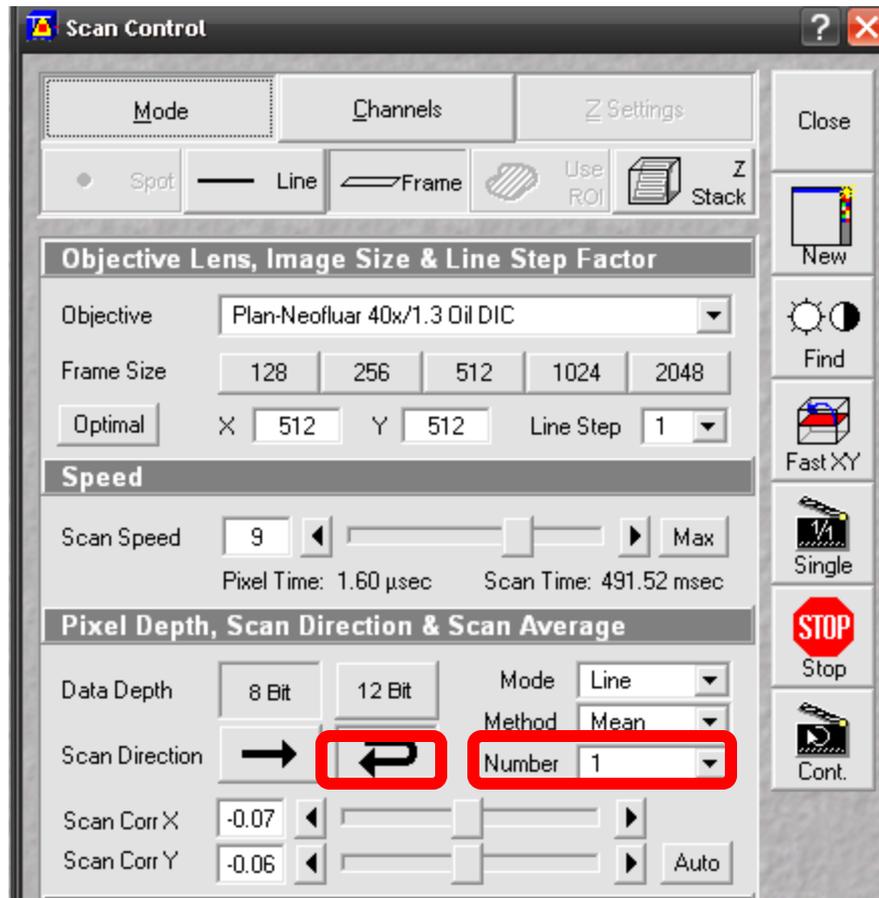


# **FRAP On the 510s**

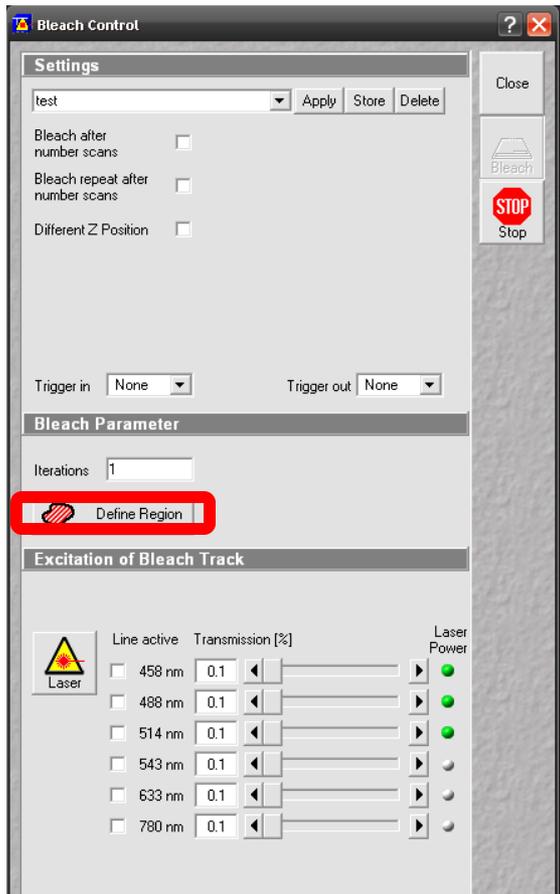
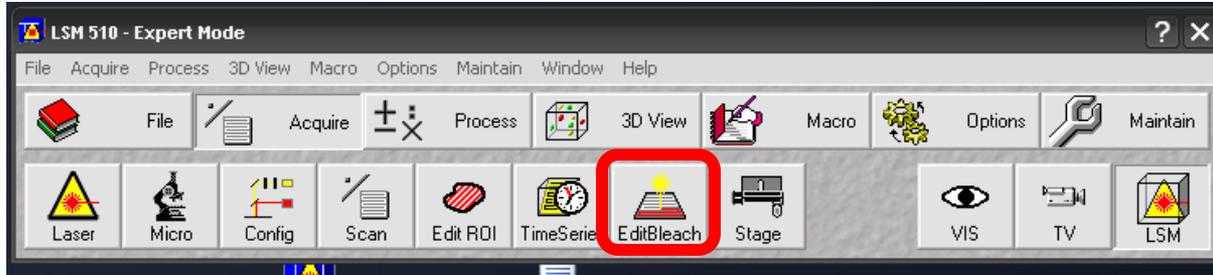
Kim Peifley

