Molecular Devices





MetaXpress: Multi Wavelength Translocation module

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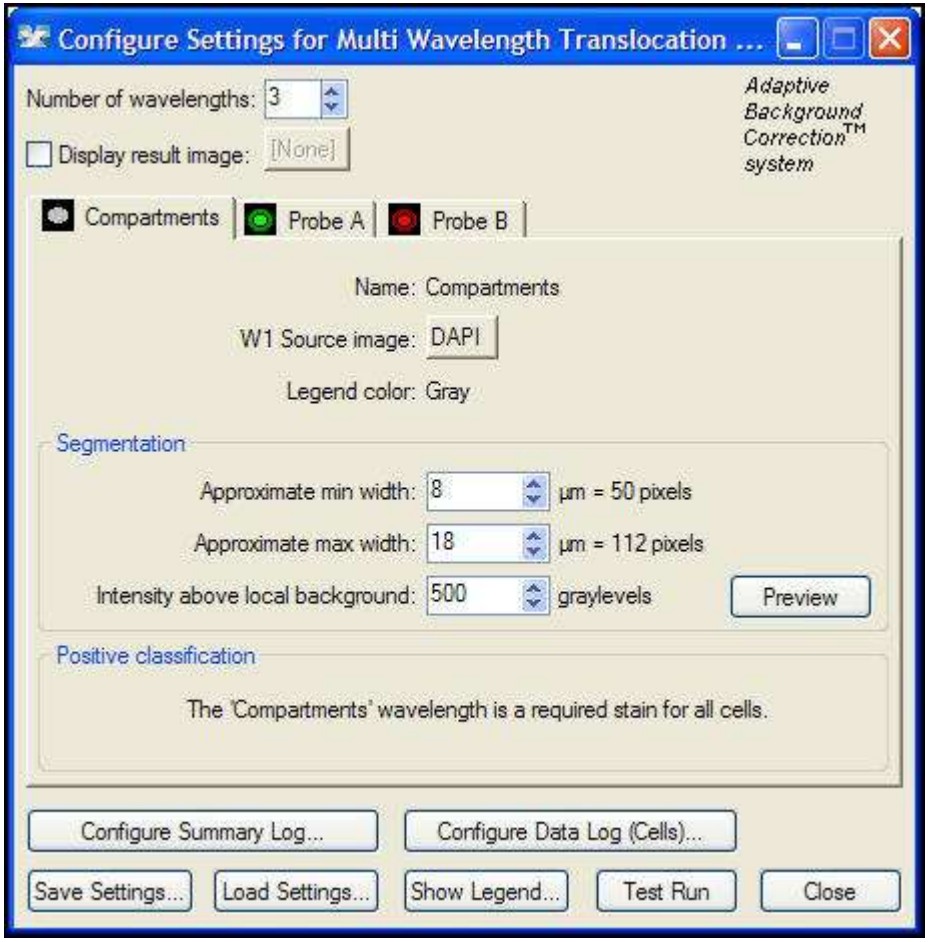
Multi Wavelength Translocation Module Overview

- Measures nuclear translocation in assays with a nuclear stain and 1-6 probes of interest
- Scores cells as positive or negative for each wavelength based on correlation with the nuclear stain
- Assigns profiles to cells based on their score in each wavelength



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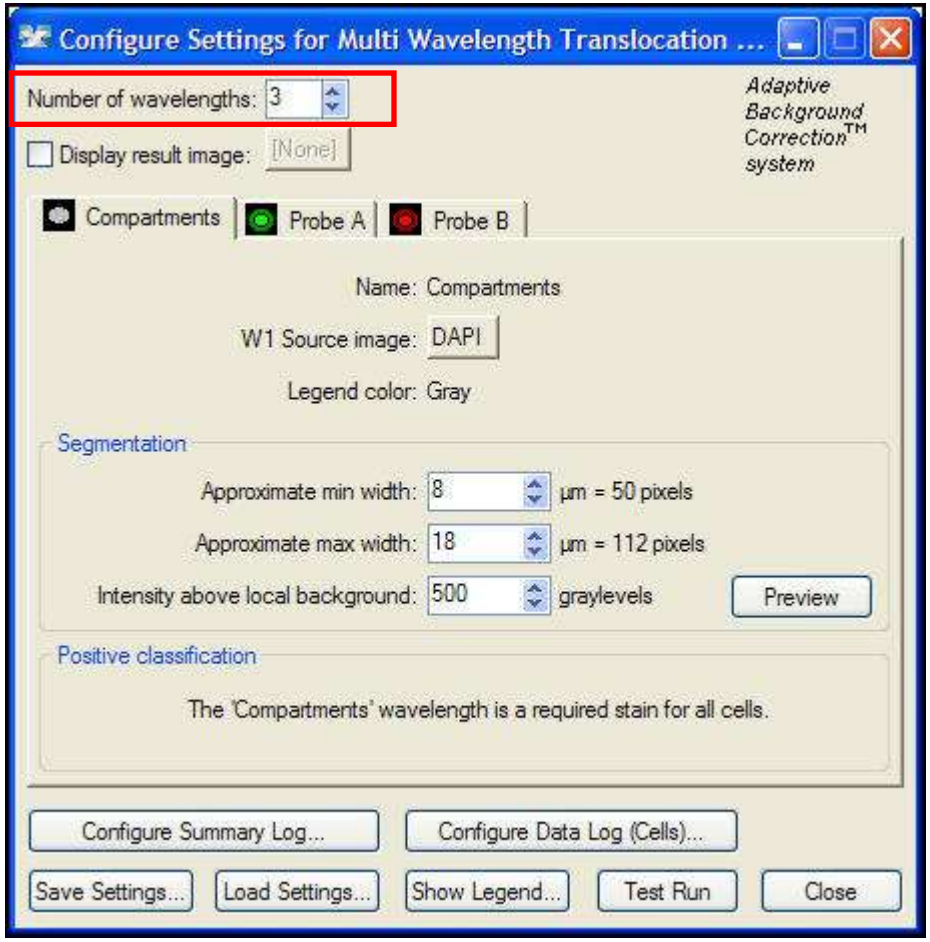
Module Settings



- **Number of wavelengths**
- This should be set to the total number of wavelengths to be analyzed (nuclei plus probes of interest).
- Up to 7 different wavelengths can be analyzed simultaneously (wavelengths may also be repeated with different settings)

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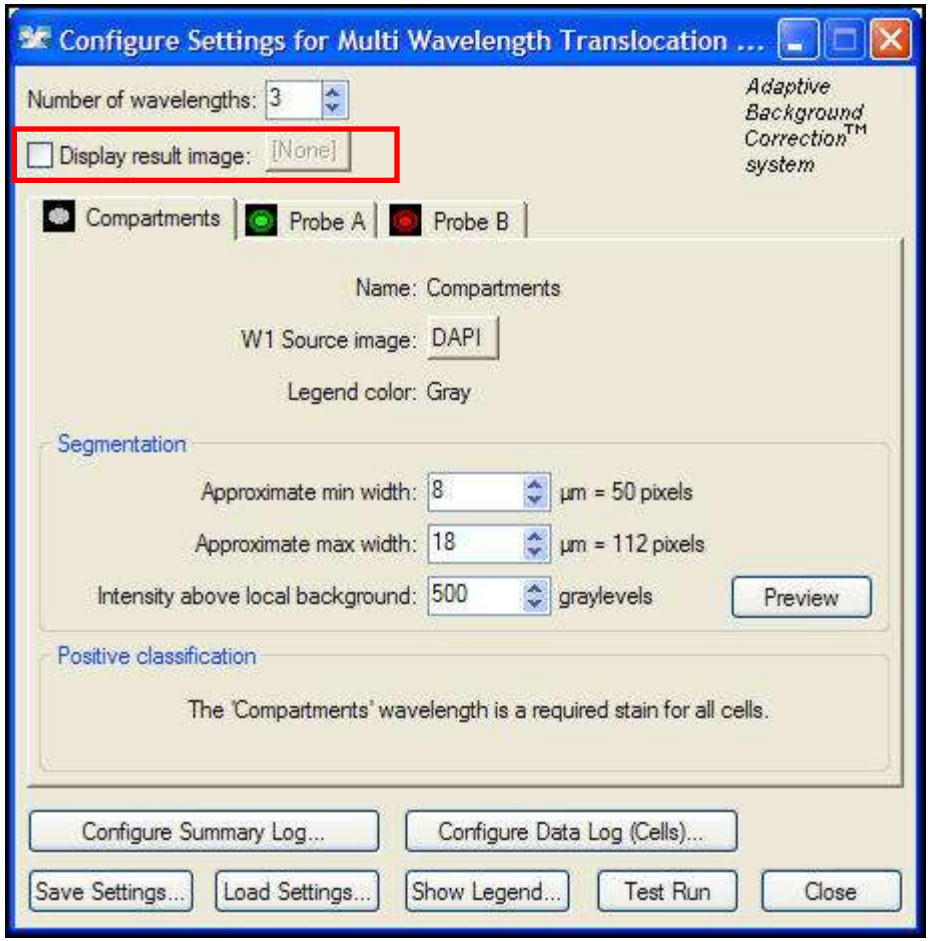
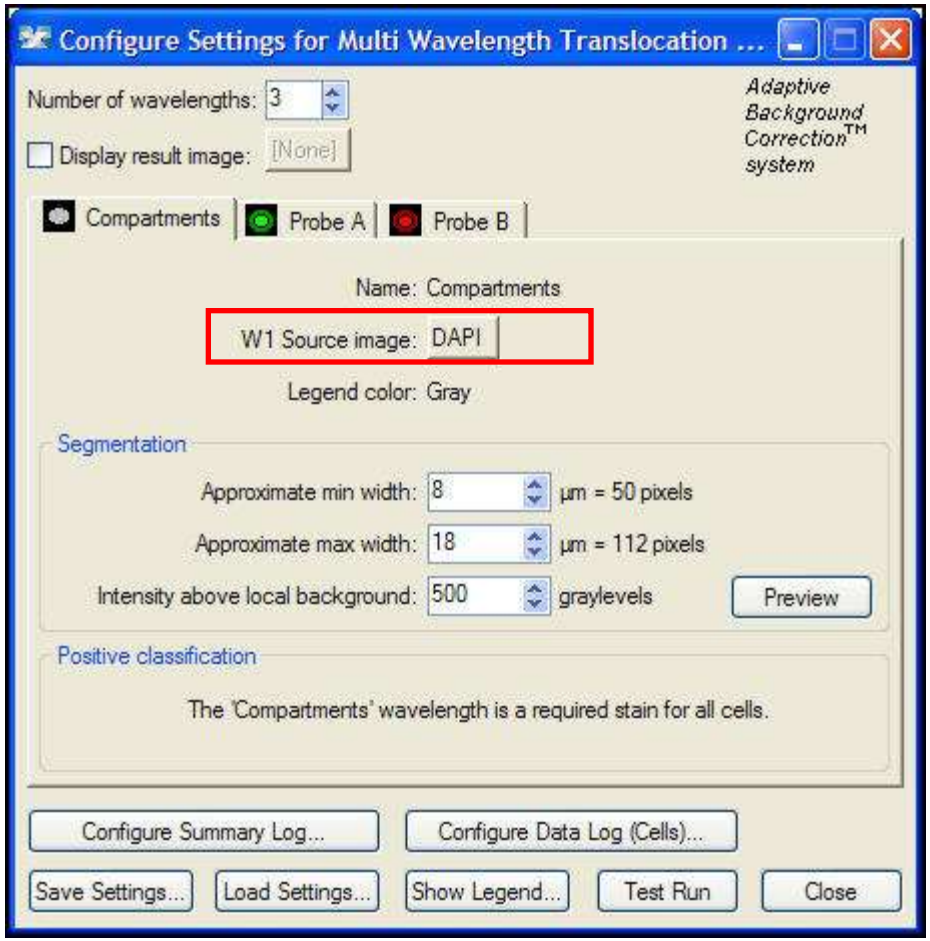
Module Settings



