



Living up to Life



Navigator

20220610_EN

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I. Navigator

You can create an overview image of the entire specimen with quick scan and use it for navigation before starting your actual experiment. Navigator enables interactive setup of tile scan and Mark & Find experiment.



Calling up

Within the “Acquisition mode” dialog field of Acquisition tab, click the “Navigator” button. The Navigator opens.

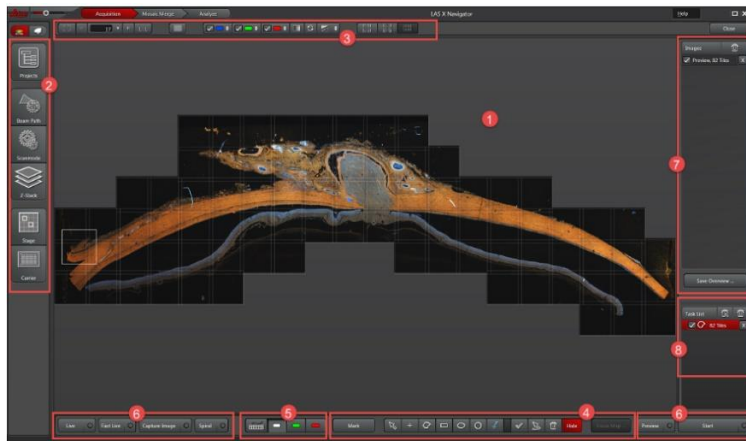
To close the Navigator again, click the “Close” button in the upper right corner.

The user interface Navigator is structured in tree steps: Acquire, Mosaic Merge and Analysis (Optional)

*Before starting the Navigator, make sure that rotation number is “0”. When Navigator is started, the rotation number automatically entered. Tiling is incorrect if a value other than value automatically entered by Navigator or 0 is entered.













II. Acquire



- ① In the viewer, the initialized stage is graphically displayed. In the viewer, you can acquire an image directory with the mouse, move the stage position and into the image section. The region for image acquisition of a frame is marked by a white box.
- ② **Tool Bar** (Ref. page 2): You can open configuration dialogs relevant to the settings of your image acquisition parameters. Clicking the icon, you can open the corresponding dialog.
- ③ **Image display** (Ref. page 3): You can select whether a channel is display or not and adjust the intensity range and color.
- ④ **Mark & Find, Scan region and Focus Map** (Ref. page 6): You can create scan regions, voxel points and voxel maps.
- ⑤ **Configuration of Control Panel** (Ref. page 4): You can change acquisition channel for which you want to set the detector gain on the Control Panel.
- ⑥ **Buttons for Image acquisition** (Ref. page 3):
- ⑦ **Image list** (Ref. page 5): In this dialog, all images for previews and images are listed. All previews that are between images are aggregated.
- ⑧ **Task List** (Ref. page 7): In this dialog, all defined scan regions and frames are listed as scan tasks.

II-1. Toolbars

Using these toolbar in left edge of the viewer, you can open configuration dialogs relevant to the settings of your image acquisition parameters. Clicking the icon, you can open the corresponding dialog.

 Projects	Project: Open the project directory
 Beam Path	Beam Path: Open the dialog for the beam path
 Scanmode	Scanmode: Open the dialog for both scan mode and XY image acquisition parameters
 Z-Stack	Z-Stack: Open the dialog for define the Z-stack. This button only displayed if you have enable the Z scan in acquisition mode.
 Time	Time: Open the dialog for the time lapse. This button only displayed if you have enable the T scan in acquisition mode.
 Carrier	Carrier: Open the dialog for selecting specimen carrier (Ref. page 12).
 Stage	Stage: Opens the dialog for setting image acquisition for the specimen overview (Ref. page 15).
 Autofocus	Autofocus: Open the dialog for configuration of autofocus. This button only displayed if you have enable the “Show Autofocus Panle” function in the acquisition mode.
 Lambda	Lambda: Open the dialog for configuration of Lambda scan. This button only displayed if you have enable the λ or Λ function in the acquisition mode.
 FLIM	FLIM: Open the dialog for configuration of autofocus. This button only displayed if you have enable the “FLIM” function.

II-2. Image

II-2-1. Image Acquisition

At the lower edge of the viewer, you can find following buttons for image acquisition.



① **Live:** Switch on the Live scan.

Function in Context menu:

-**Run Auto-Focus:** Switch on the automatic focus search.

② **Fast Live:** Enable a fast live with the setting for fast live scan from bellow “Fast Scan Settings”.

