table — Apply to Image | Close

4   Click on "Apply to image" and "Close"

5   Choose one of the available measurements

    Line
    Parallel line
    Perpendicular lines
    Rectangle
    2point Circle
    3Point Circle
    Diameter Circle
    Concentric Circle
    Polygon
    Arc
    Angle
    Point
    Remark (for adding a label with remarks)

Measure

Lock
Delete
Select
Line
Parallel
Perpendicular
Rectangle
2Point Circle
3Point Circle
Diameter Circle
Concentric Circle
Polygon
Arc
Angle
Point
Remark
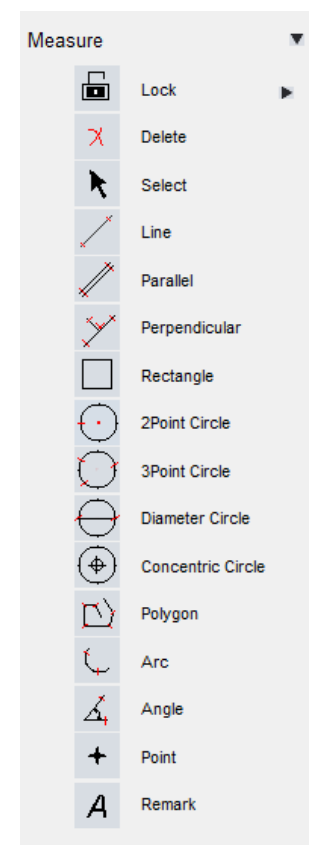
6   Perform your measurement on the image

Measurement List   -

Select   Measurements List

Export the measurement data to .txt file

Export the measurement data to Excel file

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

All measurements done on the image is kept in this table. You can export all the measurement data to the TXT or Excel file

Measurement properties
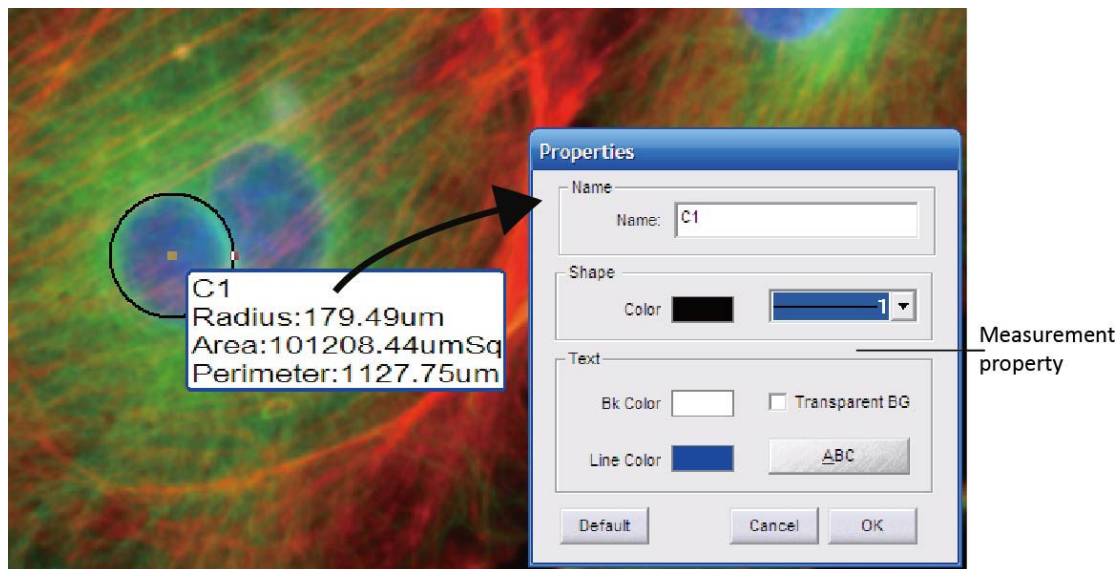
Double click on the measure to edit the properties of a measurement. You can change the measure properties like name, color, thickness, background color and the character font, etc …
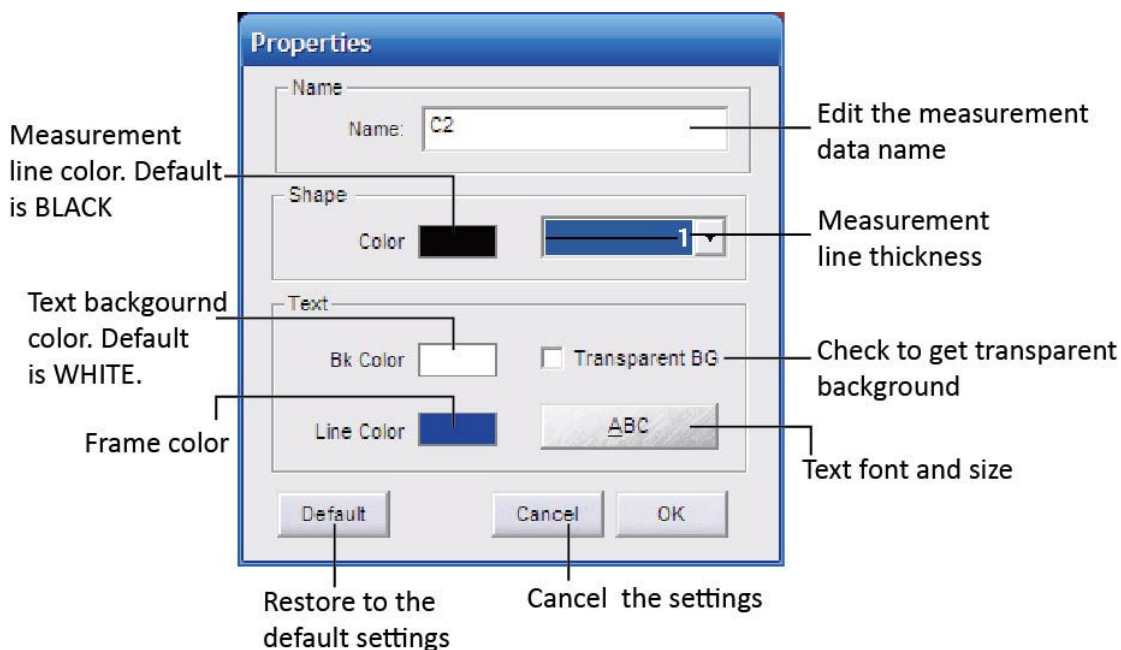
Browse tab

