Supplementary file

Using Scuba for *In Situ* Determination of Chlorophyll Distributions in Corals by Underwater Near Infrared Fluorescence Imaging

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Received: 5 December 2019; Accepted: 15 January 2020; Published: date

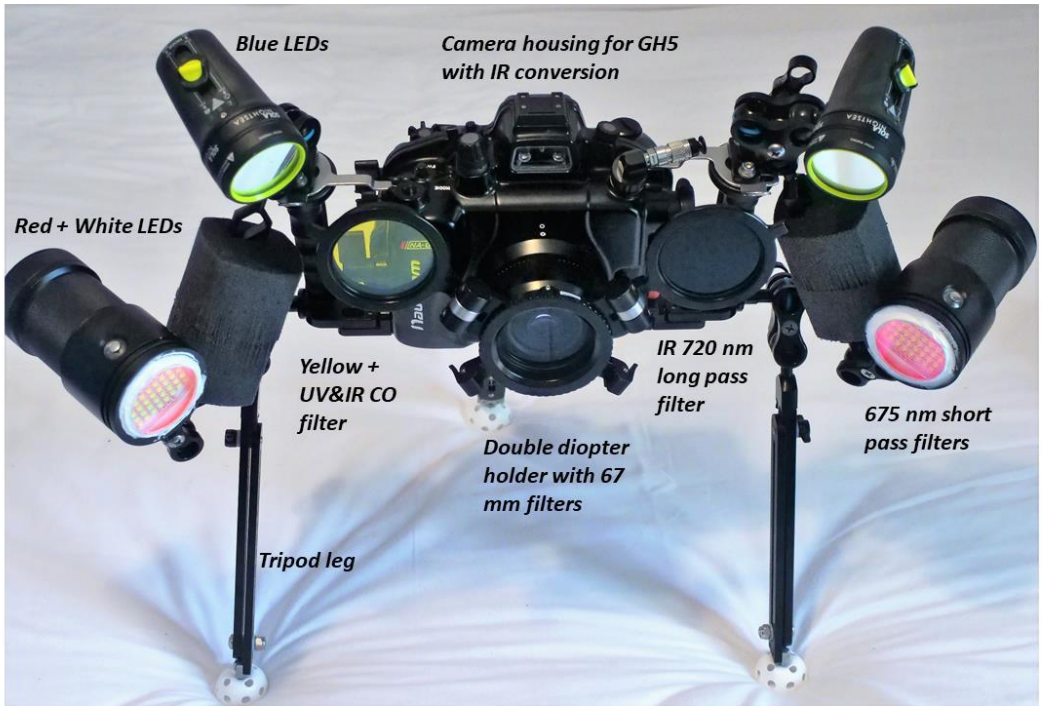

Figure 1. The imaging system used in this work showing the location of filters used. For all Chl imaging the white LEDs were used for excitation. For all GFP imaging, the blue LEDs were used. Each combination of lighting and filters required setting separate white balances before beginning.

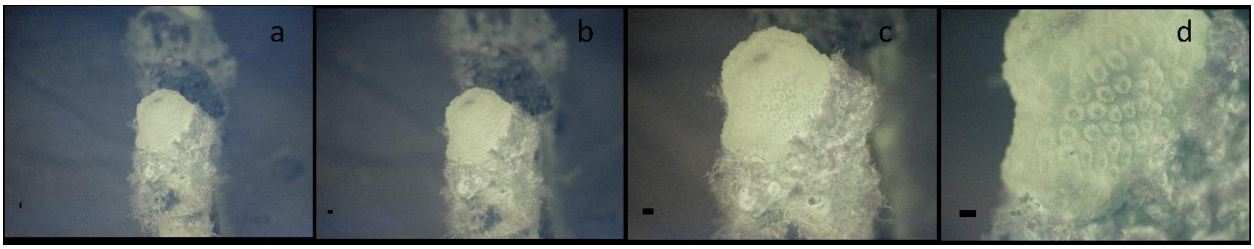

Figure 2. Imaging NIR Chl fluorescence using ambient excitation at various distances from the coral target. NIR Chl fluorescence images of a small start of *Montastraea cavernosa* imaged in daylight at a depth of about 7 m from distances of about 100 cm (a), 50 cm (b), 25 cm (c), or 10 cm (d). Additional white LED lighting had very little effect on the Chl emission intensity in the image at 100 cm.