10/29/14

1. Set up track/s you are going to use
2. Get settings for the sample. **Do not use averaging** and make sure to set scan direction to **Bidirectional**.

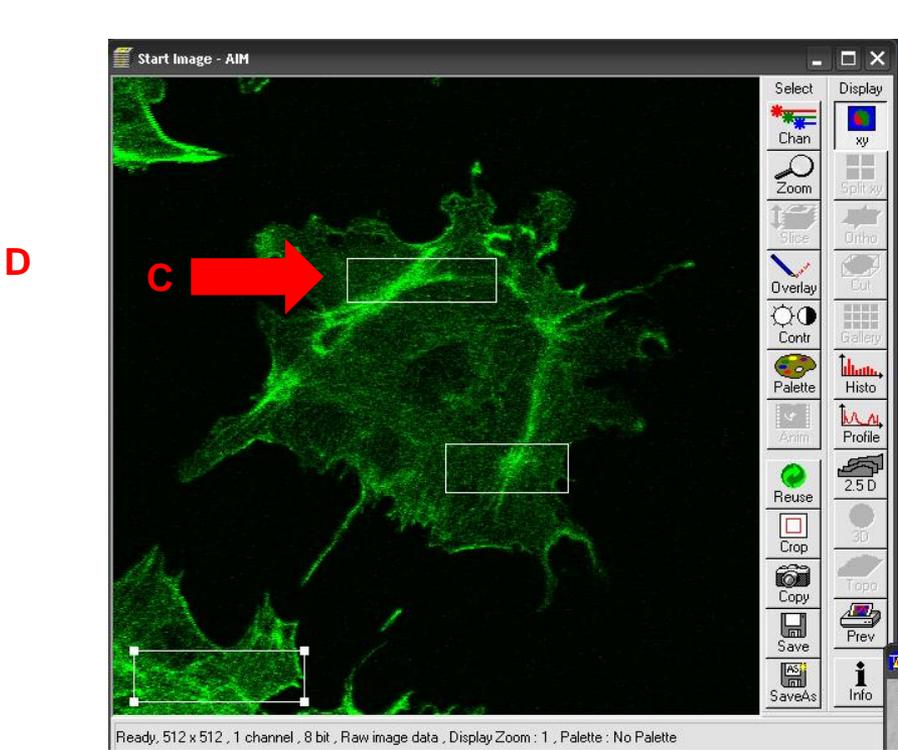
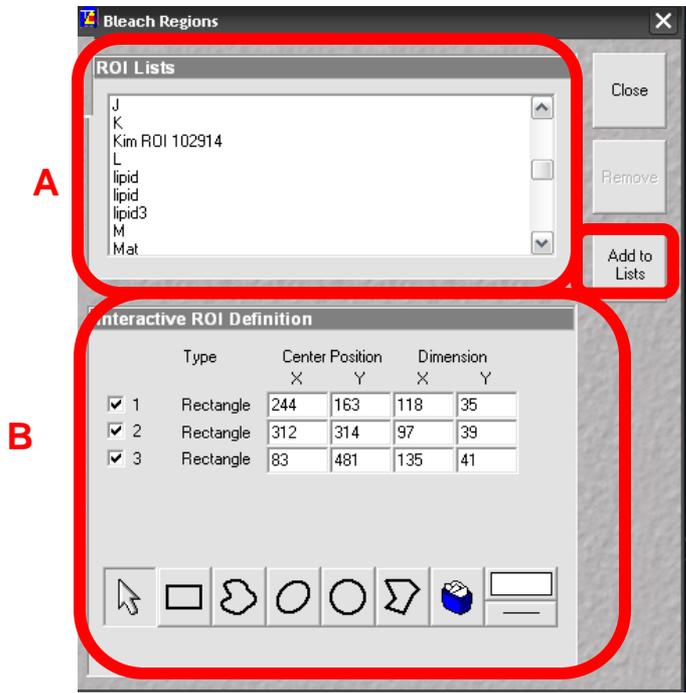


