ISTITUTO NAZIONALE DI RICERCA METROLOGICA

Microplastics Analyzer

User's manual

Date

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1. Introduction

This document is a user guide for the web application *Microplastics Analyzer* developed to analyze images of microplastics (MPs) acquired via optical microscopy. The main functions are image segmentation and extraction of the MPs geometric features.

The application was created within the framework of the METROFOOD project, an international research infrastructure aimed at advancing metrology in food and nutrition. For more information about the project, please visit: https://www.metrofood.it/.

The tool was designed to address the growing issue of plastic contamination, particularly the presence of microplastics in food matrices, which poses emerging risks to both environmental and human health. Its main purpose is to support the characterization of microplastics, especially within workflows that incorporate Raman spectroscopy. Users can choose among several segmentation methods, including both classical image processing algorithms and a convolutional neural network (CNN) for instance segmentation. The CNN is based on the Mask R-CNN architecture and performs inference using custom-trained weights developed by the authors specifically for microplastic image analysis. To facilitate microplastic characterization, the application also provides the extraction of key morphological parameters commonly used in the field. These include the major axis length of the Legendre-fitted ellipse, the Feret diameter, the area of each detected particle, and more.

Although optimized for images acquired under specific conditions (as shown in the provided examples), the program is flexible and can also be tested on a variety of image types, including those not strictly related to microplastics.

Authors

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2. Using the tool

2.1. Access

The Microplastics Analyzer is accessible via a web interface. The tool allows users to upload images directly from their local device. Optional metadata files (.h5 and .mapx) can also be loaded to automatically extract instrument information regarding spatial calibration and offset.

2.2. Usage

Upon opening the tool in the browser, users are presented with a vertically organized interface composed of the following sections:

- User guide
- Upload image and ROI selection
- Info
- Analysis Settings
- Save Options

The interface is supplemented by two final buttons, one to start the analysis and one to download the results.

The sections are described in detail from sub-Section 2.2.1 to sub-Section 2.2.5. Sub-Section 2.2.6 explains how to correctly fill in the fields in the browser and start the analysis. Details are provided on how the analysis process works and under what conditions it can work best. Finally, sub-Section 2.2.7 presents a detailed list of all possible exportable files.

2.2.1 User guide

In this section, users can download this guide by clicking on the *Download User Guide* button.

2.2.2 Upload image and ROI selection

Users can upload an image in .JPG, .PNG, or .BMP format (note: currently .TIF/.TIFF formats are not yet supported).

Once uploaded, the image is displayed on screen and a rectangular region of interest (ROI) can be interactively selected by clicking and dragging the mouse. The selection of the ROI is mandatory if *Partial Image* option has been selected in the *Analysis* section.

2.2.3 Info

Users can select whether to load the image only or additional files to automatically fill in the *Ratio pixel/micron* ratio and *Offset* values. The following options are available:

- *Image file only* to consider the image file only;
- + *.h5 file* to also load the metadata provided by Horiba Raman spectroscope (available only for LabSpec6 version or compatible versions);
- + *.mapx file* for loading metadata provided by the ThermoFisher spectroscope (OMNICix).

The .h5 and .mapx files should only be read out in order to automatically derive the pixel/micron scale factor (i.e. the conversion factor from pixels to microns) and the spatial offset of the sample. Correct reading of this metadata is not guaranteed if the files relate to software versions other than those tested. Please note that other metadata is not extracted. The pixel/micron scale factor can be manually specified with the *Ratio pixel/micron* field by inserting an integer or decimal number (e.g., 2 or 0.5). To specify the spatial offset of the sample, enter the coordinates in square brackets in the *Offset* field, e.g. [*x*,*y*] with *x* and *y* being integers or decimals, considering that they are in microns if the pixel/micron scale factor has been entered, otherwise refer to pixels.

<u>OSS</u>: The *Info*, *Pixel/micron ratio* and *Offset* sections have been included to allow comparison with data acquired by Raman instruments.

2.2.4 Analysis Settings

This section allows the user to configure the analysis parameters, including defining the particle size range to be considered (minimum and maximum thresholds), choosing between processing the entire image or a manually selected region of interest (ROI), and selecting the segmentation method to be applied to the image.

Size threshold

In the *Size threshold* section, the size range of interest for the analysis can be specified to exclude all microplastics outside the specified range. For the definition of the range, the minimum and/or maximum can be provided in the left and right input fields, respectively. It is possible to specify either both values or only one of them.

To define an **open-ended range**:

- entering a value, for example 10, only in the left field corresponds to the interval [10,∞), meaning that only objects larger than or equal to 10 will be included;
- entering a value, for example 20, only in the right field corresponds to the interval (0,20], meaning that only objects smaller than or equal to 20 will be considered.

Alternatively, specifying both values defines a **closed interval**, for example [10,20], where only objects with sizes within the specified range will be included in the analysis.

Analysis

In the *Analysis* section, it can be chosen whether to perform segmentation on the whole image, option *Whole image*, or only on a part of it, option *Partial image*. If *Partial image* is chosen, a ROI must have been previously defined on the uploaded image in the initial *Upload image and ROI* selection section. If a ROI is not selected, the analysis cannot begin and an error message will be displayed informing the user that a ROI must be selected to proceed.

