ZEN Lite Quick Guide

For any extra questions not answered in the quick guide, you may click the question mark/help button by the minimize page button and then click an area of the screen which you have questions about. After doing this the ZEISS Help viewer guide will pop up with information on what you clicked. Once in the ZEISS Help viewer guide you may also be able to view information about different tabs in ZEN Lite.

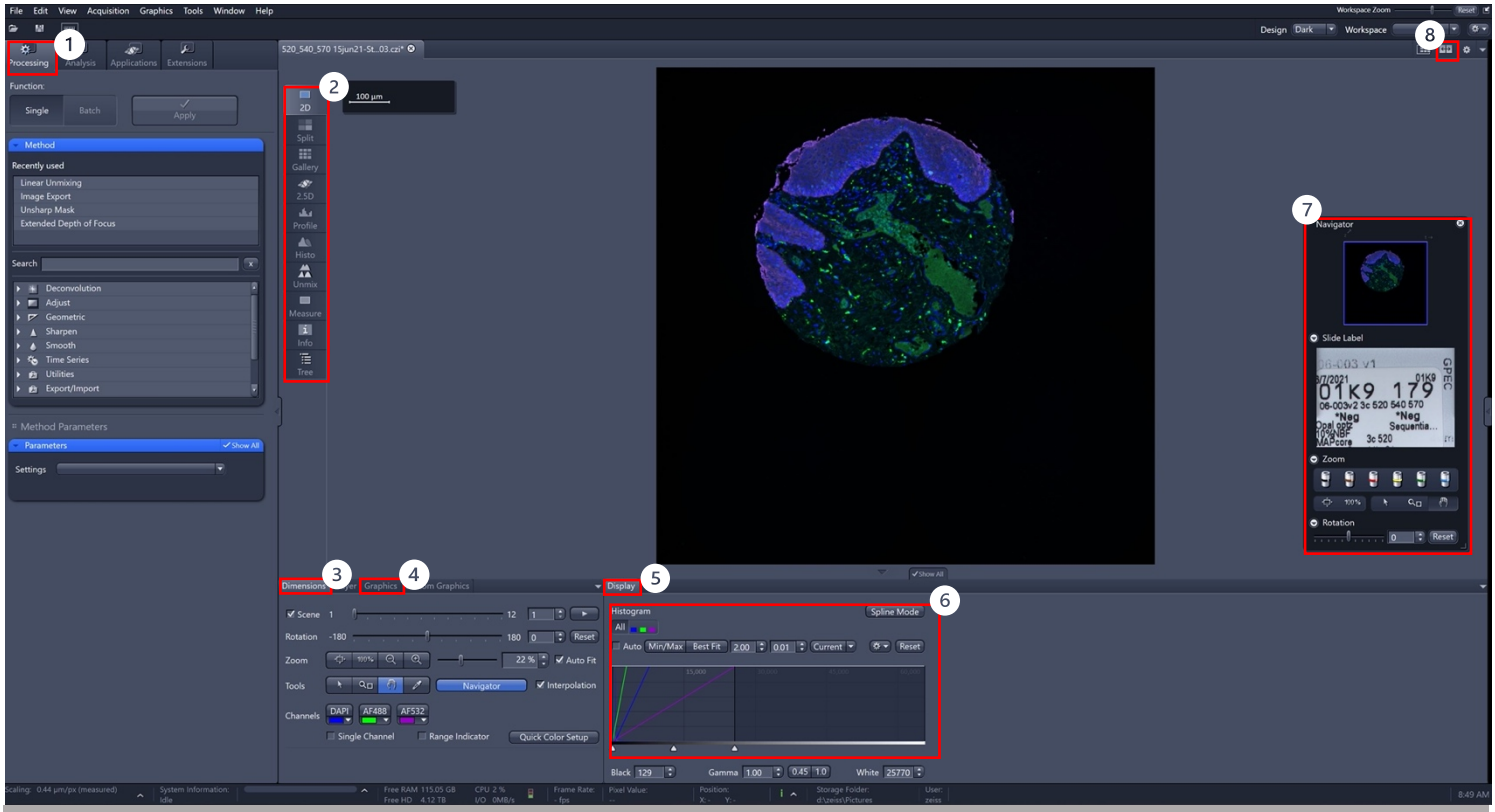
To open Zen:

1. Press the ZEN app icon.
2. Chose ZEN slidescan or ZEN image processing.
    - ZEN slidescan and image processing are very similar except that you can only use ZEN slidescan while the Axio scanner is on, available and connected. When the scanner is not on, you can still process images using ZEN image processing.

