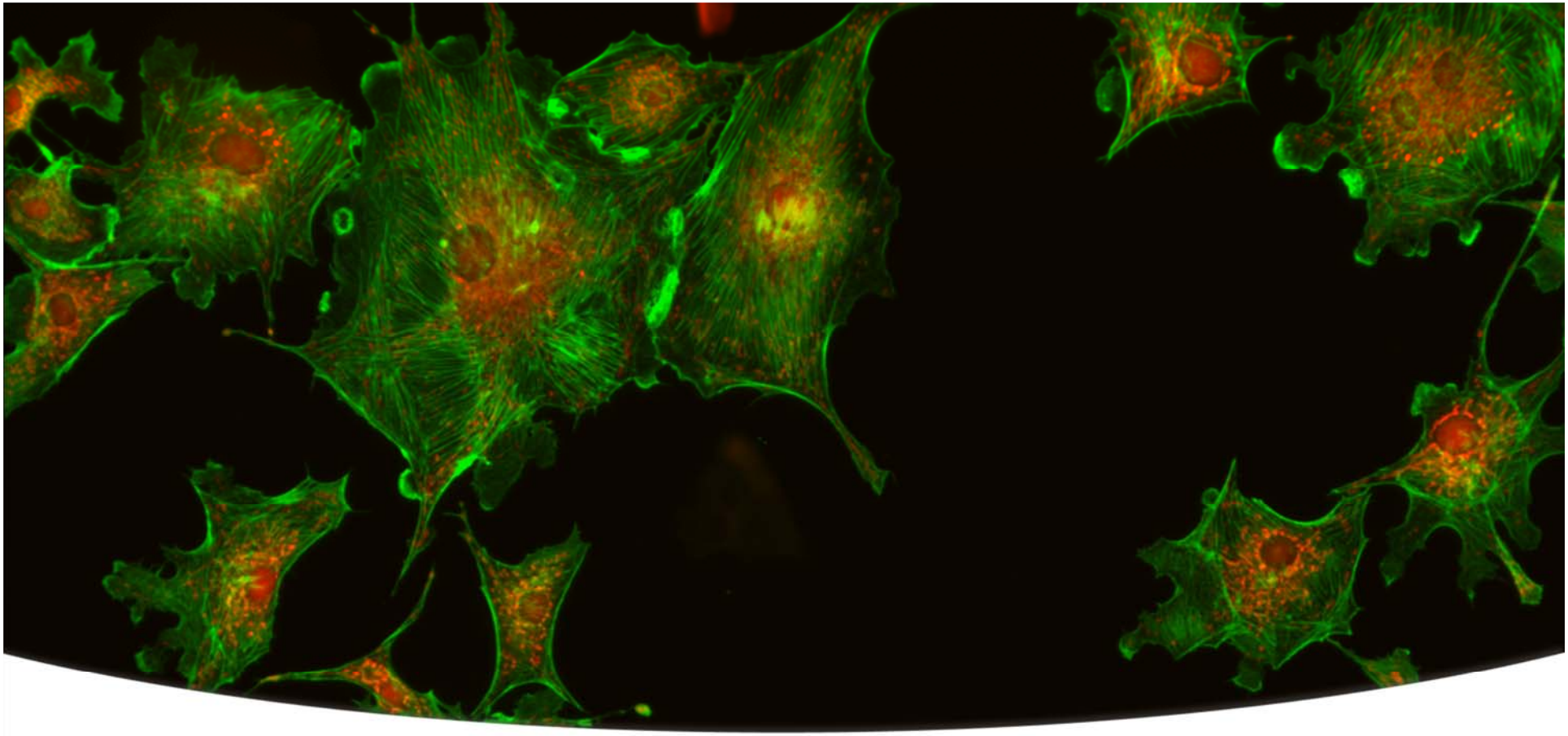
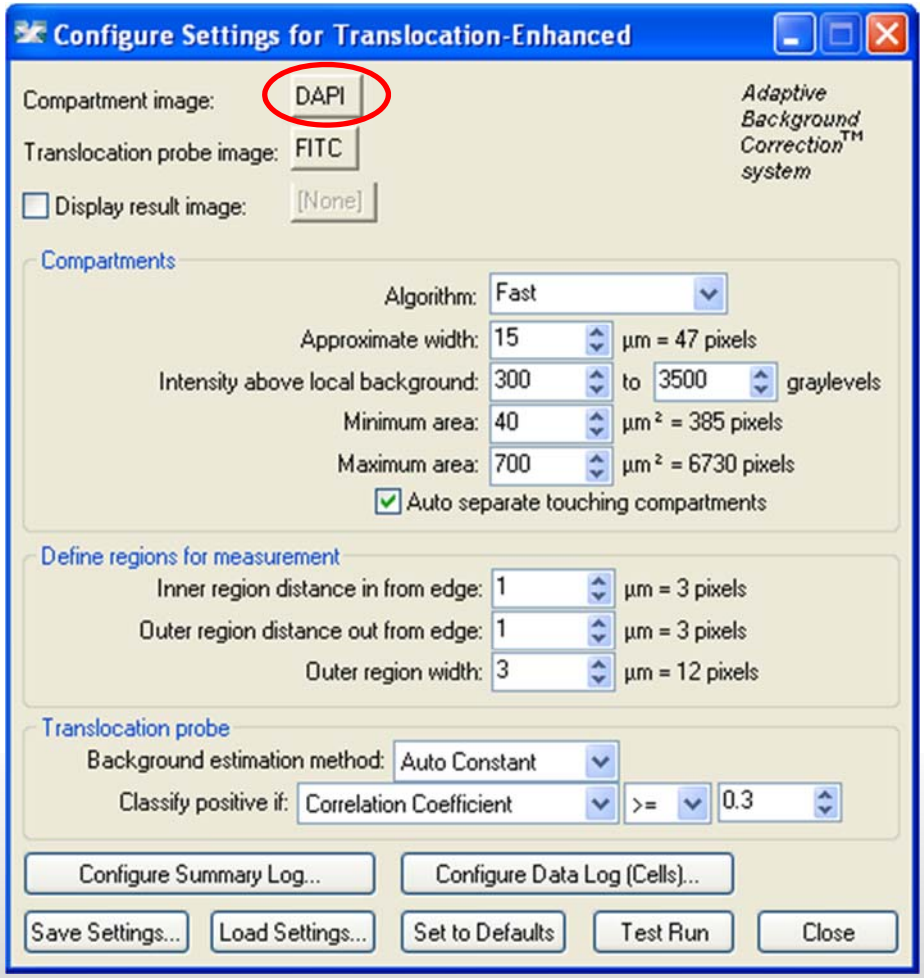


Together through life sciences.

Molecular Devices

MetaXpress® Software: Translocation – Enhanced Module

Module Settings



Compartments = Nuclei

Note that this module may also be used for measuring translocation to other organelles.

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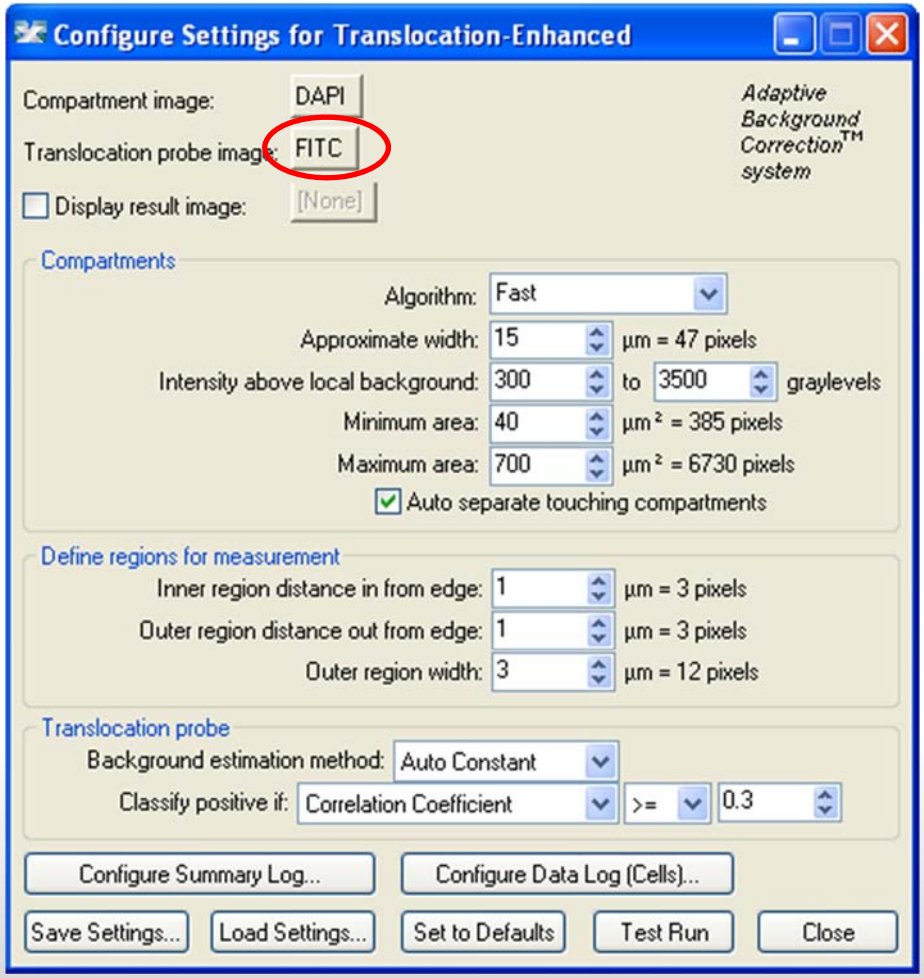
Module Settings



Translocation probe image = the marker that is moving between nucleus and cytoplasm

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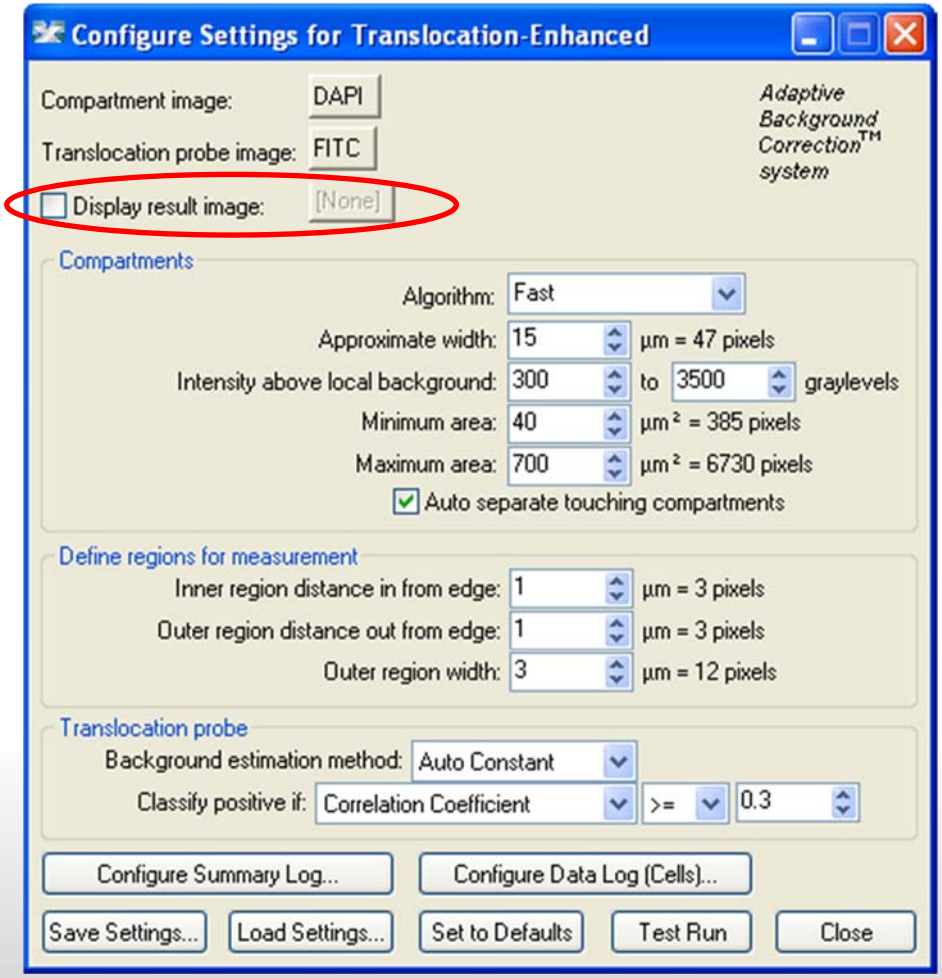
Module Settings



Leave "**Display result image**" deselected (this is generally only used when journaling)

