

ASAP guide: automation script syntax

Version 1.0

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Introduction

Operation of ASAP can be fully-automated for large data sets through the automation script; a single text file containing all parameters required to process and visualize data. This addition to ASAP is a step forward in the computational analysis required for future high-throughput super resolution microscopy.

The syntax of the automation script combines the MATLAB syntax together with exclusive wording elements to ensure easy learning and reproducibility.

Loading the automation script file

To load the automation script file, press the load automation script button on the project module. When prompted, choose an automation script (.txt) file.

The user will be notified through the command prompt whether the automation script has been successfully loaded or not.

If the automation script is not successfully loaded, the user will be notified, through the command prompt, as to where the problem lies as well as an indicator to the nature of the problem. For further information on the syntax of the produced error, refer to the **ASAP guide: description of error and warning messages**.

Refer to the next section for acquaintance with the correct syntax of the automation script.

Presence of a classification model file

A classification model (.astr) file has to be present in the project folder for the automation script to be successfully loaded.

A classification model file can only be produced through the training module which is only accessible through the GUI. For further information on ASAP's modules, refer to the **ASAP guide: user manual**.

Reference to files

In the automation script, files in the selected project folder are referred to using a unique numeric ID which can be found in the (File list.txt) file exported in the project folder following its selection.

Syntax of the automation script file

The automation script follows a single syntax throughout and obeys the following rules:

- 1. All lines starting with the % character are comments which are not parsed by the script checker.
- 2. Unless otherwise mentioned, lateral spaces are not allowed and will produce an error.
- 3. Other lines should obey one of the following syntaxes:
 - a. mainParam.subParam=entity#From->entity#To:value
 - b. mainParam.subParam=value

Refer to the next section for information on mainParam, subParam, entity#From, entity#To and value.

Parameter values

The mainParam, subParam, entity#From, entity#To and value parameters can only take specific values. The table below (divided in 8 blocks) describes the values assignable to each parameter.

mainParam	subParam	file#From	file#To	value
options	displayImages	*N1	*N2	true,false
	displayPlots	*N1	*N2	true,false
	exportRawData	*N1	*N2	true,false
	exportImages	*N1	*N2	true,false
	exportPlots	*N1	*N2	true,false

<u>Example</u>

options.displayImages=1->4:false

^{*}N1 index number of file. Has to be less than number of files in selected project folder.

^{*}N2 index number of file. Has to be less than number of files in selected project folder and more than or equal to value assigned to file#From.

Block 2				
mainParam	subParam	file#From	file#To	value
simulationParam	simulate	Leave empty	Leave empty	true,false
	simulationMode	*N3	*N4	*N5
	pixelSize	*N3	*N4	1-inf
	segment	*N3	*N4	true,false
	segmentationLevel	*N3	*N4	1-inf
	numCycle	*N3	*N4	1-inf
	photonCount	*N3	*N4	1-inf
	labelLength	*N3	*N4	0-inf
	lateralPrecision	*N3	*N4	1-inf
	numFluorophore *N1	*N3	*N4	1-inf
	numEpitope *N2	*N3	*N4	1-inf
	numStructure *N2	*N3	*N4	1-inf
	structureSize *N2	*N3	*N4	1-inf
	labelingEfficiency *N2	*N3	*N4	0.1-1
	rotationEnabled *N2	*N3	*N4	true,false

simulationParam.photonCount=1->4:1000

Notes

^{*}N1 only assignable when value of simulationMode subParam is Random.

^{*}N2 only assignable when value of simulationMode subParam is Structured.

^{*}N3 index number of file. Has to be less than number of files in selected project folder.

^{*}N4 index number of file. Has to be less than number of files in selected project folder and more than or equal to value assigned to file#From.

^{*}N5 value assignable is Random or Structured.

