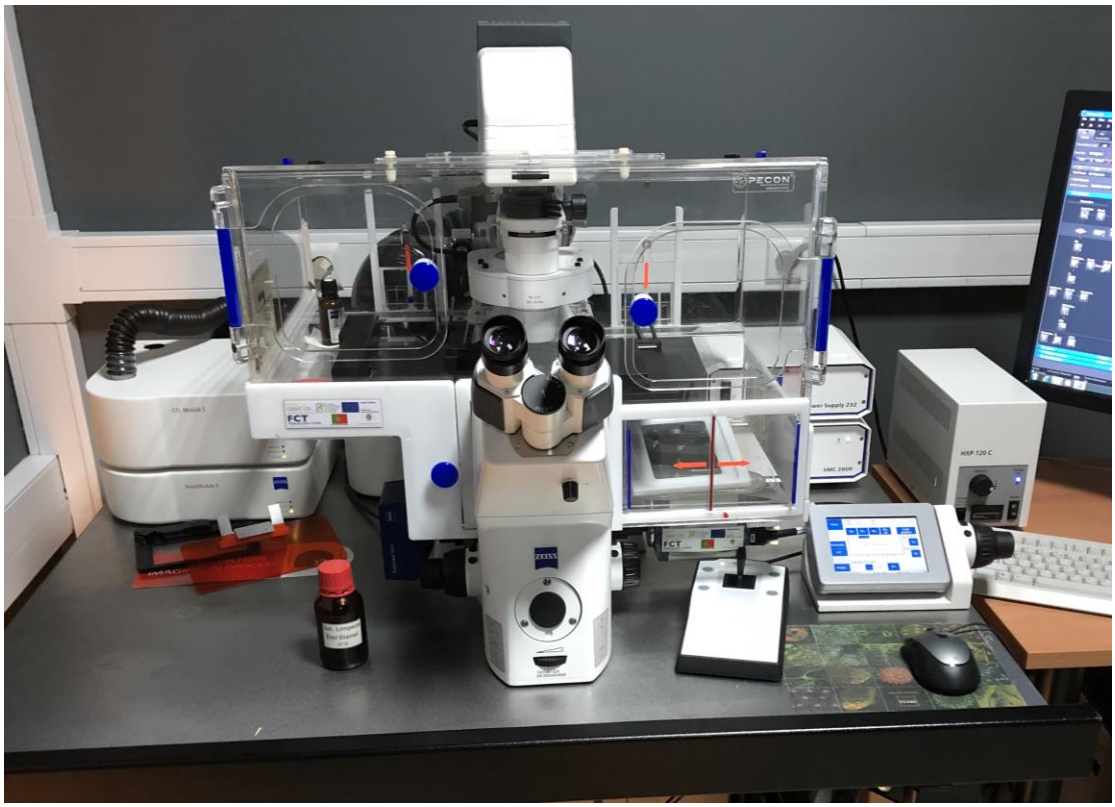
Widefield Zeiss Axio Observer.Z1
Manual/Quick guide



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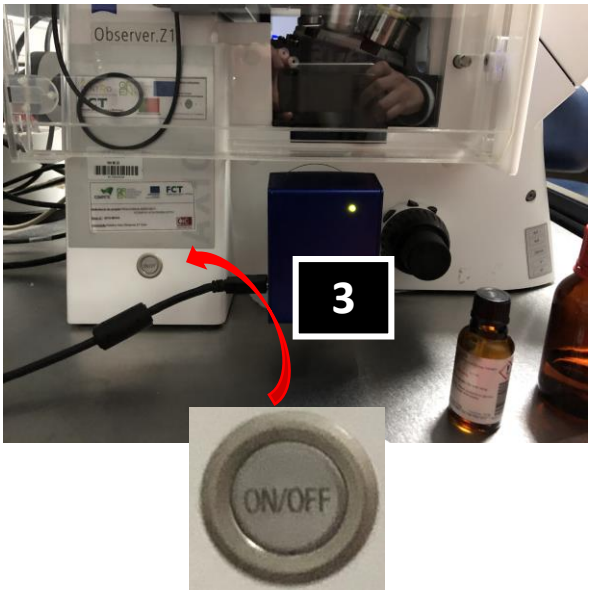
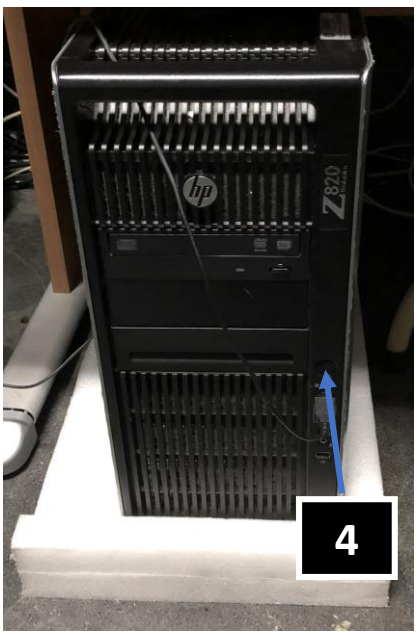
Starting the system

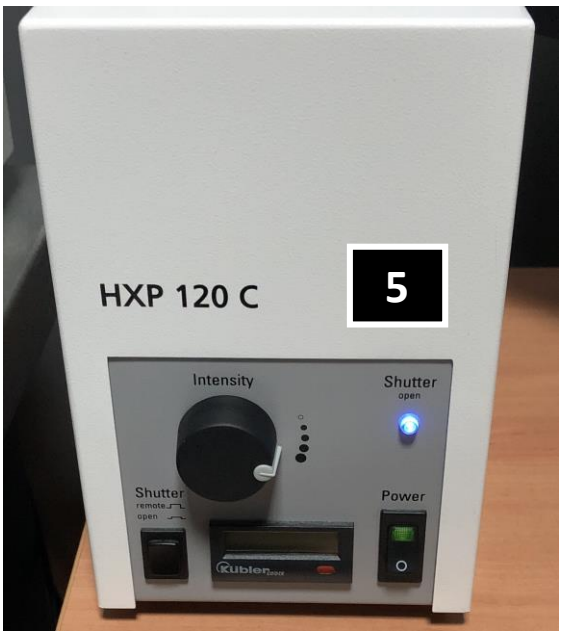


- Press the switch buttons to ON:

  - 1st Power Supply 232 **[1]**
  - 2nd SMC 2009 **[2]**

- Switch ON the microscope by pushing the 'ON/OFF' button on the left side of the system. **[3]**