Under the [Browse] tab, you can see the image File name, capturing time, data depth (bit), picture resolution and image size. It also allows to add comment to any individual image

Under the Image Tab there are some quick functions on the right hand side of the software

100.0%

—————————Rotate right

—————————Rotate left

—————————Zoom in

—————————Zoom out

—————————1:1 ratio display the image
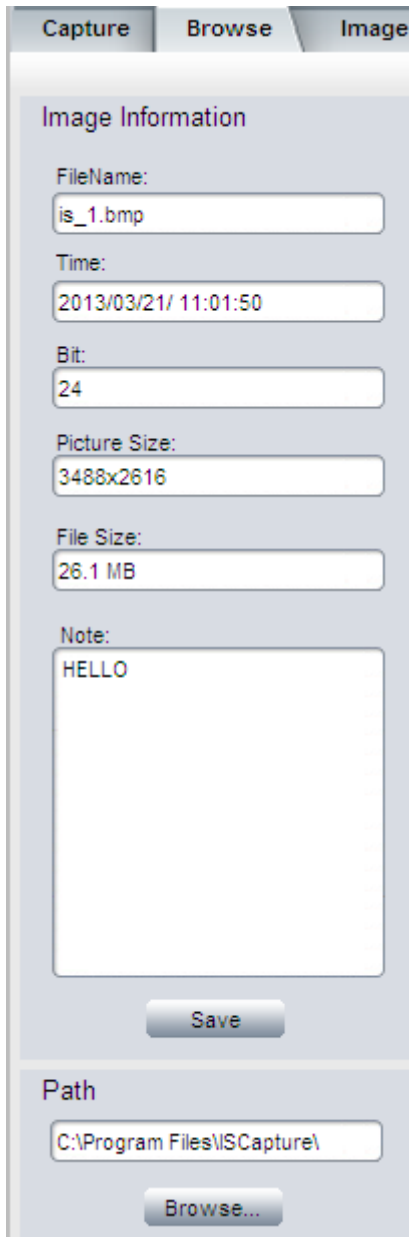
—————————Best fit the screen display

—————————Move the image to see different part of the image

—————————Cut interested area. Select interested area and double click it to confirm the selection

