ZEISS Fluorescence microscope

Introduction

Applications

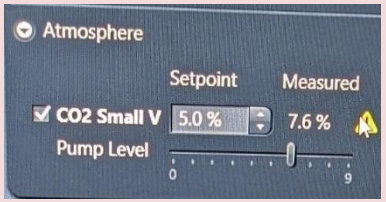
Version 2 –June 2025

D vanhecke | Adolphe Merkle Institute

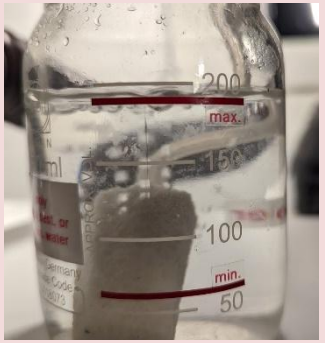
Experiment: create CO2 flow to the controller

1. Empty the water bottle and fill with Aqua Dest until the max line
2. Place the airing part under water and close the screw cap
3. On the CO2 bottle, open the main (bottle) valve completely
4. Open the manometer valve completely
5. Do not change/touch the pressure value (blue, labelled C)
6. On the Zeiss switch, open the valve,
   OPEN = the valve points down
   CLOSED = the valve is horizontal

7. In the software (right side of the screen):
   Under “Atmosphere”, tick CO2 small V



8. Pump level of 5-7 is OK. The CO2 concentration will first overshoot and then settle at the desired Setpoint.
This takes at least **30 minutes**. You should see bubbles raising in the water bottle.



Demonstration: Setup CO2 control

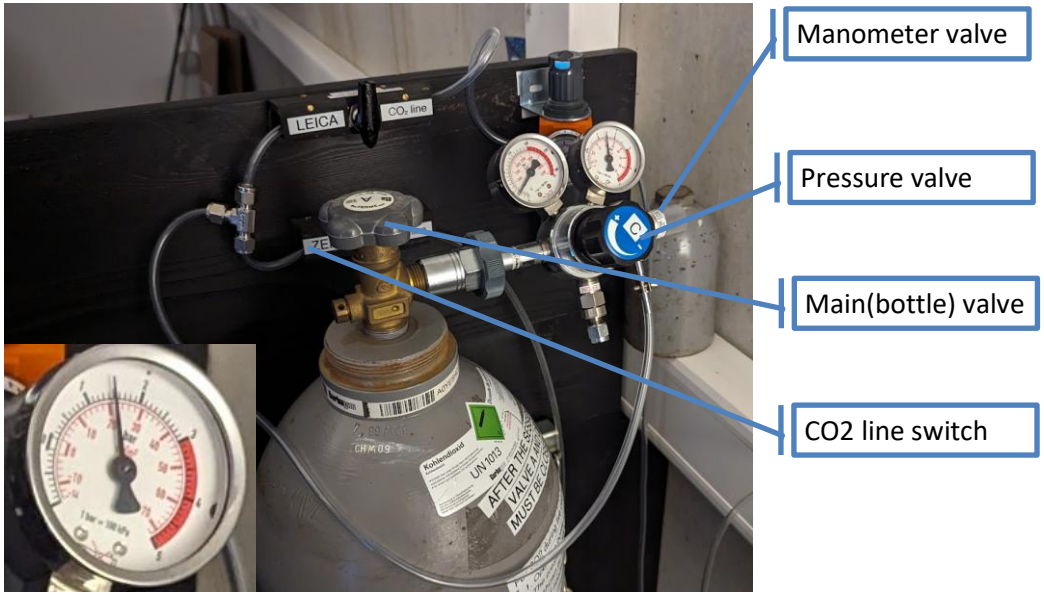
Prerequisites:

Brightfield source properly set

One fluorescence channel setup properly

Setup CO2 for live cell imaging

CO2 is delivered by the bottle in the corner. Follow these instructions to produce a 5% atmosphere in the heated insert chamber.



The output pressure should be between 1 and 2 bar. This is set by the pressure valve and **does not need to be adjusted**.

Universal rules

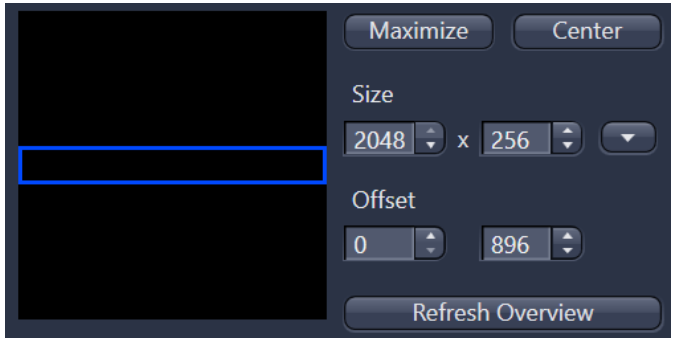
Rule 1: don't touch a control if you are not sure of the outcome of that action

Rule 2: never, ever force anything beyond finger strength

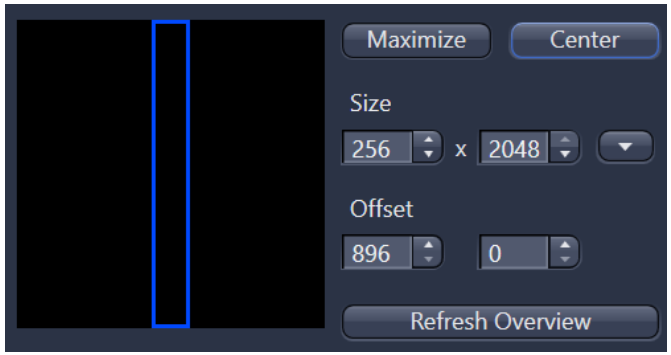
Rule 3: if in doubt ask for help

Content

Locations 5
Demonstration: Z stacks 5
Demonstration: Tiles 8
Demonstration: The tile scan viewer 17
Demonstration: Focus strategy 20
Demonstration: Tile scan viewer using focus surface defined in tiles setup 22
Demonstration: Stitching a recorded tile region 24
Demonstration: Time series 26
Demonstration: Setup CO2 control 30



