

LASAF 3.2 - 3D Viewer Manual

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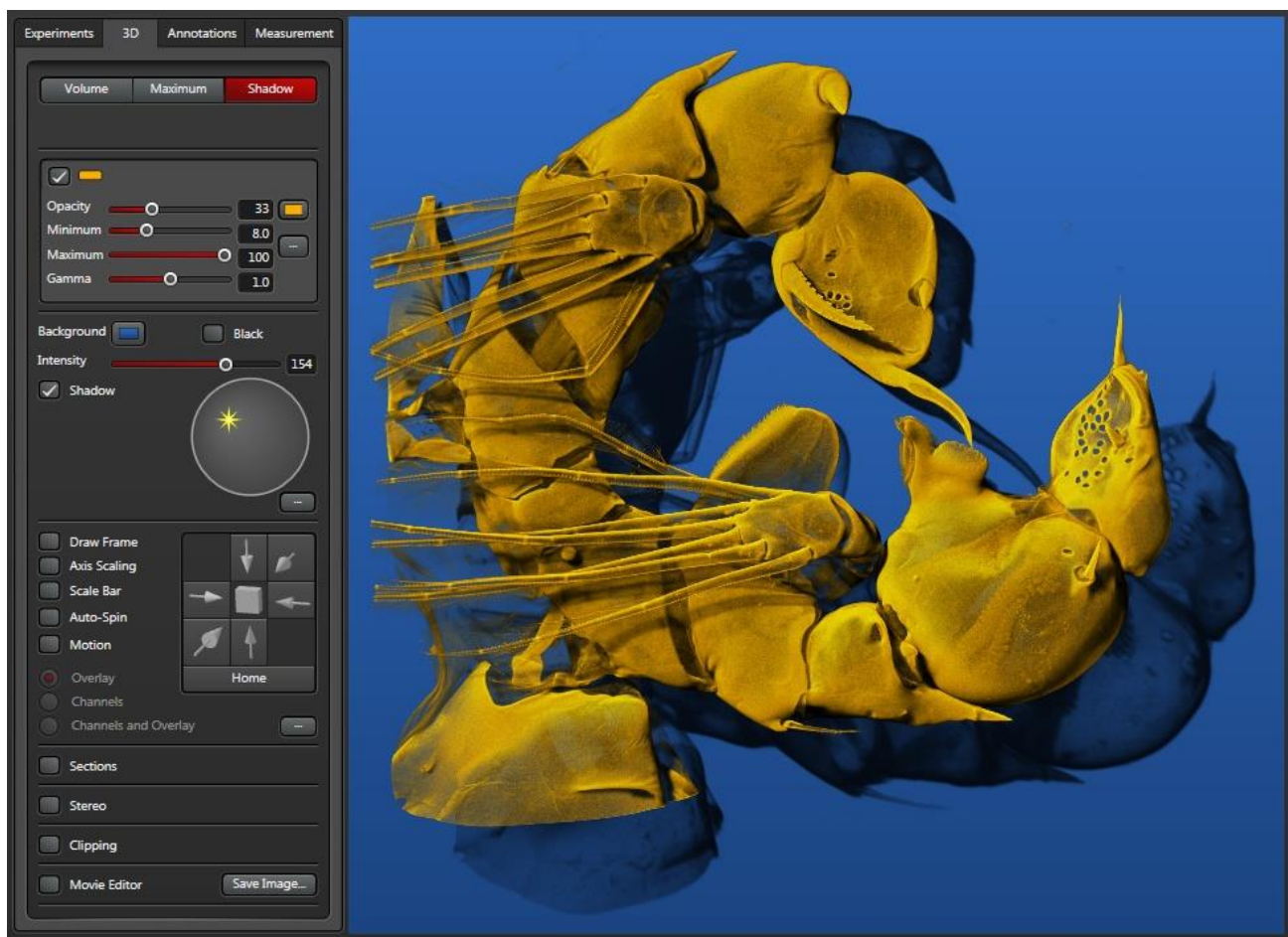
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Overview

The LASAF 3D Viewer includes functions for:

- o Visualizing image stacks with various projection procedures
- o Displaying subvolumes with clip planes
- o Creating individual images and movies from projected image stacks
- o Interactive and automatic measurements and
- o 3D image processing



Licenses

Brief Description
The LASAF 3D Viewer can be used only if you have a corresponding license.
For information about the licenses offered, refer to this page.

For LASAF 3.2, two licenses are offered for the 3D Viewer.

- LASAF AF WPF 3D Advanced Full - All functions
- LASAF AF WPF 3D Advanced Basic - Only the basic functions

Please note that the confocal license "LAS AF SP8 3D Visualisation" includes both the 3D Visualization Basic and the 3D Visualization Advanced module whereas for the Widefield/SPE/LSI systems, the Basic module and the Advanced module are separate

The license for

- LASAF AF WPF 3D Analysis

expands the 3D Viewer by adding functions for interactive measurement, automatic measurement and 3D image processing. It presupposes the existence of the licenses for LASAF AF WPF 3D Advanced Full and LASAF AF WPF 3D Advanced Basic (or the full LAS AF SP8 3D Visualisation respectively) .

The description of the individual functions contains an "only Advanced Full license" or "only 3D Analysis license" mark if the corresponding function is not available without the corresponding license.

To check for existing licenses, select Help - About in the main menu of LASAF. In the User Configuration, a dialog is opened, call up an overview dialog for the licenses using the Check Licenses button.

3D Module Startup

Brief Description

The 3D Viewer is called up with the 3D knob near the image display.

The 3D Viewer functions are called up with the *3D* button to the right next to the image display.



The current image also has to have access to the Z-dimension; otherwise, the knob is not shown.

If the *3D* button is not displayed, please check:

- Is a vertical slider for Z-index selection visible to the right alongside the image? If not, then no image stack is currently displayed.
- Is one of the [licenses](#) for the 3D Viewer available?

3D Viewer

Navigation

The Image display can be rotated, moved or its size can be changed by dragging with the mouse and using different keys and key combinations.

Rotation	Left mouse button
Size Change	Middle mouse button Mouse wheel Right mouse button + Shift key Right mouse button + Ctrl key Left mouse button + Ctrl key
Movement	Right mouse button Left mouse button + Shift key



The main views from the front, back, left, right, top and bottom can be set by clicking the corresponding arrow in the control element bar on the left of the image display.

The *Home* button returns the front view; changes to size and position are also reset.

Projection Procedure

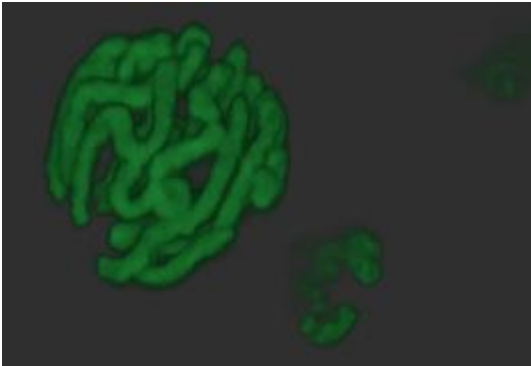
Brief Description

3D Viewer offers 4 display modes, Volume, Maximum, Depth Coding and Shadow.
The pages under Projection Procedure describe the settings that can be made for the different modes.



The upper frame on the 3D tab is used for switching between the 4 methods for generating images.

Volume

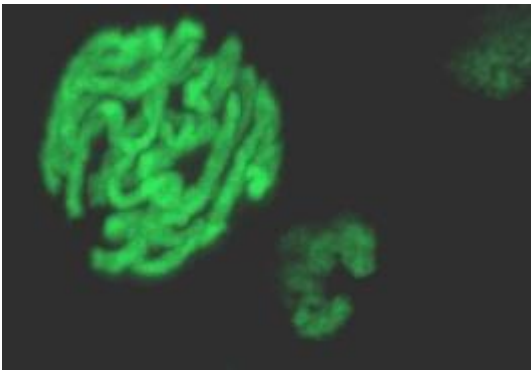


The *Volume* mode provides a display with a depth effect by offsetting transparent image planes.

Only with Advanced Full License: A display for the surface can be configured using the *Volume* mode as well.

The settings for *Volume* mode are described under [Settings in Volume Mode](#).

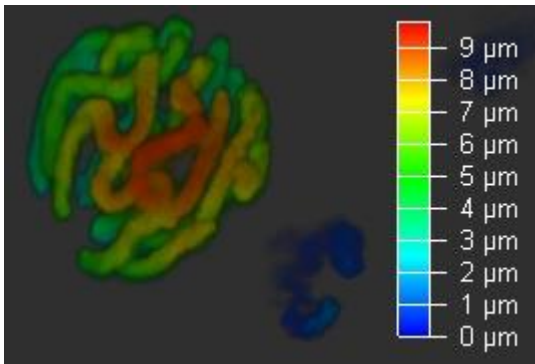
Maximum



In *Maximum* mode, the image intensity for each pixel is calculated from the maximum intensity of the pixels from all image planes. The image is similar to a X-radiograph.

The settings for *Maximum* mode are described under [Settings in Maximum Mode](#).

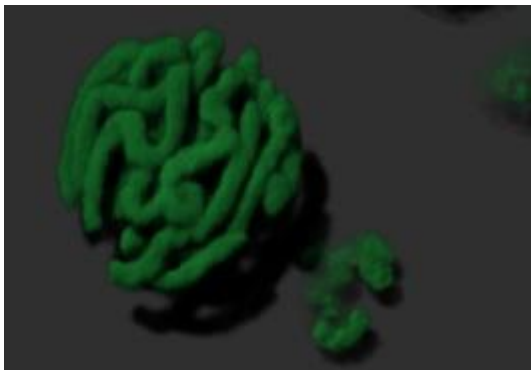
Depth Coding



In *Depth Coding* mode, the image is configured as in *Volume* mode, however, depth color codes are applied in the z-direction of the image stack. Other directions for color coding can also be configured.

The settings for *Depth Coding* mode are described under [Settings in the Depth Coding Mode](#).

Shadow - Only with Advanced Full License



In *Shadow* mode, the shadows from a light source are calculated in addition to the display from *Volume* mode.

The settings for *Shadow* mode are described under [Settings in Shadow Mode](#).

Settings in Volume Mode

Brief Description

The settings in the volume mode include channel colors, the turbidity of the specimen, the intensity range and a gamma correction.

Only with Advanced Full License: Additionally, you can select a surface representation for individual image channels with the corresponding surface properties.



Channel visibility

The display of individual channels can be switched off with the switches in the tab to the left near the channel color.

Channel mode

A display mode is selected for the respective channel with the radio button.

Depth effect is achieved through a transparent projection of image stack levels with Blend mode.

Only with Advanced Full License: With Surface Mode, the surface of the specimen is determined and displayed. The setting elements for this mode are described in [Settings in the Surface Channel Mode](#).

Only with Advanced Full License: With Mixed mode, Blend and Surface are shown simultaneously. The mode is important for the functions of the [3D clip planes](#). It can be used to apply different representations in different volume segments. See also [Channel-specific Segments](#).

Opacity

With the Opacity slider, the turbidity of the specimen is selected. With low turbidity, you can make the hidden areas of the specimen visible.

Channel color

To select the channel color, use the button behind the Opacity slider. When loading the image stack, the channel color is taken over from the image. Notes on operation for the color selection are provided in [Configuring Color and Brightness](#).

Minimum and maximum

With minimum and maximum, the displayed intensity range of the data of the image stack is selected. The unit is percent. Both values are automatically determined when loading a image stack into the 3D Viewer.

The Minimum setting is to be selected greater than the background intensity, because otherwise, visible dark areas are shown for the lower intensities.

The Maximum setting is configured when loading the image to the maximum intensity in the image stack.

Gamma

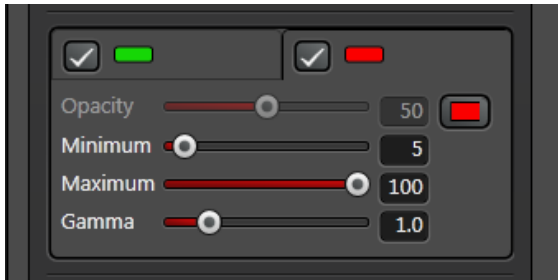
With the gamma slider, a gamma correction of the image intensity can be configured. The operating principle corresponds to that of the gamma controller for the 2D image display window.

Settings in the Maximum Mode

Brief Description

The settings in the maximum mode include channel colors, the intensity range and a gamma correction.

In comparison to the volume mode, the settings for channel mode and turbidity are not possible in maximum mode.



Channel visibility

The display of individual channels can be switched off with the switches in the tab to the left near the channel color.

Channel color

To select the channel color, use the button behind the Opacity slider. When loading the image stack, the channel color is taken over from the image. Notes on operation for the color selection are provided in [Configuring Color and Brightness](#).

Minimum and maximum

With minimum and maximum, the displayed intensity range of the data of the image stack is selected. The unit is percent. Both values are automatically determined when loading a image stack into the 3D Viewer.

The Minimum setting is to be selected greater than the background intensity, because otherwise, visible dark areas are shown for the lower intensities.

The Maximum setting is configured when loading the image to the maximum intensity in the image stack.

Gamma

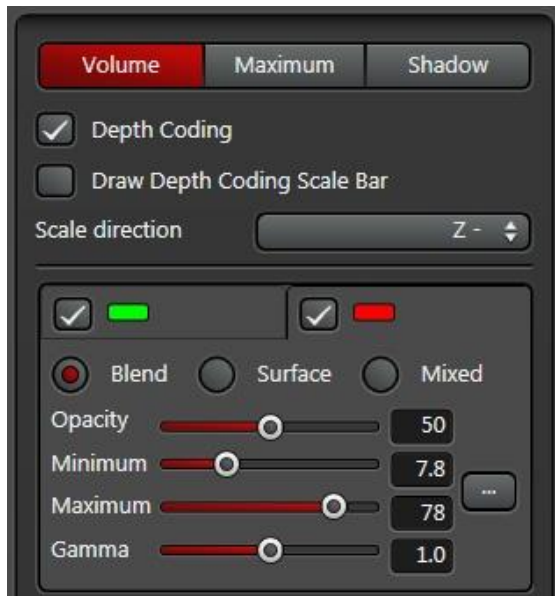
With the gamma slider, a gamma correction of the image intensity can be configured. The operating principle corresponds to that of the gamma controller for the 2D image display window.

Settings in the Depth Coding Mode

Brief Description

Compared to the volume mode settings, no color selection is provided, because the color for visualizing the position is used. Additionally, the color coding spatial direction can be selected and displayed in a scaling bar.

There is a color coding corresponding to the position within the volume in the depth coding mode. Therefore, a blue, green, and red color table is used. In this mode, therefore, no selection of colors is possible.



Scale direction

With Scale Direction, the rule for how the colors are assigned in the spatial direction is selected. In the settings X+, X-, Y+, Y-, Z+ and Z- are the colors assigned along one of the coordinates of the image stack. The character "+" indicates that the color table is applied in positive X-, Y- or Z- direction. With the character "-", the colors are assigned in the opposite direction.

With the two modes, Front to Back and Back to Front, the color selection is not done using the image stack coordinates but rather using the distance from the observer.

Draw Depth Coding Scale Bar

With the switch Draw Depth Coding Scale Bar, the color table can be shown with specification of the allocation of the colors to the distance in the image.

