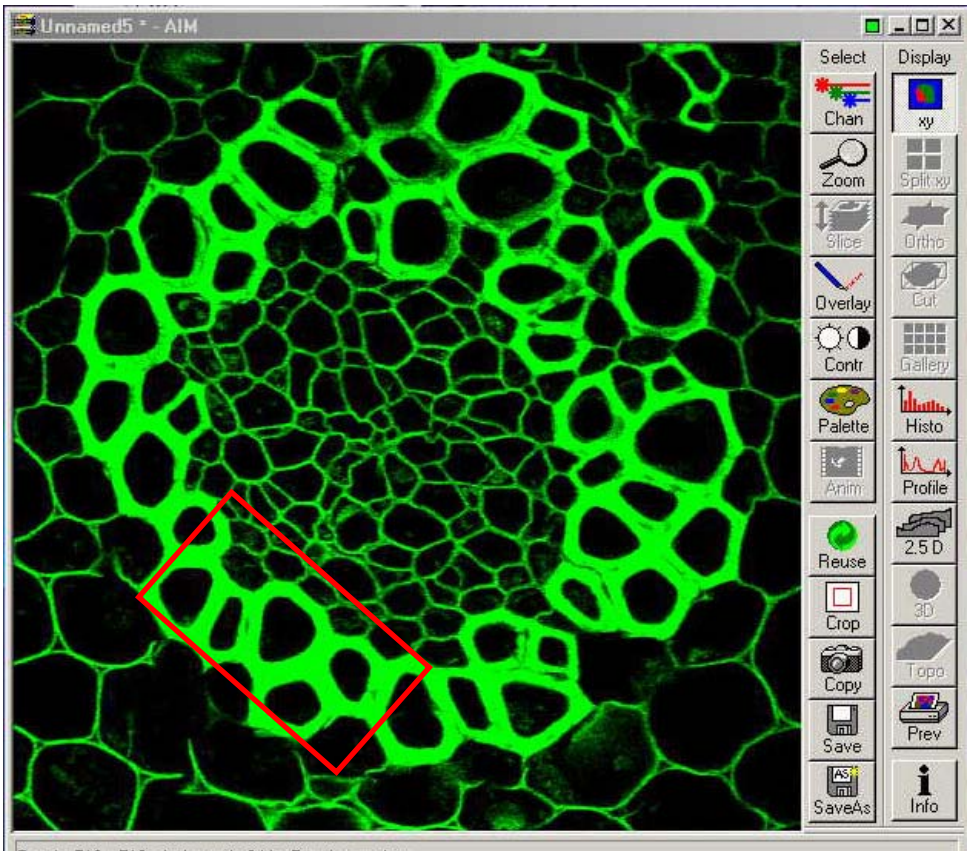
How to optimize the images during the experiments?

During the imaging experiment, you can always evaluate the quality of the images through your eyes. Many times, however, this empirical method could not let you to fully utilize the ***dynamic range*** of detectors in our Zeiss system to optimize the quality of your images. For example, in the images shown below, fluorescence intensity in a large section of the sample (as defined) is too high and saturates the detector so that the variations in the intensity in the areas could not be demonstrated.



What is the dynamic range of a detector?

For a detecting system, either a camera or a PMT detector, there is always a limited range for the sensitivity of the detecting system, which we call "***the dynamic range***". For example, a PMT detector can only detect the variations in the signal intensity within 1 to 100. If several signals come into the detector with signal intensities of 100, 105, 110, and 128, most of which are higher than the maximum (100) of the intensity the detector can recognize. In this case, the detector will read them to us as 100, 100, 100, and 100 (saturation). Apparently, the information contained in the variations in the intensity is lost. Similarly, if the signal come with low intensities of 0.5, 0.3 and 0.25, the detector will read the signal as 1, 1, and 1 as all of signals are lower than the lower threshold (1) of the detector. Consequently, all the information lost during the experiments due to the limitation of the dynamic range of the detector will not be recovered afterwards no matter how hard you work with imaging software like Photoshop.

Therefore, it is important that we do the things right in the first place, namely, during the experiments.