Same readout speed as 2048 x 2048, ie. 100 frames per second (FPS) at 16 bit; No advantage compared to full chip



Same readout speed as 256 x 256, ie. 800 frames per second (FPS) at 16 bit; 8x faster compared to full chip

Summary of achievable time lapse speeds:

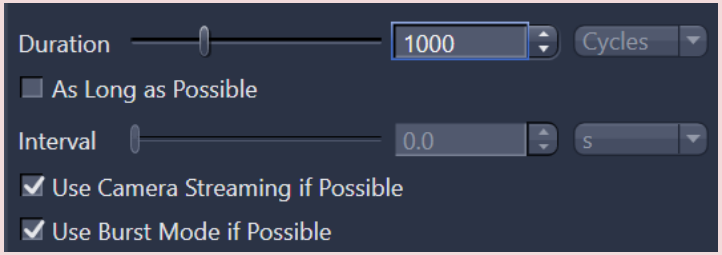
SENSOR REGION	16-BIT (FPS)	8 BIT (FPS)
2048 X 2048 PX	100	200
1024 X 1024 PX	200 (estimated)	400 (estimated)
512 X 512 PX	400 (estimated)	800 (estimated)
256 X 256 PX	800 (estimated)	1600 (estimated)
128 X 128 PX	1600 (estimated)	3200 (estimated)
64 X 64 PX	3200 (estimated)	6400 (estimated)

mode is especially useful for capturing ultra-fast events or when you need to maximize the temporal resolution of your acquisition. The exposure time settings in your setup are no longer relevant: the camera will just grab frames and send them to the PC. **The limiting factor is the chip readout rate**, not the transfer rate but.

Repeat! Be careful: you will now record really enormous data in very, very short time

Experiment: set up camera streaming

- Provided you have set up As above



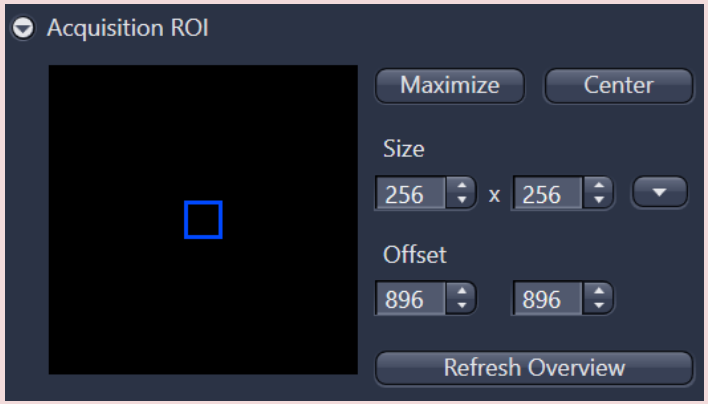
- Tick: “Use burst Mode if possible”

To reach higher speeds, you need to crop the readout area of the chip (the transfer rate is no longer the limiting factor):

- Find the “Acquisition mode” window and unfold the Acquisition ROI tab

- Reduce the Size. Ideally are Powers of 2 (1024, 512, 256, 128, 64). The ROI (in blue) Does not need to be square (rectangular is also OK)

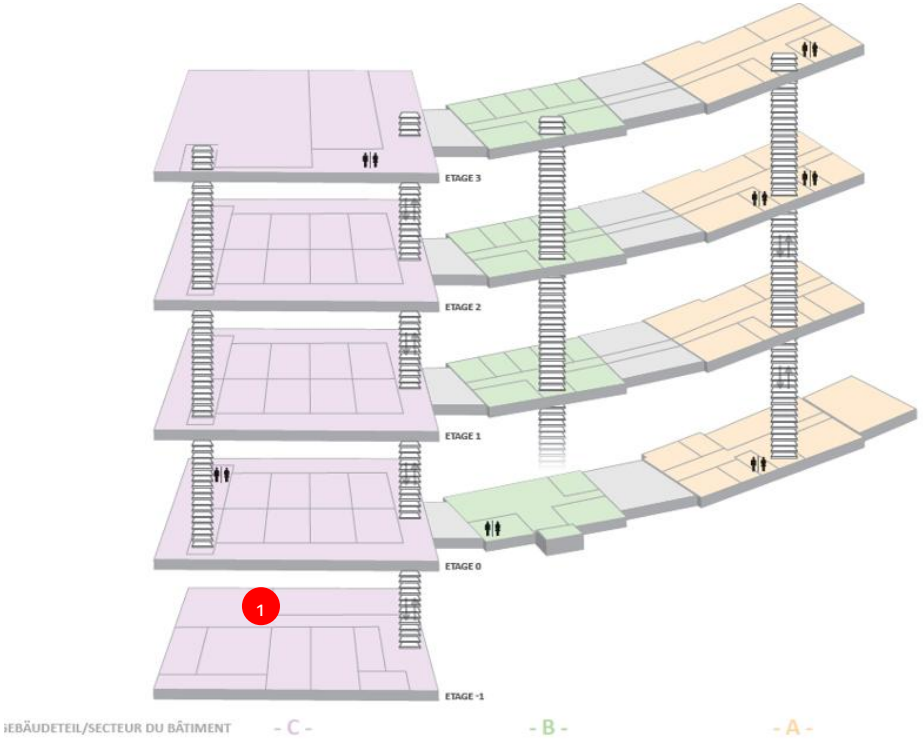
- Readout is vertical, meaning that Portrait rectangles will be Faster than landscape



Locations

The instruments can be found here:

- Laser scanning microscope (room C0141)



Demonstration: Z stacks

Prerequisites:

Brightfield source is properly set

Fluorescence channel(s) set up properly

Action:

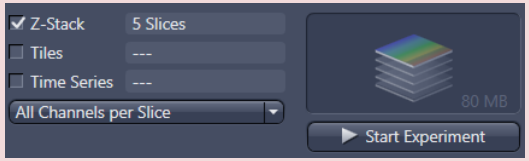
Acquire a Z stack

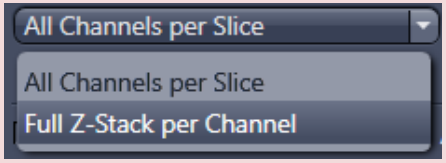
Z-stacks in non-confocal settings

This is not a confocal microscope: the Z resolution is rather poor compared to a confocal LSM, but Z-stacks are possible in reflected mode. Z-stacks in transmitted mode (brightfield, DIC) will not give 3D results. Do not use transmitted light for Z stacks!

