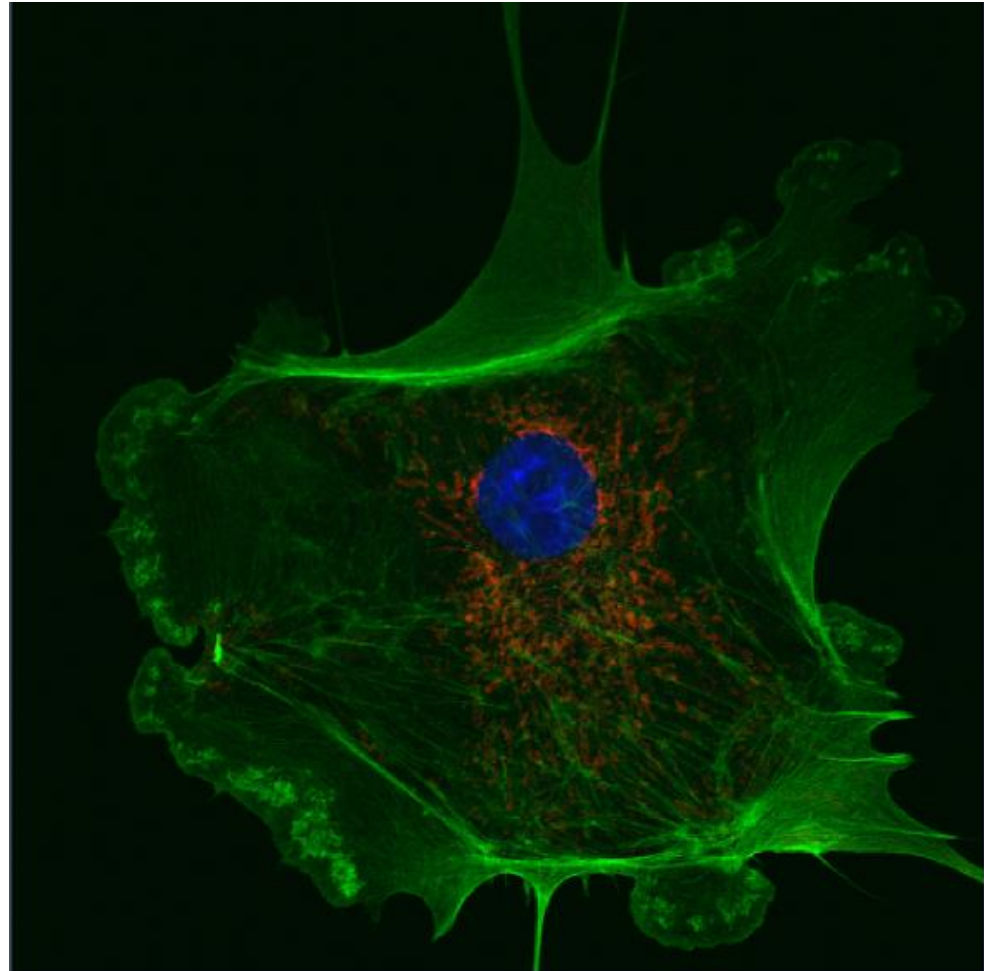


FRAP on LSM710 and LSM780

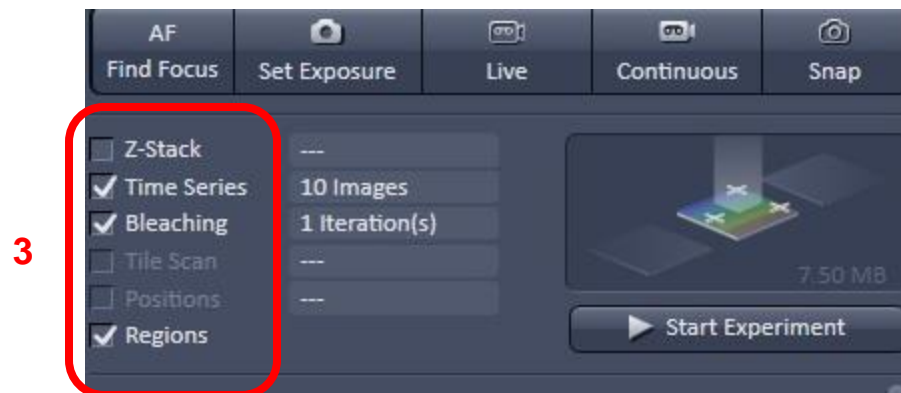
