Zeiss 880 User Guide

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Starting

1. Main switch ON
2. System PC ON
3. Components ON
4. Fluorescence lamp (If you will use it)
5. PC

Do not touch other buttons!

Start Zen Black edition software

a) Image Processing
b) Start System – for using microscope

Choose the objective

1. Choose *Microscope* (**1**) on control panel and select objective (It can be also done in ZEN software)
2. Drop immersion on the objective, if it is necessary (10x, 20x dry, 40x water, 60x, 100x oil)

Insert the sample

1. Pull the upper part of the microscope away and open the door
2. Go down with objective using focus knob, objective should not touch the glass
3. Fix the sample into the insert (holder). Cover glass should be from the bottom side.
4. Insert (holder) should be tightly fastened in the microscope (not moving).
5. Pull the microscope upper part back

Sample focusing - *Locate*

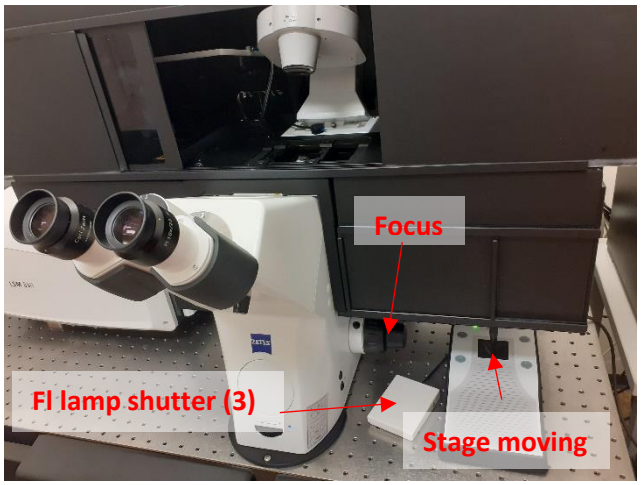
Microscope is in standard epifluorescence mode (using BF or fluorescence lamp)

1. select irradiation source (**1**):

   BF button (bright field) – transmission light mode

   Buttons *DAPI*, *GFP*… - epifluorescence mode

2. Open both shutters (If you are using the fl lamp)
   a. In the software (**2**) (*Microscope Control*)
   b. Switch under the microscope (**3**)



3. Focus sample

   Recommended way of focusing:

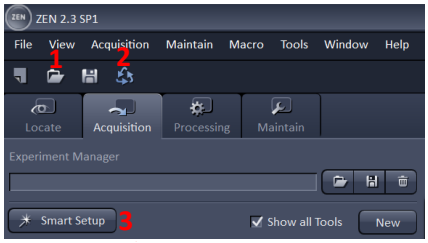
   Carefully place the objective as close to the cover glas as you can (chack the distance by your eye while you are turning the focus knob)

   Than look into eyepiece and slowly move the objective down (away from cover glass). You should find the focus plane in a while.

Sample scanning –*Acquisition*

Microscope is in the confocal mode (using lasers)

Microscope setting (lasers, filters and detectors)



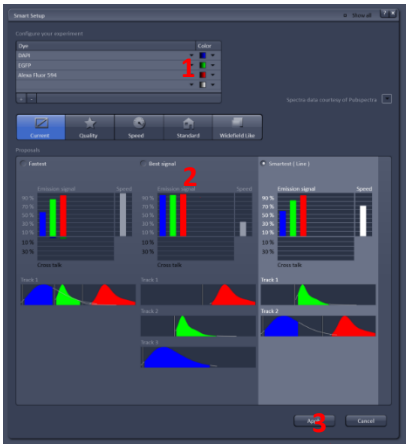
You can load the saved settings (**1**)

You can use the setting from saved image (*Reuse* button*,* (**2**))

The reuse button can be also found below the opened picture

You can use *Smart Setup* (**3**) (automatic settings)

You can set it manually (*Imaging Setup*, advanced users)