Function in Context menu:

-**Run Auto-Focus:** Switch on the automatic focus search.

-**Fast Scan Settings:** Open the Fast Scan Settings dialog.

③ **Capture Image:** Acquires a frame of the current stage position using all channels. The acquisition region is marked by a white box.

Function in Context menu:

-**Run Fast Image Capture:** Start the scan with the settings for fast scan.

-**Run Precise Image Capture:** Start the scan with default settings.

-**Run Auto-Focus:** Switch on the automatic focus search.

-**Button start Fast Image Capture:** Start the scan with fast scan settings.

-**Fast Scan Setting:** Open the Fast Scan Settings dialog.

④ **Spiral:** Start the spiral scan and create a preview image (see right figure). The start point is the current stage position.

Function in Context menu:

-**Run Smart Spiral:** The spiral scan ends at the edge of the specimen if no contrast is found any longer.

-**Run Find Object Outline Scan:** In this scan, the edge of the specimen is searched for based on the contrast. The stage first move to the left, that is, the initial search is for edge on the right.

-**Run Fast Spiral:** Start the spiral scan with the settings for the fast scan.

-**Run Precise Spiral:** Start the spiral scan with the default settings.

-**Button starts Fast Spiral:** Start the spiral scan with settings for the fast scan.

-**Fast Scan Setting:** Open the Fast Scan Settings dialog.

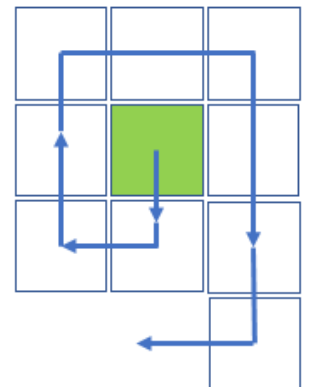
⑤ **Preview:** Craete a preview image in the current focal plane for plotted scan region.

Function in Context menu:

-**Run Fast Preview:** Start the preview scan with the fast scan settings.

-**Run Precise Preview:** Start the preview scan with the default settings.

-**Run Auto-Focus:** Switch on the automatic focus search.



-**Button starts Fast Preview:** Start the preview scan with the fast scan settings.

-**Fast Scan Setting:** Open the Fast Scan Settings dialog.

⑥. **Start:** Start the experiment including z-stacks, time series, auto-focus and focus maps if these are defined.

The images are stored in the project directory.

Function in Context menu:

-**Run Auto-Focus:** Switch on the automatic focus search.

How to configure laser power and detector gain during scan in Navigator

Configuration of Control Panel

You can change acquisition channel for which you want to set the detector gain on the Control panel.



1. Open the configure dialog for the Control panel.

2. Detector gain is set on the Control Panel. Click on a color field to change the acquisition channel for which you want to set the detector gain.

II-2-2. Image Display Function

At upper edge of viewer, you can find following buttons for image display.



① Reset viewer zoom

② Reduce viewer zoom

③ Allow you to enter the zoom factor for the viewer manually or adjust it using slider (The slider is displayed when you click the triangle next to the input field).

④ Increase viewer zoom

⑤ Viewer zoom to 100%

⑥ Live image viewer: Switch to the LAS X viewer. Simultaneously, further buttons are displayed for the image display (Ref. next page).

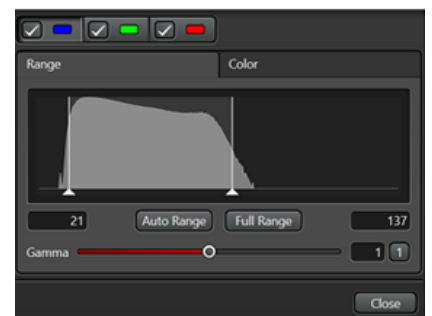
⑦ You can select whether a channel is to be displayed (enable) or not (disable). For each channel, color field with check box were displayed that can be enable or disable. Clicking the arrow let you open the dialog (shown in right figure) for adjusting the intensity range, color and gamma value. These functions were displayed separately for each channel, including channel that were defined with different settings.

⑧ Images are displayed in grayscale.

⑨ Display entire intensity range.

⑩ Auto Range: Limit the range automatically to optimal imaging. Clicking the arrow let you open the dialog with adjustment option for the Auto-Range,

⑪ Moves to the current stage position



12 Indicate all acquisition region. Using this button, you can return to your previous defined acquisition region at any time.

13 Display entire specimen carrier. This button is active only if you have selected a specimen carrier.

Live Image Viewer Function

If this function is activated, following function are available for current selected channel.



