SPINNING DISK CSU-X1 ROOM TEMPERATURE USER MANUAL

Start the spinning disk

1. Turn on the 3 power strips on the microscope table.
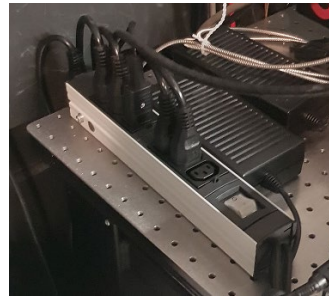
2. Turn on the PC, use the "IJM" session».

3. Desactivate safety

4. Start « *ZEN* » software (Icon on the desktop).

5. Select the tab « *Locate* ».
6. « *BF*» allows you to observe your sample in brightfield (transmission).

If you want observe in phase contrast or DIC, after select « BF » we need to choose « PH » or « DIC » in TFT screen.

7. « *DAPI* » to observe in blue.
8. « *GFP* » to observe in green.
9. « *DsRed* » to observe in orange-red.
10. « *Cy5* » to observe far red.
11. « *Lights off* » close the shutter for the transmission and the fluorescent light
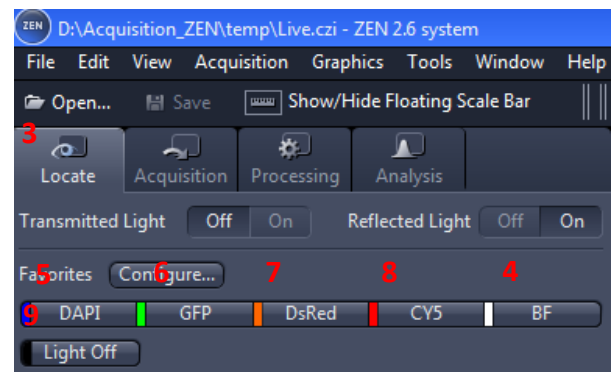
For transmitted light acquisitions, phase contrast or DIC, it is important to make adjustments on the microscope (set the Khöller illumination…)
Refer to the panels in the room of the microscope.

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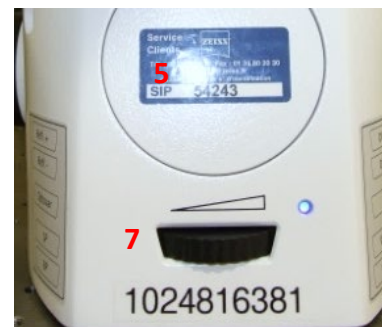
Microscope and lamp controls

Right side
1. Lowering the lens turret to the lowest position.
2. Returning the lens to the working position.
3. Macrometer screw.
4. Micrometer screw.
5. Open/Close the shutter in transmission "TL".
6. Open/Close the shutter in fluorescence "RL".

Face avant
7. Adjust the light intensity with the knob.

Le Joystick
Déplacer la platine motorisée en X et Y.
F1 for adjust speed of displacement in X and Y

TFT screen (touch screen)
1. Open/Close the Brightfield shutter.
2. Open/Close the fluorescence shutter.

In « *Microscope* », in the « *Control* » menu, there are several tabs allowing you to:
3. Choose the objective « *Objectives* ».
4. Choose the filter cube for fluorescence « *Reflector* ».
5. Add/Remove a 1.5X lens « *Optovar* ».
6. Observation in brightfield « *BF* », phase contrast « *PH* » or Normanski contrast « *DIC* ».
7. Aperture Diaphragm opening (for the köhler illumination setting)
8. Power of the halogen lamp

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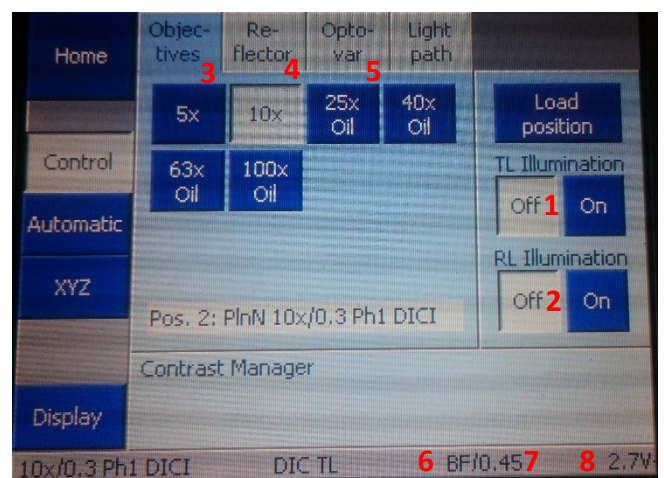
Acquire an image in single camera

