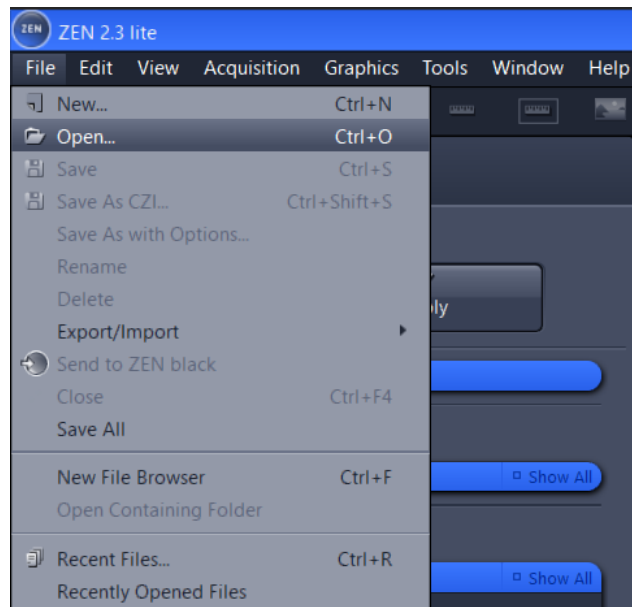


Downstream Image Processing and Analysis

A free software license is available for ZEN lite at ZEISS website (<https://www.zeiss.com/microscopy/int/products/microscope-software/zen-lite.html>). Install and use such version in your own personal computer for downstream image analysis. Although this tutorial was designed for the ZEN 2 Pro version available for operating the equipment, one may follow these procedures likewise in the free version. Please, notice that some modules exhibited in this tutorial are not available in the free version so one may need to process your images in ZEN 2 Pro available at the microscope.

Open

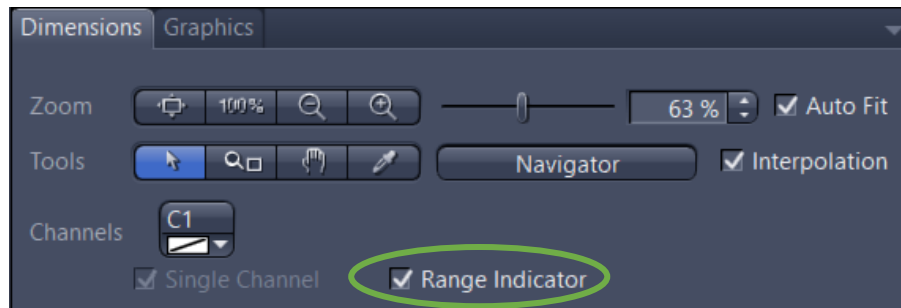
1. Go to **File -> Open**



2. Search for the .czi files previously saved in the directory dialog
3. The selected files will be displayed in the **Centre Screen Area** and **Right Tool Area**

Display Adjustments

1. In the **Dimensions** tab of the **Centre Screen Area**, activate the **Range Indicator** checkbox. This will mark in red or blue overexposed (too bright) or underexposed (too dark) areas, respectively



2. On the **Display** tab click in the **0.45** button. The display curve will be adapted to a gamma value of 0.45. This will set the optimum colour presentation. If you do not see this button activate the **Show all** mode



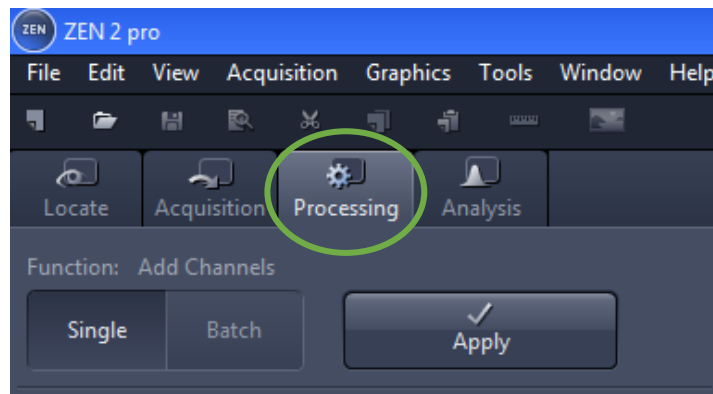
3. Move the controls left and right under the multiple curves in order to directly adjust the values for **Brightness (White)**, **Gamma**, and **Contrast (Black)** in the live image



