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Measuring coral reflectance and calculating NDVI as a proxy for chlorophyll *a* with the DIVING-PAM-II V.1

Coral Reefs

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We use this protocol and it's working

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Abstract

Reflectance measurements have been widely used in remote sensing and visual ecology work to quantify the color of surfaces and organisms objectively. The Normalized Difference Vegetation Index (NDVI) is an established method to estimate chlorophyll a (Chl a) content in various organisms and ecosystems. It is based on the spectral properties of Chl a and the reflectance it causes in the red and near-infrared spectrum. The DIVING-PAM-II from Walz GmbH comes with a spectrometer (MINI-SPEC). It allows for spectral measurements in the range of 400 – 850 nm. This range is suitable for quantifying coral reflectance and subsequently calculating the NDVI. Estimating Chl a content non-invasively allows for continuous high-resolution monitoring of signs of bleaching and pigment loss during laboratory experiments or in the field. This protocol explains how to use the DIVING-PAM-II spectrometer to obtain coral reflectance measurements and calculate the NDVI as a proxy for Chl a.

Materials

1. DIVING-PAM-II
2. Spectrometer (MINI-SPEC) + accessories
3. WinControl

Background

1 Conventional methods for measuring chlorophyll a (Chl a) content in scleractinian corals typically require sacrificing the coral to access the Chl a. The main limitation of this method is the destructive sampling, which only provides data for limited time points. Coral bleaching is associated with the expulsion of symbionts and a consequent loss of Chl pigments, causing the coral to pale (Brown, 1997; Douglas, 2003). Despite standardized methods of measuring the progression of bleaching with coral health charts (Siebeck et al., 2006), determining color changes is often subjective and lacks high resolution to allow for the detection of small optical changes. Measuring color with spectrometers offers an objective quantification method that provides detailed information about the spectral properties of a sample. When measuring color, we are in fact measuring the variation in optical properties, one of which is reflectance (Johnsen, 2016).

Spectral reflectance is a unitless ratio where light reflected from an object is compared across a wavelength range to light reflected from a standard. The standard is usually a flat, diffuse white surface which reflects light equally in all directions. Measured reflectance is dependent on the angle of incident light to the detector. Therefore, established geometries are often used for complex biological surfaces in order to allow comparing new data with previous work (Johnsen, 2016).

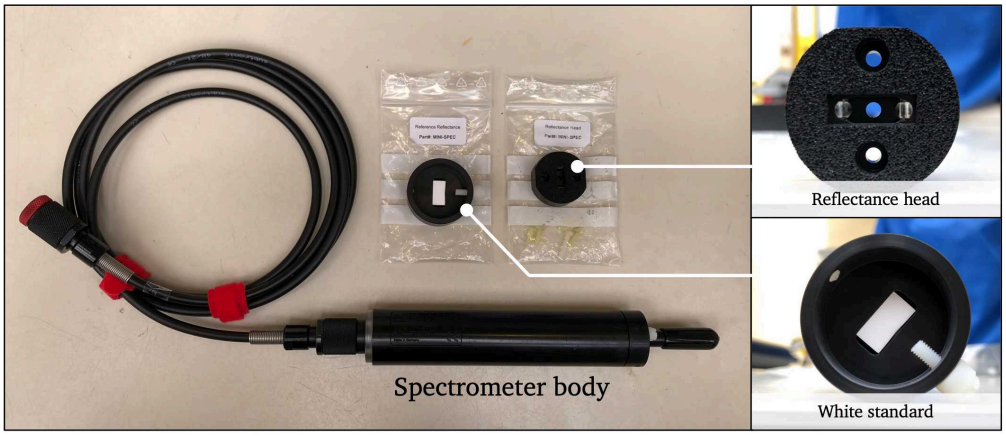
Scleractinian corals can be broadly categorized into blue and brown corals with the brown category being more common (Hochberg et al., 2004). All corals depict a relatively low reflectance between 400 and 500 nm and a higher, peaked pattern between 550 and 650 nm. The presence of Chl a creates a unique spectral shape with a prominent and narrow absorption feature near 675 nm (Hochberg et al., 2004). These properties have been used in remote sensing to estimate Chl a by calculating the Normalized Difference Vegetation Index (NDVI). It is calculated using the formula NDVI = (near-infrared - red)/(near-infrared + red) and is based on the reflectance caused by Chl a (Leal et al., 2015). The index is commonly used for terrestrial plants but has also been successfully applied to aquatic organisms. However, the application of NDVI to corals in ecological research has been scarce (Denis et al., 2024; Leal et al., 2015; Naugle et al., 2024; Rocha et al., 2013; Wijgerde et al., 2014). NDVI can be useful for monitoring Chl a content over time and detecting signs of bleaching, which alters the spectral shape due to the loss of pigments. Bleached corals will display overall higher reflectance and pigment loss will alter the spectral shape towards reflectance more similar to carbonate sand (Hochberg et al., 2003; Yamano et al., 2003). Low Chl a content will yield a low NDVI. Hitherto, only two papers have provided a calibration for Chl a vs NDVI in scleractinian corals (Denis et al., 2024; Naugle et al., 2024, see supplementary materials). Therefore, it remains unclear whether this relationship works equally well for

