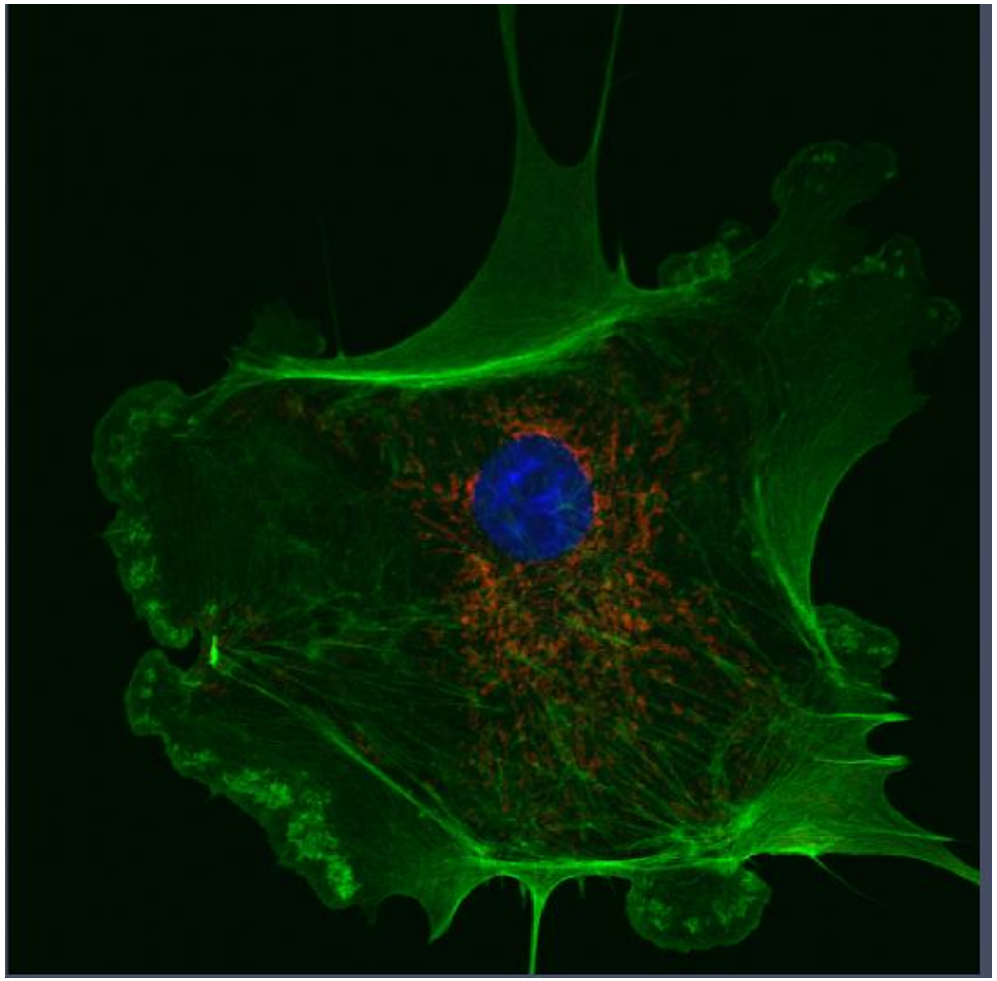
FRAP on LSM710 and LSM780

Kim Peifley

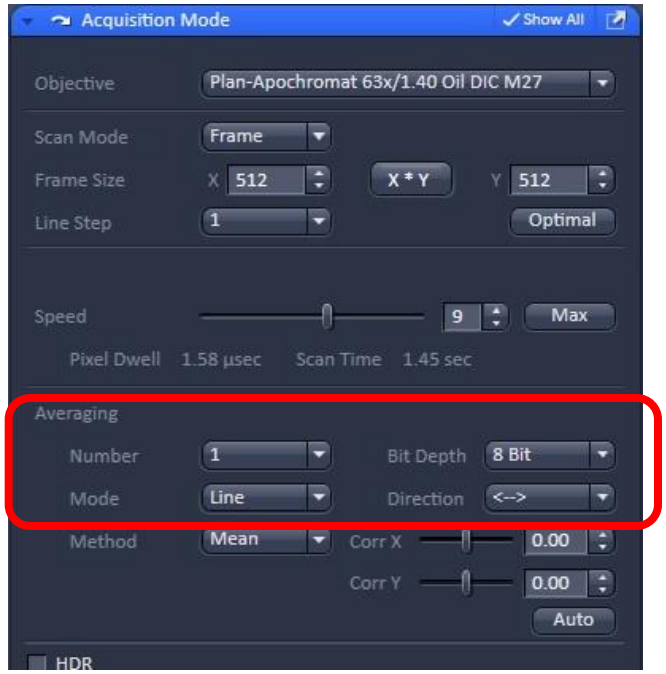
07/20/15

1. Get your image settings like normal.



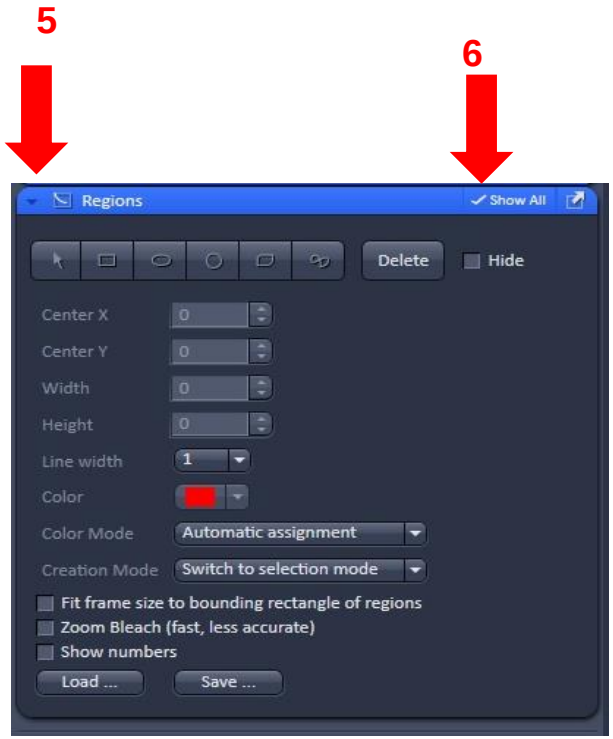
2. **In the Acquisition Mode box make sure the Averaging Number is 1 and Direction is at <-> or bidirectional.**
