ASAP

Automated Structures Analysis Program

User manual

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Introduction

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Introduction

What is ASAP?

Determining the architecture of nanoscopic structures in the cell is now possible, but requires the quantitative, statistical analysis of many thousands of subcellular assemblies. Manually classifying and analyzing the varied morphologies of those structures is very time consuming and imprecise, thereby limiting statistical inference across scales and the understanding of biological function at the molecular level. There is a strong need for a simple tool that enables rapid and unbiased detection, classification and analysis of nanoscopic biological structures from microscopy images. To fill this gap, we developed **ASAP** (Automated Structures Analysis Program); a novel, interactive and freely-available toolkit that permits automated, rapid and robust detection, classification and quantitative analysis of macromolecular structures.

Introduction

Why ASAP?

- Freely-available.
- Image based (i.e. compatible with all super-resolved microscopy methods).
- GPU- and parallel- computing accelerated image analysis.
- A variety of methods for segmentation and geometrical analysis.
- Machine learning based classification of structures.
- Geometrical-based cluster analysis.
- *In situ* statistical visualization (high quality publication-ready figures), analysis and modelling.
- Customizable easily-generable gallery of structures.

Introduction

How to operate ASAP?

- Download the R2018a MATLAB runtime compiler here.
- Install the R2018a MATLAB runtime compiler.
- Download and run the **ASAP**.exe (for Windows OS) or **ASAP**.dmg (for Mac OS) files here.
- Follow instructions ⇲.

Introduction

Examples

This manual guides the user using example image files which can be found in the ‘Examples/ASAP guide user manual’ folder to be found as attachment to the published manuscript.

Introduction

License

MIT License

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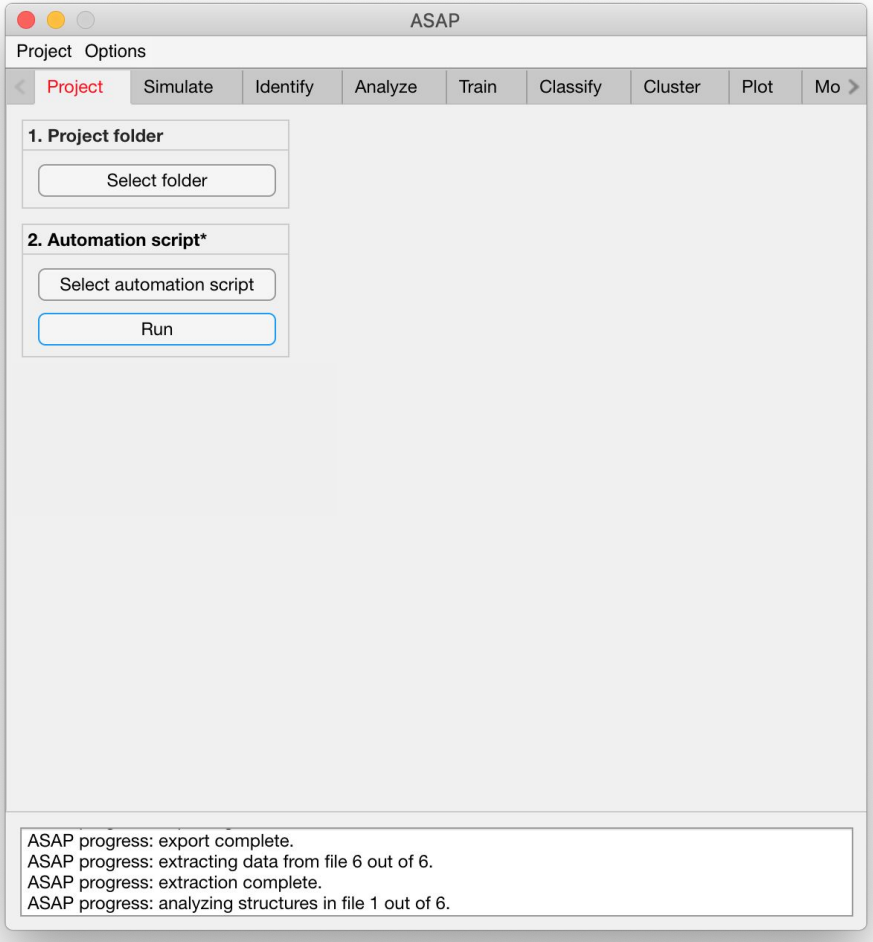
The message box



Instructions

/ All messages indicating errors, warnings and progress are displayed on the message box located underneath the main window.

/ The user is advised to refer to the **ASAP guide: description of error and warning messages** accompanying this user manual.

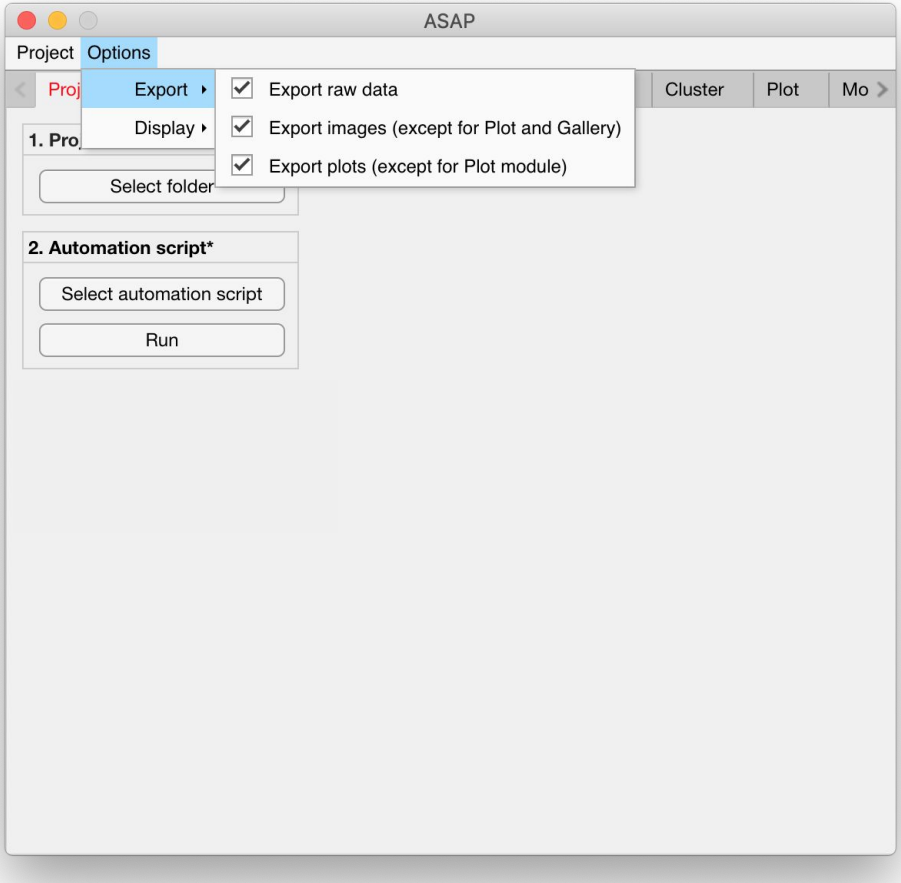
§ Note 1:
The message box has been clipped from all the consequent images of the ASAP window for clarity.

Setting options

1. Changing export settings ↘
2. Changing display settings ↘

Setting options

Changing export settings

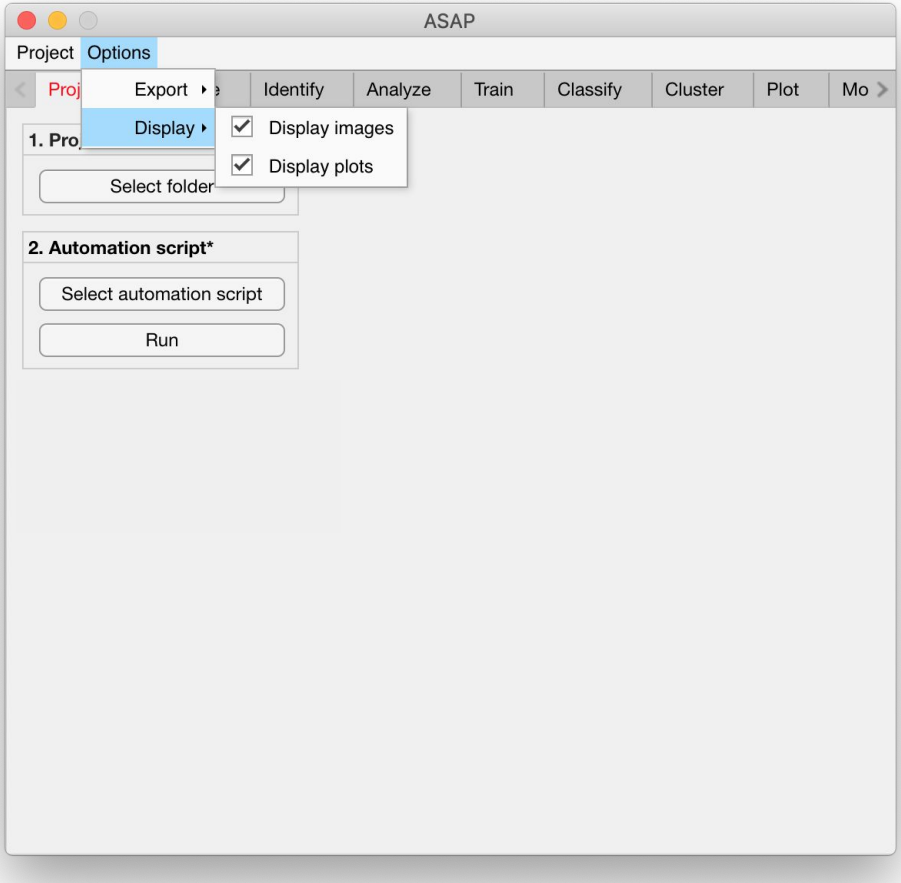


Instructions

The export process can take a long time if ASAP is exporting all raw data (as excel files), images and plots. To speed the analysis process we recommend to disable the export of the mentioned files. Files native to ASAP are always exported and do not constitute a large duration of the whole process.

Setting options

Changing display settings



Instructions

If many files are being analyzed the user may experience a slow down due to the display of an excessive number of images or plots. To speed the analysis process we recommend to disable the display of the mentioned files.

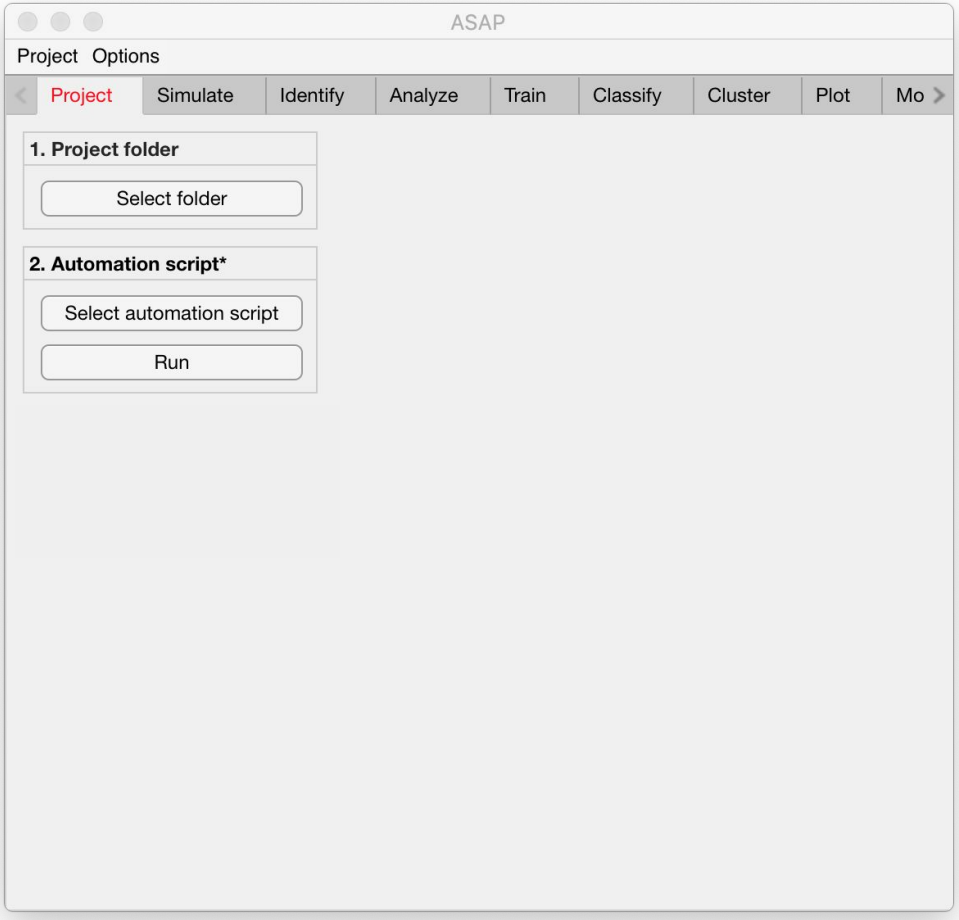
Project definition

Instructions

/ Select the ‘Project’ tab from the tabs’ bar.

/ Select a project folder containing the images to be processed by pressing the ‘Select folder’ button in the panel titled ‘1. Project folder’. The name of the folder will be shown in the text beneath.

/ The workflow of ASAP can be automated through the automation script; a single text file containing all parameters required by ASAP. To automate ASAP select an automation script (.txt) file by pressing the ‘Select automation script’ button. When the automation automation script is successfully loaded, automation is executed when the ‘Run’ button is pressed.



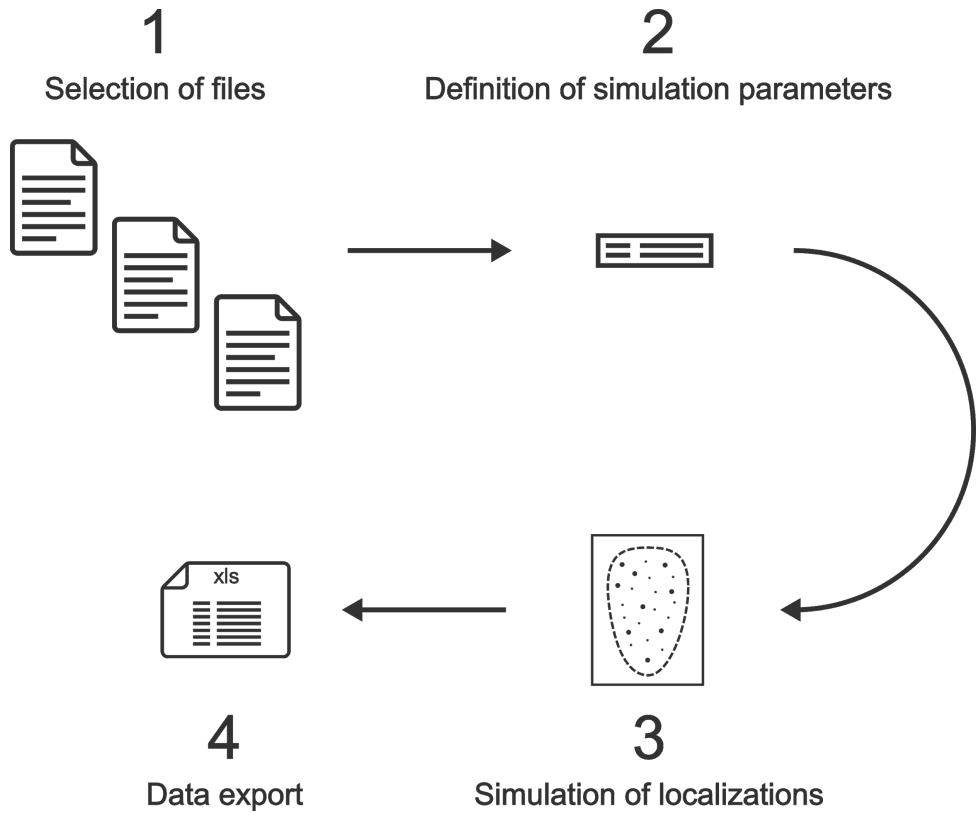
Guide ↘

Simulation of point localizations*

1. Working principle ⇲
2. Selection of files ⇲
3. Definition of simulation parameters ⇲
4. Definition of system properties ⇲
5. Sample segmentation ⇲
6. Data simulation and export ⇲

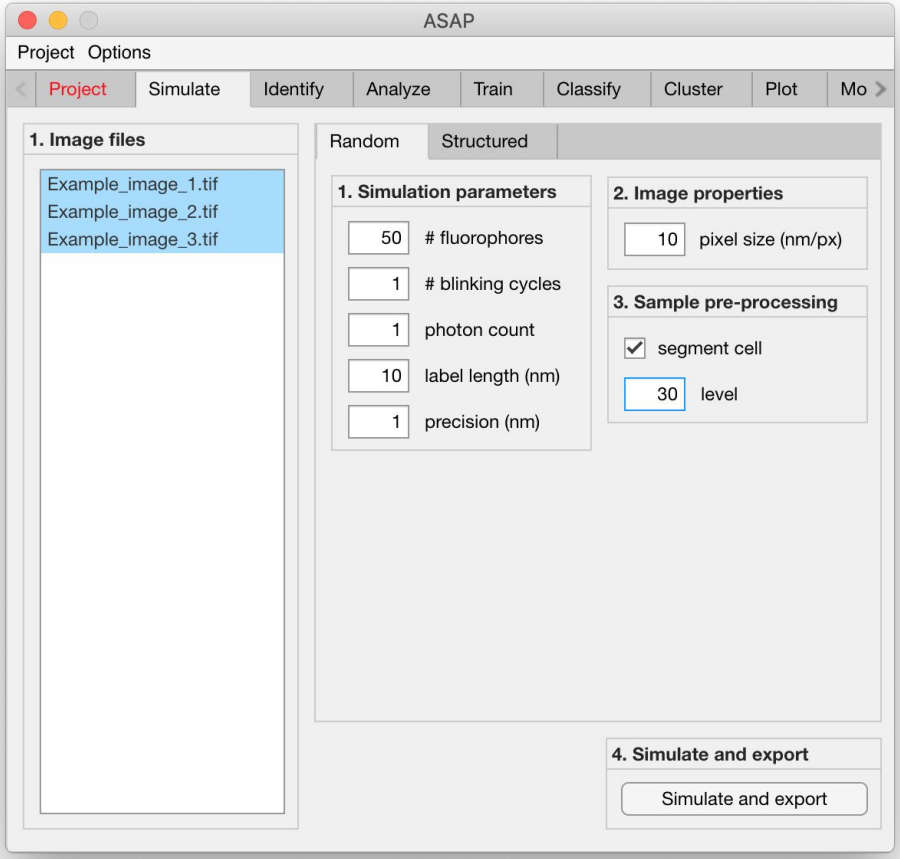
Simulation of point localizations

Working principle



Simulation of point localizations

Selection of files



Instructions

/ Select the 'Simulate' tab from the tabs' bar.

/ The listbox in the panel titled '**1. Image files**' will be populated with the names of png, jpg and tiff images located in the input folder previously selected ⇲.

/ Select files by holding the CTRL key and right-clicking the desired files in the listbox in the panel titled '**1. Image files**'. Selected files will be highlighted as shown in the figure on the right.

§ Warning 1:
Only images with the extensions png, jpg and tiff will be shown in the listbox.

Simulation of point localizations

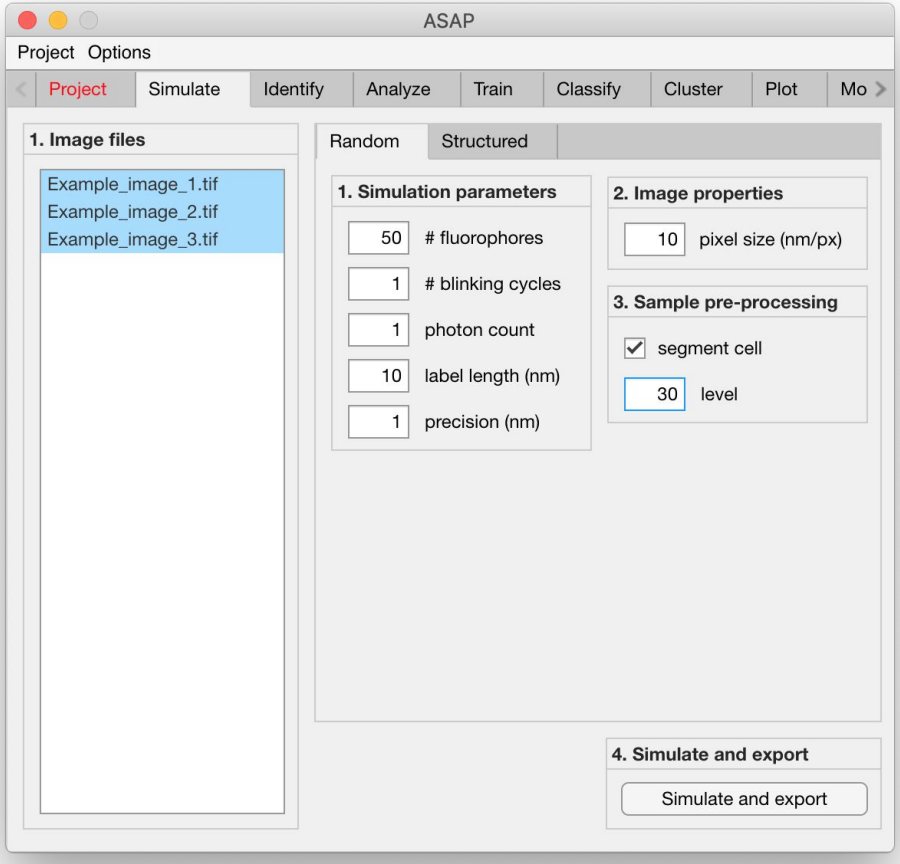
Definition of simulation parameters (1/2)

Instructions

/ Random or Structured localiations can selected to be simulated by pressing either on the Tab group labeled ‘Random’ and ‘Structured’.

/ If Random is selected, the panel titled ‘**1. Simulation parameters**’ contains 5 simulation parameters which have to be defined:

1. **# fluorophores (1000)**: total number of fluorophores in an field of view. Please note that this does not correspond to the number of localizations.
2. **# blinking cycles**: average number of blinking cycles per fluorophore.
3. **# photon count**: number of photons emitted per emission cycle.
4. **label length (nm)**: length of fluorescent label from protein..
5. **precision (nm)**: expected localization precision.



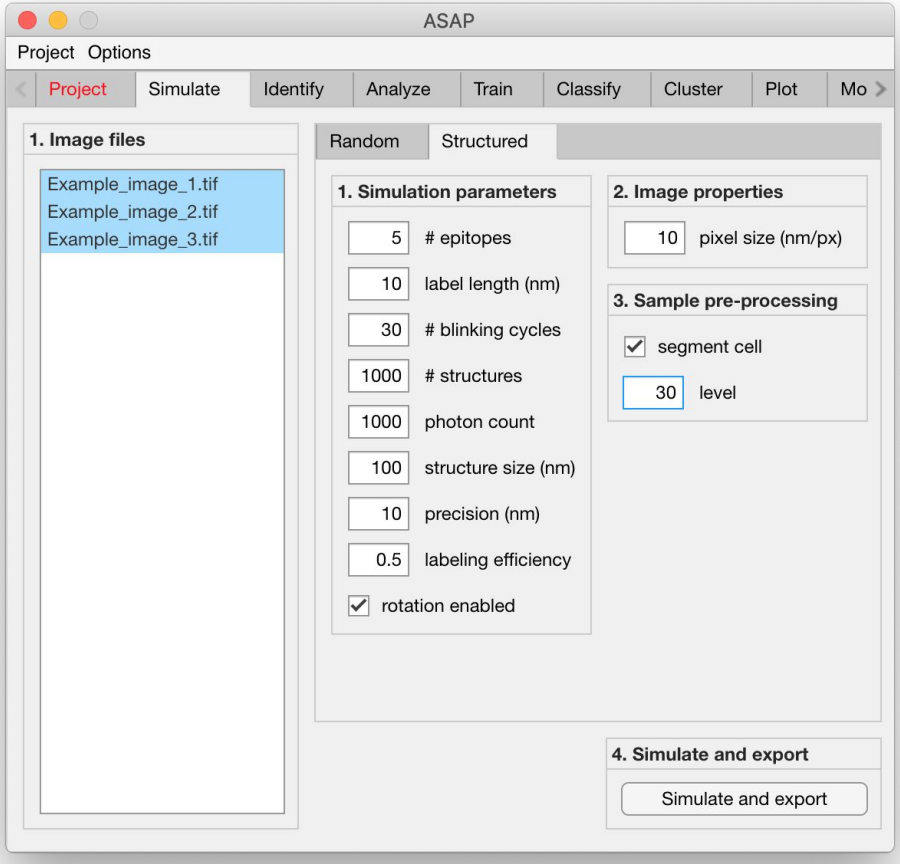
Simulation of point localizations

Definition of simulation parameters (2/2)

Instructions (contd.)

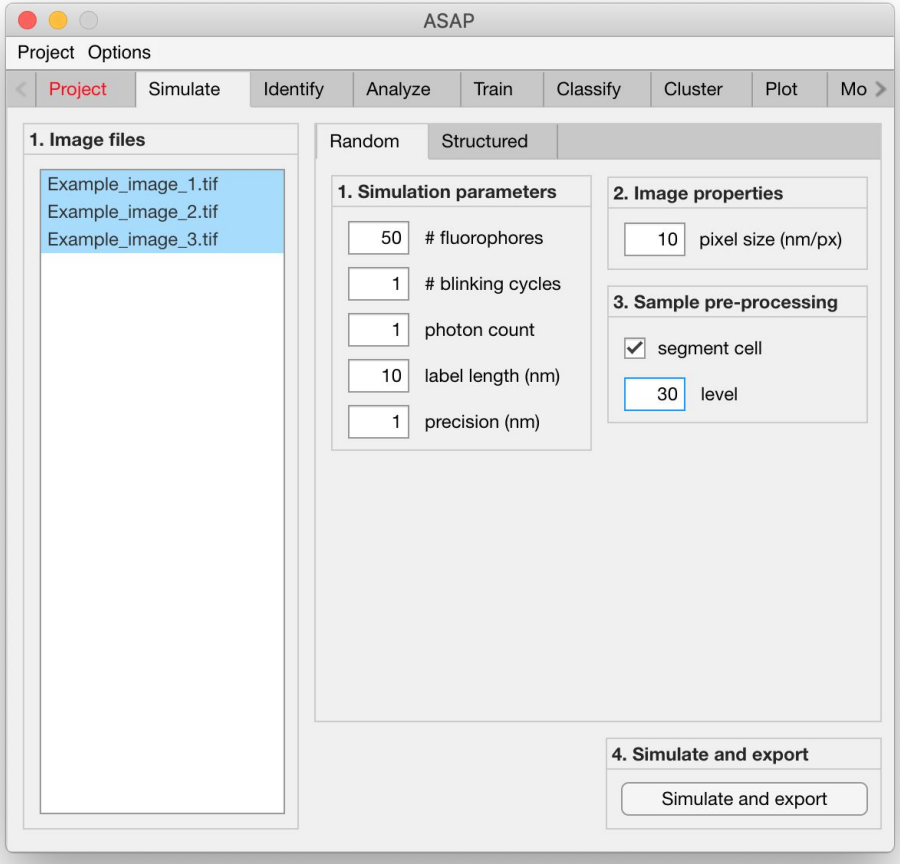
/ If Structured is selected, the panel titled '**1. Simulation parameters**' contains 9 simulation parameters which have to be defined:

1. **# epitopes**: number of discrete positions across a ring-like structure.
2. **label length (nm)**: length of fluorescent label from protein.
3. **# blinking cycles**: average number of blinking cycles per fluorophore.
4. **# structures**: number of ring-link structures in field of view.
5. **# photon count**: number of photons emitted per emission cycle.
6. **structure size (nm)**: diameter of ring-like structure in nanometers.
7. **precision (nm)**: expected localization precision.
8. **labeling efficiency**: average ratio of epitopes which are labeled.
9. **rotation enabled**: to be checked if ring-link structures are to have different orientations.



Simulation of point localizations

Definition of system properties



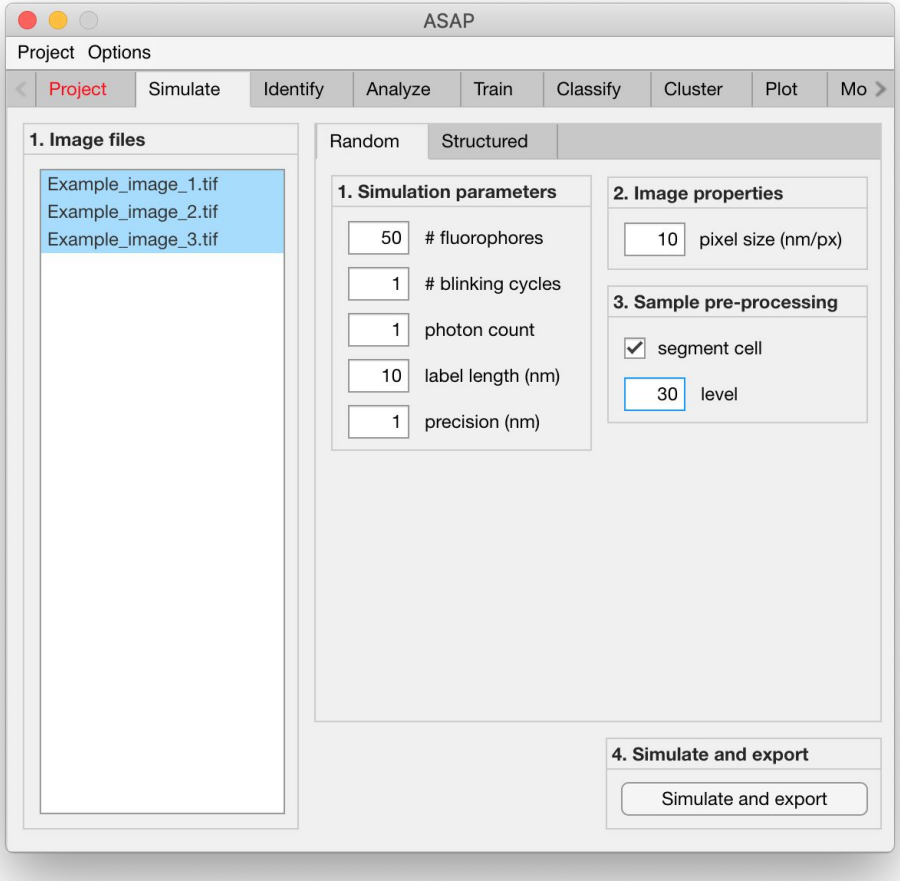
Instructions

The panel titled '**2. System properties**' contains 1 parameter which has to be specified as follows:

1. **pixel size (nm/px)**: size of 1 camera pixel (in nanometers).

Simulation of point localizations

Sample segmentation



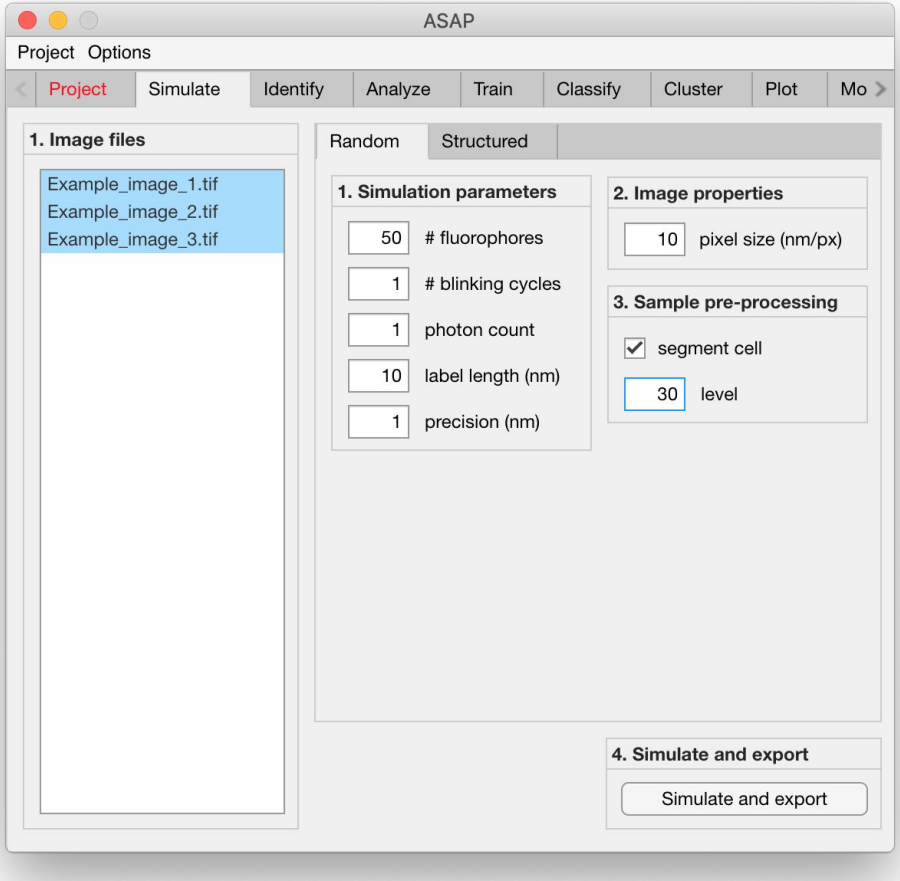
Instructions

The panel titled '**3. Sample pre-processing**' contains 2 simulation parameters, of which the first has to be at least defined:

1. **Segment cell**: to be checked if segmentation is desired.
2. **Level**: segmentation level.

