Studies of primary fragmentation using SEM-BSE images of juvenile particle cross-sections: image processing and measurement in Fiji - PASTA v.3.7 user guide

In: The PArticle Shapes and Textures Analyzer (PASTA) project, version 3.7 (February 22, 2021)
Authors: Pier Paolo Comida*, Pierre-Simon Ross#
Institut national de la recherche scientifique, 490 rue de la Couronne, Québec (Qc), G1K 9A9, Canada
***** piercomida@gmail.com, # rossps@ete.inrs.ca

Link for download: https://doi.org/10.5281/zenodo.3336335

Introduction

The PArticle Shapes and Textures Analyzer (PASTA) project allows to prepare and process SEM-BSE images of juvenile pyroclast cross-sections for magma fragmentation studies in Volcanology. It consists of i) semi-automated image preparation in Adobe Photoshop©, as described elsewhere, and ii) processing and measurement of shape factors, 2D crystallinity and 2D vesicularity in Fiji© (Schindelin et al. 2012), using the PASTA script, as described in the current user guide.

PASTA for Fiji allows the semi-automated processing of Scanning Electron Microscope (SEM)-Backscattered Electron (BSE) images of juvenile particle cross-sections and measurement of shape parameters, 2D crystallinity and 2D vesicularity. For shape factors, PASTA builds on the Liu et al. (2015) macro code, whereas the textural measurements are newly developed, as described in Comida et al. (in prep).

The script consists of three parts:

(1) automated creation of single particle images from multi-particle images;
(2) semi-automated generation of binary images for measuring shape factors and greyscale segmented images for measuring crystallinity and vesicularity;
(3) automated measurement of shape parameters, 2D crystallinity and 2D vesicularity.

Preliminary operations

If running PASTA for the first time, Fiji needs to be set up in order to work correctly. From the menu Help→Update… (Fig. 1) →"ImageJ Updater", click on "Manage update sites" and select "ResultsToExcel", then click on "Add update site" and "Apply changes". Restart Fiji.

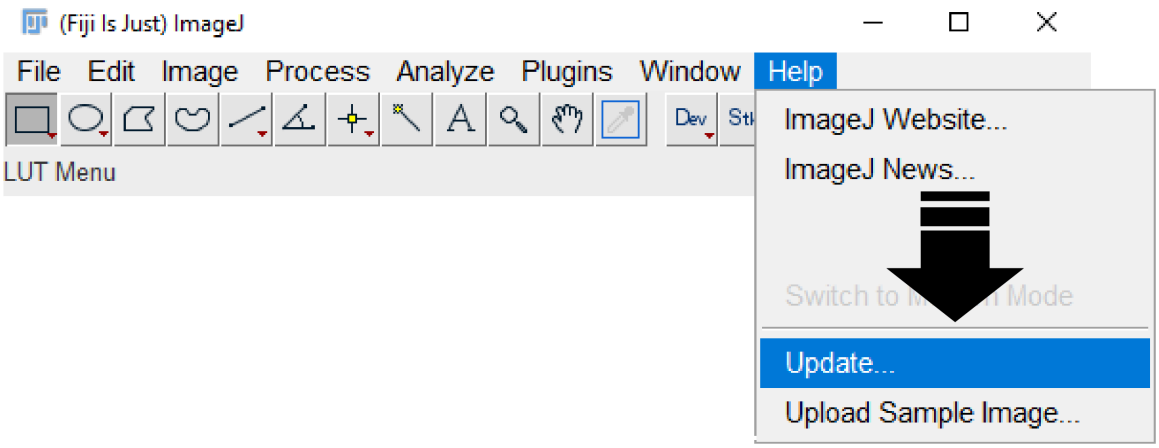

Fig. 1 Location of the "ImageJ Updater" tab to add plugins to Fiji, reachable from the "Help" menu in the toolbar.

The PASTA script can be made accessible on the "Plugins" menu, by placing the script file inside the Fiji.app\scripts\Plugins subfolder (restart of Fiji needed). Alternatively, PASTA can be launched through the script editor, which can be opened by pressing "Ctrl+Shift+N" on Windows (Cmd+Shift+N on Mac).

To optimize image processing, template folders for Microsoft Windows® machines are provided in the "Windows Template Folders" located inside the main package folder on GitHub. In what follows, folder paths and labels will correspond to those provided in the package.

