SOIL HEALTH INSTITUTE
Enriching Soil, Enhancing Life

Standard Operating Procedure

Aggregate Stability via Slaking Image Analysis: Multi-Sample Image Acquisition

Scope

This document outlines the image acquisition component of a procedure for quantifying aggregate stability indices for multiple soil samples. 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 available here 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
Digital camera (e.g., smartphone, point-and-shoot camera, or webcam)
Digital timer[1]
Petri dish (90 mm diameter, minimum 15 mm height)
Tripod or weighted cylindrical object (100-120 mm height, e.g., peanut butter jar)
Deionized water
Masking tape

[1]Alternatively, some camera apps can be set to take photos at a 10-minute interval. Open Camera is one example available for Android devices.

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

Procedure

1. **Sample collection.** For aggregate sampling procedures, see the Soil Health Institute standard operating procedure on Soil Health Sampling, Part 2) Sampling for wet aggregate stability. Avoid compressing soils during sample collection and transportation. For reference, we suggest aggregates represent the top 6 cm (2.5 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 Open Camera 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 required if you have a suitable means of capturing each of these attributes.

c. Place the tracing tablet on a flat surface that will not move abruptly if bumped, and turn on the backlight.

d. Secure the camera to the tripod or, using masking tape, secure the smartphone to the weighted cylindrical object (e.g., peanut butter jar). The camera should be pointed straight downward; an unlevel camera could give biased results.

e. Start the camera and position over the Petri dish at a height that ensures the dish containing the aggregates fills most of the camera screen (Fig. 1). Adjust the position of the camera to prevent other objects in the frame. Camera zoom settings can be adjusted but should always be set the same across batches. Optional: if using the Open Camera app, verify that the crosshairs within the app align with the guidelines around the Petri dish. Move the camera to each of the remaining five Petri dish positions to ensure consistent alignment.

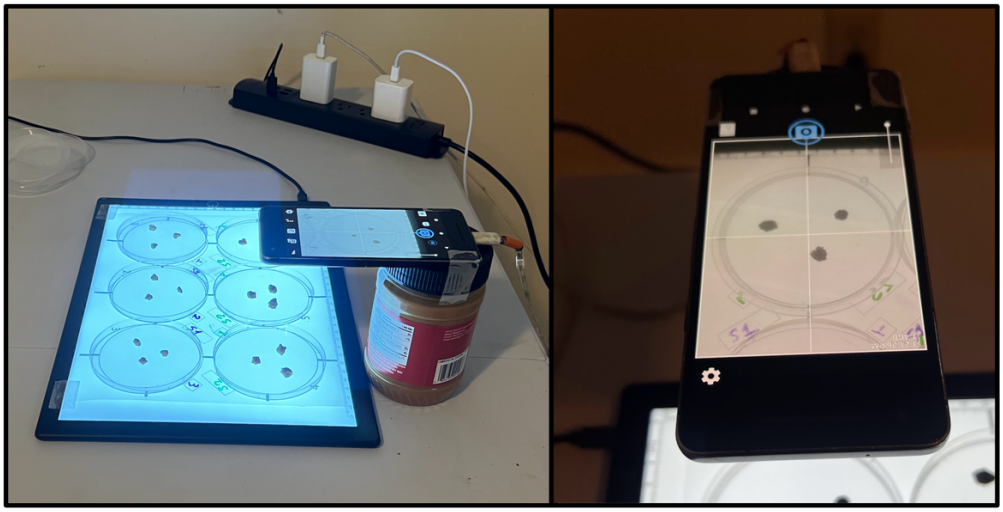


Figure 1. Phone positioned over transparency to take a series of initial and then final photos (left). Cross hairs on an example camera app are aligned with guide marks on transparency to aid in repositioning the phone (right).

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.

