

Wet Aggregate Stability by Image Quantification

Scope

This document outlines a procedure for image-based quantification of soil aggregate stability using a smartphone. Air-dried soil aggregates (4-8 mm diameter) are photographed before and after a ten-minute submersion in water in a Petri dish. The area of soil in both images is estimated automatically using a pixel thresholding algorithm in the attached R code (Appendix A). A dimensionless aggregate stability index is determined as the ratio of initial to final soil area. Values of the index range from ~0.1 to 1, with higher values indicating greater aggregate stability.

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

Optional:

8 mm (5/16 in.) sieve

4 mm (5 mesh) sieve

Forced-air drying facility (minimum temperature: 30 °C)

Transparency⁴

Dry Erase Marker⁴

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. Open Camera is one example.

³Only necessary if using a smartphone camera.

⁴Only necessary if attempting to run multiple samples at the same time.

Procedure

1. 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 in.) of the soil.
2. 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.

3. 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). Select a minimum of 9 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.
4. Place three selected aggregates in the lid of a Petri dish
5. Place the tracing table on a flat surface that will not move abruptly if bumped and turn on the backlight.
6. Place a clean Petri dish bottom on the tracing table and fill with deionized water to a minimum depth of 7 mm (~45 mL).
7. Using masking tape, secure the smartphone to the cylindrical object selected to hold the smartphone (e.g., peanut butter jar).
8. 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 and the camera will not capture any objects outside of the petri dish (Fig. 1). Adjust the position of the phone and zoom of the phone camera to prevent other objects in the frame.

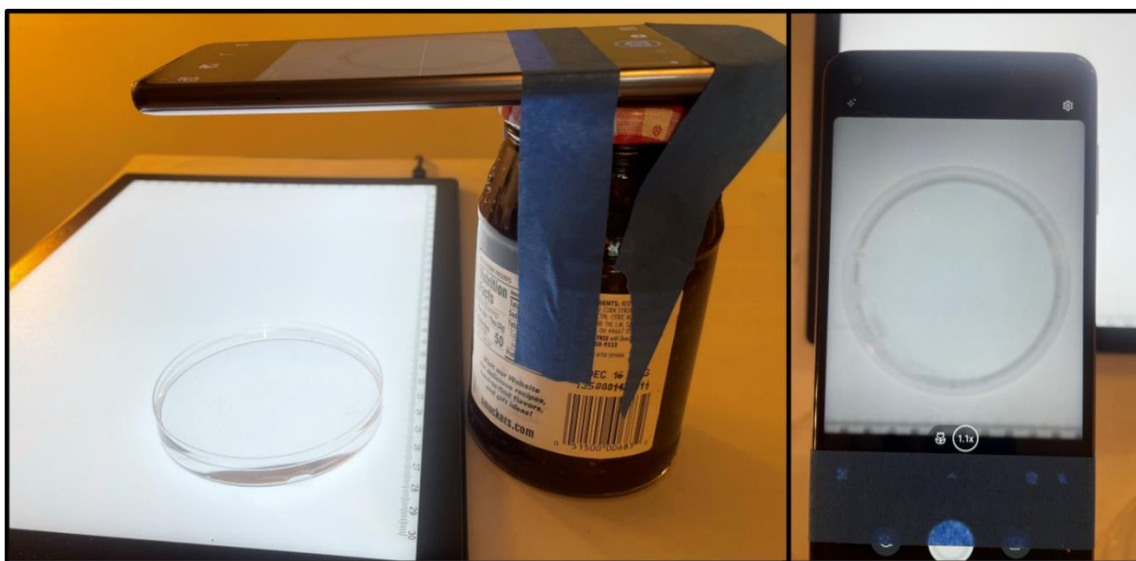


Figure 1. The phone positioned over the Petri dish (left) with no outside objects in the view (right).

9. Set a digital timer to 10 minutes.
10. Select three aggregates and gently drop them into the petri dish, taking care to drop each aggregate in a separate area of the petri dish so they will not overlap as they slake.
11. Immediately take an initial photo, start the 10- minute digital timer. Do not move the phone.