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

Module Settings: Wavelength 1



- Select the wavelength for the **Compartments** (nuclei)

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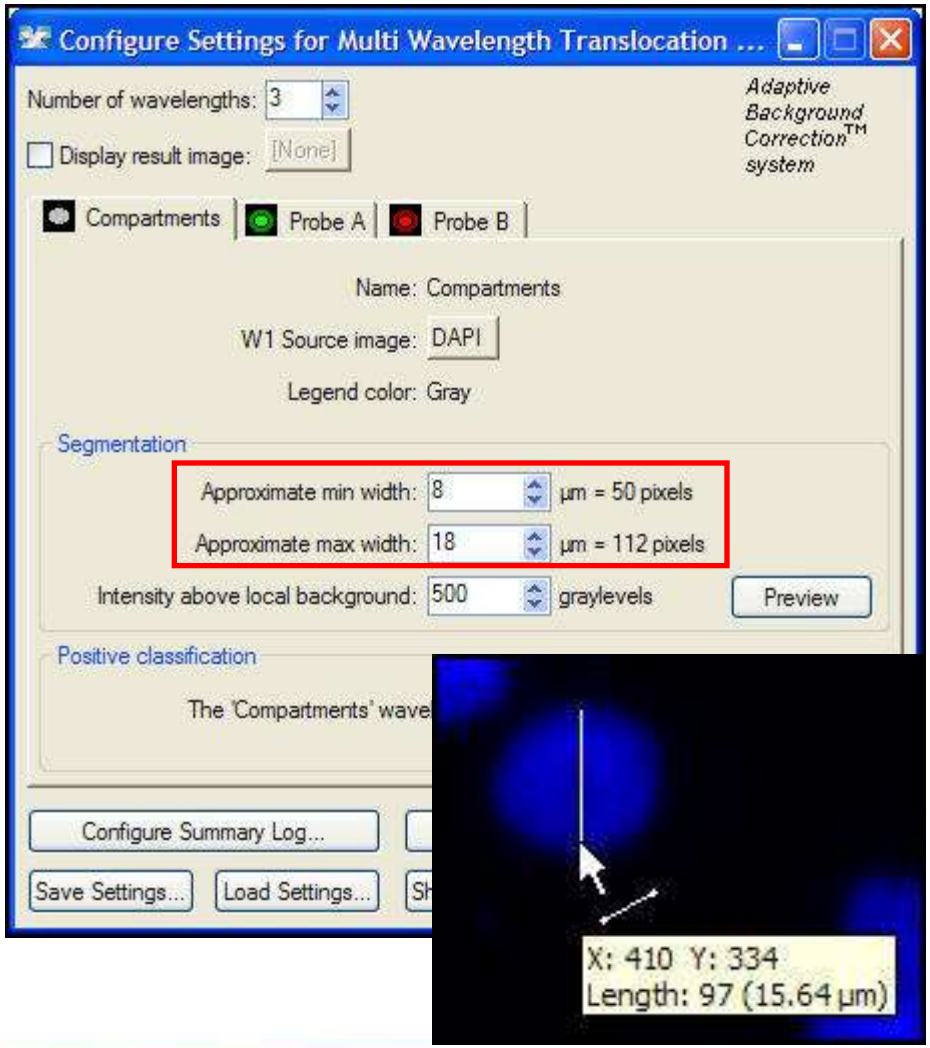
Module Settings: Wavelength 1



- **Set the Approximate min width** and **Approximate max width** for the range of nuclei that you want to detect
- The width is the short axis of a nucleus (in um)
- The region tools can be used to measure widths
- Much smaller cells will be ignored
- Much larger cells will be split

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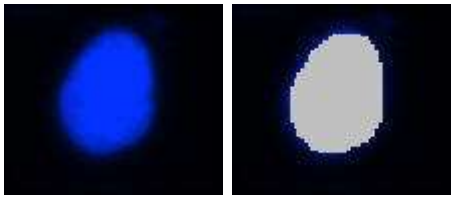
Module Settings: Wavelength 1

Effects of varying width settings



Min width too small: splits nuclei

Min width too large: omits smaller nuclei

Max width too small: may shrink nuclear boundaries

Max width too large: may slightly enlarge nuclear boundaries

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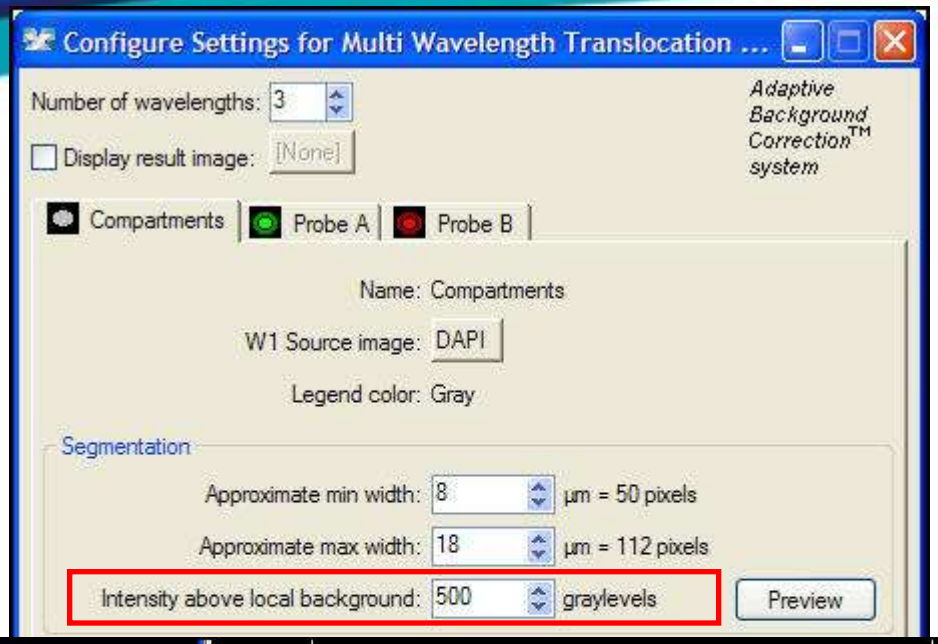
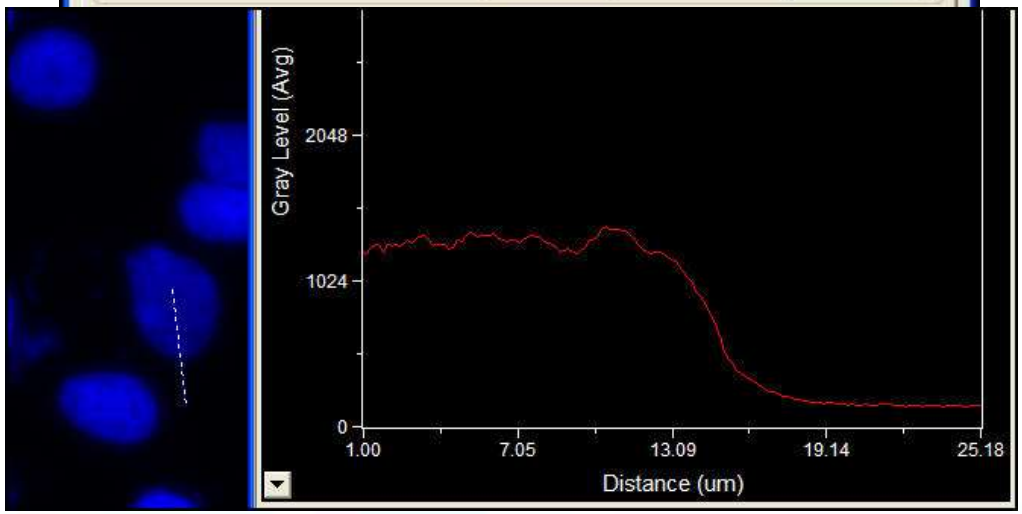
Module Settings: Wavelength 1





- The **intensity above local background** is used for finding the nuclei
- This value is a minimum and should be set slightly lower than the difference in intensity between a dim cell and its local background
- Draw a line across a cell into the background and use Measure > Linescan to determine intensity values; or simply mouse over the cell and the background and view the intensity values