Simulation of point localizations

Data simulation and export (1/2)



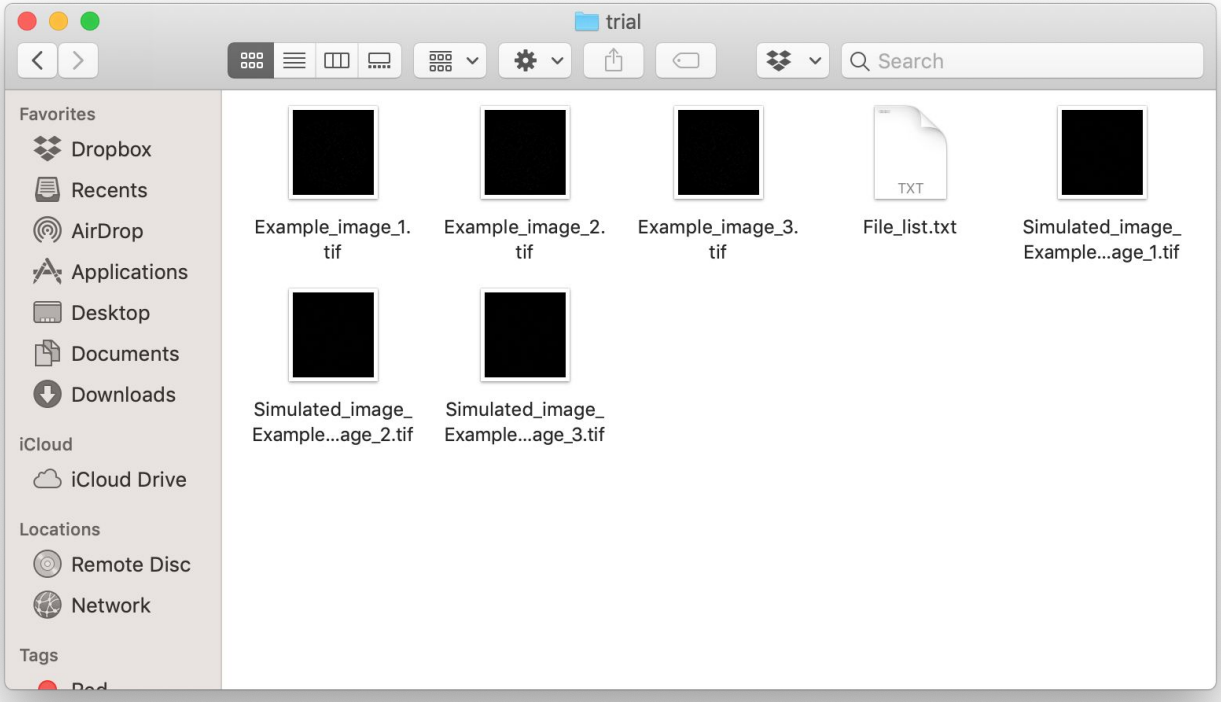
Instructions

Simulate and export data by pressing the '**Simulate and export**' button in the panel titled '4. **Simulate and export**'.

Please turn over

Simulation of point localizations

Data simulation and export (2/2)



Instructions (contd.)

The following 1 output file (per processed image) will be placed in the project folder:

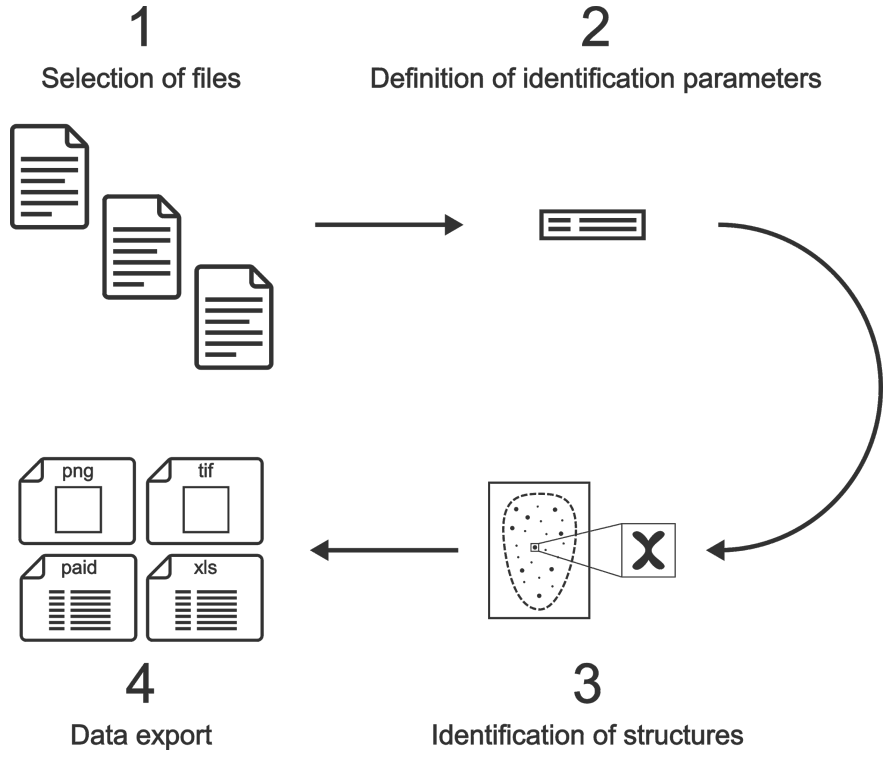
1. '**Simulated_ image_xx.tif**': an image file of the simulated localizations.

Identification of structures

1. Working principle ↘
2. Selection of files ↘
3. Definition of segmentation parameters ↘
4. Definition of image clean-up parameters ↘
5. Definition of filter parameters ↘
6. Identification of structures ↘
7. Data export ↘

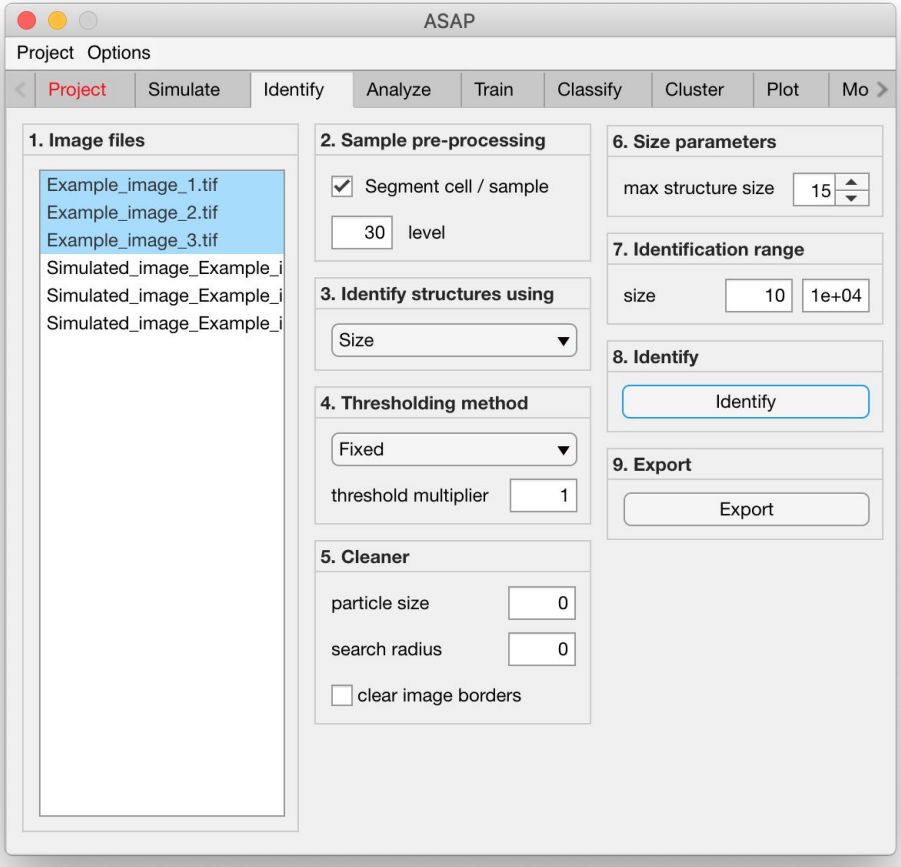
Identification of structures

Working principle



Identification of structures

Selection of files



Instructions

/ Select the 'Identify' tab from the tabs' bar.

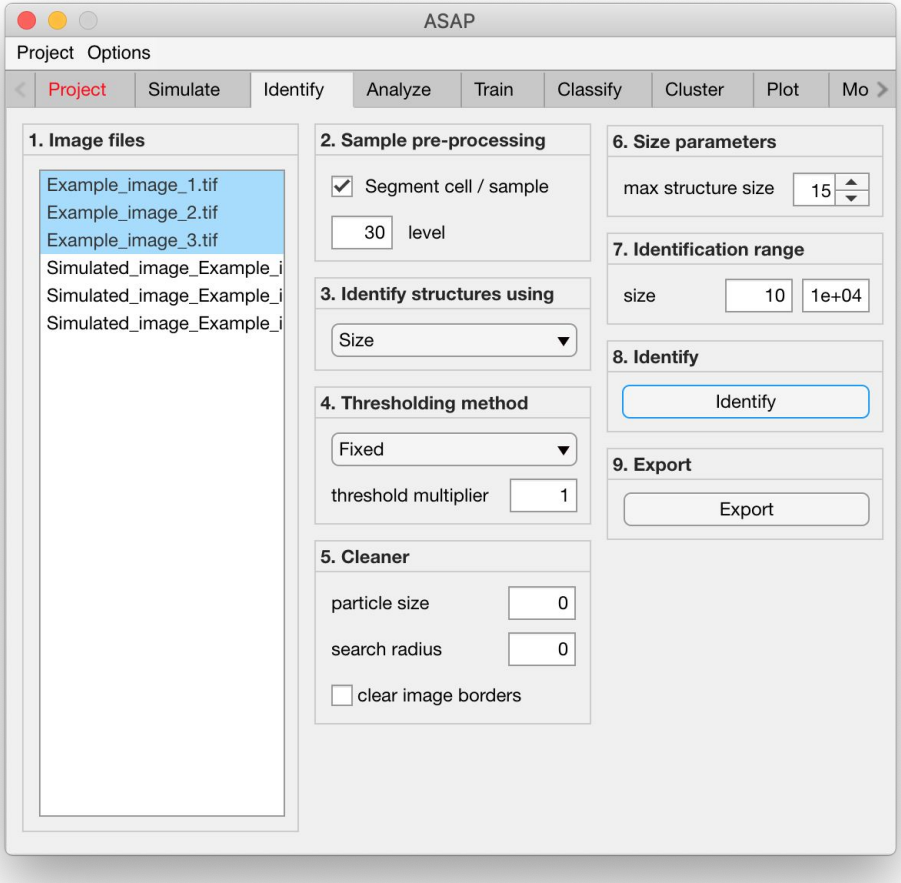
/ The listbox in the panel titled '**1. Image files**' will be populated with the names of png, jpg and tiff images located in the input folder previously selected ⇲.

/ Select files by holding the CTRL key and right-clicking the desired files in the listbox in the same panel. Selected files will be highlighted as shown in the figure on the right.

§ Note 1:
Only images with the extensions png, jpg and tiff will be shown in the listbox.

Identification of structures

Definition of segmentation parameters (1/3)



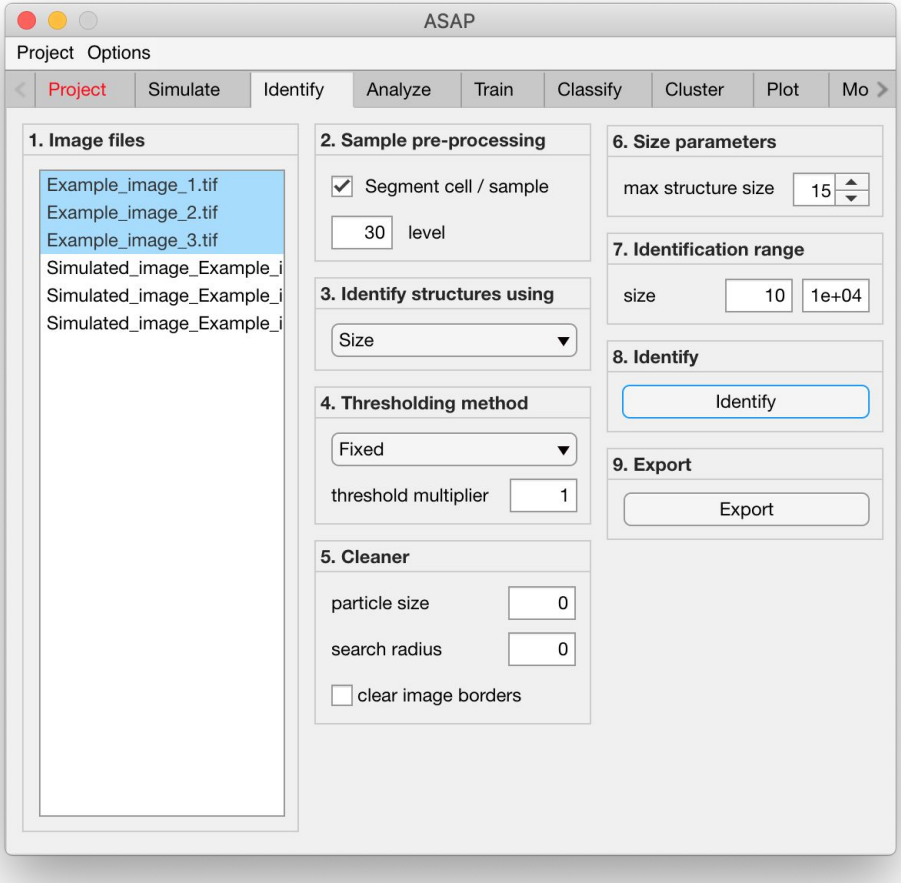
Instructions

The panel titled '**2. Sample pre-processing**' contains 2 parameters, of which the first has to be at least defined:

1. **Segment cell**: to be checked when segmentation is desired.
2. **Level**: segmentation level.

Identification of structures

Definition of segmentation parameters (2/3)



Instructions (contd.)

The panel titled '**3. Identify structures using**' contains 1 parameter which has to be defined:

1. **Connectivity / Size**: if connectivity is selected, structures are segmented (identified) based on their pixel-to-pixel connectivity (best used when the imaged structures are aggregates). If size is selected, structures are segmented based on regional density (best used when the imaged structures are sparse).

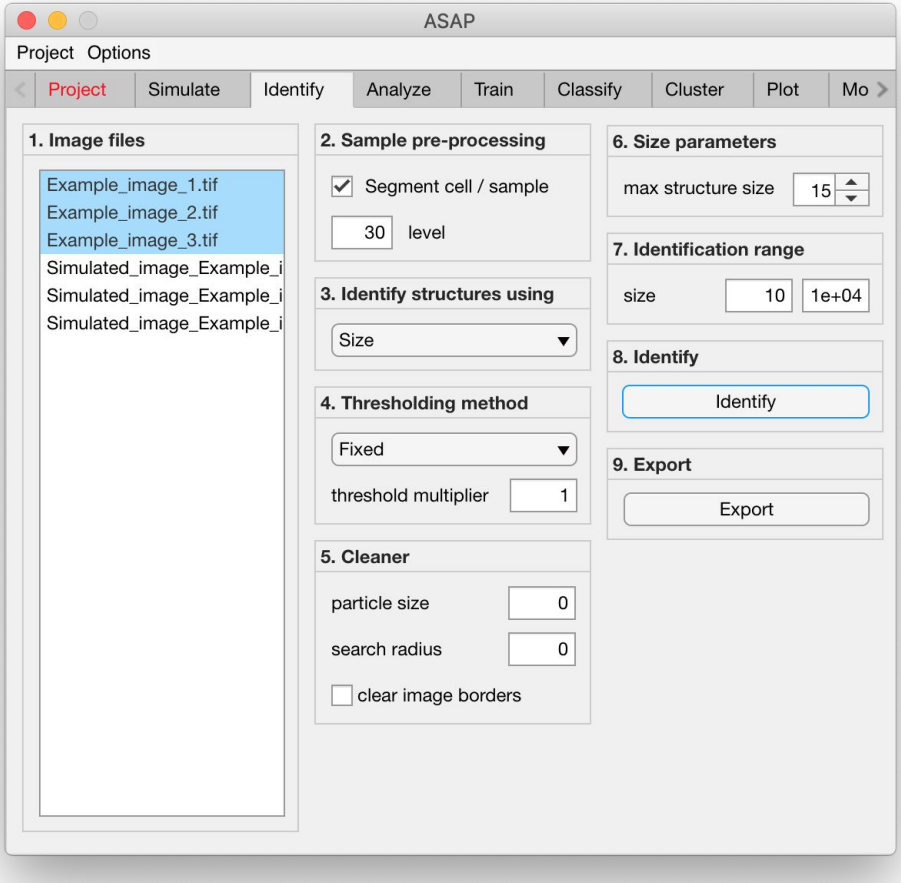
Instructions (contd.)

The panel titled '**4. Thresholding method**' contains 2 parameters which have to be defined:

1. **Fixed / Relative**: if fixed is selected, the software only considers those pixels whose intensities are higher than a global uniform threshold (best used when illumination is uniform and structures are located within a thin optical section). If relative is selected, the software only considers those pixels whose intensities are higher than a threshold value that is modulated depending on average regional intensity (best used when illumination is non-uniform and / or structures are not located within a thin optical section).
2. **Threshold multiplier**: a constant multiplier for modulating the output of the relative thresholding algorithm (best used when intensity histogram is strongly skewed).

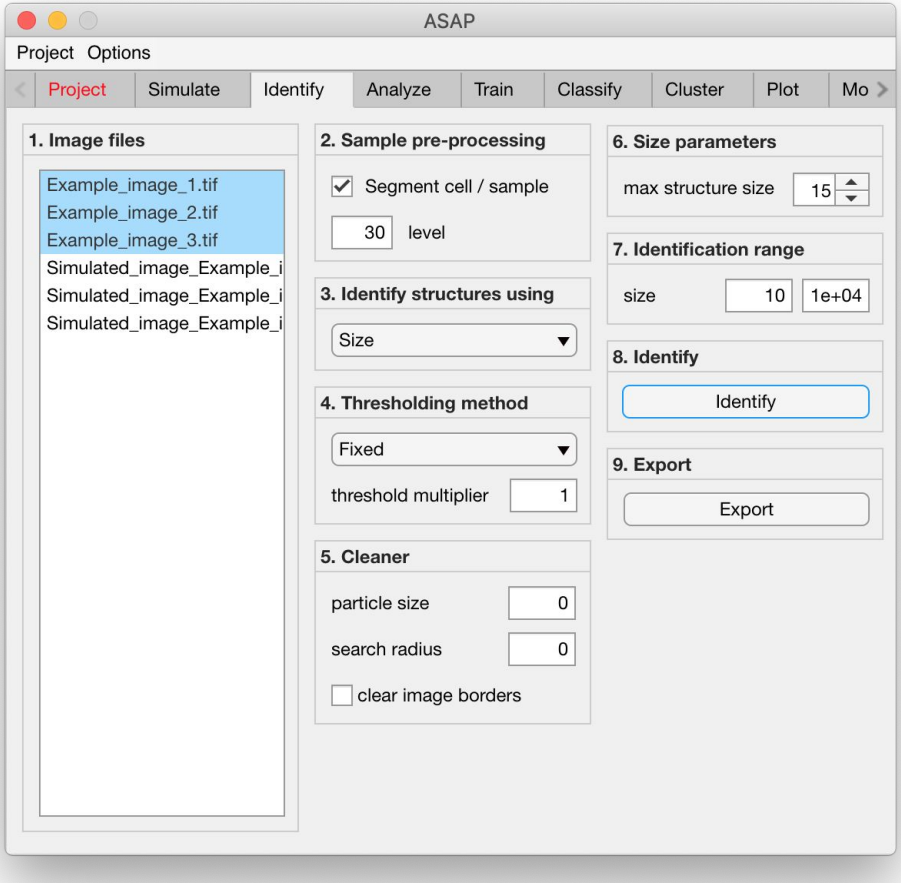
Identification of structures

Definition of segmentation parameters (3/3)



Identification of structures

Definition of image clean-up parameters



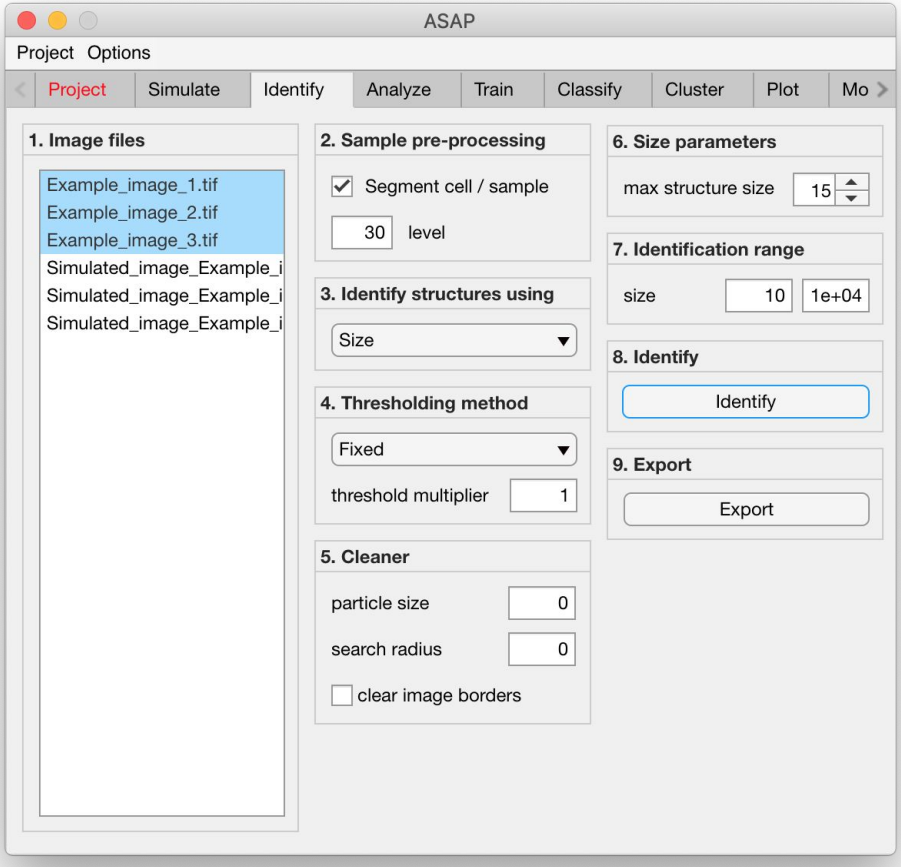
Instructions

The panel titled '**5. Cleaner**' contains 3 optional parameters:

1. **Particle size (px)**: maximum number of post-thresholding pixels to be contained in a structure for it to be discarded.
2. **Search radius (px)**: the maximum radius of a structures for it to be discarded.
3. **Clear image borders**: discards all structures which touch the borders of an image (true / false).

Identification of structures

Definition of filter parameters (1/2)



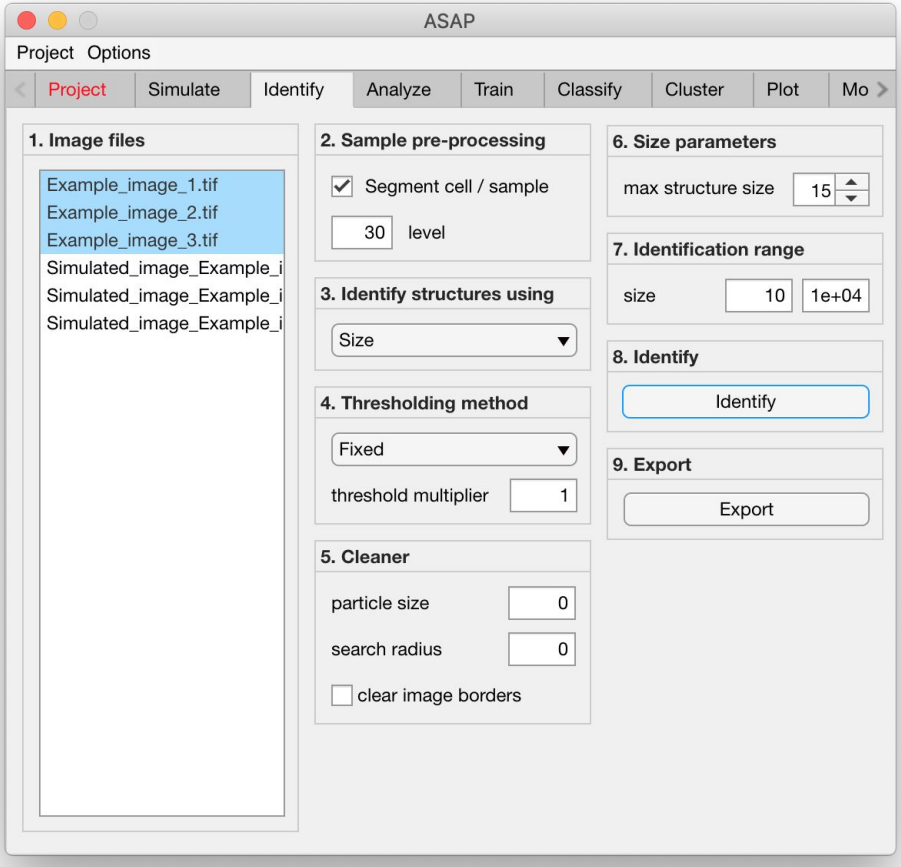
Instructions

The panel titled '**6. 'Size parameters'** contains 1 optional parameter (which is required when identifying structures using size):

1. **max structure size**: the maximum width of the underlying structures in pixels.

Identification of structures

Definition of filter parameters (2/2)

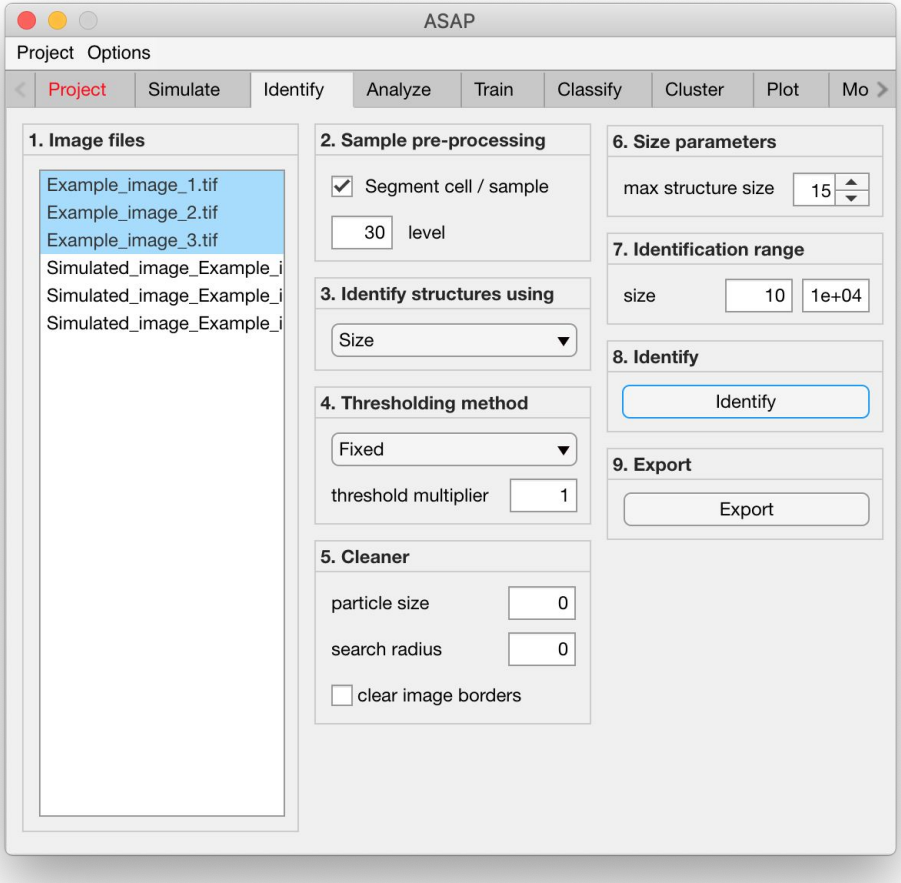


Instructions (contd.)

The panel titled '**7. Identification range**' contains 2 parameters which have to be defined:

1. **Size (px) - *left box***: lower size bound (minimum area) of structures to be filtered. Structures smaller than this value are discarded.
2. **Size (px) - *right box***: upper size bound (maximum area) of structures to be filtered. Structures larger than this value are discarded.

Identification of structures (1/2)



Instructions

Identify structures by pressing the '**Identify**' button in the panel titled '**8. Identify**'.

Please turn over

Identification of structures (2/2)

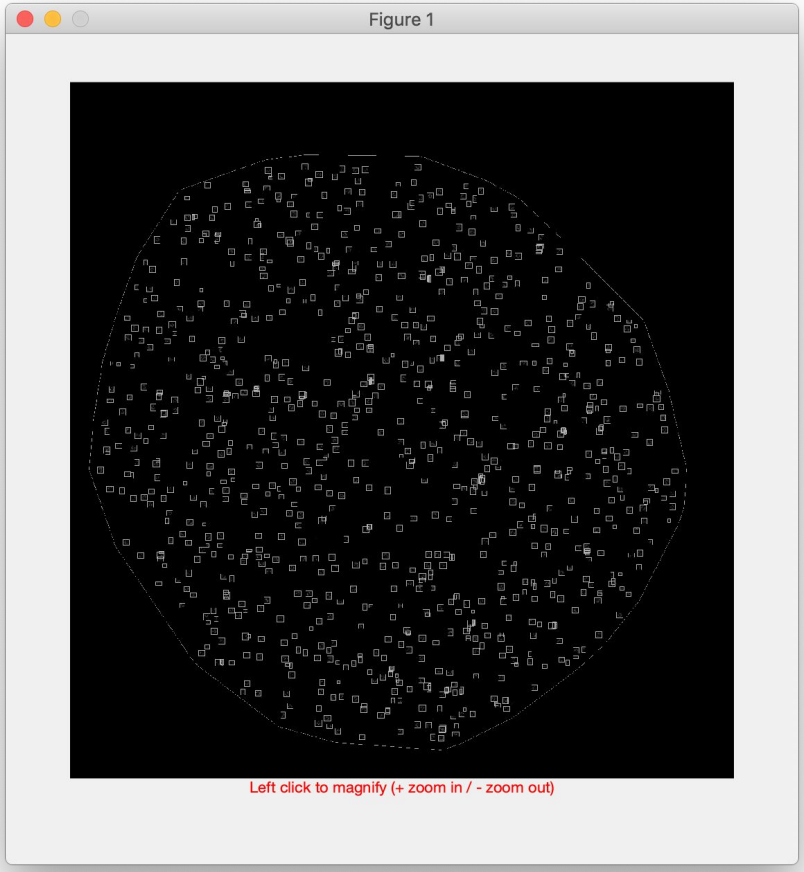
Instructions (contd.)

/ A number of figures will appear equal to the number of images being processed. A figure will similar to the one shown on the right.

/ In the figure(s), the processed image(s) will be shown with the structures identified bound within white rectangles.

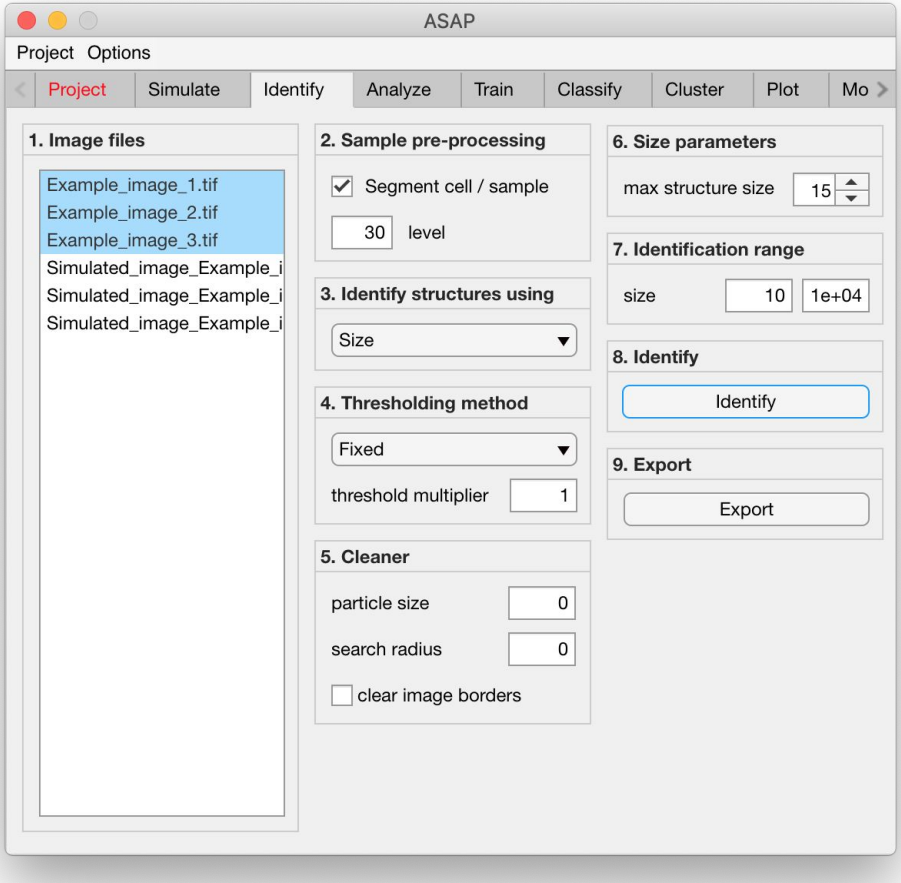
/ To magnify into the image hold the left click of the mouse at any point on the image and press the + or - to zoom in and out respectively.

