

1. How to start

1.1.Start

MikroCamLabII (referred to as MCLII) can be opened by double-clicking on the corresponding symbol.

You can also open MCLII from the Start menu and the corresponding icon.

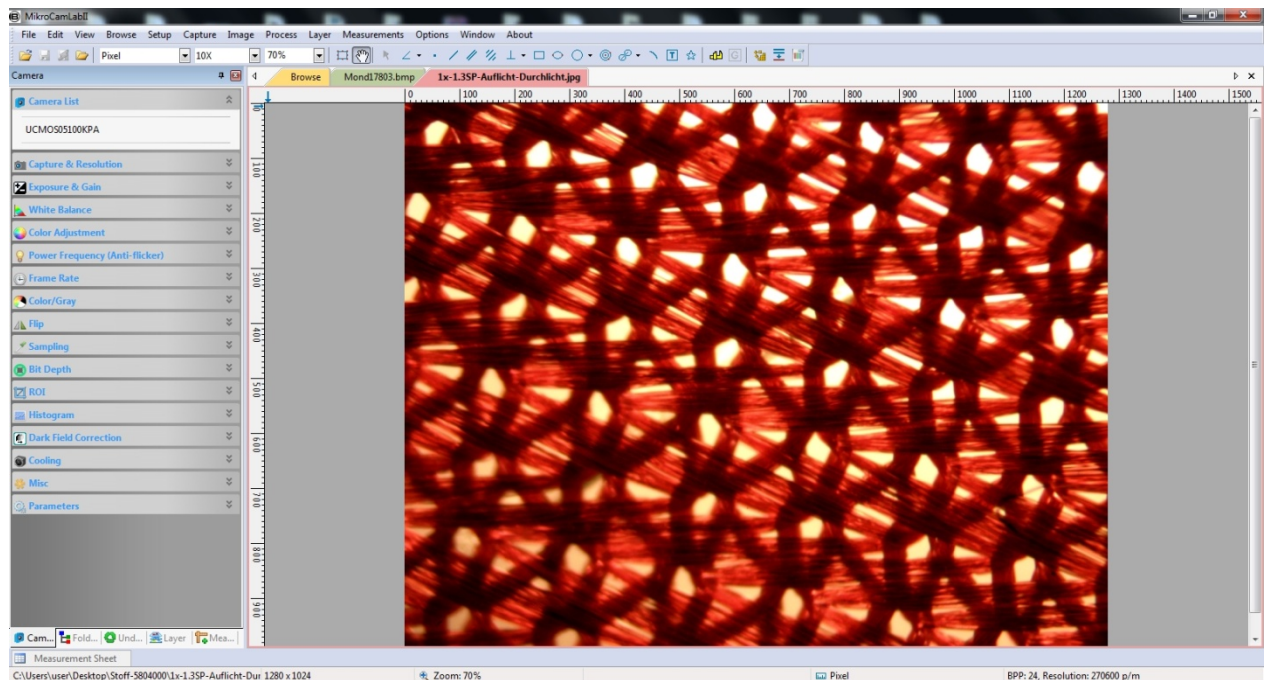
1.2 Open the image/video window

MCLII detects all installed cameras on the computer. These are listed in the camera list with the respective name.

Selecting the camera list> MikroCamXXX opens the video window with the label.

The camera is connected via the USB port of your computer / laptop.

2. Video window



2.1 Video window

A: MikroCamLabII

B: Menu

C: Toolbar

D: Camera Sidebar

E: Folders Sidebar

F: Undo/Redo Sidebar

G: Layer Sidebar

H: Measurement Sidebar

I: Measurement Sheet

J: Statusbar

K: Auto Hide Button

L: horizontal Ruler

M: Vertical Ruler

N: Frame Rate
O: Frames Captured
P: Current Video Sizes
Q: Selected Microscope Magnification
R: Current Unit
AA: Sidebar right mouse button context menu
AB: Video window right mouse button context menu
AC: Browse window right mouse button context menu
AD: Image window right mouse button context menu
AE: Frame window right mouse button context menu
AF: Double-click brings up Video properties dialog
AG: Double-click brings up Magnification dialog
AH: Double-click brings up Measurement dialog
AI: Horizontal Ruler or Vertical Ruler right mouse button context menu

2.2 How to close the video window

Double-clicking on the tab of the opened video window or by clicking on the >x in the same line closes the window.

To close the program completely, click the X in the upper right corner.

3. Image window

3.1 Image window

A: MikroCamLabII
B: Right mouse button will open the Statusbar context menu
C: The opened file name und directory
D: Double-click will open file properties dialog
E: width and height of the current image
F: Double-click will open the Scale Image dialog
G: Image Zoom ratio, double-click will zoom the image to 100%
H: Right mouse button will open the Zoom Ratio context menu
I: Currently selected Unit
J: Double-click will open the Measurement dialog
K: Double-click will open the resolution settings of the current image
L: Double-click will open the Resolution Settings
M: Right mouse button will open the Unit context menu

3.2 How to close the image window

3.2.1 Tabbed window

- a) If you have edited an image and want to close it, double-click on the tab, or click on the >X in the same line a message window. If you select **YES**, the changes are saved under the same filename and the window is closed. Clicking **NO** will close the window without further reference. **CANCEL** will abort the closing of the window.
- b) If the opened image is a snapshot of the video window, double-click on the tab, or click again on the X in the same line to open a dialog window. If you select **YES**, the **Save As** dialog opens:

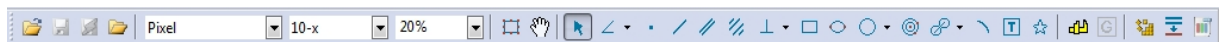
Select the drive and the folder where you want to save the image file and assign a filename. Then click **Save** to save the file with the assigned name in the selected directory. Click **CANCEL** to close the **Save As** window and return to the Picture window. Note: Selecting **Close window** -> **All** will close all open tabs.

3.2.2 MDI window

- a) For an opened and edited image window, clicking >**X** in the upper right corner will result in the same operation. The further steps are the same as for the open tab in 3.2.1 a
 - b) For a captured image from a video, clicking >**X** in the upper right corner will result in the same operation. The other steps are the same as for the open tab in 3.2.1 b
- Note: Selecting **Window** -> **Close All** also closes the video window.

4. Toolbar

When the camera is started or an image is opened, most options are activated in the toolbar. This allows quick processing.

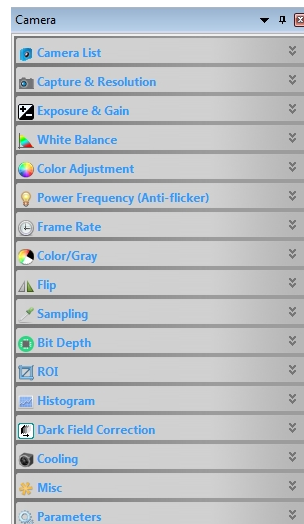


- 1: Open (CTRL + O)
- 2: Save (CTRL + S)
- 3: Quick Save
- 4: Brows (CTRL + B)
- 5: Unit
- 6: Magnification
- 7: Zoom
- 8: Video/image Select
- 9: Track (enabled if the video / image size is larger than the window)
- 10: Object Select: (enabled if an object is opened on a background layer)
- 11: Angle
- 12: Point
- 13: Line
- 14: Parallel
- 15: Two Parallel
- 16: Vertical
- 17: Rectangle
- 18: Ellipse
- 19: Circle
- 20: Annulus
- 21: Two circles
- 22: Arc
- 23: Text
- 24: Polygon
- 25: Calibration
- 26: Gray Calibration

- 27: Manual Fusion
- 28: EDF
- 29: HDR

5. Camera Sidebar

The camera sidebar is used to control the MCLII camera and contains 17 groups. The individual groups contain several functions, which can be opened by clicking on the label.



5.1 Camera List

The camera list shows all connected cameras, which are supported by MCLII. Clicking on the camera name starts the video window.



5.2 Capture & Resolution

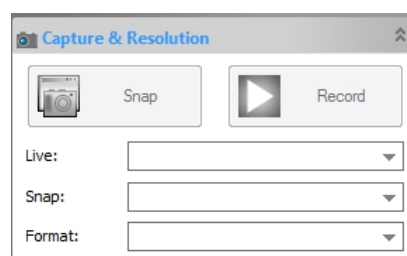
Snapshot: Draws a snapshot every time you click,

Recording: Starts a video recording in the formats wmv / asf or avi

Live: Select the video resolution

Snapshot: Select the snapshot resolution

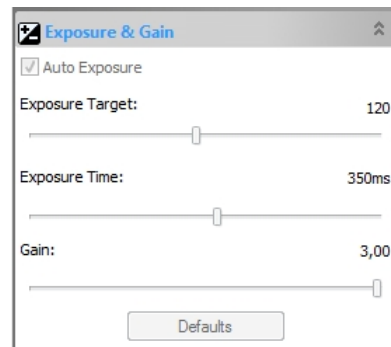
Format: Specifies the format (RGB / RAW)



5.3 Exposure & Gain

Auto exposure is preset. If you remove the hook, set to manual exposure. Here you can set the exposure time and the gain separately.

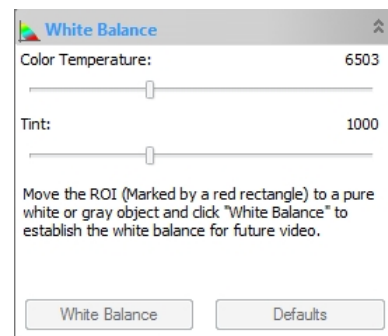
Note: High gain results in increased camera noise. A long exposure time reduces image repetition.



5.4 White Balance

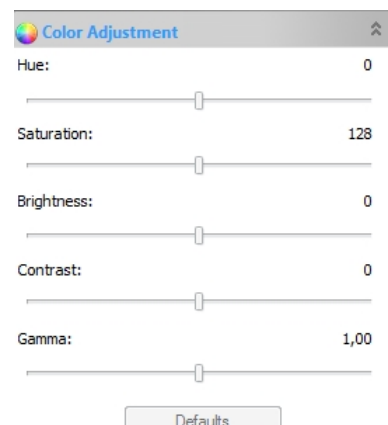
Click on the **White Balance** button to adjust the color of the camera to the specimen. You can adjust the temperature and hue manually to make further adjustments. The **Preferences** button returns the adjustments to the initial values.

Note: For Exposure and White Balance, you will be offered a frame that you can move and resize. For highly colored preparations, select an area without sample material for better adaptation.



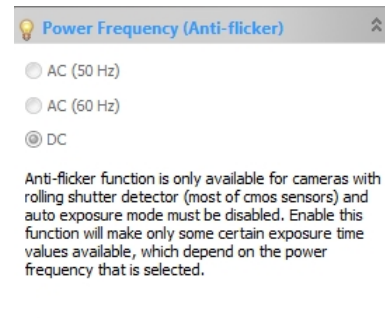
5.5 Color Adjustment

By moving the individual controls, you can adjust the image according to your needs. The **Preferences** button returns the settings to the initial values.



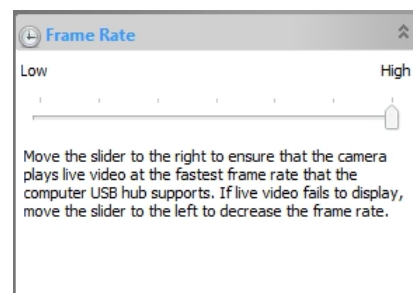
5.6 Power Frequency

If the picture is flickering, adjust the frequency of the current source. Enable the anti-flicker function.



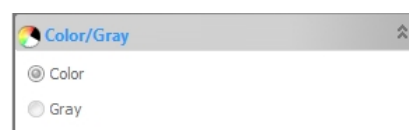
5.7 Frame Rate

Change the frame rate, depending on how your laptop / PC can process the refresh rate.



5.8 Color/Gray

Select whether to display the image in color or grayscale.



5.9 Flip

If the image is displayed inverted in the video window, you can rotate it with this function to fit the eyepiece image.



5.10 Sampling

For low resolutions, you can set to merge pixels. (Not available on all devices.)



5.11 Bit depth

Here you can adjust the depth of color in the video / image window. It can be differentiated between 8 bits and 16 bits.



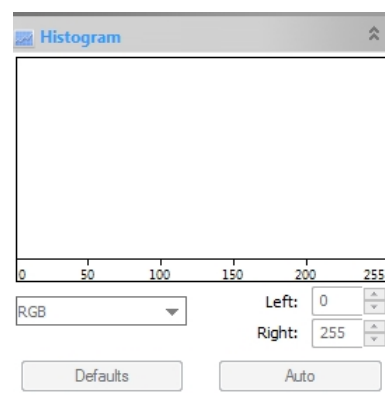
5.12 ROI (Region of Interest)

The image is surrounded by a frame which can be positioned as arbitrary. This is to cut out certain areas of the image.



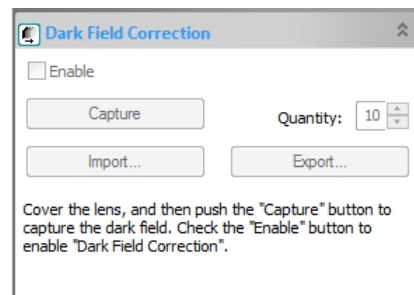
5.13 Histogram

The histogram shows the different color channels. Move the left and right bounding lines, or enter an appropriate value. The **Preferences** button returns the settings to the initial values.



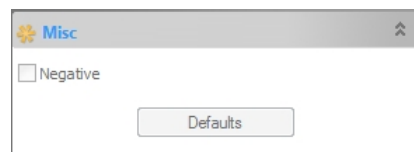
5.14 Dark Field Correction

The function can create a dark image (the camera must be covered). This image is subsequently subtracted from the actual recording. In the case of long-term exposures, the image noise or artifacts such as hot pixels can be reduced.



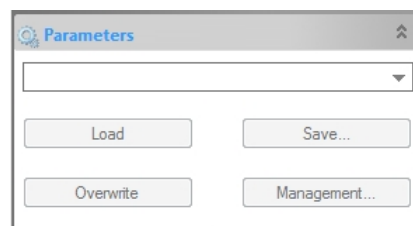
5.15 Miscellaneous

The picture / video is displayed as negative.



5.16 Parameters

If you want to save your image settings, click **Save**. Under **Management** you can edit the stored values.



6. File

6.1 Open Image: Open an existing image file

6.2 Open Video: Open an existing video file

6.3 Open Broadcast: A live video / image of another MCLII user can be displayed (only possible if the Internet connection is active)

6.4 Save: Save the current image

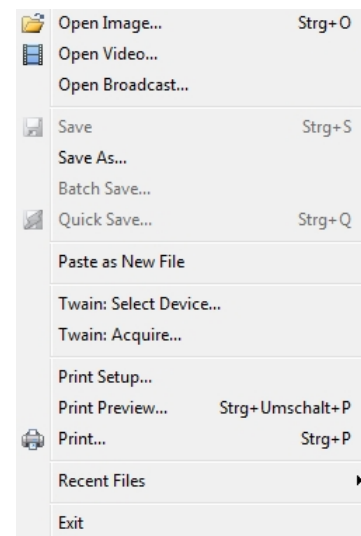
6.5 Save As: Save the current image with different name and format

6.6 Batch Save: Saves every open file with current name and file format

6.7 Quick Save: Quick save

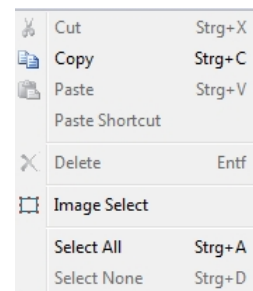
6.8 Paste as new file: Paste the image file from the clipboard as a new file

- 6.9 Twain:** Select device: Select cameras connected via a Twain interface
- 6.10 Twain:** Acquire: Image preview of a twain device
- 6.11 Print Setup:** Set up the printer
- 6.12 Print Preview:** Shows the print preview
- 6.13 Print:** Will print a open image
- 6.14 Recent Files:** Shows recently opened image files
- 6.15 Exit:** Will close MCLII



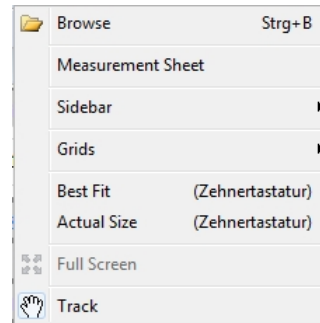
7. Edit

- 7.1 Cut:** Cut out a part of the image
- 7.2 Copy:** Copy a marked zone of the image
- 7.3 Paste:** Paste a marked zone of an image
- 7.4 Add an Abbreviation:**
- 7.5 Delete File:** Delete a marked file in the browse window
- 7.6 Select Image:** Allows you to drag a selection frame
- 7.7 Select All:** Drags a selection frame to the whole picture
- 7.8 Select None:** Cancels the selection frame



8. View

- 8.1 Browse:** Select an image from the Folders Sidebar
- 8.2 Measurement Sheet:** Shows the measured values
- 8.3 Sidebar:** Show or hide additional status bars in the left sidebar
- 8.4 Grids:** Show or hide different kind of grids
- 8.5 Best Fit:** Shows the current image with the best fit
- 8.6 Actual Size:** Shows the image in 100% size
- 8.7 Full Screen:** Shows the image on the full screen
- 8.8 Track:** The image is free to move if it is larger than the window



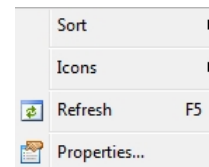
9. Browse

9.1 Sort: Sorted images in folder view according to different criteria

9.2 Icons: The icons can be shown in small or large

9.3 Refresh: Refresh image files in the current directory

9.4 Properties: Shows the properties of a marked image



10. Setup

10.1 Start/Pause: Start/Pause the Live view

10.2 Show Properties: Shows the properties of the Live view

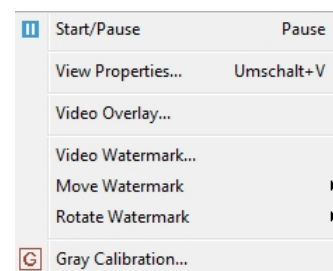
10.3 Video Overlay: Add additional fades to the image

10.4 Video Watermark: Provides the possibility to select an image file (bitmap) and insert a watermark. The watermark appears in the live image as well as in the captured video

10.5 Move Watermark: You can change the position of the Watermark in the image

10.6 Rotate Watermark: You can rotate the Watermark in the image

10.7 Gray Calibration: You can fit the brightness of the Watermark

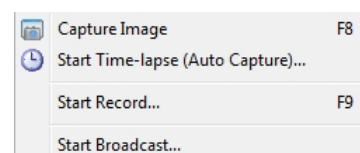


11. Capture

11.1 Capture Image: You can capture the video image

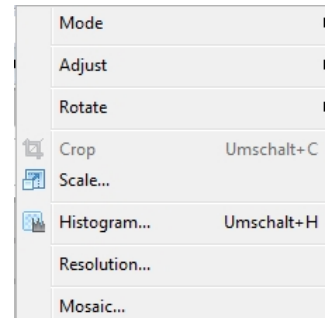
11.2 Time-Lapse: With the time-lapse function, you can automatically record multiple images at a given time interval. The time interval can be set between 2 and 3600 seconds. The maximum number of taken pictures is 9999. In addition, you can give the images a description with time and a name. Choose a suitable file format. Look for available hard disk space.