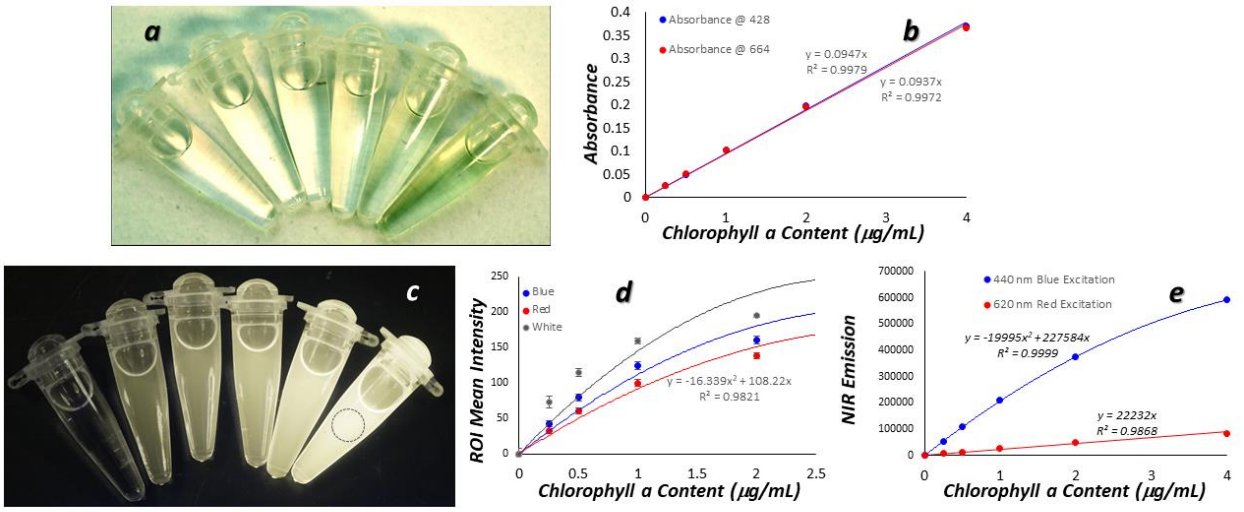

Figure S3. Quantitation of Chl by spectrophotometry, NIR fluorescence imaging and fluorometry. (a) Visual images of 6 Chla samples in MeOH ranging from 0 to 4 g/mL Chla. (b) Absorbance spectra for Chla are linear when using either blue or red absorbance peaks. (c) Chla NIR fluorescence image using white LED illumination and a 720 nm long pass CO filter over the camera lens. The circular ROI shown was used on all samples to quantify the mean fluorescence intensity within that region for production of the standard curve shown in (d). In (d) it is shown that while LED illumination of the samples in (a,c) results in the greatest NIR fluorescence emission signal, although the data with red LED illumination fits better to a 2nd order polynomial. (e) Spectrofluorometric analysis of the same samples shown in (a,c) using only the NIR portion of the emission spectrum (see Figure 1) using both blue and red light from the excitation monochromator.

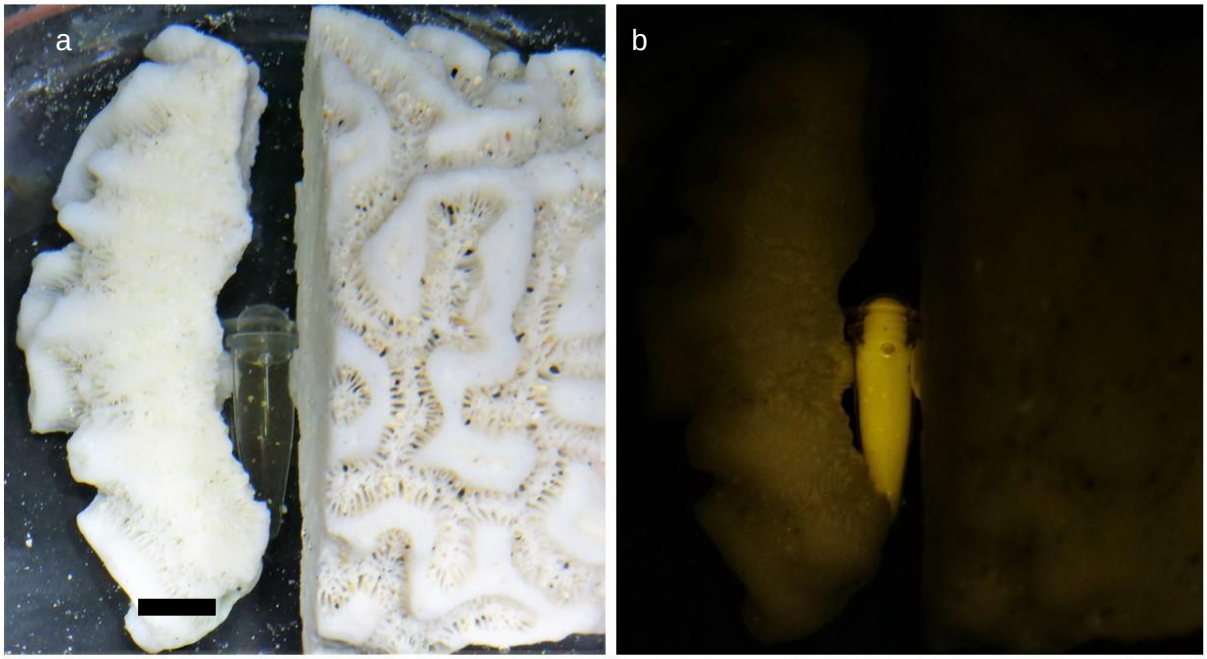

Figure S4. Virtually no NIR fluorescence is reflected from coral skeletons with our imaging system. *In vitro* control images of an old brain coral skeleton and a tube of Chla (4 g/mL, comparable to or slightly less than that in coral tissue extracts) using our imaging system. A vertical 3/8 inch section of the coral was removed from the coral with a rock saw and laid on its side. The section and the remaining skeleton were place at the bottom of a 4 L beaker of water (approximately 25 cm high) with a tube of Chla in MeOH sandwiched between them. Two custom white balance settings were prepared with and without the 720 nm long pass emission filter. The skeleton was imaged with white dive light illumination using 675 nm long pass filters over the lights as done in all *in situ* work. (a) Visible light illumination. (b) NIR fluorescence emission shows that virtually no NIR light is reflected from the coral surface with the lighting and camera settings near those used for all of the *in situ* imaging. It is interesting to note that some small specs of the coral beneath the tube of Chla, and the regions of the skeleton directly adjacent to the tube, that can be seen in the left image and appear brighter in NIR fluorescence image. This is apparently due to reflection of NIR fluorescent light emitted from the Chla in the tube. The scale bar in panel (a) is approximately 1 cm.