Supplementary Material: Fiber Features, free dedicated software for the automatic characterization of Anthropogenic Microfibers from environmental samples: Software Tutorial

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1. Software availability, documentation, and licensing

Software availability. Fiber Features (FF), version 1.0, is available for download at https://fiber.fi.mdp.edu.ar/ for Windows and Linux operating systems. Access requires users to complete a registration form, which allows the developers to monitor the number of downloads and the institutional affiliations of users. Following registration, users must submit a code generated by the software through the website to obtain an activation code. An user guide is present in Section 3 to document the software's operation and to assist users in applying its analytical features.

2. Licensing and distribution

FF is currently distributed as closed-source software and is intended for educational and research purposes. Copying or redistribution requires prior permission from the rights holders. Its use in commercial activities, incorporation into other software products, or resale requires prior written authorization. The software is provided as is, without express or implied warranties

regarding the results obtained through its use, its merchantability, or its fitness for a particular purpose. Intellectual property rights, including copyright and rights to modifications, updates, and adaptations, remain with the developers.

3. Fiber Features Software Operation

The graphical user interface (GUI) of the software (Fig. 1) is designed with four main action buttons: one for loading images, one for processing the selected fiber, one for opening the current database, and one for switching to a different working database.

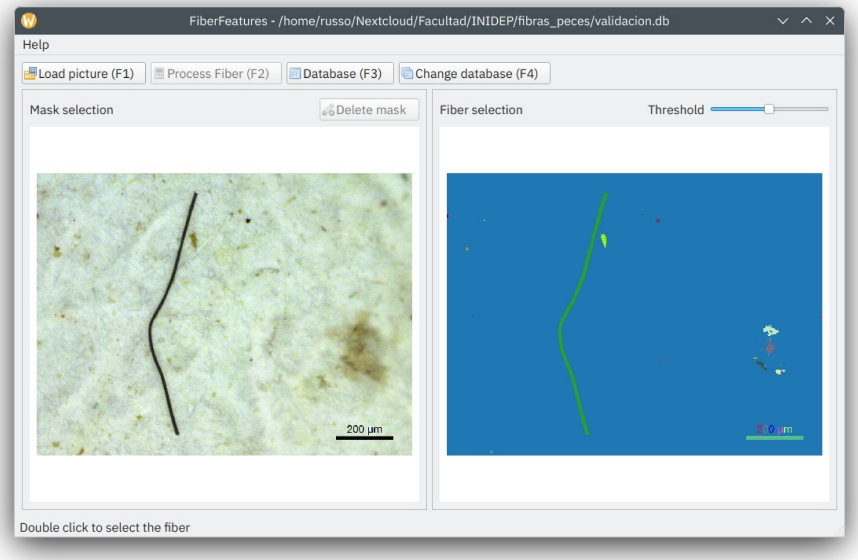


Figure 1: The graphical user interface (GUI) of the Fiber Features software.

Upon entering the workspace, the selected image is displayed on the left panel, while the right panel shows the identified objects —each assigned a false color or label for visualization purposes— based on the threshold chosen by the user, located at the top right of the interface.

Before the fiber can be processed, the user must double-click on the image on the right to select the fiber to be processed. This action places a red cross

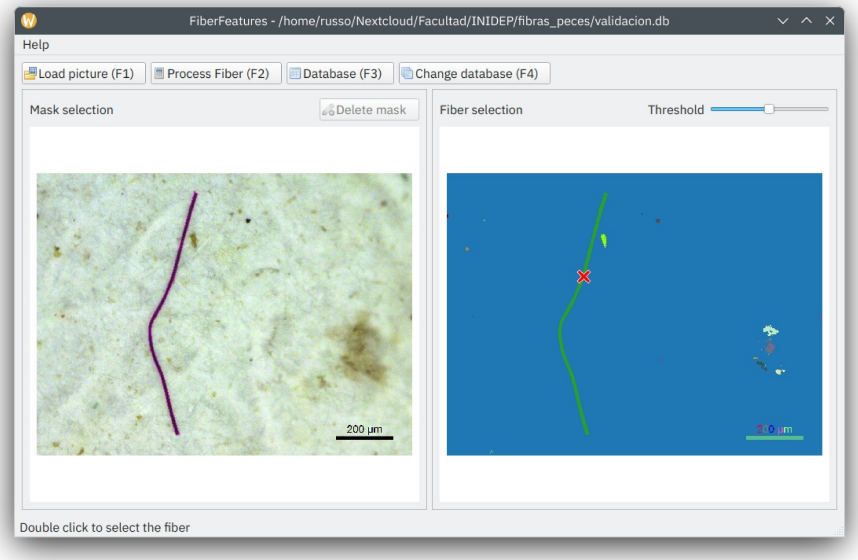


Figure 2: Conceptual diagram of maximal balls restricted to the topological skeleton of AMFs.

to indicate the object chosen within the threshold image (Figure 2), and subsequently, a pink contour is drawn around the fiber on the left image, marking its boundaries. The user can then confirm whether the selection is correct. If satisfied, the "Process Fiber" button can be clicked; otherwise, the selection process can be repeated. After processing, a new window opens showing the original image with the calculated path along the fiber, highlighted in red to indicate its length. (Figure 3).

