ASAP guide: description of parameters and selection of values

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Introduction

The wide-applicability of ASAP justifies the dependence of some of its modules on parameters input by the user. At some instances, the selection of an input value is straight-forward, at others, is not. This document guides the user in making the correct choices when selecting parameter values.

Parameters for the `Simulate` module

The simulator can be used to generate super-resolved images region resembling the convex hull of an input image. The simulated images can be visually compared to those obtained experimentally or re-input to the ASAP process workflow to retrieve quantitative information on the simulated structures and statistically compare them to those imaged.

P1   in script: `simulationMode` / in GUI: `Simulation mode`

P1.1   this parameter can take one value: `Random` or `Structured`.

P1.2   assigning `Random` generates random localizations within one or more image(s) in the selected project folder. Assigning `Structured` generates randomly-located *n-fold* rings within one or more image(s) in the selected project folder.

P1.3   if `Random` is assigned, parameters P10 – P14 are not assignable in the UI and can take dummy values in the automation script. If `Structured` is assigned, parameter P9 is not assignable.

P1.4   refer to the 'Examples/ASAP guide description of parameters examples/P1_simulationMode_examples' folder for sample images for each value.

P2   in script: `pixelSize` / in GUI: `Pixel Size`

P2.1   this parameter can take any numerical value from `1` to `inf`.

P2.2   value of parameter is internally used for mapping all other parameters provided in nanometers to pixels. Units are nanometers per pixel.

P2.3   no restricted or complementary assignments.

P2.4   no visual representation with simulated data.

P3   in script: `segment` / in GUI: `Segment`

P3.1   this parameter can take one Boolean value: `true` or `false`.

P3.2   assigning `true` allows ASAP to identify the convex hull of a cell using parameter P4 and to generate localizations within the convex hull. Assigning `false` allows ASAP to generate localizations across the whole field of view of one or more of the image(s) in the selected project folder particularly where the signal-to-noise ratio is high.

P3.3   if `true` is assigned, parameter P4 has to be assigned.

P3.4   refer to the 'Examples/ASAP guide description of parameters examples/P3_segment_examples' folder for sample images for each value.

P4   in script: `segmentationLevel` / in GUI: `Level`

P4.1   this parameter can take any numerical value from `1` to `inf`.

P4.2   assignment depends on the size of the desired convex hull. Lower values identify small convex hulls while larger values identify bigger ones. The choice depends on the size of the cell and density of localizations.

P4.3   no restricted or complementary assignments.

P4.4   refer to the 'Examples/ASAP guide description of parameters examples/P4_segmentationLevel_examples' folder for sample images for each value.

P5   in script: `numCycle` / in GUI: `# Cycle`

P5.1   this parameter can take any numerical value from `1` to `inf`.

P5.2   assignment depends on the number of emission cycles of the simulated fluorophore. A value can be experimentally obtained or else retrieved [here](#).

P5.3   no restricted or complementary assignments.

P5.4   no visual representation with simulated data.

P6   in script: `photonCount` / in GUI: `Photon count`

P6.1   this parameter can take any numerical value from `1` to `inf`.

P6.2   assignment depends on the average number of photons per emission cycle of the simulated fluorophore. A value can be experimentally obtained or else retrieved [here](#).

P6.3   no restricted or complementary assignments.

P6.4   no visual representation with simulated data.

P7   in script: `labelLength` / in GUI: `Label length`

P7.1   this parameter can take any numerical value from `0` to `inf`.

P7.2   assignment depends on the size of the label (e.g. fluorophore, quantum dot, bead etc…). Units are nanometers.

P7.3   no restricted or complementary assignments.

P7.4   refer to the 'Examples/ASAP guide description of parameters examples/P7_labelLength_examples' folder for sample images for each value.

P8   in script: `lateralPrecision` / in GUI: `Lateral precision`

P8.1   this parameter can take any numerical value from `0` to `inf`.