Before launching PASTA, place a copy of the images to be processed into the BATCH_TEMP\INPUT & OUTPUT folders, respectively.

Initial dialog box

Upon launching the PASTA script, you will be presented with a first dialog box. Select both input and output folders (found in BATCH_TEMP), and the suffix of the input images (Fig. 2).

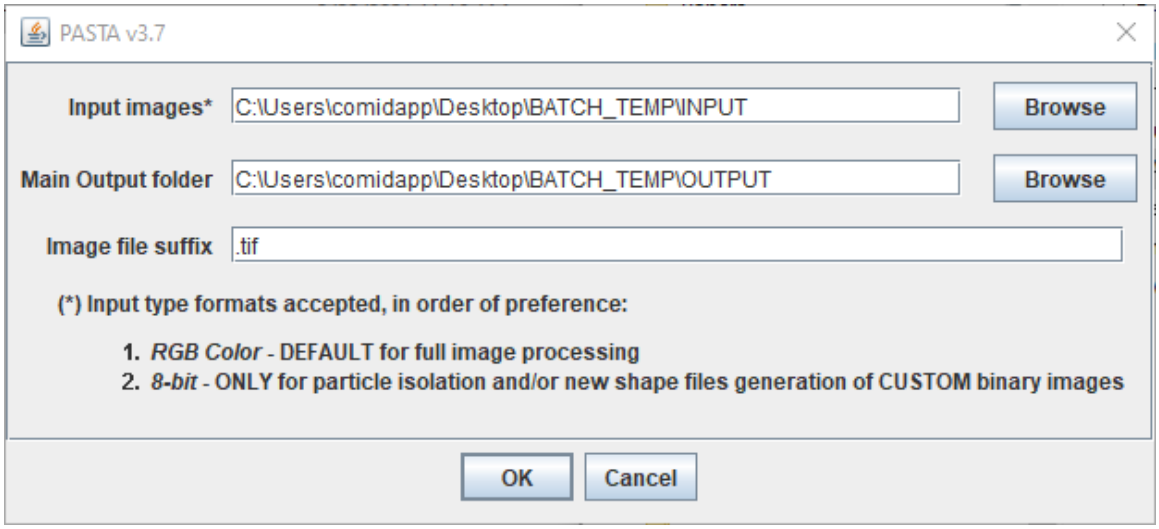

Fig. 2 View of the initial dialog box in PASTA.

Script processing settings

The next dialog box, named "Script processing settings", consists of two parts (Fig. 3). In the upper part, define which kind of operations the script will execute, such as: 1) Particle isolation for multi-particle images; 2) Image processing with generation of binary form images for shapes and greyscale segmented images for internal features; 3a) Measurements; and 3b saving the results. In the bottom part of the dialog box, set the greyscale values of the segmented false-color output images generated during processing.