NOTE: If the input image has at least one dimension larger than 7,000 pixels, the analysis will be automatically performed in Partial image mode, regardless of the selected option, to ensure optimal performance and avoid memory issues.

Segmentation

The *Segmentation with* section, permits to choose the type of segmentation for the analysis. There are four possible modes:

- *Instance segmentation* to apply the MASK R-CNN model. The model is pre-trained with weights specifically optimized for microplastic detection*.
- *Active contour* to apply the morphological geodesic active contour method (the function morphological_geodesic_active_contour, from python scikit-learn, is considered). The use of this model is proceeded by a preprocessing of the image that is optimized for dark background images. The preprocessing consists of transforming the image into a greyscale one, if the original is in color, followed by a change in contrast and brightness.
- *Autothresholding* to apply the Otsu algorithm, from the python OpenCV (Open Source Computer Vision) library. To optimize this method, the black-and-white image obtained by applying the algorithm is post-processed to eliminate the presence of noise (e.g. scattered holes or white dots) and nested contours.
- *Instance segmentation + Autothresholding* to merge the results obtained with the two methods. If there are overlapping contours, priority is given to the results from instance segmentation: the contours from thresholding are removed if there is a significant overlap with those from instance segmentation. If there are holes or discrepancies in instance segmentation, new contours are regenerated from the difference between the two masks generated by different methods.

***IMPORTANT NOTE: The MASK R-CNN model featured in this application uses weights derived specifically for this project to optimize microplastic image analysis. These custom-trained weights were developed by the authors to ensure segmentation results suitable for microplastic detection.**

2.2.5 Save options

Before starting the analysis, the user must configure the output options in the *Save Options* section of the web interface. This section allows the user to specify which files to export and how to configure them. The section is divided into four main subsections to facilitate user selection and includes:

- Segmentation;
- Result table;
- Histograms;
- Extra files for export.

Segmentation

If the option *Save image segmentation* is selected, an output image corresponding to the original image, provided as input for analysis, will be saved, with the contours obtained from the segmentation superimposed on it. With the field *Line width*, the user can specify the linewidth to customize the thickness of the line that identifies the contour of the particles (NOTE: default line width is 0.2 for images up to 1024×1024 pixels, 0.3 up to 2048×2048, and 0.4 for larger sizes). The color of the line is yellow and cannot be changed.

Result table

The user can select among all the measured parameters which are to be saved in a table to be stored in a .csv file. If the *Ratio pixel/micron* value has not been specified, the values reported are in pixels, otherwise they are automatically converted to microns. For this reason, **the unit of measurement is not reported on the table on the column names**. The parameters available for export include the main morphological descriptors typically used in microplastic characterization and are consistent with those commonly provided by commercial instruments. These include, in addition to the centroid coordinates (used to locate each particle within the image), the area, the equivalent diameter (area-based), the perimeter, the major and minor axis lengths of the fitted Legendre ellipse, the ellipse ratio, the circularity, the maximum and minimum Feret diameters, the convexity, the concavity, and the solidity.

Histograms

This section is used to select which parameters to save the histogram. For each parameter the user can specify the number of bins of the histogram and the range of values, specifying minimum and maximum. As for the .csv file, **the units of measurement are not reported**, since if the *Ratio pixel/micron* value has not been specified, the values reported are in pixels, otherwise they are automatically converted to microns.

Extra files for export

Save image segmentation with label saves an image as *Save image segmentation*, but includes labels on the image indicating the particle identification number (ID). Each label corresponds to a unique microplastic and is represented by an integer starting from 1. This number matches the row number in the accompanying .csv file where the measurement results for that specific particle are recorded. Also for this output, there is the option of selecting the linewidth to customize the thickness of the line that identifies the contour of the particles, and the default values are the same as in *Save image segmentation*.

Modified .h5 file can be selected only if the *+.h5 file* option has been flagged in the *Info* section. This option makes it possible to save an updated copy of the original .h5 file, in which the new segmented image is embedded, to guide a new spectral acquisition of the same sample using the Raman instrument (compatible only with the LabSpec6 version of Horiba). NOTE: To ensure correct alignment during the new acquisition, the sample must not have been removed or repositioned from its original holder in the Raman instrument. This is necessary to preserve the original spatial offset, as any change in position would invalidate the correspondence between the segmented image and the physical sample.

2.2.6 Start analysis

To correctly submit the form and start the analysis, the user must ensure that:

- the image has been uploaded correctly and is visible in the browser;
- in the *Info* section, an option is selected under the field *Source*;
- in *Analysis Settings* section, options are selected for both *Analysis* and *Segmentation with*;
- in the *Save Options* section, at least one output file is selected for export.

Once all required fields are completed, the user can click *Submit data for analysis* button at the bottom of the page. If any required field is missing or filled in incorrectly (e.g. invalid numeric values, or ROI not selected when *Partial image* is chosen), a warning message will appear. After clicking OK, the user is redirected back to the form to complete or correct the entries. When the form is completed correctly, the following message will appear, confirming that the data has been submitted for analysis:

Data sent successfully. Analysis started. You can use the link below to download the results once ready.

