







### MetaXpress: Multi Wavelength Translocation module

Paula Gedraitis, Ph.D.

### 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

🐲 Configure Settings for Multi V	Vavelengti	h Translocation	🗔 🗖 🔀
Number of wavelengths: 3			Adaptive Background Correction <sup>TM</sup> system
Compartments G Probe A	Probe B		1
Name:	Compartmen	its	
W1 Source image:	DAPI		
Legend color:	Gray		
- Segmentation			
Approximate min width:	8	µm = 50 pixels	
Approximate max width:	18 🗳	µm = 112 pixels	
Intensity above local background:	500	graylevels	Preview
Positive classification The 'Compartments' wave	elength is a r	equired stain for all o	cells.
Configure Summary Log Save Settings) Load Settings) S	Configure I	Data Log (Cells)	) Close



### **Module Settings**

📽 Configure Settings for Multi Wavelength Translocation 🔳 🗖	
Number of wavelengths: 3 2 Background Correction <sup>Th</sup>	1
Display result image: [None] system	
Compartments O Probe A Probe B	-
Name: Compartments	
W1 Source image: DAPI	
Legend color: Gray	
Segmentation	
Approximate min width: 8 😭 µm = 50 pixels	
Approximate max width: 18 😭 µm = 112 pixels	
Intensity above local background: 500 📚 graylevels Preview	]
Positive classification	
The 'Compartments' wavelength is a required stain for all cells.	
Configure Summary Log Configure Data Log (Cells)	
Save Settings) Load Settings) Show Legend) Test Run Close	

### 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)



### **Module Settings**

📽 Configure Settings for Multi V	/avelength	Translocation	🗖 🗖 🔀
Number of wavelengths: 3			Adaptive Background
Display result image: None			Correction <sup>™</sup> system
Compartments 💽 Probe A 🛛 🛅	Probe B		
Name:	Compartment	s	
W1 Source image:	DAPI		
Legend color:	Gray		
Segmentation			
Approximate min width:	8	µm = 50 pixels	
Approximate max width:	18 😂	µm = 112 pixels	
Intensity above local background:	500	graylevels	Preview
Positive classification			
The 'Compartments' wave	elength is a re	quired stain for all c	ells.
k			-
Configure Summary Log	Configure D	ata Log (Cells)	)
Save Settings) Load Settings) S	ihow Legend.	] Test Run	Close

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



📽 Configure Settings for Multi W	/avelength	Translocation	🗖 🗖 🔀
Number of wavelengths: 3			Adaptive Background Correction <sup>™</sup> system
Compartments O Probe A	Probe B		
Name:	Compartment	s	
W1 Source image:	DAPI		
Legend color:	Gray		
Segmentation			
Approximate min width:	8	µm = 50 pixels	
Approximate max width:	18 🗢	µm = 112 pixels	
Intensity above local background:	500 😂	graylevels	Preview
Positive classification The 'Compartments' wave	elength is a re	quired stain for all c	ells.
Configure Summary Log Save Settings) Load Settings) Si	Configure D how Legend.	ata Log (Cells)	Close

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



Configure Settings for Multi W	/avelength	Translocation	🗖 🗖 🔀
Number of wavelengths: 3			Adaptive Background Correction <sup>™</sup> system
Compartments 🔤 Probe A 🛛 🚳	Probe B		
Name: W1 Source image: Legend color:		S	
Approximate min width: Approximate max width:	-	μm = 50 pixels μm = 112 pixels	
Intensity above local background:	500 🔵	graylevels	Preview
Positive classification The 'Compartments' wave Configure Summary Log		X: 410 Y: 3 Length: 97	334 (15.64 µm)