***Smart Setup* – automatic setting**

1. Click on *Smart Setup*
2. Select the dyes from the list (**1**)
3. Choose one of three possibilities (**2**):

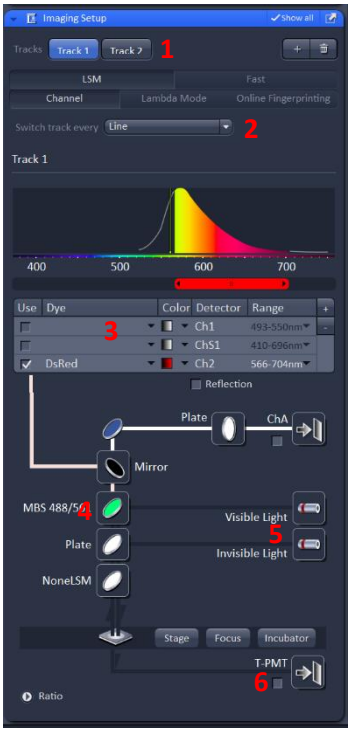
Fastest – all channels (colours) collects in 1 track

Best signal – every channel is collected separately (sequential collecting)

Smartest – compromise between previous two choices

4. Click on *Apply* (**3**)

***Imaging Setup* - advanced manual settings**



Select Track (**1**)

You can define:

Changing tracks during scanning after every line, frame or Z-Stack (**2**):

This setting remarkably influences the overall acquisition time

Detector type and range (**3**)

Fluorescence filters (**4**)

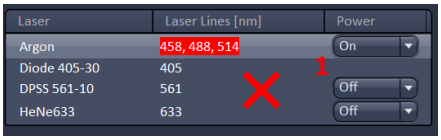
Excitation lasers (**5**)

You can use the transmission light detector (*T-PMT*) (**6**)

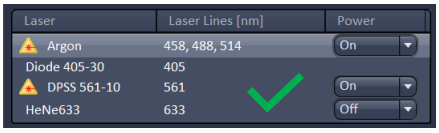
Contact technician for support If you need an advice or help!

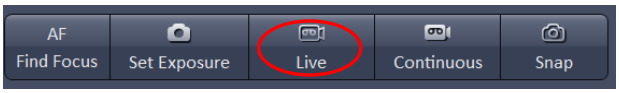
Fast scan (*Live* mode)

First fast scan for focusing and finding the area of interest



Quality of the pictures from Live scan is small!!!

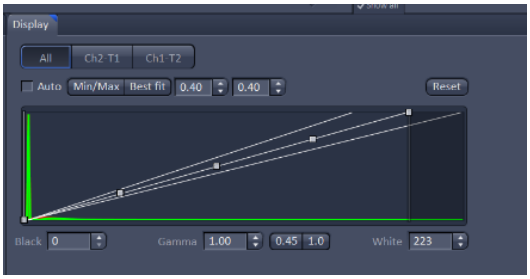


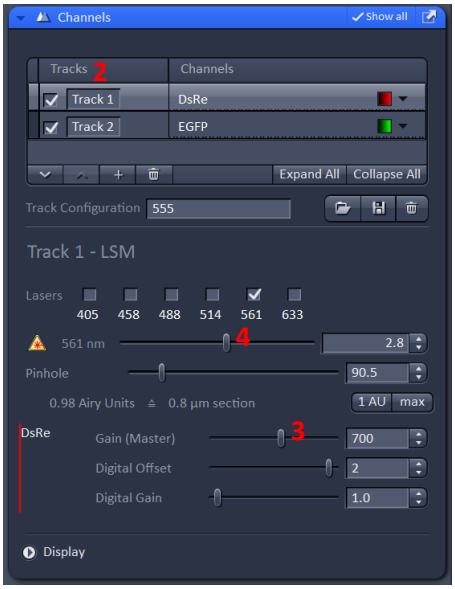


1. Turn lasers on (**1**) (if they are not on already)
2. Wait for Ar laser heating (c.a. 5 min)
   Ar laser should not be marked by red colour
3. Select only 1 track (**2**) (*Channels* window)
4. click on *Live* – fast scan
5. Focus and select the area on the sample
6. Stop scanning