11.3 Start Record: Start recording a movie (video format can be wmv/asf)

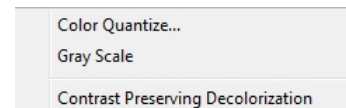


12. Image

If you choose **Image** the following dialog window will open



12.1 Modus: You can set Color Depth und Gray Scale



12.2 Adjust:

12.2.1 Brightness/Contrast: You can set Brightness and Contrast of the image

12.2.2 Color: You can set the color value

12.2.3 HMS: You can set Height/Medium/Shadow

12.2.4 Curve: You can manually correct the image with the mouse

12.2.5 Filter Color: You can set a Color Filter

12.2.6 Extract Color: Show single Color Channels from a color image

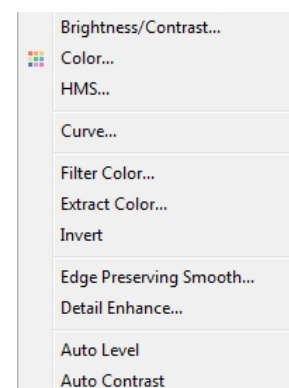
12.2.7 Invert: The image will be shown as a Negativ

12.2.8 Edge Preserving Smooth:

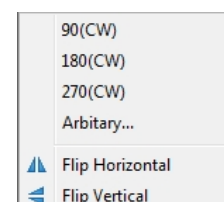
12.2.9 Detail Enhance: You can change the detailed view of the image

12.2.10 Auto Level: Auto assimilation of brightness

12.2.11 Auto Contrast: Auto assimilation of contrast

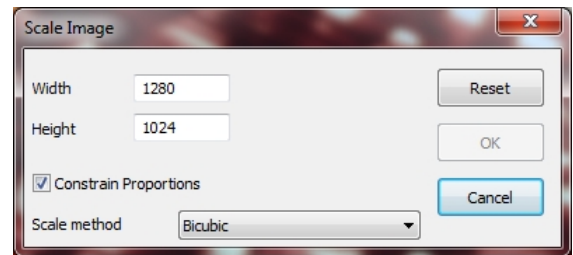


12.3 Rotate: Rotates the image in different levels

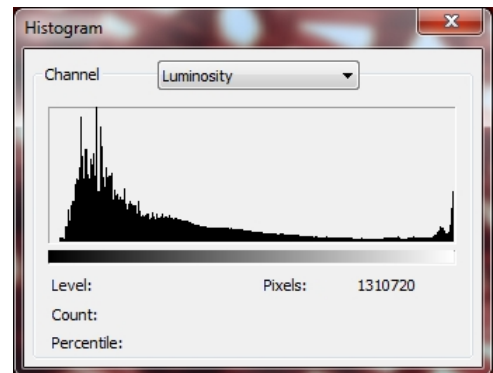


12.4 Cutting: You can cut a display detail from the image

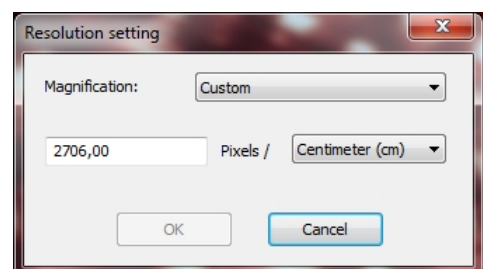
12.5 Image Scale: You can scale the image to another size



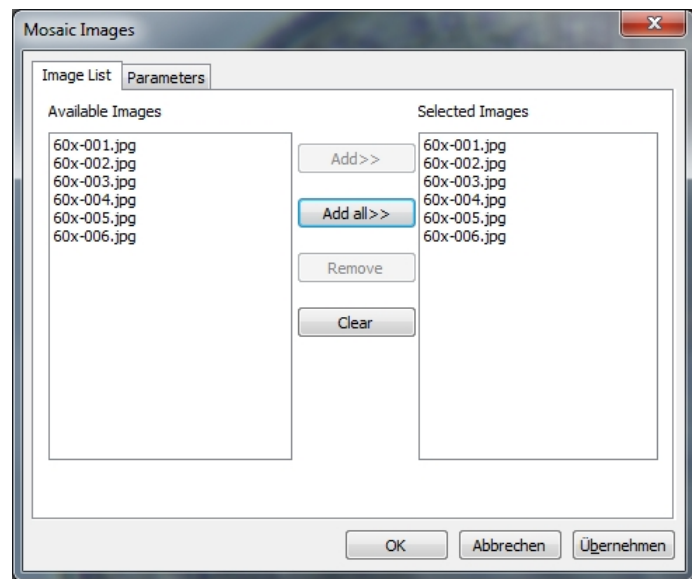
12.6 Histogram: Shows the different color channels of the image



12.7 Resolution: You can change the resolution of the image



12.8 Mosaic: You can combine open images of the same series to a new image



12.8.1 Image List:

Available Images: Shows the opened images in MCLII

Add: Add open images to the selected ones

Add All: Add all open images to the selected ones

Remove: Remove a selected image

Delete: Delete every selected image

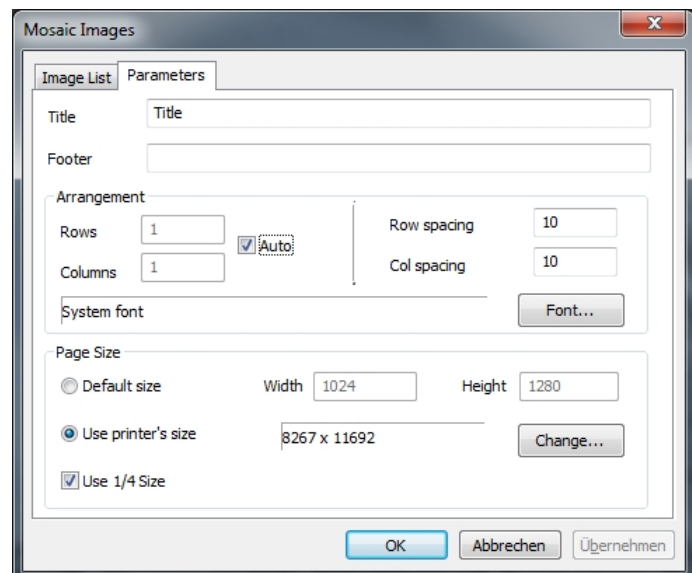
12.8.2 Parameter:

Title: Assign a individual title

Foot: Assign a individual foot

Arrangement: Distribution of the mosaic images on the page

Page Size: Page size of the mosaic images



13. Process

13.1 Assembly

This function offers the possibility to combine several images from one specimen to a more detailed one. (For this purpose, several images of an image series must be marked)

13.2 High Definition Region

This function provides the possibility to display an image as a high-contrast image and reproduces the brightness differences in detail.

13.3 EDF (Manual Fusion)

This function offers the possibility to combine images of a series using 3 different methods.

- **Maximum Contrast**
The images are combined in such a way that the final result is a picture that is as high as possible. The elements get sharper, but some details are lost. This method is the preferred of the 3 ways.
- **Weighted Average**
The images are combined in such a way to create gliding transitions.
- **FFDSSD Method**
The reference image will be determined automatically

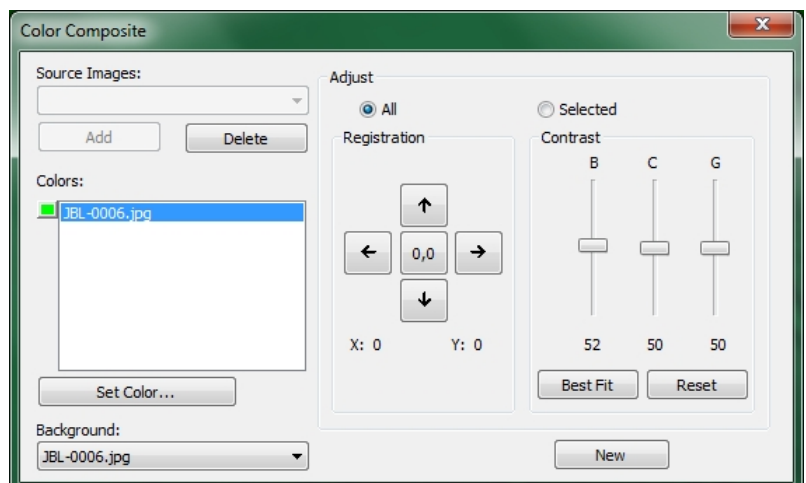
13.4 Deinterlacing

No description

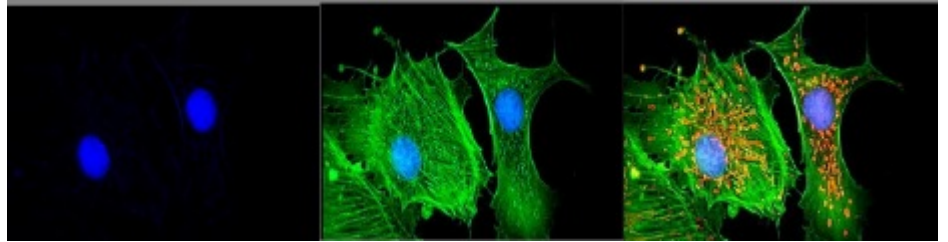
13.5 Color Composite

Use the Color Composite dialog box to create and configure color compositions with black-and-white images. (Function is only active if a B/W image is selected)

Use this function to combine grayscale images into a color compound. Each group of gray scale images which are of the same size, can be mixed in a color composite. Images of 8, 12, 16-bit integer or floating-point formats are combined into a 24-bit color format. Each input channel has individual LUT settings and a registration offset to access it with the other images.



You can also combine individual channels from a single image. Each channel is listed separately, as shown below:



Source Images: The Source Images combination field displays the images available for color mixing. The source image list is initially filled with a list of currently opened grayscale images. The size of the color composite is selected by the first input image. Once entered, the list is filtered to obtain only images of the same size as the initial selection.

Add: Click **Add** button to add an image to the mix. You are called up to associate the color to the image from the Color dialog box.

Delete: You can use this function to delete selected images from the list.

Color: The Color Box shows the images selected to Color Mixture.

To change the color in the list box, double-click the picture name (the selected color is shown in the dialog box), or highlight the name and click the Colors button. To delete colors/images from the Colors list box, first click the color/image to highlight it, and then press the Delete button.

Color Bars: The image position field on the left side of the color list shows the color assigned to each image. The Color List scrolls with the contents of the Colors field.

Set Color: This option determines the color value used for the selected channel in the final image display. When you click the Set Color button, the Select Color dialog box appears.

You can choose a color with:

- 1) Drag the color slider to the desired color in the Color Bar (Range 0 to 359°)
- 2) Click the appropriate color button on the color palette. The color bar represents a subset of possible colors (pure red, green, blue, cyan, magenta, white)
- 3) Select a dye from the list and click the "Select Dye" button.

Note: You can navigate through the Select Color dialog box by using the <Tab> and <Shift + Tab> keys to scroll through the items in the dialog box.

The intensity is determined by the image to which the color is applied. The saturation is always 100%.

The only exception is the white color, which by definition has a saturation of 0%. Selecting a value ≥ 360 (dragging the slider to the right) is a choice of white, resulting in a non-colored (gray) image.

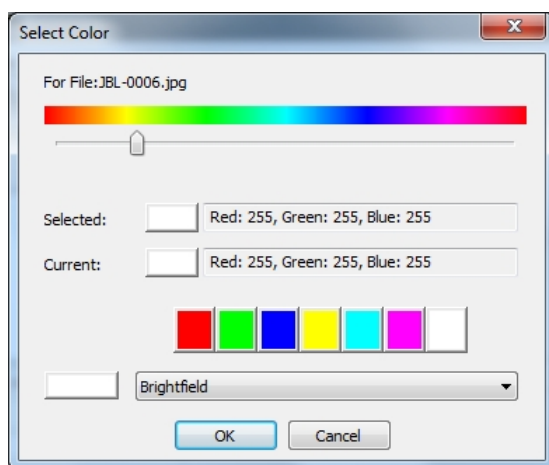
The elements of the Select Color dialog box are:

Color Bar: The Color Bar shows a rainbow displaying the colors underneath the appropriate point of the Color Value slider.

Note: If the Color Value Slider is selected/marked, then you can also control the slider with your keyboard strokes. Use the <Page Up>/<Page Down> or only the </> keys to move the slider along the Color Bar.

Selected: This color block displays the new color selected with the Color slider bar or color palette.

Select Color: The **Select Color** text field displays the corresponding numerical color value of the above-described color block. The selected color is displayed as an HSV color with the red value displayed first, followed by the green and blue values. If the color corresponds to a dye in the list, the dye name is displayed.



Currently: The **Current Color** text field shows the initially set color of the channel.

Color Palette: The Color Palette is an alternate method to select a color. When you click an item in the color palette, the color slider is set to the correct value, and the display is updated in both, the selected color block and the selected color box.

For example, if you click the white button on the color palette, the color slider is set to the right. The selected color changes to white, and the selected color text box displays white.

Adjust: The controls in this section are used to adjust the color composite contribution of each image, or to the color composite preview itself. You can customize the inserted image that is currently selected in the color list by clicking the selected button. If you want to adjust the color composite preview use the **All** button.

Registration: The registration allows you to correct errors of images caused by filter-specific optical shifts. Use the directional buttons to move the selected image in the x- and / or y-direction with respect to the rest of the input images. A click moves this channel one pixel in the specified direction. Empty areas are filled with black. Press the **0.0** key to re-center the selected image.

The registry shift for the selected channel or the overall image is displayed in the static text box below the directional buttons.

Contrast: Use the BCG slider **Best Fit** and **Reset** to adjust the contrast of the selected image.

BCG-Sliders: The brightness, contrast and gamma sliders resemble the contrast enhancement function in MicroCamLabII. You can set each channel individually or together with the **Select** or **All** buttons (to the left of the BCG sliders in the adjustment group).

Best Fit: Click this button to perform the best fit of the contrast in the selected area. The brightness and contrast settings are then set automatically (gamma is reset to 50). This method is identical to the **Best Fit** contrast setting of MCLII. The **Best Fit** function is useful for initial adjustments.

Reset: Pressing the reset button automatically resets the BCG settings. When the **All Modes** option is set to all, the BCG settings for the overall color image are reset.

Frame: Use the frame slider to adjust the frame of the selected image. (Please note the discussion of Color Composite and Image Sequences that follow this section.)

Background: Select one of the available images to display the background color in this combo box. The rest of the image planes are subtracted from the background image and display the foreground colors without mixing with the background. This is most effective for images where the background covers large areas and the other images contain smaller objects.

Background allows the other images to be displayed in front of the background images, minimizing color mixing. For example, MCLII can display a red dot on a blue background without changing the dot to magenta. If you select **None**, all images are mixed equable.

New: Click the **New** button to discard and restart the current color composite image.

The source image combination field is reset to include all grayscale images (including floating point fonts). When you select an image from the list, a new join is created with the same size as the first selection. As already mentioned, the source image list is filtered to display only images with the same size as the initial selection.

13.6 Segmentation & Count

Select Segmentation & Count if the segmentation and number of objects contained in the image should be measured. This function offers the user 6 methods. **Watershed (W)**, **OTSU Dark**, **OTSU Bright**, **RGB Histogram**, **HSV Histogram** and **Color Cube**. Initially, all methods are selectable, but as soon as one is selected, the others become inactive.

After the division, adhesions can occur. In this case, the objects can be separated manually using the **Split Objects** function.

When you have the desired results, you can use **Count Results** to view the statistics and analysis results.

After the end of the count, you have the option to return from step 2 or 3 to step 1 or 2 to repeat the split or to perform the **Split Objects** operation again. (Via the Undo / Repeat function in the sidebar)

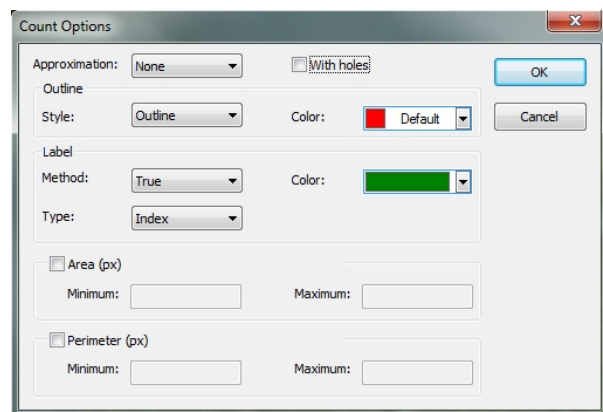
Note: If you selected **None** in the **Options** dialog box, no labels are specified for the metrics, area, or scope of the objects, regardless of the method that is selected. But **Circles** and **Ellipses** can be displayed in the results. This is why the menu is also called **Split & Count**. This function can either specify the segmentation of objects in the image, or the number of objects. In this context, we refer to **Process -> Segmentation & Count -> Watershed (W)** and the **Count Options**.

13.6.1 Watershed (W)

The Watershed method is a mathematical-morphological segmentation method based on topological theory. The basic idea captures the image as a topological geomorphology on geodesy, and each image pixel gray value indicates the height, the boundaries of each local minimum value of its impact surface as a catchment area, and the formation of catchment areas. The Watershed concept can be described as a simulation of the immersion method. A hole is drilled into every local minimum value of the surface and then the entire model is slowly immersed in water. Due to the dipping of the indentations and every local minimum, the indentation areas slowly expand outwards. From this arise two dams, which form the waterside.

The Watershed method is suitable for a relatively simple target object in the background, as well as target objects with a relatively large difference between the background images as opposed to the more complex application.

If you select **Process -> Segmentation & Count -> Watershed (W) ...**, the following note appears: The Segmentation & Count function works exclusively with 24 bits, otherwise all further menu items are deactivated. You can change the Color Bit settings to 24 bits under **Image -> Mode -> Color Quantize**.



