

**Molecular  
Devices**

Together through life sciences.

## MetaXpress® Software: *Multi Wavelength Cell Scoring Module*

Together through life sciences.

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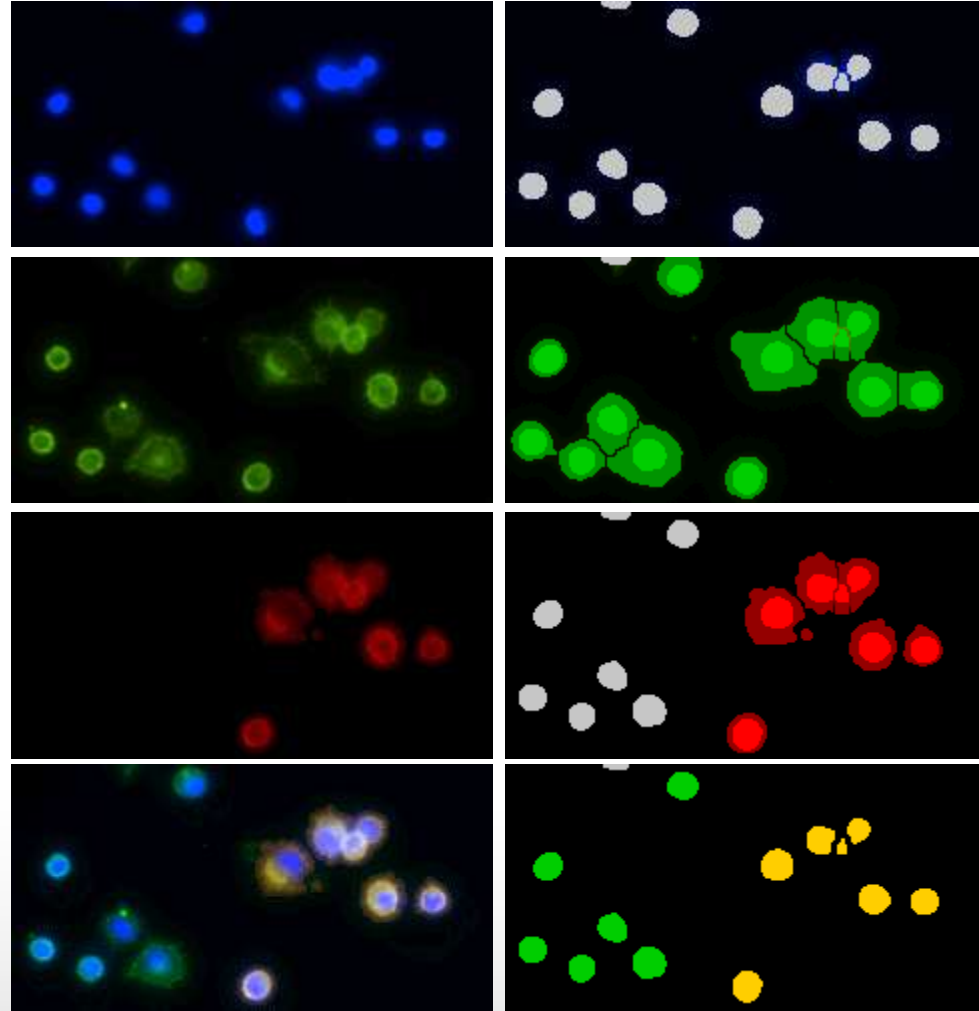


**Molecular  
Devices**

# Multi Wavelength Cell Scoring (MWCS) Module

## Overview

- The MWCS module can be used to analyze cells imaged in 1-7 wavelengths
- The first wavelength should always be a stain for all nuclei (e.g. DAPI, Hoechst, or DRAQ5)
- With just 1 wavelength, it can be used for basic cell counting
- With 2 or more wavelengths, it will score cells as positive or negative for each wavelength and assign it a “profile” of its score in each wavelength



