Method Article

Application of image analysis technique for measurement of sand grains in sediments

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ABSTRACT

This paper provides a methodological protocol for measuring diameter and other properties of mineral sand grains using an image analysis technique. The aeolian sand influx (ASI) from coastal bogs has been used to reconstruct changes in the past storminess. However, concentrations of sand grains in peat deposits, from which the ASI is calculated, tend to be low, and sieving and laser diffractometry cannot be applied. Manual counting of sands under microscope is time-consuming and less efficient because of possible human errors. This paper describes a protocol for an image analysis method used in our recent paper (Vandel et al. 2019) that overcomes those issues in sand grain measurements. After preliminary sample preparations, including loss-on-ignition and chemical pretreatments, this study uses ImageJ – a semi-automated image processing program – to analyze images captured under microscope. With proper sample size selection, pre-treatment, image capturing and image analysis settings in ImageJ described in this paper, the approach proved to be:

• objective and efficient for analysing grain-size distribution even with low concentrations of sands in samples,
• increasing reliability of the measurement and providing reproducible results by avoiding manual measuring processes, and
• non-destructive and enabling further analyses on mineral composition and other properties of sand grains.

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| Method name: | ImageJ for ASI |
| Name and reference of original Aeolian Sand Influx (ASI) Björck, S., Clemmensen, B., [1]. Aeolian sediment in raised bog deposits, Halland, SW Sweden: a new proxy record of Holocene winter storminess variation in southern Scandinavia? Holocene 14, 5, 677–688. |
| Resource availability: | ImageJ - https://imagej.nih.gov/ij/ |

Background

Grain-size analysis of mineral sands in sediments is commonly used in sedimentology and other fields of geology for various purposes [9,10]. The aeolian sand influx (ASI) obtained from peat deposits of coastal bogs has been used to reconstruct changes in the past storminess [1–3,6], and Vandel et al. [11] proves ASI to be effective in detecting major trends in the recent storm activities. When concentrations of sands are high in samples, a wide range of methods are readily available from sieving to laser diffractometry [5]. However, the amount of mineral matter in samples can be a limiting factor. The reliability of the ASI results partially depends on the number of sand grains counted. Sand concentration in peat deposits of raised bogs is relatively low; in addition volumes of sediments available for analysis are often limited. As a result the number of sand grains per sample can become small and microscopic analysis must be applied making the counting and measuring processes of grains time-consuming and prone to human errors when shape- and size-characteristics of grains, such as diameter and shape factors, are concerned.

This paper describes the protocol and methods Vandel et al. [11] used for counting and measuring shape characteristics of mineral grains (i.e., diameter, area, perimeter, width and height of the bounding rectangle, Feret’s diameter, circularity, aspect ratio, roundness and solidity) extracted from peat deposits in Estonia, using ImageJ – an open source program for image analysis. The following sections focus on the pretreatment of samples, useful tips for preparation of microscope slides to make image capture and analysis easier, and instructions and protocols in various steps of the image analysis and parameter settings with this semi-automated technique.

Subsampling and loss on ignition

Before subsampling the materials for a detailed grain-size analysis, we used a preliminary analysis of loss-on-ignition (LOI) to estimate the concentration of siliciclastic matter in peat deposits. This preliminary LOI analysis is informative to estimate the appropriate volume of sediment to be sampled from individual cores for ASI analysis. Depending on the concentration, the sample volume needs to be adjusted to avoid microscope slides with too many or too few grains during the image analysis. With too many grains, the risk of grains being stacked on slides increases, and an increased number of slides are required to complete the analysis. For example, Vandel et al. [11] show that it took up to seven slides to complete analysis of one sample and counted more than 1400 sand grains in total, when mineral matter content was high.

Samples for the preliminary LOI analysis are dried at 105 °C and then heated at 550 °C and 950 °C to constant weight at each stage, providing the proportions of organic and siliciclastic matter and carbonates per unit weight or volume of sediments [4].

For the grain-size analysis and ASI calculation, sediment volume appropriate for the ImageJ-based approach varies from 0.5 to 5 cm³, depending on the concentration of siliciclastic matter in samples based on the preliminary LOI analysis. Also, the more accurate the volume measurement, the better the concentration calculation, and thus ASI results. Vandel et al. [11] used a syringe with a cut off tip to measure sample volume of sediment. To make the ImageJ-based analysis efficient and less time-consuming, remove organic matter from samples by the LOI process at 550 °C [4].

The workflow for the preliminary LOI and LOI for ASI is as follows:

1. Preliminary LOI to estimate the siliciclastic matter content.
   - Subsample for preliminary LOI analysis.
Dry the samples at 105 °C to a constant weight.
Heat the samples at 550 °C to a constant weight.
Heat the samples at 950 °C to a constant weight.