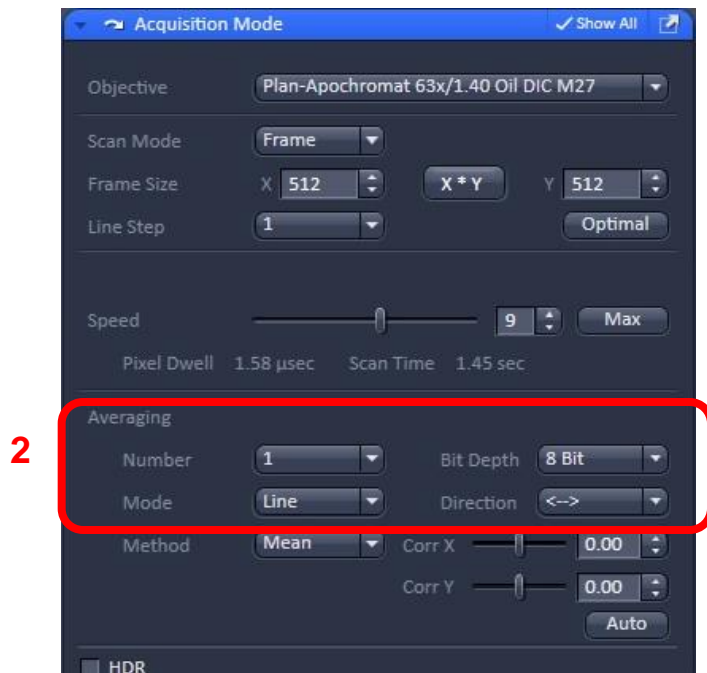
Kim Peifley

07/20/15

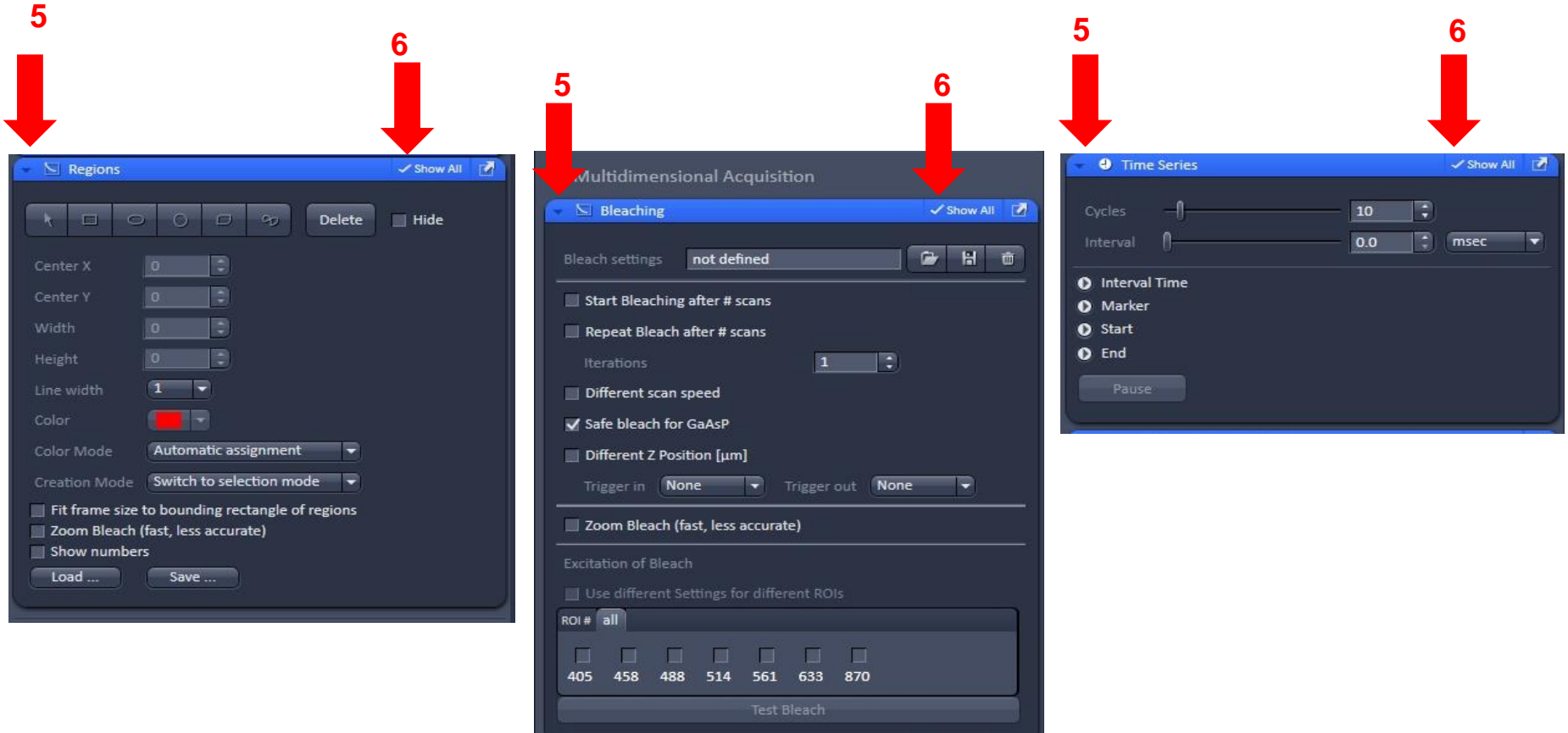
1. Get your image settings like normal.