# Module Settings

**Multi Wavelength Cell Scoring**

Number of wavelengths: 3

☐ Display result image: Segmen

Algorithm: Fast

☒ All nuclei ☐ Annexin ☐ PI

Name: All nuclei

W1 Source image: DAPI

Legend color: Gray

Stained area: Nucleus

Approximate min width: 4  $\mu\text{m}$  = 12 pixels

Approximate max width: 20  $\mu\text{m}$  = 62 pixels

Intensity above local background: 300 graylevels

Preview

**Scoring**

The 'All nuclei' wavelength is a required stain for all cells.

Configure Summary Log... Configure Data Log (Cells)...

Save Settings... Load Settings... Show Legend... Apply 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

**Multi Wavelength Cell Scoring**

Number of wavelengths: 3

☐ Display result image: Segmen

Algorithm: Fast

☒ All nuclei ☒ Annexin ☒ PI

Name: All nuclei

W1 Source image: DAPI

Legend color: Gray

Stained area: Nucleus

Approximate min width: 4  $\mu\text{m}$  = 12 pixels

Approximate max width: 20  $\mu\text{m}$  = 62 pixels

Intensity above local background: 300 graylevels

Preview

**Scoring**

The 'All nuclei' wavelength is a required stain for all cells.

Configure Summary Log... Configure Data Log (Cells)...

Save Settings... Load Settings... Show Legend... Apply Close

- Display result image
- Leave “Display result image” deselected (this is generally only used when journaling)

# Module Settings

**Multi Wavelength Cell Scoring**

Number of wavelengths: 3

☐ Display result image: Segmen

Algorithm: **Fast**

☐ All nuclei ☒ All

Name: Annexin

W2 Source image: FITC

Legend color: Blue

Stained area: Nucleus and Cytoplasm

Approximate min width: 5  $\mu\text{m}$  = 16 pixels

Approximate max width: 50  $\mu\text{m}$  = 155 pixels

Intensity above local background: 300 graylevels

Preview

Scoring

Minimum stained area: 50  $\mu\text{m}^2$  = 481 pixels

Configure Summary Log... Configure Data Log (Cells)...