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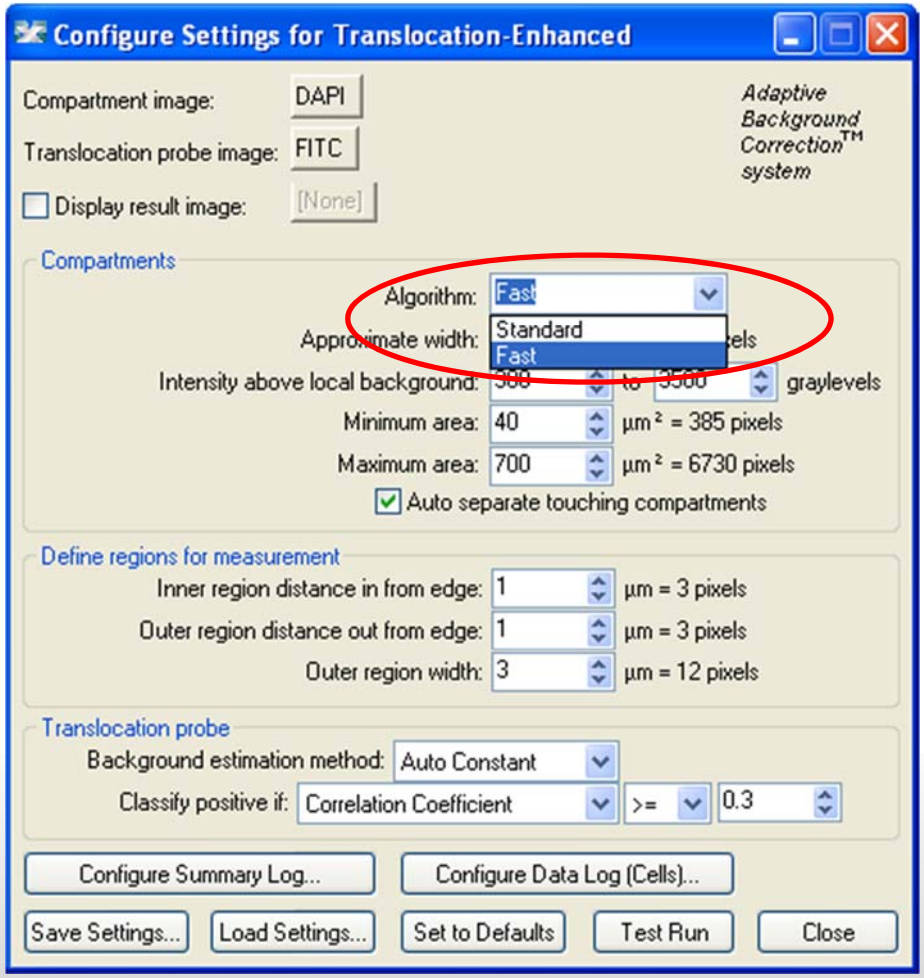
Module Settings



Algorithm

This option is only available in MetaXpress software version 4.0 and higher and determines how quickly the analysis is performed.

Fast algorithm can perform analysis up to twice as fast as **Standard**.

Both algorithms produce similar but not identical results.

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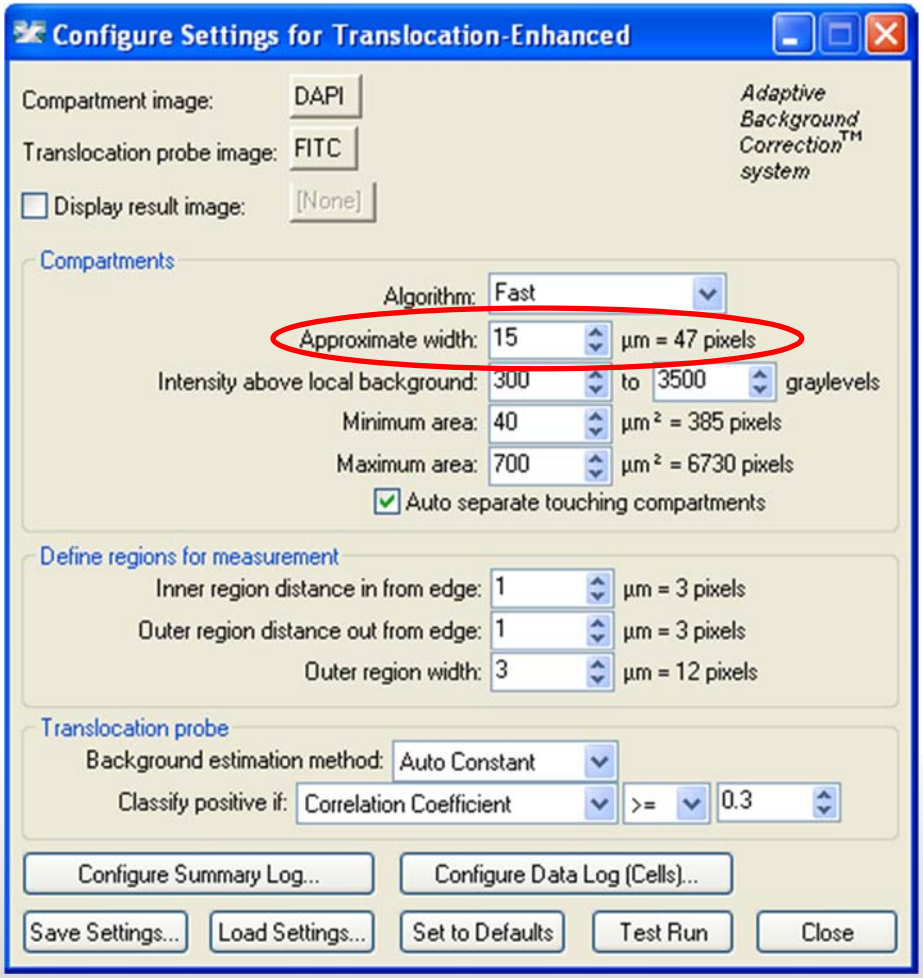
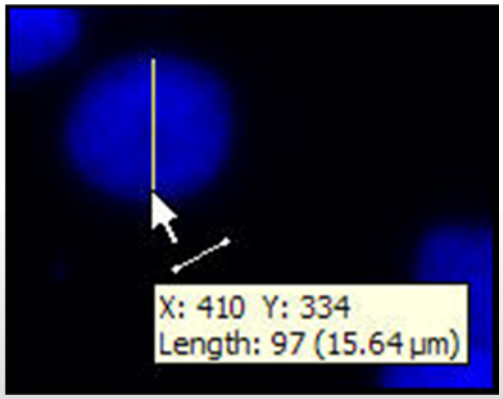
Module Settings



Approximate width should be set to the average width (short axis) of a nucleus (in um). The region tools can be used to measure widths.

Much smaller or much larger cells will be ignored.



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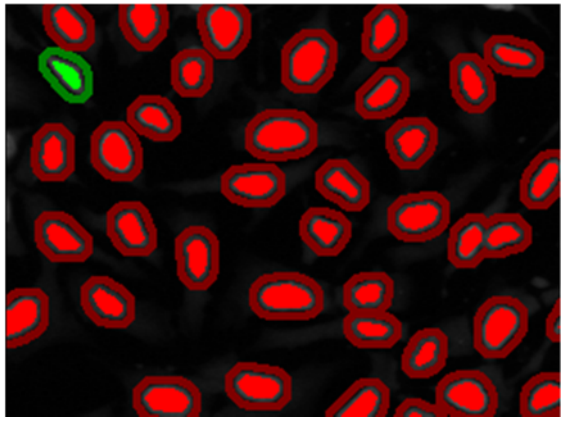
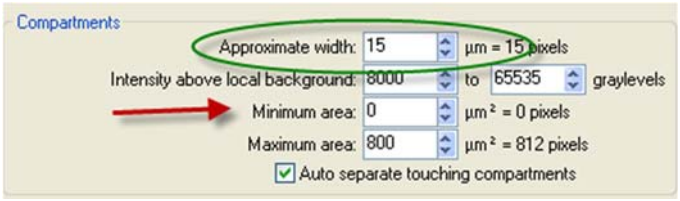
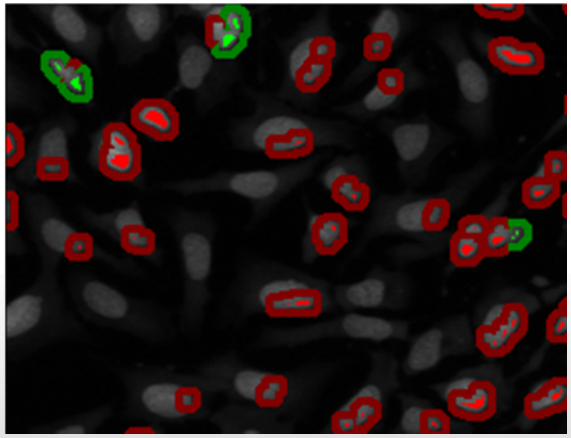
Effects of Minimum width settings



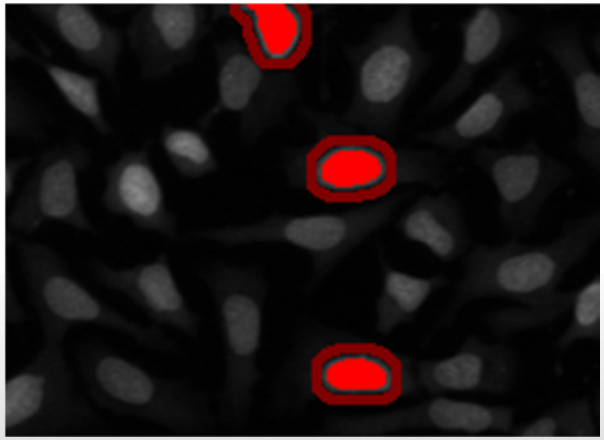
Set correctly



Note: The Minimum and Maximum area will have an impact as well!



Set too low



Set too large

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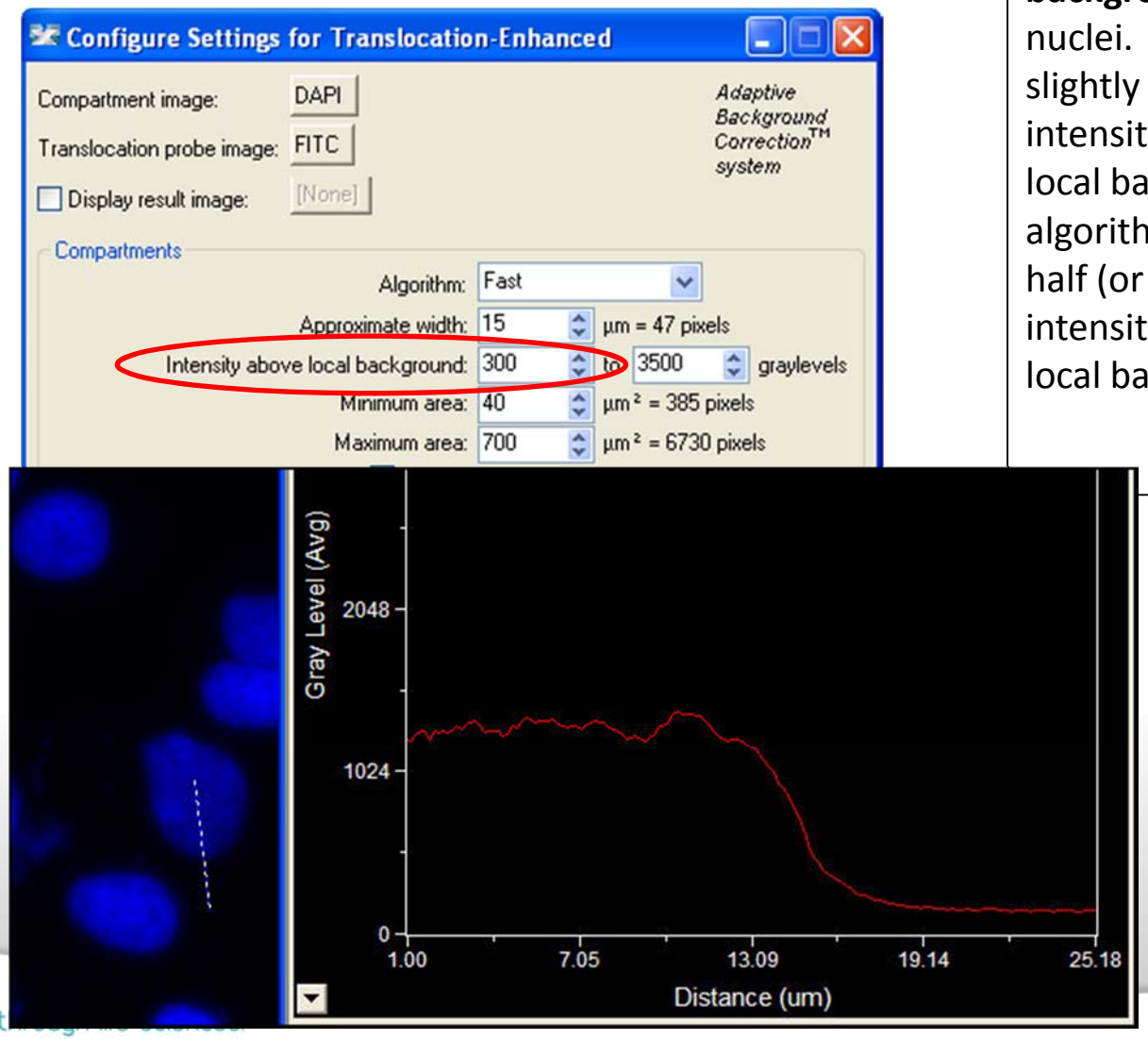
Module Settings



The **intensity above local background** is used for finding the nuclei. The **minimum** should be set slightly lower than the difference in intensity between a dim cell and its local background. For FAST algorithm, set this value to about half (or less) of the difference in intensity between a dim cell and local background.