Channel visibility

The display of individual channels can be switched off with the switches in the tab to the left near the channel color. In the Depth Coding mode, no distinction of channels by colors is possible, because the colors for position coding are used.

Channel mode

A display mode is selected for the respective channel with the radio button.

Depth effect is achieved through a transparent projection of image stack levels with Blend mode.

Only with Advanced Full License: With Surface Mode, the surface of the specimen is determined and displayed. The setting elements for this mode are described in [Settings in the Surface Channel Mode](#).

Only with Advanced Full License: With Mixed mode, Blend and Surface are shown simultaneously. The mode is important for the functions of the clip planes. It can be used to apply different representations in different volume segments. See also [Channel-specific Segments](#).

Opacity

The turbidity is selected with the slider. With low turbidity, you can make the hidden areas of the specimen visible.

Minimum and maximum

With minimum and maximum, the displayed intensity range of the data of the image stack is selected. The unit is percent. Both values are automatically determined when loading a image stack into the 3D Viewer.

The Minimum setting is to be selected greater than the background intensity, because otherwise, visible dark areas are shown for the lower intensities.

The Maximum setting is configured when loading the image to the maximum intensity in the image stack.

Gamma

With the gamma slider, a gamma correction of the image intensity can be configured. The operating principle corresponds to that of the gamma controller for the 2D image display window.

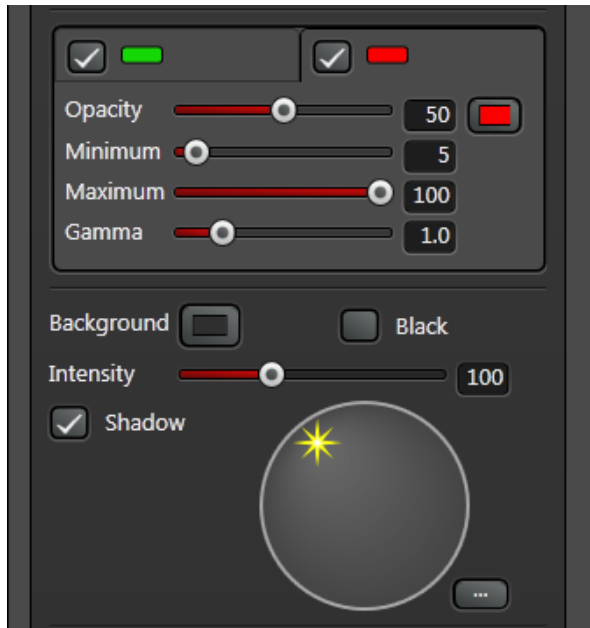
Settings in Shadow Mode

Brief Description

Only with Advanced Full License: The settings in the shadow mode include channel colors, the turbidity of the specimen, the intensity range, a gamma correction and the position of the light source for the shadow.

The shadow is always cast onto the specimen itself. The background shadow can be turned off.

Only with Advanced Full License



Channel visibility

The display of individual channels can be switched off with the switches in the tab to the left near the channel color.

Opacity

The turbidity is selected with the slider. With low turbidity, you can make the hidden areas of the specimen visible.

Channel color

To select the channel color, use the button behind the Opacity slider. When loading the image stack, the channel color is taken over from the image. Notes on operation for the color selection are provided in [Configuring Color and Brightness](#).

Minimum and maximum

With minimum and maximum, the displayed intensity range of the data of the image stack is selected. The unit is percent. Both values are automatically determined when loading a image stack into the 3D Viewer.

The Minimum setting is to be selected greater than the background intensity, because otherwise, visible dark areas are shown for the lower intensities.

The Maximum setting is configured when loading the image to the maximum intensity in the image stack.

Gamma

With the gamma slider, a gamma correction of the image intensity can be configured. The operating principle corresponds to that of the gamma controller for the 2D image display window.

Shadow

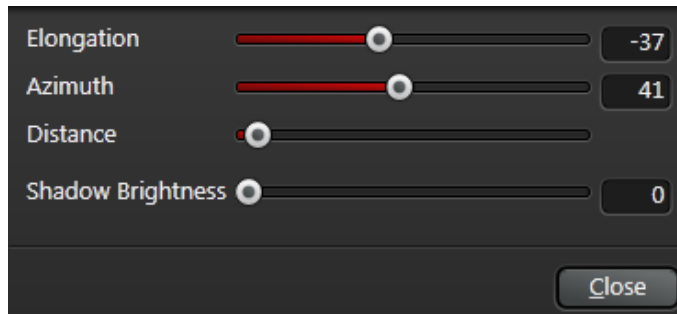
With the switch, the display of the shadow on a plane behind the specimen can be switched off.

Position of the point light source

The position of the point light source is the same for all channels. It is carried out via a circular control element. The description is found in [Setting the Position of a Point Light Source](#)

Distance and Shadow Brightness

With the ... button, two more settings can be carried out in Shadow mode.



There is a slider for the brightness of the shadow, Shadow Brightness.

The Distance slider is intended for removing the projection plane of the shadow. The left limit stop of the slider corresponds to a projection plane through the center of the displayed specimen. The right limit stop corresponds to a projection plane behind the specimen with a distance of a half space diagonal of the image stack for the specimen center.

The program prevents the projection plane of the shadow from extending into the specimen representation. In this process, the projection plane is always pushed back to the farthest corner of the image stack.

Frequently, the Z-expansion of the batch is less than the X- and Y-expansion. With the standard view from the front, a close projection plane is recommended. Therefore, the start setting is the left limit stop of the controller.

If, for example, strong rotations are used for compiling a video, you should set the controller more in the direction of the right limit stop to prevent movement of the shadow projection plane.

Settings in the Surface Channel Mode

Brief Description

Only with Advanced Full License: The settings in Surface Channel Mode contain the threshold for the generation of the surface and the surface properties.

Only with Advanced Full License



Opacity

With the Opacity slider, the turbidity of the specimen is selected. With a turbidity of less than 100, hidden areas of the specimen are visible.

Threshold

With the Threshold slider, the intensity threshold for the surface generation is defined. Image stack areas with intensity above this threshold belong to the specimen. The input is done in percent.

Specular

The color share of the surface representation, which comes into being by means of a point light source, is established by the Specular slider. In the Volume mode, the color is selectable with the button to the right alongside the slider; notes for operation with the color selection are found in [Configuring Color and Brightness](#).

Ambient

The color share of the surface representation, which comes into being by means of ambient light, is established by the Ambient slider. In the Volume mode, the color is selectable with the button to the right alongside the slider; notes for operation with the color selection are found in [Configuring Color and Brightness](#).

Shininess

With the Shininess slider, you can configure whether the surface is to be shown as smooth (high value) or rough (small value).

Position of the point light source

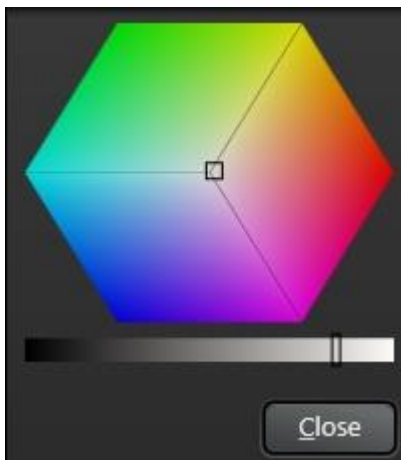
The position of the point light source is the same for all channels. It is carried out via a circular control element below the control elements of the channel properties. The description is found in [Setting the Position of a Point Light Source](#).

Configuring Color and Brightness

Brief Description

A button with a drop-down window is used to configure colors and intensities for various functions in the 3D Viewer.
The color is selected by clicking the color palette, the intensity is selected by clicking the intensity bar under the palette.

A button with a color field is always offered in the 3D Viewer to select colors and intensities for the different functions. The button opens a window with a color palette and an intensity bar.



The currently selected color is displayed in the color palette with a small black rectangle. A new color can be selected by clicking. The rectangle can also be dragged to another position with the mouse. You can accurately configure the primary colors red, green, and blue by dragging the rectangle into the corresponding corner with the mouse.

The current intensity is marked by a black rectangle in the intensity bar under the palette. Here as well, a change can be made by clicking and dragging with the mouse.

Setting the Position of a Point Light Source

Brief Description

The position of a light source for displaying shadows and surfaces is possible by clicking at the desired position in the circle.

Only with Advanced Full License

A control element for selecting the light source with a circle and a yellow star for marking the light source is used for displaying shadows and surfaces.



Another position can be set with a mouse click. Dragging the mouse is also possible.

The position of a light source is set relative to the viewing direction in the process. Selecting the center of the circle provides light from the direction that the observer sees the object.

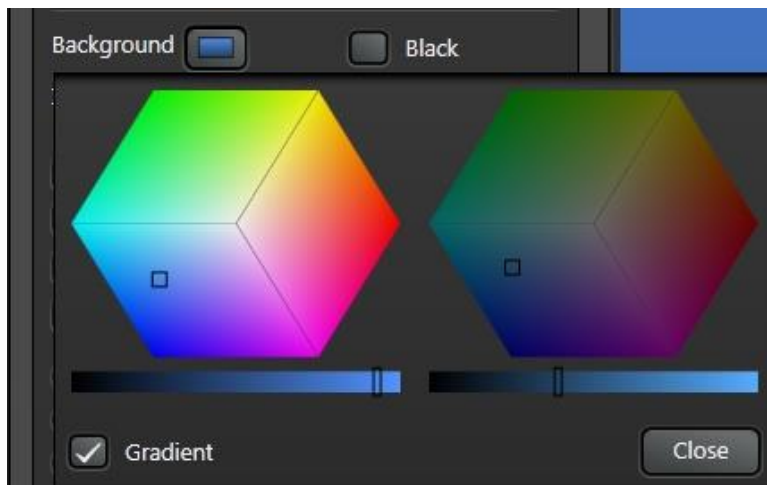
Using the ... button, you can also set it in a drop-down window using Elongation and Azimuth sliders. Elongation and azimuth are specified in degrees here.

Background

Brief Description

In addition to a background color, a color gradient for the background can also be selected. A switch provides the ability to set the background to black.

Configure the background color using the *Background* color selection element. Notes on operation for a color selection are provided in [Configuring Color and Brightness](#).



A color gradient can be configured for the background. To do so, enable the *Gradient* button. Choose the color for the upper screen edge in the left area and the color for the lower screen edge in the right area.

Black Background

The *Black* button can be used to toggle to a black background. This does not reset the previously selected background colors. This feature is particularly recommended for the Maximum mode.

Intensity

Brief Description

The intensity for displaying the data of the image stack can be continuously modified.

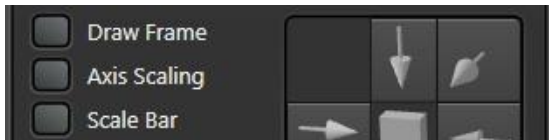


The *Intensity* slider is used to modify the brightness of the image data for all channels. The modification has no effect on the background, frame, scaling, and other display elements.

Frame and Scaling

Brief Description

You can use a switch to display a positioning frame with the border lines of the image stack volume and metric scaling.



Draw Frame

The *Draw Frame* button displays a positioning frame with the border lines of the image stack volume. Particularly when editing videos, the positioning frame is helpful for defining the orientation.

Axis Scaling

The *Axis Scaling* switch displays metric scaling for the coordinate axes x, y, and z of the image stack. The program automatically selects suitable outer border lines where the scaling is applied.

Scale Bar

The *Scale Bar* button displays a scaling bar on the left lower edge of the 3D Viewer.

Auto-Spin and Motion

Brief Description

In Auto-Spin mode, the image stack rotates in the direction the mouse is first dragged.
In Motion mode, the image stack is rotated a few degrees right and left in alternation.



Auto-Spin

The *Auto-Spin* button enables a mode that causes the image stack to rotate continuously in the direction in which the mouse is first dragged (refer also to Navigation).

Motion

The *Motion* switch enables a mode that causes the image stack to rotate continuously a few degrees left and right in alternation. This mode can provide better visual depth.

View per Channel

Brief Description
For image stacks having multiple channels, it is possible to view projections of individual channels beside one another. All of the functions of the 3D Viewer can be combined with this viewing mode.



Overlay

The *Overlay* mode enabled by default when the program is launched. All image channels are displayed in one projection.

Channels

The *Channels* radio button can be used to select a view in which projections of individual channels are displayed beside or below each other.

Channels and Overlay

In *Channels and Overlay* mode, a projection containing all channels is displayed in addition to the projections of the individual channels.

The ability to switch to this mode is enabled only if there is more than one image channel.

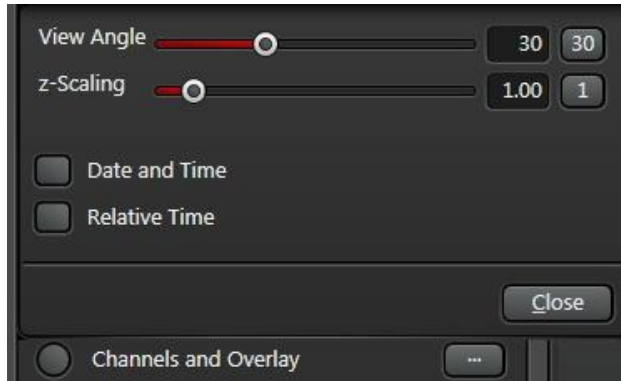
Viewing per Channel can be combined with all other functions of the 3D Viewer.

The program automatically distributes the image region based on the maximum image size.

Projection

Brief Description

Only with Advanced Full License: By changing the projection angle, effects can be achieved similar to using different focal distances in photography. A parallel projection is also configurable. Magnification of the Z-direction can be configured for image stacks of thin specimens.



View Angle

Only with Advanced Full License: The angle at which the image boundary is seen by the observer is changed using the *View Angle* slider. An angle of 0 corresponds to a parallel projection. Effects such as those from a wide-angle lens in photography can be achieved using a large angle.

Z-Scaling

For thin specimens, increasing the Z-direction can improve the 3D effect. The magnification factor is adjusted using the *Z-scaling* slider. The slider can be found in the drop-down window above the ... button next to the radio buttons for channel-based image display.

Orthogonal sections

Brief Description

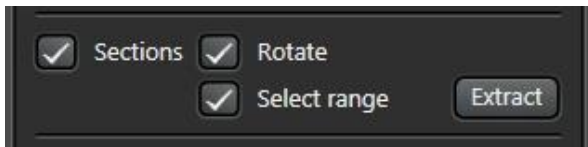
Orthogonal sections can be displayed via the *Sections* button. The clip planes can be rotated.

Rotated box-shaped image areas can be cut out.

Only with Advanced Full License

After switching on *Sections* you will find

- at the top right - the 3D projection
- at the bottom left - the clip plane with view from the front
- at the top left - the clip plane with view from the top and
- at the bottom right - the clip plane with view from the left.



Rotate

By rotating the projection at the top right, the corresponding slanted clip planes are also displayed.

Select Range

Border lines are displayed in the views of the clip planes. These lines can be moved using the mouse. In the other views for clip planes, a projection is displayed for the selected range.



Extract

The button is used to write the selected box-shaped region into a new image. The image that was cut out appears as a new entry in the Experiment tree.

Time Series

Brief Description

With time series of stacks, an image stack can be selected from the time series with the slider under the image display.