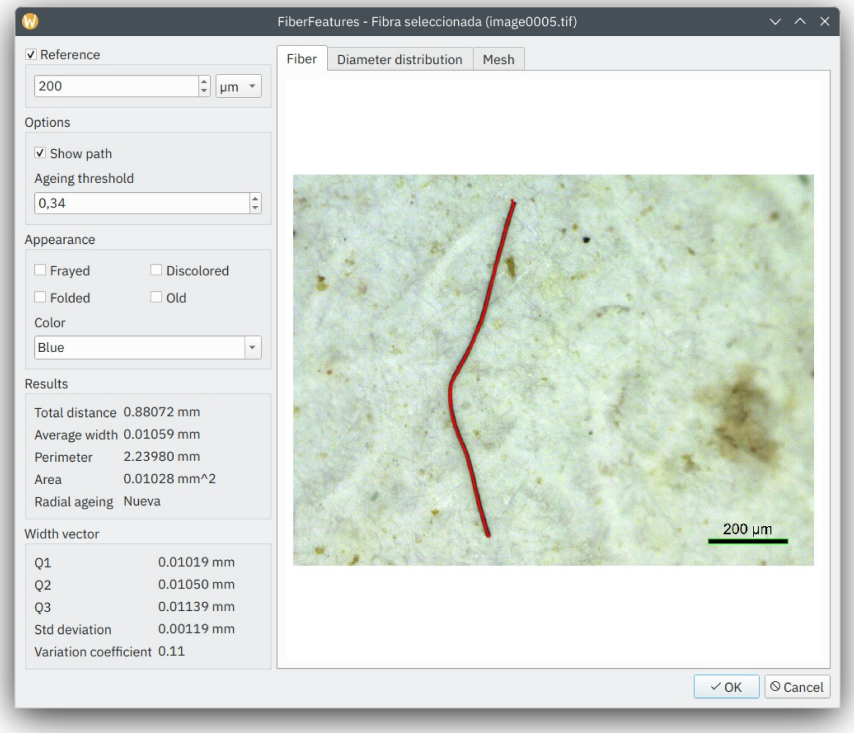


Figure 3: Conceptual diagram of maximal balls restricted to the topological skeleton of AMFs.

On the left side of the new window, the user can adjust several parameters, including the reference size (if available) to convert pixel measurements into physical units (µm, mm, or cm). The user can also choose to display or hide the computed fiber path, depending on whether they are conducting a qualitative analysis. Additionally, the ageing threshold can be adjusted as described in Section 2.2.1, and various appearance characteristics can be modified as outlined in Section 2.1. The same panel also displays the quantitative results computed by the software. These results include total length, average width, perimeter, area, automatic ageing classification, and statistical information about the fiber's width vector. All these values are expressed in millimeters.

At the top of the new panel, three tabs allow the user to change the view mode. The *Fiber* tab displays the processed image with the fiber path traced

on it. The *Diameter Distribution* tab shows a boxplot representation of the fiber width statistics (Fig. 4), while the *Mesh* tab renders a 3D visualization of the width vector, displaying distances from the center of the fiber (Fig. 5).

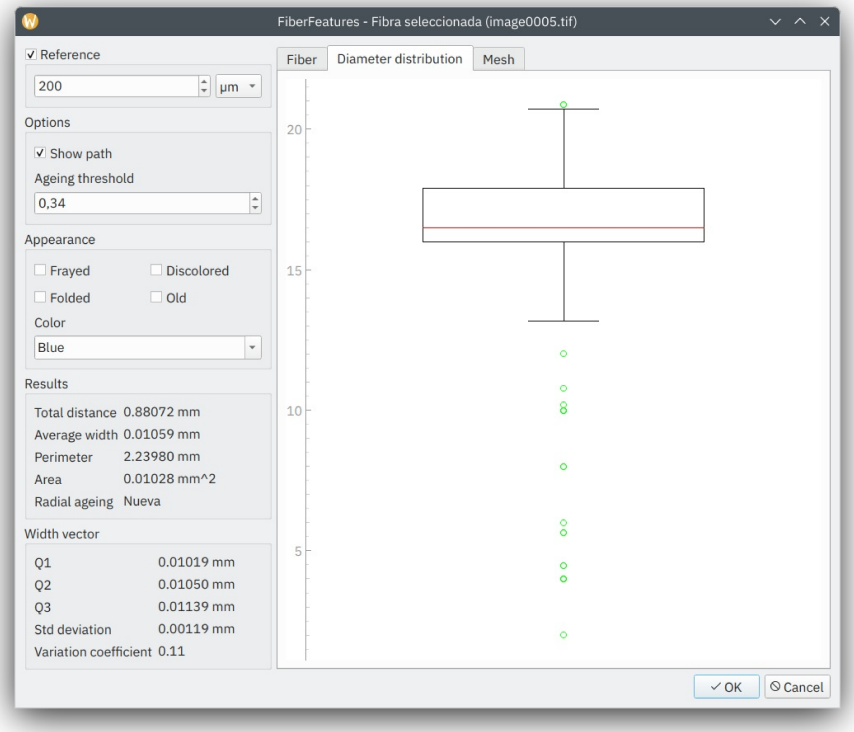


Figure 4: Conceptual diagram of maximal balls restricted to the topological skeleton of AMFs.