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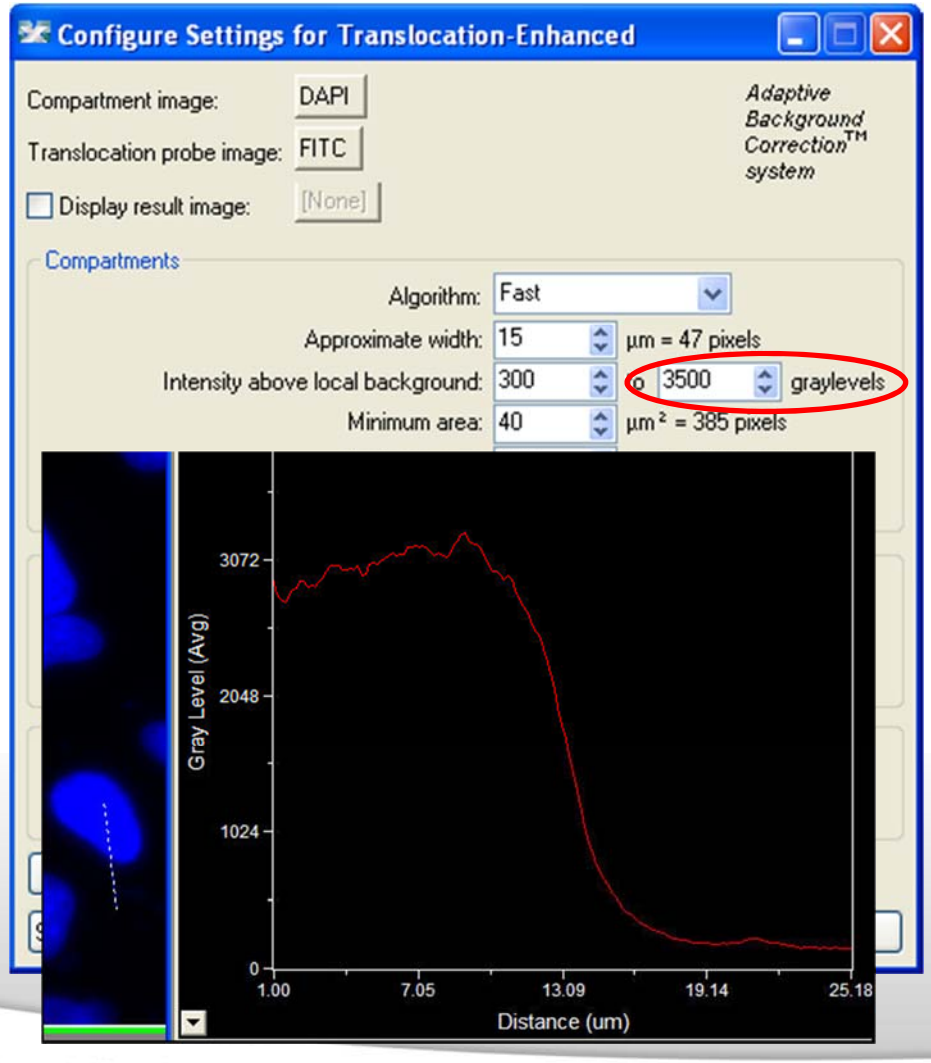
Module Settings



The **intensity above local background** is used for finding the nuclei. The **maximum** setting can be used to exclude cells with bright nuclei.

To include cells with bright nuclei, set this value to 4095 (12-bit images including IXM images) or 65535 (16-bit images including IXU images)

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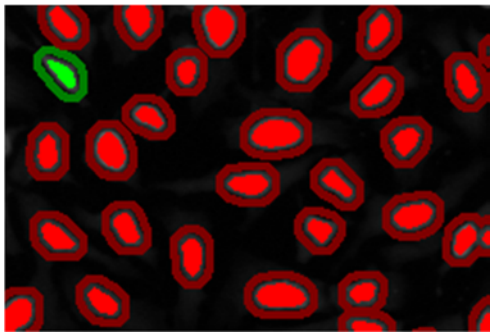
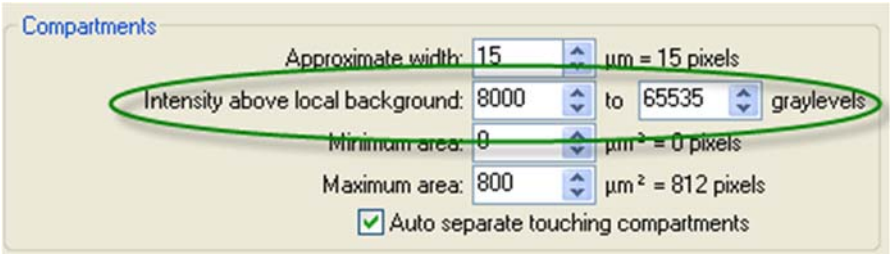
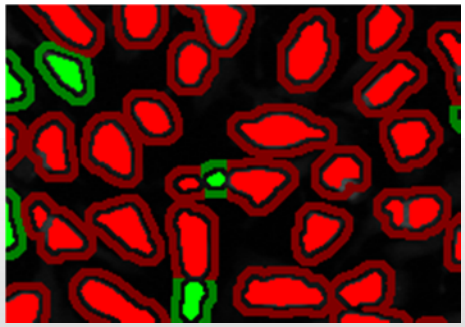
Effects of intensity settings

- Set correctly

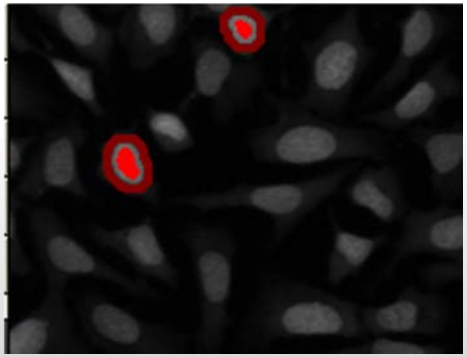




- Set too low → Compartments are too large



- Set too high → Compartments are not being detected



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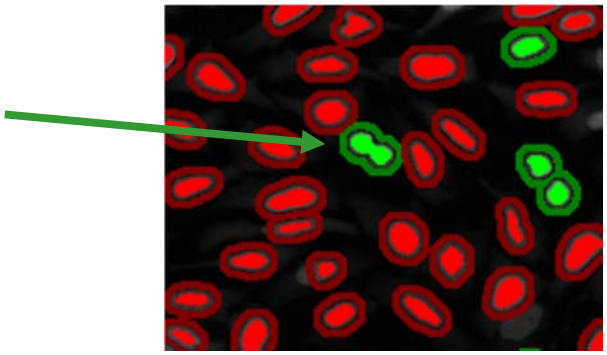
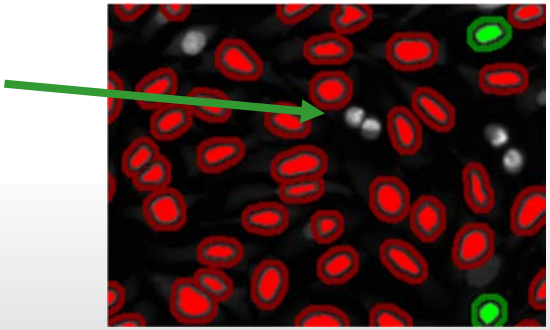
Effects of intensity settings

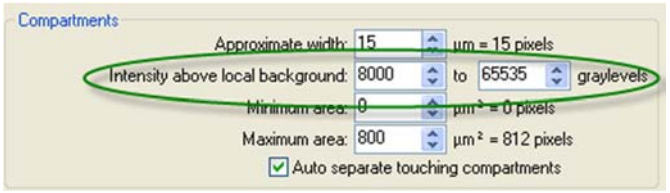
- All cells/ compartment detected



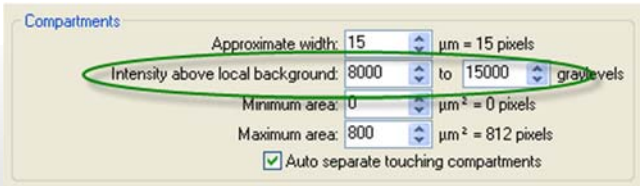
- Bright cells are being ignored



- Max intensity set to 65535 (bit dept of image
- All nucleus detected



- Max intensity set lower then the bright (mitotic) cells
- Only “normal” cells detected



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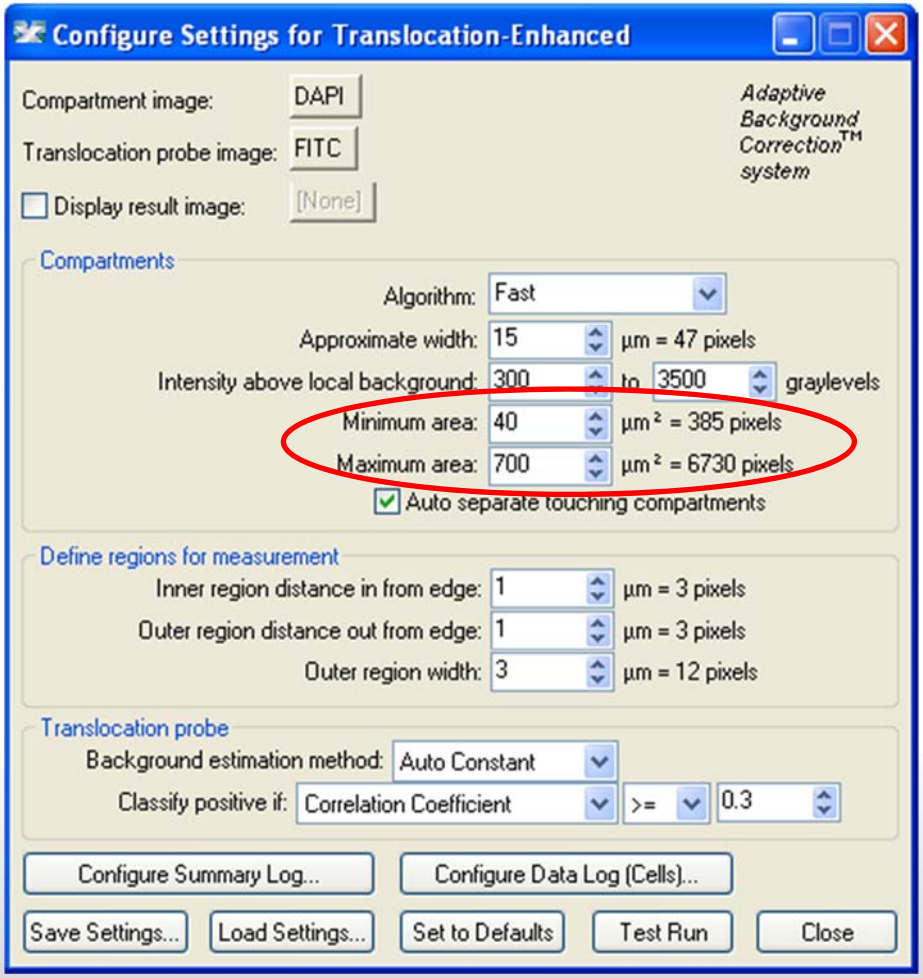
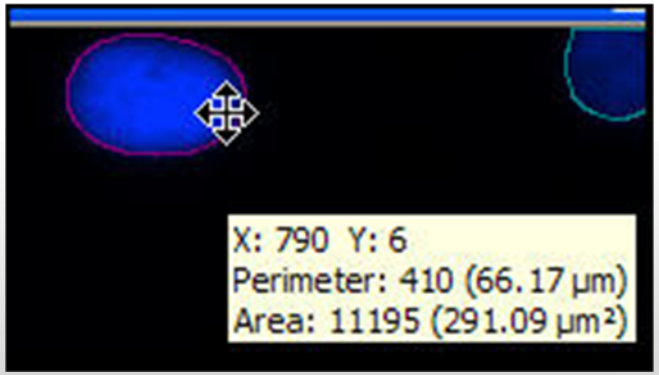
Module Settings



Translocation-enhanced allows you to set a **minimum and maximum area** for nuclei to be included in the analysis. This can be used to remove debris (too small) and large clusters (too big).

Area can be measured using the region tools.