/By hovering the pointer across the image the user can access the different parts magnified in real time.



Identification of structures

Data export (1/2)



Instructions

Export data by pressing the '**Export**' button in the panel titled '**9. Export**'.

Please turn over

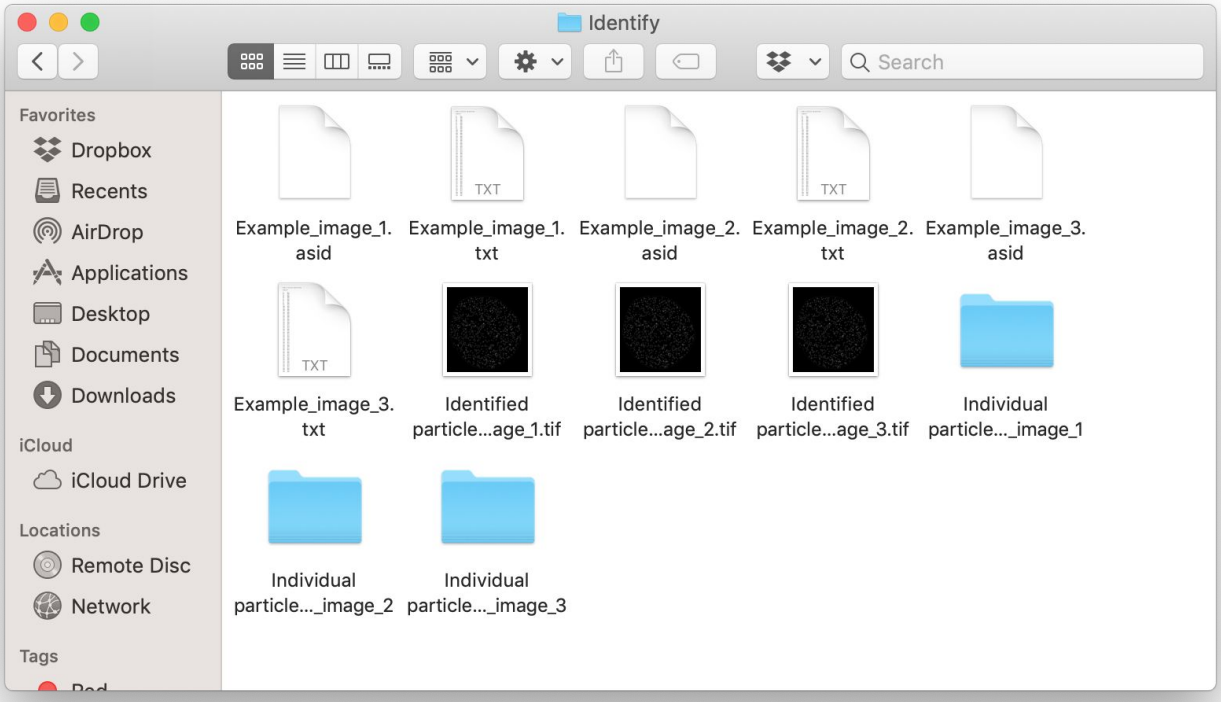
Identification of structures

Data export (2/2)

Instructions (contd.)

A folder named **'Identify'** will be created in the output folder and the following 1 output folder + 3 output files (per processed image) will be placed in the folder:

1. '**Individual particles_xx**' - ***folder***: a folder containing tiff images of all identified structures in an image.
2. **'xx.asid' - *file***: a ASAP file containing processing details used by the program for later analysis.
3. '**Identified particles_xx.tif**' **- *file***: a grayscale tiff image of the processed image with identified particles shown bound within white rectangles.
4. '**xx.txt**' **- *file***: text file containing a summary of the structures identified as well as their coordinates in the original image.

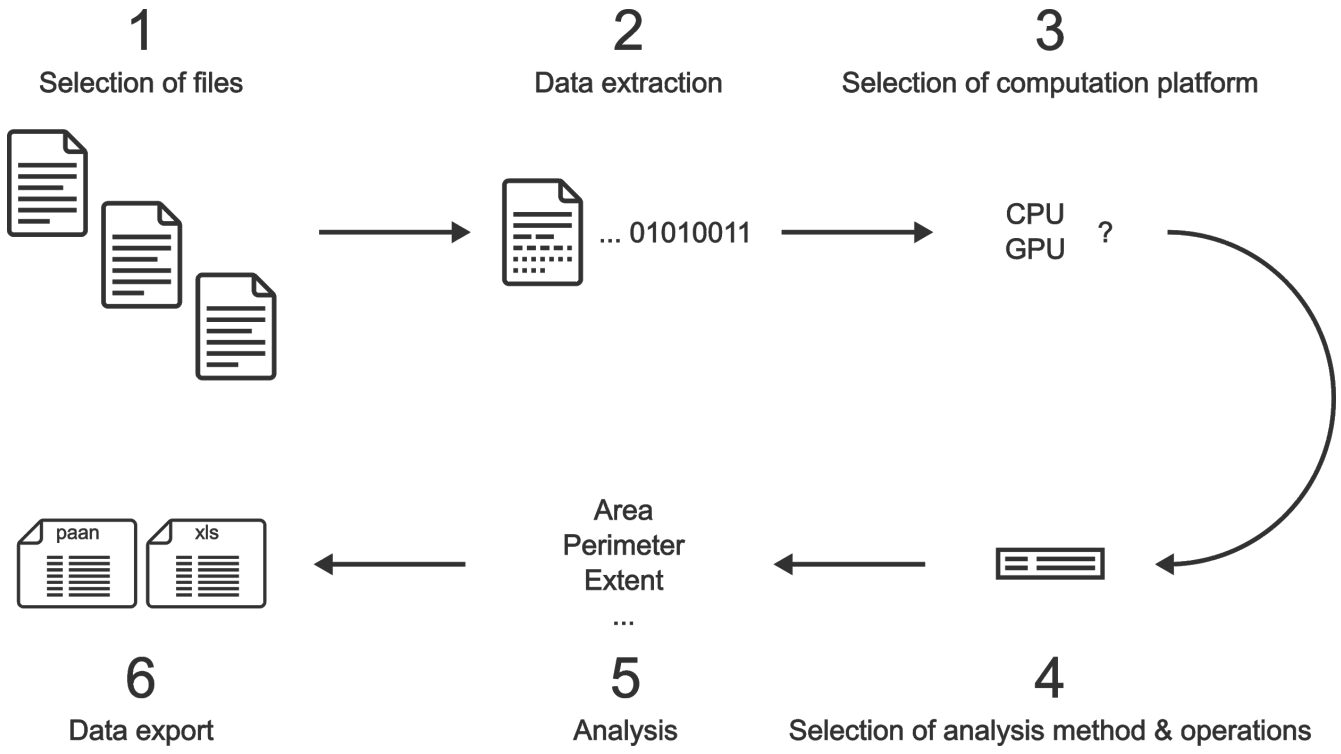


Geometrical analysis of structures

1. Working principle ⇲
2. Selection of files ⇲
3. Data extraction ⇲
4. Definition of pixel size ⇲
5. Selection of computation platform ⇲
6. Selection of analysis method ⇲
7. Selection of image operation(s) ⇲
8. Analysis and revision ⇲
9. Data export ⇲

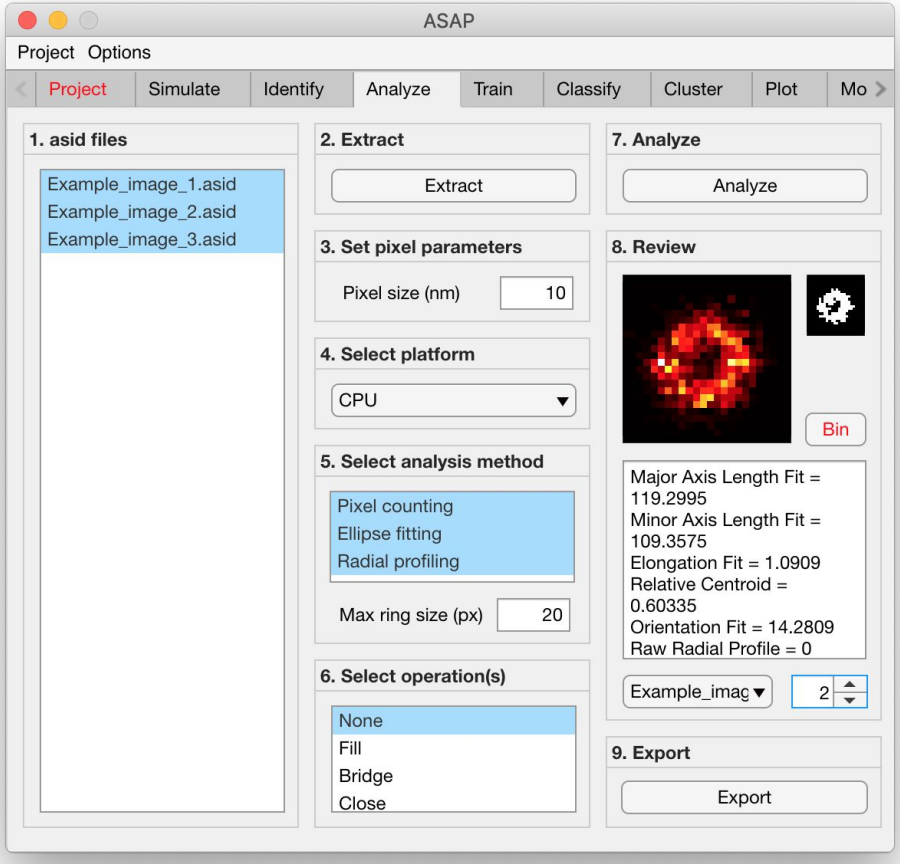
Geometrical analysis of structures

Working principle



Geometrical analysis of structures

Selection of files



Instructions

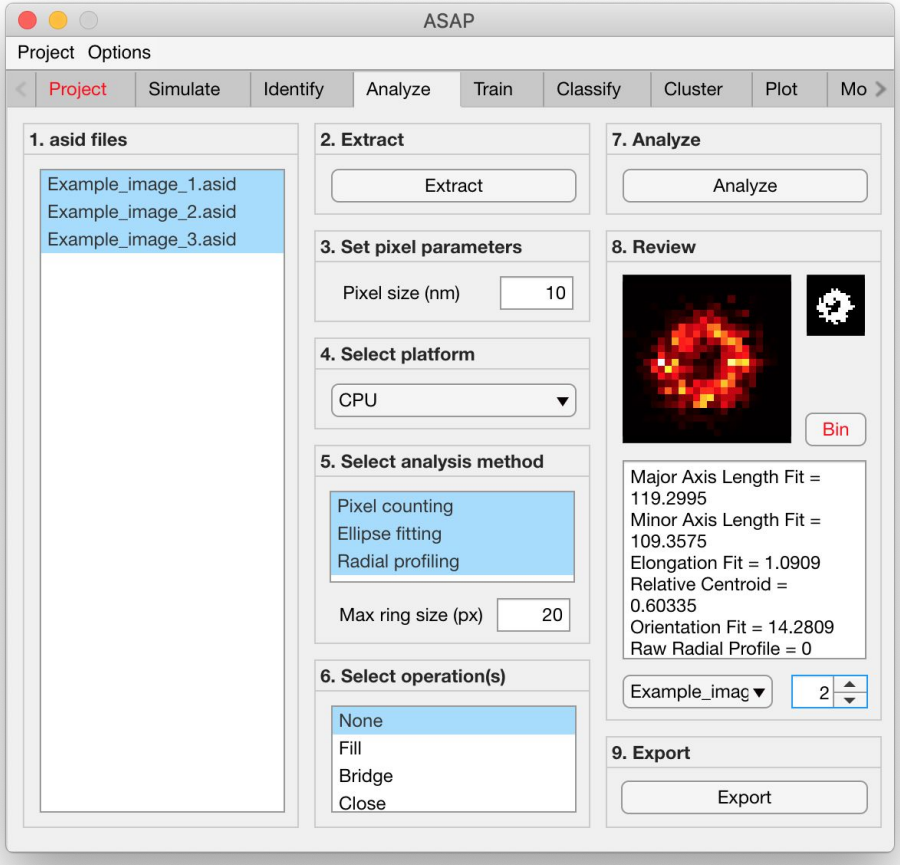
/ Select the 'Analyze' tab from the tabs' bar.

/ The listbox in the panel titled '**1. asid files**' will be populated with the names of asid files located in the input folder previously selected ⇲.

/ Select files by holding the CTRL key and right-clicking the desired files in the listbox in the same panel. Selected files will be highlighted as shown in the figure on the right.

Geometrical analysis of structures

Data extraction

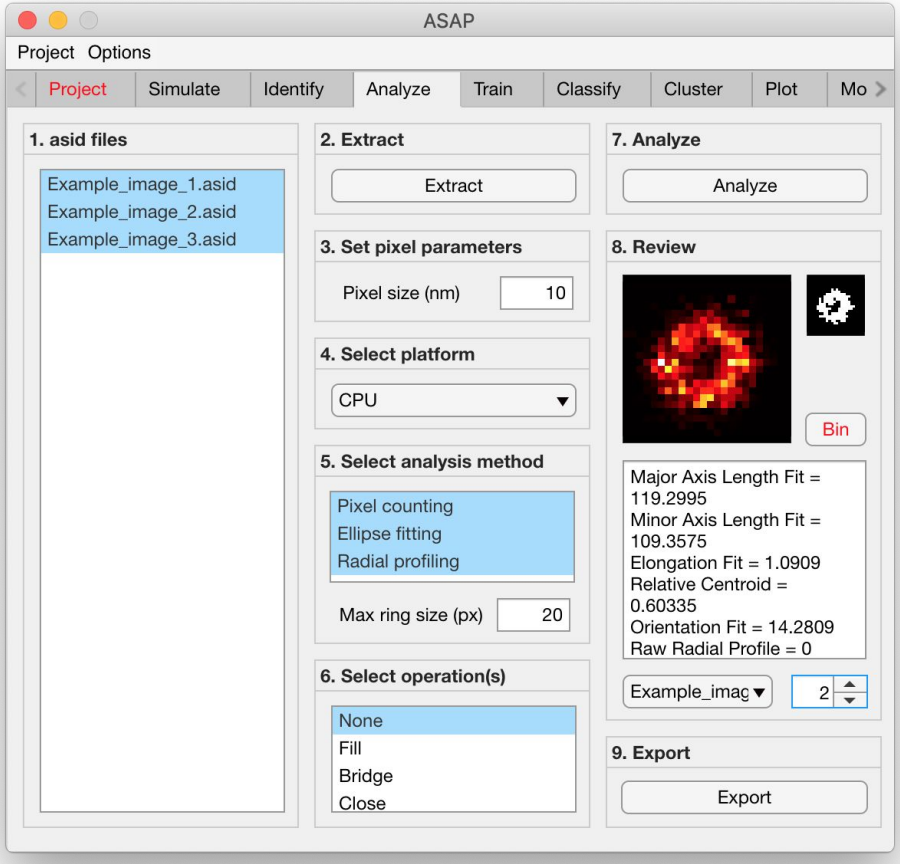


Instructions

Extract data by pressing the '**Extract**' button in the panel titled '**2. Extract**'.

Geometrical analysis of structures

Definition of pixel size



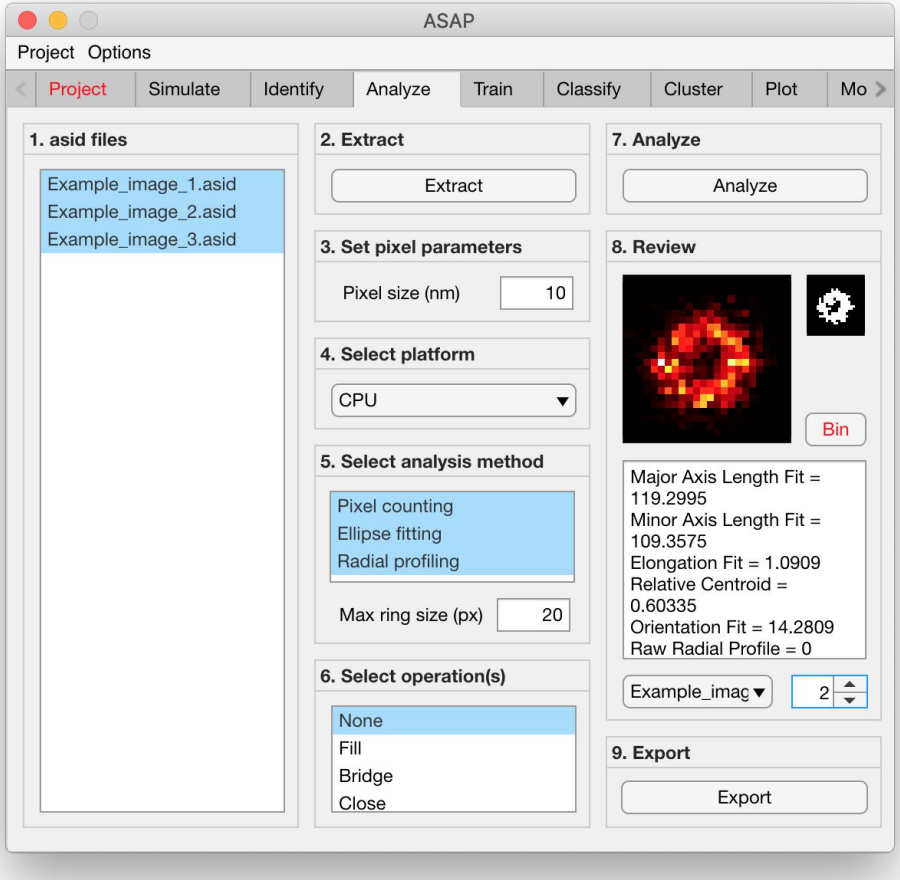
Instructions

The panel titled '**3. Set pixel parameters**' contains 1 parameter which has to be defined:

Pixel size (nm): the size of 1 pixel (in nanometers) depends on several factors, including: the actual size of 1 camera pixel and the magnification factor. A size of 10 nm is typically reported, however, consult your microscopy administrator for a precise value.

Geometrical analysis of structures

Selection of computation platform



Instructions

The panel titled '**4. Select platform**' contains 1 parameter which has to be defined:

CPU / GPU*: if GPU computing was previously enabled, the drop down menu will contain a GPU item which can be selected by the user. Otherwise, the CPU is automatically selected.

Instructions

The panel titled '**5. Select analysis method**' contains 1 parameter which has to be defined:

Pixel counting / Ellipse fitting / Radial profiling: dictates the analysis method(s) to be used. if 'Ellipse fitting' is selected, the identified structures are fitted with an ellipse and the following descriptors are reported:

1. Major axis length
2. Minor axis length
3. Orientation

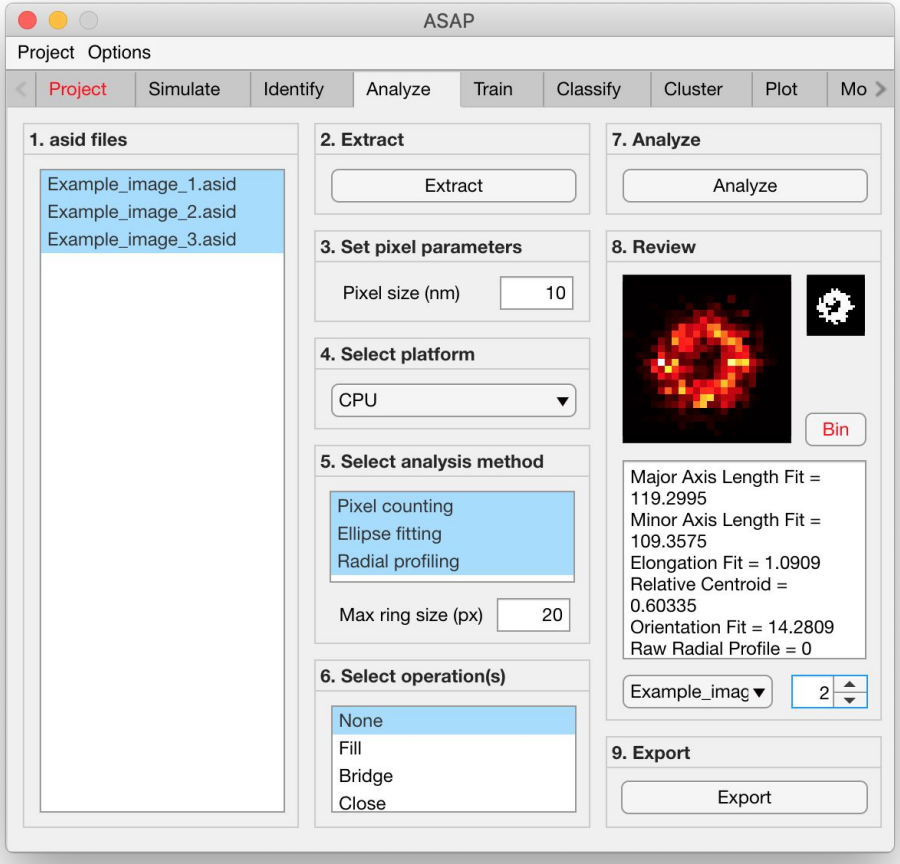
If 'Radial profiling' is selected, the number and intensity of pixels lying within a pixel-sized ring of increasing radii is counted and reported as follows:

1. Raw radial profile
2. Intensity normalized radial profile
3. Area normalized radial profile
4. Raw radial density profile
5. Intensity normalized radial density profile
6. Area normalized radial density profile

Please turn over

Geometrical analysis of structures

Selection of analysis method (1/3)



Guide ↘

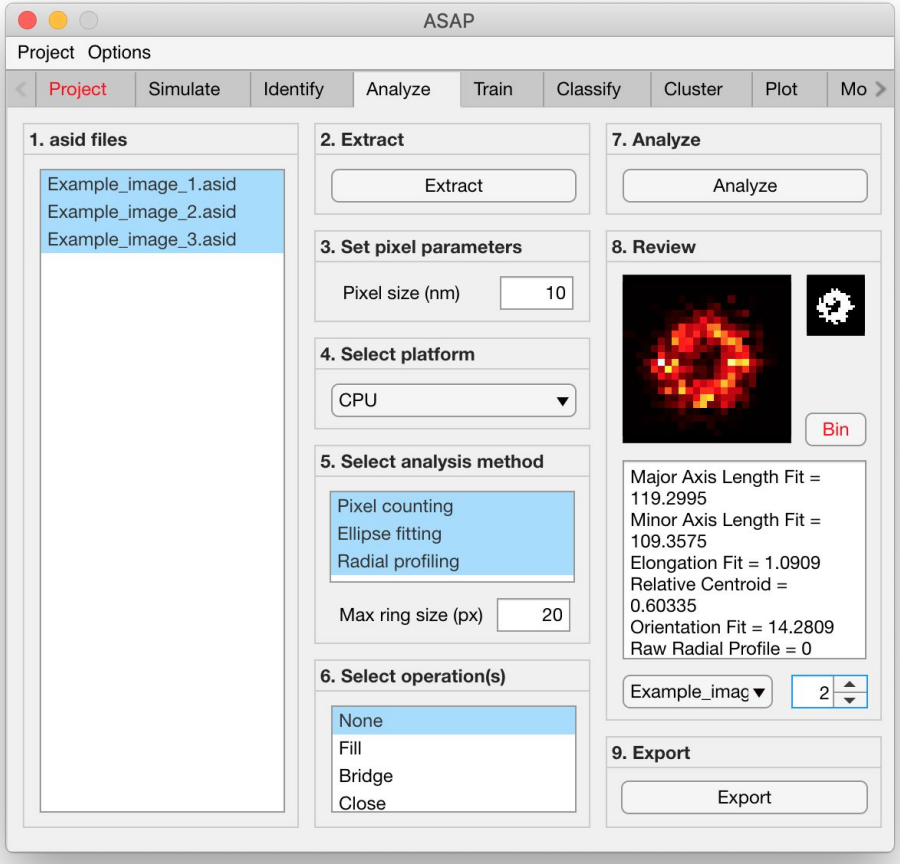
Geometrical analysis of structures

Selection of analysis method (2/3)

Instructions (contd.)

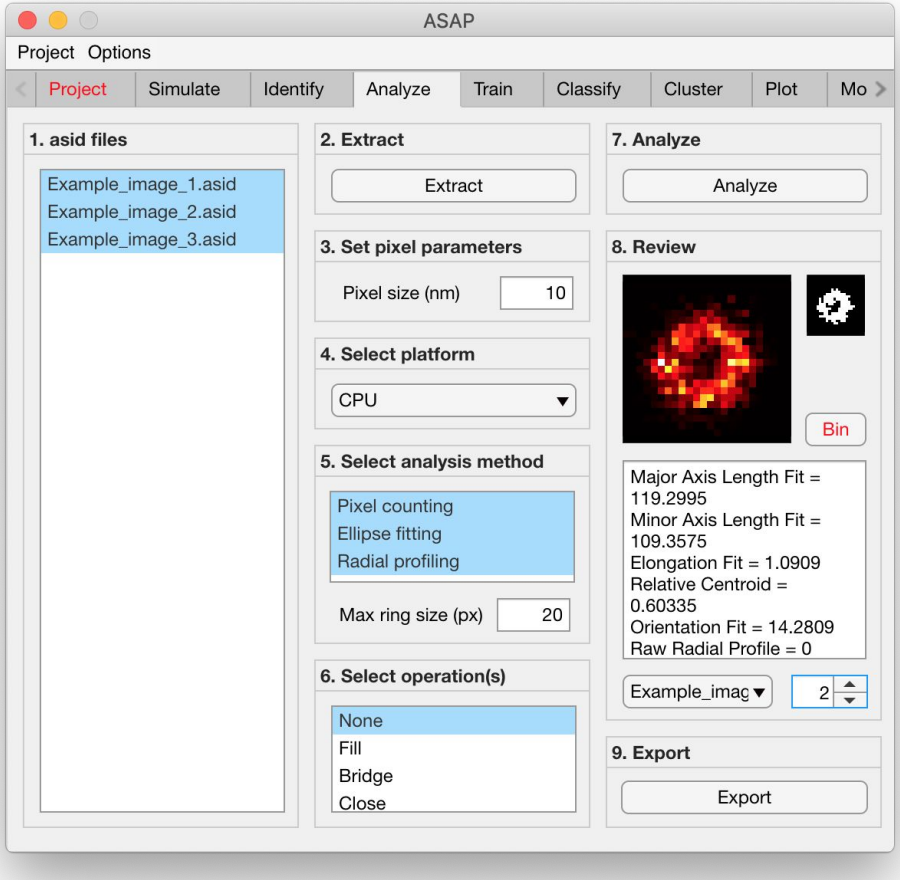
If 'Pixel counting' is selected, the following descriptors are reported:

1. Area
2. Filled area
3. Convex area
4. Perimeter
5. Euler number
6. Eccentricity
7. Solidity
8. Orientation
9. Extent
10. Major axis length
11. Minor axis length
12. Form factor
13. Roundness
14. Elongation
15. Fill ratio
16. Mean intensity
17. Number of minimas
18. Minima intensity
19. Minima eccentricity
20. Minima area
21. Minima convex area
22. Number of maximas
23. Maxima intensity
24. Maxima eccentricity
25. Maxima area
26. Maxima convex area
27. Segment total length
28. Number of intersections



Geometrical analysis of structures

Selection of analysis method (3/3)



Instructions (contd.)

/ The numeric field labeled 'Max Ring Size (px)' will be enabled if 'Radial profile was selected'. A maximum radius of the ring used for radial profiling has to be entered in this field.

§ Note: it is advised that the Max ring size be 5 pixels larger than the average radius of the underlying structures.

Geometrical analysis of structures

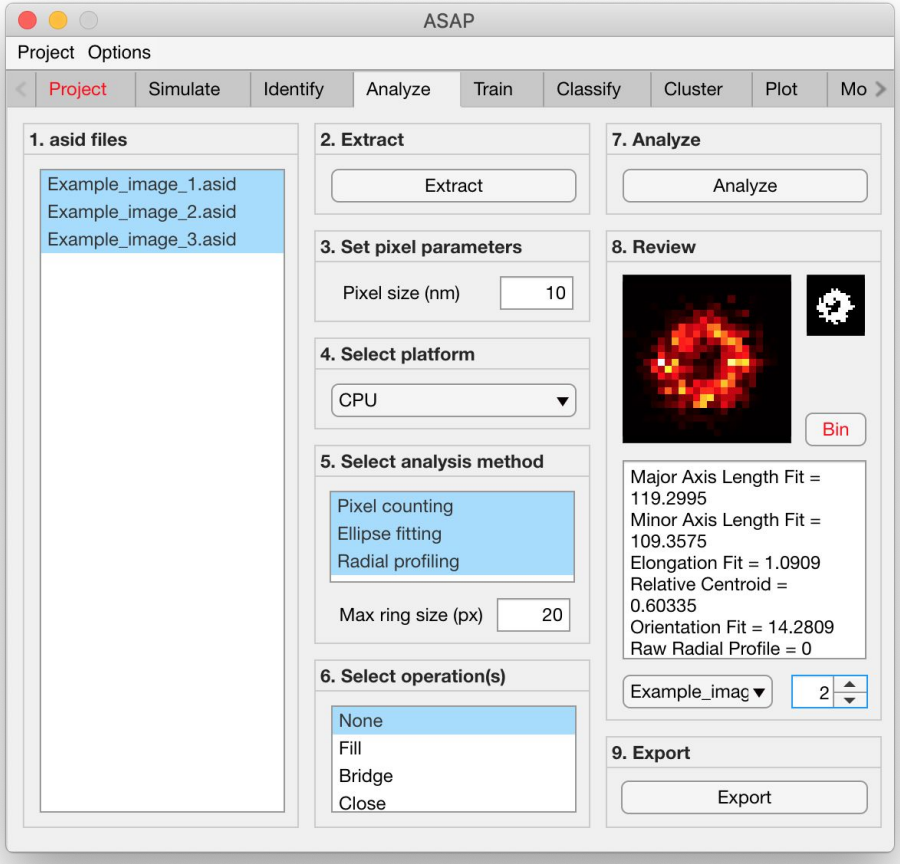
Selection of image operation(s)

Instructions

The panel titled '**6. Select operation(s)**' contains 1 optional parameter:

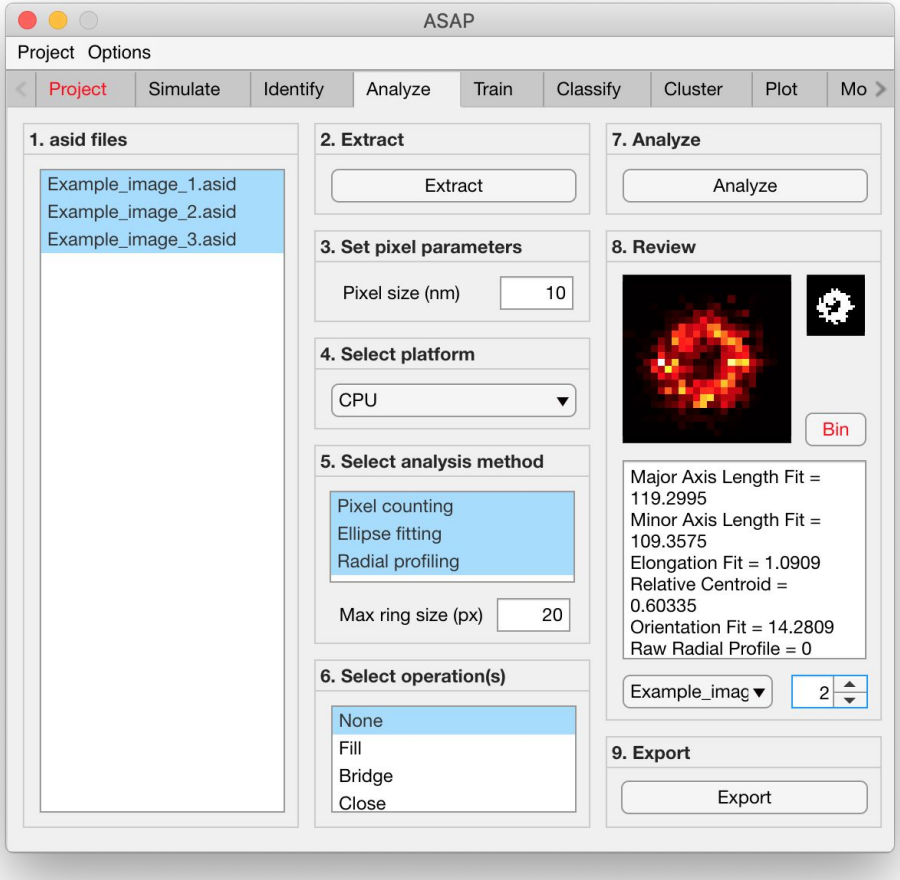
Listbox with multiple selections:

1. **None**: no operation is performed on the identified structures.
2. **Fill**: dark pixels in the thresholded images of the identified structures are filled if surrounded with bright pixels.
3. **Bridge**: bridges unconnected pixels, that is, sets dark pixels to bright if it has 2 bright unconnected pixels as neighbours
4. **Close**: dilates then erodes a thresholded image
5. **Open**: erodes then dilates a threshold image
6. **Clear**: removes isolated pixels (1 bright pixel that is surrounded by dark pixels)
7. **Rotate**: rotates a structure to align its major axis with the x axis.
8. **Center**: centers a structure in the field of view.
9. **Resize**: resizes and pads a structure to fill a 30 x 30 square pixels area.



