ASAP

## Automated Structures Analysis Program

## **User manual** John S H Danial & Ana-J Garcia-Saez

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## Guide

- 1. Introduction  $\blacksquare$
- 2. The message box rightarrow
- 3. Setting options  $\square$
- 4. Project definition  $\supseteq$
- 5. Simulation of point localizations  $\supseteq$
- 6. Identification of structures  $\supseteq$
- 7. Geometrical analysis of structures  $\supseteq$
- 8. Classifier training  $rac{}$
- 9. Classification of structures  $\supseteq$
- 10. Cluster analysis  $\supseteq$
- 11. Statistical and visual analysis  $\square$
- 12. Montage construction  $\square$

## Introduction

- 1. What is ASAP?  $\supseteq$
- 2. Why ASAP? rightarrow
- 3. How to operate ASAP?  $\supseteq$
- 4. Examples rightarrow
- 5. License ⊾

## 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 <u>here</u>.
- 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  $\square$ .

# 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

	ons							
Project	Simulate	Identify	Analyze	Train	Classify	Cluster	Plot	Мо
1. Project fo	older							
Se	elect folder							
2. Automati	ion script*							
Select a	utomation scri	ipt						
	Run							
ASAP progr	ess: export co	mplete						
ASAP progr	ess: export co	mplete.	e 6 out of 6.					
ASAP progr	ess: export co ess: extracting ess: extraction ess: analyzing	data from fil complete.						

#### 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.

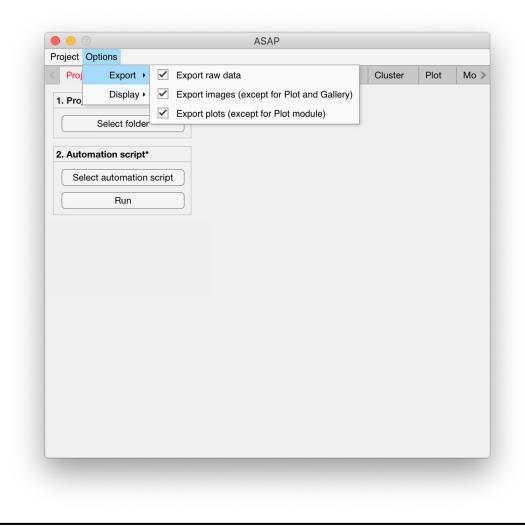
#### § Note 1:

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

## **Setting options**

- 1. Changing export settings rightarrow
- 2. Changing display settings rightarrow

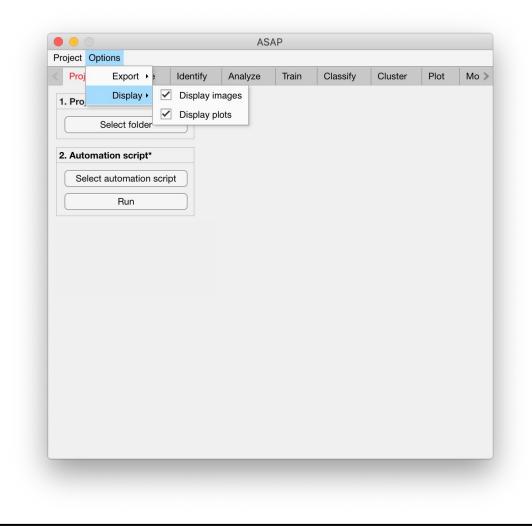
### Setting options Changing export settings



#### 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.

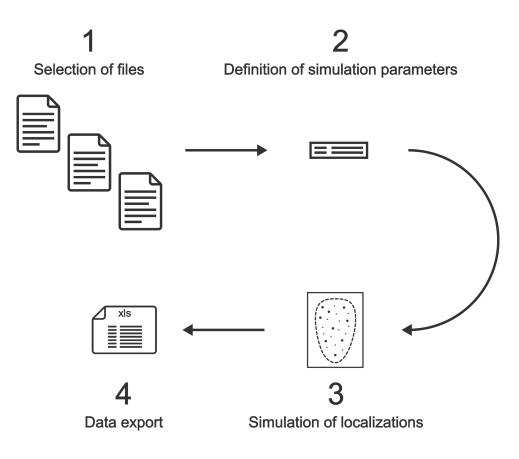
/ The workflow of ASAP can be automated through the automation script; a single text file containing all parameters required by ASAP. To automate ASAP select an automation script (.txt) file by pressing the 'Select automation script' button. When the automation automation script is successfully loaded,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

Project	Simulate	Identify	Analyze	Train	Classify	Cluster	Plot	Мо
_								
1. Project fo	bider							
Se	elect folder							
2. Automati	ion scrint*							
Select a	utomation scr	ipt						
	Run							

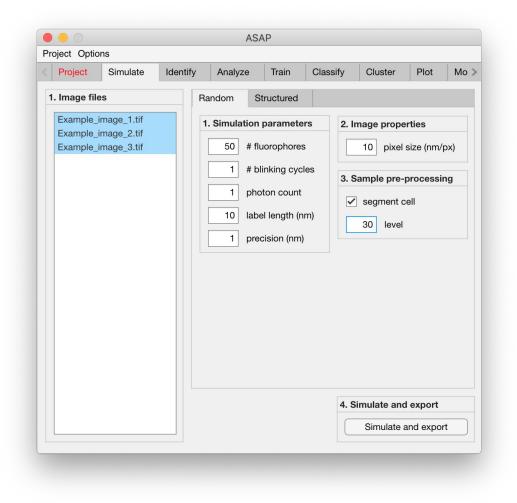
## Simulation of point localizations\*

- 1. Working principle  $\supseteq$
- 2. Selection of files rightarrow
- 3. Definition of simulation parameters  $\square$
- 4. Definition of system properties  $\supseteq$
- 5. Sample segmentation rightarrow
- 6. Data simulation and export rightarrow

# 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 凶.

/ 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 <sup>(1/2)</sup>

#### 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.

	ons		1	1			1	1
Project	Simulate	Identify	Analyze	Train	Classify	Cluster	Plot	Mo >
. Image fil	es	Ra	andom St	tructured				
Example_	image_1.tif image_2.tif image_3.tif	1	1 # bl 1 pho 10 labe	parameter lorophores inking cycl ton count el length (nr cision (nm)	es 3. s	ample pre-	size (nm/p processir	
					4. S	imulate and	l export	

### Simulation of point localizations Definition of simulation parameters <sup>(2/2)</sup>

#### 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.

Project Simulate	Identify	Analyze	Train	Classify	Cluster	Plot	Mo
Image files         Example_image_1.tif         Example_image_2.tif         Example_image_3.tif		. Simulation         5       # e         10       lab         30       # b         1000       # s         1000       ph         100       str         100       pre         0.5       lab	Structured paramete pitopes el length (n plinking cyc tructures oton count ucture size ecision (nm) eling efficie enabled	(nm) 3. 5	Sample pre-	size (nm/j processii	
				4. S	<b>Simulate and</b>		t

# Simulation of point localizations Definition of system properties

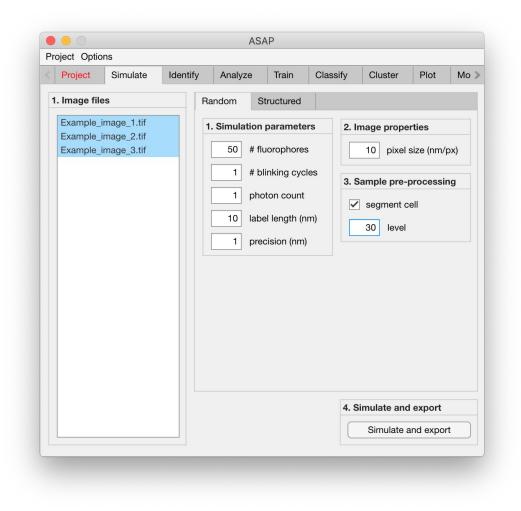
Project	Simulate	Identify	Analyze	Train	Classify	Cluster	Plot	Mo
1. Image fil Example_ Example_		R	andom S . Simulation 50 # fli 1 # b 1 phc 10 lab	tructured	rs 2. II es 3. S m)	mage proper	rties size (nm/p processin	×)
					4. S	<b>imulate and</b> Simulate a	-	

#### 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 <sup>(1/2)</sup>

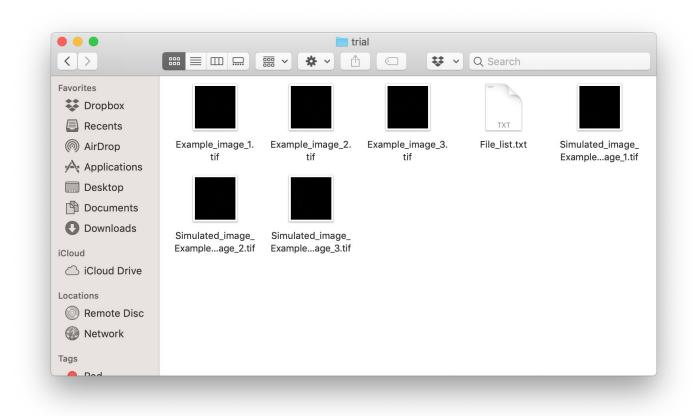
roject Opti		1						1
Project	Simulate	Identify	Analyze	Train	Classify	Cluster	Plot	Mo >
1. Image fi	les	R	andom S	structured				
Example	_image_1.tif _image_2.tif _image_3.tif		1 # b 1 pho 10 lab	parameter uorophores linking cycl oton count el length (ni cision (nm)	es 3. S	imulate and Simulate and	size (nm/p processir ell d export	Ig

#### 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 <sup>(2/2)</sup>



#### Instructions (contd.)

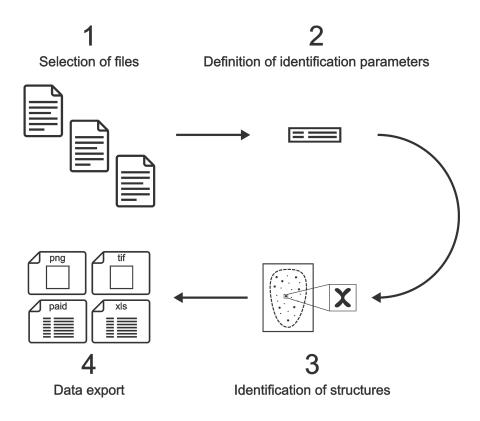
The following 1 output file (per processed image) will be placed in the project folder:

1. **'Simulated\_image\_xx.tif'**: an image file of the simulated localizations.