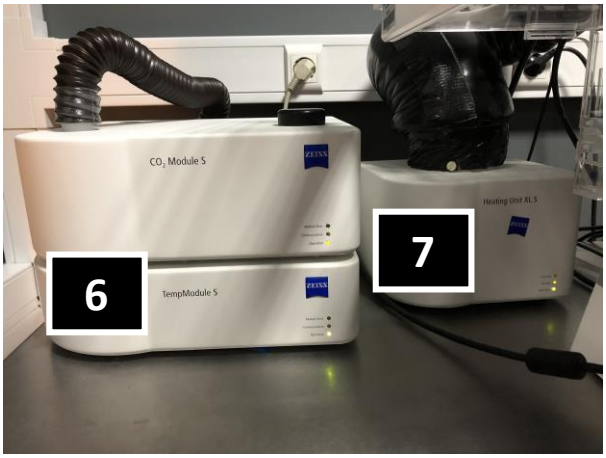


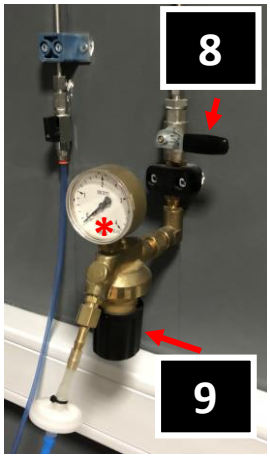


- Turn on the PC workstation **[4]**
- If you will be using fluorescence turn on the fluorescence lamp **[5]**

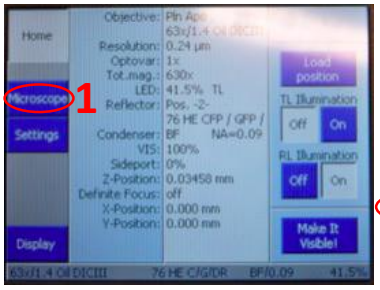
Switching ON temperature/humidity/CO2 (when needed)

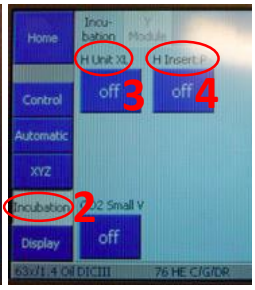
Turn ON the temperature for the incubator, the Insert (if necessary), the humidifier and CO2 (if necessary) **1h** before starting acquisition





1. Turn on the 'TempModule S' **[6]** and the 'Heating Unit Xl-S' **[7]** (buttons in the back of the boxes).

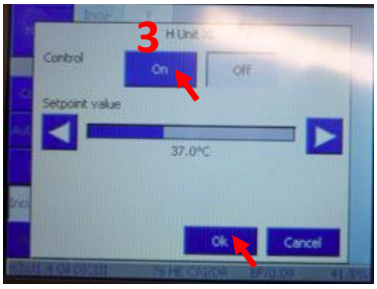




2. Choose the desired temperature using:

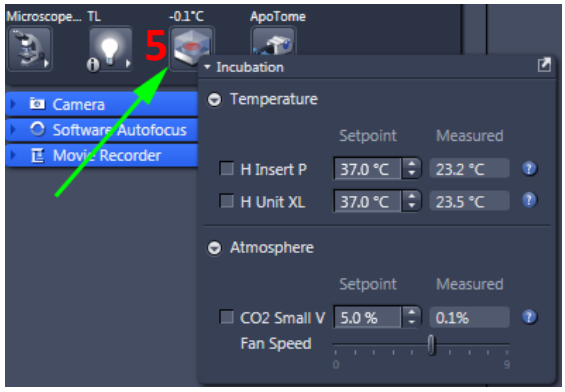
→ **<u>the touchpad</u>:**

- Go to Microscope **[1]**
- Press Incubation **[2]**
- Press:
  - H Unit XL **[3]**, Press On and press ok
  - H Insert P **[4]** Press On and press ok
  - H Dev Humid, Press On and press ok



→ **<u>Using the software</u>:**

- Go to Locate/Light Path/Expand the incubation menu **[5]** and click the box to switch all the devices on.



3. Switch on the CO2 controller

- Rotate the CO2 handle to a vertical position **[8]** .
- Turn the air compressed bottle valve (black) **[9]** clockwise until you obtain a pressure of 1 Bar in the exit of the pressure regulator (right pressure gauge *).
- Go to Locate/Light Path/Expand the incubation menu **[5]** and click the box to switch all the devices on.

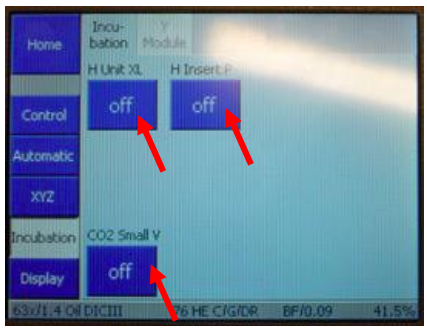
Switching off the system

1. Save your data on D:/Users/PI folder/"your folder".

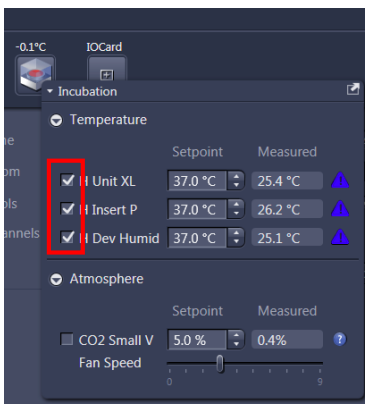
2. Clean the objectives (x40, x63 an x100) with LENS TISSUE ONLY and cleaning solution.

3. Check if the Observer is in use in less than 1 hour after the end of your session. Leave the fluorescence lamp ON **only** if the next user comes in less than 45/60 minutes. ***If there is no one after you:***

4. Switch off Incubation (if you turned it ON):



   - With the screen:
     - Press CO2 Small V, Turn it off and press ok
     - Press H Unit XL, Turn it off and press ok
     - Press H Insert P, Turn it off and press ok
     - Press H Dev Humid, Turn it off and press ok



   - Or with the software:
     Go to Locate/ Light Path/ Expand the incubation menu and unclick the boxes to switch all the devices off.

5. Exit Zen software.

6. Turn the fluorescence lamp off.

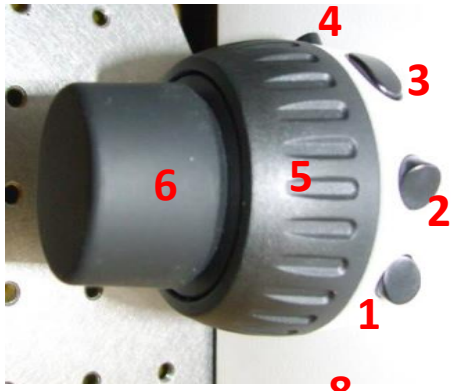
7. Burn your data onto a CD, DVD or MICC external drive. <u>Do not use USB keys</u> to transfer data as it put the computer at risk of viruses.