Approximations

"Approximations" include 3 options:

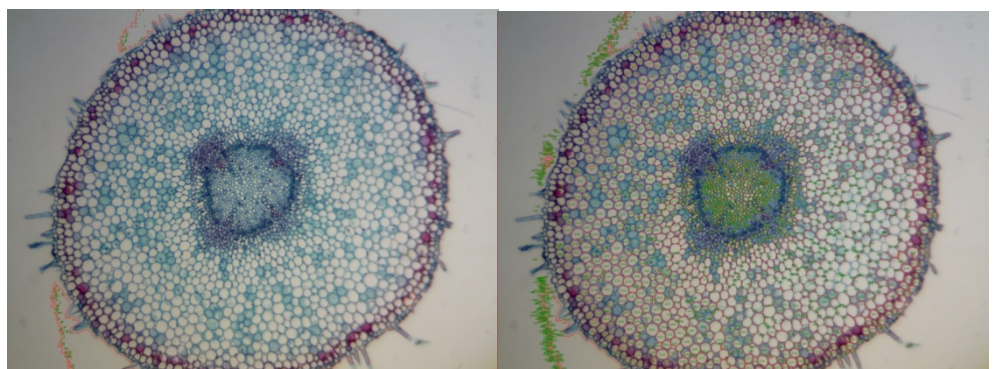
None: The actual outline of the segmented object is drawn.

Circle: A circular outline is drawn. The radius of each object is contained in the results of the count.

Ellipse: Contours are drawn in the form of ellipses. The axes of each object are included in the results of the count.

With holes

In the **With holes** check, the segmented object outlines are marked with color. Meanwhile, the holes are marked with color and their parameters are indicated in the picture (the holes are marked in grayscale, or similar to the background color). The left image is the result of segmentation without the **With holes** function, the right with the function. The **With Holes** function is usually used to obtain hole parameters.



Outline

"Style" includes 3 options:

Outside: The objects are marked by outlines. Its color can be determined by the user.

Filled: The objects are filled with custom color.

None: The objects are not marked by any outlines.

Label

"Method" includes 3 options:

XOR: The objects are marked with the color indicated for XOR pixels.

True: The objects are marked by the color you specify.

False: The objects are not highlighted.

Note: If you select **False**, no labels are specified for the metrics, area, or scope of the objects, regardless of the method that is selected. But **None**, **Circles** and **Ellipses** are displayed in the image. This is why the menu is called **Segmentation & Count**.

Color: The custom color is used to replace the color of the label. If you selected **XOR**, the color selection is not active, but the program marks the divisions in the current XOR pixel color.

"Type" includes 3 options:

Index: The listed objects are marked with numbers.

Surface: The object surface is marked by an outline.

Scope: The scope of the object is marked by outline.

Area

Minimum: Determine the lower limit of the area. That means, if the object area is smaller than the lower limit value, it will not be registered.

Maximum: Determine the upper limit of the area. That means, if the object area is larger than the upper limit value, it will not be registered.

Perimeter

Minimum: Determine the lower limit of the perimeter. That means, if the object perimeter is smaller than the lower limit value, it will not be registered.

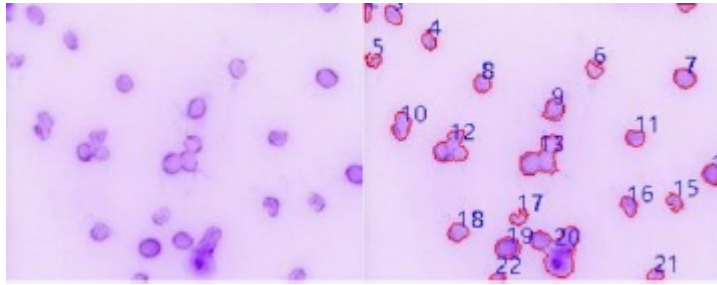
Maximum: Determine the upper limit of the perimeter. That means, if the object perimeter is larger than the upper limit value, it will not be registered.

13.6.2 OTSU Dark

OTSU was developed in 1979 by Japanese scientists and it is a method for determining an adaptive threshold, also known as the Otsu method. The method is based on the gradation characteristics of the image and divides the image into the background and the target. As larger the OTSU between the background and the target is, as greater the difference between the two images is. The wrong part of the background, or the wrong part of the target, will result in smaller differences between the two parts. Therefore, the maximum OTSU means the minimum probability of mis-classification.

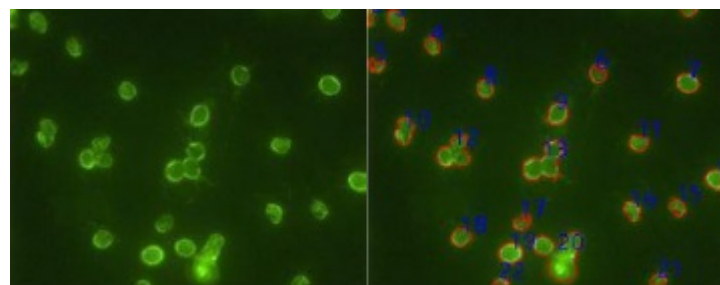
In the OTSU Dark segmentation method, the darker object is segmented from a light background using the OTSU algorithm. The counting options correspond to those of the Watershed method.

The image below shows a bright background with dark objects (left) and the result of the segmentation (right) as a reference.



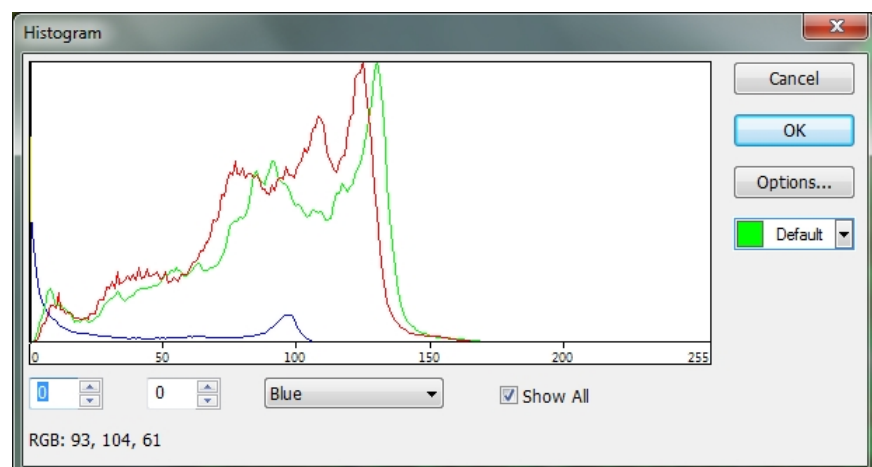
13.6.3 OTSU Bright (B)

In the OTSU Bright segmentation method, the lighter object is segmented from a dark background using the OTSU algorithm. The counting options correspond to those of the Watershed method.



13.6.4 RGB Histogram

The RGB histogram segmentation is based on the image histogram in which the image pixels are color-processed in a specific area in the histogram. The corresponding range can be defined by two vertical lines, or by values in the processing fields. The color that is representing the segmented region can be determined by the color selection. For a 24-bit true-color image, the segmentation can be performed by the respective R, G, B channels. The histogram window is displayed via **Process -> Segmentation & Count -> RGB Histogram**. Each function is given as follows:



The left value in the selection area of the histogram window corresponds to the position of the left vertical in the histogram. This can be changed by entering a value and the confirmation with the Enter key.

The right value in the selection area of the histogram window corresponds to the position of the right vertical in the histogram. This can be changed by entering a value and the confirmation with the Enter key.

The currently displayed color channel in the histogram can be selected here. It can be selected between R (red), G (green) and B (blue).

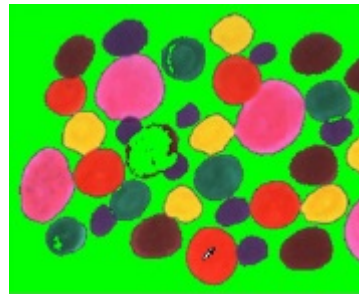
All color channels (RGB) are displayed in the histogram. If this is not selected, only one of the color channels is displayed.

Specifies the color in which the segmented pixels will be displayed in the image. The default is green.

The Counting options are opened via the Options button. The counting options correspond to those of the Watershed method.

Example of RGB Histogram:

1. Open an image, then select **Process -> Segmentation & Count -> RGB Histogram**. The histogram window is displayed. When you move the mouse to the image area, the mouse pointer is displayed as a color selection cursor (pipette).

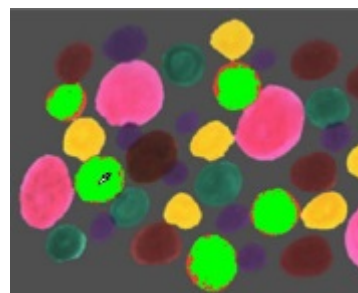


2. When you move the color selection cursor over the image, the RGB values are displayed in the bottom bar of the histogram window.
3. To set the target exactly, you can set the RGB values of the desired pixels in the initial interval. The RGB values can be reset to the initial interval as follows:

R initial interval: 200 ~255

G initial interval: 0 ~ 60

B initial interval: 0 ~ 60



4. When you move the color selection cursor back to any pixel, the RGB values are now displayed as 237, 70, and 45 in the example.

It is quickly found that the G-channel, here defined at 60, is too small to segment the pixel. The initial interval for G can be set from 0- 60 to 0- 80 to segment pixels in the vicinity.

Note: If the background pixel of a single channel is within the initial interval, the segmentation is not changed. Only if all three channels of the background pixels are within the initial intervals, the pixels can be segmented.

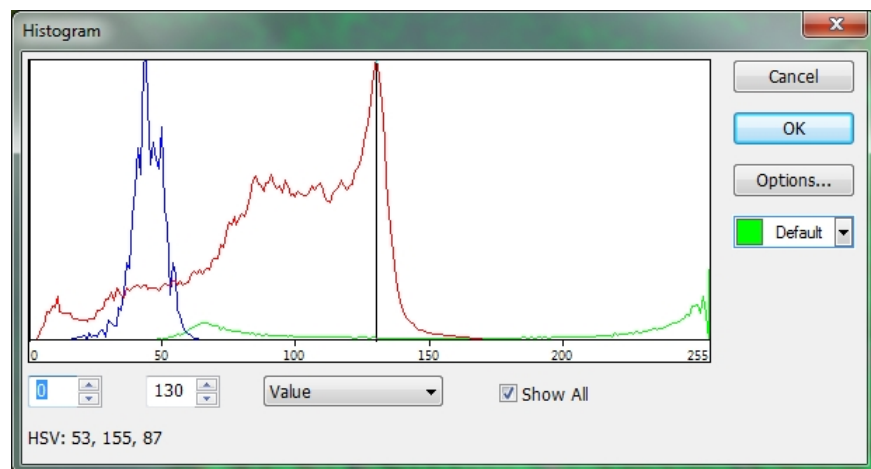
The segmentation effect caused by the readjustment of the initial interval of the G channel entry is shown below.

5. Repeat step 4 and adjust the initial interval of each RGB channel to achieve optimal segmentation.

13.6.5 HSV Histogram

The HSV histogram segmentation is based on the image histogram, in which the image pixels are color-processed in a specific area in the histogram. The corresponding range can be defined by two vertical lines, or by values in the processing fields. The color representing the segmented region can be determined by the color selection. For a 24-bit true-color image, the segmentation can be performed by the respective H, S, V channels.

The histogram window is displayed via **Process -> Segmentation & Count -> HSV Histogram**. Each function is given as follows:



The left value in the selection area of the histogram window corresponds to the position of the left vertical in the histogram. This can be changed by entering a value and the confirmation with the Enter key.

The right value in the selection area of the histogram window corresponds to the position of the right vertical in the histogram. This can be changed by entering a value and the confirmation with the Enter key.

The currently displayed channel in the histogram can be selected here. It can be selected from H (Hue), S (Satiation), and V (Value).

All channels (HSV) are displayed in the histogram. If this is not selected, only one of the channels will be displayed.

Specifies the color in which the segmented pixels will be displayed in the image. The default is green.

The Counting options are opened via the Options button. The counting options correspond to those of the Watershed method.

Example of RGB Histogram:

1. Open an image and select **Process -> Segmentation & Count -> HSV Histogram**. The histogram window is displayed. When you move the mouse to the image area, the mouse pointer is displayed as a color selection cursor (pipette).
2. When you move the color selection cursor over the image, the HSV values are displayed in the bottom bar of the histogram window.
3. When you move the mouse to the background area, the HSV values in the example are at 214, 87, 228. To set the target exactly, you can set the HSV values of the desired pixels in the initial interval. The HSV values can be reset to the initial interval as follows:

R Initial Interval: 60~200

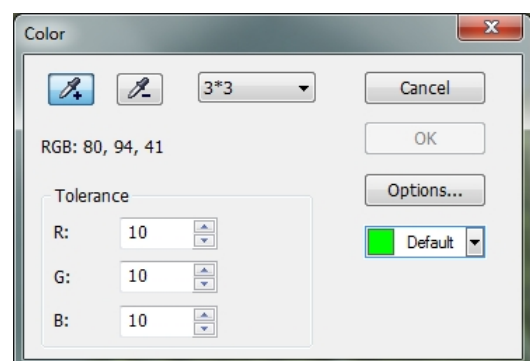
G Initial Interval: 90 ~130

B Initial Interval: 0 ~210

4. When you move the color selection cursor back to any pixel, the RGB values are now displayed as 194, 79, and 212 in the example.
It is quickly found that the S-channel, defined here at 90 ~ 130, is too small to segment the pixel. The initial interval for S can be set to 90 ~ 130 and 60 ~ 130 to segment pixels in the vicinity. (Note: If the background pixel of a single channel is within the initial interval, the segmentation is not changed. Only if all three channels of the background pixels are within the initial intervals, the pixels can be segmented.) The segmentation effect caused by the readjustment of the initial interval of the S channel entry is shown below.
5. Repeat step 4 and adjust the initial interval of each HSV channel to achieve optimal segmentation.

13.6.6 Color Cubic

Process> Segmentation & Count> Color Cubic opens a window named **Color**.



At this moment, the color pipette is activated by default. Click on the image to set a color value in the selected area as the basic value. MCLII will segment the image by selecting the pixel within the basic tolerance range and mark it with the currently selected color. By repeatedly clicking the target pixel, MCLII can continuously insert new pixels within the basic tolerance range into the target, thereby achieving continuous segmentation. If some

unwanted pixels have been added to the segmentation data, the decolorization pipette can be used to remove the unwanted pixels.

This process is very similar to the color pipette function. This means that the average color value in the pixel mask area is also set as the basic value by a click with the pipette in order to wipe the segmentation data which lie within the basic tolerance color range. (This can already be used to divide segmented areas again.) Also describes this process, only easier)

Colorizing Pipette: Collect average color value of the selected pixel mask area as basic value and add pixels that are within the basic-tolerance range of the segmentation data.

Decolorizing Pipette: Collect average color value of the selected pixel mask area as basic value and deconstruct the segmentation data is within the basic tolerance range.

Mask area: When colorizing or decolorizing with the pipette, a mask area is required to calculate the average color value of the clicked image point. The color value of the mask area is used as the basic value. Color values within the basic measurement tolerance range can be added to or removed from the segmentation data. The default mask range is **3x3**, other options are **1x1**, **5x5**, **7x7**.

The average RGB values of the mask area.

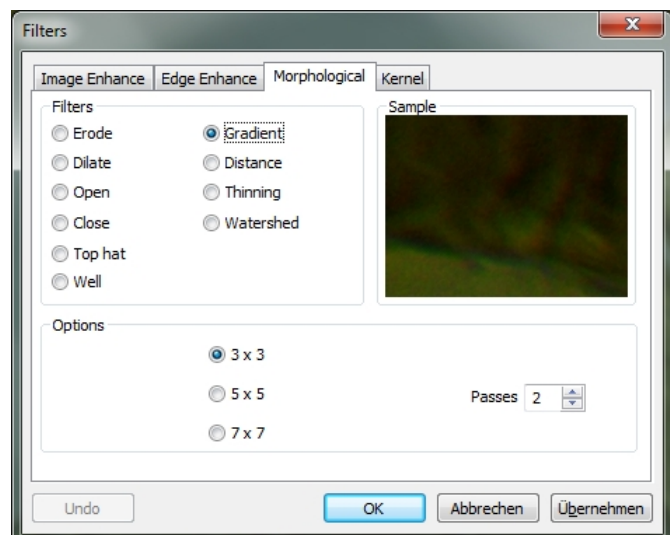
Tolerance: There are three tolerance values: R, G and B. The user can perform fast and accurate object segmentation by selecting a suitable tolerance value. The standard R, G and B tolerance values are 10.

This is used to select the color of the segmented area. The default color is green.

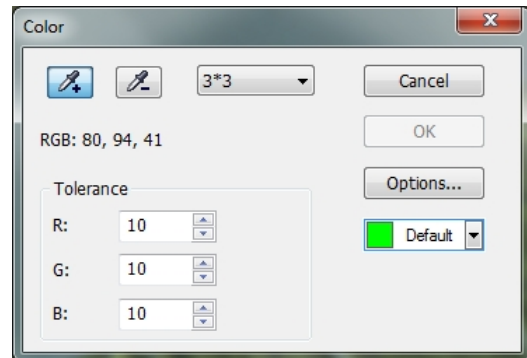
When you click on the **Options** button, the window with the counting options opens. The displayed options are the same as for **Process -> Segmentation & Count -> Watershed (W)**. For more detailed information, see **Process -> Segmentation & Count -> Watershed (W)**.

Here's an example to illustrate the Color Cube process.

1. Open an image file
2. Due to the inevitable noise in the image, an image smoothing operation should first be carried out. Various algorithms ensure smooth image operation. This is done as follows: After selecting **Process -> Filter -> Morphological**, the following menu will open. Click **OK** and the smoothed image is displayed as a preview in the right window.



3. Select **Process -> Segmentation & Count -> Color Cube ...**, then the following menu will open.

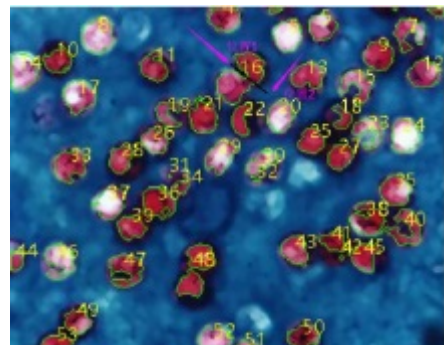


4. At this moment, the area is checked by the color pipette. Click the pixel you want to segment. That is, select the color range you are interested in. If an unwanted color range is included in the segmentation area, select the Decolorizing pipette and click the unwanted pixels so they are removed. When the segmentation process is complete, click **OK** and the segmentation & count is executed.
5. Click Options to select Outline, Label, and so on. The primary segmentation is completed.

13.6.7 Split Objects

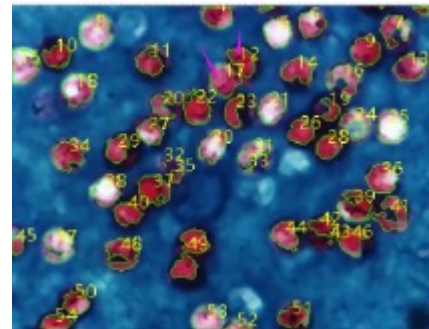
After segmentation, it can be found that a particle is not a single object, but two separate objects. However, they were calculated as a single object. The selection of split objects corrects this problem. Object splitting with **Split Objects** is explained below. (Function is only active after one of the previous functions, e.g., Watershed, has been performed)

Move the mouse to the object you want to separate. Press the left mouse button and draw a straight line.