Set up a Z-stack experiment

1. To set up a Z-stack, tick the option Z-stack in the experiments





2. You get the option:
   - All channels per slice
   - Full Z-stack per channel

Ensure you select **Full Z-stack per channel.** This is mandatory[1]

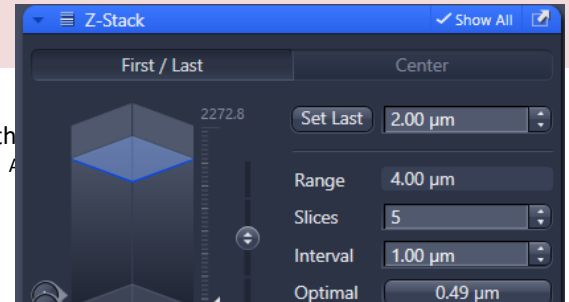
3. Find the Z-stack window (in multidimensional acquisition)

Set First/Last:

allows to set a Z-range (Centre sets the central plane of the stack)

Range

(info) the distance between the first



[1] The rationale is to put the least strain on th

2. High speed time lapse using camera streaming

Camera streaming:

the camera continuously acquires images with minimal delay between frames[2]. Camera streaming is typically used for very fast, single-channel acquisitions.

Careful: you will record enormous data in very short time

For example:
- full chip (2048x2048px),
- 10 ms (100 frames per second)

Result: 8 GB in 12 seconds (!)

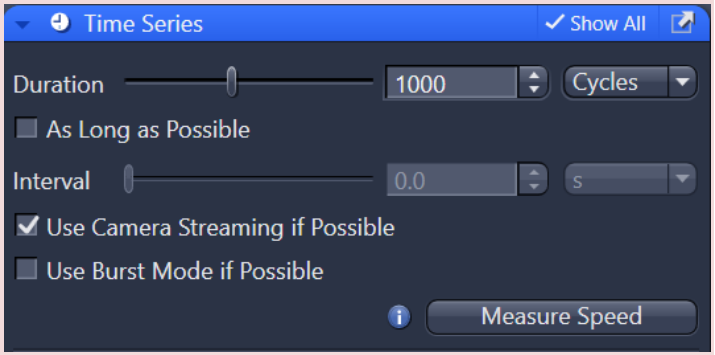
Experiment: set up camera streaming

- Provided you have set up your imaging properly

- Make sure you have only 1 channel activated (=ticked)

- In the time series window, Setup as shown above and Tick: "Use camera Streaming if possible"

- Start the experiment



3. Ultra high speed time lapse using burst mode (and Camera link USB 3)

Burst mode is a post-acquisition option. The burst mode is only available if no interval is set and 2D data is acquired. It processes and transfers data most efficiently. Burst

[2] As opposed to triggering each image individually.

Demonstration: Time series

Prerequisites:

Brightfield source is properly set

One fluorescence channel is setup properly

Set up video recording with one or multiple fluorescence or transmitted light sources

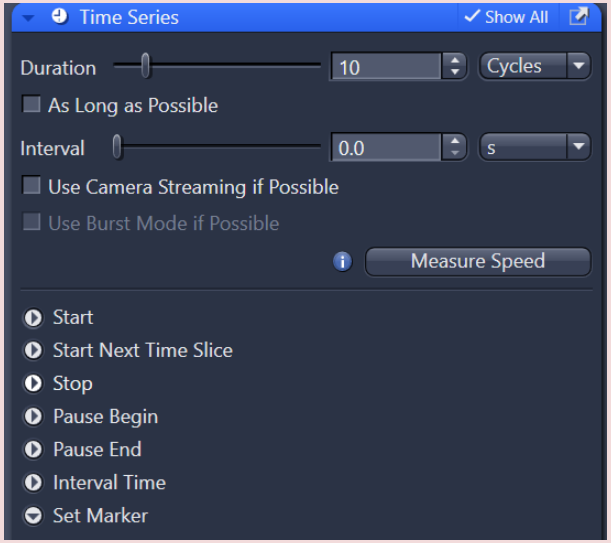
The system can provide video speeds up to 100 frames per second in full chip readout (2048 x 2048 px) and more than 1000 of frames per second when cropping the chip.

1. Full chip time lapse recording

This is the situation where frame speed is not key, you will end up with about 1 image per second, irrelevant of the exposure time settings.

Experiment

The setup is fairly simple. Assuming the time per image is set by your setup (in the Channels window).



1. Define:
- the number of cycles (or infinite)
- The interval between two cycles

2. Click “start experiment” to start the time lapse

You can semi-program starts, stops, pauses, loops (in switches, ...). We do not have an Input/Output card installed, so external triggers are – with the current setup – not possible.

and last slice along the axial axis

Slices
The number of steps along the axial axis, i.e. the number of images you will end up with

Interval
The distance between adjacent slices Along the axial axis. Use the Optimal button to apply the Nyquist criterion.

Start Auto configuration
finds the Range based on the software autofocus.

Demonstration: Tiles

Prerequisites:

Brightfield source properly set

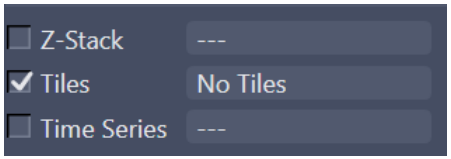
Fluorescence channel(s) setup properly

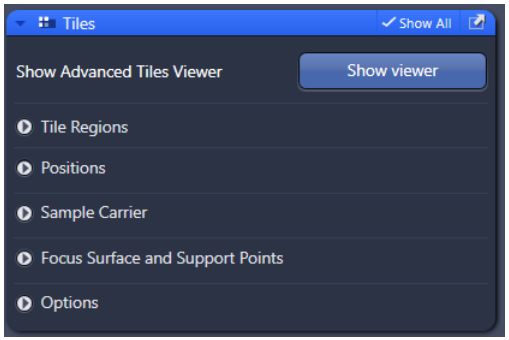
Action:

Acquire a larger area with tiling

Using tiles and positions

Tick the option Tiles. A new window (collapsed)

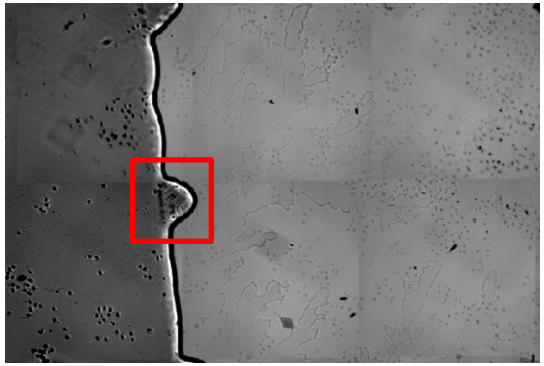


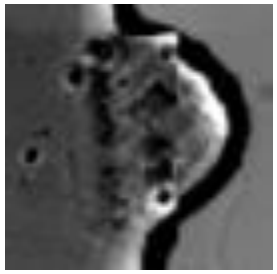


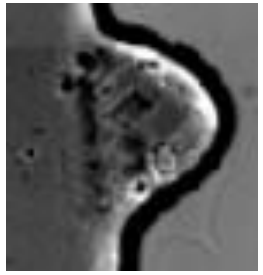
A new window appears in the list: Tiles.

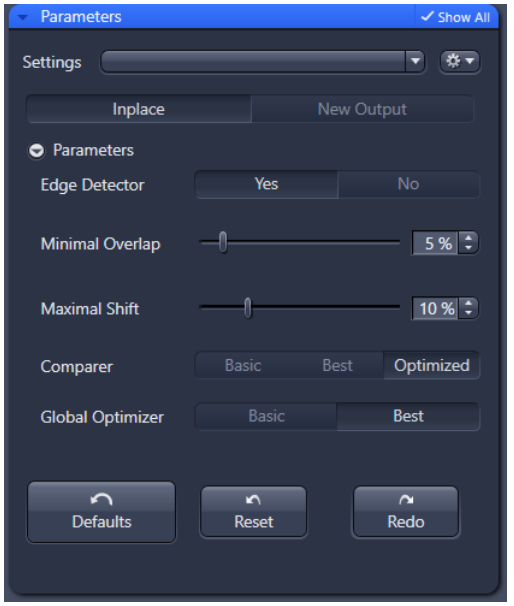
(For the viewer, see below)

Icon	Meaning	Description
	Selected	Represents the selected container (blue border)
	Live navigator	Shows the current stage positionincluding live image. To move: double click anywhere
	Tile region	Represents the tile regions in the stage view (red grid)

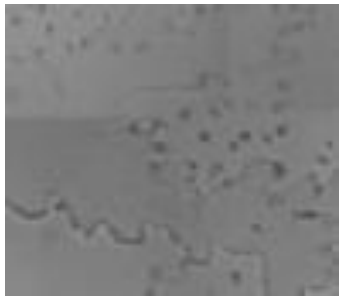








Before After



To minimize the edge effects:

- Use black reference (page **Error! Bookmark not defined.**)
- Use shading correction (page **Error! Bookmark not defined.**)

Demonstration: Stitching a recorded tile region

Prerequisites:

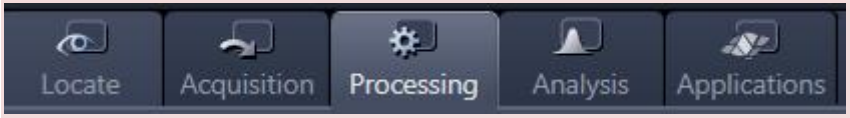
Tile region recorded

Post processing allows for stitching the pieces together into 1 image

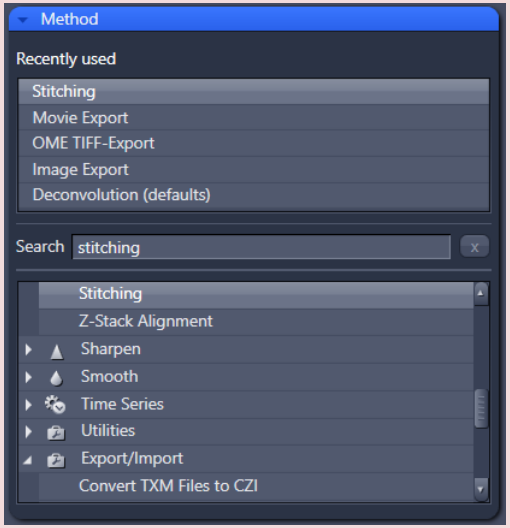
The stitching is based on mechanical movements of the stage, which are precise up to around 1 µm. the 5-10% overlap is key here. If there is no overlap between the tiles, a proper stitching will not be achieved.

Experiment: Software-based correction of small mismatches of the tiles

- Move to the tab “Processing”



- Under Method, search for “stitching”
(it usually features in the recently used list)



Unless you know exactly what you are doing, use the default parameters:

- inplace
- Edge detector yes
- 5% overlap
- 10% maximal shift
- Compared Optimised
- Best global optimiser

Press Apply (at the top)

	Positions	Represents positions in the stage view (yellow +)
	Position array	Represents the position arrays in the stage view
	Local support point	Yellow dot with circle
	Global support point	White dot with circle

1. Tile regions

Open the Tile regions in the Tiles window. The list will display all tile regions (TL) currently active (currently none).

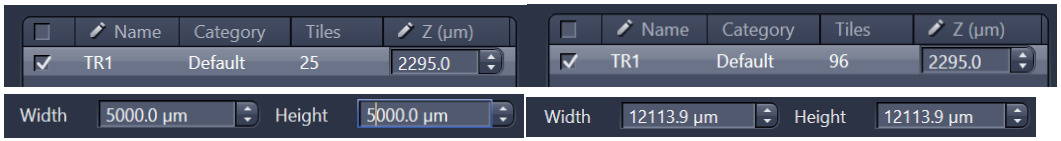
Use the joystick to move to a position and add the position by pressing **F9**. The tile region will be a rectangular form with the current field of view at its center. The number of images (tiles) can be changed by altering the width and heigth of the region (see below).

