Protocol for Collecting Hyperspectral Reflectance Data for Flowers, Fruits, Bark, and Leaves of Tropical Forest Plant Species

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Study summary

Title	Building the basis for automated species identification of tropical plants from hyperspectral data
Methodology	Purposive sampling, observational study
Sampling-phase duration	Initial funding is for 1 year of sampling; extended to 2 years
Study site	Barro Colorado Island (BCI), Colón, Panamá
Objectives	- Collect and publish a large, high-quality dataset of spectral reflectance of flowers, fruits, leaves, and bark for tree and liana species of Central Panama, in part to serve as a resource for calibration and validation of remote sensing. Given prior measurements of leaf reflectance, focus especially on collecting data for flowers and fruits. - Quantify within-species variation and between-species distinctiveness in the collected data, and their utility for taxonomic classification.
Number of plants	At least 150 species, ideally 3 individuals per species and organ.
Inclusion criteria for plants	- Woody plant species - Preference for canopy species and those common at the study site - Individuals should, ideally, be at least 50 m apart
Funding	- Smithsonian Institution Life on a Sustainable Planet Pathfinder grant, with P.I, H.C. Muller-Landau - Simons Foundation award 429440 to the Smithsonian Tropical Research Institute. - NASA for loan of the ASD FieldSpec spectrometer used for the measurements.

Glossary of terms

Spectral measurement: Measurement of spectral reflectance of a given sample using a spectrometer.
Plant: A single tree or liana which may be the source of one or more samples.
Sample: A single collection of tissue or organ taken from a plant to measure in a specific day, typically consisting of multiple flowers of a given plant or multiple fruits, etc. Each sample is assigned a unique **SampleID** code within a FieldMaps form.
Subsample: A distinct subsample of a sample. Each sample should ideally be used to create 5 distinct subsamples, each of which is measured separately.
Subview: A particular view of a subsample, for example internal or external view of a capsule, aril vs. seed. Many samples have only one subview, others have multiple subviews.
Measurement type: How the sample is measured, for example, leaf clip, contact, near-contact.
SpectraID: A unique identifier for each spectral measurement. Each measurement is associated with a given plant, sampleID, subsample, subview, and measurement type. Each white reference measurement also has a unique spectraID. Each spectraID will has its own spectral reading file (e.g. 2024_03_02_0002.asd).

Methods

Sample collection in the field

Supplies

- Zip-loc bags
- Portable cooler with ice
- Sharpie marker
- GPS / iPad with GPS
- Field notebook

Sampling protocol. Fruit, flower, leave and bark samples for organ level measurements are collected opportunistically from fallen material on the forest floor. These samples should be of freshly fallen material, unless noted otherwise. Where there is an opportunity to do so, collection of fresh samples directly from living plants is preferred, except for bark which could be destructive for the plant.

For this project, field forms were created on ArcGIS and downloaded for use with the FieldMaps app on an iPad with GPS capabilities. The following information is recorded for every collected sample:

- Sample ID, unique to every sample, generated automatically within the field form and cannot be changed.
- The location, recorded as the center of the collection area.
- The date and time.
- The sample type, fruit/flower/leaf/bark.
- The sample state: immature, mature, or senescent.
- If the individual plant is tagged, record the tag number and if relevant (and feasible) the mapped plot in which the plant is located.
- Photos of the sample (and associated plant stem and crown when known), in part to serve as a reference for species identification.
- The species (6 letter code, 4 letter code and Binomial name), which can be added later after consultation with an expert botanist.
- Who collected the sample.
- Who identified the sample.

When making a collection, take photographs of the sample, then place it in a zip-top bag. On the bag, record the unique sampleID number and the date with a permanent marker. Store the samples in a Portable cooler containing ice or an ice-pack. Where different organ types or organs of different sample states are collected from the same tree, record these as different samples. Samples of the same organ from the same species should be taken at least 50 m apart to avoid collecting multiple samples from the same individual.

Naming convention for files. Each day's field sample data should be exported and saved as a separate csv file, named YYYY_MM_DD_Field_Form.csv.

Measurement of spectra of organ-level samples in the lab

The text on measurement methods here is based on instructions provided by Yoseline Angel and the Technical Report from Danner, *et al.* (2015).

1. Equipment and supplies

- ASD Field Spec 4 (Figure 1**A**).
- Contact probe for ASD Field Spec with covered with Lambertian* material (Figure 1**B**).
- Charging cable (lined with Lambertian material) to connect probe to Spec (Figure 1**C**).
- Panasonic CF-53 laptop with relevant software and has been calibrated (Figure 1**D**).
- Cable to connect ASD Field Spec to Computer (Figure 1**E**).
- Black box lined with Lambertian material (Figure 1**F**).
- Clamp lined with Lambertian material (Figure 1**G**).
- Flat stand lined with Lambertian material (Figure 1**H**).
- White reference plates (Figure 1**I,J**).
- Leaf-clip accessory (Figure 1**K**).