The processing time can last from a few seconds to a few minutes depending on the size of the image and the type of segmentation chosen. The optimal file size, in pixels, is 1024×1024 or smaller; larger images are divided into 1024×1024 sub-images, which are analyzed sequentially in random order. For memory efficiency, a maximum limit of 10,000 particles is imposed.

Please note that the analysis of files with .mapx extension may require a significant amount of time and is therefore not recommended for very large images**.**

Immediately after the message confirming successful data upload, the following message will appear under the *Submit data for analysis* button:

The analysis has started. Use the following code to download your results:

followed by the identifying name of the zip file containing the results. The file name is structured as follows: it begins with *file_* and continues with a unique numerical code identifying the analysis.

To download the data, simply paste the full name of the zip file into the *Enter the file name* space and click on the *Download ZIP* button. If the file is not yet ready, no file will be downloaded. You can access the interface again at a later time and download the file directly without having to repeat the analysis.

2.2.7 Output files

The names of the files that can be exported share a common base, consisting of the word *file_* followed by an identification number associated with the user session, and a descriptive part is added to this base, indicating the type of output generated. Taking *file_000* as an example of the base of the name, the file structure is as follows:

- **Save image segmentation** ⇨ .png file with name *file_000_contours*;
- **Select table data** ⇨ .csv file with name *file_000_results_tabl*e;
- **Select histogram** ⇨ .png file for each histogram stored with the name *file_000_featurename_histogram* with *featurename* that identifies the parameter, e.g. file_000_area_histogram.png, file_000_minimum_feret_diameter_histogram.png;
- **Modified .h5 file** ⇨ .h5 file with name *file_000_file_new_segmentation*;
- **Save image segmentation with label** ⇨ .png file with name *file_000_contours_label*.png, e.g. file_000_segmentation_label.png.

If errors occur during pipeline execution and the analysis cannot be completed correctly, the output will consist of a single .txt file named file_000_analysis_failed.txt. This file lists the possible causes of the error, along with suggested solutions to resolve the issue. However, if an error occurs while creating one of the files to be saved, it will not be included in the zip folder that is downloaded.

3. Application examples

3.1. Guided Image Analysis Procedure

3.1.1. Upload image and ROI selection

Figure 1 shows an example of how to use the user interface after the image has been correctly loaded and the area of interest selected, marked by a red rectangle. The image used for this example analysis is acquired in dark-field mode and has a resolution of 1024×1024 pixels. It depicts microplastics deposited on a silicon filter, whose holes are visible in the background. After selecting the ROI, the coordinates (in pixels) of the upper left vertex from which the selection began are displayed below the image, along with the width and height of the selected area.