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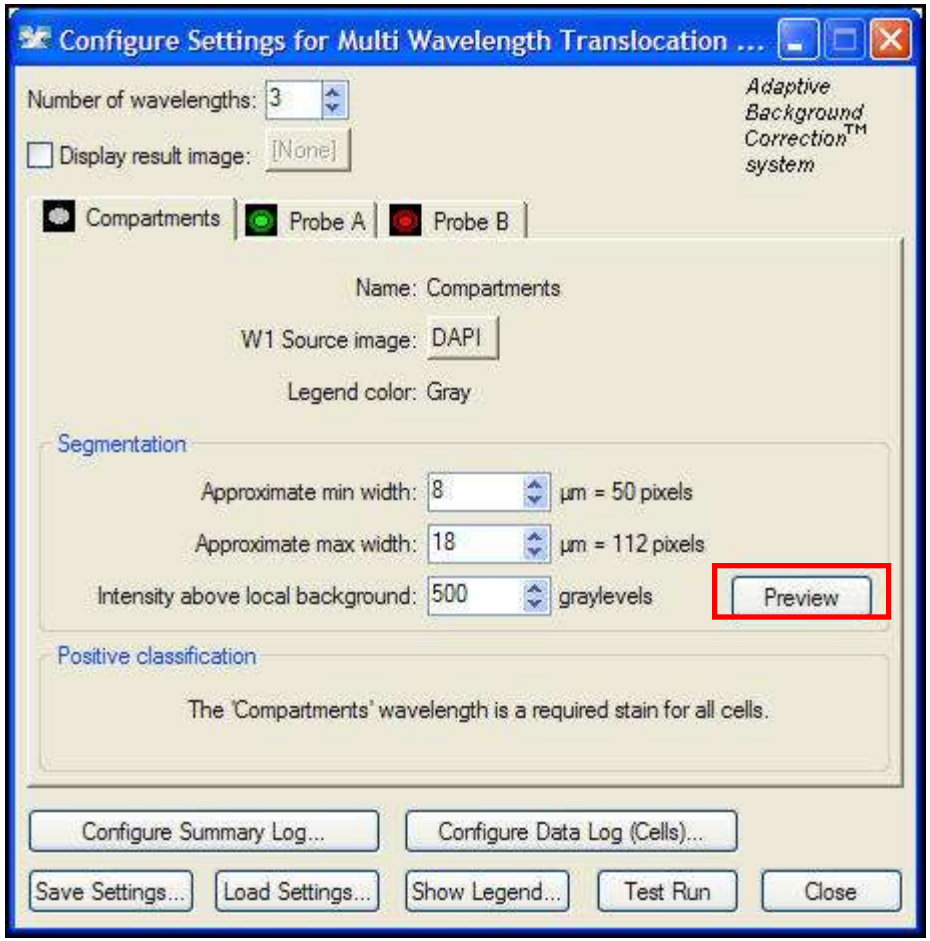
Module Settings: Wavelength 1



- Click on **Preview** to test settings for the current wavelength only

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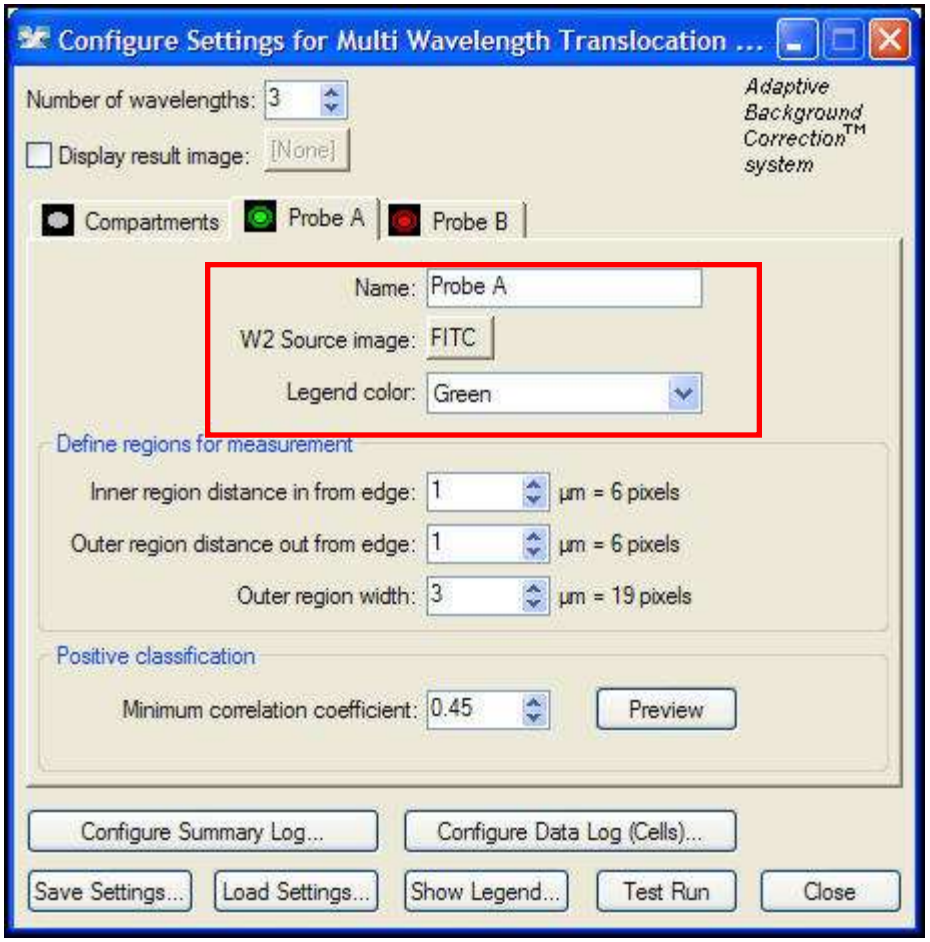
Module Settings: Wavelength 2



- **Define W2:**
- Enter a name and select wavelength and legend color for your 2nd wavelength (probe A in this example)

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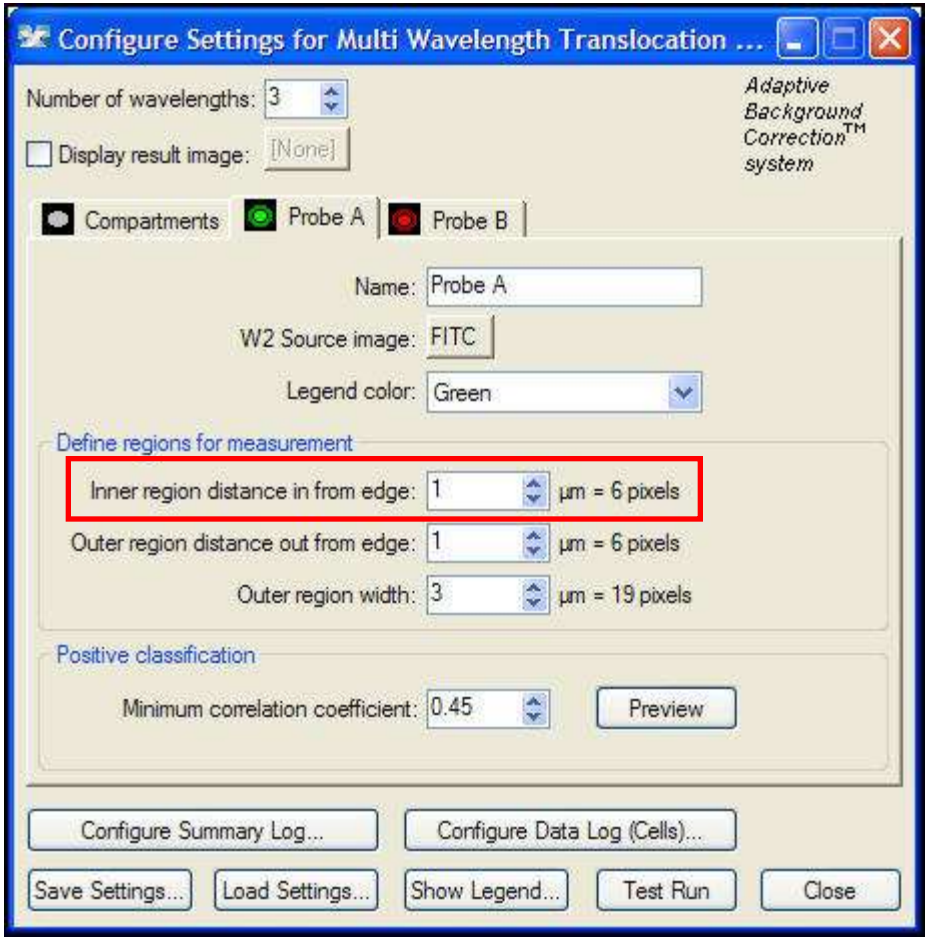
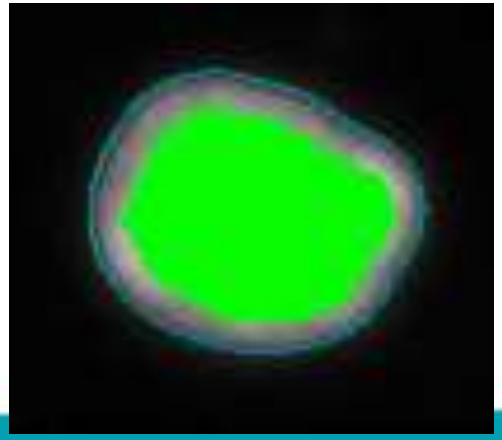
Module Settings: Wavelength 2



