

Single Cell Classifier - Plugin documentation

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July 2020

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1 Introduction

Single Cell Classifier (SCC) is an ImageJ/Fiji plugin that aims to classify cells based on features related to their nuclei and their neighbors. The plugin detects nuclei by using the StarDist plugin, measure information for each cell and then classify them.

The plugin is designed to work on multi-channels image and any custom model for segmentation and classification.

2 Installation

The plugin can installed in Fiji by following those steps:

1. Download and install Fiji from here.
2. Download the plugin jar file and put it in the plugin folder of Fiji.
3. Start Fiji.
4. Select ‘Help/Update...‘ from the menu bar.
5. Click on the button ‘Manage update sites‘.
6. Scroll down the list and tick the checkboxes for update sites ‘IJPB-plugins‘, ‘CSBDeep‘ and ‘StarDist‘, then click the ‘Close‘ button. (If one of the site is missing, click ‘Update URLs‘ to refresh the list of update sites.)
7. Click on ‘Apply changes‘ to install the plugin.
8. Restart Fiji.

3 Nuclei detection

The plugin allows the detection of nuclei by using the StarDist plugin (see Fig. 1).

3.1 Detect nuclei of an open image

To detect the nuclei of an open image you can click in *Plugins/Single Cell Classifier/Detect/Detect Nuclei*.

3.1.1 Parameters

Config Choose between built-in configuration or custom user configuration (parameter **Config (.json) from File**).

Output Type Choose between a generation of the nuclei in the ROI Manager or in a label image.

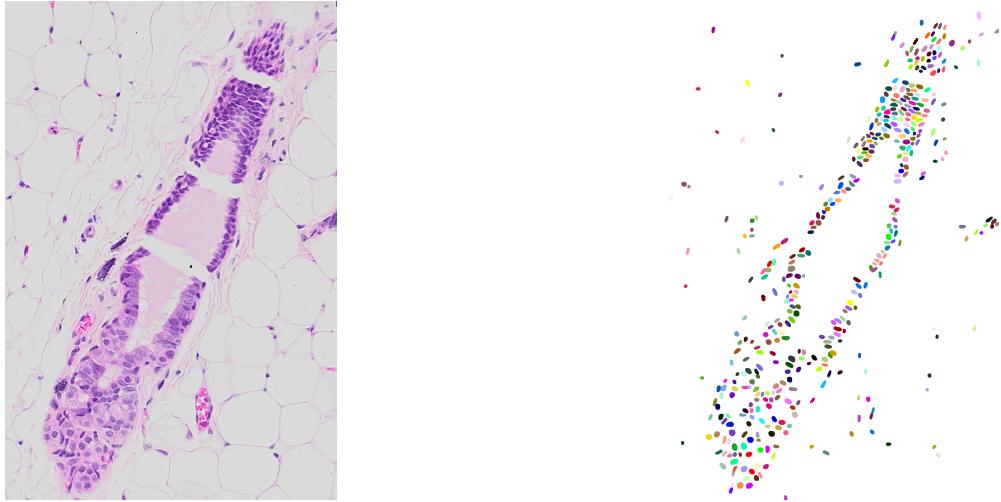


Figure 1: Detected nuclei label (right) of the source image (left)

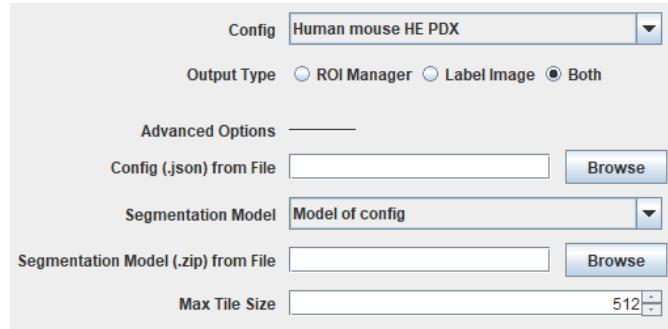


Figure 2: Detect Nuclei parameters

Segmentation model Choose between the default model of the configuration, a built-in model (including StarDist built-in models) and a custom user model (parameter **Segmentation model (.zip) from File**).