1. Activate safety

2. Select the tab « *Acquisition* ».
3. Choose « single cam ».
4. Open the menu « *Channels* ».
5. Check the line(s) with your fluorophores of interest
6. Select one channel (light grey).
7. Make a « *Live* ».
8. Adjust laser power
9. Adjust the exposure time
10. Repeat for each channel you want.
11. Make a « *Snap* » to image the checked channels
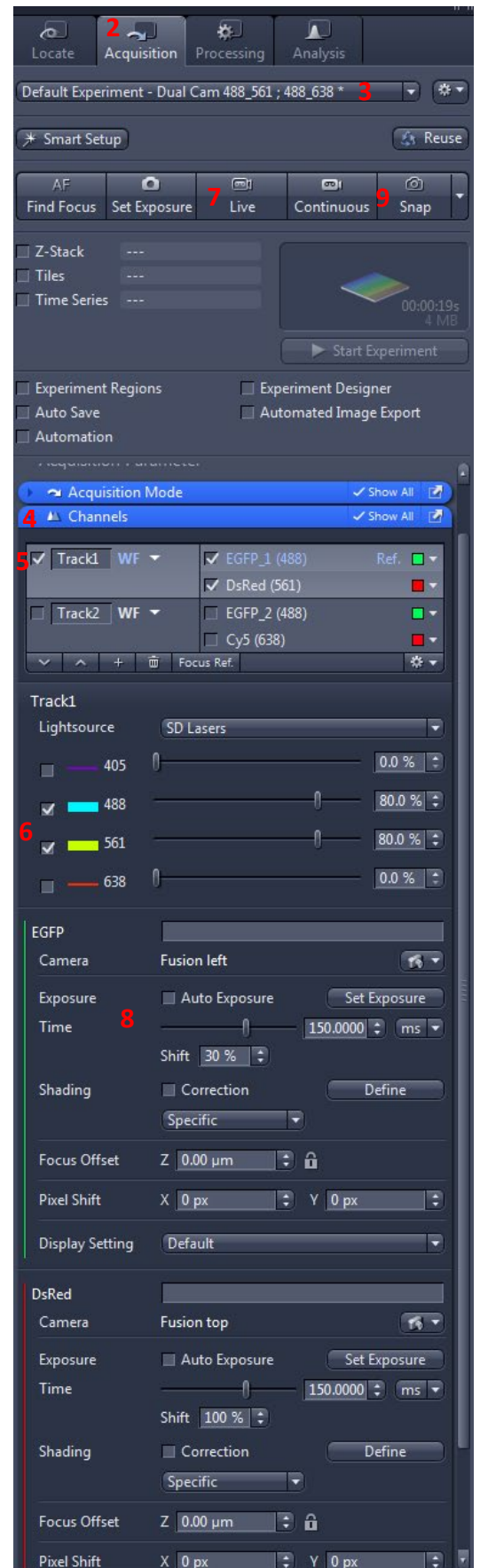
Acquire an image in Dual camera

1. Activate safety

2. Select the tab « *Acquisition* ».
3. Choose « *Dual Cam* ».
4. Open the menu « *Channels* ».
5. Check the line with your fluorophores of interest
6. Make a « live »
7. Adjust laser power
8. Adjust exposure time
9. Make a « *Snap* » to image the checked channels

