

Standard Operating Procedure

Wet Aggregate Stability: Multi-Sample Image Acquisition

Scope

This document outlines the image acquisition component of a procedure for analyzing aggregate stability of multiple soil samples using a smartphone. Soil aggregates (4-8 mm diameter) are photographed in an air-dried state, immediately upon submersion in water, and after ten minutes of submersion. This image acquisition procedure is designed to process two soil samples in triplicate (i.e., six Petri dishes) per ~20-minute batch. An accompanying document <u>available here</u> describes the use of the free and open-source R package SlakeItEasy to process images generated through this procedure and calculate aggregate stability indices.

Equipment

Tracing tablet with backlight Paper bag or tray Smartphone camera¹ Digital timer² Petri dish (90 mm diameter, minimum 15 mm height) Weighted cylindrical object to hold phone horizontally (100-120 mm height, e.g., peanut butter jar)³ Deionized water Masking tape

¹Any digital camera may be used in place of a phone camera ²Alternatively, some camera apps can be set to take photos at a 10-minute interval. <u>Open Camera</u> is one example available for Android devices. ³Only necessary if using a smartphone camera

Optional: 8 mm (5/16 in.) sieve 4 mm (5 mesh) sieve Forced-air drying facility (minimum temperature: 30 °C)

Procedure

- Sample collection. For aggregate sampling procedures, see the Soil Health Institute standard operating procedure on Soil Health Sampling, Part 2) <u>Sampling for wet aggregate stability</u>. Avoid compressing soils during sample collection and transportation. For reference, we suggest aggregates represent the top 6 cm (2 in.) of the soil.
- 2. Sample preparation.
 - a. Air-dry the sample of soil aggregates by transferring them to a paper bag or spreading them out on a tray. If drying at elevated temperatures (30-40 °C), a minimum drying



time of 24 hours is sufficient. If drying under ambient conditions, a minimum drying time of 72 hours is recommended. Forced air circulation is recommended to remove humidity. Soils that form very strong aggregates upon drying (e.g., Andisols) should be dried at lower temperatures (<25 °C). When in doubt, consult the sample submitter about whether your soils form very strong aggregates when dried.

b. Aggregates should be 4 to 8 mm in diameter. If aggregates and clods larger than 8 mm are present, break them up by hand along natural planes of weakness (i.e., do not crush aggregates). For each soil sample to be processed (i.e., two samples per 20-minute batch), select a minimum of nine aggregates between 4 and 8 mm in diameter; 5 to 6 mm diameter is ideal. Avoid aggregates with flat faces created during sample extraction. Sieves may expedite the aggregate selection process, but sieving is not required. Retain remaining aggregates in case additional analyses are required later, and discard material less than 4 mm.

3. Setup.

a. Optional: If using the <u>Open Camera</u> Android app, select the gear icon to set the following settings.

These three settings should be saved between batches and only need to be specified once:

Repeat mode: 20x Repeat interval: 1 min Camera preview > show a grid > crosshair

If desired, specify a prefix for image files (e.g., per batch, sampling location, etc.) by clicking "More camera controls..." and then "Save photo prefix."

- b. Print a Petri dish template onto a transparency and tape it on the surface of the tracing tablet. The key features of the transparent template are (1) a unique ID for each of the six Petri dish positions (2) guidelines around each Petri dish to realign the camera crosshairs for each image, and (3) a space to label each sample. The template is not strictly required if you have a suitable means of achieving each of these functions.
- c. Place the tracing tablet on a flat surface that will not move abruptly if bumped, and turn on the backlight.
- d. Using masking tape, secure the smartphone to the cylindrical object selected to hold the smartphone (e.g., peanut butter jar).
- e. Start the camera application and position the smartphone over the Petri dish at a height that ensures the dish that will contain the aggregates takes up most of the camera screen (Fig. 1). Adjust the position of the phone and zoom of the phone camera to prevent other objects in the frame. Optional: if using the Open Camera app, verify that the crosshairs within the app align with the guidelines around the Petri dish. Move the cylindrical object with the phone to each of the remaining five Petri dish positions to ensure consistent alignment.