Max Tile Size The maximal size of a tile for the StarDist segmentation.

3.2 Detect nuclei of a batch of images

To detect the nuclei labels of all the images of a directory you can click in *Plugins/Single Cell Classifier/Detect/Detect Nuclei (Batch)*. They will be exported in another directory with the same name as their source.

3.2.1 Parameters

Config Choose between built-in configuration or custom user configuration (parameter **Config (.json) from File**).

Source Directory The directory of the sources images.

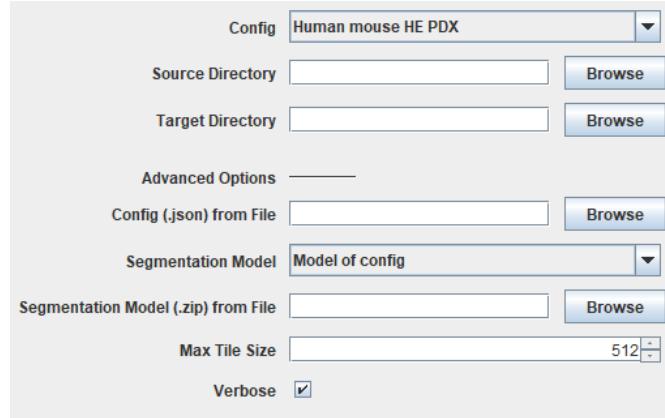


Figure 3: Detect Nuclei (Batch) parameters

Target Directory The directory of the generated nuclei labels images.

Segmentation model Choose between the default model of the configuration, a built-in model (including StarDist built-in models) and a custom user model (parameter **Segmentation model (.zip) from File**).

Max Tile Size The maximal size of a tile for the StarDist segmentation.

4 Cells detection

Cells can be estimated from the nuclei label and the source image (see Fig. 4).

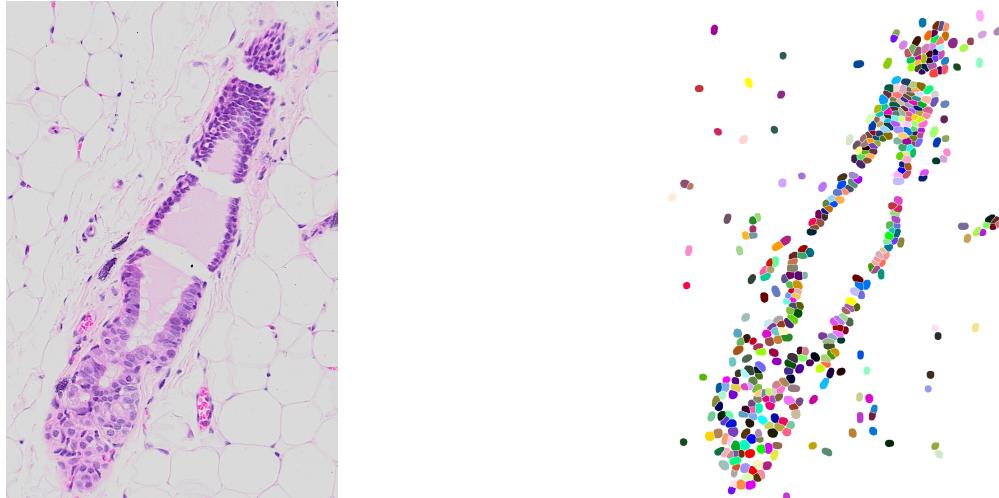


Figure 4: Detected cells label (right) of the source image (left)

4.1 Detect cells of an open image

To detect the cells of an open image you can click in *Plugins/Single Cell Classifier/Detect/Detect Cells*.