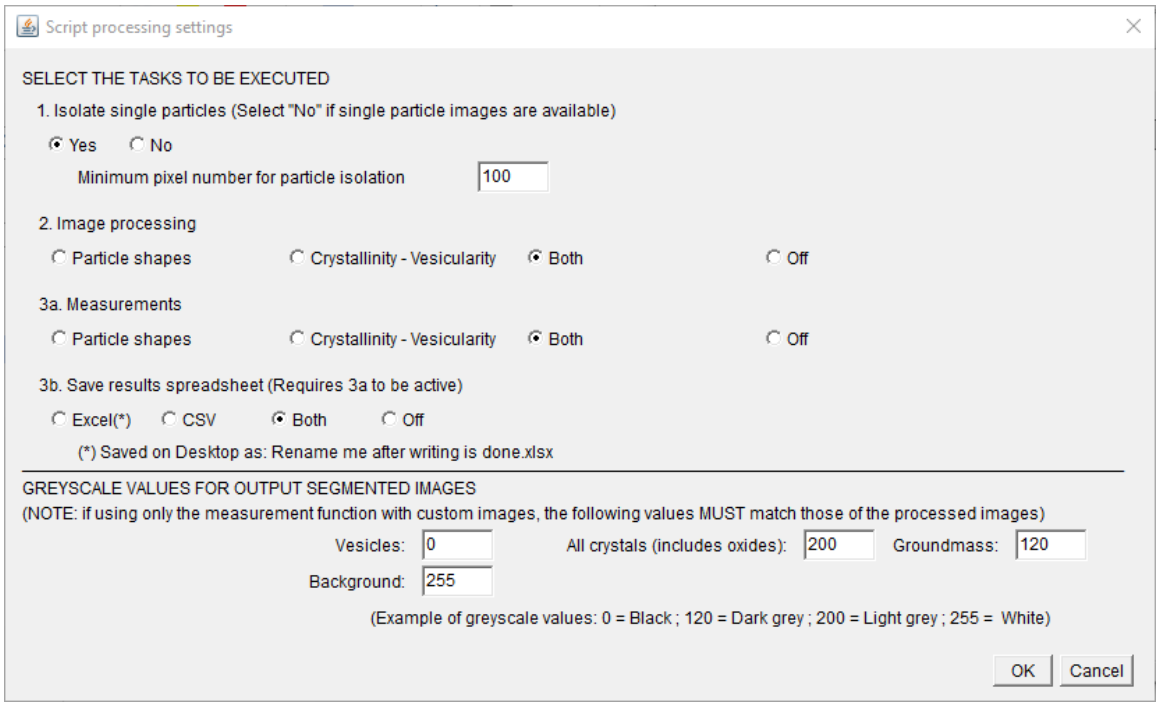

Fig. 3 View of the "Script processing settings" dialog box.

Step 1 - Isolate single particles

Particle isolation requires, as input files, multi-particle images featuring a homogeneous color inter-particle area (i.e., the "background"), such as those obtained through image preparation in Photoshop provided with this package (see separate Photoshop user guide). A minimum pixel number for particle isolation can be defined, in order to filter possible noise in the image which will be otherwise considered (and therefore isolated) as a particle. When "Isolate single particles" in the "Script processing settings" tab is enabled (i.e., "Yes" is ticked), pick the inter-particle background color on the first image of the stack (Fig. 4).

Fig. 4 Picking the color of the inter-particle area (i.e., the "background") to perform particle isolation. Note. The image has been cropped to save space.

Once the correct value is confirmed (Fig. 5), the script will generate single particle images from the whole stack of input images, saved into BATCH_TEMP\OUTPUT\RGB_Singles. Moreover, a "map" of each multi-particle image is saved in BATCH_TEMP\OUTPUT\Input_Drawings, containing a numbered outline of each particle, for traceability.

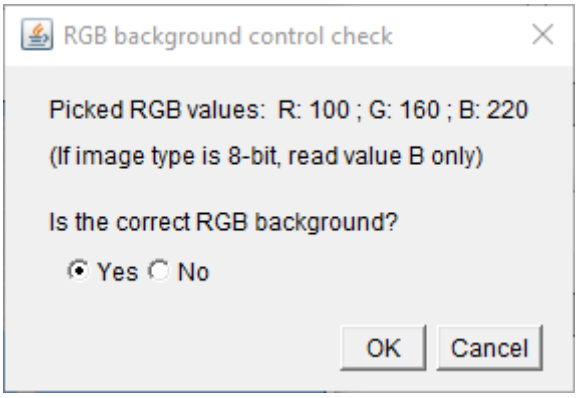

Fig. 5 Checking background color for particle isolation.

Step 2 – Image processing

Particle shapes

When "Particle shapes" is ticked in step 2 of the "Script processing settings" of PASTA, binary files suitable for particle shape measurements will be generated for each single particle image. This happens automatically, hidden from view. During each iteration, each single particle image generated from Step 1 is thresholded to binary, characterized by a 100% black (RGB 0) particle area surrounded by a 100% white (RGB 255) background. The new binary particle image is saved in BATCH_TEMP\OUTPUT\FORM.

Crystallinity – Vesicularity

Overview

When "Crystallinity-Vesicularity" is ticked in step 2 of the "Script processing settings" of PASTA, the script will semi-automatically create segmented false-color greyscale images suitable for the measurement of 2D crystallinity and 2D vesicularity. This is the only step in the PASTA script for Fiji which requires significant user input. Using the RBG single particle images, the goal is to identify grey levels typical of the vesicles, oxide crystals, and of several types of grey crystals, so that these features can be segmented into a false-color greyscale image. This can be done as a batch (on a stack of images) or if required, particle by particle.