## **Identification of structures**

- 1. Working principle  $\supseteq$
- 2. Selection of files rightarrow
- 3. Definition of segmentation parameters  $rac{}$
- 4. Definition of image clean-up parameters  $\supseteq$
- 5. Definition of filter parameters  $rac{1}{2}$
- 6. Identification of structures  $rac{}$
- 7. Data export  $\supseteq$

# Identification of structures Working principle



# Identification of structures Selection of files



/ 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 □.

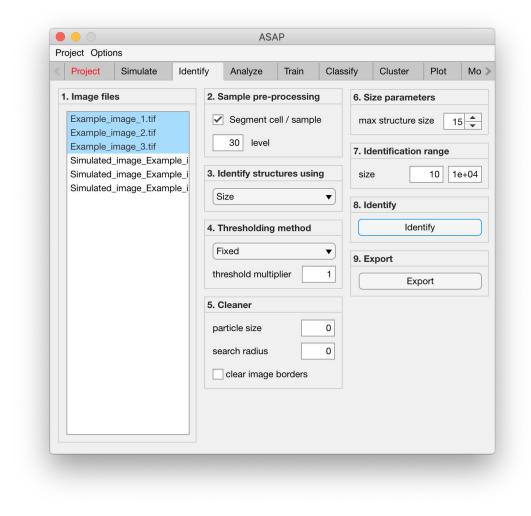
/ Select files by holding the CTRL key and right-clicking the desired files in the listbox in the same panel. Selected files will be highlighted as shown in the figure on the right.

#### § Note 1:

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

Project	Simulate	Identify	Analyze	Train	Class	sify	Cluster	Plot	Мо
1. Image fil	es	2. 5	ample pre-p	processing	J	6. Si	ze paramet	ers	
Example_ Example_ Simulated Simulated	image_1.tif image_2.tif image_3.tif I_image_Exam I_image_Exam I_image_Exam	ple_i 3. I ple_i 8 4. 1 F th 5. 0 pa	Segment c 30 level dentify struct ize 'hresholding ixed ixed cleaner rticle size arch radius clear image	method		7. Id size	x structure s entification e [] entify [der cport Exp	range 10 1 htify	5 ÷

### Identification of structures Definition of segmentation parameters <sup>(1/3)</sup>



#### 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 <sup>(2/3)</sup>

#### 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).

Project Simulate Ident	tify Analyze	Train	Classify	Cluster	Plot	Мо
1. Image files	2. Sample pre-p	processing	6. 9	Size parame	ters	
Example_image_1.tif Example_image_2.tif Example_image_3.tif Simulated_image_Example_i Simulated_image_Example_i Simulated_image_Example_i	<ul> <li>Segment of 30 level     </li> <li>Identify struct Size     </li> <li>Identify struct</li></ul>	tures using method	7.1 g si • 8.1	Export	n range	5 <b>*</b>

### Identification of structures **Definition of segmentation parameters** <sup>(3/3)</sup>

#### 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).

Project	Simulate	Identify	Analyze	Train	Class	ify	Cluster	Plot	Mo
Example_ Example_ Simulated Simulated	es image_1.tif image_2.tif image_3.tif _image_Exam _image_Exam _image_Exam	ble_i ble_i ble_i <b>3. I</b> ble_i <b>4. 1</b> <b>F</b> th	Sample pre-r Segment c 30 level dentify struct Size Thresholding rixed reshold multi Cleaner article size earch radius clear image	ell / sampletures usin	e	ma 7. Id siz	lentify Ider xport	size 1 1 range	5 <b>*</b> e+04

### Identification of structures Definition of image clean-up parameters

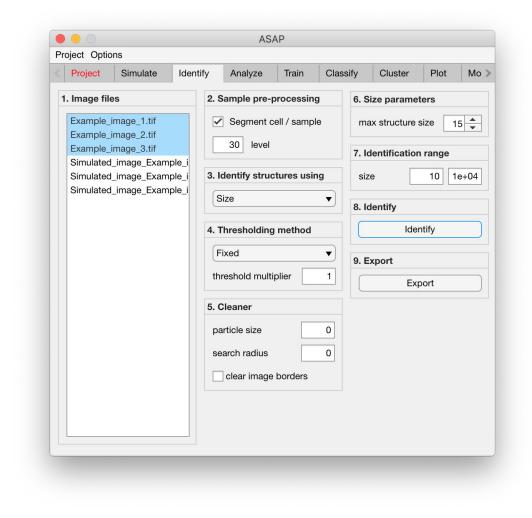
< Project	Simulate	Identify	Analyze	Train	Classi	fy Cluste	er Plot	N
1. Image files	\$	2. 5	Sample pre-p	rocessing	9	6. Size para	meters	
Simulated_	nage_2.tif	a. 1     ble_i     a. 1     ble_i	Segment of     Segment of     adots     a	method		max structu 7. Identifica size 8. Identify 9. Export		15 <b>1</b> e+0

#### 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 <sup>(1/2)</sup>



#### 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 <sup>(2/2)</sup>

Project Simulate Ident	ify	Analyze	Train	Class	sify	Cluster	Plot	Мо	
1. Image files	2. Sa	mple pre-p	rocessing	J	6. Size parameters				
Example_image_1.tif Example_image_2.tif Example_image_3.tif Simulated_image_Example_i Simulated_image_Example_i Simulated_image_Example_i	3. Ide Size 4. The Fixe three 5. Cle parti sear	r <b>esholding</b> ed shold multip	method		7. ld size	lentify Ider xport	10 1	5 <b>•</b> e+04	

#### 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

<	Project	Simulate	Identify		Analyze	Train	Class	sify	Cluster	Plot	Мо
1.	. Image file		2	2. S	ample pre-p			6. Size parameters			
	Example_ Example_ Simulated Simulated	image_1.tif image_2.tif image_3.tif _image_Exam _image_Exam _image_Exam	ple_i 3 ple_i 4	Si 4. T Fi thr 5. C pa	Segment ce 30 level Jentify struct ize hresholding xed eshold multip reaner rticle size arch radius clear image	method		7. ld size	x structure s entification e local entify lder kport Exp	10 1	5 ÷

#### Instructions

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

Please turn over

# Identification of structures

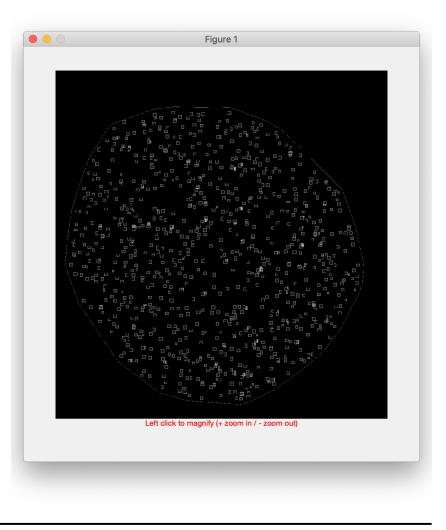
#### Instructions (contd.)