If there is nothing to see on Live scan:

1 Focus
2 Select Track (**2**)
3 Rise *Gain* (*Channels* window) (**3**)
detector sensitivity, generally 500-800
4 Select shorter range on *Histogram* (below live window)





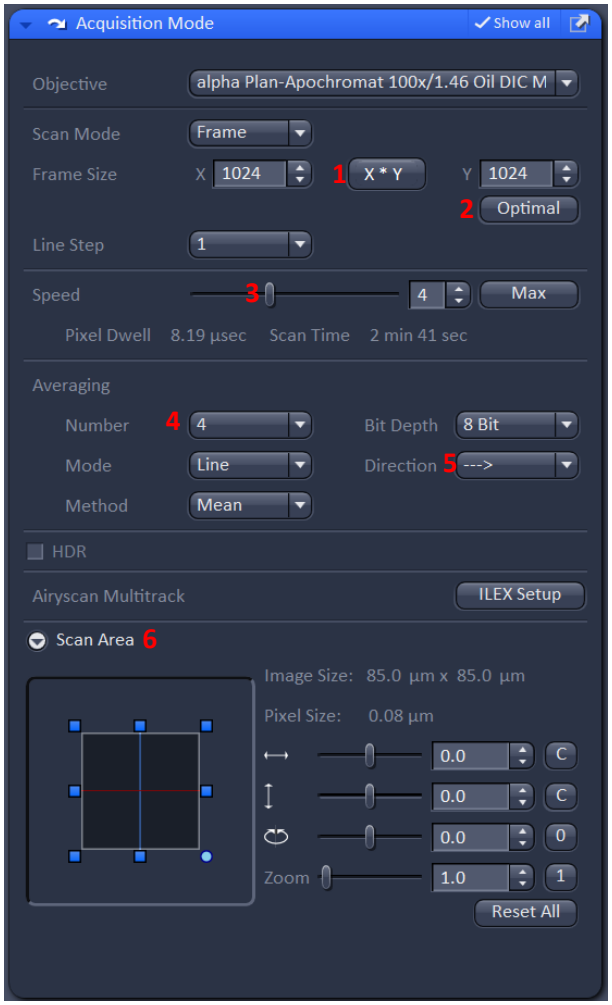
5. Check/rise laser intensity, generally 2 (*Channels* window) (**4**)
   remember bleaching or sample degradation
6. Check microscope setting (lasers, filters, detector….)
7. Call for support

***Live* scanning is suitable only for focusing!**
The quality of the image is very low due to high scan speed and low resolution.

Scanning parameters (detector sensitivity, laser intensity…) for final image should be set in *Continuous* mode!

Scanning parameters setting (*Acquisition Mode*):

These parameters influence the *Continuous* scanning mode and final snap.



1. *Frame Size* (Image Resolution)

   *X*Y* button – sets a standard value of resolution (**1**)

   Optimal – sets highest resolution achievable by chosen objective (**2**)

   Do not use higher resolution than optimal. Quality of the image will not rise, but scan time will be significantly prolonged.

   You can use lower resolution for saving time.

2. Scan *Speed* (**3**)

   The lower speed, the better image quality

   (generally between 5-7)

   Pixel Dwell – time the laser stays on 1 pixel

   Scan Time – time needed for scanning 1 image

3. *Averaging* (**4**)

   Enhancing the signal/noise ratio

   Recommended if *Gain* is high, especially above 800

   Generally 2-4, more snaps significantly prolong scan time

   It is recommended to use averaging right before taking the final snap. If you want to save time, do not use averaging during working in Continuous mode.

4. Scanning *Direction* (**5**)

   ---> One-directional: more precise, slower

   <---> Bi-directional: less precise, faster

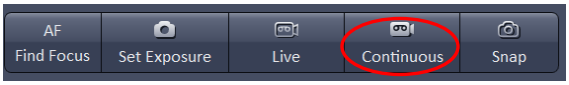
5. *Scan Area* (**6**)

   Allows using *Zoom* or view field shifting or rotation

Detector sensitivity setting (*Channels window*)

This setting must be performed for every Track separately!

