PROTOCOL: High-fidelity Leaf Spectral Reflectance and Transmittance (350-2500 nm)

(i) Equipment

- Analytical Spectral Devices (ASD Inc., Boulder CO USA) spectroradiometers with custom detectors and customized exit slit to maximize signal-to-noise performance; Range: 350-2500 nm; Sampling: 2.4 nm FWHM reported at 1 nm; Radiometric calibration +/- 1 nm; Customized cooling for humid forest conditions; Long-duration 9000 mAH batteries.
- Licor-1800 integrating spheres for hemispherical reflectance and transmittance measurements covering the 350-2500 nm spectra range at 2.4 nm FWHM; Interior sphere walls and port plugs are refitted with Spectralon™ (Sphere Optics Inc., Durham, NH USA) coating.
- Custom-built sphere light sources producing one solar irradiance power orthogonal to leaf surface and supporting the 350-2500 nm measurements; Custom heat exchangers and brightness/voltage regulators.
- Weather-resistant palmtop computer running Windows XP; Software for operating spectrometer and viewing spectra.
- Spectranomics leaf optics processing software.

(ii) Consumable materials

- Spectralon™ (Sphere Optics Inc., Durham, NH USA) reference standard
- Dust- and lint-free clothes
- Compressed air
- Plastic tarps

(iii) Sample preparation

- For details on the collection method, see leaf collection protocol on the Spectranomics website.
- Following top-of-canopy branch collection, the selected branches are kept in shade and cool conditions for up to 15 minutes (often much less), while delivered to the spectrometer. The spectrometer is stationed within minutes of the collection tree, often in the forest on a tarp.
- Leaves are selected from branches to represent the state of the tree at the time of collection. These leaves may show signs of herbivory, epiphyll growth, and endophytes. Only mature, fully-expanded leaves are taken for spectroscopy; new undeveloped leaves are avoided. Six leaves are taken from the uppermost surface of the branch volume, thereby representing the topmost canopy foliage.

(iv) Measurement procedure

- Each leaf is placed facing inward and tangent to the leaf sample port of the integrating sphere. Leaves are placed in the sphere so that the field-of-view (approx. 9 mm dia.) is focused at the adaxial leaf surface during reflectance measurements. The leaf is placed in the sample holder to present an amount of leaf and vein material that is roughly proportional to the leaf-and-vein area found throughout the leaf. The large mid-vein is avoided entirely.
- Each leaf collection includes a measurement of the Spectralon reference standard. A reference standard is measured for every leaf measured in the Spectranomics database in order to maximize data quality.
- Reference radiance: The light source is placed in the reference position on the sphere to directly illuminate the Spectralon reference standard. The reference radiance is measured with the leaf in the sample port, which is not

directly illuminated during reference measurements. The reference spectrum is a 50-spectrum (350-2500 nm) scan.

- Reflectance radiance: Holding the leaf in the same position as during the reference measurement, the light source is moved to the reflectance position on the sphere, thereby directly illuminating the leaf sample. A 50-spectrum radiance measurement is collected.
- Transmittance radiance: Maintaining the leaf in the original position, the light source is moved to the transmittance position on the sphere, thereby directly illuminating the abaxial side of the leaf. Light passes through the leaf and into the sphere space. A 50-spectrum radiance measurement is collected.
- In addition to leaf measurements, the 'stray light' scattering within the sphere is measured by illuminating the sample port (reflectance mode) with no sample present. A similar measurement is made with transmittance mode.

(v) **Data preparation and finalization**

- Leaf hemispherical reflectance is calculated using the equation:

  $Refl = [(I_{refl}-I_{stray})*R_{ref}] / (I_{ref}-I_{stray})$

  where I_{refl} is the radiance measured when the sample is in the port and the light is in the reflectance position; I_{stray} is the radiance measured when no sample is in the port and the light in the reflectance position; R_{ref} is the reflectance (not radiance) of the Spectralon reference material; I_{ref} is the radiance measured when the light shines directly upon the Spectralon reference standard.

- Leaf hemispherical transmittance is calculated using the equation:

  $Tran = [(I_{tran}-I_{stray})*R_{ref}] / (I_{ref}-I_{stray})$

  where I_{tran} is the radiance measured when the sample is in the port and the light is in the transmittance position.

- Following reflectance and transmittance calculations, the energy closure is assessed and corrected using the method provided by:

  Merzlyak, M.N., Chivjunova, O.B., Melo, T.B., and Naqvi, R. 2002. Does a leaf absorb radiation in the near infrared (780-900 nm) region? A new approach to quantifying optical reflection, absorption and transmission of leaves. Photosynthesis Research 72:263-270.

- A manual auditing step is done to ensure that all computed reflectance and transmittance spectra are free from errors, and that final quality is sufficiently high to warrant inclusion of the spectra in the database.

- Following spectral calibration, reflectance/transmittance calculation and manual audit, the mean and standard deviation spectra are calculated for each specimen.