/ 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 <sup>(1/2)</sup>

< Project	Project Simulate Iden		tify Analyze Train Cla		Class	ify	Cluster	Plot	Mo
1. Image files			2. Sample pre-processing			6. Size parameters			
Example_ Example_ Simulated	image_1.tif image_2.tif image_3.tif I_image_Exam I_image_Exam I_image_Exam	ple_i ple_i 3 ple_i 4	Segment of 30 level . Identify struct Size . Thresholding Fixed threshold multi . Cleaner particle size search radius clear image	y method		7. Id size 8. Id	x structure s entification entify Iden cport Exp	range 10 1 tify	5 ÷

#### Instructions

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

Please turn over

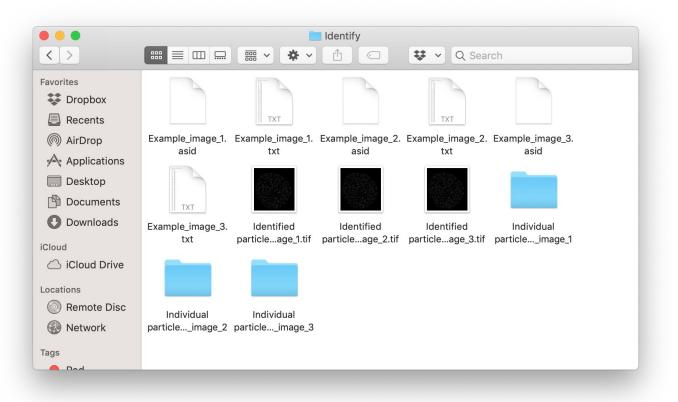
### Identification of structures Data export <sup>(2/2)</sup>

#### 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.
- 'xx.asid' file: a ASAP file containing processing details used by the program for later analysis.
- 'Identified particles\_xx.tif'

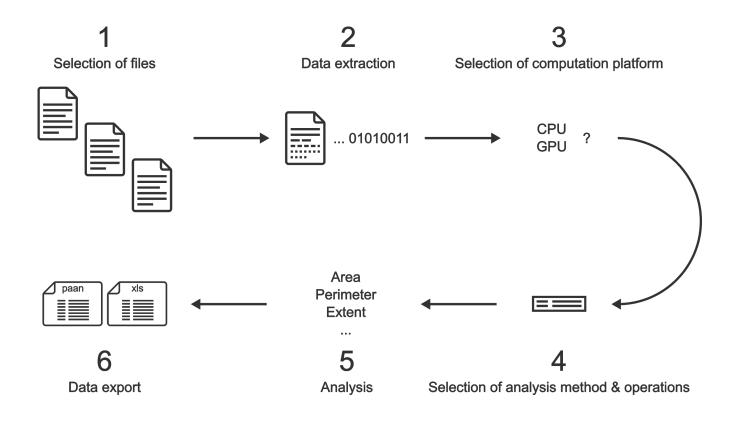
   file: a grayscale tiff image of the processed image with identified particles shown bound within white rectangles.
- 4. 'xx.txt' *file*: text file containing a summary of the structures identified as well as their coordinates in the original image.



## **Geometrical analysis of structures**

- 1. Working principle  $\supseteq$
- 2. Selection of files rightarrow
- 3. Data extraction  $rac{}$
- 4. Definition of pixel size rightarrow
- 5. Selection of computation platform  $rac{}$
- 6. Selection of analysis method rightarrow
- 7. Selection of image operation(s)  $\supseteq$
- 8. Analysis and revision  $\square$
- 9. Data export  $\supseteq$

## Geometrical analysis of structures Working principle



## Geometrical analysis of structures Selection of files



/ 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 rightarrow.

/ 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.

oject Optic	ons		AS	AP						
Project	Simulate	Identify	Analyze	Train	Classify	Cluster	Plot	Мо		
1. asid files		2.	Extract		7. /	Analyze				
1000 - 100 Colored	image_1.asid image_2.asid		Ext	ract		Analyze 8. Review				
	image_3.asid	3.	Set pixel par	ameters	8. F					
			Pixel size (nm	ו)	10	1		Ċ,		
		4.	Select platfo	rm		1 B	1.			
			CPU		•			Bin		
		5.	Select analys	sis metho	d N	/lajor Axis Le	ength Fit =			
			Pixel counting Ellipse fitting	1	Ň	19.2995 /inor Axis Le 09.3575	ength Fit =	-		
			Radial profiling	g	E	Iongation Fit				
			Max ring size	(px)	20	0.60335 Drientation Fi Raw Radial P	it = 14.280	09		
		6.	Select opera	tion(s)	E	xample_ima	ig 🔻 🛛	2		
			None Fill			Export				
			Bridge Close		9.1		port			
			01036							

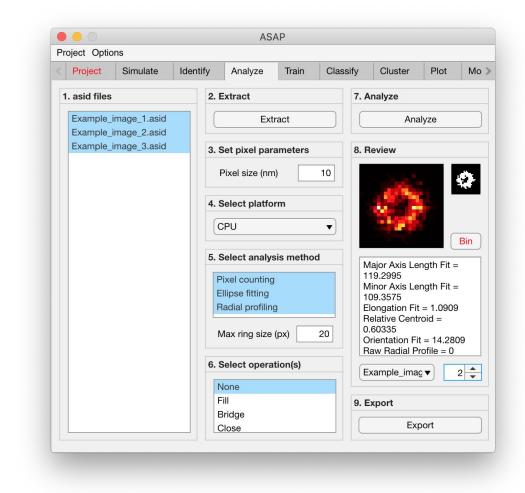
## Geometrical analysis of structures **Data extraction**



#### 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.

### Geometrical analysis of structures Selection of analysis method <sup>(1/3)</sup>

#### 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 <sup>(2/3)</sup>

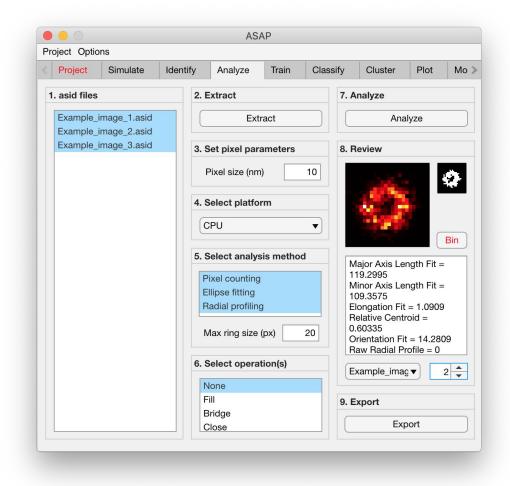
#### Instructions (contd.)

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

roject Options		7.5/	AP							
Project Simulate	Identify	Analyze	Train	Classif	fy	Cluster	Plot	Mo >		
1. asid files	2. E	xtract			7. Analyze					
Example_image_1.asid Example_image_2.asid		Extract				Analyze				
Example_image_3.asid	3. S	et pixel para	ameters		8. Re	eview				
	F	Pixel size (nm	)	10				Ċ,		
	4. S	elect platfo	rm			4 B				
	C	PU						Bin		
	5. S	elect analys	is method	ł	Ma	ajor Axis Le	ngth Fit =			
		ixel counting lipse fitting			Mi	9.2995 nor Axis Le 9.3575	ngth Fit =			
		adial profiling	9		Elc	ongation Fit				
	N	lax ring size	(px)	20	0.6 Or	50335 ientation Fit aw Radial Pr	:= 14.280	09		
	6. S	elect opera	tion(s)		Ex	ample_ima		2		
	N	one			9. Ex	ort				
		ridge lose					oort			

### Geometrical analysis of structures Selection of analysis method <sup>(3/3)</sup>



#### 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

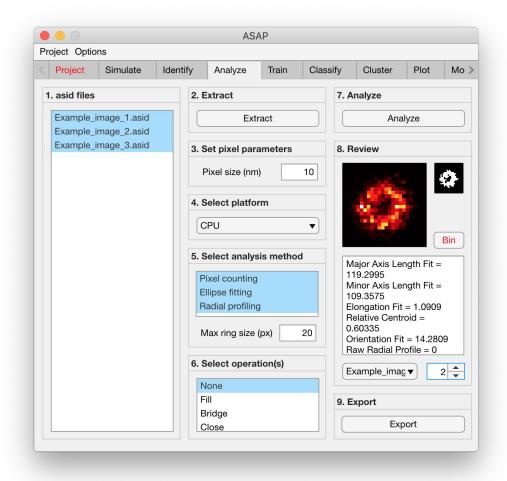
The panel titled '6. Select operation(s)' contains 1 optional parameter:

#### 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
- 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.

Project	Simulate	Identify	Analyze	Train	Classif	fy	Cluster	Plot	Мо	
1. asid files		2.	Extract			7. Ana	lyze			
Example_im Example_im Example_im	nage_2.asid			ract		Analyze				
Example_in	lage_0.asia		Set pixel para Pixel size (nm		10	8. Rev	iew		0	
			Select platfo	rm	▼		2		Bin	
		F	Select analys Pixel counting Ellipse fitting Radial profiling		d	Major Axis Length Fit 119.2995 Minor Axis Length Fit 109.3575 Elongation Fit = 1.090 Relative Centroid =			-	
			Max ring size	(px)	20	0.60 Orie		t = 14.280	09	
		6. 9	Select opera	tion(s)		Exar	nple_ima	g 🔻 🛛	2	
			lone							
			-ill Bridge			9. Exp	ort			
			Close				Exp	oort		

### Geometrical analysis of structures Analysis and revision <sup>(1/2)</sup>



#### Instructions

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

### Geometrical analysis of structures Analysis and revision <sup>(2/2)</sup>

#### 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.

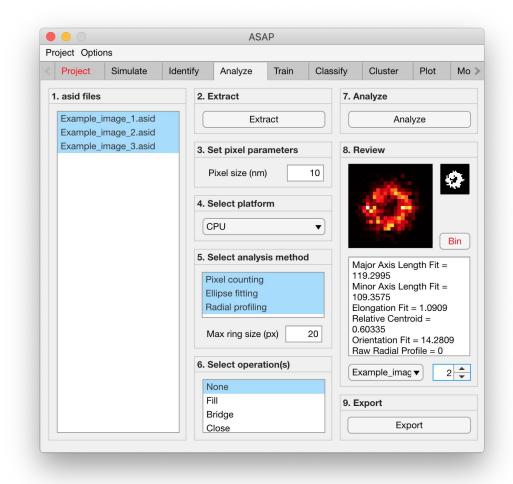
/ The **drop down menu** in same panel can be used to access the analyzed files. Selecting a different file from the drop down menu will result in the text box and large & small in-app figures updating accordingly.