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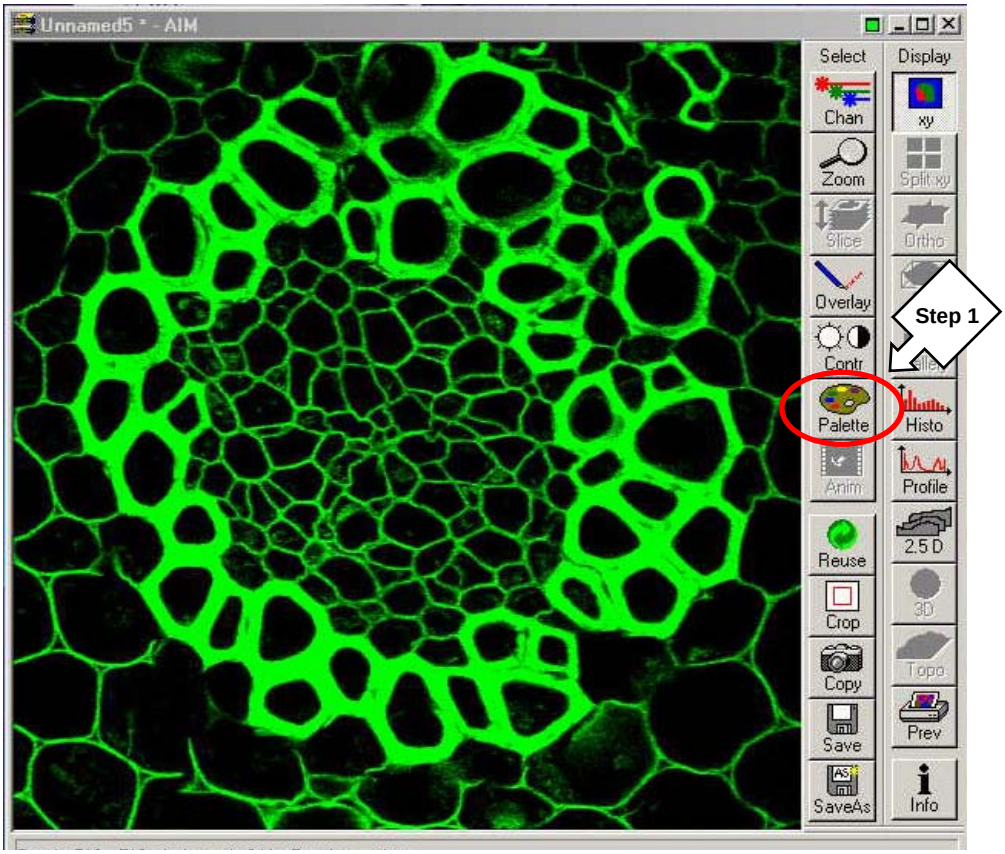
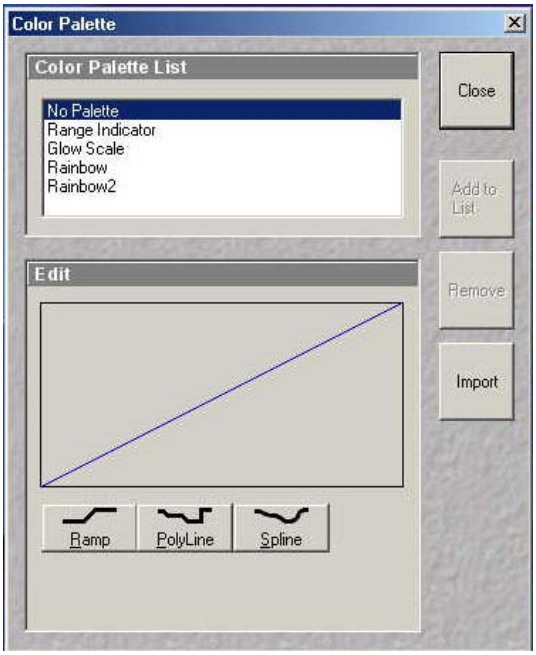
To fully take advantage of the dynamic range of the detector so that you can optimize the quality of the images, take the following steps:

Step 1 Select ***Palette*** in the image window



A ***Colo Palette*** window appears:



