NGEE Tropics - ENSO leaf spectral collection

This list covers the basic instruments, accessories, and protocols needed for collecting spectral data in support of the ENSO campaign including pairing with diurnal and CO2 response curve gas exchange measurements

Instruments & Equipment

- Instruments:
    - ASD FieldSpec Pro, FieldSpec3 or FieldSpec4
    - Spectra Vista Corporation (SVC) HR-1024i
    - Spectral Evolution PSR+
    - *Any others?*
- Equipment
    - Leaf clip and/or plant-probe assembly
    - Instrument / leaf-clip, probe batteries
    - Battery chargers
    - External illumination lamps (where applicable, e.g. SE PSR+)
    - Laptop, tablet, or handheld controlling computers
    - Thumb drive with installers for each instrument (backup)
    - Spectralon standards
    - Low reflective black cloth
    - Leaf temperature thermocouple (e.g. Omega HH801B)
    - Small folding table and shade canopy
    - Barcodes (to match gasex samples)
    - sample envelopes (to match gasex samples)
    - Spare parts and tools, where applicable (e.g. bulbs)

Advance Preparation

1. Ensure instrument is recently calibrated (<2 years)
2. Ensure you have the most recent instrument calibration files on hand and if applicable installed in the software directory (e.g. SE, ASD). SVC and Spectral Evolution cal files are stored on the instrument
3. Ensure most recent software and firmware has been loaded on controlling computers and instruments
4. Ensure all Spectralon reflectance standards have been cleaned of grit and debris before each campaign (minimal) or each day (preferred) using 240 waterpoof sandpaper under running water. See Spectralon handling manual located here: https://drive.google.com/open?id=0B_qFBZ6hyQLccWsyU0xJRW5yZVk

NGEE Tropics - ENSO spectral collection protocol for non gasex leaves

Use this simpler protocol for any leaves that you only want spectra on, or that you are using together with measurements that are not temperature sensitive. To better standardize we will use instrument specific plant probes without leaf-clip assemblies (when applicable) to collect the leaf spectra together with optically dark (<5% reflective) black cloth (provided)

ASD specific settings
Dark current: 10+
White Reference: 15+ (more is better for higher SNR)
Number of averages: 5+ (more is better for higher SNR)

SVC HR-1024i specific settings (see https://docs.google.com/document/d/1ighE2hbVJLdW3gdJeCSSGcS9kjb-OTQG0uhqX5Kdlpc/edit?usp=sharing for more information)

Set the "Optic" to FIBER1
Specify Total Scan Time (Sec): set this to 1
Select "Auto Integration"

SE PSR+ specific settings
Set the foreoptic to Raw DN
Set Scan Time to 5

Warm up procedures

1. Turn on instrument and warm up for 15 minutes
2. Turn on leaf clip or plant/probe lamp and warm up for 5 minutes
3. Turn on controlling computer and connect to instrument
4. Setup daily directory for spectral measurements on controlling computer
5. Setup first sample filename

Steps:

1. Create a sample filename prefix for each leaf or sample; should allow for easy averaging of all subsamples to create an average spectra per leaf. For NGEE we should be using barcodes (e.g. BNL10111). All spectrometer software programs append a set of auto numbers to the end of the filenames to differentiate multiple spectral measurements per sample
2. Take a photograph of the leaf with a cell phone or other camera with the barcode in view.
    a. For small leaves or if interested in collecting leaf dimensional data, place a small ruler or length standard within the frame of the photograph. We will later use this to estimate leaf area, length, width, etc
3. Collect a white reference - Place the lens of the leaf clip / plant probe flat against the external Spectralon reflectance standard hold the standard in place until the reference

collection is complete. It is important to confirm that the lens of the probe is completely covered by the white spectralon material

   a. *Remember to record which spectral puck you are using each day*

4. Place leaf sample on top of the black cloth material on the sample table and position the lens of the probe over the desired section so that it is completely filled by the leaf. It is best to avoid the mid-vein, when possible.
   a. For smaller, narrow leaves position the lens of the probe so that the leaf is centered within the lens. For most plant probes this is sufficient to get a reasonable spectral measurement, including the HR-1024i, FieldSpec, and PSR+
5. Hold the lens of the probe firmly and flat against the leaf; try to avoid creasing or leaf folding which will cause erroneous spectral measurements or unrealistically high NIR reflectance (by increasing scattering)
6. Collect the spectral measurement
   a. If collecting spectra for an exact point on a leaf then collect at least 2 more spectra of the same location without releasing;
   b. Else move the probe to another position on the leaf and collect another spectra
7. Measure the leaf 2-5 times to get a good leaf average; collect more if a mistake is made on any of the sub samples. More sub samples results in cleaner spectra
8. Detach / save leaf for later analysis (e.g. SLA, CHN, NSCs, etc), when applicable