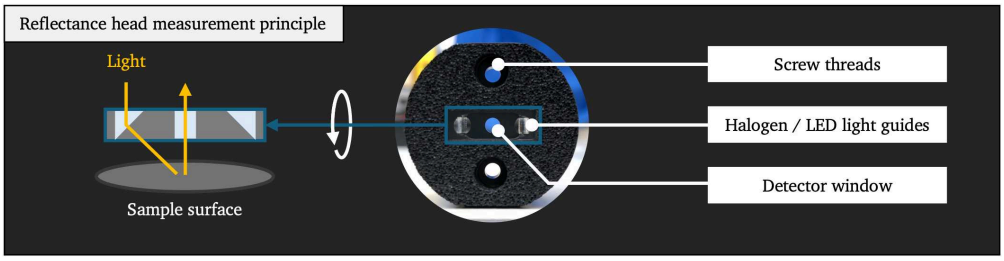
all coral species, and users of this protocol are advised to keep this in mind and ideally perform their own calibrations.

The DIVING-PAM-II from Walz GmbH comes with a spectrometer (MINI-SPEC) which allows to easily measure spectral reflectance in laboratory experiments and in the field (Walz, 2023). It obtains spectral measurements in the range of 400 - 850 nm and is suitable for calculating the NDVI. The reflectance head uses a common geometry (45°) to obtain the measurement (Walz, pers. communication), which is favorable for comparisons with previous work (Johnsen, 2016). Furthermore, the spectrometer is submersible, simplifying its use in aquatic environments. In summary, the application of NDVI on corals with the aid of the DIVING-PAM-II has the potential to non-invasively monitor coral health and obtain high-resolution data over time without the disadvantages of conventional methods.

Calibration of the spectrometer

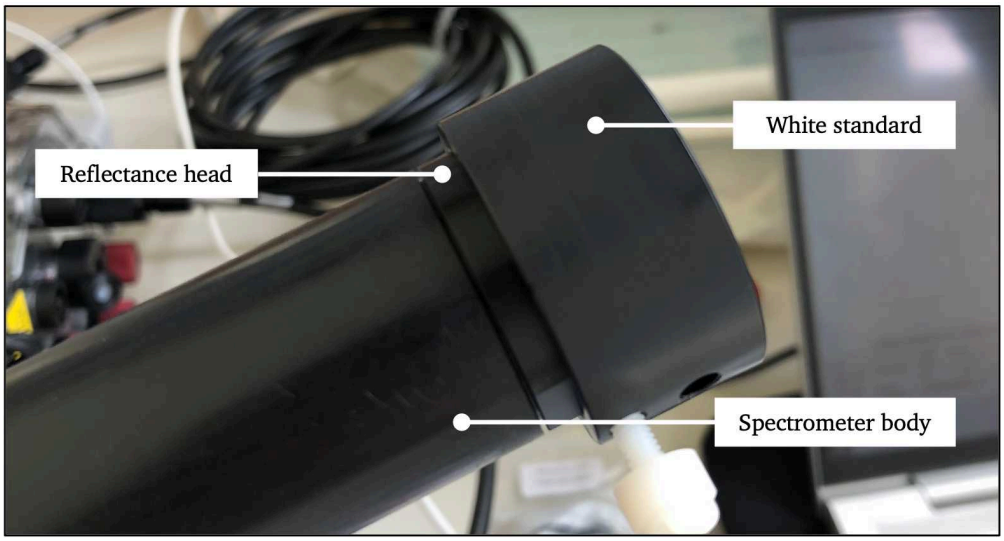
2 Configure the spectrometer for reflectance measurements by attaching the reflectance head to the spectrometer body (see Walz manual, Seventh Edition, April 2023, Fig. 9, page 21).





