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Quantifying RGB-Based Color Score in Corals

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Protocol status: Working

This protocol has been evaluated in Hackerott et al (2025), published in Coral Reefs.

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Keywords: color score in coral, quantitative color score protocol, based color score, color score, quantifying rgb, coral, coral reef, bleaching severity, blue intensity

Abstract

This protocol describes the steps required for photo capture, image processing, and data analysis to quantify color scores based on standardized red, green, and blue intensities, which can be used as metrics of coral bleaching severity. This is the quantitative color score protocol evaluated in [Hackerott et al. \(2025\)](#), published in Coral Reefs.

Guidelines

To maximize comparability, use the same color standards (e.g., same rolls of red, green, and blue electrical tape) across measurement sessions (e.g., sites, timepoints, species, etc.).

Include comparison groups (e.g., control vs treatment, pre- vs post-heating) to evaluate color changes as a metric of bleaching severity.

While color scores are quantitative, they are also relative. Therefore, it is important to conduct the PCA-LDA approach on the entire dataset in which comparisons will be made (e.g., comparing bleaching severity across species or sites).

This specific method was validated using *Acropora cervicornis*, staghorn corals, under artificial lighting on land (described in [Hackerott et al, 2025](#)). Use with other species or under other conditions may require additional validation.

Materials

- Digital camera
- White background (e.g., dive slate)
- Red, green, and blue color standards (e.g., electrical tape)
- Computer with [ImageJ software](#)
- Analysis pipeline [datafiles and scripts](#)

Safety warnings

- ! This specific method was validated using *Acropora cervicornis*, staghorn corals, under artificial lighting on land (described in [Hackerott et al, 2025](#)). Use with other species or under other conditions may require additional validation.



Before start

To maximize comparability, use the same color standards (e.g., same rolls of red, green, and blue electrical tape) across measurement sessions (e.g., sites, timepoints, species, etc.).

Include comparison groups (e.g., control vs treatment, pre- vs post-heating) to evaluate color changes as a metric of bleaching severity.

While color scores are quantitative, they are also relative. Therefore, it is important to conduct the PCA-LDA approach on the entire dataset in which comparisons will be made (e.g., comparing bleaching severity across species or sites).