If you press the left button, the cursor should not touch the target. The starting point of the separation line should be outside the target to be separated. Move the cursor to the right side of the target. The end point of the separation line should also be outside the target to be separated. Release the left mouse button when this operation is completed. A separation line is now displayed between the two contiguous objects. After you release the left mouse button, the image is counted again by MCLII.

If you disable or select the **Split Objects** menu again, the separator line is dimmed. After the dividing line has been hidden, the **Split Objects** process is completed. The final result is shown below.



13.6.8 Count Results

With **Count Result** you have the possibility to display the statistical results of the image. The result window displays the index, center, area and perimeter. The units of the statistical results are displayed as selected by the user. If you click on Index, Center, (radius (main and secondary axes or none) in this case), the results are sorted by size (ascending or descending). If you click on a specific element from the list, it is displayed in the image. Meanwhile, index, or area and perimeter, are automatically blanked out. (Function is only active after one of the previous functions, e.g., Watershed, has been performed)

Result			
Index	Center	Area	Perimeter
1	(813,50, 1940,50)	0,50	3,41
2	(1910,00, 1939,00)	1,00	4,83
3	(1603,05, 1933,86)	5,50	13,07
4	(740,00, 1932,50)	0,50	8,24
5	(1154,00, 1924,50)	3,00	8,83
6	(738,75, 1924,00)	3,00	10,83
7	(1908,50, 1915,00)	2,50	9,41
8	(902,50, 1925,50)	256,50	69,36
9	(863,00, 1916,50)	4,00	26,49
10	(1618,50, 1906,50)	5,00	10,83
11	(860,50, 1904,00)	1,50	5,41
12	(1616,00, 1898,50)	3,50	29,07
13	(1612,83, 1891,83)	3,50	12,24
14	(1238,00, 1897,00)	31,00	44,14
15	(706,00, 1874,00)	16,50	34,38
16	(701,50, 1868,50)	0,50	3,41
17	(1735,50, 1855,50)	0,50	3,41
18	(1855,00, 1855,00)	1,00	4,83

Export Close

The center column in the result is relevant for the approximation option in the Options dialog box. When **Circle** is selected, the radius of the circle is displayed as a radius column, and the main and minor axis of the ellipse is listed as an axis column when selecting **Ellipse**. If you select **None**, no column is displayed.

If you click on **Export**, the statistical result is exported together with the image into an Excel sheet for further analysis and processing (Excel is not a component of MCLII and is additionally required).

13.7 Denoise

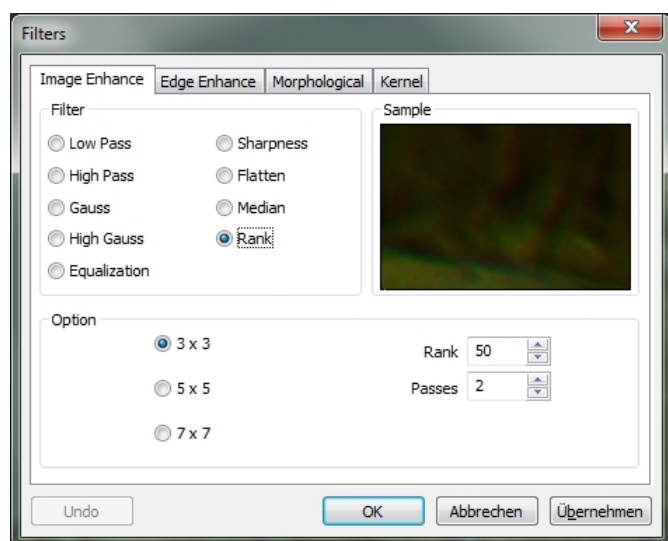
13.8 Sharpening

13.9 Color Assignment

13.10 Filter

Select **Filter** to apply one of the numerous filter functions in MCLII to the active image. You can also create custom filters and apply them using the filter commands. Each group of filters has its own property page or tab, whereby the filter type and the size can be selected. The filtered results are almost always written to the active image. You can use the Undo / Redo command to remove filter operations that have been applied.

13.10.1 Image Enhance



Low Pass: Use this filter to remove high-frequency information from the image (this has the effect of blurring sharp edges). The low-pass filter replaces the center pixel with the average value from its surroundings. This filter can also be used to remove noise.

High Pass: Use this filter to improve high-frequency information. The high-pass filter replaces the center pixel with a folded value, which increases its contrast significantly to its surroundings. The high-pass filter leaves only elements with high contrast.

Gauss: Use this filter to soften an image by turning off high-frequency information with a Gauss function. This has the effect of blurring sharp edges. The Gauss filter is similar to the low-pass filter, but it degrades the image less than the low-pass filter.

High Gauss: Use this filter to improve the detail. This process is similar to the blurred masking technique (see sharpness filter), but it brings less noise into the picture. A Gaussian curve kernel is used. Available in 7x7 and 9x9 kernel sizes.

Equalization: Use this filter to improve the contrast, based on the histogram of the local environment.

Sharpness: Use this filter to improve fine details or refocus an image. The sharpness filter sharpens the image with the blurred masking technique.

Flatten: no description

Median: Use this filter to remove the impulse noise from an image. The median filter replaces the center pixel with the mean value in the environment.

Rank: Use this filter to remove impulse noise from an image. The pixels in the kernel are in order of intensity, and the pixel in this range is selected for comparison. For example, there are 25 pixels in a 5x5 kernel. A percentage of 95% would choose the second largest pixel for comparison. If the difference between the selected pixel and the center pixel is greater than the threshold, the filter replaces the value of the center pixel with the value of the selected pixel.

Options

When one of the filters is selected, the following options are displayed:

3x3: creates a more subtle filter effect

5x5: creates a more moderate filter effect

7x7: creates a more extreme filter effect

Passes: Filter is applied multiple times on the image. When a filter is applied multiple times, its effect is enhanced by each pass. An image softened by a pass of the low-pass filter is again softened by a second pass.

Strength: Enter a value of 1-10 that determines the filter effect on the image. A value of 10 specifies the full strength (100%) of the filtered result applied to each pixel. Values less than 10 reduce the intensity of the filter. A value of 1 indicates that only 10% of the difference between the filtered pixel value and the original pixel value should be applied. A value of 2 indicates that 20% of the difference should be applied, and so on.

Rank: This value specifies which pixel is used in the sorted field to replace the central pixel. Pixels in the field are sorted in ascending order. The pixels are indexed from 0 to kernel size x kernel size-1. Pixel index 0 corresponds to the lowest pixel value.

Rank is expressed as a percentage of the indexes (kernel size x kernel size-1). A 50% rank means the center of the field. 0% Rank means the lowest index (lowest gray value) and 100% rank means the highest index (highest gray value).

If the **Equalization Filter** is selected, the options refer to the histogram distortion.

Linear: This option distributes the histogram linearly over the intensity scale. This function creates a contrast-rich image with the highest possible dynamic range.

Exponential: This option focuses the histogram at the top of the scale. This function produces a contrast-rich image. It is useful to increase the contrast in a very dark image.

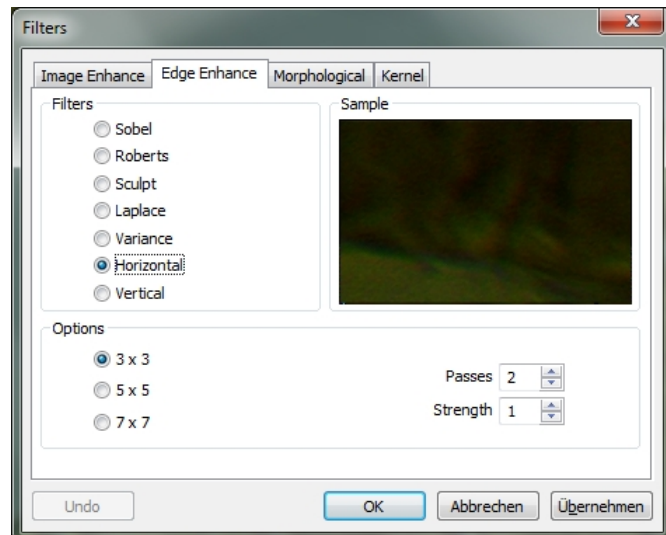
Logarithmic: This option focuses the histogram at the bottom of the scale. This function produces high contrast in an image with a low dynamic range. It tends to obscure the overall picture. It is useful to increase the contrast in a very bright picture.

Best Fit: This option optimizes the values for the image. The results are achieved by stretching the local histogram to maximize the contrast between the brightest and darkest pixels in the local window area.

Window: Image pixel statistics (minimum, maximum, histogram, average, standard deviation, etc.) are calculated on a small window of the image. These measurements are

used to derive the local contrast for this region of the image. In short, a range of windows x windows around each pixel is all that is taken into account in changing the intensities in the image. Larger windows produce smoother results, while smaller windows more closely track small details.

13.10.2 Edge Enhance



Sobel: Use this filter to improve only the edges in an image. The Sobel Filter applies a mathematical formula to a 3x3 environment to locate and highlight its edges.

Roberts: Use this filter to improve fine edges in an image. The Roberts filter is not a convolution filter. It is a mathematical formula on a 4x4 environment to produce its effects. The upper left pixel in the environment is replaced.

Sculpt: Use this filter to apply a sculptural effect to the image.

Laplace: No description

Variance: No description

Horizontal: Use this filter to detect and highlight horizontal edges.

Vertical: Use this filter to detect and emphasize vertical edges.

Options

If one of the **Edge Enhance** filter is selected, the options are based on kernel size and filter strength. The following options are displayed:

3x3: Select 3x3 kernel to create a more subtle filter effect

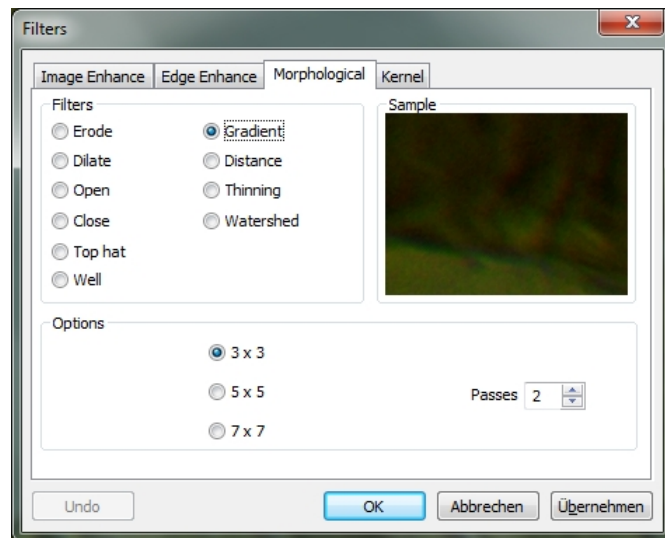
5x5: creates a more moderate filter effect

7x7: creates a more extreme filter effect

Passes: Filter is applied multiple times on the image. When a filter is applied multiple times, its effect is enhanced by each pass. An image softened by a pass of the low-pass filter is again softened by a second pass.

Strength: Enter a value of 1-10 that determines the filter effect on the image. A value of 10 specifies the full strength (100%) of the filtered result applied to each pixel. Values less than 10 reduce the intensity of the filter. A value of 1 indicates that only 10% of the difference between the filtered pixel value and the original pixel value is to be applied. A value of 2 indicates that 20% of the difference should be applied, and so on. If Sobel or Roberts is selected, no other options are available.

13.10.3 Morphological



Erode: Use this filter if you want to change the size of the objects in the image. The filter reduces the edges of the bright objects and increases the edges of the dark objects.

Dilate: Use this filter if you want to resize the objects in the image. The filter expands bright objects and reduces dark objects.

Open: Use this filter when you want to change the shape of objects in the image. Suppose the image contains bright objects on a dark field. Then the filter balances the object contours, separates closely connected objects, and removes small dark holes.

Close: Use this filter when you want to change the shape of objects in the image. Suppose the image contains bright objects on a dark field. Then, the filter fills gaps and enlarges projections to connect objects that are close to each other.

Top hat: Use this filter to recognize and emphasize dots or grains that are brighter than the background. For this processing there are 3 kernel sizes. Click the option box to change the kernel size to the value that most closely matches the size of the kernels.

Well: Use this filter to recognize and emphasize dots or grains that are darker than the background. For this processing there are 3 kernel sizes. Click the option box to change the kernel size to the value that most closely matches the size of the kernels.

Gradient: Use this filter to enhance the edges in an image.

Distance: Use this filter to show the distances of pixels within drops to the outer boundaries of these drops. After the distance filter is applied, the background is black (i.e., pixels having

a value of 0). Only the range within the drop has nonzero values (becomes white). The values of each pixel within the drop are a count of the shortest distance from that pixel to the edge of the drop. Thus, all pixels along the drop edge have a value of 1 (since they are one pixel from the edge of the droplet); Pixels having a distance of 2 from the edge have the value 2, and so on. This creates a distance map of the image. The distance filter does not work with True Color images. If you want to use the distance filter with a true color image, the image must be converted to grayscale.

Thinning: Use this filter to reduce an image to its skeleton. When selecting the filter, the threshold value must be determined which determines whether a pixel is part of the subject or part of the background. The thinning filter does not work with True Color Images. If you want to thin the True Color image, it must first be converted to a grayscale image.

Watershed: Use this filter to separate objects that touch each other. The infiltration filter reduces objects until they disappear. Then they are enlarged again, but without touching. The turn-point filter cannot separate objects in True Color images. If you want to separate objects in a True Color image, the image must first be converted to a grayscale image.

Options

When one of the Zoom, Expand, Open, or Close filters is selected, the options apply to the kernel size and shape. The following options are available:

2x2 Square: Select this option to use the 2x2 square kernel configurations.

3x1 Row: Select this option to use the 3x1 row kernel configurations.

1x3 Column: Select this option to use the 1x3 column kernel configurations.

3x3 Cross: Select this option to use the 3x3 cross kernel configurations.

5x5 Circle: Select this option to use the 5x5 circle kernel configurations.

7x7 Circle: Select this option to use the 7x7 circle kernel configurations. This is a two-pass filter, accomplished using a 5x5 circle followed by a 3x3 cross.

11x11 Circle: Select this option to use the 11x11 circle kernel configurations. This is a three-pass filter, accomplished by using a 5x5 circle followed by another 5x5 circle, followed by a 3x3 cross.

Passes: Set the number of filter iteration.

Note: The circular kernels are more effective in round objects (cells, grains, etc.) because their circular configuration preserves the circular shape of the objects better than square configurations.

If one of the Top Hat, Well, or Gradient filters is selected, the options are based on kernel size and shape. The following options are available:

3x3: Select this option to use the 3x3 square kernel configurations.

5x5: Select this option to use the 5x5 square kernel configurations.

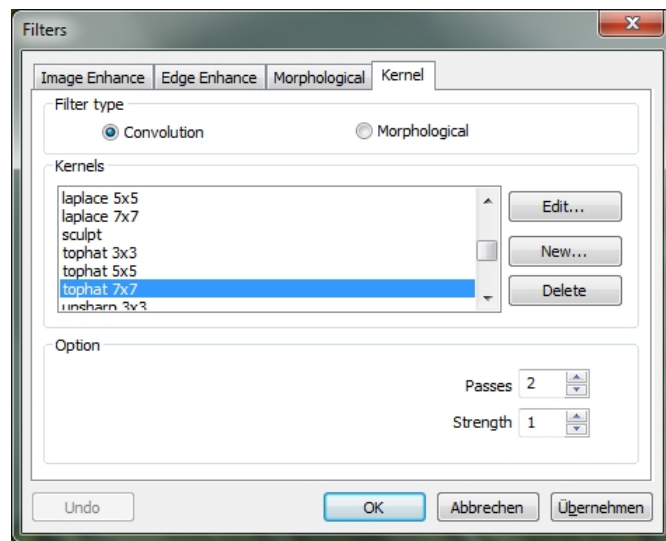
7x7: Select this option to use the 7x7 square kernel configurations.

If one of the Watershed, Thinning, or Distance filters is selected, the options refer to the threshold. The following options are available:

Threshold: Enter a percentage value of 1-100 indicating the intensity value to binarize the image. For example, a threshold value of 50% on a gray scale image would set all values ≤ 127 to 0 (black) and all values ≥ 128 to the maximum value for this image class (white).

13.10.4 Kernel

The kernel page allows you to edit the kernel files for the morphological and convolution filters.



Note: The HighPass, LowPass, Laplace, and Unsharp kernels are used by the HighPass, LowPass, Laplace, and Sharpen options listed in the Image Enhancements dialog box (there is no difference between these options - The two methods ultimately make the same). Because these kernel files are essential to the operation of these filter options, they must not be deleted or renamed.

Filter Type: Select the filter type you want to modify.

Edit ...: Select "Edit" to modify the settings of the various kernels.

Name: In this window, you can change the name of the corresponding kernel.

Kernel Size: In this window you can change the size of the corresponding kernel.

Fill: Click this button to fill each element of the kernel with a specific value. The Fill dialog box appears. You can enter a value between 0 and 10. The fill key is useful to set all coefficients to the same value. You can then change the coefficients that require the same value.

Offset: The pixel whose value is changed is usually the center pixel. However, you can change any pixel. MCLII marks the pixel to be changed by placing a marker.

New ...: Select **New** to create a new filter core. The **Edit Kernel** dialog is displayed. The functions correspond to those of **Edit** (described above), except that the file name for the new kernel file must be specified.

Delete: Deletes the selected kernel.

Options: The choice in this case depends on the kind of filter that is selected.

13.11 Image Stacking

The **Image Stacking process** is a form of speckle imaging commonly used to obtain high quality images from a number of short exposures with varying image shifts. It has been used in astronomy for several decades and is the basis for image stabilization in some cameras. The method involves calculating the differential shifts of the images. This is easily achieved in astronomical images, as they can be aligned with the stars. Once the images are aligned, they are averaged. It is a basic principle of statistics that the variation in a sample can be reduced by averaging the individual values. When using an average, the signal-to-noise ratio should be increased by a factor of the square root of the number of images.

The Image Stacking method can also be used for the biological and fluorescent microscope image to capture the image-to-noise ratio (SNR) and the dynamic ranges. That is why we introduce the image stacking process in MicroCamLabII.

The MCLII image stacking method introduces advanced image matching techniques for twisted, shifted, and scaled images. The user can record a short video to obtain a high-quality stacked image without taking picture rotation, displacement, and scaling between a series of images in the video. The steps of the image stacking process are as described below:

1. Select **Process -> Image Stacking**. Then, as shown below, a Windows window opens.
2. Select a video file from the open directory. In this case, we open "test.avi" for the demonstration. Click "Open" to open the file.

