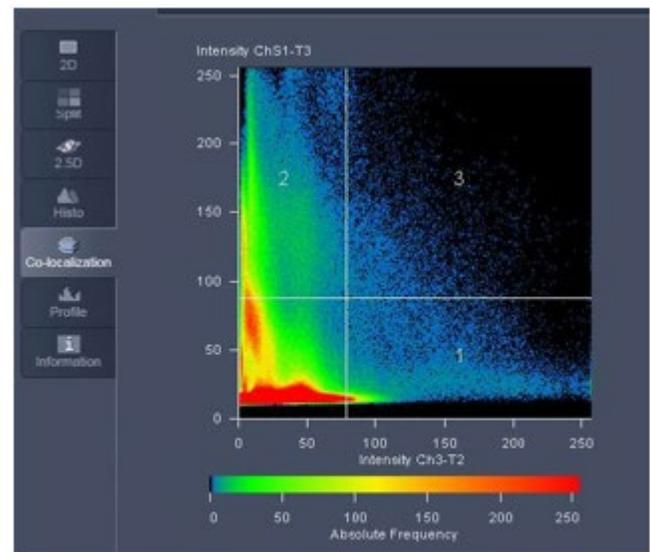


Colocalization

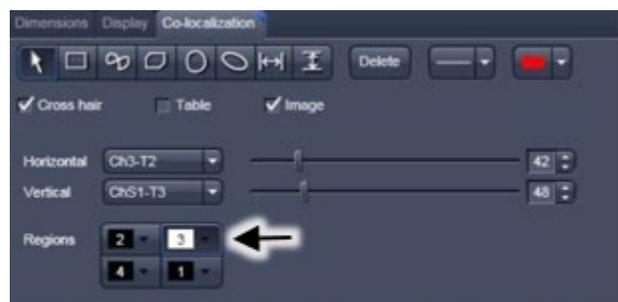
In fluorescence microscopy, colocalization refers to observation of the spatial overlap between two (or more) different fluorescent labels, each having a separate emission wavelength, to see if the different "targets" are located in the same area of the cell or very near to one another.

Setup

1. During/after image acquisition, select "Colocalization" from the left-hand tabs in the image display window. This tab will only appear if the image has at least two channels.
2. Every pixel in the image is plotted in the displayed scatter diagram based on its intensity level from each channel. The color in the scatterplot represents the number of pixels that are plotted in that region.
3. Select which channel you want on each axis of the scatterplot. Note that only two channels can be selected for colocalization analysis.
4. Four quadrants are designated in the software by use of the crosshairs. The crosshairs can be turned on (check Crosshair in Zen) and centered anywhere in the scatterplot by clicking on the crosshairs and then moving them with the mouse (see below for notes on accurately setting the crosshairs).
5. In Regions (Colocalization tab), select Quadrant 3 and set to white for easier visualization of the pixels exhibiting colocalization. The other three quadrants can also be false colored in the same fashion.



Colocalization image window shows intensity-based scatter diagram divided into 4 quadrants



Colocalization tab - selection of colocalized regions for visualization

Four Quadrants in Scatterplot

- *Quadrant 1*: pixels in the Channel designated as x axis with high intensities.
- *Quadrant 2*: pixels in the Channel designated as y axis that have high intensities.
- *Quadrant 3*: pixels with high intensity levels in both channels. These pixels are considered to be colocalized.
- *Quadrant 4* (lower left): pixels that have low intensity levels in both channels; these pixels are referred to as background and are not taken into consideration for colocalization analysis.

Setting the Crosshair

Use single-labeled control samples:

1. Image the double label experimental sample first.
2. Image each single label control sample separately, with the exact same settings (2 channels).
3. On the scatterplot for the first control sample (e.g. Green), the pixel distribution of the green-only population is displayed. Set the horizontal crosshair just above this population. Note the exact Y-value for the horizontal crosshair.
4. On the scatterplot for the second control sample (e.g. Red), the pixel distribution of the red-only population is displayed. Set the vertical crosshair just above this population. Note the exact X-value for the vertical crosshair.
5. Use these exact (X,Y) coordinates on the scatterplot for the double label experimental sample.

Important Notes

- All imaging parameters that influence the intensity of the image should remain the same for all data acquisition. This includes: objective lens, laser power, light path settings, acquisition speed, detector settings
- The type of mounting media should also be kept constant as different types may exhibit varying levels of autofluorescence or affect the intensity levels of the fluorophores.
- Imaging through different types of material, such as a glass coverslip versus a plastic bottom dish, will affect the intensity of the resulting image.