



# **ZEISS Lightsheet Z.1**

## Quick Guide: Tile Scanning



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### Background Information:

Lightsheet Z.1 Tile Scan is a tool that assists with the setup of a tiling experiment with ZEN 2014 for Lightsheet Z.1 software. A Tiling Experiment acquires images or Z-stacks (tiles) which are adjacent in x and y direction to each other with or without a designated overlap. The resulting images or Z-stacks cover an uninterrupted area in x and y.

Following the workflow described in this document, the experiment will result in individual files for each tile.

As a prerequisite one must be able to acquire images and Z-stacks with ZEN for Lightsheet Z.1 and be familiar with the usage of the Multiview tool window.

The Tile Scan Tool uses the functionality of the Multiview tool window. Hence the experiment will result in the acquisition of Views, and as such the dimension is saved in the metadata, recognized and handled by ZEN for Lightsheet Z.1. The following definitions associated with this tool will be used in this document:

### Definition of a view:

- a view is a Z-stack at a certain position
- the position is defined by its x,y,z and angle value
- the Z-stack; its dimension, interval and number of slices; is defined in the Z-Stack tool window

### Definition of a group:

- a group can consist of one or more views (= group of views)
- a group will result in one file; or in case time, views, illumination or channel are split, a master file and its associated files for each timepoint/view/illumination/channel are generated

### Result of the experiment:

The experiment will produce a number of views (see definition above), each can be saved as an individual file. This might be necessary in order to stitch individual Z-stacks

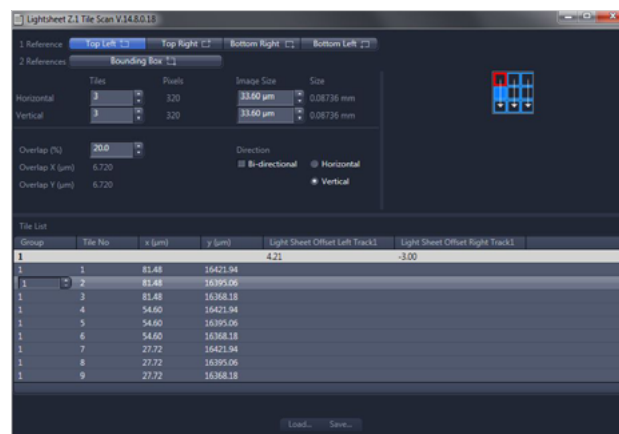


Figure 1 Tile Scan Tool Window

together to one image, which cannot be done in ZEN for Lightsheet Z.1 but needs 3rd party software such as arivis Vision 4D™ (recommended) or freeware such as Fiji.

### Tile Scan Tool Window

The Tile Scan Tool software opens in an external tool window that is partitioned into three fields (Fig. 1).

The upper left part contains input fields to set up and define the tiling experiment and holds information on the size of the resulting area in x and y direction.

In the upper right is a graphical display of the tiles in blue as they are arranged in x and y. The reference positions are labelled with a red frame, the sequence of acquisition is marked with white arrows.

During acquisition the first tile of an experiment is always the upper left corner, independent of the chosen reference points. In the lower part of the tool window is a table, the **Tile List**, which displays the views in the sequence as they will be imaged in the experiment. This list is updated, based on the settings in the upper left field.

In this list, groups and light sheet offset can be generated and edited.

**Workflow:**

1. Open ZEN for Lightsheet Z.1 and place your sample in the system for imaging.
2. Find and position your sample with the **Locate Sample** tool window and switch to the **Acquisition tab**.
3. Setup the **Light Path** to image your sample, define the necessary tracks. Proceed with adjusting the settings in the **Acquisition** tool window and the **Channels** tool window for images or Z-stacks.
4. Activate the **Multiview** checkbox in the **Multidimensional input field** (Fig.2). This will activate the Z-Stack and Group function at the same time. The Z-Stack and Group option cannot be deactivated individually, and are greyed out.

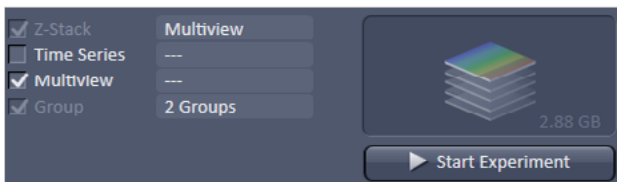


Figure 2 Multidimensional input field

5. The **Multiview – Setup** tool window (Fig.3) and the **Z-Stack** tool window are now available.
6. One or two reference points need to be created as a basis for the setup of the Tiling experiment. Additionally, the dimensions of the Z-stack are defined in the next steps. Using one or two reference points is explained individually in the following sections.