Be cautious when attaching the screws as the plastic may weaken over time, potentially causing the head to snap off and leaving the thread inside the spectrometer body.

3 Attach the white standard (i.e. highly reflective reference material) to the reflectance head and tighten the screw.

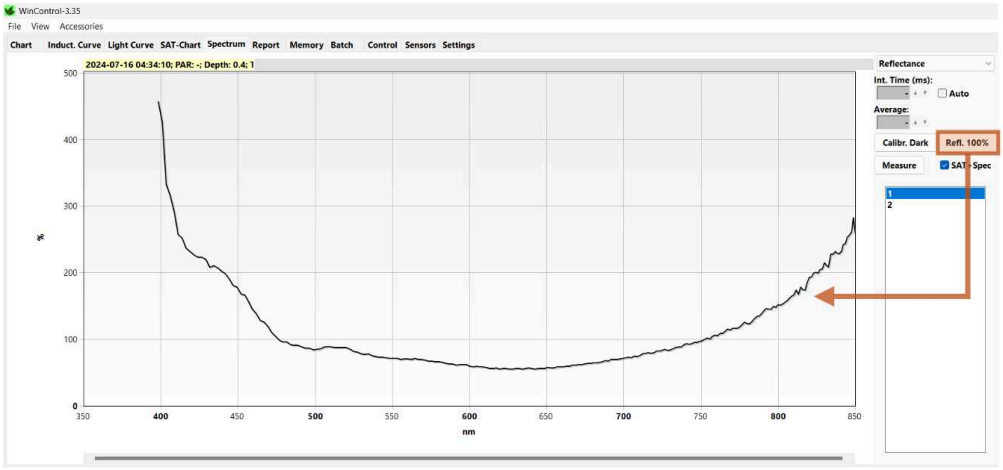


Do not touch the white surface, keep away dirt, and store the white standard in a protective casing to maintain its optical properties.

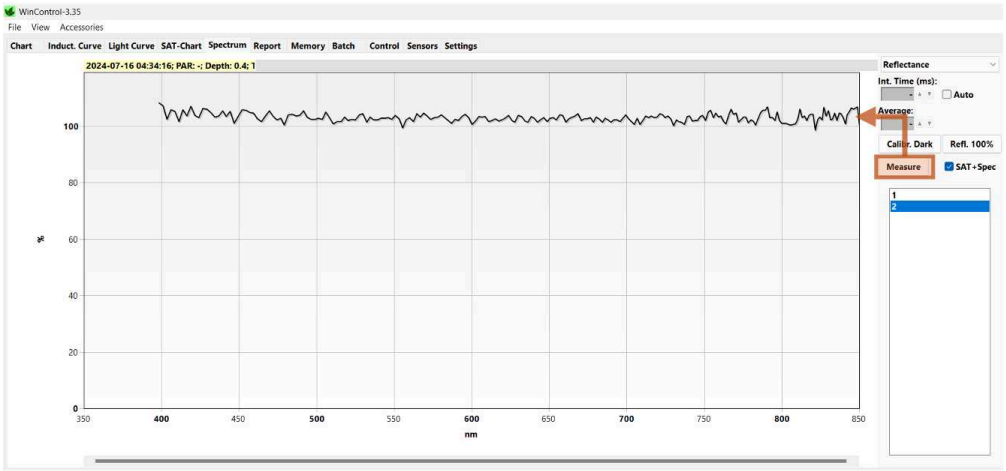
4 Connect the PAM to the computer, open **WinControl**, and navigate to the **Spectrum** tab.