Block 3				
mainParam	subParam	file#From	file#To	value
identificationParam	identify	Leave empty	Leave empty	true,false
	segment	*N3	*N4	true,false
	segmentationLevel	*N3	*N4	1-inf
	identificationMode	*N3	*N4	*N5
	thresholdMode	*N3	*N4	*N6
	thresholdMultiple	*N3	*N4	0-inf
	clearBorder	*N3	*N4	true,false
	maxClearParticleSize	*N3	*N4	0-inf
	maxClearSearchRadius	*N3	*N4	0-inf
	clusterParamD *N1	*N3	*N4	1-inf
	clusterParamS *N1	*N3	*N4	1-inf
	maxStructureSize *N2	*N3	*N4	1-inf
	minSize	*N3	*N4	0-inf
	maxSize	*N3	*N4	0-inf

identificationParam.identificationMode=1->2:Density;3->4:Size

Notes

^{*}N1 only assignable when value of identificationMode subParam is Density.

^{*}N2 only assignable when value of identificationMode subParam is Size.

^{*}N3 index number of file. Has to be less than number of files in selected project folder.

^{*}N4 index number of file. Has to be less than number of files in selected project folder and more than or equal to value assigned to file#From.

^{*}N5 value assignable is Connectivity or Size.

^{*}N6 value assignable is Fixed or Relative.

Block 4				
mainParam	subParam	file#From	file#To	value
analysisParam	analyze	Leave empty	Leave empty	true,false
	pixelSize	*N2	*N3	1-inf
	analysisPlatform	*N2	*N3	*N4
	analysisMethod	*N2	*N3	*N5
	maxRingSize *N1	*N2	*N3	1-inf
	operations	*N2	*N3	*N6

analysisParam.analysisMethod=1->4:Pixel counting, Radial profiling

Notes

*N1 only assignable when value, or one of value(s), of analysisMethod subParam is Radial profiling.

*N2 index number of file. Has to be less than number of files in selected project folder.

*N3 index number of file. Has to be less than number of files in selected project folder and more than or equal to value assigned to file#From.

*N4 value assignable is CPU or GPU.

*N5 values assignable are Pixel counting, Ellipse fitting and / or Radial profiling. If more than one value is to be assigned they should be separated by the , character.

*N6 values assignable are either None or one or more of the following: Fill, Bridge, Close, Open, Clean, Rotate, Center and / or Resize.

Block 5				
mainParam	subParam	file#From	file#To	value
classificationParam	classify	Leave empty	Leave empty	true,false
	assignmentNum	*N2	*N3	0-inf
	shapeBefore *N1	*N2	*N3	*N4
	shapeAfter *N1	*N2	*N3	*N5
	descriptor *N1	*N2	*N3	*N6
	includeSecondBound	*N2	*N3	true,false
	*N1	*N2	*N3	0-inf
	firstBound *N1	*N2	*N3	0-inf
	secondBound *N1	*N2	*N3	*N7
	firstEquality *N1	*N2	*N3	*N7
	secondEquality *N1			

classificationParam.assignmentNum=1->4:3
classificationParam.shapeBefore=1->4:Line/Dot/Arc,Ring,Arc
classificationParam.shapeAfter=1->4:Dot/Ring/Line

Notes

*N3 index number of file. Has to be less than number of files in selected project folder and more than or equal to value assigned to file#From.

*N4 following rules are obeyed:

- 1. Values assignable are shape names as inserted during the training process in the Train module of the GUI.
- 2. Number of groups of shape names assignable should be equal to the value of the assignmentNum subParam.
- 3. Number of shape names assignable per group is limited to number of shapes.
- 4. Shape names should be separated by the , character.
- 5. Groups should be separated by the / character.

*N5 following rules are obeyed:

- 1. Values assignable are shape names as inserted during the training process in the Train module of the GUI.
- 2. Number of groups of shape names assignable should be equal to the value of the assignmentNum subParam.
- 3. Number of shape names assignable per group is limited to one shape.
- 4. Groups should be separated by the / character.