/ 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.

1	ct Optio	1					1		1	-		
P	roject	Simulate	Ident	tify	Analyze	Train	Classify	Cluster	Plot	Мо		
. a	sid files			2. E	xtract		7.	Analyze				
Example_image_1.asid Example_image_2.asid					Ext	ract		Analyze				
	Example_image_2.asid			3. S	et pixel par	ameters	8.	8. Review				
				P	'ixel size (nm	)	10			¢		
				4. S	elect platfo	rm		1 B				
				С	PU		•			Bin		
				5. S	elect analys	sis metho	d I	Major Axis Le	ngth Fit =			
					xel counting lipse fitting			119.2995 Minor Axis Le	ength Fit =	-		
					adial profiling	g		109.3575 Elongation Fit Relative Cent		9		
				N	ax ring size	(px)	20	0.60335 Orientation Fi Raw Radial P	t = 14.280	09		
				6. S	elect opera	tion(s)	(	Example_ima	g▼	2		
					one							
				Fi	ll ridge		9.	Export				
					lose			Ex	port			

### Geometrical analysis of structures Data export <sup>(1/2)</sup>

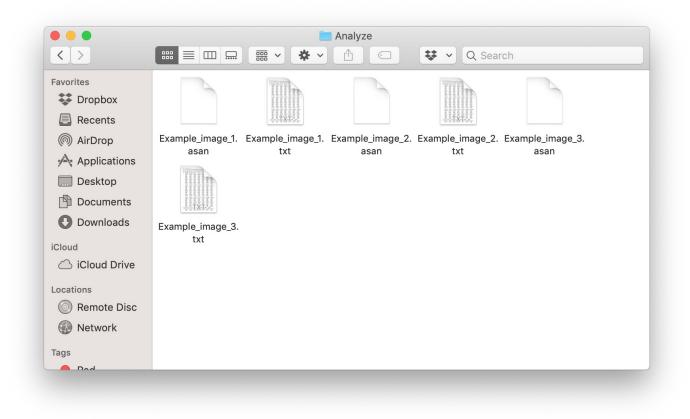


#### Instructions

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 <sup>(2/2)</sup>



#### Instructions (contd.)

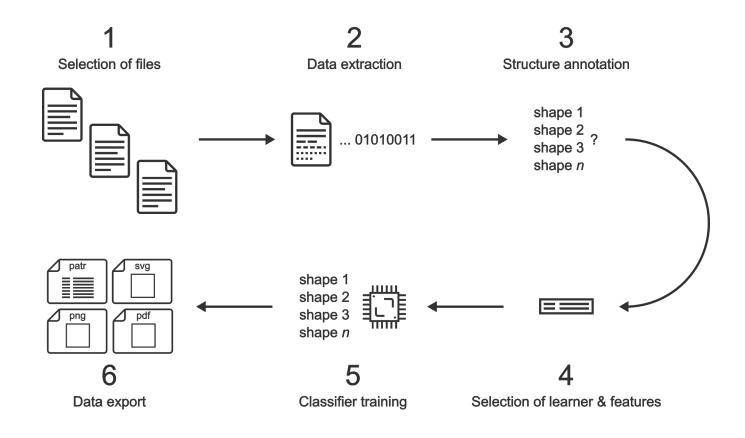
A folder named '**Analyze**' will be created in the output folder and the following 2 output files (per processed image) will be placed in the folder:

- 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  $\supseteq$
- 2. Selection of files rightarrow
- 3. Data extraction rightarrow
- 4. Structure annotation  $\square$
- 5. Selection of learner and features  $\supseteq$
- 6. Classifier training raining
- 7. Data export 🗵

# Classifier training Working principle



### Classifier training Selection of files



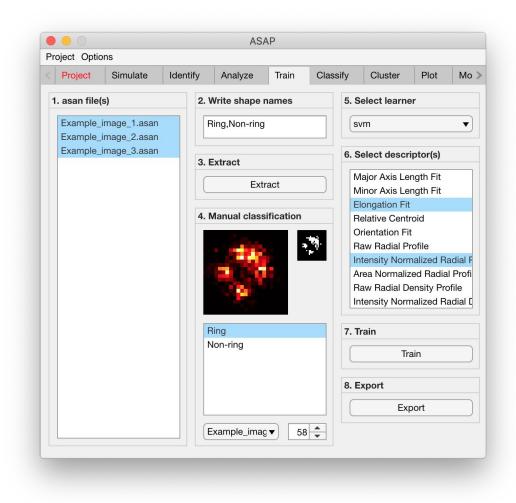
/ Select the 'Train' tab from the tabs' bar.

/ 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.

\$	Project	Simulate	Identify	/	Analyze	Train	Class	ify	Cluster	Plot	Mo		
1	. asan file(	s)		2. Write shape names					5. Select learner				
	1000 - 100 -	image_1.asan image_2.asan		Ring,Non-ring					'n		•		
Example_image_3.asan				3. Extract				6. S	elect descri	iptor(s)			
				Extract  4. Manual classification					Major Axis Length Fit Minor Axis Length Fit				
									Elongation Fit Relative Centroid				
							<b>*</b> ).		rientation Fit aw Radial Pr	-			
						-			tensity Norn				
					- e -			Ra	ea Normaliz aw Radial De tensity Norn	ensity Pro	ofile		
				Ring					7. Train				
				N	on-ring				Tra	ain			
								8. E	xport				
									Exp	port			
				E	xample_imag	▼ 58							

## 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.

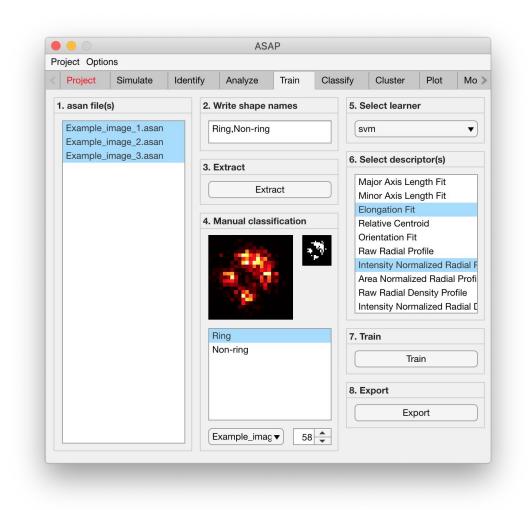
/ The names of the shapes will be displayed in the **text box** in the same panel.

/ 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.

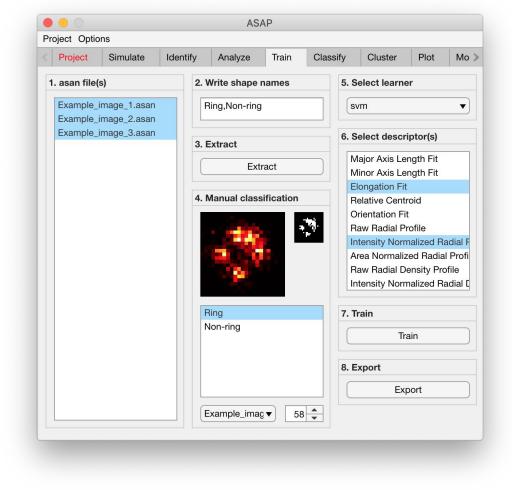
/ The **numeric spinner** in the same panel contains the IDs of the structures belonging to the file selected in the drop down menu. Scrolling the spinner will result in the large & small in-app figures updating accordingly.

/ 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

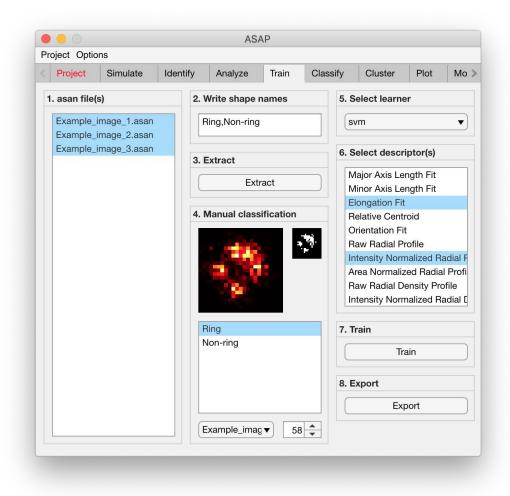


#### Instructions

/ 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 <sup>(1/2)</sup>



#### 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:

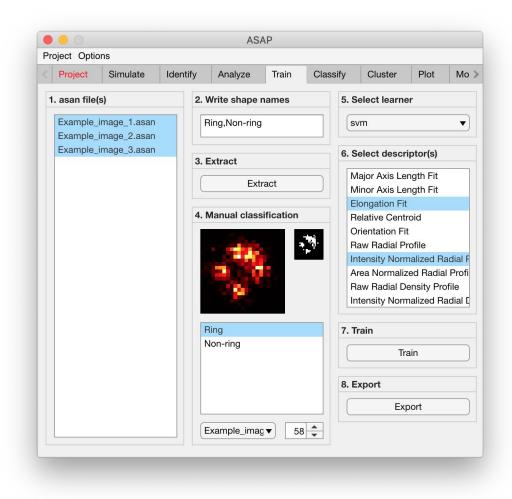
- 1. Increase the number of annotated structures  $\mathfrak{L}$ .
- 2. Select another learner  $\supseteq$ .
- Modify or expand the set of features used for discrimination ≥.

and, re-run the classifier.