3. **Check the Bleaching box. The Time series and Regions boxes will automatically be checked too.**

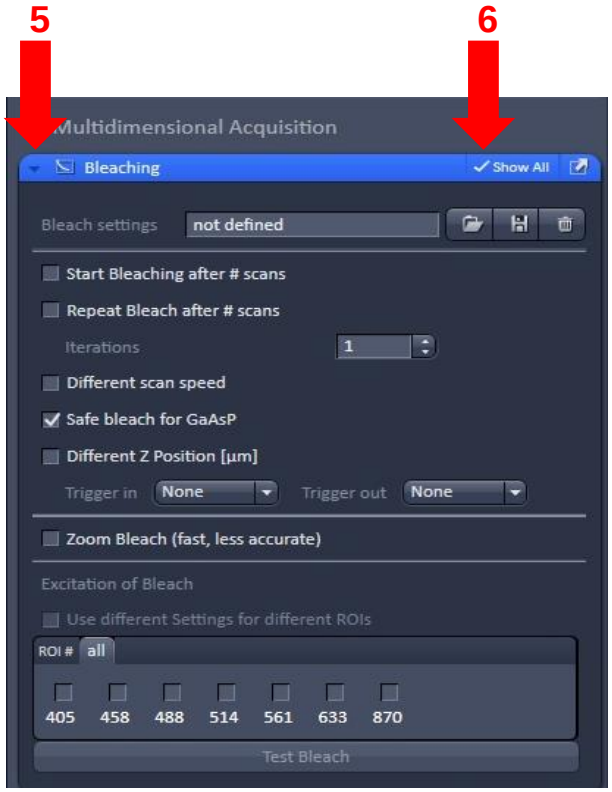
2



3

4. **You will now see the Regions, Bleaching and Time Series boxes.**
5. **Toggle on each box to open them.**
6. **Make sure the Show All box is checked so you can see all the options.**