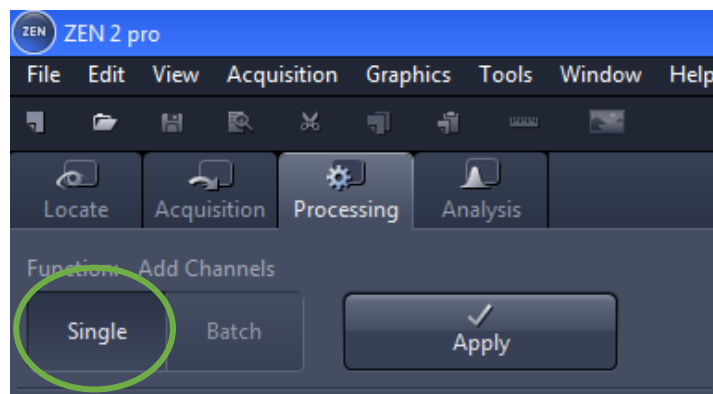
- 1 - Contrast (black point) control
- 2 - Gamma control
- 3 - Brightness (white point) control

Channel Combine or Image Overlay

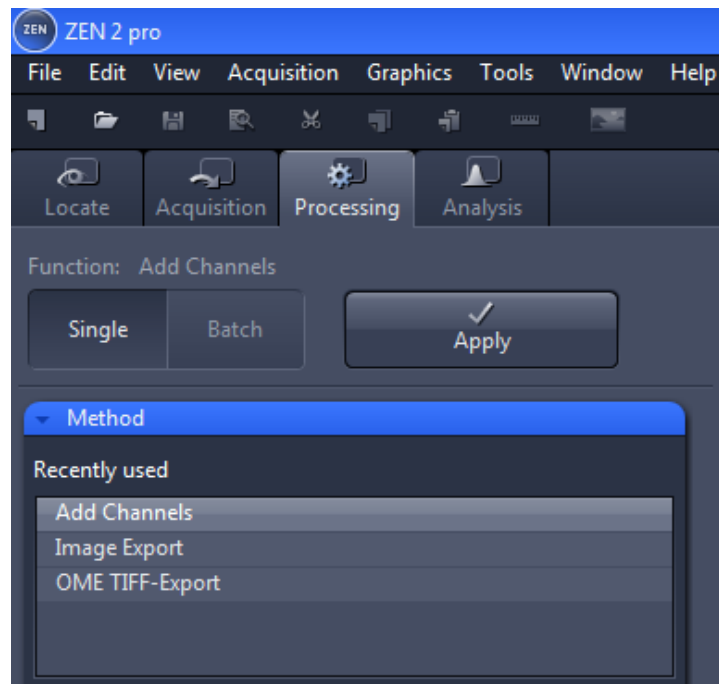
1. Go to the **Processing** tab



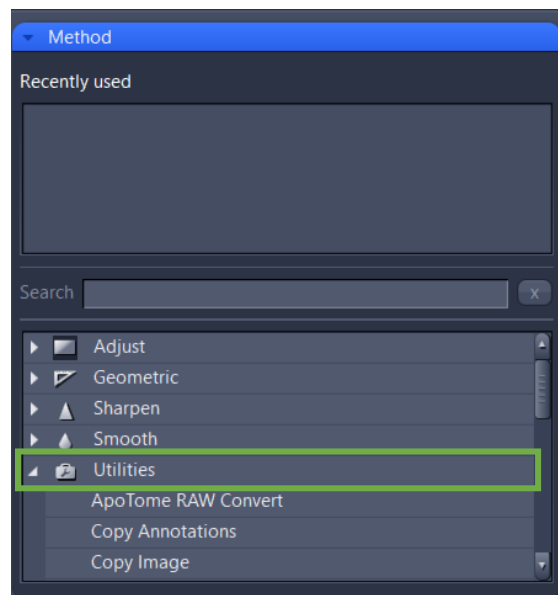
2. Click in the **Single** button



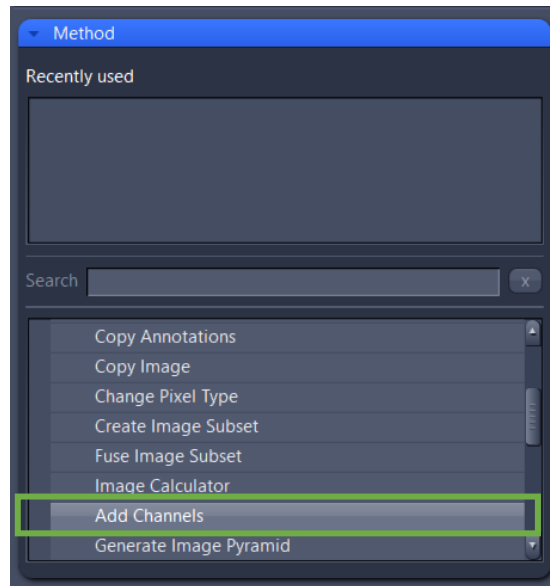
3. Click in the blue header of the **Method** tool