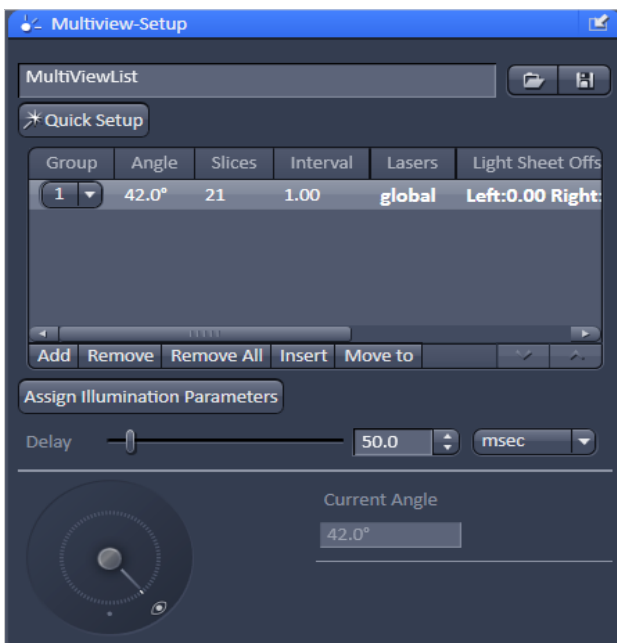


Figure 3 Multiview-Setup tool window

**Tile Scan with One Reference Point:**

1. Decide which area you wish to cover with the tile scan experiment by moving the sample with the **System Navigator** or the Ergo Drive Operating Panel. Use the position information given for x and y by the System Navigator window (Fig. 4) to estimate the total size of the area.
2. Choose the zoom and illumination settings in the **Acquisition** Mode and the **Channels** tool window.
3. Define a Z-stack with the **Z-Stack** tool window at the deepest (thickest) part of the sample within the tile scan area. Make sure to set the desired interval for the experiment. All Z-stacks of a Tile experiment have the same number of z-slices.
4. Move the sample to a position which you want to use as the reference for the tiling. This position should be the top left, the top right, bottom left or bottom right Z-stack of the tile experiment.
5. After selecting a reference position, check and adjust the light sheet offset (**Channels tool** window) for the needed illumination sides. These settings can be changed later on as well.
6. Continue and press the **Add** button in the Multiview tool window. The position with the defined Z-stack is added to the multiview list as a view.
7. Press the button with the disc display to save the multiview list of the reference position.
8. Choose a name and location to save the multiview list (.mvl file) in the opened Windows (OS) window.
9. Launch the Tile Scan Tool using the Desktop Icon. (Fig. 5, see Installation on how to generate such a Desktop Icon if not present).
10. The Tile Scan Tool opens as an individual software window, which is operated independently from ZEN for Lightsheet Z.1