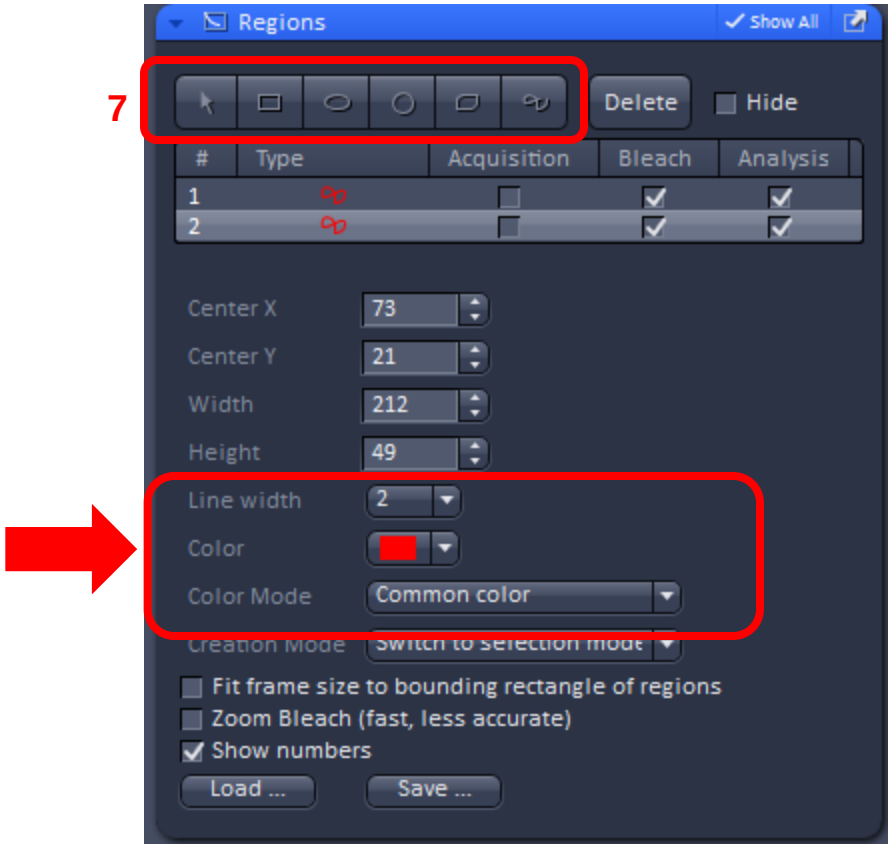


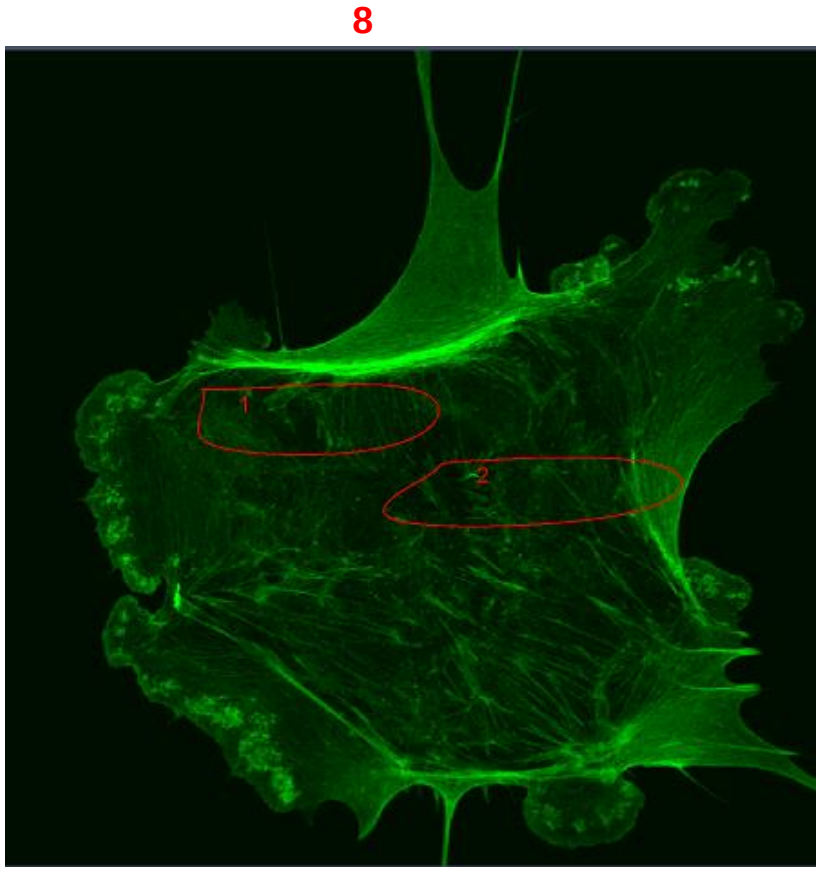


Using just the single color you wish to bleach:

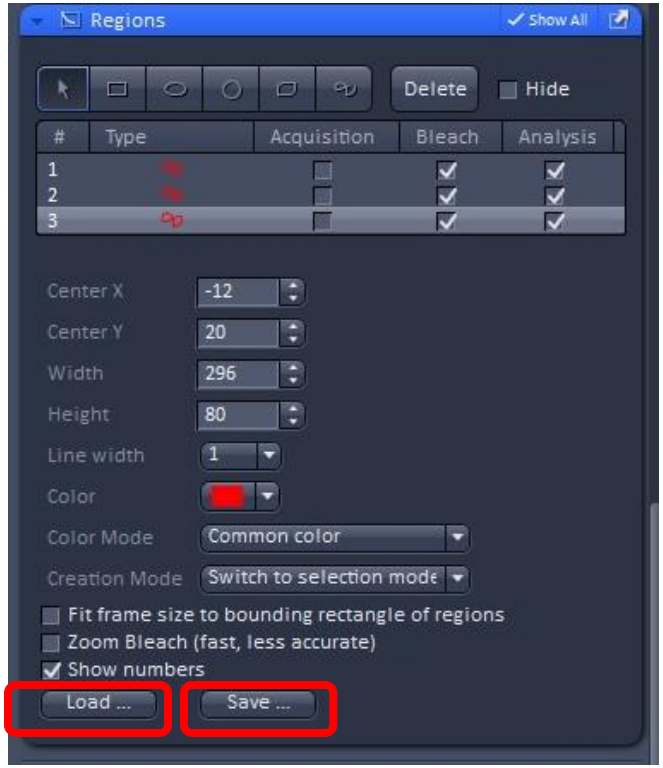
7. **Select the shape you wish to draw your regions.**
8. **Draw the regions.**