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.



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.]

The image shows the 'Regions' software interface on the left and a fluorescence image on the right. The interface includes a toolbar with drawing tools (arrow, rectangle, ellipse, circle, polygon, lasso) and a table with columns for #, Type, Acquisition, Bleach, and Analysis. Below the table are input fields for Center X, Center Y, Width, and Height. A red arrow points to the 'Line width' (set to 2), 'Color' (set to red), and 'Color Mode' (set to 'Common color') settings. The fluorescence image on the right shows a green-stained cell with two red regions labeled 1 and 2, with a red '8' above it.

#	Type	Acquisition	Bleach	Analysis
1	sp	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
2	sp	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

Center X: 73
Center Y: 21
Width: 212
Height: 49
Line width: 2
Color: [Red]
Color Mode: Common color
Creation Mode: Switch to selection mode
 Fit frame size to bounding rectangle of regions
 Zoom Bleach (fast, less accurate)
 Show numbers
Load ... Save ...

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

Save ROI Configurations

Regions ✓ Show All

Delete Hide

#	Type	Acquisition	Bleach	Analysis
1		<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
2		<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
3		<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

Center X: -12
Center Y: 20
Width: 296
Height: 80
Line width: 1
Color: ■
Color Mode: Common color
Creation Mode: Switch to selection mode

Fit frame size to bounding rectangle of regions
 Zoom Bleach (fast, less accurate)
 Show numbers

Load ... Save ...

Save As

Save in: 062415 FRAP Screen Shots

ROI Filter for 062415 first try.ovl

File name:

Save as type: Overlay files

Save

Open

Look in: 062415 FRAP Screen Shots

ROI Files for 062415 first try.ovl

File name:

Files of type: Overlay files

Open

Cancel

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

#	Type	Acquisition	Bleach	Analysis
1		<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
2		<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>

10

12

13

Bleaching

Bleach settings: jjs

Start Bleaching after # scans: 2 of

Repeat Bleach after # scans

Iterations: 50

Different scan speed

Safe bleach for GaAsP

Different Z Position [μm]

Trigger in: None Trigger out: None

Zoom Bleach (fast, less accurate)