Instructions

You are first presented with an information box ("Image segmentation for 2D crystallinity – 2D vesicularity – Intro") detailing the segmentation sub-steps to be carried out on the RGB single particle images (Fig. 6). The generation of false-color greyscale segmented particle images occurs through two recursive actions, which also enable the browsing of the entire stack of "input" RGB particle images.

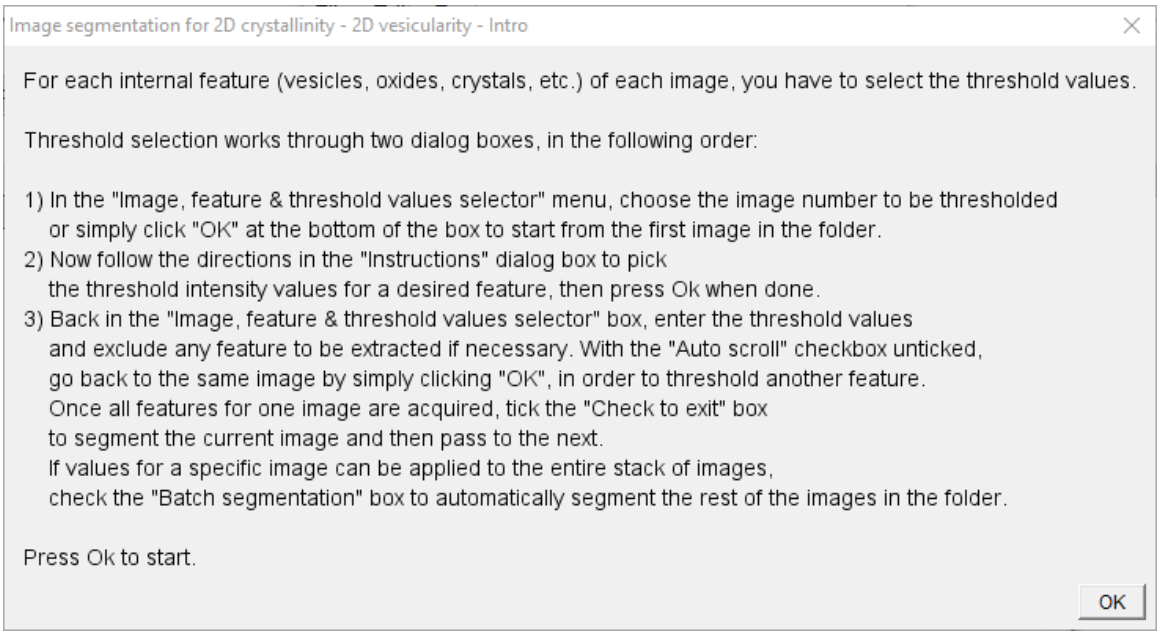

Fig. 6 Instructions of image segmentation for the generation of false-color greyscale particle images for crystallinity and vesicularity measurements.

After viewing the main instructions (Fig. 6), the "Image, features & values selector" dialog box opens along with a preview of the selected image to be thresholded. The dialog box consists of four parts (Fig. 7).

(1) In the top part (the "Image selector"), select the image to work with. By ticking the "Auto scroll" checkbox you can view, after each recursion, the next image in the folder. Alternatively, you can browse through the entire "RGB_Singles" image folder by moving the scroll bar, or inserting the image number manually. Note: you can additionally have both the "RGB_Singles" folder and images physically open on one side of the screen, for better browsing.

(2) In the second part, you must select the features to be segmented among those actually present in the images (such as vesicles, oxide crystals, different types of grey crystals), as well as defining the minimum pixel size and personalized label for each feature. Based on tests and in general agreement with previous studies (Shea et al. 2010), we suggest a minimum (default) size of 4 pixels for vesicles and oxides, and 20

pixels for grey crystals. Such minimum values yield relatively clean refined (segmented) images while accurately depicting internal features without underestimating their relative abundance.