### 3. Click the Edit Bleach button to get the Bleach Control Box

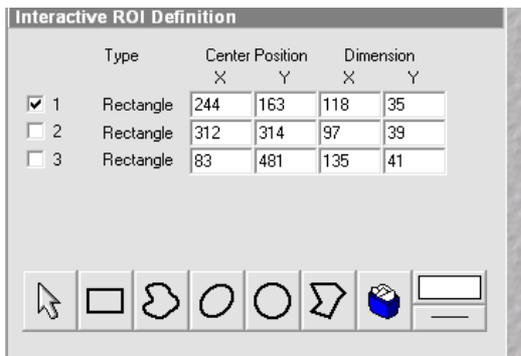


### 4. In Bleach Parameter box click define region

5. Select region(s) from ROI Lists Box (A) or Create ROIs by clicking on the shape (B) and then drawing the shape on the image (C). Note: You should draw ROIs for the whole imaging session and save as one ROI file. This allows you to just check the ROI you wish to use each scan. All ROIs should be horizontal rectangular boxes to provide for faster scan time.



6. If you are creating new ROIs click Add to Lists (D), give it a name then click OK.



First you will need to determine the number of iterations and intensity of laser. This next step is to test your settings.

7. Select only one region to test.

8. Bleach Control Box settings:

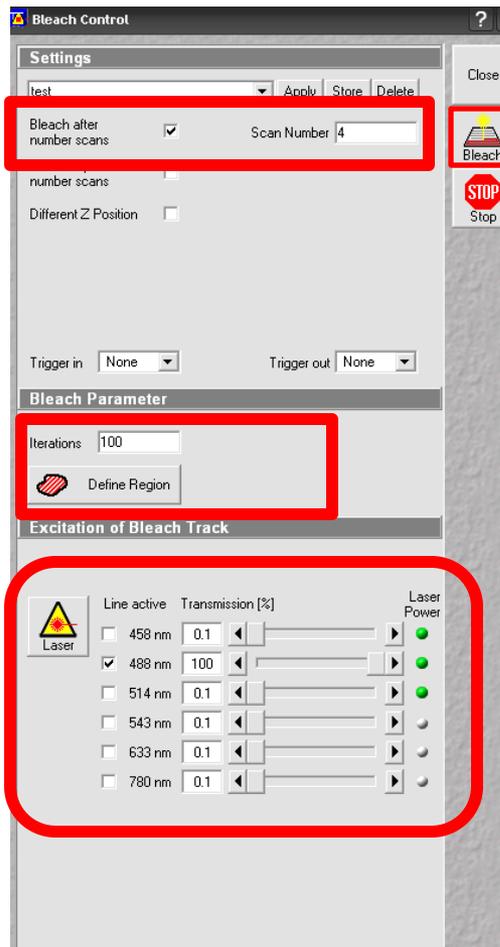
a) Check the Bleach after number scans box.

b) Enter Scan Number [In this example it is 4]

c) Under Bleach Parameter enter the number of Iterations. [In this example it is 100].

d) In Excitation of Bleach Track select the laser line or lines you are using for bleaching and the power of the laser line(s). [In this example the 488nm laser line at 100%.]

e) Click Bleach to start the experiment.