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Acquire a Z-stack serie
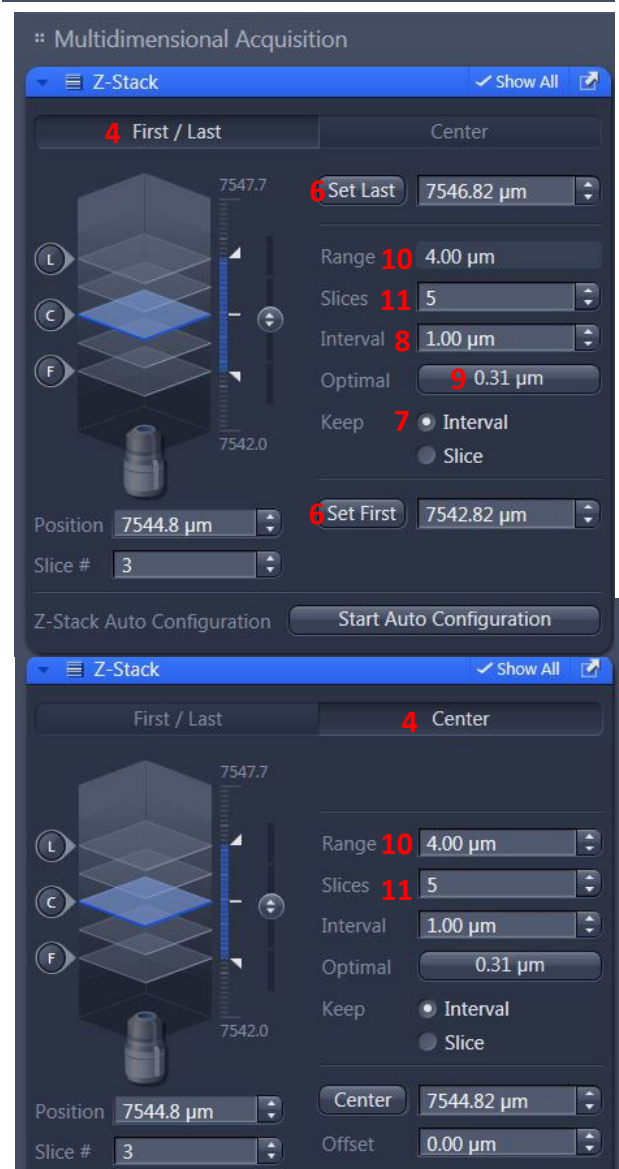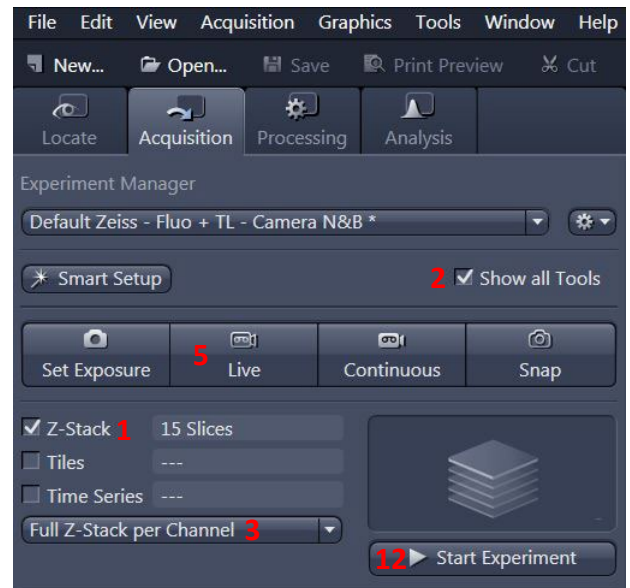
Stack acquisition

1. Check the « *Z-Stack* » option.
2. Check « *Show all Tools* ».
3. For multicolor acquisitions, choose the way you want to switch channels: make the entire z-stack of one channel, then switch to the other one « *Full Z-Stack per Channel* » or all channels per plane « *All Channels per Slices* ».

Two acquisition mode exists: you can define the top and the bottom of your stack, or define only the center of you stack.

4. In the menu « *Z-Stack* » Check the tab « *First/Last* »
5. Make « *Live* ».
6. « *Set First* » defines one extremity of you stack and « *Set Last* » the other one

OR

4. In the menu « *Z-Stack* » Check the tab « *Center* ».
5. Make « *Live* ».
6. Define the center of your stack with « *Center* ».

7. « *Interval* » must be checked to ensure a fixed interval value.
8. Enter the wanted interval value in µm.
9. Too choose the optimal resolution, you can use the « *Optimal* » option.
10. « *Range* » indicates the thickness of your stack.
11. The number of planes can be changed in « *Slices* ».
12. Start the acquisition by using « *Start Experiment* ».