- 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



### Effects of varying width settings

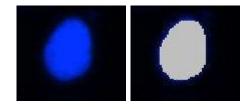
Min width too small: splits nuclei

Min width too large: omits smaller nuclei

Max width too small: may shrink nuclear boundaries

Max width too large: may slightly enlarge nuclear boundaries











Number of wavelengths: 3			Adaptive Background Correction <sup>TM</sup> system
	Compartmen	ts	
W1 Source image: Legend color: Segmentation			
Approximate min width:		µm = 50 pixels	
Approximate max width: Intensity above local background:			Preview
(GAY) Ievel (Article			
1024 -			
0	7.05	13.09 Distance (um)	19.14 2

- 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



📽 Configure Settings for Multi Wa	avelength Translocation 🗐 🗖 🔀
Number of wavelengths: 3	Adaptive Background Correction <sup>™</sup> system
Compartments 0 Probe A	Probe B
Name: C	Compartments
W1 Source image:	DAPI
Legend color: G	Gray
Segmentation	
Approximate min width:	8 🗢 μm = 50 pixels
Approximate max width:	18 🔿 µm = 112 pixels
Intensity above local background:	500 📚 graylevels Preview
Positive classification The "Compartments" wavele	length is a required stain for all cells.
	Configure Data Log (Cells) now Legend) Test Run Close
Contro containgo) [2000 containgo] [201	

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



📽 Configure Settings for Multi V	Wavelength Translocation	🗖 🗖 🔀
Number of wavelengths: 3	Probe B	Adaptive Background Correction <sup>TM</sup> system
	Probe A	<b>- </b>
W2 Source image:	FITC	
Legend color:	Green	
<ul> <li>Define regions for measurement</li> </ul>		
Inner region distance in from edge:	1 🗢 µm = 6 pixels	
Outer region distance out from edge:	1 🗢 µm = 6 pixels	
Outer region width:	3 µm = 19 pixels	
Positive classification Minimum correlation coefficient:	0.45 Preview	]
Configure Summary Log	Configure Data Log (Cells) Show Legend)	Close

### • Define W2:

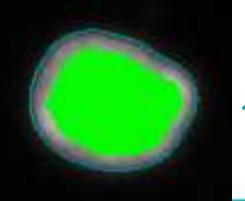
 Enter a name and select wavelength and legend color for your 2nd wavelength (probe A in this example)



📽 Configure Settings for Multi Waveler	ngth Translocation 🔳 🗖 🔀
Number of wavelengths: 3	Adaptive Background Correction <sup>™</sup> system
Compartments 🧧 Probe A	1
Name: Probe A	
W2 Source image: FITC	
Legend color: Green	×
- Define regions for measurement	
Inner region distance in from edge: 1	🗢 µm = 6 pixels
Outer region distance out from edge: 1	🛫 µm = 6 pixels
Outer region width: 3	拿 µm = 19 pixels
Positive classification Minimum correlation coefficient: 0.45	Preview
Configure Summary Log Configu Save Settings Load Settings Show Leg	ure Data Log (Cells) end) Test Run Close

# • 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.



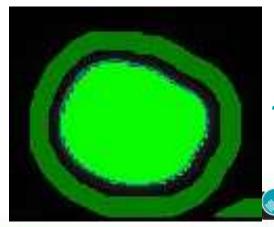


Molecular

📽 Configure Settings for Multi W	avelength Translocation	🗖 🗖 🔼 '
Number of wavelengths: 3		Adaptive Background Correction <sup>™</sup> system
Compartments 💿 Probe A	Probe B	T
Name:	Probe A	
W2 Source image:	FITC	
Legend color:	Green 💌	
- Define regions for measurement		
Inner region distance in from edge:	1 🗢 µm = 6 pixels	
Outer region distance out from edge:	1 💭 µm = 6 pixels	
Outer region width:	3 💭 µm = 19 pixels	
Positive classification Minimum correlation coefficient:	0.45 😭 Preview	
Configure Summary Log Save Settings	Configure Data Log (Cells) how Legend) Test Run	Close