After opening ZEN slidescan to open a file:

1. Go to the file tab and press New image.
2. Press the folder icon (Open…) **or** press Ctrl+O to select an image from your files to open on ZEN slide scan.

To open a scanned image that has been scanned and is on your ZEN magazine, right click on the image and select “Open image(s)”.



1 Processing tab
2 Image views tab
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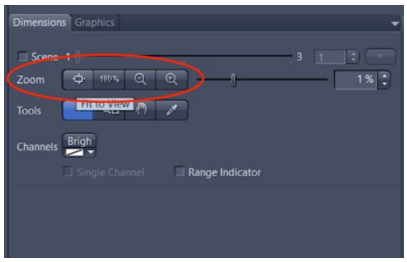
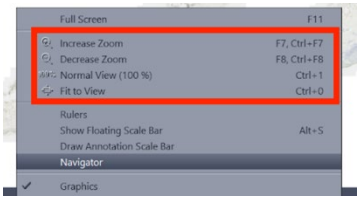
Using the Image views Tab:

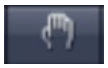
Within the image views tab, you can switch between different image views by selecting the corresponding tab in the list. Additionally, within this tab there is the **"Info View"**, which allows you to view extensive information about your image. The information is organized into sections including the General section, where you can edit the title, description, and comments for the image, the File Information section, and the Image Dimensions section. In addition to this, there is also the Acquisition Information section where you can see information including what filters and beam splitters were used to scan the image.

To zoom in and out:

	You may use the mouse scroll wheel to scroll into and away from your image.
	The Dimensions tab found beneath the image will have a Zoom section that allows you to alter the zoom, set to Normal view (100%) or fit the image to the screen ("Fit to View").
	You may also use the navigator tab to select the image zoom settings (1x, 2.5x, 5x, 10x, 20x or 40x). ○ To open the navigator tab, right click on the open image and select "Navigator" so that a checkmark appears next to it.
	By right clicking the image, you may also increase or decrease the zoom, set to Normal view (100%) or have the image fit to view.
	The "zoom rectangle" found in the tools in the dimensions tab also allows for the user to zoom into a specific area within the image by drawing a rectangle in the region of interest.

To move the Image around:

- Once zoomed in, the horizontal and vertical scroll bars around the image may be used to move around from one area to another.



- Additionally, in the tools tab, the "Panning zoomed image" tool can be selected, allows for movement around the image by clicking and dragging.

Dimensions Tab

Scene:

- Sometimes, when a slide has more than one tissue section (ex. TMA) the image will contain various "scenes" and separate each section. For example, within a TMA, each core will be considered a different scene.
    - Created scenes will be labelled left to right from top to bottom. Due to different mounting technique it may be important to disregard scenes while comparing different TMA scans as what may be considered the third scene in one TMA might not be the third scene in a different TMA.
    - To switch between scenes: you may change the scan region by changing the number by scene or scrolling down the bar by scene.

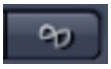
Changing channels:

When working with IF images with different channels, you may turn different channels on and off by simply clicking on the channel option (ex. DAPI) in the **Dimensions tab**. This can allow for the visualization of only one channel at a time or different combinations of channels. The different channels are defined by the different beam splitters and filters used for scanning.

Graphics Tab

In the graphics tab beneath the image, there are many tools that can be used to edit the image to add more information to it for presentation.

	**Drawing a Region of interest-** this tool allows for a rectangle to be drawn around any ROI in the image. This allows for the creation of subset images by holding the Ctrl key while moving the mouse cursor within the ROI. Then keep pressing the left mouse key while dragging the mouse outside of the ROI. This will create a subset image containing the outlined ROI and any scale bars the original image may have.
	**Drawing Text-** this tool allows for the addition of text to annotate the image anywhere within the image. Once the field for the text is drawn, you may start typing in the text.
	**Insert Scale Bar-** When selected, a scale bar is automatically inserted into the bottom right corner of the image. This scale bar can be moved to another section of the image and the length can be modified by selecting the scale bar and dragging it to modify the length.