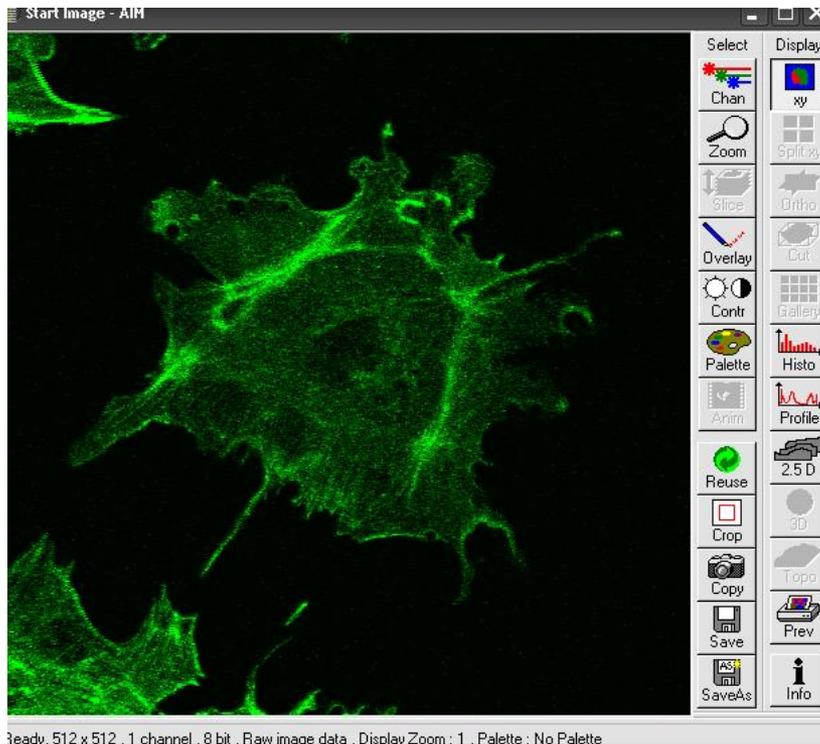


You will not see anything on the screen while the bleaching is occurring. You will see the laser flashing from the objective to know that it is bleaching.

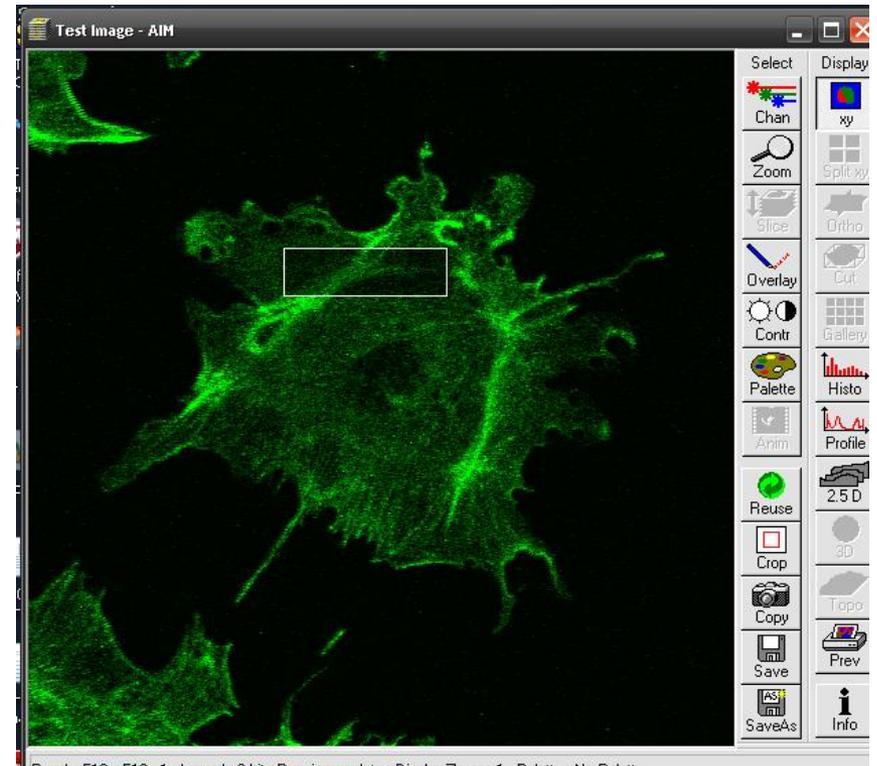
9. Take an image to test out your conditions.