* Lambertian material is dark black and absorbing in all wavelengths; it can be cloth, masking tape, or foil. We used the following:

- https://www.thorlabs.com/thorproduct.cfm?partnumber=BKF12
- https://www.thorlabs.com/thorproduct.cfm?partnumber=BK5
- https://www.thorlabs.com/thorproduct.cfm?partnumber=T743-2.0

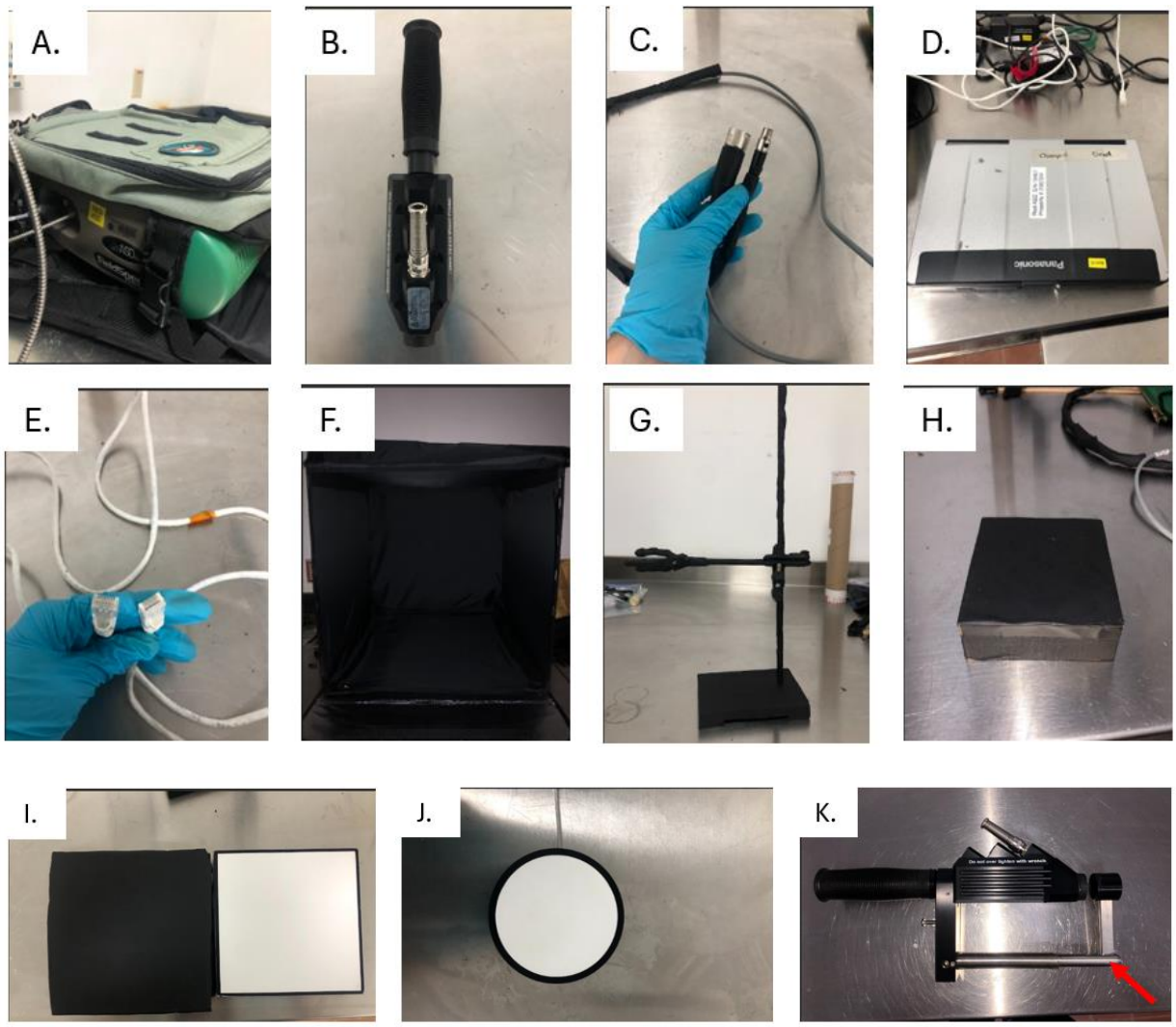


Figure 1. Equipment for laboratory measurements. A. ASD Field Spec 4 spectrometer. B. Contact Probe accessory. C. Charging cable for contact probe. D. Calibrated laptop for saving spectral measurements. E. Cable to connect spectrometer to laptop. F. Black box covered in Lambertian material. G. Clamp covered with Lambertian material. H. Flat stand to hold samples and covered with Lambertian material. I. White reference plate for near-contact measurements. J. White reference plate for contact measurements. K. Leaf-clip accessory.

2. Instrument set up

Attach power supply cable to the slot (Figure 2). This must be done at least 20 minutes before the first measurement is taken. The spectrometer can either be charged directly from wall plug or from batteries. There is a separate cable for each; both cables are plugged into the same point on the spectrometer. Switch on the spectrometer, by pressing the switch on the side next to where the power supply is plugged in. A whirring noise indicates the spectrometer is on.

