

Citation: Nguyen DH, Zhou T, Shu J, and Mao JH (2013). "Quantifying chromogen intensity in immunohistochemistry via reciprocal intensity." *Cancer InCytes* 2(1):e.

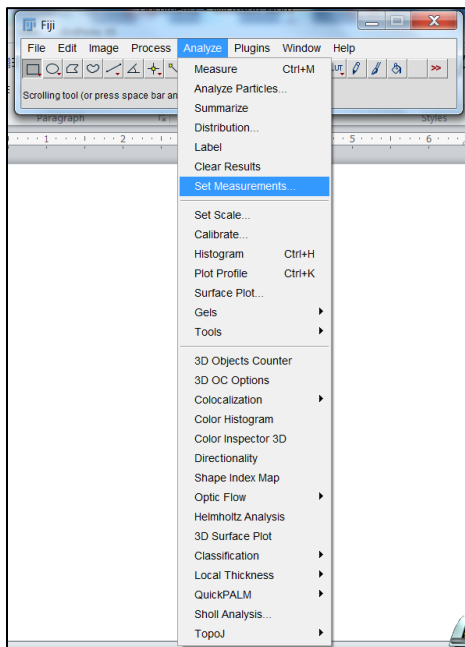
Protocol for Measuring Reciprocal Intensity of a Color Image Using Fiji

Note: This protocol describes how to manually measure the area, mean, mode, and median intensity of an area/shape that is drawn within an image. It does not discuss the following:

- a) How many areas should be measured in each image.
- b) What control areas or other images should be measured in order to obtain a background subtraction, if desired.

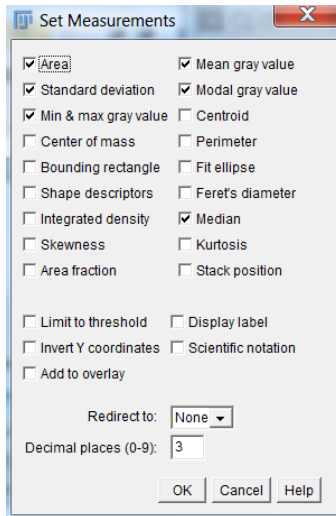
Protocol

1. Open Fiji
2. Pick what features to measure by clicking "Analyze" and then clicking on "Set Measurements".
(See below image)

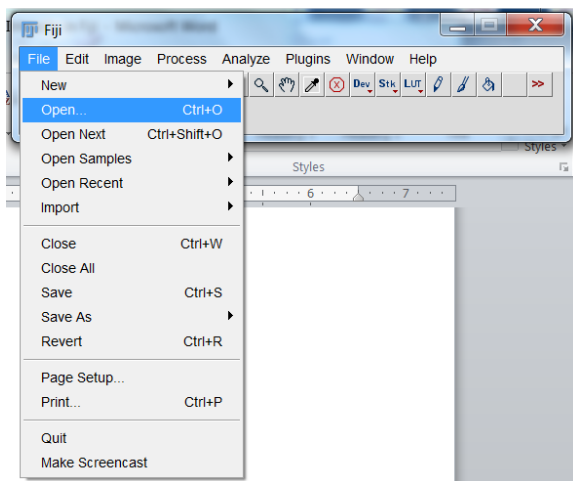


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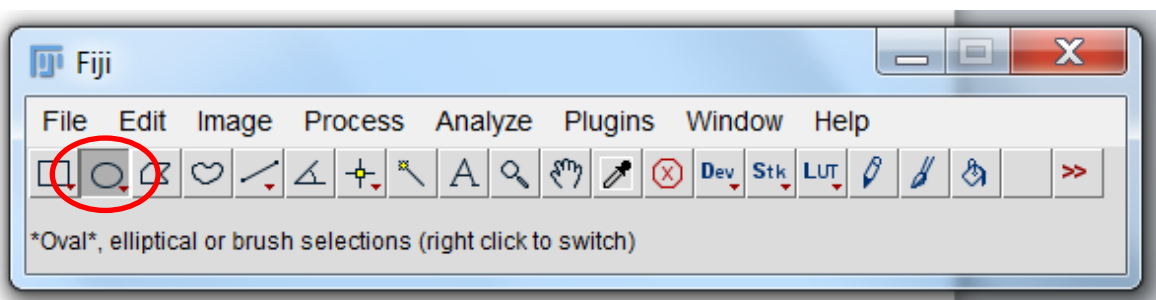
3. Check off the boxes that are marked in the below image. Then click "OK".