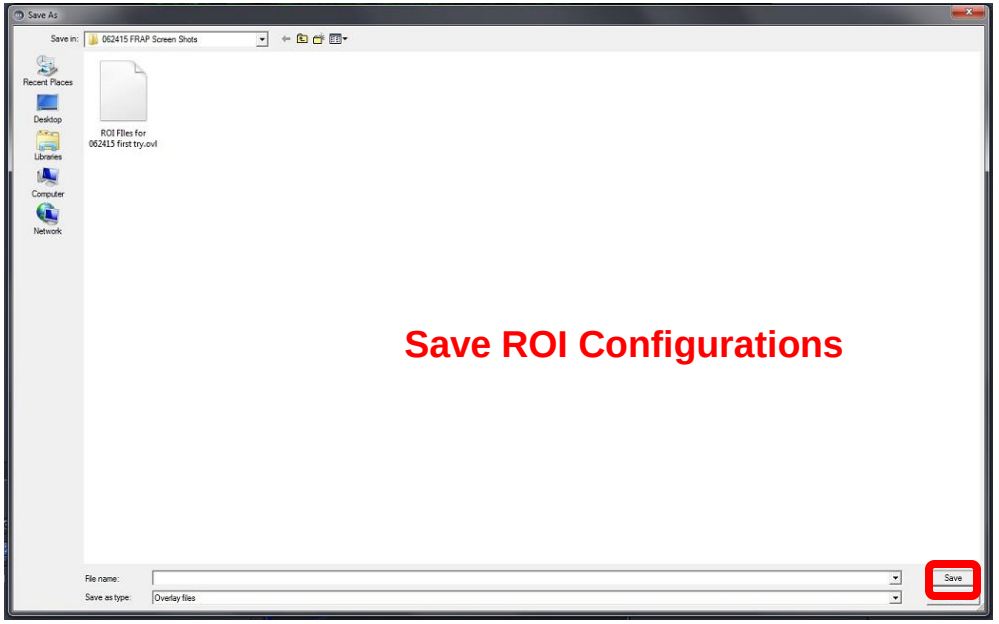
Note: The software automatically assigns a different color to each region and uses a default line width of 1. For purposes of visibility all regions in this tutorial are the same color and a line width of 2. If you wish to have the same color just select the color in the Color drop down box and then select Common color in the Color Mode box. The line width can also be changed in this section. [See red arrow.]



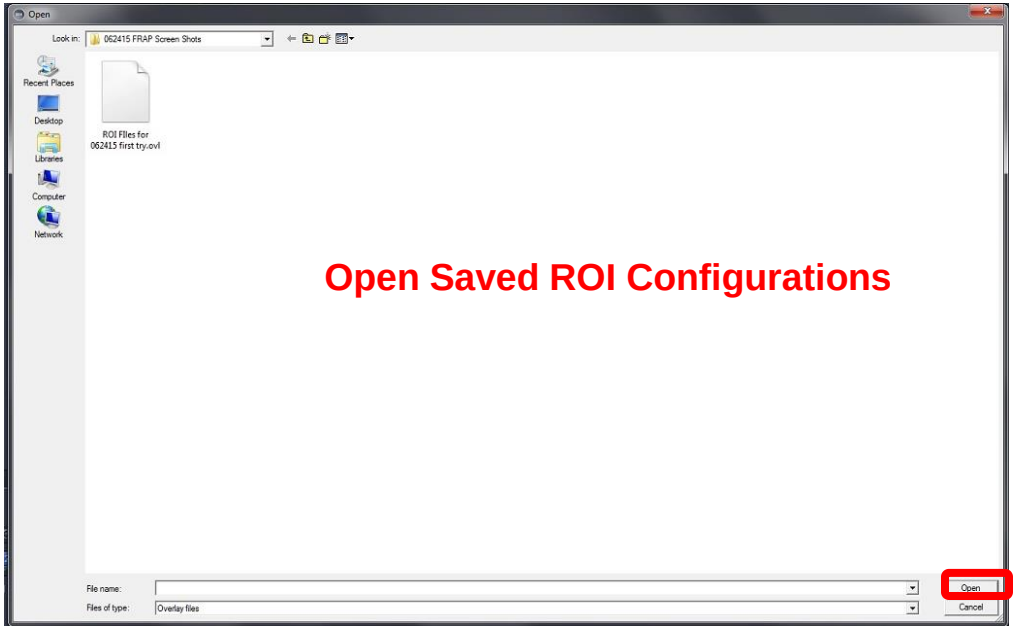


If you want you can save your ROI configurations by clicking Save. To reuse them just click Load.





Save ROI Configurations



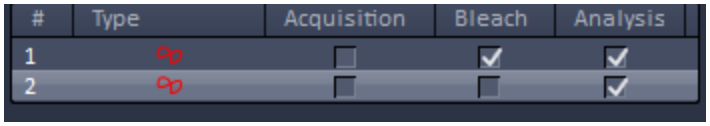
Open Saved ROI Configurations

You will first need to determine the number of iterations and intensity of the laser needed to bleach your sample. These next steps are running that test. You start with low iterations and high laser power.

