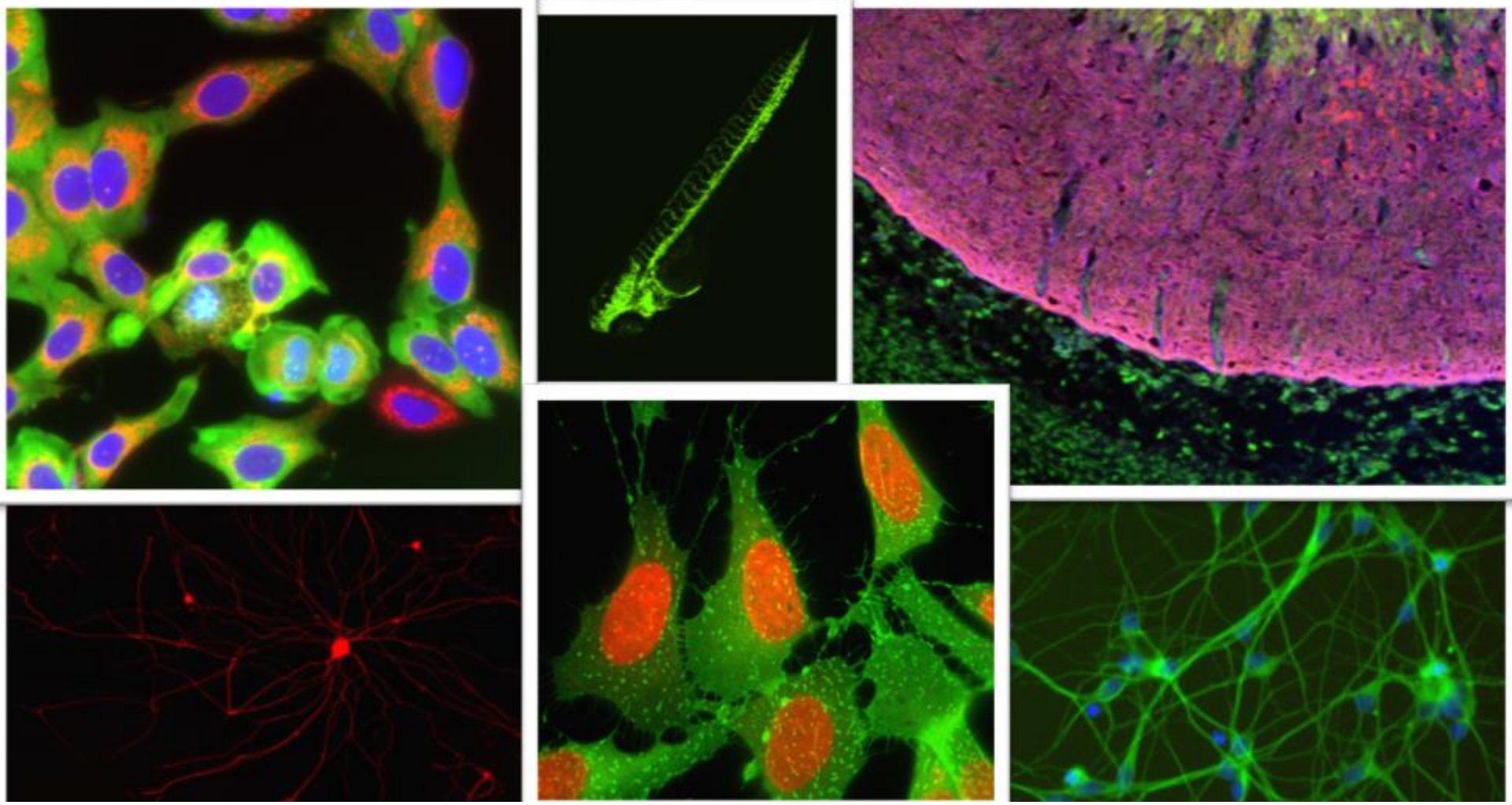


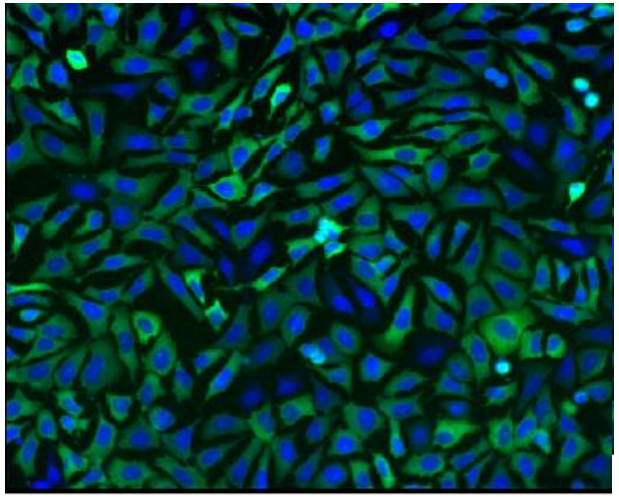
Molecular Devices

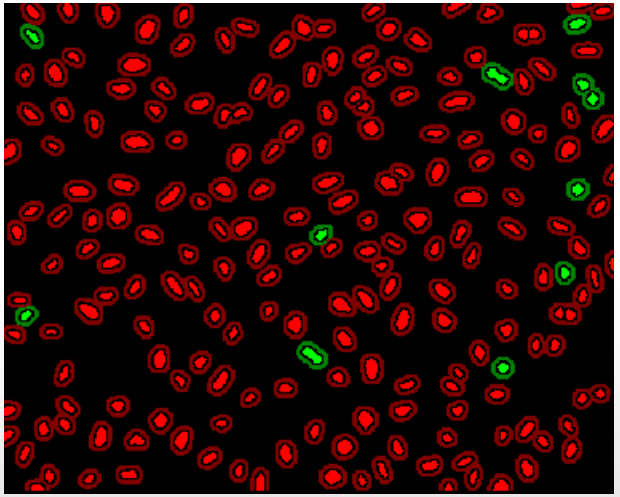
Together through life sciences.