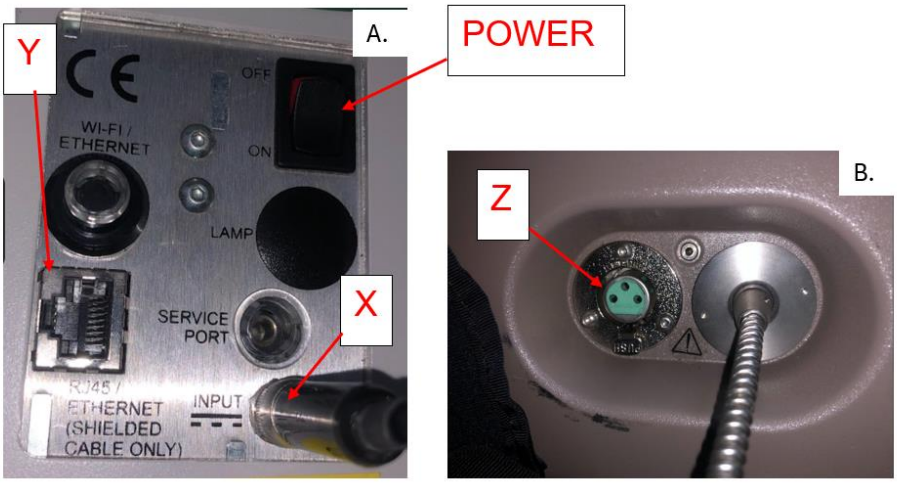


Figure 2. ASD Field spec4 Connector Panel. A. Panel containing power switch, charging port and computer connection Port. B. Panel containing probe connector pot and fiber optic cable.

3. Black box and contact probe set up

For all samples, near-contact and full contact laboratory measurements are taken using ASD Field spec 4 with plant probe. This is done in a black box lined with special Lambertian material. All cables and other hardware inside the box are also covered with this material. Carefully remove and unravel the fiber optic cable from the case netting on the spectrometer. Attach the grey cable charging cable to the spectrometer by pushing into slot Z (Figure 2) until a click is heard. Thread the fiber optic cable and grey charging cable through the hole at the top of the black box; then close the flap to fully seal.

Attach the fiber optic cable and charging cable to the contact probe sensor: On the contact probe (Figure 3**A,B**) unscrew silver metal attachment and then remove grey plastic attachment. Thread the silver metal attachment and then grey plastic attachment onto the end of the fiber optic cable (Figure 3**C**). Slot the plastic attachment back into the original position on the contact probe and push fiber optic cable as far as it goes. Re-screw the silver metal attachment to secure sensor in place. Attach the grey cable (Figure 3**A**) to the contact probe.

Place a laboratory clamp that has been covered in black Lambertian tape on the right side of the box and clamp the handle of the spectrometer contact probe (now covered with black material) so that the sensor is pointing down and the points at which the cables are attached face the back of the black box set-up. Place a flat stand to hold white reference plate or sample underneath the probe. The height of the clamp should be adjusted for each measurement so the distance between the sample and the probe is consistent. Switch on the contact probe light by pressing the button on the front of the contact probe. The resulting set up should replicate Figure 3**D-F,** showing the probe without the cover.

Leaf-clip measurement set-up Leaf-clip measurements are done on select samples of flowers and leaves which consist of softer tissue. These measurements do not have to be done in the Black box. Measurements are done with the plant probe accessory, as with near-contact and contact measurements, in addition to the leaf clip attachment.

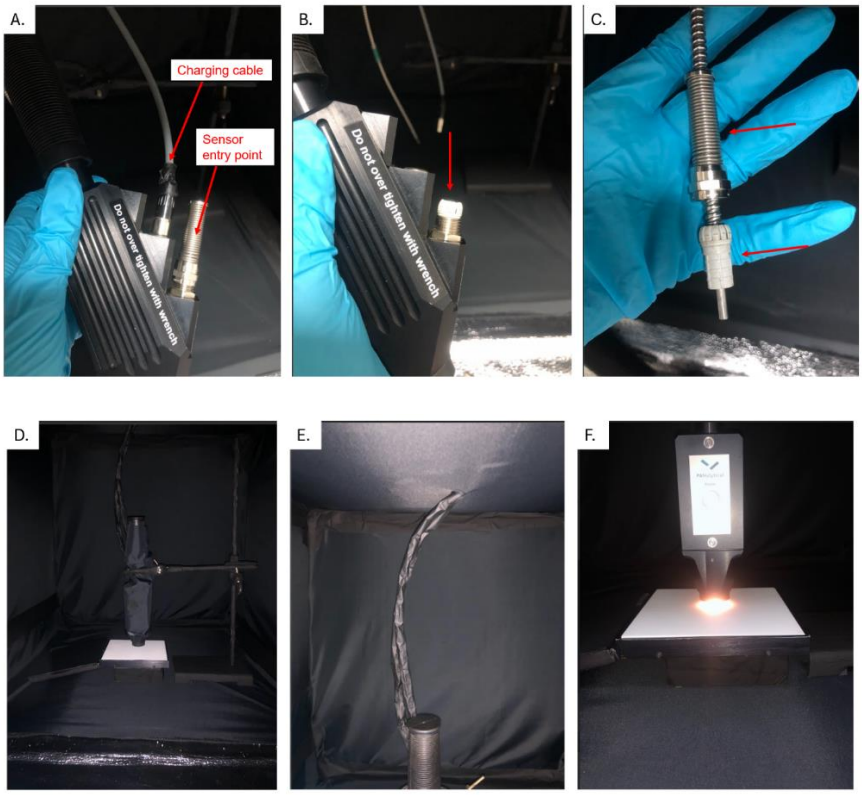


Figure 3. Contact probe and black box set up. A-C. Connecting power cable and Fiber optic sensor cable to the contact probe. D-E. Black box set up with equipment covered in Lambertian material. F. Probe and white sensor set up for near-contact measurements (without cover).