### Classifier training Classifier training <sup>(2/2)</sup>



## Classifier training Data export (1/2)

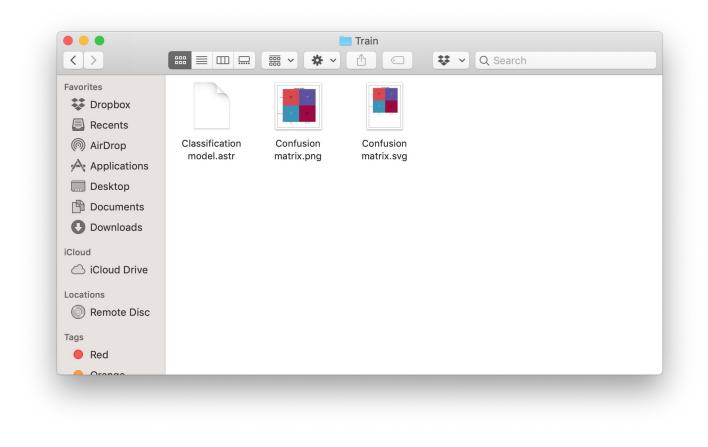


#### Instructions

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

Please turn over

### Classifier training Data export <sup>(2/2)</sup>



#### Instructions (contd.)

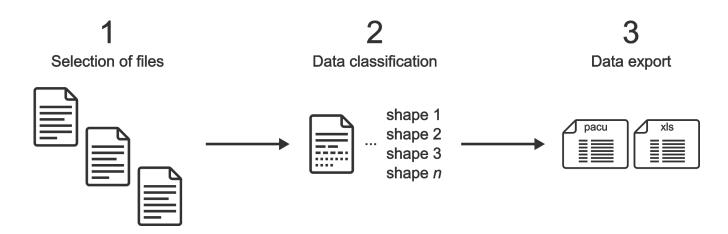
A folder named '**Train**' will be created in the output folder and the following 3 output files will be placed in the folder:

- 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  $\supseteq$
- 2. Selection of files  $\square$
- 3. Data extraction  $\square$
- 4. Structure reassignment rightarrow
- 5. Structure classification and revision  $\supseteq$
- 6. Data export 🗵

# Classification of structures Working principle



## Classification of structures **Selection of files**



/ 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..

Project	Simulate	Identify	Analyze	Train	Classif	y Cluster	Plot	Мо		
1. asan file	s)	3.	Extract			5. Classify				
100 C 100	image_1.asan image_2.asan		Ext	ract		Classify				
	image_3.asan	4.	Re-assign			6. Review				
			Assignment	# 1				¢2:		
		5	Shape					÷.,		
			Non-ring			- 19 C				
			Ring			- A		Bin		
			Re-assig	a to		Non-ring				
			Bin	110	•	Ring				
		F	eature							
			Elongation	Fit	•					
		E	Bounds		_	Example_ima	g▼	3 🐥		
			>▼		0	7. Export				
			□ > ▼		0	Ex	port			

## Classification of structures **Data extraction**



#### Instructions

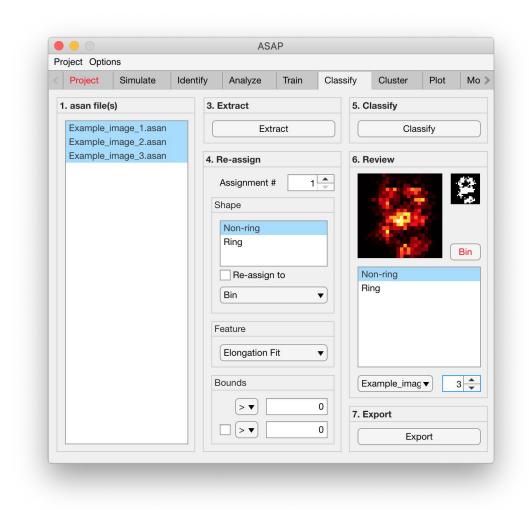
Extract data by pressing the 'Extract' button in the panel titled '3. Extract'.

#### Instructions

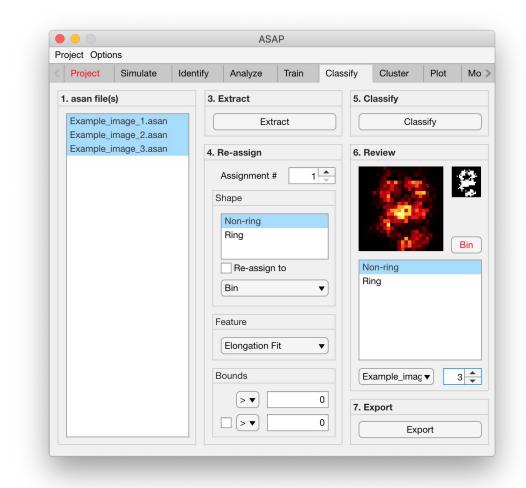
To re-assign shapes based on hard limits imposed on the extracted features:

- 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.
- 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.

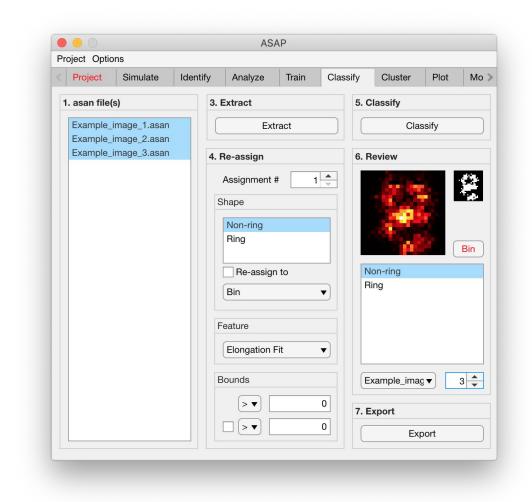
/ The names of the shapes will be displayed in the **text box** in the same panel.

/ 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.

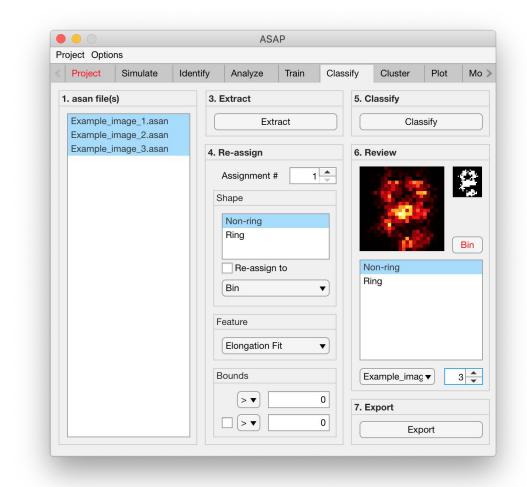
/ The **numeric spinner** in the same panel contains the IDs of the structures belonging to the file selected in the drop down menu. Scrolling the spinner will result in the large & small in-app figures updating accordingly.

/ 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 <sup>(2/2)</sup>



### Classification of structures Data export <sup>(1/2)</sup>

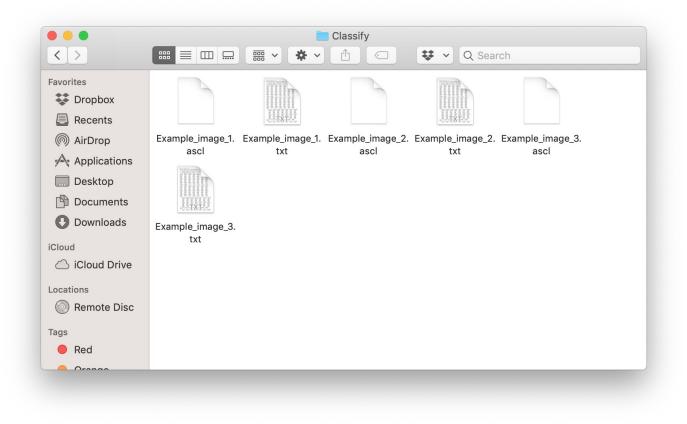


#### Instructions

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

Please turn over

### Classification of structures Data export <sup>(2/2)</sup>



#### Instructions (contd.)

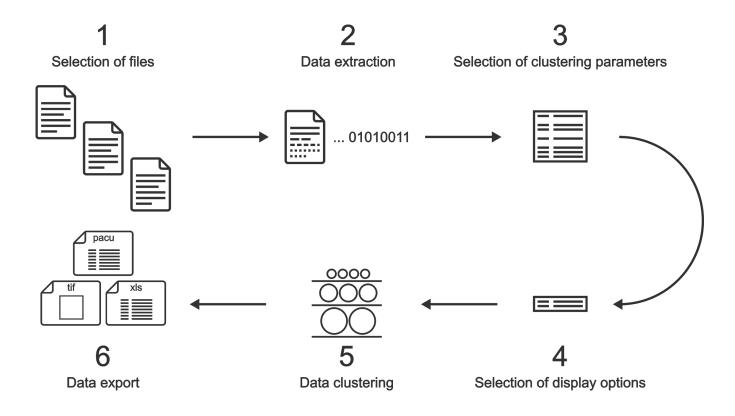
A folder named **'Classify'** will be created in the output folder and the following 2 output files (per processed image) will be placed in the folder:

- 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.