MetaXpress® Software:
Translocation Module

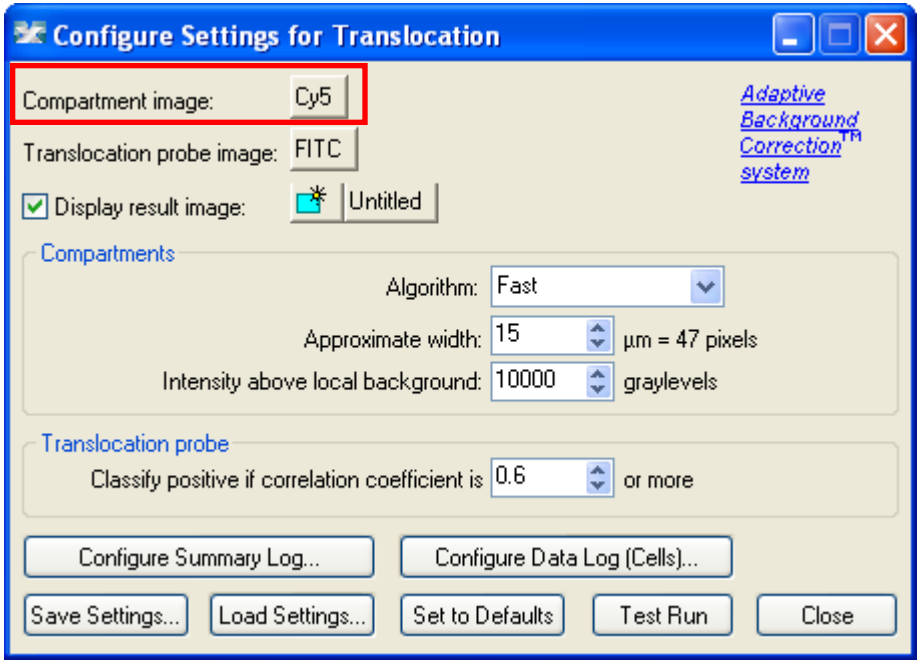
Translocation

- Translocation module can be used to measure intensity movement from one compartment to another (for instance the nucleus to cytoplasm)





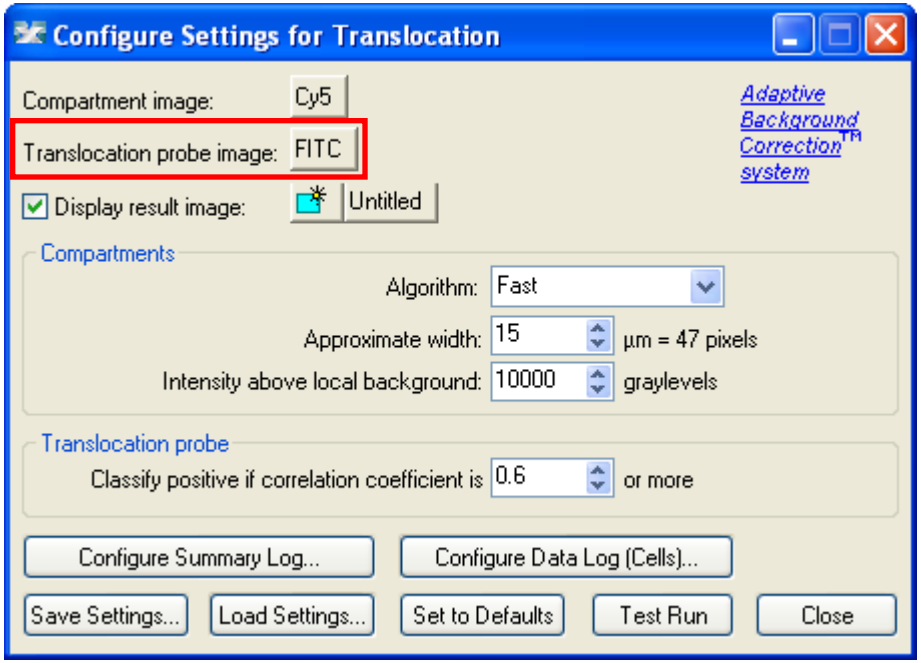
Module Settings



Compartment image = Nuclei

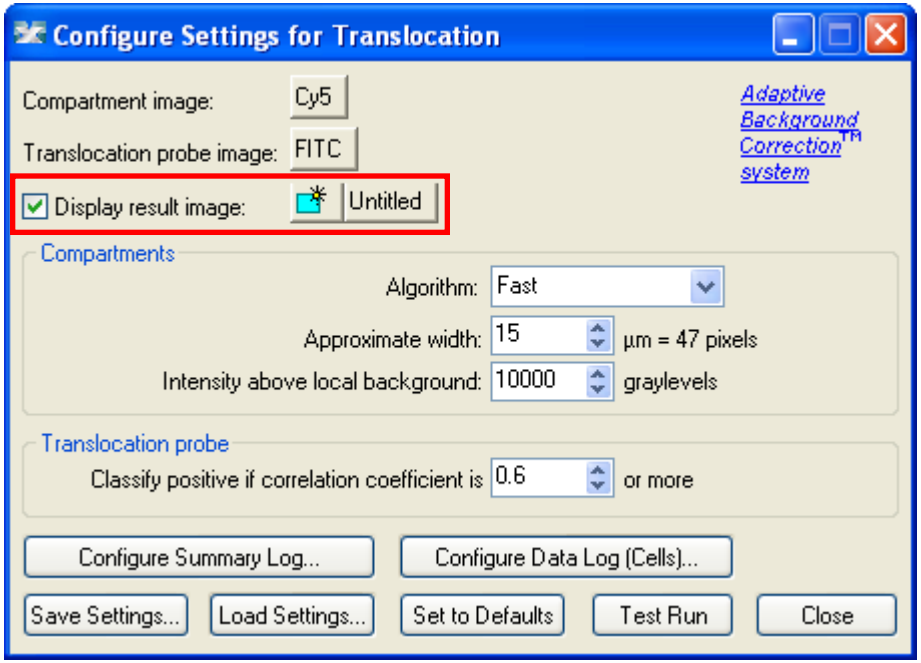
Note that this module may also be used for measuring translocation to other organelles.

Module Settings



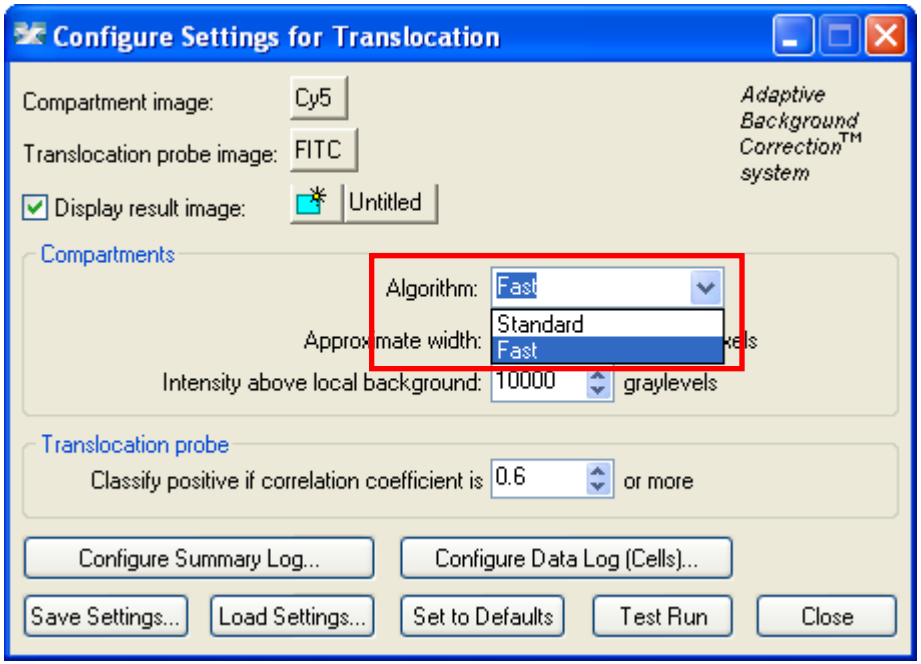
Translocation probe image = the marker that is moving between nucleus and cytoplasm

