

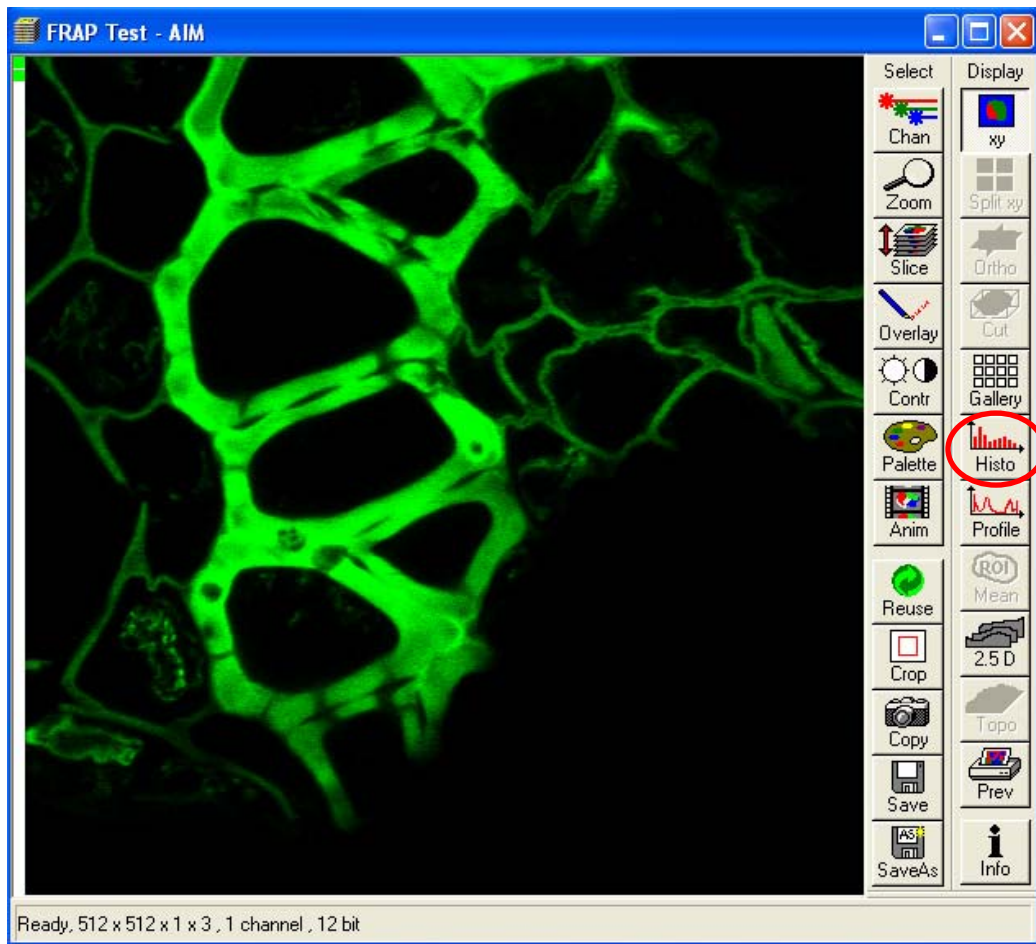


How to quantify the changes in fluorescence intensity in your images

Many times you may want to know if the expression level of a protein in your sample changes under different conditions. For example, people have applied electrical stimulations to the brains of mice to see the changes in amount of specific protein in neurons, a method to examine the activation of specific signaling pathway. The following procedure allows you to quantify (or to ratio) the changes in the expression level of a protein in terms of the changes in fluorescence intensity of dye molecules tagged to the protein.

Step 1 Open the image from your database.