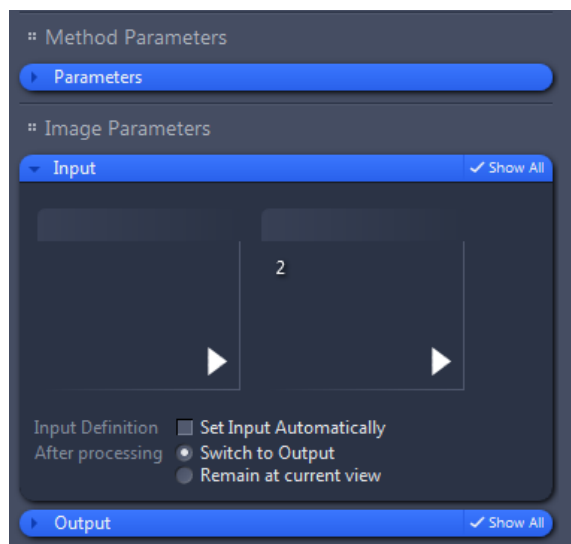
4. Click in the **Utilities** group to display the dropdown list of tools included in this category



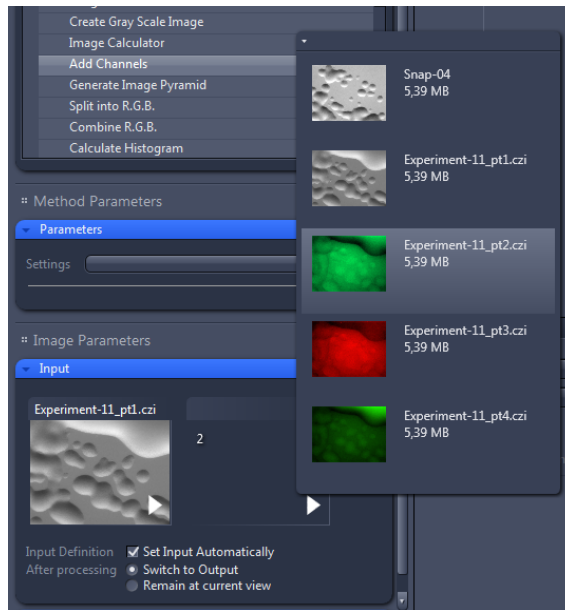
5. Search for the **Add Channel** tool and click in it



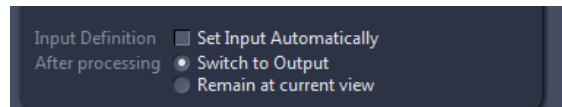
6. Click in the blue header of the **Input** tool



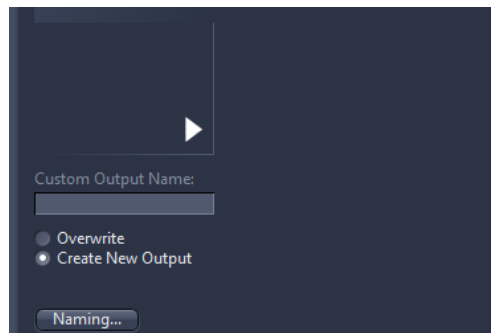
7. Select images of channels you would like to combine. To do so, click in the small preview image within the **Input** tool. You will see a preview of all open images. To select an image, just click in it

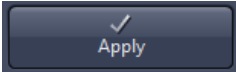


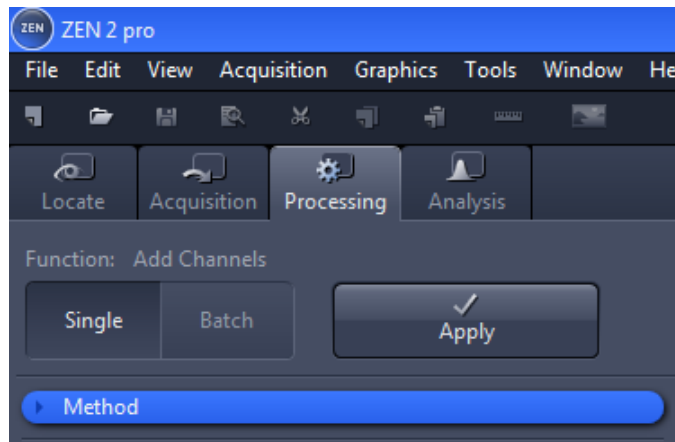
8. Yet in the **Input** tool, select the **Switch to Output** option only



9. Click in the blue header of the **Output** tool and select the **Create New Output** option only. Additionally, you can name the new image as you like by clicking in the **Naming** button