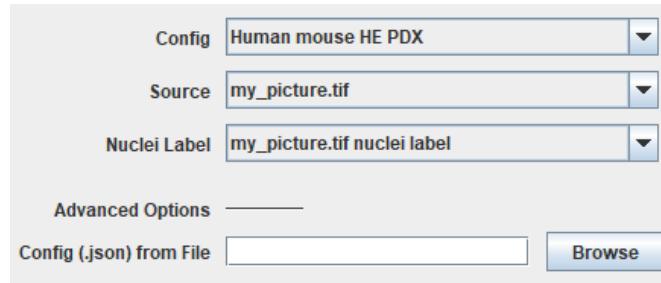


Figure 5: Detect Cells parameters

4.1.1 Parameters

Config Choose between built-in configuration or custom user configuration (parameter **Config (.json) from File**).

Source The source image.

Nuclei Label The nuclei label image associated to the source image.

4.2 Detect cells of a batch of images

To detect the cells labels of all the images of a directory you can click in *Plugins/Single Cell Classifier/Detect/Detect Cells (Batch)*. They will be exported in another directory with the same name as their source.

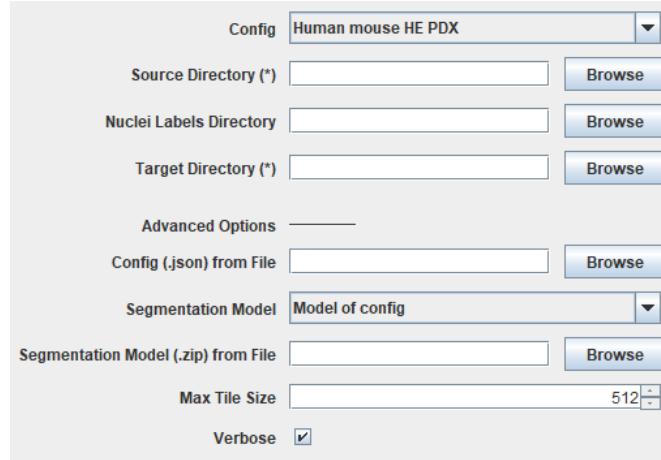


Figure 6: Detect Cells (Batch) parameters

4.2.1 Parameters

Config Choose between built-in configuration or custom user configuration (parameter **Config (.json) from File**).

Source Directory The directory of the sources images.

Nuclei Labels Directory The directory of the nuclei label images. This parameter is optional and the labels will be computed if let undefined.

Target Directory The directory of the generated cells label images.

Segmentation model Choose between the default model of the configuration, a built-in model (including StarDist built-in models) and a custom user model (parameter **Segmentation model (.zip) from File**).

Max Tile Size The maximal size of a tile for the StarDist segmentation.

5 Measurements

Many features can be computed from the detection of the nuclei and the cells such as shape, texture or context information.

5.1 Measure features of an open image

To obtain the features of an open image and their illustration you can click in *Plugins/Single Cell Classifier/Measurement/Measure Information*.

5.1.1 Parameters

Config Choose between built-in configuration or custom user configuration (parameter **Config (.json) from File**).

Output Type Choose between the generation of the features in a Results Table or in a csv file (parameter **Output File (.csv)**).

Show nuclei ROI Select if generate the nuclei in the ROI Manager.

Generate images Select which features are illustrated as images.

Segmentation model Choose between the default model of the configuration, a built-in model (including StarDist built-in models) and a custom user model (parameter **Segmentation model (.zip) from File**).

Max Tile Size The maximal size of a tile for the StarDist segmentation.