3. Then an **Image Stacking** window opens in which the processing progress is displayed.
4. If you click **Cancel**, the process is canceled. After stacking, a stacked image is displayed in a new window for further processing. In the stacked image, the signal-to-noise ratio is significantly improved. There are some black edges on the stacked image, because the image in the video is moved. Image areas which do not have corresponding pixels in the reference image are marked with a 0 (we select the first image in the video file as a reference image).

Note: MCLII supports the following image stacking formats: wmv, asf, avi, mp4, m4v, 3gp, 3g2, 3gp2, 3gpp, mov, mkv, flv, rm, rmvp.

In the image stacking process, MCLII records the first frame as a reference image. That is, the first frame is very important and it should be ensured that this frame is the right picture scene. The following frames should have a larger overlap range than the first frame.

13.12 Line Profile

Select **Process -> Line Profile** to visualize how pixels are distributed along a selected line by graphically displaying the number of pixels at each color intensity step.

Select **Measurements -> Line -> Any Line / Horizontal or Vertical Line** to draw a line on the image. If you select **Process -> Line Profile** again, a line profile graph is displayed. (procedure can also be executed in reverse):



Line profile: In a line profile, the X-axis represents the spatial scale and the Y-axis represents the intensity values ranging from 0 to 255. If the image is a gray scale image, the gray value is displayed. If the image is an HSV color image, the R, G, and B values are displayed separately with the corresponding R, G, and B color.

Background: Open the Windows color dialog to set the background color of the profile window.

Title: Use this command to give the line profile a title.

Recording: Recording the picture line profile as a new picture

Copy: Copy the line profile image to a clipboard.

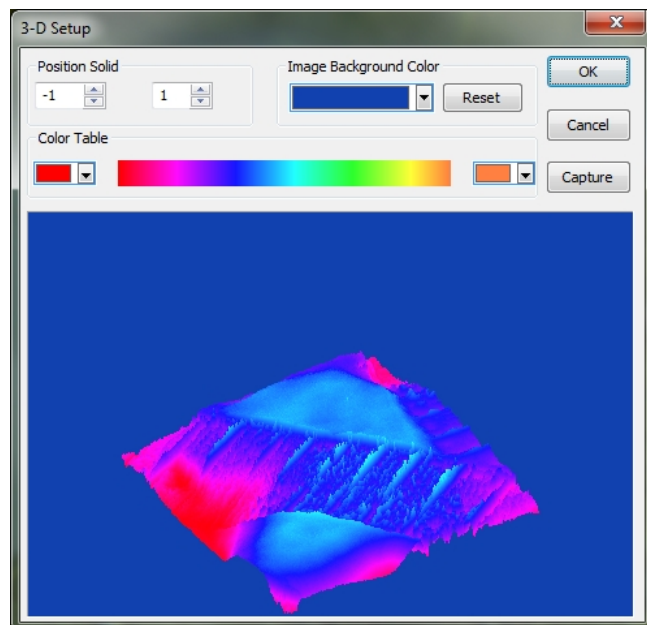
Save As: Save the Line Profile image in bmp format.

13.13 Surface Plot

Note: This option is only enabled by using a gray scale image

Select **Process -> Surface Plot** (or 3-D Setup). The function creates a 3D representation of the surface intensity of an image. When selecting the "Surface Plot" command, note that X = image, where Y = image height and Z = pixel is the gray value.

In the view window, you can adjust the elevation and rotation of the image by dragging the mouse.



Position Solid: The left input window displays the relative position of the entire image in the view window whose default value is 0.5. The right input window indicates the relative height of the Z scale display, the default value of which is 1.

Reset: Resets the values in the input windows **Position Solid** to the default values.

Image Background Color: Choose this option to select a color to adjust the background color of the viewport.

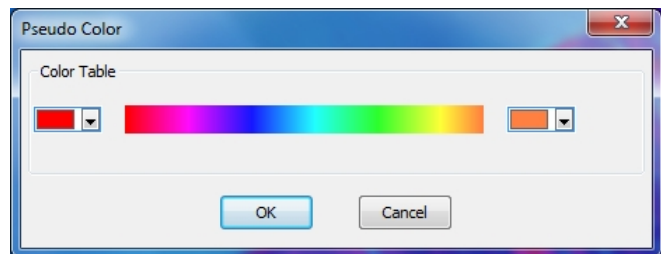
Capture: Capture the active image in the view window as a new image.

Color Table: Select the colors to map the gray values found in the surface drawing. The button at each end of the color panel opens the color dialog by selecting the start and end colors of the area. (Please refer to **Pseudo Color** for more information)

13.14 Pseude Color

Note: This option is only enabled by using a grey scale image.

Select **Process -> Pseudo Color** to colorize the active monochromatic image. This is used to highlight certain features in a greyscale image, such as displaying all densities above a particular point in red, or the recorded thermal information. All temperatures below a certain point can be displayed in blue.



When a monochromatic image is processed with the **Pseudo Color** function, a special palette has to be created which the monochromatic image is to be displayed with. Due to the pseudo-coloring of an image, the pixel value in the image is not changed (it does not convert an image into the correct color or palette). It simply associates a pseudo color palette with the image that interprets the gray scale values in the image as a color.

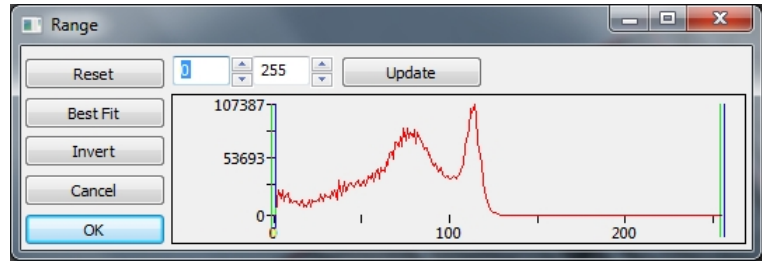
Pseudo color images are very similar in structure to the palette-class images, but there are a few important differences. First, the pixel values in a pseudo-color image actually represent continuous tone intensity information, palette image pixels not carrying any intensity signals. Second, a color palette image contains a color palette table that is actually part of the image file.

Various colors can be selected to display the gray values. The buttons at each end of the color strips display the color dialog separately, which allows the start and end colors of the area to be selected.

13.15 Range

You can use the **Process -> Range** command to adjust the intensity levels of the image to increase the contrast and increase the display in low light situations. Select the **Range** command to open the corresponding dialog.

Two vertical markers show the upper and lower limits of the intensity levels. These markers can be moved with the mouse by the Drag & Drop method. For a color image, the histogram will display the red, green, and blue values with appropriate color lines.



Two Edit controls specify the values of the intensity levels. Select **Up / Down** buttons to increase or decrease these values. All values between 0 and the lower limit are black, and all values between the upper limit and the upper end of the scale are white. These two processing values, which correspond to the two vertical markers, indicate the upper and lower limit values of the intensity stages. The default values are 0 and 255.

Reset: Use the **Reset** button to reset the black and white values to the high and low end of the dynamic range.

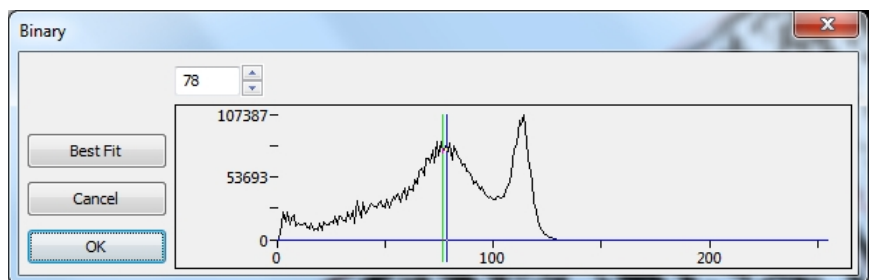
Best Fit: The **Best Fit** button automatically adjusts the intensity levels to the optimum value and instructs MCLII to optimize the brightness and contrast values for the respective image.

Invert: The Invert button reverses the color of the image.

Update: Update will refresh the display Range with the most current image information.

13.16 Binary

Binary is a kind of gray-level process. If the gray of the pixel is larger than the predetermined threshold, the pixel color is changed to white. Otherwise the pixel color is changed to black. Although some information may be lost during the process, it is an important step for other processes.



The curve in the binary dialog shows the gray distribution of the image.

The line in the dialog shows the threshold value. Drag the line to change the threshold, or change the value in the input field (in the upper left corner of the dialog) to move the line.

The input field shows the current value position of the line. If you change the value, the line is moved at the same time;

Click **Best Fit** to apply the automatic threshold to the image. An automatic threshold is used to display the image in the optimal gray scale ratio.

14. Layer

14.1 About Layer

MCLII **Layer** works like several layers of slides, where the original background remains visible. Each film layer contains different information, which gives an overall picture. These layers can be added and removed as desired.

You can use layers to perform tasks. For example, to perform the measurement on the image without polluting the image and save it for further analysis, adding text to an image, or adding vector graphic shapes.

14.2 Organizing Layers

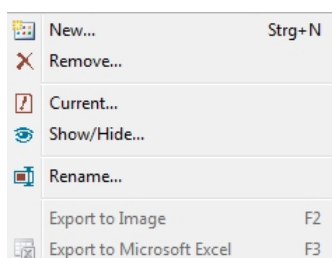
A new image has a single layer called Background Layer. The number of additional layers that you can add to an image is limited only by the memory of your device. You work with layers in the sidebar. The **Layer Sidebar** helps you organize and manage the layers, just like the **Layer Menu**.

14.3 Layers for non-destructive measurement and label

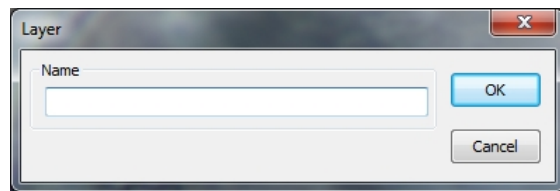
Instead of directly editing pixels, you can label and measure the image without changing the underlying pixels. Since no pixels are changed, you can easily remove the measurement objects and labels.

14.4 Layer Menu & Sidebar

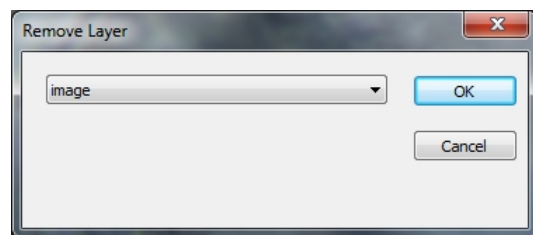
The **Layer Menu** and **Sidebar** contain the same functions. In the sidebar, you can access them with the right mouse button.



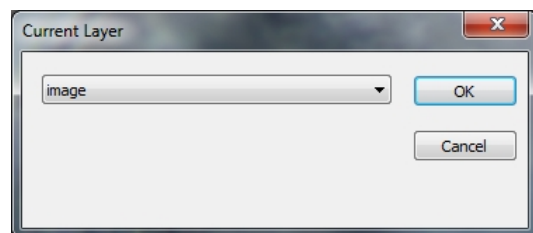
New: Setup a new Layer



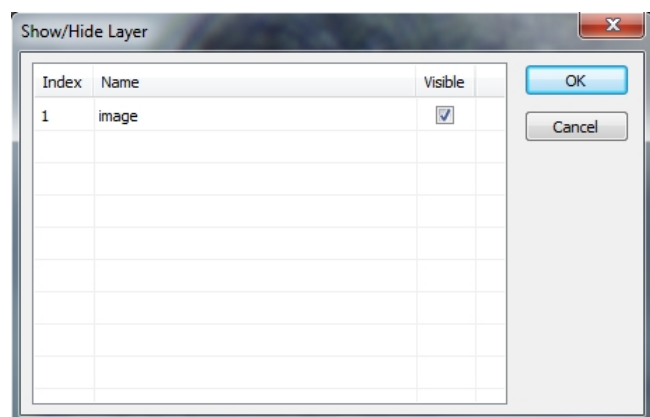
Remove: Remove the non-current Layer



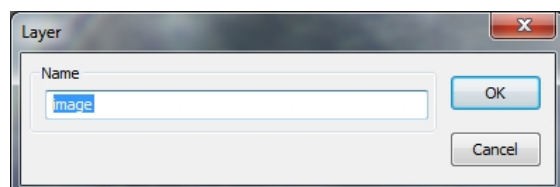
Current: Set the selected Layer as the Current Layer



Show/Hide: Set the non-current Layer Visible or Invisible.



Rename: rename a Layer



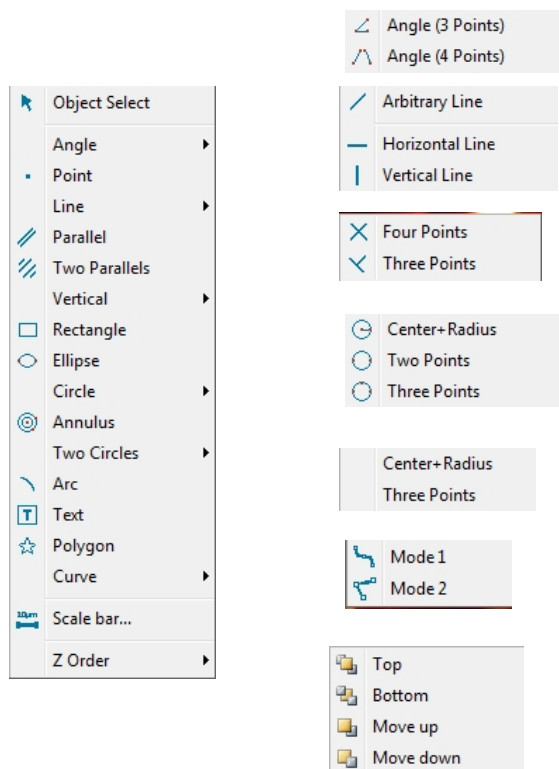
Export to Image: Export all the Layers measurement Objects to the image. This cannot be removed anymore.

Export to Microsoft Excel: Export the image and all the Objects on the Current Layer to the Excel File (Excel is not a component of MCLII and is additionally required).

15. Measurements

With the **Measurement Menu** items you can easily measure the image with many geometric shapes.

MCLII uses the Layer technique to perform the measurements. This does not result in any contamination or damage to the image pixels. The Measurement menus and their submenus are shown below.



15.1 Object Select

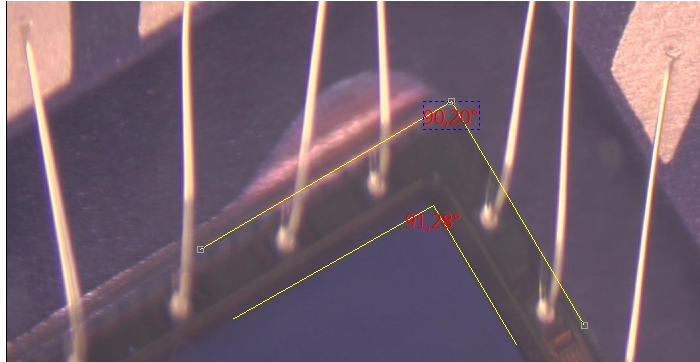
The Option **Measurements -> Select Objects** or the corresponding button in the toolbar is activated only if a new measurement is carried out via the background level.

After the measurement has been performed on the respective level, select this menu to select the objects.

1. The object is selected by clicking on it.
2. Select a group of objects by placing them in a rectangular area with the object selection command (left mouse button and dragging), or by pressing the Shift key and clicking the objects with the left mouse button until all the desired objects are selected.

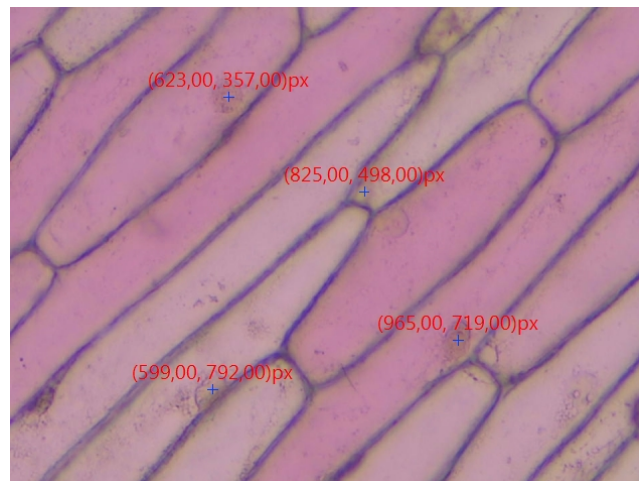
15.2 Angle

1. Move the mouse to the beginning point of the angle. Select the point by clicking with the left mouse button.
2. Now move the mouse to the vertex of the angle and select it again by clicking the left mouse button.
3. Finally, move the mouse to the end point of the angle and select the end point again by clicking with the left mouse button. A label is displayed near the vertex.



15.3 Point

Move the mouse to the displayed point and click with the left mouse button to highlight it. Now the point is marked with a label and the X and Y coordinates are displayed.



15.4 Line

15.4.1 Arbitrary line

Draw Arbitrary Line between two points on the specified Layer.

1. Select **Measurements -> Line -> Arbitrary Line**
2. Move the mouse to the beginning of the line and click the left mouse button to select the point.
3. Move the mouse to the end point of the line and click again with the left mouse button to select the point. You will now see a line labeled L1 and length.



15.4.2 Horizontal Line

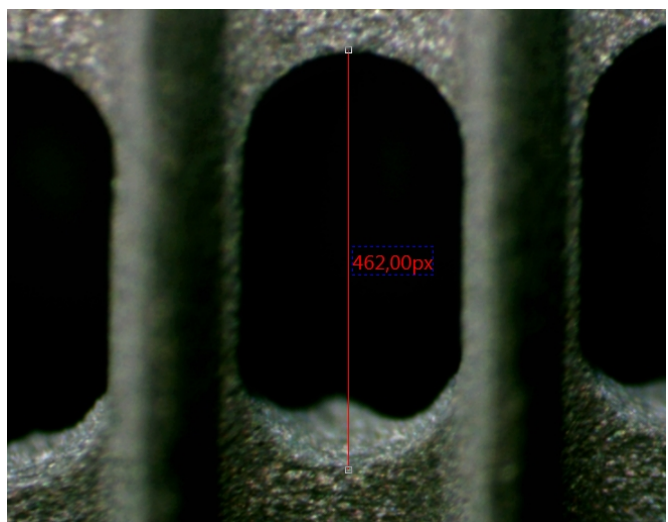
Draw a horizontal line between two points on the specified plane.

The starting point, as in the case of the Arbitrary Line, is defined by a click with the left mouse button. Now the line can be drawn automatically to the horizontal. The end point is determined by a click with the left mouse button.