### 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.

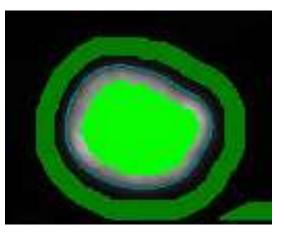


2 um out

🐲 Configure Settings for Multi W	avelength Translocation	🗖 🗖 💌
Number of wavelengths: 3		Adaptive Background Correction <sup>™</sup> system
Compartments 🙆 Probe A	Probe B	1
Name:	Probe A	
W2 Source image:	FITC	
Legend color:	Green 💌	
Define regions for measurement		
Inner region distance in from edge:	1 🗢 µm = 6 pixels	
Outer region distance out from edge:	1 💭 µm = 6 pixels	
Outer region width:	3 💭 µm = 19 pixels	
Positive classification Minimum correlation coefficient:	0.45 🗢 Preview	
Configure Summary Log Save Settings	Configure Data Log (Cells) how Legend Test Run	Close

# • Define regions for measurement:

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



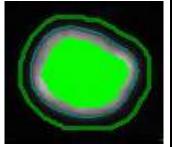
2 um in and out



📽 Configure Settings for Multi Wave	length Translocation 🗐 🗖 🔀 🏾
Number of wavelengths: 3	Adaptive Backgrownd Correction™ system
Compartments Probe A	e B
Name: Prob	e A
W2 Source image: FITC	
Legend color: Gree	en 💌
Define regions for measurement	
Inner region distance in from edge: 1	🗢 µm = 6 pixels
Outer region distance out from edge: 1	🗢 µm = 6 pixels
Outer region width: 3	🗢 µm = 19 pixels
Positive classification Minimum correlation coefficient: 0.45	Preview
	figure Data Log (Cells) Legend) Test Run Close

# • 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

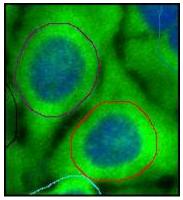


5 um Molecular Devices

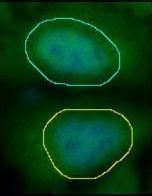
📽 Configure Settings for Multi W	avelength Translocation	🗖 🗖 🔼
Number of wavelengths: 3 🛟		Adaptive Background Correction <sup>TM</sup> system
Compartments 🙆 Probe A	Probe B	T
Name:	Probe A	
W2 Source image:	FITC	
Legend color:	Green 💌	
- Define regions for measurement		
Inner region distance in from edge:	1 🗢 µm = 6 pixels	
Outer region distance out from edge:	1 💭 µm = 6 pixels	
Outer region width:	3 🗢 µm = 19 pixels	
Positive classification		
Minimum correlation coefficient:	0.45 Preview	
Configure Summary Log	Configure Data Log (Cells)	
Save Settings	how Legend	Close

### • 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)



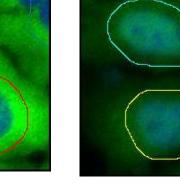
### Positive classification:

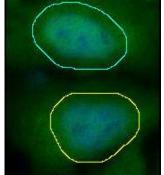
- **Correlation Coefficient -** This is the Pearson's correlation coefficient of the pixel intensity of the probe of interest and the nuclear stain in the entire cell region (nucleus + gap + cytoplasm)
- This is typically the most robust method for classifying translocation
- 1.0 is perfect correlation (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)







### Module Settings: Additional wavelengths

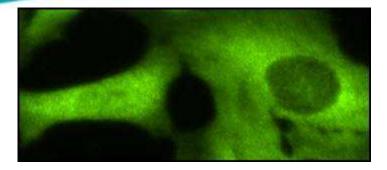
📽 Configure Settings for Multi W	/avelength Translocation	🗖 🗖 🔀
Number of wavelengths: 3 🛟		Adaptive Background Correction <sup>™</sup> system
Compartments O Probe A	Probe B	1
Name:	Probe B	
W3 Source image:	Texas Red	
Legend color:	Red	
- Define regions for measurement		
Inner region distance in from edge:	1 💭 µm = 6 pixels	
Outer region distance out from edge:	1 💭 µm = 6 pixels	
Outer region width:	3 🔅 µm = 19 pixels	
Positive classification Minimum correlation coefficient:	0.3 🗘 Preview	
Configure Summary Log	Configure Data Log (Cells)	
Save Settings	how Legend Test Run	Close