10. Adjust if necessary. Play with the iterations and laser power and which laser line[s] to use to get what you want.

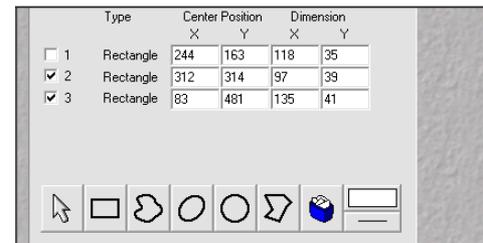
Before Bleach



After Bleach



11. Go back to the Bleach Regions box: Uncheck the test ROI and check the ROIs you wish to use for the experiment.



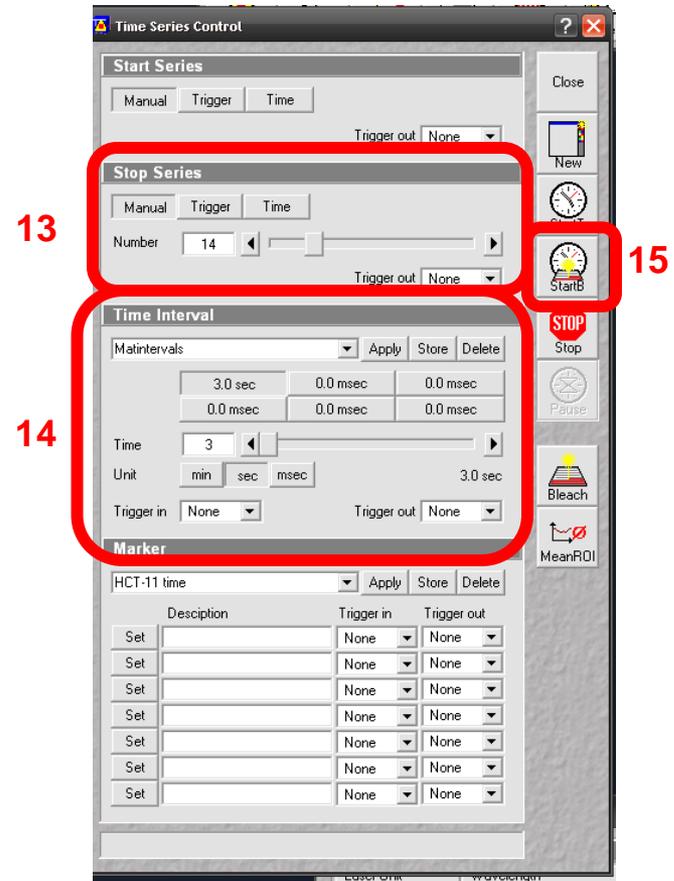
12. After bleaching your sample you will need to take images of the whole view field so you now need to go in to the Time Series window.