4. Computer set-up

Ensure computer is switched on and has sufficient charge or is charging. Connect the computer to the spectrometer:

- For a **wireless connection**: At the bottom right hand of the screen click on 'wireless' icon and select the wireless identity of the spectrometer you are using.
- For a **direct connection**: Alternatively, plug the white cable directly from laptop to spectrometer slot Y (Figure 2**A**).

Check the fibre optic cable by clicking on the cable symbol in the taskbar. This will open a window, tick all three boxes which correspond to each sensor in the spectrometer and select okay (Figure 4). A green light indicates the computer is connected to the spectrometer. If a red light appears, restart the computer and set up again. If you are using a wireless connection and a red light continues to appear then switch to non-wireless connection using the cable.

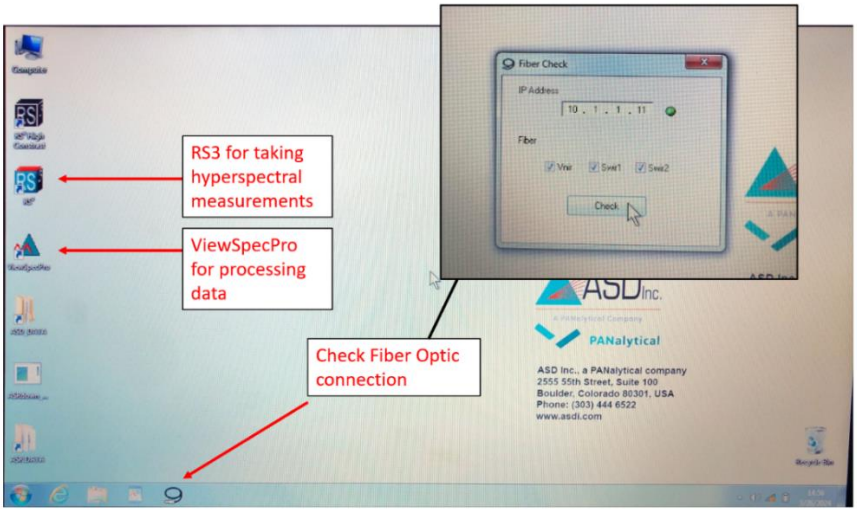


Figure 4. Desktop display with arrows demonstrating the senor connection check process and the relevant software.

5. Software set-up using RS3 package for taking spectrometer readings

Launch the instrument software by double-clicking the RS3 icon in the desktop. Open Control/Adjust configuration in the toolbar and fill out following details (**Figure 5A—B**):

- **Fore optic type**: Bare cable.
- **Number of samples**: Number of readings taken and averaged per saved readings. For laboratory measurements select 25.
- **Black reference**: always enter 4 x number of sample. For laboratory measurements select 100.
- **White reference**: equal to number of samples. For laboratory measurements select 25.

Select Ok to accept details and close window.

Open Control/spectrum save in the toolbar and fill out the following details (Figure 5**C**):

- **Path name**: Select the folder in the raw data file that corresponds to that date.
- **Base name**: The base name is the same for every reading on a given day. Input this base name into the 'SpectralFileStem' column of File reference Table. Each reading carries a file with the following naming convention:

YYYY_MM_DD_ConsecutiveNumberInFourDigits.FileType
2024_03_14_0001.asd

- **Starting spectrum number**: 00000
- **Number of files per sample**: 1

Select OK to accept details and close window. At the top of the screen ensure 'Bare cable' is selected.