5 Select **Refl. 100%** to establish the 100% reflection signal. The calibration result is shown in the graph (The dark current is measured in the factory and stored in the flash memory. It should therefore not need additional calibration. Dark current refers to the electrical noise that is inherent to the spectrometer's detector when no light is present. Its calibration is crucial to account for this baseline noise. If necessary, the dark current can

be newly established by fully darkening the probe and pressing **Calibr. Dark**).



6 **Measure** while the white standard is still attached. The result should display a line of 100% reflectance across the entire wavelength range.



7 The PAM stores the calibrated reference signal and uses it for all subsequent measurements (Walz, personal communication).

We encountered a problem in our DIVING-PAM-II where the reference signal gets lost after turning the device off. The consequence is that measured spectra are not saved upon downloading the data even though the PAM screen displays accurate reflectance.

As a workaround, leave the PAM turned on until you finish measuring and calibrate the white standard newly before measuring again. Turning the PAM off after performing the measurements keeps the measured spectra.

8 Remove the white standard and disconnect the PAM from the laptop to proceed with subsequent measurements.

Measuring coral reflectance

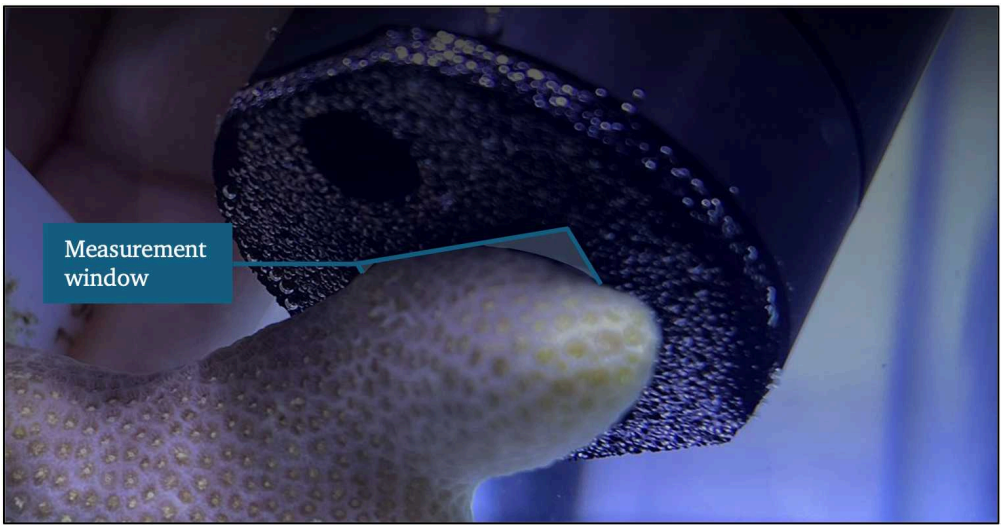
9 Change the operation mode on the PAM to **Reflectance**. PAM screen path: Main Menu → Sensor → Spectrometer → Operation Mode.

10 Navigate down from the main screen to reach the Spectrometer window.

11 Darken the room to reduce interference with other light sources (in the field, interference can be limited by covering the entire measurement area with the probe).

12 Submerge the spectrometer in the water and verify that no air bubble blocks the detector window or LED light guides. Bubbles may appear on the protective foam.

13 Position the reflectance head onto the coral so the measurement window covers the measurement area.



Light is only turned on for demonstrational purposes here.

14 Press **SPEC** to measure. The spectrometer should flash and the screen display the measured spectrum. Repeat the measurement for two more points of interest on the coral fragment. Data of triplicates will be pooled to represent one fragment.