P8.2   assignment depends on localization precision of the imaging system. Can be assumed or measured from the localization statistics or using Fourier Ring Correlation.

P8.3   no restricted or complementary assignments.

P8.4   refer to the 'Examples/ASAP guide description of parameters examples/P8_lateralPrecision_examples' folder for sample images for each value.

P9   in script: `numFluorophore` / in GUI: `# Fluorophore`

P9.1   this parameter can take any numerical value from `1` to `inf`.

P9.2   assignment depends on approximate number of fluorophores in field of view or convex hull which depends on the size of structures and labelling density. Value can be estimated through visual comparison of simulated image with that experimentally obtained.

P9.3   if assigned, parameters P10 – P14 do not have to be assigned.

P9.4   no visual representation with simulated data.

P10   in script: `numEpitope` / in GUI: `# Epitope`

P10.1 this parameter can take any numerical value from `1` to `inf`.

P10.2 assignment depends on number of epitopes in simulated ring. Epitopes are located equidistantly across the circumference of the ring. Parameter can be tuned for rings to resemble the structure of biological machineries such as the nuclear pore complex.

P10.3 if assigned, parameter P9 does not have to be assigned.

P10.4 refer to the 'Examples/ASAP guide description of parameters examples/P10_numEpitope_examples' folder for sample images for each value.

P11   in script: `numStructure` / in GUI: `# Structure`

P11.1 this parameter can take any numerical value from `1` to `inf`.

P11.2 assignment depends on number of rings to be simulated within field of view or convex hull. Value can be arbitrary or estimated from super-resolved image.

P11.3 if assigned, parameter P9 does not have to be assigned.

P11.4 no visual representation with simulated data.

P12   in script: `structureSize` / in GUI: `Structure size`

P12.1 this parameter can take any numerical value from `1` to `inf`.

P12.2 assignment depends on size of rings to be simulated. Units are nanometers.

P12.3 if assigned, parameter P9 does not have to be assigned.

P12.4 refer to the 'Examples/ASAP guide description of parameters examples/P12_structureSize_examples' folder for sample images for each value.

P13   in script: `labelingEfficiency` / in GUI: `Labeling efficiency`

P13.1 this parameter can take any numerical value from `0.1` to `1`.

P13.2 assignment depends on the average ratio of epitopes that are labeled. Value can be estimated from acquired images or else assumed.

P13.3 if assigned, parameter P9 does not have to be assigned.

P13.4 refer to the 'Examples/ASAP guide description of parameters examples/P13_labelingEfficiency_examples' folder for sample images for each value.

P14　in script: `rotationEnabled` / in GUI: `Rotation enabled`

P14.1　this parameter can take one Boolean value: `true` or `false`.

P14.2　assignment depends on whether all rings should have same orientation, or, be allowed to take on any arbitrary rotation.

P14.3　if assigned, parameter P9 does not have to be assigned.

P14.4　no visual representation with simulated data.

Parameters for the `Identify` module

The identification module can be used to segment or identify nanoscopic structures in a super-resolved image. The segmented structures are re-fed into the ASAP workflow to further processing and analysis. Parameter P15 and associated parameters are of particular importance to the operation of this module.

P15　in script: `identificationMode` / in GUI: `Identification mode`

P15.1　this parameter can take one value: `Connectivity`, `Density` (OBSELETE) or `Size`.

P15.2　assigning `Connectivity` is useful when the pixels in the underlying structures are connected to each other (see below for example). Assigning `density` is useful when the pixels in the underlying structures are not connected and structures are present at high densities (see below for example). Assigning `Size` is useful when the pixels in the underlying structures are not connected and structures are present at moderately high to low densities.

P15.3　if `Density` is assigned, parameters P23 and P24 have to be assigned. If `Size` is assigned, parameter P25 has to be assigned.

P15.4　refer to the 'Examples/ASAP guide description of parameters examples/P15_identificationMode_examples' folder for sample images for each value.