Excitation of Bleach

Use different Settings for different ROIs

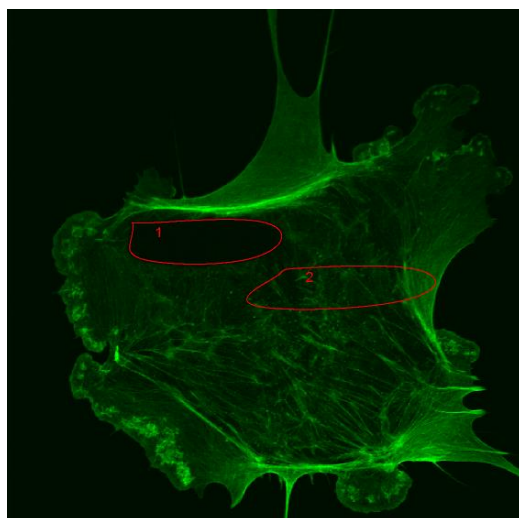
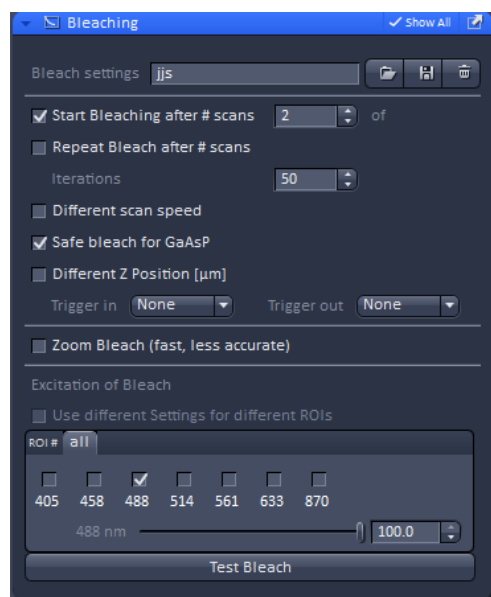
ROI #: all

405 458 488 514 561 633 870

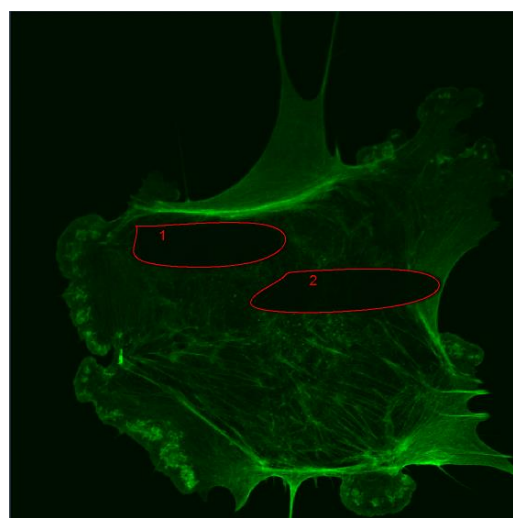
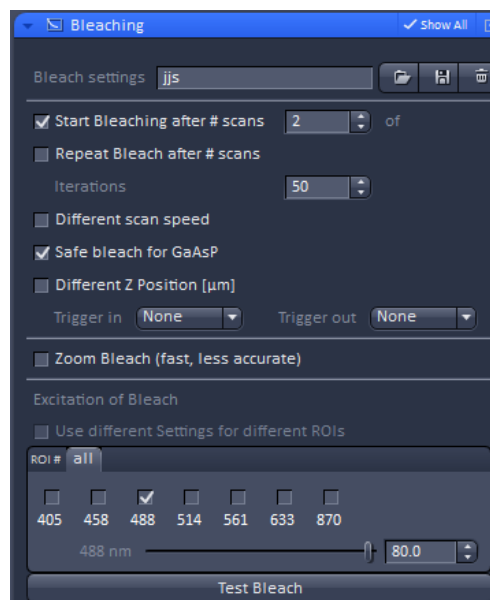
488 nm 100.0