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Display the stack
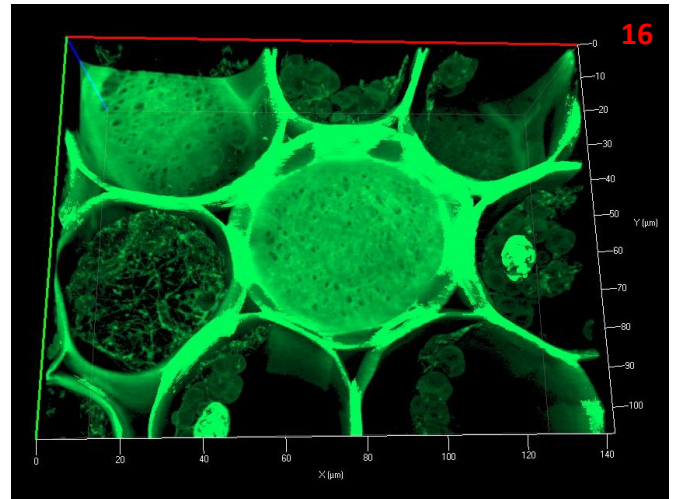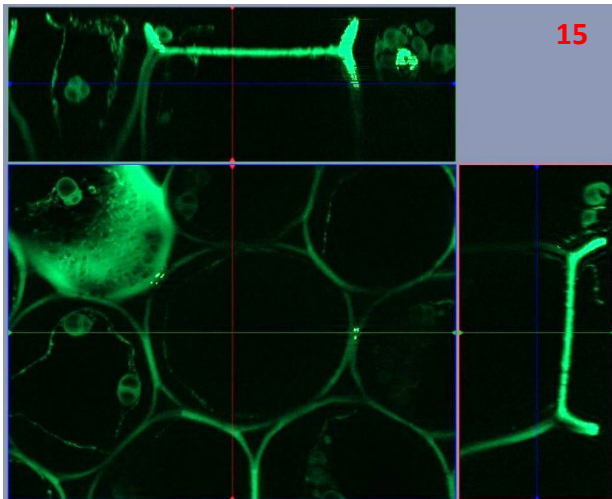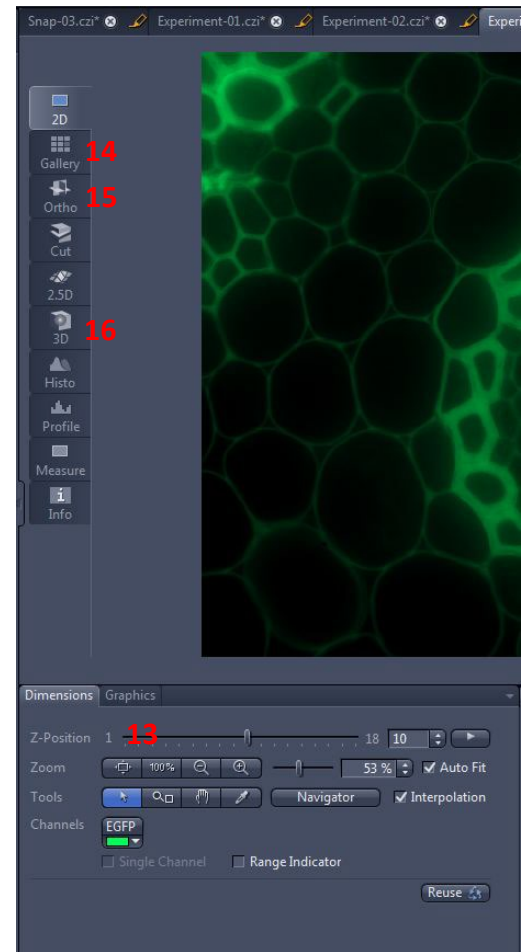
Your stack is displayed in the ZEN interface at the end of the acquisition.

In the tab « *Dimensions* » :

13. « *Z-Position* » allows you to see the different planes of the stack.
14. « *Gallery* » displays a set of all the images composing the stack.
15. « *Ortho* » allows an orthogonal view of your stack.
16. « *3D* » allows a 3D reconstruction of your stack.

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Acquire a time serie

1. Check the « *Time Series* » option.
2. Open the menu « *Time Series* ».
3. Define the time interval between two images.
4. Choose the number of cycles.

5. Warning: The time the microscope make to acquire a z-stack (or tile) has to be taken in account in the chosen interval. You can measure it by clicking on « Measure Speed», the value will be shown in the « Interval » rubric

6. You can minimize the time interval by checking « *Use Camera Streaming if Possible* ».

Your time serie is displayed in the Zen interface at the end of the acquisition.