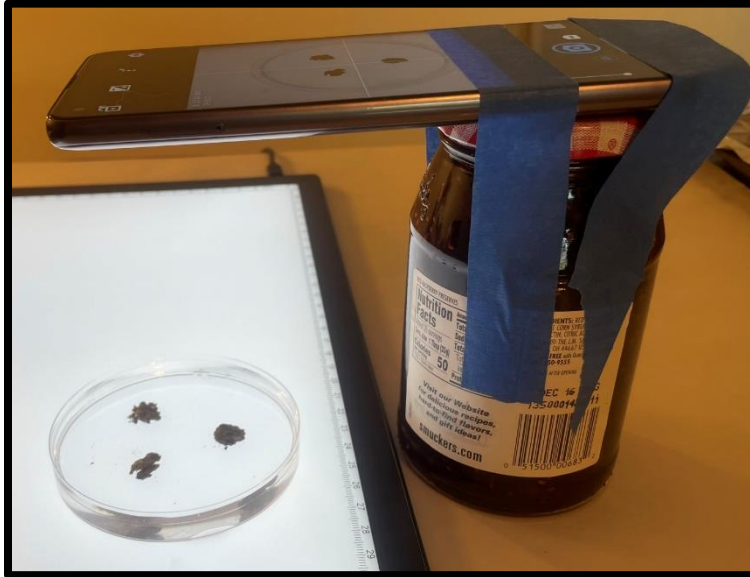


Figure 2. Phone positioned over aggregates after first photo is taken.

12. Once the 10 minutes have elapsed, take a second, final photo.
13. Download the initial and final photo to a computer. You will need to know which file is the initial photo and which file is the final photo so that you can use the R code to quantify aggregate stability.
14. Change file paths as indicted in the R code and run the R code located in Appendix A. The code calculates the aggregate stability index using the following equation:

$$STAB_{10} = \frac{A_0}{A_{600}}. \quad [\text{Eq.1}]$$

Where A_0 is the initial aggregate area, A_{600} is the final aggregate area, and $STAB_{10}$ is the 10-minute stability index value.

QA/QC

1. Assess coefficient of variation from triplicate petri dishes. If coefficient of variation is greater than 30%, rerun sample.

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

We recommend calculating the mean of 9 aggregates (3 aggregates in each of three Petri dishes) to represent the aggregate stability of a point or zone soil sample. Because we have found $STAB_{10}$ to be lognormally distributed, we recommend using a geometric rather than an arithmetic mean. Additional replicates will further decrease coefficients of variation.

This SOP may be modified to run multiple samples simultaneously (Figure 3). If you are moving the phone to take photos of different petri dishes simultaneously, be sure to label each photo correctly. We have found using a transparency to label to be very useful for quality control. Labels must be cropped out prior to running the image recognition analysis located in Appendix A.

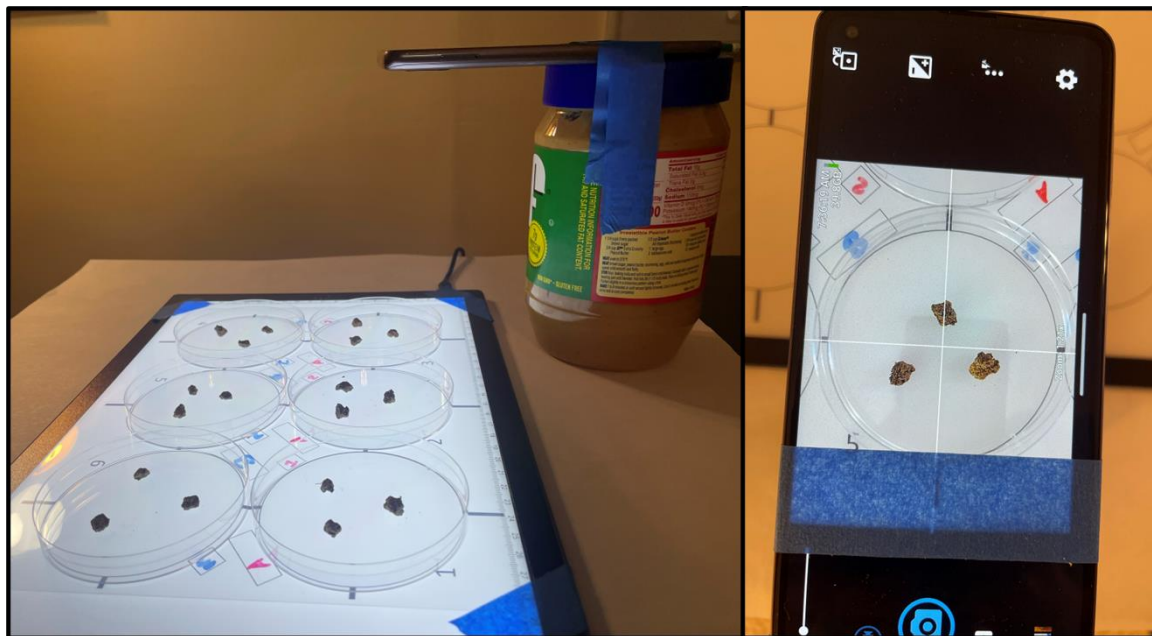


Figure 3. Phone positioned over transparency to take a series of initial and then final photos (left). Cross hairs on an example camera app are aligned with guide marks on transparency to aid in repositioning the phone (right).