1. Select one Track (**1**)
2. Start scanning in *Continuous* mode
3. Mark *Range Indicator* (*Dimensions* bookmark under live window)



Display oversaturated and undersaturated pixels.

Saturated pixels are red, only few red pixels (not areas) should be present on the image.

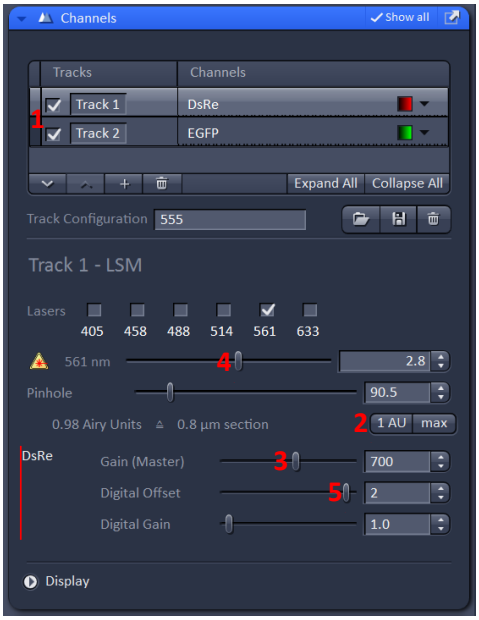
4. Tune detector sensitivity:

   Set suitable *Pinhole* diameter (button *1AU*) (**2**). It could be wider for weak 2D samples, but not for Z-Stack.

   Set *Gain* value (detector sensitivity, generally between 500 and 800) (**3**) to see only few red pixels (not areas)

   More *Gain* = more noise, especially if *Gain* is higher than 800. Can be compensated by *Averaging* (*Acquisition Mode* window) right before the final snap

   It is also possible to rise laser power (**4**) in the case of weak signal and high noise.



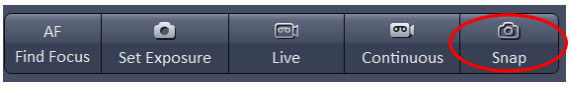
   This should be performed only for stable samples
   Most common laser power is 2%. High laser power (above 10%) can cause autofluorescence, bleaching or sample and dye degradation.

5. Tune *Digital Offset* if it is necessary (only few blue pixels should be present) (**5**)
6. Unmark *Range Indicator* and stop *Continuous* scan
7. Repeat this procedure for every Track