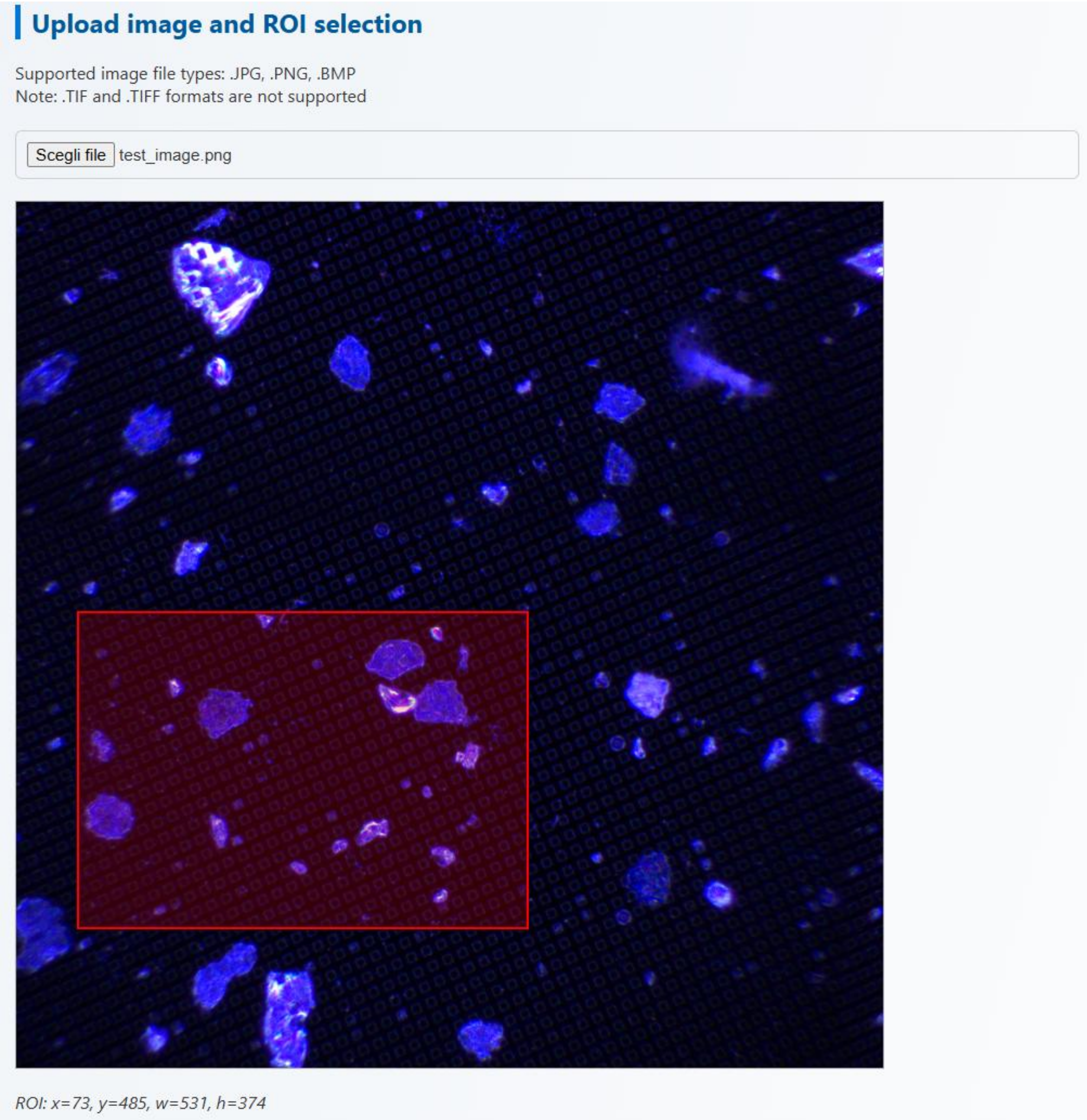

Figure 1. Detail of the "Upload image and ROI selection" section of the user interface. The example shows a successful image upload: the selected image is displayed below the upload button. The red rectangle represents the ROI manually selected by the user, with the coordinates and dimensions of the ROI are shown below the image. Specifically, x and y indicate the coordinates of the upper left corner, while w and h represent the width and height of the rectangle, respectively.

3.1.2. Info and Analysis Setting

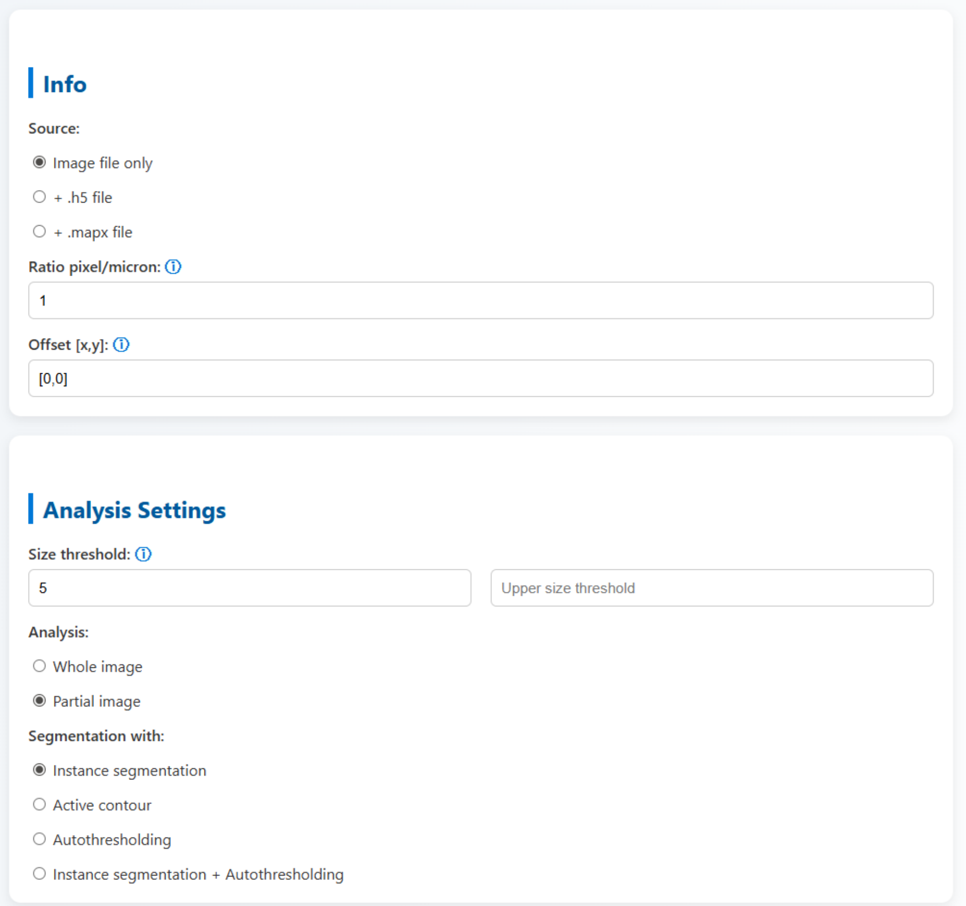

Figure 2. Detail of the "Info" and "Analysis Settings" sections of the user interface, showing an example of how to complete the various fields and select the options.

As reported in Figure 2, in the example shown, the analysis is configured for the image file only (as indicated by the *Image file only* option in the *Info* section), with the following settings:

- *Ratio pixel/micron* = 1, which corresponds to the default value and is used here to keep the measurements in pixels;
- *Offset* = [0, 0], also corresponding to the default value.

