# Photo Quantification Method – Protocol

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Calibrate Image

1. Run in MATLAB
2. Open 🡪 file “CalibrateImageA031108.m”
3. Click RUN
4. Type “R” in the Command Window, then hit ENTER 🡪 this selects a reference image rather than a linear approximation
5. Hit ENTER again 🡪 this selects the number of gray level bars for each image (20 total)
6. Select reference image, or the file “REFERENCEIMAGE.jpg”, then click OK
7. Select photo to calibrate, then click OK
8. Hit ENTER in the Command Window 🡪 this selects the rectangle width in pixels (standard = 25)
9. Press any key to continue
10. You will now select the gray level bars on the reference image 🡪 select gray level bar “A” and continue down to gray level bar “19”
11. Once completed, press ENTER within the Command Window to select the rectangle width for the image to calibrate
12. Repeat step 10 for this image
13. You have now calibrated an image based on the reference 🡪 to save, press “Y” within the Command Window 🡪 you will need this for the latter half of the quantification method
14. Additionally, Figure 3 contains the calibration parameters from the calibrated image 🡪 to save, press the save icon in the upper left 🡪 SAVE AS .JPG, NOT .FIG

\*\*If you receive an error, re-run the script again. If an error still occurs, try a duplicate photo – this should solve the issue\*\*

Analyze Intensity

1. Run in MATLAB
2. Open 🡪 file “AnalyzeIntensity071108.m”
3. On line 16, you can change the amount of selecting points 🡪 for example, instead of 20, you want 30 points (NumPoints=30;)
4. Click RUN
5. Click OK
6. Select the calibrated image, then click OK
7. You may now randomly select the number of points you assigned on the coral fragment
8. After selecting random points, the 10 x 3 double file named “RGB” will contain the intensities of each spectrum based on the selected points 🡪 copy these into an Excel spreadsheet or other form of documentation

\*\*If an error occurs, re-run the script and try again – sometimes MATLAB doesn’t like the points you select\*\*