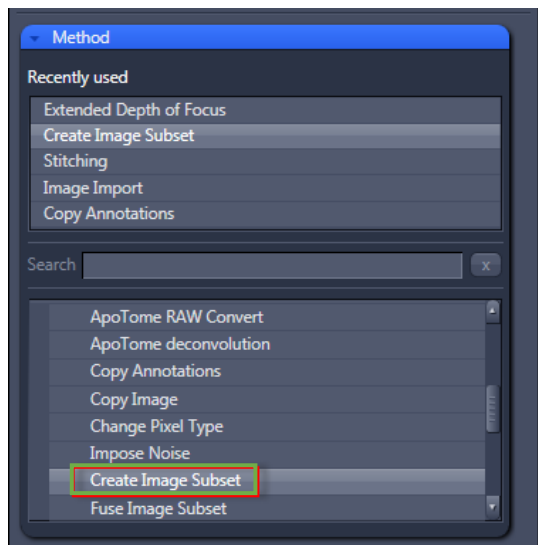


10. Finally, click in the **Apply**  button. The image combined will be displayed in the **Centre Screen Area**.

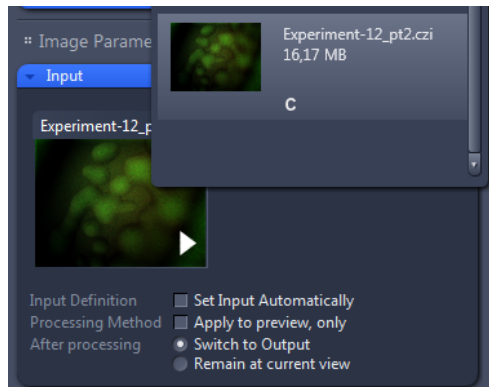


Extracting Single Fluorescence Images of a Multichannel Image

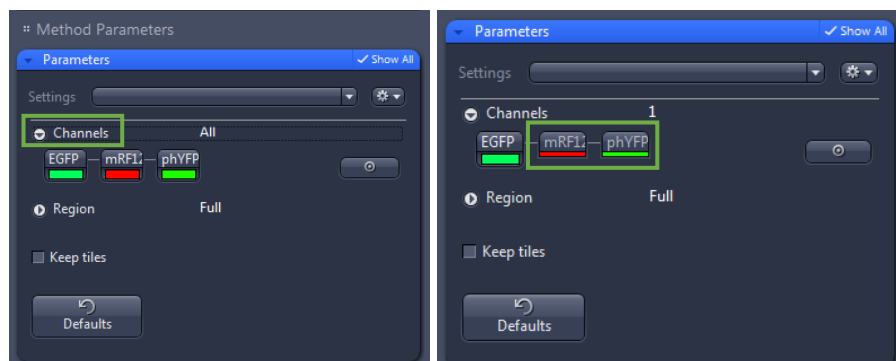
1. Select the **Processing** tab. Open the **Method** tool and under **Utilities** select the entry **Create Image Subset**

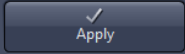


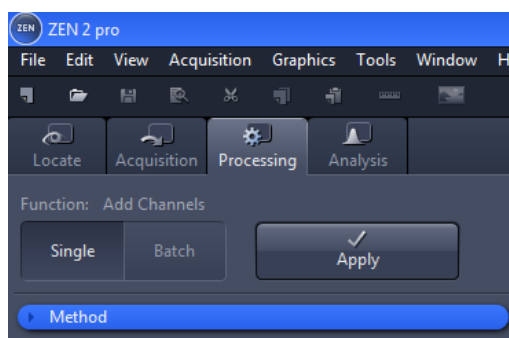
2. In the **Image Parameters** group, open the **Input** tool and select the multichannel image as input



3. In the **Method Parameters** group, open the **Parameters** tool and click in the **Channels** entry. For instance, deactivate the red and green/yellow fluorescence channels by clicking in the corresponding channels to extract only a single green fluorescence image