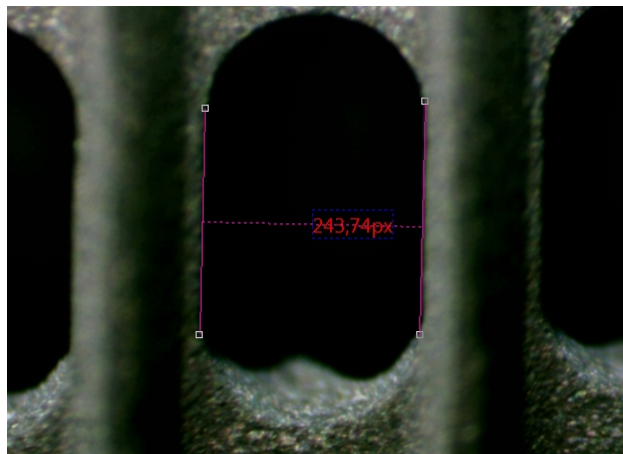
15.4.3 Vertical Line

Draw a vertical line between two points on the specified object. The starting point is set again by clicking with the left mouse button. Now the line can be drawn automatically to the vertical. The end point is determined by a click with the left mouse button.



15.5 Parallel

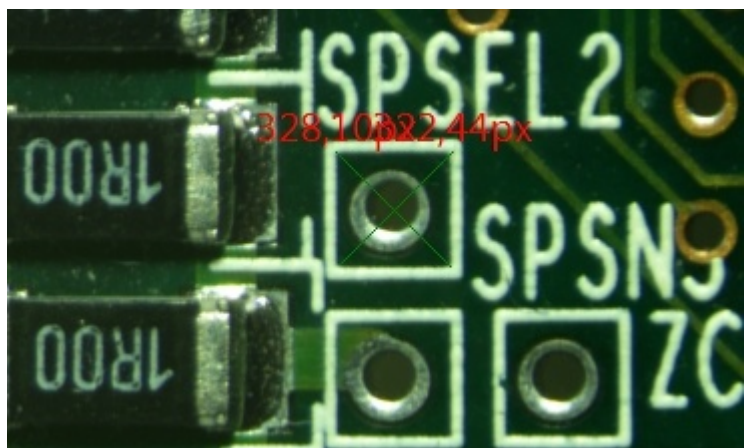
1. Select **Measurement -> Parellel**
2. Move the mouse and click with the left mouse button to select a point.
3. Move the mouse to the desired end point and highlight it again with the left mouse button.
4. Now move the mouse to the starting point of the parallels and then highlight it with the left mouse button.
5. If you now drag the mouse to the end point, the line can only be drawn as a parallel to the first line. Click the left mouse button again to set the end point.
6. To cancel the current operation, click the right mouse button once.



15.6 Vertical

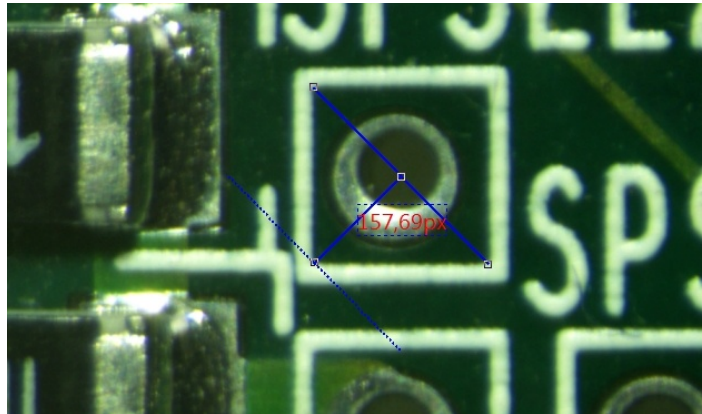
15.6.1 Four points

1. Move the mouse and select the starting point with the left mouse button.
2. Move the mouse and select the second point with the left mouse button. The two points are now connected by a line.
3. Move the mouse to select the third point.
4. If you now move the mouse around the fourth point, the line can only be drawn vertically (depending on the orientation of the first line) to the first line (points 1 and 2).



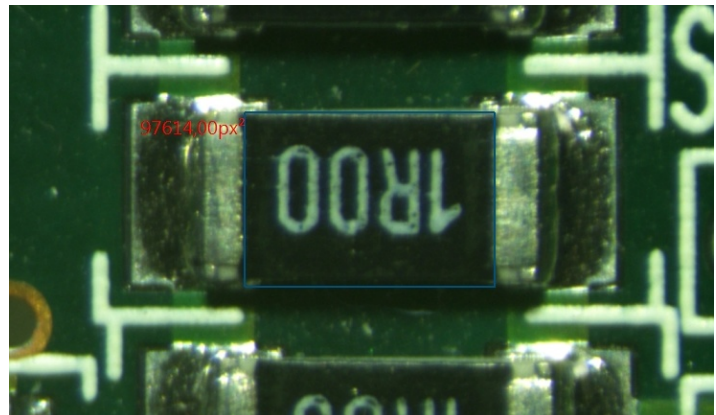
15.6.2 Three Points

1. Move the mouse and select the starting point with the left mouse button.
2. Move the mouse and select the second point with the left mouse button. The two points are now connected by a line.
3. If you move the mouse again to set the third point, the second line can only be drawn vertically (depending on the orientation of the first line) to the first line.



15.7 Rectangle

1. Move the mouse to the start point of the rectangle and click with the left mouse button.
2. Move the mouse to a second point to align the rectangle.

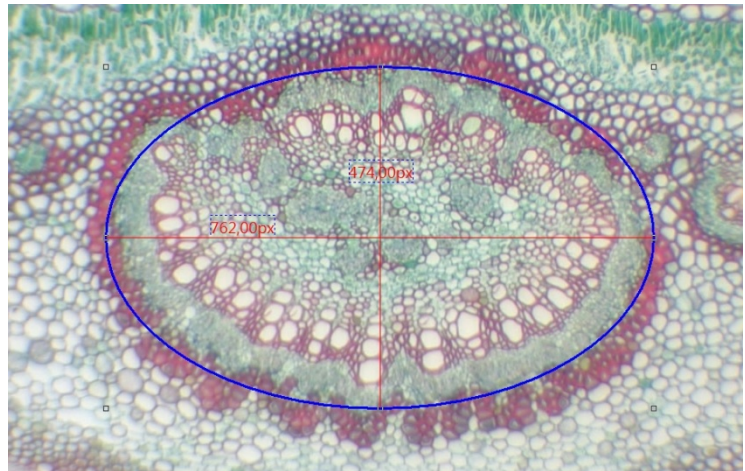


15.8 Round Rectangle

1. Use this function to measure a rounded rectangle.
2. Select **Measurement -> Round Rectangle** to highlight the round rectangle.
3. Move the mouse to the start point and click the left mouse button to highlight the point.
4. Move the mouse to a second point to set the size of the round rectangle, and then highlight the point with the left mouse button. You will now see a round rectangle named Rr1 and height and width in two dimensions.

15.9 Ellipse

1. Select **Measurement -> Ellipse**
2. Move the mouse to select the first point with the left mouse button.
3. Move the mouse to the second point to highlight it again with the left mouse button.
The ellipse is drawn by the movement with the mouse.
4. If the ellipse does not completely overlay the object on the image, select **Measurements**
-> **Select Object** to adjust the ellipse.



15.10 Circle

15.10.1 Center + Radius

Select **Measurements -> Circle -> Center + Radius** to draw a circle with center and radius function on the corresponding layer. Move the mouse to the center of the circle and click the left mouse button. The selected point marks the center of the circle and can be resized by moving the mouse.



15.10.2 Two points

Select **Measurements -> Circle -> Two Points** to draw a circle with two points on the corresponding level. Move the mouse to the edge of the circle to be drawn and click

the left mouse button. The selected point marks the edge of the circle and can be resized by moving the mouse in size and orientation.



15.10.3 Three points

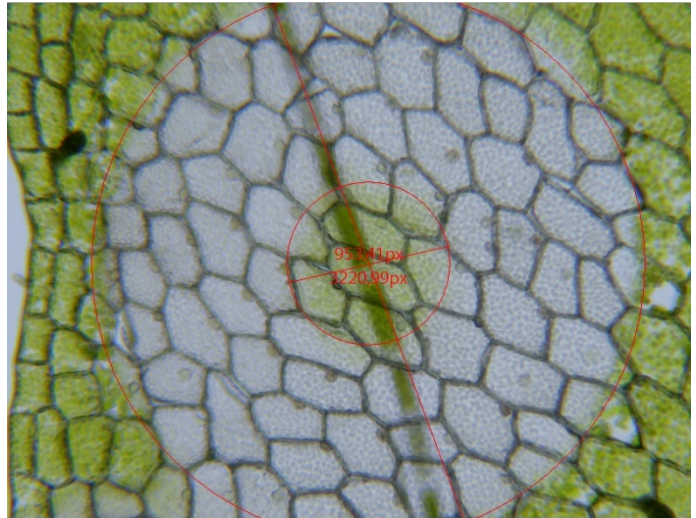
Select **Measurements** -> **Circle** -> **Three points** to draw a circle with three points on the corresponding level.



15.11 Annulus

1. Move the mouse to the center of the ring and click the left mouse button to highlight the center point.
2. Move the mouse to resize the ring to the perimeter of the object, and then click the left mouse button again.

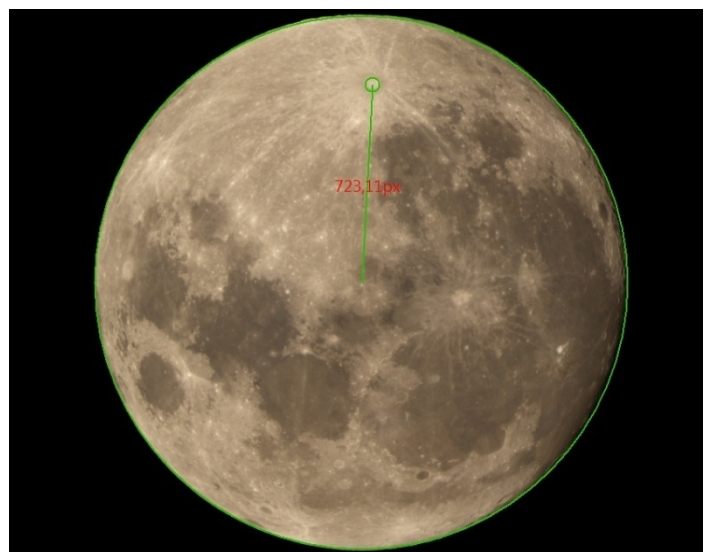
3. Move the mouse again to adjust the second ring to the inner diameter of the circle. Now you will see the diameter of the two drawn rings.



15.12 Two Circles

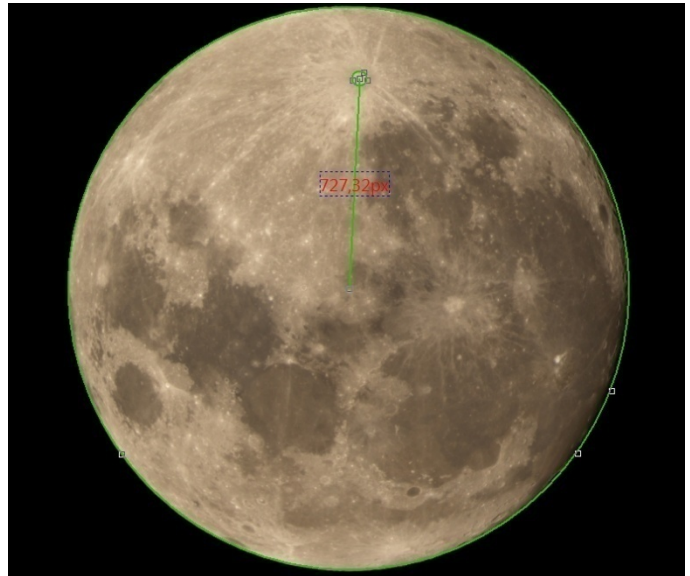
15.12.1 Center + Radius

1. Move the mouse to the center of the circle and click the left mouse button to highlight the center point.
2. Now move the mouse to adjust the size of the circle to the perimeter of the object, and click the left mouse button again.
3. Now move the mouse to the center of the second circle and click the left mouse button again to select the center point and then resize it by moving the mouse. After both circles have been drawn, a connection is drawn between the two circle centers.



15.12.2 Three points

Draw two circles with the three-point method at the appropriate level. After both circles are drawn, a connection is drawn between the two circle centers.



15.13 Arc

Select **Measurements** -> **Arc** to draw one or more Arcs on the appropriate layer.

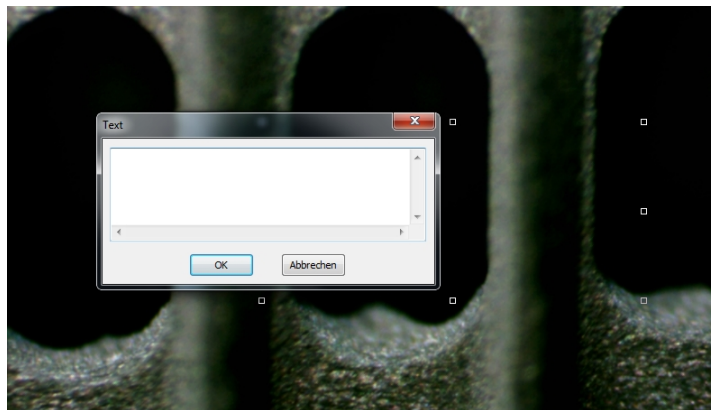
1. Move the mouse to the start point of the arc and click the left mouse button to select the first point.
2. Move the mouse to another point of the arc (at best to the center of the arc) and click again with the left mouse button to highlight the point.
3. Move the mouse to a third and last point of the arc and click the left mouse button. The line now adjusts to the selected points and becomes a bow.



15.14 Text

Select **Measurements** -> **Text** to insert a text field at the appropriate level.

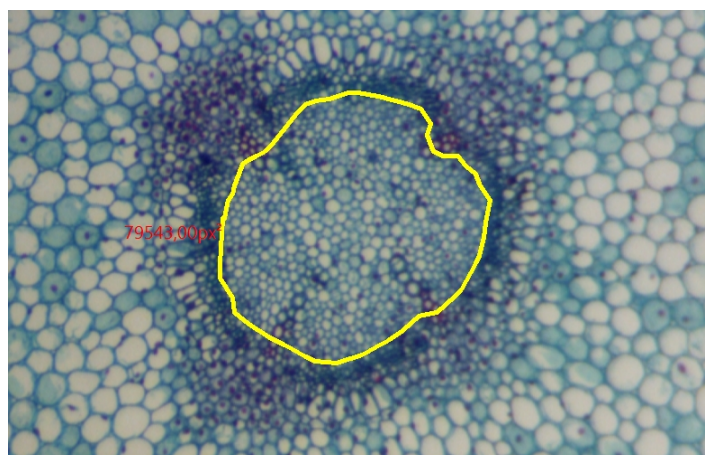
1. Move the mouse to the start point of the text window and click the left mouse button to select the point.
2. Move the mouse to another point and click the left mouse button again to set the size of the text window.
3. Then a text window opens in which you can insert the desired text. The settings for the font, size and color can be adjusted via the sidebar.



15.15 Polygon

Select **Measurements** -> **Polygon** to draw a shape around a polygon at the appropriate level.

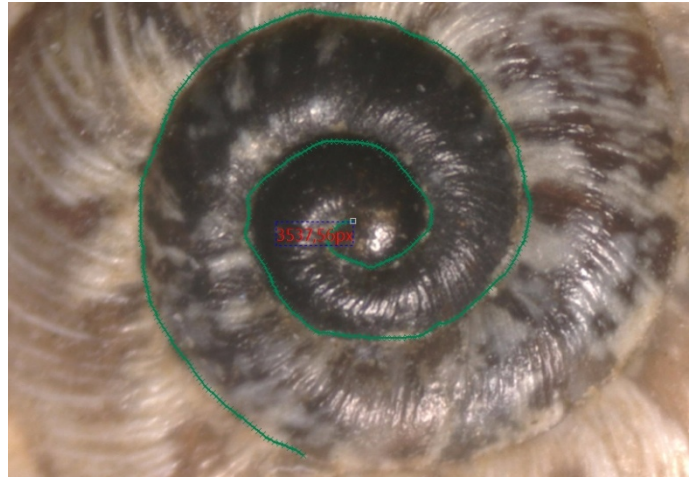
1. Move the mouse to any starting point of the object and click the left mouse button to select the point.
2. Move the mouse to another point of the polygon and click again with the left mouse button to highlight the point. The marked points are connected by a line. Repeat this procedure as many times as necessary until the object is completely outlined. The narrower the dots are, the closer the outline becomes.
3. When the outline is complete, close the polygon function with the right mouse button.



15.16 Curve

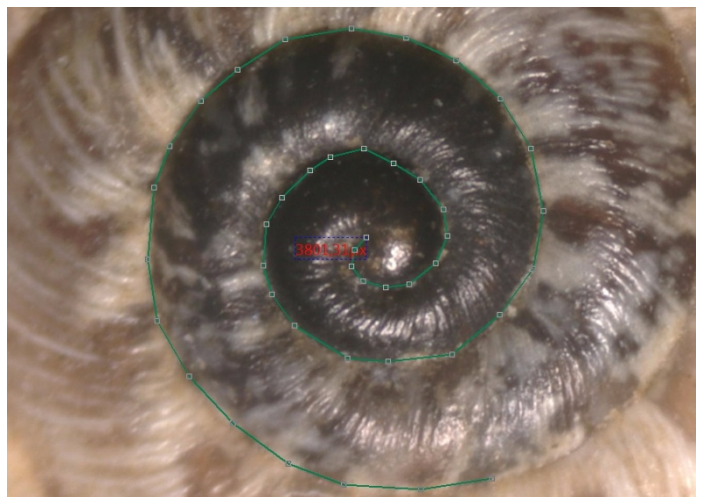
15.16.1 Mode 1

1. Move the mouse to the desired starting point and hold down the left mouse button.
2. Use the left mouse button to move the mouse along the test object until it is completely framed.



15.16.2 Mode 2

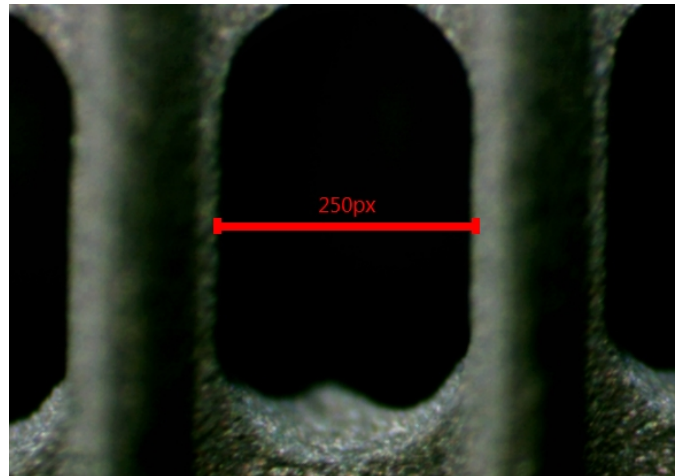
1. Move the mouse to the starting point of the measurement object and click the left mouse button.
2. Move the mouse to the next point and click the left mouse button again. Repeat this process as often as necessary until the object is measured (operation similar to the polygon). The shorter the distances between the set points, the more accurate the measurement becomes.