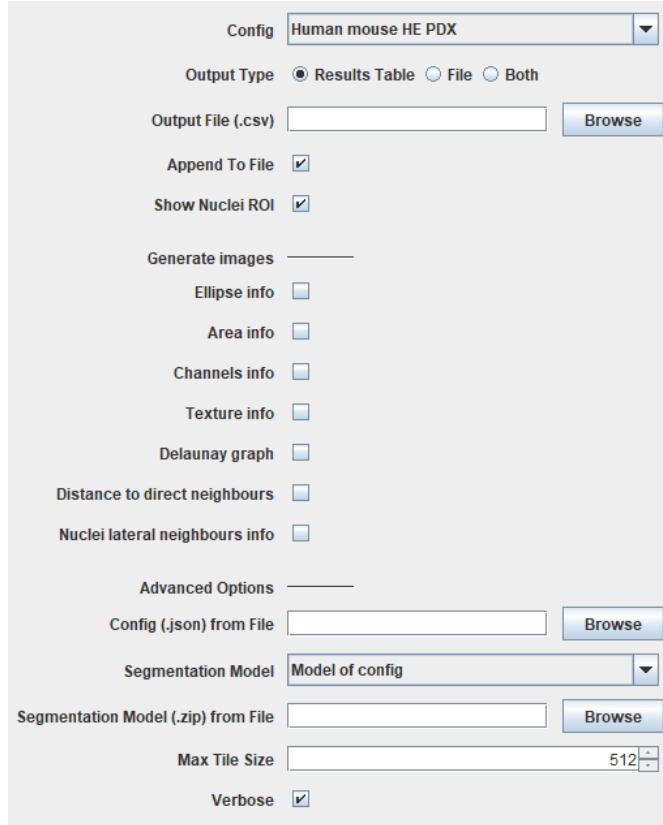


Figure 7: Measure Information parameters

5.2 Collect features of a batch of images

To collect the features of all the images of a directory you can click in *Plugins/Single Cell Classifier/Measurement/Collect Information (Batch)*.

5.2.1 Parameters

Config Choose between built-in configuration or custom user configuration (parameter **Config (.json) from File**).

Source Directory The directory of the sources images.

Nuclei Labels Directory The directory of the nuclei label images. This parameter is optional and the labels will be computed if let undefined.

Cells Labels Directory The directory of the cells labels images. This parameter is optional and the labels will be computed if let undefined.

Classes Mask Directory The directory of the classes mask images. This parameter is optional and no class column will be added if let undefined.

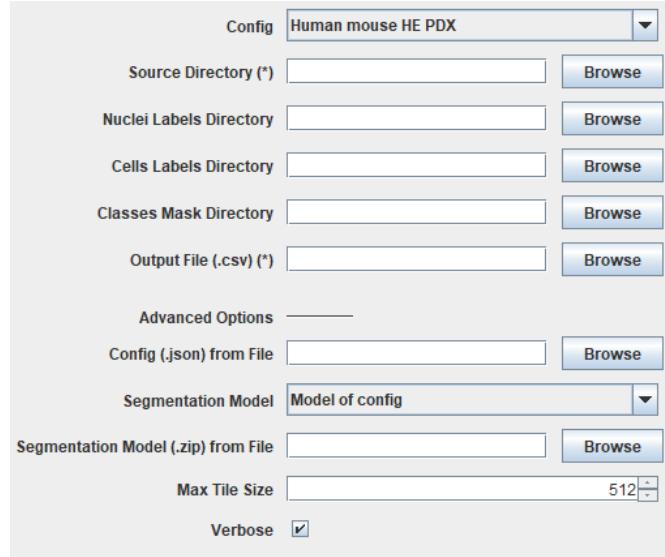


Figure 8: Collect Information (Batch) parameters

Output File (.csv) The output path of the csv file that contains the features of all the images.

Segmentation model Choose between the default model of the configuration, a built-in model (including StarDist built-in models) and a custom user model (parameter **Segmentation model (.zip) from File**).

Max Tile Size The maximal size of a tile for the StarDist segmentation.

6 Classification

The main function of this plugin is the classification (see Fig. 9).