	**Draw Arrow-** This tool allows for arrows to be drawn in the image after being selected by simply clicking and dragging in the image to create the desired arrow.
	**Draw Rectangle-** This tool allows for a rectangle of any size/dimensions to be drawn in the image. The rectangle will remain in the image and is by default parallel to the edges of the image.
	**Draw Circle-** This tool allows for a circle of any size to be drawn in the image.
	**Draw Spline Contour-** This tool allows for any shape/free contour within the image. You may choose to define the corner points of the shape by a series of clicks, **or,** you may trace the contour by keeping the left mouse key pressed. To close the shape/contour right click your mouse.

Selection Chart

The selection chart allows us to control and organize some of the elements added by the graphics tools.

	**Visibility-** selection allows to either hide or show the selected graphic element.
	**Fix Position-** Locks the graphic element in place so that it can no longer me modified until the "fix position" option is deselected.
	**Save-** allows for the selected graphic element(s) to be saved and used for other images.
	**Load-** allows the loading of an already existing graphic to the image.
	**Delete-** deletes the selected graphic.

To edit the contrast and Brightness (Using the Display Histogram):

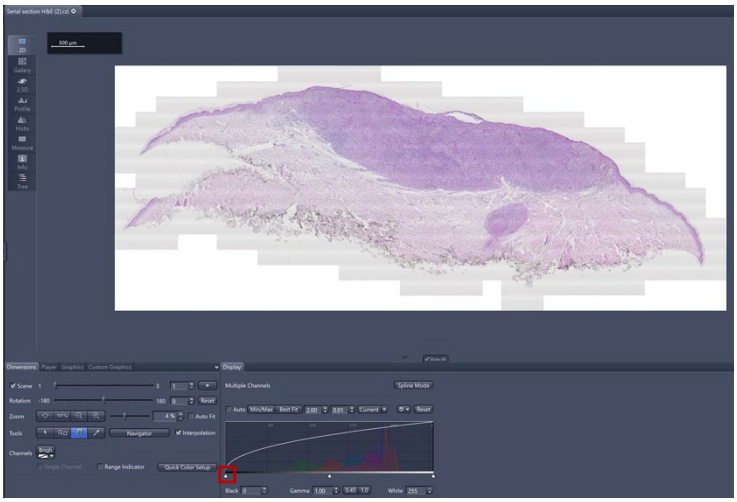
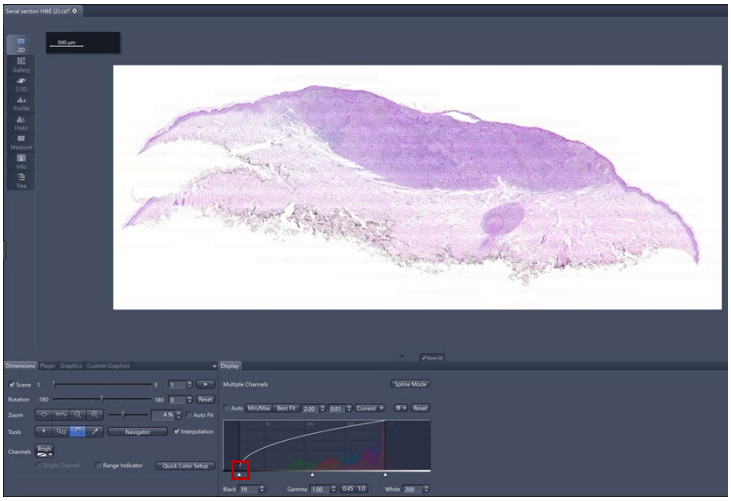
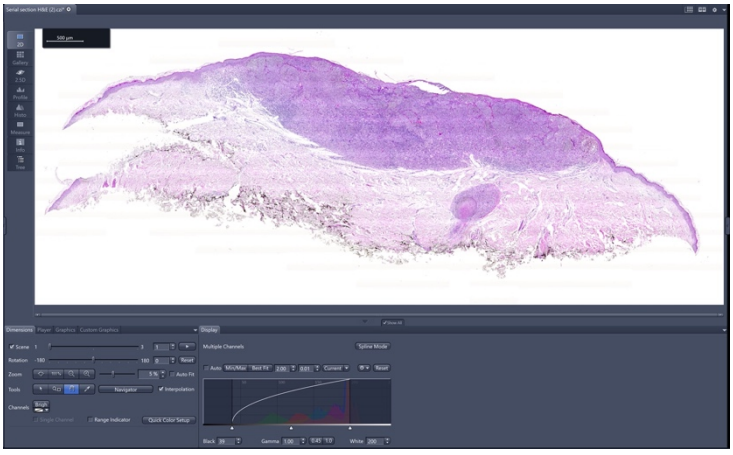
The display Histogram shows the brightness distribution of the pixels present from all channels simultaneously. In this histogram, the X-axis represents the brightness, and the Y-axis represents the relative frequency. Each channel has its own display characteristic curve.