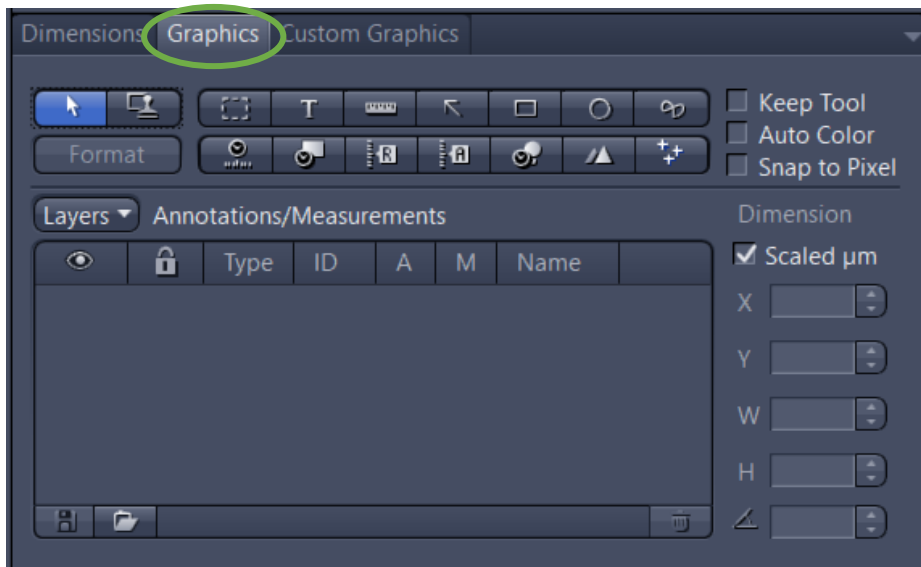




4. Finally, click in the **Apply**  button. The single channel image will be displayed in the **Centre Screen Area**

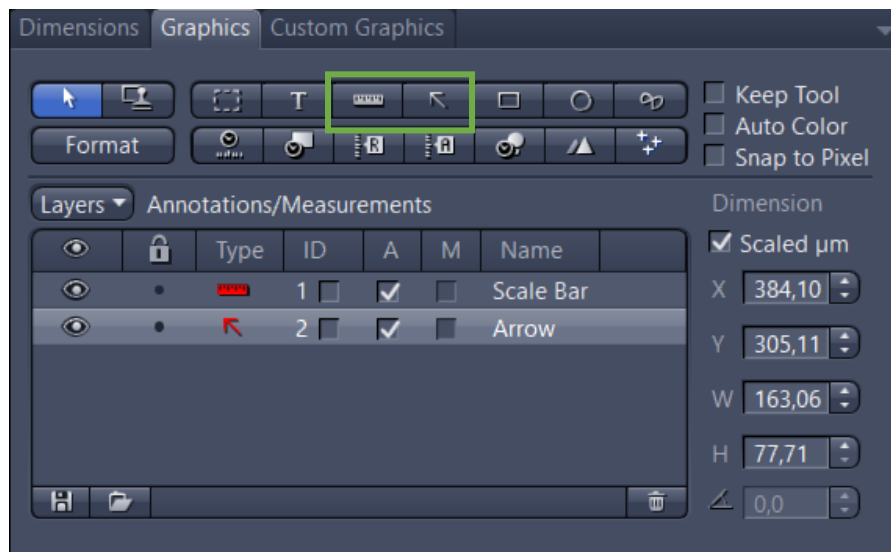


Add Scale Bar or Arrows

1. In the **Center Screen Area**, select the **Graphics** tab



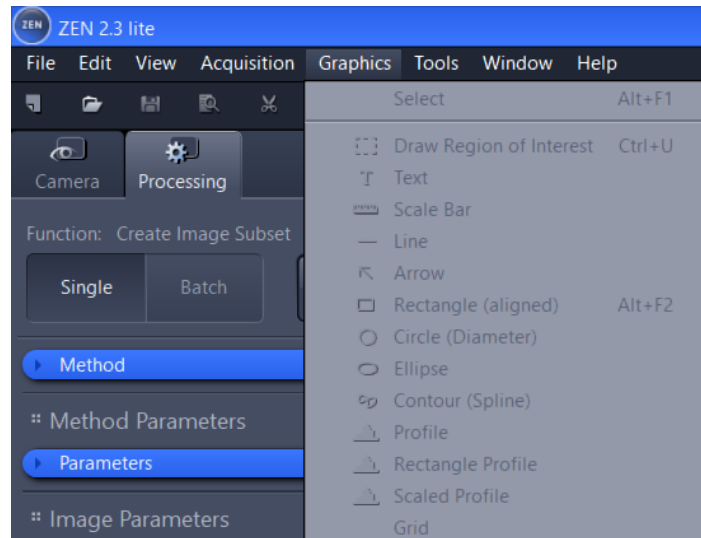
2. Click in the **Scale Bar**  or **Draw Arrow**  buttons. The scale bar will appear directly in the image and in the **Annotations/Measurements** as an entry. To set an arrow, the button will turn blue and then you can draw an arrow over the image



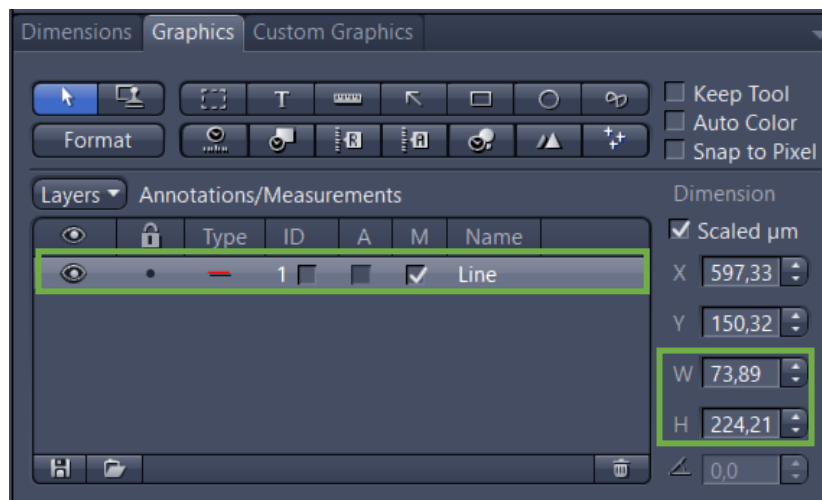
You can access to numerous formatting possibilities by right-clicking in the annotation (scale bar, arrow, etc). This will open the context menu. Select **Format Graphical Elements...** in this dialog