*N6 values assignable depend on value of the analysisMethod subParam of the analysisParam mainParam. If value is:

- 1. Pixel counting, value assignable is Area, Filled Area, Convex Area, Perimeter, Euler Number, Eccentricity, Solidity, Orientation, Extent, Major Axis Length, Minor Axis Length, Form Factor, Roundness, Elongation, Fill Ratio, Mean Intensity, Minima Number, Minima Intensity, Minima Eccentricity, Minima Area, Minima Convex Area, Maxima Number, Maxima Intensity, Maxima Eccentricity, Maxima Area, Maxima Convex Area, Segment Total Length, Segment Number Intersections Or Objects.
- 2. Ellipse fitting, value assignable is Major Axis Length Fit, Minor Axis Length Fit, Elongation Fit, Relative Centroid or Orientation Fit.

*N7 following rules are obeyed:

- 1. Value assignable is >, < or =.
- 2. Number of groups of assigned equalities should be equal to the value of the assignmentNum subParam.

^{*}N1 only assignable when value of assignmentNum subParam is greater than 0.

^{*}N2 index number of file. Has to be less than number of files in selected project folder.

- Number of equalities assigned per group is limited to one equality.
 Groups should be separated by the / character.

Block 6				
mainParam	subParam	file#From	file#To	value
clusteringParam	cluster	Leave empty	Leave empty	true,false
	shape	*N2	*N3	*N4
	descriptor	*N2	*N3	*N5
	clusteringMode	*N2	*N3	*N6
	clusterAll	*N2	*N3	true, false
	numClustersKnown	*N2	*N3	true, false
	numClusters *N1	*N2	*N3	1-inf

clusteringParam.clusteringMode=1->4:Centroid

Notes

- *N1 only assignable when value of clusterNumKnown subParam is true.
- *N2 index number of file. Has to be less than number of files in selected project folder.
- *N3 index number of file. Has to be less than number of files in selected project folder and more than or equal to value assigned to file#From.
- *N4 values assignable are shape names as inserted during the training process in the Train module of the GUI.
- *N5 values assignable depend on value of the analysisMethod subParam of the analysisParam mainParam. If value is:
 - 1. Pixel counting, value assignable is Area, Filled Area, Convex Area, Perimeter, Euler Number, Eccentricity, Solidity, Orientation, Extent, Major Axis Length, Minor Axis Length, Form Factor, Roundness, Elongation, Fill Ratio, Mean Intensity, Minima Number, Minima Intensity, Minima Eccentricity, Minima Area, Minima Convex Area, Maxima Number, Maxima Intensity, Maxima Eccentricity, Maxima Area, Maxima Convex Area, Segment Total Length, Segment Number Intersections Or Objects.
 - 2. Ellipse fitting, value assignable is Major Axis Length Fit, Minor Axis Length Fit, Elongation Fit, Relative Centroid or Orientation Fit.

*N6 value assignable is Centroid or Gaussian Mixture.

Block 7				
mainParam	subParam	graph#From	graph#To	value
plottingParam	plot	Leave empty	Leave empty	true,false
	length	Leave empty	Leave empty	0-inf
	width	Leave empty	Leave empty	0-inf
	fontSize	Leave empty	Leave empty	0-inf
	numGraphs	Leave empty	Leave empty	0-inf
	includeBinned	*N1	*N2	true,false
	normalize	*N1	*N2	true,false
	addLegend	*N1	*N2	true,false
	flip	*N1	*N2	true,false
	addBox	*N1	*N2	true,false
	split	*N1	*N2	true,false
	files	*N1	*N2	*N3
	label	*N1	*N2	*N4
	binNum	*N1	*N2	*N5
	shapes	*N1	*N2	*N6
	rowNum	Leave empty	Leave empty	*N7
	yDescriptor	*N1	*N2	*N8
	xDescriptor	*N1	*N2	*N9
	map	*N1	*N2	*N10
	subType	*N1	*N2	*N11
	fit	*N1	*N2	*N12
	equation	*N1	*N2	*N13
	xLine	*N1	*N2	-inf-inf
	yLine	*N1	*N2	-inf-inf