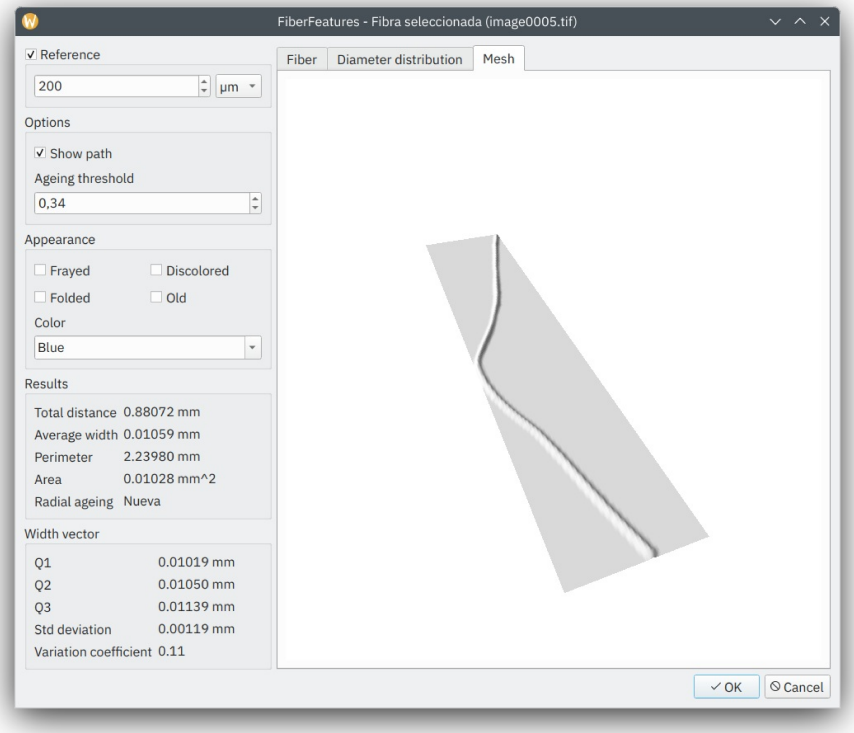


Figure 5: Conceptual diagram of maximal balls restricted to the topological skeleton of AMFs.

Once the processing is complete and the user clicks *OK*, the results and images are automatically saved to an SQLite database [1] which can be accessed via the application interface. The *Database* button, located in the main window (Fig. 1), opens a dialog displaying all entries in the current working database (Fig. 6).

[1]SQLite is a C-language library that implements a small, fast, self-contained, high-reliability, full-featured, SQL database engine. https://www.sqlite.org

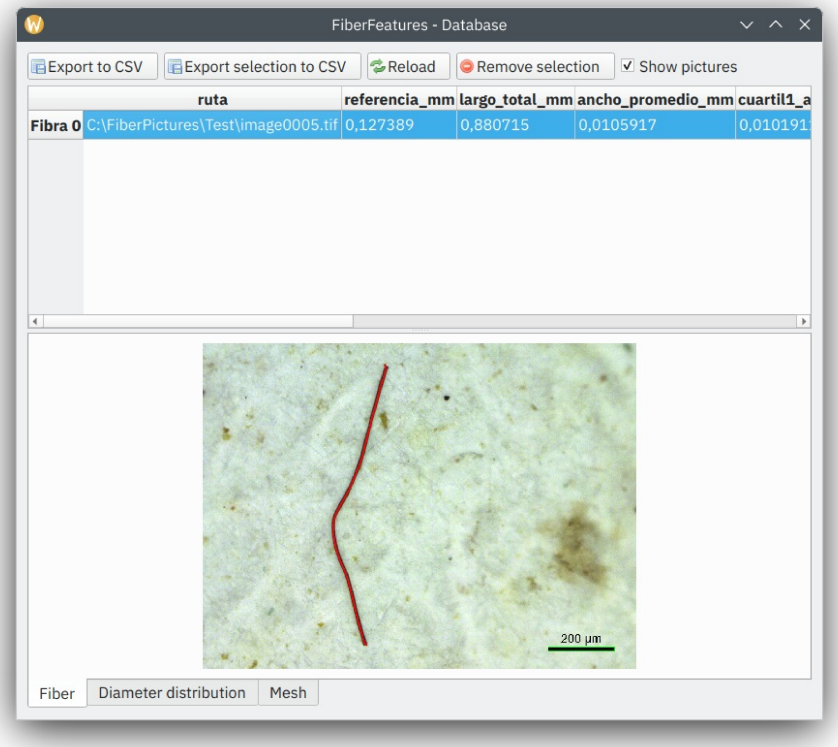


Figure 6: Conceptual diagram of maximal balls restricted to the topological skeleton of AMFs.