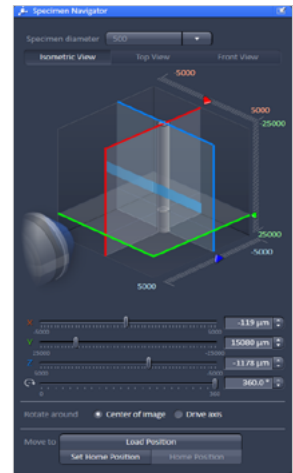


Figure 4 Specimen Navigator

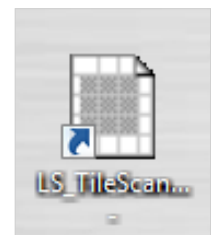


Figure 5 Tile Scan Desktop Icon

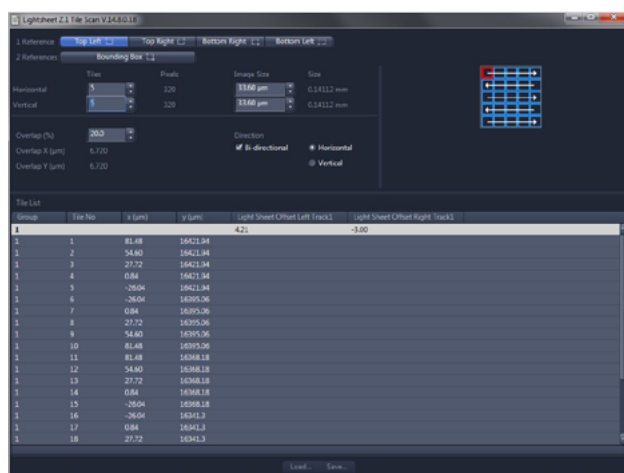


Figure 6 Lightsheet Z.1 Tile Scan Tool window

11. Fill in the correct **Image Size** in  $\mu\text{m}$  for Horizontal (x) and Vertical (y) in the respective fields. These values are displayed in the Acquisition Mode window of ZEN for Lightsheet Z.1.
  - ☞ This value is dependent on the **Frame Size** and **Zoom settings** – if these are changed afterwards, the Tile Scan will be flawed.
12. Press the **Load...** button on the bottom of the window to find and open the previously saved multiview list (.mvl file) with the reference position.
13. The loaded position will be displayed in the first row of the **Tile list**.
14. Define which position within the tile scan the loaded reference point should mark, use the 1 Reference buttons to choose between **Top Left**, **Top Right**, **Bottom Right** or **Bottom Left**. The reference position, hence this tile, is displayed in red on the right hand side display.
15. Chose how many tiles are needed in horizontal and vertical direction, to cover the field of the sample/structure. Type in these values in the boxes named **Tiles** for **Horizontal** and for **Vertical** direction. All tiles are displayed in blue, but the reference position is labelled with a red frame. The positions needed to acquire the tiles are added to the Tile List in the lower half of the tool window.
16. If the tiles should be stitched together after acquisition, make sure to define an appropriate **Overlap (%)** by using the respective box. Values of 10–20 % normally work well. The respective overlap in micrometers is displayed below the spin box.
17. With the options **Horizontal**, **Vertical** and **Bi-directional** you can decide in which order the tiles will be acquired. Each choice results in a different sequence for the entries in the created Tile List, displayed in the table.
18. When one entry of the Tile List is marked/ highlighted with a mouse click, the corresponding square is highlighted (square is filled in blue) in the displayed tile scan graphic at the right hand side.
19. **Groups** and the according **light sheet offset values** can be edited optionally in the Tile Scan tool. Please see the description of this workflow below (Group definition and light sheet offset).
20. When the tiling experiment has been set up as desired, save the created list by pressing the **Save...** button at the bottom of the tool window.
21. Chose a folder in the opened Windows (OS) window and save the .mvl file with a recognizable name.
22. Return to ZEN for Lightsheet Z.1 software.
23. **Remove all** entries in the Multiview list of the Multiview-Setup tool with the according button.
24. Open the created multiview list (.mvl file), holding the tiling experiment, by pressing the **folder icon** in the Multiview tool window.
25. The multiview list is loaded into the multiview table and all views are displayed as entries.
26. If it is likely that the sample will move due to the stage-movement during the experiment, it is possible to define a pause in between the view acquisition. This can reduce unwanted movement of instable/ shaky samples. Use the **Delay** slider or box in the Multiview-Setup tool window and define the unit of time.
27. Open the **Separate Files** tool window (Fig. 7) and activate the checkboxes of **View** and **Illumination**. This will save all views and the respective illumination sides in separate files, which maybe necessary to stitch the tiles and generate one image with third party software.

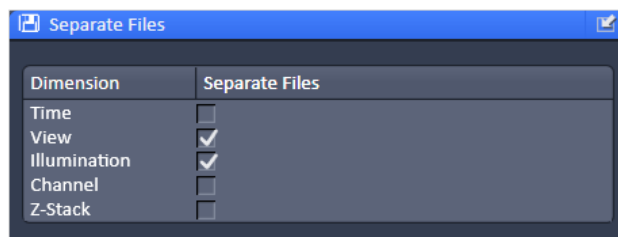


Figure 7 Separate Files tool window

28. Start the experiment by pressing the **Start Experiment** button. A Windows (OS) window opens for you to define the folder in which the experiment will be saved.
29. Name the file and press the save button. The acquisition will start.
30. As a result of the experiment there will be one master file which is the first view and the illumination from the left hand side. All additional files are numbered, e.g. Filename(1), sequentially.