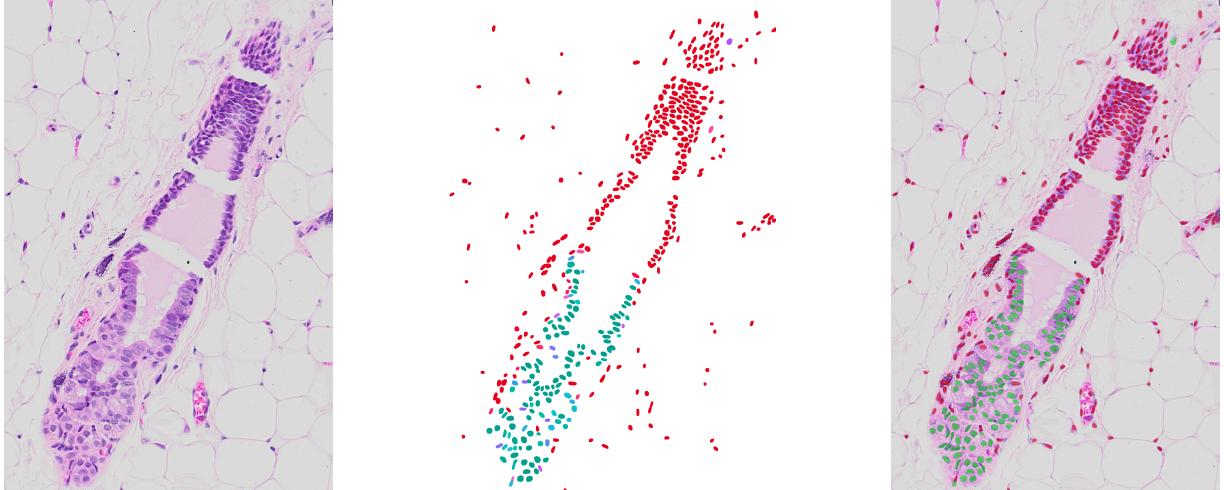


Figure 9: Classified image (right) and class probability (middle) of the source image (left)

6.1 Classification of an open image

To classify an open image you can click in *Plugins/Single Cell Classifier/Classification/Classify*.

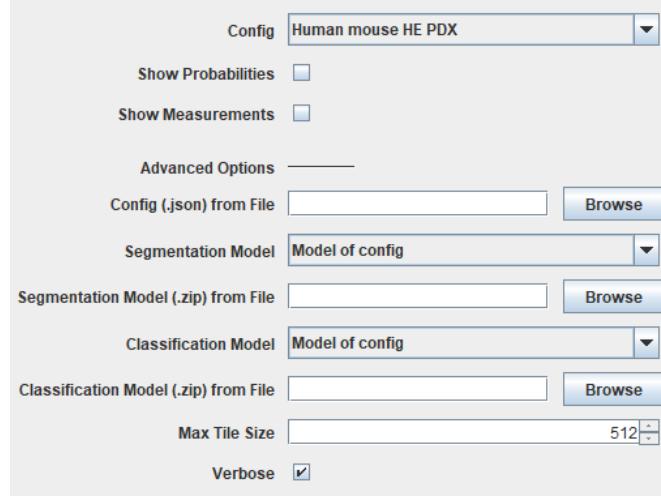


Figure 10: Classify parameters

6.1.1 Parameters

Config Choose between built-in configuration or custom user configuration (parameter **Config (.json) from File**).

Show Probabilities Select if generate an image that illustrate the probabilities, each class will be represented by one channel.

Show Measurements Select if generate a results table with the features and the classification.

Segmentation model Choose between the default model of the configuration, a built-in model (including StarDist built-in models) and a custom user model (parameter **Segmentation model (.zip) from File**).

Classification model Choose between the default model of the configuration, a built-in model and a custom user model (parameter **Classification model (.zip) from File**).

Max Tile Size The maximal size of a tile for the StarDist segmentation.

6.2 Classification of a batch of images

To classify all the images of a directory you can click in *Plugins/Single Cell Classifier/Classification/Classify (Batch)*.