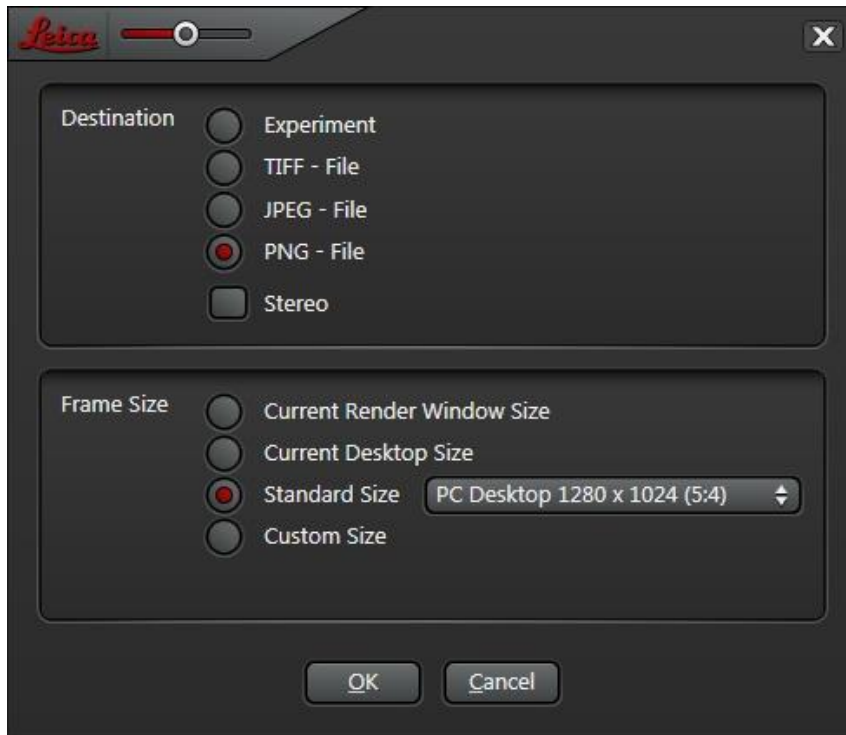
In the case of time series, a slider is displayed under the image display. The image is calculated for the time series index selected there. The control element nearby to the left can be used to automatically change the time series index, like in the 2D-image display.

Only **License Advanced Full**: For time series, there are other functions available during movie editing. You can find the description under movies with time series.

Save and Export

Brief Description
Projections can be appended as a new image for the current experiment or exported into an image file.

Use the Save Image... button to call up the dialog for selecting the settings.



Destination

When selecting the Experiment radio button, the image in the Experiment tree is appended under the current experiment.

If you would like to write directly in an image file, select the appropriate file format with one of the other three radio buttons.

Stereo

The Stereo switch can be used to activate production of a stereoscopic image file. The format used for this is referred to by image display programs for stereoscopic images as "Side by Side, Right Image First". For more information on the Stereo topic, refer to the [Stereo](#) section.

Frame Size

In *Current Render Window Size* mode, an image with the number of pixels is produced as it is currently used in the image display.

In *Current Desktop Size* mode, an image is generated that fits exactly on the primary monitor.

In *Standard Size* mode, a list of common image sizes is available.

In *Custom Size* mode you can enter the horizontal and vertical number of pixels.

Stereo

Brief Description

Only with Advanced Full License: Stereo image viewing can be selected for different types of monitors and glasses. This page contains, along with information on operation, notes on suitable technology and their installation. These can be found under [Stereo operation with shutter glasses](#).

With stereo operation, images are calculated separately for the right and left eyes. Due to double calculation, the image display is also slower by up to a factor of two.

Passive glasses with color foils, some passive monitors with polarization filters and polarization glasses, and some active monitors and shutter glasses are supported. Please observe the notes under [Hardware and Software Requirements](#) and [Stereo operation with shutter glasses](#).

With the Stereo switch, the stereo mode is activated. With the ... button, additional settings can be configured.



Mode

There are four modes available to display stereo images:

- Red / Cyan:** This mode is intended for glasses with color foils. Adjust the glasses so that the red foil is in front of the left eye. If, due to design, this is not possible, you can also reverse the stereo images with the *Eye Distance* slider.
- Quad-Buffer:** This mode is intended for systems that support stereo display via the OpenGL interface. Please observe the notes under [Hardware and Software Requirements](#) for suitable technology. If the program recognizes that stereo display is possible via OpenGL, then this mode is used as a start setting.
- Horizontal Interlaced:** This mode is intended for monitors with polarization layer and polarization glasses if lines following one another on the monitor have different polarization respectively.
- Vertical Interlaced:** This mode is intended for monitors where columns following one another on the monitor have different polarization.

Eye Distance

The intensity of the stereo effect can be set with this slider. The best setting depends on the distance from the monitor observer and from the pixel distance of the monitor. The default value of 30 must only be changed in rare cases.

Select a sharp image stack with a lot of content for setting. Select in alternation a view from the front and a view from the side. Set the slider so that the thickness of the stack when viewing from the front and viewing from the side appear equal.

View Point

With the *View Point* slider, the distance of the stereo image relative to the monitor surface can be set. With small values, the stereo image is shown in front of the monitor surface. The default setting is selected in such a way that the image appears approximately behind the monitor surface, which is less strenuous for the observer.

3D Clip Planes

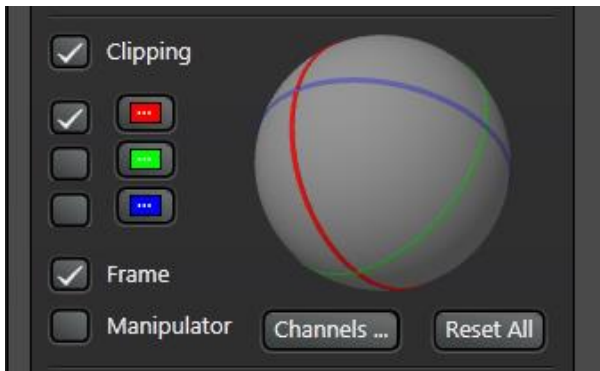
Visibility of Volume Segments

Brief Description

Up to three clip planes can be selected for viewing internal areas of the specimen. The visibility of volume segments is activated and deactivated by clicking the sphere.

Only with Advanced Full License

The *Clipping* button has to be enabled to use clip planes.



Up to three clip planes can be activated via the switches on the left next to the colored buttons.

The clip planes are displayed in the image with a colored frame. The color corresponds to the color of the button next to the switch of the plane,

On the sphere, the clip planes are also displayed with this color. When the specimen rotates, the sphere also rotates accordingly.

By clicking a volume segment on the sphere, the display of the data in the corresponding volume segment can be activated and deactivated again.



The display of the limit frame of the clip plane can be activated and deactivated using the Frame switch.

Navigation

Brief Description

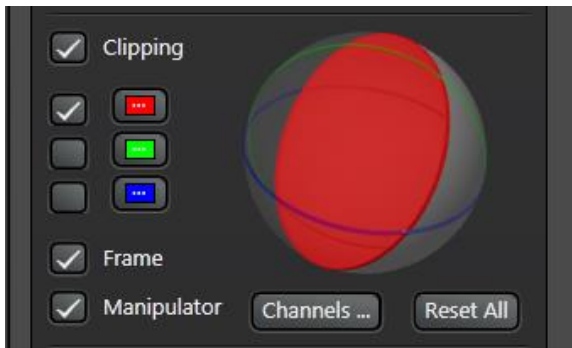
The position and orientation of clip planes can be modified using the mouse.

Only with Advanced Full License

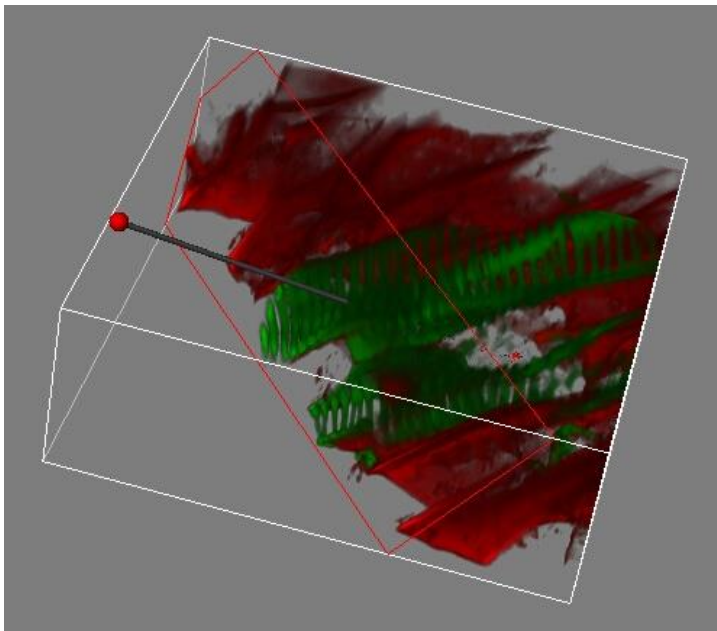
Move

When you hover the mouse over one of the colored border edges of a clip plane, the shape of the mouse cursor changes to that of an arrow. Drag the mouse to shift the clip plane.

Changing Orientation



To change the orientation of a clip plane, first enable the Manipulator switch. A gray needle with two colored spheres appears on the screen. Adjust the orientation of the clip plane by dragging the spheres with the mouse.



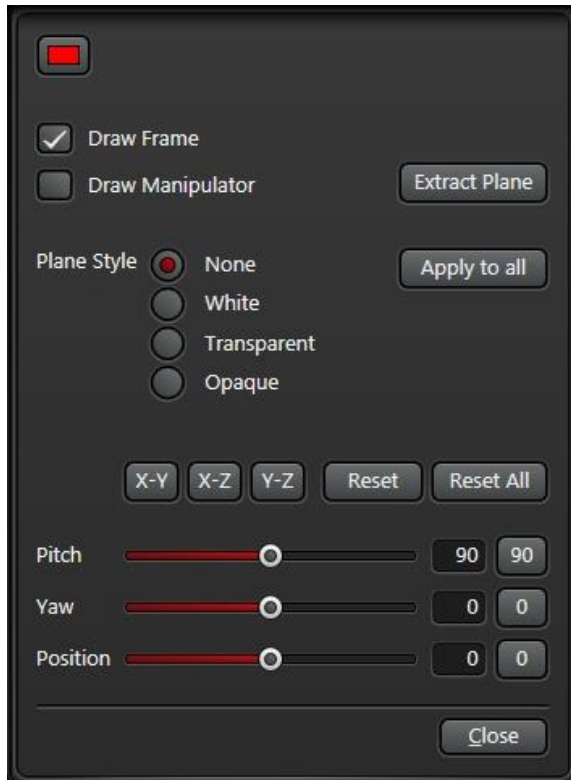
Characteristics of Planes

Brief Description
Display, position, and orientation can be configured for the individual planes via a drop-down window.

Only with Advanced Full License

Next to the switches for the clip planes there is a button ... with a color mark.

Pressing the button returns a window with the control elements for displaying the corresponding clip plane.



Frame Color

The colored button can be used to change the color of the limit frame of the clip plane. Details concerning color selection are on the [Configuring Color and Brightness](#) page.

Draw Frame

The display of the colored border edge is activated and deactivated using the *Draw Frame* button.

Draw Manipulator

The display of the manipulation needle for orienting the clip plane is activated and deactivated using the Draw Manipulator switch. The steps for modifying the orientation are described on the [Navigation](#) page.

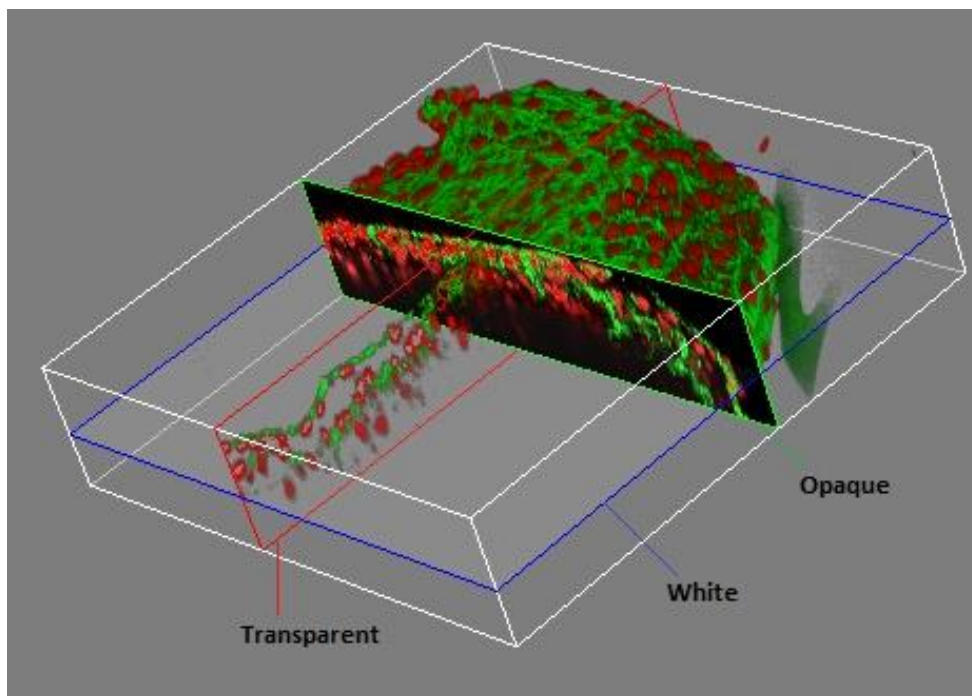
Extract Plane

The *Extract Plane* button is used to write the clip plane into a new image. The new image appears in the Experiment tree.

Plane Style

The *Plane Style* radio buttons can be used to modify the display of the individual planes.

- None:** The surface of the plane is not depicted.
- White:** The surface of the plane is depicted as transparent white.
- Transparent:** The surface of the plane is depicted corresponding to the image intensity. If the image intensity is low, the corresponding image area is transparent.
- Opaque:** The surface of the plane is depicted corresponding to the image intensity. If the image intensity is low, the corresponding image area is dark.



Apply to All

The *Apply to All* button can be used to transfer the Plane Style configuration to all clip planes.

Position and Orientation

The operation for modifying the orientation and position with the mouse in the image are described on the [Navigation](#) page. Additionally, these operations can also be carried out via the *Pitch*, *Yaw*, and *Position* sliders.

The three buttons, X-Y, X-Z, and Y-Z, return clip planes parallel to the limit surfaces of the volume through the center of the volume.

The *Reset* button can be used to restore the default orientation and position from the program start. Here, the orientation is different for the individual planes. The *Reset All* button can be used to configure the default orientation and position for all planes from the program start.

Channel-specific Segments

Brief Description

The cutting away can be shut off for individual channels.

Different channels can be displayed in different segments.

Surface and transparent projection can be differently selected for channels and segments.

These functions can be accessed via the *Channels...* button.