8. Shut down the computer.

9. Turn off the switch buttons:
   - 1st SMC 2009 **[2]**
   - 2nd Power Supply 232 **[1]**

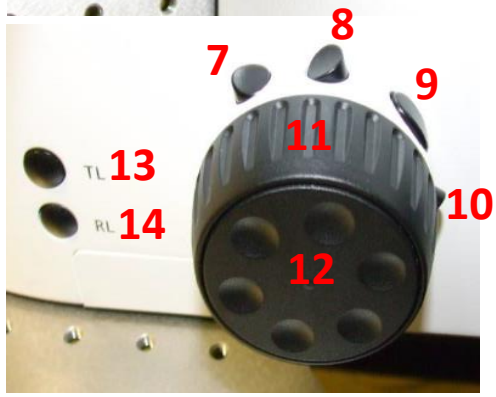
10. Write the information in the logbook

Microscope stand presentation



Left side

- **Not a necessary action [1].**
- Change light path (ocular or camera) **[2].**
- Open/Close the fluorescence shutter **[3].**
- Filter cube change for fluorescence (2 back buttons) **[4].**
- Macrometric knob **[5].**
- Micrometric knob **[6].**



Right side

- Take the objectives down at the lowest position **[7].**
- Take the objective back up to its working position **[8].**
- Open/Close the transmission shutter **[9].**
- Change objectives (2 back buttons) **[10].**
- Macrometric knob **[11].**
- Micrometric knob **[12].**
- Open/Close the brightfield shutter in '*TL*' **[13].**
- Open/Close the fluorescence shutter in '*RL*' **[14].**

Front side

Light intensity adjustment (wrench adjuster) **[15].**

The Joystick

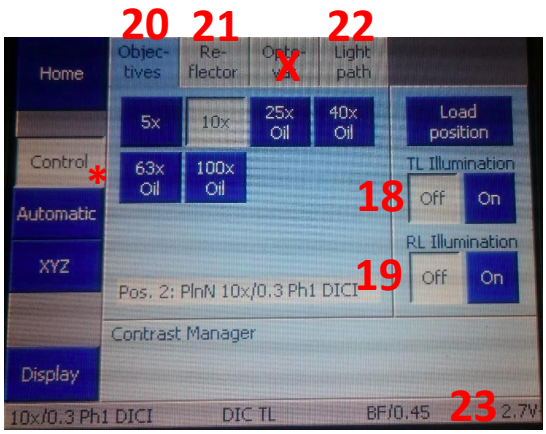
Move the motorized stage in X and Y.
Press the joystick button F1 **[16]** to move faster/slower.

The CMOS camera (ORCA Flash 4.0)

Turn the camera ON before starting the software:
- switch the button to ON **[17]**

TFT screen (touch screen)

- Open/Close the Brightfield shutter **[18]**
- Open/Close the fluorescence shutter **[19]**
- In '*Microscope*', in the '*Control*' menu*, there are several tabs allowing you to:
  - Choose the objective '*Objectives*' **[20]**
  - Choose the filter cube for fluorescence '*Reflector*' **[21]**
  - Choose the light path **[22]**.
  - Power of the halogen lamp **[23]**.



Start Zen / Observation with the oculars

- Start the '*ZEN*' software (Icon on the desktop).

- When Zen opens you will be under the '*Locate*' tab **[1]**, this is where you can view your sample through the eyepiece;

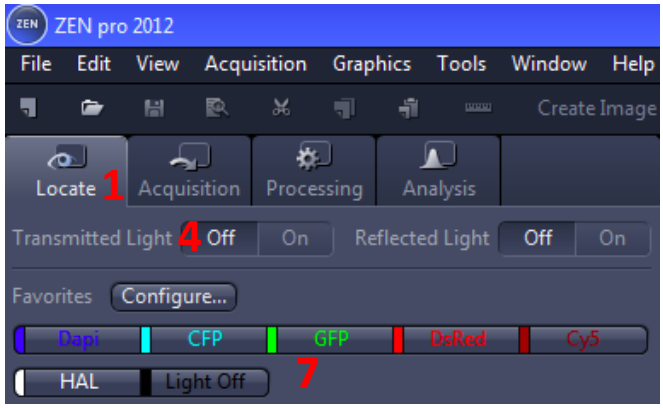
- Lower down the objective a bit before loading a slide; Position the sample right above the objective (**coverslip facing the objective**) and adjust the microscope to see a focused image through the eyepiece;

- Choose your objective by clicking on the objective button **[2]** (or touch screen); this will bring up a list of objectives currently on the microscope; please remember to add oil when appropriate;

- To check your sample using Transmitted Light, switch to the desired lamp **[3]** and turn on respective shutter: using the software (shortcut buttons TL **[4]),** touch screen or shortcuts on the microscope;

- To check sample using fluorescence turn on respective shutter using the software **[5]** (touch screen or shortcut buttons on the microscope) and choose filter using:
  - Touch screen (Microscope / Control / Reflectors)
  - filter button **[6]**; this will bring up a list of filters currently on the microscope;
  - quick access buttons **[7]**,
    '*DAPI*' to observe in blue.
    '*GFP*' to observe in green.
    '*DsRed*' to observe in orange-red.
    '*Cy5*' to observe in far red.
    '*Light off*' close the shutter for the transmission and the fluorescent light.

- Once you focus, close the fluorescence shutter and go to acquisition tab.



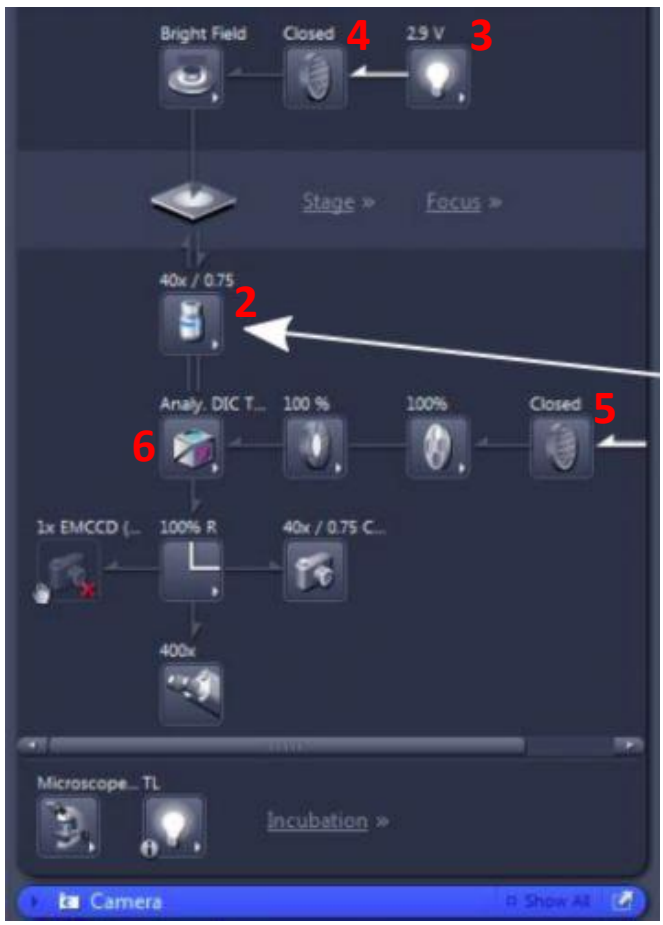
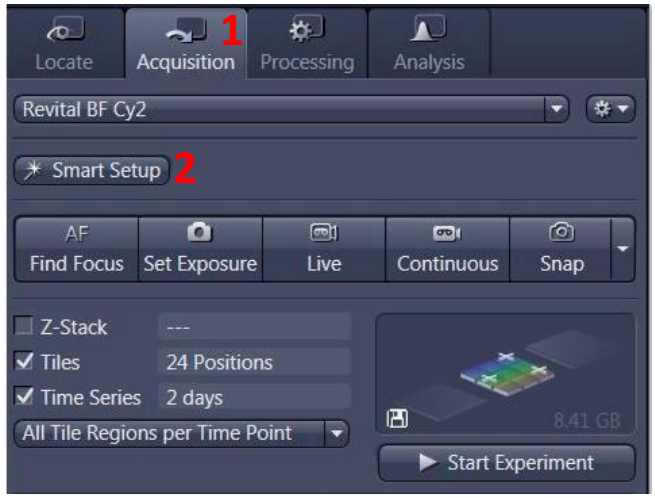
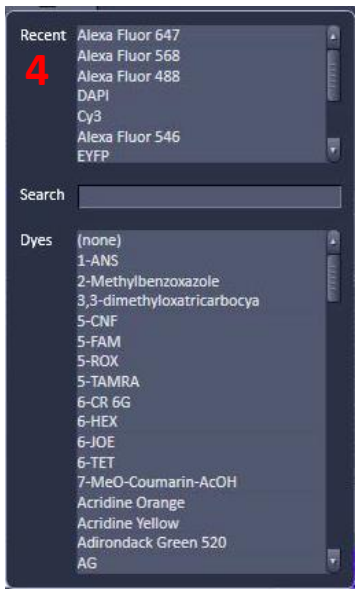