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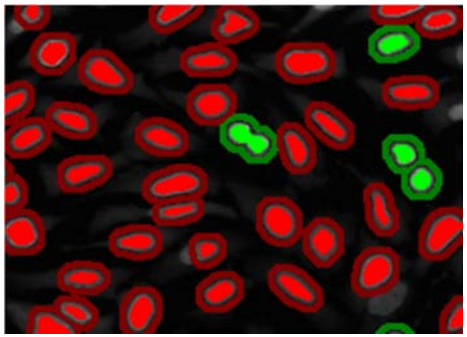
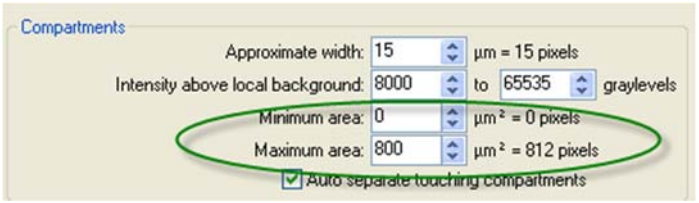
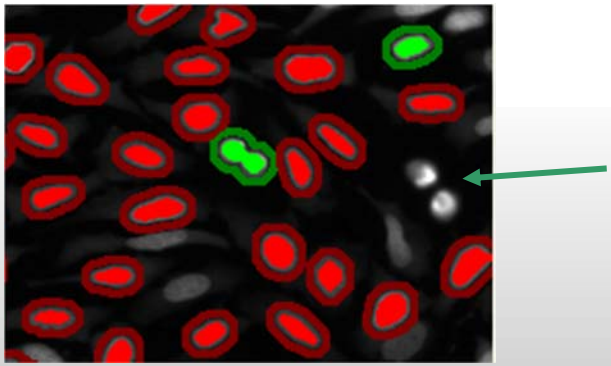
Effects of minimum and maximum area

- Min= 0, Max=800
- Detect all compartment/ cells

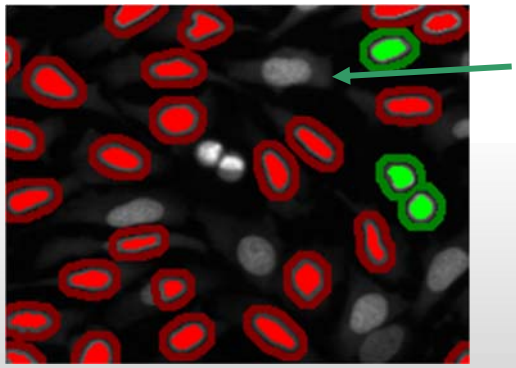




- Min= 150, Max=800
- Ignores small compartments/ cells



- Min= 0, Max=250
- Ignores large compartments/ cells



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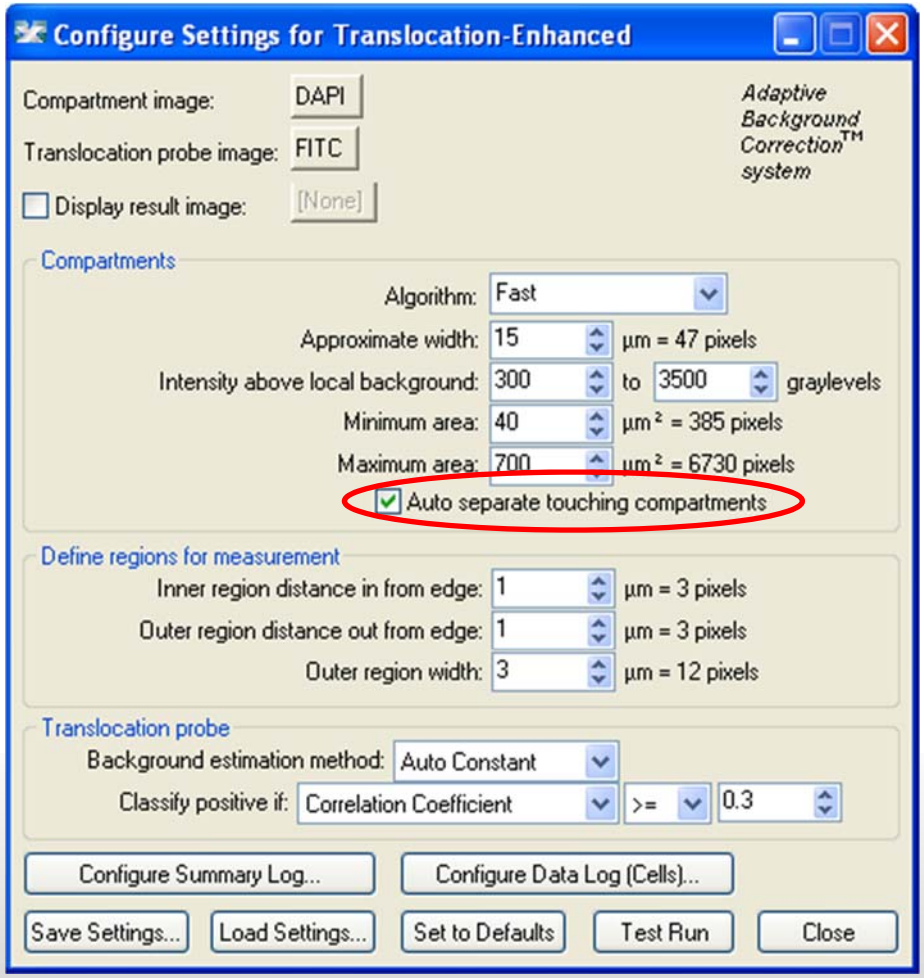
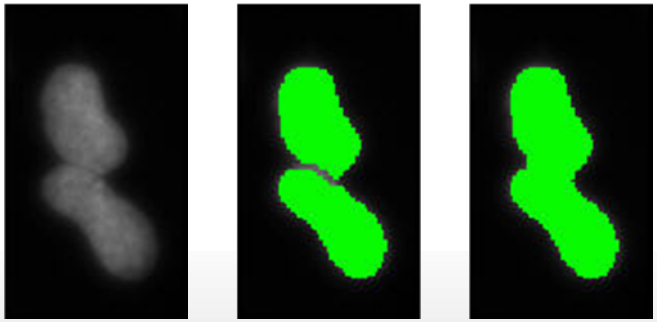
Module Settings



Select "**Auto separate touching compartments**" to split touching nuclei.

The module looks for round blob-shaped objects and the shape is used to determine the splitting.



Auto separate option: On Off

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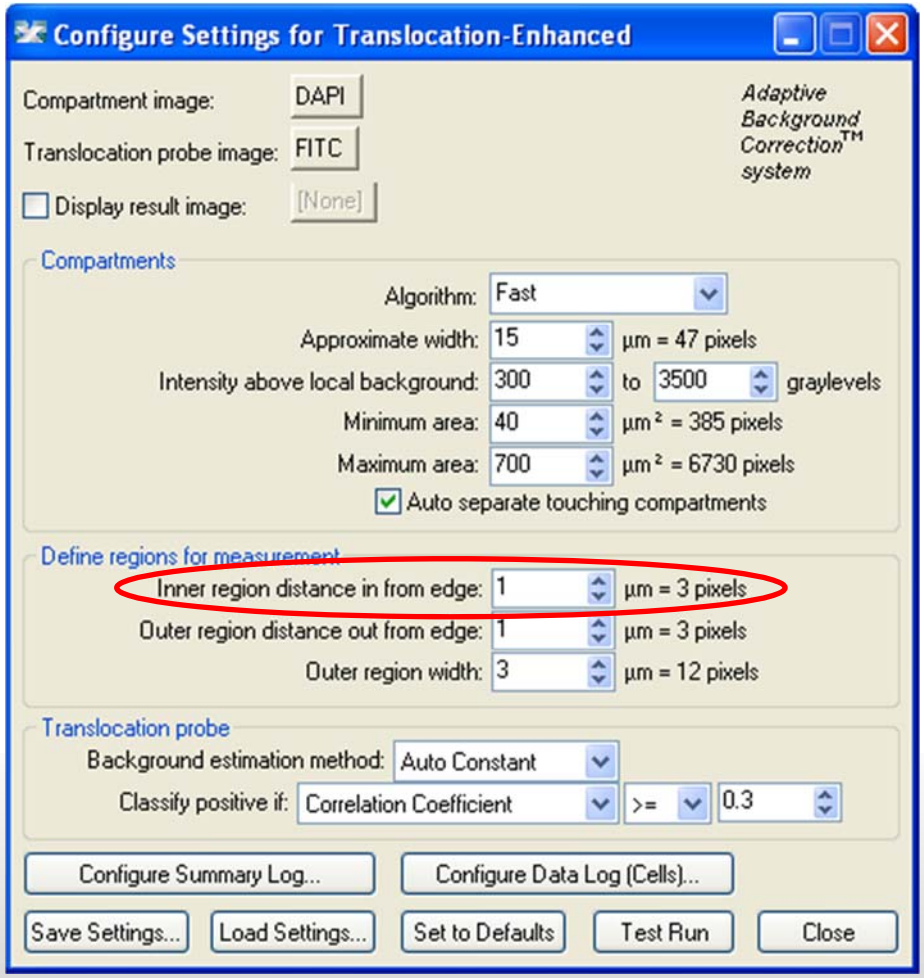
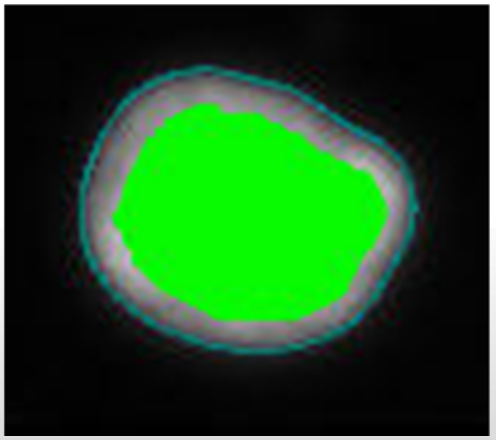
Module Settings



Define regions for measurement:

The “inner region” can be set to be shrunk in inner region from the detected nucleus to avoid boundary effects. 1 um is the default setting used in the Translocation module.



2 um in

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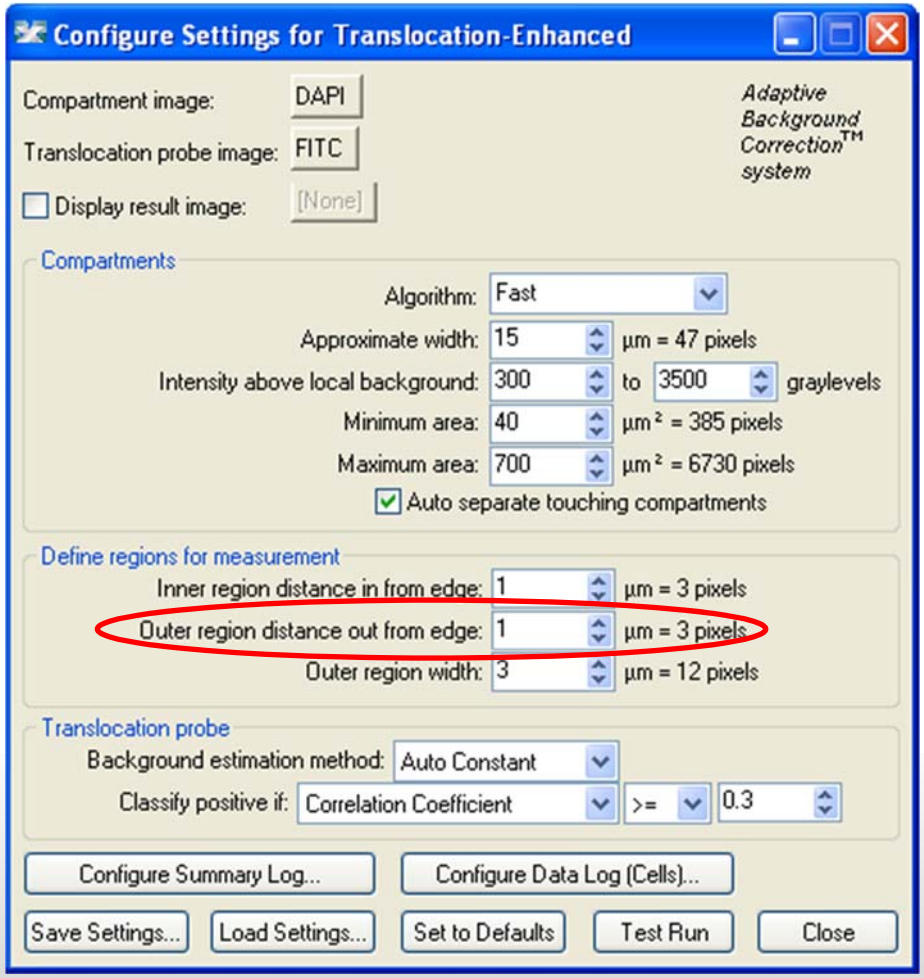
Module Settings



Define regions for measurement:

The "outer region" can be set to be expanded out from the detected nucleus to avoid boundary effects. 1 um is the default setting used in the Translocation module.

2 um out

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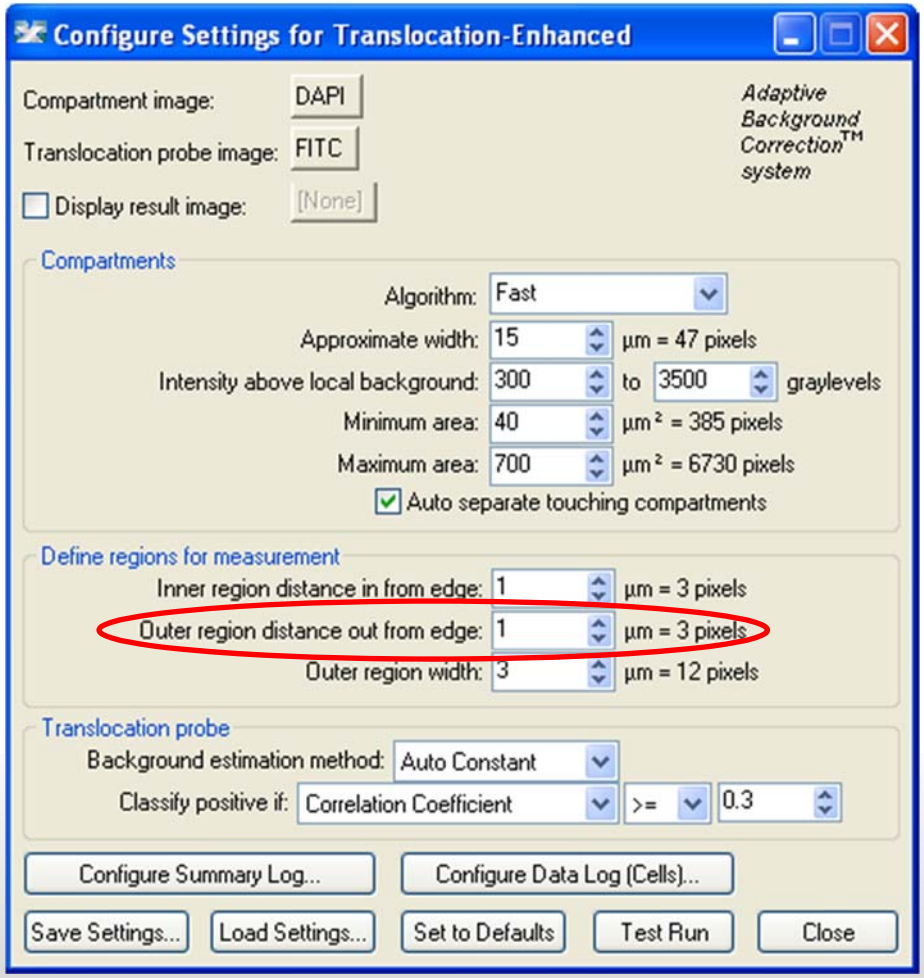
Module Settings



Define regions for measurement:

Typically the inner region and outer region distances are both utilized.