—————————Restore to original image

—————————Delete image

—————————Print out image

—————————Browse image folders

—————————Save image

—————————Save as...
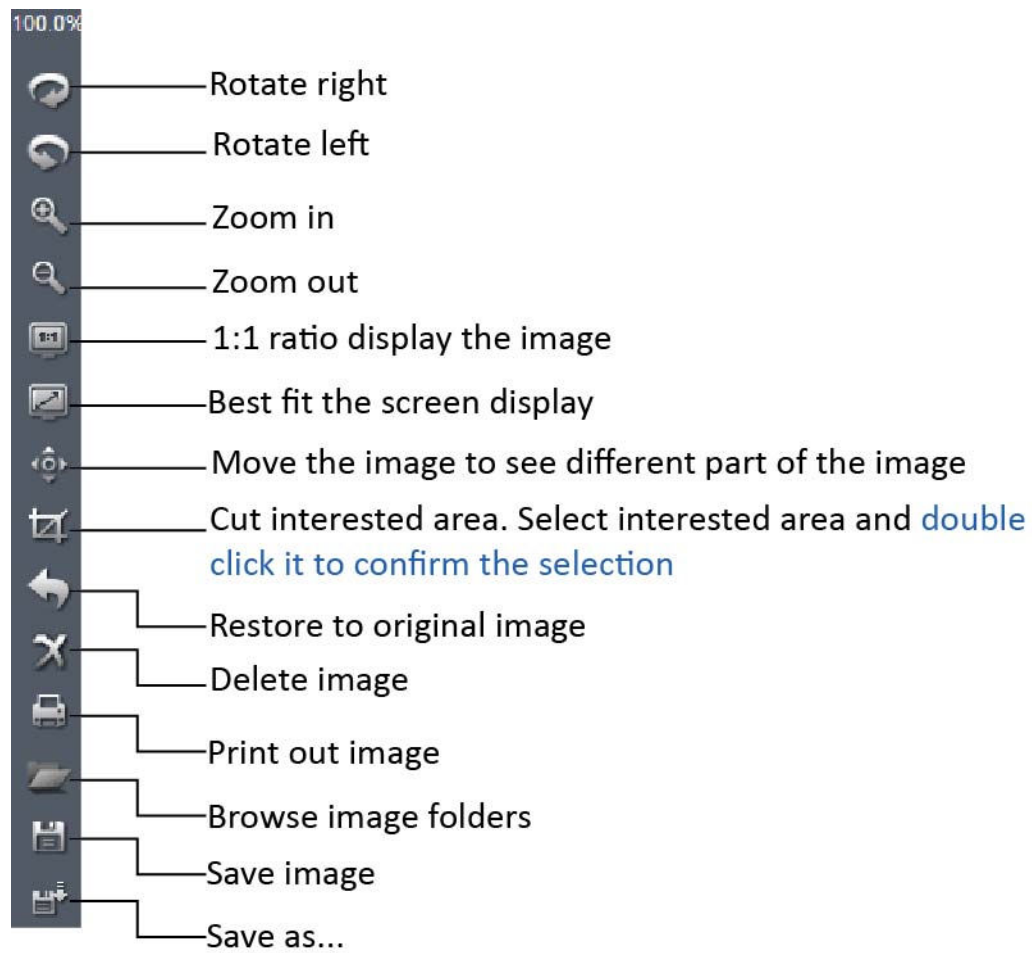
Image Processing

CAUTION

When you click [Apply], all the changes are applied to the image ! These changes **CAN NOT BE RECOVERED**

Provide some basic stilled image processing functions and allows to extend the Depth of Focus.