P16　in script: `segment` / in GUI: `Segment`

P16.1　this parameter can take one Boolean value: `true` or `false`.

P16.2　assigning `true` allows ASAP to identify the convex hull of a cell using parameter P4 and to segment structures within the convex hull. Assigning `false` allows ASAP to segment structures across the whole field of view of one or more of the image(s) in the selected project folder particularly where the signal-to-noise ratio is high.

P16.3　if `true` is assigned, parameter P16 has to be assigned.

P16.4　no visual representation with simulated data.

P17　in script: `segmentationLevel` / in GUI: `Level`

P17.1　this parameter can take any numerical value from `1` to `inf`.

P17.2　assignment depends on the size of the desired convex hull. Lower values identify small convex hulls while larger values identify bigger ones. The choice depends on the size of the cell and density of localizations.

P17.3　no restricted or complementary assignments.

P17.4　see P4.4.

P18   in script: `thresholdMode` / in GUI: `Threshold mode`

P18.1 this parameter can take one value: `Fixed` or `Relative`.

P18.2 assignment depends on the intensity levels across the field of view. Assigning `Fixed` is useful when the sample is homogenously illuminated and structures are located within one thin optical section. Assigning `Relative` is useful when the sample is not homogenously illuminated and / or structures are scattered across a large depth of field.

P18.3 no restricted or complementary assignments.

P18.4 no visual representation with simulated data.

P19   in script: `thresholdMultiplier` / in GUI: `Threshold multiplier`

P19.1 this parameter can take any numerical value from `0` to `inf`.

P19.2 assignment depends on the intensity levels across the field of view. ASAP generates an intensity threshold value which serves well the purpose of segmenting bright from dim pixels. Nevertheless, with sample which are highly-dense or containing high fluorescence background, the user may wish to tweak the automatically generated threshold value.

P19.3 no restricted or complementary assignments.

P19.4 refer to the 'Examples/ASAP guide description of parameters examples/P19_thresholdMultiplier_examples' folder for sample images for each value.

P20   in script: `clearBorder` / in GUI: `Clear border`

P20.1 this parameter can take one Boolean value: `true` or `false`.

P20.2 if assigned `true`, structures located close to the image border are removed to prevent further processing of incomplete structures. If assigned `false`, structures located close to the image border are not removed.

P20.3 no restricted or complementary assignments.

P20.4 no visual representation with simulated data.

P21   in script: `maxClearParticleSize` / in GUI: `Particle size`

P21.1 this parameter can take any numerical value from `0` to `inf`.

P21.2 this parameter is used to remove single unconnected localizations which do not belong to a larger structure. ASAP searches a region as large as the value assigned to parameter P22 for the minimum number of bright pixels assigned to this parameter centered around a bright pixel. If not found, the bright pixel at the center is removed from the image.

P21.3 if assigned, parameter P22 has to be assigned.

P21.4 no visual representation.

P22   in script: `maxClearSearchRadius` / in GUI: `Search radius`

P22.1 this parameter can take any numerical value from `0` to `inf`.

P22.2 this parameter is used to remove single unconnected localizations which do not belong to a larger structure. ASAP searches a region as large as the value assigned to this parameter for the minimum

number of bright pixels assigned to parameter P21 centered around a bright pixel. If not found, the bright pixel at the center is removed from the image.

P22.3 if assigned, parameter P21 has to be assigned.

P22.4 no visual representation.

P23 in script: `clusterParamD` / in GUI: `D` (OBSELETE)

P23.1 this parameter can take any numerical value from `1` to `inf`.

P23.2 this parameter is equivalent to the minPoints parameter of the [DBSCAN](#) algorithm. Please refer to original publication for further assistance.

P23.3 if assigned, parameter P24 has to be assigned and parameter P15 has to be assigned `Density`.

P23.4 no visual representation.

P24 in script: `clusterParamS` / in GUI: `S` (OBSELETE)

P24.1 this parameter can take any numerical value from `1` to `inf`.