- **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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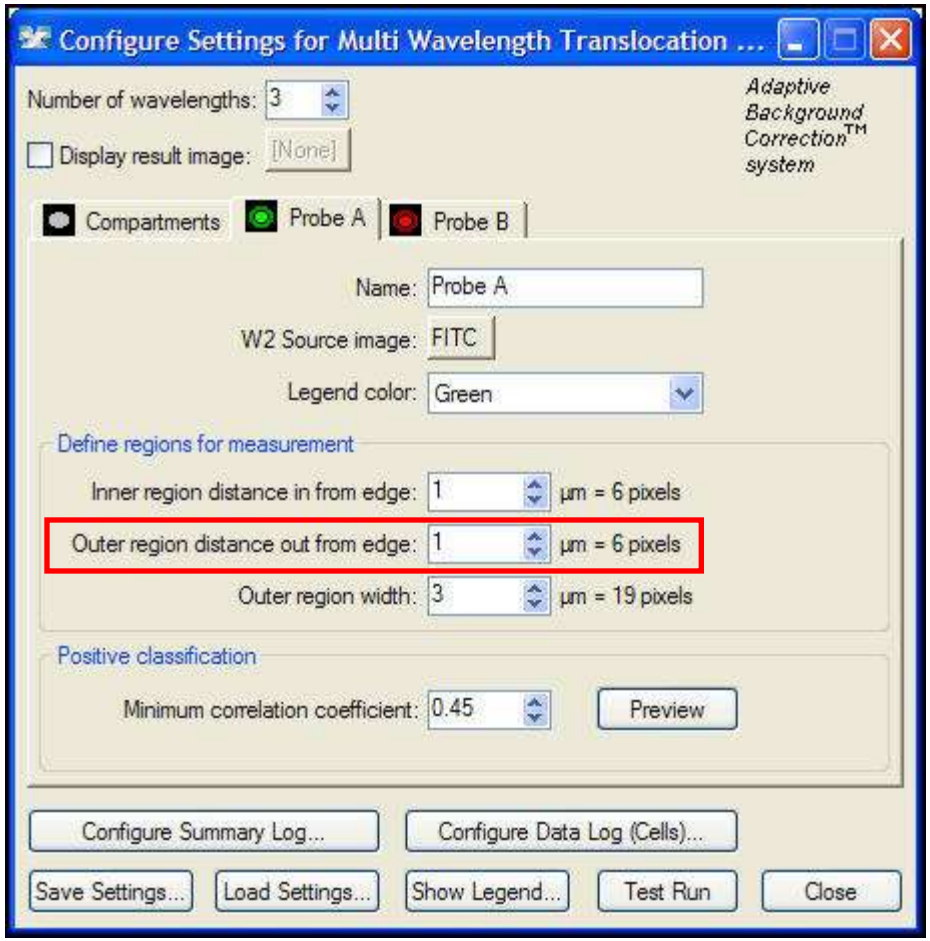
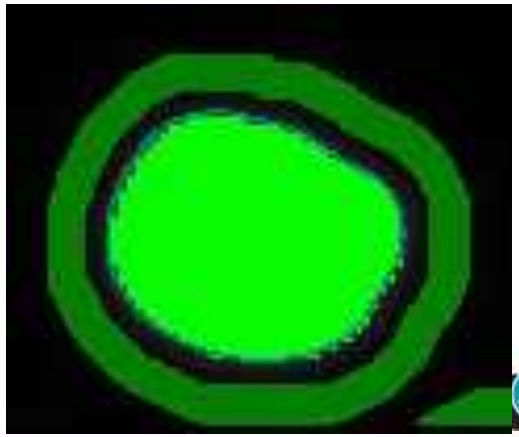
Module Settings: Wavelength 2



- **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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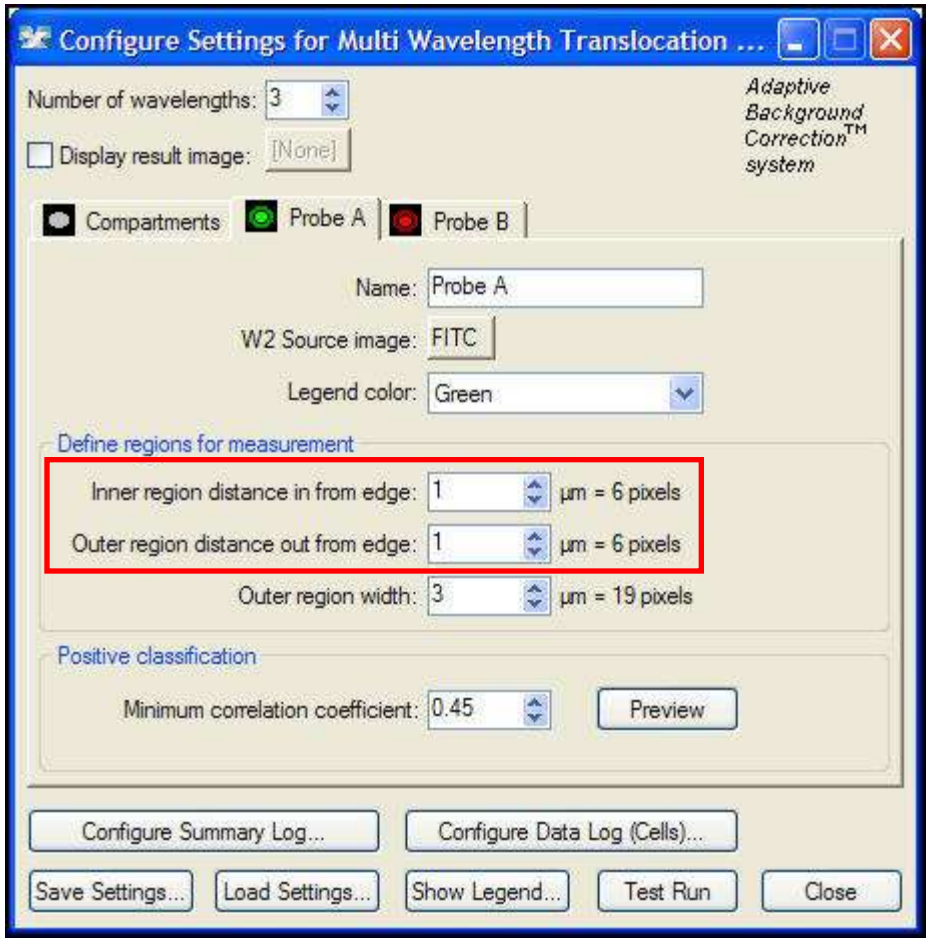
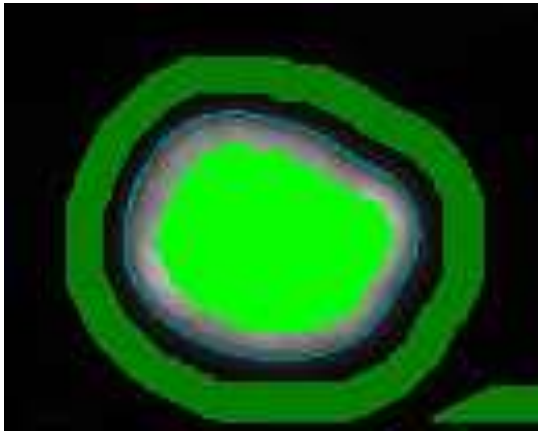
Module Settings: Wavelength 2



- **Define regions for measurement:**
- Typically the inner region and outer region distances are both utilized.



2 um in and out

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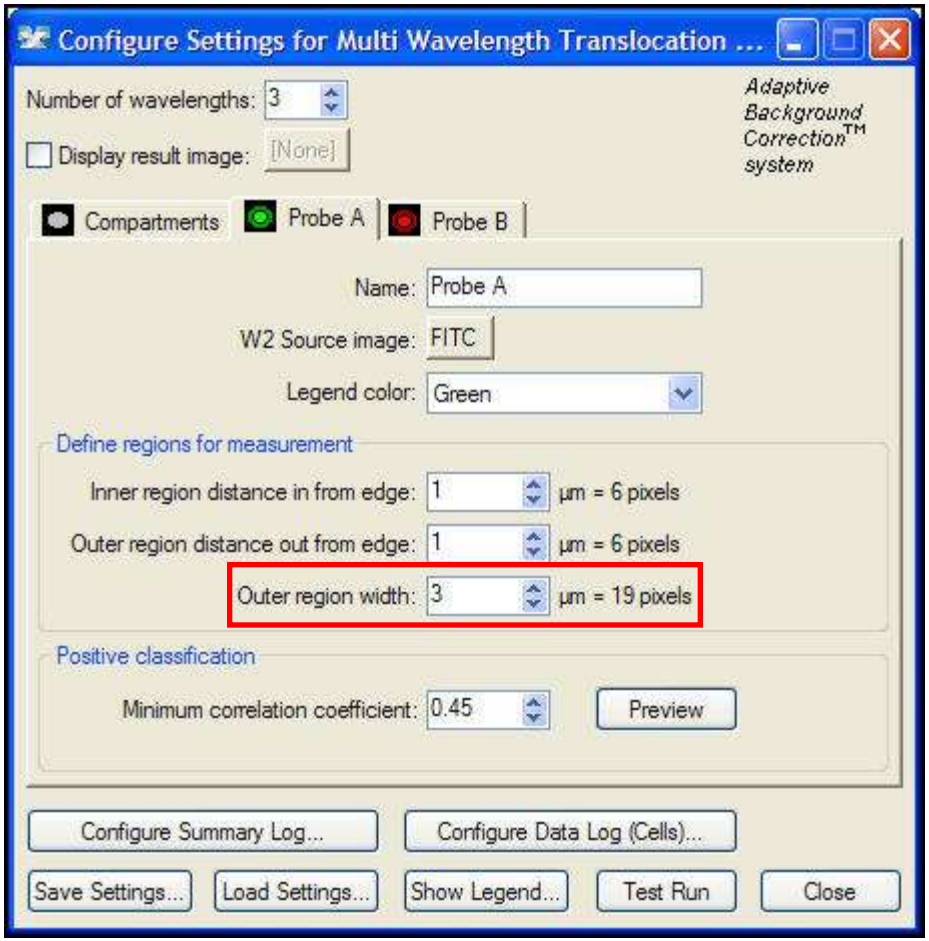
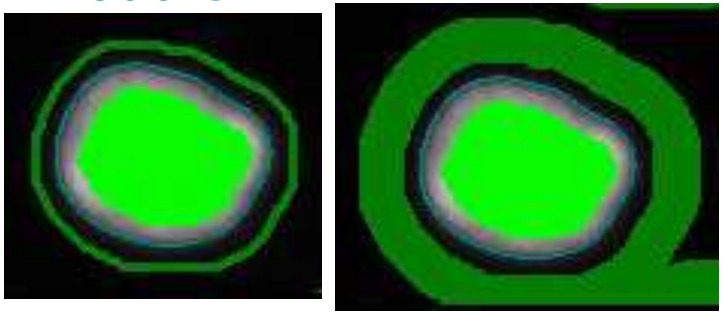
Module Settings: Wavelength 2