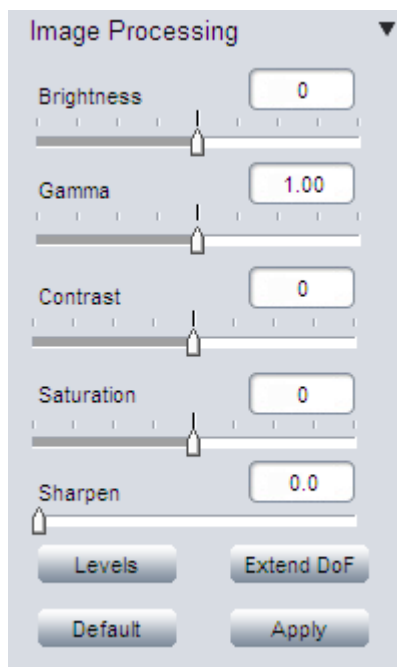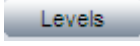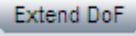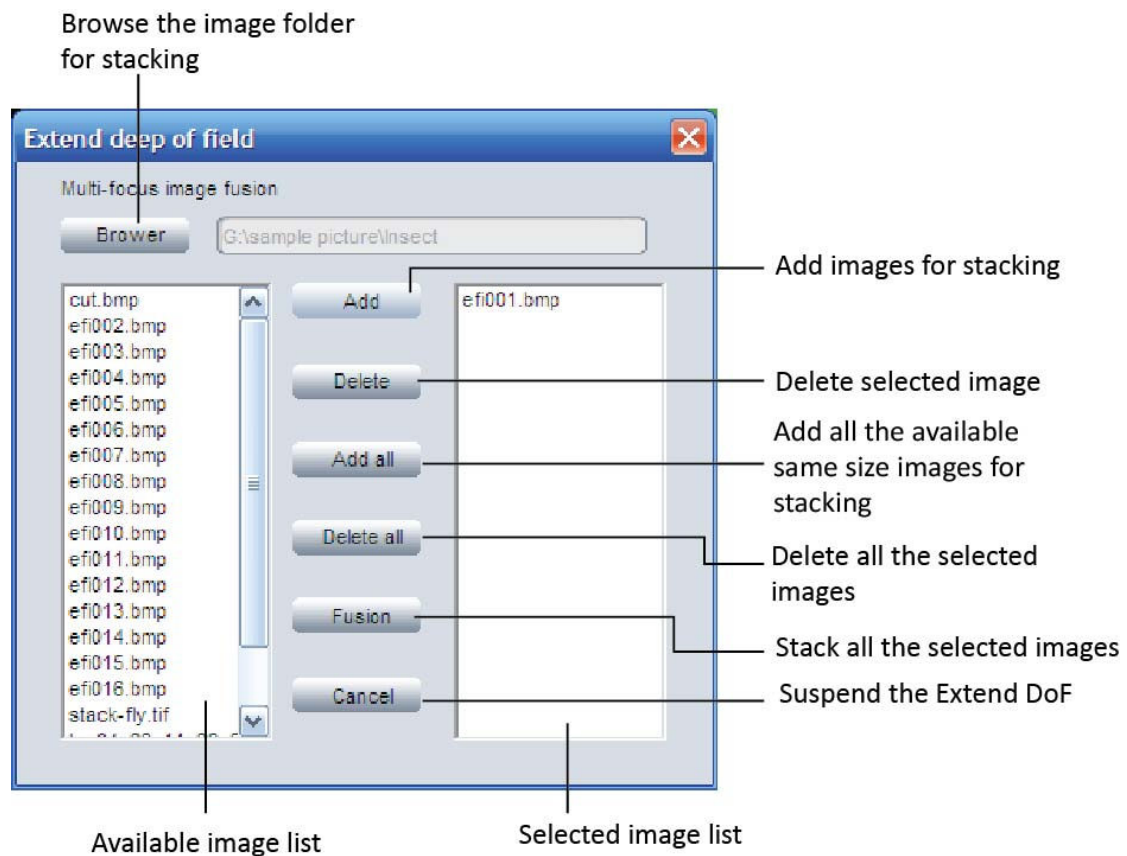|	
Brightness	Adjust captured image brightness. Default brightness = 0
Gamma	Adjust captured image gamma. Default gamma = 1.00
Contrast	Adjust contrast. Increase the contrast, the shadows become darker and the highlights brighter. Decrease the contrast, the highlights grow dim and the dark areas lighten 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)
Default	Restore Brightness, Gamma, Saturation, Sharpen and levels back to the default value
Apply	Confirm to apply all the settings to the image.