Name
can be altered by double LMB

Categories
can be created and assigned

Width & Height

will affect the number of tiles to be scanned for the tile region.



Verify

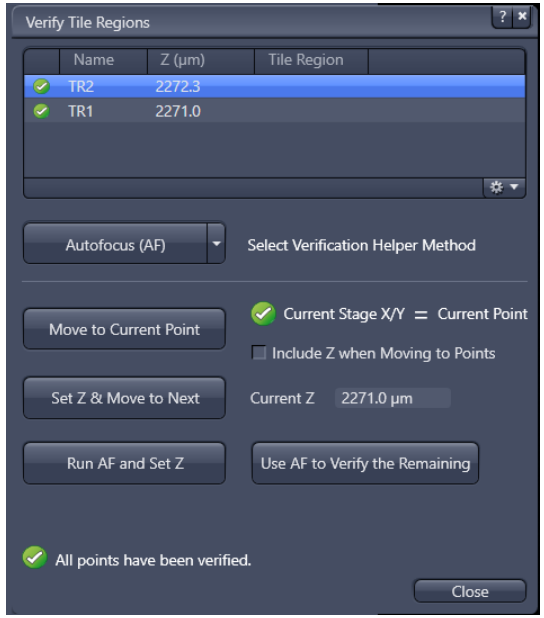
Checks for focus differences.

Experiment: Verify tile Regions

- Verification Helper Method: Autofocus (AF = Software autofocus).

- Cycle through the entries (Set Z & Move to next, you can activate Live) and Run AF and set Z. This will set the Z value for the specific point using the autofocus.

- To cycle automatically through all points, use "Use AF to Verify the remaining"



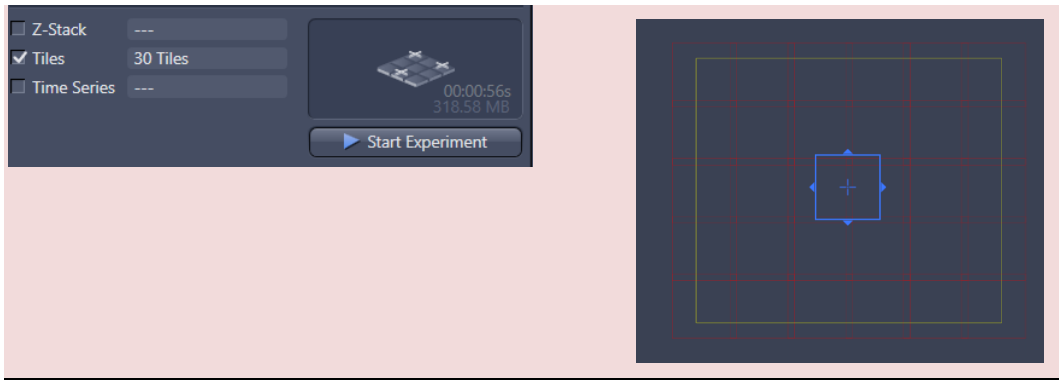
2. Positions

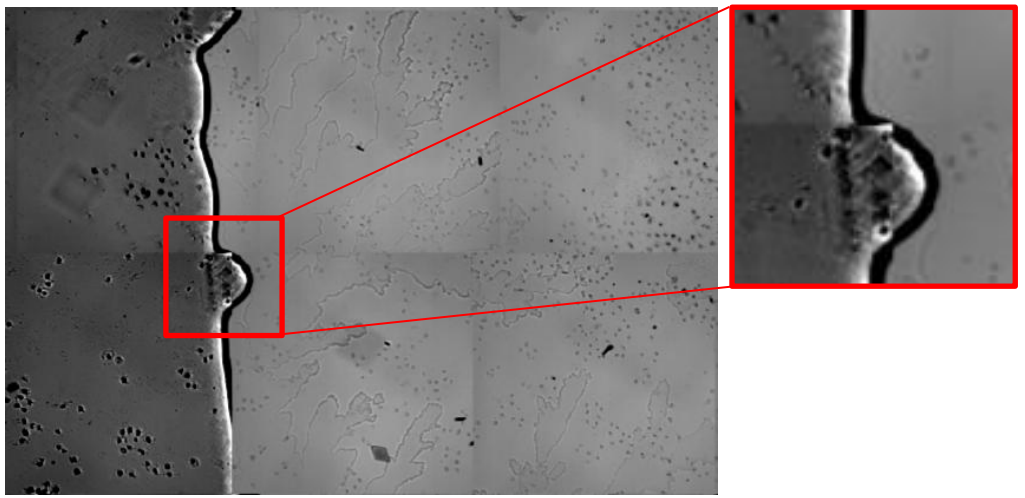
In a similar way to the Tile Regions, this list will display all positions (P) currently active. Use the joystick to move to a position and add the position by pressing **F10**.

In a similar way to the Tile Regions, you can verify (or find) the correct Z position of the points.

3. Sample Carrier

You can select and calibrate (and even create your own) sample carriers.





The result shows a stitched region, but between the tiles, there are mismatches. To correct this, see the Stitching a recorded tile region below.

Demonstration: Tile scan viewer using focus surface defined in tiles setup

Prerequisites:

Tile region defined

Support points allow to adjust the focus over your tile scan

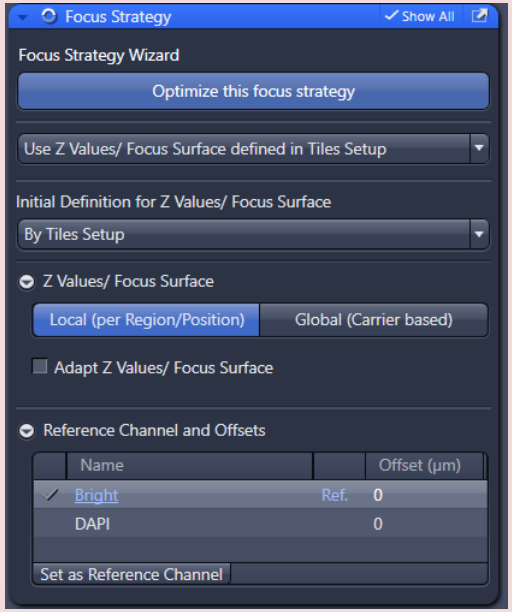
Experiment: Focus strategy with support points

- In the Tile region list or in the viewer, select the relevant Tile region (one must be selected).