This configuration ensures that the analysis is only applied to the loaded image and retains all measurements in pixels, so the origin of the image coordinate system is in the top left corner. For the segmentation settings, a size threshold of 5 is chosen as the lower limit, meaning that only microplastics with a major axis length greater than 5 pixels are included in the analysis (since the pixel-to-micron ratio is set to 1, the size is interpreted in pixels). The partial analysis option is selected, and the chosen segmentation method is *Instance segmentation*.

3.1.3. Output file selection

Figure 3 shows an example of how to complete the data saving window. In this example, all parameters for the .csv file are selected, along with three histograms and the two segmentation images— without and with labels.

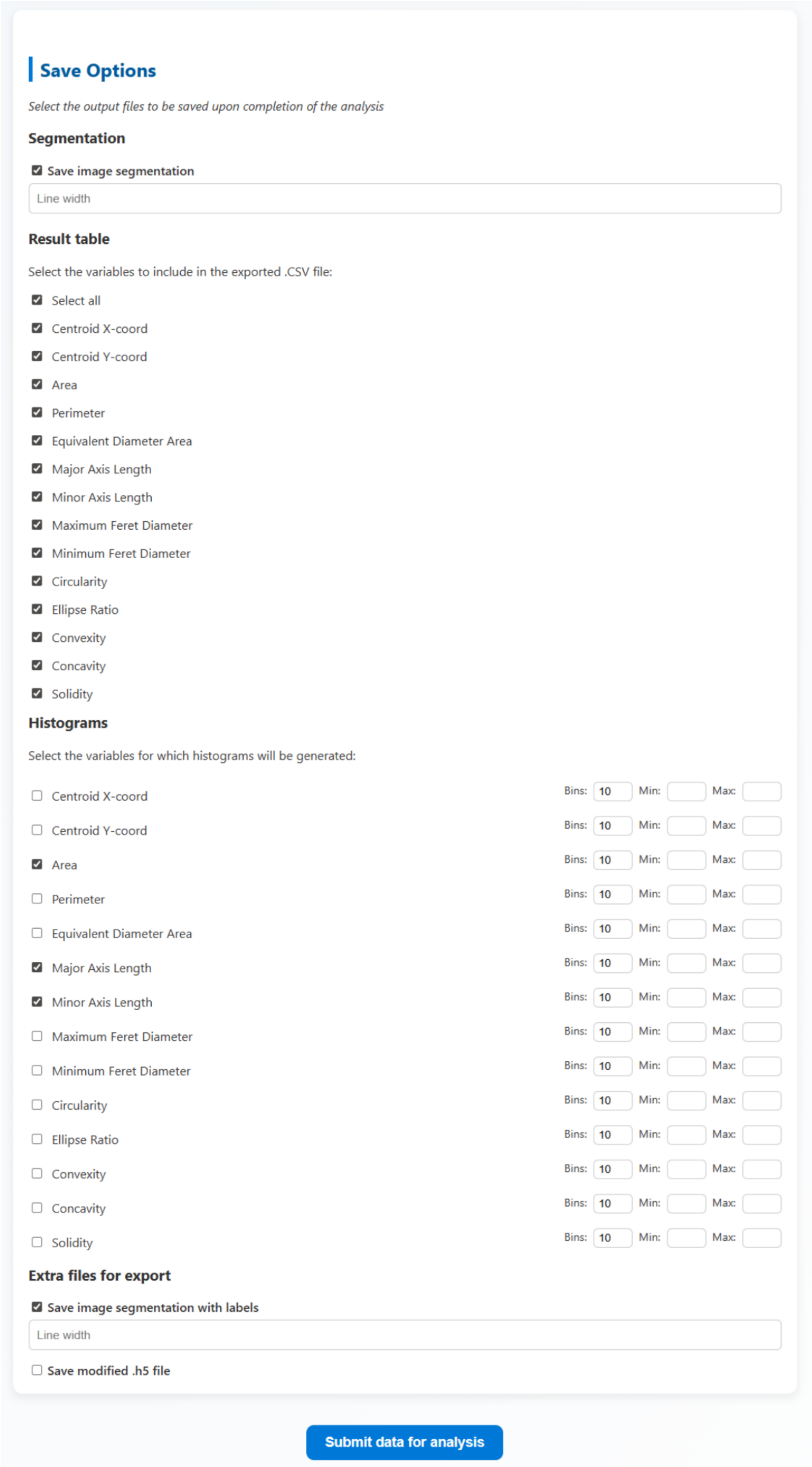

Figure 3. Detail of the "Save Options" section, showing an example of how to fill the fields and select the options.

3.1.3. Output files

The selected output files are shown in the following images. The image with the segmentation results is shown in The image with the segmentation results is shown in Figure 4 on the left, while on the right are details of the same image—at the top—and the segmentation image with labels—at the bottom—showing the area corresponding to the ROI.

The three histograms relating to area, major axis length, and minor axis length are shown in Figure 5, while a portion of the results table with the selected measurements is shown in Figure 6.