Geometrical analysis of structures

Analysis and revision (1/2)



Instructions

Analyze data by pressing the '**Analyze**' button in the panel titled '**7. Analyze**'.

Geometrical analysis of structures

Analysis and revision (2/2)

Instructions (contd.)

/ Grayscale images of the identified structures will be displayed in the **large in-app figure** in the panel titled '**8. Review**'.

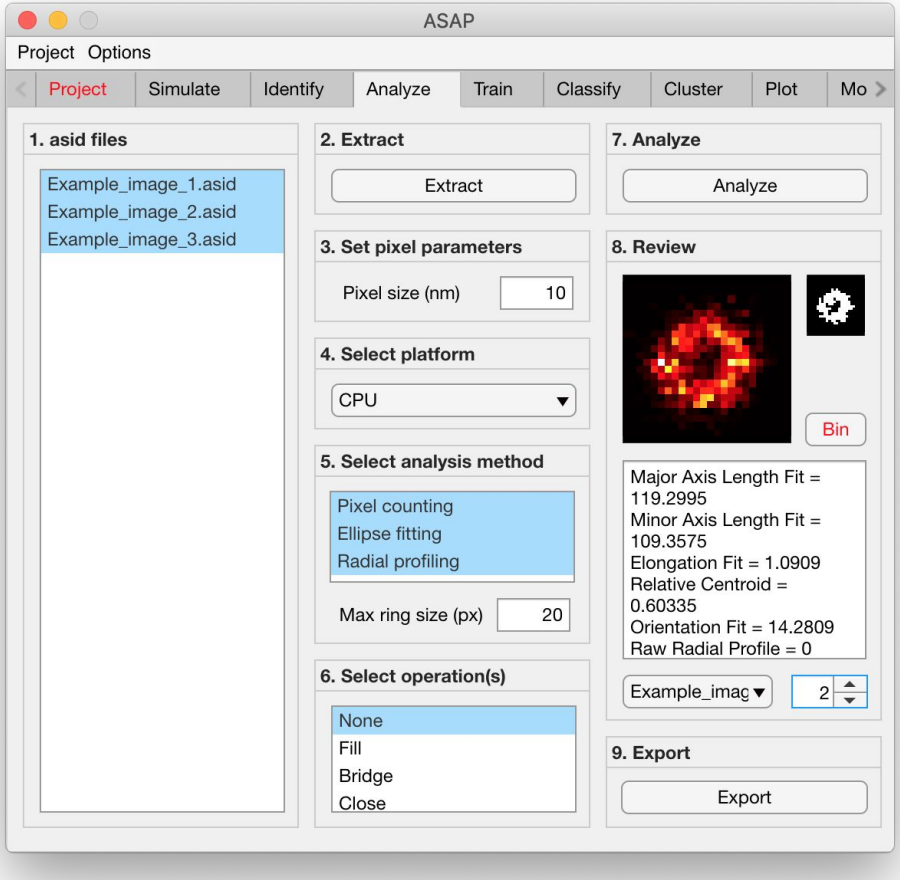
/ Binary images of the identified structures will be displayed in the **small in-app figure** in the same panel.

/ The analyzed parameters will be displayed in the **large text box** in the same panel.

/ The **drop down menu** in same panel can be used to access the analyzed files. Selecting a different file from the drop down menu will result in the text box and large & small in-app figures updating accordingly.

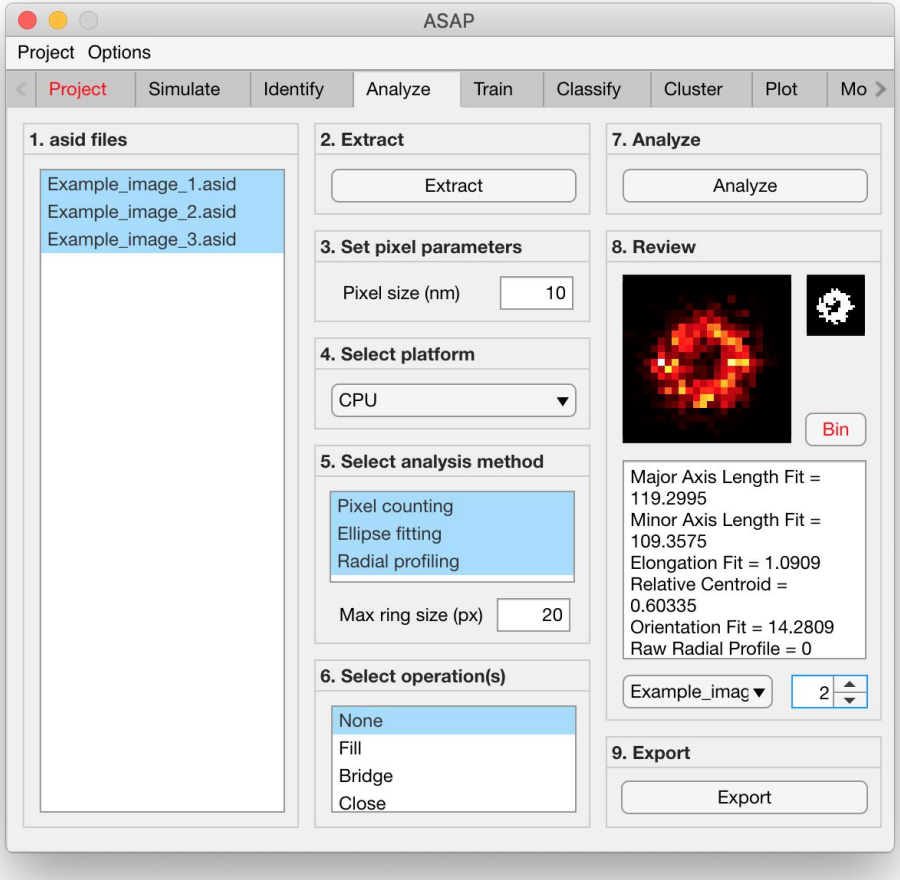
/ The **numeric spinner** in the same panel contains the IDs of the structures belonging to the file selected in the drop down menu. Scrolling the spinner will result in the text box and large & small in-app figures updating accordingly.

/ To bin a structure press the button labeled '**Bin**' in the same panel.



Geometrical analysis of structures

Data export (1/2)



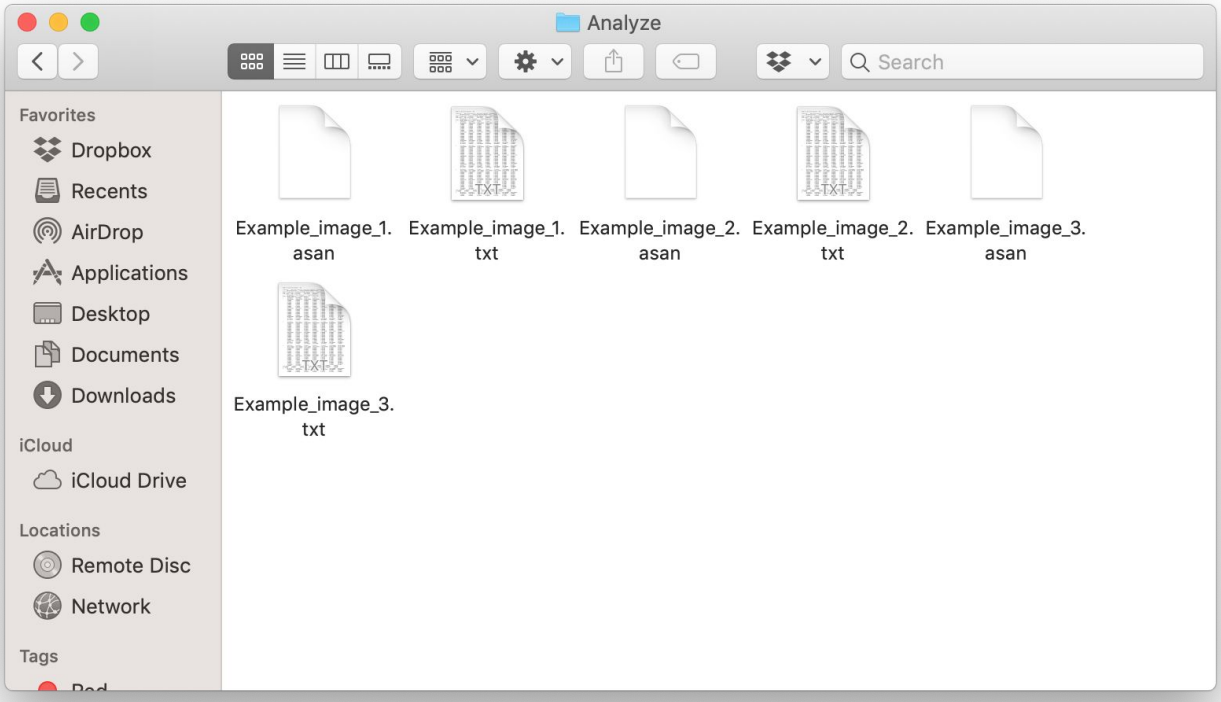
Instructions

Export data by pressing the '**Export**' button in the panel titled '**9. Export**'. The gray light on the right-hand side should lit orange followed by green (for 1 second) when processing is complete then back to gray.

Please turn over

Geometrical analysis of structures

Data export (2/2)



Instructions (contd.)

A folder named **'Analyze'** will be created in the output folder and the following 2 output files (per processed image) will be placed in the folder:

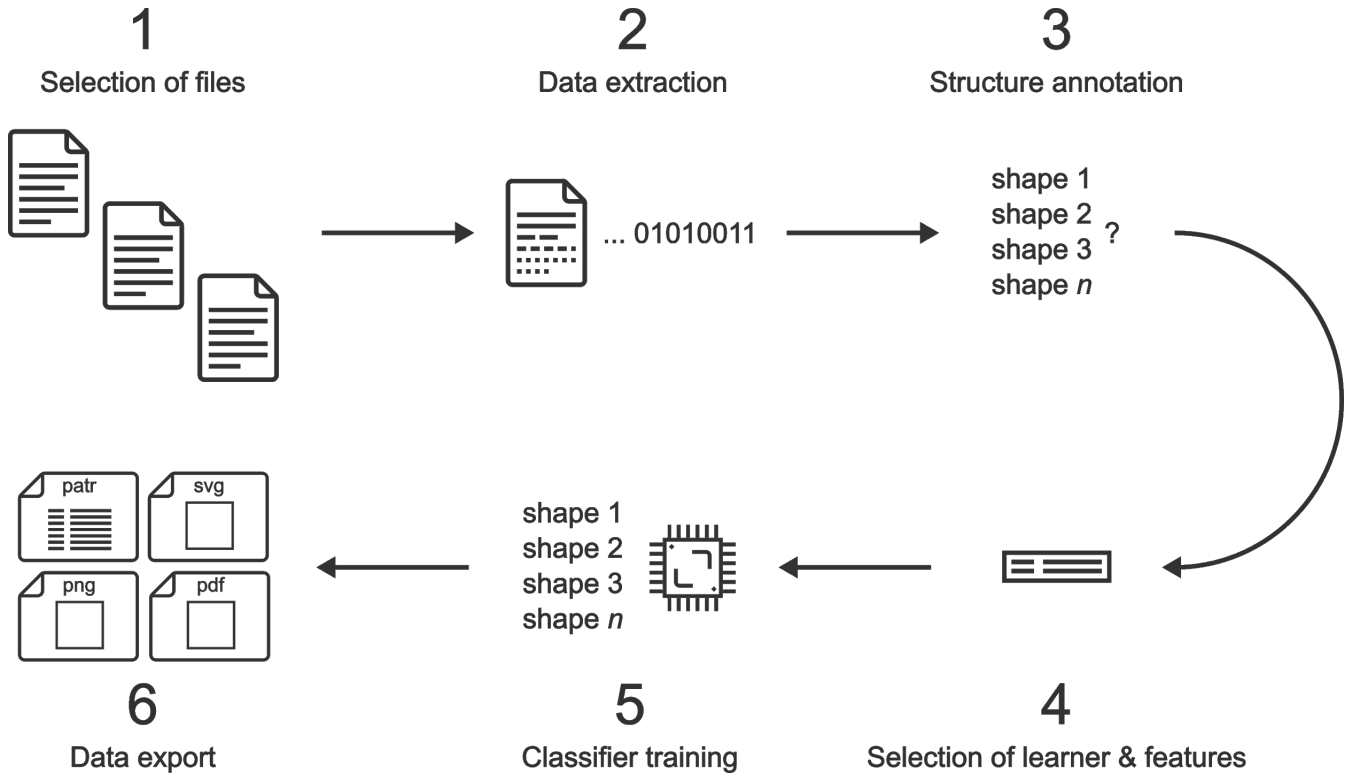
1. '**xx.txt**' - ***file***: text file containing a summary of the structures analyzed as well as their parameters.
2. **'xx.asan' - *file***: a ASAP file containing processing details used by the program for later analysis.

Classifier training

1. Working principle ↘
2. Selection of files ↘
3. Data extraction ↘
4. Structure annotation ↘
5. Selection of learner and features ↘
6. Classifier training ↘
7. Data export ↘

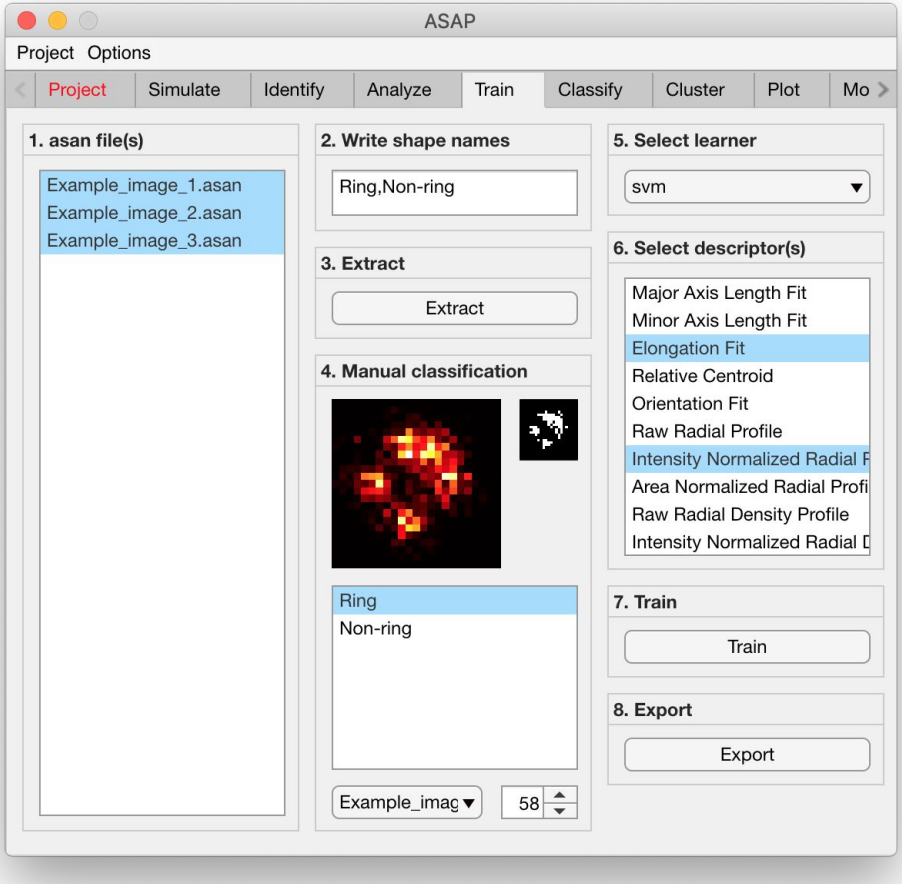
Classifier training

Working principle



Classifier training

Selection of files



Instructions

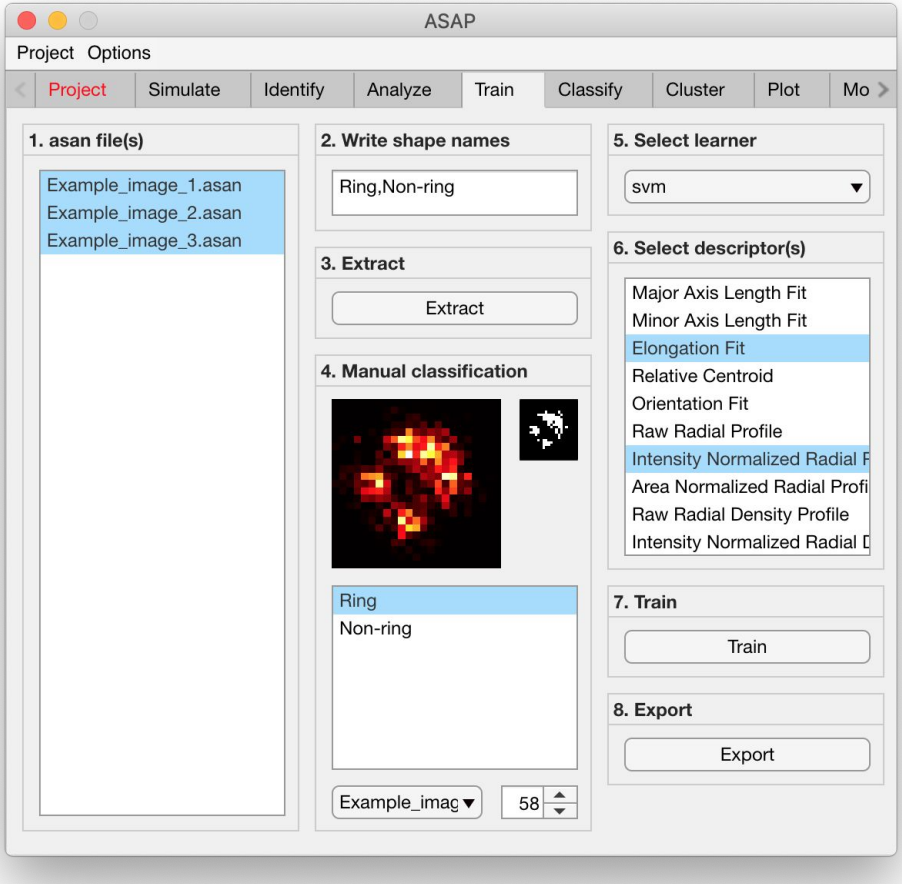
/ Select the 'Train' tab from the tabs' bar.

/ The listbox in the panel titled '**1. asan files**' will be populated with the names of asan files located in the input folder previously selected ↘.

/ Select files by holding the CTRL key and right-clicking the desired files in the listbox in the same panel. Selected files will be highlighted as shown in the figure on the right.

Classifier training

Data extraction



Instructions

/ Write the names of the shapes which you would the software to recognize in the text box in the panel titled '**2. Write shape names**' separated by commas.

/ Extract data by pressing the '**Extract**' button in the panel titled '**3. Extract**'.

Instructions

/ Grayscale images of the identified structures will be displayed in the **large in-app figure** in the panel titled '**4. Manual classification**'.

/ Binary images of the identified structures will be displayed in the **small in-app figure** in the same panel.

/ The names of the shapes will be displayed in the **text box** in the same panel.

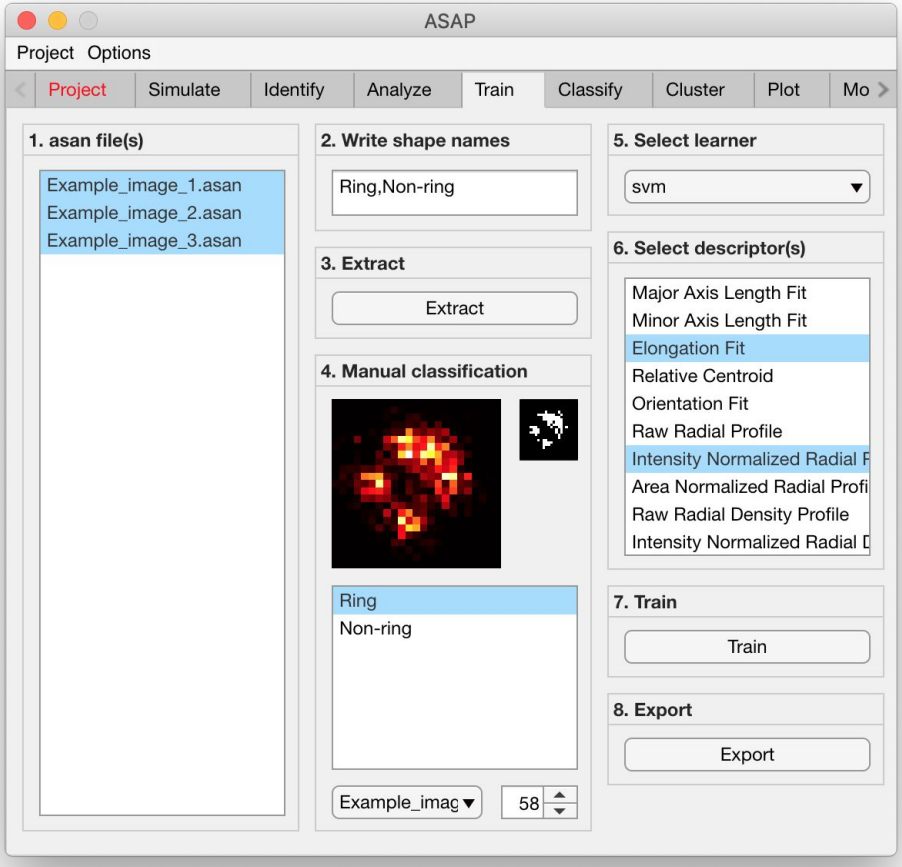
/ The **drop down menu** in the same panel can be used to access the analyzed files. Selecting a different file from the drop down menu will result in the large & small in-app figures updating accordingly.

/ The **numeric spinner** in the same panel contains the IDs of the structures belonging to the file selected in the drop down menu. Scrolling the spinner will result in the large & small in-app figures updating accordingly.

/ Annotate the currently displayed structure by selecting the shape it corresponds to in the listbox. Display next structure by scrolling the spinner and selecting the corresponding shape accordingly.

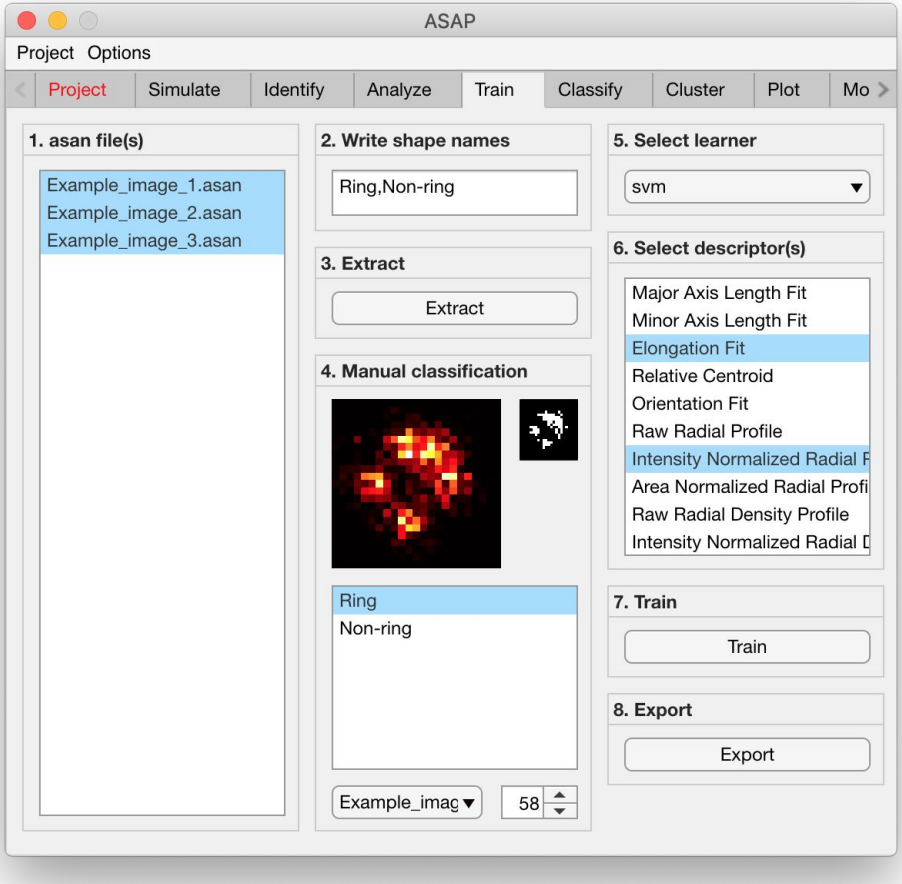
Classifier training

Structure annotation



Classifier training

Selection of learner and features



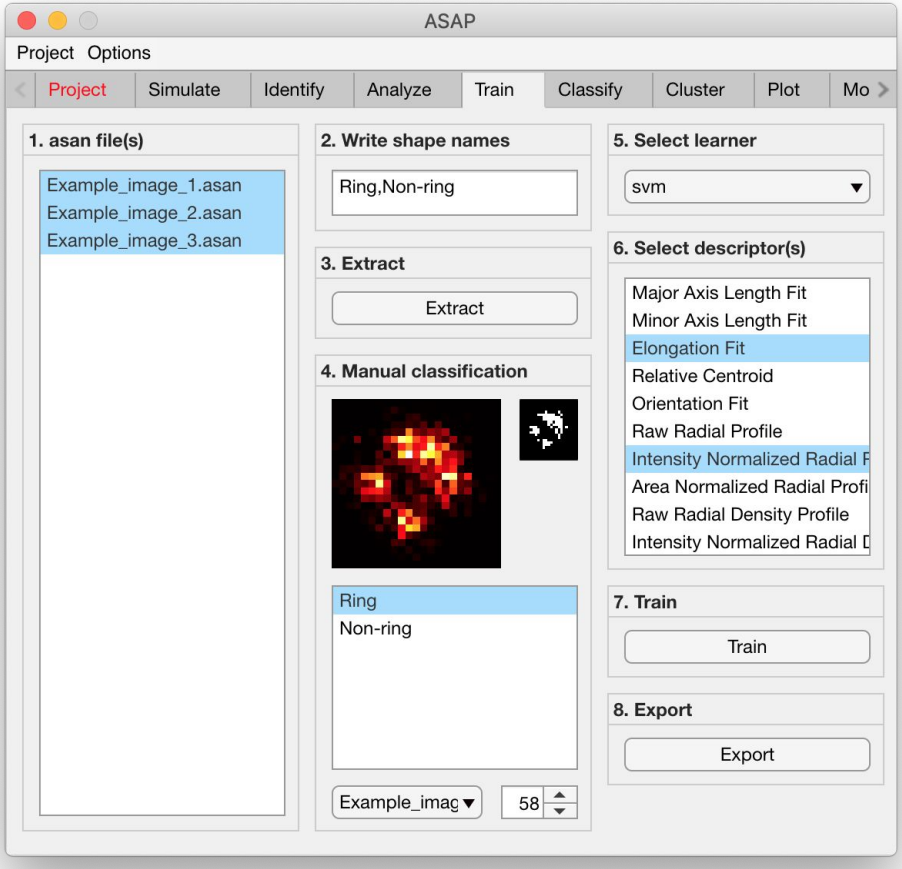
Instructions

/ Select a learner from the **drop down menu** in the located in the panel titled '**5. Select learner**'. More information on the different learners can be found here ↘.

/ Select the most important features that discriminates between the different shapes from the listbox in the panel titled '6. Select descriptor(s)'. We advise to select as few features as possible then expand the set of features for improvement.

Classifier training

Classifier training (1/2)



Instructions

Train the classifier by pressing the '**Train**' button in the panel titled '**7. Train**'.

Please turn over

Instructions (contd.)

/ A figure similar to the one shown on the right will be displayed.

/ The shown matrix is known as the *confusion matrix*. The confusion matrix visually represents the proportion of structures belonging to a certain group of shapes is being classified as to belonging to the same group or groups.

/ A diagonal value (i.e. one that is shared between the same annotated group and the classified group) should be the highest amongst all values in the intersecting row and column.

/ The overall accuracy of the trained classifier can be assessed by comparing the accuracy value shown on top of the matrix with the desired accuracy.

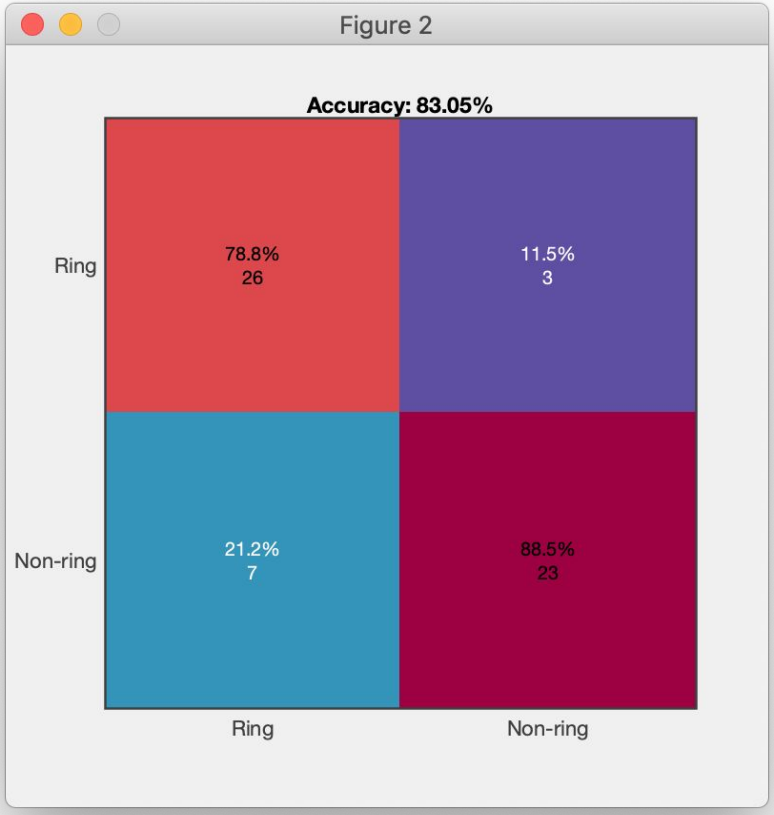
/ If the displayed accuracy is lower than expected, the user can:

1. Increase the number of annotated structures ⇲.
2. Select another learner ⇲.
3. Modify or expand the set of features used for discrimination ⇲.

and, re-run the classifier.

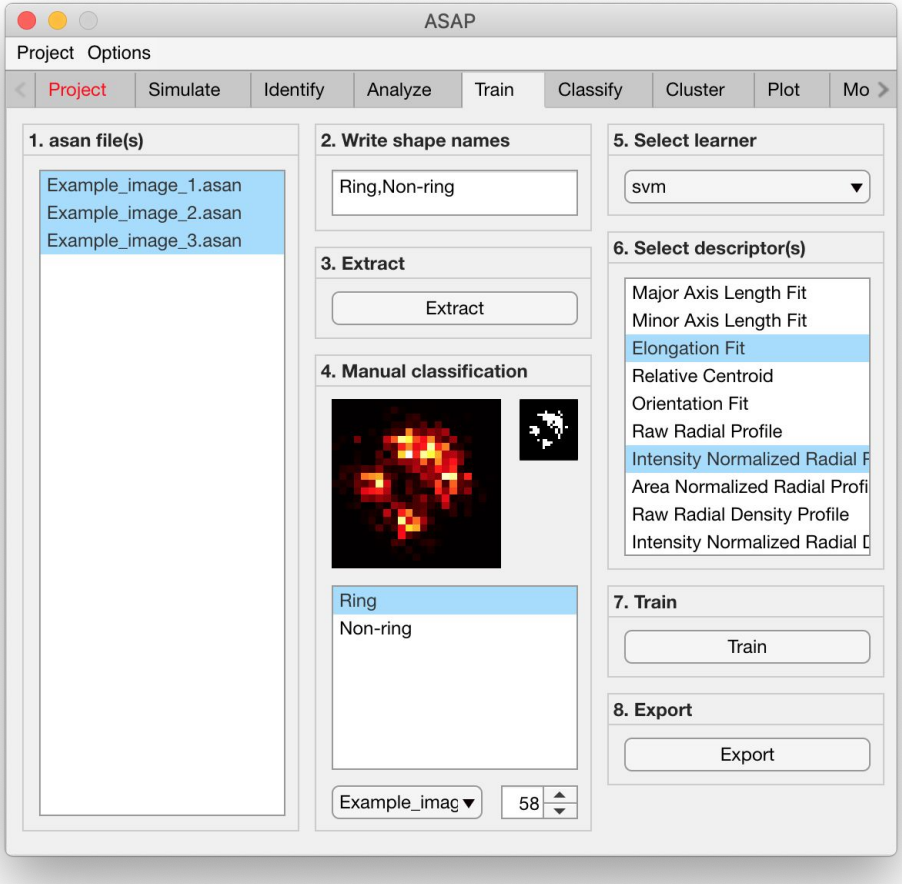
Classifier training

Classifier training (2/2)



Classifier training

Data export (1/2)



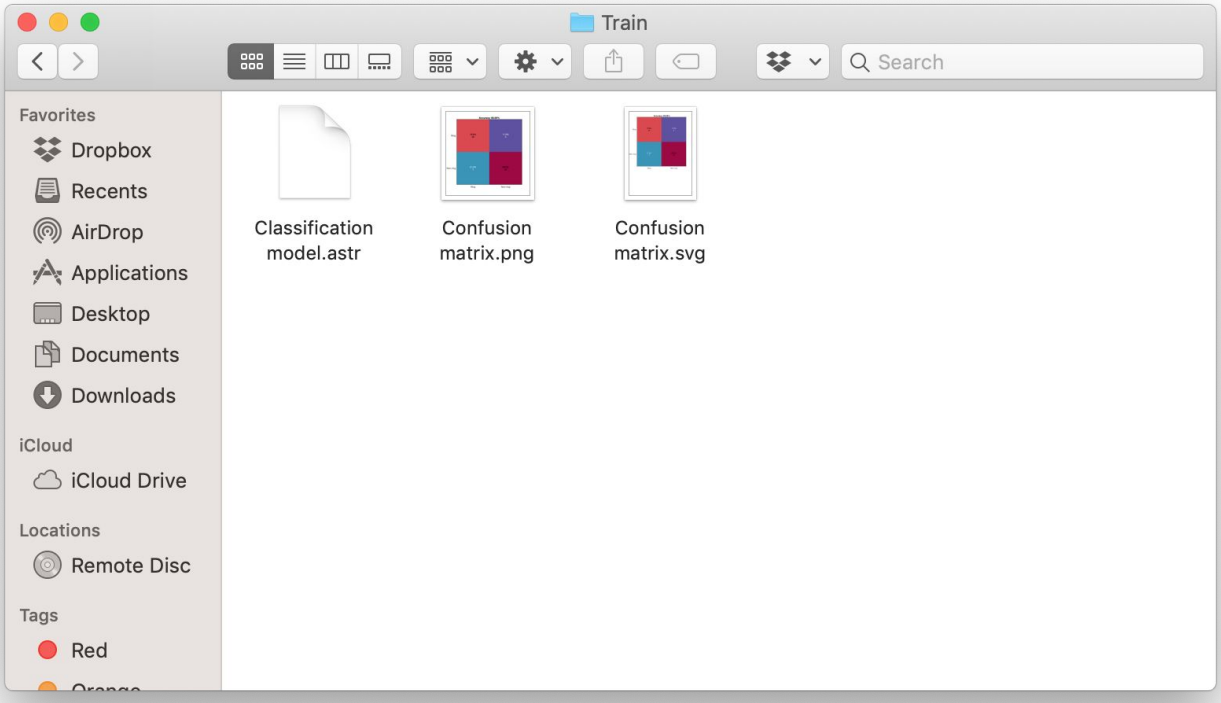
Instructions

Export data by pressing the '**Export**' button in the panel titled '**8. Export**'.