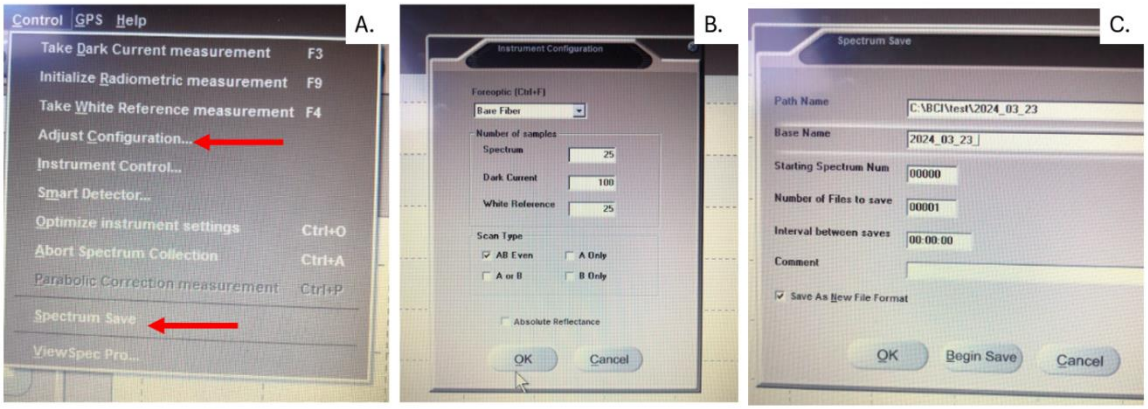
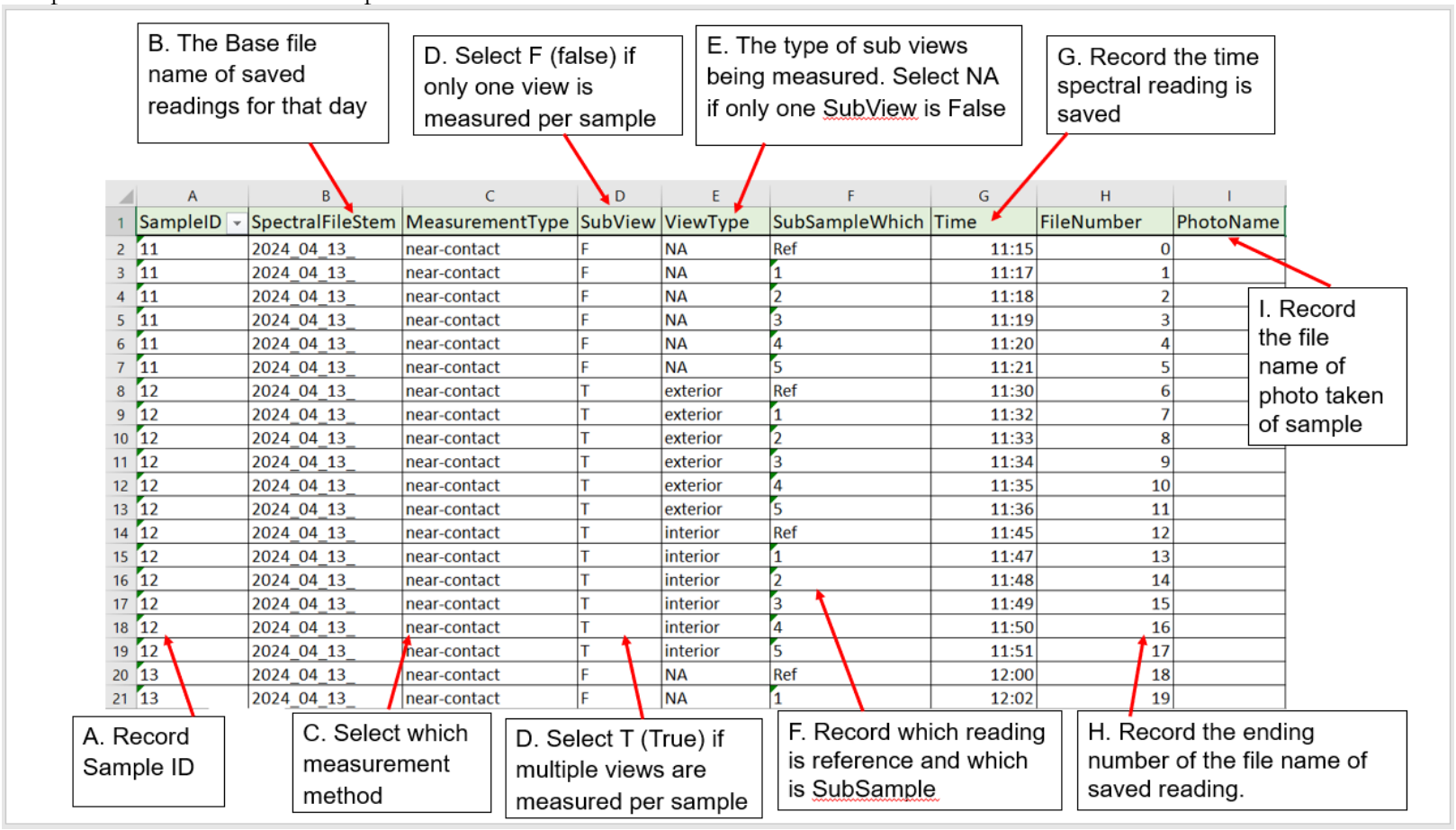


Figure 5. RS³ software configurations made prior to measurements. A Drop-down menu indicating relevant sections. B Adjust instrument configuration. C Spectrum Save.

Tracking measurements

For measurements, you will need a Lab Table to track which spectral reading (and associated file) corresponds to which sample. See **Figure 6** for detailed description of Lab Table. A template for this can be found saved as Lab_table_template.xlsx. Open on a personal computer or device next to the spectrometer.



	A	B	C	D	E	F	G	H	I
1	SampleID	SpectralFileStem	MeasurementType	SubView	ViewType	SubSampleWhich	Time	FileNumber	PhotoName
2	11	2024_04_13_	near-contact	F	NA	Ref	11:15	0	
3	11	2024_04_13_	near-contact	F	NA	1	11:17	1	
4	11	2024_04_13_	near-contact	F	NA	2	11:18	2	
5	11	2024_04_13_	near-contact	F	NA	3	11:19	3	
6	11	2024_04_13_	near-contact	F	NA	4	11:20	4	
7	11	2024_04_13_	near-contact	F	NA	5	11:21	5	
8	12	2024_04_13_	near-contact	T	exterior	Ref	11:30	6	
9	12	2024_04_13_	near-contact	T	exterior	1	11:32	7	
10	12	2024_04_13_	near-contact	T	exterior	2	11:33	8	
11	12	2024_04_13_	near-contact	T	exterior	3	11:34	9	
12	12	2024_04_13_	near-contact	T	exterior	4	11:35	10	
13	12	2024_04_13_	near-contact	T	exterior	5	11:36	11	
14	12	2024_04_13_	near-contact	T	interior	Ref	11:45	12	
15	12	2024_04_13_	near-contact	T	interior	1	11:47	13	
16	12	2024_04_13_	near-contact	T	interior	2	11:48	14	
17	12	2024_04_13_	near-contact	T	interior	3	11:49	15	
18	12	2024_04_13_	near-contact	T	interior	4	11:50	16	
19	12	2024_04_13_	near-contact	T	interior	5	11:51	17	
20	13	2024_04_13_	near-contact	F	NA	Ref	12:00	18	
21	13	2024_04_13_	near-contact	F	NA	1	12:02	19	