Histogram correction

Click on [Levels] to get the image histogram of the image. This represent the intensity distribution of all the pixels of the image
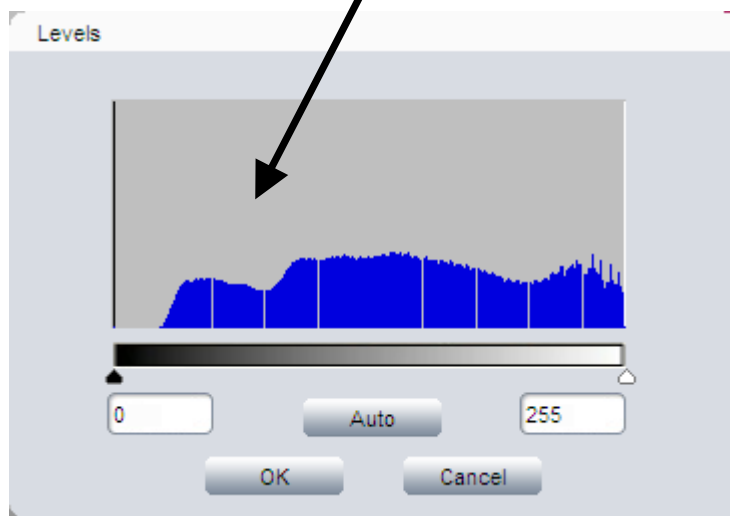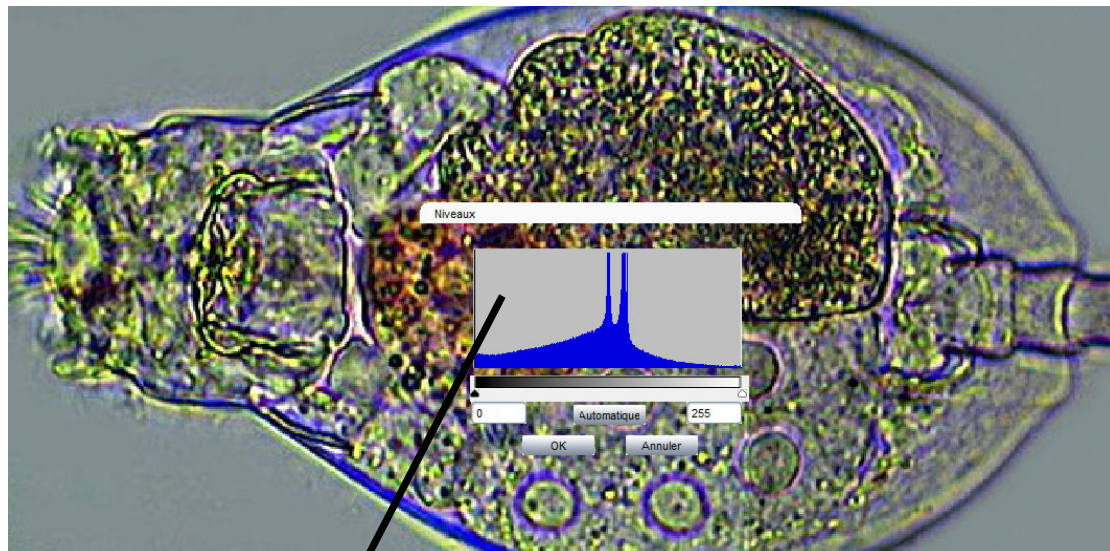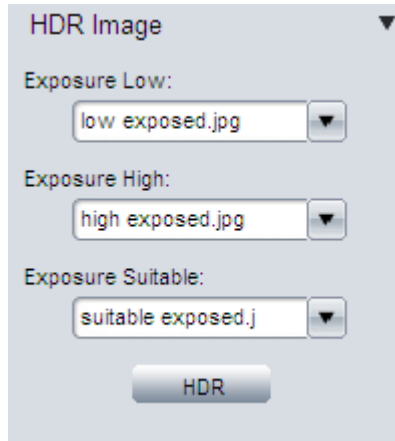

You can move the back cursor to the right and move the white cursor to the left in order to correct the contrast by applying a mathematical transformation on each pixel of the image

Extend depth of focus or stacking of images

Click [Extend DoF] Extend DoF to get the dialog for stacking images. Select the images to stack and apply the function.

Browse the image folder
for stacking

Extend deep of field

Multi-focus image fusion

Brower   G:\sample picture\Insect

Add images for stacking

cut.bmp
efi002.bmp
efi003.bmp
efi004.bmp   Delete   Delete selected image
efi005.bmp
efi006.bmp   Add all the available
efi007.bmp   Add all   same size images for
efi008.bmp   stacking
efi009.bmp
efi010.bmp   Delete all   Delete all the selected
efi011.bmp   images
efi012.bmp
efi013.bmp   Fusion   Stack all the selected images
efi014.bmp
efi015.bmp   Cancel   Suspend the Extend DoF
efi016.bmp
stack-fly.tif