2 um in and out

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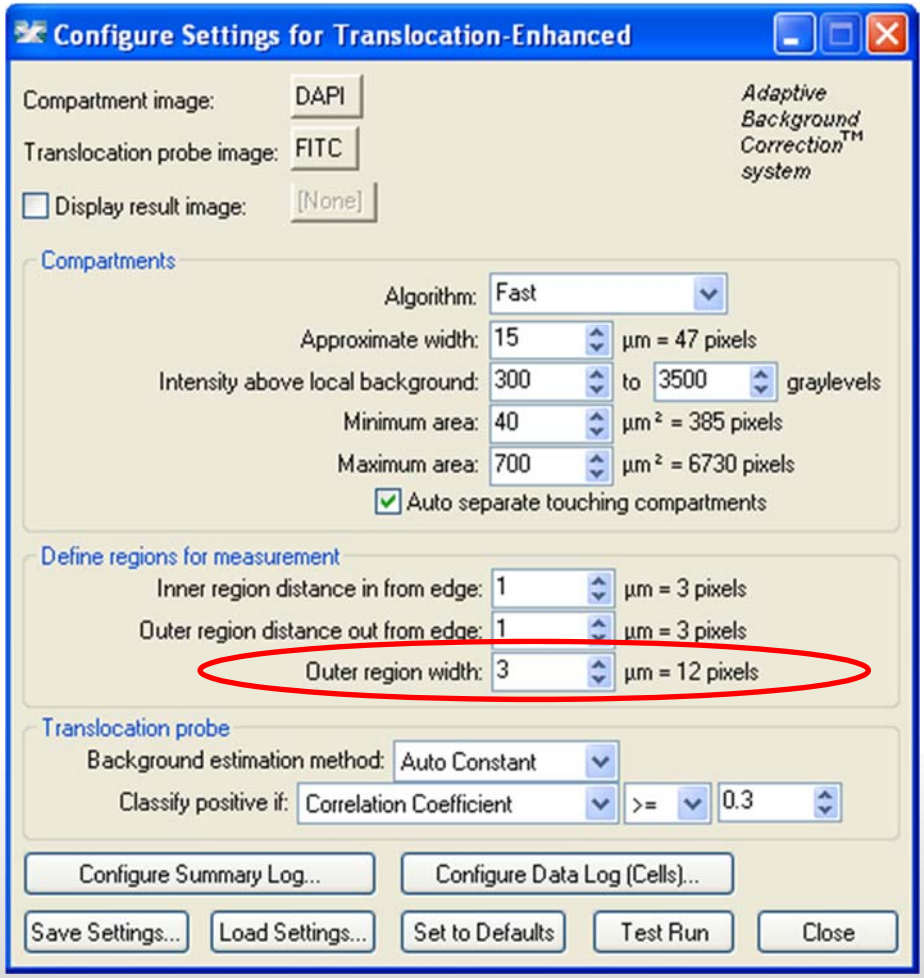
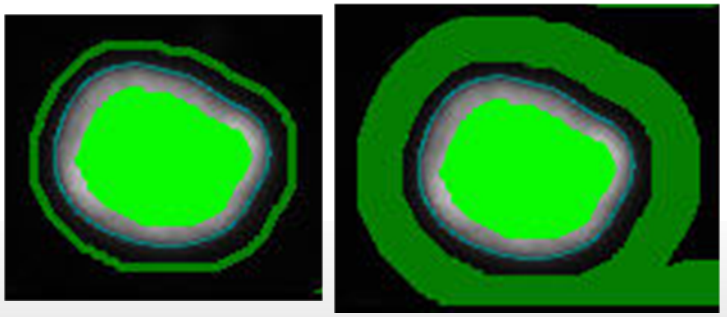
Module Settings



Define regions for measurement:

The outer region width can be adjusted for the specific cell type (and degree of confluency). 3 um is the default setting used in the Translocation module.



1 um 5 um

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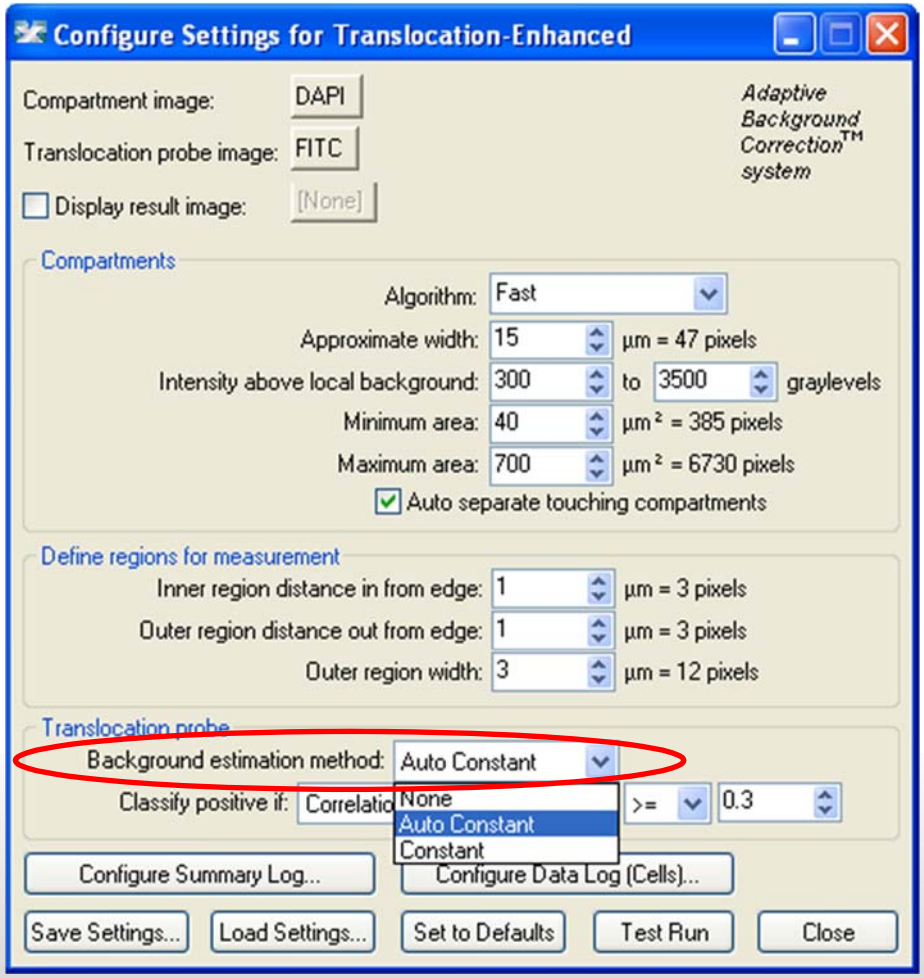
Module Settings



Background estimation method:

The background intensity is subtracted from the probe intensities before measurements are performed and recorded.

None: no background subtraction is performed

Auto Constant: an average background value is calculated for each image and subtracted

Constant: you input a fixed background intensity to be subtracted

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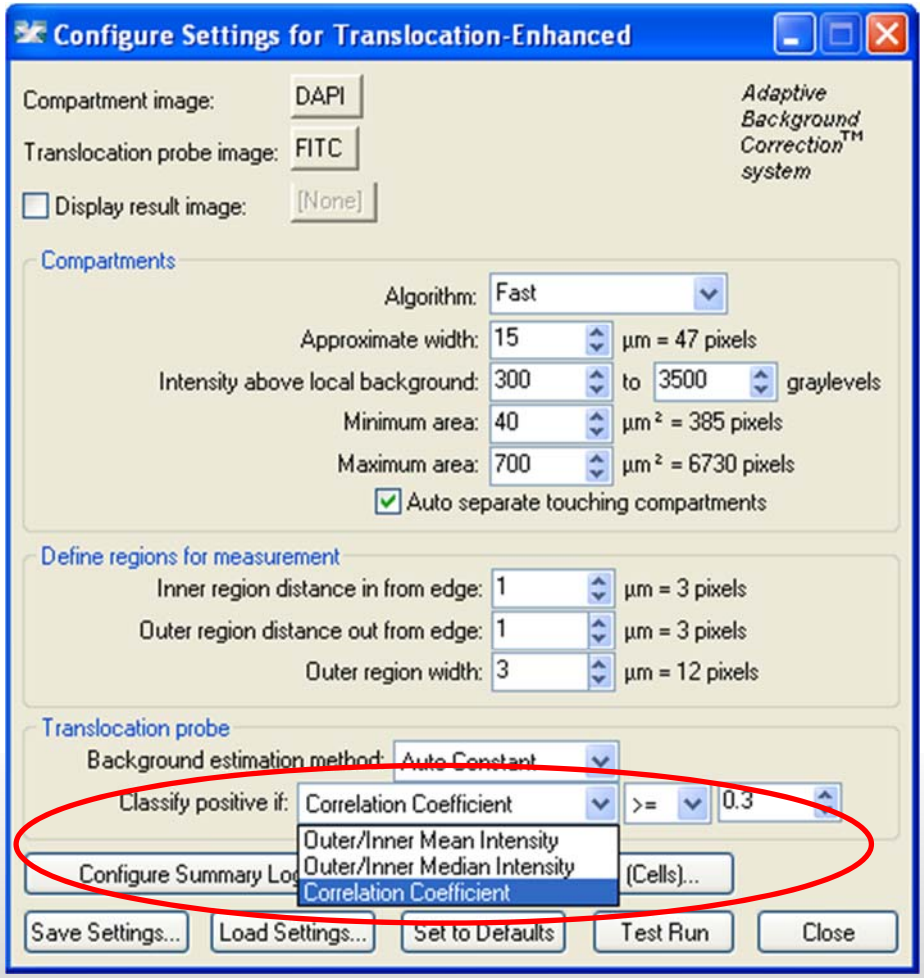
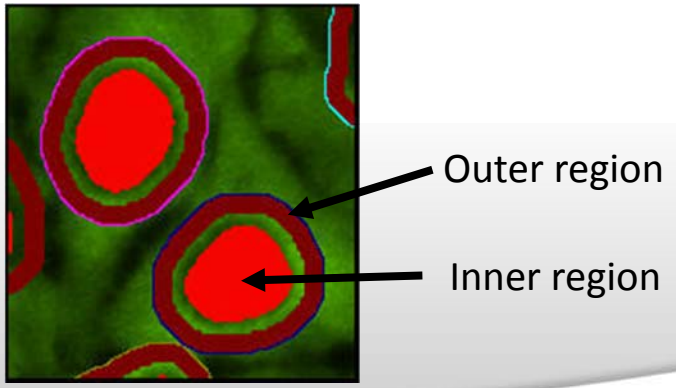
Module Settings



Cell classification:

Typically, positive cells are those with nuclear staining (marked green). Negative cells are those with cytoplasmic staining (marked red).