1 Live image viewer: Switch to the LAS X viewer. Simultaneously, further button are displayed for the image display.

2 The image displayed take place in the color-coding of the channels

3 Images displayed in glayscale

4 The image display take place using a look-up table, in which the grayscale values for overexposed pixels are displayed in red and underexposed pixels are displayed in blue (For camera system).

5 The image display take place using a look-up table “Glow O and U”, in which the grayscale values for overexposed pixels are displayed in bleu and underexposed pixels are displayed in green.

6 All channels are displayed superinposed in one image

7 All channel are displayed side-by-side

8 All channel and overlay are displayed side-by-side

9 The image displayed with or without “Smoothing” (Default setting is “Smoothing” ON).

II-2-3. Image list

At right edge of the screen, you can find dialog (right figure). In this dialog, all images previews (Preview) and images (Image and Serises) are listed. All previews that are between the images are aggregated. You can exclude individual images and previews. These are ignored when saving “Save Overview”.

1 To exclude images when saving the overview, select the entry and uncheck the corresponding check box.

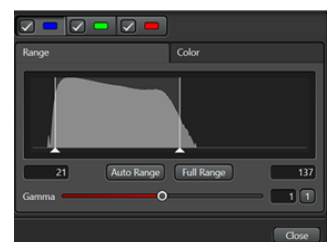
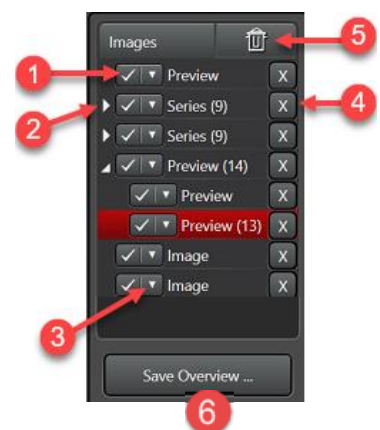
2 Clicking the triangle that points to the right open the folder for an image series. One entry is shown each individual image.

3 For each image, series and preview, you can click triangle to open a dialog (right figure) for adjusting the intensity range, color and gamma value.

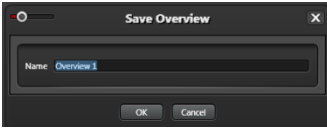
4 To remove images, select the entry and click **X** button

5 To remove all images, click the trash can icon.

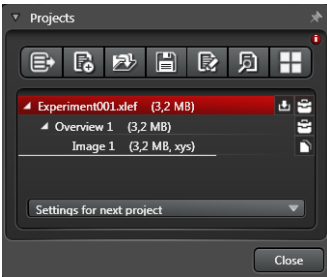
6 Save Overview. You can save all frames of the overview.



1. Click the “Save Overview...” button. The following dialog opens.



2. Enter a designation under “Name” and click “OK”. All images from the navigator are saved for the project. Hidden images are not saved.



II-2-4. Mark & Find, scan region and Focus map

At lower edge of screen, you find following buttons for creating scan regions, voxels and voxel maps.



- ① Marks the current position (X, Y, Z) on your specimen and create an image. You use this function for Mark & Find experiment.
- ② Selection tool. It allows you to select one or more plotted regions.
- ③ Marks the current position (X, Y) and creates an image.
- ④ Polygon tool. The polygon tool can be used like a free-hand tool.
- ⑤ Rectangle tool.
- ⑥ Ellipsis tool.
- ⑦ Circle tool.
- ⑧ Magic wand. Detects boundary of the object and plots a scan region that completely encloses the object.
- ⑨ Focus Map. Sets the voxel point (Ref. page 10)
- ⑩ Auto-Focus point. Sets the a focus point for autofocus search. If the autofocus is only to be carried out at a single position, refer also Advance Manual. The focus search is run on nearest tile.
- ⑪ The selected plotting tool remains active so that you can plot additional regions using the same drawing tool. If the function disable, the selection tool will automatically become active again after plotting done.
- ⑫ Selection tool for all plotted scan regions.
- ⑬ Remove all selected regions
- ⑭ You can hide individual scan regions. These will not consider when saving using “Save Overview” (see Above).
- ⑮ Opens the dialog for the determine the Focus Map (Ref. page 10)

II-2-5. Task list

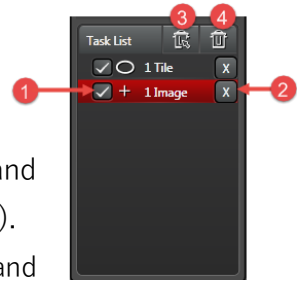
At lower edge of screen, you find following dialog. In this dialog, all defined scan regions and frames are listed as scan tasks. Individual regions and images can be excluded from image acquisition in the task list. These regions and images are not considered in the preview (Preview) and image acquisition (Start).

