IKOSA®
Application Documentation

Application Name	Cytosolic and Nuclear Translocation
Version	3.0.0
Documentation Version	16.12.2021 - 1
Input Image(s)	2D / Time Series Images; Grayscale, (8/16 bit)
Input Parameter(s)	Regions of interest (optional)
Keywords	gene regulation, signal transduction, protein expression, HEK, RBL, EAHY, fluorescence, in-vitro, single cell analysis, live cell imaging, microscopy, transcription factor, nuclear import
Short Description	Measurement of intensity and intensity ratios of fluorescence protein expression in the nucleus and cytosol of detected cells imaged by fluorescence microscopy.
References / Literature	For more information regarding the assay check e.g. https://www.mdpi.com/1422-0067/21/12/4410; Reference laboratory: Medical University of Graz, Biophysics: Klaus Groschner, Rainer Schindl;

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IKOSA® Image Analysis

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Application Description

This application automatically detects cells in fluorescent microscopy images that are used during research of gene regulation and signal transduction. For each cell, heterologous fluorescence protein expression is automatically measured by measuring cytosol and nucleus intensity and calculating intensity ratios (intensity cytosol/intensity nucleus). The application was developed and tested with images showing HEK, RBL, and EAHY cells. This analysis can also be performed on timelapse recordings (Time Series) uploaded as 8 or 16-bit multipage TIFF files.

In the following, the requirements for an accurate analysis are given and the output of the application is described.

Further Information

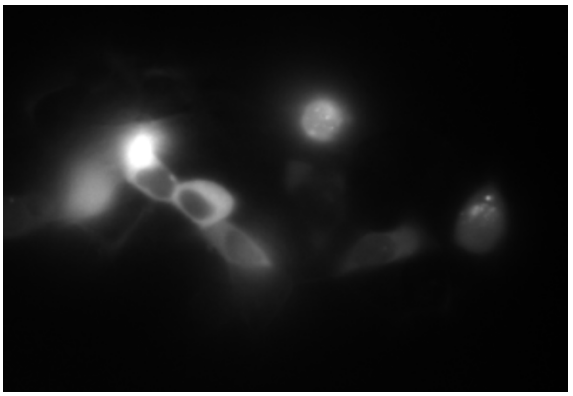
If you have any questions regarding this application or if you want to know if your specific type of images can be analyzed, please get in touch with us at support@kmlvision.com. Also, if you have requests or ideas regarding additional image analysis applications that you would require, please get in touch with us at support@kmlvision.com.

For more information, please visit www.ikosa.ai.

Requirements

Input Image(s)

Input for this application is the following image data:

No.	Image data	Type of image	Color Channels	Color Depth (per channel)	Size [Px]	Resolution [µm/Px]
#1	2D or Time Series	2D or Multipage TIFF	1 (Gray)	8 Bit or 16 Bit	WSI formats: arbitrary Standard images: max. 25,000 x 25,000	0.15 - 0.25
	Image Content: Fluorescent microscopy image showing (single) HEK, RBL, or EAHY cells, typically taken with 40x or 20x magnification. **Additional requirements:** None **Examples:** 					

For all images, the following requirements apply:

- The illumination must be constant throughout the image(s).
- The sample must be in focus, i.e. no blurry regions in image(s).

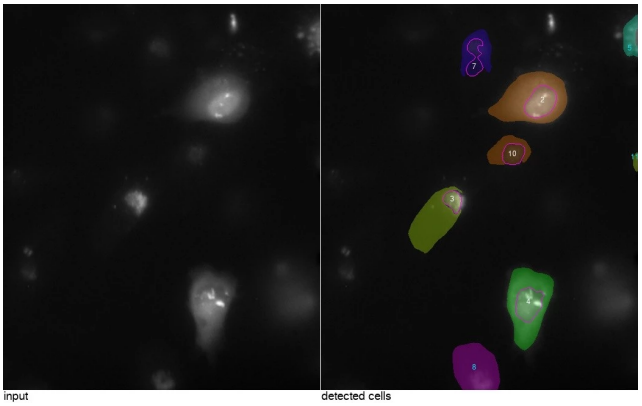
Input Parameter(s)

No additional input parameters are required for this application.

As an optional parameter, a single or multiple regions of interest (ROIs) can be defined in which the analysis should be performed (‘inclusion ROIs’).