Figure 6 Lab Table template to link saved spectral files to SampleID

6. Sample selection and sample preparation for near-contact and full contact measurements

Samples are placed on black cardboard plates covered in Lambertian foil material marked with a black disk indicating the outer dimension of the contact probe (**Figure 7**)**.** Prepare five plates for each sample as sub samples. Sample preparation depends on the dimensions of the focal flowers or fruits:

- For small/medium sized samples, layer multiple flowers/fruits (or clumps thereof) from the sample on each black disk (**Figure 7**), so that in each case the area of the disk is completely covered.
- For large samples in which a single sample is greater than the black disk: place a single flower/fruit from the sample on each black disk.

Take a photo of each sample from above. Within the photo include a small piece of paper with the sub sample number (1-5) and the ID number and take a photo of each sample from above. Record the photo file number in the Lab Table.

Figure 7. Sample plate and five Subsample plates for one SampleID.

7. Calibrating sensor

For **near-contact measurements:** place the white reference panel (Figure 1**I**) on the sample stand and adjust the height so the plant probe is close to touching the surface of the reference plate.
For **contact measurements**: place the white reference panel (Figure 1**J**) on the sample stand and adjust the height so the plant probe is pressing the surface of the reference plate. Close the front flap of the black box and thoroughly secure Velcro in place. Make sure there is no light escaping from the edges of the box, and that the room lights are off.
For **leaf-clip measurements**: the sensor is calibrated using the white reference panel that is attached to the leaf clip. Ensure the white reference side is facing the sensor and close the clip.

For all measurements: Begin by taking a dark reference, click on the DC icon underneath the toolbar (Figure 88). Wait until bar under 'dark reference' on the left of the screen has reached 100 %.

Next carry out the optimization through clicking on OPT underneath the toolbar (**Figure 8**). Wait until the software states 'optimized' at the bottom right-hand side of the screen and the spectrum displays a raw radiance spectrum. To set the white reference, click on WR underneath the toolbar (**Figure 8**). Wait until the bar under 'white reference' on the left side of the screen has reached 100 % and the spectrum displays a flat line on 1. Save this reference by clicking *space bar* and record the file number of this reference and the time in the Lab Table.

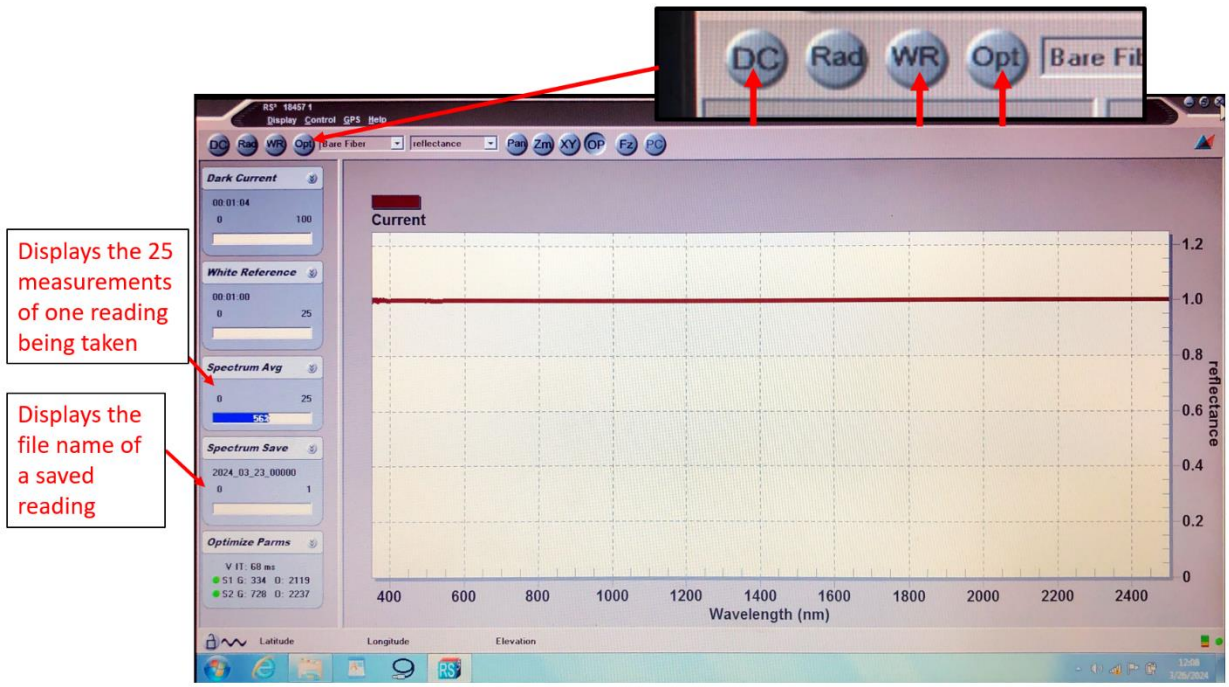


Figure 8. RS³ software display showing current reflectance reading and arrows indicating steps to calibrate sensor.