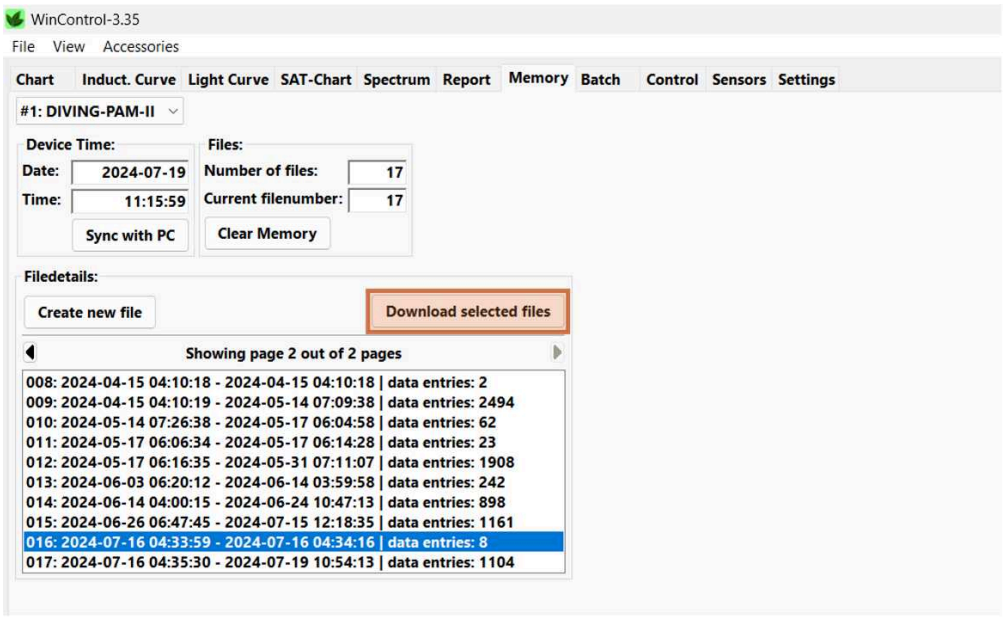
15 Note down the respective coral identifier for subsequent data processing.

Processing the data

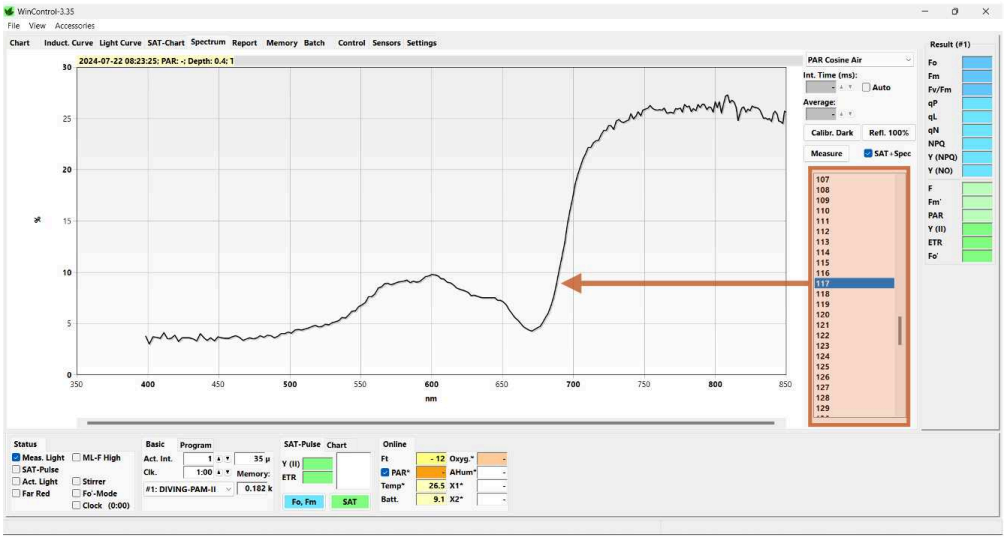
16 Connect the PAM to **WinControl**.