① To exclude the a region or an image from image acqisition, select the entry and uncheck the corresponding check box.

② To remove a region or image from image acquisition, select the entry and click the corresponding **X** button.

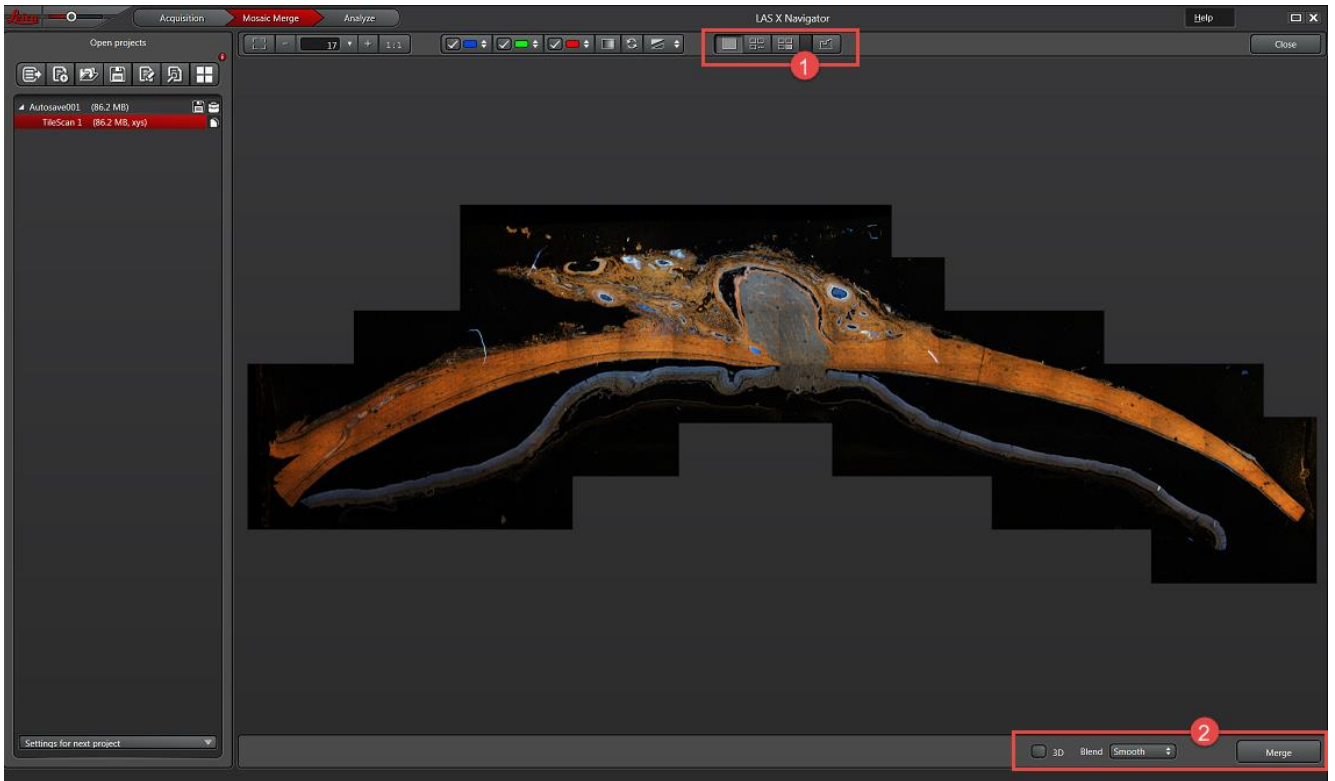
③ To remove the selected regions and images, click this icon.

④ To remove all regions and images and thereby empty, click this trash can icon.



III. Mosaic Merge

In this step you can merge mosaic regions into one image.



- ① Image display
- ② Merge

III-1 Image Display

Some of image display are described in “Acquisition” step (Ref. page 4). In addition, the following buttons are available under “Mosaic Merge”.



- ① Overlay. All channels are displayed superimpose in one image.
- ② All channels are displayed side-by-side.
- ③ All channels and overlays are displayed separately side-by-side
- ④ The image displayed with or without “Smoothing” (Default setting is “Smoothing” ON).