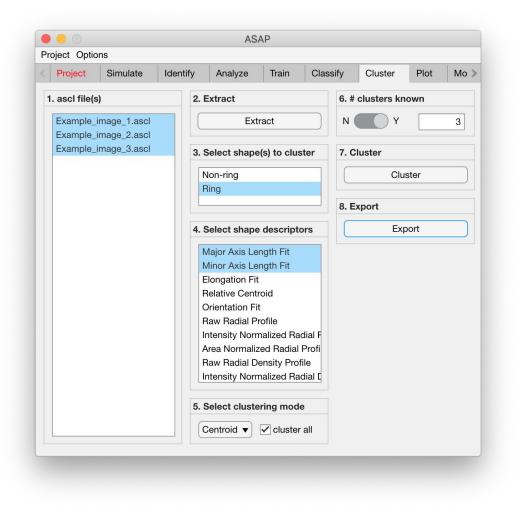
### **Cluster analysis**

- 1. Working principle  $\supseteq$
- 2. Selection of files  $\square$
- 3. Data extraction rightarrow
- 4. Selection of clustering parameters  $\supseteq$
- 5. Data clustering  $rac{}$
- 6. Data export 🗵

# Cluster analysis Working principle



## Cluster analysis Selection of files



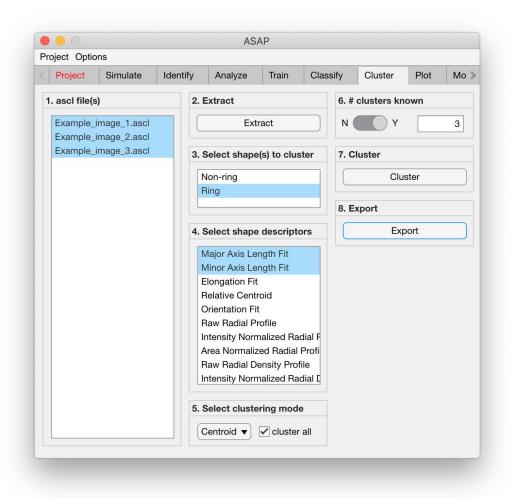
#### Instructions

/ 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 rightarrow.

/ 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

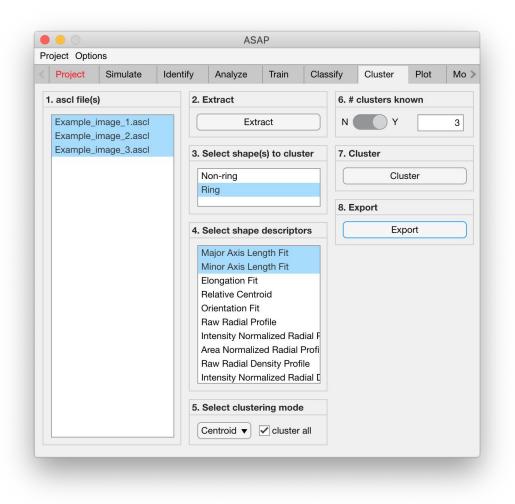


#### Instructions

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

#### Cluster analysis

## Selection of clustering parameters (1/4)

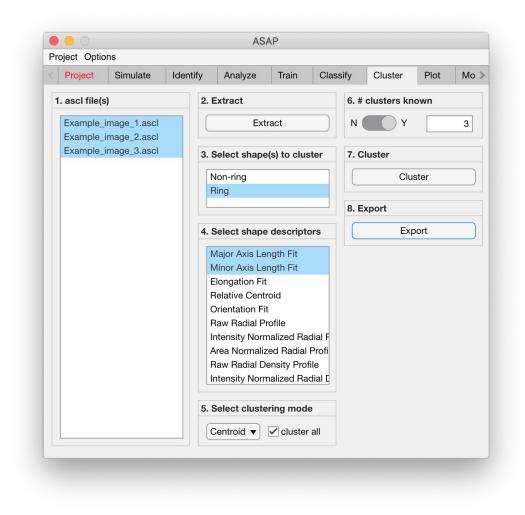


#### Instructions

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 <sup>(3/4)</sup>

#### 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.

1	Dustant	Simulate	Lala and if	c	A	Tusta	Olasai	٤.	Oliveter	Dist	Mo	
	Project	Simulate	Identif	ry	Analyze	Train	Classi	ту	Cluster	Plot	IVIO	
1.	ascl file(s	5)		2. E	xtract			6. # clusters known				
	Example_	image_1.ascl			Ext	ract		N	Y		3	
	Example_	image_2.ascl										
	Example_	image_3.ascl		3. S	elect shape	(s) to clus	ter	7. CI	uster			
				N	on-ring				Clus	ster		
					ing							
								8. Ex	port			
				4. S	elect shape	descripto	ors		Exp	ort		
					ajor Axis Le	•						
					inor Axis Le ongation Fit	-						
					elative Centr							
				0	rientation Fit							
				R	aw Radial Pi	ofile						
					tensity Norn							
					rea Normaliz							
					aw Radial D							
				In	tensity Norn	nalized Rac						
				5. S	elect cluste	ring mode	•					
				С	entroid 🔻	<ul> <li>cluster</li> </ul>	all					

## Cluster analysis Selection of clustering parameters <sup>(4/4)</sup>

## Instructions (contd.)

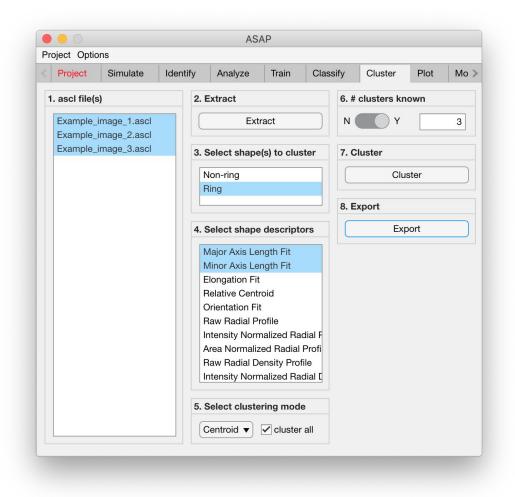
/ If the number of clusters is known *a priori*, pull the switch in the panel titled '**6. # clusters known**' to the position labeled '**Y**'. If otherwise, pull the switch to the position labeled '**N**'.

/ 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.

Project	Simulate	Identify	Analyze	Train	Classify	Cluster	Plot	Mo		
l. ascl file(	s)	2. 1	Extract		6.	6. # clusters known N Y 3 7. Cluster				
	image_1.ascl image_2.ascl		Ext	ract						
	image_3.ascl	3. 5	Select shape	e(s) to clus	ter 7.					
			lon-ring			Cluster				
		F	Ring		8.	Export				
		4. 5	Select shape	descripto	ors	Exp	oort			
			/lajor Axis Le /linor Axis Le	•						
			Elongation Fit Relative Cent							
			Drientation Fi							
			Raw Radial P							
			ntensity Norr							
			Area Normaliz							
			Raw Radial D ntensity Norr							
		5. 5	Select cluste	ering mode	)					
			Centroid 🔻	✓ cluster	all					

## Cluster analysis Data clustering <sup>(1/3)</sup>



#### Instructions

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

## Cluster analysis Data clustering <sup>(2/3)</sup>

## Instructions (contd.)

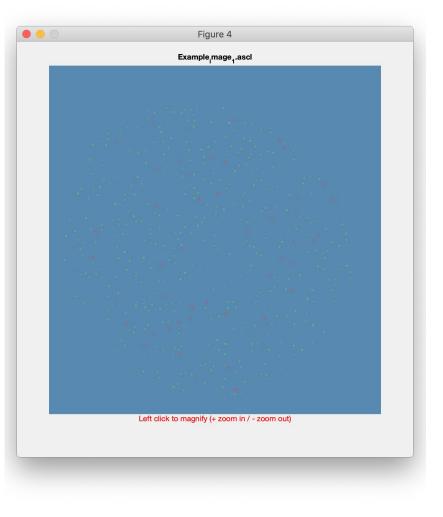
/ If the user selects to show the clustered images, a number of figures equal to the number of processed files and similar to the one shown on the right will be displayed.

/ 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.



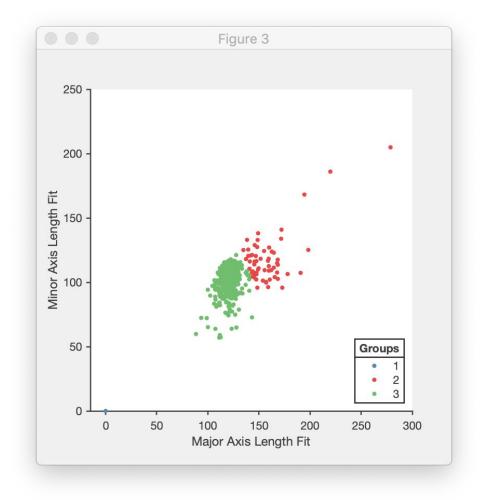
## Cluster analysis **Data clustering** <sup>(3/3)</sup>

### Instructions (contd.)

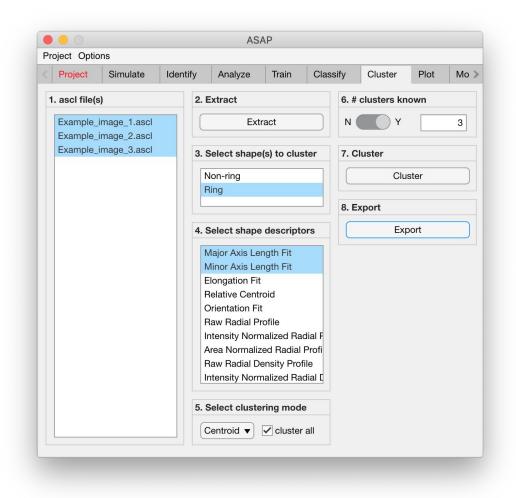
/ If the user selects to cluster all data, 1 plot similar to the one shown on the right will be displayed. The *clustering plot* maps structures across 2 similar or different dimensions (i.e. shape descriptors) and groups them into different clusters according to the chosen clustering mode.