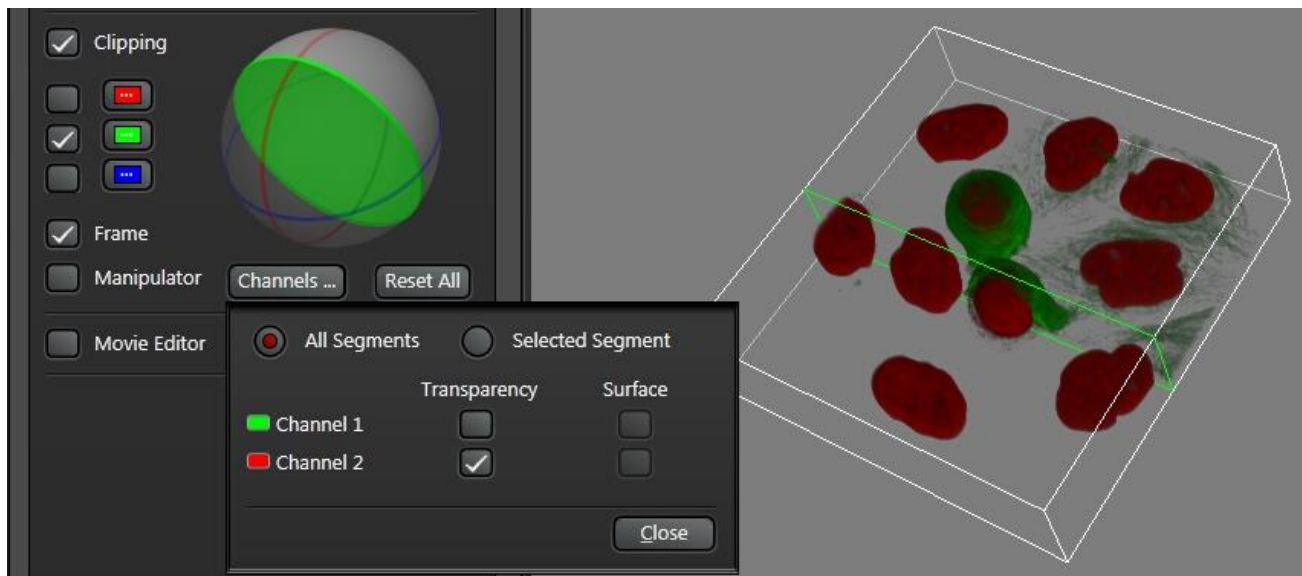
Only with Advanced Full License

The *Channels...* button can be used to drop down a window with functions for selecting the display mode for individual channels and segments.

In *All Segments* mode you can determine whether individual channels will be displayed nonetheless in the segments that have been cut away. If a switch in the matrix is switched on, the channel in the corresponding display mode is also displayed in the segments that have been cut away.

The choice between *Transparency* and *Surface* is possible if the corresponding mode has been selected for the projection procedure; details on selecting the projection modes are included in [Settings in Volume Mode](#) and [Settings in Depth Coding Mode](#). *Transparency* and *Surface* are available when selecting *Mixed mode for the projection* procedure.

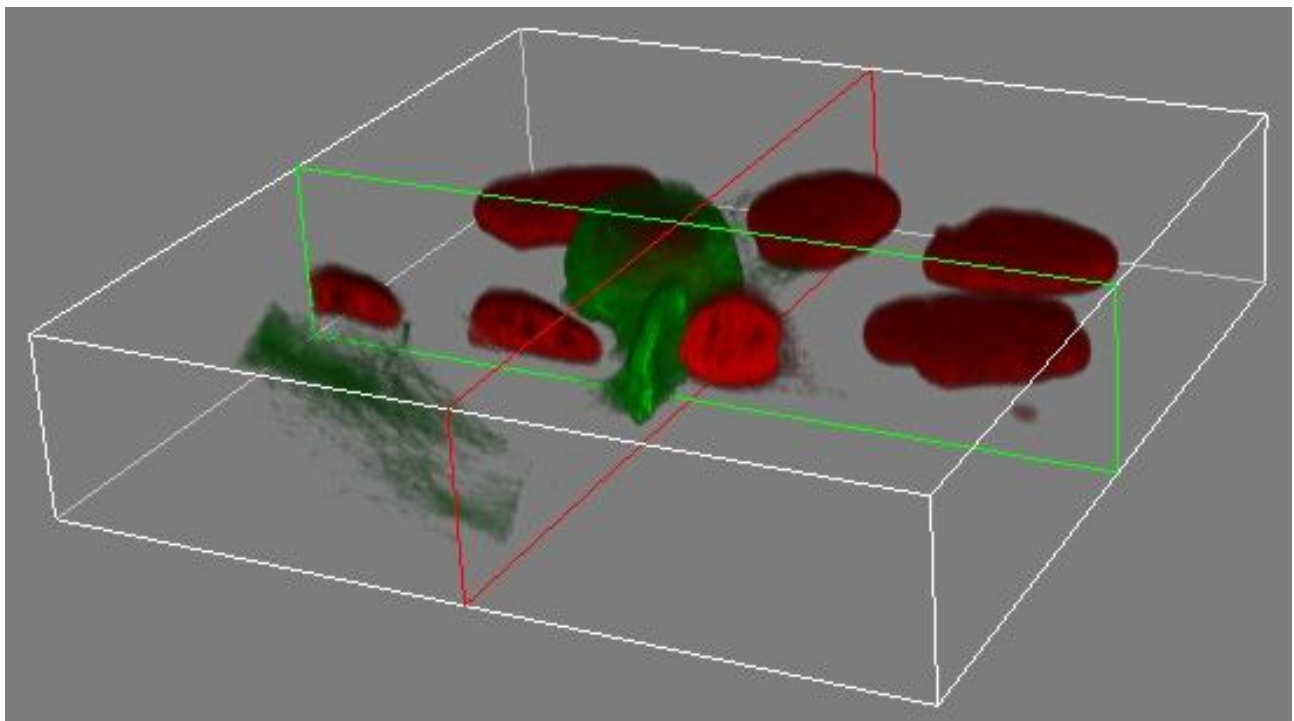
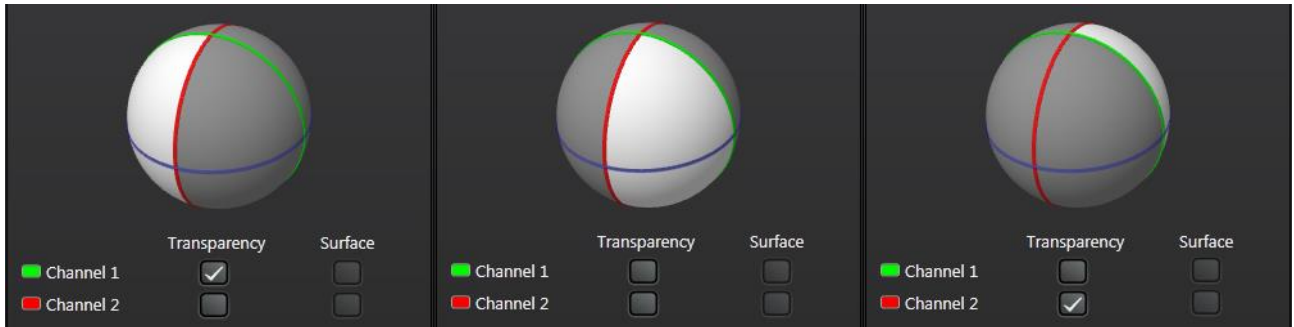
The figure shows how the red channel can be displayed even if the front segment is switched off.



In *Selected Segment* mode you can define that different channels will be displayed in different segments with different modes.

A segment is selected with the sphere. Then the channels and modes can be configured for the segment.

The figure contains an example for how none of the channels can be shown in the front segment and a different channel can be shown in each of the two neighboring segments.

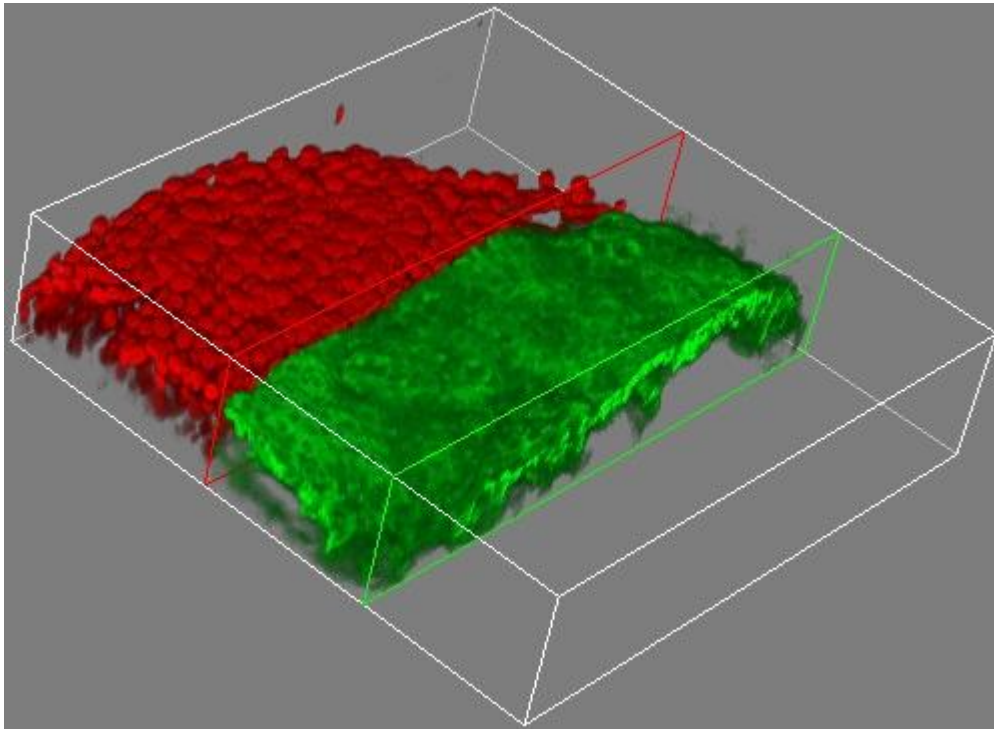


Parallel Clip Planes

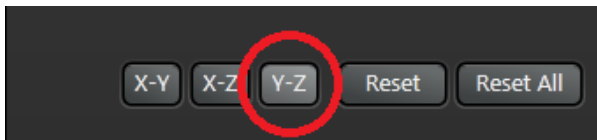
Brief Description

In conjunction with creating videos, the use of multiple parallel clip planes provides good insight into the structure of the specimen for multiple image channels.

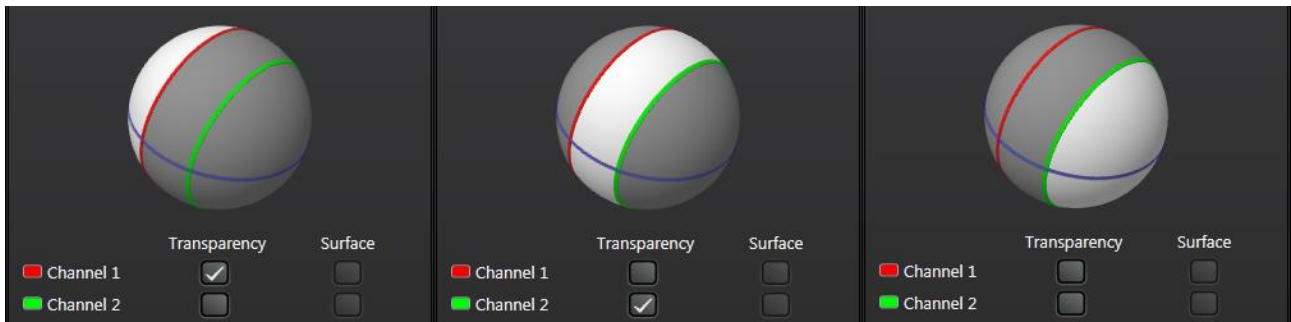
Only with Advanced Full License



To produce parallel clip planes, select the same orientation for multiple clip planes in the drop-down window for the respective plane. The details for operation are included on the [Characteristics of Planes](#) page.



In the *Channels...* drop-down window you can then select the channels for the individual segments. The details for operation are included on the [Channel-specific Segments](#) page.



3D Movie Editor

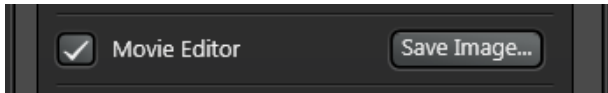
Start and Licenses

Brief Description
The Movie Editor only has its full functionality with an <i>Advanced Full</i> license.
The control elements are attached on the bottom edge of the window. They are visible after being activated with the <i>Movie Editor</i> or <i>Movie</i> switch.

All of the Movie Editor's functions are only available with the Advanced Full license. Multiple keyframes can be defined here. When creating the video, the properties are interpolated from the keyframes. This results in a fluid video.

Start and end frames can be set with the Advanced Basic license. The properties of these two frames are interpolated.

The control elements for this are displayed with the *Movie Editor* button for the Advanced Full license or the *Movie* button for the Advanced Basic license.



Keyframes

Brief Description

Only with Advanced Full License: Keyframes are added using the Add or Rotate buttons.
 Only with Advanced Basic License: The start and end frames of a video are set using the Set Start and Set End buttons.

Only with Advanced Full License

Start by using 3D Viewer to configure which frame the video is to start with. Press the *Add* button. A marking for the first frame is added by the program at the start of the bar on the right side.

Modify the settings in the 3D Viewer for another frame intended to be part of the movie and press the *Add* button again. Continue setting keyframes this way. Each time you press the *Add* button you receive a marking on the bar on the right side.



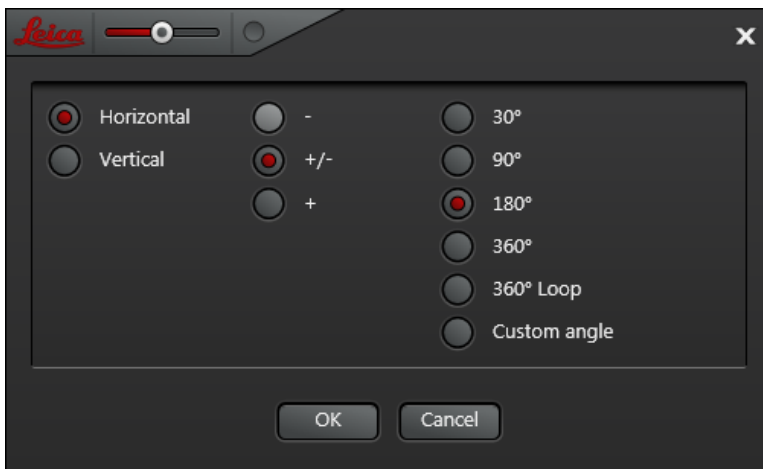
Only with Advanced Full License: Keyframes can also be set as fixed, pre-defined rotations using the *Rotate...* button. Select the axis of rotation in the first column, the direction of rotation in the second column and the swivel angle in the third column.

The +/- entry provides a starting keyframe originating from the current view for negative rotation by the selected angle. The movement then goes from the initial view to the view with the positive swivel angle.

Example: Using the settings Horizontal, +/- and 90°, you set a movement with a view from the left from the initial view to a view from the right.

The keyframes set this way are appended to existing keyframes.

The 360° Loop entry is an exception. Here the process starts by removing all already existing keyframes. Then keyframes for rotation are added. If a video of this type were set to loop the result would be a continuous rotation around the set axis.



Only with Advanced Basic License

Start by using 3D Viewer to configure which frame the video is to start with. Press the Set Start button. Then configure the last frame of the video and press the Set End button.