plottingParam.plot=true

plottingParam.numGraphs=9

plottingParam.file=1->6:1->3;7->9:4-4

plottingParam.label=1->3:Label-X;4->6:Label-Y;7->9:Label-Z

Notes

*N1 minimum number of graphs or index number of graph less than or equal to the value assigned to the numGraphs subParam.

*N2 maximum number of graphs or index number of graph less than or equal to the value assigned to the numGraphs subParam.

*N3 value assignable has to range between 1 and number of files in the selected project folder.

*N4 any text value assignable.

*N5 only assignable when value of yDescriptor subParam is Events. Any numeric value 0-inf is assignable.

*N6 values assignable are shape names as inserted during the training process in the Train module of the GUI.

*N7 value assignable has to range between 1 and value assignable to numGraphs subParam.

*N8 values assignable depend on value of the analysisMethod subParam of the analysisParam mainParam. If value is:

1. Pixel counting, value assignable is Area, Filled Area, Convex Area, Perimeter, Euler Number, Eccentricity, Solidity, Orientation, Extent, Major Axis Length, Minor Axis Length, Form Factor, Roundness, Elongation, Fill Ratio, Mean Intensity, Minima Number, Minima Intensity, Minima Eccentricity, Minima Area, Minima Convex Area, Maxima Number, Maxima Intensity, Maxima Eccentricity, Maxima Area, Maxima Convex Area, Segment Total Length, Segment Number Intersections Or Objects.

- 2. Ellipse fitting, value assignable is one of the following: Major Axis Length Fit, Minor Axis Length Fit, Elongation Fit, Relative Centroid Or Orientation Fit.
- 3. Radial profiling, value assignable is one of the following: Raw Radial Profile, Intensity Normalized Radial Profile, Area Normalized Radial Profile, Raw Radial Density Profile, Intensity Normalized Radial Density Profile Or Area Normalized Radial Density Profile.

Additionally, the values Events or Count can be assigned.

*N9 values assignable depend on value of the analysisMethod subParam of the analysisParam mainParam and the yDescriptor subParam. If value is:

- 1. Pixel counting, value assignable is Area, Filled Area, Convex Area, Perimeter, Euler Number, Eccentricity, Solidity, Orientation, Extent, Major Axis Length, Minor Axis Length, Form Factor, Roundness, Elongation, Fill Ratio, Mean Intensity, Minima Number, Minima Intensity, Minima Eccentricity, Minima Area, Minima Convex Area, Maxima Number, Maxima Intensity, Maxima Eccentricity, Maxima Area, Maxima Convex Area, Segment Total Length, Segment Number Intersections Or Objects.
- 2. Ellipse fitting, value assignable is Major Axis Length Fit, Minor Axis Length Fit, Elongation Fit, Relative Centroid or Orientation Fit.
- 3. Radial profiling, value assignable is one of the following: Distance.

Additionally, the value Label can be assigned if the value assigned to the yDescriptor subParam is Count or any of the Pixel counting or Ellipse fitting descriptors.

*N10 values assignable is lch, matlab, brewer1, brewer2, brewer3, brewer_pastel, brewer dark, brewer paired, d3 10, d3 20, d3 20b or d3 20c.