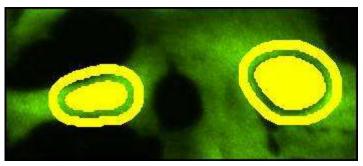
### • Wavelength 3:

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



## **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	

# **Module Settings**

📽 Configure Settings for Multi V	Vavelength	Translocation	🗖 🗖 🔀
Number of wavelengths: 3			Adaptive Background Correction <sup>™</sup> system
Compartments G Probe A	Probe B		
Name:	Compartment	ts	
W1 Source image:	DAPI		
Legend color:	Gray		
Segmentation			
Approximate min width:	8	] µm = 50 pixels	
Approximate max width:	18 🗳	] µm = 112 pixels	
Intensity above local background:	500 😂	graylevels	Preview
Positive classification			
The 'Compartments' wave	elength is a re	equired stain for all c	ells.
Configure Summary Log	Configure D	ata Log (Cells)	)
Save Settings) Load Settings	õh <mark>ow Leg</mark> end.	) Test Run	Close

### Show Legend

• Optionally name each profile

Wavelengths:	
Compartments	~
O Probe A	
🧑 Probe B	~
Profiles: Custom Profile Double negative A positive	Names:
B positive	



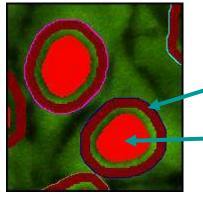
# Module Settings – General Settings

📽 Configure Settings for Multi Wavelength Translocation 🔳 🗖 🔀			
Number of wavelengths: 3			Adaptive Background Correction <sup>™</sup> system
Compartments O Probe A	Probe B		1
Name:	Compartment	s	
W1 Source image:	DAPI		
Legend color:	Gray		
- Segmentation			
Approximate min width:	8	µm = 50 pixels	
Approximate max width:	18 💲	µm = 112 pixels	
Intensity above local background:	500 ᅌ	graylevels	Preview
Positive classification The 'Compartments' wave	elength is a re	quired stain for all c	ells.
Configure Summary Log Save Settings) Load Settings) S	Configure D how Legend.	ata Log (Cells)	Close

- Configure Summary Log

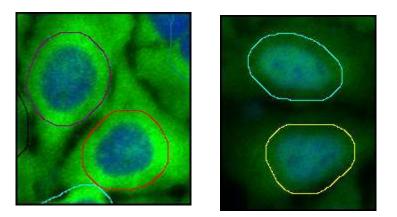
   select site-by-site
   measurements
- Configure Data Log select cell-by-cell measurements
- Save Settings save analysis parameters to database
- Load Settings load saved analysis parameters
- Set to Defaults restore default analysis parameters
- Test Run test all settings together and display cellby-cell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

### **Regions for Measurements**



### Outer region

Inner region



### 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



- Mean Compartments Area
- Mean Integrated Compartments Intens
- Mean Average Compartments Intens
- % Positive W2
- % Positive W3
- Positive W2
- Positive W3
- Scoring Profile 1--
- Scoring Profile 12-
- Scoring Profile 1-3
- Scoring Profile 123
- Correlation Coefficient W2
- Correlation Coefficient W3
- Integrated Inner Intensity W2
- Integrated Inner Intensity W3
- Integrated Outer Intensity W2
- Integrated Outer Intensity W3
- Average Inner Intensity W2
- Average Inner Intensity W3
- Average Outer Intensity W2
- Average Outer Intensity W3
- Background Intensity W2
- Background Intensity W3
- Mean Marked Area W2
- Mean Marked Area W3
- Mean Integrated Marked Intensity W2
- Mean Integrated Marked Intensity W3
- Mean Average Marked Intensity W2
- Mean Average Marked Intensity W3