- **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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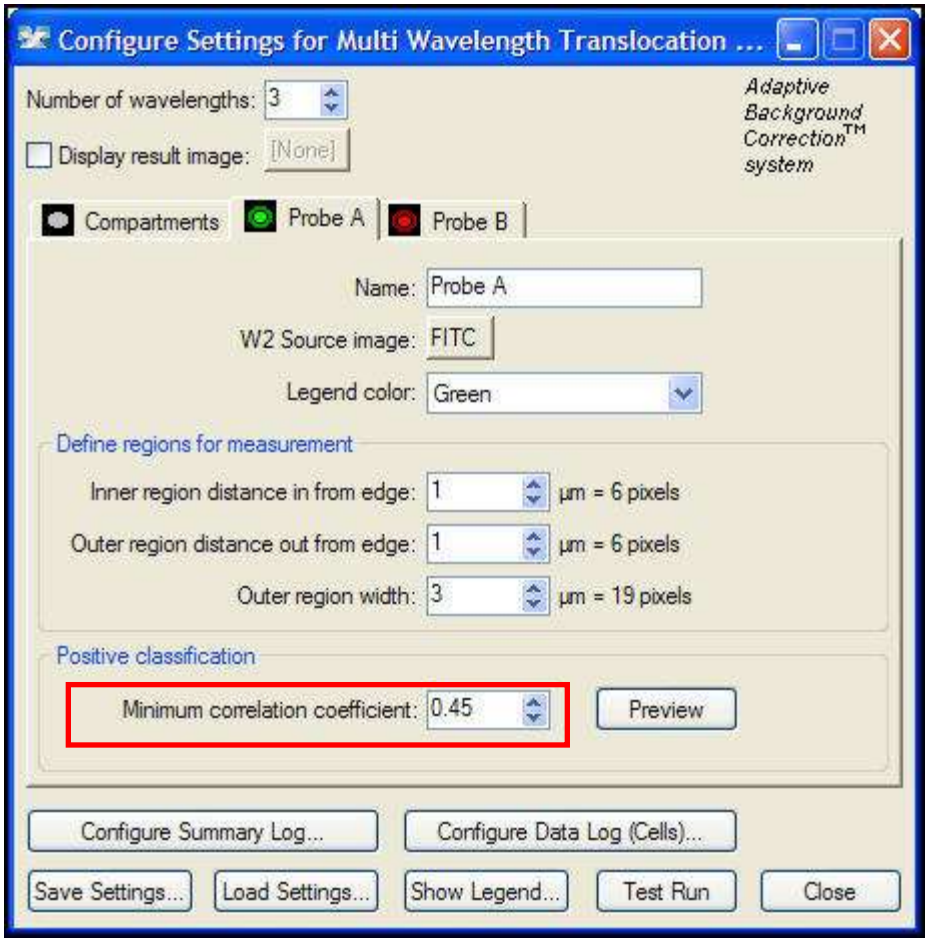
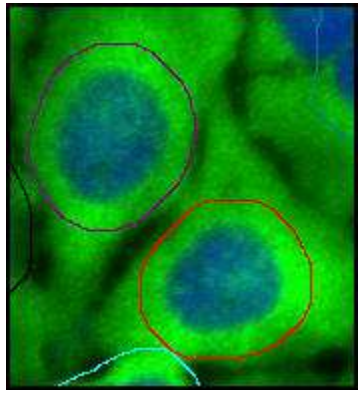
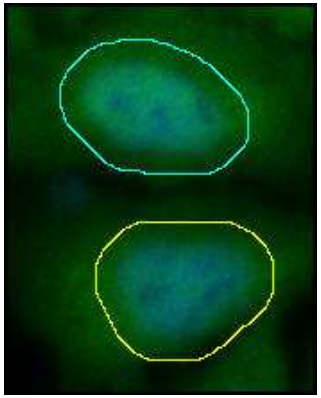
Module Settings: Wavelength 2



- **Positive classification:**
- Positive cells for this probe are those with nuclear staining. Negative cells for this probe are those with cytoplasmic staining.



Negative (cytoplasmic)



Positive (nuclear)

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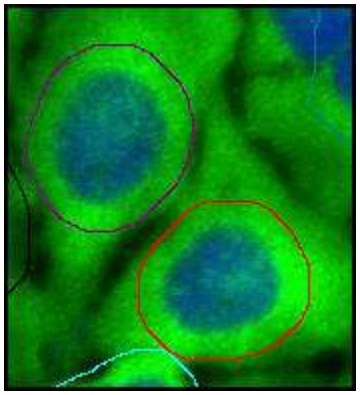
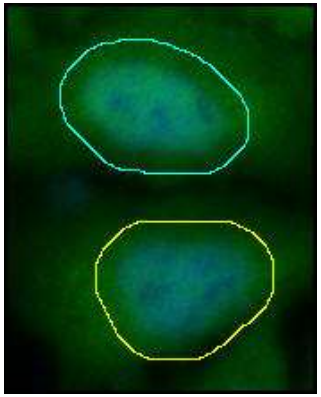
Module Settings: Wavelength 2

- **Positive classification**:
- **Correlation Coefficient -** This is the Pearson's correlation coefficient of the pixel intensity of the probe of interest and the nuclear stain 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



Negative (cytoplasmic)



Positive (nuclear)

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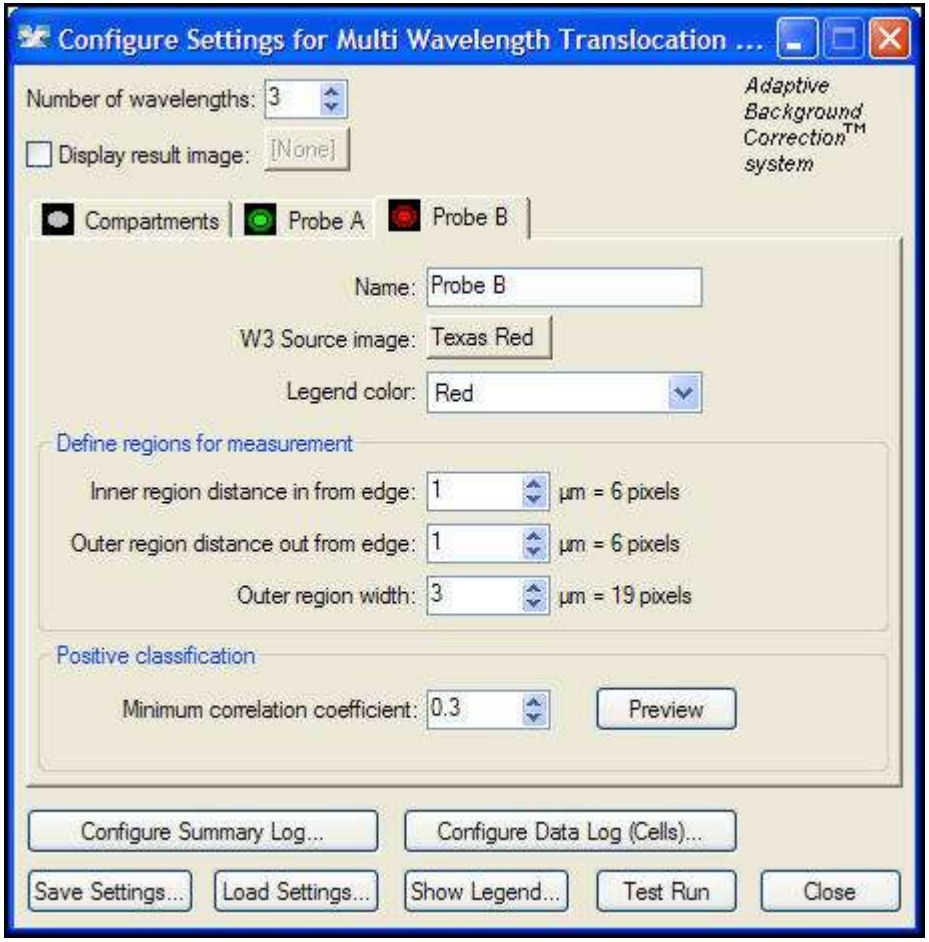
Module Settings: Additional wavelengths



- **Wavelength 3:**
- Follow the same steps to configure the settings for wavelength 3 (probe B in this example)

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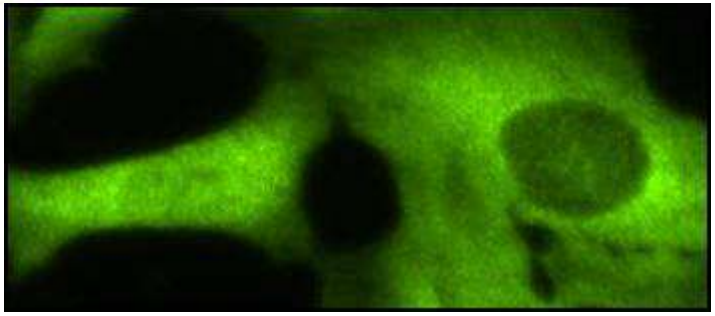
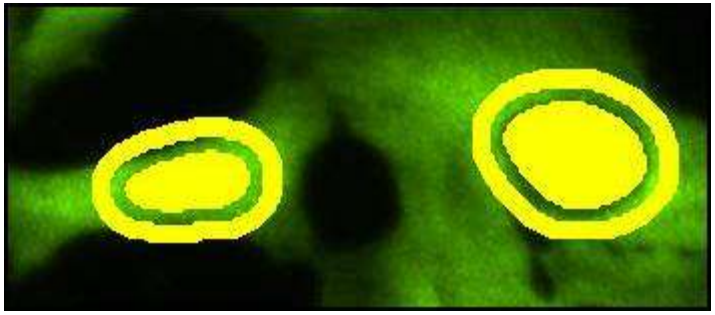
Module Settings





- **Setting Cell classification cutoffs**:
- Test module on positive and negative controls
- Use the interactive cellular results table to view the individual correlation or results for positive and negative cells
- An image showing both phenotypes makes it easy to compare results

Cell: Correlation Coefficient W2	Cell: Correlation Coefficient W3
-0.543586	-0.543574
-0.0849507	-0.084916
0.268204	0.268217
-0.872282	-0.872281
-0.618995	-0.618988
0.673702	0.673709
-0.635529	-0.635505
-0.852296	-0.852296
-0.262854	-0.262858
-0.277377	-0.277375
-0.859471	-0.859467
-0.103183	-0.103184
-0.409432	-0.40944
0.490158	0.49016
-0.0994213	-0.0994328
-0.760795	-0.760797
-0.508837	-0.508822
0.0343354	0.0343394
-0.780436	-0.780431
-0.532925	-0.532945
-0.681289	-0.681297
-0.692857	-0.692865
-0.717771	-0.717781