Save Settings... Load Settings... Show Legend... Apply Close

## 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

**Multi Wavelength Cell Scoring**

Number of wavelengths: 3

☐ Display result image: Segmen

Algorithm: Fast

☒ All nuclei ☐ Annexin ☐ PI

Name: All nuclei

**W1 Source image: DAPI**

Legend color: Gray

Stained area: Nucleus

Approximate min width: 4  $\mu\text{m} = 12$  pixels

Approximate max width: 20  $\mu\text{m} = 62$  pixels

Intensity above local background: 300 graylevels

Preview

**Scoring**

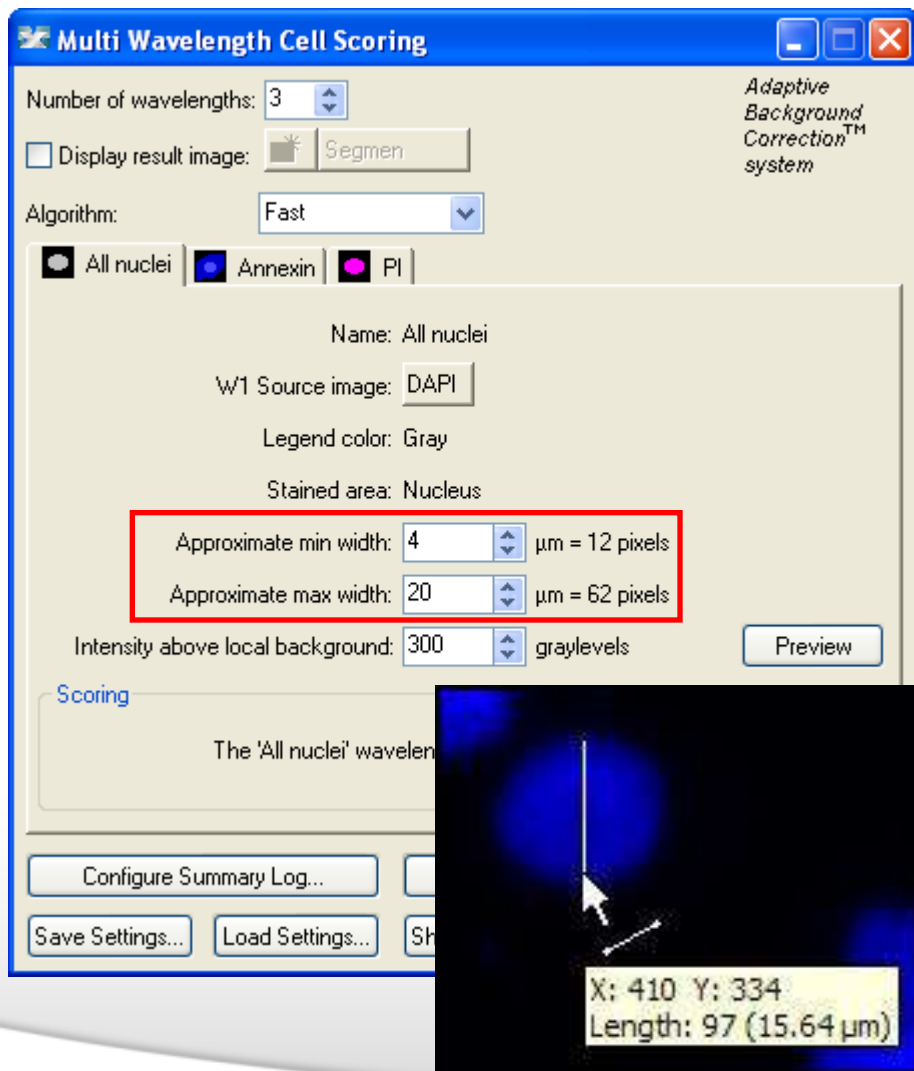
The 'All nuclei' wavelength is a required stain for all cells.

Configure Summary Log... Configure Data Log (Cells)...

Save Settings... Load Settings... Show Legend... Apply Close

- **Wavelength 1 (W1)**
- Select the wavelength for the **nuclei** (required)

# Module Settings

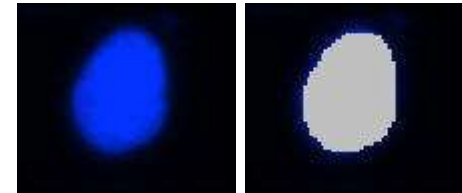


## Wavelength 1 (W1)

- 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  $\mu\text{m}$ )
- The region tools can be used to measure widths
- Much smaller cells will be ignored
- Much larger cells will be split