- Move down in the tiles window to the Focus surface and support points (How to place the support points was detailed above).

- In the window Focus strategy, select “Use Z values/Focus surface defined in tile setup”.

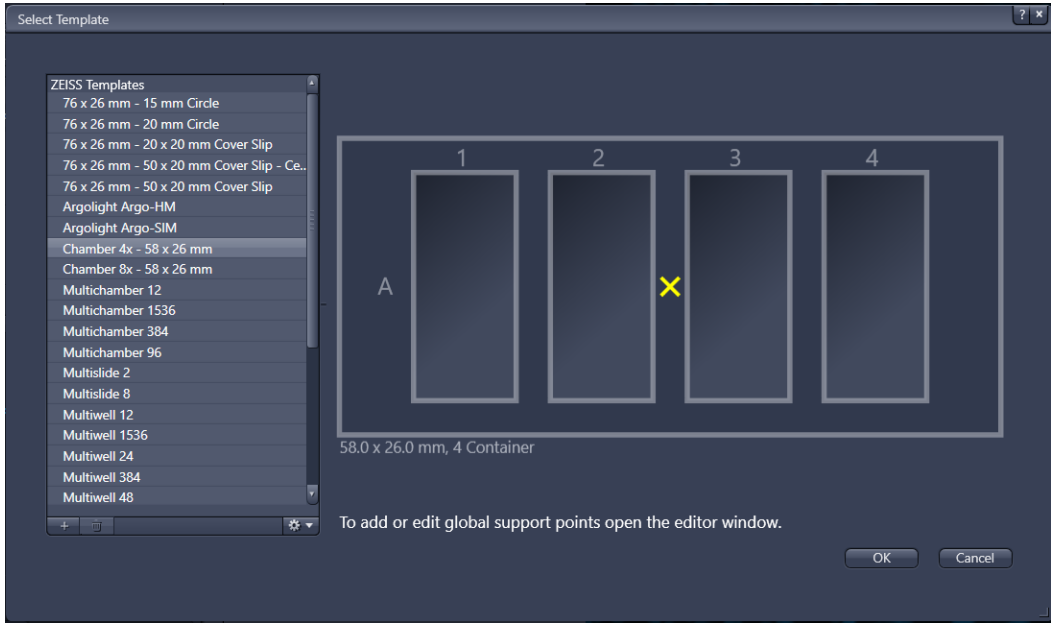
- Choose between your own local focus points (yellow dots) or - if a carrier is specified – global support points of the carrier.



Note: click “Optimise this focus strategy” for an extensive wizard to fine-tune your strategy. The detailed explanation of this wizard is beyond the scope of this manual.

With all support points verified, start the recording by clicking “start experiment”

Click “select” to get the graphical overview of the sample carriers.



Experiment: Calibrate the sample carrier

- Press Calibrate. The workspace changes into a four step wizard.

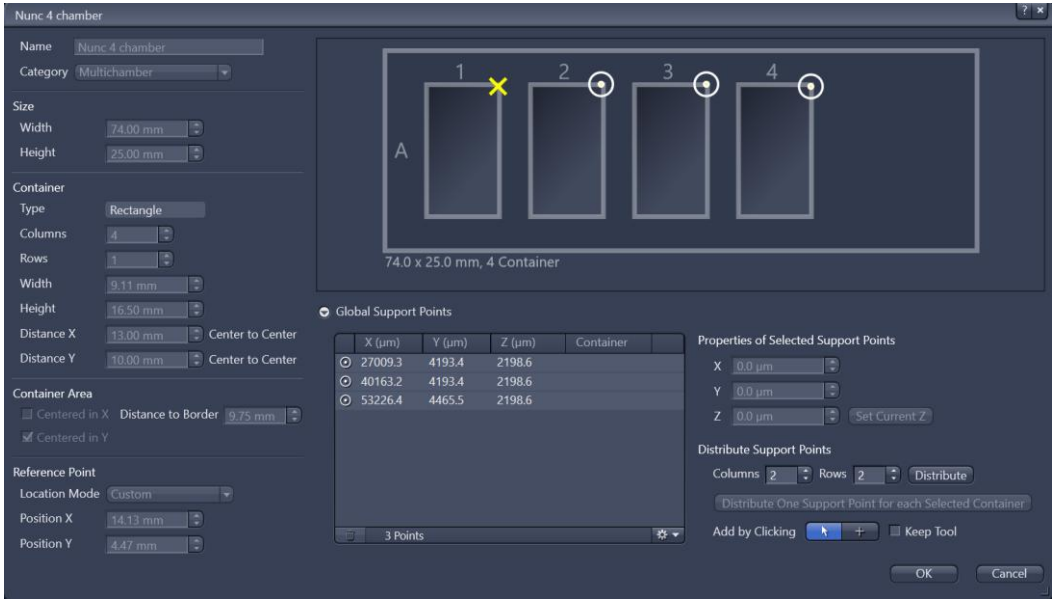
Step 1: select the 10X lens. Click next.

Step 2: select the illumination. Typically, Transmitted light will be the choice.

Step 3: calibrate the stage.

Edit or create carriers

Click the  button next to Select...

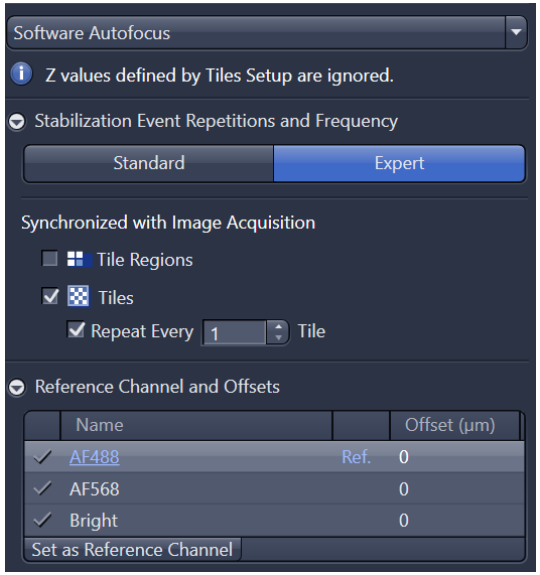


A detailed explanation of the carrier edit tool is beyond the scope of this manual. Search for “edit sample carrier dialogue” in help > Contents... (or CTRL + F1).