This calibration, including the optimization and white reference measurement, is repeated before every sample. The dark reference only needs to be calibrated at the beginning of all the measurements.

8. Taking sample spectral measurements

For **Near-contact measurements**: Place the disk holding the sub sample on the flat surface underneath the contact probe. Adjust the height of the clamp so the probe is near-touching the sample and the light from the contact probe is covering the area of the sample to be measured (corresponding with the area covering the black disk on the black tray). Close the front flap of the black box and thoroughly secure Velcro in place. Make sure there is no light escaping from the edges of the box and that the room lights are off (**Figure 9A**).

For **Contact measurements**: Place the disk holding the sub sample on the flat surface underneath the contact probe. Adjust the height of the clamp so the probe is pressing down on the sample. Aim to press down so a limited amount of light is visible where the probe is in contact with the sample

(**Figure 9B**). Close the front flap of the black box and thoroughly secure Velcro in place and ensure that the room lights are off.

For **Leaf-clip measurements**: Ensure the dark panel side of the leaf clip is facing the sensor. Place the soft tissue sample on the dark panel so that over 80 % of the area is covered by sample. Close the leaf clip so the sample is held in place against the probe and there is no light visible where the probe is in contact with the sample (**Figure 9C**).

For **All measurements**: Once the spectrum displayed on the screen reaches stability, click *space bar* to save the reference reading. Note down the file name and the time of the spectrum in the Lab Table. Repeat these steps with the other four sub samples so that in total there are five readings (one for each sub sample) and one reference file saved per sample for each measurement type. Note down the file names (Figure 8) with associated sample ID and which sub sample in the Lab Table.

Return the white reference plate to the clamp stand and observe the spectral reading to ensure the reference panel still displays a reflectance at 1 across all the wavelengths.

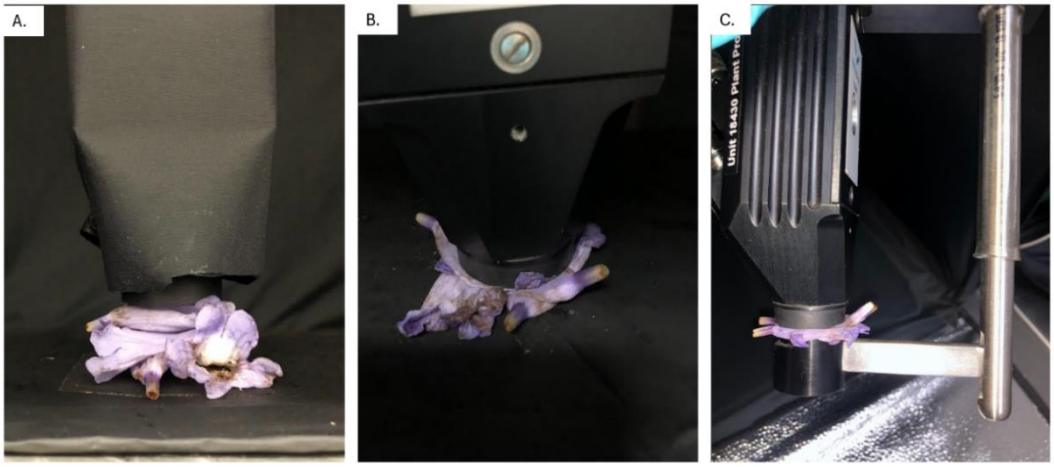


Figure 9. Different measurement types for organ level spectral measurements. A. Near-contact measurements. B. Contact measurements. C. Leaf-clip measurements.

Cases of multiple views with separate spectral measurements per sample. In cases where the sample displays two or more contrasting visual colours at different points across the organ, you may wish to carry out two or more independent sets of five sub sample measurements. Follow the same protocol as above but at different views so there are multiple sets of five sub samples per sample

In the Lab Table, note T (True) in the 'SubView' column to indicate multiple views are being measured. In the 'View' column, select from the type sub categories of view are being measured.

9. Data processing: Processed in ViewSpecPro software

Launch processing software by double clicking on ViewSpecPro icon (Figure 4). Open Set-up/Input directory and select file the raw file of that date containing the data to be processed (**Figure 10A—B**). When asked whether the output directory should be the same as input by default, select No (**Figure 10C**). Open Set/up/Output directory and select the corresponding file for that date in the processed folder where the data will be generated. Select 'file' which will take you to the input

directory where all data is selected and press okay. Data can be seen listed in the software. Highlight all data, select process/Reflectance (Transmission) as shown in **Figure 11A**.