#### **Tile Scan with Two Reference Points (Bounding Box):**

1. Two references for the tile experiment define the area to be covered by the experiment.
2. Decide which part of the sample you wish to image with the tile scan experiment by moving the sample and inspecting the desired area.
3. Chose the zoom and illumination settings in the **Acquisition** Mode and the **Channels** tool window.
4. Define a Z-stack with the **Z-Stack** tool window at the deepest (thickest) part of the sample within the tile scan area. Make sure to define the desired interval for the experiment. All Z-stacks of a Tile experiment have the same number of z-slices.
5. Move the sample to a position which you want to use as the first reference for the tiling. This position defines one corner of the tile area.
6. Check and adjust the light sheet offset (**Channels** tool window) for the wanted illumination sides. These settings can be changed later on as well.
7. Press the **Add** button in the Multiview tool window. The position with the previously defined Z-stack is added to the multiview list as a view.
8. Move the sample to a position which you want to use as the second reference point. This position should be located diagonally from the first reference.
9. Press the **Add** button in the Multiview tool window. The position with the previously defined Z-stack is added to the multiview list.
10. Two entries are now in the multiview list of the Multiview tool window. For both views the dimensions of the Z-stack must be identical.
11. Press the button with the disc to save the multiview list.
12. Chose a name and location to save the multiview list (.mvl file) in the opened Windows (OS) window.
13. Launch the **Tile Scan Tool** with the Desktop Icon. (Fig. 5, see Installation on how to generate such a Desktop Icon if not present).
14. The Tile Scan Tool opens as an individual window, which is operated independently from ZEN for Lightsheet Z.1
15. Fill in the correct **Image Size** in  $\mu\text{m}$  for Horizontal (x) and Vertical (y) in the respective fields. These values are displayed in the **Acquisition Mode** window of ZEN main software.
  - ☞ This value is dependent on the **Frame Size** and **Zoom settings** – if these are changed afterwards, the Tile Scan will be flawed.
16. Press the **Load...** button on the bottom of the window to find and open the previously saved multiview list (.mvl file) with the reference positions.
17. The loaded positions will be displayed in the first two rows of the Tile List.
18. To use both positions as references, press the **Bounding Box** button of the 2 References option.
19. It is calculated how many tiles in horizontal and vertical direction are needed to cover the defined field, with the set Image Size. These positions are present in the **Tile List**. The number of tiles are displayed in the boxes named **Tiles** for **Horizontal** and for **Vertical** direction. The values are greyed out and cannot be changed. All tiles are shown in blue in the graphical display, but the reference positions are labelled with a red frame.
20. If the tiles should be stitched together after acquisition, make sure to define an appropriate **Overlap (%)** by using the respective box. Values of 10 – 20 % work normally well. The respective overlap in micrometers is displayed below the box.
21. Changing the value for the overlap will influence the number of tiles needed to cover the area, defined by the two references.
22. With the options **Horizontal**, **Vertical** and **Bi-directional** you can decide in which order the tiles will be acquired. Each choice results in a different sequence for the entries in the created Tile List.
23. When one entry of the Tile List is marked/ highlighted with a mouse click, the corresponding square is highlighted (square is filled in blue) in the displayed tile scan at the right hand side

24. **Groups** and the corresponding **light sheet offset values** can be edited optionally in the Tile Scan tool. Please see the description of this workflow below.
  25. When the tiling experiment has been set up as desired, save the created list by pressing the **Save...** button at the bottom of the tool window.
  26. Chose a folder in the opened Windows (OS) window and save the .mvl file with a recognizable name.
  27. Return to ZEN software for Lightsheet Z.1.
  28. **Remove all** entries in the Multiview list of the Multiview-Setup tool with the according button.
  29. Open the created multiview list (.mvl file), holding the tiling experiment, by pressing the **folder icon** in the Multiview tool window.
  30. The multiview list is loaded into the multiview table and all views are displayed as entries.
  31. If it is likely that the sample will move due to the stage-movement during the experiment, it is possible to define a pause in between the view acquisition. This can reduce unwanted movement of instable/ shaky samples. Use the **Delay** slider or box in the Multiview-Setup tool window and define the unit of time.
  32. Open the **Separate Files** tool window (Fig. 7) and activate the checkboxes of **View** and **Illumination**. This will save all views and the respective illumination sides in separate files, which may be necessary to stitch the tiles and generate one image with third party software.
  33. Start the experiment by pressing the Start Experiment button. A Windows (OS) window opens for you to define the folder in which the experiment will be saved.
  34. Name the file and press the save button. The acquisition will start.
  35. As a result of the experiment there will be one master file which is the first view and the illumination from the left hand side. All additional files are numbered, e.g. Filename(1), sequentially.
- ☞ Make sure that the chosen **Direction** in the Tile Scan tool (Horizontal, Vertical, Bi-directional) will result in a list of positions along which the light sheet offset will need to change. Light sheet offset values can only be changed by using the group functionality of the multiview list.
- Up to 10 groups can be defined in one multiview list
  - Views of one group will always be imaged in a sequence, then the views of the next group will be imaged
  - Light sheet offset and Laser power can be set individually for each group
  - One light sheet offset value is possible in one view (Z-stack)