Please turn over

Classifier training

Data export (2/2)



Instructions (contd.)

A folder named **'Train'** will be created in the output folder and the following 3 output files will be placed in the folder:

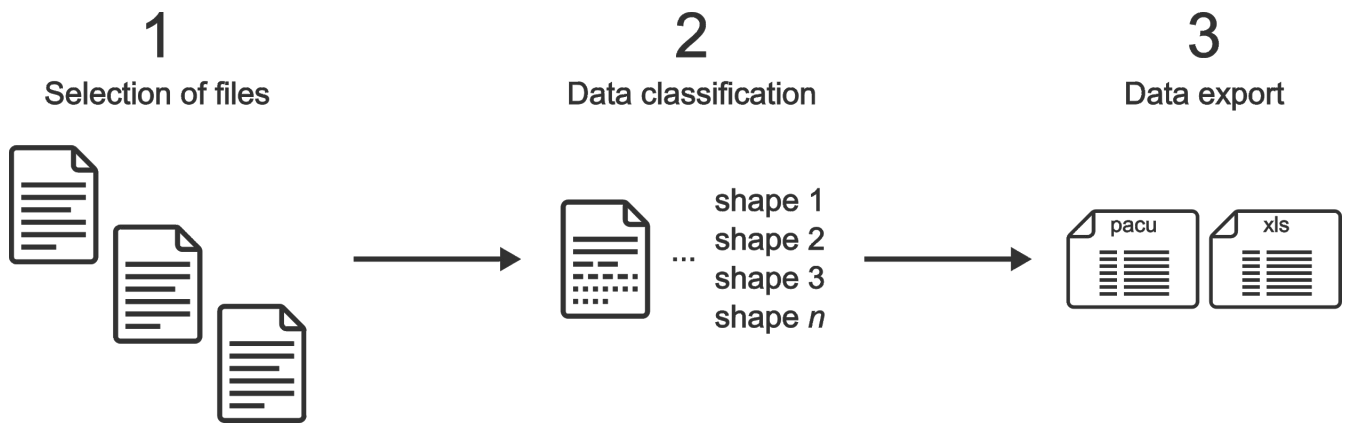
1. '**Confusion matrix.png**' - ***file***: png file of the confusion matrix.
2. '**Confusion matrix.svg**' - ***file***: svg file of the confusion matrix.
3. **'Confusion matrix.astr' - *file***: a ASAP file containing the classifier details used by the program for later analysis.

Classification of structures

1. Working principle ↘
2. Selection of files ↘
3. Data extraction ↘
4. Structure reassignment ↘
5. Structure classification and revision ↘
6. Data export ↘

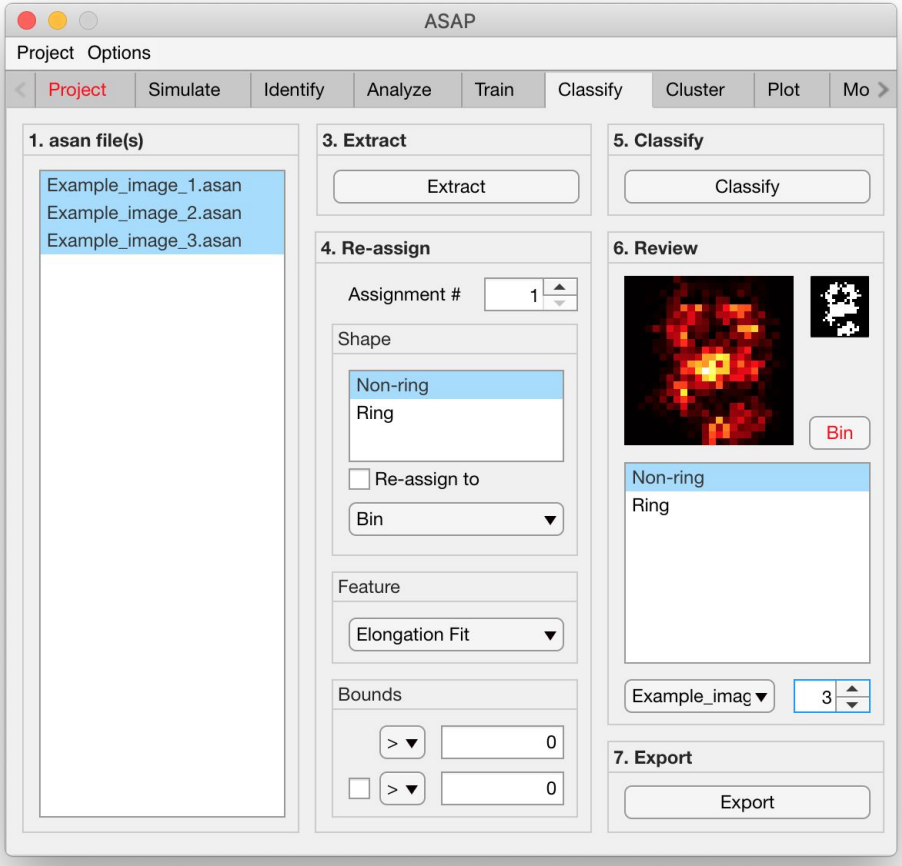
Classification of structures

Working principle



Classification of structures

Selection of files



Instructions

/ Select the 'Classify' tab from the tabs' bar.

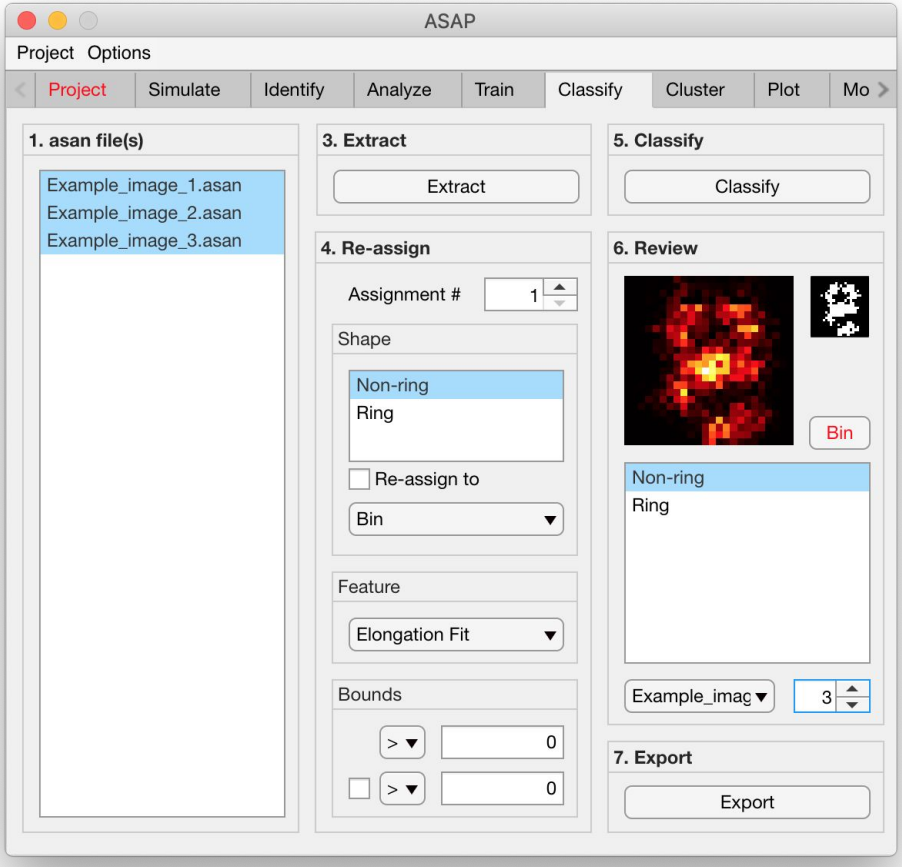
/ The listbox in the panel titled '**1. asan files**' will be populated with the names of asan files located in the input folder previously selected ↘.

/ Select files by holding the CTRL key and right-clicking the desired files in the listbox in the same panel. Selected files will be highlighted as shown in the figure on the right.

§ Note1:
A classification model .astr file located in the project folder will be automatically read..

Classification of structures

Data extraction



Instructions

Extract data by pressing the '**Extract**' button in the panel titled '**3. Extract**'.

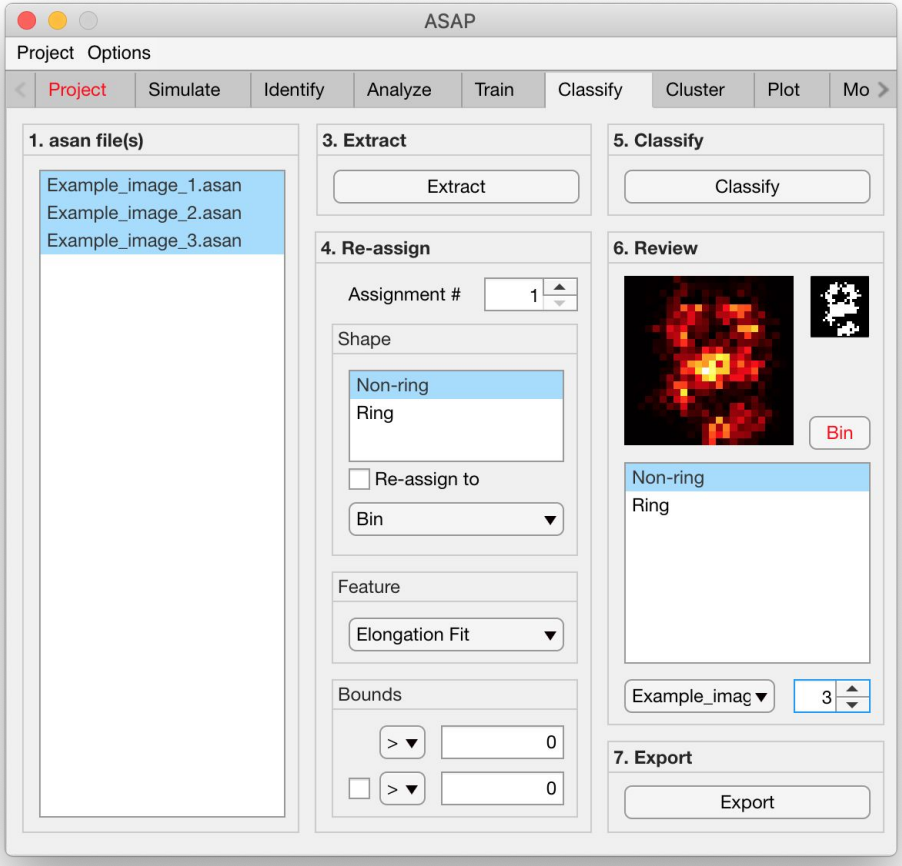
Instructions

To re-assign shapes based on hard limits imposed on the extracted features:

1. Select an assignment number by changing the spinner value labeled ‘Assignment #’.
2. Select 1 or more shape(s) from the listbox located in the subpanel titled ‘Shape’ to be assigned from.
3. Check the checkbox labeled ‘Re-assign to’ to activate the current assignment.
4. Select a shape, or bin, from the dropdown menu located in the subpanel titled ‘Shape’ to be assign to.
5. Select a feature from the dropdown menu located in the subpanel titled ‘Feature’.
6. Select bounds for the selected feature. Within those bounds a structure is re-assigned, otherwise not.
7. At least 1 bound has to be assigned, using an equality and number in the subpanel titled Bounds. To assign a second bound, check the checkbox in the same panel and assign the parameters accordingly.

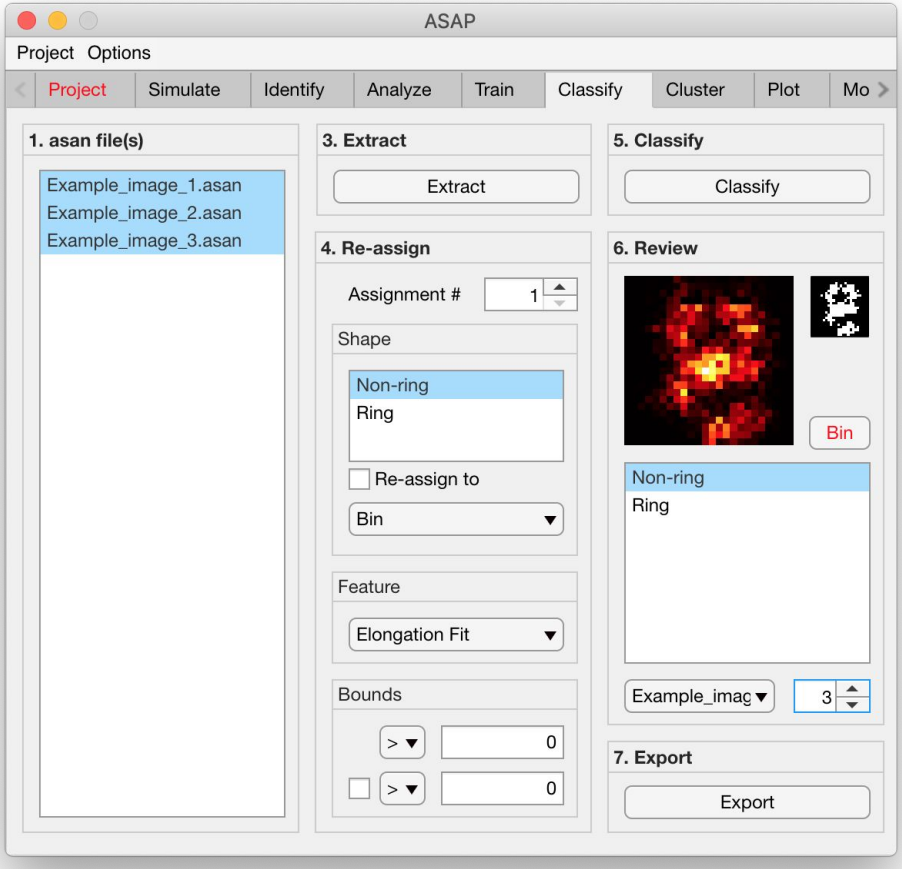
Classification of structures

Structure reassignment



Classification of structures

Structure classification and revision (1/2)



Instructions

Classify all structures by pressing the '**Classify**' button in the panel titled '**5. Classify**'.

Please turn over

Instructions (contd.)

/ Grayscale images of the classified structures will be displayed in the **large in-app figure** in the panel titled '**6. Review**'.

/ Binary images of the classified structures will be displayed in the **small in-app figure** in the same panel.

/ The names of the shapes will be displayed in the **text box** in the same panel.

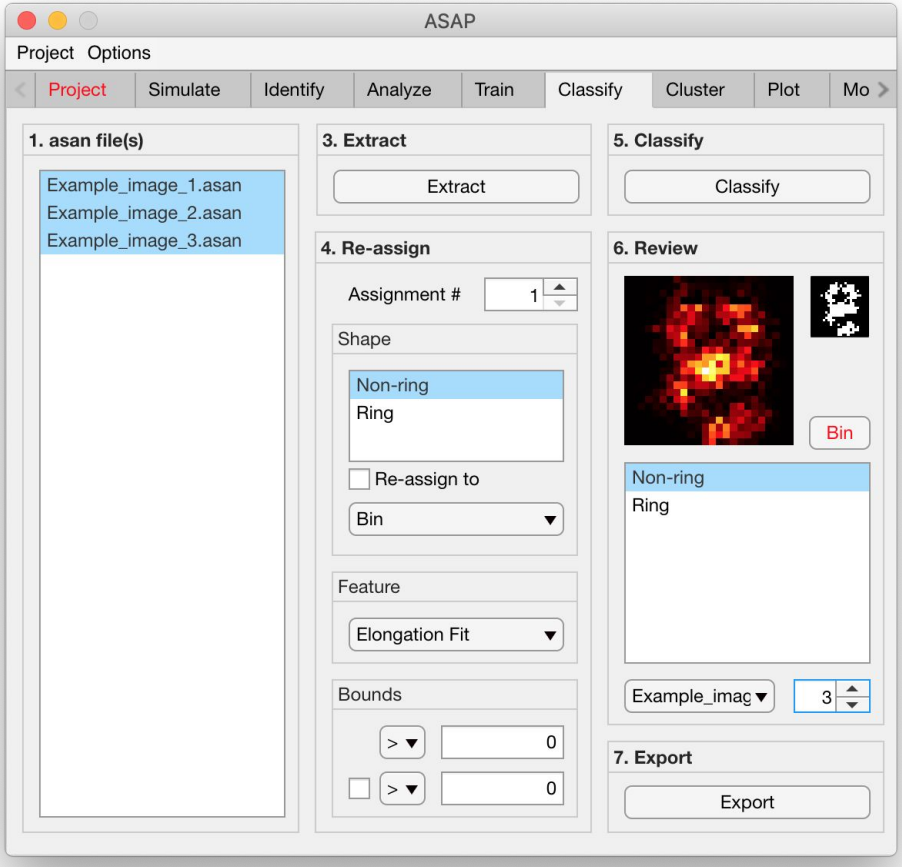
/ The **drop down menu** in the same panel can be used to access the classified files. Selecting a different file from the drop down menu will result in the large & small in-app figures updating accordingly.

/ The **numeric spinner** in the same panel contains the IDs of the structures belonging to the file selected in the drop down menu. Scrolling the spinner will result in the large & small in-app figures updating accordingly.

/ Review the currently displayed structure by selecting the shape it corresponds to in the listbox if the highlighted shape is incorrect. Display next structure by scrolling the spinner and repeating process.

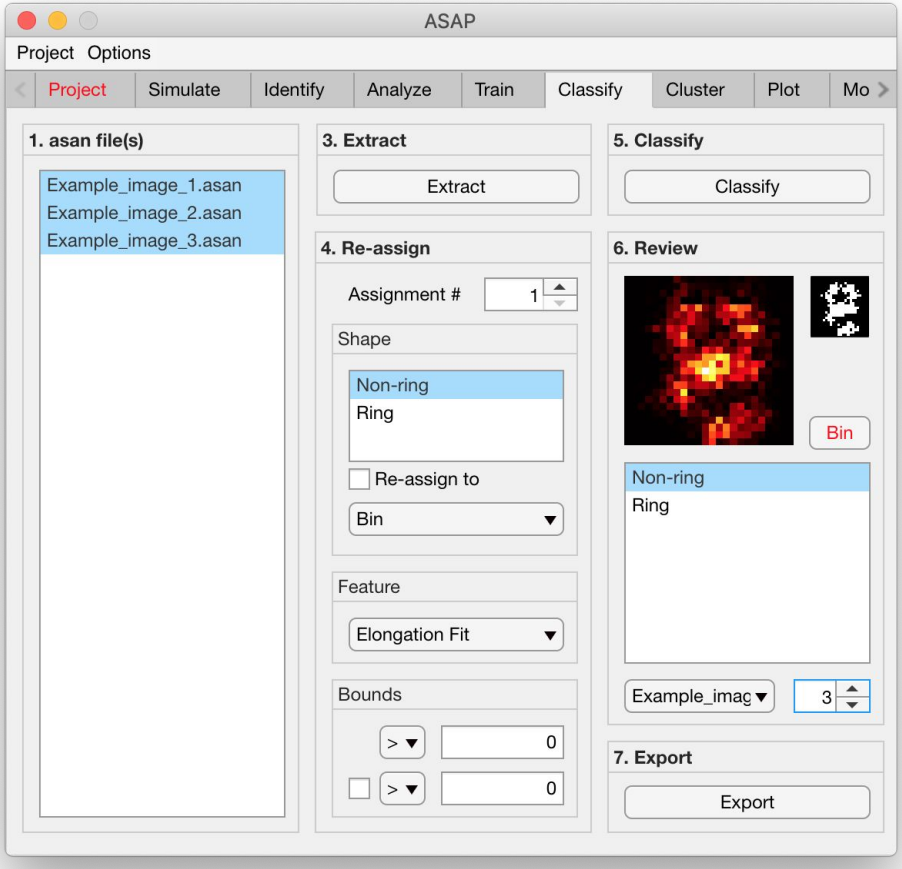
Classification of structures

Structure classification and revision (2/2)



Classification of structures

Data export (1/2)



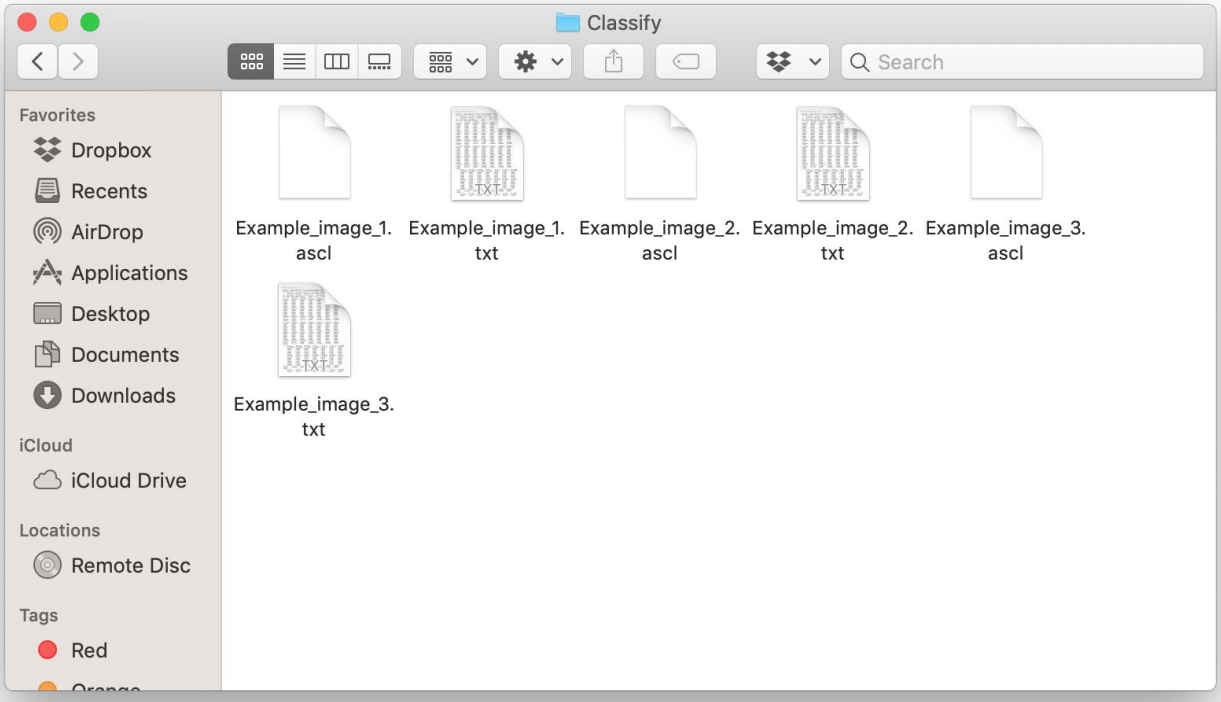
Instructions

Export data by pressing the '**Export**' button in the panel titled '**7. Export**'.

Please turn over

Classification of structures

Data export (2/2)



Instructions (contd.)

A folder named **'Classify'** will be created in the output folder and the following 2 output files (per processed image) will be placed in the folder:

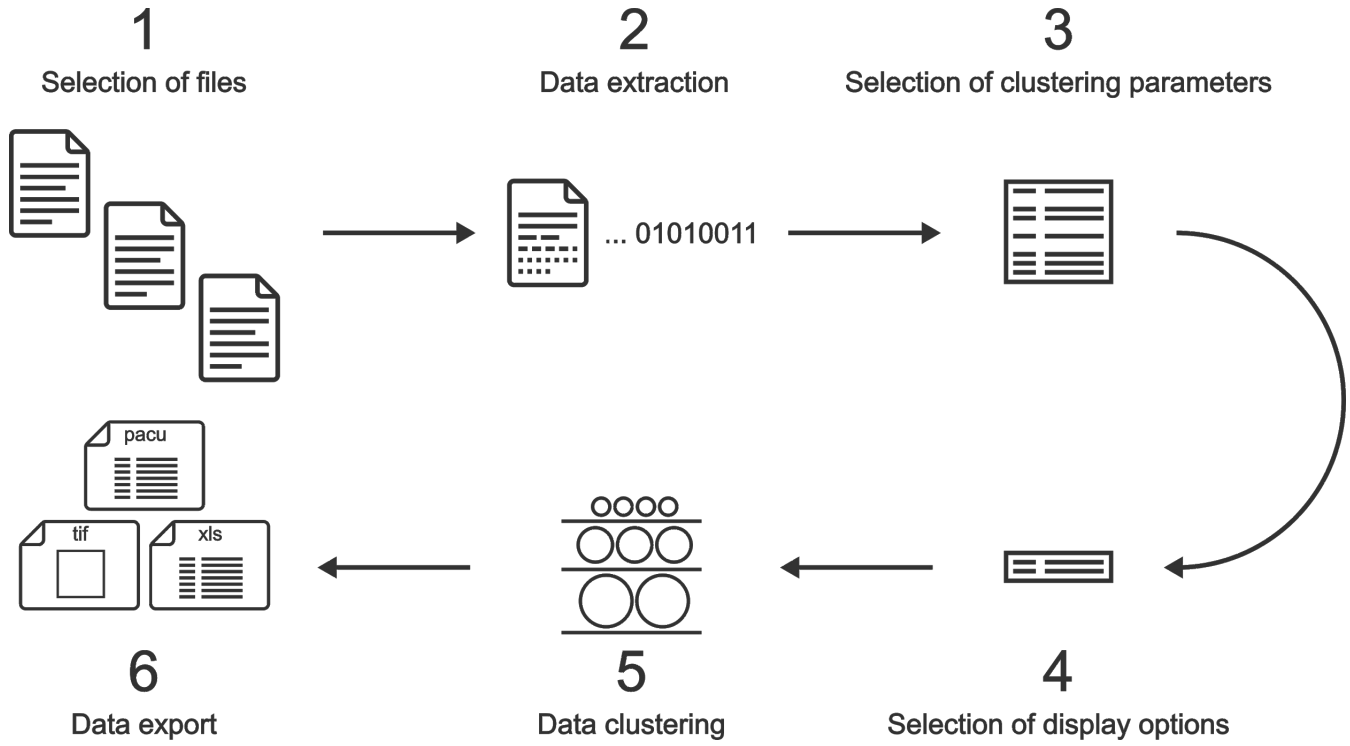
1. '**xx.txt**' - ***file***: text file containing a summary of the structures classified as well as their parameters.
2. **'xx.ascl' - *file***: a ASAP file containing classification details used by the program for later analysis.

Cluster analysis

1. Working principle ⇲
2. Selection of files ⇲
3. Data extraction ⇲
4. Selection of clustering parameters ⇲
5. Data clustering ⇲
6. Data export ⇲

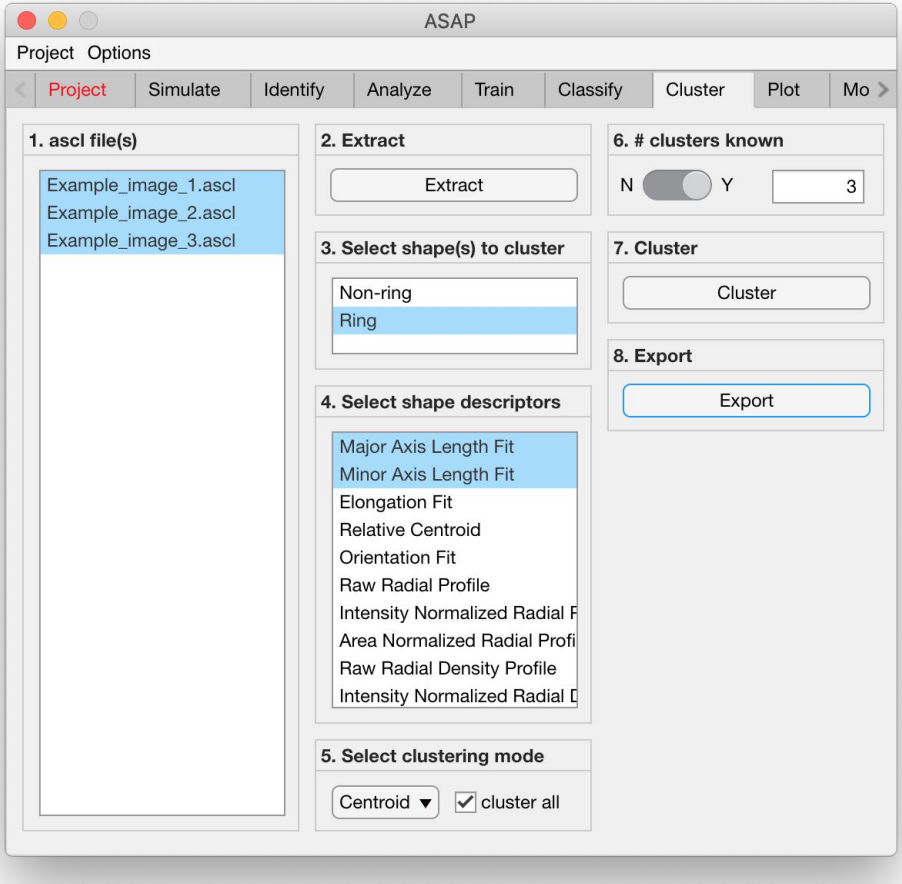
Cluster analysis

Working principle



Cluster analysis

Selection of files



Instructions

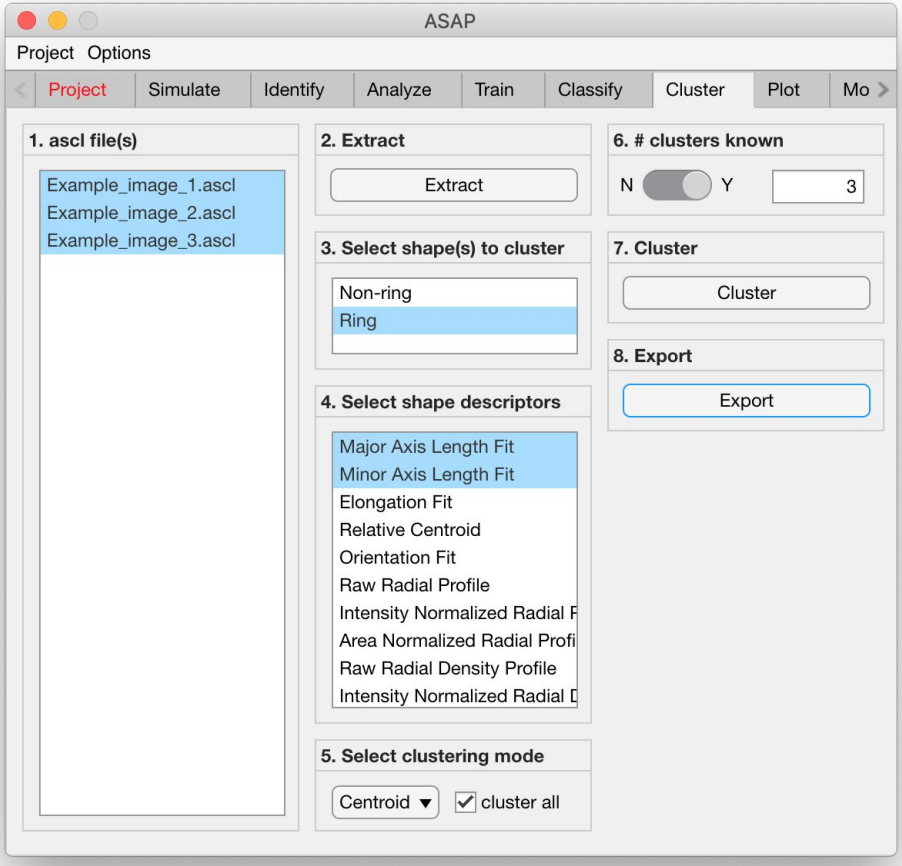
/ Select the 'Cluster' tab from the tabs' bar.