*N11 value assignable depends on value of xDescriptor and yDescriptor subParam:

- 1. If value assigned to the xDescriptor is Distance, value assignable is Scatter, Line, Smooth or Summary.
- 2. If value assigned to the xDescriptor is Label and value assigned to yDescriptor is Count, value assignable is Aligned bars or Stacked bars.
- 3. If value assigned to the xDescriptor is Label and value assigned to yDescriptor is one of the Pixel counting or Ellipse fitting descriptors, value assignable is Box plot, Violin or Jittered.
- 4. If value assigned to the yDescriptor is Events, value assignable is Bar, Stacked bar, Point, Line, or Stairs.
- 5. If value assigned to the xDescriptor is one of the Pixel counting or Ellipse fitting descriptors and value assigned to yDescriptor is one of the Pixel counting or Ellipse fitting descriptors, value assignable is Scatter, Line, Smooth or Summary.

*N12 value assignable depends on value of xDescriptor and yDescriptor subParam:

- 1. If value assigned to the xDescriptor is Distance, value assignable is None, Linear, Quadratic, Exponential, Polynomial, Or Custom.
- 2. If value assigned to the xDescriptor is Label and value assigned to yDescriptor is Count, value assignable is None.
- 3. If value assigned to the xDescriptor is Label and value assigned to yDescriptor is one of the Pixel counting or Ellipse fitting descriptors, value assignable is None
- 4. If value assigned to the yDescriptor is Events, value assignable is None, Density or Normal.
- 5. If value assigned to the xDescriptor is one of the Pixel counting or Ellipse fitting descriptors and value assigned to yDescriptor is one of the Pixel counting or Ellipse fitting descriptors, value assignable is None, Linear, Quadratic, Exponential, Polynomial, or Custom.

*N13 only assignable when value fit subParam is Custom. Refer to **ASAP guide: user manual** for further information on syntax of the equation.

Block 8				
mainParam	subParam	image#From	image#To	value
montagingParam	montage	Leave empty	Leave empty	true,false
	numImages	Leave empty	Leave empty	0-inf
	imageSize	Leave empty	Leave empty	0-inf
	fontSize	Leave empty	Leave empty	0-inf
	includeBinned	*N1	*N2	true,false
	label	*N1	*N2	*N3
	scale	*N1	*N2	0-inf
	file	*N1	*N2	*N4
	shape	*N1	*N2	*N5
	rowNum	*N1	*N2	*N6

montageParam.montage=true
montageParam.numImages=6

montageParam.label=1->1:label-X;3->3:label-Y;5->5:label-Z

montageParam.rowNum=0

Notes

*N1 minimum number of images or index number of image less than or equal to the value assigned to the numImages subParam.

*N2 maximum number of images or index number of image less than or equal to the value assigned to the numImages subParam.

*N3 any text value assignable.

*N4 value assignable has to range between 1 and number of files in the selected project folder.

*N5 values assignable are shape names as inserted during the training process in the Train module of the GUI.

*N6 value assignable has to range between 1 and value assignable to numImages subParam.