III-2 Merge



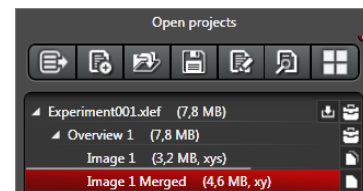
- ① **3D**: When the function is switched on, images are aligned with each other not only in XY-directions but in the Z-direction as well. The function requires long calculation times and deactivated by default because of this.
- ② **Blend**: In this dropdown menu, you can select the calculation method for overlapping mosaic images.
 - None**: The pixels in the area of overlap are neither differentiated nor weighted. The pixels of the lower partial image are used for the calculation. This option is suitable if the areas of overlap fit exactly over each

other, that is, if they are identical.

-**Smooth:** The intensity of the pixels in the area of overlap is weighted depending on the distance between the pixel and the center of the corresponding partial image. The smaller the distance to the center of the image, the higher the weighting. This method attains smooth transitions between the partial images. This method is not suitable if a maximum projection is to be carried out afterwards, because then the transitions appear as a gray reticule due to the intensity averaging.

-**Statistical:** The pixel of either the upper or lower image is used for the calculation. The selection is random; the probability of selection depends on the distance between the pixel and the center of the corresponding partial image. The smaller the distance to the center of the image, the higher the probability. This option is advisable if a maximum projection is to be carried out afterwards, because the full intensity of each pixel is to be included in the calculation.

③ Start the merging of mosaic images. The result image is stored in the project directory. The ending “Merge” is added to the file name.



IV. Advanced Functions

IV-1. Focus Map

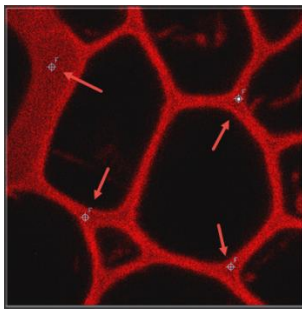
The focus map makes it possible to keep the specimen in focus at every scan position when running a Tile Scan or Mark & Find experiment. The focus map is created in advance from the focus values of predefined focal points. The focus position of any other point can be calculated through the interpolation of these map values. The higher the number of the default points, the higher the accuracy of predictability and the better the overall focus. If your system is equipped with a hardware-based focus stabilizer (AFC), an AFC map is created at the same time.

To create a focus map, follow these steps:

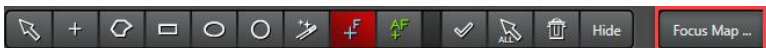
1. Click the Focus Map Point button at the lower edge of the screen. The button turned red.



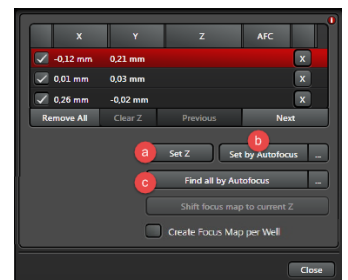
2. Click in sequence the positions in the viewer that you want to define as focal points. Set approximately four points depending on specimen structure; the minimum is two. The focal points are marked in the image.



3. Click the Focus Map ... button.



The following dialog opens. Here you can find a table entry with the corresponding x and y values for each focal point.



4. Select the first focal point or the first entry from the table.

5. Define the focal plane for the first point. You have several options for setting the z-positions of the focal points:

- a) **Set Z:** Search for the focal plane manually in live mode and click this button.
- b) **Set by Autofocus:** Clicking this button makes the autofocus function search the focal plane automatically.
- c) **Find all by Autofocus:** Clicking this button makes the autofocus function automatically search the focal planes for all defined focal points in sequence. Whenever you have defined a focal point, this button is renamed “Find remaining by Autofocus”. Thus you can carry out the focus search for the remaining focal points automatically.

The found z-values are entered in the Z column.

If your system is equipped with a hardware-based focus stabilizer (AFC), the respective offset to the referenced boundary surface (cover glass, specimen slide, bottom of a well plate, etc.) is entered in the AFC

column. In this way, the AFC map is created simultaneously with the focus map. The focal plane(s) is/are set. The value for the found z-position is displayed in the table.

