Dimensional-morphological and quantitative analysis of fossil ostracods through static image analysis with automated optical scanning microscopy

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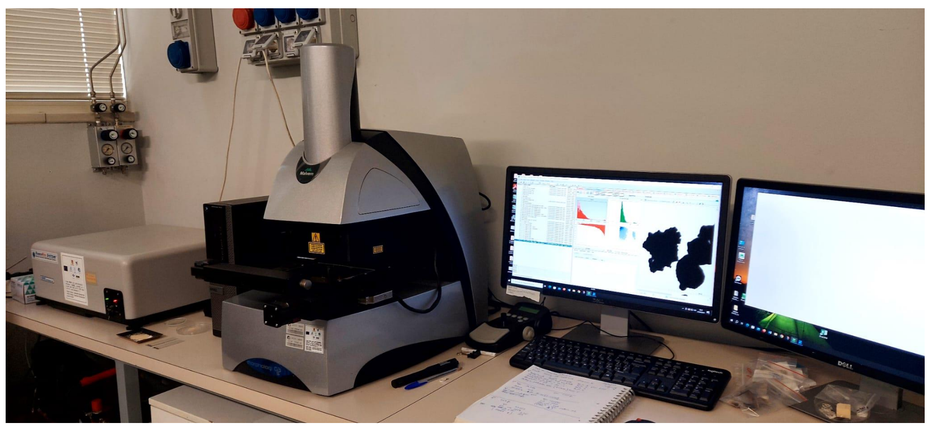
Traditionally, the observation and extraction of ostracods from sediments are performed manually with the aid of a stereomicroscope at low magnifications (from x20 to x500), working on dry sediments. However, this method is time-consuming. To overcome this limitation, a quantitative technique using computerized optical scanning and image analysis has been introduced. This automated approach allows for the discrimination, counting, and quantification of fossil ostracod valves present in a sample without manual separation by the operator. Automated scanning applied to optical microscopy provides quantitative analyses in significantly less time than manual methods and with excellent quality. Light microscopic images of ostracod valves could be complicated due to the convexity of the valves (need of focus stacked images). Also, some key morphological details such as marginal pore canals could be very helpful for taxonomical analyses.

Dispersion and Automatic Scanning of the Sample



The instrument used for the automated analysis of size and shape is the **Malvern Morphologi G3ID system** from Malvern Panalytical (UK).

The analyser consists of:

- A Nikon G200 optical microscope
- A motorized three-dimensional scanning system (x-y-z)
- A lighting system for transmission and reflection in dark field, bright field, and polarization
- Fluorite optics suitable for analyses in the Visible and NIR (near infrared) range

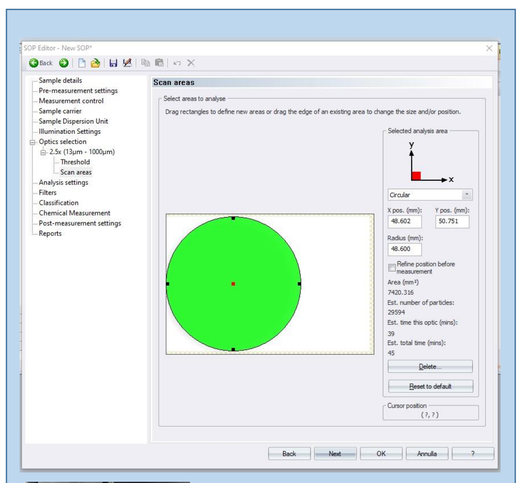
The microscope is designed for the analysis of solid particles on optical supports (slides, plates), in dispersion (cell), or directly on sampling filters. The optics are equipped with a polaroid system (polarizer/analyser) that can be manually integrated into the microscope. The instrument is managed by a software capable of filtering and/or classifying parameters based on size versus shape.
The scanning station is equipped with piezoelectric motors that move in the x-y-z directions with a precision of 0.1 microns. The instrument handles the acquisition of particle images, with which it performs morphological analysis and extracts morphometric and granulometric data. It uses an integrated system for sample dispersion with adjustable positive pressure from 0.1 to 5 bar.

Additionally, the optical system includes a RAMAN spectrometer from Kaiser Electronics (USA), integrated into the optical system via a single-mode optical fiber. The Raman spectrometer has a power output of 20 mW, variable, with a wavelength of 735 nm, and its beam is coaxial with the observation optics. This setup allows, through software and filters and/or classifications, the capture of Raman spectra of individual particles to chemically identify and classify their species.