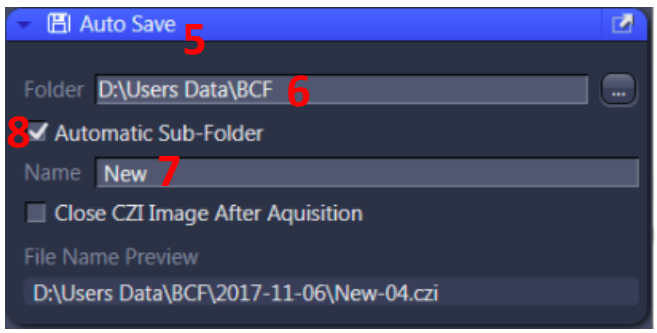
Image Acquisition

- Go to Acquisition tab **[1]**;
- Use **Smart setup** to define channels: Click on '*Smart Setup*' **[2]**
- To add a channel click on the [+] button at the '**Configure your experiment'** section **[3]**. The **Add dye or contrasting method** list appears **[4]**.
- Double-click on the desired fluorophore or **TL Brightfield** under **Contrast methods** for transmitted. Repeat for further channels.
- Click **ok**
- Alternatively, you can load a saved image which includes the desired channels and acquisition settings: open "your" folder, open a previous image and click [redacted] in the '*dimensions*' tab below the image





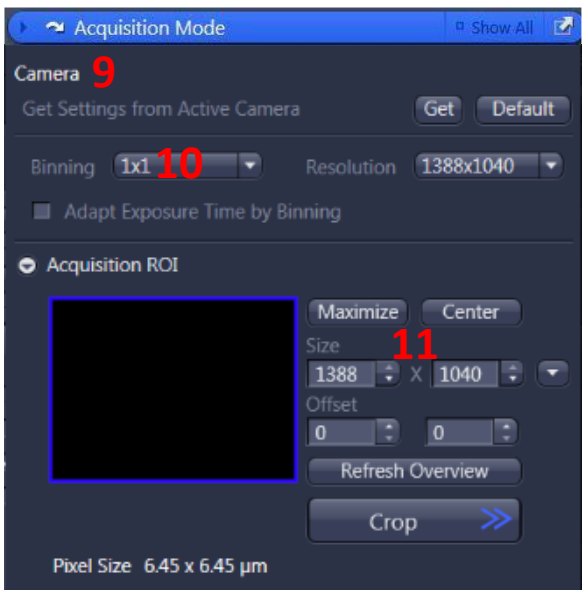
- It is strongly recommended to use **Auto save** for time lapse experiments. The **Auto Save tool [5]** will appear in the **Applications** section below
- Choose the folder your files will be saved in and give a name **[6]**.
- This will be used as a prefix in case more than one files are created before altering the **Name [7]** field and automatic numbering will be appended.
- Save your files in:
  D:\Users data\PI name\User name
- Checking **Automatic sub-folder** will create a folder YYYY-MM-DD where your files will be saved **[8]**.



Check settings:

'Acquisition Mode' Tab

- **In Camera** section **[9]:**
  - Usually no binning is required (1x1) **[10]** and maximum ROI is acquired (Resolution/Size 1388x1040) **[11]**



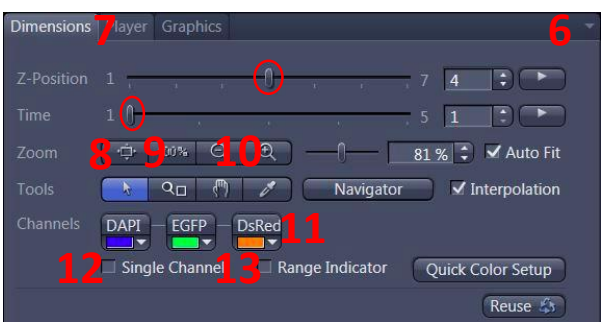
‘Channels’ Tab

- Open the menu **Channels [1].**
- The line(s) with your chosen fluorophores of interest should be checked (✓)
- Select one channel (light grey).
- Make a ‘*Live*’ **[2]**. This will open a Live image tab in the center section. Use the ***tabs below the image [6]:** Dimensions to control zoom and choose to display in **range indicator** and Display to change brightness/contrast.
- Set the exposure time manually by entering the wanted number or moving the corresponding cursor **[4]** or automatically by clicking ‘*Set Exposure*’ **[3]**. Do it for every channel of interest.
- Make a ‘*Snap*’ to image the checked ✓channels **[5]**.
- If you do not **auto save**, save the file using the **Images and documents** tool at the right panel. Save file as native **.czi** which contains all the experiment parameters and could be requested by any reviewer at time of publication. Always backup and keep the native file.
- A file is not saved if it has an “orange pencil” after its name.

*Tabs below the image **[6]**. :

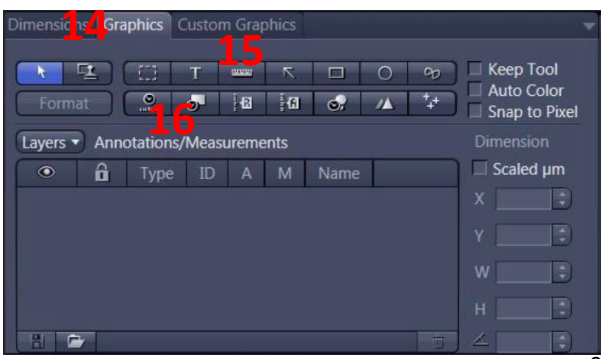
Dimensions Tab [7]

- Display a plane in the z-stack, or a time position by entering the wanted number or moving the corresponding cursor.
- Adjust the image to the screen size **[8]**.
- Adjust the pixel size of the image to the pixel size of the screen **[9]**.
- Enlarge or reduce the image **[10]**.
- Show/Hide the color (channel) on the screen **[11]**.
- See only one channel at a time **[12]**.
- Show the levels of gray and the saturation **[13]**. ✓‘*Range indicator*’ to monitor saturation (avoid red pixels which are saturated)



Graphics Tab [14].

