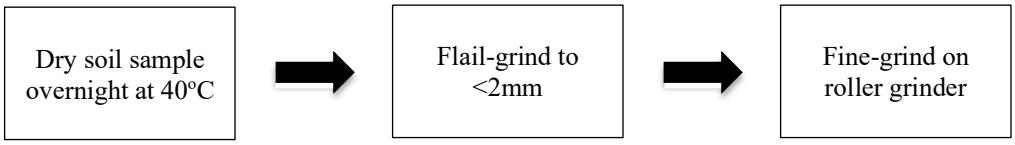
Procedure for Spectra Acquisition via DRIFTS

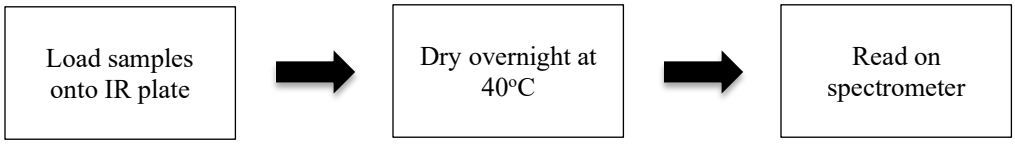
Procedure Overview

This protocol describes the procedure for acquiring spectra from soils via diffuse reflectance infrared Fourier transform spectroscopy in the mid infrared region (DRIFTS) in a high-throughput framework. Before DRIFTS analysis, soil samples require two grinding steps. This first step is drying soils in an oven at 40°C and then flail-grinding to <2 mm. (See accompanying protocol for procedure.)

Sample Preparation



Quantification



This procedure assumes samples have already been dried and ground to <2 mm within the last month. If soil grinding occurred over a month ago, then soils should be oven-dried overnight before samples are finely ground. Nitrile gloves should be worn throughout the procedure, as contaminants will affect spectra.

Materials

Sample Preparation/Grinding

- Nitrile Gloves
- 20 mL glass scintillation vials
- Avery Labels
- Funnel
- 5-gram soil scoop
- Cylindrical stainless-steel grinding bars (3 per vial)
- Roller grinder
- Soil drying oven at 40°C and 12–14% relative humidity
- Tweezers

Plating

- Spatula
- Weighing paper
- Aluminum sample plates
- 0.25 mL sample cups (Fisher Sci. Cat. No. 22-170-161)
- Small paint brush
- Carboard covered container for drying

Scanning

- Soil drying oven at 100ºC
- Desiccator and Drierite desiccant
- Potassium Bromide (KBr)
- Infrared Spectrometer

Detailed Procedure

I. Sample Preparation and Grinding

1. Using the accompanying DRIFTS plate layout excel template, input sample IDs in the 'Sample List' tab. This step automatically populates sample IDs in the 'Plate Layout' tab.
2. Using Sample IDs from 'Sample List' tab, create and print Avery labels using the mail merge function in MS Word.
3. Label 20 mL glass scintillation vials with Avery self-adhesive labels.
4. Using a funnel, scoop 5-6 grams of soil into corresponding scint vial. Strive to get a representative sample. Ensure ground soil is mixed with scoop and use a full scooping motion that captures soil from a cross section of the sample.
5. Insert 3 stainless-steel grinding bars. Cap vial tightly. Ensure foam disc is in cap to fully seal vial.
6. Transfer vials to roller grinder in sequential order, laying on side.
7. Grind at 60 rpm (setting '30' on dial) for 16 hours.
8. Transfer vials back to original scintillation vial box in the same sequential order. Ensure labels did not peel off during grinding. If so, tape label to vial with clear tape.
9. Remove grinding bars from vials with tweezers and set aside to be DI rinsed and dried.

II. Plating Samples

1. Print paper copy of each plate to be loaded from the 'Plate Layout' tab in excel template.
2. Fill out operator and date information on paper plate layout, ensuring sample IDs on layout match with soil samples about to be loaded.
3. Scoop, tap-tap, scrape:
   a. Using a spatula, scoop enough sample from vial to fill sample cup. Sample cup should be overflowing with soil.
   b. Gently tap the bottom of the sample cup twice to settle soil.
   c. Slowly scrape off excess with straight edge of spatula. It is critical that soil surface is level and smooth. Do not to leave gaps in surface, do not leave large soil particles on surface, do not pack down sample. Ideally this is accomplished with a single smooth motion. If soil surface in sample cup isn't smooth, simply dump sample back into vial and repeat step 3.
   d. Once soil is level, clean the shoulder with a small paint brush.
   e. If salvaging excess is desired, place a piece of weighing paper beneath the sample cup during this step and return excess to scintillation vial.
4. Carefully place sample cup into labelled DRIFTS plate according to plating template.
5. Continue to next sample and repeat process of loading. It is critical that sample IDs and plate position reflects what is illustrated in paper template. Check and double-check often that samples are being properly loaded.

6. Once loaded, place the plate in a cardboard box to protect samples from air flow in dryer, cover loosely and place in drying oven at 40°C overnight to drive off any remaining moisture.
7. Transfer KBr to a beaker, leave in a high temperature oven at 100°C overnight along with desiccant.

III. Spectra Acquisition

1. Transfer dried plates, KBr, and desiccant to desiccator in Williams Hall lab. During the scanning process plates can be stored up to eight hours before being returned to dehydrator.
2. Wait for the KBR to cool down (~15 min) in the desiccator. Then, scoop, tap-tap, scrape KBr in the cup and place cup into position 1 of each plate. KBr is a highly hygroscopic material and therefore this procedure should be only done for plates to be read in the next 4 hours or twice a day. New KBr from the high temperature oven should be used for the second batch of the day following step 1.
3. Spectra are acquired using an X,Y Autosampler equipped with a deuterated triglycine sulfate (DTGS) detector coupled with a Nicolet iS50 spectrometer.
    a. Omnic
        i. Change experimental setup to 8 cm^{-1} wavenumber resolution and 24 co-added scans in absorbance mode.
        ii. Enable collect background at >1000 minutes.
        iii. Add H_2O reference. In Omnic—Edit > Options > Process > Browse H_2O reference. Search for in Computer files: Local Disk (C:) > PIKE_Technologies > H_2O reference > Open
    b. AutoPro
        i. Select 24 well profile.
        ii. Oversampling motion 4 random points, 3 mm diameter in the central position of each well, disable average scans. If less than a full plate, adjust point map to reflect.
        iii. Rename and save to appropriate box folder.
        iv. Load plate into the spectrometer (before loading, make sure the cups are leveled in the plate to avoid the plate getting stuck).
        v. Measure the background, by selecting 'Background'. This will allow software to account for (i.e., subtract out) reflectance from water and CO_2.
        vi. Measure samples, by selecting 'Scan'.
    c. Return KBr to beaker, discard sample cups, and wipe plate if necessary. KBr supply can be reused multiple times but should be replaced at regular intervals or if contaminants are detected.

IV. Spectra Processing and Saving

1. Spectra are automatically saved to the hard drive (Local Disk (C:) > PIKE_Technologies > Spectra) as .SPA files.
2. After saving, .SPA files should be dragged into Omnic window, selected, then saved as .CSV to the appropriate data file on Box.

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