/ The listbox in the panel titled '**1. ascl files**' will be populated with the names of ascl files located in the input folder previously selected ↘.

/ Select files by holding the CTRL key and right-clicking the desired files in the listbox in the same panel. Selected files will be highlighted as shown in the figure on the right.

Cluster analysis

Data extraction

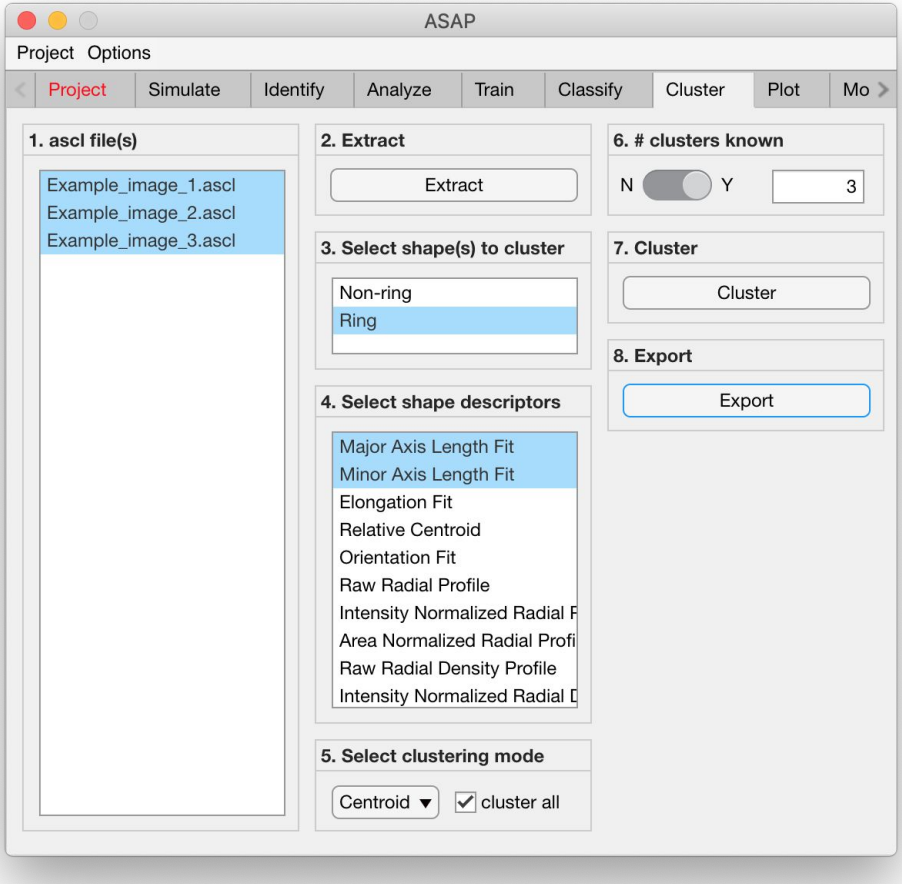


Instructions

Extract data by pressing the '**Extract**' button in the panel titled '**2. Extract**'.

Cluster analysis

Selection of clustering parameters (1/4)

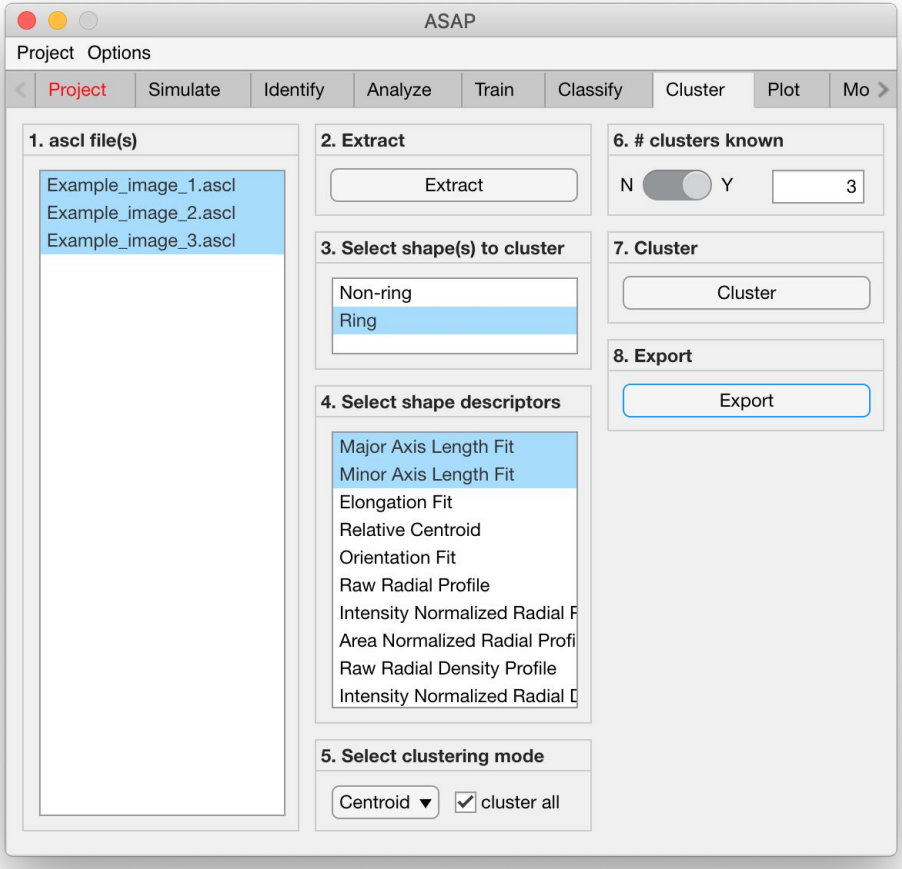


Instructions

Select 1 or more shapes for clustering from the **listbox** in the panel titled '**3. Select shape(s) to cluster**'.

Cluster analysis

Selection of clustering parameters (2/4)

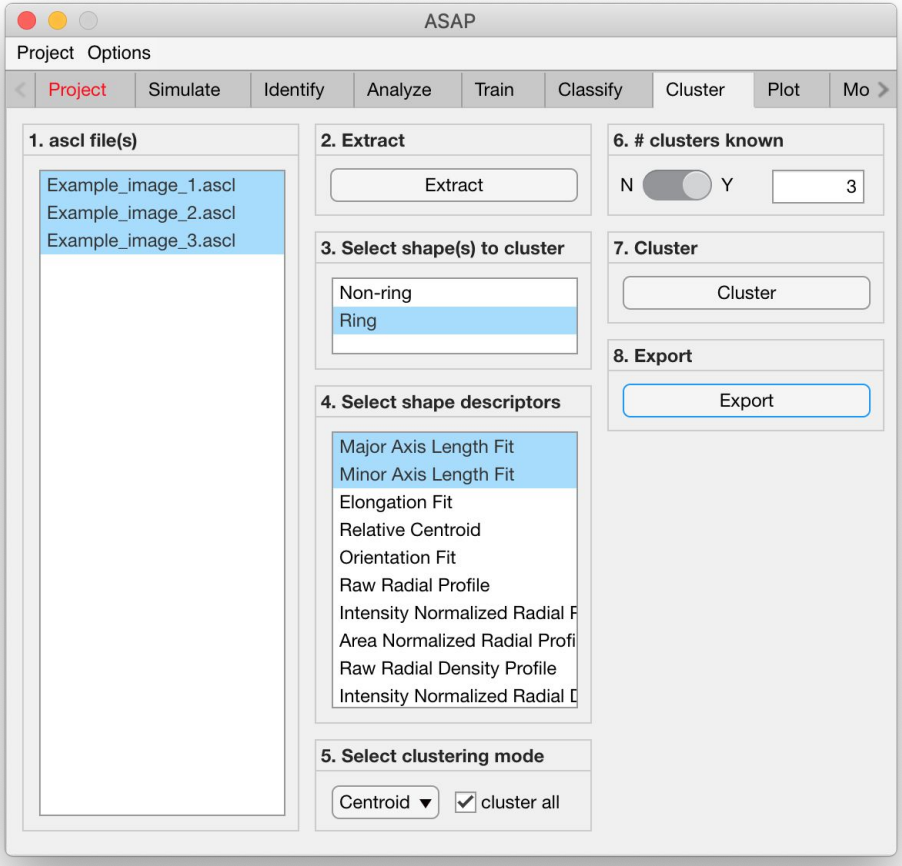


Instructions (contd.)

Select a maximum of 2 shape descriptors for the cluster analysis from the **listbox** in the panel titled '**4. Select shape descriptors**'

Cluster analysis

Selection of clustering parameters (3/4)



Instructions (contd.)

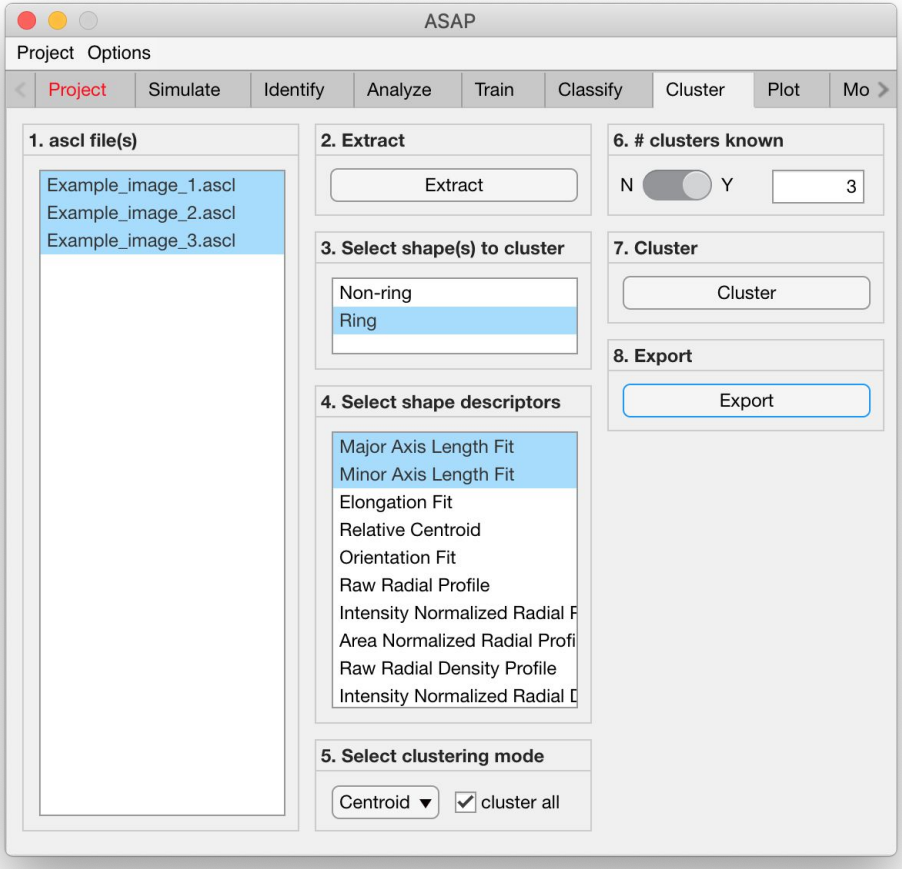
/ Choose a clustering mode from the **drop menu** in the panel titled '**5. Select clustering mode**'. The following modes (algorithms) are available:

1. **Centroid**: deploys a *k-means* algorithm ↘ for data clustering.
2. **Gaussian mixture model**: deploys an *expectation maximization* algorithm ↘ for data clustering.

/ To use data from all the selected files in the clustering process, check the checkbox labeled '**cluster all**' in the same panel.

Cluster analysis

Selection of clustering parameters (4/4)



Instructions (contd.)

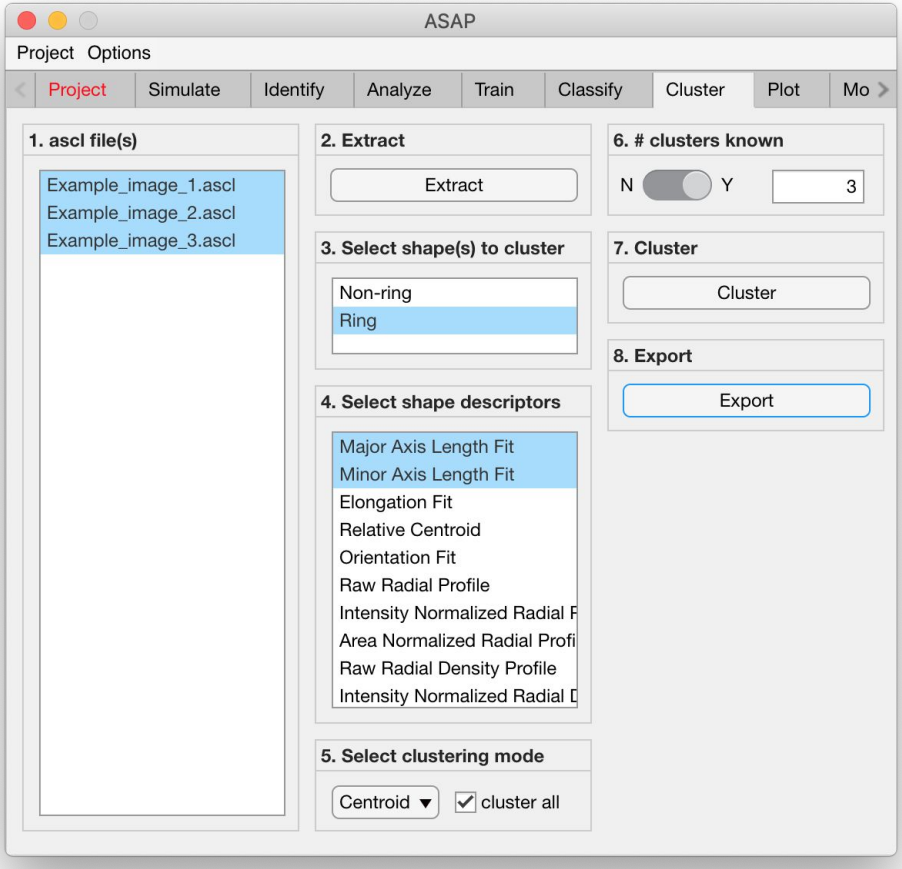
/ If the number of clusters is known *a priori*, pull the switch in the panel titled '**6. # clusters known**' to the position labeled '**Y**'. If otherwise, pull the switch to the position labeled '**N**'.

/ If the number of clusters is known, a numeric edit field will be enabled on the right side where the user can enter the known number of clusters.

/ If the number of clusters is unknown, an optimal number of clusters will be automatically calculated.

Cluster analysis

Data clustering (1/3)



Instructions

Cluster the data by pressing the '**Cluster**' button in the panel titled '**7. Cluster**'.

Please turn over

Cluster analysis

Data clustering (2/3)

Instructions (contd.)

/ If the user selects to show the clustered images, a number of figures equal to the number of processed files and similar to the one shown on the right will be displayed.

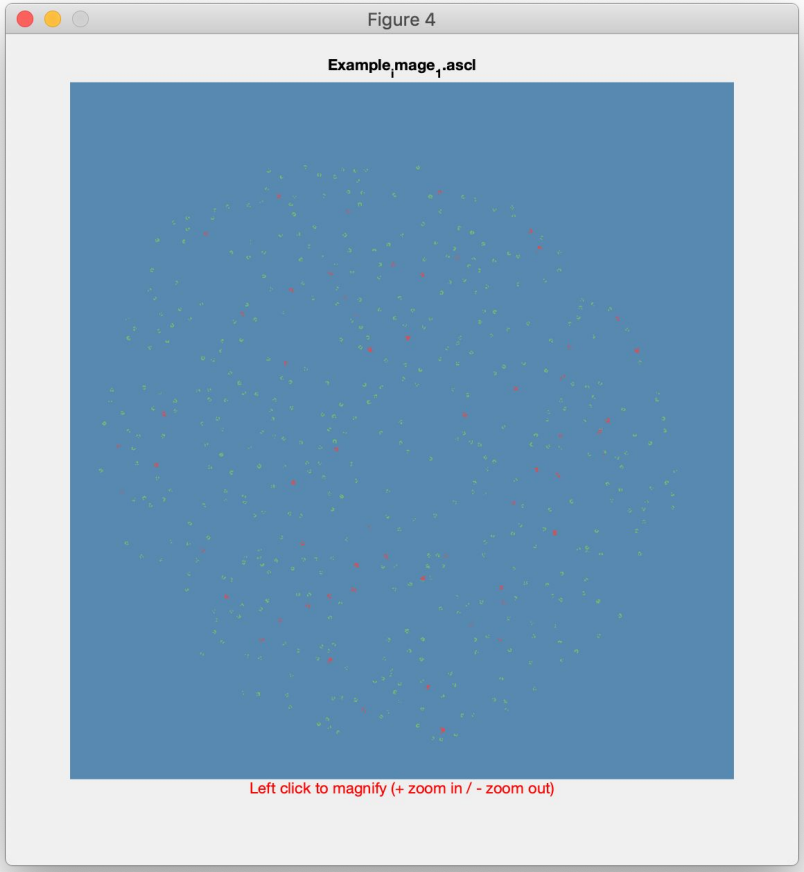
/ Figures are titled with the name of the processed file.

/ Each structure is color-coded according to its cluster affiliation.

/ To magnify into the image hold the left click of the mouse at any point on the image and press the + or - to zoom in and out respectively.

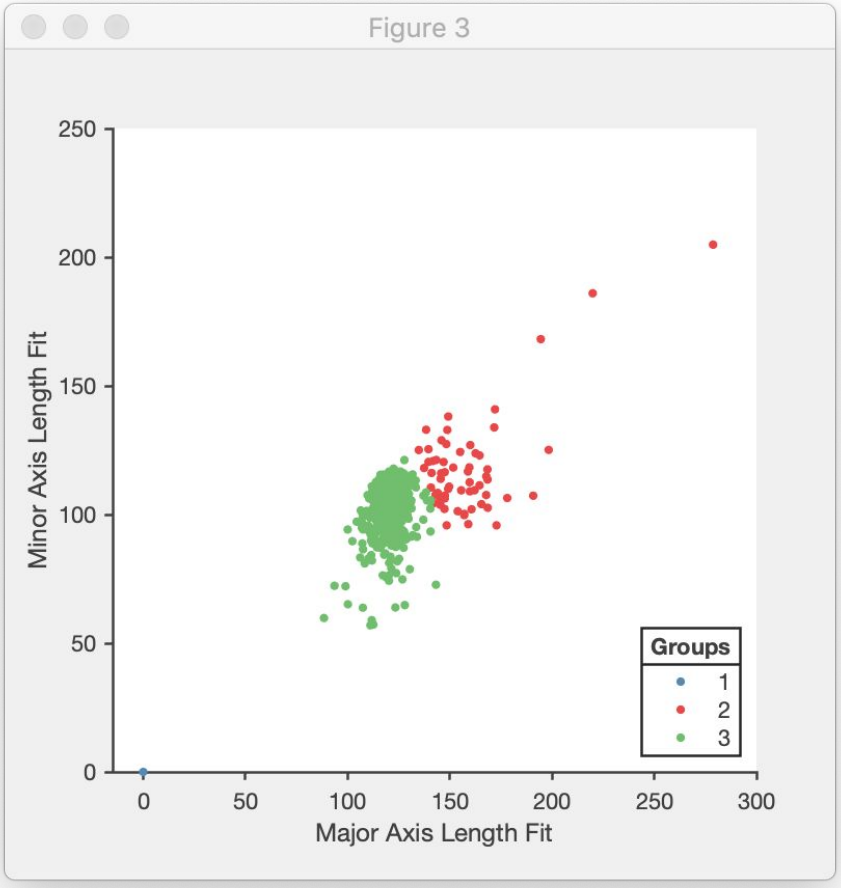
/ By hovering the pointer across the image the user can access the different parts magnified in real time.

Please turn over



Cluster analysis

Data clustering (3/3)



Instructions (contd.)

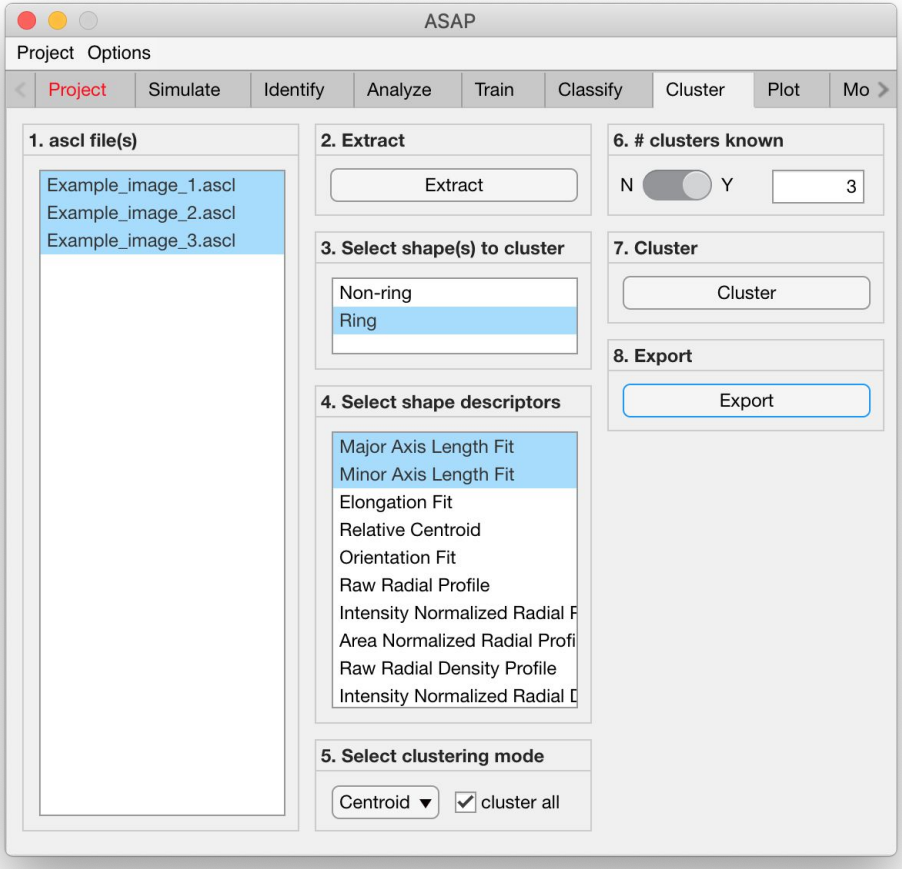
/ If the user selects to cluster all data, 1 plot similar to the one shown on the right will be displayed. The *clustering plot* maps structures across 2 similar or different dimensions (i.e. shape descriptors) and groups them into different clusters according to the chosen clustering mode.

/ Plots are titled with the name of the processed file.

/ If the user does not select to cluster all data, a number of plots equal to the number of processed files and similar to the plot shown on the right will be displayed.

Cluster analysis

Data export (1/2)



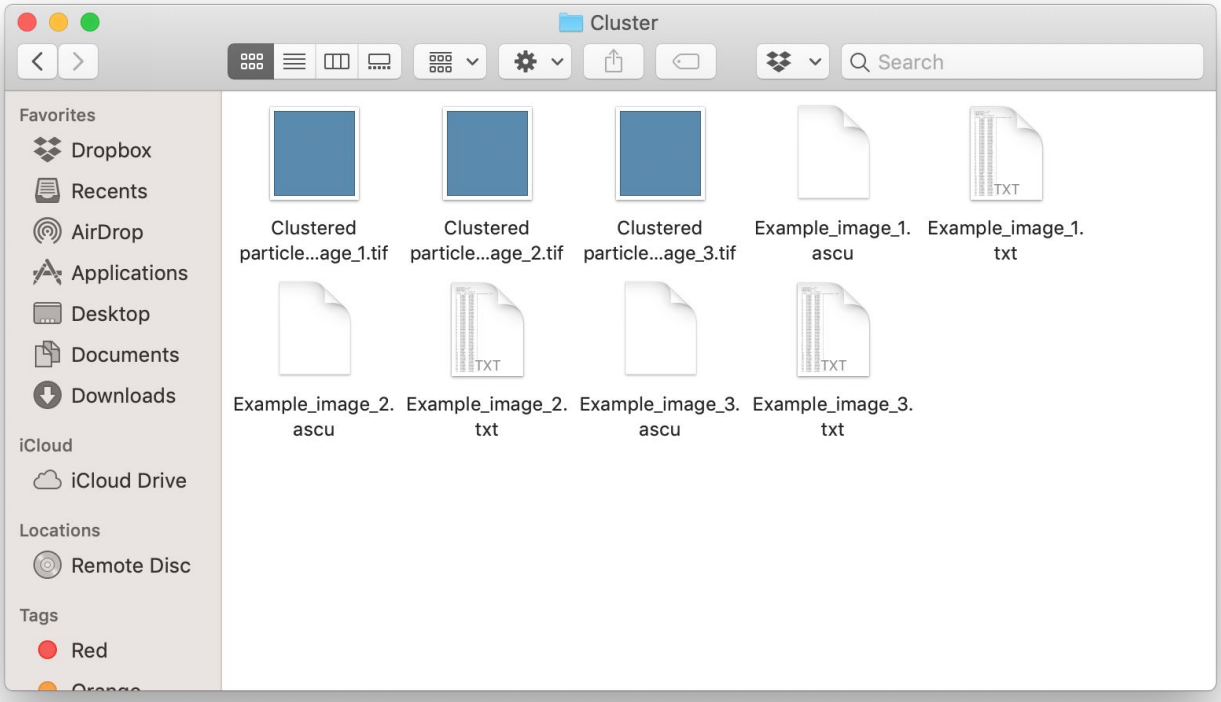
Instructions

Export data by pressing the '**Export**' button in the panel titled '**8. Export**'.

Please turn over

Cluster analysis

Data export (2/2)



Instructions (contd.)

A folder named **'Cluster'** will be created in the output folder and the following 3 output files (per processed image) will be placed in the folder:

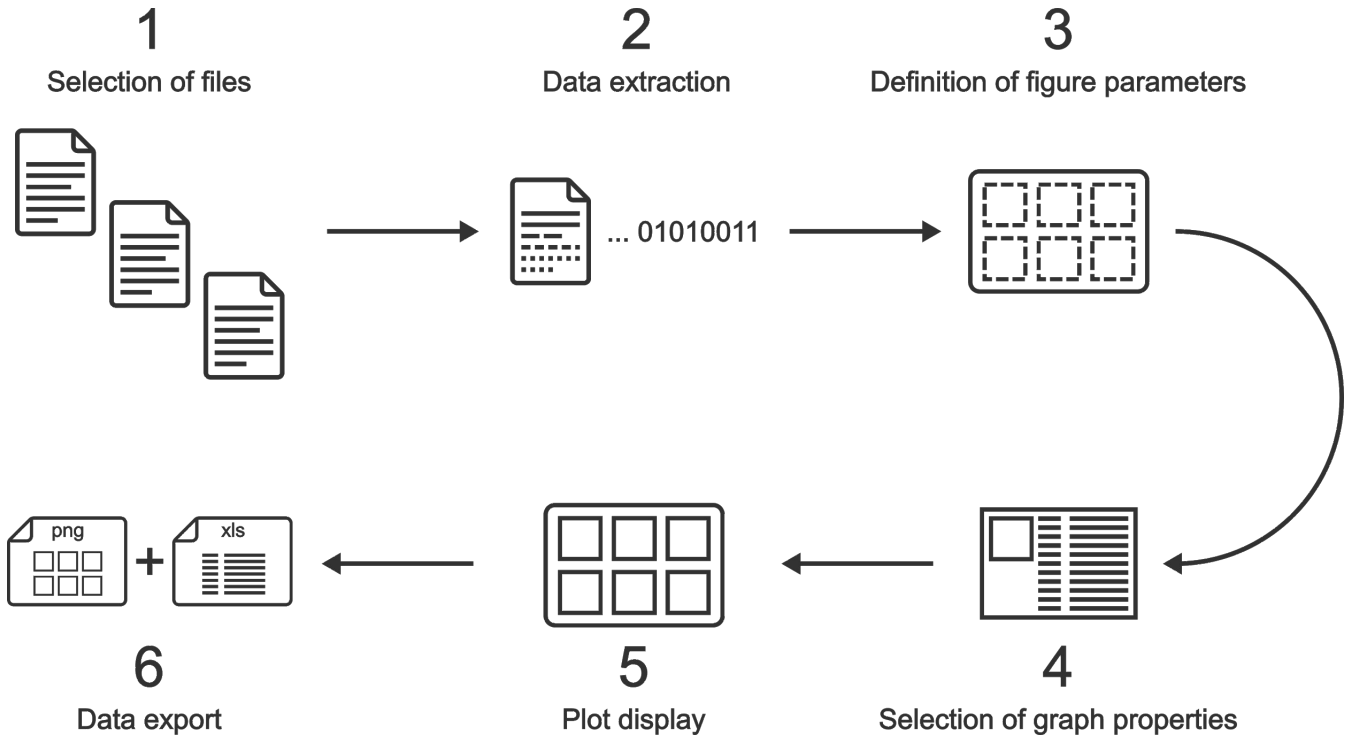
1. '**xx.txt**' - ***file***: text file containing a summary of the structures clustered as well as their parameters.
2. **'xx.ascu' - *file***: a ASAP file containing clustering details used by the program for later analysis.
3. '**Clustered particles_xx.tif**' - ***file***: tiff file of the clustered image.

Statistical and visual analysis

1. Working principle ⇲
2. Selection of files ⇲
3. Data extraction ⇲
4. Definition of plot properties ⇲
5. Selection of graph properties ⇲
6. Data plotting ⇲
7. Data export ⇲

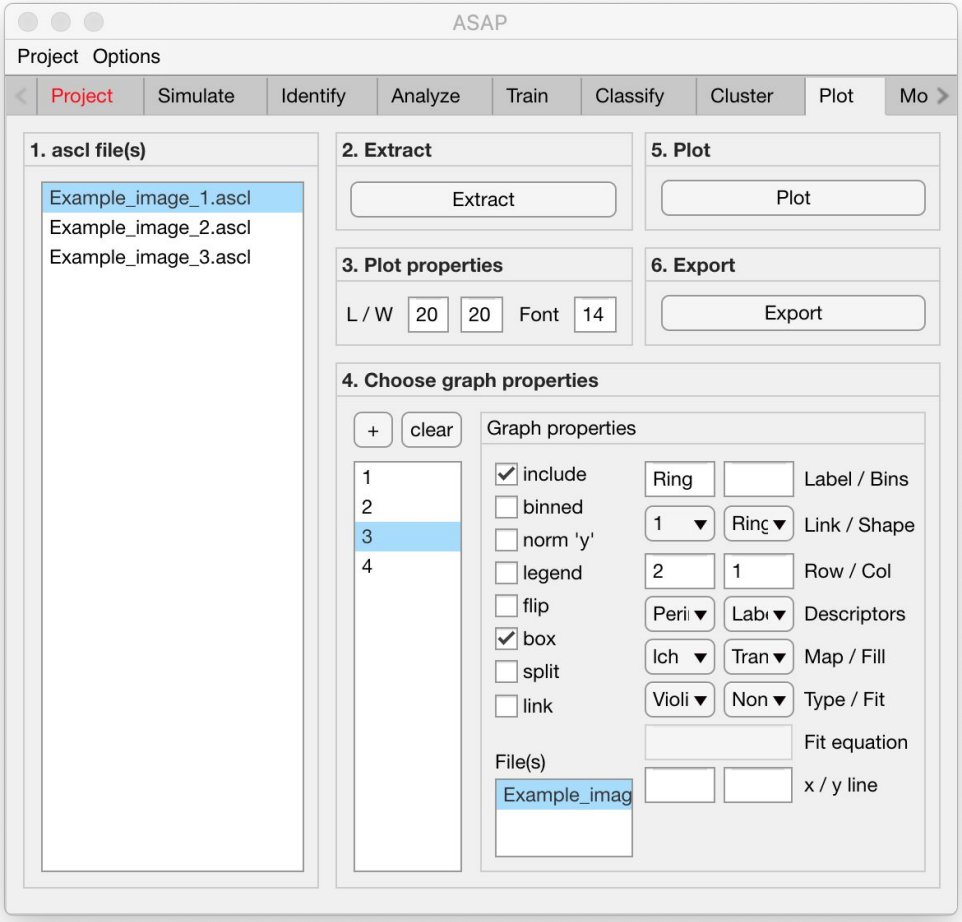
Statistical and visual analysis

Working principle



Statistical and visual analysis

Selection of files



Instructions

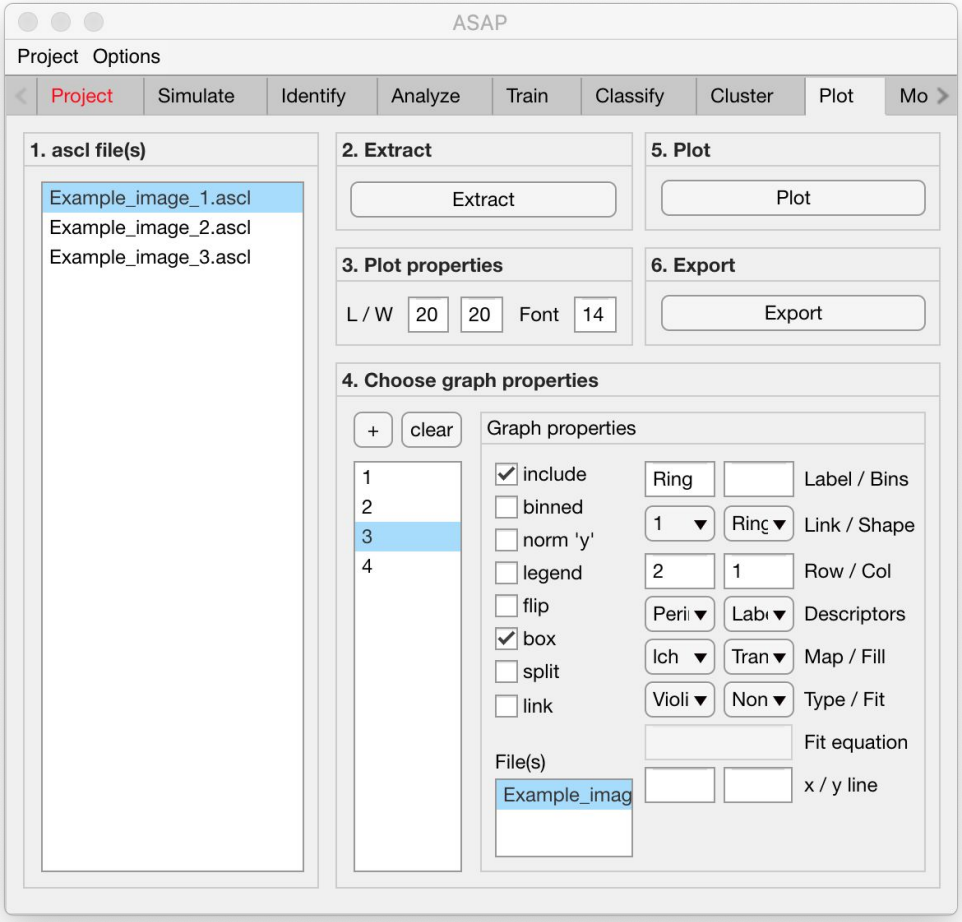
/ Select the 'Plot' tab from the tabs' bar.