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

Appendix A

#This sample code is Appendix A for the Soil Health Institute's Standard Operating Procedure for Wet Aggregate Stability by Image Quantification.

#The code was last updated on May 16th, 2022. Email erieke@soilhealthinstitute.org with questions.

#The code uses two images of a petri dish containing three soil aggregates. One image is taken at the start and one is taken at the end of a 10 minute

#submersion in water. The code calculates the mean aggregate stability for the three aggregates in the petri dish by 1)cropping the images, 2)converting them to

#grayscale, 3) converting to binary by using a threshold obtained using Otsu's method (this value represents the cutoff value between water and aggregate pixels)

#and then 4) dividing the total initial area of aggregates (not individual aggregate areas) by the total final are of aggregates. In step 5), an example of how to save data is given.

#You will need to update file paths to locate the images on your computer lines and to save output.

```
library(EImage)#Helpful resource to download EImage  
https://bioconductor.org/packages/release/bioc/html/EImage.html
```

```
library(dplyr)
```

```
# path to folder where results are/will be stored -- need forward slash
```

```
results_path <- 'C:/Users/SlakeltEasy_results/'
```

```
# read in the initial and final images and plot to visualize
```

```
# you don't have to plot, but it can be helpful to confirm you have the correct image
```

```
img_Initial <- readImage("C:/Users/Slake it easy/Images/InitialTestImage.jpg")
```

```
plot(img_Initial)
```

```
img_Final <- readImage("C:/Users/Slake it easy/Images/FinalTestImage.jpg")
```

```
plot(img_Final)
```

```
#get the image dimensions to inform where to crop
```

```
dim(img_Initial)
```

```
dim(img_Final)
```

1) crop images so they only contain the three aggregates you intend to analyze.

```
#It is good practice to leave the sample ID and replicate number in the image
```

```
#If you already cropped your images before reading in, you need to rename them.
```

```
#To rename your image, you need to comment out lines 39 and 41 and uncomment lines 34 and 35 and run them:
```

```
img_Initial_crop <- img_Initial
```

```
img_Final_crop <- img_Final
```

```
#No other aggregates should be in the image and no petri dish edges should be present.
```

```
#you may have to guess and check to get the right crop.
```

```
img_Initial_crop <- img_Initial[350:870, 250:650,]#
```

```
plot(img_Initial_crop)#plot to see if you got a good trim, if not, change numbers and try again. Note that images have three bands and the first is plotted by default. We will average over bands at the end.
```

```
img_Final_crop <- img_Final[370:900, 250:700,]
```

```
plot(img_Final_crop)
```

#2 make images gray scale

```
colorMode(img_Initial_crop) <- Grayscale
```

```
colorMode(img_Final_crop) <- Grayscale
```

#3 get the threshold values for the images so we can make them binary, these are essentially the cutoffs for what values will be classified as soil

```
threshold_Initial <- otsu(img_Initial_crop)
```

```
threshold_Final <- otsu(img_Final_crop)
```

```
#make binary versions of the images by selecting values less than the threshold (found in lines 48 and 49) and then summing the pixels that are
```

```
#greater than the threshold (lines 48 and 49). These are done for each of the three image bands.
```

```
img_binary_Initial <- EImage::combine( mapply(function(frame, th) frame <= th,  
getFrames(img_Initial_crop), threshold_Initial, SIMPLIFY=FALSE) )
```

```
plot(img_binary_Initial)
```

```
img_binary_Final <- EImage::combine( mapply(function(frame, th) frame <= th,  
getFrames(img_Final_crop), threshold_Final, SIMPLIFY=FALSE) )
```

```
plot(img_binary_Final)
```

```
area_Initial <- apply(img_binary_Initial, MARGIN = 3, sum, na.rm = T)
```

```
area_Final <- apply(img_binary_Final, MARGIN = 3, sum, na.rm = T)
```

#4 The stability index for this petri dish is initial area divided by the final area. The mean is taken across the three image bands.

```
StabilityIndex<-mean(area_Initial/area_Final)
```

```
StabilityIndex
```

#5 Example of how to save results

```
#This will name results with the year, month, day, hour, and minute
```

```
processing_datetime <- format(Sys.time(), "%Y%m%d%H%M")
```

```
#this will save the value to a CSV
```

```
write.csv(StabilityIndex, paste0(results_path, 'slaking_image_results_', processing_datetime, '.csv'),  
row.names = F)
```