P24.2 this parameter is equivalent to the epsilon parameter of the [DBSCAN](#) algorithm. Please refer to original publication for further assistance.

P24.3 if assigned, parameter P23 has to be assigned and parameter P15 has to be assigned `Density`.

P24.4 no visual representation.

P25 in script: `maxStructureSize` / in GUI: `Structure size`

P25.1 this parameter can take any numerical value from `1` to `inf`.

P25.2 assignment depends on the maximum size of the underlying structures (see below for example with 100 nm (10 pixels) wide ring structures). A few pixels added are recommended for best results. Units are pixels.

P25.3 if assigned, parameter P15 has to be assigned `Size`.

P25.4 refer to the 'Examples/ASAP guide description of parameters examples/P25_maxStructureSize_examples' folder for sample images for each value.

P26 in script: `minSize` / in GUI: `Area` (lower bound)

P26.1 this parameter can take any numerical value from `0` to `inf`.

P26.2 assignment depends on the minimum size of a structure to be removed. Can be used to reject very small structures which are deemed false localizations. Units are pixels.

P26.3 value assigned has to be smaller than or equal to the value assigned to parameter P27.

P26.4 no visual representation with simulated data.

P27 in script: `maxSize` / in GUI: `Area` (upper bound)

P27.1 this parameter can take any numerical value from `0` to `inf`.

P27.2 assignment depends on the maximum size of a structure to be removed. Can be used to reject very large structures. Units are pixels.

P27.3 value assigned has to be larger than or equal to the value assigned to parameter P26.

P27.4 no visual representation with simulated data.

Parameters for the `Analyze` module

The module can be used to geometrically analyze the structures present in the image(s) found in the selected project folder. ASAP presents several analysis modes which are described here. The output of this module is a set of descriptors, or parameters, for each structure which can be used stand alone or latter during the classification process to discriminate between different structures.

P28   in script: `pixelSize` / in GUI: `Pixel size`

P28.1 this parameter can take any numerical value from `1` to `inf`.

P28.2 value of parameter is internally used for mapping all other parameters provided in nanometers to pixels. Units are nanometers per pixel.

P28.3 no restricted or complementary assignments.

P28.4 no visual representation with simulated data.

P29   in script: `analysisPlatform` / in GUI: `Analysis platform`

P29.1 this parameter can take one value `CPU` or `GPU`.

P29.2 if `CPU` is assigned, analysis operations are distributed across the CPU cores for processing. If `GPU` is assigned, analysis operations are performed over the GPU. If `GPU` is selected and the running PC does not contain a suitable GPU, ASAP will default to the CPU.

P29.3 no restricted or complementary assignments.

P29.4 no visual representation with simulated data.

P30   in script: `analysisMethod` / in GUI: `Analysis method`

P30.1 this parameter can take one or more value(s) `Pixel counting`, `Ellipse fitting` or `Radial profiling`.

P30.2 assignment depends on the topology of the underlying structure, the ratio of the size of a structure to the pixel size and the requested data for analysis and training. If assigned `Pixel counting`, ASAP counts bright pixels and deduces a list of geometrical parameters (also called descriptors) from those. This method is suitable for large and connected structures. If assigned `Ellipse fitting`, ASAP fits a free-ellipse to bright pixels and deduces a reduced list of descriptors. This method is suitable for unconnected structures which have a circular or elliptical topology. If assigned `Radial profiling`, ASAP draws sub-nanometer concentric circles centered around the underlying structures and calculates the absolute and relative number of bright pixels between each concentric circle (also called radial profile). The radial profile can be later used for mathematical fitting to derive a number of parameters describing the underlying geometry with sub-nanometer accuracy. This method is suitable connected and unconnected circular topologies.

P30.3 if assigned `Pixel counting` or `Ellipse fitting` parameter P31 cannot be assigned. If assigned `Radial profiling`, parameter P30 has to be assigned.

P29.4 no visual representation with simulated data.