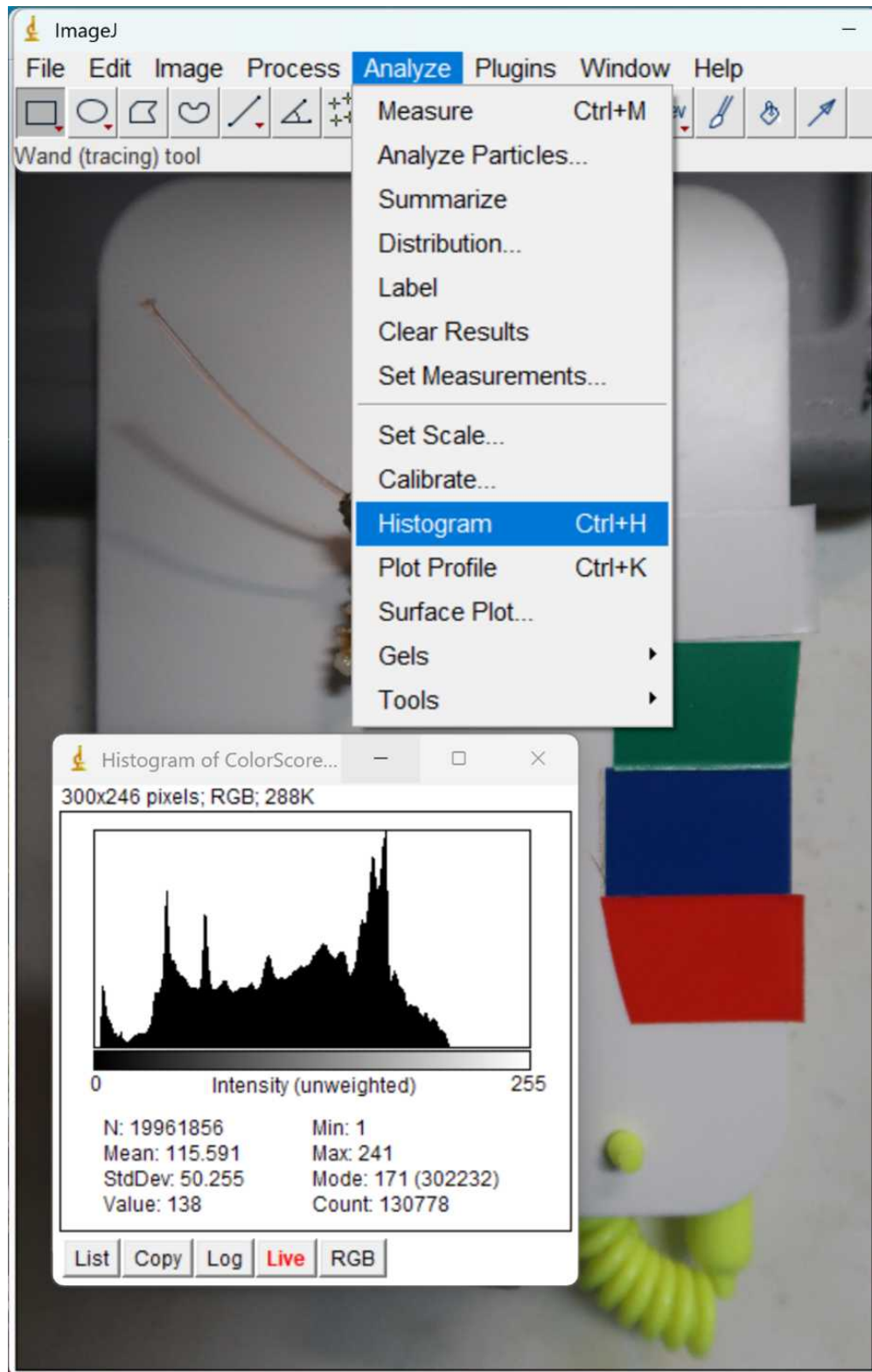
Photo Capture

- 1 Place the coral fragment on the white background with the RGB color standards visible. Ideally, position the coral to maximize the fragment surface area that is visible.
- 2 Avoid shadows or glares on the coral or the color standards. It is critical that the coral fragment is under the same light conditions as the color standards.
- 3 Limit water droplets on the color standards.
- 4 Capture the photo from in front of the coral fragment. If bleaching is occurring in patches, it may be appropriate to capture photos from additional angles or faces for an averaged color score across the full fragment.