/ The listbox in the panel titled '**1. ascl files**' will be populated with the names of ascl files located in the input folder previously selected ↘.

/ Select files by holding the CTRL key and right-clicking the desired files in the listbox in the same panel. Selected files will be highlighted as shown in the figure on the right.

Statistical and visual analysis

Data extraction

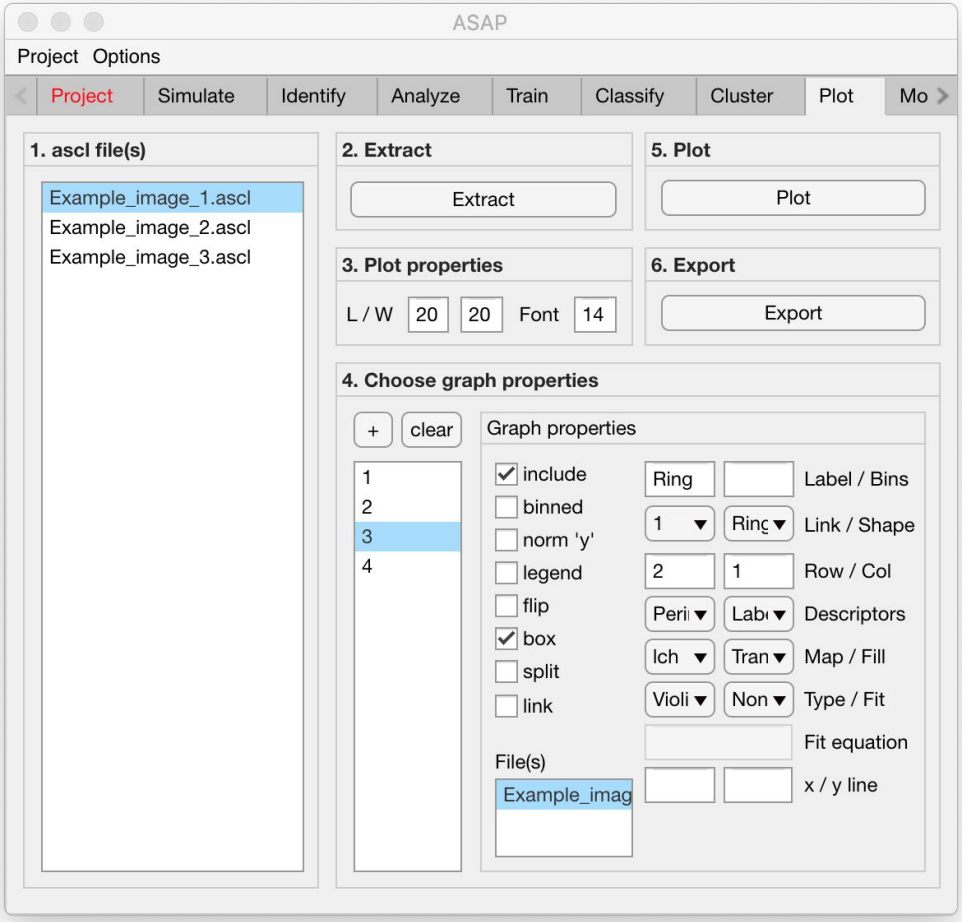


Instructions

Extract data by pressing the '**Extract**' button in the panel titled '**2. Extract**'.

Statistical and visual analysis

Definition of plot properties



Instructions

/ The length, width and text size of the figure plot can be defined by setting numeric edit fields labeled '**L/W**' and '**Font**' respectively.

/ The units of the length and width are in centimeters.

/ The unit of the font size is in pixels.

Statistical and visual analysis

Selection of graph properties (1/6)

Instructions

/ Each plot (figure) is composed of smaller subplots positioned at specific rows and columns.

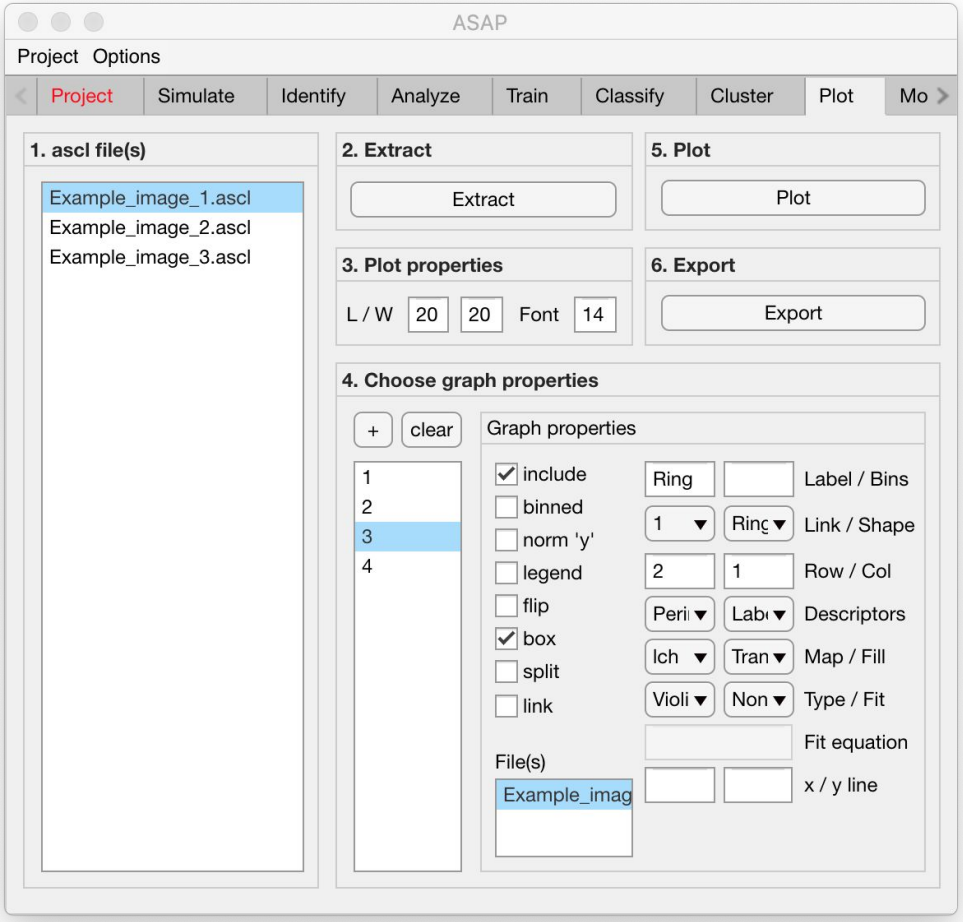
/ Each subplot is composed of 1 or more graphs visually representing numeric data.

/ To create a graph press the button labeled '**+**' located on the top-left corner in the panel titled '**4. Choose graph properties**'.

/ The large listbox located underneath in the same panel will be populated with the name of the created graph as shown in the figure on the right.

/ Multiple graphs can be created at once by pressing the '**+**' button multiple times.

/ To clear (remove) all graphs and start from scratch press the button labeled '**clear**' in the same panel.



Instructions (contd.)

/ To define what is represented by one graph, select the graph name from the listbox earlier referred to. Once selected the graph name will be highlighted.

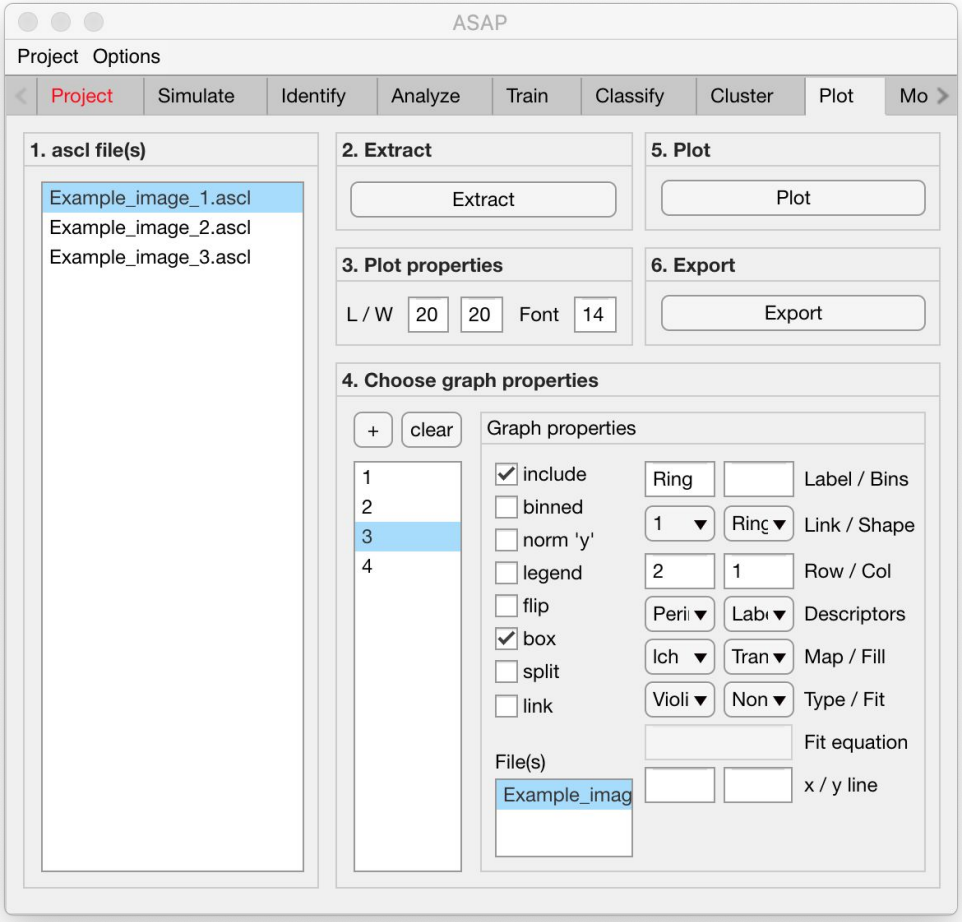
/ For each graph / plot, the following properties can be modified, defined or selected:

1. **Graph property:** to include the selected graph in a plot, check the checkbox labeled '**include**'.
2. **Graph property**: to include structures previously binned during analysis and classification in the selected graph, check the checkbox labeled '**binned**'.
3. **Plot property**: to normalize the y - axis (i.e. plot the probability instead of the number of counts), check the checkbox labeled '**norm 'y'**'.
4. **Plot property:** to include a legend for 1 or more graphs, check the checkbox labeled '**legend**'.
5. **Plot property:** to flip the x - and y - axes, check the checkbox labeled '**flip**'.

Please turn over

Statistical and visual analysis

Selection of graph properties (2/6)



Instructions (contd.)

6. **Plot property**: to include a bounding box around a plot, check the checkbox labeled '**box**'.
7. **Plot property**: to split graphs belonging to the same subplot into different sub subplots, check the checkbox labeled '**split**'.
8. **Graph property**: to link one graph to another graph (i.e. include two graphs in the same plot) select the graph name of the *mother* graph to which the selected *child* graph should be linked to from the drop down menu labeled '**Link**' and check the checkbox labeled '**link**'. Once the checkbox is checked, the name of the *mother* graph will be modified to include an (*).

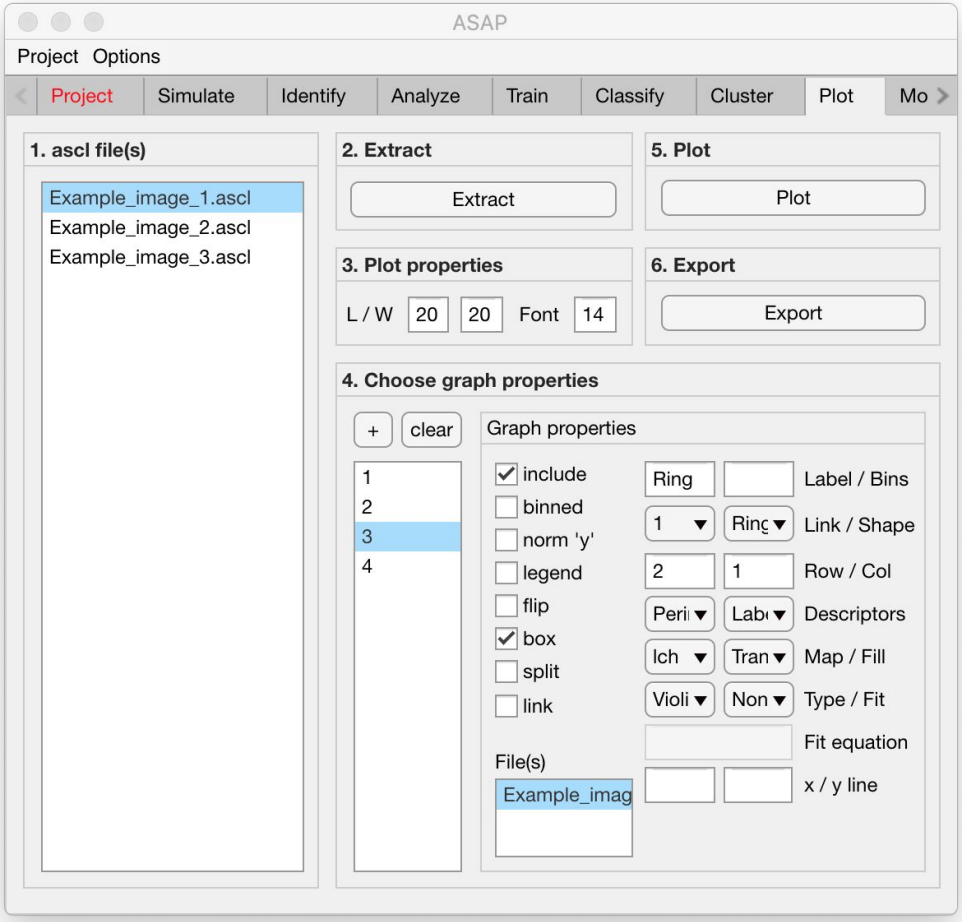
§ Warning 1:
Do not link a mother graph to a child / mother graph. Only child to mother graph linking is permitted.

§ Warning 2:
Do not check the '**link**' checkbox prior to selecting the linked to graph.

Please turn over

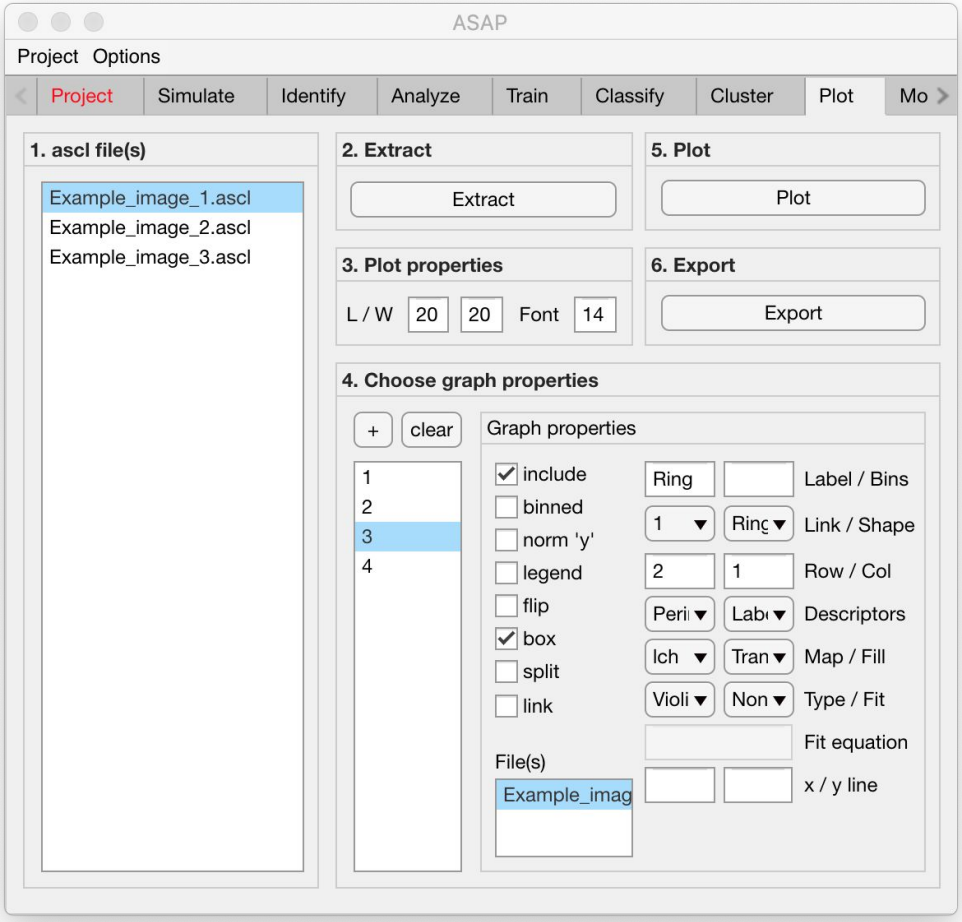
Statistical and visual analysis

Selection of graph properties (3/6)



Statistical and visual analysis

Selection of graph properties (4/6)



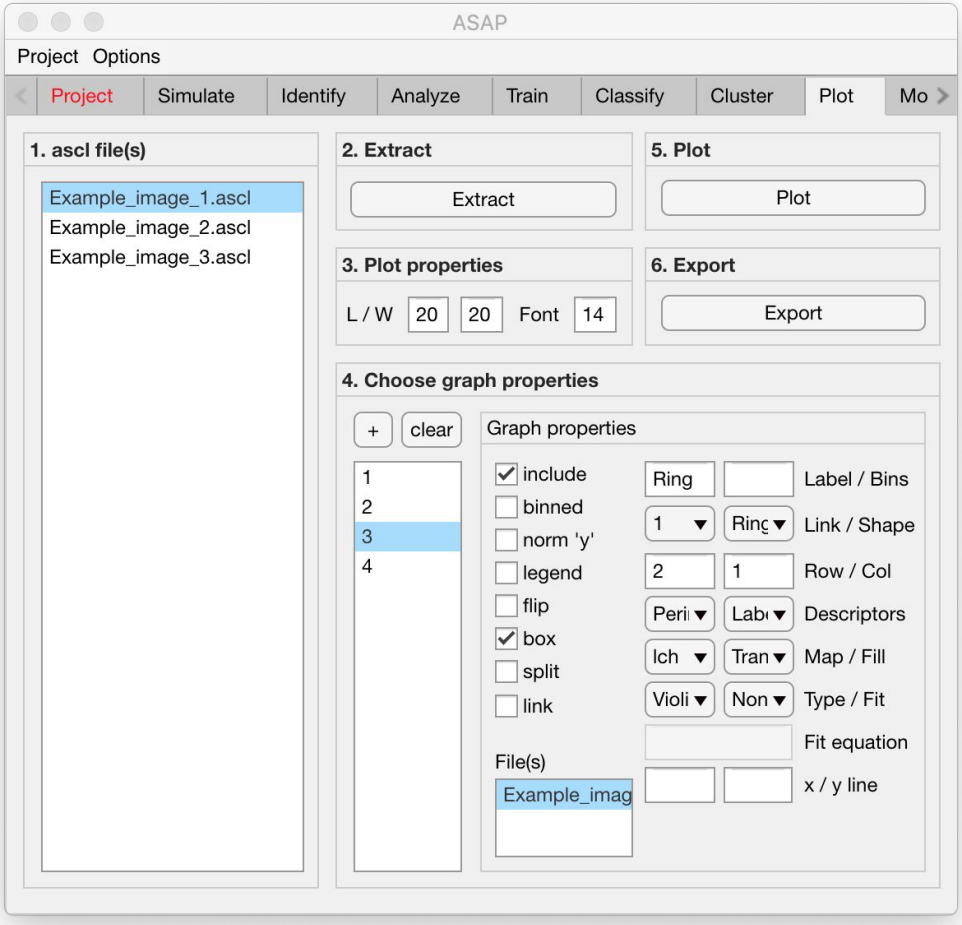
Instructions (contd.)

9. **Graph property:** select 1 or more file(s) from the listbox labeled '**File(s)**'.
10. **Graph property:** to include a *sample* label type a label in the text edit field labeled '**Label**'.
11. **Graph property**: to manually set the number of bins of a histogram, type the number of bins in the text edit field labeled '**Bins**'. The input number will only be processed when the plotted graph is a histogram.
12. **Graph property:** select a shape from the dropdown menu labeled '**Shape**'.
13. **Graph properties:** enter the row and column numbers of the plot to which the currently selected graph belongs.

Please turn over

Statistical and visual analysis

Selection of graph properties (5/6)



Instructions (contd.)

15. **Graph properties**: select the 2 descriptors to be plotted from the 2 dropdown menus labeled '**Descriptors**'.
16. **Plot property**: to change the set of colors used for plotting a plot, select a *color map* from the drop down menu labeled '**Map**'.
17. **Plot property**: to change the filling for histogram(s), bar plot(s) and box plot(s) select a filling from the dropdown menu labeled '**Fill**'.
18. **Plot property**: select a plot type from the dropdown menu labeled '**Type**'.

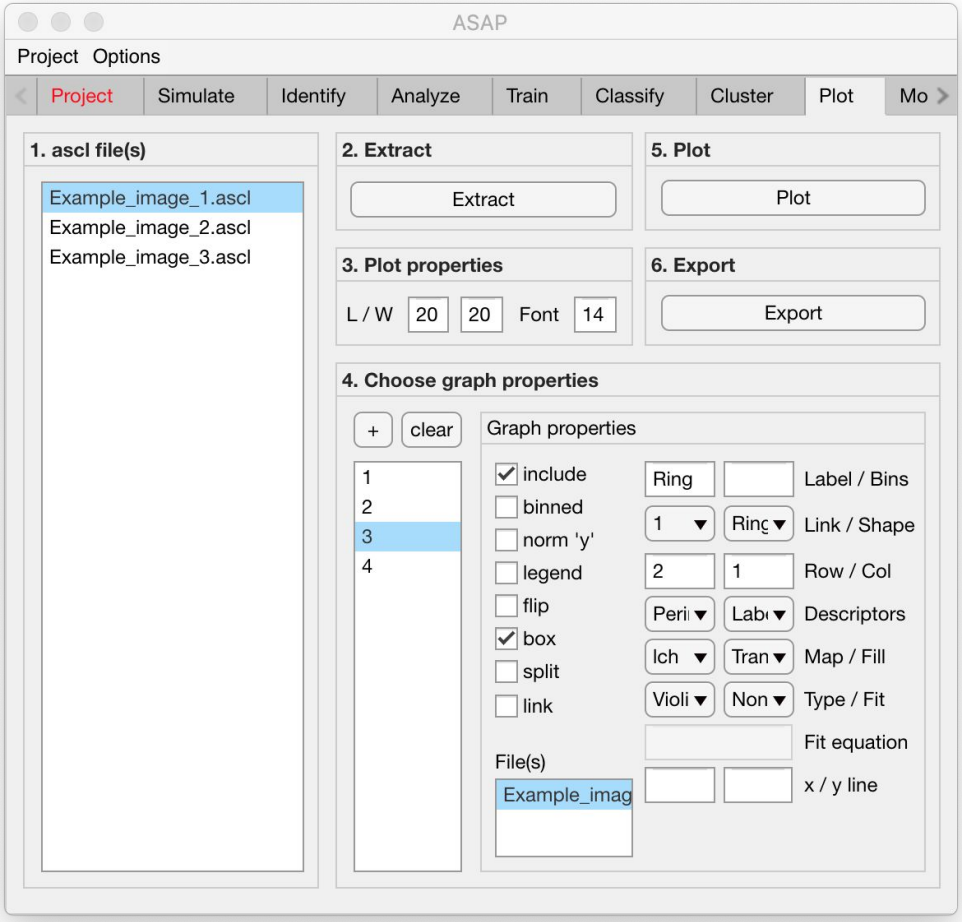
Please turn over

Statistical and visual analysis

Selection of graph properties (6/6)

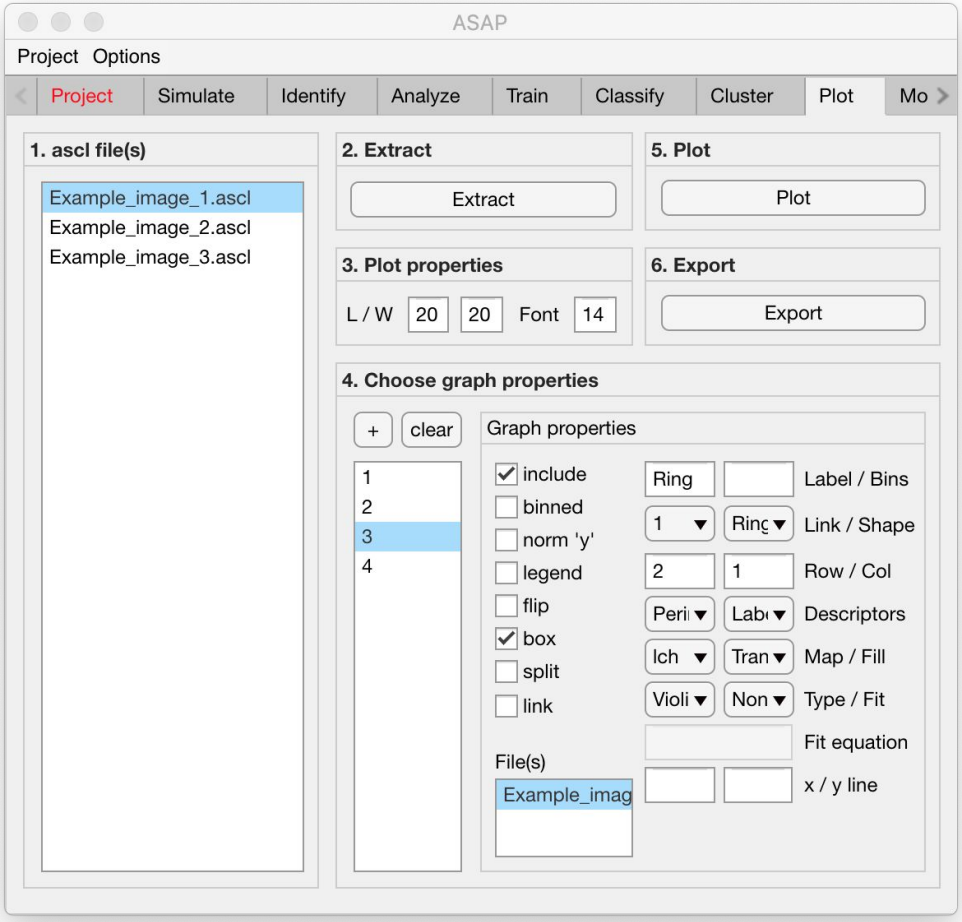
Instructions (contd.)

19. **Plot property**: select a fitting curve from the dropdown menu labeled '**Fit**'. If 'custom' is selected, the edit field labeled '**Fit equation**' will be enabled.
20. **Plot property**: type a fitting equation and starting points between rectangular brackets in the text edit field labeled '**Fit equation**' if enabled:
    a. a * x + b [1 1]
    b. ln(x / sqrt(2))
    c. a * x ^ 3 + x ^ 2 + x [10]
    d. x / ((x ^ 2) + m) [3]
    e. erf((x + d - s) / l * 2) [0.1 10 100]
21. **Plot properties**: to draw a vertical dashed line at a specific x - and / or y - value(s), type the x - and / or y - values in the text edit fields labeled x / y line.



Statistical and visual analysis

Data plotting (1/2)



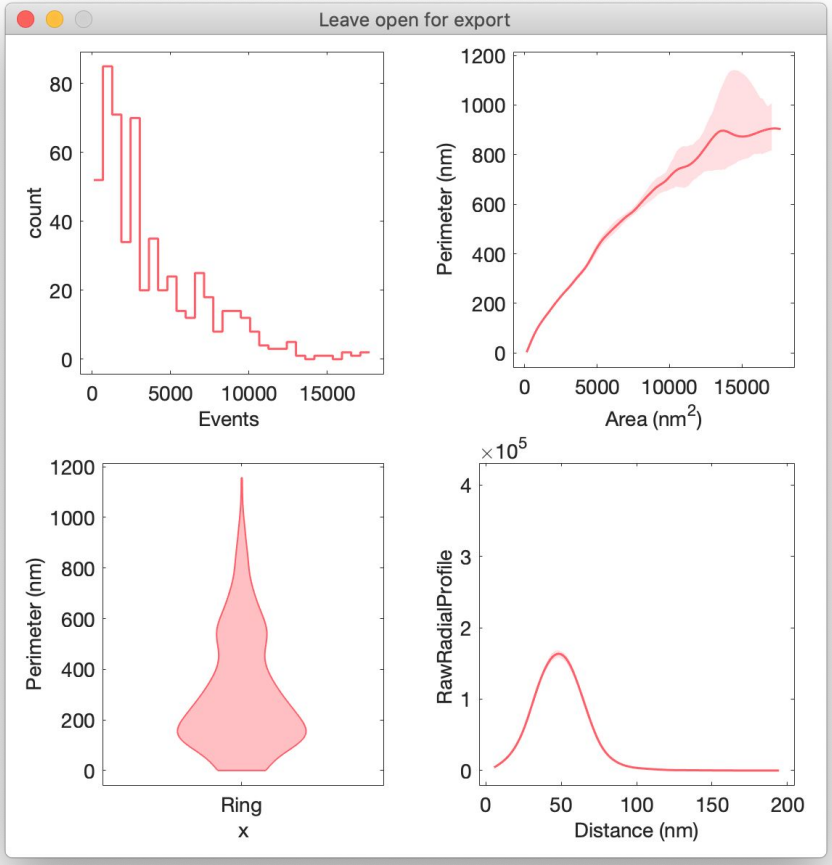
Instructions

Plot the data by pressing the '**Plot**' button in the panel titled '**5. Plot**'.

Please turn over

Statistical and visual analysis

Data plotting (2/2)



Instructions

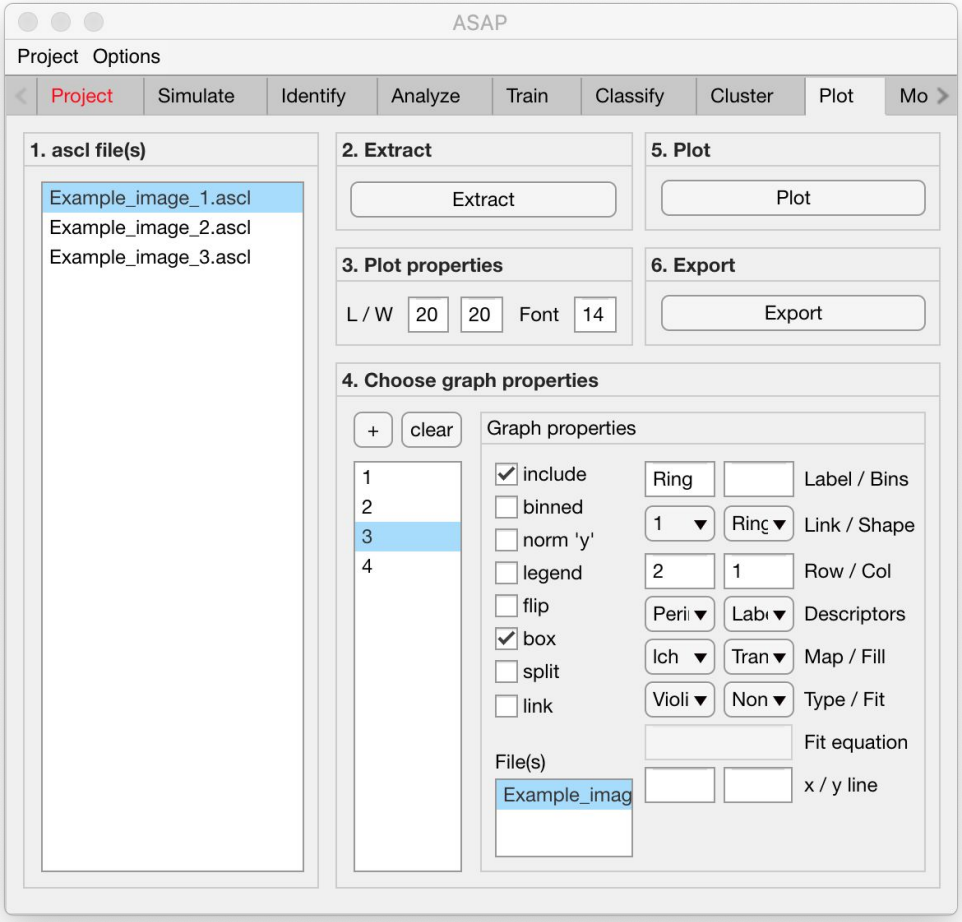
A figure containing a plot, as the one shown on the right, will be displayed.