Use the graphics tab to add annotations, e.g., scale bar **[15]**. or relative time **[16]**. Press format or right click on the annotation to change format



Display Tab [17]

In this tab are the contrast options:

- Change image brightness and contrast by selecting one or All channel(s) (color) **[18]** and moving the line defining the limits of gray levels.
- Adjust the contrast automatically (Min/Max, Best Fit) **[19]**.
- Reset the contrast **[20].**

Note that in order to compare images it is absolutely imperative to change the parameters to exactly the same levels, i.e., the values of Black, Gamma, White should be the same.

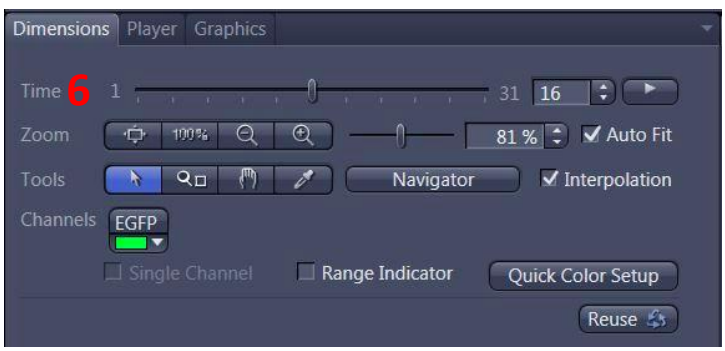
Multidimensional Acquisition (Time Series, Z-stack and Tiles)

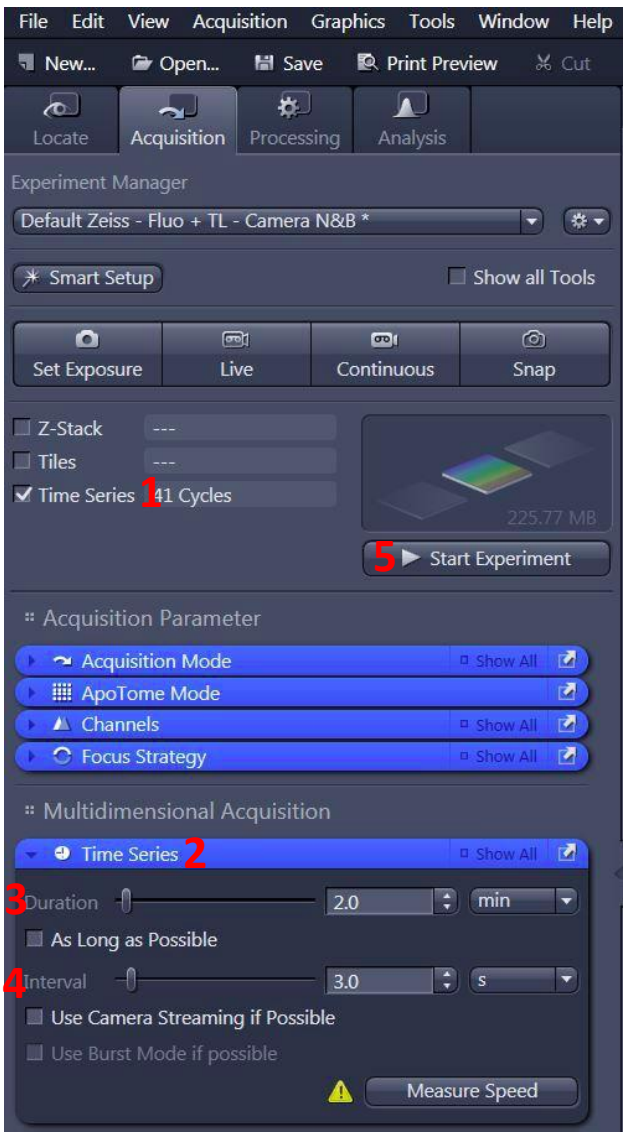
Time Series

- To do a time series, first click on Time Series **[1]**.
- The Time Series tab will appear under the Multidimensional Acquisitions on the left side. **[2]**
- Expand the Time Series window and Select:
  - Duration of the experiment (number of cycles) **[3]**
  - Interval between acquisitions **[4]**
- Click on '*start experiment*' **[5]**

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

- In the '*Dimensions*' tab you can look at the different time point of your acquisition '*Time*' **[6]**.



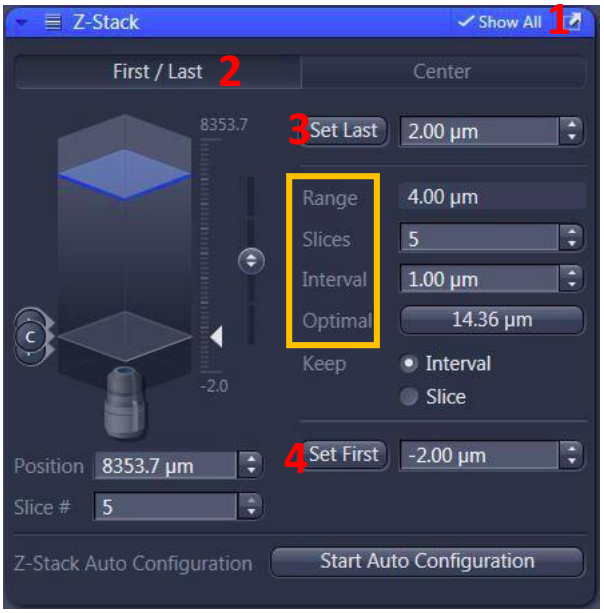


Z-Stacks

- To make a z-stack first tick Z-Stack (under the experiment manager); scroll down on the left until you see a Z-Stack box (ensure Show All is enabled **1**).

- 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*'.

- There are two ways of setting a z-stack:



First/Last

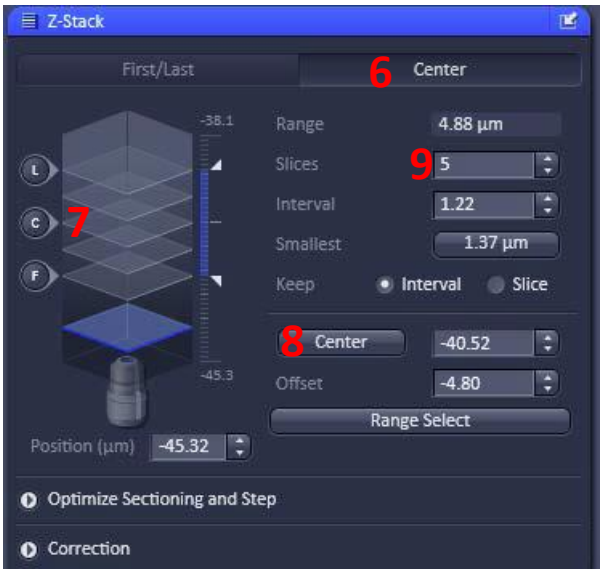
- Select '*First/Last*' imaging Mode **[2]**.

- While fast scanning using Live, move the focus to the top of your sample and click '*Set Last*' **[3]**.

- Now move the focus to the bottom of your sample and click '*Set First*' **[4]**.

- Click on '*Start Experiment*' **[5]**.