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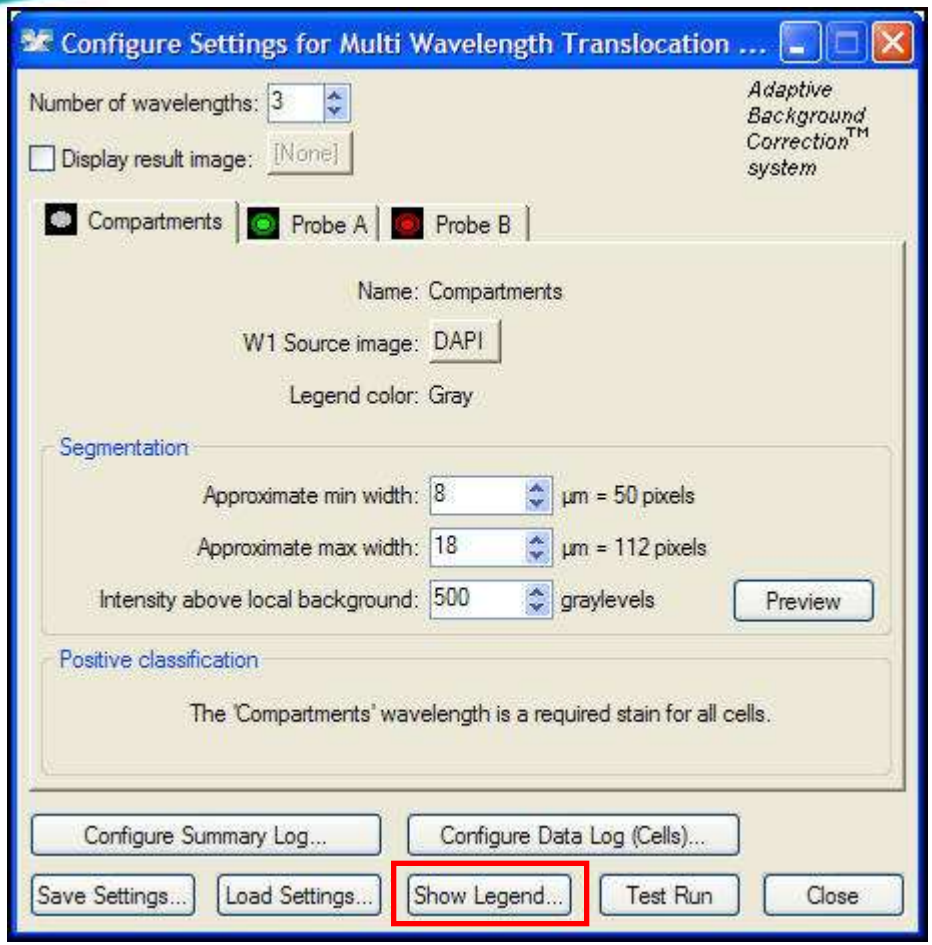
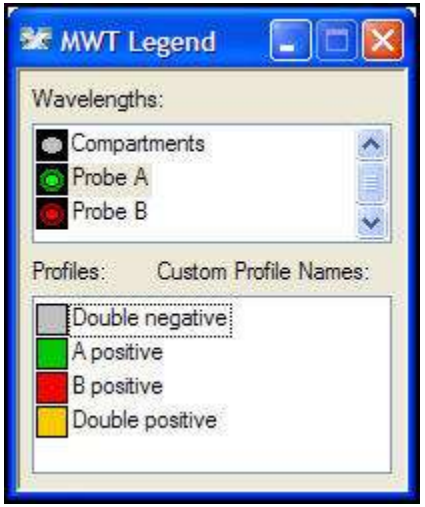
Module Settings



- **Show Legend**
- Optionally name each profile



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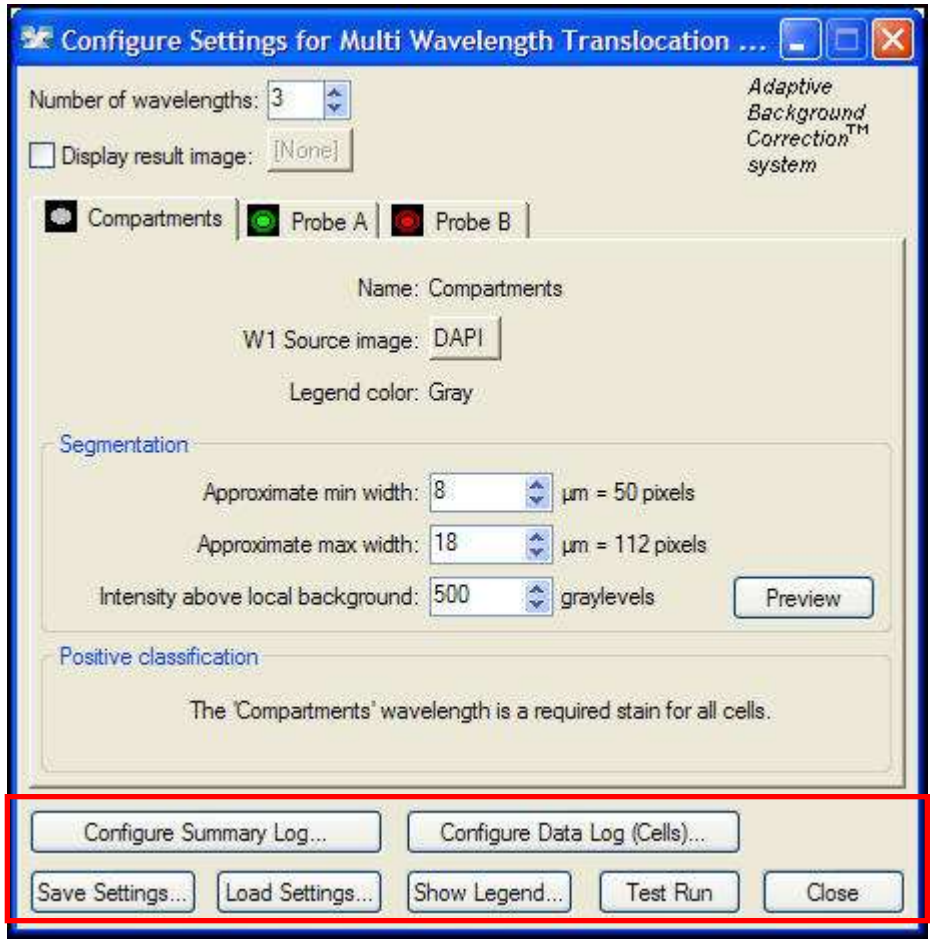
Module Settings – General Settings



- **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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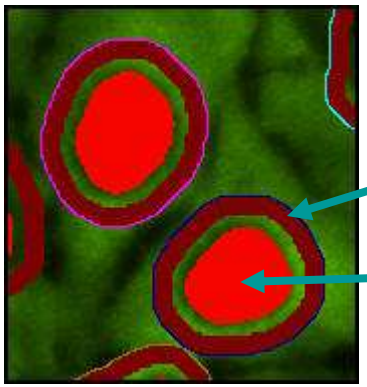
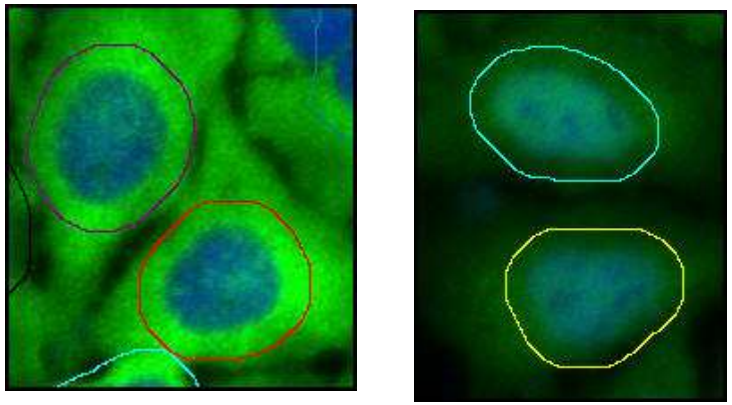
Regions for Measurements





Area for correlation coefficient

- **Regions**
- **Outer region**: corresponds to cytoplasm (minus gap at boundary)
- **Inner region**: corresponds to nucleus (minus gap at boundary)
- **Marked area**: corresponds to inner + outer regions
- Area for **correlation coefficient**: inner region + gap + outer region
- Note that the regions are defined separately for each wavelength

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Summary Data (site-by-site measurements)

- **Total cells:** Total number of nuclei (cell count)
- **Mean Compartment Area:** The average nuclear area (in um^2)
- **Mean Integrated Compartment Intensity:** The total pixel intensity of the nuclear stain over the nuclear area, divided by the number of cells
- **Mean Average Compartment Intensity:** The average pixel intensity of the nuclear stain over all the nuclear areas in the image

Molecular Devices

Summary Data (site-by-site measurements)

- **Positive W2:** The number of cells positive for nuclear translocation in wavelength 2 (probe A) as defined by correlation with the nuclear stain over the cell region
- **Positive W3:** The number of cells positive for nuclear translocation in wavelength 3 (probe B) as defined by correlation with the nuclear stain over the cell region
- **% Positive W2:** The number of positive W2 cells divided by the total number of cells, times 100
- **% Positive W3:** The number of positive W3 cells divided by the total number of cells, times 100

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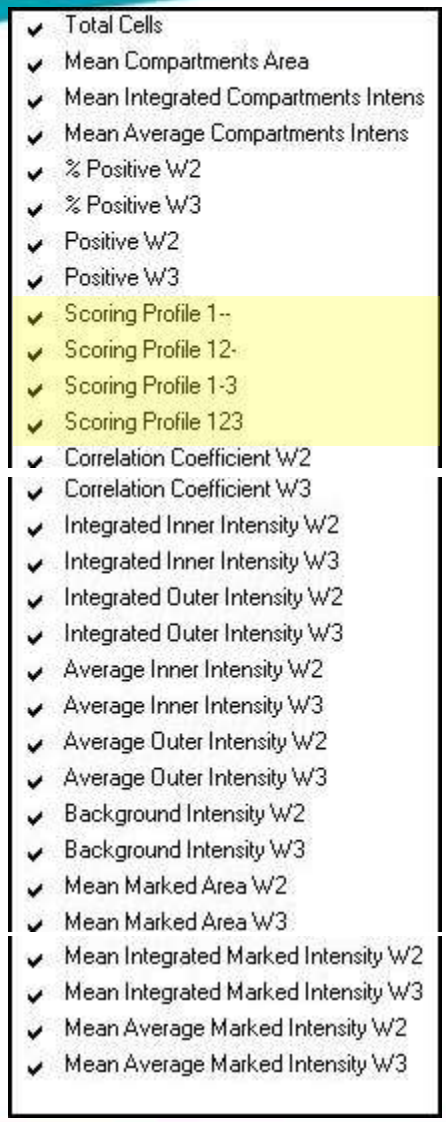
Summary Data (site-by-site measurements)



- **Scoring Profile 1--:** The number of cells negative for nuclear translocation in both wavelengths 2 and 3
- **Scoring Profile 12-:** The number of cells positive for nuclear translocation in wavelength 2 and negative for nuclear translocation in wavelength 3
- **Scoring Profile 1-3:** The number of cells negative for nuclear translocation in wavelength 2 and positive for nuclear translocation in wavelength 3
- **Scoring Profile 123:** The number of cells positive for nuclear translocation in both wavelengths 2 and 3