There are two options to define groups and set individual light sheet offsets for different groups:

#### 1. In ZEN for Lightsheet Z.1 Multiview-Setup tool window

This method is recommended for a small number of tiles.

- 1.1 Save the multiview list as it is defined in the Tile Scan tool.
- 1.2 Load the multiview list with the tiling experiment in the multiview tool window.
- 1.3 Use the **Move to** button to move through the list and check the light sheet offset values for accuracy.
- 1.4 If changes are needed use the dropdown menu in the **Group** column to start a new group (Fig.8).
- 1.5 Change the light sheet offset for the illumination sides in the **Channels tool window**. Click on the view in the multiview list to mark the corresponding group and afterwards press the **Assign Illumination Parameters** button, to use these values for the newly formed group.
- 1.6 Move forward in the multiview list, and change all following entries to the newly created group.
- 1.7 Repeat the process of generating a new group and assigning the views to this group until all necessary groups are created.
- 1.8 If it is likely that the sample will move due to the stage-movement during the experiment, it is possible to define a pause in between the view acquisition. This can reduce unwanted movement of instable/shaky samples. Use the **Delay** slider or box in the Multiview-Setup tool window and define the unit of time.

#### Group definition and Light Sheet Offset:

When a tiling experiment covers a large area, a single light sheet offset value (for each illumination side) may not be ideal for all Z-stacks. In this case it is possible to separate the list of views in groups. For each group an individual light sheet offset can be defined.

- 1.9 Open the **Separate Files** tool window (Fig. 7) and activate the checkboxes of **View** and **Illumination**. This will save all views and the respective illumination sides in separate files, which may be necessary to stitch the tiles and generate one image with third party software.
- 1.10 Start the experiment by pressing the **Start Experiment** button. A Windows (OS) window opens for you to define the folder in which the experiment will be saved.
- 1.11 Name the file and press the save button. The acquisition will start.

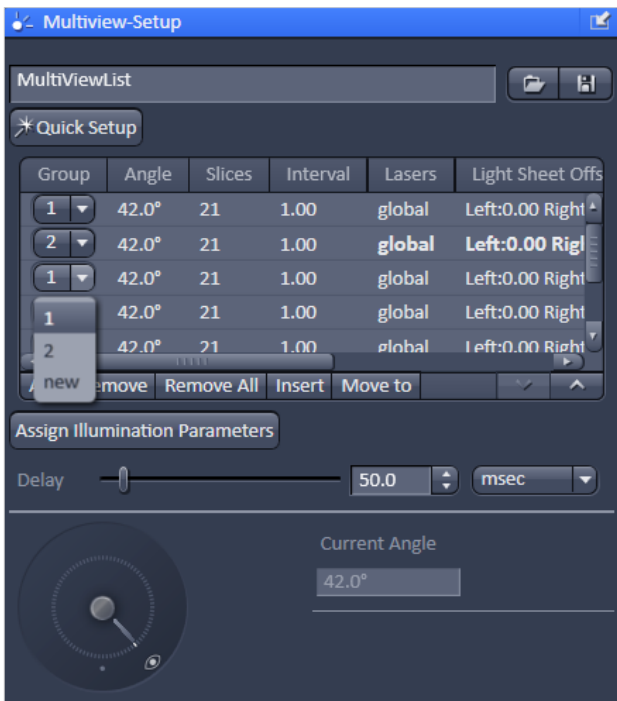


Figure 8 Multiview-Setup Tool with views and groups

## 2. In Tile Scan Tool, Tile List

- This method is recommended for a large number of tiles.
- 2.1 Before using the multiview list in the **Tile Scan tool** window, the groups and light sheet offset values can be defined in the Tile Scan tool window.
  - 2.2 Save the multiview list as it is defined in the Tile Scan tool by pressing the **Save...** button.
  - 2.3 Chose a folder in the opened Windows (OS) window and save the .mvl file with a recognizable name.
  - 2.4 Important: do not close the Tile Scan tool but leave the window with the just saved **Tile List** open.
  - 2.5 Load the multiview list with the tiling experiment in the multiview tool window.