Module Settings



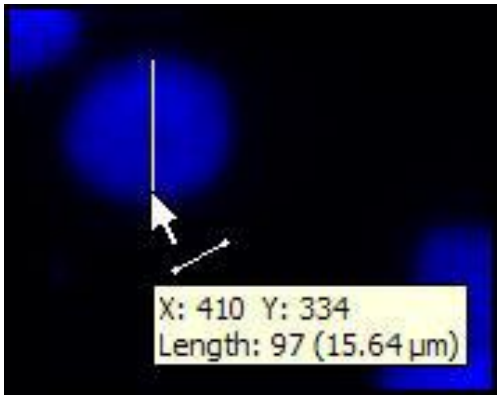
Leave "**Display result image**" deselected (this is generally only used when journaling)

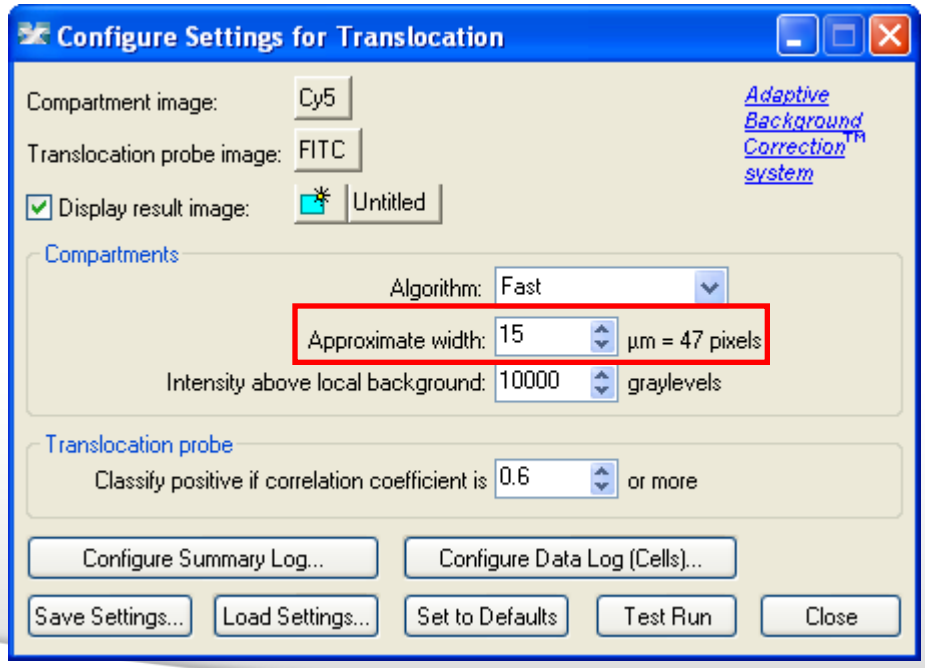
Module Settings



- **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.

Module Settings

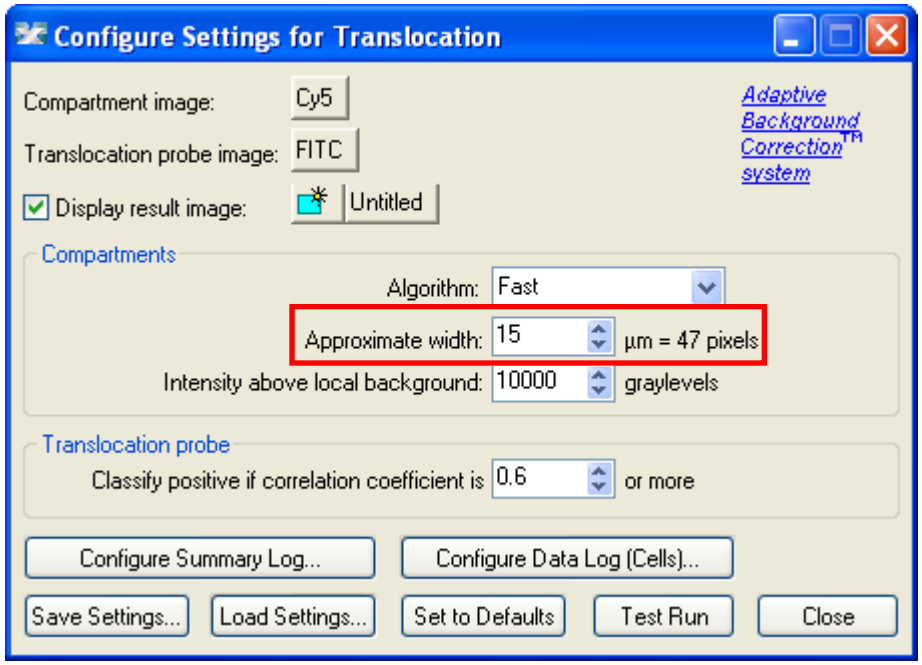


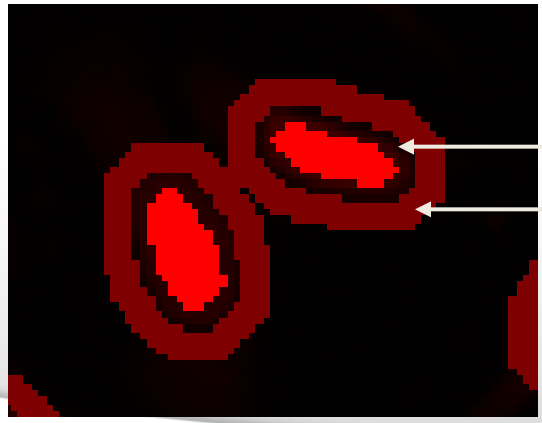


Approximate width should be set to the average width (short axis) of a nucleus (in um). The region tools can be used to measure widths.

Much smaller or much larger cells will be ignored.