P31　in script: `maxRingSize` / in GUI: `Max ring size`

P31.1　this parameter can take any numerical value from `1` to `inf`.

P31.2　assignment depends on the maximum size of the underlying structures. This parameter describes the size of the largest concentric ring to draw centered around a structure for the purpose of obtaining a radial profile. This parameter does not affect the analysis process but will affect the way you visualize the radial profile in the `Plot` module.

P31.3　if assigned, parameter P30 has to be assigned `Radial profiling`.

P31.4　no visual representation with simulated data.

P32　in script: `operations` / in GUI: `Operations`

P32.1　this parameter can take one or more value(s) `None` or `Fill`, `Bridge`, `Close`, `Open`, `Clean`, `Rotate`, `Center` or `Resize`.

P32.2　assignment depends on the morphology of the underlying structures. See [here](#) for further information on the different operations.

P32.3　no restricted or complementary assignments.

P32.4　no visual representation with simulated data.

Parameters for the `Classify` module

This module takes as input the analyzed structures output file (`.asan`) as well as the classification model file (`.astr`) produced by the `Train` module and classifies all registered structures accordingly. When the size of the processed data set is small, the structures present within are highly heterogenous or the quality of the image(s) is inferior, the classifier may fail to appropriately classify the structures. To mitigate this problem, the user could manually assign structures automatically classified to an incorrect shape using the re-assignment tool available in this module. The user can perform multiple re-assignments to ensure that the majority of the registered structures have been appropriately classified.

P33　in script: `assignmentNum` / in GUI: `Assignment #`

P33.1　this parameter can take any numerical value from `0` to `inf`.

P33.2　assignment depends on the number of re-assignments required to appropriately classify registered structures. Note that, in script, this parameter describes the total number of assignments requested which is different in the GUI where the parameter describes the assignment Id.

P33.3　no restricted or complementary assignments.

P33.4　no visual representation with simulated data.

P34　in script: `shapeBefore` / in GUI: `Shape before`

P34.1　this parameter can take more than one value equivalent to the names of the shapes as inserted in the `Train` module.

P34.2　assignment depends on the shapes for which a re-assignment is requested. This parameter takes as value the names of the shapes to be re-assigned.

P34.3　no restricted or complementary assignments.

P34.4 no visual representation with simulated data.

P35 in script: `shapeAfter` / in GUI: `Shape after`

P35.1 this parameter can take one value equivalent to the name of one of the shapes as inserted in the `Train` module.

P35.2 assignment depends on the shape to which a re-assignment is requested. This parameter takes as value the name of one shape to which re-assignment will be made. As an example, the classifier may have mis-classified rings as discs. For re-assignment purposes, this can be set to `Disc`.

P35.3 no restricted or complementary assignments.

P35.4 no visual representation with simulated data.

P36 in script: `descriptor` / in GUI: `Descriptor`

P36.1 this parameter can take one value depending on value assigned to parameter P30. If value assigned to parameter P30 is `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`. If value assigned parameter P29 is `Ellipse fitting`, value assignable is `Major Axis Length Fit`, `Minor Axis Length Fit`, `Elongation Fit`, `Relative Centroid` or `Orientation Fit`.

P36.2 assignment depends on which descriptor re-assignment is to be made. As an example, the classifier may have mis-classified rings as discs. For re-assignment purposes, this can be set to `Minima number`.

P36.3 no restricted or complementary assignments.

P36.4 no visual representation with simulated data.

P37 in script: `lowerBound` / in GUI: `Bounds` (upper text field)

P37.1 this parameter can take any numerical value from `0` to `inf`.

P37.2 assignment depends on which value for the assigned descriptor re-assignment is to be made. As an example, the classifier may have mis-classified rings as discs. For re-assignment purposes, this can be set to `1`.

P37.3 no restricted or complementary assignments.

P37.4 no visual representation with simulated data.

P38 in script: `lowerEquality` / in GUI: `Bounds` (upper equality dropdown menu)

P38.1 this parameter can take one value >, < or =.