Results

Files

No.	File type	Content and Description
1	csv	*results.csv*: A csv file containing the overall analysis results for the input image or all inclusion ROIs.
2	csv	*results_01_cells.csv*: A csv file containing the analysis results for all detected cells in the input image or inclusion ROIs.
3	jpg	*results_vis/vis.jpg* (2D image, no ROI), or *results_vis/t<time-step>.jpg* (time step *<time-step>* of time series, no ROI), or *results_vis/<roi-id>.jpg* (2D image, ROI *<roi-id>*), or *results_vis/t<time-step>_<roi-id>.jpg* (time step *<time-step>* of time series, ROI *<roi-id>*): A visualization of the analysis result for a specific time step for either the whole image (if no inclusion ROIs selected for analysis) or each individual inclusion ROI. Each visualization includes two parts: ● *input* ○ preprocessed (contrast stretched) version of the input image. ● *detected cells* ○ prediction visualization. ○ numbers show the object (cell) IDs. ○ numbers are displayed in cyan colour if the cell is homogeneous (cytosol without nucleus). ○ nuclei are surrounded by a border in magenta. ○ coloured areas outside the border indicate the cytosol of a cell. ○ The coloured area inside the border indicates the nucleus of a cell. ○ If no border is shown, the cell does not have a nucleus and the coloured area indicates the cytosol area only. Example: 

4	json	*roiMeta.json*: A json file containing all information regarding the ROIs defined for the analysis job to ensure reproducibility. The file is empty if no ROIs were defined for analysis.
5	jpg	*rois_visualization.jpg* or *t<time-step>_rois_visualization.jpg*: An overview visualization to show locations of all analyzed ROIs for the 2D image or time step *<time-step>* of a time series. This file is only created if inclusion ROIs were defined for analysis.
6	json	*jobResultBundleMeta.json*: A json file containing all information regarding the analysis job (application name and version, project, etc.) to ensure reproducibility. This file is only included if bundled or merged analysis jobs are downloaded.

Please note:

- For inclusion ROIs that are partially outside of the image, the ROIs are cropped to the areas that are inside of the image.
- For inclusion ROIs that are completely outside of the image, no analysis is performed, however, they are still listed in corresponding result files..
- *<roi-id>* is generated automatically by the application according to the creation date of ROI. The location of a ROI with a specific *<roi-id>* within an image can be seen in the file *rois_visualization.jpg*. ROIs that are completely outside of the image are not shown in this file.
- All visualizations are downscaled to 25 megapixels (MP) if the original image or inclusion ROI is larger than 25 MP.
- **Attention:** Results for a specific region of the same image may vary when performing an analysis on the whole image or ROIs that include this region.

Description of files

File no. 1: Single csv-file with the following content (*results.csv*):
If one or more time steps (of a Time Series) were specified, the results in a specific row refer to the time step specified in the corresponding column.
If one or more ROIs were specified, the results in a specific row refer to the ROI specified in the corresponding columns, otherwise (empty ROI columns) the results refer to the whole image.

Col. no.	Column name	Examples	Value range	Description
1	t	3	1 -	Time step, i.e. the position of the image in the time series.
2	roi_id	ROI-03	ROI-01 -	<roi-id> starting from “ROI-01”. Empty, if no inclusion ROI is specified and the whole image was analyzed.
3	roi_name	“central”	text	Custom text to identify the ROI. Empty, if no inclusion ROI is specified and the whole image was analyzed.
4	roi_size [Px^2]	1212212	1 -	Size of the ROI that was analyzed in pixels^2. The size of the whole image is given if no inclusion ROI is specified and the whole image was analyzed.
5	total_nr_of_cells	3	0 -	Total number of detected cells.
6	nr_active	1	0 -	Number of active cells (nucleus detected that has higher intensity than cytosol).
7	nr_inactive	1	0 -	Number of inactive cells (nucleus detected that has lower intensity than cytosol).
8	nr_homogeneous	1	0 -	Number of homogeneous cells (no nucleus detected in cytosol).

File no. 2: Single csv-file with the following content (*results_01_cells.csv*):
If one or more time steps (of a Time Series) were specified, the results in a specific row refer to the time step specified in the corresponding column.
If one or more ROIs were specified, the results in a specific row refer to the ROI specified in the first columns, otherwise (empty ROI columns) the results refer to the whole image.