Module Settings





Gap

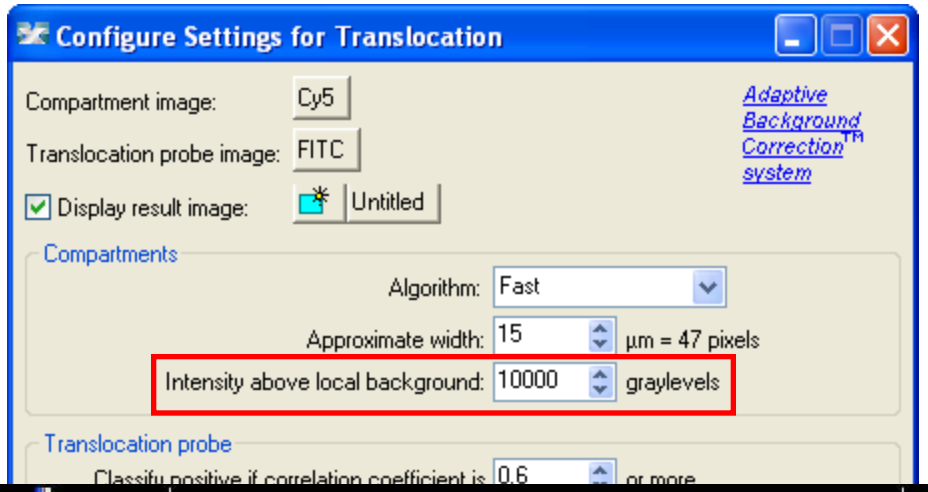
Ring

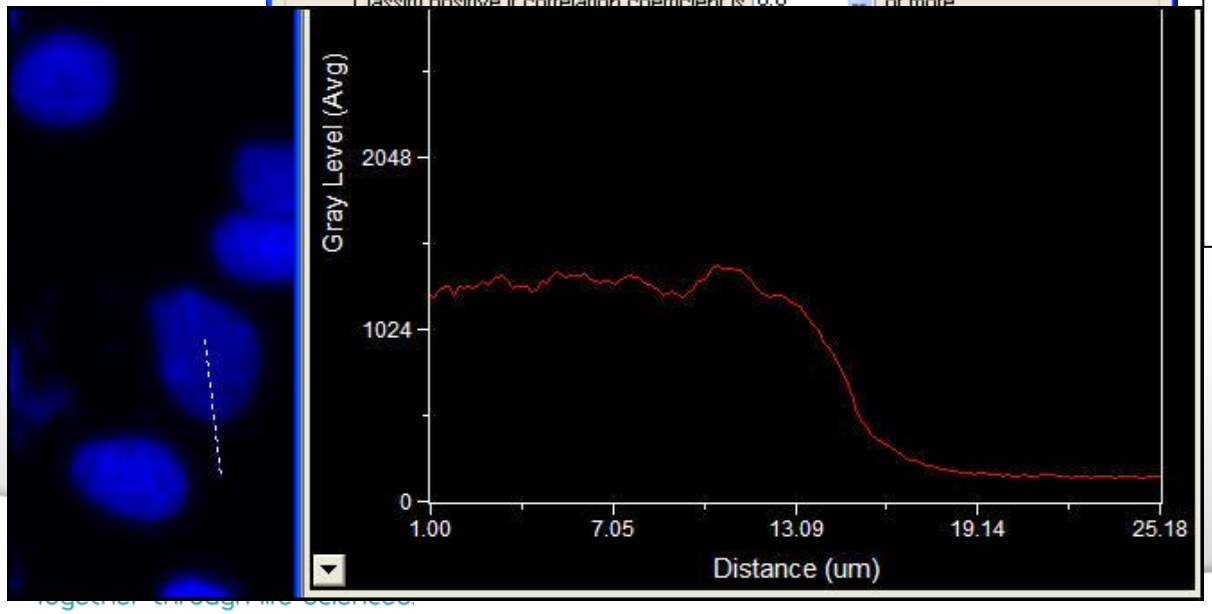
Defaults:

Width ring: is always 1/3 of the Appropriate width.

Gap: is always 3 pixels wide.

Module Settings



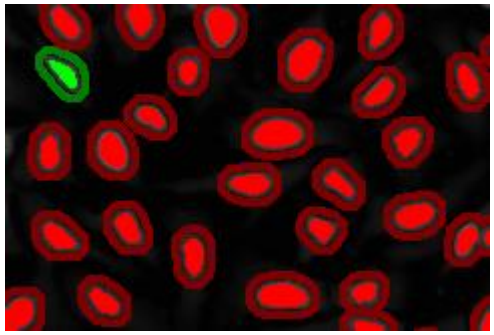


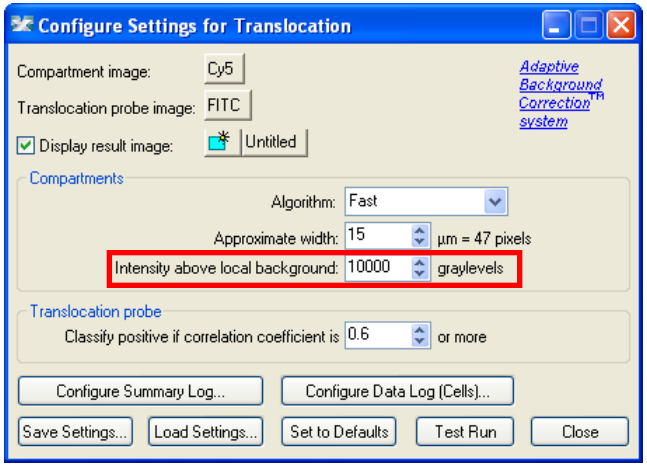
The **intensity above local background** is used for finding the nuclei. The **value** should be set slightly lower than the difference in intensity between a dim cell and its local background. For FAST algorithm, set this value to about half (or less) of the difference in intensity between a dim cell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