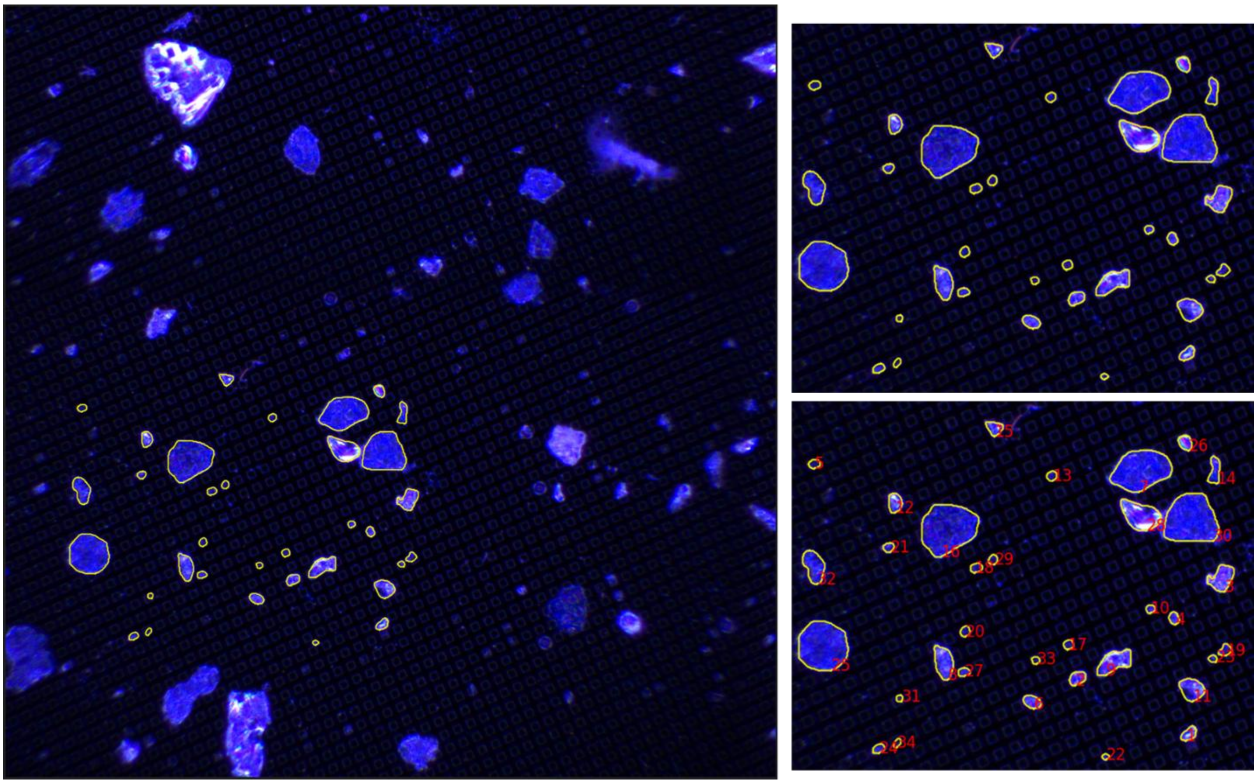

Figure 4. Segmentation results. On the left, the complete image with the contours obtained from segmentation defined by the yellow curves. On the right, the ROI with the segmentation results showing only the contour (top) and also the labels (bottom).

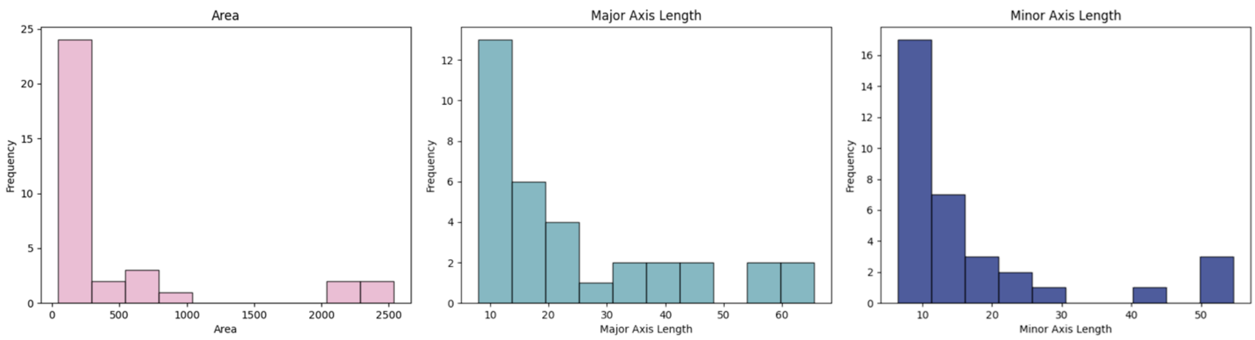

Figure 5. Histograms of, from left to right, area, major axis length and minor axis length.