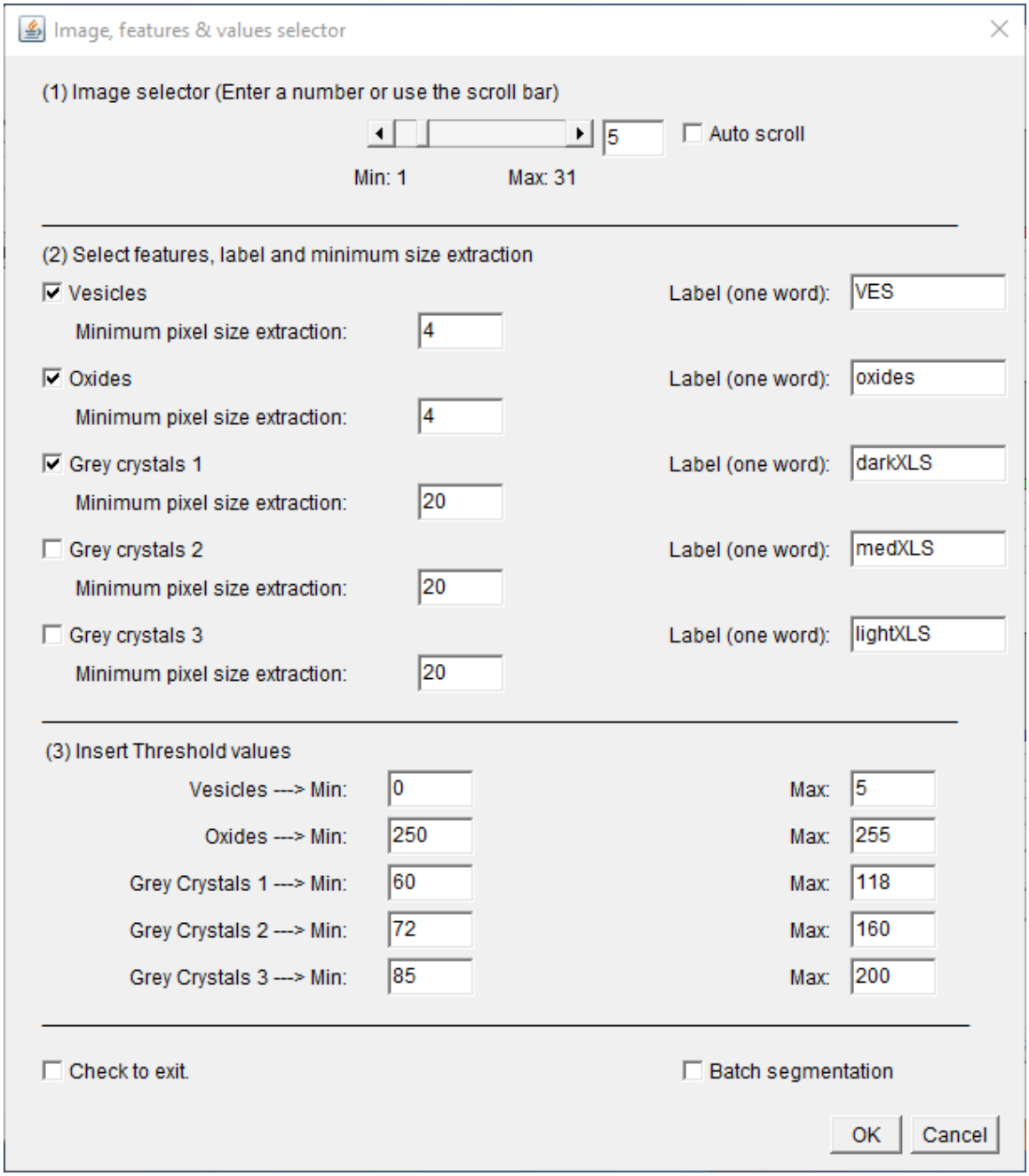

Fig. 7 View of the "Image, features & values selector" dialog box.

(3) In the third part, you can manually insert the greyscale range values for each feature to be processed. The threshold values to be entered here will be determined on a representative image, or on each image, as part of the second recursive action, as described later. The default values, ranging from 0 to 255, are purely indicative, although vesicles and oxides will normally tend to be black and white in SEM-BSE images, respectively. It is worth noting that the greyscale values for a specific feature will be considered only if that feature is selected in (2). The distinction of these fields from the upper part is wanted, as it helps the user minimizing possible errors due to insertion in the wrong field.

(4) The bottom part serves multiple purposes. By simply pressing "Ok", the script opens the image number displayed in the image selector, allowing you to threshold the internal phases of the particle (see next section). If "Check to exit" is ticked, the script creates the segmented file corresponding to the last image opened, saved in BATCH_TEMP\OUTPUT\SEGMENTED. Finally, when both "Check to exit" and "Batch segmentation" are selected, the script applies the current settings to the whole stack of RGB single particle images.