Molecular Devices

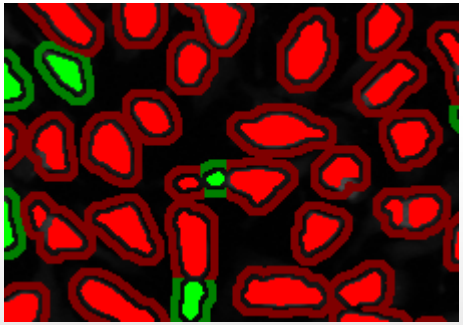
Effects of intensity settings

- Set correctly

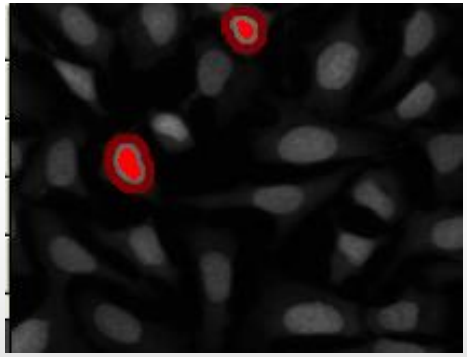




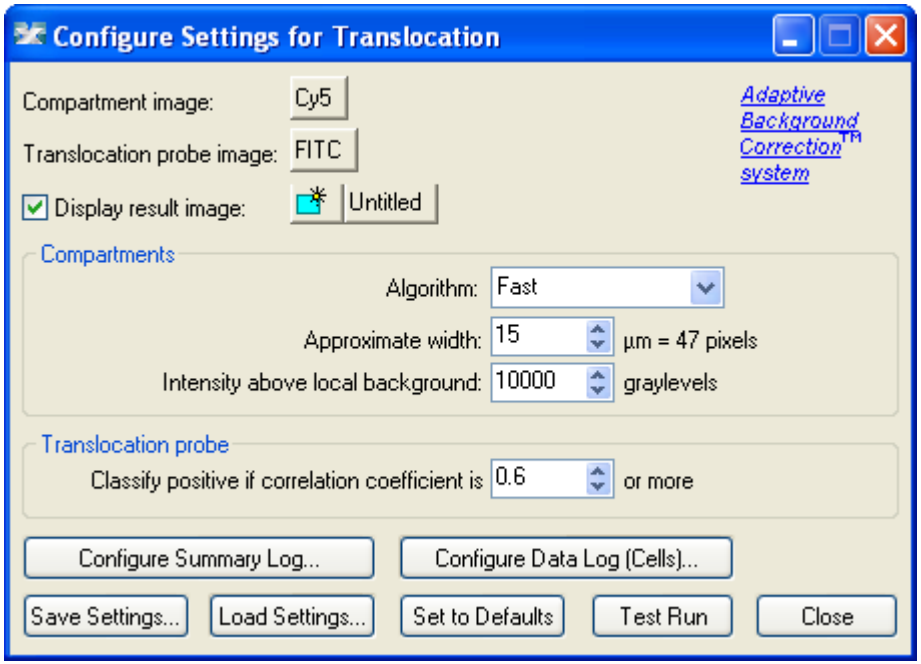
- Set too low → Compartments are too large



- Set too high → Compartments are not being detected



Module Settings



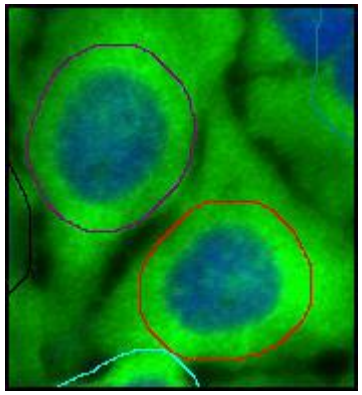
Background estimation method:

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

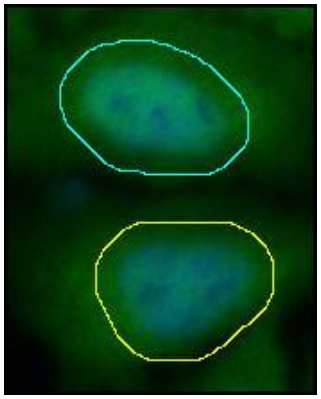
Auto Constant: an average background value is calculated for each image and subtracted

In the translocation-enhanced module you have also the options: Constant and None

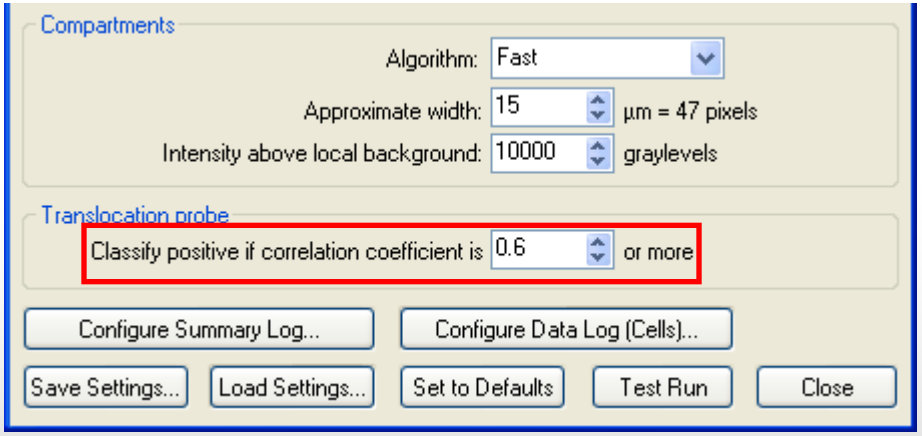
Module Settings



Negative
(cytoplasmic)



Positive
(nuclear)



Cell classification:

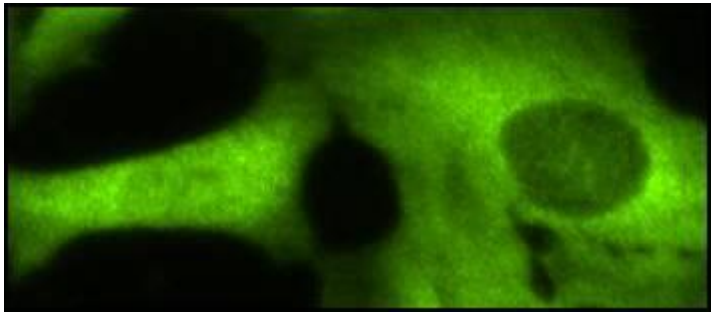
Correlation Coefficient: This is the Pearson's correlation coefficient of the pixel intensity of the two stains in the entire cell region (nucleus + gap + cytoplasm). This is typically the most robust method for classifying translocation.

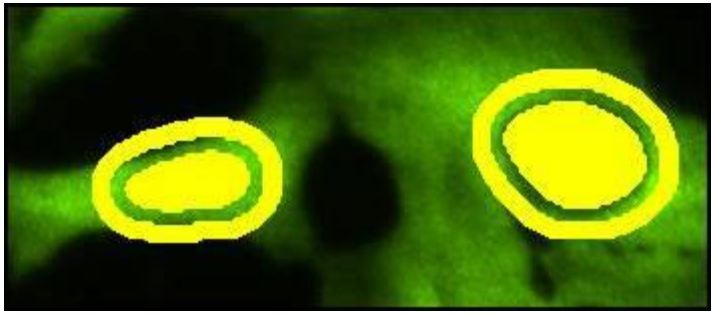
1.0 is perfect correlation (the two stains perfectly overlap)

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

0 indicates the stains are independent

Module Settings





Setting Cell classification cutoffs:

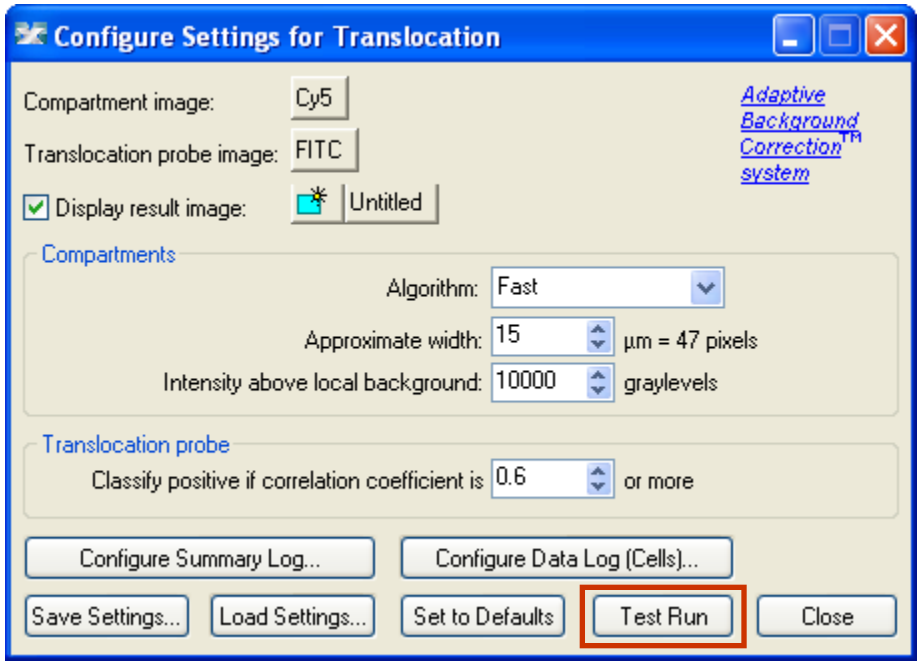
Test module on positive and negative controls.