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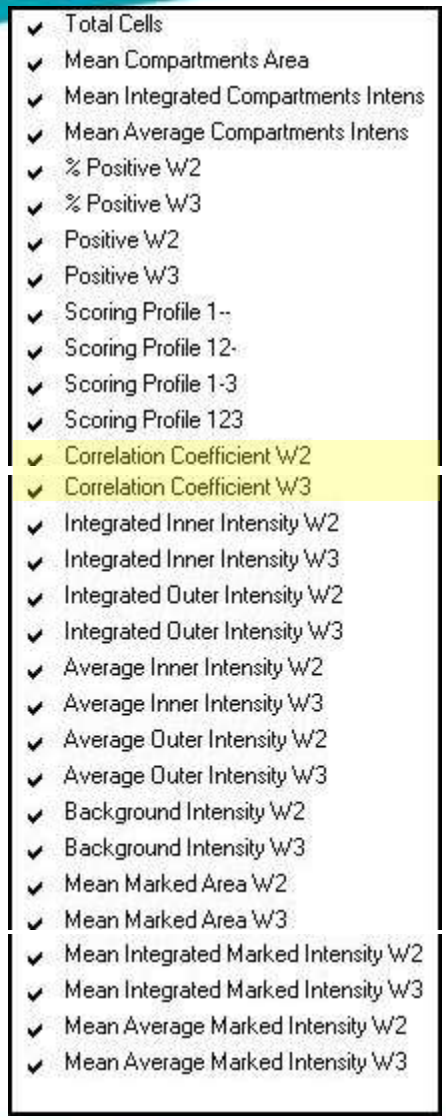
Summary Data (site-by-site measurements)



- **Correlation Coefficient W2:** The Pearson's correlation coefficient between the nuclear stain and wavelength 2 over all of the pixels located in all of the cell regions (nuclei + gaps + cytoplasms) in the site

- **Correlation Coefficient W3:** The Pearson's correlation coefficient between the nuclear stain and wavelength 3 over all of the pixels located in all of the cell regions (nuclei + gaps + cytoplasms) in the site

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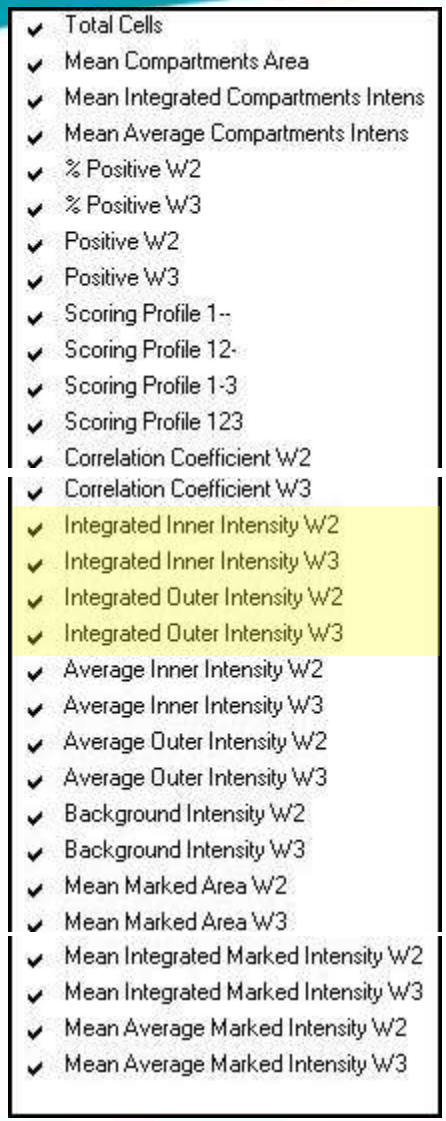
Summary Data (site-by-site measurements)



- **Integrated Inner Intensity W2:** The total pixel intensity of wavelength 2 (probe A) in all the inner W2 regions for the site after background subtraction (note this correlates with cell count)
- **Integrated Inner Intensity W3:** The total pixel intensity of wavelength 3 (probe B) in all the inner W3 regions for the site after background subtraction (note this correlates with cell count)
- **Integrated Outer Intensity W2:** The total pixel intensity of wavelength 2 (probe A) in all the outer W2 regions for the site after background subtraction (note this correlates with cell count)
- **Integrated Outer Intensity W3:** The total pixel intensity of wavelength 3 (probe B) in all the outer W3 regions for the site after background subtraction (note this correlates with cell count)

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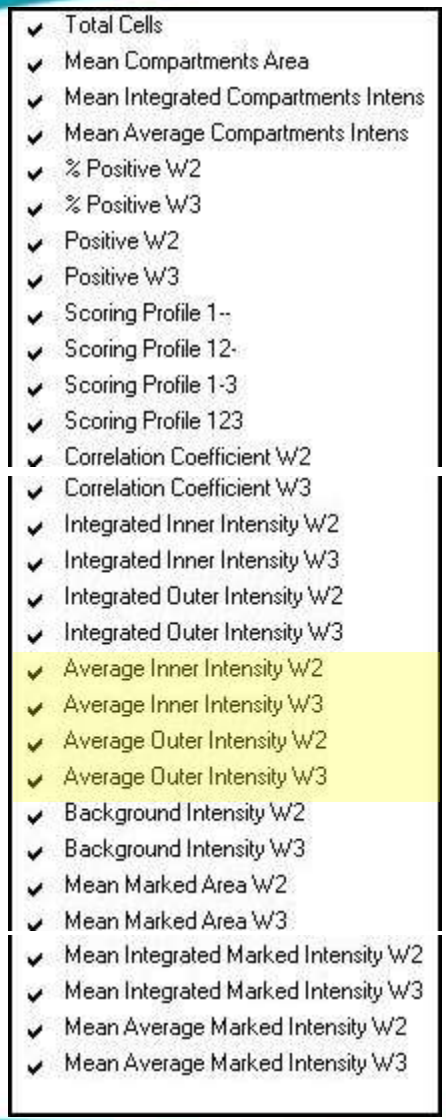
Summary Data (site-by-site measurements)



- **Average Inner Intensity W2:** The average pixel intensity of wavelength 2 (probe A) in all the inner W2 regions for the site after background subtraction (independent of cell count)
- **Average Inner Intensity W3:** The average pixel intensity of wavelength 3 (probe B) in all the inner W3 regions for the site after background subtraction (independent of cell count)
- **Average Outer Intensity W2:** The average pixel intensity of wavelength 2 (probe A) in all the outer W2 regions for the site after background subtraction (independent of cell count)
- **Average Outer Intensity W3:** The average pixel intensity of wavelength 3 (probe B) in all the outer W3 regions for the site after background subtraction (independent of cell count)

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Summary Data (site-by-site measurements)

- **Background Intensity W2:** The average background pixel intensity of the wavelength 2 (probe A) image. This is the value that has been subtracted from other W2 intensity measurements.
- **Background Intensity W3:** The average background pixel intensity of the wavelength 3 (probe B) image. This is the value that has been subtracted from other W3 intensity measurements.
- **Mean Marked Area W2:** The total area covered by inner + outer regions as defined for wavelength 2, divided by the total cell count
- **Mean Marked Area W3:** The total area covered by inner + outer regions as defined for wavelength 3, divided by the total cell count

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Summary Data (site-by-site measurements)

- **Mean Integrated Marked Intensity W2:** The total pixel intensity of wavelength 2 (probe A) in the inner + outer W2 regions minus background, divided by the total cell count
- **Mean Integrated Marked Intensity W3:** The total pixel intensity of wavelength 3 (probe B) in the inner + outer W3 regions minus background, divided by the total cell count
- **Mean Average Marked Intensity W2:** The average pixel intensity of wavelength 2 (probe A) across all of the inner + outer W2 regions in the image minus background
- **Mean Average Marked Intensity W3:** The average pixel intensity of wavelength 3 (probe B) across all of the inner + outer W3 regions in the image minus background

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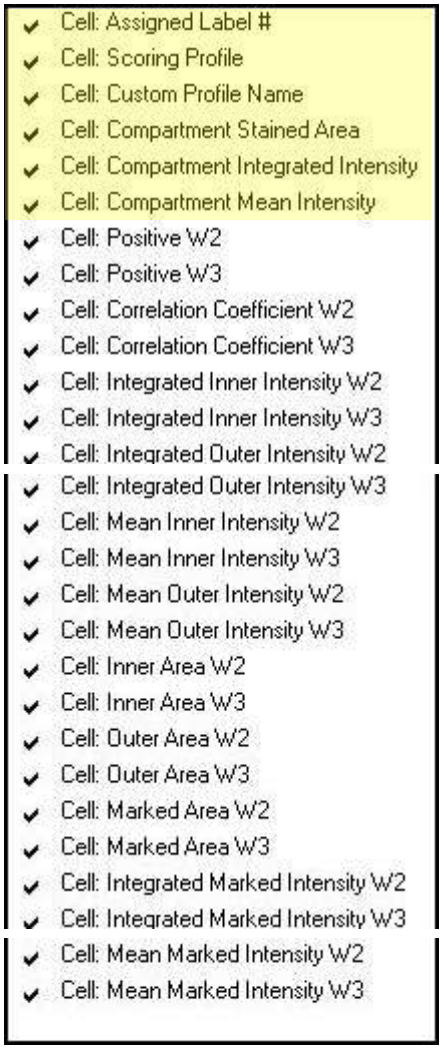
Cell Data (cell-by-cell measurements)



- **Cell: Assigned Label #** – Cell label number (1 through total cell number)
- **Cell: Scoring Profile** – The profile for this cell, i.e. "1—" or "12-" or "1-3" or "123"
- **Cell: Custom Profile Name** – The user-defined profile for this cell, as specified in the legend (e.g. "A positive", "Double positive")
- **Cell: Compartment Stained Area** – Total square microns of the nucleus
- **Cell: Compartment Integrated Intensity** – Total pixel intensity of the nuclear stain in the nucleus
- **Cell: Compartment Mean Intensity** – Average pixel intensity of the nuclear stain in the nucleus