Test Bleach

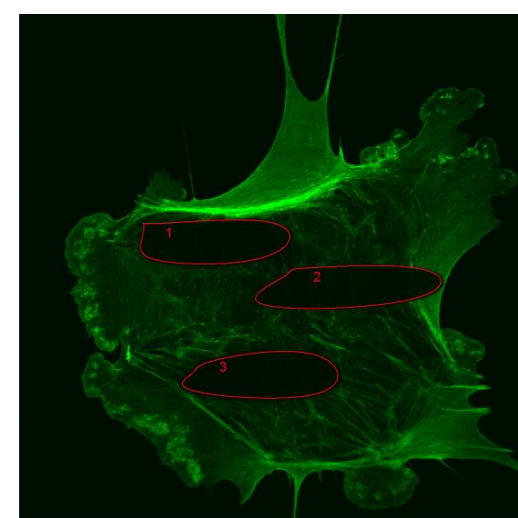
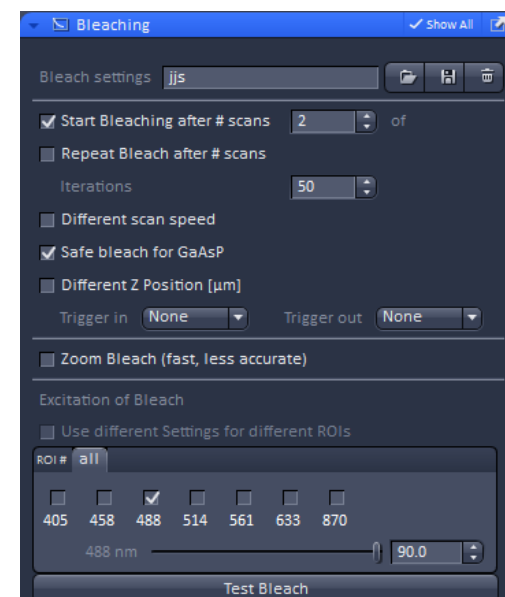
Test Region 1: 50 Iterations, 100% Laser Power