- **Total cells:** Total number of nuclei (cell count)
- Mean Compartment Area: The average nuclear area (in um<sup>2</sup>)
- 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



- Mean Compartments Area
- Mean Integrated Compartments Intens
- Mean Average Compartments Intens
- % Positive W2
- % Positive W3
- Positive W2
- ✓ Positive W3
- ✓ Scoring Profile 1--
- ✓ Scoring Profile 12-
- Scoring Profile 1-3
- Scoring Profile 123
- Correlation Coefficient W2
- Correlation Coefficient W3
- Integrated Inner Intensity W2
- Integrated Inner Intensity W3
- Integrated Outer Intensity W2
- Integrated Outer Intensity W3
- Average Inner Intensity W2
- ✓ Average Inner Intensity W3
- Average Outer Intensity W2
- Average Outer Intensity W3
- Background Intensity W2
- Background Intensity W3
- ✓ Mean Marked Area W2
- Mean Marked Area W3
- Mean Integrated Marked Intensity W2
- Mean Integrated Marked Intensity W3
- Mean Average Marked Intensity W2
- Mean Average Marked Intensity W3

- 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



- Mean Compartments Area
- Mean Integrated Compartments Intens
- Mean Average Compartments Intens
- % Positive W2
- % Positive W3
- ✓ Positive W2
- Positive W3
- Scoring Profile 1--
- Scoring Profile 12-
- Scoring Profile 1-3
- Scoring Profile 123
- Correlation Coefficient W2
- Correlation Coefficient W3
- Integrated Inner Intensity W2
- Integrated Inner Intensity W3
- Integrated Outer Intensity W2
- Integrated Outer Intensity W3
- Average Inner Intensity W2
- Average Inner Intensity W3
- Average Outer Intensity W2
- Average Outer Intensity W3
- Background Intensity W2
- Background Intensity W3
- ✓ Mean Marked Area W2
- Mean Marked Area W3
- Mean Integrated Marked Intensity W2
- Mean Integrated Marked Intensity W3
- Mean Average Marked Intensity W2
- Mean Average Marked Intensity W3

- 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



- Mean Compartments Area
- Mean Integrated Compartments Intens
- Mean Average Compartments Intens
- ✓ % Positive W2
- ✓ % Positive W3
- ✓ Positive W2
- ✓ Positive W3
- Scoring Profile 1--
- ✓ Scoring Profile 12-
- Scoring Profile 1-3
- Scoring Profile 123
- Correlation Coefficient W2
- Correlation Coefficient W3
- Integrated Inner Intensity W2
- Integrated Inner Intensity W3
- Integrated Outer Intensity W2
- Integrated Outer Intensity W3
- Average Inner Intensity W2
- ✓ Average Inner Intensity W3
- Average Outer Intensity W2
- Average Outer Intensity W3
- Background Intensity W2
- ✓ Background Intensity W3
- Mean Marked Area W2
- Mean Marked Area W3
- Mean Integrated Marked Intensity W2
- Mean Integrated Marked Intensity W3
- Mean Average Marked Intensity W2
- Mean Average Marked Intensity W3