The second part of the recursive action allows you to threshold the selected image for each feature of interest (vesicles, oxides, grey crystals). You are presented with a view of the image, accompanied by an "Instructions" box for assistance and the "Threshold color" command tab (Fig. 8). To threshold a feature, for example plagioclase crystals, start by setting the "Color space" to RGB on the Threshold color tab, then click on "Original" to reset any selection present on the image. Using the "magnifying glass" tool, zoom on a detail of the feature that visually encompasses the typical greyscale range of the selected phase. Finally, using one of the four selection tools provided, contour a representative area of the selected phase and then click on "Sample" (Fig. 8).

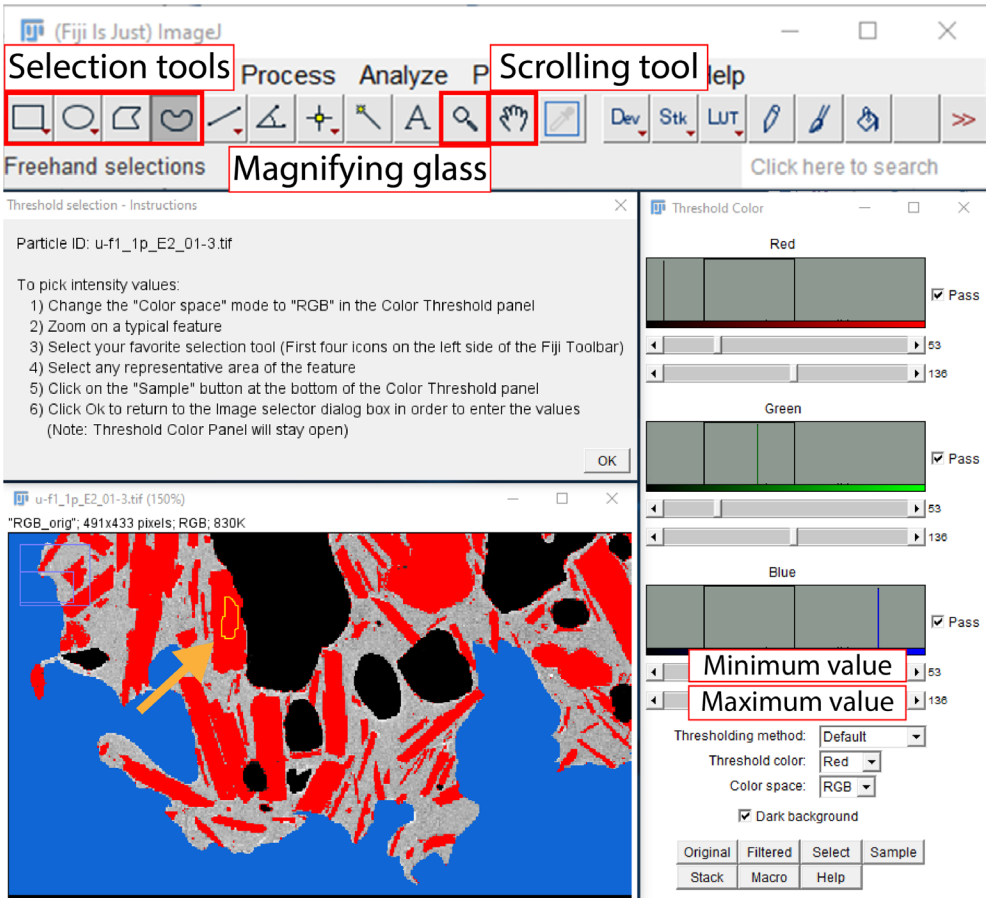

Fig. 8 Thresholding workspace and operations. The most commonly used tools with PASTA are highlighted within the red boxes. Instructions for thresholding are provided in the upper left box below the Fiji menu bar. Bottom left image shows the grey crystal phase (here plagioclase, temporarily displayed in red) thresholded using the freehand selection tool (yellow contour line highlighted by the arrow). The right vertical box is "Threshold color" tab, showing both minimum and maximum thresholding values for the selected phase.