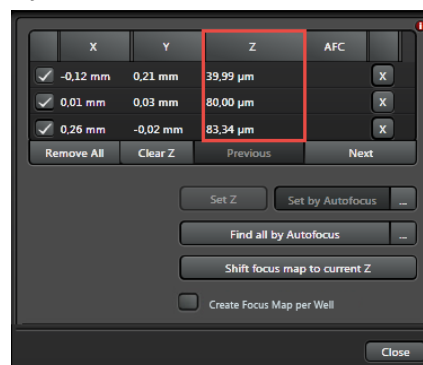
If you want to work with the autofocus function, please note that you must carry out the settings for the autofocus in the Autofocus dialog field (Ref. Advance manual). The dialog field is displayed only if you have enabled the Show autofocus panel function in the Acquisition Mode dialog field. Make sure that the same z-actuator is set for the autofocus search in the Autofocus dialog field under Focusdrive as in the Z-Stack dialog field.

6. Click the “Next” button if you have selected “Set Z” or “Set by Autofocus” in the previous step. The stage moves to the next defined focal point. Proceed to step 9 if you have selected “Find all by Autofocus” in the previous step.

7. Define the focal plane for the next focal point by clicking “Set Z” or “Set by Autofocus”.

8. Repeat steps 6 and 7 for all additional focal points.

The values for the z-positions for all focal points are displayed in the table.



9. Close the dialog by clicking the “Close”.

You can correct the Z-value later.

Remove All: Deletes all defined focal points. You can redefine your entire focus map.

Clear Z: Deletes all z-values for the defined focal points.

Previous: Moves the stage to the previous focal point. Now you can redefine the focal plane for this point.

For multi-slide specimen carriers, you can create an individual focus map for each specimen slide. To do so, check the box for Create Focus Map per Well. For image acquisition, only the focus points on the respective specimen carrier are used.

Moving Focus point:

Select “selection tool” and click the focal point you want to move.



Removing Focus point:

① Select “selection tool” and click the focal point you want to delete.

② Click “Delete selected tool”



Reuse Focus Map after Focus drift:

If you have created a focus map and there is reason to believe that the objective has since changed or focus

drift has occurred, you can continue using the focus map:

Focus a spot on your specimen.

Click the “Shift focus map to current Z” button. The system automatically moves all focal points by the found z-offset. This retains the focus map; only the position in the z-direction changes.


IV-2. Z-stacks with Different Settings

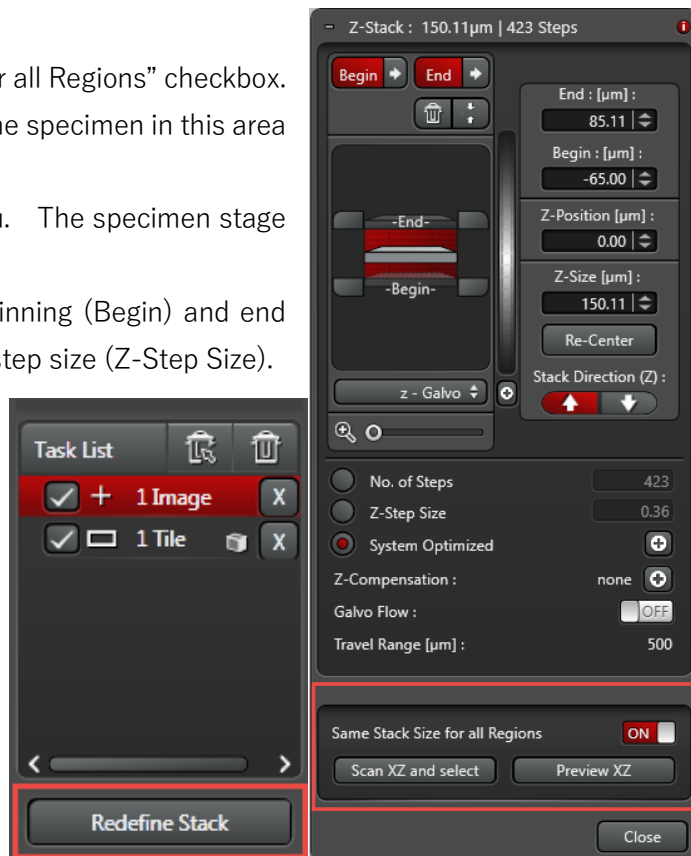
1. In the Z-Stack toolbar field, disable the “Same stack for all Regions” checkbox. This makes it possible to adapt a z-stack specifically to the specimen in this area in any xy-position.

2. Selecting the position in the Positions dropdown menu. The specimen stage automatically moves to this position.

Set the parameters for the z-stack in this position: Beginning (Begin) and end (End) of the z-stack, number of steps (Nr. of Steps) and step size (Z-Step Size).

Approach the next position and define the z-stack for this position.

Click the “Redefine Stack” button in the “Task List” dialog field to apply the modified settings to the new position. The  icon indicate redefined position.