# Module Settings

## Effects of varying width settings



Min width too small: splits nuclei



Min width too large: omits smaller nuclei

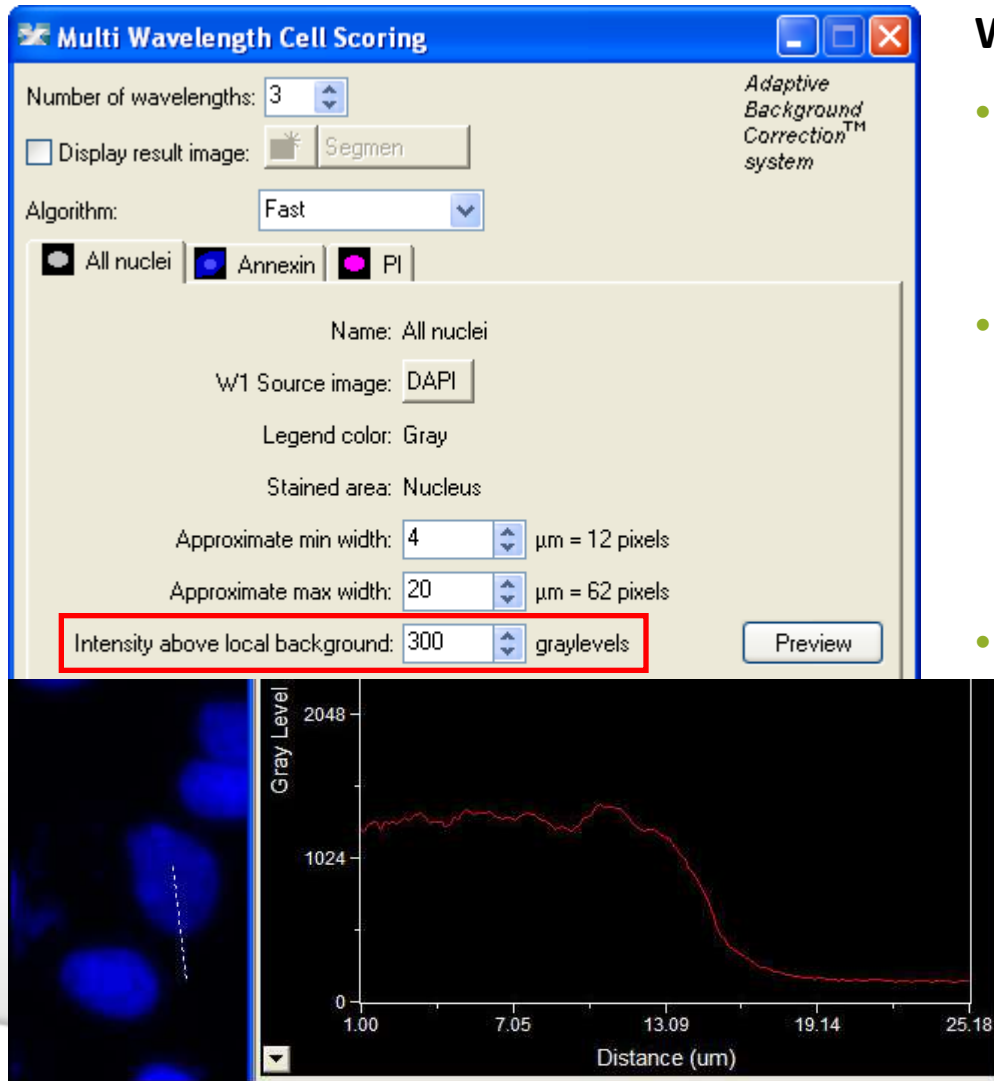
Max width too small: may shrink nuclear boundaries



Max width too large: may slightly enlarge nuclear boundaries



# Module Settings



## Wavelength 1 (W1)

- 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

# Module Settings

**Multi Wavelength Cell Scoring**

Number of wavelengths: 3

☐ Display result image: Segmen

Algorithm: Fast

☒ All nuclei ☒ Annexin ☒ PI

Name: All nuclei

W1 Source image: DAPI

Legend color: Gray

Stained area: Nucleus

Approximate min width: 4  $\mu\text{m}$  = 12 pixels

Approximate max width: 20  $\mu\text{m}$  = 62 pixels

Intensity above local background: 300 graylevels

**Preview**

**Scoring**

The 'All nuclei' wavelength is a required stain for all cells.

Configure Summary Log... Configure Data Log (Cells)...

Save Settings... Load Settings... Show Legend... Apply Close

## Wavelength 1 (W1)

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

# Module Settings

**Multi Wavelength Cell Scoring**

Number of wavelengths: 3

☐ Display result image: Segmen

Algorithm: Fast

☒ All nuclei ☒ Annexin ☒ PI

**W2 Source image:**

Name: Annexin

W2 Source image: FITC

Legend color: Blue

Stained area: Nucleus and Cytoplasm

Approximate min width: 5  $\mu\text{m}$  = 16 pixels

Approximate max width: 50  $\mu\text{m}$  = 155 pixels

Intensity above local background: 300 graylevels

Preview

**Scoring**

Minimum stained area: 50  $\mu\text{m}^2$  = 481 pixels

Configure Summary Log... Configure Data Log (Cells)...