Distance Measurements

1. Go to **Graphics** -> **Line**



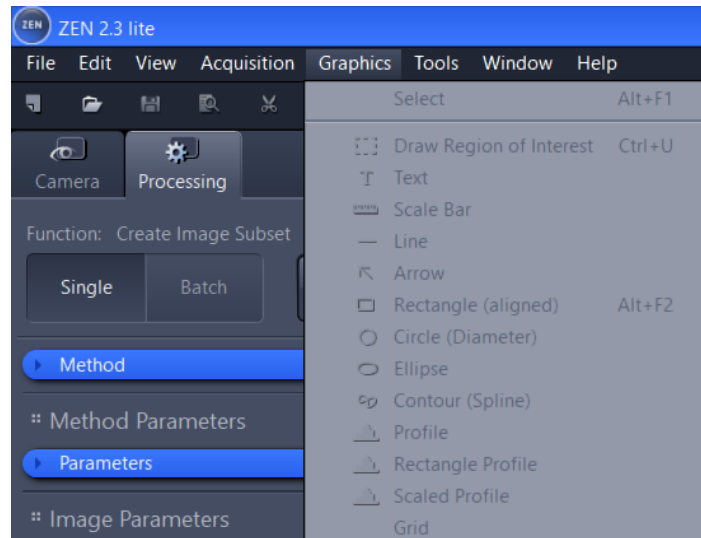
2. Draw the line over the object you would like to measure
3. In the **Center Screen Area**, select the **Graphics** tab. An entry of the **Line** type will appear together with the positions and sizes (these measurements are not the distance length between the two-point edges of the line)



4. To display the distance length, box the **M** column and the measurement will be displayed in the image under the line

Cropping Region of Interest (ROI)

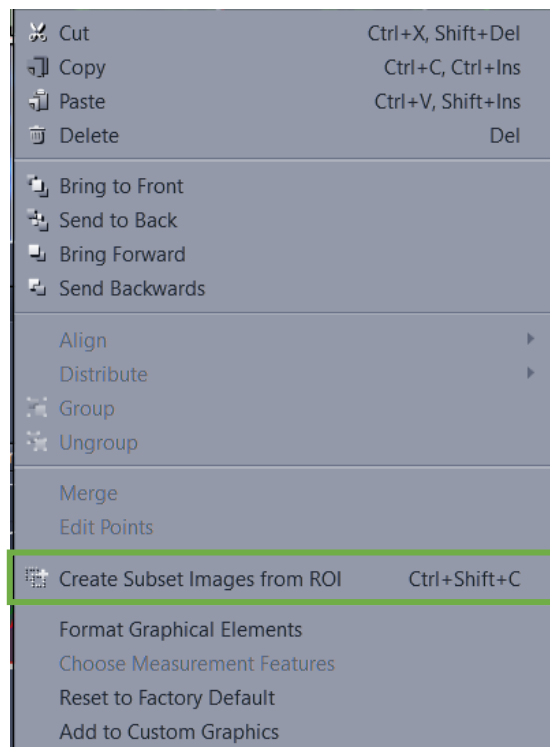
1. Go to **Graphics** -> **Draw Region of Interest**