9. **Select only one region to test.**
10. **In Bleaching Box Check Start Bleaching after # scans and select a number. [In this example it is 2.]**
11. **Select the number of Iterations you wish to do. [In this example 50].**
12. **In Excitation of Bleach select the laser you wish to use and the laser power [Here we are using the 488nm laser at 100%].**
13. **Click Test Bleach.**
14. **Click Snap to see image of your test.**

Repeat this until you find the iterations that bleaches the sample then try lowering the laser power until you get the ideal iterations and laser power. You may need to use several regions for this so remember to uncheck and check the appropriate Bleach boxes.

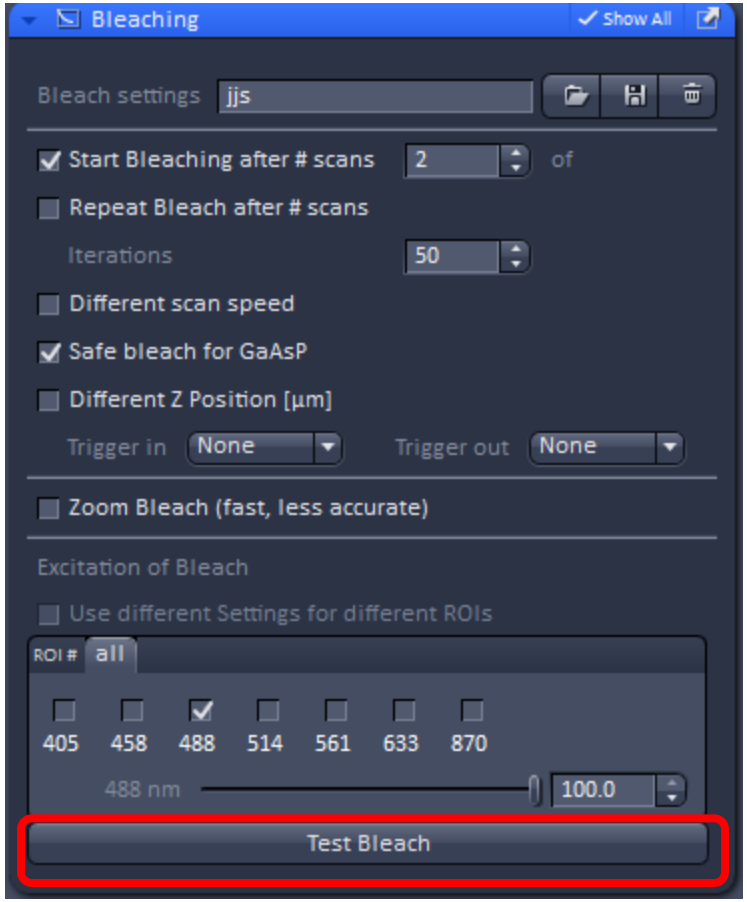
9



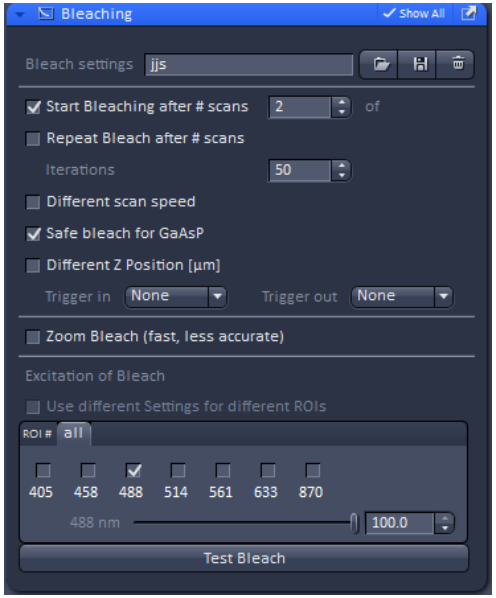
10

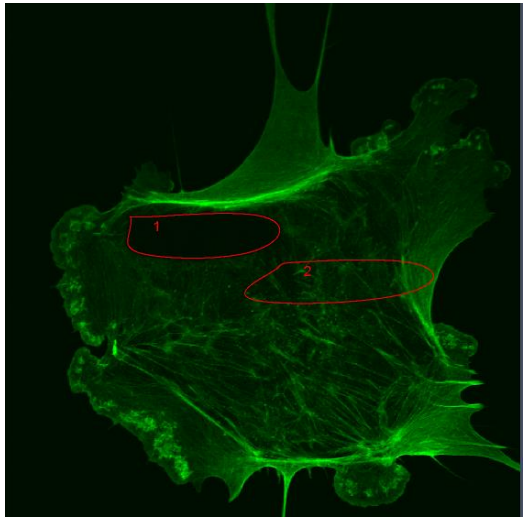
12

13

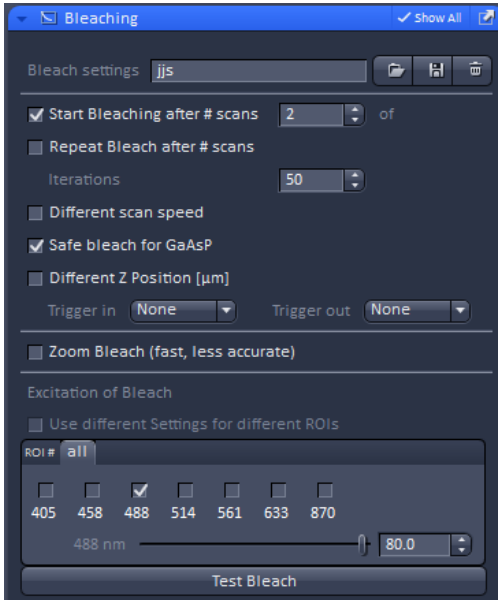


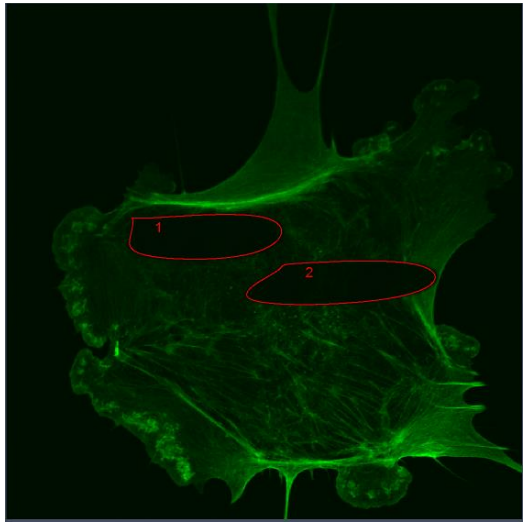
Test Region 1:
50 Iterations, 100% Laser Power



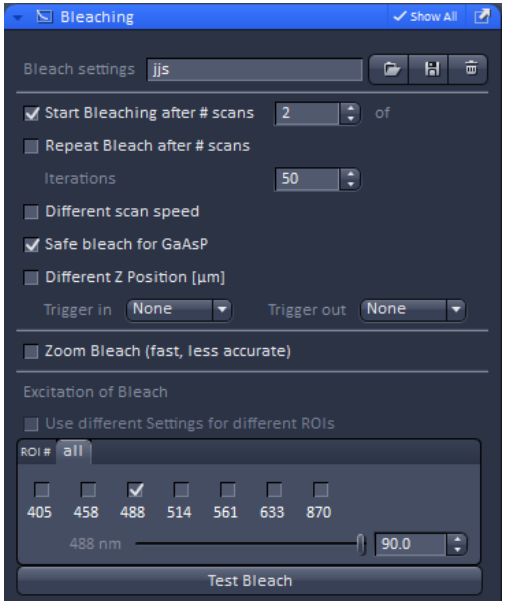


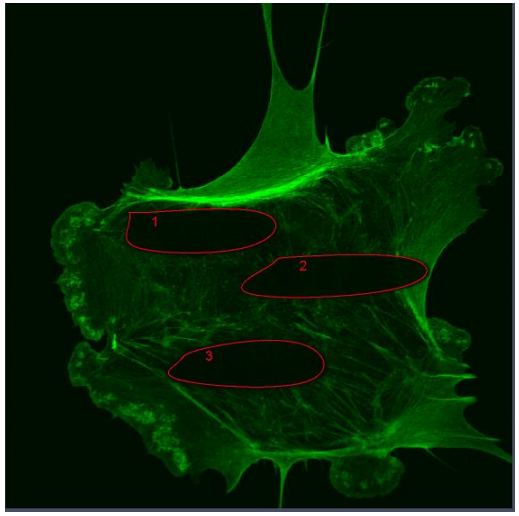
Test Region 2:
50 Iterations, 80% Laser Power