Note: Both operations are completed with the right mouse button.

15.17 Scale Bar

Select the Scale Bar to add a scale (in pixels) to the image. There is no description available in the english manual.



15.18 Z Order

Top: Change the position of the selected object to the top of all objects in the current layer.

Bottom: Change the position of the selected object to the lowest point of all objects in the current layer.

Move Up: Change the position of the selected object to the top of the two at the current level.

Move Down: Change the position of the selected object to the lower point of the two on the current level.

Notes for the measurements:

- To check or modify the parameters of an object, select a single object. The Sidebar with the details of appearance, calculation and coordinates is activated automatically. Here you can edit the corresponding object parameters to change their properties.
- The appearance and the coordinates in the sidebar are only activated when a single object is selected. The calculation is for a single object, or it multiplies all selected objects. The calculated elements depend on the selected object type (the type can be the same or different)
- To cancel or finish a measurement, click the right mouse button.

16 Options

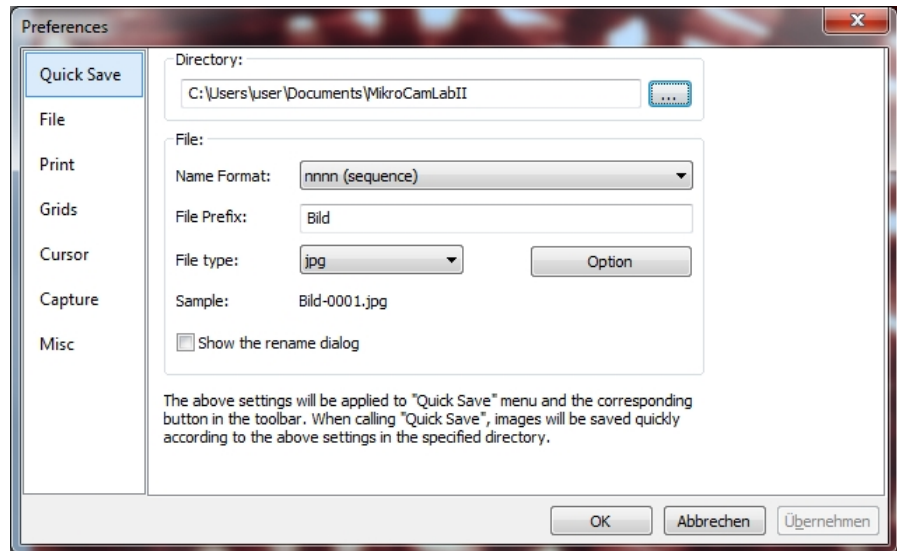
16.1 Preferences

In the presets, there are 7 different sections in the side bar of the window. These are quick storage, file, print, grid, cursor, recording and miscellaneous.

16.1.1 Quick Save

The **Quick Save** menu or icon is activated when a new image has been captured with the camera, or a picture window is created by selecting **File> Paste as New File**.

File -> Quick Save can save the file quickly, without specifying file directory, file name and file format. The settings are specified in **Options> Preferences> Quick Save**.



Directory: Specifies the file path in which folder the image files are stored in **Quick Save**.

Name Format: Select the file name to be used for Quick Save.

A number sequence (nnnn) and various date formats (e.g., yyyyymmdd) may be selected.

File Prefix: When creating file names for a series of images, enter a filename prefix for fast storage. This prefix is used with the name format to form a final filename paradigm.

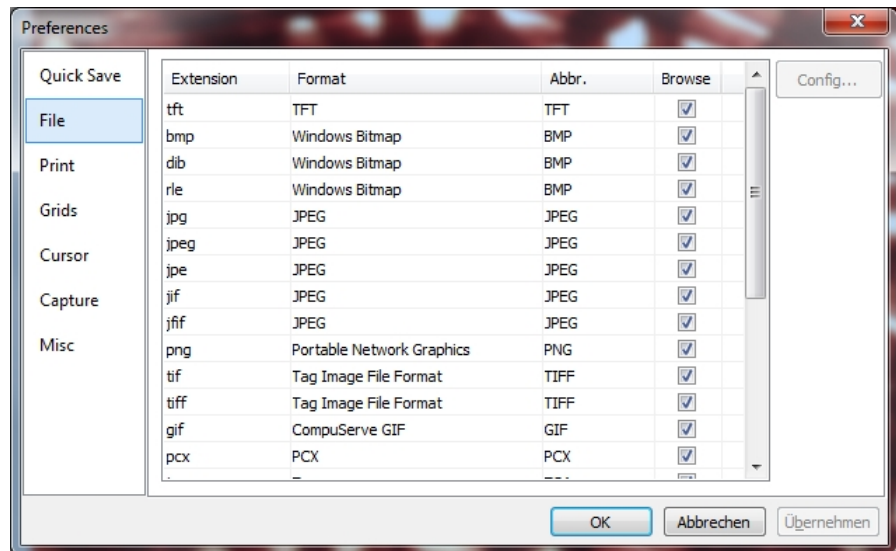
File Type: In this combo box you can select the format in which the image should be saved (BMP, JPG, PNG, TIF). Click on the option button to set the various parameters for the encoding of the file (for the BMP format, the option is disabled. **See File -> Save As** for details on the format encoding method); If there are objects on the screen, the file type is selected as MCLII file type (* .tft)".

Sample: The final name is indicated to the right of the sample label.

Show the rename dialog: The file can be renamed after this term. When this item is selected, a rename dialog appears after selecting **File -> Quick Save**, or click the **Quick Save** icon on the toolbar. The new name can be specified again according to the request.

16.1.2 File

You can extend a file for the specified file format and its abbreviation to determine whether or not it is displayed in the **Browse** window (the checked format can be displayed in the Browse window).



Extension: Used to identify file extension.

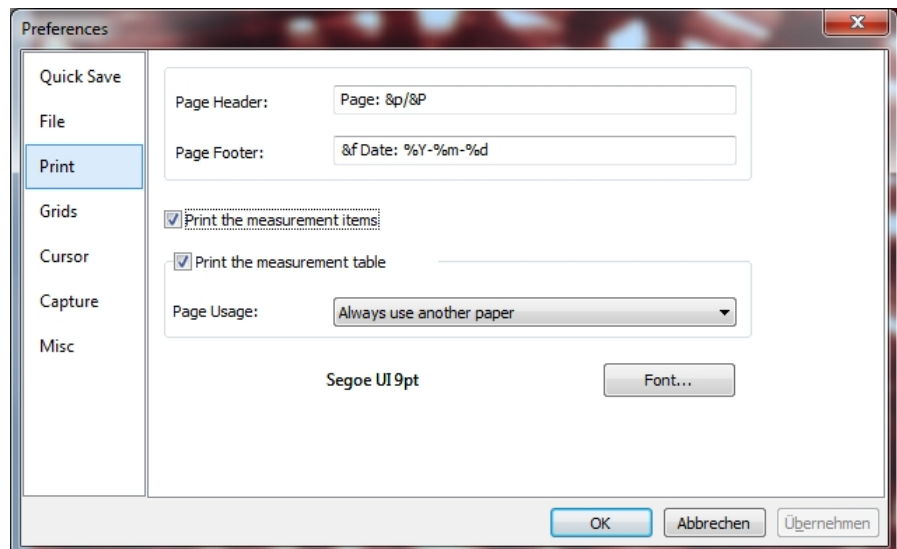
Format: The English name of the file format.

Abbr: Abbreviation of the file format.

Browse: To determine whether the file format can be displayed in the "Browse" window.

Configuration: To configure the image file as an option for JPEG, Portable Network Graphics, Tag Image File Format, CompuServ, JPEG 2000 Standard, JPEG Codestream. For more information, see the **File -> Save As** dialog box on the **Option** button.

16.1.3 Print



You can set the Page Header and Page Footer in the following format:

& F: filename
& P: Current page
& P: All pages
% A: Full weekday name
% B: Abbreviated month name
% B: Full month name
% C: Date and time plot for locale
% D: day and month as decimal
% H: Time in 24 hour format (00 - 23)
% I: Time in 12h. Format (01 -12)
% J: Anniversary as decimal number (001 - 366)
% M: Month as decimal (01 - 12)
% M: Minute as decimal (00 - 59)
% P: Current local time A.M: /P.M.
% S: second as decimal
% U: Year week as decimal number, Sunday as first weekday (00 - 53)
% W: Weekday as decimal number (0 - 6, Sunday corresponds to 0)
% W: Year week as decimal number Monday as first weekday (00 - 53)
% X: Date representation for current locale
% X: Time representation for current locale
% Y: Year without century as decimal (00 - 99)
% Y: Year without century as decimal number
% Z% Z: Timezone abbreviation, only if timezone is known
%%: Percentage sign

For example: If you select **& f Date:% Y-% m-% d**, the file name and date are printed in the yyyy-mm-dd format in the header line.

If you select Page: **& p / & P**, 01/11 is printed in the footer (Assuming the current page is page 1 and the page is 11).

Print measurement items: If the measurement object overlays the image superimposed, check these objects on the image. Measurement sheet on the side;

Print measurement chart: If the measurement object is superimposed on the image, check whether it prints these objects. Measurement sheet on the side with;

Always use different paper: print the measurement data on a new page;

Auto: Print the measured data directly behind the image (if the image and the measurement data can be arranged on a single page), on a separate page (if not);

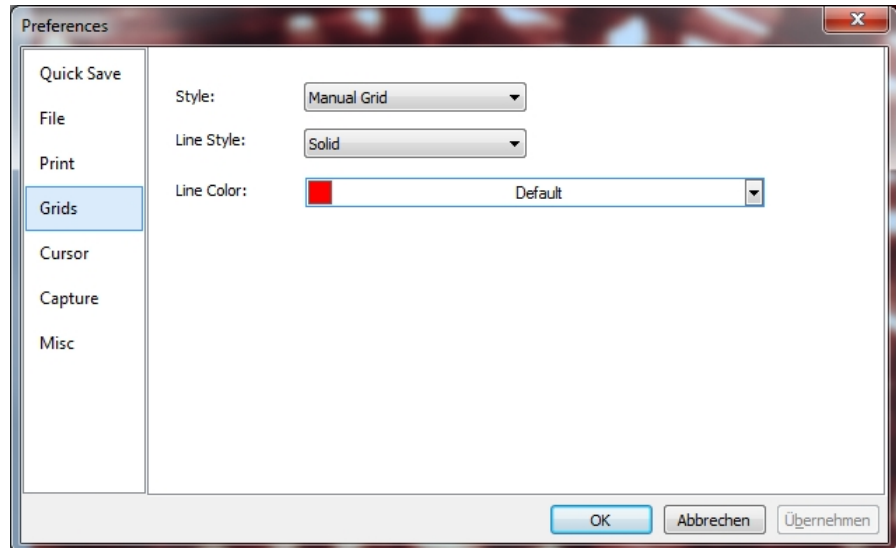
Never use other paper: print the data sheet directly behind the image page;

Font: Select the font for the data.

16.1.4 Grids

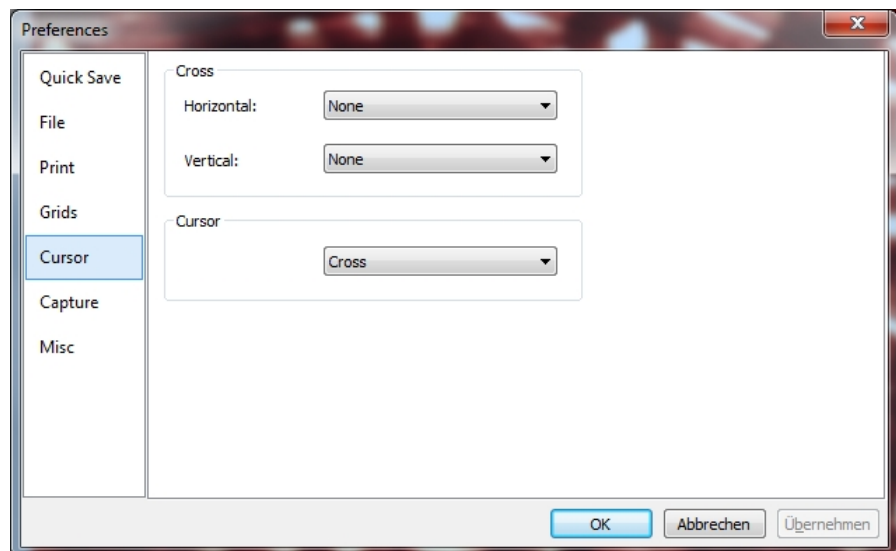
When you select **View -> Grid -> Settings**, or **Options -> Preferences -> Grids**, the same settings are displayed.

There you can select the grid style, the line style and the line color for the grid.



16.1.5 Cursor

This command will set the mouse cursor for the video and image window operations.



Select the horizontal cross in:

None (Window Standard), Single (Single Line), Double (1 pixel), Double (3 pixels), Double (5 pixels), Double (7 pixels) and Double (9 pixels) formats.

Select the vertical cross in:

None (Window Standard), Single (Single Line), Double (1 pixel), Double (3 pixels), Double (5 pixels), Double (7 pixels) and Double (9 pixels) formats.

Single means Simple Line. Double means two parallel lines. 1 pixel means that the line space between the two lines is 1 pixel in the distance. The other sizes also have this meaning.

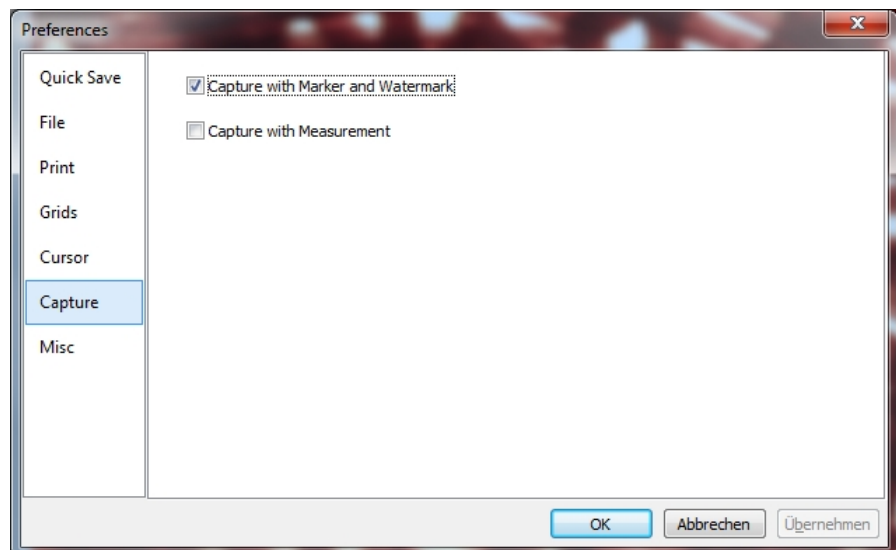
Select the cursor shape from cross, point and zero.

In the following figure, a cursor with a double horizontal and vertical line is intersected with 9 pixels (for the alignment application the longer one) and the cursor is defined as a cross.

16.1.6 Capture

If you want to capture an image from the video filter with many superimposed measurement objects, the following setting should be defined first.

1. If you select **Options -> Preferences**, the window below appears
2. Now you can choose between **Capture with Marker and Watermark** and **Capture with Measurement**.



16.1.7 Misc

The **Miscellaneous** item is mainly used to control MCLII UI. It contains the settings:

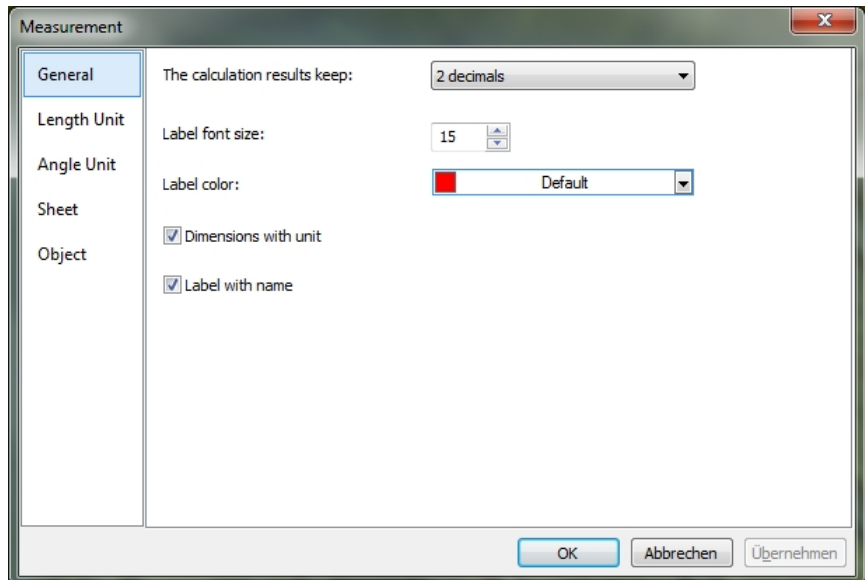
MicrCamLabII Warnings for special file formats
Sidebars
UI Style
Language
Enable GPU accelerated image processing
Privacy

16.2 Measurement

Select **Options** -> **Measurement**

16.2.1 General

The calculation results keep: The selection allows you to set the calculation results to 1-6 decimal places. The default is 2 decimal places.



Label font size: Font size and kind for the measurement label

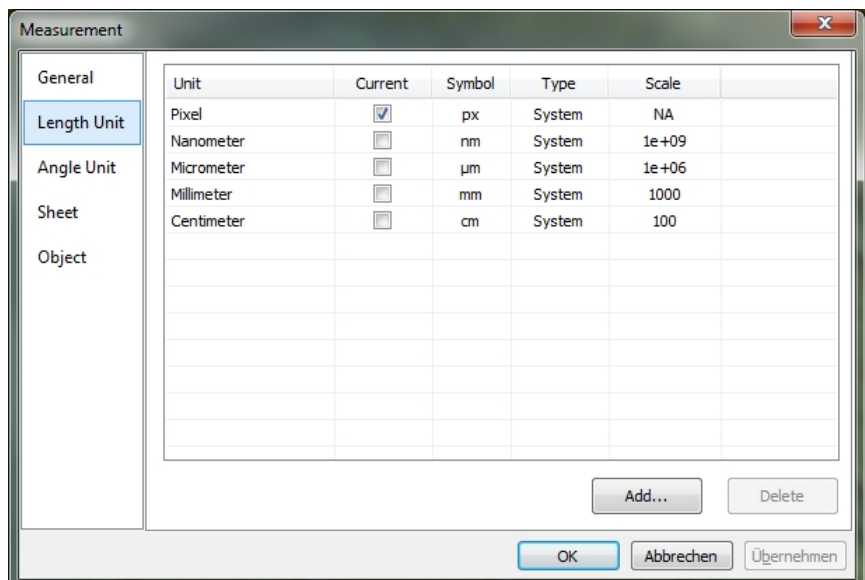
Label color: Color of measurements und labels

Dimensions with unit: If this function is selected, the dimension is displayed together with the unit of measurement objects. If the function is not selected, only the dimension with the measurement objects is displayed.