- Range: thickness of the z-stack in µm
- Slices: number of planes in the z-stack
- Interval: step (in µm) in between planes
- Optimal: Optimal interval in between planes to adapt to the Nyquist criterion according to channels and microscope configuration



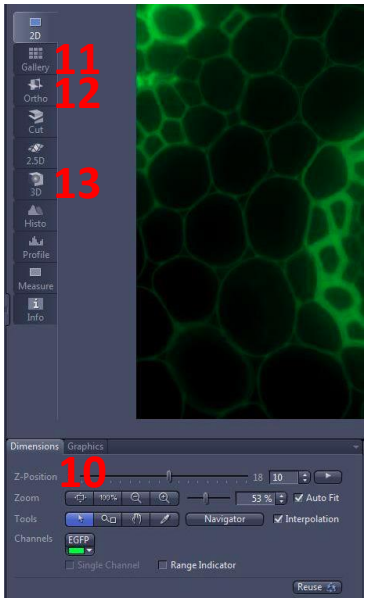
Center

- Select '*Center*' imaging Mode **[6]**.

- Click on Center then find the center of the sample you would like to image. Click on '***C***' **[7]** to take a Snap of the center image to make sure you are in the right spot. Click '*Center*' **[8]** to confirm this is the slice in the middle of the stack.

- Set your interval and check your number of slices **[9]**. Make sure you have an odd number of slices

- Click on '*Start Experiment*' **[5]**.

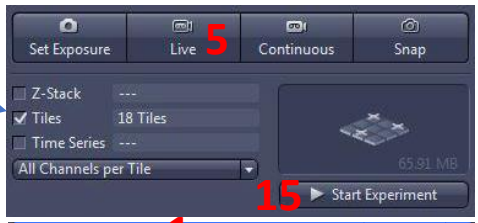


Display the stack

Your stack is displayed in the ZEN interface at the end of the acquisition. In the tab '*Dimensions*':

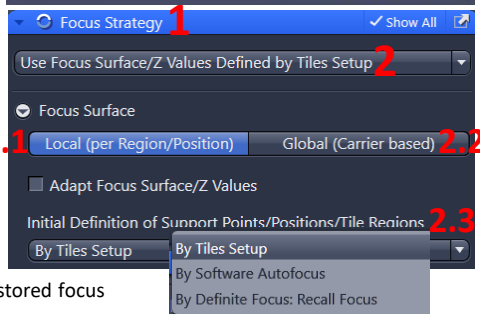
- '*Z-Position*' allows you to see the different planes of the stack **[10]**.
- '*Gallery*' displays a set of all the images composing the stack **[11]**.
- '*Ortho*' allows an orthogonal view of your stack **[12]**.
- '*3D*' allows a 3D reconstruction of your stack **[13]**.

Tile Scan



- To create mosaic images of large areas of your sample select **Tile Scan** from the **Experiment Manager**.

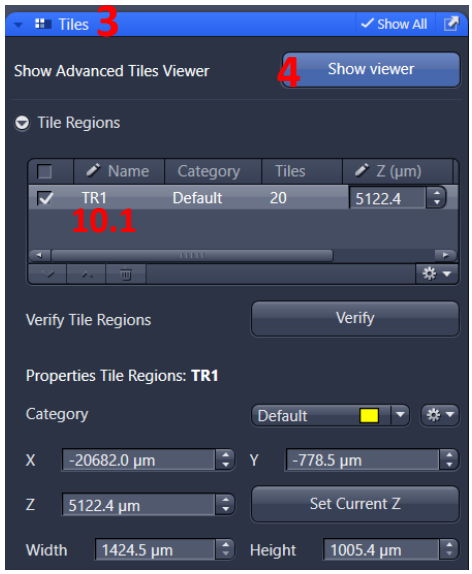
- Open the menu Focus Strategy **[1]**; Select '*Use Focus Surface/Z Values Defined by Tiles Setup*' **[2]**.
  - **Local [2.1]** – For positions: The Z value of each position is used; For tile regions: The Z values are interpolated from a local focus surface
  - **Global [2.2]** – The Z values of tiles and positions are interpolated from a global carrier based focus

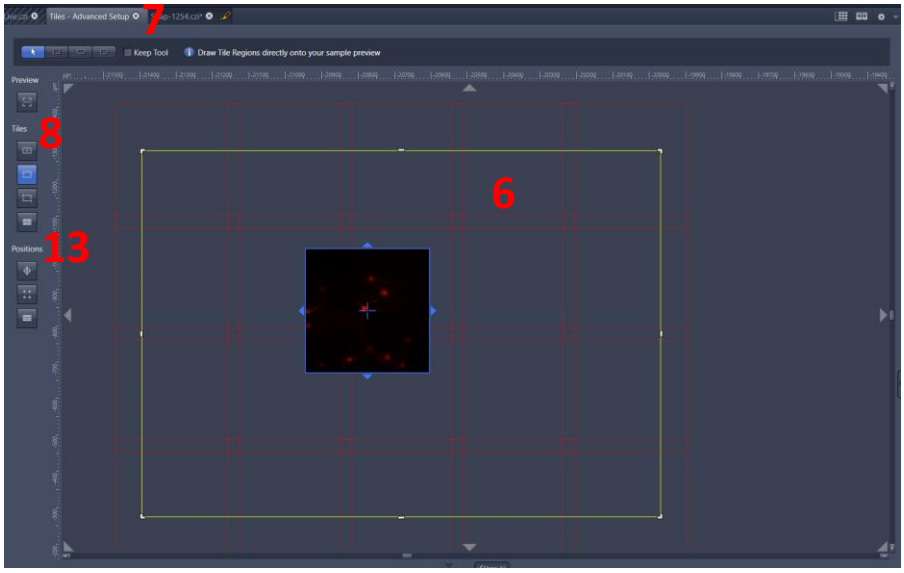


NOTE: Initial Definition of Support Points/Positions/Tile regions **[2.3]**
- **By tiles setup** – The fixed z values from tiles setup are used as the initial values
- **By Software Autofocus** - The initial z values are determined by a separate software autofocus run on each support point/position/tile region
- **By Definite Focus: Recall Focus** – The initial z values are determined by recalling the previously stored focus (distance to coverglass surface) with the definite focus on each support point/position/tile region.

- Open the window Tiles **[3]**. Click on '*Show Viewer*' **[4]**. Make '*Live*' **[5]**.

- The navigation space is displayed in the center of the software interface **[6]**.