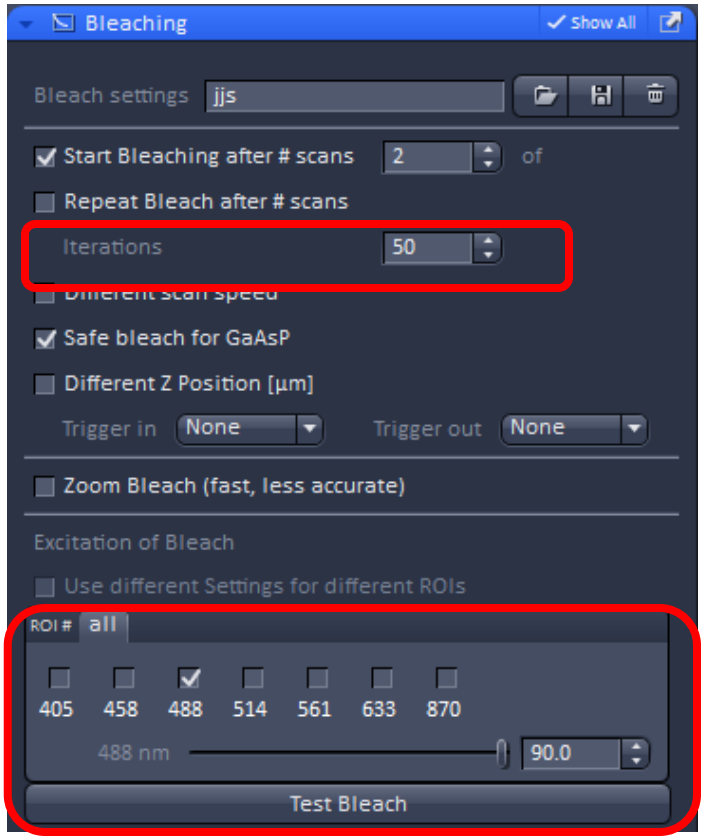
Test Region 3:
50 Iterations, 90% Laser Power

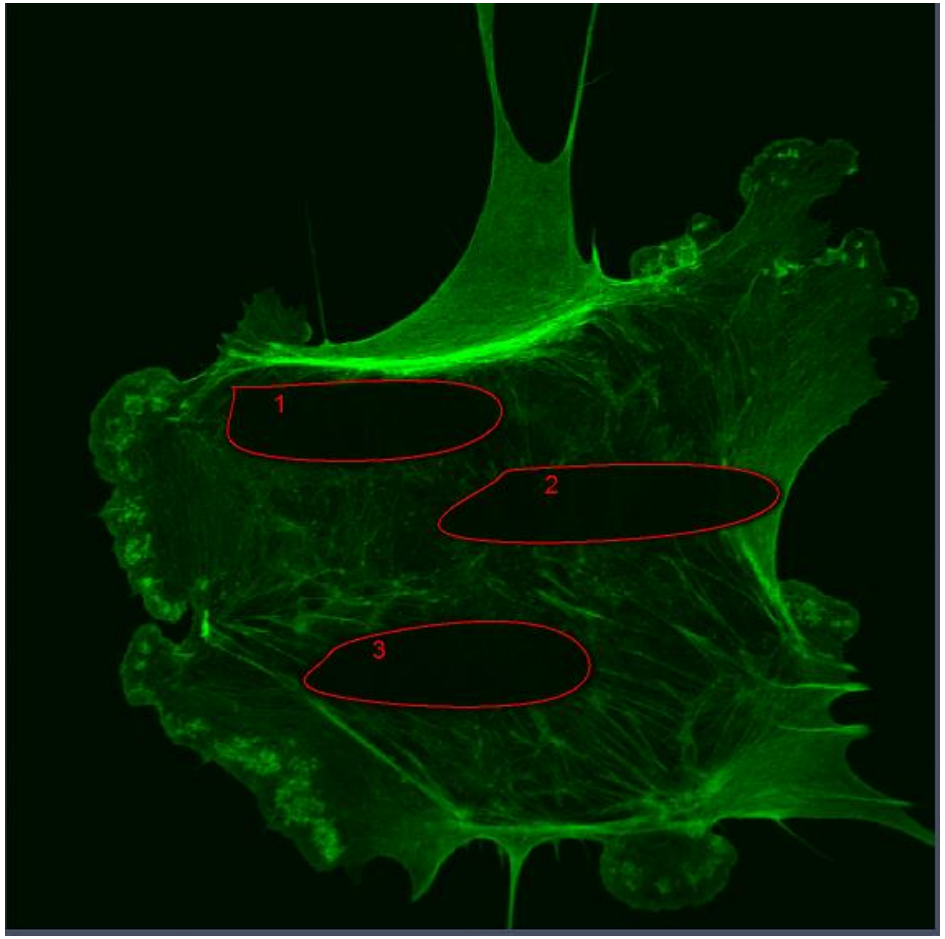




Based on these tests we determined the best settings were:

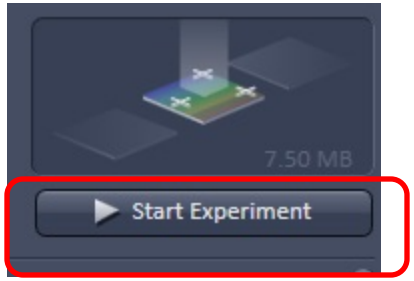
50 Iterations and 90% laser power.