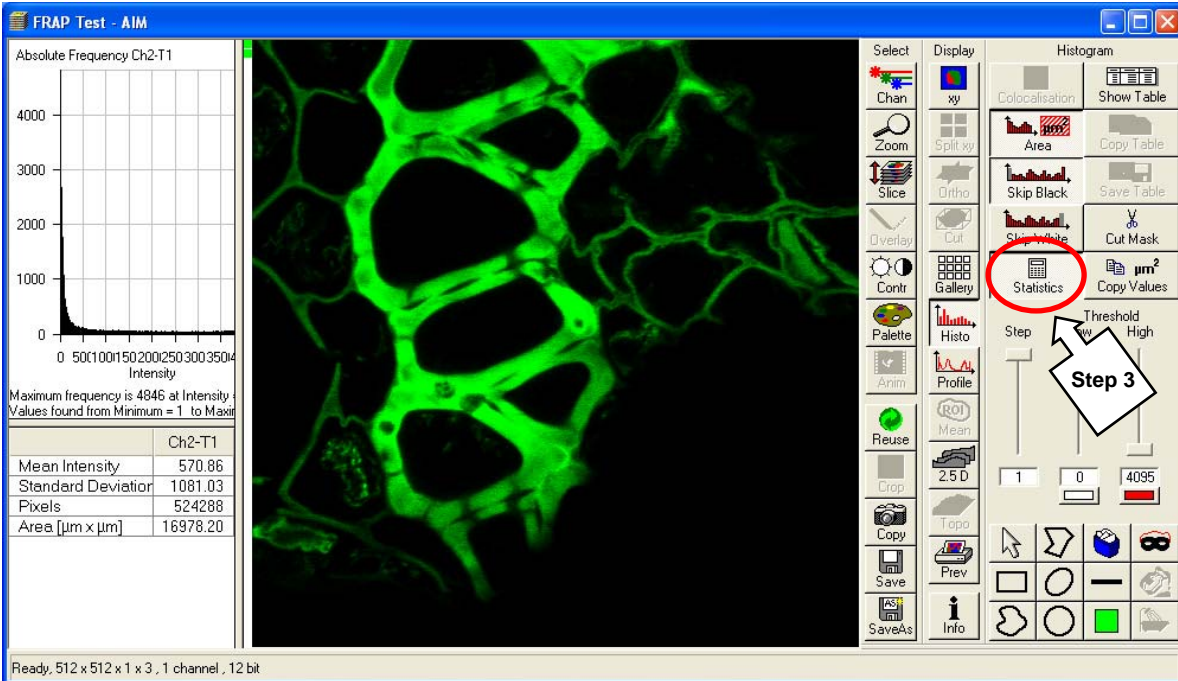
Step 2 Click button *Histo*.





Step 3 Click button **Statistics**

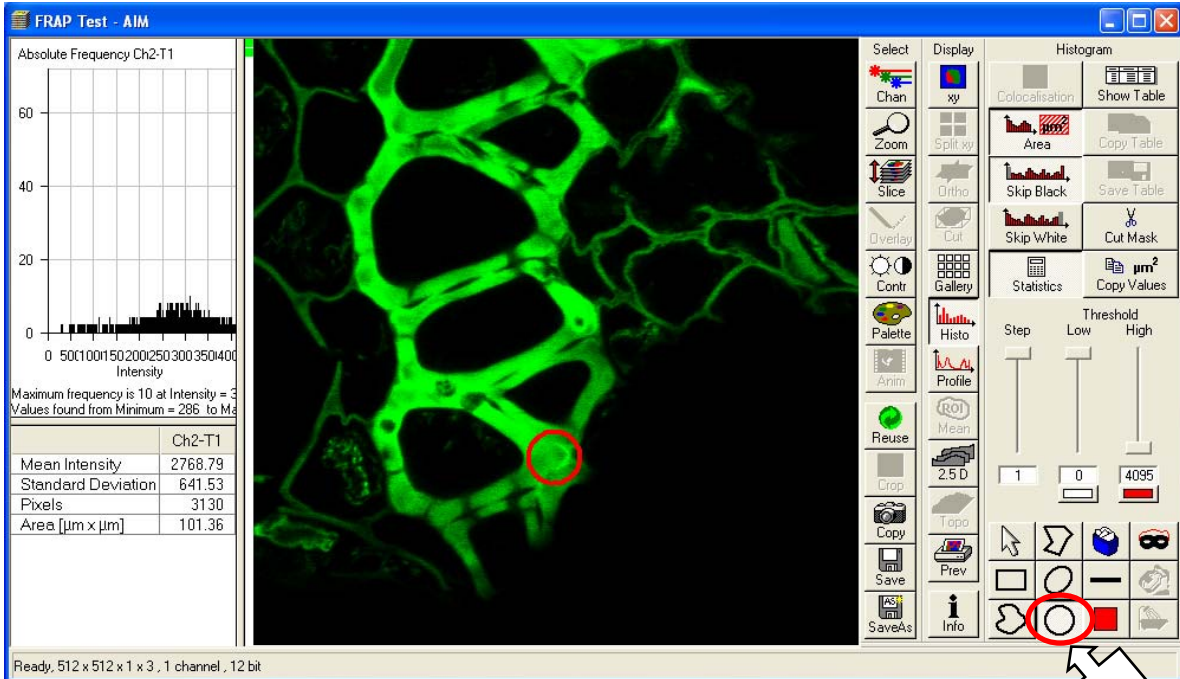
If you look at the left side of the diagram, you can find the *Mean Intensity* of the entire image in the table below the histogram.





However, you may want to concentrate on the region where you can find most of the expressed protein in your image. For this purpose:

Step 4 Select a button with a shape you want to define the region of interest (circle in this example) and draw a circle in the image where you are interested. The *mean intensity* shown in the table reflects the number only in the region you selected.



Step 4

Step 5 Repeat the above steps for another image of your samples with a different expression level of the protein, say, due to an electrical stimulation. Now you can obtain a ratio of the intensities from the two images reflecting the changes in the expression level of the protein under different conditions.