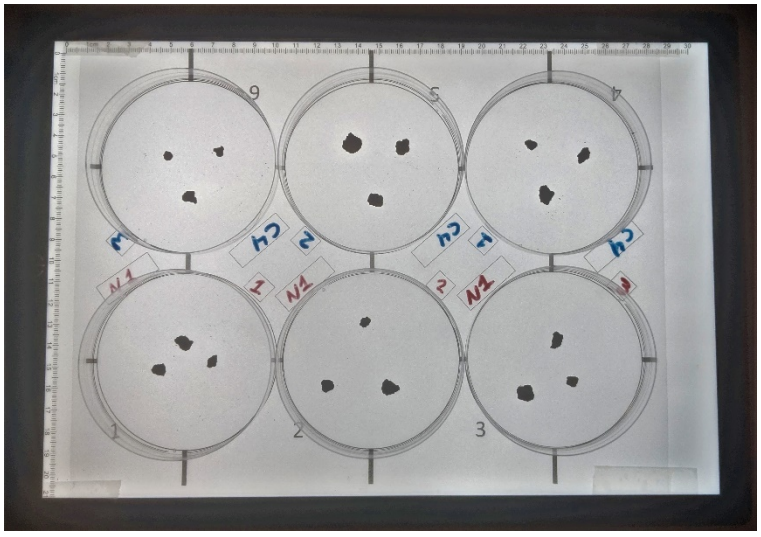


Figure 2. Position and labeling of Petri dishes for two soil samples in triplicate.

4. **Image acquisition.**
    a. Capture initial images. Set the camera holder against the edge of the tracing 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 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 for replacement.
    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 previously positioned for the initial image, and gently 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 Petri 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.
    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 Petri dish and capture the final image of the second set of aggregates (11 minutes since the timer started). Repeat for the remaining sets of aggregates. The digital timer should be close to 15 minutes when the final image is captured for the sixth set of aggregates.
5. **Image sorting.** To analyze images using the SlakeItEasy R package, images need to be transferred from the camera to a computer. Create an empty folder for each batch of images to be analyzed (e.g., one folder for all the procedures conducted in one 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).

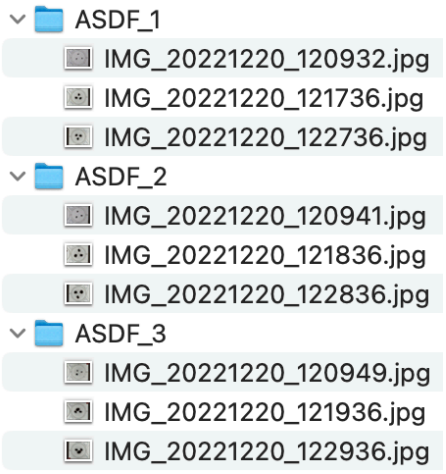
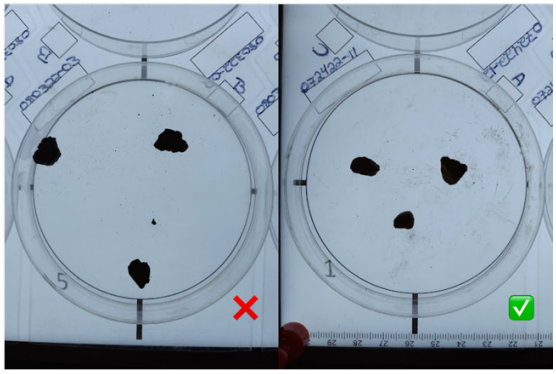


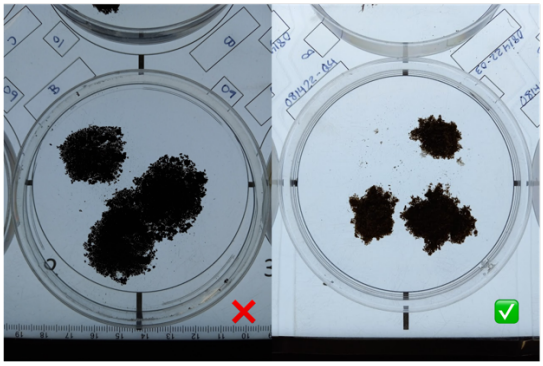
Figure 3. File structure for image analysis, with one folder containing three images per Petri dish.

Comments

1. Keep the tablet surface and Petri dishes free of debris. Loose material within the Petri dish area can lead to biased results.
2. If you notice glare in the images from overhead lighting, try increasing the light intensity of the tracing table or repositioning your workstation.
3. The space between aggregates and the Petri dish edge must allow soils to slake without touching the edge.



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



5. If no handling-stable aggregates are present in a sample upon arrival, report “no aggregates present” as a result. This situation can arise in coarse-textured soils with poor soil structure.
6. 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.
7. 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.
8. Exercise caution when modifying image files. If renaming files, make sure to retain the date and time of image acquisition in each filename. SlakeItEasy is designed to process images as collected, so there is no need to preprocess images (e.g., cropping or rotating) prior to analysis.

Reference

1. 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

This SOP was developed by SHI for SHI communication. For any specific questions, contact Dr. Liz Rieke, erieke@soilhealthinstitute.org.