	A	B	C	D	E	F	G	H	I	J
1	ID	Centroid X-coord	Centroid Y-coord	Area	Perimeter	Equivalent Diameter	Major Axis	Minor Axis	Maximum Feret Diameter	Minimum Feret Diameter
2	1	502	820	180	51.69848481	15.13879513	20.017853	11.713625	19.52331939	12.0208149
3	2	381	761	206	52.627417	16.19528778	19.450057	13.615214	19.20833153	15
4	3	534	660	532	99.254834	26.02620675	32.235314	22.779761	32.11790778	25.4911747
5	4	484	697	121	39.79898987	12.41217084	14.041546	11.039723	13.73171512	11.62755299
6	5	101	533	100	35.55634919	11.28379167	13.136819	9.7023703	12.75774275	10
7	6	334	787	213	54.87005769	16.46815178	20.592514	13.179609	20.30664916	15
8	7	445	541	2118	182.509668	51.92996587	65.559501	41.654099	67.85985558	43.82692719
9	8	240	747	561	96.76955262	26.72615544	37.524197	19.406663	38.65177874	21
10	9	419	743	645	110.4264069	28.65727667	43.062434	20.386007	43.92220395	22.8708725
11	10	459	688	72	28.72792206	9.57461473	10.301165	8.9142108	9.867117107	9

Figure 6. Detail of the result table.

3.2. Recommended vs. non-recommended image types

The tool is designed to analyze high-resolution images containing well-defined objects. Images should not include elements with excessively blurred contours, as this may compromise the accuracy of segmentation. The instance segmentation and self-segmentation models have been trained to process microplastic images on dark and bright backgrounds. The active contour model, on the other hand, is primarily optimized for dark background images. All three segmentation methods perform best when analyzing images with clearly separated objects. In the presence of grouped or overlapping particles, it is recommended to use the instance segmentation model for better separation.

To guide users toward optimal use of the tool, below are some examples illustrating the conditions under which the implemented segmentation models offer good results. For completeness, a representative example of a case in which the tool may not function correctly is also provided.

Optimal results with all segmentation methods

Figure 7 shows a case in which all segmentation methods achieve excellent results, namely on images with a dark background where the microplastics present are well defined, clearly distinguishable, and scattered throughout the image.

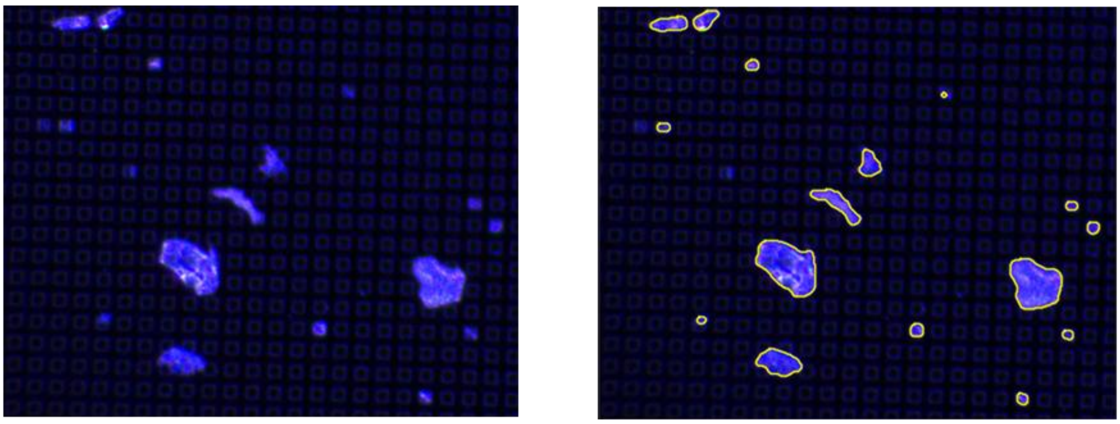

Figure 7. Left, original image. Right, results obtained with the autothresholding method

Best performance with the instance segmentation method

The instance segmentation method demonstrated the highest effectiveness under challenging conditions, such as the presence of microplastic agglomerates (Figure 8, top and middle panels), high background noise (Figure 8, middle panel), and bright-field imaging (Figure 8, bottom panel). In these scenarios, image complexity and poor particle separation hinder the performance of traditional segmentation techniques. In contrast, instance segmentation can accurately detect and isolate individual particles, even in cases of overlap or suboptimal image quality.