IV-3. Carrier

In this dialog, you can select specimen carriers and configure specimen carriers on your own that you want to use for your experiment.

None: No specimen carrier

Slide: Slide

Dish: Petri dish

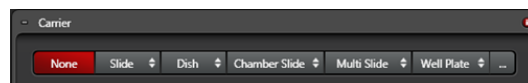
The following four functions are available only with the LAS X Assay Editor license:

Chamber Slide: Chamber specimen slide

Multi Slide: Combination specimen carrier. Stage plate for a combination of various specimen slides that you can configure under ..., refer to Configuring the .

Well Plate: Well plate

...: For the configuration dialog for a user-defined specimen carrier, see Configuring the Specimen Carrier.

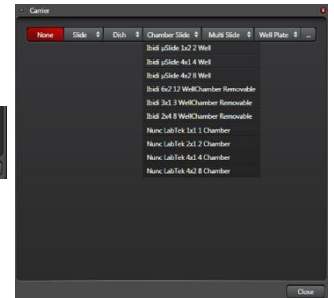


Using the respective dropdown menus, you can select the specimen carrier you used: If you have selected a specimen carrier, it will be displayed in the viewer. In addition, two more buttons are displayed in the dialog:

-**Align**: Opens a dialog window for aligning the specimen carrier (see below, Aligning the Specimen Carrier, page)

This is necessary if the view of the specimen carrier in the viewer does not match the actual specimen carrier.

-**Well Coverage**: Opens a dialog window for selecting specific specimen chambers (Ref. page 14, Setup Chamber Acquisition Regions).



Aligning the Specimen Carrier

In this dialog, you can align your selected specimen carrier. This is necessary if the view of the specimen carrier in the viewer does not match the actual specimen carrier.

1. Select the desired specimen carrier from the corresponding dropdown menu.
2. Click the Align button in the Carrier dialog field.

The Carrier dialog field is displayed. Depending on the setting under “Options ...”, different numbers of points and chambers are offered for alignment.

In order to use other chambers for alignment, you can also edit the chamber coordinates.

The first point to be adjusted has a yellow outline, the others are displayed in gray.



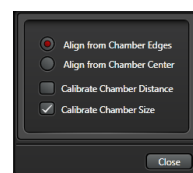
3. Click Options

A dialog opens, in which you set up how the alignment is to be carried out.

Select one of the two options:

-**Align from Chamber Edges**: Alignment refers to the chamber edge.

-**Align from Chamber Center**: Alignment refers to the chamber center.



Moreover, further options for alignment are available to you:

-**Calibrate Chamber Distance**: Additionally, the distance between the chambers is calibrated. Enable this function when aligning a specimen carrier of a given type for the first time.

-**Calibrate Chamber Size**: Additionally, the size of the chamber is calibrated.

This function is only available for the Align from Chamber Edges option. Enable this function when aligning a specimen carrier of a given type for the first time.

4. Close the “Options...” dialog by clicking Close.

In the viewer, the position of the point to be aligned is indicated by a red crosshair within an individual image.

5. If the crosshair is not in the desired position, move the mouse to the position that is to match the point. The current mouse position is indicated by a red crosshair.

6. Double-click the assumed position to display an image at that spot.

Repeat this step until you have found the correct position.

7. Then click Next.

The aligned item is now displayed in green. The next point to be aligned will be outlined in yellow. Proceed accordingly.

If the next point is in a different chamber and at a large distance from the previous one, you will receive the following warning message:

8. Decide how you want to proceed and click the corresponding button.

When the stage has moved to the next position (manually or automatically), the red crosshair is displayed in the new position in an individual image.

9. For aligning all other points, follow the same process you did for the first.

You also have the following options:

-**Reset:** Reset all aligned points to start over with alignment.

-**Previous:** Return to the previous point to align it again.

10. When you have aligned all points, click Done.

This button is only displayed when there are no more points to be aligned.

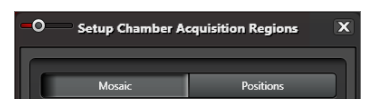
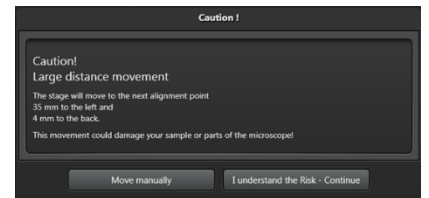
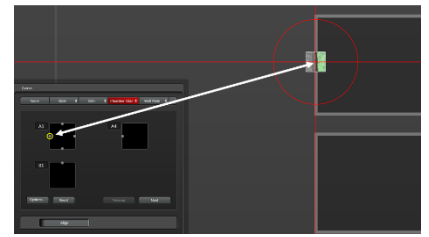
The dialog closes. The specimen carrier is now aligned, and you can start your experiment.