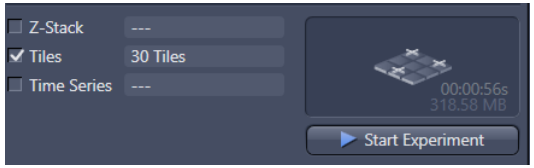
4. Focus surface and support points

At least one tile region must be defined. Each support point defines a Z-height and will correct for the non-horizontal position of your sample. There are local support points (defined by you) and global support points (defined by the carrier you chose, if you did). There are two approaches:

- Add single support points: Place support points one-by-one manually (recommended for very random topography with many changes in Z)
- Add multiple support points: Place an array of support points automatically (faster)



In the top left, click Start Experiment



3. Use Z values / Focus surface defined in the Tiles setup

See below (used in the tiles viewer)

Demonstration: Focus strategy

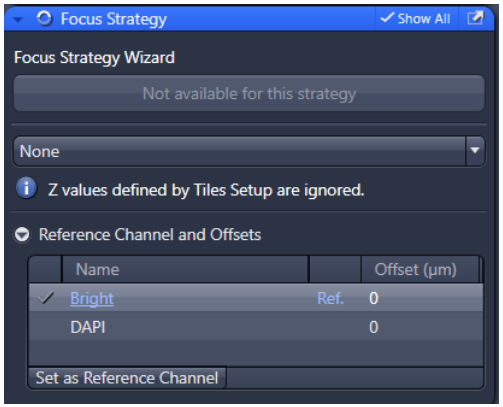
Prerequisites:

Tile region defined

Support points may not be needed for flat or small tile regions

1. Without a focus strategy

In the focus strategy, select None. Z values by the tile setup will be ignored



2. With Software autofocus

This will use the autofocus as defined in the autofocus window. Can be set for every Tile, or every Tile region (and then, where within this tile).

Add single support Point

will save the current position (or the centre of the Tile) as a support point, which you can Z-define using the Verify button.

Experiment: Add single support points (manual)

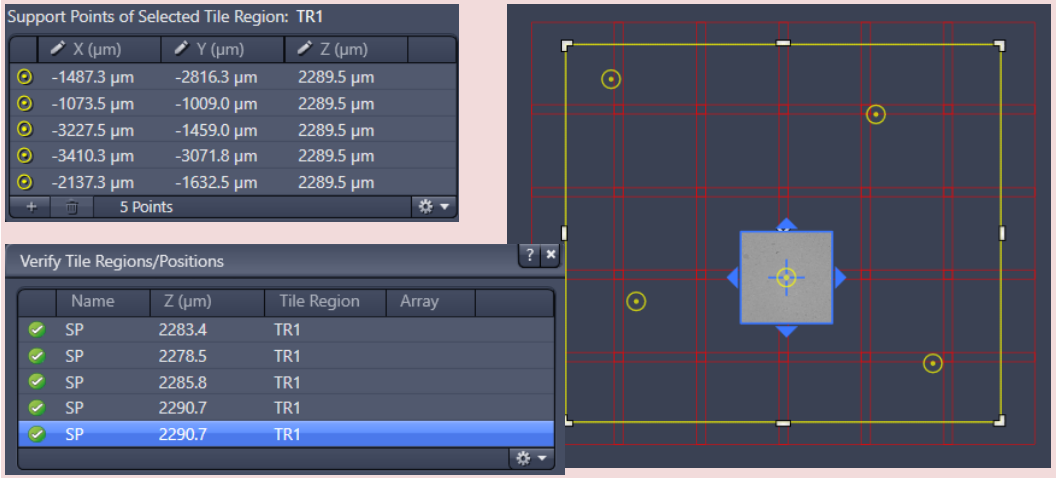
- Move to a position on your tile scan (LMB double click).

- Make sure the tile region is elected (LMB on tile region)

- Click current position in Tiles > Foxus Surface and Support points

- Repeat for several points in your tile region.

- Under the support points list: verify support points.
Click Use AF to Verify the remaining. (autofocus will cycle through all points and focus them)



Add multiple Support points

will define an array of support points automatically.

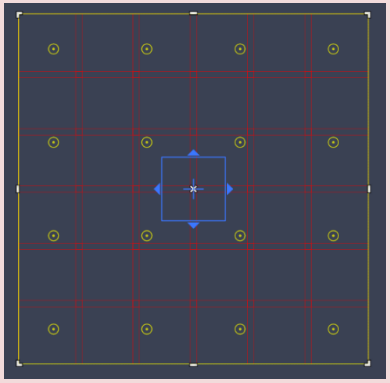
Generic: best for rectangular tile regions. The more heterogeneous your sample is (in Z), the more columns and rows you should choose.

Onion Skin: best for round tile regions. The more heterogeneous your sample is (in Z), the higher the density should be. Margin controls how close support points can be placed along edges of the tile region. 1 means: in the outer tiles of the tile region. Max. The maximum number of support points to be placed (5% density of very large tile regions can create a number of support points that are no longer feasible to work with).

Experiment: Automatic support points with generic method

The support points will be placed in a rectangular array. This is great for square or rectangular tile regions, but not for circular regions (underrepresentation of the outer parts).

Set the number of columns / Rows and click generate



Verify the Z positions as explained in the manual support points.

3. Setup tile regions by setting up marker positions

This is helpful for large areas where you cannot see the boundary in the viewer. Ideally, the obect has a more or less defined shape (e.g. circular). The idea is to search (live) the boundaries (e.g. top. Bottom left and right) and define these. A tile region will then be created using these boundary positions.