17 Navigate to the **Memory** tab.

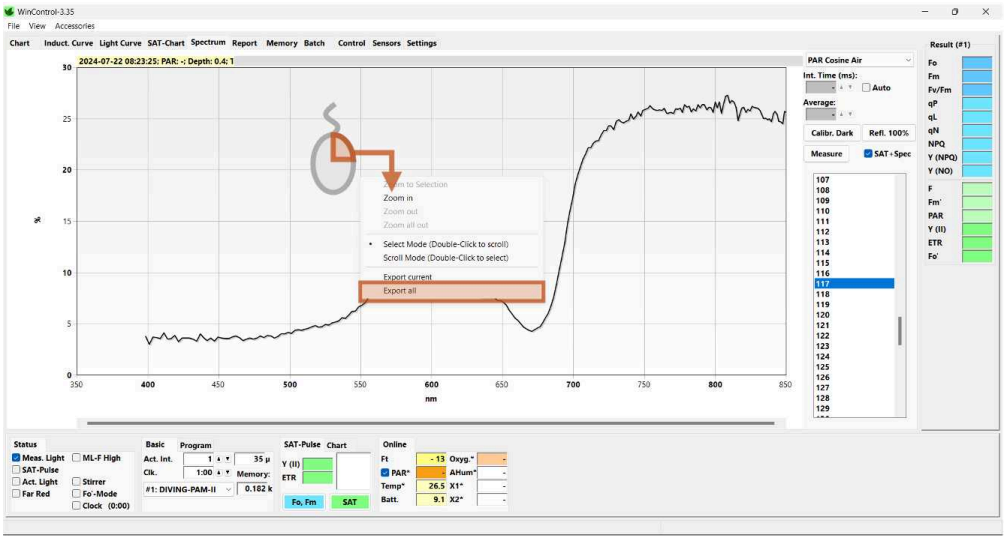
18 Download the file that contains your measurements.



19 In the presence of spectral data, the **Spectrum** window is active. Navigate to the Spectrum window. The right panel contains a list of your measurements. Selecting a measurement displays the respective spectrum in the graph.

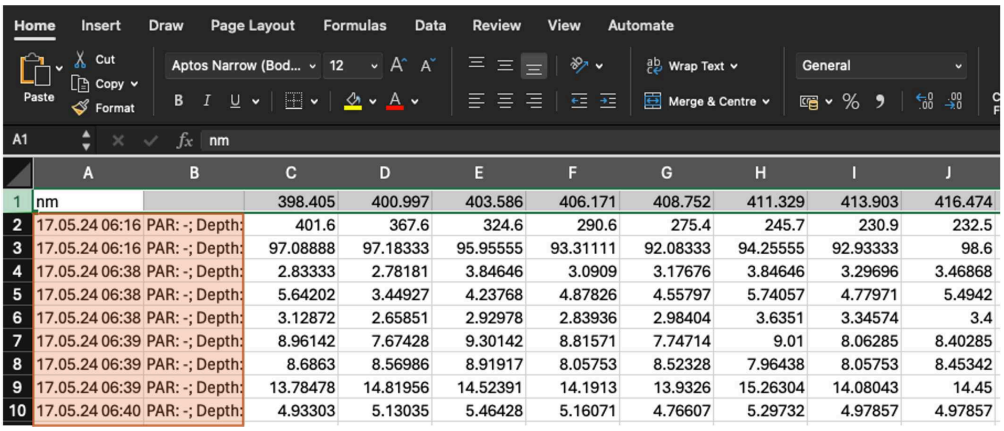


20 Download the spectral data by right-clicking into the graph and selecting **Export all**. The downloaded file contains all spectra listed underneath a row containing the wavelengths (nm). The first two entries represent our calibration and white standard measurements.



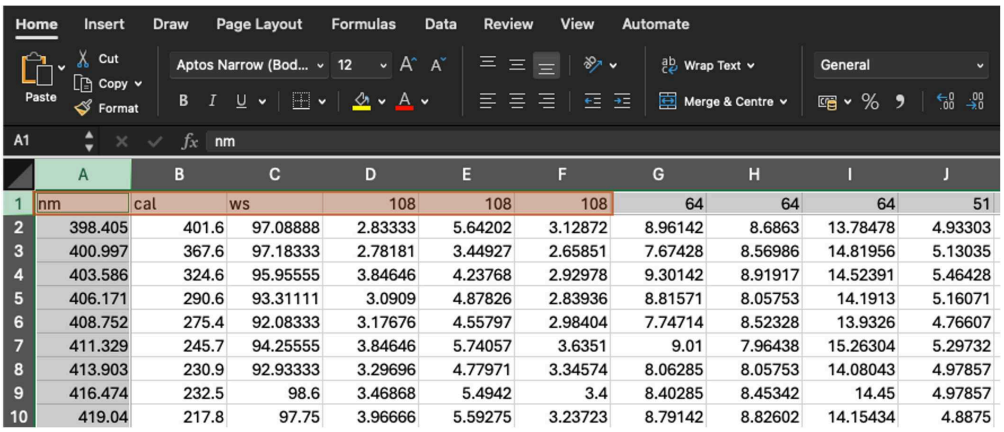
21 *The following steps should be adjusted as needed for individual requirements.*

Remove the time annotations and enter the coral identifiers to the respective entries.