P38.2 assignment depends on which value for the assigned descriptor re-assignment is to be made. As an example, the classifier may have mis-classified rings as discs. For re-assignment purposes, this can be set to =.

P38.3 no restricted or complementary assignments.

P38.4 no visual representation with simulated data.

P39 in script: `upperBound` / in GUI: `Bounds` (lower text field)

P39.1 this parameter can take any numerical value from `0` to `inf`.

P39.2 assignment depends on which value for the assigned descriptor re-assignment is to be made. As an example, the classifier may have mis-classified rings as discs. For re-assignment purposes, this can be set to `1`.

P39.3 no restricted or complementary assignments.

P39.4 no visual representation with simulated data.

P40 in script: `upperEquality` / in GUI: `Bounds` (lower equality dropdown menu)

P40.1 this parameter can take one value >, < or =.

P40.2 assignment depends on which value for the assigned descriptor re-assignment is to be made. As an example, the classifier may have mis-classified rings as discs. For re-assignment purposes, this can be set to =.

P40.3 no restricted or complementary assignments.

P40.4 no visual representation with simulated data.

Parameters for the `Cluster` module

When the structures within one shape category are heterogenous in size, those can be further clustered into different groups. The `Cluster` module allows the user to cluster structures belonging to one or more shape category into different clusters based on one or two descriptor(s). This tool is useful when coming to answer biological questions about the number of oligomeric states of a protein of interest or drawing conclusions on the effect of one protein's macro-structure on another.

P41 in script: `shape` / in GUI: `Shapes`

P41.1 this parameter can take more than one value equivalent to the names of the shapes as inserted in the `Train` module.

P41.2 assignment depends on which shape is requested for clustering.

P41.3 no restricted or complementary assignments.

P41.4 no visual representation with simulated data.

P42 in script: `descriptor` / in GUI: `Descriptor`

P42.1 this parameter can take one value depending on value assigned to parameter P30. If value assigned to parameter P30 is `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`. If value assigned parameter P29 is

Ellipse fitting, value assignable is `Major Axis Length Fit`, `Minor Axis Length Fit`, `Elongation Fit`, `Relative Centroid` or `Orientation Fit`.

P42.2 assignment depends on which shape descriptor is best for clustering data.

P42.3 no restricted or complementary assignments.

P42.4 no visual representation with simulated data.

P43   in script: `clusteringMode` / in GUI: `Clustering mode`

P43.1 this parameter can one value `Centroid` or `Gaussian mixture`.

P43.2 assignment depends on which algorithm is best-suited for clustering the data. Refer [here](#) for more information on the k-means and Gaussian Mixture Model clustering algorithms.

P43.3 no restricted or complementary assignments.

P43.4 no visual representation with simulated data.

P44   in script: `clusterAll` / in GUI: `Cluster all`

P44.1 this parameter can take one Boolean value: `true` or `false`.

P44.2 since the performance of the clustering algorithms is proportional to the size of the data set, this parameter offers the user the possibility to pool all the data for clustering. Data is pooled when parameter is assigned `true`.

P44.3 no restricted or complementary assignments.

P44.4 no visual representation with simulated data.

P45   in script: `clusterNumKnown` / in GUI: `# Cluster known`

P45.1 this parameter can take one Boolean value: `true` or `false`.

P45.2 if assigned `true`, the user indicates that the number of clusters is known. If assigned `false`, the user indicates that the number of clusters is unknown and should be inferred from the data.

P45.3 if assigned `true`, parameter P46 has to be assigned.

P45.4 no visual representation with simulated data.

P46   in script: `clusterNum` / in GUI: `# Clusters`

P46.1 this parameter can take any numerical value from `1` to `inf`.

P46.2 assignment depends on a priori knowledge of the number of clusters present in the underlying sample and for the shapes to be clustered.

P46.3 if assigned, parameter P45 has to be assigned `true`.

P46.4 no visual representation with simulated data.