Preview

<p>Brief Description</p> <p>With the <i>Preview</i> button, the movie can be examined before it is stored. Only Advanced Full <i>License</i> : By clicking in the bar on the right side, an image from the movie is preprocessed and displayed.</p>

In the *Frames* input field, define how many frames the movie should contain altogether. The number is usually much greater than the number of established keyframes.

A movie can also be saved so that, after the last image, you are returned to the start image by interpolation. Select the corresponding mode in the drop-down list field to the right alongside the input field for the number of images.

- Single:** The movie is saved just as it was configured. With repeat playback, there is a jump from the last image to the first.
- Yo-Yo:** After the last image, the images are again appended in reverse order. The movie can be played continuously without jumping.
- Loop:** Only with Advanced Full License: After the last image was reached, images are quickly inserted in order to return to the first image by the shortest way possible. The movie can be played continuously without jumping.

In *Yo-Yo* mode, a saved movie receives twice as many images as was indicated in *Frames*. In *Loop* mode, it is a maximum of twice as many images. The exact number in *Loop* mode is dependent on the number of keyframes.

Only with Advanced Full License: The bar on the right side is subdivided by dark, vertical lines that each a frame of the movie. If you click on an image marked as such in the bar, the program calculates this image and displays it.



With the *Preview* button, you can allow a continuous preview of the movie to play. With large image stacks, however, the program reduces the quality of the display. In contrast, a movie is always generated by the program in the highest quality.

Correcting and Adding Keyframes

Brief Description

Only with *Advanced Full License*: Keyframes can also subsequently be replaced with the *Replace* button. Additional keyframes can be inserted with the *Add* button. Individual keyframes can be removed with the *Remove* button.

Only with Advanced Full License

Modifying Keyframes

Keyframes can subsequently be changed. To do so, first select the keyframe that you would like to change in the bar on the right side by clicking on the white keyframe marking.

Then modify the view with the 3D Viewer. Finally, press the *Replace* button.



Move keyframes

The position of a keyframe can be changed by dragging the keyframe marking to the right side in the bar.

Inserting keyframes

To do so, first select the position where you have to insert the keyframe by clicking on the corresponding position in the bar on the right side. The small white mark shows the position. Then modify the view with the 3D Viewer. Then press the *Add* button. A new keyframe is then inserted at the marked position.

Removing keyframes

Select the keyframe that you would like to remove in the bar on the right side by clicking on the white keyframe marking. Then press the *Remove* button.

Remove all keyframes

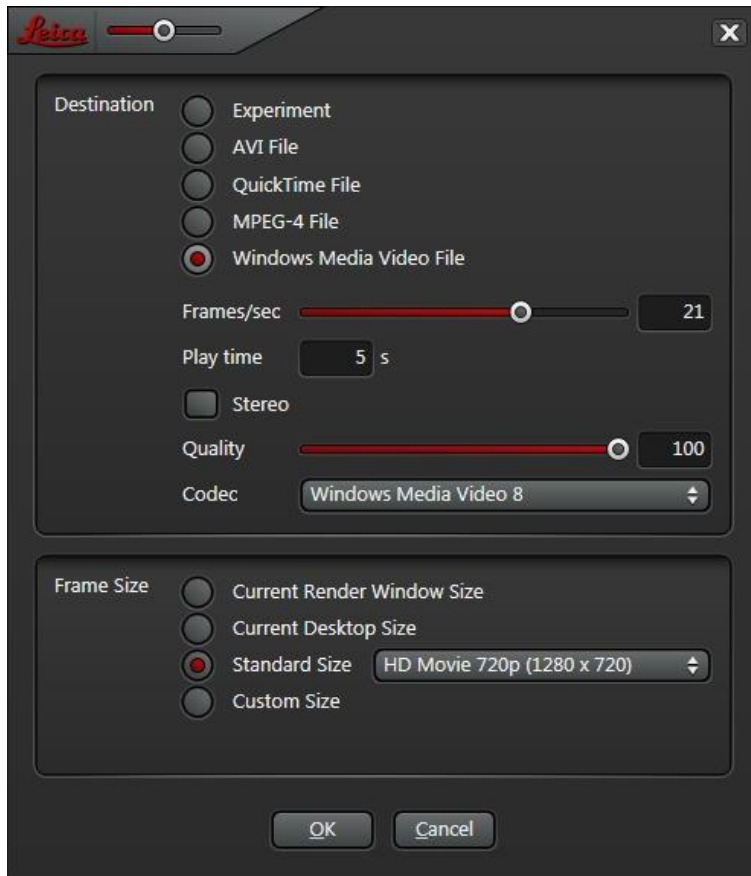
Then press the *Clear* button.

Saving Movies

Brief Description

Videos can be saved appended to a new image series in the experiment or directly in a movie file.

The *Save...* button calls up a dialog window where the destination and settings for an image series or movie file are specified.



Destination

When selecting the Experiment radio button, the image series in the Experiment tree is appended under the current experiment.

If you would like to write directly to a video file, select the appropriate file format with one of the other four radio buttons. Additional information on the movie file formats can be found at the end of this page.

Frames/sec

The *Frames/sec* slider is only visible if you are saving to a movie file. The set number of frames per second is written in the image file metadata. The programs for playing the videos use the information to determine the playback speed.

Play time

The playing time of the movie is displayed in seconds. Instead of selecting *Frames/sec*, the playback speed can also be specified by entering a playing time in this edit field.

Stereo

The *Stereo* button can be used to activate production of a stereoscopic movie file. The format used for this is designated by playback software for stereoscopic movies as "Side by Side, Right Image First". Saving in stereo format is only offered for the AVI and Windows Media Video formats.

Please note that playing stereo movies is only possible with hardware and software that is intended for and specially configured for this application. Also read the page [Hardware and Software Requirements](#).

Quality

The *Quality* slider can influence the size of a movie file in Windows Media Video and QuickTime format. At low values, smaller files can be made at the cost of lower quality compared to the movie's original frames.

We recommend keeping it at the right limit position and working with lower quality only if the file is too large.

Codec

The compression type for a movie file in Windows Media Video format can be selected with the drop-down *Codec* list field.

Windows Media Video 9 Advanced is the latest and Windows Media Video 8 is the oldest compression type. Quality and compression rate differ only slightly with different types. Not all media players support all types.

Use Compression

Movies can only be saved uncompressed in full quality in the AVI and QuickTime formats. You can also specify lossy encoding for these formats with the Use Compression switch.

With the AVI format, select compression type and parameters (codecs) in an additional dialog. This window is provided by the operating system and takes the installation compression procedure into account.

Frame Size

In *Current Render Window Size* mode, a movie with the number of pixels per frame is produced as it is currently used in the image display.

In *Current Desktop Size* mode, a movie is generated that fits exactly on the primary monitor.

In *Standard Size* mode, a list of common movie and image formats is available.

In *Custom Size* mode you can enter the horizontal and vertical number of pixels. Please note that for movie file formats and compression types, there are limitations on the selection of a horizontal and vertical number of pixels. For more information, refer to the following paragraph on the movie file formats.

Movie file formats

AVI is the native file format for Microsoft Video for Windows. The files can be played with Microsoft Windows Media Player. The format is limited to movie file sizes up to 2 GB. The horizontal and vertical number of pixels has to be even. The 3D Viewer corrects the number of pixels input automatically in order to maintain this rule. Compression methods can be installed in the operating system in the form of "Codec Packs". There can be additional limitations for the respective compression method. Please check whether a movie created can also actually be played if you use AVI compression.

QuickTime (.mov file extension) is the native Apple QuickTime file format. The files can be played with Apple QuickTime Player.

MPEG-4 (mp4 file extension) is a video file format that was standardized by the International Organization for Standardization (ISO). The files can be played with the newer versions of Microsoft Windows Media Player and Apple QuickTime Player. The maximum number of pixels is 1920 horizontally and 1080 vertically. The horizontal and vertical

number of pixels has to be even. The 3D Viewer corrects the number of pixels input automatically in order to maintain this rule. MPEG-4 thus cannot be used to play videos without scaling in full screen mode on monitors with more than 1920 x 1080 pixels.

Windows Media Video (.wmv file extension) is the successor of the AVI format, and is used in all newer products from Microsoft. The files can be played with Microsoft Windows Media Player. The horizontal and vertical number of pixels has to be even. The 3D Viewer corrects the number of pixels input automatically in order to maintain this rule.

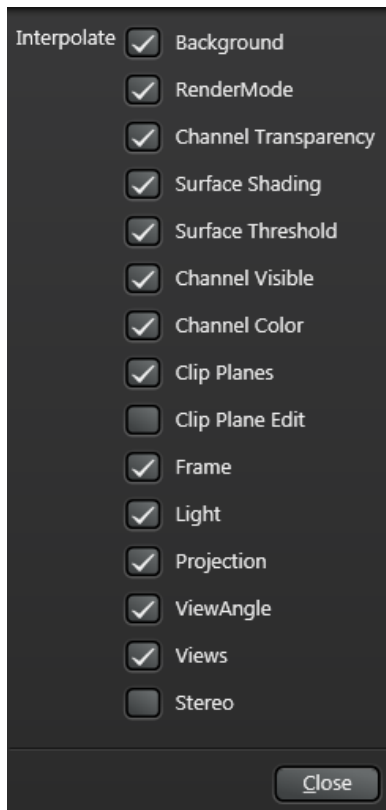
Settings

Brief Description

Settings that can be varied in the video are collected in groups. The modification of the settings for a group can be turned off. The switches are reached via the *Settings...* button.

If accessories such as the positioning frame or manipulator of the clip plane orientation are used during the compilation of a video, these can disrupt elements in the final video. The Movie Editor allows you to determine later which of the settings in the video are varied.

The *Settings...* button can be used to open a window in which you can determine which settings of the 3D Viewer can be modified during production of a video.



The settings from the user interface of the 3D Viewer were grouped as well. The following table contains a brief description of the allocation of the settings for the groups.

Background	Background color and slope Shadow intensity in Shadow mode Offset of the shadow in Shadow mode
Render Mode	Only with Advanced Full License: Toggling the Volume, Maximum, Depth Coding, and Shadow modes

Channel Transparency	Channel properties: Opacity, Minimum, Maximum and Gamma
Surface Shading	Only with Advanced Full License: Channel properties for the Volume - Surface mode: Opacity, Specular Intensity, Specular Color, Ambient Intensity Ambient Color Shininess
Surface Threshold	Only with Advanced Full License: Channel property for the Volume - Surface mode: Threshold
Channel Visible	Only with Advanced Full License: Switch-on status of the channels (switches in the tabs of the channels)
Channel Color	Color of the image channels
Clip Planes	Only with Advanced Full License: Properties of the clip planes: Switch, Position and Orientation, Plane Style, Frame color of the clip planes and segments that were cut away
Clip Plane Edit	Only with Advanced Full License: Adjusting aid for clip planes: Draw Frame and Draw Manipulator
Frame	Only with Advanced Full License: Draw Frame and Draw Scaling
Light	Position of the light source: Elongation and azimuth
Projection	Rotation, shift, and zoom
View Angle	Only with Advanced Full License: Projection angle: View Angle
Stereo	Only with Advanced Full License: Properties of the stereo display Mode, Eye Distance and View Point

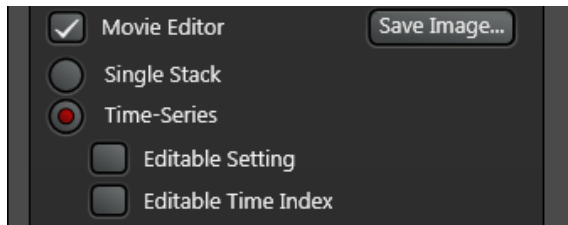
Movies with Time Series

Brief Description

Only with *Advanced Full* License: For time series stacks, movies can be generated via the time points with equal or different settings. Additionally, there are additional setting elements under the switch *Movie Editor*.

For time series, five different types of movies are created:

- 1) A movie for a time index such as with an individual image stack.
- 2) A movie about the complete time series with an adjustment. The number of images in the movie corresponds to the number of time points of the series.
- 3) A movie about the complete time series with different settings. The number of images in the movie corresponds to the number of time points of the series.
- 4) A movie about a partial area of the time series with an adjustment.
- 5) A movie about a partial area of the time series with different settings.



Single Stack

In this mode, a movie for a time index is generated. The time index is adjustable with the slider under the image display.

Time Series

In this mode, a movie is generated on multiple stacks of the time series. The setting of the switch *Editable Setting* and *Editable Time Index* determine what type of time series can be created in the movie.

Editable Setting

If the option is switched on, the settings for the view in the movie can be modified. With that, you can proceed as with the compilation of a movie for an individual image stack.

Editable Time Index

If the option is switched on, you can specify the keyframes from the time series index during the compilation of the keyframes. Also select the time series index with the slider under the image display.

If the option is switched off, the number of images in the movie corresponds to the number of time points in the series.

3D Annotations

Brief Description

Annotations can be used to indicate interesting image regions.

Annotations are saved with the LIF file image and are therefore available when the file is reopened.

Only with Advanced Full License

To insert a comment, press the *New Annotation button*.

Then, with a mouse click, mark a point on the specimen you wish to comment on in the 3D projection.

Then mark the upper left corner of the desired text field position with an additional mouse click.

Use the mouse to drag the text field from the bottom right to the desired size, and conclude the operation with a mouse click.

You can enter header and text in the text input fields in the bar on the left edge of the window.

3D Measurement

Interactive measurement of target range, angle, surface and volume

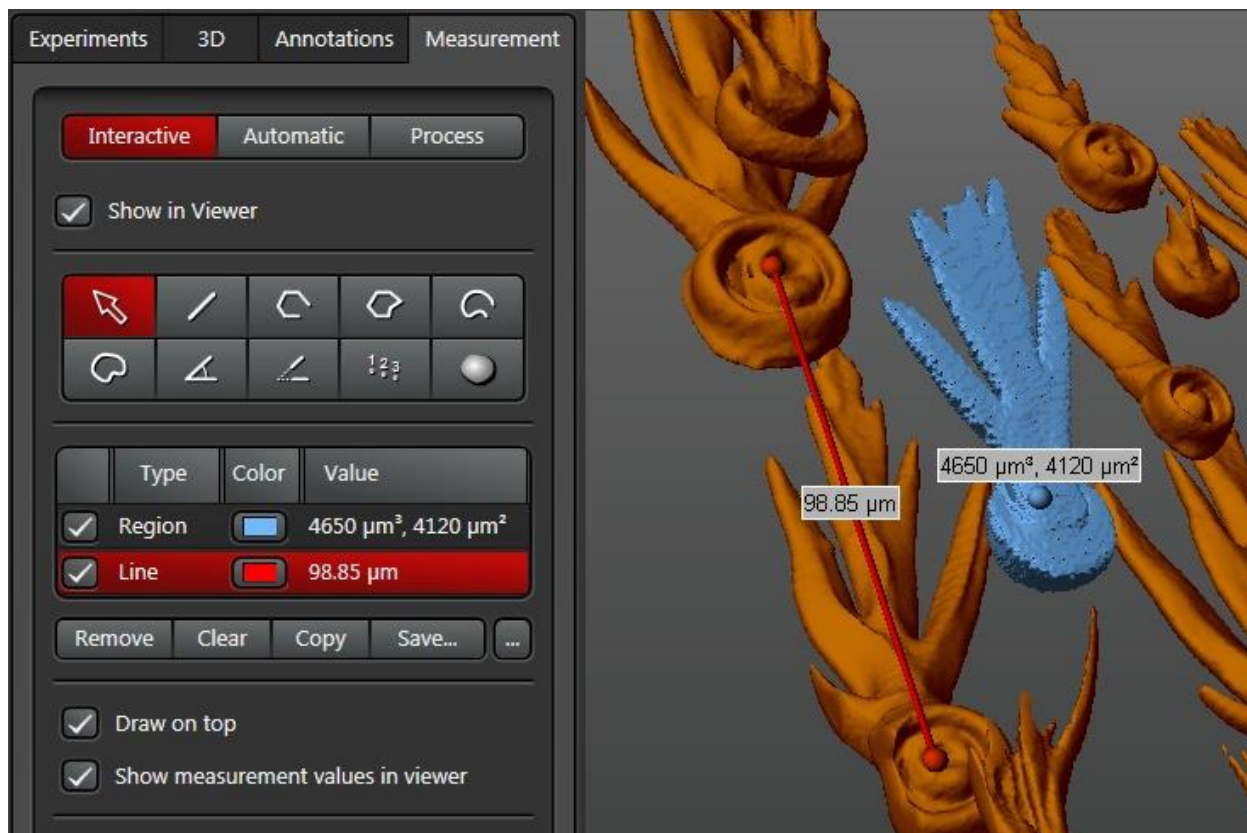
Brief Description

On the *Measurement* tab, target ranges and angles can be measured by drawing in the image.