7. In the « *Dimensions* » tab you can look a the different time point of your acquisition « *Time* »

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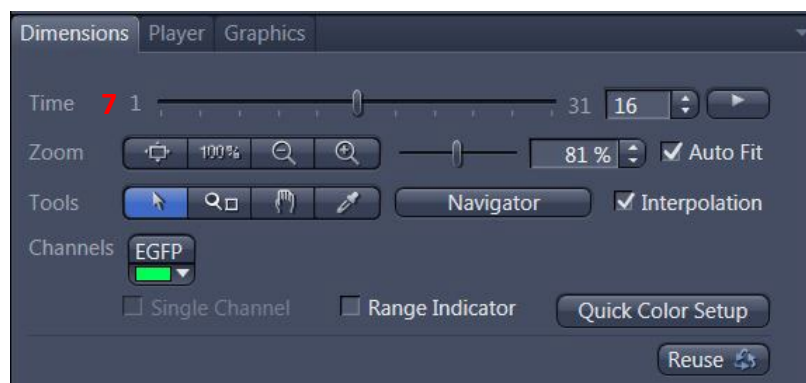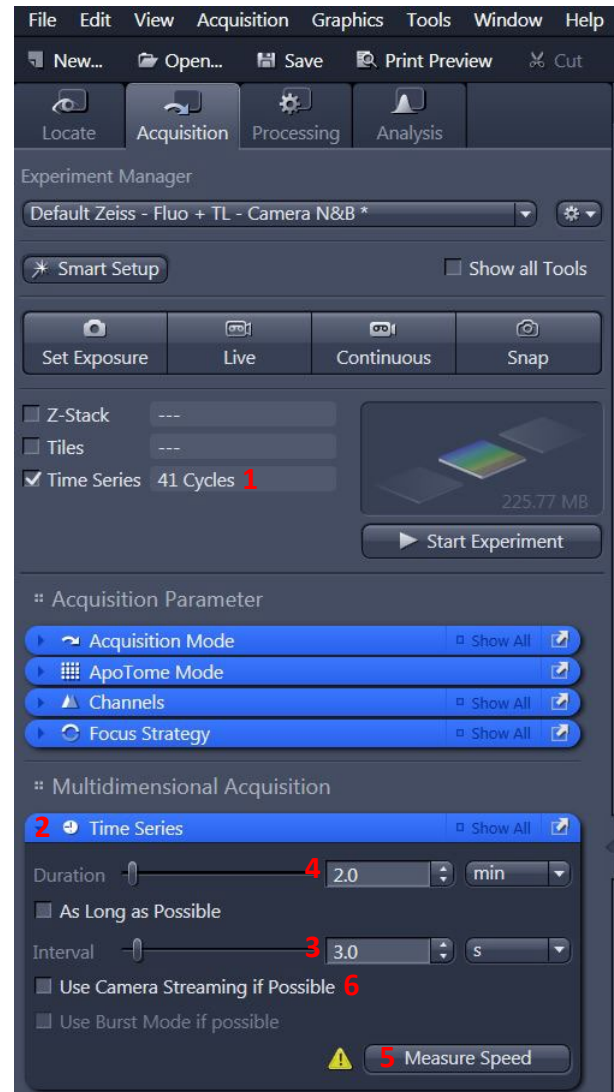
Multiple stage positions