Outer / Inner Mean Intensity: This is the ratio of the average pixel intensity of the probe in the outer region (cytoplasm) to the inner region (nucleus)



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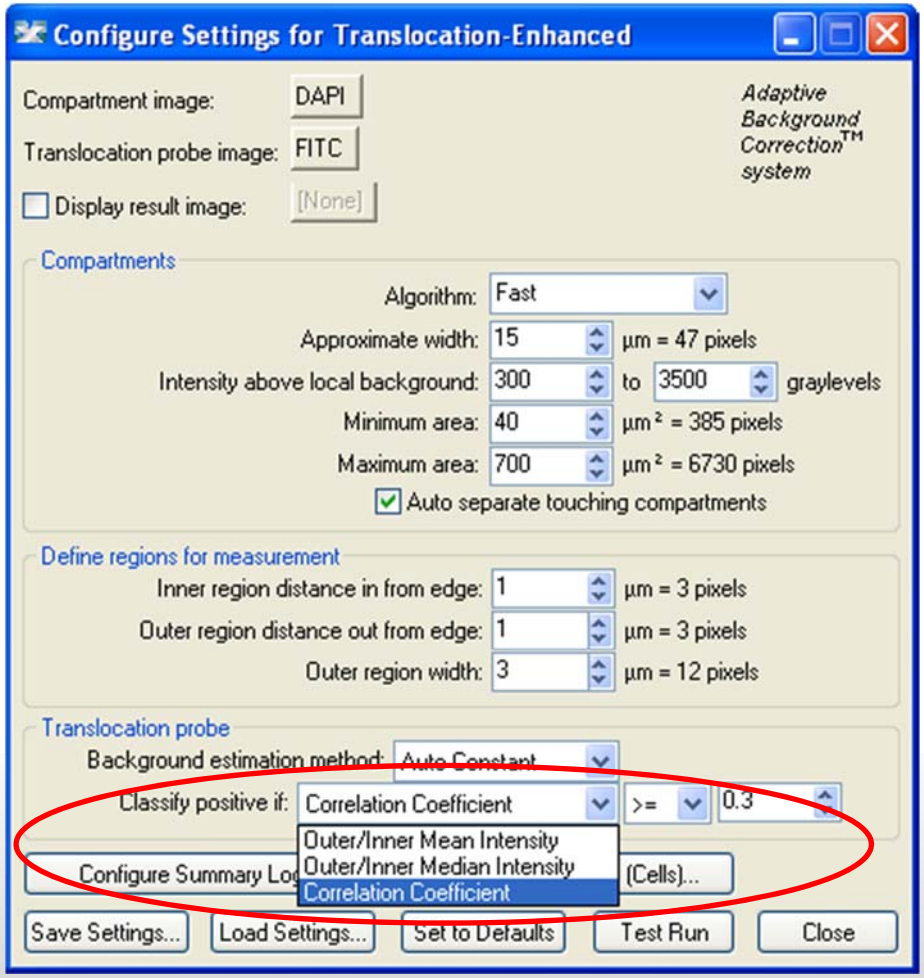
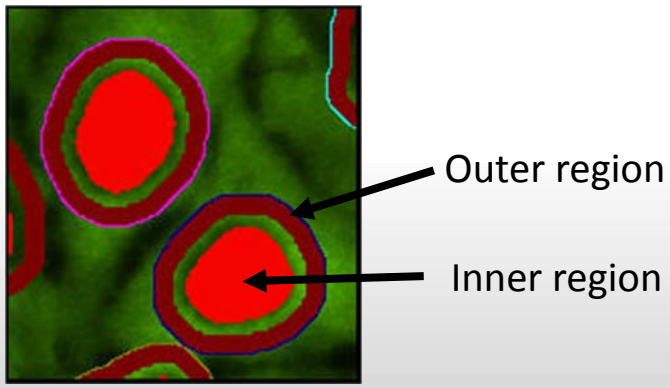
Module Settings



Cell classification:

Outer / Inner Median Intensity:
This is the ratio of the middle pixel intensity of the probe in the outer region (cytoplasm) to the inner region (nucleus). Median is less likely than the mean to be skewed by outlying bright/dim pixels.



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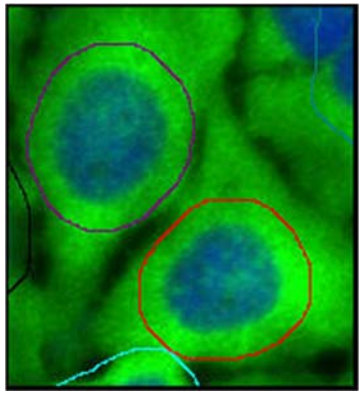
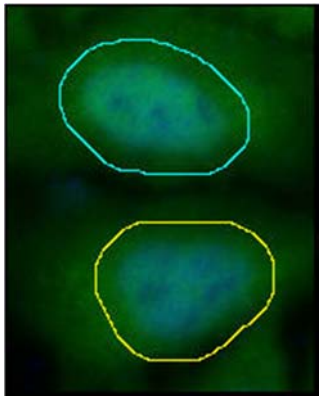
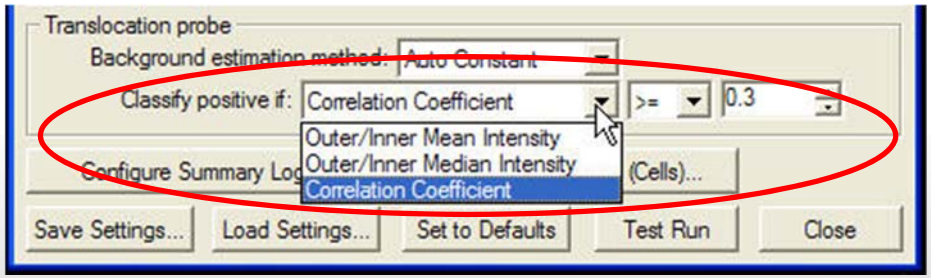
Module Settings



Negative
(cytoplasmic)



Positive
(nuclear)



Cell classification:

Correlation Coefficient: This is the Pearson's correlation coefficient of the pixel intensity of the two stains in the entire cell region (nucleus + gap + cytoplasm). This is typically the most robust method for classifying translocation.

1.0 is perfect correlation (the two stains perfectly overlap)

-1.0 is perfect anti-correlation (the two stains never overlap)

0 indicates the stains are independent

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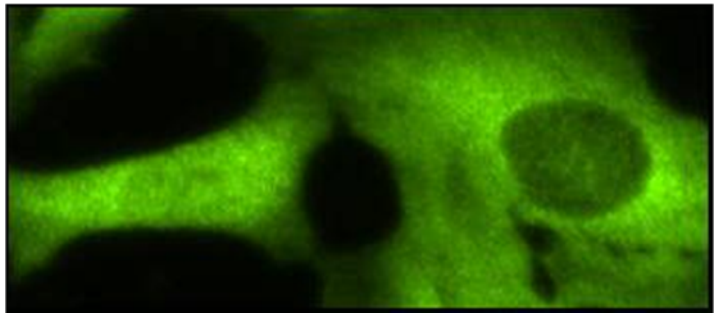
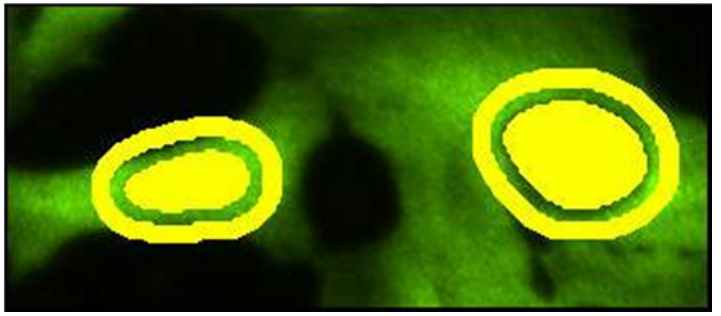
Module Settings





Setting Cell classification cutoffs:

Test module on positive and negative controls.

Use the interactive cellular results table to view the individual correlation or intensity ratio results for positive and negative cells.

An image showing both phenotypes makes it easy to compare results.

Cell: Outer/Inner Mean Intensity	Cell: Outer/Inner Median Intensity	Cell: Inner/Outer Mean Intensity	Cell: Inner/Outer Median Intensity	Cell: Correlation Coefficient
2.45225	2.78571	0.407788	0.358974	-0.750299
1.24792	1.26404	0.801333	0.791111	-0.473332
0.917899	0.911544	1.08944	1.09704	-0.133511
0.853035	0.880068	1.17229	1.13628	0.324409
2.3273	2.52778	0.429682	0.395604	-0.745756
1.38996	1.44051	0.719446	0.6942	-0.45814
2.19401	2.32195	0.455786	0.430672	-0.664952
2.39232	2.35507	0.418005	0.424615	-0.752218
1.72638	1.63306	0.579246	0.612346	-0.441223
1.19731	1.08209	0.835203	0.924138	-0.203629
1.88186	2.005	0.531389	0.498753	-0.792422
0.907879	0.985294	1.10147	1.01493	0.112205
1.91809	1.89589	0.521352	0.527457	-0.674677
0.452187	0.404901	2.21147	2.46974	0.519238
1.74348	1.63874	0.573567	0.610224	-0.61115
1.66259	1.83203	0.60147	0.545842	-0.62916

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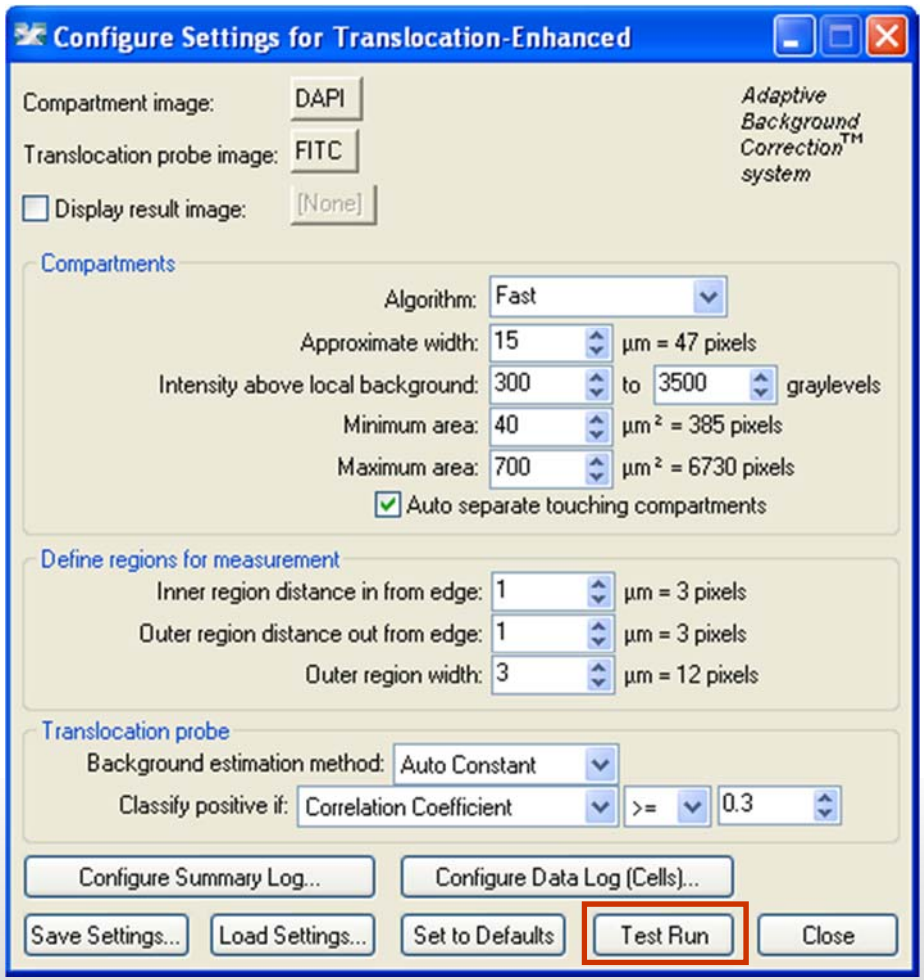
Module Settings



- Select **Test Run** to view the cell segmentation
- Change settings if needed
- Save the settings

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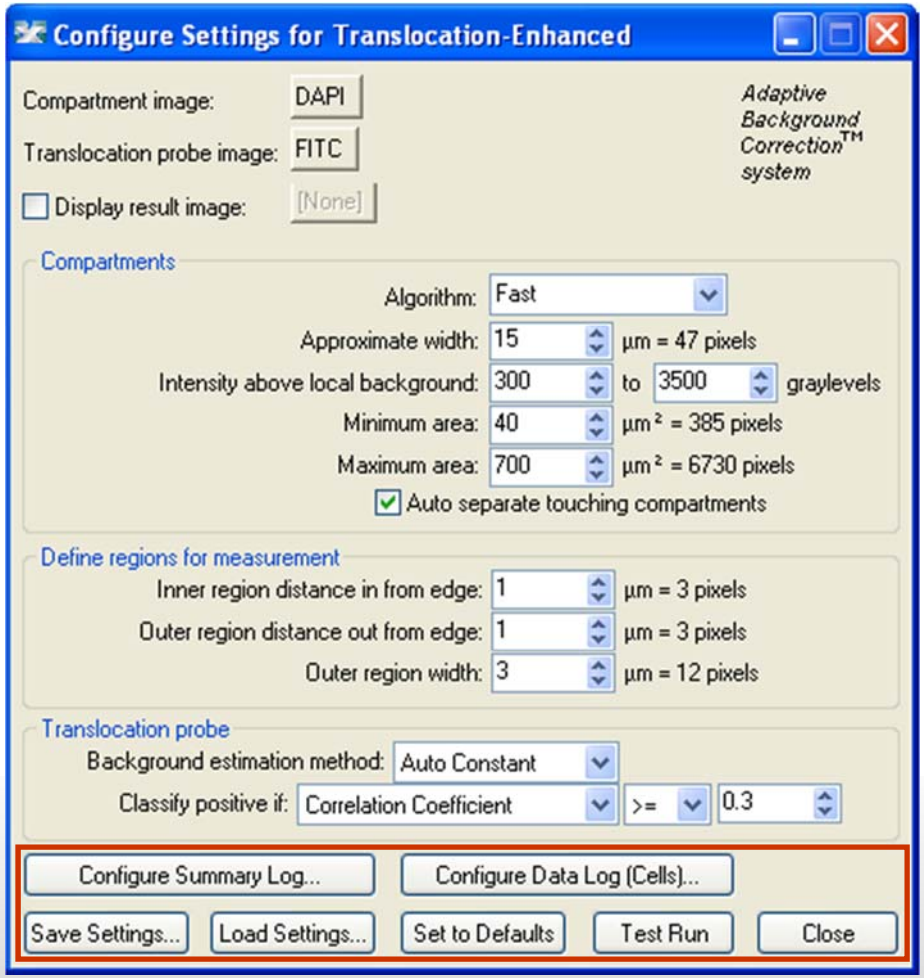
Module Settings – General Settings