	A	B	C	D	E	F	G	H	I	J
1	nm		398.405	400.997	403.586	406.171	408.752	411.329	413.903	416.474
2	17.05.24 06:16	PAR: -; Depth:	401.6	367.6	324.6	290.6	275.4	245.7	230.9	232.5
3	17.05.24 06:16	PAR: -; Depth:	97.08888	97.18333	95.95555	93.31111	92.08333	94.25555	92.93333	98.6
4	17.05.24 06:38	PAR: -; Depth:	2.83333	2.78181	3.84646	3.0909	3.17676	3.84646	3.29696	3.46868
5	17.05.24 06:38	PAR: -; Depth:	5.64202	3.44927	4.23768	4.87826	4.55797	5.74057	4.77971	5.4942
6	17.05.24 06:38	PAR: -; Depth:	3.12872	2.65851	2.92978	2.83936	2.98404	3.6351	3.34574	3.4
7	17.05.24 06:39	PAR: -; Depth:	8.96142	7.67428	9.30142	8.81571	7.74714	9.01	8.06285	8.40285
8	17.05.24 06:39	PAR: -; Depth:	8.6863	8.56986	8.91917	8.05753	8.52328	7.96438	8.05753	8.45342
9	17.05.24 06:39	PAR: -; Depth:	13.78478	14.81956	14.52391	14.1913	13.9326	15.26304	14.08043	14.45
10	17.05.24 06:40	PAR: -; Depth:	4.93303	5.13035	5.46428	5.16071	4.76607	5.29732	4.97857	4.97857

22 Transpose the data to have the wavelength and coral identifiers as column headers (cal = calibration, ws= white standard).



	A	B	C	D	E	F	G	H	I	J
1	nm	cal	ws	108	108	108	64	64	64	51
2	398.405	401.6	97.08888	2.83333	5.64202	3.12872	8.96142	8.6863	13.78478	4.93303
3	400.997	367.6	97.18333	2.78181	3.44927	2.65851	7.67428	8.56986	14.81956	5.13035
4	403.586	324.6	95.95555	3.84646	4.23768	2.92978	9.30142	8.91917	14.52391	5.46428
5	406.171	290.6	93.31111	3.0909	4.87826	2.83936	8.81571	8.05753	14.1913	5.16071
6	408.752	275.4	92.08333	3.17676	4.55797	2.98404	7.74714	8.52328	13.9326	4.76607
7	411.329	245.7	94.25555	3.84646	5.74057	3.6351	9.01	7.96438	15.26304	5.29732
8	413.903	230.9	92.93333	3.29696	4.77971	3.34574	8.06285	8.05753	14.08043	4.97857
9	416.474	232.5	98.6	3.46868	5.4942	3.4	8.40285	8.45342	14.45	4.97857
10	419.04	217.8	97.75	3.96666	5.59275	3.23723	8.79142	8.82602	14.15434	4.8875

23 Save the sheet as a CSV file. The data is now ready for processing in *R*.

Calculating the NDVI

24 Use the following formula to calculate the NDVI for the respective spectra where R_{670} and R_{750} represent the average diffuse reflectance in the red and near-infrared wavelength regions:

$$NDVI = \frac{(R_{750} - R_{670})}{(R_{750} + R_{670})}$$

The wavelength that best represents your data might slightly differ from this example. Most literature refers to using 675 nm for the red region (Leal et al., 2015; Rocha et al., 2013; Wijgerde et al., 2014). However, to get the best fit for your data, choose the Chl a absorption peak (i.e. trough in reflectance) from your spectra, usually between 660 - 680 nm (Galvao et al., 2000).

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