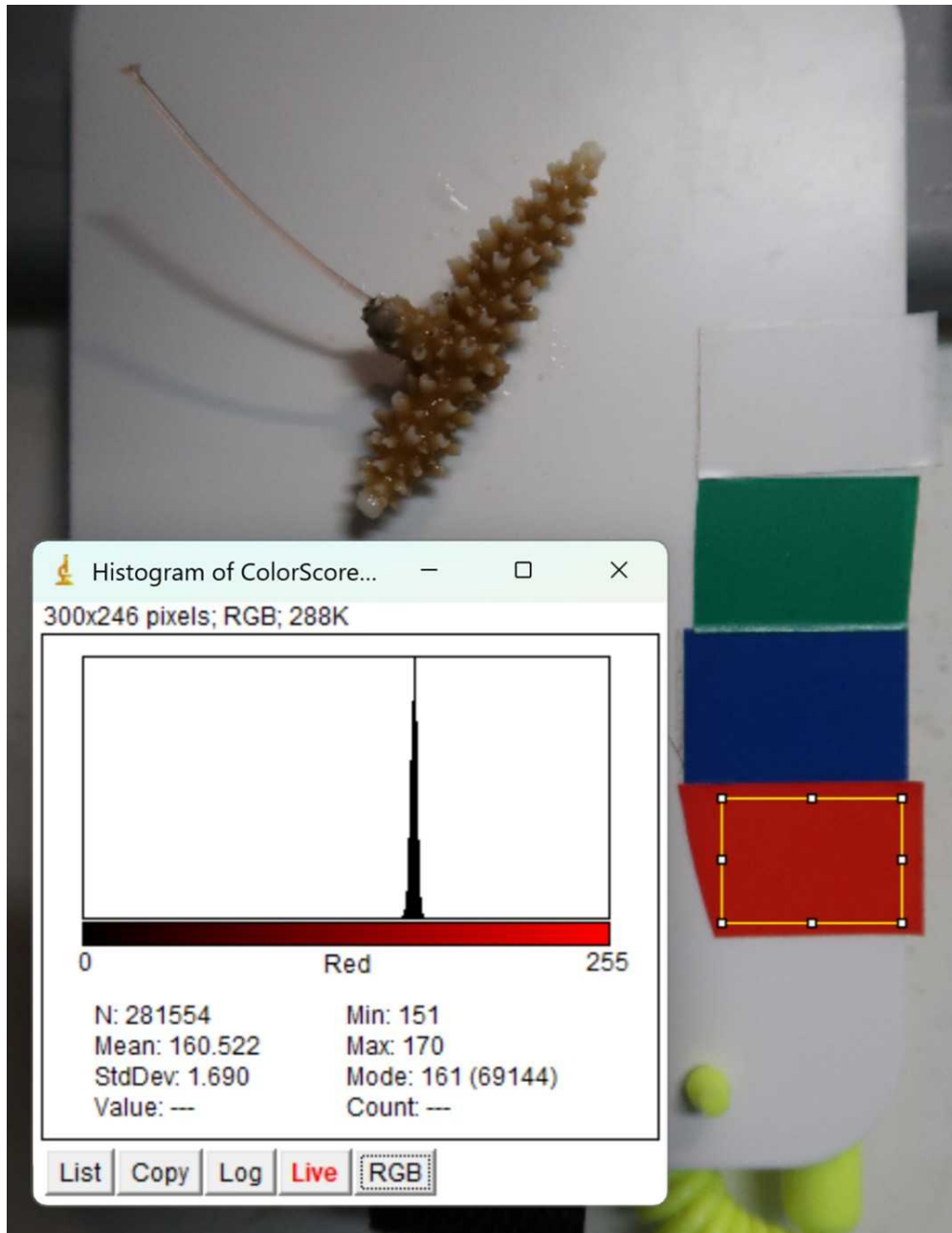


Image Processing

- 5 Open the ImageJ software. Select File > Open and select the first image to be processed.
- 6 Select Analyze > Histogram and a third window with the intensity histogram will appear. Select the Live button to activate.



- 7 Use the cursor to select a square that encompasses most of the red color standard tape. Click the RGB button until the intensity spectrum being shown is Red only. Record the mean value as the Red Standard value for that photo.



- 8 Repeat Step 7 for the green and blue color standard tapes, making sure to click the RGB button to record the corresponding color intensity (i.e., green intensity for the green standard, blue for blue).



- 9 To measure the coral, select the Freehand Selections button. Trace the entire coral fragment. Avoid including borders, shadows, and non-coral sections (e.g., crimps, monofilament, plugs, etc.). It is better to be slightly inside the border of the fragment rather than including outside border colors (such as the white background) or shadowed edges in the measurement area.
- 10 After the entire fragment is traced, click through the RGB options and record the mean red, green, and blue intensities as the Red, Green, and Blue Coral values.

Data Analysis

- 11 Calculate the standardized colors for each coral by dividing the Coral value by the corresponding Standard value (e.g., Red Coral / Red Standard).
- 12 Obtain the color score using the PCA-LDA approach detailed in [this GitHub repository](#). Briefly, perform a PCA on the standardized colors, then use PC 1, 2, and 3 as predictors of the treatment group (e.g., control vs heated, baseline vs bleaching, etc.). Save the LD1 scores as the quantitative color score.
- 13 Color scores may need to be inverted in their signs (* -1) for intuitive interpretation (i.e., control > heated) and an adjuster constant may need to be added to make all scores positive (e.g., add 20 to all scores).
- 14 Color scores can then be visualized and analyzed using similar methods as those used for other bleaching metrics (e.g., cell density, chlorophyll concentration, etc.) to compare levels of bleaching severity between treatment groups.

Protocol references

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