**Protocol - Coral Color Analysis**

1. Calibration of images with Adobe Photoshop
2. Quantification of color intensity (RGB channels)
3. Statistical analysis in R (not described here)
4. IMAGE CALIBRATION

Requires: your images, Adobe Photoshop

NOTE: If your images have a grayscale (20 or more grays) in them, you can use the Winters et al. macro Calibration to calibrate your photos instead of this procedure. Read in their instructions how to do it.

1. Open Photoshop on the computer where you have downloaded your photos.
2. Open your first image in Photoshop.
3. Go to ‘Window’ and select ‘Info’. This will open a small window that reports Red, Green, and Blue (RGB) channel info for whatever point in your photo you select.
4. To select a point, go to the left-hand side bar menu and select the ‘eyedropper tool’ image.
5. Choose a white point on your image. Make sure it is a point that is consistent across all images, in the same relative location and lighting. This will be your calibration point.
6. Hold down shift and click the white point. The eyedropper tool will sample the RGB channels of that point. You can see these results in the Info window.
7. Go to the top menu and click ‘Image’ -> ‘Adjustments’ -> ‘Levels’. This will open the Levels window.
8. Click on the white eyedropper icon. This is on the left of the Levels window near ‘Options’.
9. Click with the eyedropper on the white point that you chose before. You will see a new column of RGB channel values appear next to the ones you sampled before in the Info window.
10. Keep clicking until all of these new values read ‘255’. If the three channels all equal 255, that means that ‘white’ point has been calibrated to be ‘pure white’.
11. Save the calibrated image as a JPEG.
12. Repeat the process for every photo you wish to analyze.
13. QUANTIFYING COLOR INTENSITY

Requires: your calibrated images, MATLAB, macro “AnalyzeIntensity”, from Winters et al., your data spreadsheet

1. Open MATLAB
2. You will need to use the AnalyzeIntensity macro. If you already have a copy, go to ‘Open File’ and open AnalyzeIntensity. If you do not have it, go to <https://www.sciencedirect.com/science/article/pii/S0022098109003700> (Winters et al. article and supplementary data). Download the zip file in Appendix A Supplementary data. This contains the AnalyzeIntensity code. Also in the Appendix is a Word document containing instructions for how to use AnalyzeIntensity. Download this as well and read it.
3. Once you have opened AnalyzeIntensity in MATLAB, click ‘Run’ under the green arrow to run the program.
4. A window will pop up asking you to choose the number of pictures to analyze (default=2), the number of points per picture to analyze (default=10) and the size of the quadrats (default=25 pixels by 25 pixels). I used all of these default settings except the number of pictures. I chose to analyze one picture at a time in order to make sure the results were matched with the correct picture.
5. Follow the procedures outlined in the Winters et al. document. After you put in your preferences in step 4, choose the picture/s to analyze.
6. Pick 10 (or however many you choose for your protocol) points on the coral, semi-random. In this experiment I tried to avoid points near the base of the coral (which was usually not alive) or any points that were in dark shadows or exposed to glare. Be mindful that you are trying to give an accurate, random sampling of the color of the coral.
7. Go back to the main MATLAB window. On the right hand side you will see a sidebar with some of the data that has just been gathered. Find the three consecutive rows that are labeled R, G, and B, somewhere about halfway down the side.
8. Double click on each of these rows to open them in another tab in MATLAB. You will see each channel opens its own spreadsheet in which the intensity reading from each point is organized.
9. Copy and paste this data from MATLAB into your master spreadsheet. Be sure to keep track of which data came from which color channel.
10. Run AnalyzeIntensity again to restart this process with another picture or set of pictures. Continue until you have gathered data from every photo you wish to analyze.

REFERENCES:

Winters G, Holzman R, Blekhamn A, Beer S, Loya Y. (2009). Photographic assessment of coral chlorophyll contents: implications for ecophysiological studies and coral monitoring. *Journal of Experimental Marine Biology and Ecology* **380**: 25-35.

See Appendix for macros. Can use this link to find online: <https://www.sciencedirect.com/science/article/pii/S0022098109003700>