- **Configure Summary Log** – select site-by-site measurements
- **Configure Data Log** – select cell-by-cell measurements
- **Save Settings** – save analysis parameters to database
- **Load Settings** – load saved analysis parameters
- **Set to Defaults** – restore default analysis parameters
- **Test Run** – test all settings together and display cell-by-cell results for this site

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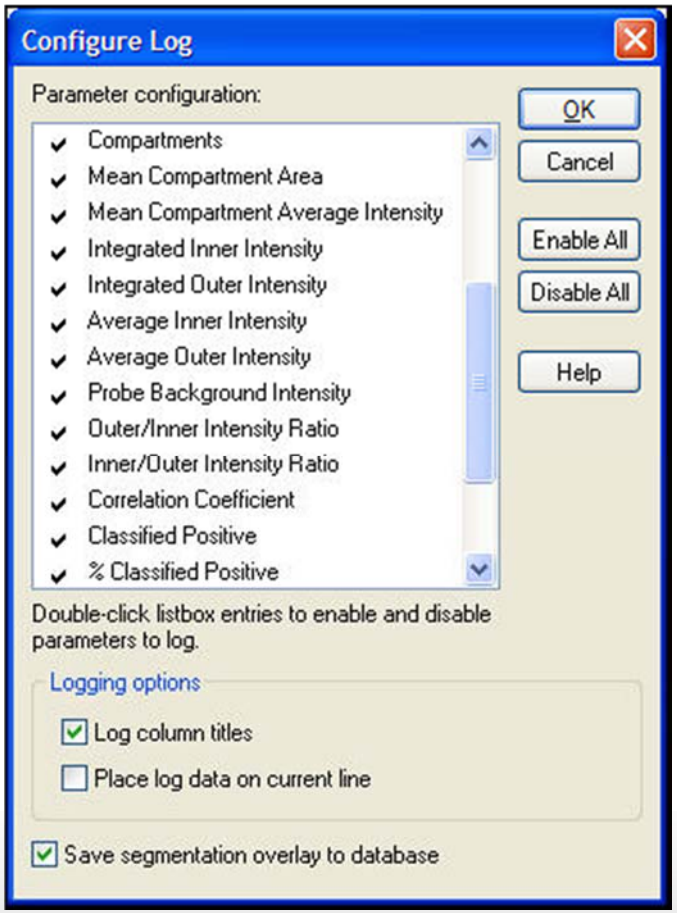
Summary Data (site-by-site measurements)



Compartments: Total number of nuclei (cell count)

Mean Compartment Area: The average nuclear area (in um^2)

Mean Compartment Average Intensity: The average pixel intensity of the nuclear stain over the nuclear area

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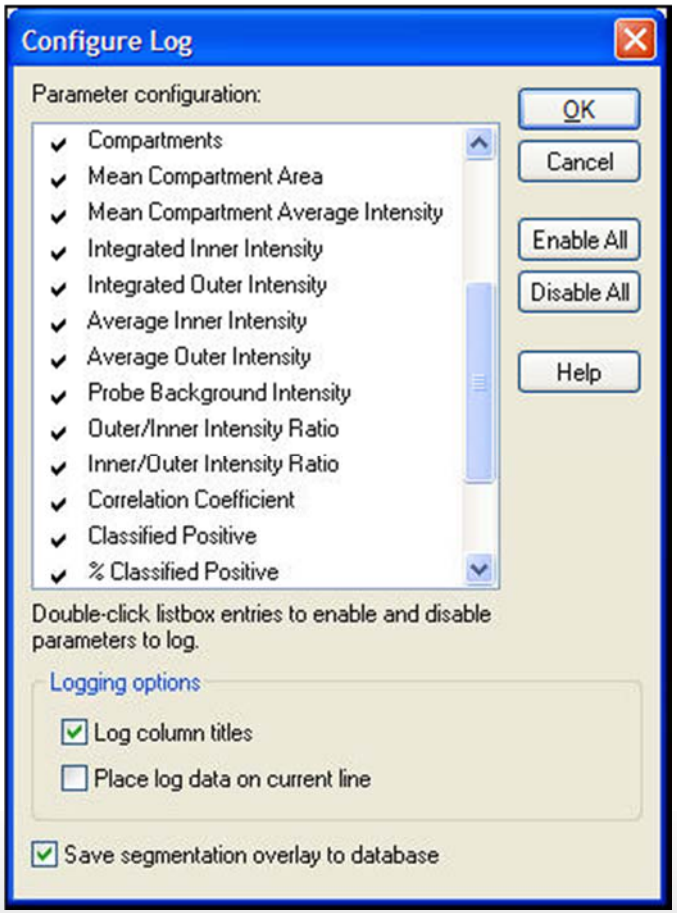
Summary Data (site-by-site measurements)



Integrated Inner Intensity: The total pixel intensity of the probe in all the inner regions for the site after background subtraction (note this correlates with cell count)

Integrated Outer Intensity: The total pixel intensity of the probe in all the outer regions for the site after background subtraction (note that this correlates with cell count)

Average Inner Intensity: The average pixel intensity of the probe in all the inner regions for the site after background subtraction (independent of cell count)

Average Outer Intensity: The average pixel intensity of the probe in all the outer regions for the site after background subtraction (independent of cell count)

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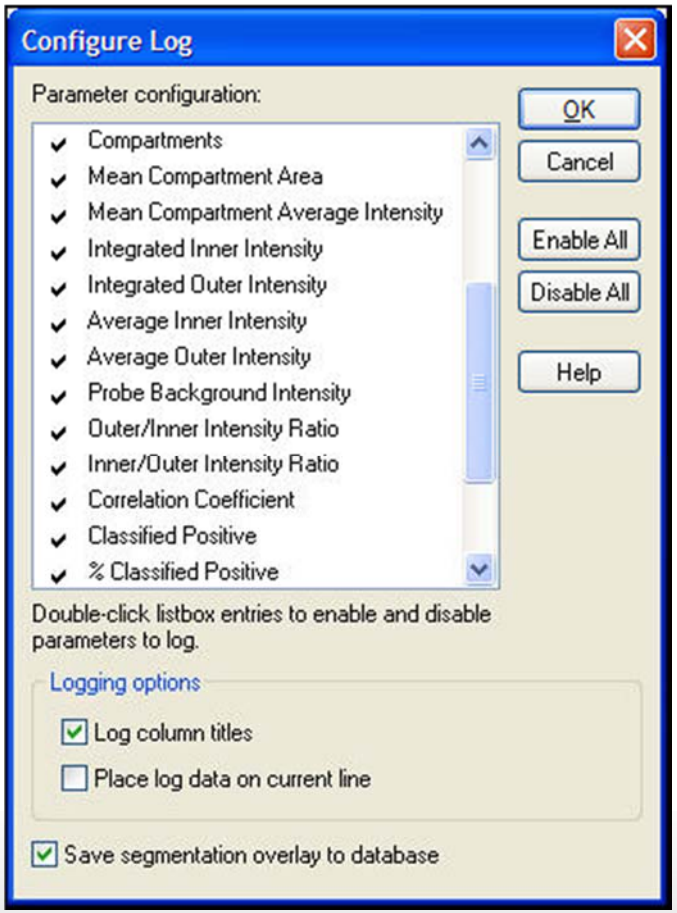
Summary Data (site-by-site measurements)



Probe Background Intensity: The average background pixel intensity of the probe image. This is the value that has been subtracted from other intensity measurements if the "Auto Constant" option was chosen

Outer/Inner Intensity Ratio: The average pixel intensity of the probe in all outer regions (minus background) divided by the average pixel intensity the probe in of all inner regions (minus background)

Inner/Outer Intensity Ratio: The average pixel intensity of the probe in all inner regions (minus background) divided by the average pixel intensity of the probe in all outer regions (minus background)

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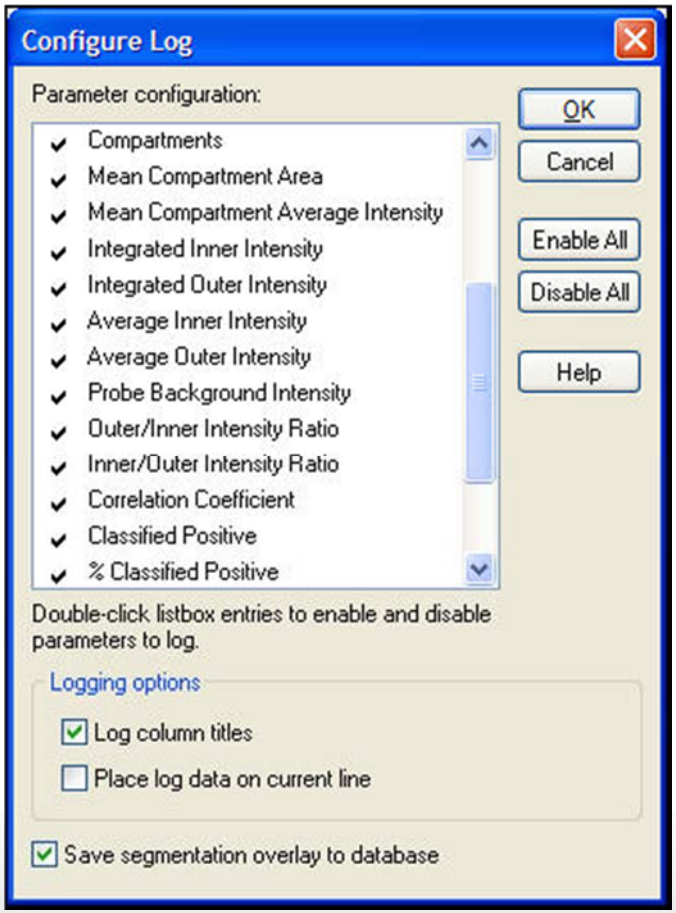
Summary Data (site-by-site measurements)



Correlation Coefficient: The Pearson's correlation coefficient between the two stains over all of the pixels located in all of the cell regions (nuclei + gaps + cytoplasm) in the site

Classified Positive: The total number of cells classified as positive for translocation

% Classified Positive: The number of cells classified as positive for translocation divided by the total cell count, times 100

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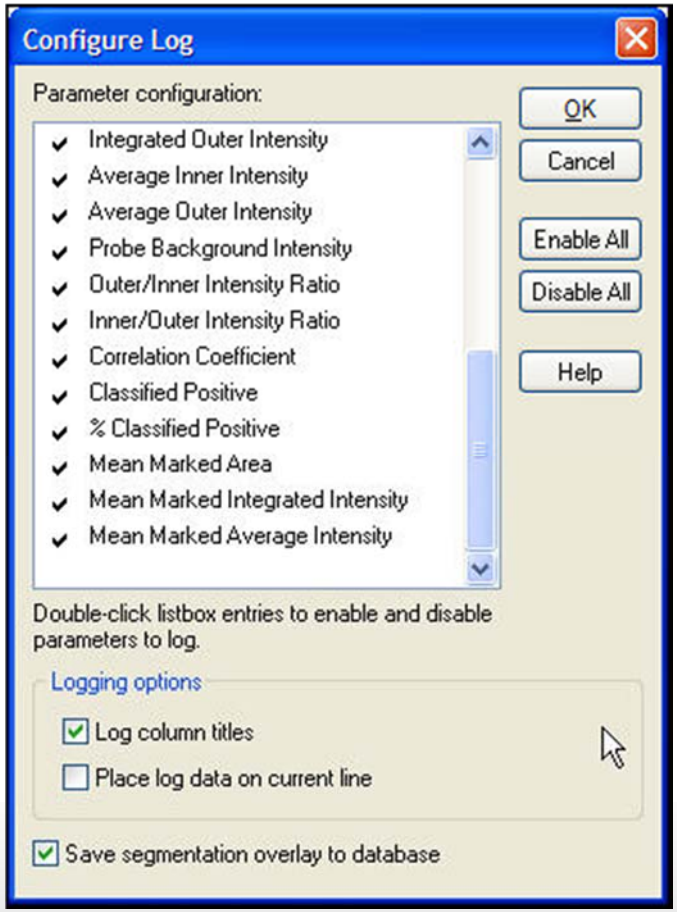
Summary Data (site-by-site measurements)



Mean Marked Area: The total area in um^2 covered by inner + outer regions, divided by the total cell count (Compartments)

Mean Marked Integrated Intensity: The total pixel intensity of the probe in the inner + outer regions minus background, divided by the total cell count (Compartments)

Mean Marked Average Intensity: The average pixel intensity of the probe across all of the inner + outer regions in the image minus background