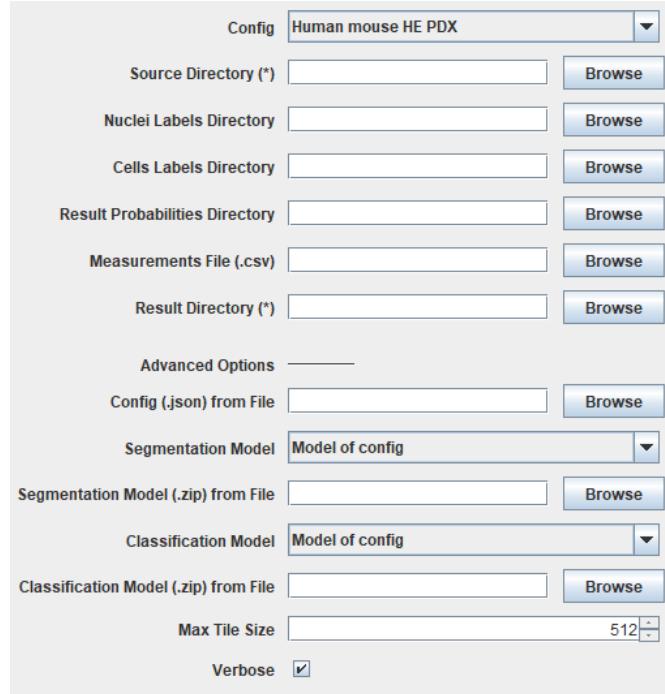


Figure 11: Classify (Batch) parameters

6.2.1 Parameters

Config Choose between built-in configuration or custom user configuration (parameter **Config (.json) from File**).

Source Directory The directory of the sources images.

Nuclei Labels Directory The directory of the nuclei label images. This parameter is optional and the labels will be computed if let undefined.

Cells Labels Directory The directory of the cells labels images. This parameter is optional and the labels will be computed if let undefined.

Result Probabilities Directory The directory of the resulting probabilities images. This parameter is optional and the probabilities will not be saved if let undefined.

Measurements File (.csv) The path of the csv file were the features and the classification are stored. This parameter is optional and the measures will not be saved if let undefined.

Results Directory The directory of the generated classified images.

Segmentation model Choose between the default model of the configuration, a built-in model (including StarDist built-in models) and a custom user model (parameter **Segmentation model (.zip) from File**).

Classification model Choose between the default model of the configuration, a built-in model and a custom user model (parameter **Classification model (.zip)** from File).

Max Tile Size The maximal size of a tile for the StarDist segmentation.

7 Utilities

7.1 Config Creator

Most of the commands in this plugin are using a configuration file, this file contains a lot of parameters for both the segmentation, the measurements and the classification. To create your own configuration file you can click in *Plugins/Single Cell Classifier/Utilities/Config Creator*.

