Installing and Using "PhytolithsBatch" in ImageJ

Install

If you don't already have ImageJ on your computer download one from the websites:

http://imagej.nih.gov/ij/download.html for ImageJ

To install "PhytolithsBatch" and "Phytoliths_" in ImageJ

Copy the files "PhytolithsBatch.ijm," "Phytoliths_.class" and "Phytoliths_java" into ImageJ's plugins folder. The directory should look something like C:\Program Files\ImageJ\plugins. For those who want to know, the "PhytolithsBatch.ijm" file is the macro that helps you measure all the image files you have in a folder you choose, the "Phytoliths_.class" file is the program that does the measurements and the "Phytoliths_java" file is the program that fully installs "Phytolith_.class.

1) Open the ImageJ program and click on the "Plugins" button. Click on either the "Install" or the "Compile and Run" buttons under the Plugins menu (not the one under the "Macros" menu) and choose the "Phytoliths_.java" file that you copied into ImageJ's Plugins folder. (Again, you should find it in a directory that looks something like C:\Program Files\ImageJ\plugins). After the program installs or compiles close ImageJ and restart it.
2) Click on the "Plugins" button. The files "Phytoliths" and "PhytolithsBatch" should both show up under the "Plugins" menu.

Prepare images for analysis

1) If you have not already done so, place all the images of the phytoliths you want to measure using PhytolithsBatch in a single folder. All the images in a given folder should be at the same magnification for calibration purposes.

Run PhytolithsBatch

1) Open ImageJ
2) Under the ImageJ Plugins menu click on "PhytolithsBatch."
3) When prompted select the folder in which you saved the images you want to measure. (Note, The program will automatically create a subfolder called "BinaryImages" in this folder where it will store the binary counterparts of your phytoliths as you measure them. If you already had a "BinaryImages" subfolder, it will create a new subfolder named "BinaryImages(1)."")
4) ***Select Startup Options*** A dialog will then pop up allowing you to choose to start with the first image in the folder or a different image if you choose. If you check the box for

"Choose Save Directory," in this dialog box it will allow you to choose to save elsewhere the automatically created "BinaryImages" subfolder, but will not delete the "BinaryImages" folder that was just created.

5) The image you chose to start with will now be pulled up.
6) ***Calibration*** Note: You will need an image with a calibration scale or micron bar on it to calibrate. You can set the calibration for your measurements at any time during a measurement session, but you must do it before finishing the measurements on your last image. We recommend calibrating first thing so we don't forget. As you close the program you will prompted to calibrate just in case you did forget. If you do not calibrate measurements will be given in pixels. To calibrate:
    a) Using the line selector tool, draw a line the length of your calibration scale or micron bar. Next select the "Analyze" menu button and choose "Set Scale." Enter the known distance of your calibration scale or micron bar in the "Known distance" window then enter the unit of length (usually um) in the "Unit of length" window. Finally check the "Global" box (this keeps the calibration set for all images until ImageJ is closed), then press OK.
    b) All measurements of images in this file will now be calibrated to this scale as you make the measurements.
7) ***Identify Phytoliths to be measured*** You must now identify the phytoliths you want to measure on the open image by tracing around them. To do so, select the paint brush tool (default color should be black and the brush width 2, if it is not, double click on the paintbrush tool. A menu will open where you can select the color and width of the paintbrush. Choose black and set the width to 2 pixels). Next outline or trace the phytolith you want to measure using the paintbrush tool by drawing around outside the edges of the phytolith (be careful not to paint over any part of the actual phytolith, you only want to trace around the phytolith). If the image is difficult to see you can adjust brightness and contrast using Image>Adjust>Brightness/Contrast. You can also enlarge the image for tracing using the magnifying glass tool. If you make a mistake you can undo it using the Edit>Undo buttons. When you are done tracing all the phytoliths you want to measure in this image click OK. You will then be presented with a list of options.
    a. Option "Done: Continue With Images" This will display a pop-up asking you to fill in the background black. Use the flood-fill tool (paint bucket) to do this. The program will then invert this image to be black images on a white background. It will then save your outlines as a binary image file (in the BinaryImages subfolder it created) and make measurments of all the phytoliths you have traced. The program will then open up the next image in the folder for you to measure.
    b. Option "Done: Analyze Now - Skip Subsequent Images" As with the above, This will display the pop-up asking you to fill in the background black. As above, use the flood-fill tool to do this. The program will then save your outlines as a binary image file and measure the phytoliths you have traced, but it will not open up

anymore images for measurements. You will only choose this option if there are no other images you want to measure in this folder.

c. Option “Skip This Image” This will close the current open image without changing it or saving anything you have drawn on the image and then it will move to the next image.
d. Option “Redo” This will clear everything you’ve done on the image so far and let you restart your tracing.
e. Option “Redo Previous Image” This will pause the image you are on, and allow you to select an Image you previously skipped or were “done” with (but note that if you started in the middle of your images it will not allow you to redo images before the one you started on). It will open the file you want to redo and when you finish tracing and select okay it will give you 3 options.
    i. Option “Done: Replace Image” This will save your new traced image as a binary file in place of the old one you did or skipped.
    ii. Option “Skip” This will ignore anything on the current image, close it out, and go back to the previous image you were on at the point you left it. It will not replace or change the old image you were redoing.
    iii. Option “Redo” This will erase everything on the current image and let you start tracing the original image again.

When you finish making measurements on all the images in the folder you are working on or if you select “Done: Analyze Now - Skip Subsequent Images”, the program will automatically run the morphological analysis and display all the measurements of all the phytoliths you traced to be measured in the folders images. The results will be automatically saved in the BinaryImages subfolder the program created.

8) Save Results All the images in BinaryImages should have been analyzed and the “Results Table” should contain all the measurements. You can save it somewhere besides Binary Images by clicking “Save” under the “File” menu.

9) ***Access Results*** To open the Results file in another program, open the containing file and right click on Results. Select Open With… and select the appropriate program. (We typically use Excel. You can set the default so that it always opens this way.)

Below is a chart listing and defining the measurements the program makes.

MORPHOMETRIES OF SIZE

Area	Simple area of the feature.
Convex area	Area within a taut string around the feature.
Perimeter	Length of the feature boundary.
Convex perimeter	Length of a taut string around the feature.
Length	Longest cord within the feature.
Fiber length	Length of the feature along its medial axis.
Width	The minor dimension of the feature. Equal to the diameter of the smallest circle through which the feature may pass.
Equivalent diameter	Diameter of a circle with the same area as the feature.
Inscribed radius	Radius of largest circle that can be drawn in the feature.

MORPHOMETRIES OF SHAPE

Form factor	Equals 4 x Area x π/Perimeter, it is 1.0 for a perfect circle and diminishes for irregular shapes.
Roundness	Equals 4 x Area/π x Length^2, it is 1.0 for perfect circle and diminishes with elongation of the feature.
Convexity	Ratio of convex perimeter to perimeter; it is 1.0 for a perfectly convex shape and diminishes if there are surface indentations.
Solidity	Ratio of area to convex area; it is 1.0 for a perfectly convex shape, diminishes if there are surface indentations.
Compactness	Ratio of the equivalent diameter to the length.
Aspect ratio	Equals length/width
Elongation	Equals fiber length/width.
Curl	Equals length/fiber length.