1. Check « *Tiles* ».
2. Open the menu « *Tiles* ».
3. Open the rubric « *Positions* ».
4. Click on « *Advanced Setup* » to display the navigation space that will allow you to set your positions.
5. Make « *Live* ».
6. On the navigation space, double click where you want to acquire an image, or use the joystick to move the stage.
7. Once positioned, click on the arrow next to the « *Positions* » rubric.
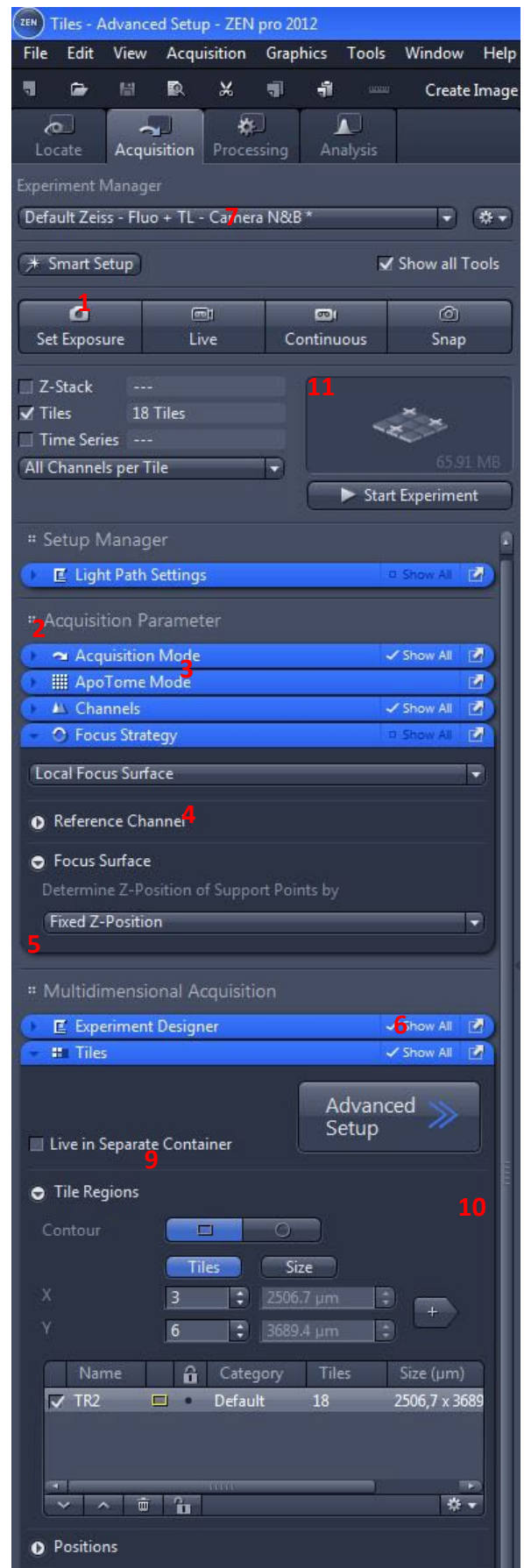   Repeat this operation every time you want to save a new position.
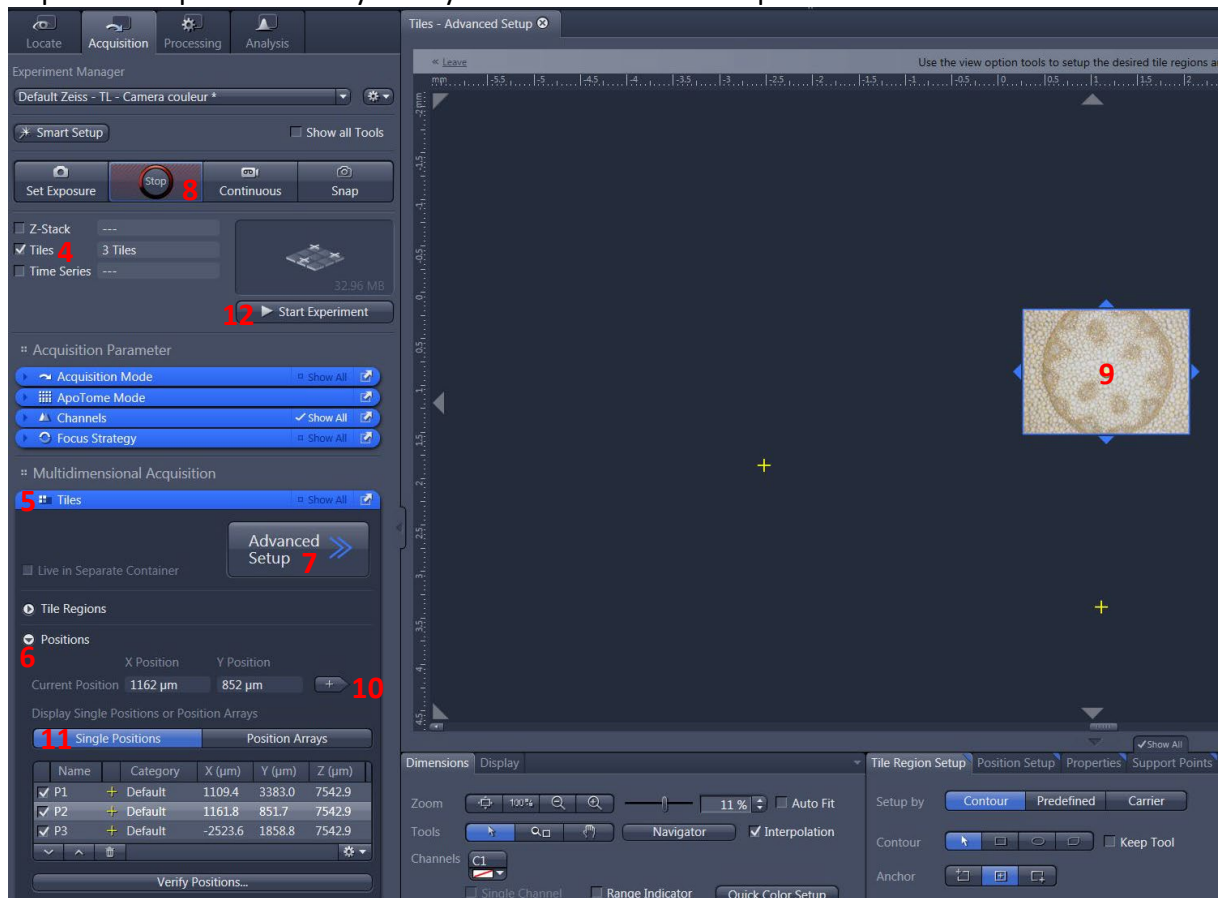
8. In the tab « *Single Positions* », you can see all the positions that you have saved.
9. Click on « *Start Experiment* ».
   It's possible to use different format of well-plates. Don't hesitate to ask to the imaging facility engineers for advices.