The pixels within the selected greyscale range will temporarily turn red on the image, allowing you to check if the thresholding for this particular phase was successful. Several attempts might be needed to obtain a representative thresholding. Once satisfied, you have two choices:

(1) Memorize the minimum and maximum grey levels for this feature (53 to 136 for plagioclase on Fig. 8; for greyscale features this range is the same in all three RGB

color channels); click "Ok" on the instruction box to return to the "Image, features & values selector" dialog box; and insert the minimum and maximum intensities there before going back to the image to threshold the next feature

(2) Stay on the image to threshold the other features, writing down the minimum and maximum values for each feature on a piece of paper.

Do not threshold the groundmass, as this will be computed automatically upon generation of the segmented particle image by subtraction of the crystals and vesicles from the whole particle area. Lastly, it is worth noting that when the "Auto scroll" check box (Fig. 7) in the "Image, features & values selector" dialog tab is ticked, clicking "Ok" on the instruction tab (Fig. 8) will increase the number in the "Image selector" by one unit, therefore opening the next image for thresholding. This option is useful during batch segmentation, when defining inclusive greyscale range values for each specific feature valid throughout the whole stack of images.

Step 3 – Measurements

The measurement of shape factors, 2D crystallinity and 2D vesicularity are done automatically by PASTA, respectively on the binary form images and the false-color greyscale segmented files. The main output parameters are particle area, particle perimeter, area and perimeter of the convex hull bounding the particle, axial ratio, solidity, convexity, form factor, 2D vesicularity (both in pixels and %), and 2D crystallinity (both in pixels and %). 2D vesicularity and 2D crystallinity are calculated as the number of black and grey (crystal) pixels divided by the total surface area of the particle, respectively. By default, the measurements are saved both as an Excel spreadsheet and a comma-separated values (CSV) file. The former, obtained using the plugin "ResultsToExcel", will be located directly on the computer's Desktop and named temporarily "Rename me after writing is done.xlsx". The CSV file is saved in the main output folder. Once measurements are completed, the user is asked to choose a label for the .csv data file and the summary log.

Flexibility of PASTA

Although the script was developed to process ready-to-use, SEM-BSE multi-particle images of juvenile particle cross-sections generated by pre-processing in Adobe Photoshop© (see Photoshop_Image_Prep_User_Guide provided in this package for details), it could also be utilized under different scenarios, as listed below:

a) If you already have single particle greyscale or color images available, simply disable "Isolate single particles" in the "Script processing settings" dialog box to use existing single particle images as input files for the next steps;
b) Multi-particle greyscale or color images with custom intensities for background and particle can be isolated by simply enabling "Isolate single particles". Note that, in this case, the inter-particle background *must* have a unique color intensity, different than those within the particles area;
c) If single particle binary images are available, they can be used directly to measure shapes in step 3. In the "Script processing settings" dialog box, untick everything in steps 1 and 2 and only select "Particle shapes" in step 3. Note that the input binary files *must* have a 100% white (RGB 255) background and 100% black (RGB 0) particle area. If this is not the case, you can convert such files by running image processing for particle shapes only;
d) If segmented greyscale single particles images are already available (e.g. from another software), you can similarly skip steps 1 and 2 and go straight to step 3, selecting "Crystallinity-Vesicularity" under "Measurements". However, the intensity values for vesicles, crystals, groundmass and background MUST match those inserted at the bottom of the "Script processing settings" dialog box;

References

Comida PP, Ross P-S, Dürig T, White JDL, Lefebvre NS (in prep) Standardized analysis of juvenile pyroclasts in comparative studies of primary magma fragmentation; 2. Choice of size fraction and method optimization for particle cross-sections. Bull Volcanol

Liu EJ, Cashman KV, Rust AC (2015) Optimising shape analysis to quantify volcanic ash morphology. GeoResJ 8:14-30

Ross P-S, Dürig T, Comida PP, Lefebvre NS, White JDL, Andronico D, Thivet S, Eychenne J, Gurioli L (in prep) Standardized analysis of juvenile pyroclasts in comparative studies of primary magma fragmentation; 1. Overview and workflow. Bull Volcanol

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez J-Y, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A (2012) Fiji: an open-source platform for biological-image analysis. Nature Methods 9:676

Shea T, Houghton BF, Gurioli L, Cashman KV, Hammer JE, Hobden BJ (2010) Textural studies of vesicles in volcanic rocks: An integrated methodology. J Volcanol Geotherm Res 190(3):271-289