Add   efi001.bmp

Available image list   Selected image list

HDR High Dynamic Range imaging

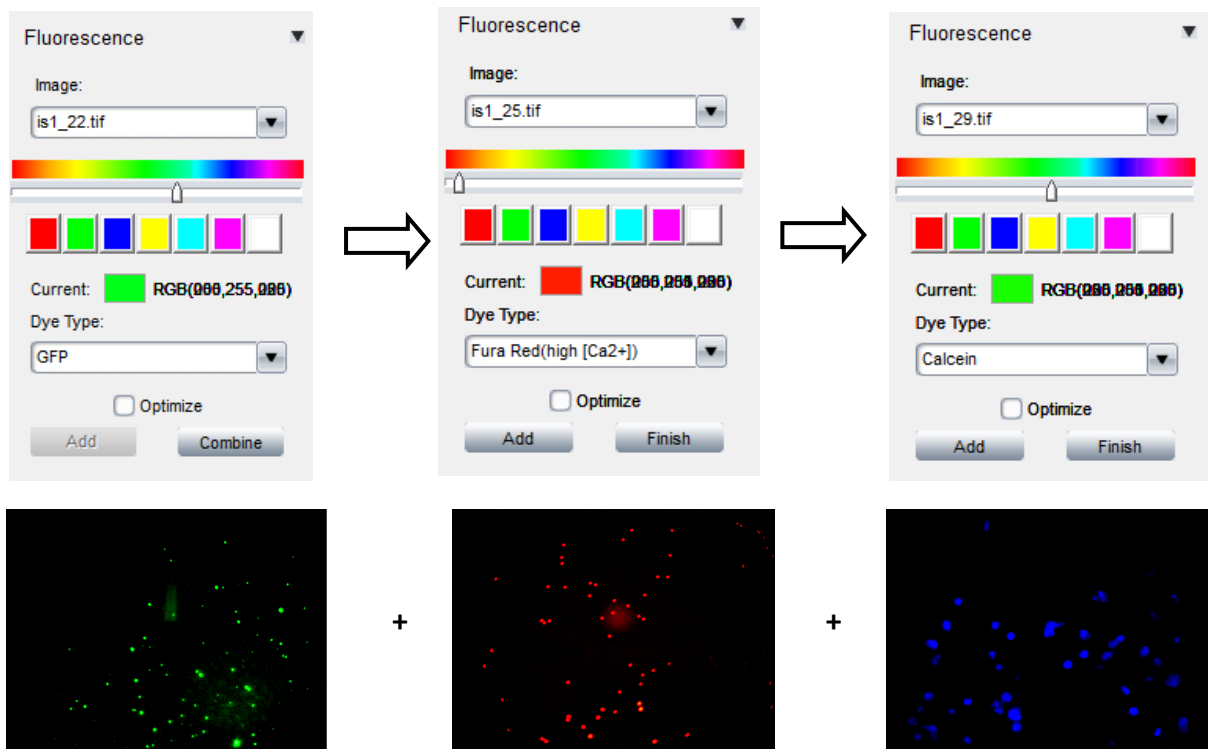High Dynamic Range (HDR) image function is used to get higher dynamic image

- Take pictures of the same scene with different exposure times and load them in ImageFocus 4.0

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

- Click [HDR] button to combine different exposed images into one. The generated HDR image will be named as "hdr_image"

Image - Fluorescence

This function is used to assign an image taken with fluorescence with a fluorophore and to combine different images into one combined image

Note that all mages MUST have the same size in order to be combined   !

a. Select the first image   (exemple below: is2_22.tif)
b. Select from the list a fluorophore that have been used for this image
    You can also select a color instead of the name of the fluorophore
c. Click 'Combine'

| Is1_22.tif | is1_25.tif | is1_29.tif |
| GFP | Fura Red | Calcein Blue |

d. Select the next image (example is2_25.tif)   **=**
e. Choose from list a fluorophore or a color
f. Click 'Add'

g. Repeat steps d to f for each image to combine
h. To end the operation, click 'Finish'
    The resulting image is called 'combine'
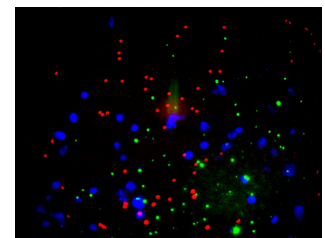
combine.tif

 The optimize checkbox can be also selected during the combination