Only 3D Analysis license

You can get to the functions for interactively measuring target ranges, angles, surfaces and volume using the Measurement, Interactive Mode tab.

Displaying in Surface mode is recommended for measuring. However, the program also supports the measuring functions in the other modes.



Select a display element using the button band:



Line - The distance between the line's end points is measured.



Lines - The total length along multiple consecutive lines is measured.



Polygon - The perimeter is measured. If the nodes are roughly in one plane, the area is also measured.



Freehand line - The extent is measured. If the nodes are roughly in one plane, the area is also measured.



Angle - The angle between two consecutive lines is measured.



Angle, two lines - The angle between two lines is measured.



Counter - The number of selected points is counted.



Region - The program determines the region where all voxels have an intensity value above the specified threshold and are contiguous with the selected point. The intensity threshold can be modified using the *Intensity Threshold* controller under the list. Volume and surface are measured.



Selection - The nodes can be moved.

Clicking on the starting point in the image view highlights the starting point with a small sphere. Then select the end point or the next point for the display element by clicking. You can finish selecting points for the Lines, Polygon, Freehand Line and Counter display elements by right-clicking or double-clicking.

The table displays the measurements. Highlighting a list entry marks the small sphere for the corresponding display element in red.

Remove

In order to remove a list entry, highlight the entry and press the Remove button.

Clear

All of the list's entries can be deleted using the Clear button.

Copy

All of the entries in the list can be copied to the clipboard using the Copy button.

Save...

All of the entries in the list can be written to a file using the Save... button.

Draw on top

With this button you can always make covered display elements visible.

Show measurement values in viewer

With this button you can fade in the display of the measurement values in the 3D Viewer.

Automatic Measurement

Brief Description

A wizard is available for automatic measurement and is used for carrying out common processing steps.

The settings can be saved and loaded under *Configuration* for use with other images.

An export for further processing for measurements with spread sheet software is provided in the last step of the wizard.

Only 3D Analysis license

The wizard contains seven steps. The step is selected in the list field.



Steps that are not relevant for a particular image can be skipped.

For steps 1 through 5, the wizard switches the settings for the displayed channel and 3D display mode to the compatible setting for this step.

At any time you can change over to the 3D tab and change all other settings of the 3D Viewer.

The last-selected settings are saved with the image in the LIF file and therefore are available again for resuming work on the image.

For a new image, the last-used settings are used.

The measurement settings can also be saved and loaded for use with other images (see section [Automatic Measurement – Configuration](#)).

[Step 1 - Channel](#)

[Step 2 - Image Processing Pre-Filter](#)

[Step 3 - Adjust Threshold](#)

[Step 4 - Binary Processing Pre-Filter](#)

[Step 5 - Binary Image Editing](#)

[Step 6 - Measurements](#)

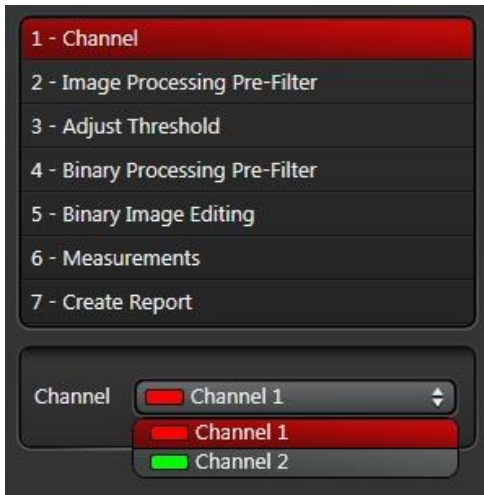
[Step 7 - Create Report](#)

[Measurements](#)

Steps of Workflow

Step 1 - Channel

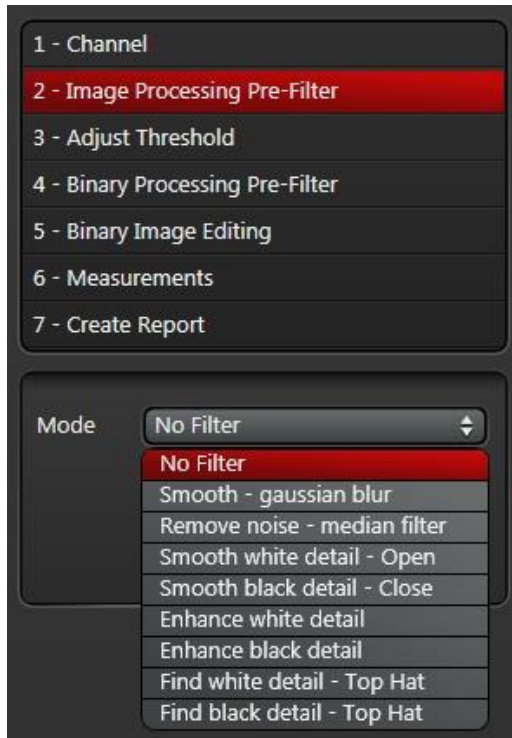
In the combination list field, select the image channel for which the measurements are to be determined.



For steps 1 to 5, only the selected channel is displayed in the 3D Viewer.

Step 2 - Image Processing Pre-Filter

In this step you can carry out gray-scale value image processing.



All filters with exception of the first two (Gauss and Median) offer as a parameter the radius of a spherical structure element, which influences the intensity of the filtering. Alternatively, more intense filtering can also be achieved by the *Iterations* parameter; the program works faster when filtering is intense, but the geometry of the structure element is no longer determined accurately.

Detailed information about the morphological filters used is compiled in the [Morphological Filter](#) section.

Smooth - Gaussian blur

The Gauss filter removes high-frequency components of the image and thereby smooths the transitions at the specimen edges and removes noise. The filter behavior can be adjusted continuously.

The Sigma setting is the width of the filter function in multiples of a voxel.

Intense filtering can lead to falsification of the specimens, since the filter then moves the specimen contours.

Remove noise - median filter

The Median filter removes noise by sorting the intensities of the adjacent voxels and selection of the value in the middle of the sorted list.

The *Size* setting is the number of adjacent voxels in each of the dimensions x, y and z.

Smooth white detail - Open

The Open filter removes small connections between specimens and removes small protrusions on the specimen surface. It retains the specimen contour to the greatest extent possible.

Smooth black detail - Close

The Close filter connects specimens that are close together and closes small notches on the specimen surface. It retains the specimen contour to the greatest extent possible.

Enhance white detail

A Dilate filter is used, which enlarges bright areas and smooths the surface.

Enhance black detail

An Erode filter is used, which reduces bright areas and smooths the surface.

Find white detail - Top Hat

The filter highlights small, bright structures. It can also be used for identifying small specimens that are inside of larger, darker specimens.

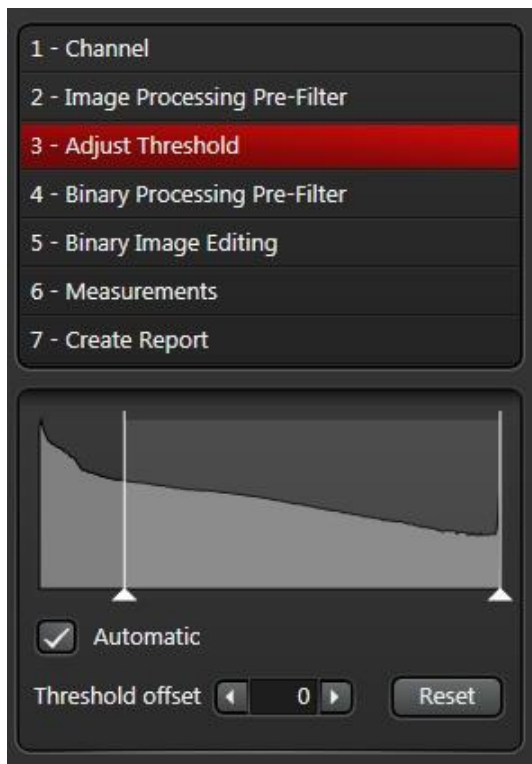
Find black detail - Top Hat

The filter highlights small, dark structures.

If you want to use more than one of the filters, you can use the corresponding filter functions in the [Process](#) part of the program for the corresponding images.

Step 3 - Adjust Threshold

In this step, a binary image for distinguishing between specimens and background is generated using intensity thresholds.



All voxels with an intensity value between the two selected threshold values, including these threshold values, belong to one of the specimens to be measured.

For fluorescence images, the background is dark and the specimens are bright. All you need is a lower threshold and to set the upper threshold to the maximum intensity.

A histogram is displayed for this step. The marking lines for the thresholds over the histogram can be dragged for setting the thresholds. The result is displayed in the 3D Viewer.

Reset

The Reset button triggers automatic determination of the lower threshold in accordance with the method from Otsu. The criterion here is the minimum dispersion for the background and specimen intensity (Nobuyuki Otsu. 1979. "A threshold selection method from gray-level histograms". IEEE Trans. Sys., Man., Cyber. 9 (1): 62–66). The upper threshold is set to the maximum intensity,

Automatic

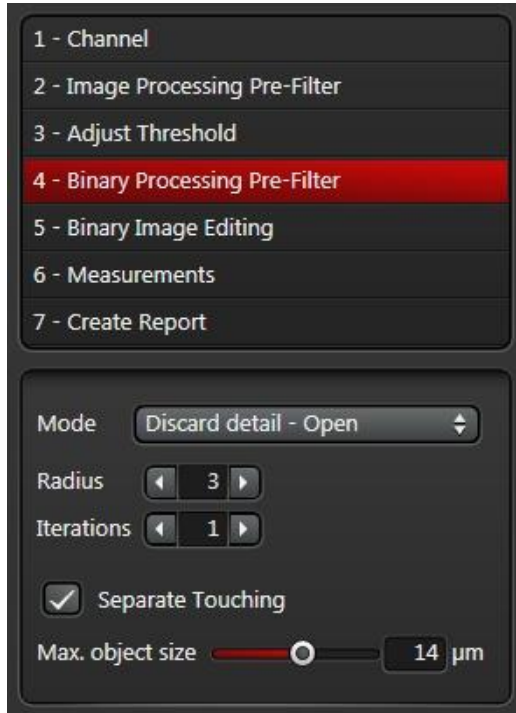
If Automatic is enabled, a deviation from the automatically determined threshold value can be defined. The setting defined in this way can then be used for other images.

In this mode, the upper threshold is always set to the maximum intensity and is not adjustable.

Step 4 - Binary Processing Pre-Filter

In most cases the generated binary image from the previous steps still is not perfect. In this step image processing filters can be used to make corrections.

Various morphological filters are provided under Mode. The parameters for the intensity of the filtering are *Radius* and *Iterations*, as for [Step 2 - Image Processing Pre-Filter](#).



Discard Detail - Open

The Open filter removes small specimens. The volume of the remaining specimens remains approximately the same.

Combine Detail - Close

The Close filter fills holes on the surface. The volume remains approximately the same.

Binary Erode

The Erode filter separates touching specimens. However, the volume of the specimens is also reduced.

Binary Dilate

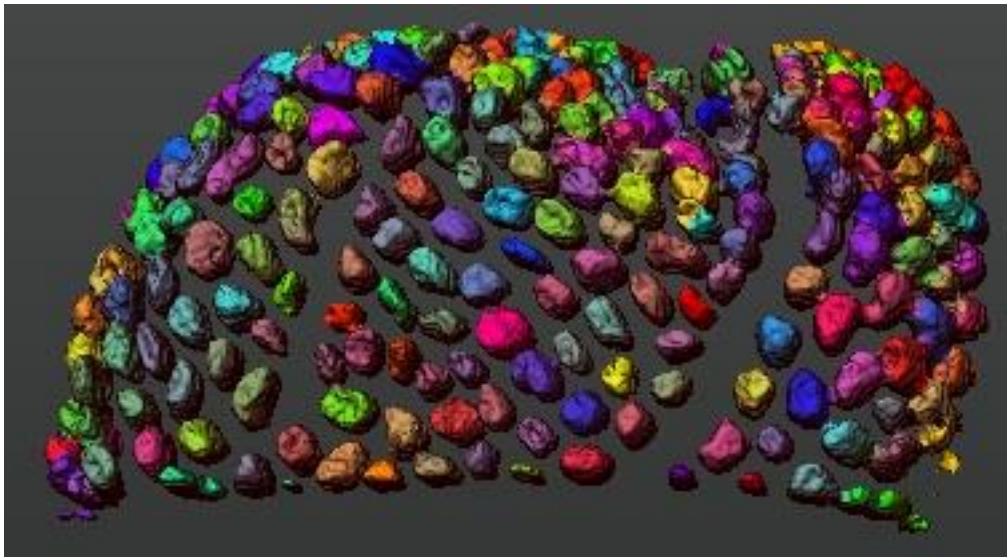
The Dilate filter smooths the surface. However, the volume of the specimens is increased.

Separate Touching

If there are still connections between the specimens after all the image processing filters, the Separate Touching function can be used.

A prerequisite, however, is that the specimens have approximately the same size,

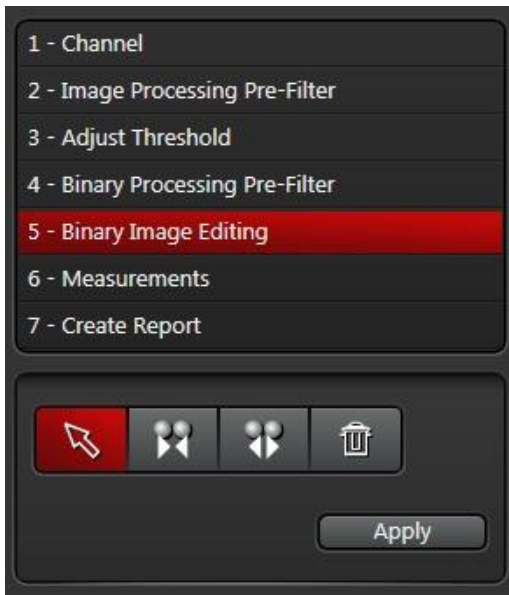
Separate Touching determines disconnection points at the concave regions of the specimen surface. The specimens separated by this are displayed in different colors for neighboring specimens.