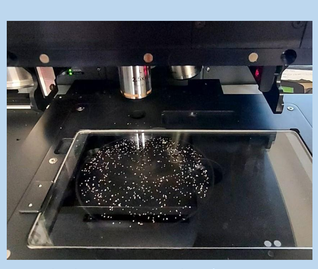
Samples treatment and preparation

The sediment samples are:

1. dried in an oven to remove residual moisture
2. possibly digested in hydrogen peroxide
3. washed with 63 µm sieves and dried again in the oven
4. randomly poured over a transparent support in the Malvern Morphology system
5. automatic dispersion under pressure of a known quantity of powder on a transparent support (glass and/or quartz)
6. counting and photographing the granules
7. image analysis of each particle
8. once the necessary distinctions are made, the nature of the detected particles is verified (only if necessary) using a RAMAN probe
9. determination of the number of valves contained in the sample



Integrated dispersion chamber



Sample dispersion on glass/quartz

Creation of a Standard Operating Procedure (SOP)

Selection of the area of analysis

Image acquisition

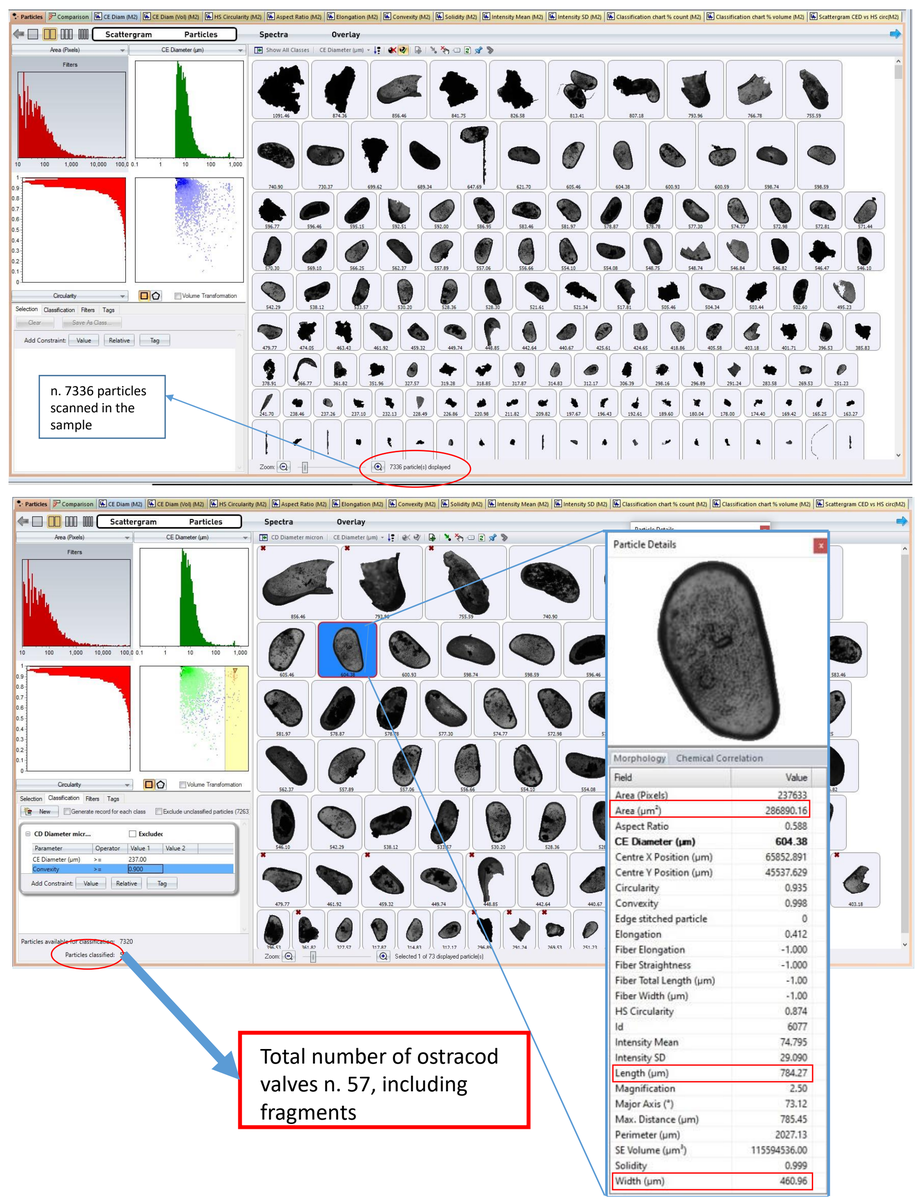
Data analysis

The counting of the valves can be carried out by classifying specific granulometric and morphological parameters such as:

- CE Diameter
- Convexity
- Area (micron)
- Length
- Circularity
- Width

Acquisition of images of all the particles that make up the total sample. =7336

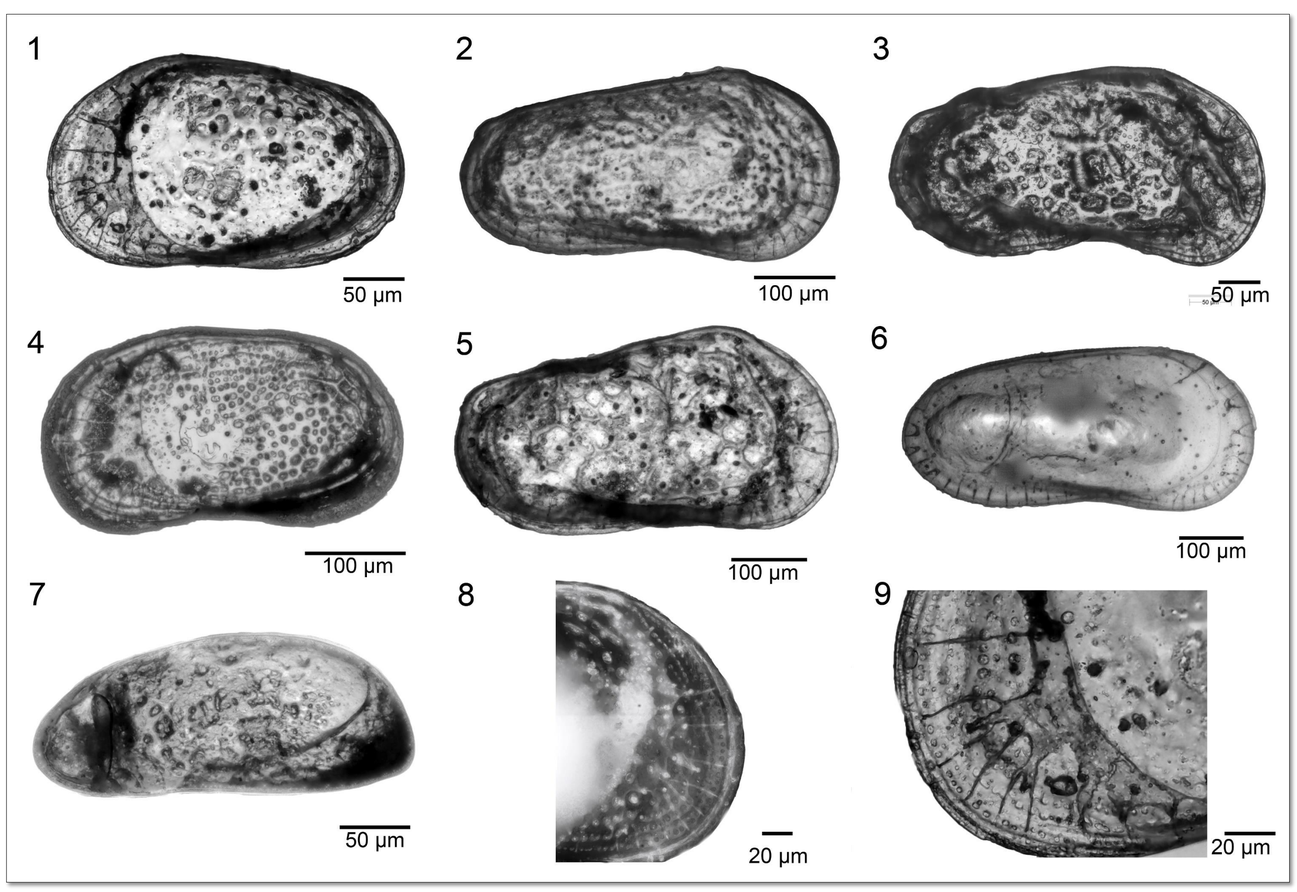
Selecting parameters for filtering and ranking:
-CE Diameter (µm)
-Convexity



For this sample, n. 46 ostracods, including fragments were manually counted

Imaging of ostracod valves

The **Malvern Morphologi G3ID** system includes high resolution microscope with a Nikon CFI 60 brightfield / darkfield system that ensures quality particle images.
The camera is equipped with the following magnifications: 2.5X 5X 10X 20X 50X.



It is particularly suitable for ostracods because each specimen can be photographed at:

- different magnifications
- different lighting conditions (episcopic, diascopic, brightfield, darkfield, with polarisers, in grey scale or colour).

The instrument generates Z-stacked images that can be immediately checked for their quality.
This allows to change the light-magnification-stacking options depending on the scope of the captured image.

Examples of images captured by the **Malvern Morphologi G3ID .**

All ostracod valves come from cores drilled during the IODP EXP. 381

(Corinth Gulf, Greece)

1. *Tuberoloxoconcha* sp., LV, external view, Sample 21.02 m - Site M0078
2. *Amnicythere* aff. *malva*, RV, external view, Sample 52.30 m - Site M0078
3. *Callystocythere littoralis*, LV, internal view, Sample 105.4 m - Site M0078
4. *Pseudolimnocytere* sp., RV, internal view, Sample 176.64 m - Site M0078
5. *Euxinocythere virgata*, LV, internal view, Sample 157.33 m - Site M0078
6. *Amnicythere cymbula*, RV, external view, Sample 77.22 m - Site M0080
7. *Microcythere* sp., LV, external view, Sample 5.62 m - Site M0080
8. Detail of *Tuberoloxoconcha aielloi*, RV, external view, Sample 54.71 m - Site M0078
   Anterior marginal pore canals of a RV on a black surface.
9. Detail of *Tuberoloxoconcha aielloi*, LV, external view, Sample 21.02 m - Site M0078
   Anterior marginal pore canals of a LV on a transparent surface.