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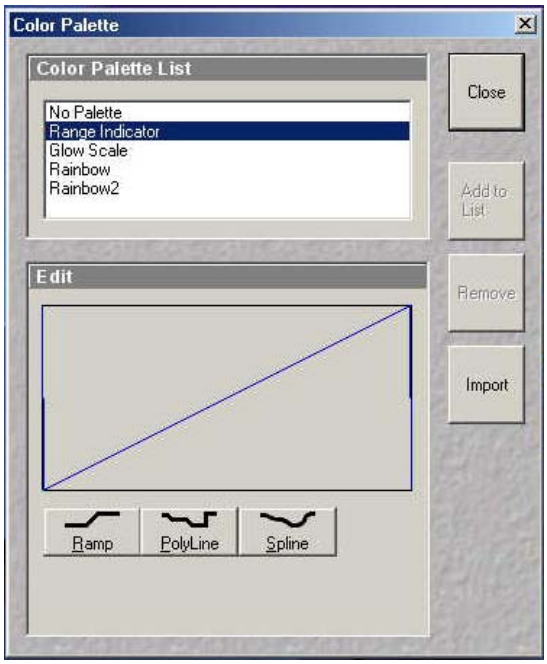
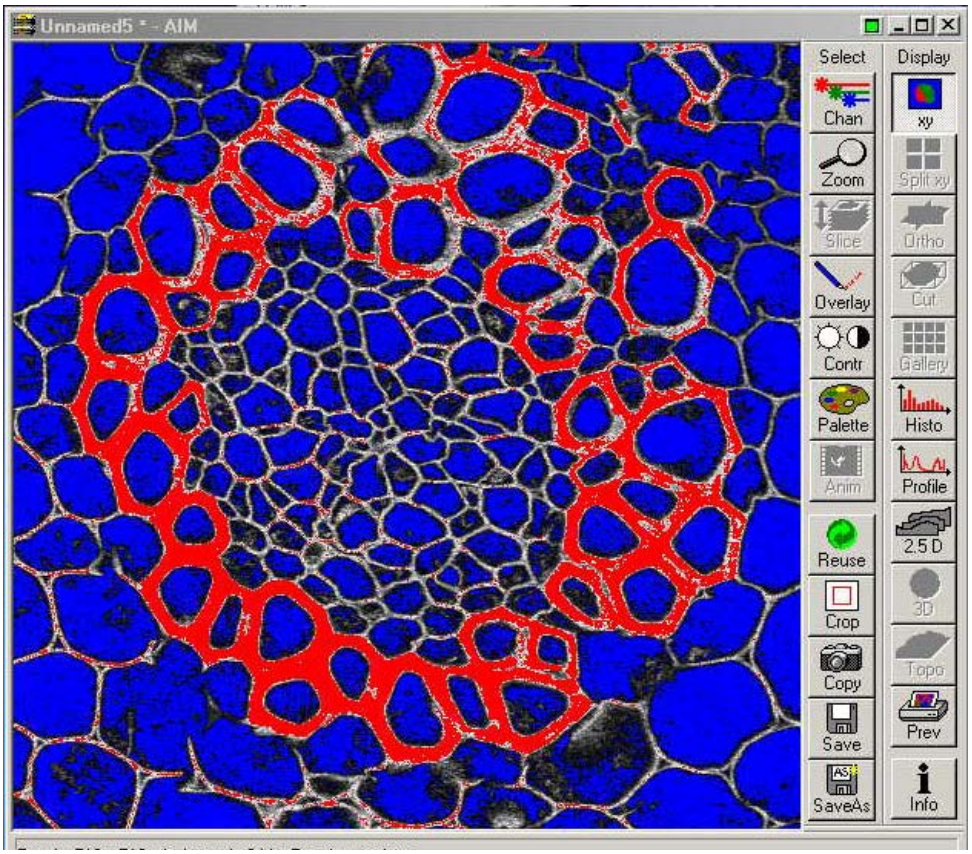
Step 2 Select ***Range Indicator***