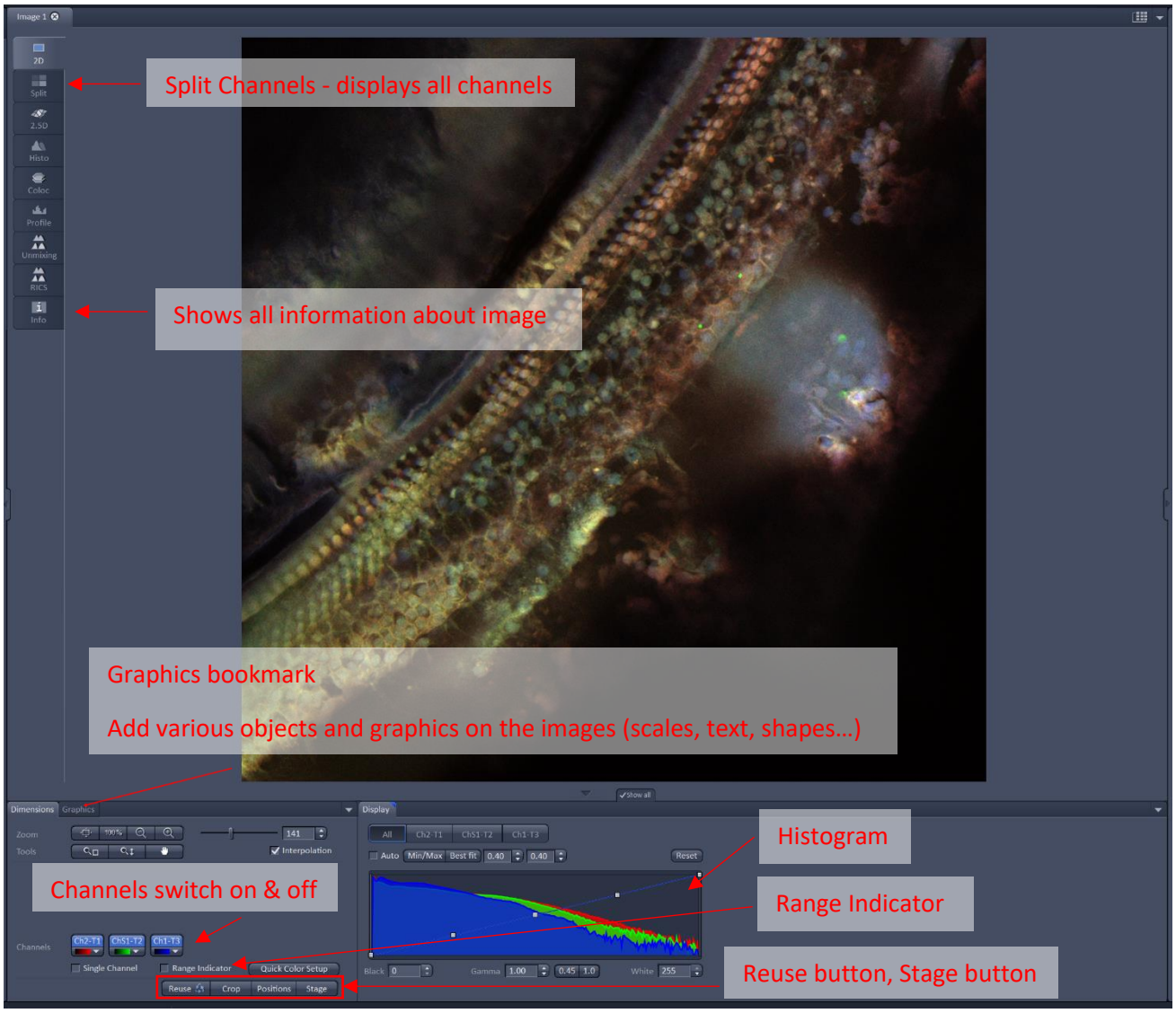
Final Snap

Check *Range Indicator* before taking the final image. Detector sensitivity should be checked especially when pinhole diameter, laser power or scan speed was changed

1) Select all *Tracks* you want to have on the final image
2) Use *Averaging* if the *Gain* is high
3) Take final image using the button *Snap*

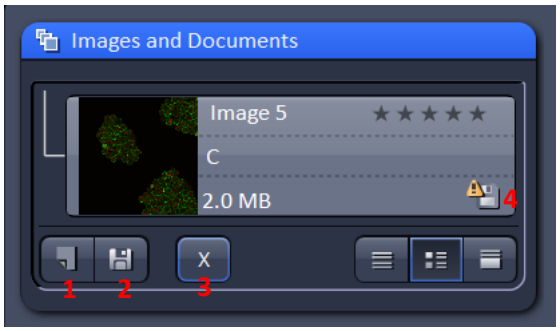


Scanning parameters are the same as in the Continuous mode



Saving Images

Images and Documents window (right site of the screen)



Icon with the paper sheet opens new scanning window (**1**)

Icon with flop saves image (**2**)

Icon with cross closes the image without saving (**3**)

Unsaved images show warning picture (**4**)

Folder for saving (Zeiss880): Z:/Tento Počítač/Zeiss880/*Oddělení(Lab Number)/Jméno(Name)*

Ending

Sample removing

1. Pull out upper part of microscope
2. Remove sample
3. Clean objectives (use ethanol and cleaning tissues to remove the immersion oil), clean the room
4. Pull down the upper part of the microscope, close the front door and turn the microscope light off.