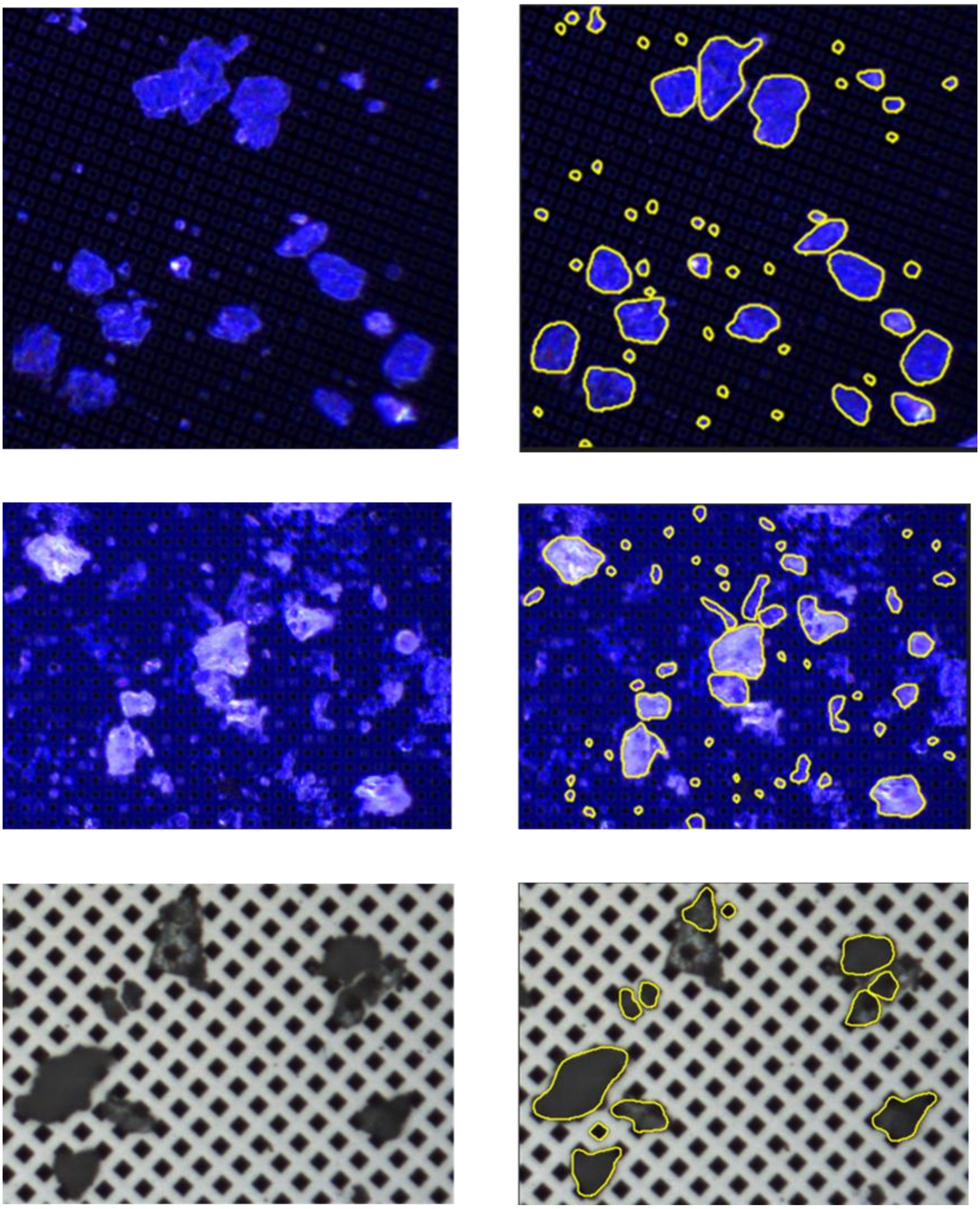

Figure 8. Left, original images. Rigth, results obtained with the instance segmentation method.

Suboptimal performance

Segmentation methods show limited effectiveness when applied to blurred images, as the lack of edge definition compromises the identification of particle boundaries. The problem is further exacerbated in bright-field acquisition mode, where contrast is typically lower and background noise higher. An example of this condition is shown in Figure 9, where the combination of blurring and bright-field imaging significantly reduces the performance of the segmentation results.

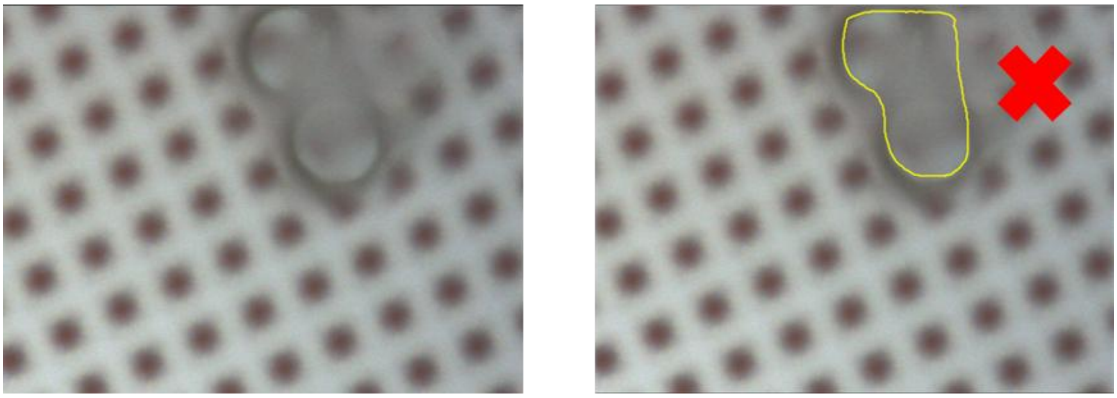

Figure 9. Example of the type of image for which segmentation may fail. On the left, the original image. On the right, the result using the instance segmentation method (the red cross indicates that the method failed).

Furthermore, it is recommended not to use images or select ROI with very small dimensions. Extremely small dimensions may not contain sufficient spatial information for the models to correctly identify and segment individual particles/objects. As an initial test, it is recommended to use the automatic thresholding method to see if the image can be analyzed correctly.