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Tile scan

Tile scan acquisition

1. Check « *Tiles* ».
2. Open the menu « *Focus Strategy* ».
3. Choose the option « *Local Focus Surface* ».
4. Select « *Fixed Z-position* ».
5. Open the menu « *Tiles* ».
6. Click on « *Advanced Setup* ».
7. Make « *Live* ».
8. The navigation space is displayed in the center of the software interface. Double click where you want to acquire an image, or use the joystick to move the stage
9. In the menu « *Tile Regions* », click on « *Tiles* » and enter the dimension of the desired tile. Your current position will correspond to the center of the tile.
10. Click on « + », the tile is then displayed on the navigation space.
11. Click on « *Star Experiment* » to start the acquisition.

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Focus correction

There are two ways to correct the focus on the entire tile. It has to be done before any acquisition.

12. Click on « *Support Points* » below the navigation space.
13. Enter the number of positions on wich you wish to correct the focus.
14. Click on « *Distribute* ».
15. Yellow circles will be distributed in the tile.
16. Double click on a yellow circle, make a « *Live* », set the focus and click on « *Set Current Z* » to save it. Repeat for each yellow circle.

OR

17. Double click on the position where you wish to correct the focus. Adjust the focus.
18. Click on « *Add Support Point at Current Stage and Focus position*», a yellow circle will appear.
19. Repeat the process on at least 5 positions.
20. Click « *Start Experiment* » to start the acquisition.

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Tile scan finalization in fluorescence after the acquisition

For this option, the checkbox "online stitching" has to be unselected during the acquisition (see above).

1. Click on the tab « *Processing* ».
2. Click on « *Single* ».
3. Open the menu « *Method* ».
4. Go to the « *Geometric* » rubric and select « *Stiching* ».
5. Open the menu « *Input* » and select the tile you want to stitch.
6. Open the menu « *Parameters* ».
7. Click on « *New Output* ».
8. Check « *Fuse Tiles* ».
9. If the shading is not correct, click on « *Correct Shading* » and select the « *Automatic* » mode.
   If you have several colors (channels) or a z-stack:
10. Open the menu « *Select dimension reference for stitching* ».
11. Click on « *All by reference* » and select a channel that will be considered as the reference.
12. Choose the z that will be considered the reference.
13. Click on « *Apply* ».
14. The adjusted image is called « *IP- Stitching* ».

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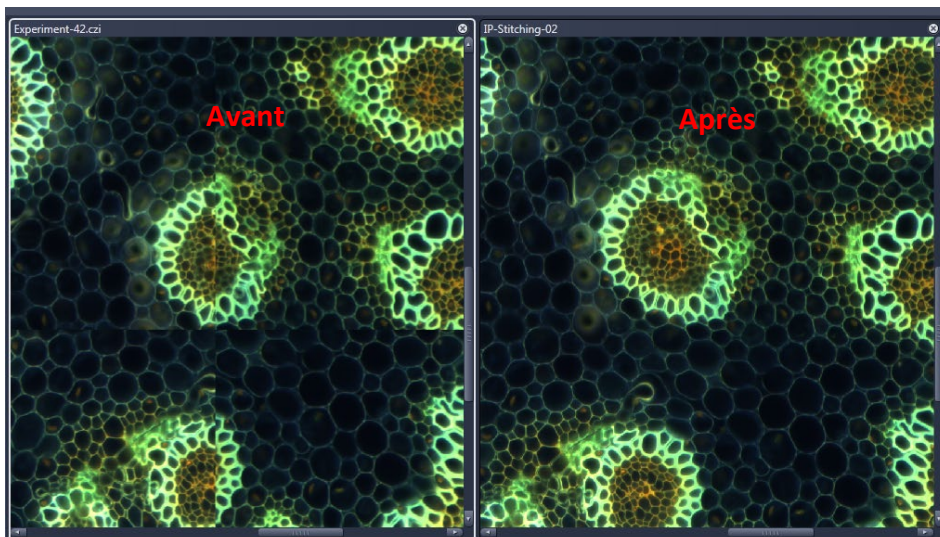
Graphic tools

In the tab « *Dimensions* »