Figure 1. Phone positioned over transparency to take a series of initial and then final photos (left). Cross hairs on an example camera app are aligned with guide marks on transparency to aid in repositioning the phone (right).

f. Place empty Petri dishes in all six positions. Aggregates for a single soil sample will be analyzed in triplicate (i.e., three separate Petri dishes), so two unique soil samples can be analyzed per batch of six. Place three aggregates of the first sample in a triangle in each of three Petri dishes. Repeat for the second sample in the remaining three Petri dishes. Label each Petri dish with its respective sample ID by writing on the transparency with a dry-erase marker (Fig. 2) or placing a printed label near the Petri dish. Ensure that the sample ID and Petri dish position number are legible within the smartphone camera app.



Figure 2. Position and labeling of Petri dishes for two soil samples in triplicate.



4. Image acquisition.

- a. Capture initial images. Set the phone holder against the edge of the tablet above the Petri dish labeled "1" and align the crosshairs with the guidelines. Double-check that the sample ID and Petri dish number are visible in the camera app and click to take a photo. (If using Open Camera, click again to cancel the timer.) Repeat for the remaining five Petri dishes. Carefully remove dishes containing samples from the tablet, maintaining them in order so you'll know where to put them back.
- b. Fill six new Petri dishes with deionized water to a minimum depth of 7 mm (~45 mL). The water level in each Petri dish must be sufficient to fully submerge the selected aggregates. Place water-filled dishes on tablet. If you notice debris floating in the dishes, dump in the waste receptacle, wipe clean, rinse, and refill.
- c. Initiate slaking. With the camera back in place above the first Petri dish (crosshairs aligned with guidelines and sample ID/replicate number visible within image), pick up the three aggregates that you had had in that position for the initial image, and drop them into the water-filled petri dish. Start the digital timer, and immediately click to take an image; if using Open Camera, image capture will activate a timer that will count down from 60 seconds.
- d. Move and align the camera with the guidelines around the second Petri dish. When the digital timer approaches 60 seconds (i.e., ~20 seconds remaining on the Open Camera timer), pick up the next three aggregates. Carefully drop them into the second Petri dish with <5 seconds remaining. After the image is obtained (one minute since the timer started), move and align the camera with the guidelines around the third petri dish. In the same way, drop the third set of aggregates into the third dish when the digital timer reaches two minutes (<5 seconds remaining on Open Camera timer). Repeat this process for the fourth, fifth, and sixth Petri dishes.</p>
- e. Move the phone holder back to the first Petri dish and realign the crosshairs. You'll need to wait five minutes until the final image is obtained for the first set of aggregates. Note: if using Open Camera, four extra images will be collected in this five-minute interval (at six, seven, eight, and nine minutes since the timer started); these images are not required for analysis. Use the digital timer to keep track of total elapsed time. Take the final image of the first set of aggregates at 10 minutes, then move and align the camera with the second dish and capture the final image of the second Petri dish (11 minutes since the timer started). Repeat for the remaining samples. The digital timer should be close to 15 minutes when the final image is captured for the sixth Petri dish.
- 5. Image sorting. To analyze images using the SlakeItEasy R package, images need to be transferred from the smartphone to a computer. Create an empty folder for each set of images to be analyzed (e.g., one folder per day or week). Within the folder you created, create one empty folder for each Petri dish, and name each folder following the convention



SampleID_PetriDishNumber (e.g., ASDF_1 for sample ASDF in Petri dish position 1). Move the initial image of air-dry soil, the image of soil collected immediately upon submersion in water, and the final image of soil after ten minutes of slaking into the corresponding folder. Verify that you have three folders per sample ID, each containing three images (Fig. 3).



Figure 3. File structure for image analysis, with one folder containing three images per Petri dish.

Comments

1. The space between aggregates and the Petri dish edge must allow soils to slake without touching the edge.



2. The space between aggregates must allow soils to slake without touching one another.





- 3. If aggregates break during handling prior to submersion, the initial image of air-dry soil is no longer valid, and the procedure must be repeated for that replicate.
- 4. The time between the second and third image should be ten minutes. Inconsistency in image timing will make results unreliable. A tolerance (e.g., 10 minutes +/- 30 seconds) can be specified during image processing in the SlakeItEasy R package.

Reference

 Fajardo, M., McBratney, A. B., Field, D. J., & Minasny, B. (2016). Soil slaking assessment using image recognition. *Soil and Tillage Research*, 163, 119–129. https://doi.org/10.1016/j.still.2016.05.018

NOTE

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