Experiment: Set up marker positions

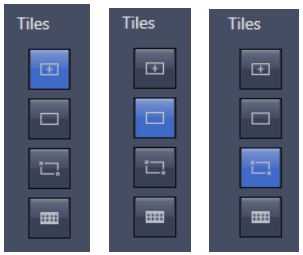
- Activate a live view. Search for a first marker (e.g. top left) and press +

- Then find a second corner (.e.g bottom right) and click +

- A new tile region will be created.

- Adjust size and position of needed.

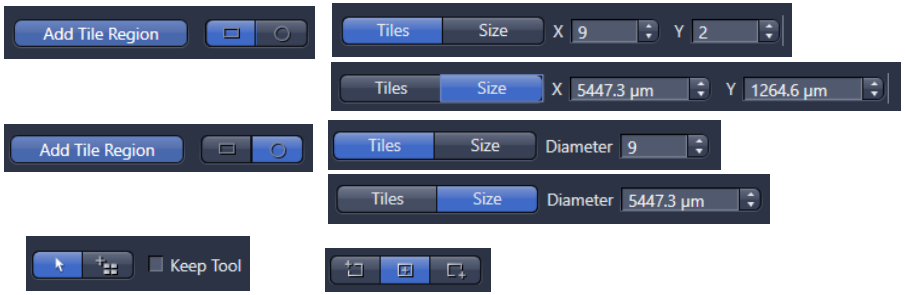
There are 4 ways to define tile regions:



- Left: Predefined size
- Middle: contour drawing
- Right:Marker positions

1. Set up tile regions from a predefined size

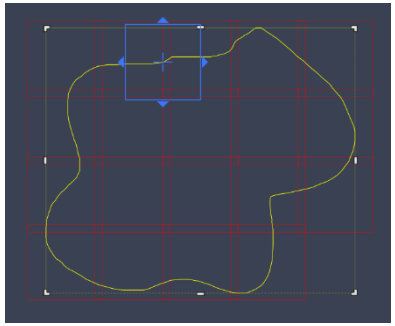
Use a rectangular or round predefined size, set as # tiles or in size.



Then click “Add region”. An entry appears in the tile region list.

2. Set up tile regions by drawing a contour

Pick a drawing setting and draw a region.

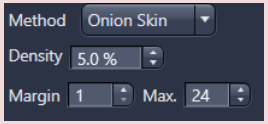


Note: use RMB to close the free contour drawing

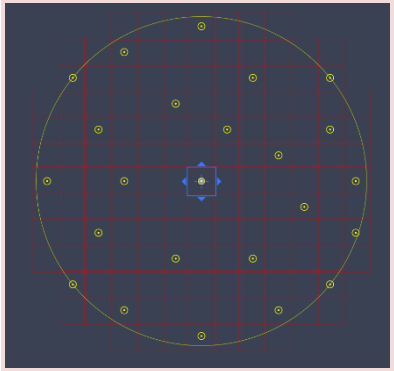
Experiment: Automatic support points with Onion skin

Density: 5% means that 5% of the tiles will be used for focusing

Margin: how many images from the edge the focus will be done



Max: limits the number of focus points for very large sets



Click verify to set the focus points of all support points (or not, then just proceed, but no focus will be performed)

Verify

See above. Sets the Z-height of each support point

Properties of support points

Set current X/Y/Z: set the current xyz position of the selected support point

Set current Z: set the current z position of the selected support point

Interpolation Degree:

Sets the mathematics used to interpolate the Z-heights between the support points

Add multiple support points (automated)

For the automatic placement, two options are possible: generic and onion skin

5. Options

It is not advised to alter these settings. In short, the defaults are:

Tile overlap:	10%
Travel in tile Regions:	Meander (fastest, most flexible)
Tile regions/positions:	Sort by Y, then X
Carrier Wells/Container:	Meander

Tick:

- Use Stage speed from stage control
- Use Stage acceleration from stage control
- Image Pyramid During acquisition

Untick:

- Move focus to load position between regions/Positions
- Split scenes into separate files

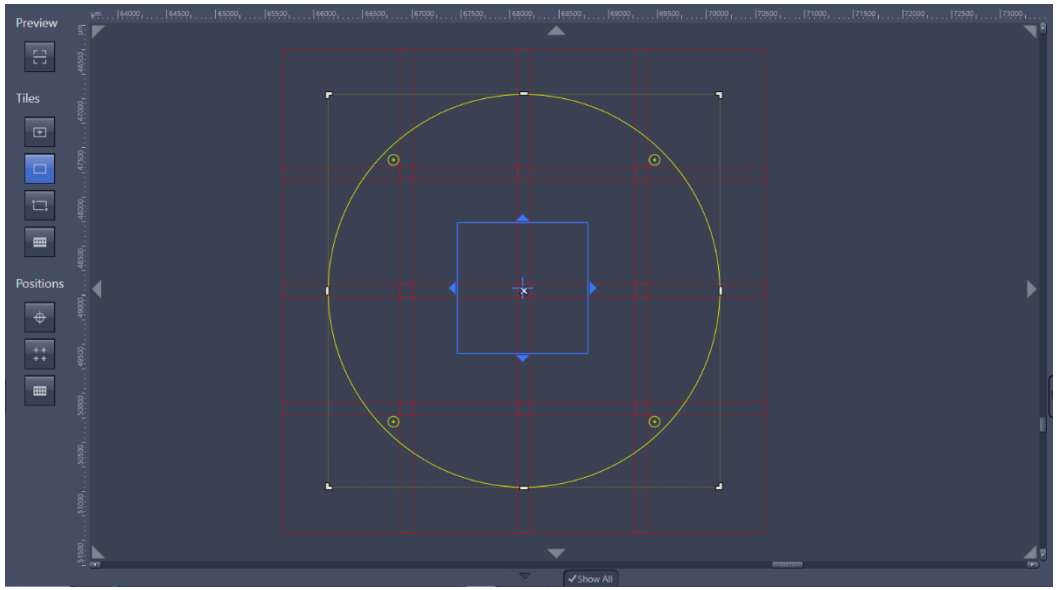
Demonstration: The tile scan viewer

Prerequisites:

Good illumination

Define a tile scan in the viewer

Open the viewer using the tile scan menu.



Tile scan viewer with 1 tile region (round, yellow circle, tiles as red lines), 4 local support points (yellow dots), 1 live navigator (blue)