Label with name: If you select **Label with name**, a prefix is added before the measurement object number. The prefix for the other object can be specified in the measurement page bar where an item is named **Name**.

16.2.2 Length Unit

Here you can select the Length Unit for the measurement object on one layer.



Unit: The unit defined by MCLII. This cannot be deleted.

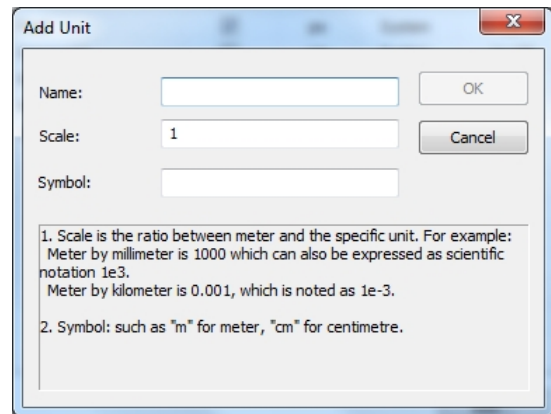
Current: The selected unit.

Type: Type of unit. This can be specified as **System** (defined by MCLII) or **User** (defined by the user).

Symbol: Abbreviation of the unit.

Scale: Represents the ratio of **meter to unit**. For example, if the unit is μm , then **meters in μm** should be 100.000, the scale should be $1\text{e} + 6$;

Add Unit: The user can also define own units. Click the **Add** button and the **Add Unit** dialog box opens. Here you can enter the units **Name** and **Symbol** in the fields. In the example shown, we enter the name decimeter and the symbol dm. The scale for meters in decimeter is 10. Click OK to add or cancel the entry.



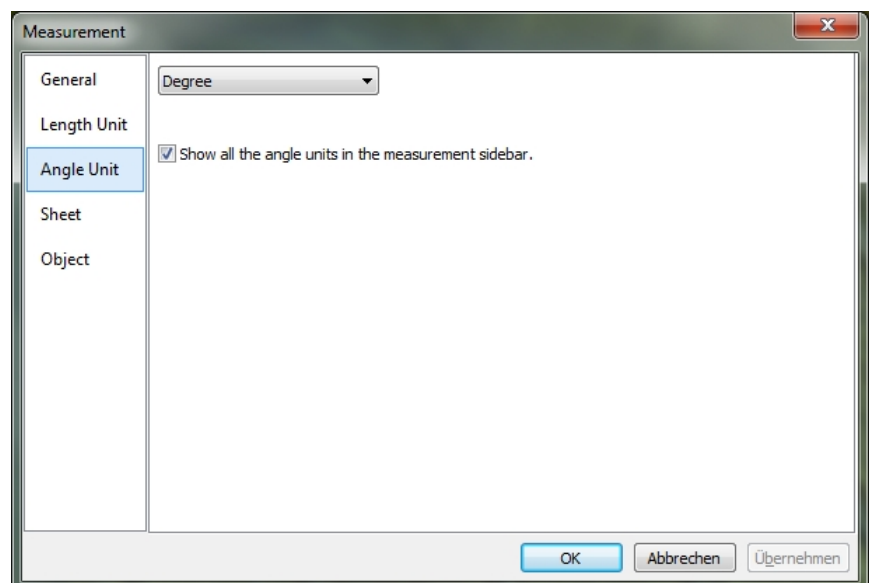
The 'Add Unit' dialog box contains the following fields and controls:

- Name:** A text input field.
- Scale:** A text input field containing the value '1'.
- Symbol:** A text input field.
- Buttons:** 'OK' and 'Cancel' buttons.
- Help Text:**
 1. Scale is the ratio between meter and the specific unit. For example: Meter by millimeter is 1000 which can also be expressed as scientific notation 1e3. Meter by kilometer is 0.001, which is noted as 1e-3.
 2. Symbol: such as "m" for meter, "cm" for centimetre.

The specified type is now user. This means that this unit is not defined by the system, but by the user.

16.2.3 Angle Unit

On the **Angle Unit** page you can select Radian, Degree, or PI as the angle unit. Display all the angular units in the measurement sidebar to display all of the objects listed as a reference in the scale bar as shown above.

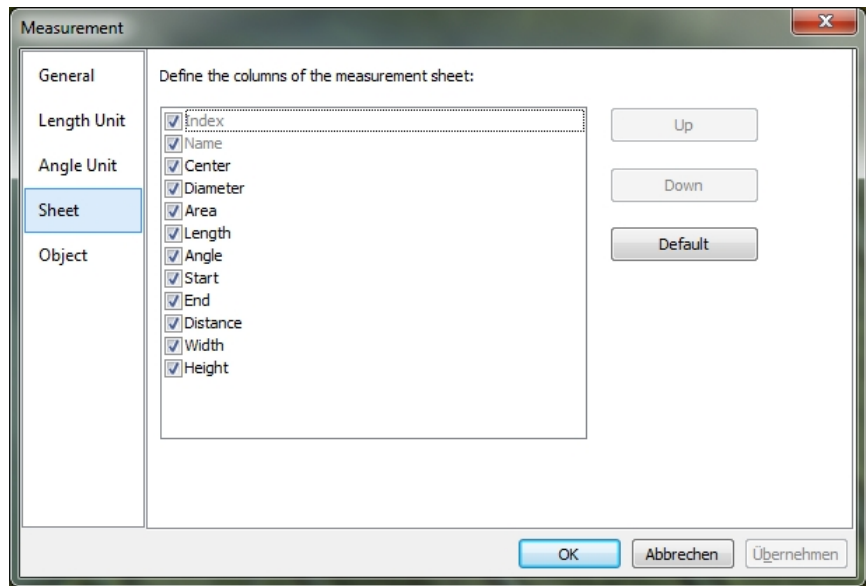


The 'Measurement' dialog box features a sidebar with the following options: General, Length Unit, Angle Unit (highlighted), Sheet, and Object. The main area includes:

- A dropdown menu for the angle unit, currently set to 'Degree'.
- A checkbox labeled 'Show all the angle units in the measurement sidebar.' which is checked.
- Buttons at the bottom: 'OK', 'Abbrechen', and 'Übernehmen'.

16.2.4 Sheet

Select **View -> Measurement Sheet** to understand the functions.



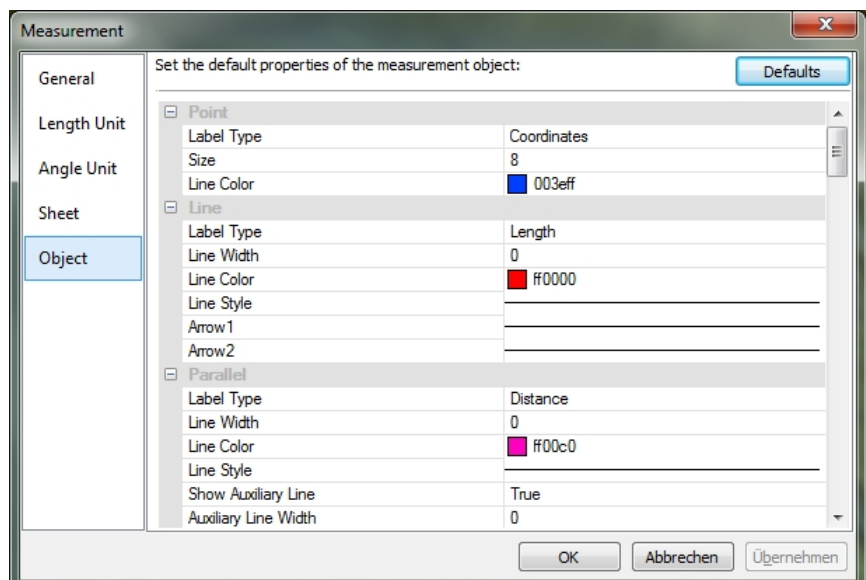
1. To select the dimensions, click on the corresponding position. The up or down buttons are activated.
2. By selecting or unselecting the units, they are displayed or blanked in the Measurement Sheet.
3. By clicking on **Preferences**, the information is reset to the default. **Index** and **Name** remain unaffected.

Note: The position index and the name are always grayed out and cannot be deselected. This means that these two elements cannot be changed.

16.2.5 Object

Object lists the features of point, line, parallel, two-parallel, vertical, angle, rectangle, circular, arc, annulus, two-circle, ellipse, polygon, Scales and text.

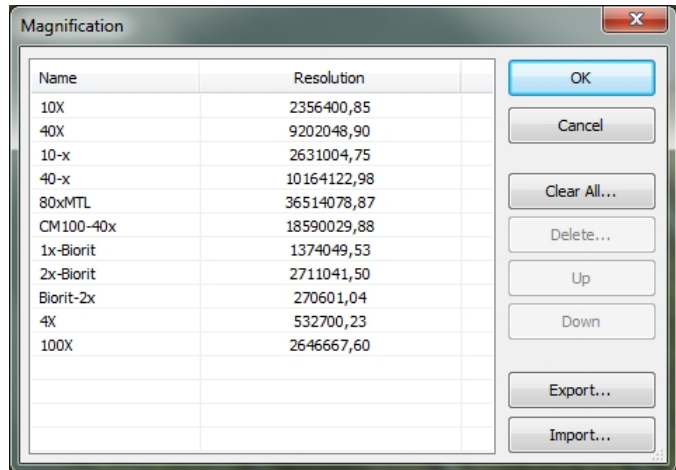
By clicking **Preferences**, all changes are reset to the default.



16.3 Magnifications

Select **Options -> Magnification** to manage the calibrated magnifications.

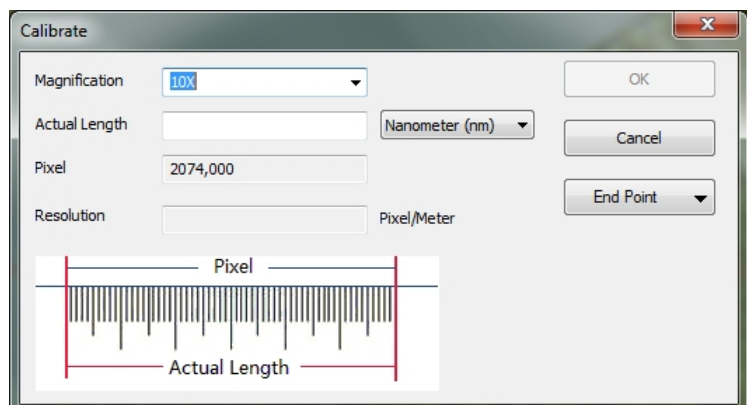
For example, if the 4x, 10x, 40x, 100x magnifications were defined, the magnification dialog should look as follows.



1. If you select a resolution in the list, the **Delete ...** button is activated and you can delete it. The **Up** and **Down** buttons are also activated.
2. Select **Delete All** to delete all specified magnifications.
3. If you want to use the magnification later, you should first make a backup.
4. Click **Export ...** to save the magnifications in a media. The file extension is then *. Magn file.
5. After the new installation is complete, the magnification can be imported by selecting **Options -> Magnification ...** and clicking Import to load the previously saved *.magn file.
6. When everything is OK, press **OK** to close the magnification dialog box.

16.4 Calibrate

1. Open MCLII
2. Connect the camera to the computer and the microscope.
3. Start the camera (in this case, UCMOS00350KPA)
4. Switch the microscope object to 10x and place the TS-M1 micrometer in the center of the microscope field. Try to focus the ruler. Set the unit to **Pixels**, the video resolution to the maximum (2048x1536 for UCMOS03100KPA) and the zoom ratio to 100%.

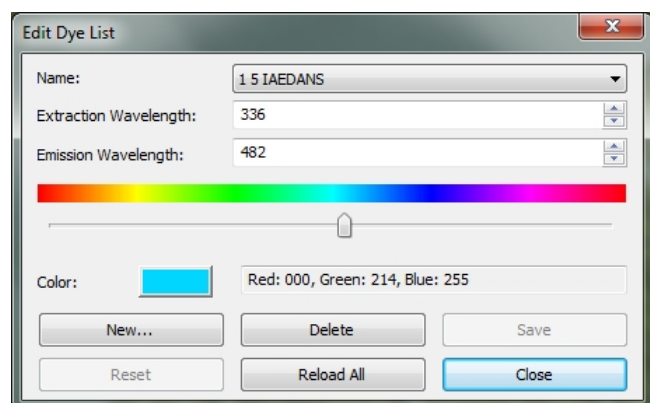


5. If you select **Options -> Calibration ...** or click on the corresponding symbol in the toolbar, a red line with a pixel number and 0.000 μ m length will be superimposed on the video window. At the same time, a dialogue called **Calibration** is displayed in the video window.
6. Try to align the two ends of the red line with the video ruler scale (try to pull the red line as long as possible to maintain the calibration accuracy);
7. Enter the current microscope lens or select it in the magnification field. The current magnification is 10x.
8. Read the superimposed (by the red line) micro-ruler length and enter it in the field **Current length**. This current length is also displayed in the middle of the red line just behind the pixel number. Simultaneously, the resolution in the resolution field is simulated and displayed.
9. When everything is ok, click **OK** to complete the calibration. The 10x magnification is available in the magnification dropdown combo box on the toolbar of the video window.
10. If you want to perform the measurement under this magnification, first select 10x in the above magnification drop-down combo box. Then, select Unit from the Unit drop-down list box, which is just to the left of the expansion drop-down combo box. (The user can also choose from the menu **Options -> Measurements ...**) A dialog with the name Measurement is called up. Click on the **Unit of Length** to check the unit of the current position.
11. Now you can measure the objects with the selected unit.
12. The selected resolution can be stored for future measurements. Once the measurements have been made on this video, the objects and resolution in the image can be saved with MicroCamLabII file format (*.tft) for future applications.
13. The further magnification (4x, 40x, 100x) can be defined as well as the above steps. So when you switch the microscope lens, you only need to select the magnification on the toolbar to perform the measurement.

16.5 Edit Dye List

The Dye List can be used for the **Process -> Color Composite** function. The new Dye List Feature in MCLII allows you to select from a list of Dyes or to add their own custom Dyes to the list. The list of colors (toupview.dye) is stored in **C: / ProgramFiles / TubeTek / ToupView**.

If you select **Options -> Edit Dye List ...**, the **Edit Dye List** dialog box opens:



The list contains all the definitions that are in the current field of view. When this function is used to select a particular dye, the list displays the currently selected dye. You can also select a color from the drop-down list.

Name: The list contains all the definitions that are in the current field of view. You can select a different dye from the drop-down list, and all other controls are updated to display the properties of the new color.

Extraction Wavelength: Displays the default wavelength for your dye. You can adjust the wavelength by entering a new value.

Emission wavelength: This field displays the emission wavelength for the selected dye. You can set the emission wavelength by entering a new value. The standard color changes in response to the changes in the emission wavelength.

Color: Use the slider to adjust the emission wavelength for your dye. This also defines the default color. (You can also use the Color key to select a specific color using a standard Windows Color Selection dialog). The color changes in response to the changes in the emission wavelength. Here you can change the color associated with the selected color, or set a custom color. The default color is linked to the wavelength selection for your dye. If you change the emission wavelength, the displayed color is the default color for that wavelength. To specify a custom color, you should set the wavelength first and then edit the color.

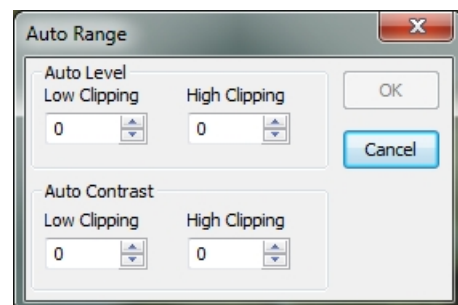
When you click **New**, a new color is created, which opens a dialog box named New Dye. Enter your new Dye name and click **OK** to exit the dialog, click **Cancel** to abort the entry. You are now returned to the color list and the new name is the default name in the name list. Try to set the emission wavelength, extraction wavelength, and color for your new dye.

Save: Click Save to save your color selection.

Close: Click Close to close the hue selection.

16.6 Auto Correction

Set the low and high clipping for the **Image -> Settings -> Auto Level** and **Image -> Adjust -> Auto Contrast** settings. The default value is 0.5% for both, but MicroCamLabII suggests that this value should be between 0 and 0.0999.

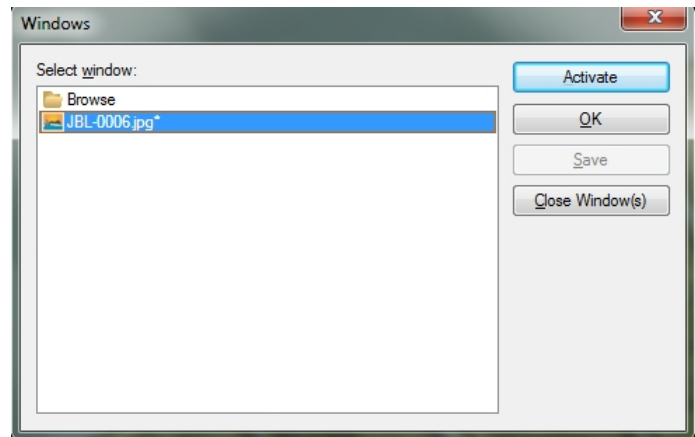


See **Image -> Settings -> Auto Level** and **Image> Adjust> Auto Contrast** for more details.

17. Window

17.1 Close All

Select **Window -> Close All** to close all open windows (including the camera) in MCLII. If changes have not been saved, you will be asked if you want to save them, and then you can select the direction.



17.2 Reset Window Layout

When you select this command, the MCLII window will be reset to the original. The reset will take effect after restarting.

17.3 Window...

Select **Window -> Window ...** opens a dialog box where you can manage the currently open windows.

The dialog allows you to manage large lists of open windows. Let's say you have eight windows open, but want to close four of them scattered through the list.

1. Select **Window -> Window**
2. Select the windows you want to close. Hold Ctrl or Shift (Shift) key to select more than one at a time. In the example, four windows are selected.
3. Click Close Window to close the selected window. The remaining windows are excluded from the operation and opened for further processing.

18. Help

18.1 Help Content

Select **Help -> Help Contents** to open the MCLII Help file. The help file is a PDF document. If your computer does not install the PDF Reader, download the program from the following pages: <http://www.adobe.com/downloads>

19. **About**

Select **About** -> **About** to view the corresponding information about MicroCamLabII, including MCLII version, compatibility, built date, and its developer www, etc.

If you click on the URL link, you will be taken to the address of the camera manufacturer. If you have problems with the camera or MCLII, please contact your supplier.