If there is another user after me:

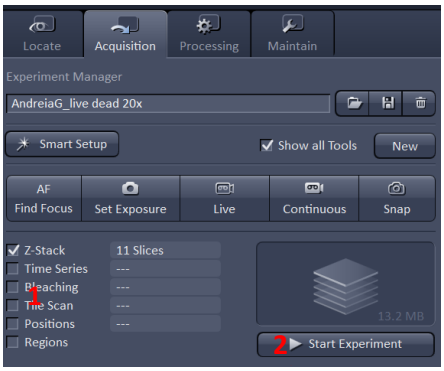
1. Save and close images
2. Switch Ar to Standby mode, do not turn it off!!!
2. Do not close ZEN software
3. Turn fluorescence lamp off

If I am the last person using microscope:

1. Turn Ar laser off (in ZEN software)
2. Save and close images
3. Close ZEN software and PC
4. Wait 5 min until Ar laser fan turn off
5. Turn Components, System PC and Main switch off

Write your name into logbook, put everything on right place and turn the light of.

Experiments



Zen SW provides several predefined experiments, which can be combined arbitrarily:

Z-Stack, Time Series, Bleaching, Tile Scan, Positions, Regions

The Experiment window will appear after marking experiment box (**1**)

Use *Start Experiment* button for acquisition (**2**)

Z-stack

Snaps series of scans (slices) along z – axis

The thickness of the slice is determined by pinhole diameter (pinhole is thin shutter in front of the detector). The thinner pinhole = the thinner slice. Pinhole diameter (slice thickness) influent the overall resolution in the z-axis. The thinner slice = more slices contained in Z - stack = better resolution.

The suitable pinhole diameter for reasonable z-axis resolution can be set by 1AU button (Channels window). Better resolution can be achieved using lesser pinhole diameter, but the signal intensity is remarkably reduced. Lesser pinhole diameters are useful only for bright samples.

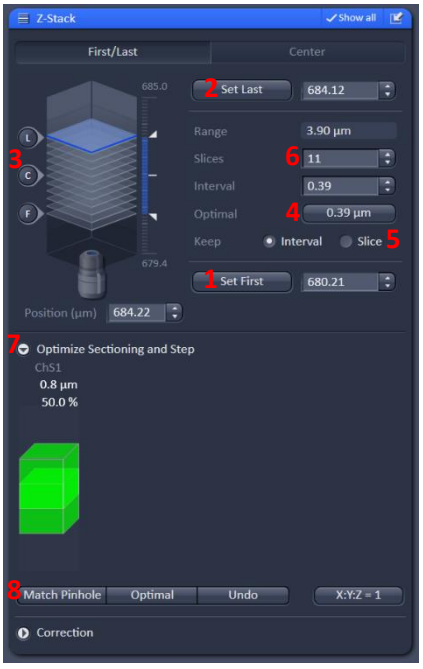
If you are not interested in z-axis resolution, wider pinhole diameter can be utilized. The overall acquisition time is then reduced.

The slices in Z-stack must overlap in order to include whole area of interest. The recommended overlap is 50%.

Z-Stack setting:

Scanning parameters and detector sensitivity should be set in continuous mode before starting Z-Stack acquisition. Detector sensitivity should be set on the brightest slice.

Always check *Range Indicator* after Pinhole diameter change.



Z-Stack range

1. Mark *Z-Stack* in the list of experiments
2. Start *Live* scan
3. Go to the *Z-stack* window
4. Define the first position using focus knob
5. Use *Set First* button (**1**)
6. Define last position using the focus knob
7. Use *Set Last* button – Z-Stack range is then defined (**2**)
8. *C* button displays central slice (**3**)
9. Stop *Live* scanning

Setting for best Z-axis resolution (pinhole is defined):

10. Set the pinhole diameter using *1AU* button (*Channels* window) or use slide for lower value.
11. Set the slice number using the *Optimal* button (**4**) (set 50% slice overlap).
12. Check *Range Indicator* and correct the *Gain* value
13. Start Experiment