- 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



- Mean Compartments Area
- Mean Integrated Compartments Intens
- Mean Average Compartments Intens
- ✓ % Positive W2
- % Positive W3
- Positive W2
- ✓ Positive W3
- ✓ Scoring Profile 1--
- ✓ Scoring Profile 12-
- Scoring Profile 1-3
- Scoring Profile 123
- Correlation Coefficient W2
- Correlation Coefficient W3
- Integrated Inner Intensity W2
- Integrated Inner Intensity W3
- Integrated Outer Intensity W2
- Integrated Outer Intensity W3
- ✓ Average Inner Intensity W2
- ✓ Average Inner Intensity W3
- Average Outer Intensity W2
- Average Outer Intensity W3
- Background Intensity W2
- Background Intensity W3
- Mean Marked Area W2
- Mean Marked Area W3
- Mean Integrated Marked Intensity W2
- Mean Integrated Marked Intensity W3
- Mean Average Marked Intensity W2
- Mean Average Marked Intensity W3

- **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)



- Mean Compartments Area
- Mean Integrated Compartments Intens
- Mean Average Compartments Intens
- ✓ % Positive W2
- % Positive W3
- Positive W2
- ✓ Positive W3
- ✓ Scoring Profile 1--
- ✓ Scoring Profile 12-
- Scoring Profile 1-3
- ✓ Scoring Profile 123
- Correlation Coefficient W2
- Correlation Coefficient W3
- Integrated Inner Intensity W2
- Integrated Inner Intensity W3
- Integrated Outer Intensity W2
- Integrated Outer Intensity W3
- Average Inner Intensity W2
- Average Inner Intensity W3
- Average Outer Intensity W2
- Average Outer Intensity W3
- Background Intensity W2
- ✓ Background Intensity W3
- Mean Marked Area W2
- Mean Marked Area W3
- Mean Integrated Marked Intensity W2
- Mean Integrated Marked Intensity W3
- Mean Average Marked Intensity W2
- Mean Average Marked Intensity W3

- 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)



- Mean Compartments Area
- Mean Integrated Compartments Intens
- Mean Average Compartments Intens
- % Positive W2
- ✓ % Positive W3
- Positive W2
- Positive W3
- Scoring Profile 1--
- ✓ Scoring Profile 12-
- ✓ Scoring Profile 1-3
- Scoring Profile 123
- Correlation Coefficient W2
- Correlation Coefficient W3
- Integrated Inner Intensity W2
- Integrated Inner Intensity W3
- Integrated Outer Intensity W2
- Integrated Outer Intensity W3
- Average Inner Intensity W2
- Average Inner Intensity W3
- ✓ Average Outer Intensity W2
- Average Outer Intensity W3
- Background Intensity W2
- ✓ Background Intensity W3
- Mean Marked Area W2
- Mean Marked Area W3
- Mean Integrated Marked Intensity W2
- Mean Integrated Marked Intensity W3
- Mean Average Marked Intensity W2
- Mean Average Marked Intensity W3

- 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



- Mean Compartments Area
- Mean Integrated Compartments Intens
- Mean Average Compartments Intens
- % Positive W2
- % Positive W3
- Positive W2
- ✓ Positive W3
- ✓ Scoring Profile 1--
- ✓ Scoring Profile 12-
- Scoring Profile 1-3
- Scoring Profile 123
- Correlation Coefficient W2
- Correlation Coefficient W3
- Integrated Inner Intensity W2
- Integrated Inner Intensity W3
- Integrated Outer Intensity W2
- Integrated Outer Intensity W3
- Average Inner Intensity W2
- Average Inner Intensity W3
- ✓ Average Outer Intensity W2
- Average Outer Intensity W3
- Background Intensity W2
- ✓ Background Intensity W3
- ✓ Mean Marked Area W2
- Mean Marked Area W3
- Mean Integrated Marked Intensity W2
- Mean Integrated Marked Intensity W3
- Mean Average Marked Intensity W2
- Mean Average Marked Intensity W3