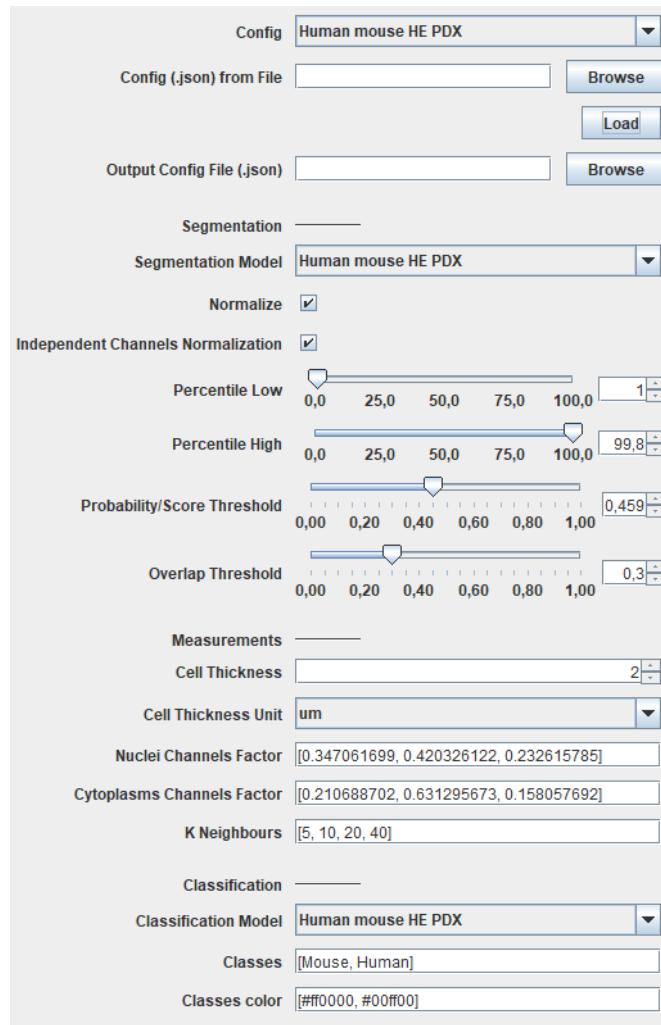


Figure 12: Config Creator parameters

7.1.1 Loading parameters

It is possible to fill all the parameters based on an existing configuration file.

Config Choose between built-in configuration or custom user configuration (parameter **Config (.json) from File**).

7.1.2 Parameters

Output Config File (.json) The path of the new config file that will be generated.

Segmentation Model Choose the segmentation model selected by default for the configuration file.

Normalize Select if the source image should be normalized before the segmentation.

Independent Channels Normalization Select if the normalization of the source image should be done independently for each channels or jointly.

Percentile Low/High Boundaries applied when normalizing, allow to reduce the outliers.

Probability/Score Threshold A threshold used by StarDist, higher values lead to fewer segmented objects, but will likely avoid false positives.

Overlap Threshold A threshold used by StarDist, higher values allow segmented objects to overlap substantially.

Cell Thickness The thickness of the cell used for the cells detection.

Cell Thickness Unit The unit of the cell thickness, can be in pixel or in micrometer, in this case it will automatically use the calibration of the given source image.

Nuclei/Cytoplasms Channels Factor The factors applied on the channels of the source image to convert it into gray level fro Nuclei/Cytoplasms.

K Neighbors The number of neighbors observed at the same time when collecting measurements.

Classification Model Choose the classification model selected by default for the configuration file.

Classes The name of the classes that will be classified.

Classes color The color associated to the classes (example in Fig. 9).

7.2 Compute best RGB factor of a batch of image

It is possible to compute the best RGB factor to convert a RGB image to a gray level by using the Principal Component Analysis. To compute the factors of all the images of a directory you can click in *Plugins/Single Cell Classifier/Utilities/Compute Best RGB Factor*, the factors will be then displayed in a Results Table.

The factors can be computed on the whole image or only on some regions defined by a mask.

Figure 13: Compute Best RGB Factor parameters

7.2.1 Parameters

Source Directory The directory of the sources images.

Mask Directory The directory of the mask images, can be a label (check only the value 0). This parameter is optional and the factors will be computed on the whole image if let undefined.

Invert Mask Select if the value 0 is positive or negative when looking at the mask. When checked, we will only look at the image were the values are 0 on the mask.

7.3 Get the gray level of an open image

To get the gray level of an image like the plugin would when measuring its information you can click in *Plugins/Single Cell Classifier/Utilities/Get Gray Level*.

Figure 14: Get Gray Level parameters

7.3.1 Parameters

Config Choose between built-in configuration or custom user configuration (parameter **Config (.json) from File**).

Weight Type Choose the factors used for the conversion to gray level.

7.4 Get the saturation level

To get the saturation of an image you can click in *Plugins/Single Cell Classifier/Utilities/Get Saturation*.