Col. no.	Column name	Examples	Value range	Description
1	t	3	1 -	Time step, i.e. the position of the image in the time series.
2	roi_id	ROI-03	ROI-01 -	<roi-id> starting from “ROI-01”. Empty, if no inclusion ROI is specified and the whole image was analyzed.
3	roi_name	“central”	text	Custom text to identify the ROI. Empty if no inclusion ROI is specified and the whole image was analyzed.
4	roi_size [Px^2]	1212212	1 -	Size of the ROI that was analyzed in pixels^2. The size of the whole image is given if no inclusion ROI is specified and the whole image was analyzed.
5	object_id	5	1 -	ID of cell corresponding to id in visualization of ROI or image.

6	I_bckg	229.1	0 -	Image background intensity (determined as minimum intensity).
7	I_cyt	479.7	0 -	Cytosol(es) area mean intensity.
8	I_nuc	411.8	0 -	Nucleus area mean intensity.
9	I_cytbckg	250.7	0 -	Cytosol(es) area mean intensity with subtracted background intensity (I_cyt - I_bckg).
10	I_nucbckg	182.8	0 -	Nucleus area mean intensity with subtracted background intensity (I_nuc - I_bckg).
11	R_nuc_cyt	0.73	0 -	Ratio between nucleus and cytosol mean intensity with subtracted background intensity (I_nucbckg / I_cytbckg). If cell is homogeneous (no nucleus), R_nuc_cyt is set to 1.
12	is_active	1	1 / 0	Boolean indicator if cell is active (I_nuc > I_cyt).
13	is_inactive	0	1 / 0	Boolean indicator if cell is inactive (I_nuc < I_cyt).
14	is_homogeneous	0	1 / 0	Boolean indicator if cell is homogeneous. “1” if no nucleus area is detected.
15	area_cell [Px^2]	132	1 -	Area of detected cell in Pixels^2.
16	bbox_area_cell [Px^2]	175	1 -	Area of bounding box of detected cell in Pixels^2.
17	perimeter_cell [Px]	78.5	0 -	Perimeter of detected cell in Pixels.
18	circularity_cell	0.91	0 -	Circularity factor of detected cell; circularity = 4*pi*area/(perimeter^2). The circularity of a circle is 1.
19	eccentricity_cell	0.96	0 - 1	Eccentricity of the ellipse that has the same second-moments as the region of the cell. The eccentricity is the ratio of the focal distance (distance between focal points) over the major axis length. When it is 0, the ellipse becomes a circle.
20	area_cyt [Px^2]	102	1 -	Area of detected cytosol in Pixels^2.
21	bbox_area_cyt [Px^2]	142	1 -	Area of bounding box of detected cytosol in Pixels^2.
22	perimeter_cyt [Px]	52.3	0 -	Perimeter of detected cytosol in Pixels.
23	circularity_cyt	0.74	0 -	Circularity factor of detected cytosol; circularity = 4*pi*area/(perimeter^2). The circularity of a circle is 1.
24	eccentricity_cyt	0.91	0 - 1	Eccentricity of the ellipse that has the same second-moments as the region of the cytosol. The eccentricity is the ratio of the focal distance (distance between focal points) over the major axis length. When it is 0, the ellipse becomes a circle.
25	area_nuc [Px^2]	30	1 -	Area of detected nucleus in Pixels^2. Empty, if the cell is detected as homogeneous (no nucleus detected).

26	bbox_area_nuc [Px^2]	46	1 -	Area of bounding box of detected nucleus in Pixels^2. Empty, if the cell is detected as homogeneous (no nucleus detected).
27	perimeter_nuc [Px]	12.5	0 -	Perimeter of detected nucleus in Pixels. Empty, if the cell is detected as homogeneous (no nucleus detected).
28	circularity_nuc	1.02	0 -	Circularity factor of detected nucleus; circularity = 4*pi*area/(perimeter^2). The circularity of a circle is 1. Empty, if the cell is detected as homogeneous (no nucleus detected).
29	eccentricity_nuc	1.23	0 - 1	Eccentricity of the ellipse that has the same second-moments as the region of the nucleus. The eccentricity is the ratio of the focal distance (distance between focal points) over the major axis length. When it is 0, the ellipse becomes a circle. Empty, if the cell is detected as homogeneous (no nucleus detected).

Error Information

If an image analysis job fails, an error code and error message are returned. Failed analysis jobs are listed in the *Failed* tab of the *Analysis Jobs* section on the *Image Analysis* page on IKOSA®. For more information regarding the errors, please check the Application Errors Documentation.