You can click on "All" (default- on top of the histogram) and move the histogram curve to alter the setting of all the channels of the image. Alternatively, you can click on specific channels besides "All" and alter the curves separately. By hovering your mouse pointer on top of the colour field displays the channel name.

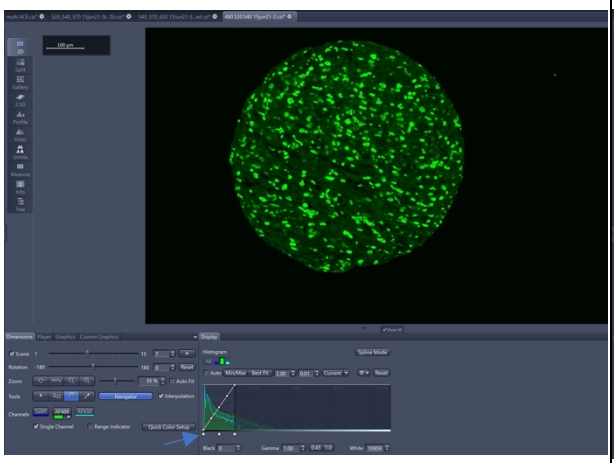
By altering the display characteristic curve, you can influence the **contrast** of the image by limiting the black (left) and white (right) value. To do this you can move the mouse pointer on the corresponding adjustment handles on the bottom edge of the display histogram.

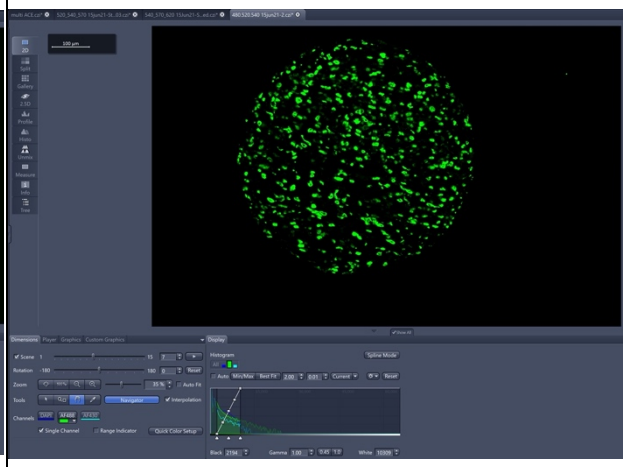
By altering the curvature of the display characteristic curve you alter the gamma value (what we perceive as **brightness**). By moving the mouse pointer to the second or fourth small rectangle on the curve and clicking on it to move it up and down, you can alter the gamma value. Alternatively, by clicking the middle rectangle you can also move the whole display curve to alter the brightness.

- **With Brightfield images:**

	Brightfield images often have a darker background surrounding the tissue. To remove this, simply slide the left display curve arrow to limit the black value of the image.
	As seen in this image, by altering the curve and removing some of the black value, the dark background is no longer visible.
	Adjusting the left and right display curve arrows (black and white respectively) can also increase the contrast of the image, as seen in the image to the left.

- **With Fluorescent images:**





When working with fluorescent images, there is often some autofluorescence or background fluorescence in the image. This background is normally fainter than the real fluorescence and thus can be mostly removed by adjusting the left display curve arrow and moving it slightly to the right. It is important to be cautious, however, when doing this since it runs the risk of removing true signal if not careful. Therefore, it's important to assess the stain critically to determine what is real and what is background.

To Export Images

- In the processing tab under "Methods", you can click the search box and type in "Export" to find the Image export option. This will open up the method parameters where you can edit where the file will be exported to, the file type, quality and size of the image in addition to other features. For examples, one of the features that you can choose is to export fluorescent images as individual channels or a combination of different channels. When working with images with Z stacks,
- You will need to export the image as either .tiff (for publication) or .jpeg (for presentation) as the original. czi will contain a lot of extra unnecessary metadata. Once you have completed this, you can press "Apply" on the top of the Processing tab and your image will be exported.

Create a Multiimage

By clicking on this icon found on above the right corner of the magazine or image you are viewing you may open a multi-image where you can drag two different images from the gallery (displayed on your right)