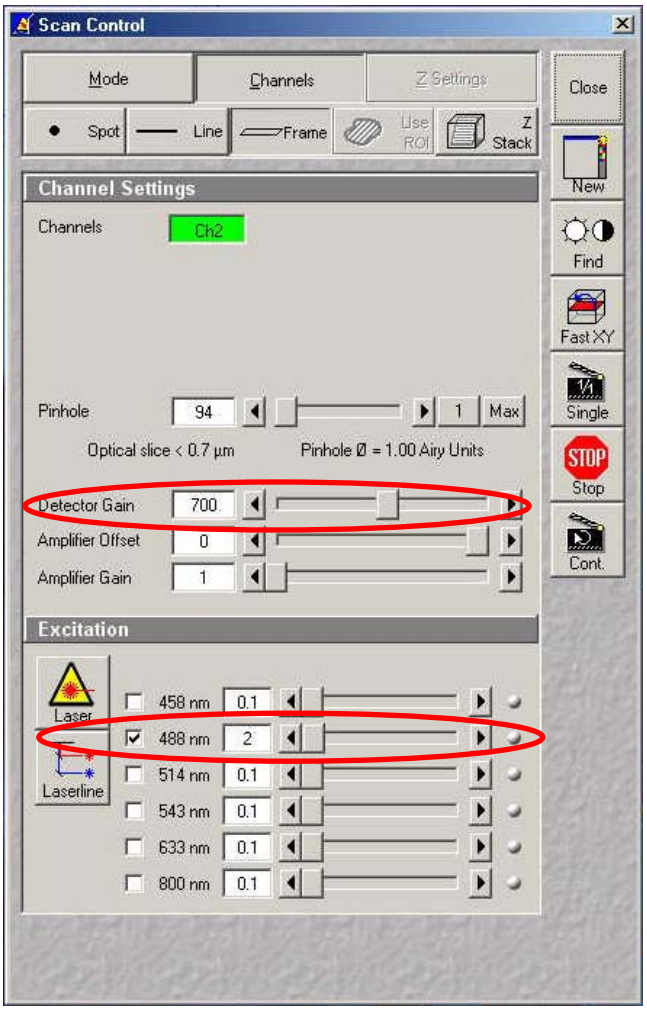
Now the image turns into one with two colors of ***red*** and ***blue***.



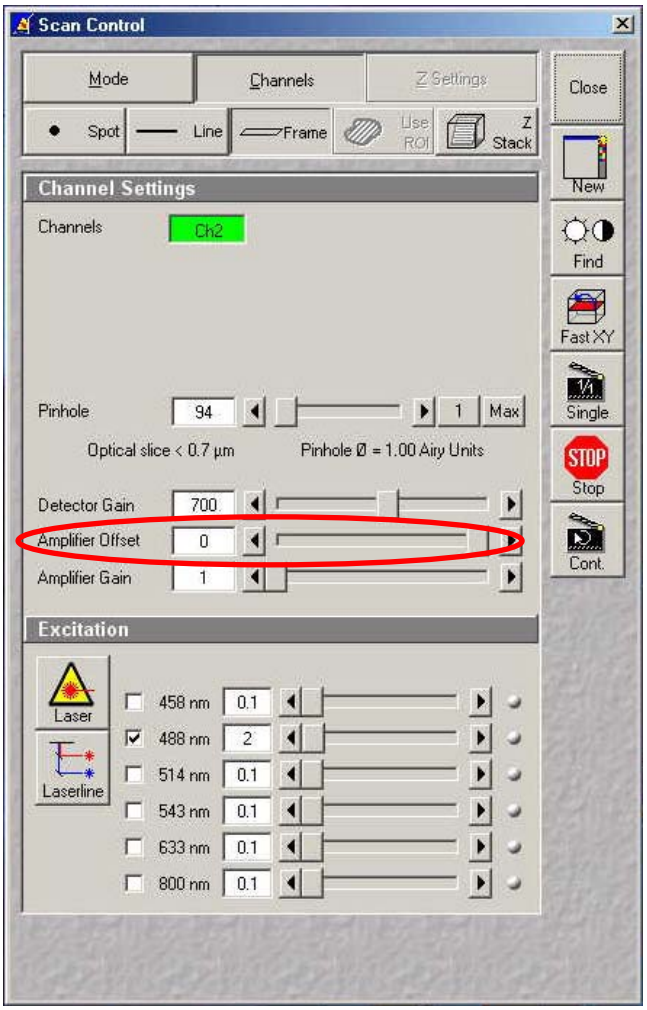
In this two-color image, the red areas represent the pixels with the highest fluorescence intensity the detector can detector. If we see a large area of red color, this indicates that this part of the sample emit fluorescence with a flat strongest intensity. Apparently, this is due to the saturation of the detector. Similarly, the blue area represents the pixels with the lowest intensity the detector can sense. If we see a large area of blue color that means signal in that area is very likely lower than the minimum threshold of the detector.

Therefore, to optimize the quality of the image, our goal is to reduce the size both of the red area and the blue area.

Step3 To reduce the size of red area, decrease either the laser power or the detector gain in the ***Scan Control*** window.



Step 4 To reduce the size of blue area, increase the ***Amplifier Offset*** in the Scan Control window.



