**Soil and Water Conservation Laboratory**

**Standard Operating Procedure**

**Sherman 230, Soil and Water Conservation Laboratory, UH Mānoa**

**Visible Near Infrared (VNIR) Diffuse Reflectance Spectroscopy of Dried and 2 mm Sieved Soil Samples (& Plant Tissues) with the Agrispec**

**Purpose:**

To scan soil samples in the visible and near infrared wavelengths to create spectral libraries and for chemometric analyses.

**Soil Sample Preparation (Also applies to plant tissues)**

**1.** Soil sample preparation for this analysis is accomplished by oven drying soil samples at 105oC for 24 hours in one of the ovens in Sherman 230. Once the samples have been dried they should be sieved with a 2 mm sieve (see cabinet underneath the Agrispec for the sieves). This will reduce problems due to soil moisture and presence of macroorganic matter, pebbles, coral/shell, or other non-soil material.

**2.** As moisture interferes with collection of accurate and reproducible spectra, if archived soils have been sitting in the lab for long periods, they should be re-dried in the oven at 105oC for 24 hours prior to scanning. Dried samples should be removed from the oven and placed in the larger desiccators to cool for at least 1 hour before scanning. Do NOT leave dried samples out or uncovered after drying as they will absorb moisture from the humidity in the lab air.

**AgriSpec Instrument Operation and Scanning**

**1.** Be VERY careful and gentle with the FIBER OPTIC CABLE. See signs on the lab bench and cable itself. This part of the instrument cost about $2500 and can be easily damaged by bending/kinking the cable at sharp angles, dropping it on the benchtop or floor, or placing heavy objects on top of it such as books, hands, etc.

**2.** Turn on the spectrometer and let it warm up for at least an hour prior to scanning. This will allow the instrument’s temperature to stabilize and allow you to collect reproducible scans. It is good to do this first before you get your soils out of the oven, etc. Follow the power rules provided by ASD when switching on and off the instrument/laptop computer (e.g., make sure to turn on the spectrometer before turning on the laptop). See page 20 of the Indico Pro user Manual for more information on this topic.

**3.** Turn on the laptop. Ignore usual messages that pop-up about anti-virus software, etc.

**4.** Open the Indico Pro software package on the laptop. Go to:

 *Start Menu → All Programs → ASD Programs → Indico Pro*

Select *File → New Project* or open an existing project file. See page 25 of Indico Pro user manual for more detailed instructions.

 \*Make sure the project is set to use 30 co-added scans, not the default of 10

**5.** Use the Spectralon (white reference) to calibrate the instrument before starting to scan soil samples.

 -Make sure sample cup, top of glass, and Spectralon reference are free of dust

 -Carefully set Spectralon reference on top of instrument

-Within Indico Pro, select *Spectrum → Take Baseline*. Hit ‘okay’ in pop-up window.

-You should see a straight baseline across the Project Graph at 1.00 (100% reflectance). There will be some noise at the very start and end of the spectrum.

-Zoom in on Y-axis by going to *Display → Axes*, check ‘autoscale’ under Reflectance

\*If you do not get a reading with 1.00 when collecting the white reference, come and see Dr. Bruland to troubleshoot. Don’t proceed will collection of sample spectra until you have a collected a proper white reference.

-Put Spectralon reference carefully to the side on a kimwipe.

**6.** Place the soil samples or a representative subsamples in the scanning vial.

 -Roll soil sample vial gently just a bit to mix up particles if they have settled.

 -Pour some into scanning vial, making sure that the soil completely covers all of the glass window of the vial and that no light from the mug light can pass through gaps in the soil. Don’t touch the glass of the vial or leave fingerprints on it. This can also interfere with the reflectance.

 -Place scanning vial on instrument.

**7.** Scan the soil sample

-Hit *Spectrum → Take a Scan*. Click ‘okay’ in pop-up window.

\*You will need to decide on how many replicates to take at different angles. This may be two replicates at two different angles, three at three angles (down, center, up), or some other variation. Talk to Dr. Bruland about this to decide on a final protocol.

-*Spectrum → Save* to save file

-Repeat measurement for other angles

\* Check for variation in the individual scans from the 2-4 replicates for each individual soil to see if any need to be re-run.

**8.** Place the soil back into the sample container (remember that VNIR DRS is a non-destructive analysis technique and we want to save samples for future use).

**9.** Clean the scanning vial with compressed air. This can be accomplished by spraying a blast of compressed air onto the vial to remove soil particles and then gently wipe the glass clean and dry both the glass and the rest of the vial with kimwipes.

**10.** Repeat steps 7, 8, & 9 for next sample

**11.** Recalibrate the instrument with Spectralon (white reference) at a minimum of every 30 minutes (Step 5).

**12.** When done, need to convert ASD files to SPC and/or ASCII.

 *Run → ASD to SPC →* select files

 -save in reflectance, no derivatives, enter file name, etc.

 -files will be saved in C://Program Files/ASD/Project/…

**13.** Save project file and exit Indico Pro

**14.** Shut down computer

**15.** Turn off instrument if not using again soon

**Other Notes:**

Leave the instrument running during periods of scanning that stretch over the same day or multiple days as powering on and off can impact reflectance values. If you are not going to use the Agrispec for a period longer than a week, then go ahead and power it down until the next use.