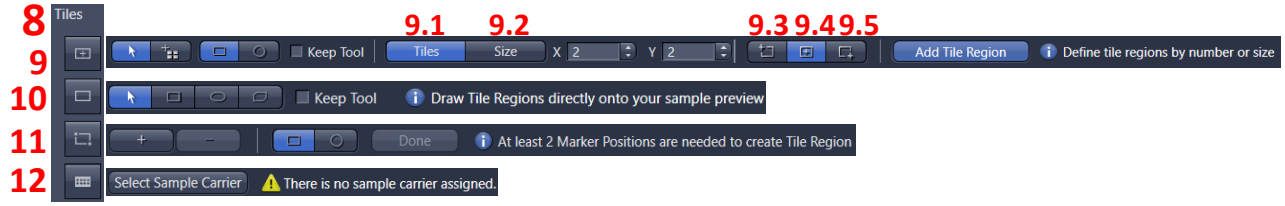
- In '*Tiles-Advanced Setup*' **[7]**, choose a desired '*Tile option*' **[8]**:

- "Setup new tile regions from a predefined size" **[9]**
  - The predefined tile regions are defined by specifying a number of tiles (columns and rows). The absolute size depends on the current tile size. 9.1
  - The predefined tile regions are defined by specifying an absolute area on the stage. The columns and rows are calculated to cover that area. 9.2
  - The tile regions are defined by the top left anchor position. 9.3
  - The tile regions are defined by the top center anchor position.9.4
  - The tile regions are defined by the bottom right anchor position. 9.5

- "Setup new tile regions by drawing a contour" **[10]**(e.g. rectangle, oval, free-drawing and draw a region in the image; the marked area will show up as a new tile region in the region list **[10.1]**);

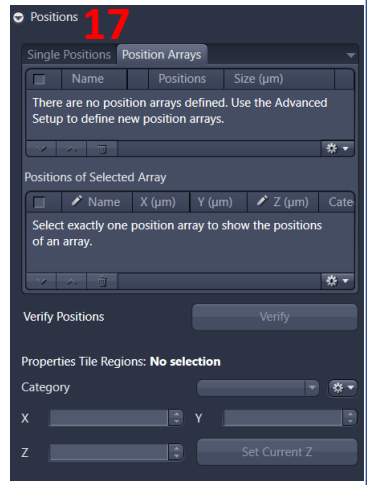
- "Setup new tile regions by specifying two ore more marker positions, e.g, the top-left and bottom-right center of the desired region" **[11]**;

- "Setup new tile regions from a underlying sample carrier" **[12]**.

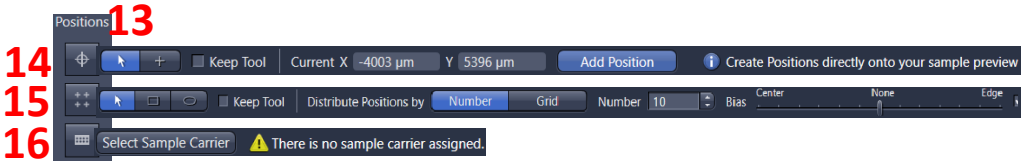


• In *'Tile Advanced Setup'* **[7]**, you can also choose the *'Positions'* option **[13]**:

-"Setup new position by selecting the location with the mouse cursor" **[14]**,
-"Setup new position by defining an array of positions within a drawing shape **[15]**;
-"Setup new positions from an underlying sample carrier" **[16]**.

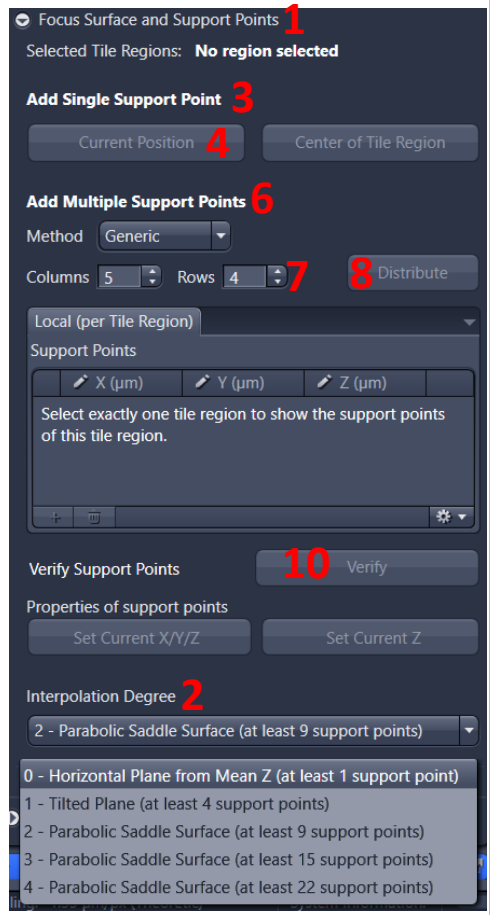


• The chosen positions will show up in the positions list (menu under the tile tab – left **[17]**.)



Support Points

If your sample carrier does not consist of an even flatness on the imaging surface (which most likely will be the case), you need to tell the software reference points, so-called Global Support Points, in order to approximate for an even surface flatness. The necessary amount of Global Support Points is thereby determined by the degree of interpolation the software is supposed to calculate. In order to know how many support points you should add in the next step, please first go to **Focus Surface and support points [1]** *(menu below positions)* in the Tiles section and choose the degree of interpolation **[2].** As a general rule of thumb, you choose more points if your area of interest is quite large.

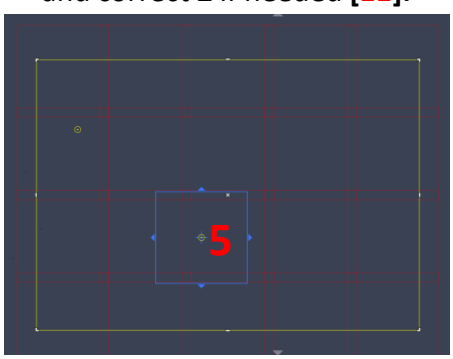


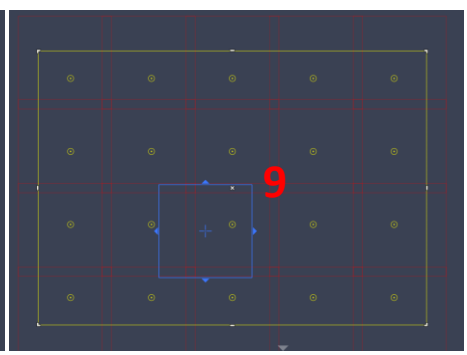
• Correct the focus on the entire tile. It has to be done before any acquisition in *'Focus Surface and Support points'* (menu below positions) **[1]**. You have 2 options:

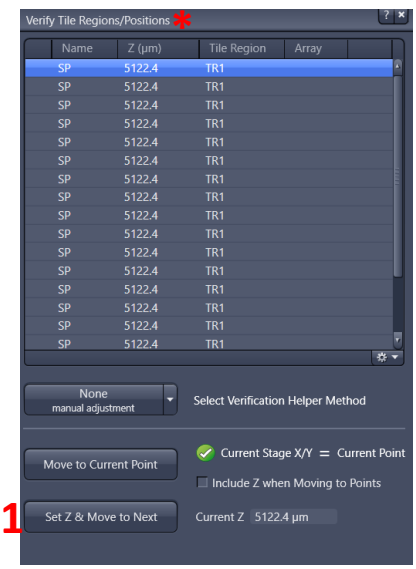
- **Add single Support Point [3]** - Double click on the position where you wish to correct the focus. Do *'live'* and adjust the focus. Click on *'Current Position'* **[4]** in the position menu**,** a yellow circle will appear **[5].** Repeat the process on at least 5 positions.