Example automation script

The following is an example automation script which can be ran on the 3 images provided in the 'Examples/ASAP guide example automation script files' folder. The folder also contains a classification model file. Please note that neither the classification model nor the intended analysis is representative of real-world analysis and that the produced data is by no means useful. However, this automation script and the accompanying files are merely provided for guidance. A copy of the automation script text file can be found in the same folder.

```
% Template script for automating ASAP
엉
% Copyright -
% John S. H. Danial (2018).
% danial@is.mpg.de
% Please note: a classification model .astr has to be copied in the project
folder.
% Please note: only microscopy-generated image files should be located in the
project folder.
%% setting options
options.displayImages=1->6:false
options.displayPlots=1->6:false
options.exportRawData=1->6:true
options.exportImages=1->6:true
options.exportPlots=1->6:false
%% setting simulation parameters
simulationParam.simulate=false
simulationParam.simulationMode=1->3:Structured
simulationParam.pixelSize=1->3:10
simulationParam.segment=1->3:true
simulationParam.segmentationLevel=1->3:30
simulationParam.numCycle=1->3:30
simulationParam.photonCount=1->3:4000
simulationParam.labelLength=1->3:10
simulationParam.lateralPrecision=1->3:10
% simulation parameters for the (Random) simulation mode. Can be left empty if
% the (Structed) simulation mode is selected
simulationParam.numFluorophore=1->3:0
% simulation parameters for the (Structured) simulation mode. Can be left empty
% the (Random) simulation mode is selected
simulationParam.numEpitope=1->3:10
simulationParam.numStructure=1->3:1000
simulationParam.structureSize=1->3:100
simulationParam.labelingEfficiency=1->3:0.5
simulationParam.rotationEnabled=1->3:true
%% setting identification parameters
identificationParam.identify=false
identificationParam.identificationMode=1->6:Size
identificationParam.segment=1->6:true
identificationParam.segmentationLevel=1->6:30
identificationParam.thresholdMode=1->6:Fixed
identificationParam.thresholdMultiplier=1->6:1
identificationParam.clearBorder=1->6:false
identificationParam.maxClearParticleSize=1->6:0
identificationParam.maxClearSearchRadius=1->6:0
identificationParam.clusterParamD=1->6:1
identificationParam.clusterParamS=1->6:1
```

```
identificationParam.maxStructureSize=1->6:15
identificationParam.minSize=1->6:10
identificationParam.maxSize=1->6:10000
%% setting analysis parameters
analysisParam.analyze=false
analysisParam.pixelSize=1->6:10
analysisParam.analysisPlatform=1->6:CPU
analysisParam.analysisMode=1->6:Pixel counting, Ellipse fitting, Radial profiling
analysisParam.maxRingSize=1->6:20
analysisParam.operations=1->6:None
%% setting classification parameters
classificationParam.classify=false
classificationParam.assignmentNum=1->6:1
classificationParam.shapeBefore=1->6:Ring
classificationParam.shapeAfter=1->6:Ring
classificationParam.descriptor=1->6:Area
classificationParam.includeSecondBound=1->6:false
classificationParam.firstBound=1->6:0
classificationParam.secondBound=1->6:0
classificationParam.firstEquality=1->6:<</pre>
classificationParam.secondEquality=1->6:<</pre>
%% setting clustering parameters
clusteringParam.cluster=true
clusteringParam.shape=1->6:Ring
clusteringParam.descriptor=1->6:Elongation Fit
clusteringParam.clusteringMode=1->6:Centroid
clusteringParam.clusterAll=1->6:true
clusteringParam.numClustersKnown=1->6:true
clusteringParam.numClusters=1->6:2
%% setting plotting parameters
plottingParam.plot=true
plottingParam.length=20
plottingParam.width=20
plottingParam.fontSize=14
plottingParam.numGraphs=4
plottingParam.includeBinned=1->4:false
plottingParam.normalize=1->4:false
plottingParam.addLegend=1->4:false
plottingParam.flip=1->4:false
plottingParam.addBox=1->4:true
plottingParam.split=1->4:false
plottingParam.files=1->4:1->6
plottingParam.label=1->4:
plottingParam.binNum=1->4:5
plottingParam.shapes=1->2:Ring;3->3:non-ring;4->4:Ring
plottingParam.rowNum=2
plottingParam.xDescriptor=1->4:Distance
plottingParam.yDescriptor=1->4:Raw Radial Profile
plottingParam.map=1->4:1ch
plottingParam.fill=1->4:All
plottingParam.subType=1->2:Scatter;3->4:Smooth
plottingParam.fit=1->4:None
plottingParam.equation=1->4:
plottingParam.xLine=1->4:
plottingParam.yLine=1->4:
```

%% setting montage parameters

montagingParam.montage=true
montagingParam.numImages=600
montagingParam.imageSize=200
montagingParam.fontSize=14
montagingParam.includeBinned=1->600:false
montagingParam.label=1->600:
montagingParam.scale=1->600:50
montagingParam.file=1->600:1
montagingParam.shape=1->600:Ring
montagingParam.rowNum=20