Usually too many disconnection points are identified. With the "Max. object size" slider you can define a maximum object size, up to which disconnection points with a small joining surface are not taken into account.

Step 5 - Binary Image Editing

If incorrectly separated specimens still remain after all processing steps, you can separate and combine individual specimens in this step.



Display elements for the Combine and Separate functions are available. The operations are not carried out and displayed in the 3D Viewer until the Apply button is pressed.

Combine

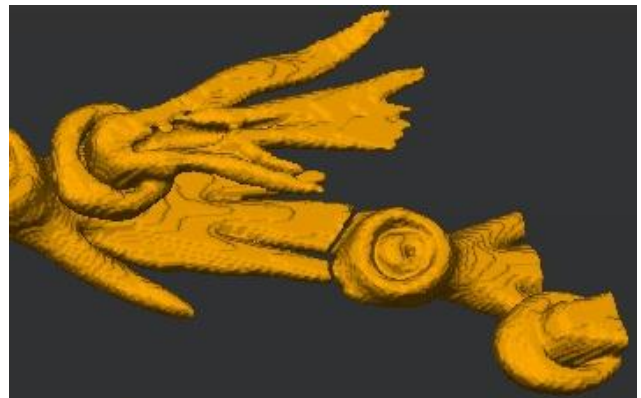
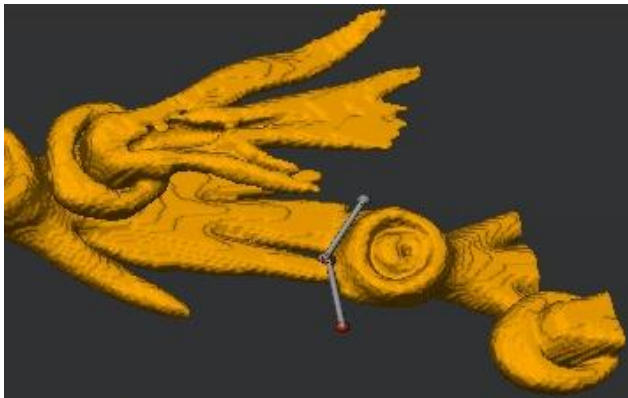


Draw lines between the specimens you want to combine.

Separate



Draw lines at the places where you want to separate the specimens.



Step 6 - Measurements

When changing to step 6, a table with the measurements is displayed at the bottom right.

The meaning of the individual metrics is described under [Measurements](#).

Objects

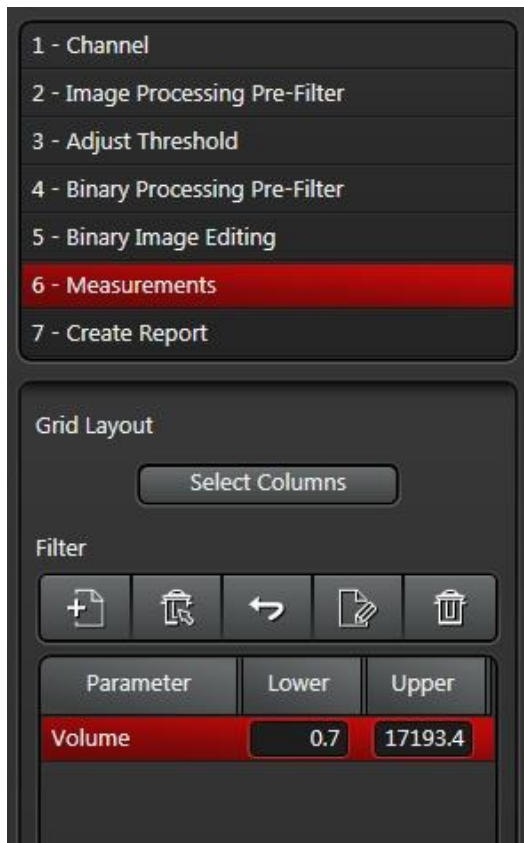
The measurements for each of the specimens are listed under this tab.

A mouse click on the column header in the table sorts the specimens according to the metric in this column. The order of the columns can be changed by dragging a column header horizontally.

After selecting a table row by mouse click, the corresponding specimen is also displayed in the 3D Viewer. Multiple table rows can be selected using the control and shift keys.

Statistics

The statistical data for the metrics are listed under this tab.



Select Columns

The button calls up a dialog with which the displayed table columns can be selected.

Filters

Filters can be used to define value ranges for the display. Only specimens with measurements in this range will be displayed in the table and taken into account by the Export function ([Step 7 - Create Report](#)).



Calls up a dialog for selecting the metrics according to which the filtering will occur.



Removes an individual filter. To do so, first select the filter you want to delete in the table.



Resets all filter areas to the default values for the corresponding metrics.



Sets all filter areas to the minimum to maximum of the measured values for the corresponding metrics.

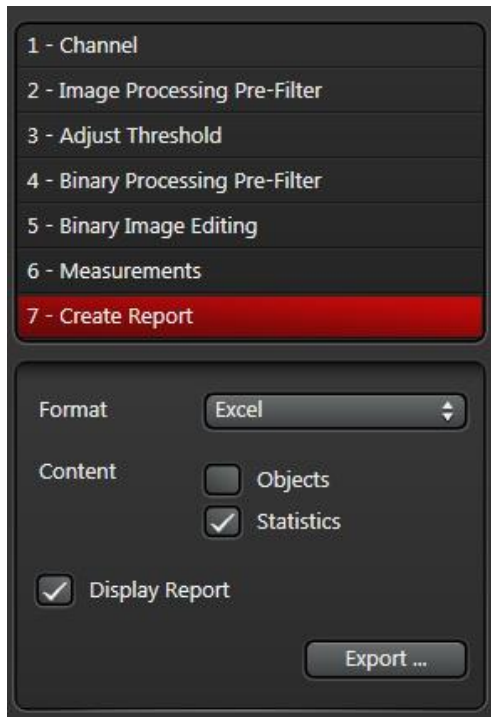


Removes all filters.

In the edit fields under Lower and Upper, enter the value range for the table rows to be displayed.

Step 7 - Create Report

In this step the measurements can be exported as a CSV or Excel file for spreadsheet programs.



Format

Available file formats for the export are Microsoft Excel with the filename extension `xlsx` and CSV (comma-separated values).

Content

Here you can decide which of the results tables are exported.

When exporting in the Excel format and selecting both options, one spreadsheet is created for specimen measurements and one for statistics.

Display Report

When installing programs, links to filename extensions are registered via the operating system.

If the Display Report option is enabled, LAS AF starts after exporting the program that is registered for the corresponding filename extension and opens the exported file there.

Measurements

Coordinate System

The coordinate system used can be displayed via the *Axis Scaling* setting element (see section [Frame and Scaling](#)). For a front view the coordinate origin is located on the left, top front.

Centroid X, Centroid Y, Centroid Z

Centroid X, Y or Z is the x-, y- or z-coordinate of the center of mass of the measured region in μm .

Bounds X, Bounds Y, Bounds Z

Bounds X, Y or Z is the difference between the largest x-, y- or z-coordinate value and the smallest x-, y- or z-coordinate value of the measured region in μm .

Voxel Count

Voxel Count is the number of voxels in the measured region.

Volume

Volume is that of all voxels in the measured region in μm^3 .

Volume Filled

Volume Filled is the volume of all voxels in the measured region and in the holes that are completely encompassed by the region in μm^3 .

Surface Area

Surface Area Filled is the surface of the measured region in μm^2 , including the surface of the holes that are completely encompassed by the region.

Surface Area Filled

Surface Area Filled is the surface of the outer shell of the measured region in μm^2 .

Sphericity

The ratio of the surface of a sphere with the same volume as the measured region to the measured surface is determined.

The sphericity returns values in the range from 0 to 1. A sphere has a sphericity of 1.

$$\text{Sphericity} = \frac{\sqrt[3]{\pi * (6 * \text{Volume Filled})^2}}{\text{Area Filled}}$$

Diameter

The diameter of a sphere is determined with the same volume as the measured region.

$$\text{Diameter} = \sqrt[3]{\frac{6 * \text{Volume Filled}}{\pi}}$$

Unit: μm

Ellipsoid Major, Mean, Minor

An ellipsoid is determined with the same geometric moments as the measured region around its geometric center of mass.

Ellipsoid Major is the length of the longest main axis of this ellipsoid.

Ellipsoid Minor is the length of the shortest main axis of this ellipsoid.

Ellipsoid Mean is the length of the medium-length main axis of this ellipsoid.

Unit: μm

Feret Maximum, Feret Maximum Azimuth, Feret Maximum Elevation

The line segment with the greatest length is determined from the quantity of all line segments between corner points of all voxels in the measured region.

Feret Maximum is the length of this line segment in μm .

Feret Maximum Azimuth is the orientation angle of this line segment after projection into the x-y plane in the range from 0 to 180°.

Feret Maximum Elevation is the elevation angle of this line segment with respect to the x-y plane in the range from -90 to +90°.

Feret Minimum, Feret Minimum Azimuth, Feret Minimum Elevation

The two parallel planes with minimum distance are determined, between which all corner points of all voxels in the measured region lie.

From the quantity of all the shortest distance line segments joining these two planes, one is chosen at random.

Feret Minimum is the length of this line segment in μm .

Feret Minimum Azimuth is the orientation angle of this line segment after projection into the x-y plane in the range from 0 to 180°.

Feret Minimum Elevation is the elevation angle of this line segment with respect to the x-y plane in the range from -90 to +90°.

Feret Ratio

The dimensionless ratio

$$\text{Feret Ratio} = \frac{\text{Feret Minimum}}{\text{Feret Maximum}}$$

is determined.

Minimum, Maximum, Mean

The minimum, maximum or mean of the gray-scale values of all voxels in the measured region is determined and output.

Unit: Gray-scale value

Sum

The sum of the gray-scale values of all voxels in the measured region is determined and output.

Unit: Gray-scale value

Standard Deviation

The standard deviation of the gray-scale values of all voxels in the measured region is determined and output.

Unit: Gray-scale value

Morphological filters

This section includes detailed information about the morphological filters used.

Structure element

Almost all morphological filters used use the Erosion and Dilation basic operations and thus are based on proximity operations. The proximity is specified by a binary mask designated as a structure element. For the filters used, the structure element is determined by all voxels with a distance in voxels to the result voxel that is smaller or equal to the *Radius* value. *Radius* is a setting value for the corresponding filters.

Erosion sets the intensity for each of the result voxels to the largest intensity value of the adjacent voxels in the structure element and Dilation sets the intensity to the smallest intensity value.

Binary images

After binarization using the Thresholding function, all that remains are the 0 values (belongs to no specimen) and Maximum Intensity (belongs to one of the specimens).

Then the binary Erosion is an OR operation for the intensities of the voxels in the structure element and the binary Dilation is an AND operation.

When a binary image is present is known only within the wizard for [Automatic Measurement](#). For the filters under [Process](#) there is a Binary button. If this is enabled, the program uses the binary operations. If the source image is a gray-scale image, however, all intensity values greater than 0 are treated as if they had maximum intensity.

Open

The Open filter executes first an Erode operation and then a Dilate operation.

Close

The Close filter executes first a Dilate operation and then an Erode operation.

White Top Hat

The White Top Hat filter subtracts the result image of the Open filtering from the intensity of the original image.

Black Top Hat

The Black Top Hat filter subtracts the intensity of the original image from the result image of the Close filtering.

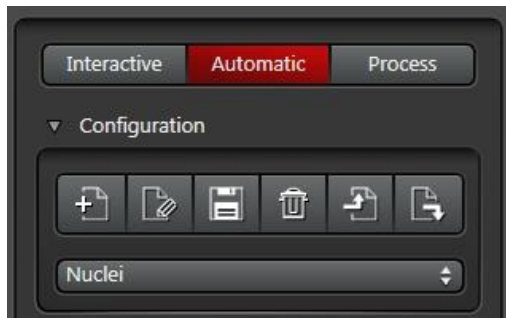
Iterations

Depending on the image size, the efficiency of the morphological operations can be improved with respect to calculation speed by repeated execution of the single operations as opposed to using a larger structure element. The program provides the Iterations parameter for this. The resulting structure element deviates slightly from the spherical structure element described above.

For the Open or Close operations, first this number of Erosion or Dilation operations is executed and then this number of Dilation or Erosion operations.

Configuration

All settings in the measuring process, which is defined via the wizard, can be saved via the Configuration frame and applied to other images.



The program keeps a list of the measurement programs for each user. It is displayed in the drop-down list field. When a list entry is selected, the corresponding measurement program is loaded.



The current settings are saved in a new list entry.



The name of the selected list entry can be changed.



The current settings are saved in the selected list entry. The list entry is overwritten in the process.



The selected entry is removed from the list.



The current settings are saved in a file. Then a selection window for the file name appears.



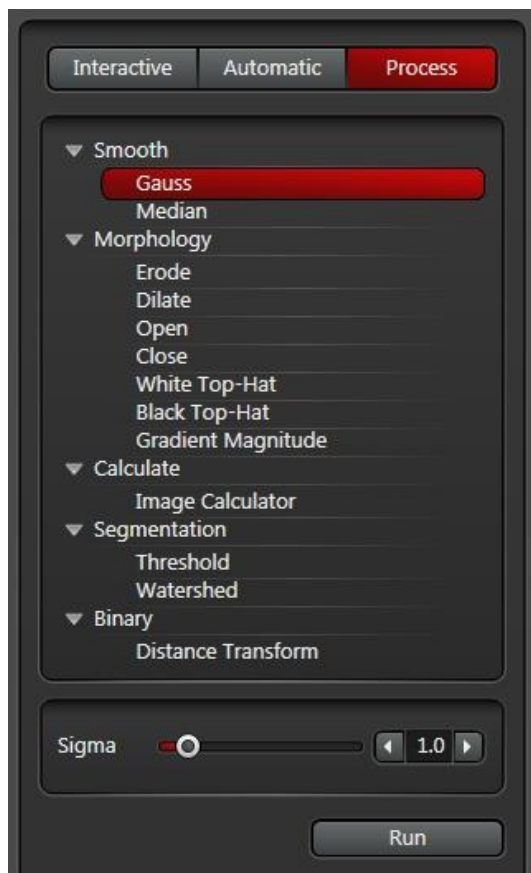
The settings are loaded from a file.

Process

Brief Description
Various editing functions are available in Process mode as a supplement to the wizard for Automatic Measurement.

Only 3D Analysis license

Note: Image regions can be cut out in the view for [Orthogonal sections](#) and under clip planes (see [Characteristics of Planes](#)).



Select the editing function in the frame with the tree.

A frame appears under this with the settings for the respective editing function.

Use the *Run* button to start execution of the function.

If there is a long execution time, a progress bar appears at the lower edge of the window, where you can cancel the execution as needed.

Smooth - Gauss

The Gauss filter removes high-frequency components of the image and thereby smooths the transitions at the specimen edges and removes noise.

The Sigma setting is the width of the filter function in multiples of a voxel.

Smooth - Median

The Median filter removes noise by sorting the intensities of the adjacent voxels and selection of the value in the middle of the sorted list.

The Size setting is the number of adjacent voxels in each of the dimensions x, y and z.