- 2.6 Use the **Move to** button in the **Multiview-setup** tool window to move through the list and check the light sheet offset values for accuracy or
- 2.7 Use the Specimen Navigator to move within the sample. Type in the values for x and y coordinates (z is defined by the Z-stack) of the Tile List for a certain position of the Tile Scan tool into the according boxes of the **Specimen Navigator**. Press enter and the sample will be moved to that position.
- 2.8 Press the **Continuous** button to check and adjust if necessary the **light sheet offset values** in the **Channel tool window** for the needed tracks and illumination sides according to the live image.
- 2.9 If you want to change the light sheet offset value in the **Tile Scan tool window**, click on the entry representing the current position and change the value in the **Group** column (Fig. 9). This entry and all further entries will be assigned to the newly generated group (here Group 2, Fig.10).
- 2.10 The first row of the group, which is marked in grey, holds the **light sheet offset** values. To change them, click in the columns **Light Sheet Offset Left** or **Light sheet Offset Right** and type in the values from the **Channel tool window** into the appearing boxes (Fig. 10).

Group	Tile No	x (µm)	y (µm)	Light Sheet Offset Left Track1	Light Sheet Offset Right Track1
1	1	733691004.00	-3492815327	5.63	-3.1
1	2	733690604.00	-3492815327		
1	3	733690204.00	-3492815327		
1	4	733690204.00	-3492815487		
1	5	733690604.00	-3492815487		
1	6	733691004.00	-3492815487		
2	7	733691004.00	-3492815487		

Figure 9 Tile List. Editing of Groups

- 2.11 Repeat the process as described in 2.6 – 2.10 until you reach the last entry in the Tile List.
- 2.12 When the tiling experiment is has been set up as desired, save the created list again by pressing the **Save...** button at the bottom of the tool window.
- 2.13 Chose a folder in the opened Windows (OS) window and save the .mvl file with a recognizable name.
- 2.14 Return to ZEN software for Lightsheet Z.1.

Group	Tile No	x (µm)	y (µm)	Light Sheet Offset Left Track1	Light Sheet Offset Right Track1
2	1	733691004.00	-3492815327	5.20	-3.50
2	2	733690604.00	-3492815327		
2	3	733690204.00	-3492815327		
2	4	733690204.00	-3492815487		

Figure 10 Tile List. Editing of Light Sheet Offset Columns



- 2.15 **Remove all** entries in the Multiview list of the Multiview-Setup tool with the according button.
- 2.16 Open the created multiview list (.mvl file), holding the tiling experiment with the edited light sheet off-set values, by pressing the **folder icon** in the Multiview tool window.
- 2.17 The multiview list is loaded into the multiview table and all views are displayed as entries.
- 2.18 If it is likely that the sample will move due to the stage-movement during the experiment, it is possible to define a pause in between the view acquisition. This can reduce unwanted movement of instable/shaky samples. Use the **Delay** slider or box in the Multiview-Setup tool window and define the unit of time.
- 2.19 Open the **Separate Files** tool window (Fig. 7) and activate the checkboxes of **View** and **Illumination**. This will save all views and the respective illumination sides in separate files, which may be necessary to stitch the tiles and generate one image with third party software.
- 2.20 Start the experiment by pressing the Start Experiment button. A Windows (OS) window opens for you to define the folder in which the experiment will be saved.
- 2.21 Name the file and press the save button.  
The acquisition will start.

**Installation:**

The Tile Scan tool is an external software tool window, which is not part of the ZEN for Lightsheet Z.1 main software. Hence it is installed independently, after ZEN for Lightsheet Z.1 has been installed.

1. Copy the LS\_TileScan 14.8.23.zip file onto your PC for system control and/or the Storage and Analysis PC of your Lightsheet Z.1 system.
2. Extract the LS\_TileScan 14.8.23.zip file into the ZEN folder (C:\ZEN).  
☞ It is crucial to use this location for the LS\_TileScan.exe file to work.
3. As a result you have the additional file: LS\_TileScan.exe in the ZEN folder.
4. Click onto this file with the right mouse button to open a windows menu.
5. Choose the option **Send to** and here **Desktop (Create Shortcut)**.
6. A shortcut is created on the desktop (Fig.5), this allows fast access to the Tile Scan Tool.





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