/ 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 <sup>(1/2)</sup>



### Instructions

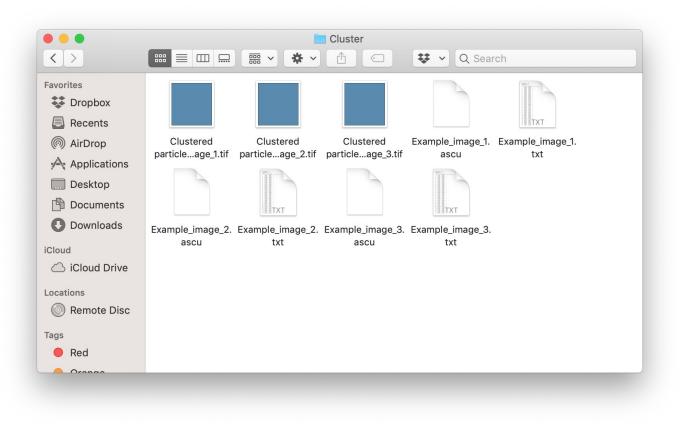
Export data by pressing the 'Export' button in the panel titled '8. Export'.

## Cluster analysis Data export <sup>(2/2)</sup>



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:

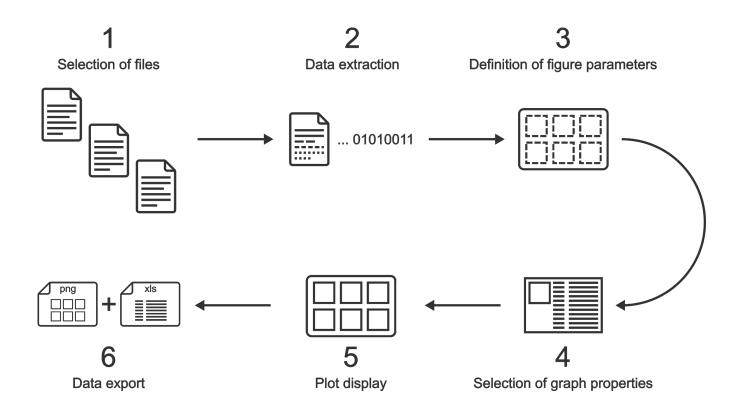
- 1. **'xx.txt'** *file*: text file containing a summary of the structures clustered as well as their parameters.
- 2. **'xx.ascu' file**: a ASAP file containing clustering details used by the program for later analysis.
- 'Clustered particles\_xx.tif' - file: tiff file of the clustered image.



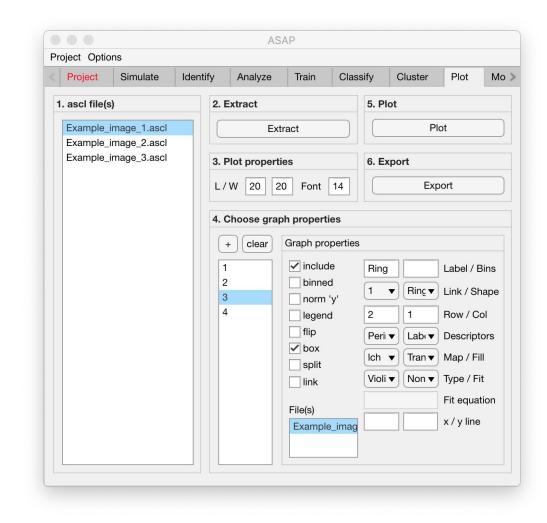
## Statistical and visual analysis

- 1. Working principle  $rac{}$
- 2. Selection of files  $rac{}$
- 3. Data extraction rightarrow
- 4. Definition of plot properties  $\supseteq$
- 5. Selection of graph properties  $\square$
- 6. Data plotting rightarrow
- 7. Data export  $rac{}$

# Statistical and visual analysis Working principle



## Statistical and visual analysis Selection of files



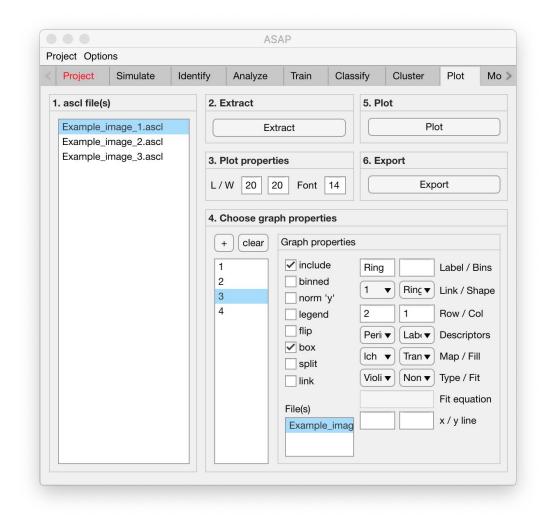
### Instructions

/ 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 rightarrow.

/ 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.

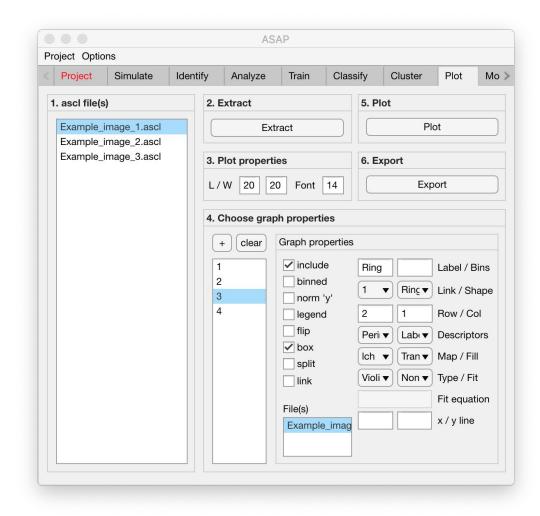
## Statistical and visual analysis Data extraction



### 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 <sup>(1/6)</sup>

#### 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.

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### 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 <sup>(2/6)</sup>

Project	Simulate	Identify	Analyze	Train	Classi	ify	Cluster	Plot	Мо
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### 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 <sup>(3/6)</sup>

Project	Simulate	Identify	Analyze	Train	Class	ify	Cluster	Plot N	
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## Statistical and visual analysis Selection of graph properties <sup>(4/6)</sup>

#### Instructions (contd.)

- 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.

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## Statistical and visual analysis Selection of graph properties <sup>(5/6)</sup>

#### 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**'.

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## Statistical and visual analysis Selection of graph properties <sup>(6/6)</sup>

### Instructions (contd.)

- 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.
   Plot property: type a fitting.
- 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) / I \* 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.

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				File(s) Example	e_imag			x / y line		

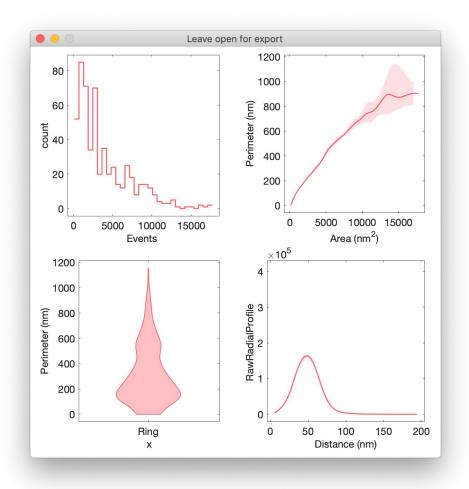
# Statistical and visual analysis Data plotting <sup>(1/2)</sup>

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### Instructions

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

# Statistical and visual analysis Data plotting <sup>(2/2)</sup>



### 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.

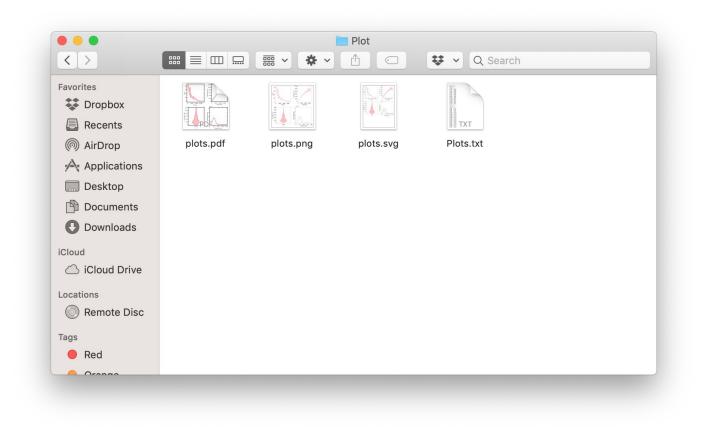
# Statistical and visual analysis **Data export** <sup>(1/2)</sup>