Save Settings... Load Settings... Show Legend... Apply Close

*Adaptive Background Correction™ system*

## Wavelength 2 (W2)

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

# Module Settings

**Multi Wavelength Cell Scoring**

Number of wavelengths: 3

☐ Display result image: Segmen

Algorithm: Fast

☒ All nuclei ☒ Annexin ☒ PI

Name: Annexin

W2 Source image: FITC

Legend color: Blue

**Stained area: Nucleus and Cytoplasm**

Approximate min width: 5  $\mu\text{m}$  = 16 pixels

Approximate max width: 50  $\mu\text{m}$  = 155 pixels

Intensity above local background: 300 graylevels

Preview

**Scoring**

Minimum stained area: 50  $\mu\text{m}^2$  = 481 pixels

Configure Summary Log... Configure Data Log (Cells)...

Save Settings... Load Settings... Show Legend... Apply Close

*Adaptive Background Correction™ system*

## Wavelength 2 (W2)

- Define **Stained area**:
  - Nucleus (stain matches W1 area)
  - Cytoplasm (stain is surrounding but not including W1 area)
  - Nucleus and Cytoplasm (stain encompasses W1 and surrounding area)

# Module Settings

**Multi Wavelength Cell Scoring**

Number of wavelengths: 3

☐ Display result image: Segmen

Algorithm: Fast

☒ All nuclei ☒ Annexin ☒ PI

Name: Annexin

W2 Source image: FITC

Legend color: Blue

Stained area: Nucleus and Cytoplasm

Approximate min width: 5  $\mu\text{m} = 16$  pixels

Approximate max width: 50  $\mu\text{m} = 155$  pixels

Intensity above local background: 300 graylevels

Preview

Scoring

Minimum stained area: 50  $\mu\text{m}^2 = 481$  pixels

Configure Summary Log... Configure Data Log (Cells)...

Save Settings... Load Settings... Show Legend... Apply Close

*Adaptive Background Correction™ system*

## Wavelength 2 (W2)

- Set the **Approximate min width** and **Approximate max width** for the stain of interest
- For a nuclear stain, use the same settings as W1
- For a cytoplasmic or nuclear + cytoplasmic stain, measure the whole cell the same way you measured the nucleus for W1

# Module Settings

**Multi Wavelength Cell Scoring**

Number of wavelengths: 3

☐ Display result image: Segmen

Algorithm: Fast

☒ All nuclei ☒ Annexin ☐ PI

Name: Annexin

W2 Source image: FITC

Legend color: Blue

Stained area: Nucleus and Cytoplasm

Approximate min width: 5  $\mu\text{m}$  = 16 pixels

Approximate max width: 50  $\mu\text{m}$  = 155 pixels

**Intensity above local background: 300 graylevels**

Preview

**Scoring**

Minimum stained area: 50  $\mu\text{m}^2$  = 481 pixels

Configure Summary Log... Configure Data Log (Cells)...

Save Settings... Load Settings... Show Legend... Apply Close

Adaptive Background Correction™ system

## Wavelength 2 (W2)

- **Intensity above local background** for the stain of interest is used to distinguish positive from negative cells
- Measure the stain intensity vs background just as for W1
- Check both positive and negative control images to help set a suitable cutoff

# Module Settings

**Multi Wavelength Cell Scoring**

Number of wavelengths: 3

☐ Display result image: Segmen

Algorithm: Fast

☒ All nuclei ☒ Annexin ☒ PI

Name: Annexin

W2 Source image: FITC

Legend color: Blue

Stained area: Nucleus and Cytoplasm

Approximate min width: 5  $\mu\text{m}$  = 16 pixels

Approximate max width: 50  $\mu\text{m}$  = 155 pixels

Intensity above local background: 300 graylevels

**Preview**

**Scoring**

Minimum stained area: 50  $\mu\text{m}^2$  = 481 pixels

Configure Summary Log... Configure Data Log (Cells)...