§ Warning 1:
The user is required not to close the figure if it will be exported.

Guide ⇲

Statistical and visual analysis

Data export (1/2)



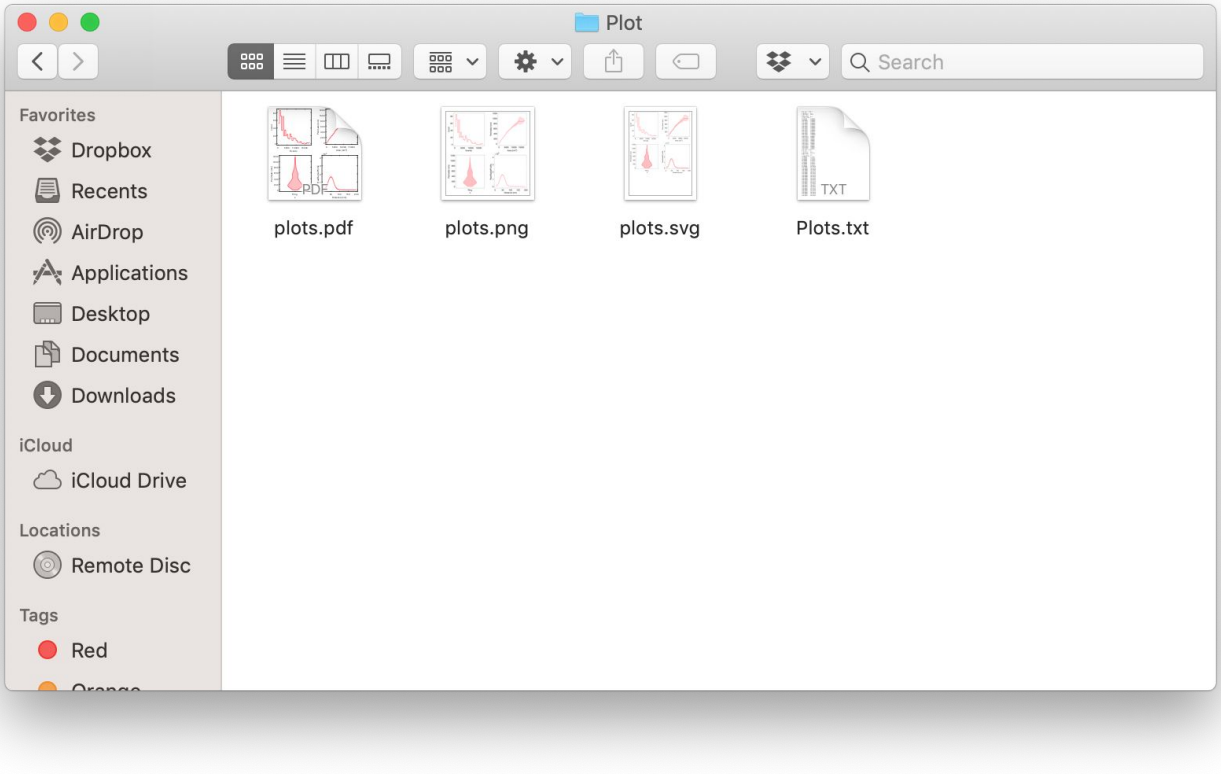
Instructions

Export data by pressing the '**Export**' button in the panel titled '**6. Export**'.

Please turn over

Statistical and visual analysis

Data export (2/2)



Instructions (contd.)

A folder named **'Plot'** will be created in the output folder and the following 4 output files will be placed in the folder:

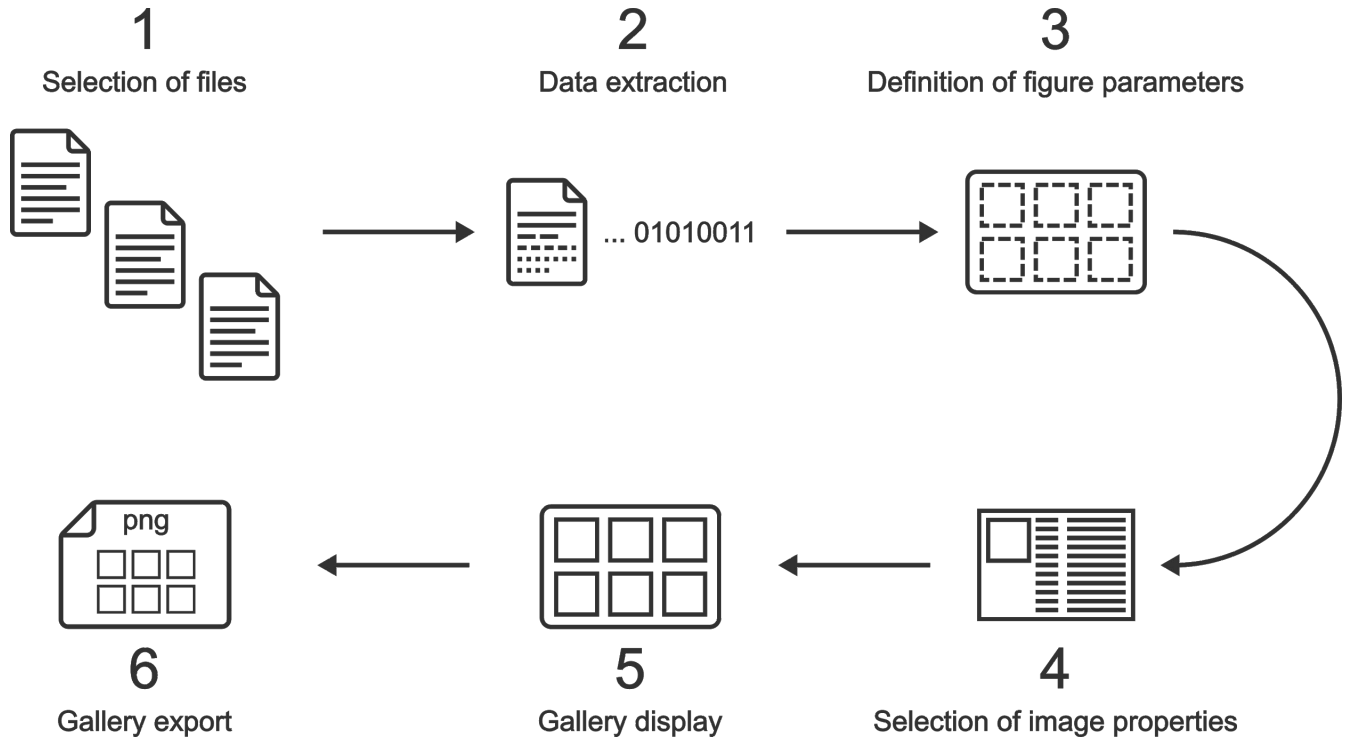
1. '**plots.png**' - ***file***: png file of the plot.
2. '**plots.pdf**' - ***file***: pdf file of the plot.
3. '**plots.svg**' - ***file***: svg file of the plot.
4. '**xx.txt**' - ***file***: text file containing a summary of the graphs including relevant parameters.

Montage construction

1. Working principle ⇲
2. Selection of files ⇲
3. Data extraction ⇲
4. Definition of montage properties ⇲
5. Selection of montage parameters ⇲
6. Montage display ⇲
7. Montage export ⇲

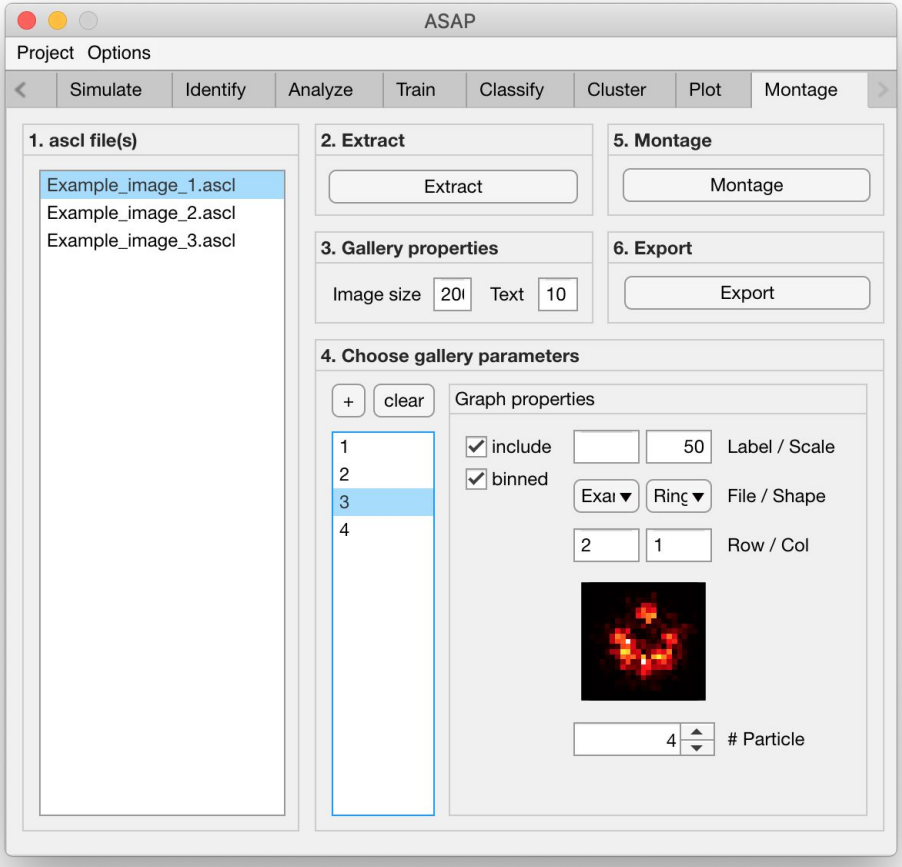
Gallery construction

Working principle



Montage construction

Selection of files



Instructions

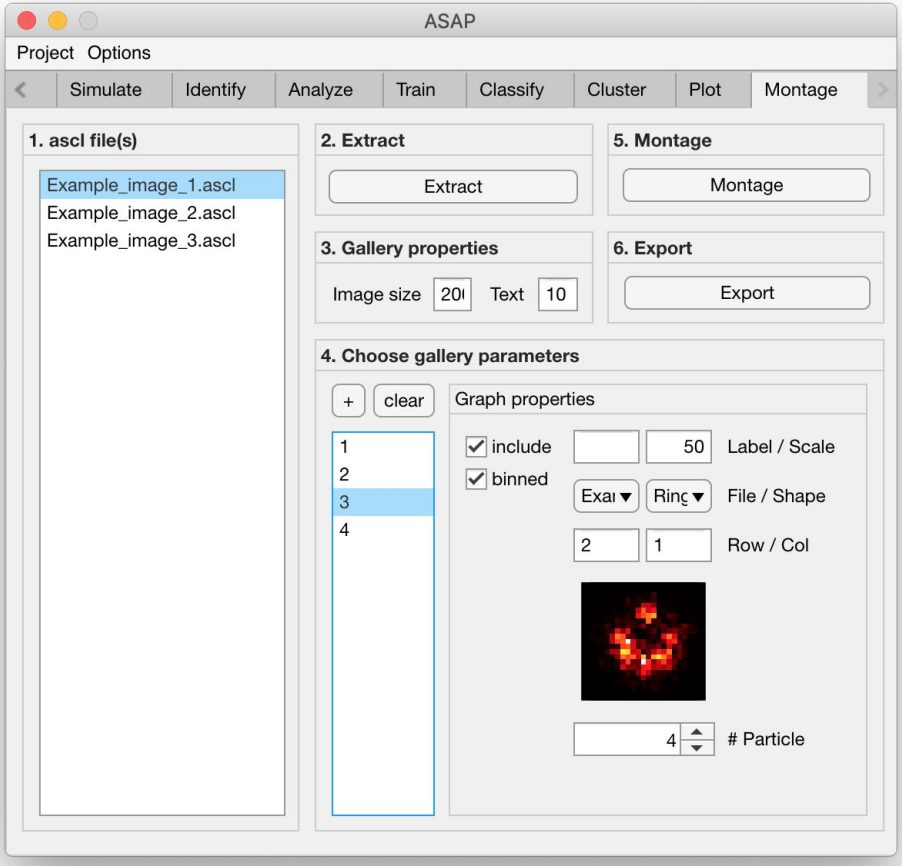
/ Select the 'Montage' tab from the tabs' bar.

/ The listbox in the panel titled '**1. ascl files**' will be populated with the names of ascl files located in the input folder previously selected ⇲.

/ Select files by holding the CTRL key and right-clicking the desired files in the listbox in the same panel. Selected files will be highlighted as shown in the figure on the right.

Montage construction

Data extraction

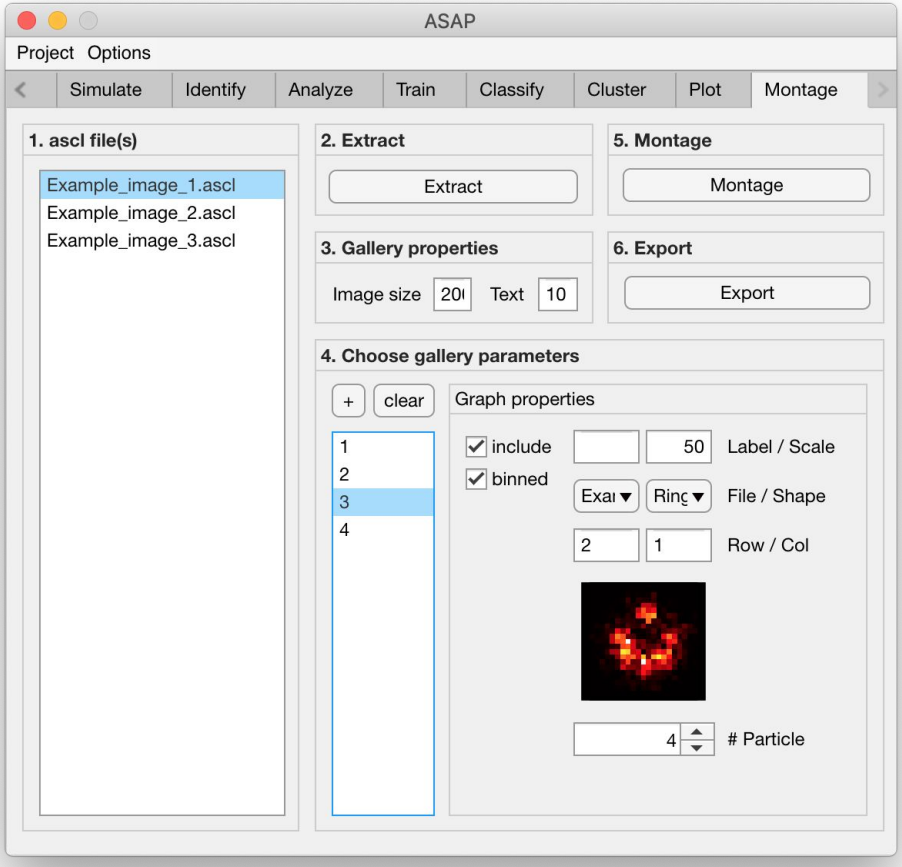


Instructions

Extract data by pressing the '**Extract**' button in the panel titled '**2. Extract**'.

Montage construction

Definition of montage properties



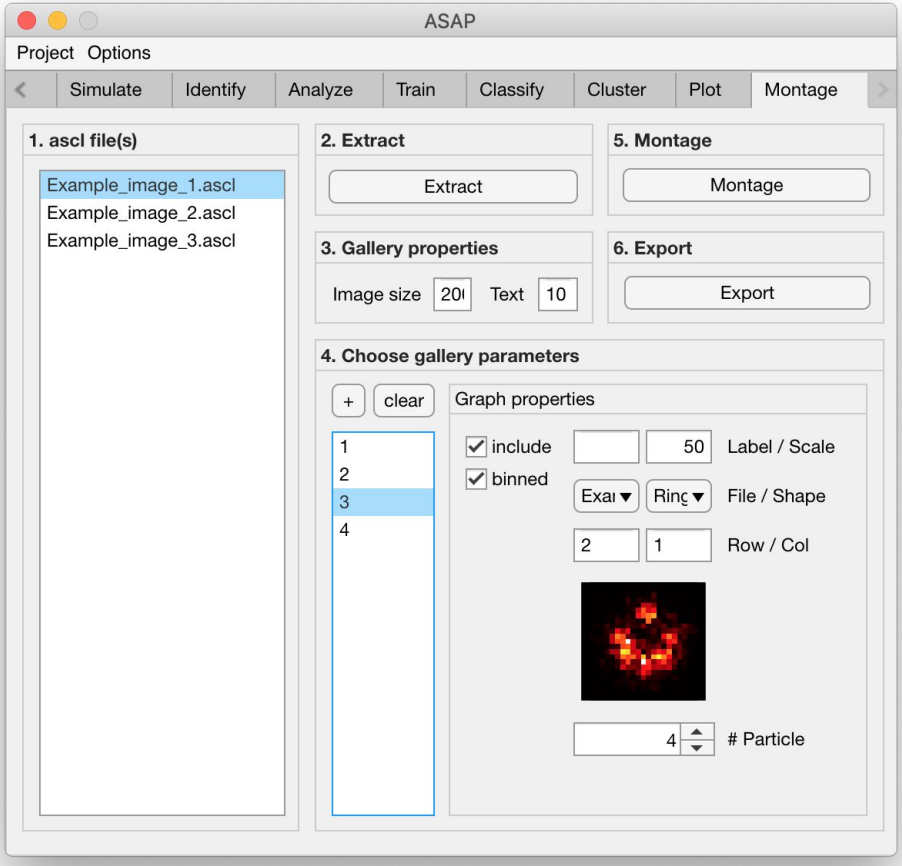
Instructions

/ The size of single image and text size in the gallery can be defined by setting the numeric edit fields labeled '**Image size**' and '**Text**' respectively in the panel titled '**3. Gallery properties**'.

/ The units of the text size is in pixels.

Montage construction

Selection of montage parameters (1/3)



Instructions

/ Each gallery (figure) is composed of smaller images positioned at specific rows and columns.

/ To add images press the button labeled '**+**' located on the top-left corner in the panel titled '**4. Choose gallery parameters**'.

/ The large listbox located underneath in the same panel will be populated with the name(s) of the add images, or *particles*, as shown in the figure on the right.

/ Multiple images can be added at once by pressing the '**+**' button multiple times.

/ To clear (remove) all graphs and start from scratch press the button labeled '**clear**' in the same panel.

Montage construction

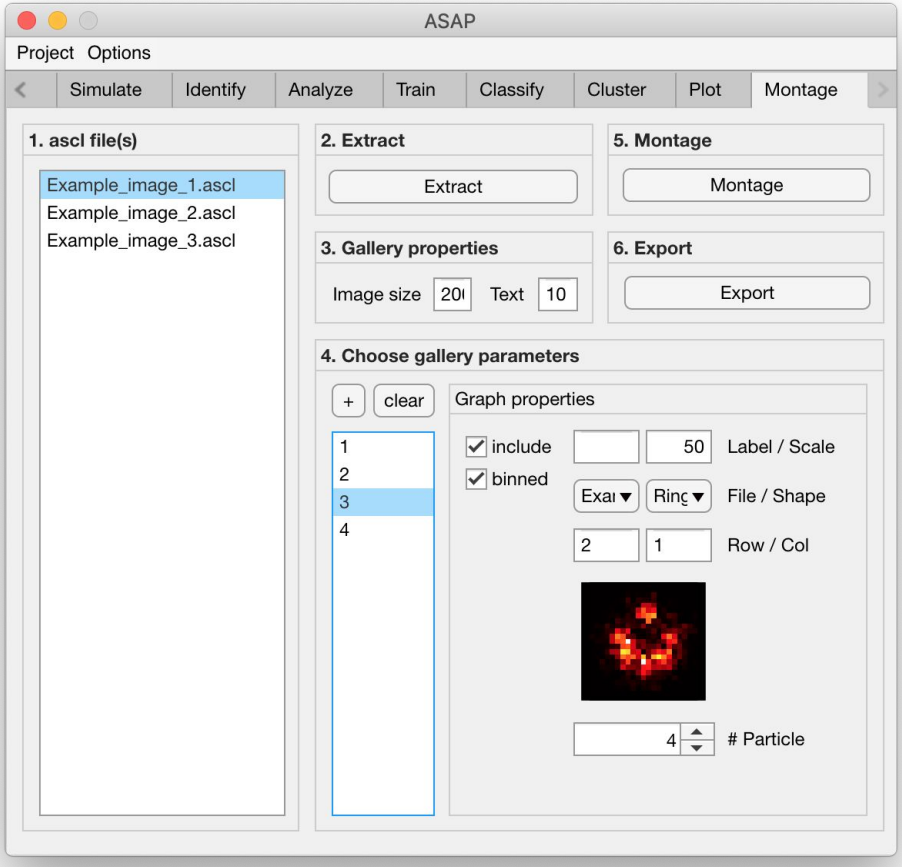
Selection of montage parameters (2/3)

Instructions (contd.)

/ To define what is represented by one image, select the image name from the listbox earlier referred to. Once selected the image name will be highlighted.

/ For each image, the following parameters can be modified, defined or selected:

1. **Image property:** to include the selected image in the gallery, check the checkbox labeled '**include**'.
2. **Image property:** to include binned structures in the gallery, check the checkbox labeled '**binned**'.
3. **Image property:** to label the selected image in the gallery, type a label in the text edit field labeled '**Label**'.
4. **Image property:** to add a scale bar to the selected image in the gallery, type the length of the scale bar (in nanometers) in the text edit field labeled '**Scale**'.



Please turn over

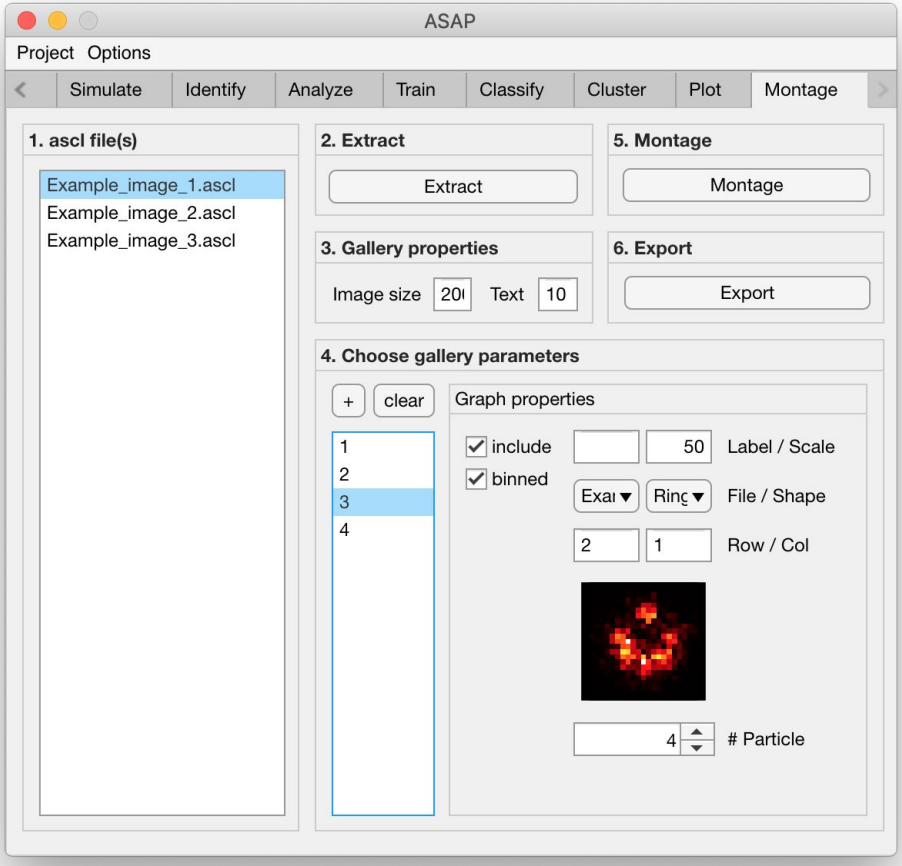
Montage construction

Selection of montage parameters (3/3)

Instructions (contd.)

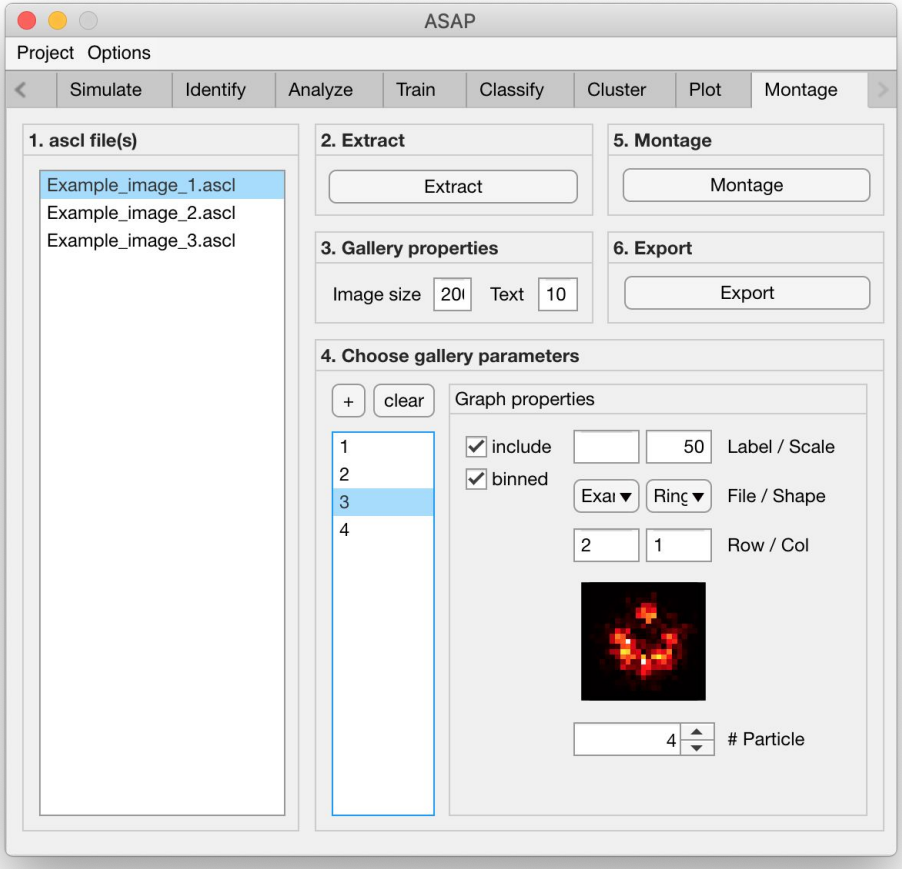
5. **Image property:** select a file from the dropdown menu labeled '**File**'.
6. **Image property:** select a shape from the dropdown menu labeled '**Shape**'.
7. **Image properties:** select the row and column to which the selected image belongs from the dropdown menus labeled '**Row**' and '**Col**' respectively.
8. **Image property:** scroll through the structures in the selected file and of the selected shape by modifying the value of the spinner labeled '**# Particle**'. The in-app figure located above the spinner will be updated to show the selected structure.

§ Warning 1:
The row and column values of one image should not coincide with the row and column values of another. Ensure this condition is satisfied to prevent error propagation.



Montage construction

Montage display (1/2)



Instructions

Display the gallery by pressing the '**Montage**' button in the panel titled '**5. Montage**'.

Please turn over

Montage construction

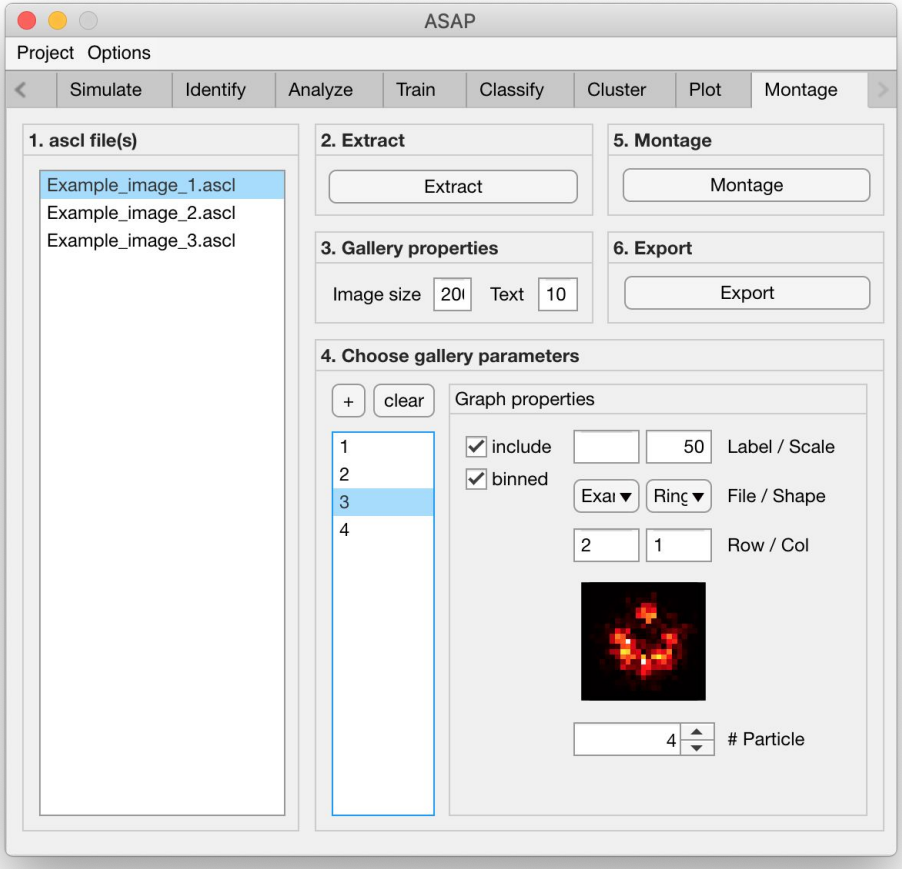
Montage display (2/2)

Instructions

A figure containing a montage, as the one shown on the right, will be displayed.

Montage construction

Montage export (1/2)



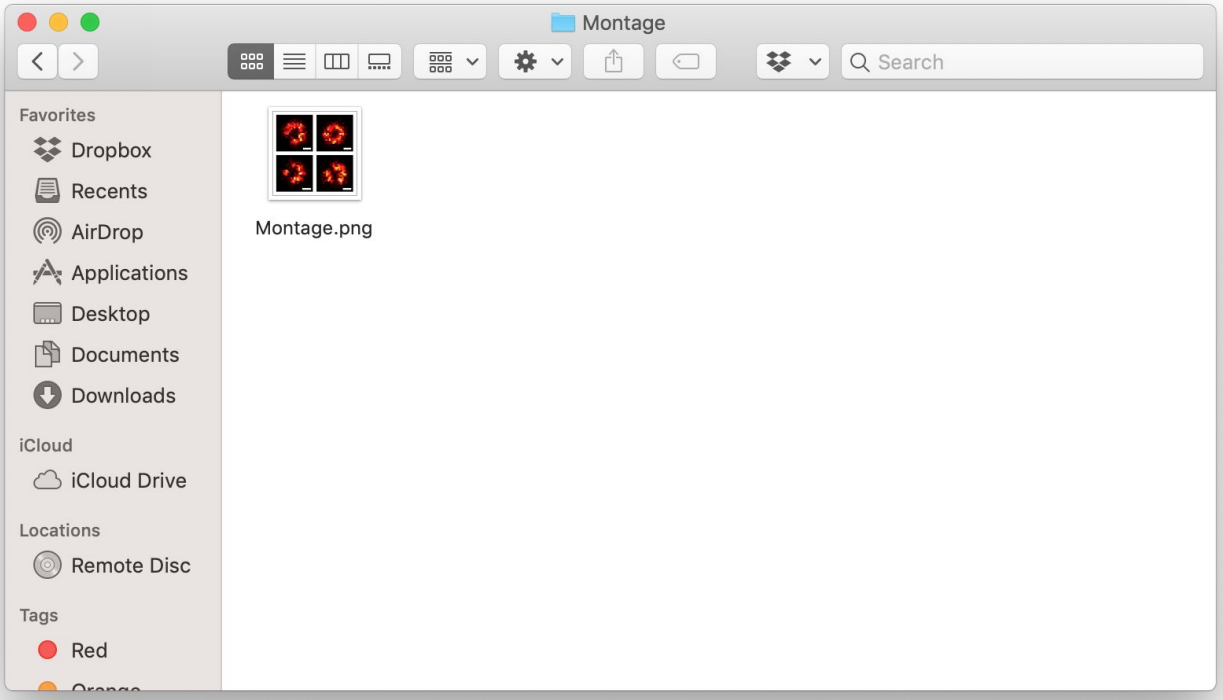
Instructions

Export the gallery by pressing the '**Export**' button in the panel titled '**6. Export**'.

Please turn over

Montage construction

Montage export (2/2)



Instructions (contd.)

A folder named **'Montage'** will be created in the output folder and the following output file will be placed in the folder:

1. '**Montage.png**' - ***file***: png file of the gallery.