2. Draw a square or rectangular region over the region of interest

3. Right-click in the drawn region. This will open the context menu. Select **Create**

Subset Images from ROI in this dialog

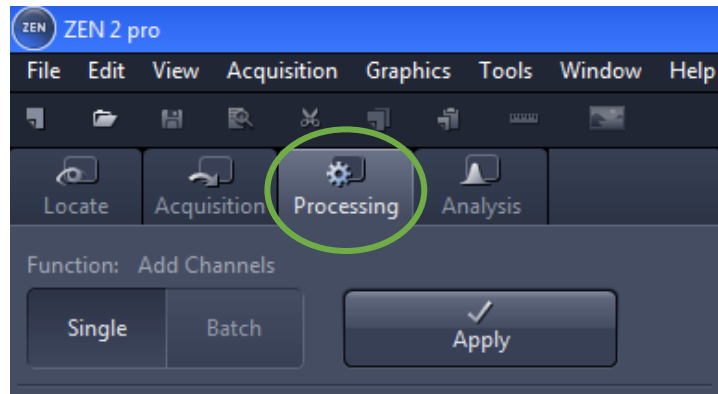


4. The selected region will be displayed as a new image in the **Centre Screen Area**.

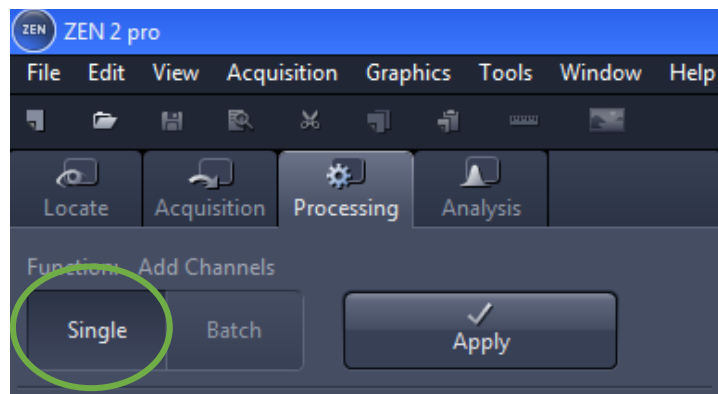
Besides, the image document will appear in the right-side **Right Tool Area**

Export

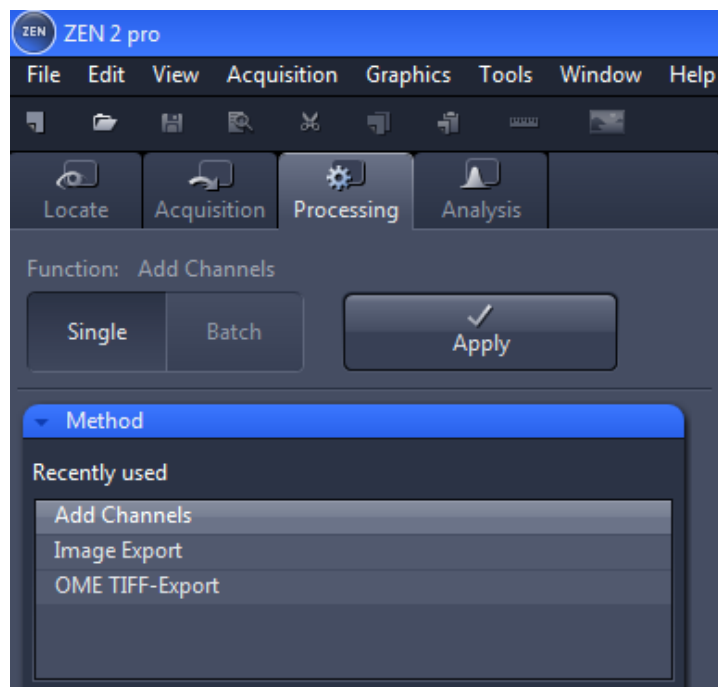
1. Go to the **Processing** tab



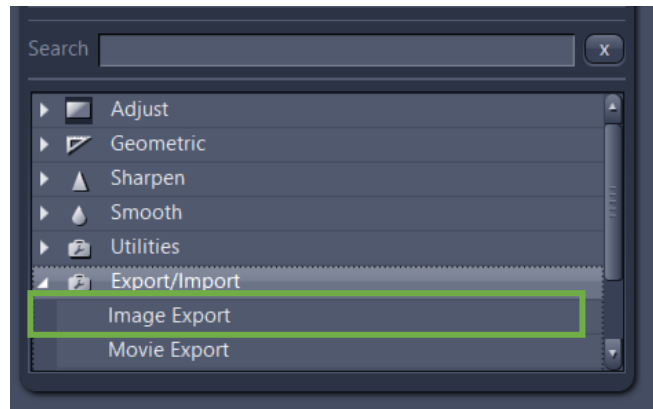
2. Click in the **Single** Single button



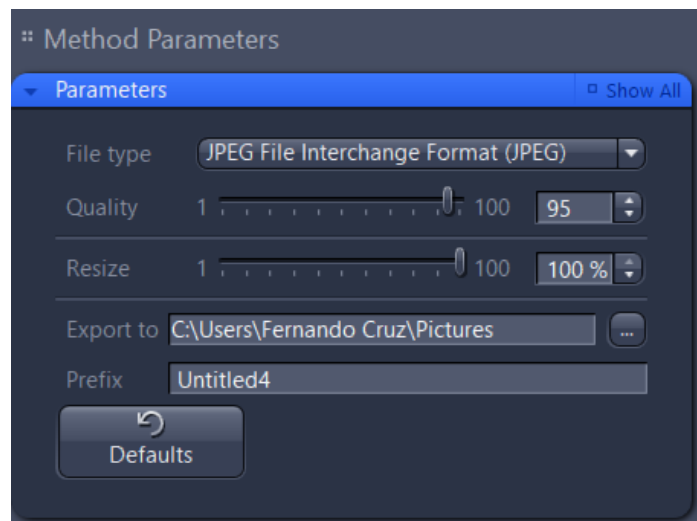
3. Click in the blue header of the **Method** tool