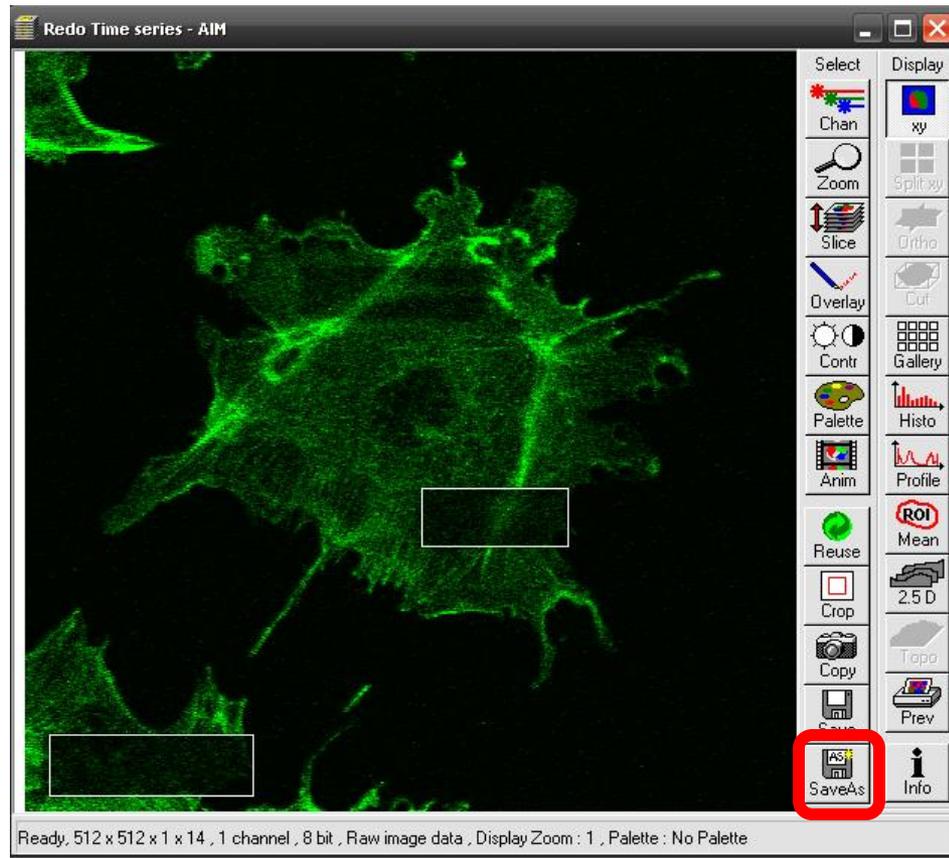
13. Determine the total number of images you need – pre-bleach + post-bleach. This is entered in the Number box under Stop Series. In this example we have 4 images pre-bleach + 10 images post-bleach = 14 images.

14. Determine how much time you want between images and enter that in the Time Interval Box. In this example it is 3 seconds. Double check that you have selected the correct unit – min, sec or msec.