2. LOI for ASI

Based on the preliminary LOI results subsample 0.5 to 5 cm³ of untreated sediment for ASI analysis.
Heat the ASI samples at 550 °C (LOI 550) to a constant weight.

Next, the residue of LOI 550 goes through a chemical pretreatment process.

Chemical pretreatment

The residue of LOI 550 is treated on a 63 μm mesh (or similar, Vandel et al. [11] used 65 μm mesh) with HCl to remove carbonates and with KOH to remove diatoms and clean the sample. Also, the redundant silt and clay fractions are removed from the sample during the pretreatment, leaving only the sand fraction (>63 μm) that is needed for the image analysis.

The workflow for the chemical pretreatment is as follows:

1. Place a 10×10 cm mesh inside a glass funnel by folding it to conical shape.
2. Pour the residue of LOI 550 on the mesh.
3. Add 4–5 ml of 10% HCl gradually drop by drop to the sample and let it soak around 10–15 min.
4. Treat the sample with distilled water (approximately 50 to 100 ml) afterwards with care, not to spread the sands all over the mesh.
5. Add 4–5 ml of 10% KOH gradually drop by drop to the sample and let it soak around 10–15 min.
6. Treat the sample with distilled water (approximately 50 to 100 ml) afterwards and wash the remaining sample to the bottom part of the conical mesh.
7. Place the funnel with the sample in a glass beaker and dry the sample at 45 °C for ca. 40 min.

Slide preparation and analysis

The workflow for microscope slide preparation is as follows:

1. Add one drop of immersion oil to the microscope slide (we used immersion oil with a refractive index of 1.534). The immersion oil makes the grains more easily distinguishable from the background (i.e., the grains will be darker using water) and also makes it possible to identify the mineral composition [8].
2. Make sure there are no visible air bubbles in the immersion oil drop; if air bubbles occur, replace the oil drop. This is important, because grains tend to be stacked around the air bubbles, making the analysis process more time-consuming. See the "Manual improvement of images" section below.
3. With a soft brush, transfer the grains from the mesh to a smooth black lauder (dark background allows to see the grains). Pouring grains directly from the mesh to the microscope slide can result in loss of grains; Vandel et al. [11] used black, folded paper as a lauder for transferring the grains.
4. Pour the grains from the lauder carefully on the oil drop.
5. Use a small soft brush to wipe all the grains from the lauder onto the oil drop.
6. Place the cover glass on the slide. Be careful not to create air bubbles under the cover glass; apply extra oil on the edge if necessary. Seal the edges between slide and cover glasses with a glass glue to prevent oil outflow.
7. Skim systematically through the slide and capture images of every grain or grain cluster.

Use dark field microscopy to obtain clear and well-defined images of grains. Vandel et al. [11] used a modified Leica DM75OP petrographic microscope (5 × 10 magnification) and captured images with a digital microscope camera (Olympus SC100) with the Olympus CellSens program. For best results the grains should be well illuminated and focused with clearly defined edges.
Image

ImageJ is an open source program for image processing and analysis [7]. It enables automated measurements of a set of images by running the macros (macros are workflow protocols that can be executed by ImageJ) with a set of commands and specified parameters under given protocols. In order to obtain grain-size data from captured images two analytical protocols are set and executed: Macro1 and Macro2. Macro1 converts images to black and white (B&W), and Macro2 measures the number and various properties of grains and exports the measured data. Instructions on how to operate ImageJ are available online: https://imagej.nih.gov/ij/.

Macro1

ImageJ parameters for Macro1:

```java
run("8-bit");
setAutoThreshold("Default dark");
//run("Threshold...");
setThreshold(65, 255);
//setThreshold(65, 255);
setOption("BlackBackground", false);
run("Convert to Mask");
```

With this macro setting we convert the original image into a B&W image (i.e. white background with black grains). The “setThreshold(65, 255)” command determines the thresholds by which the pixels are either converted to black or white. By setting the first threshold value higher or lower we can adjust the brightness level at which the pixels are converted to black (Fig 1). When the grains are properly illuminated moving the threshold does not affect the outcome much (Fig 1.A and B). Images with over- or under-illuminated grains are more sensitive to the changes in threshold values. When the value is lower, less-illuminated objects will be converted to black, but also the halo around illuminated grains is turned to black and will be later measured as grain area (Fig 1.C and D). When
the value is set higher, the edges of well- and over-illuminated grains are defined better; however, it is more likely that poorly illuminated grains will not be partly or entirely converted to black objects or have poorly defined edges (Fig 1. C and D). Based on our samples and experience “65, 255” is the optimal set of the threshold values that gives the best results with the least manual fine-tuning in later stages.