NGEE Tropics - ENSO spectral collection protocol for diurnal & *A*-Ci / A-Q leaves

This protocol is for the measurement of leaf reflectance on samples that have had parallel measurements of diurnal leaf level photosynthesis, stomatal conductance, leaf water potential or A-Ci (CO2) response curves in support of the NGEE-Tropics ENSO campaign. The goal is for this protocol to be used at the NGEE Tropics ENSO core sites to collect data every 4-6 weeks through the duration of the ENSO event. To better standardize we will use instrument specific plant probes without leaf-clip assemblies (when applicable) to collect the leaf spectra together with optically dark (<5% reflective) black cloth (provided). Note this protocol is relevant for any leaf with a corresponding physiological or biochemical measurement that is temperature sensitive or with leaves that have a corresponding A-Q curve (light response curve)*

ASD specific settings
Dark current: 10+
White Reference: 15+ (more is better for higher SNR)
Number of averages: 1-2 (Ensure target measurement time of less than 2 seconds to maintain leaf temperature)

SVC HR-1024i specific settings (see https://docs.google.com/document/d/1ighE2hbVJLdW3gdJeCSSGcS9kjb-OTQG0uhqX5KdIpc/edit?usp=sharing for more information)

Set the "Optic" to FIBER1
Specify Total Scan Time (Sec): set this to 1
Select "Auto Integration"

SE PSR+ specific settings
Set the foreoptic to Raw DN
Set Scan Time to 5

Warm up procedures

1. Turn on instrument and warm up for 15 minutes
2. Turn on leaf clip or plant/probe lamp and warm up for 5 minutes
3. Turn on controlling computer and connect to instrument
4. Setup daily directory for spectral measurements on controlling computer
5. Setup first sample filename

Steps:

1. Create a sample filename prefix for each leaf or sample; should allow for easy averaging of all subsamples to create an average spectra per leaf. For NGEE we should be using barcodes (e.g. BNL10111). All spectrometer software programs append a set of auto

numbers to the end of the filenames to differentiate multiple spectral measurements per sample

2. Take a photograph of the leaf with a cell phone or other camera with the barcode in view.
    a. For small leaves or if interested in collecting leaf dimensional data, place a small ruler or length standard within the frame of the photograph. We will later use this to estimate leaf area, length, width, etc
    b. For gasex leaves that do not fully fill the 2x3 chamber, make sure the photograph shows the gasket markings and contains a known length standard or a metric ruler
3. Collect a white reference - Place the lens of the leaf clip / plant probe flat against the external Spectralon reflectance standard hold the standard in place until the reference collection is complete. It is important to confirm that the lens of the probe is completely covered by the white spectralon material
    a. *Remember to record which spectral puck you are using each day*
4. Measure the leaf temperature on the leaf abaxial surface to maintain consistency with the LiCor 6400 using a thermocouple (Omega HH801B, preferred) or IR gun; try to minimize handling of the leaf (which can heat it up) or moving it too much out of its resting position (if still attached to the twig) which will influence the temperature of the leaf.
    a. If using the leaf thermocouple, make sure not to hold the thermocouple wire and keep your and at a sufficient distance as to not influence the leaf temperature measurement. Try to avoid direct sunlight during measurement
    b. If using the IR gun, make sure you are measuring the abaxial side and not placing the leaf in direct sunlight
5. Record the leaf temperature you measured before the collection of spectra
6. Place leaf sample on top of the black cloth material on the sample table and position the lens of the probe over the desired section so that it is completely filled by the leaf. It is best to avoid the mid-vein, when possible.
    a. For smaller, narrow leaves position the lens of the probe so that the leaf is centered within the lens. For most plant probes this is sufficient to get a reasonable spectral measurement, including the HR-1024i, FieldSpec, and PSR+
7. Hold the lens of the probe firmly and flat against the leaf; try to avoid creasing or leaf folding which will cause erroneous spectral measurements or unrealistically high NIR reflectance (by increasing scattering)
8. Collect the spectral measurement
    a. If collecting spectra for an exact point on a leaf then collect at least 2 more spectra of the same location without releasing;
    b. Else move the probe to another position on the leaf and collect another spectra
9. Measure the leaf 2-4 times to get a good leaf average; collect more if a mistake is made on any of the sub samples. More sub samples results in cleaner spectra
10. Immediately re-measure leaf temperature on the abaxial surface and record leaf temperature after spectra collection
11. If gasex has not yet been measured, you can now match the temperature in the LiCor to that measured on the leaf (use the average);

12. Or if relying on a temperature function to scale V_{cmax} (for example) to the temperature during spectra, detach the leaf and save for later analysis (e.g. SLA, CHN, NSCs, etc)

*Required: Thermocouple or IR thermometer for measuring abaxial leaf temperature before and after spectral collection