15. Click Start B to start bleach.

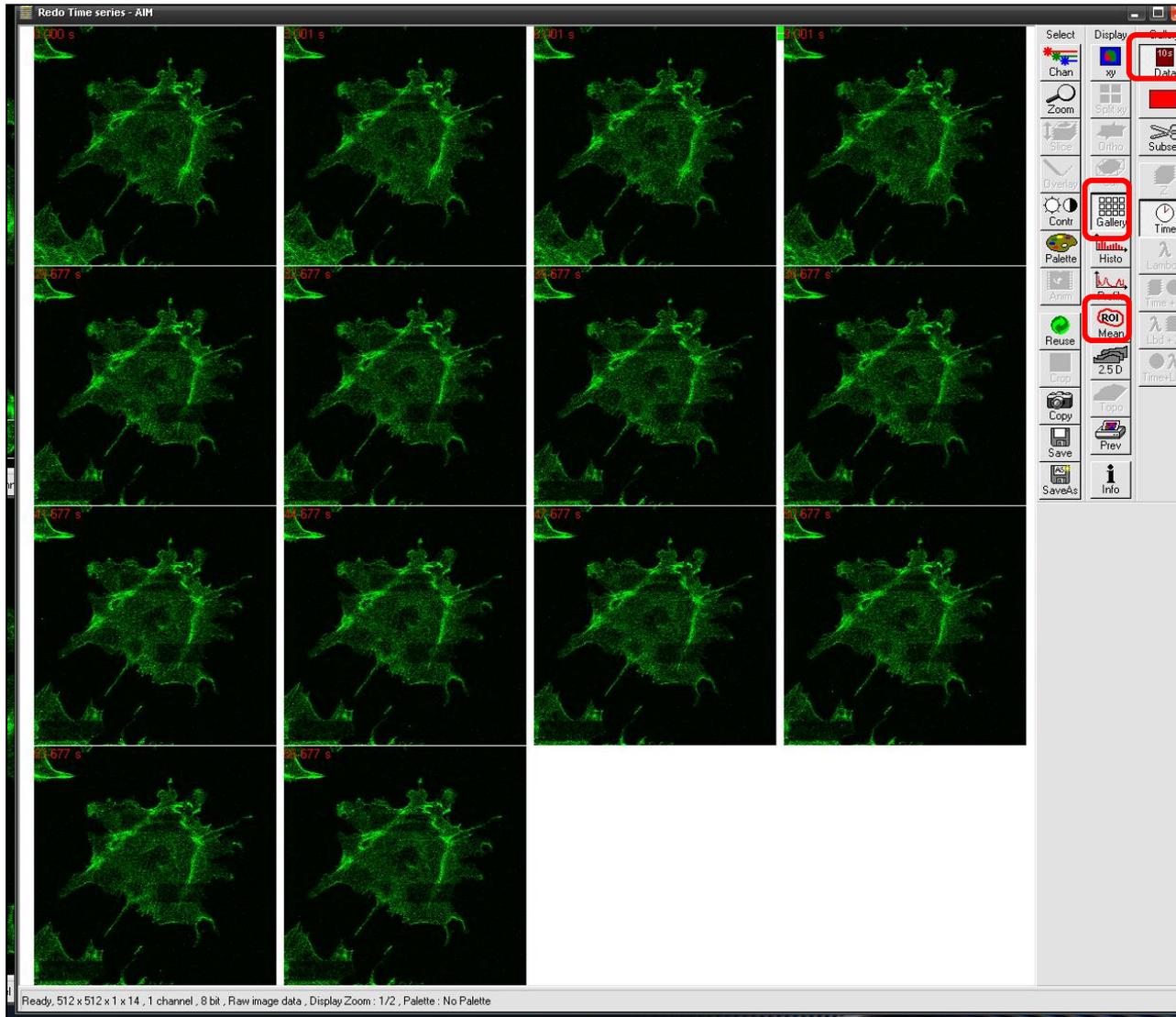


## 16. Save Image



## Additional Information:

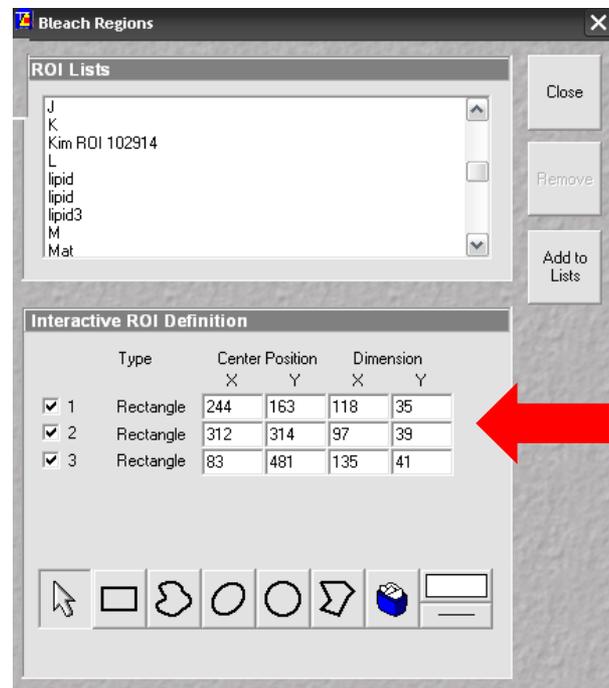
When you are in Gallery mode you can click the Data button to add time each image was taken. The Mean ROI button will measure intensity in each region.



## Additional Information Continued:

Users will normally want to save ROIs. Saving ROIs is described in Step 6. If you reuse the ROI but move it's location and wish to save those new locations you need to resave them.

If you wish to put the ROIs into an image in analysis you either have to do the analysis on the microscope or in Position and Dimension box you need to write down the locations so you can re-find them in analysis.



ROI locations