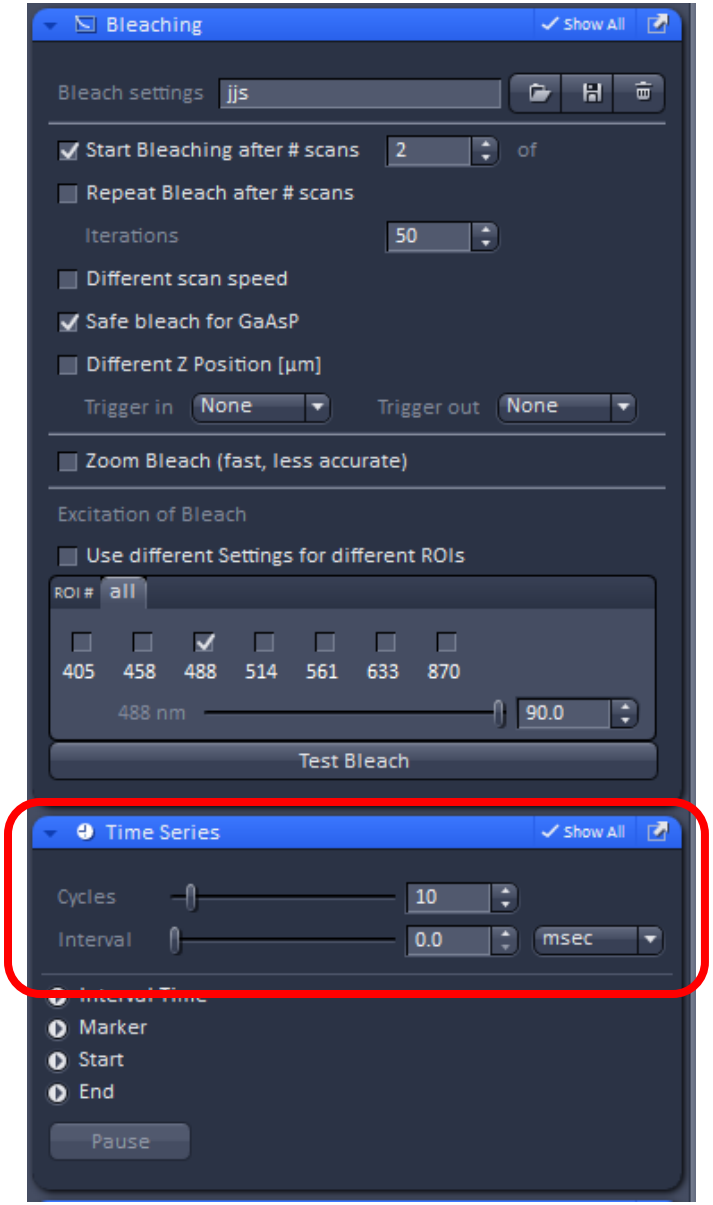
Now move to your experiment area and draw the regions you wish to bleach.

15. **In the Time series box set the number of cycles and the Interval between the cycles. In this case 10 Cycles with 0 [zero] msec Interval.**
16. **Click Start Experiment.**

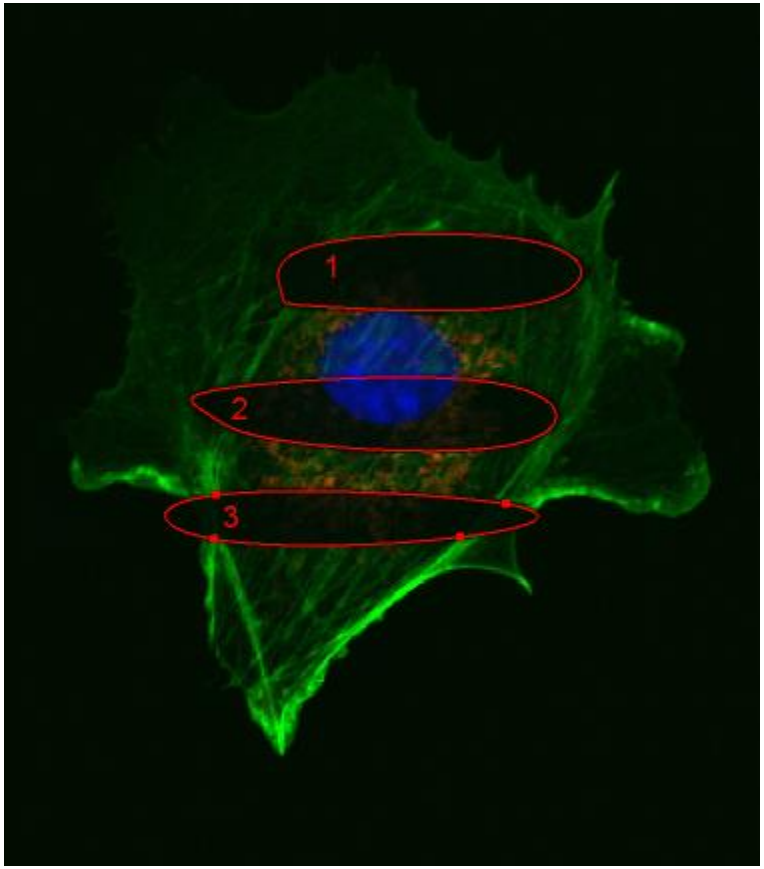


16

15



Final 3 color image with bleached regions.



In gallery mode you can see all 10 scans. The first two scans then the before bleaching then the 8 following scans showing the bleached region.