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				<pre>_ logonal _ flip _ box _ split _ link File(s) Example_</pre>		Peri ▼ Ich ▼ Violi ▼	Labı▼ Tran▼ Non▼	Descripto Map / Fill Type / Fit Fit equations x / y line	rs

### Instructions

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

## Statistical and visual analysis **Data export** <sup>(2/2)</sup>



## Instructions (contd.)

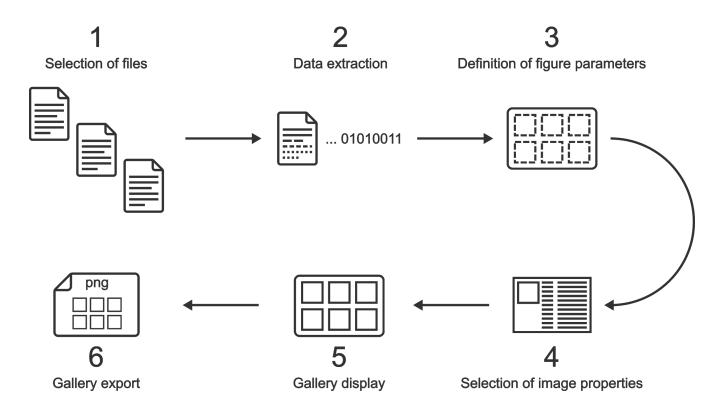
A folder named '**Plot**' will be created in the output folder and the following 4 output files will be placed in the folder:

- 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.

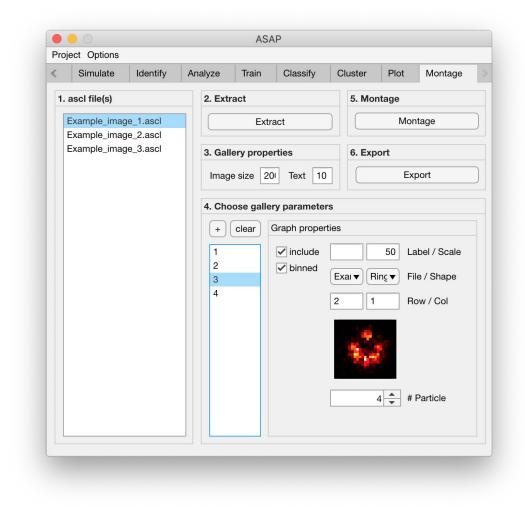
## **Montage construction**

- 1. Working principle  $\supseteq$
- 2. Selection of files rightarrow
- 3. Data extraction rightarrow
- 4. Definition of montage properties  $\supseteq$
- 5. Selection of montage parameters  $\supseteq$
- 6. Montage display ⊾
- 7. Montage export  $rac{}$

# Gallery construction Working principle



## Montage construction Selection of files



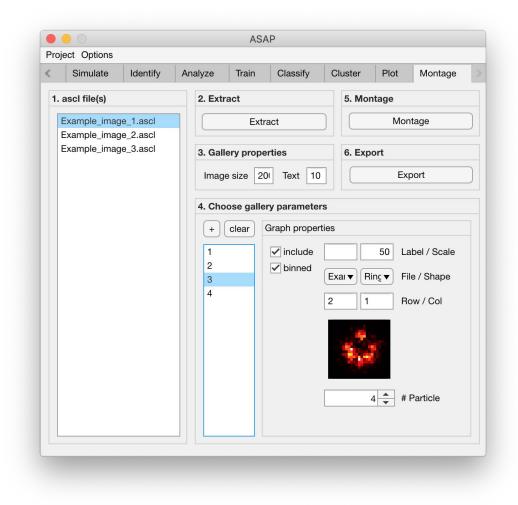
### Instructions

/ 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 rightarrow.

/ 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.

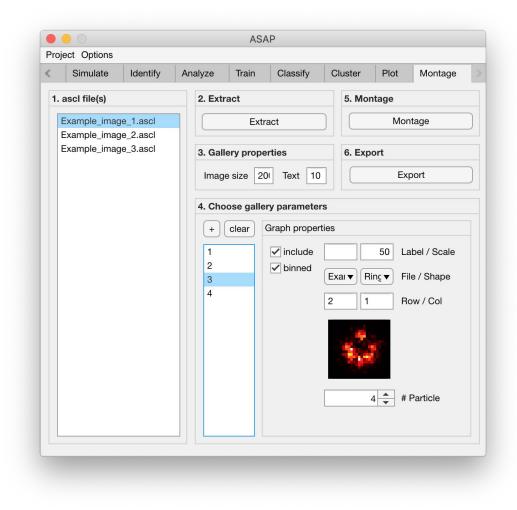
## Montage construction Data extraction



### 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 <sup>(1/3)</sup>

### 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.

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## Montage construction Selection of montage parameters <sup>(2/3)</sup>

### 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**'.

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## Montage construction Selection of montage parameters <sup>(3/3)</sup>

#### Instructions (contd.)

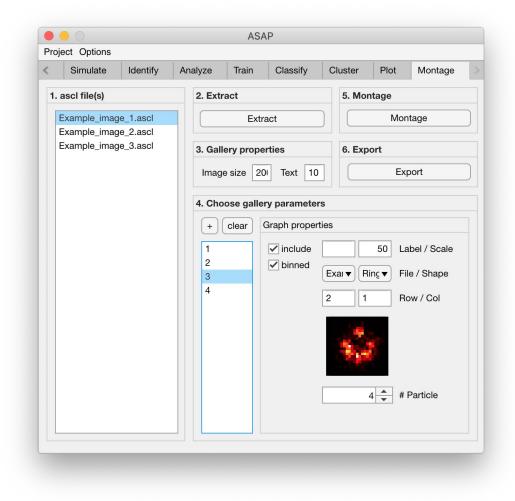
- 5. **Image property:** select a file from the dropdown menu labeled '**File**'.
- 6. **Image property:** select a shape from the dropdown menu labeled '**Shape**'.
- 7. Image properties: select the row and column to which the selected image belongs from the dropdown menus labeled 'Row' and 'Col' respectively.
- 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.

Simulate Identify	Analyze	Train	Classify	Cluste	r Plot	Montage
simulate Identity . ascl file(s) Example_image_1.ascl Example_image_2.ascl Example_image_3.ascl	2. Extr	act Extra ery prope e size 20 ose galler	act	5. N 6. E	Montage Mo Export Expo	Montage ntage aport abel / Scale ile / Shape tow / Col Particle

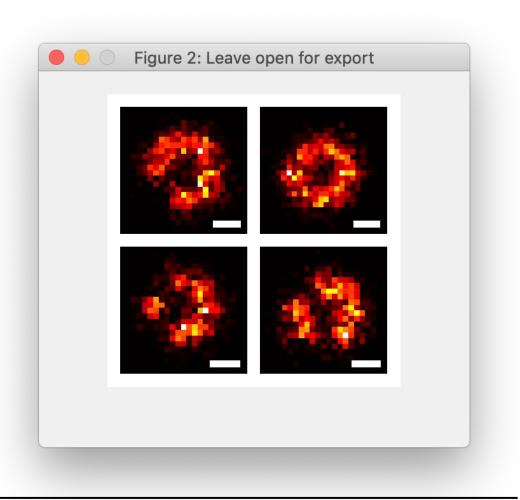
## Montage construction Montage display <sup>(1/2)</sup>



### Instructions

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

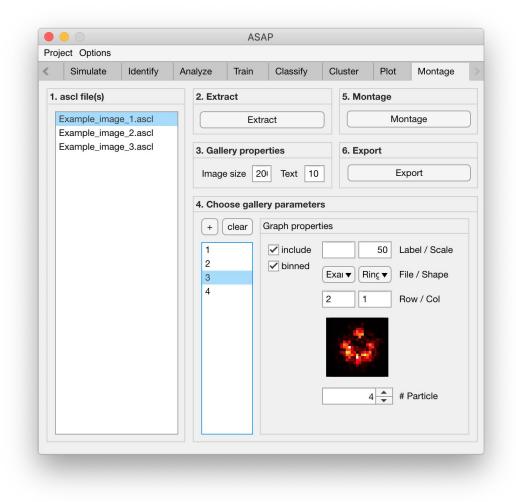
Montage construction Montage display <sup>(2/2)</sup>



### Instructions

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

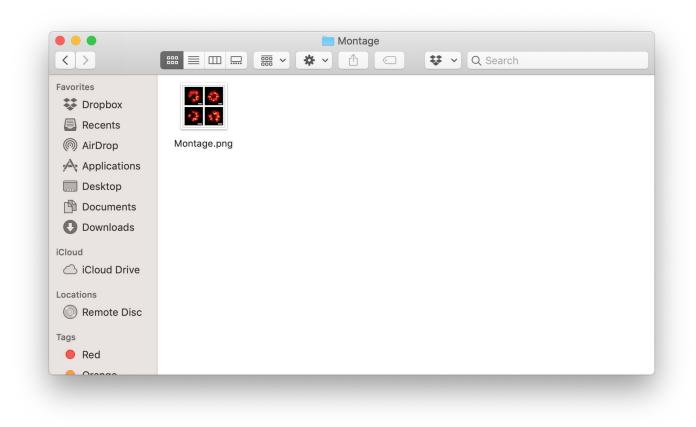
## Montage construction Montage export <sup>(1/2)</sup>



#### Instructions

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

## Montage construction Montage export <sup>(2/2)</sup>



### 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.