Setting using slice number (if you are not interested in resolution and want to save time):

7. Mark *Slice* (**5**) in the *Z-Stack* window
8. Set number of slices in Z-Stack (**6**)
9. Open *Optimize Sectioning and Step* (**7**) and use *Match Pinhole* button (**8**) (set pinhole to 50% overlap)
10. Check *Range Indicator* and correct the *Gain* value
11. Start experiment

Multi-channel (colour) Z-Stack advice

Tracks can be switched after every *Track* (snap) or every *Z-Stack* (*Imaging Setting* window)

Switching after every Z-Stack remarkably shorten the acquisition time

Usable for fixed samples, which did not change in time

Pinhole should be set for the central channel

If there is red, green and blue channel, use green for setting

In the *Channels* window select central track and use *Match Pinhole* (**8**) button

Don't forgot to check *Range Indicator* before starting

Select only one Track for setting Z-Stack range, all Tracks should be marked right before final acquisition

Tile Scan (Mosaic)

Creates mosaic image by stitching the neighbour snaps together.

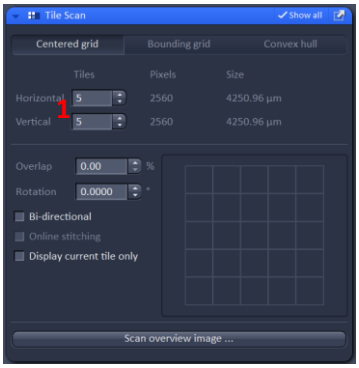
Suitable for large samples (bigger than view field) or for overview images.

Scanning parameters and detector sensitivity should be set in continuous mode before starting Tile Scan acquisition. Detector sensitivity should be set on the brightest snap.

Three modes of Tile Scan, use one of three bookmarks:

Cantered grid

Final image is directed by predefined lattice.

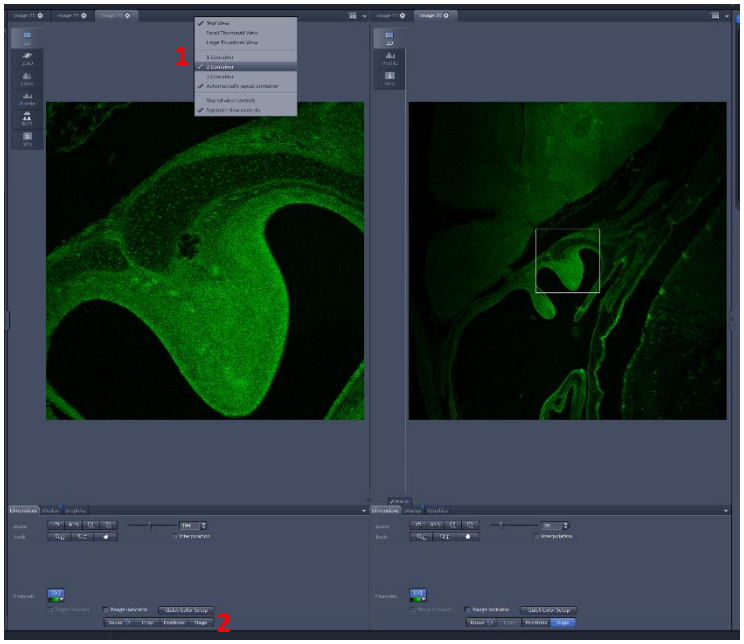


1.Define the number of vertical and horizontal snaps (for example 3x3, 4x5, maximum 100x100) (**1**)

2. Start experiment

Suitable for overview images

How to create an overview image:



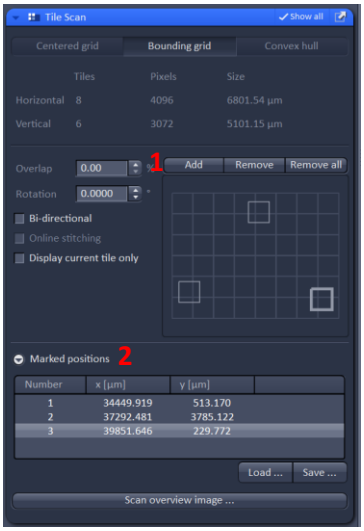
a) Scan overview image in *Cantered gird* mode (do at last 5 x 5 Tile Scan)
b) Open second scanning window (click to the area which is placed right from the image name and choose 2 containers) (**1**)
c) Move Tile scan into the second empty window
d) Click on the *Stage* button (**2**). The Tile Scan is than converted to overview image. White square shows actual position of scanning. This square can be moved by single-click onto the desired position.
e) Go back to the first scanning window and start scanning. Scanned position can be changed by clicking on the overview image.
f) Selected positions can be saved in the *Positions* experiment or they can by use for defining *Bounding grid* or *Convex Hull Tile Scan* experiment
g) *Stage* button change the Overview image into the common Tile Scan

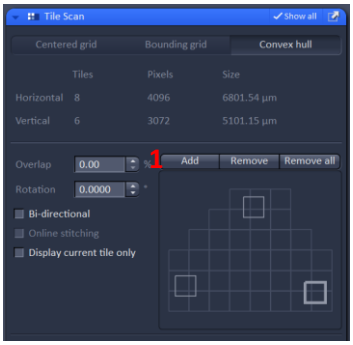
Bounding Grid, Convex Hull

The gird is defined by selected positions (see pictures on right side)

Add button defines the selected border images (**1**)

List of marked positions (**2**)





Comments:

The stitching mistakes are possible in the case of weak or homogenous samples

If you move with the sample during the Overview image using (because of immersion change for example), the selected positions will probably not match to the real positions. It is recommended to make new Overview image after the sample is moved.

Time series

Collects series of images during the time.

Basic setting:

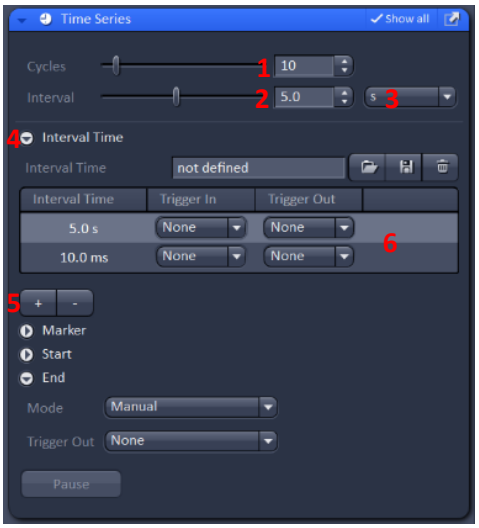
1. Mark *Time Series* in the list of experiments
2. Set number of cycles (scans) (**1**)
3. Set interval between cycles (**2**)

   Interval = scan time + rest time

   Time unit setting (**3**)

Multiple interval setting:

1. Open Interval Time area (**4**)
2. Add interval (**5**)
3. Interval can be manually switched during the experiment (highlight interval by mouse click) (**6**)



Positions

Snaps series of images on predefined positions

It is very practical to make an overview image for defining positions (see Tile Scan experiment), but it is not necessary.

1) Make an overview image (see Tile Scan experiment)
2) Mark Position experiment
3) Go to the desired position on overview image (double click on image), or move to this position using joystick
4) Add position/s to the list (**1**)
5) Use Start Experiment Button

Position experiment stores also z-coordinate, so it is possible to do z-stack on multiple positions.

Regions

Scans sample only in selected regions

1) Mark Regions experiment
2) Select region (you can use different shapes) (**1**)
3) Draw the ROI (region of interest) on image (**2**)
4) Select actions for every ROI (**3**)
    a) Acquisition – common image scanning
    b) Bleach – bleach selected region (further setting in Bleaching experiment)
    c) Analysis – select which region will be used for analysis
5) Start it by snap (not Start experiment button!)