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.

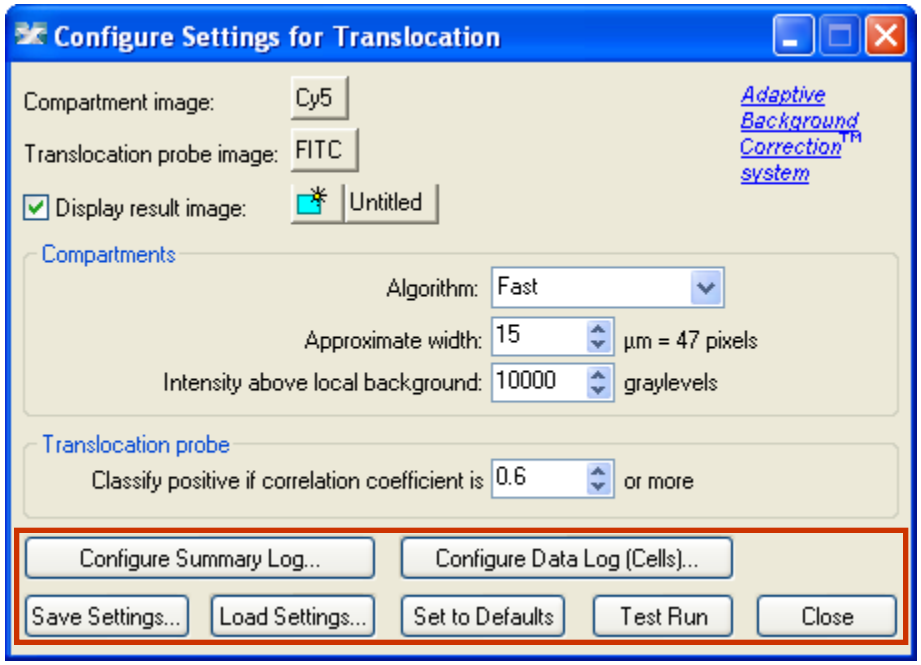
Cell: Mean Outer Intensity	Cell: Median Outer Intensity	Cell: Correlation Coefficient	Cell: Classification
2749.37	2585	0.235741	0
4157.58	4385	-0.176836	0
7100.5	5961	0.721798	1
6238.77	7140	0.295065	0
4788.17	5602.5	-0.161206	0
2260.92	1045.5	0.634542	1
3031.64	2836.5	-0.0434752	0
2407.08	1482	0.126382	0
384.993	527.5	-0.253856	0
2907.29	2391	0.129046	0
4879.95	4923	-0.569827	0
0	0	-0.0852586	0
1739.99	1908	-0.318487	0

Module Settings



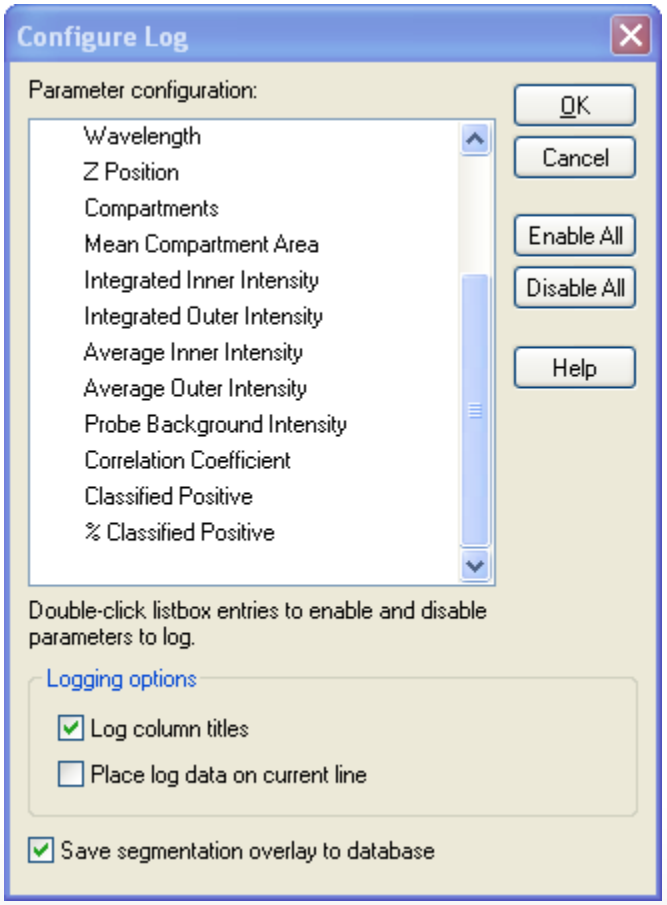
- Select **Test Run** to view the cell segmentation
- Change settings if needed
- Save the settings

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

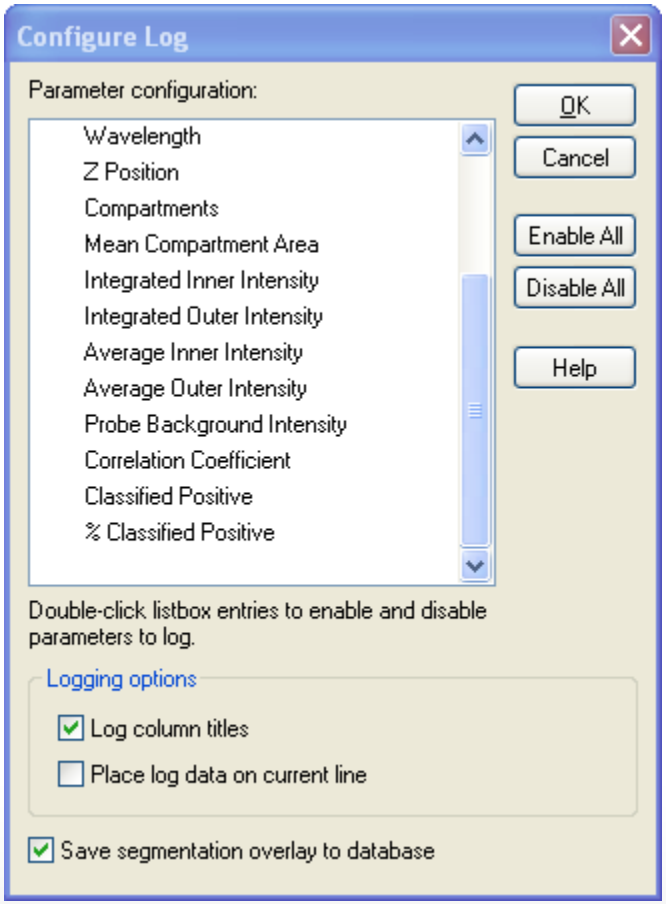
Summary Data (site-by-site measurements)