Test Region 2: 50 Iterations, 80% Laser Power

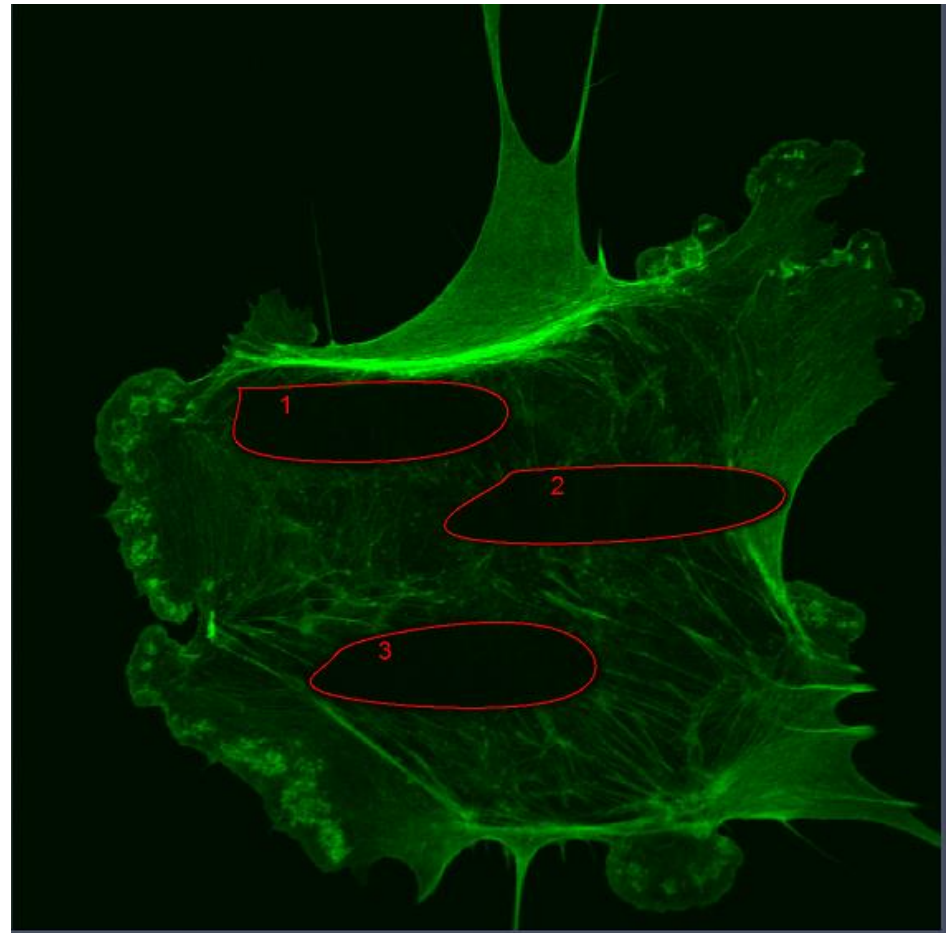
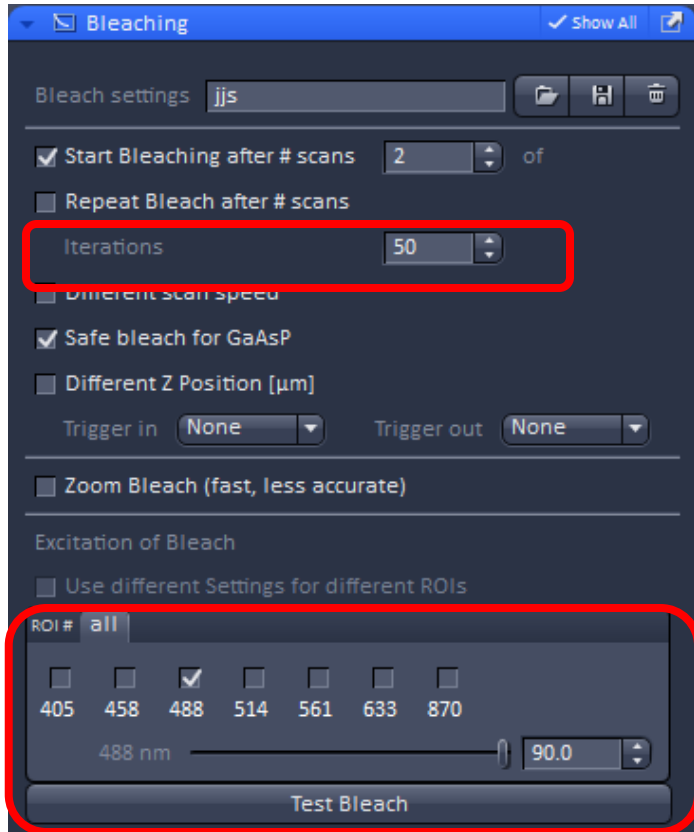


Test Region 3: 50 Iterations, 90% Laser Power



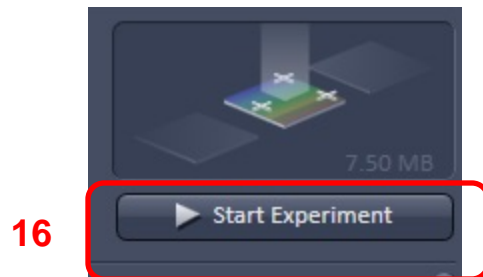
Based on these tests we determined the best settings were:

50 Iterations and 90% laser power.

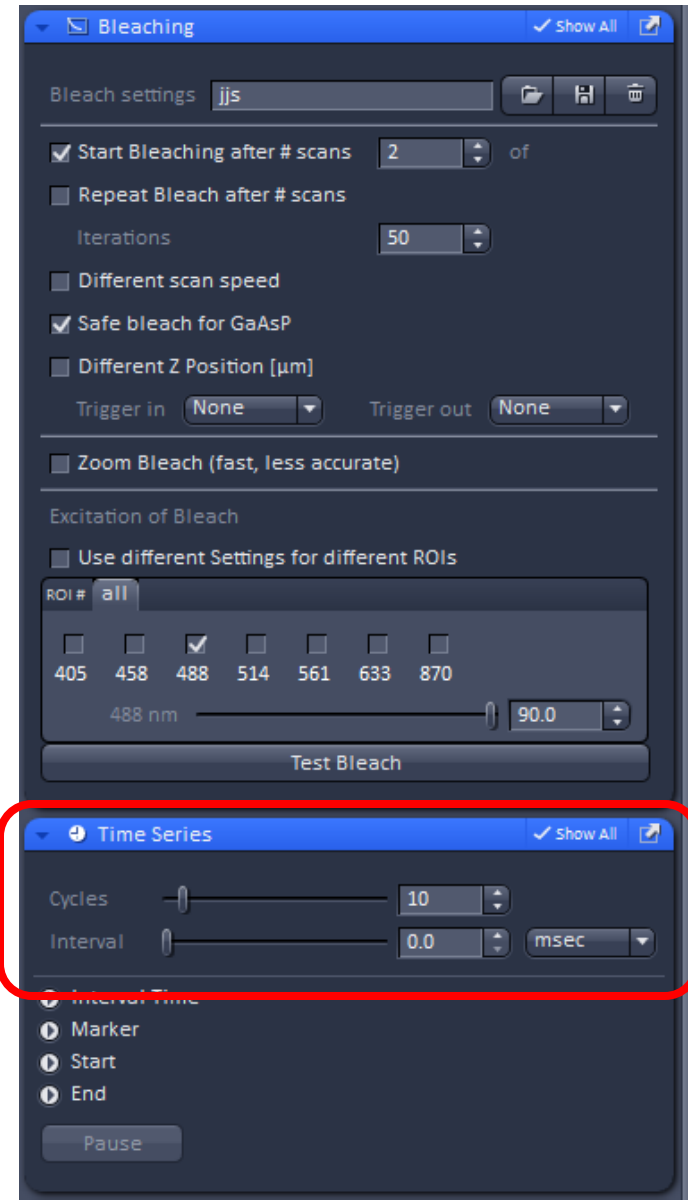


Now move to your experiment area and draw the regions you wish to bleach.

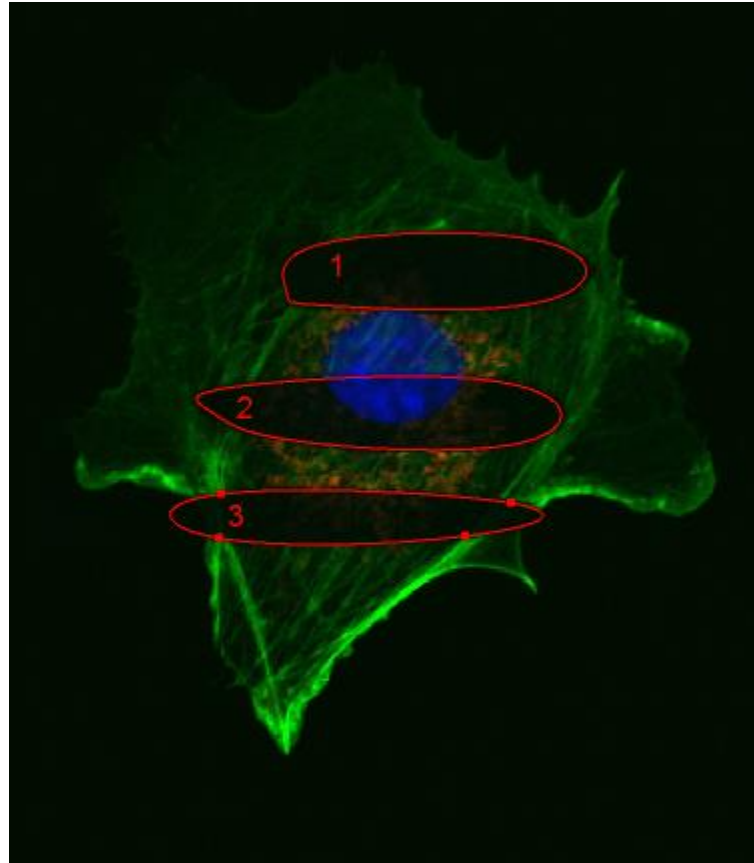
15. In the Time series box set the number of cycles and the Interval between the cycles. In this case 10 Cycles with 0 [zero] msec Interval.
16. Click Start Experiment.



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.

Bleaching