**Macro2**

ImageJ parameters for Macro2:

```
run("Set Scale...", "distance=1.650 known=1 pixel=1 unit=\mu m global");run("8-bit");
setAutoThreshold("Default");
//run("Threshold...");
setThreshold(0, 147);
//setThreshold(0, 147);
setOption("BlackBackground", false);
run("Convert to Mask");
run("Set Measurements...", "area mean standard modal min centroid center perimeter bounding fit shape feret’s integrated median skewness kurtosis area_fraction stack display redirect=\None decimal=3");
run("Analyze Particles...", "size=3100-Infinity show=Outlines display exclude include in_situ");
```

Macro2 sets the scale in such a way that 1.65 pixels on the image equal 1 micrometer; the scale in *ImageJ* needs to be calibrated for each microscope. The threshold in Macro2 determines the pixel brightness level with which they are counted as objects. Although the analysed images are in B&W, some gray pixels can occur around the grains; therefore the second threshold number should be set around 150 (based on our samples and experience the threshold value around 150 is optimal for the best results). The setting `size=3100-infinity`, determines the object area from which the objects are counted and measured; 3100 square micrometers is the approximate area of a round grain 63 micrometer in diameter [11]. Macro2 lets *ImageJ* accept the following conditions:

1. every object must be clearly separated from each other; if several grains are linked by even one pixel they are counted as one object.
2. the objects are measured based on their outer contour, and empty/white areas inside an object will be counted as part of the object.
3. every object that is connected to the edge of the image will not be counted or measured as the whole object is not captured in the image.

**Manual improvement of images**

The efficiency of the pretreatment, the composition of the sample, quality of the slide preparation and image capturing settings all affect the quality of captured images and reliability of the data produced afterwards. It is essential to skim through all the B&W images before running Macro2 to avoid issues potentially affecting the measurements and final outcome. The easiest images to analyze are those in which the grains are properly illuminated and focused, separated from each other, and the sample is free of debris (Fig 2.A). These kinds of images can be run through Macro2 with no further action needed.

It is common that some grains are stuck together and linked in B&W images after running Macro1 (Fig 2.B). As two linked objects are regarded as one when running Macro2, the grains must be manually separated by drawing a white line between them for *ImageJ* to count and measure each grain separately. Depending on the mineral composition, sand grains are not always clear and well illuminated (Fig 2.C). The grains can be out of focus and hard to separate from the black background. After running Macro1 those grains can be partly or entirely missing in the B&W image. In order to measure the grains, their borders need to be redrawn manually by tracing the original image.
Fig. 2. Various scenarios and image processing solutions when working with image analysis of mineral grains: (A) clear grains with no overlapping and no debris; (B) clear grains with partial overlapping; (C) under-illuminated grains of different extent with some overlapping; (D) clear grain with some debris connected to the edge of the slide; (E) partly over saturated and overlapping grains connected with debris; and (F) heavily stacked grains with various illumination levels on the edge of the immersion oil drop. From left to right: originally captured images; images converted to black and white in ImageJ by running Macro1; manually processed black and white images; images of measured grains by ImageJ after running Macro2. Scales vary among images.

Depending on the sample composition and the pretreatment efficiency, some sediment debris can be left in pretreated samples (Fig 2.D) and stuck to mineral grains. These debris needs to be removed manually from the B&W image by erasing or, like on the example D on Fig. 2, separating them from the grain by drawing a white line between them. In case the parts of the debris are larger than 63 micrometers in area, they need to be connected to the edge of the image with a black line so the ImageJ does not count or measure them. Another way is to cut the debris in smaller particles using white lines while separating them from the grains (Fig 2.E).

Fig 2.F shows an image where a large number of grains are stacked together at the edge of a drop of immersion oil. This happens when the amount of added immersion oil is not enough and air pockets are formed between the slide and cover glasses. This issue can be avoided by applying extra oil on the edge of the cover glass when preparing the microscope slide (see "Slide preparation and analysis" paragraph). When there are too many grains together it is difficult to have them all well-illuminated in the captured image. Lighting condition can be altered by rotating the slide under the microscope, but some grains would still be poorly illuminated. Also, the edge of the immersion oil can distort the image of grains. In this case, a lot of manual altering of the image is needed in the B&W stage. Overall, it is essential not to add too many grains on one slide and to avoid air bubbles in the immersion oil while preparing the slides.
Calculating the diameter of mineral grains

With Macro2, ImageJ produces a data table that records all the measured parameters for characterizing the size and shape of all the grains counted. Various shape factors (e.g., circularity, aspect ratio, roundness and solidity) and size measurements (e.g. area, perimeter, width and height of the bounding rectangle, and Feret’s diameter) are recorded. Vandel et al. [11] calculated the average diameter of a sand grain $D$ by $D = 2\sqrt{\frac{A}{\pi}}$, where $A$ represents the projection area of the grain.

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Declaration of Competing Interest

None.

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