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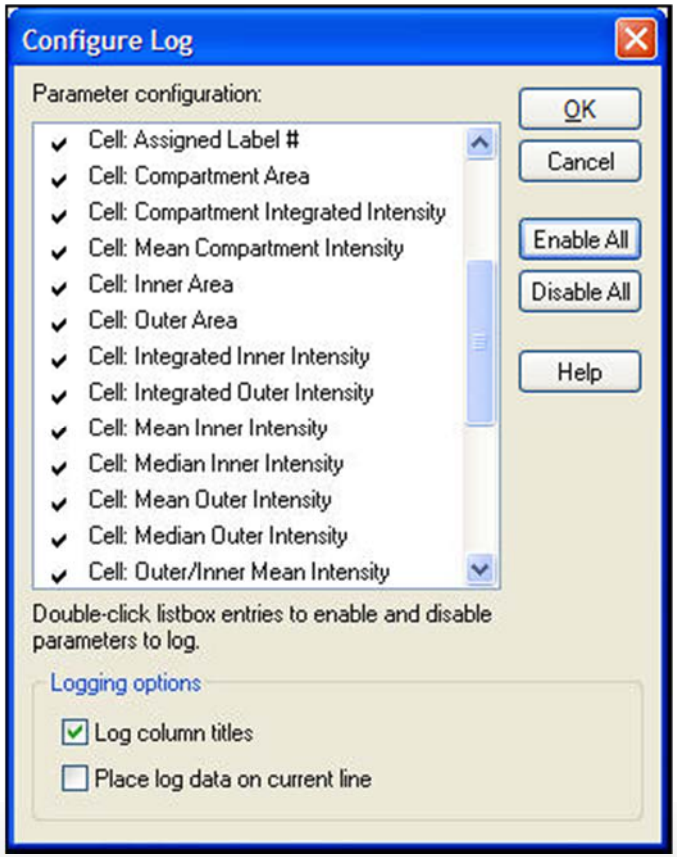
Cell Data (cell-by-cell measurements)



Cell: Assigned Label # – Cell label number (1 through total cell number)

Cell: Compartment Area – Total square microns of the nucleus

Cell: Mean Compartment Intensity – Average pixel intensity of the nuclear stain in the nucleus

Cell: Inner Area – Total square microns in the inner region

Cell: Outer Area – Total square microns in the outer region

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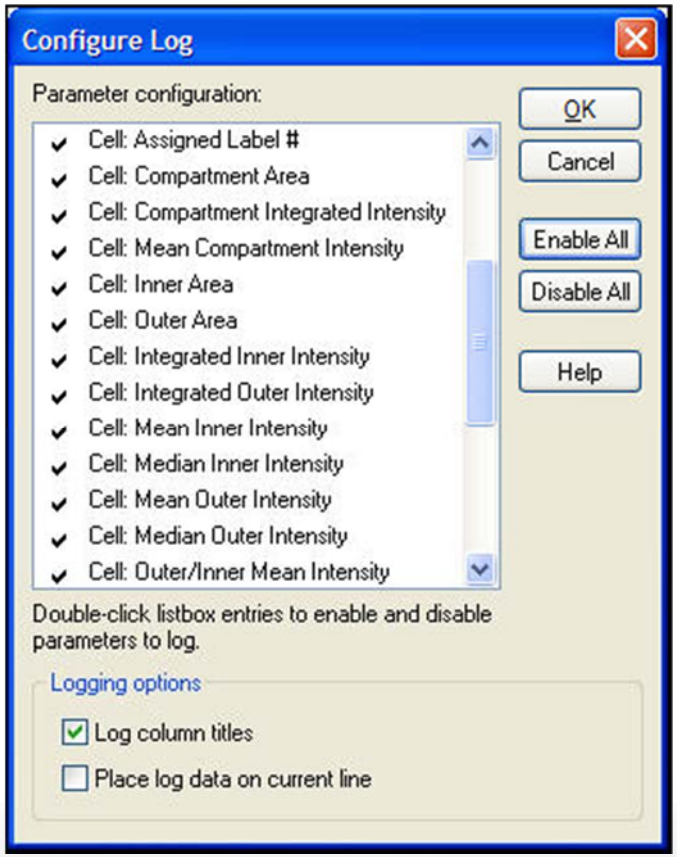
Cell Data (cell-by-cell measurements)



Cell: Integrated Inner Intensity – The total pixel intensity of the probe in the inner region minus background

Cell: Integrated Outer Intensity – The total pixel intensity of the probe in the outer region minus background

Cell: Mean Inner Intensity – The average pixel intensity of the probe in the inner region minus background

Cell: Median Inner Intensity – The median (middle) pixel intensity value of the probe in the inner region minus background

Cell: Mean Outer Intensity – The average pixel intensity of the probe in the inner region minus background

Cell: Median Outer Intensity – The median (middle) pixel intensity value of the probe in the outer region minus background

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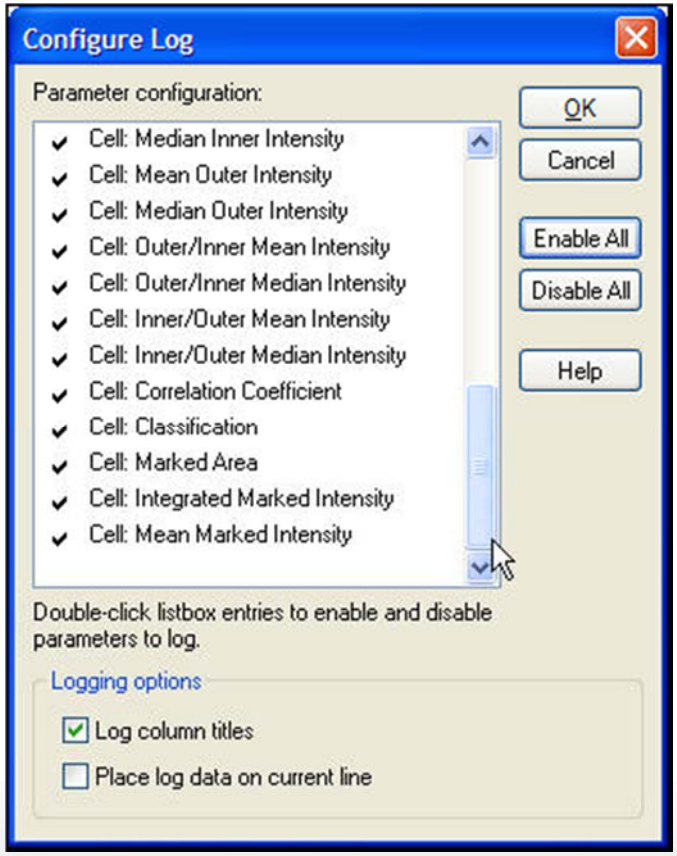
Cell Data (cell-by-cell measurements)



Cell: Outer/Inner Mean Intensity – The average pixel intensity of the probe in the outer region (minus background) divided by the average pixel intensity of the probe in the inner region (minus background)

Cell: Outer/Inner Median Intensity – The median pixel intensity of the probe in the outer region (minus background) divided by the median pixel intensity of the probe in the inner region (minus background)

Cell: Inner/Outer Mean Intensity – The average pixel intensity of the probe in the inner region (minus background) divided by the average pixel intensity of the probe in the outer region (minus background)

Cell: Inner/Outer Median Intensity – The median pixel intensity of the probe in the inner region (minus background) divided by the median pixel intensity of the probe in the outer region (minus background)

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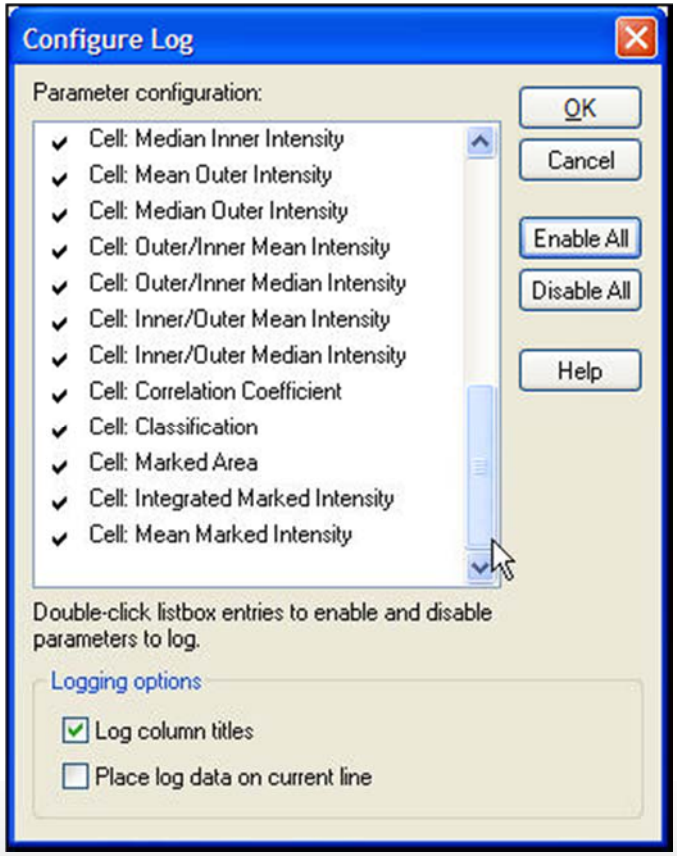
Cell Data (cell-by-cell measurements)



Cell: Correlation Coefficient – The Pearson's correlation coefficient between the intensities of the two stains for all pixels in the cell region (nucleus + gap + cytoplasm). The value ranges from -1 (anti-correlated) to 1 (correlated).

Cell: Classification – 1 for positive translocation classification (nuclear staining), 0 for negative translocation classification (cytoplasmic staining)

Cell: Marked Area – The sum of the inner and outer areas.

Cell: Integrated Marked Intensity – The total pixel intensity of the probe in the inner + outer areas (minus background)

Cell: Mean Marked Intensity – The average pixel intensity of the probe in the inner + outer areas (minus background)

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Translocation vs Translocation Enhanced Settings

- Translocation makes some assumptions that can duplicate in Translocation- Enhanced

1. Intensity above local background.
   - Set max to max bit dept of the image
   - 4095 for 12 bit image (ImageXpress Micro, Discovery-1)
   - 63535 for 16 bit image (ImageXpress Ultra, ImageXpress Micro XL, mageXpress 5000A)
2. Area
   - Minimum: play with the value to get the same number of compartments/ cells
3. Auto separate toughing compartments
   - Selected
4. Inner and outer distance from Edge
   - Enter values that represent 1 pixel
5. Outer region width
   - Enter a value that represent: 1/3 of the appropriate width for the compartment and subtract 1 pixel.
   - For instance is the width is 15 pixels, then enter 4 ((15/3) -1)
6. Background estimation method:
   - Select Auto Constant
7. Classify positives
   - Select Correlation Coefficient
   - Select >=

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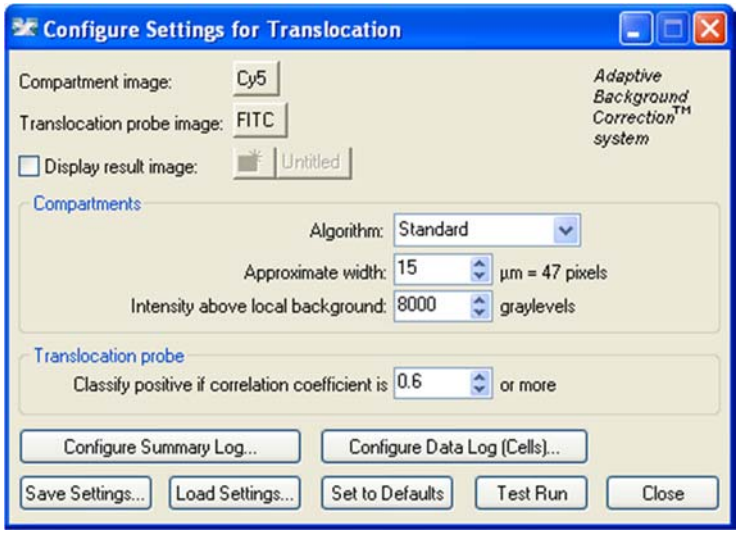
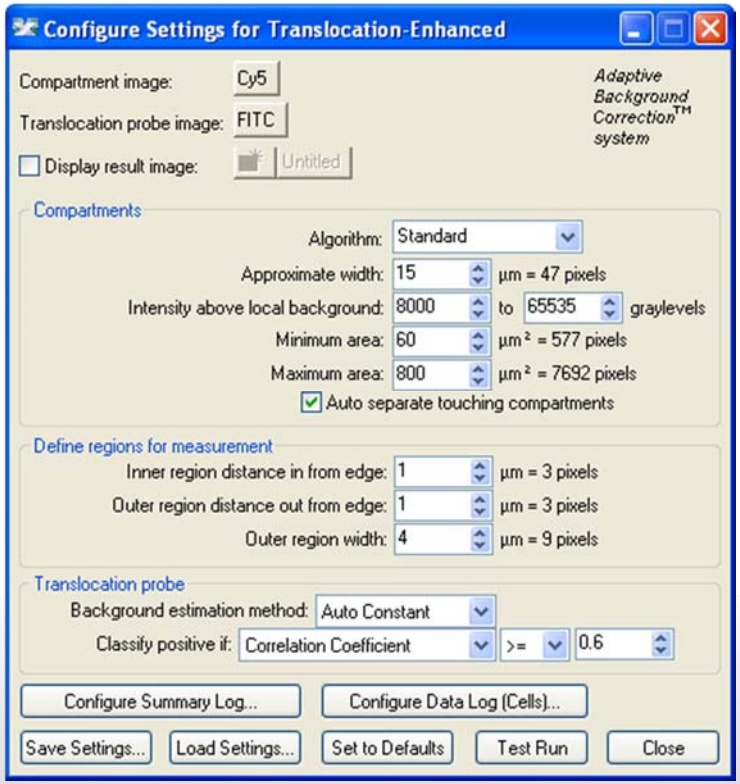
Translocation vs Translocation Enhanced Settings

- These settings gave identical results





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