Microscopy QuickTip – Confocal Imaging - Speed

The pictures we take on microscopes are *raster* images, which means that they are made up of a grid of picture elements, or *pixels*. In a regular widefield fluorescence microscope the number and arrangement of pixels in our images are determined by the arrangement of picture elements on the image sensor (the electronic "film" of the camera). In confocal microscopes, however, there is no camera as we generally understand it. Instead, an image is reconstructed by scanning a laser from point to point in a grid pattern over our sample and detecting the amount of light collected from each of those points in turn. The controls on the confocal allow us to change the number of points in our grid, the size of the grid, how long we stay at each point, etc. This great degree of control we have over the confocal allows us to optimise the way an image is acquired to suit our experimental needs.

Starting from an image optimized for resolution, lets see how we can speed up image acquisition:

1. Reduce the Frame Size - this gives you a lower resolution image by increasing the distance between each pixel thus losing more information.
2. Increase the Line Step - lowers the resolution by regularly missing vertical lines in the image then using software interpolation to fill in the missing information.
3. Increase the Scan Speed – decreases the Pixel Dwell time or the amount of time the laser spends at each pixel, decreasing the Signal/Noise ratio.
4. Use Bi-Directional scanning to halve the Scan Time, use Auto to correct for image aberrations.
5. Increase the zoom to image just the region in which you are interested, and then recalculate your Optimal image size and Increase the Scan Speed.
6. Check the Scan Time here to see how long it will take to acquire your image after making each adjustment.

7. Tick the Regions tickbox, then from the Regions control select a rectange, circle etc and draw a Region of Interest (ROI) on your image that contains only what you're interested in. Make sure you have "Acquisition" ticked, along with "Fit frame size to bounding rectangle of regions". Once you've made your adjustments confirm the Scan Time in the Acquisition Mode Control #6.
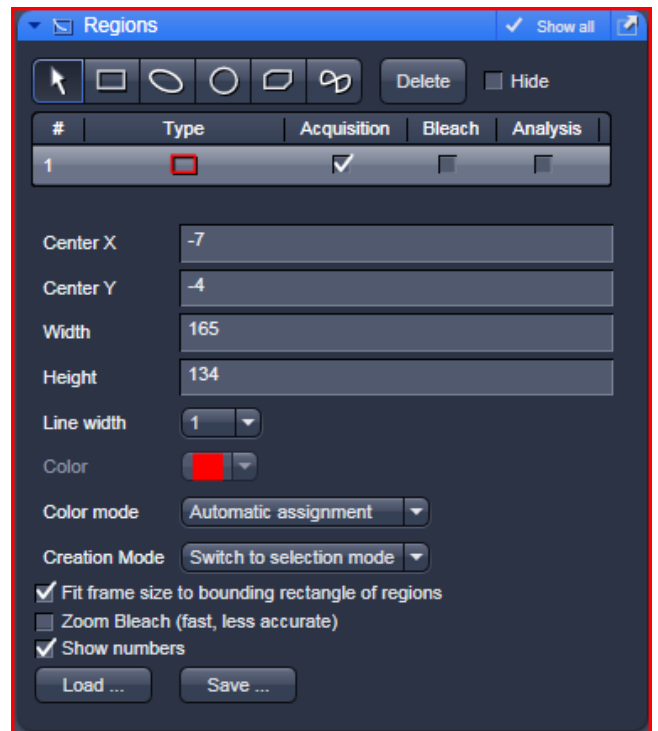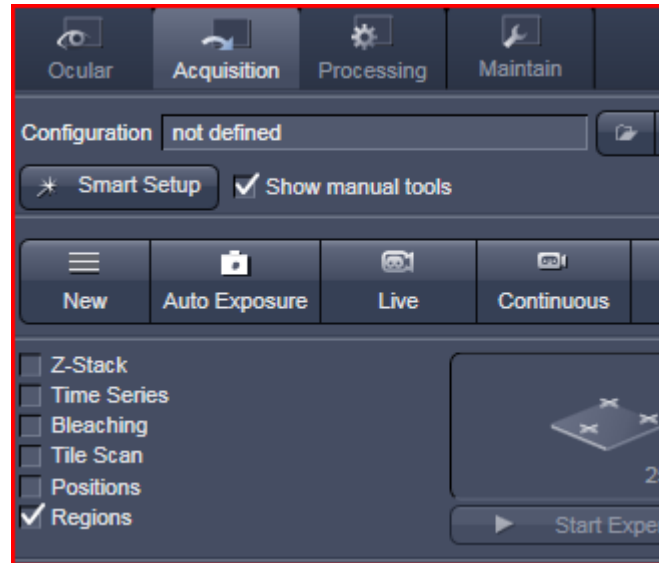
Use the techniques either singly or in combination to boost your acquisition speed.

Do keep in mind that there is a trade-off here: higher acquisition speeds generally mean lower-quality images; but the difference between a two minute acquisition time and a seventy millisecond acquisition time can be significant!