The alignment is saved for the respective type of specimen carrier and can be reused for the next experiment with the same type of specimen carrier.

Setup Chamber Acquisition Regions

In this dialog, you can configure the settings for the acquisition of a partial region. You define a certain area within a scan region to be used for preview and image acquisition. For chamber specimen holders, you define the area for all selected chambers.

You have two options of defining these areas within a scan region.



Mosaic: On the Mosaic tab, you set the portion of the partial region from the entire scan region or of the specimen carrier.

-**Fill Factor:** Here you can set the percentage of the entire scan region (filling factor) to be acquired.

-**Rows and Columns:** Here, for chamber specimen holders, you can set the number of the rows and columns to be acquired.



Positions: On the Positions tab, you can set the distribution of the individual images within the scan region.

Number: Number of scan fields

Random: Random distribution: If you enable the function, further options are available for you:

-**Can Overlap:** If the function is activated, the scan fields may overlap; if the function is deactivated, overlapping is not permitted.

-**Same Pattern:** If the function is activated, in all the sample chambers the same pattern for the scan field distribution is created.

If the function is deactivated, each sample chamber receives an individual scan field distribution.

Bias: Position of the scan fields

-**Center:** In the center of the scan region.

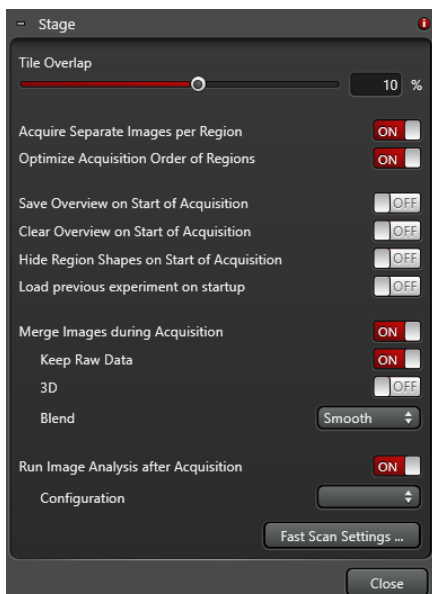
-**None:** No preference.

-**Edge:** On the edge of the scan region.



IV-4. Stage

In this dialog, you can configure the following settings for your experiment:



Tile Overlap: Using the slider or directly entering the value, you can define the percentage overlap between the individual partial images. A value of 10% is entered by default. You can also set negative values. In that case, a distance is maintained between the individual partial images in the overview. This is helpful for creating a quick overview.

Acquire Separate Images per Region: If the function is switched ON, separate images are acquired for each scan region.

Optimize Acquisition Order of Regions: If the function is switched on (ON), the sequence of acquisition of the individual scan regions is determined by the shortest travel paths of the specimen stage. Otherwise, the scan regions are processed in the sequence given in the task list.

Save Overview on Start of Acquisition: If the function is switched ON, the overview image is saved at the beginning of the image acquisition by clicking Start.

Clear Overview on Start of Acquisition: If the function is switched ON, the overview image is deleted at the beginning of the image acquisition by clicking Start.

Hide Region Shapes on Start of Acquisition: If the function is switched ON, the drawn scan regions are not indicated during acquisition. This function is deactivated by default.

Load previous experiment on startup: Reloads the IPS for the image acquisition parameters and the beam path settings at the first opening of LAS X Navigator after restarting LAS X, refer also to Instrument Parameter Settings (IPS) .

Merge Images during Acquisition: If the function is switched ON, the images are automatically merged after acquisition.

-**Keep Raw Data:** If the function is switched ON, the unmerged frames are stored.

-**3D:** If the function is switched ON, the images will be aligned with each other not only in the xy-directions but also in the z-direction.

-**Blend:** Here you can select the calculation method for the overlap of mosaic images. Refer also to Tilescan.

Run Image Analysis after Acquisition: If the function is switched ON, image analysis starts immediately after acquisition. This function is available only with the 2D Analysis or 3D Analysis license.

Configuration: This dropdown menu is displayed only if Run Image Analysis after Acquisition is switched on. Select a configuration for image analysis here.

Fast Scan Settings ...: Opens the Fast Scan Settings dialog. Here you can configure the fast scan settings.

Close: Closes the dialog.