- **Add Multiple Support Points [6]** – Choose the number of columns and rows **[7]** of the tile area and click distribute **[8]**. Several yellow circles will appear according to the chosen parameters **[9]** (e.g image - tile 5x4 choose 5 columns and 4 rows and you'll have a support point in each tile).

• After adding all the support points, you can/should verify all the positions. Click 'verify' **[10]** (a window appears*) and correct **Z** if needed **[11].**







• Click on *'Star Experiment'* to start the acquisition.

• After acquisition click **Processing** and use the **stitching** tool to merge the image tiles together into a single image (see next page).

Stitching a Tile Scan

- Click on the Processing tab

- Under Method **[1]** click on '*stitching*' **[2]** (if it is not in your recently used list then it can be found under Geometric heading).

- Click on the image you would like to stitch (click on white arrow and select from the list **[3]**) and then click on '*Select*'.

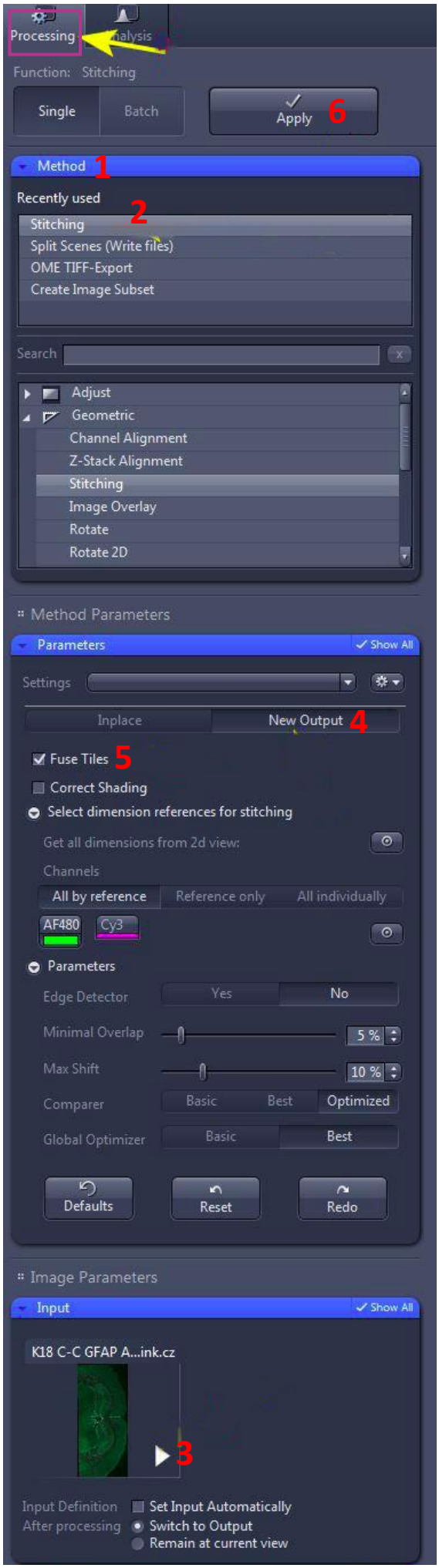
- Set output type in the Parameters tab to:
  A. New Output **[4]**
  B. Fuse Tiles **[5]**
  (This only needs to be done at the start of each session)

- Other parameters are mostly default, but can be adjusted for best alignment and stitching.

- Next click on '*Apply*' **[6]**, this will stitch your image for you. The stitched image will be displayed as a separate image.

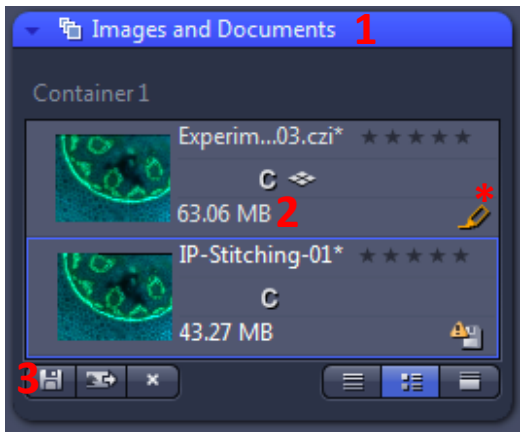
- Finally save your file, it will have the original image name with an "_Stitch" at the end.

NOTE: it is worth keeping the original raw version as well as the fused stitched in case you want to re-use your settings (you can't re-use a fused file) and also re-stitch.



Saving Images

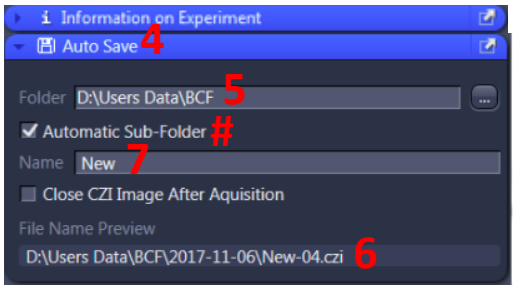
- All documents created in a session are listed in the *'Images and Documents'* **[1]** tool on the right-hand side of the workspace. This list also gives an estimation of the size on disk of each dataset **[2]**. (A file is not saved if it has an "orange pencil"* after its name)



- To save an Image you can click the *'save'* **[3]** button in the '*Images and Documents'* tool: you can save one by one or select all at the same time and 'save selected'. 'File --> Save' can also be used from the software menu.

- Save your files in: **D:\Users data\PI name\User name**
  - This will save the image as a native*.CZI file, which can be opened by Zen, FIJI (ImageJ) or Imaris.
  - It is best to keep the CZI file as your original data because it contains all the experiment parameters and could be requested by any reviewer at time of publication. (Always backup and keep the native file).
  - If you need the image for a powerpoint presentation or want to open it in photoshop you can export your CZI files as TIF files - see next page.

- You can also use '*Auto save*' tool**.** In fact, it is strongly recommended to use it for tile experiments. The *'Auto Save'* tool **[4]** is in the '*Applications*' section



- Choose the folder your files will be saved in and give it a name **[5]**.

- This will be used as a prefix in case more than one files are created before altering the *'Name'* **[6]** field and automatic numbering will be appended.

- Save your files in: **D:\Users data\PI name\User name**

- Checking *'Automatic sub-folder'***#** will create a folder YYYY-MM-DD where your files will be saved **[7]**.

Exporting *.CZI files as TIFs

Images saved as CZI throughout a session can be saved as batch to TIFF as follows:

- Choose the '*Processing*' tab and click the *'Batch'* **[8]** button (single images in the current workspace can be exported by choosing Single).
- In the '*Batch Method*' tool choose *'Image Export'* **[9]**.
- Under '*Method Parameters*' open the '*Parameters*' **[10]** tool. Choose the output file type to be TIFF **[11]**. You can then choose to convert the image to 8-bit **[12]** and choose the compression type (the default is '*LZW*', but usually we choose '*none*') **[13]**. Here you can also choose to apply any display mapping to the final image (note your raw data will be lost!). You can also burn annotations and merge channels.
- Choose the destination directory **[14]** for the exported images and note the '*Create Folder'* **[15]** option. Check this option if you want every new image to have a new folder. This is useful for multiple channels or Z-Stacks.
- Click *'Apply'* **[16]** to convert the batch of images.