- 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



#### ✓ Cell: Assigned Label #

- ✓ Cell: Scoring Profile
- Cell: Custom Profile Name
- Cell: Compartment Stained Area
- Cell: Compartment Integrated Intensity
- Cell: Compartment Mean Intensity
- ✓ Cell: Positive W2
- ✔ Cell: Positive W3
- ✓ Cell: Correlation Coefficient W2
- ✓ Cell: Correlation Coefficient W3
- Cell: Integrated Inner Intensity W2
- Cell: Integrated Inner Intensity W3
- Cell: Integrated Outer Intensity W2
- Cell: Integrated Outer Intensity W3
- ✓ Cell: Mean Inner Intensity W2
- ✓ Cell: Mean Inner Intensity W3
- ✓ Cell: Mean Outer Intensity W2.
- ✔ Cell: Mean Outer Intensity W3
- ✓ Cell: Inner Area W2
- ✓ Cell: Inner Area W3
- ✓ Cell: Outer Area W2
- ✔ Cell: Outer Area W3
- ✓ Cell: Marked Area W2
- ✓ Cell: Marked Area W3
- Cell: Integrated Marked Intensity W2
- Cell: Integrated Marked Intensity W3
- ✓ Cell: Mean Marked Intensity W2
- ✓ Cell: Mean Marked Intensity W3

- 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 userdefined 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



#### ✓ Cell: Assigned Label #

- ✓ Cell: Scoring Profile
- Cell: Custom Profile Name
- Cell: Compartment Stained Area
- Cell: Compartment Integrated Intensity
- Cell: Compartment Mean Intensity
- ✔ Cell: Positive W2
- ✓ Cell: Positive W3
- ✓ Cell: Correlation Coefficient W2
- ✓ Cell: Correlation Coefficient W3
- Cell: Integrated Inner Intensity W2
- Cell: Integrated Inner Intensity W3
- ✓ Cell: Integrated Outer Intensity W2
- Cell: Integrated Outer Intensity W3
- ✓ Cell: Mean Inner Intensity W2
- ✓ Cell: Mean Inner Intensity W3
- ✓ Cell: Mean Outer Intensity W2.
- ✓ Cell: Mean Outer Intensity W3
- ✓ Cell: Inner Area W2
- ✓ Cell: Inner Area W3
- ✓ Cell: Outer Area W2
- ✓ Cell: Outer Area W3
- ✓ Cell: Marked Area W2
- ✓ Cell: Marked Area W3
- ✓ Cell: Integrated Marked Intensity W2
- Cell: Integrated Marked Intensity W3
- ✓ Cell: Mean Marked Intensity W2
- ✔ Cell: Mean Marked Intensity W3

- 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).



#### ✓ Cell: Assigned Label #

#### ✓ Cell: Scoring Profile

- Cell: Custom Profile Name
- Cell: Compartment Stained Area
- Cell: Compartment Integrated Intensity
- ✔ Cell: Compartment Mean Intensity
- ✔ Cell: Positive W2
- ✓ Cell: Positive W3
- ✔ Cell: Correlation Coefficient W2
- ✔ Cell: Correlation Coefficient W3
- Cell: Integrated Inner Intensity W2
- Cell: Integrated Inner Intensity W3
- 🧹 Cell: Integrated Outer Intensity W2
- Cell: Integrated Outer Intensity W3
- ✓ Cell: Mean Inner Intensity W2
- ✓ Cell: Mean Inner Intensity W3
- ✓ Cell: Mean Outer Intensity W2
- ✔ Cell: Mean Outer Intensity W3
- ✓ Cell: Inner Area W2
- ✓ Cell: Inner Area W3
- ✓ Cell: Outer Area W2
- ✓ Cell: Outer Area W3
- ✓ Cell: Marked Area W2
- ✓ Cell: Marked Area W3
- ✓ Cell: Integrated Marked Intensity W2
- Cell: Integrated Marked Intensity W3
- ✓ Cell: Mean Marked Intensity W2
- ✔ Cell: Mean Marked Intensity W3