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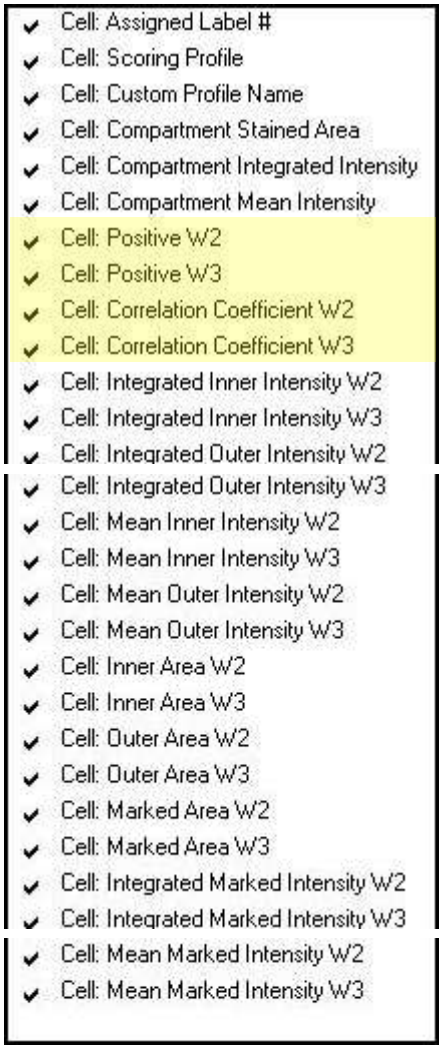
Cell Data (cell-by-cell measurements)



- **Cell: Positive W2** – Classification of the cell by correlation coefficient as positive for translocation (nuclear staining) in wavelength 2 (value of 1) or negative for translocation (cytoplasmic staining) in wavelength 2 (value of 0)
- **Cell: Positive W3** – Classification of the cell by correlation coefficient as positive for translocation (nuclear staining) in wavelength 3 (value of 1) or negative for translocation (cytoplasmic staining) in wavelength 3 (value of 0)
- **Cell: Correlation Coefficient W2** – The Pearson's correlation coefficient between the intensities of the nuclear stain and wavelength 2 (probe A) for all pixels in the cell region for W2 (nucleus + gap + cytoplasm). The value ranges from -1 (anti-correlated) to 1 (correlated).
- **Cell: Correlation Coefficient W3** – The Pearson's correlation coefficient between the intensities of the nuclear stain and wavelength 3 (probe B) for all pixels in the cell region for W3 (nucleus + gap + cytoplasm). The value ranges from -1 (anti-correlated) to 1 (correlated).

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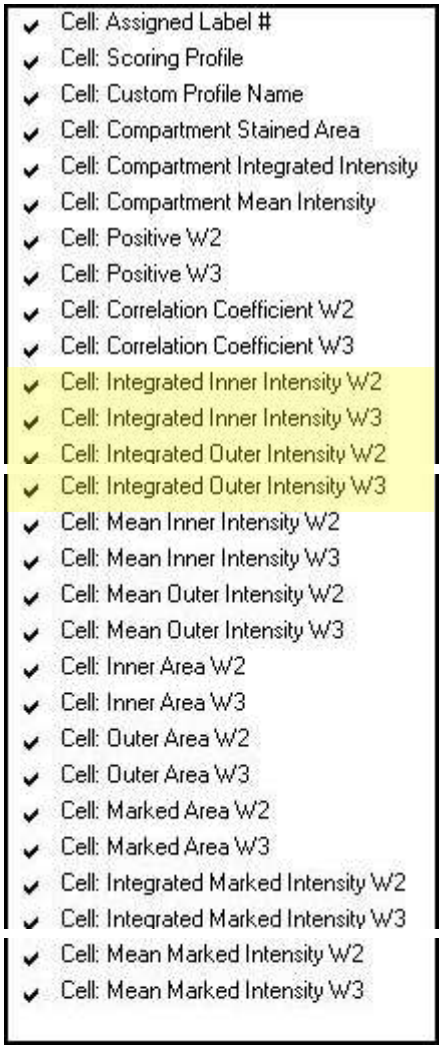
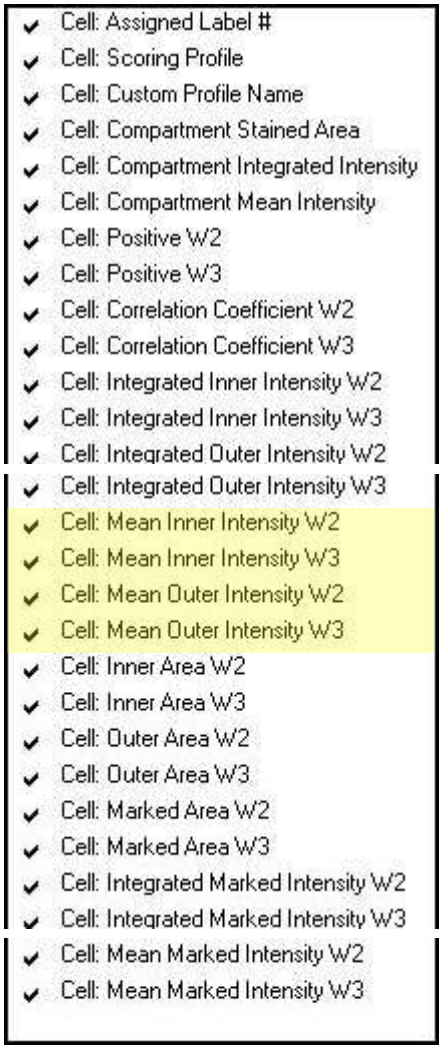
Cell Data (cell-by-cell measurements)



- **Cell: Integrated Inner Intensity W2** – The total pixel intensity of wavelength 2 (probe A) in the inner W2 region minus background
- **Cell: Integrated Inner Intensity W3** – The total pixel intensity of wavelength 3 (probe B) in the inner W3 region minus background
- **Cell: Integrated Outer Intensity W2** – The total pixel intensity of wavelength 2 (probe A) in the outer W2 region minus background
- **Cell: Integrated Outer Intensity W3** – The total pixel intensity of wavelength 3 (probe B) in the outer W3 region minus background

Molecular Devices

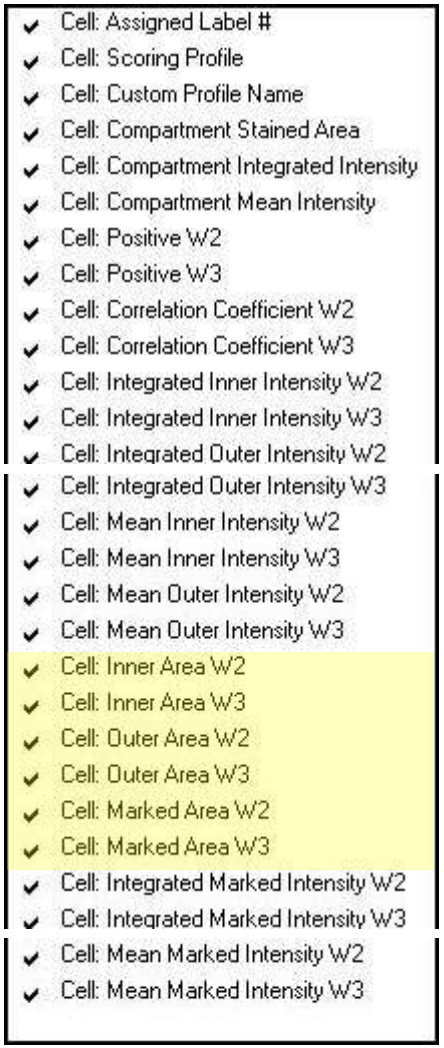
Cell Data (cell-by-cell measurements)



- **Cell: Mean Inner Intensity W2** – The average pixel intensity of wavelength 2 (probe A) in the inner W2 region minus background
- **Cell: Mean Inner Intensity W3** – The average pixel intensity of wavelength 3 (probe B) in the inner W3 region minus background
- **Cell: Mean Outer Intensity W2** – The average pixel intensity of wavelength 2 (probe A) in the outer W2 region minus background
- **Cell: Mean Outer Intensity W3** – The average pixel intensity of wavelength 3 (probe B) in the outer W3 region minus background

Molecular Devices

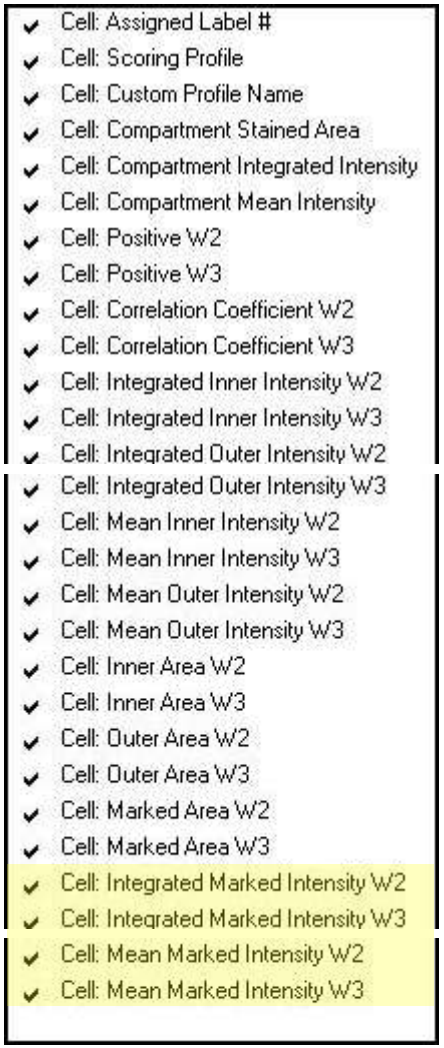
Cell Data (cell-by-cell measurements)



- **Cell: Inner Area W2** – Total square microns in the inner W2 region
- **Cell: Inner Area W3** – Total square microns in the inner W3 region
- **Cell: Outer Area W2** – Total square microns in the outer W2 region
- **Cell: Outer Area W3** – Total square microns in the outer W3 region
- **Cell: Marked Area W2** – The sum of the inner and outer W2 areas
- **Cell: Marked Area W3** – The sum of the inner and outer W3 areas

Molecular Devices

Cell Data (cell-by-cell measurements)



- **Cell: Integrated Marked Intensity W2** – The total pixel intensity of wavelength 2 (probe A) in the inner + outer W2 areas (minus background)
- **Cell: Integrated Marked Intensity W3** – The total pixel intensity of wavelength 3 (probe B) in the inner + outer W3 areas (minus background)
- **Cell: Mean Marked Intensity W2** – The average pixel intensity of wavelength 2 (probe A) in the inner + outer W2 areas (minus background)
- **Cell: Mean Marked Intensity W3** – The average pixel intensity of wavelength 3 (probe B) in the inner + outer W3 areas (minus background)

Molecular Devices

Molecular Devices





Thank You