Compartments: Total number of nuclei (cell count)

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

Summary Data (site-by-site measurements)



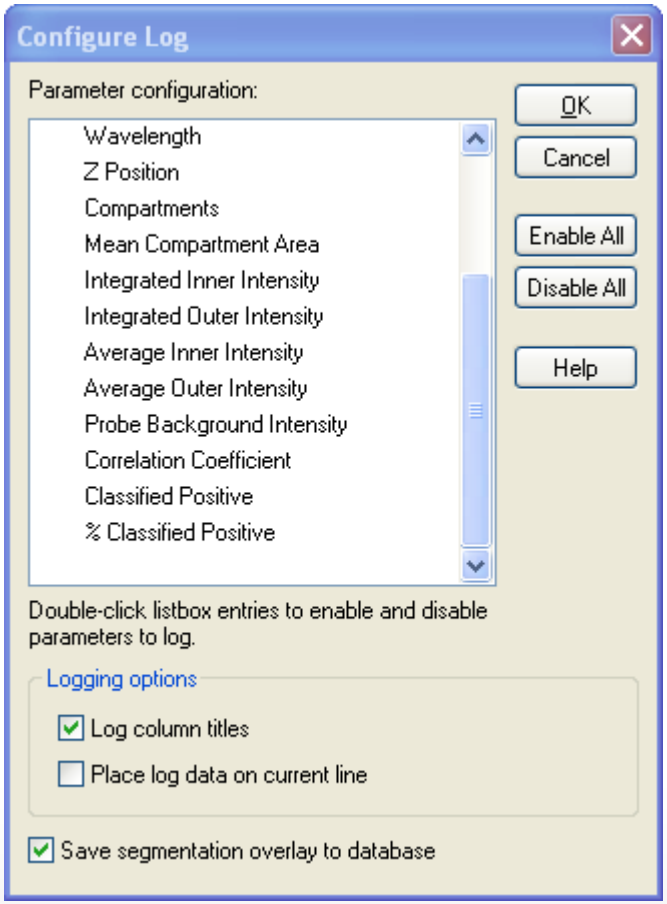
Integrated Inner Intensity: The total pixel intensity of the probe in all the inner regions for the site after background subtraction (note this correlates with cell count)

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)

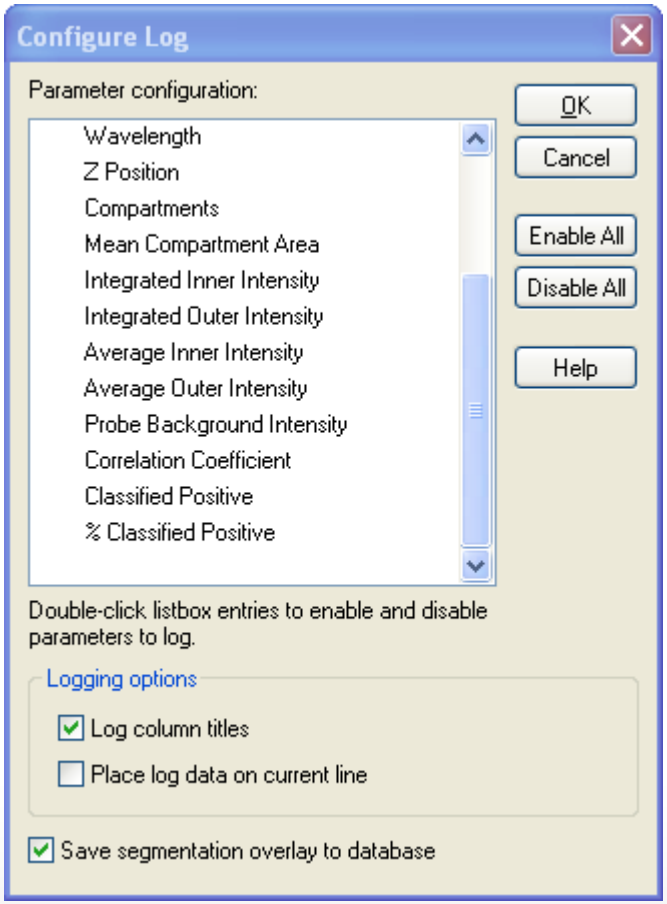
Average Outer Intensity: The average pixel intensity of the probe in all the outer regions for the site after background subtraction (independent of cell count)

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

Summary Data (site-by-site measurements)

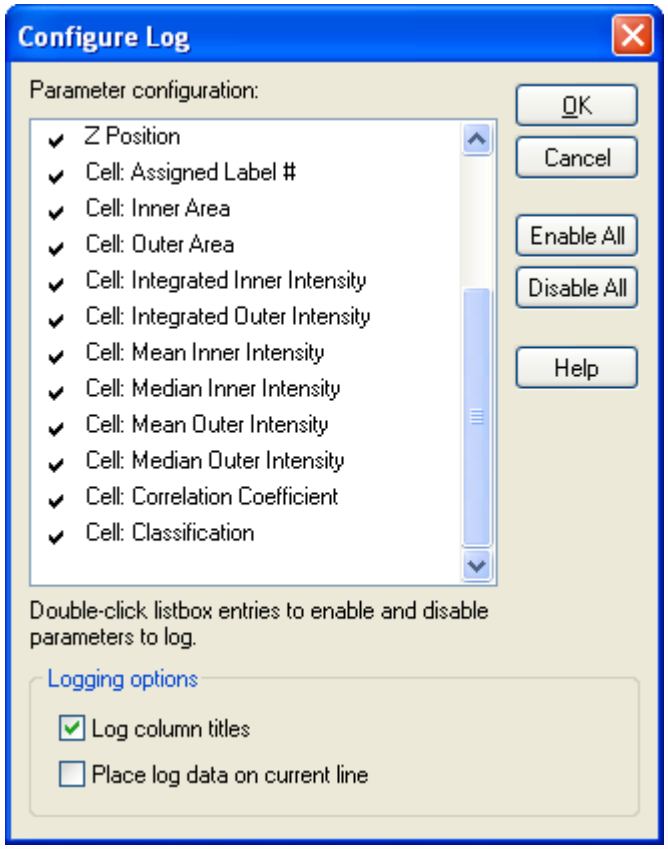


Correlation Coefficient: The Pearson's correlation coefficient between the two stains over all of the pixels located in all of the cell regions (nuclei + gaps + cytoplasm) in the site

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

Cell Data (cell-by-cell measurements)