Morphology - Erode, Dilate, Open, Close, White Top-Hat and Black Top-Hat

These filters are described in the [Morphological Filter](#) section.

Morphology - Gradient Magnitude

The Gradient Magnitude filter determines the intensity difference Dx, Dy and Dz of the adjacent voxels in the x-, y- and z-directions and calculates a resulting intensity rise with

$$g = \sqrt{(\Delta x)^2 + (\Delta y)^2 + (\Delta z)^2}$$

as a new intensity value in the result image. The filter is frequently used for preprocessing in connection with the watershed segmentation.

The setting value is the distance to the adjacent voxels used in the x-, y- and z-directions in the unit voxel.

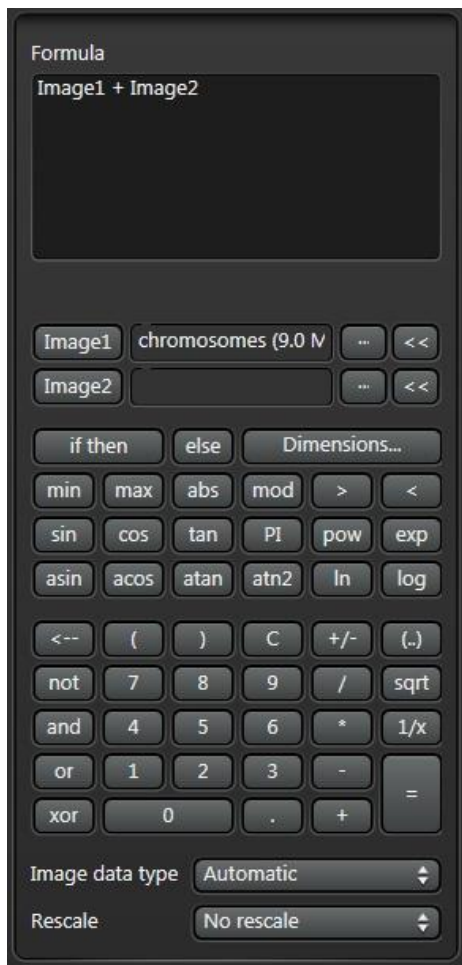
Calculate - Image Calculator

An image calculator is provided for the calculation of images by pixels with arithmetic and boolean operators.

The source images can be selected with the "<<" button to select the image just shown in the 3D Viewer. Use the "..." button to reach the selection from the Experiment tree.

The Image1 and Image2 buttons are used to enter the image operands into the formula.

Please note that the pocket calculator always works with intensities in the value range from 0 to 1, not with gray-scale values (0 to 255 or 0 to 65535).



Segmentation - Threshold

The Threshold function executes segmentation based on the lower and upper intensity threshold as in the wizard for 3D measurement. The operation is described there under [Step 3 - Adjust Threshold](#).

Binary - Distance Transform

For a binary image, the Distance Transform function calculates the shortest distance of the voxels with an intensity value other than 0 to the closest voxel with an intensity value of 0.

Hardware and Software Requirements



Important!

In no case does Leica Microsystems CMS GmbH provide a warranty or bear liability for direct, indirect, coincidental, or consequential damages that may arise from hardware and software components that have not been supplied by Leica Microsystems CMS GmbH.

The requirements for the 3D Viewer to function are:

- Windows 7 operating system, 64-bit and
- Graphics card with driver for OpenGL version 3.3 or driver for Direct3D 10.0 with shader model 4.

The 3D Viewer first checks the requirements with respect to the OpenGL driver. Direct3D is used only if these are not met.

The NVIDIA Quadro 600 graphics card was thoroughly tested for Leica Microsystems systems. Although the 3D Viewer was developed using various graphics cards from NVIDIA and AMD/ATI, Leica Microsystems CMS GmbH Support can provide only limited support in connection with other graphics cards.

Stereo operation with shutter glasses

Important!



In no case does Leica Microsystems CMS GmbH provide a warranty or bear liability for direct, indirect, coincidental, or consequential damages that may arise from hardware and software components that have not been supplied by Leica Microsystems CMS GmbH.

The stereo operation with shutter glasses with the 3D Viewer and with other programs requires specially developed hardware and software. Only a few products from electronics and PC manufacturers for stereo operation with shutter glasses are functional and intended for use in conjunction with the 3D Viewer.

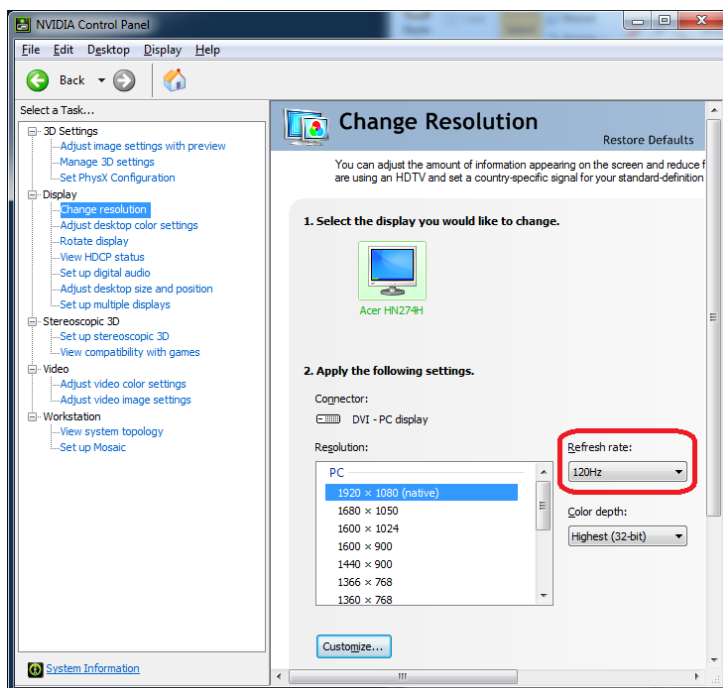
Stereo operation with shutter glasses was developed for the NVIDIA 3D Vision technology with NVIDIA Quadro graphics cards. NVIDIA 3D Vision can be used only together with special monitors and graphics cards developed for it. The list of products is on the NVIDIA web page, <http://www.nvidia.com/>, under 3D Vision.

With 3D Viewer in QuadBuffer stereo mode, the stereo image is displayed in a window. This operating mode is reserved for the graphics card series for Quadro workstations. The GeForce for home users is only intended for the stereo operation in full-screen mode and can therefore not be used for stereo operation with the 3D Viewer.

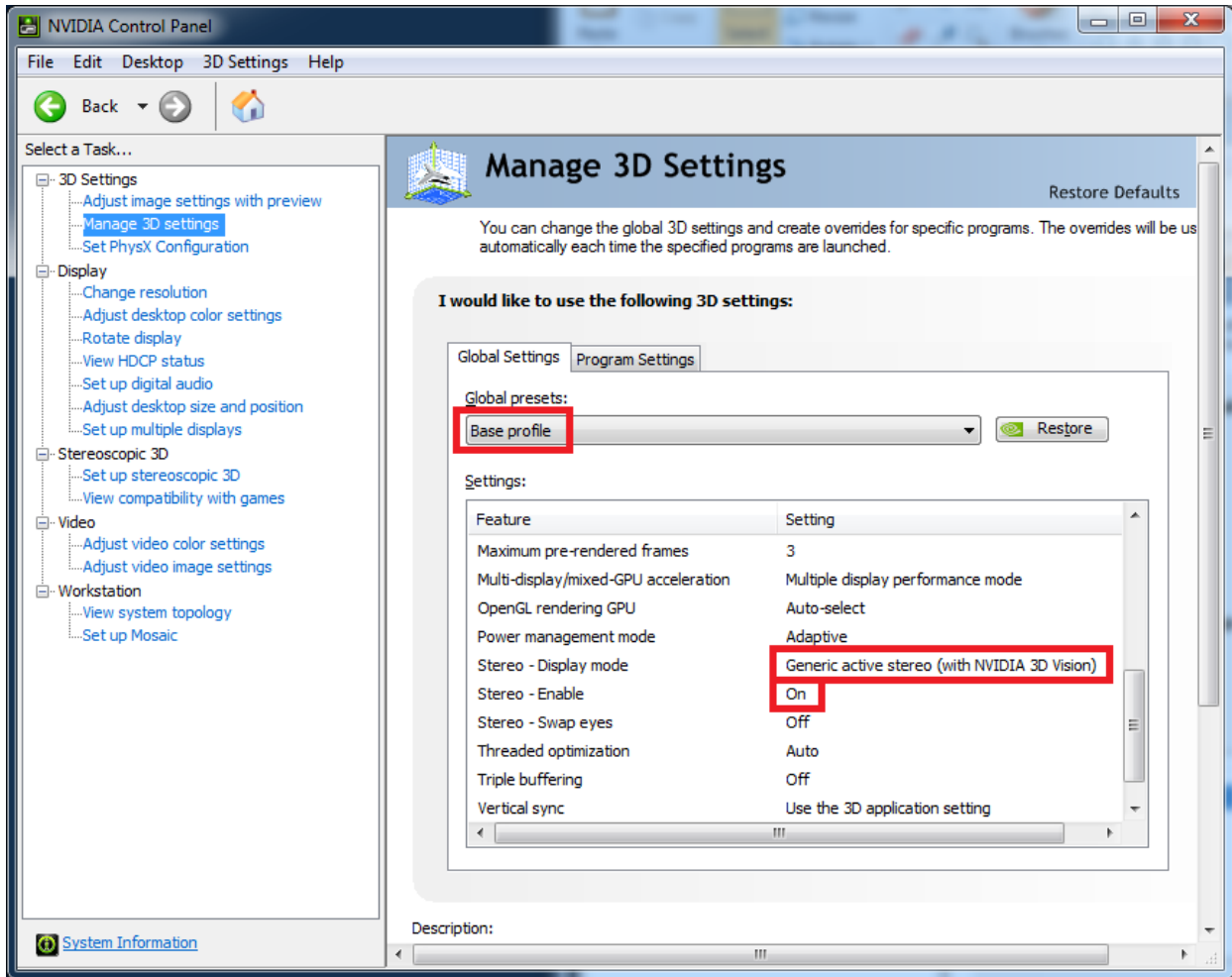
Taking quick development in the stereoscopic solutions field into account, new solutions will have been introduced since the drafting of this text. The 3D Viewer simply requires an OpenGL driver with support from QuadBuffer Stereo in window mode, and is therefore not absolutely limited to the selected technique. In any case of technology that deviates from the recommendation, check by means of a test installation before you invest in this technology.

The following is a description for the setup of stereo monitors when using the NVIDIA Quadro 600 graphics card used by Leica Microsystems microscope systems.

- 1) Open the NVIDIA Control Panel via the context menu on the desktop.
- 2) Select a screen refresh rate of 110 Hz or 120 Hz under Display - Change Resolution.



3) Under 3D Settings - Manage 3D Settings, select the Base profile entry in the global list. Set Stereo - Display mode to Generic active Stereo (with NVIDIA 3D Vision) and Stereo - Enable to On.

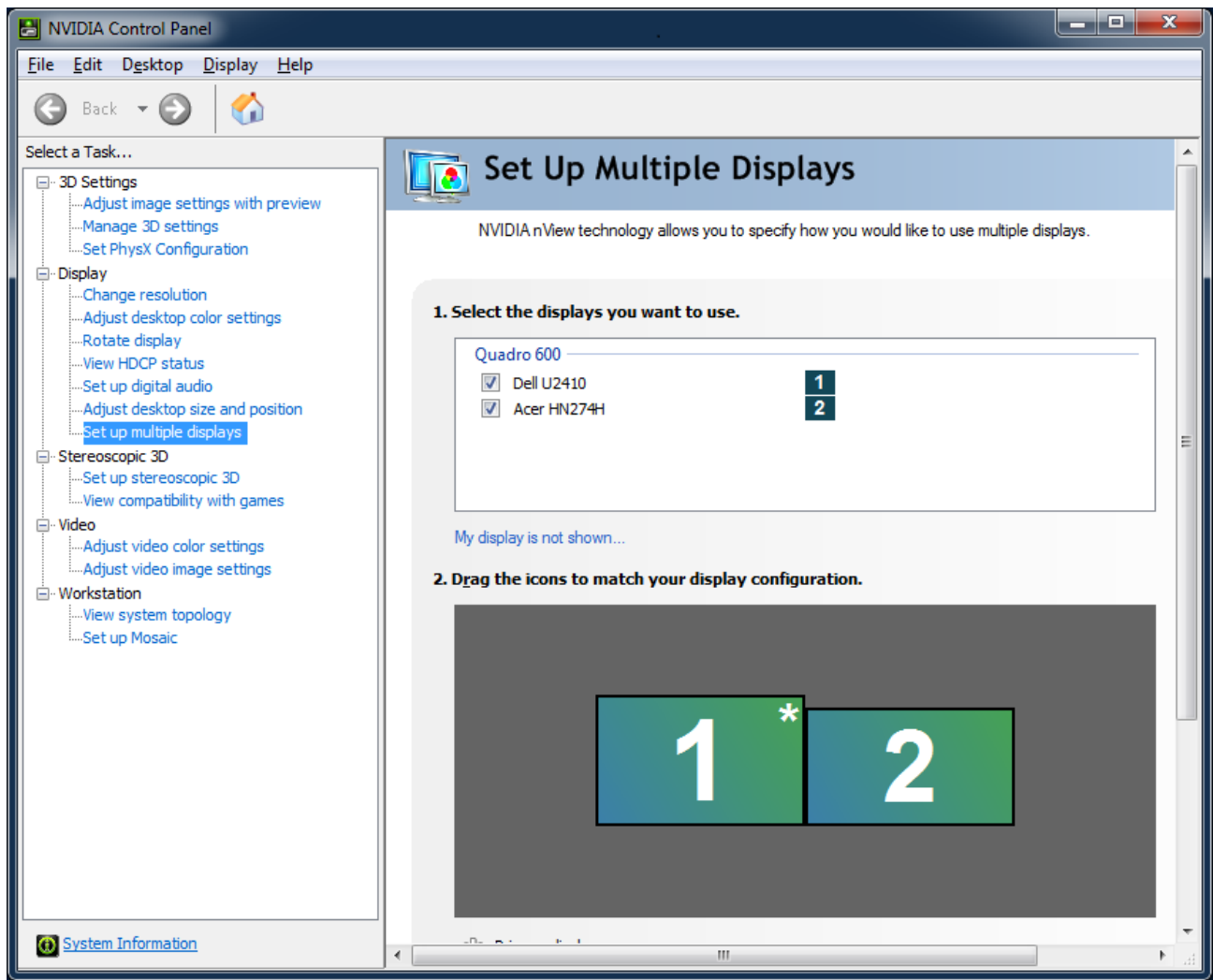


If you do not find the corresponding settings in the NVIDIA Control Panel, check whether your monitor is intended for 3D Vision operation. If this is the case, you have to install a newer driver.

Equipping a stereo monitor for NVIDIA 3D Vision with two different monitors

When using a stereo monitor and a normal monitor, you connect stereo monitor via a DVI-D cable and the normal monitor via a DisplayPort cable.

Set up the normal monitor as the primary monitor. To do so, call up the NVIDIA Control Panel. Under Display - Set up multiple displays, call up the Make primary context menu item with a right mouse click across the normal monitor graphics.



If you later connect a monitor, please ensure that the screen refresh rate of the stereo monitor is set to 110 Hz or 120 Hz.