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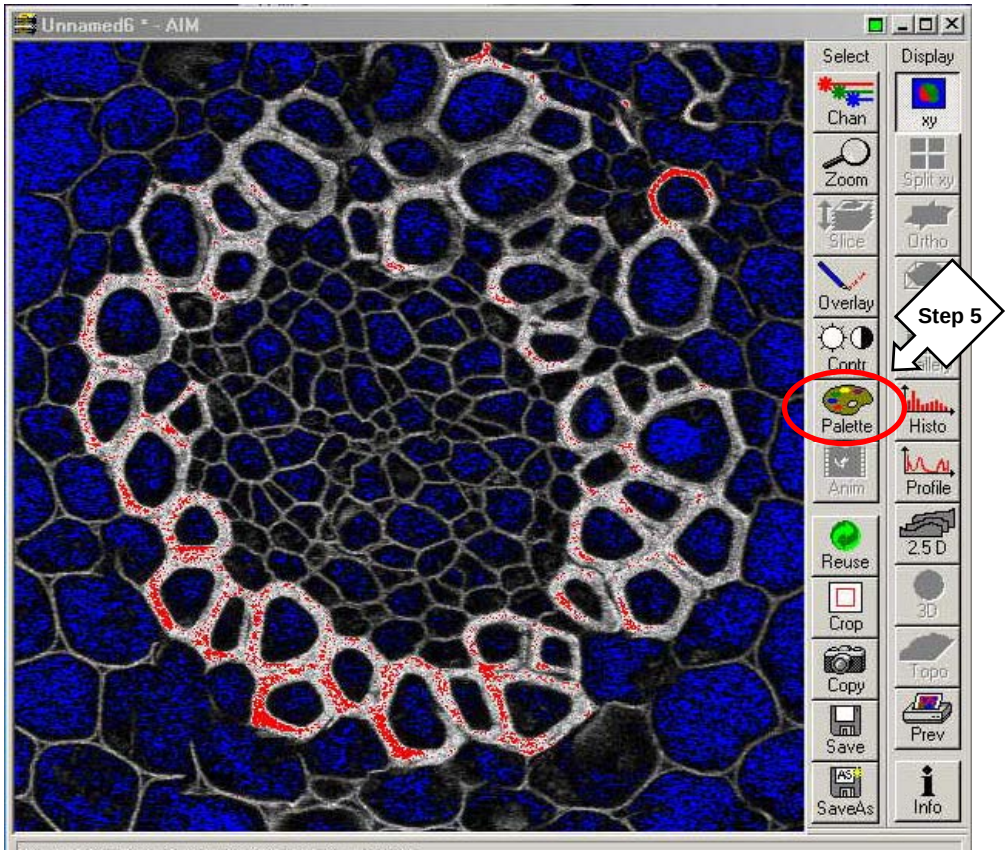
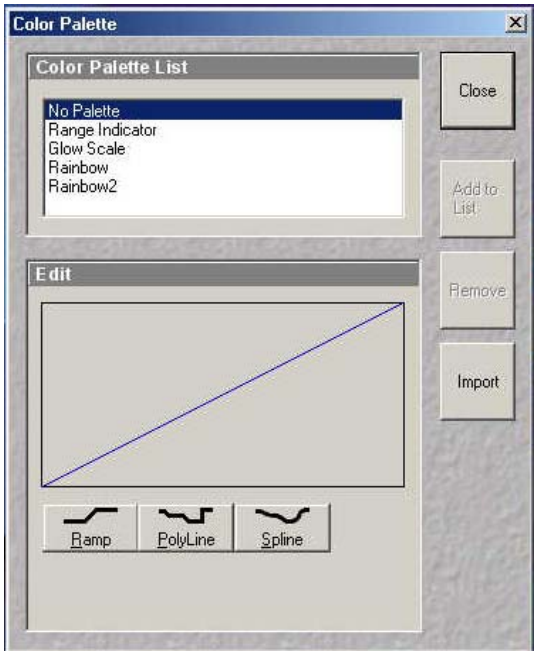
After **Step 3** and **Step 4**, a resulted image has a look shown below, namely, what we want is a little bit **red** color and a little bit **blue** color.



Step 5 Now select ***Palette*** again and choose ***No Palette*** in the ***Color Palette*** window



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The optimized image appears. Compare this new image with the one at the beginning of the experiment, you will see the variation in the intensity of the signal in the area where the signal was too high so that saturation appeared.