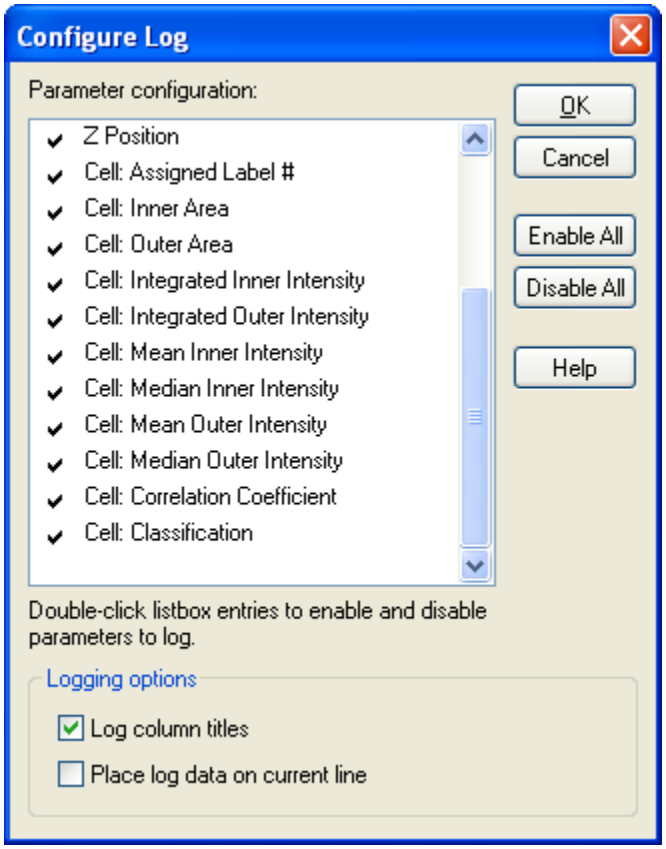


Cell: Assigned Label # – Cell label number (1 through total cell number)

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

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

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

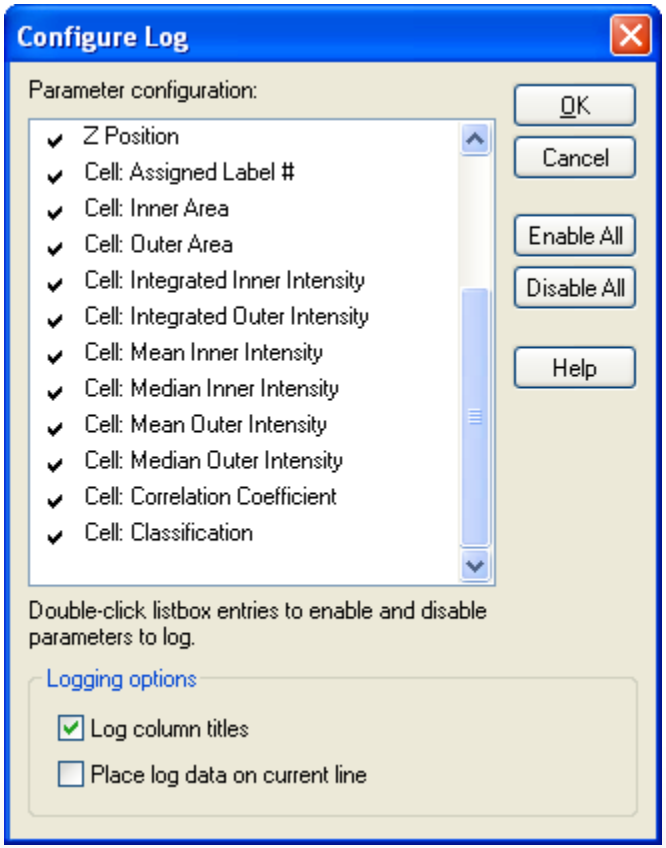
Cell: Mean Inner Intensity – The average pixel intensity of the probe in the inner region minus background

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

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)

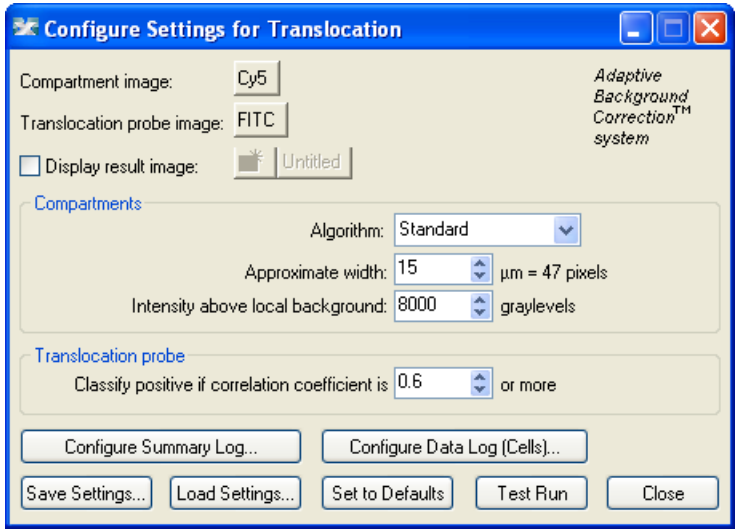
Translocation vs Translocation Enhanced Settings

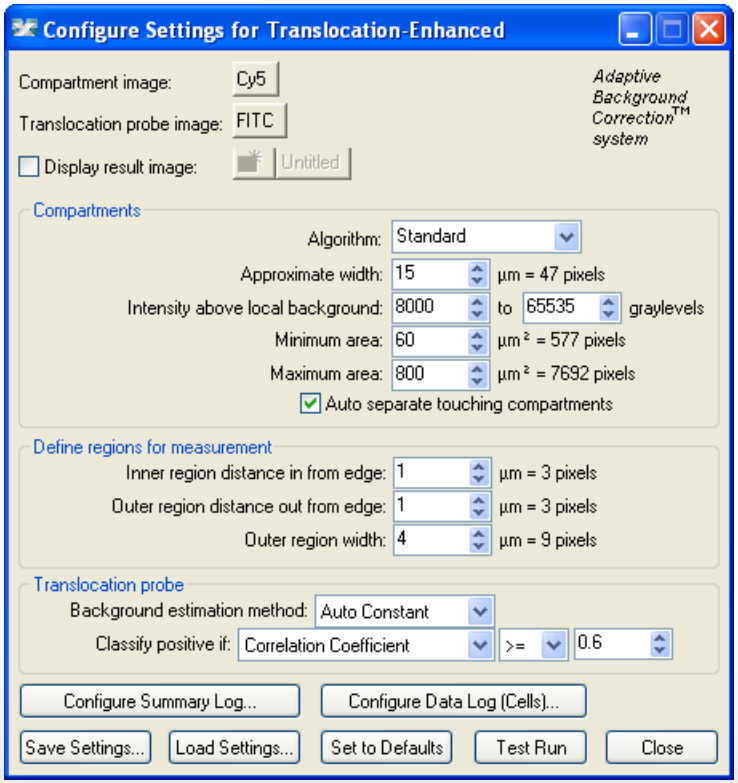
- Translocation makes some assumptions that can be duplicate in Translocation-Enhanced

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 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 >=

Translocation vs Translocation Enhanced Settings

- These settings gave identical results





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