4. Open image file.

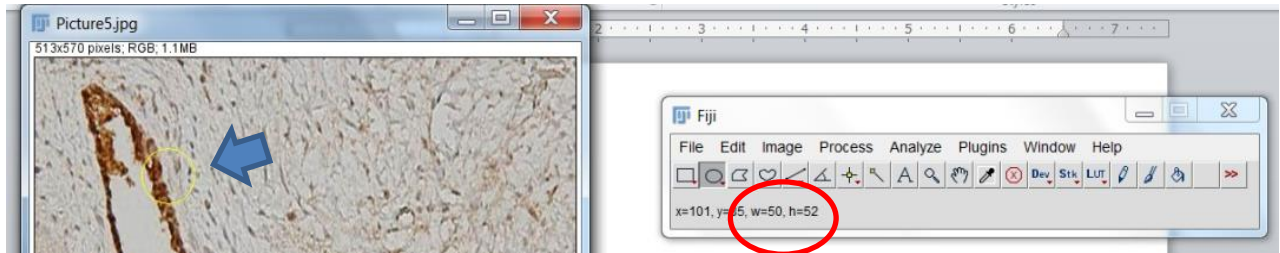


5. Pick the desired shape to draw. A circle or square is easiest. (See below image; red circle)

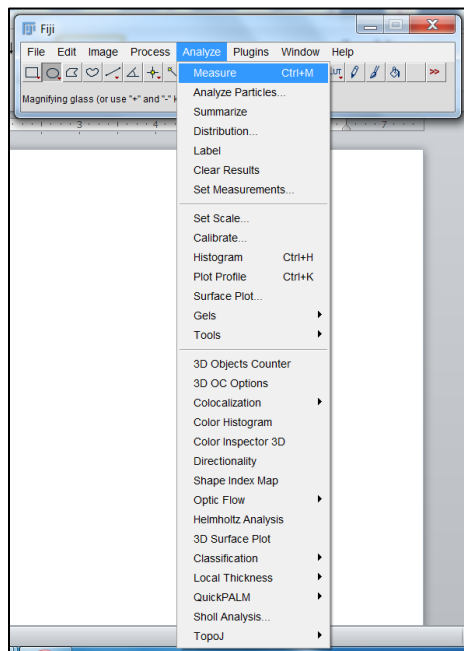


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6. Draw a circle (See blue arrow in image) around the area of interest. Pay attention to the dimensions of the circle as you click down on the image and drag the mouse. Write down the dimensions so that you use the same-sized circle for every measurement in an image and across different images. (See below image; red circle)

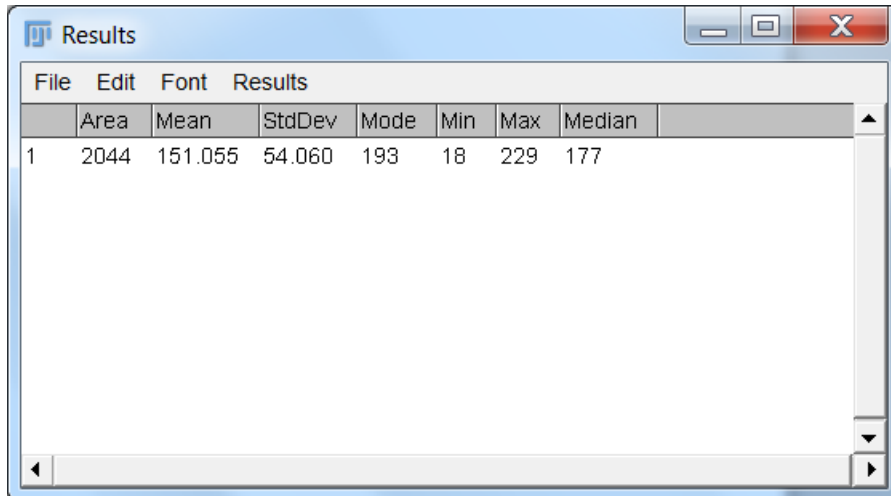


7. Measure intensity of the color within the circle by clicking "Control+M" or click on the following menu item: "Analyze" → "Measure" (See below image).



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8. A window will appear that contains the values of each feature that you checked off in step 2. These numbers can be highlighted with the mouse, copied, and pasted into Excel.



	Area	Mean	StdDev	Mode	Min	Max	Median
1	2044	151.055	54.060	193	18	229	177

9. In order to obtain the "reciprocal intensity" for each area in an image that was measured the value of in the "Mean" or "Median" column of the image in step 8 must be subtracted from 250. See the original paper in *Cancer InCytes Magazine* for more details.