

## Preparing for Colocalization

Below (in no particular order) are some simple processing tasks you may need to carry out in order to prepare your images for colocalization measurements.

**A. Converting 8-bit grayscale to 16-bit grayscale:** In the main menu, click on Process → Arithmetic to bring up the arithmetic window. Select one of your channels as the first source, and the constant value 0 as the second source. Select 16-bit as the format for your result, select “addition” as the function, and click on “Apply”. Save your new 16-bit result and repeat for the other channel.

**B. Converting one RGB image to individual grayscale images:** In the main menu click on Display → Color Separate. Select the desired image from the drop-down list and, if desired, give names to the new grayscale images. Click on “Apply”.

**C: Aligning channels:** If there is a systematic shift between the two channels, you can bring them into register using the Color Align tool. This procedure will work for individual grayscale images and single RGB images.

- Click on Display → Color Align to open up the dialog box and preview window
- Select the Red and Green images (or the single RGB image) from the list of open files
- Select “48-bit” as the output format (note that this is not the default)
- Drag the little black preview square to move the previewed area over a useful part of the image. You can zoom in or out in the preview window if desired.
- Check the box next to the channel you want shifted.
- Move the horizontal and vertical scroll bars to shift the selected channel – the result is shown in the preview window.
- When you’re happy with the alignment, click “Apply”.

Unfortunately, this procedure produces an overlay (RGB) image, which is not what you want for colocalization. So, you need to re-create separate red and green files from this overlay by following the conversion procedure in **B**.

You should now have separate 16-bit grayscale images for the red and green channels that are aligned and ready for colocalization analysis.