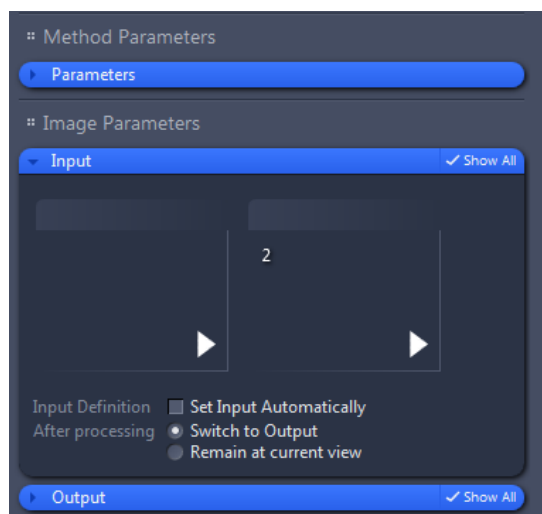
4. In the **Method** tool, open the **Export/Import** group and select the **Image Export** method



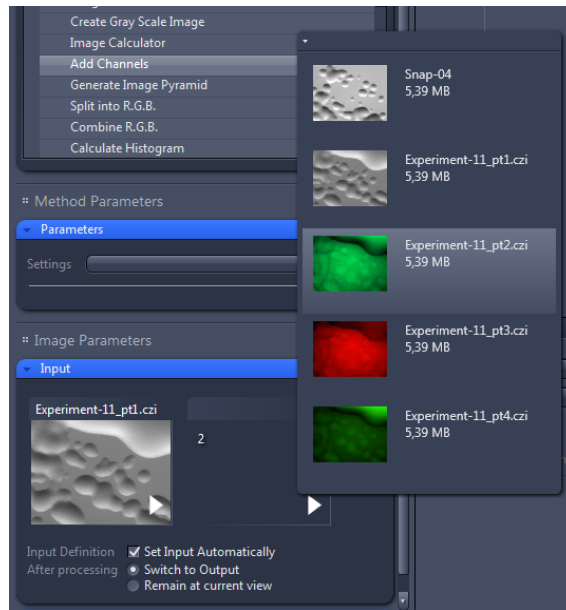
5. Under **Method Parameters** group, select the **Parameters** tool, and set the desired export settings, such as file type, quality, export folder, ...



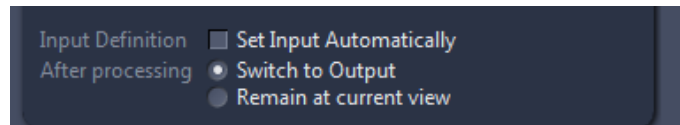
6. Click in the blue header of the **Input** tool



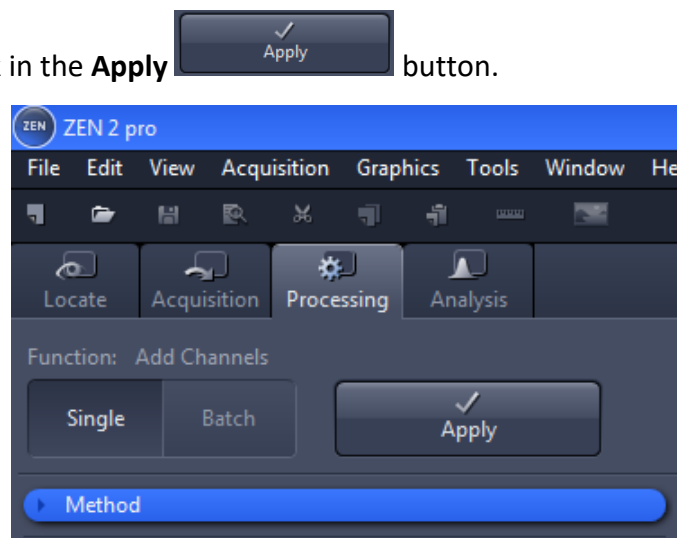
7. Select the images you would like to export. To do so, click in the small preview image within the **Input** tool. You will see a preview of all open images. To select an image, just click in it



8. Yet in the **Input** tool, select only the **Switch to Output** option



9. Finally, click in the **Apply** button.



Closing ZEN 2.1

1. To end ZEN 2.1 software, go to **File** -> **Exit**. Alternatively, you can press **ALT + F4** on your keyboard or click in the **Close** icon of the program bar.

!!!Note: If you haven't saved your files, the **Save/Keep Documents** dialog will open before the program closes. Select files you want to save or unselect files you don't want to save.

Save only documents that are actually yours, and not pre-set experiments that belong to others or light path settings of the favorites filter sets.