1. Display a plane in the z-stack, or a time position by entering the wanted number or moving the corresponding cursor.
2. Adjuste the image to the screen size.
3. Adjust the pixel size of the image to the pixel size of the screen.
4. Enlarge or reduce the image.
5. Show/Hide the color (channel) on the screen.
6. See only one channel at a time.
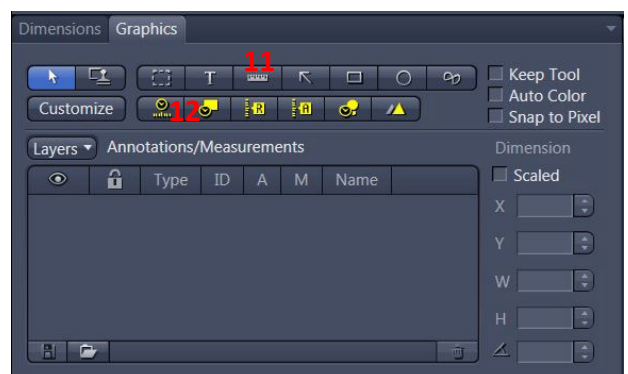7. Show the levels of gray and the saturation.
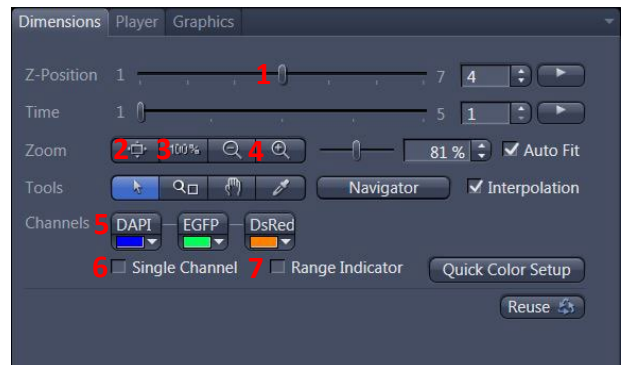
In the tab « *Display* » are the contrast options.

8. Choose the color you want to modify or « *All* » to modify all of them.
9. Adjust the contrast automatically.
10. Reset the contrast.

In the tab « *Graphics* » are the annotation options.

11. Show the scale bar.
12. Show the time.

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How to save your data

Save your experimental configuration
1. Click on the parameter logo.
2. Select « *Save As* ».
3. Rename your configuration and save it.
4. Load the wanted configuration by opening the menu below « *Experiment Manager* » and select your configuration.
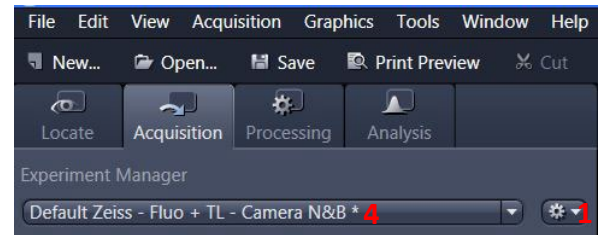
Save images taken
5. All of your acquisitions are displayed on the right in the colunm « *Images and Documents* ».
6. Double click on the image to save.
7. Click on the diskette icon.
8. Rename tour images.
9. Save them in the folder « *Users* » on the Data Hardrive. All images should be saved in the pathway users/year/month/day/your name.
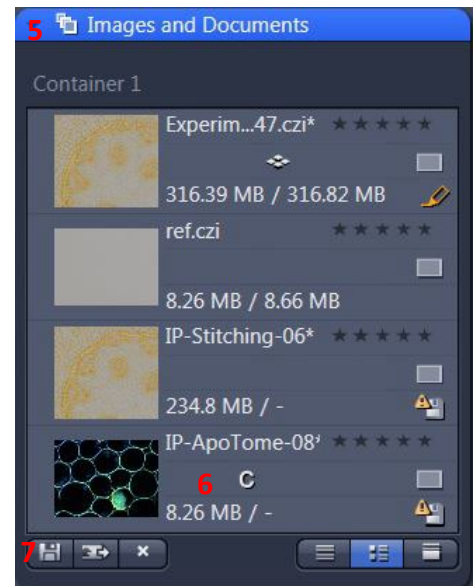
Switch off the system

1. Lower the objectives, clean the front lens and on the side with optical paper.
2. Exit from the software « *ZEN* ».
3. Transfer your data on your hardrive.

Check the booking site. If there is no one after you:
4. Turn off the computer.
5. Turn off the microscope with the button on the left side.
6. Turn off multi-sockets powers.

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