- 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



#### ✓ Cell: Assigned Label #

#### ✓ Cell: Scoring Profile

- Cell: Custom Profile Name
- Cell: Compartment Stained Area
- Cell: Compartment Integrated Intensity
- ✓ Cell: Compartment Mean Intensity
- ✔ Cell: Positive W2
- ✔ Cell: Positive W3
- Cell: Correlation Coefficient W2
- ✔ Cell: Correlation Coefficient W3
- Cell: Integrated Inner Intensity W2
- Cell: Integrated Inner Intensity W3
- ✓ Cell: Integrated Outer Intensity W2
- Cell: Integrated Outer Intensity W3
- ✓ Cell: Mean Inner Intensity W2
- ✓ Cell: Mean Inner Intensity W3
- ✓ Cell: Mean Outer Intensity W2
- ✓ Cell: Mean Outer Intensity W3
- ✓ Cell: Inner Area W2
- ✓ Cell: Inner Area W3
- ✓ Cell: Outer Area W2
- ✓ Cell: Outer Area W3
- ✓ Cell: Marked Area W2
- ✓ Cell: Marked Area W3
- ✔ Cell: Integrated Marked Intensity W2
- Cell: Integrated Marked Intensity W3
- Cell: Mean Marked Intensity W2
- ✔ Cell: Mean Marked Intensity W3

- 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



#### ✓ Cell: Assigned Label #

- ✓ Cell: Scoring Profile
- Cell: Custom Profile Name
- Cell: Compartment Stained Area
- Cell: Compartment Integrated Intensity
- ✓ Cell: Compartment Mean Intensity
- ✔ Cell: Positive W2
- ✔ Cell: Positive W3
- ✔ Cell: Correlation Coefficient W2
- ✓ Cell: Correlation Coefficient W3
- Cell: Integrated Inner Intensity W2
- ✓ Cell: Integrated Inner Intensity W3
- ✓ Cell: Integrated Outer Intensity W2
- Cell: Integrated Outer Intensity W3
- ✓ Cell: Mean Inner Intensity W2
- ✓ Cell: Mean Inner Intensity W3
- ✓ Cell: Mean Outer Intensity W2
- ✓ Cell: Mean Outer Intensity W3
- ✓ Cell: Inner Area W2
- ✓ Cell: Inner Area W3
- ✓ Cell: Outer Area W2
- ✓ Cell: Outer Area W3
- ✓ Cell: Marked Area W2
- ✓ Cell: Marked Area W3
- Cell: Integrated Marked Intensity W2
- Cell: Integrated Marked Intensity W3
- ✓ Cell: Mean Marked Intensity W2
- ✔ Cell: Mean Marked Intensity W3

- 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



#### ✓ Cell: Assigned Label #

- ✓ Cell: Scoring Profile
- Cell: Custom Profile Name
- Cell: Compartment Stained Area
- Cell: Compartment Integrated Intensity
- Cell: Compartment Mean Intensity
- ✔ Cell: Positive W2
- ✔ Cell: Positive W3
- Cell: Correlation Coefficient W2
- ✓ Cell: Correlation Coefficient W3
- Cell: Integrated Inner Intensity W2
- Cell: Integrated Inner Intensity W3
- Cell: Integrated Outer Intensity W2
- Cell: Integrated Outer Intensity W3
- ✓ Cell: Mean Inner Intensity W2
- ✓ Cell: Mean Inner Intensity W3
- ✓ Cell: Mean Outer Intensity W2.
- ✓ Cell: Mean Outer Intensity W3
- ✓ Cell: Inner Area W2
- ✓ Cell: Inner Area W3
- ✔ Cell: Outer Area W2
- ✔ Cell: Outer Area W3
- ✓ Cell: Marked Area W2
- ✓ Cell: Marked Area W3
- ✓ Cell: Integrated Marked Intensity W2
- Cell: Integrated Marked Intensity W3
- ✓ Cell: Mean Marked Intensity W2
- ✓ Cell: Mean Marked Intensity W3

- 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)















# **Thank You**