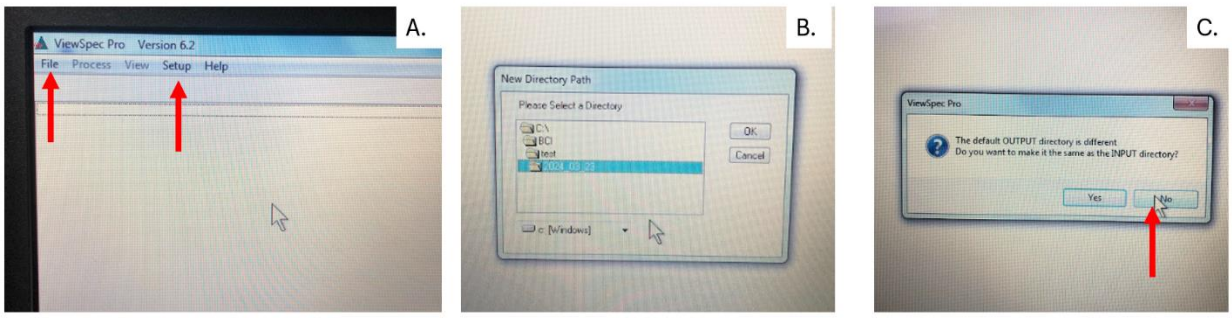


Figure 10. ViewSpecPro software configuration to load files for spectral data processing. A Menu bar indicating relevant section. B-C Selecting file directory.

Exporting data Once data is processed, in the same programme select all, open process/ASCII Export. Fill out the following details (**Figure** Figure 11**B**).

- Select 'reflectance'
- Select 'None'
- Field separator: Tab
- Select: 'column'
- X-axis: select 'wavelength nm'
- Select 'output to single file'

This generates a single .txt file containing all the processed measurements in the output directory.

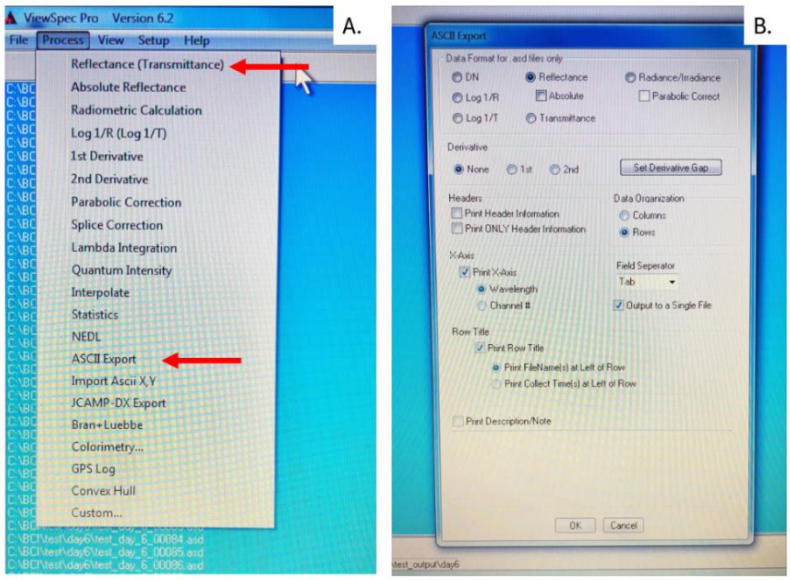


Figure 11. A Drop-down menu indicating relevant sections for spectral data processing. B Details for exporting spectral files.

Sampling schedule

Sample collection in the field is done at least once every week, year round, with more days of collection in seasons when there is more flowering and fruiting. Year-round, high-frequency, collection is important because species vary in the seasonal timing of their flowering, fruiting, leafing, and leaf fall, the duration of such events for any given plant or species is often short, and such events occur year round.

Data management and publication

All data will be published within one year of data collection in data publications at the Smithsonian Tropical Data Portal hosted by DataOne: https://smithsonian.dataone.org/portals/tropical. All individuals contributing substantially to data collection or data management will be included as coauthors or in the acknowledgments. Coauthor status requires substantial contributions representing at least 300 hours of work. These data publications will be published under a CC BY license (https://creativecommons.org/licenses/by/4.0/), meaning any subsequent use of these data must cite (give credit to) the originating data publication and authors. Authorship on scholarly publications analyzing these data will follow accepted guidelines for authorship such as those stated by the journal *Science* at https://www.science.org/content/page/science-journals-editorial-policies#authorship Additional details regarding data management plan are given in the separate data management plan.

Study personnel and roles

Helene C. Muller-Landau is the principal investigator, who designed the study, wrote the grant proposals funding the research, and supervises the ongoing data collection and analysis. Yoseline Angel trained the team in measurement methods and guided the protocol development. Daria Lipsky, Juan Camilo Osorio Ospina, and Lily Pitcher contributed to data collection, data management, data analysis, and the drafting of this written protocol. Pablo Ramos contributed to data collection and coordination of the field work. David Brassfield, Osvaldo Calderon, Andres Hernandez and Omar Hernandez contributed to taxonomic identification of plant samples. Juan Camilo Osorio Ospina made the FieldMaps data collection form.

References

Danner, M.; Locherer, M.; Hank, T. & Richter, K. (2015): Spectral Sampling with the ASD FieldSpec 4 – Theory, Measurement, Problems, Interpretation. *EnMAP Field Guides Technical Report, GFZ Data Services*. http://doi.org/10.2312/enmap.2015.008