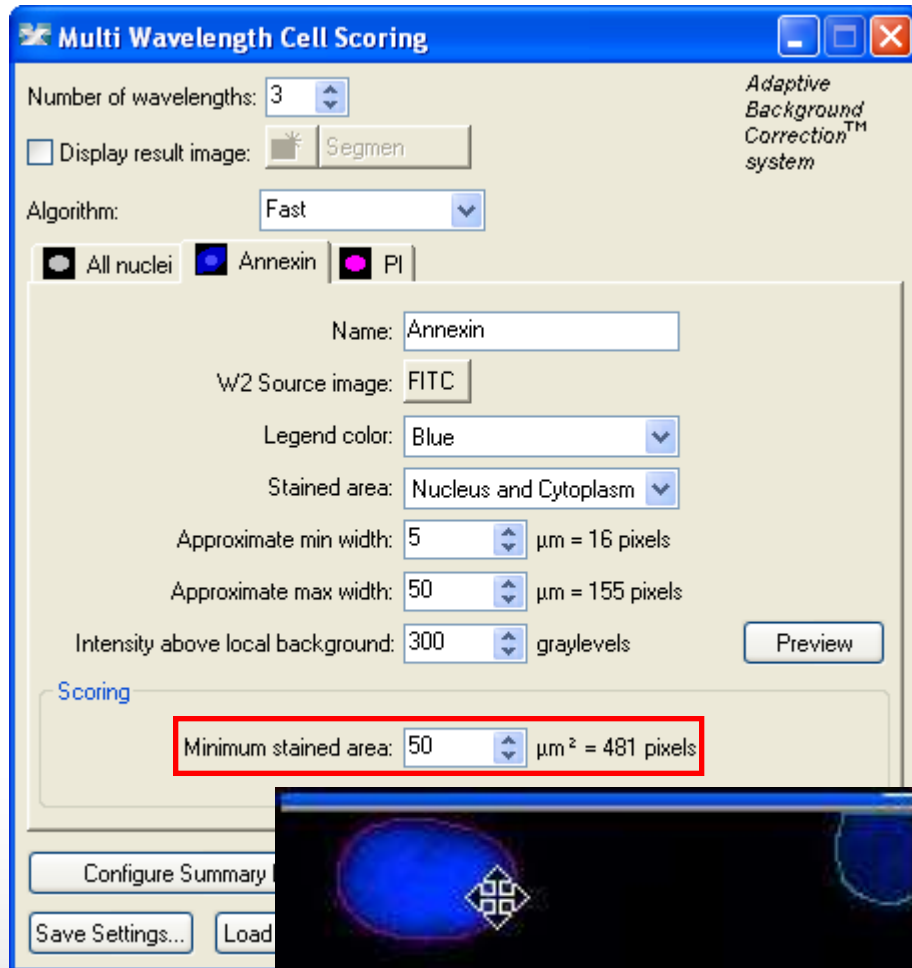
Save Settings... Load Settings... Show Legend... Apply Close

*Adaptive Background Correction™ system*

## Wavelength 2 (W2)

- Click on **Preview** to test settings for the current wavelength only
- Note that this may not accurately segment the cells as this Preview does not use the nuclear staining
- This is most useful for testing the Intensity settings for this wavelength

# Module Settings



## Wavelength 2 (W2)

- **Minimum stained area** is an additional criteria for separating positive from negative cells
- To be scored as positive, a cell must be stained across this area (or larger)
- This helps eliminate false positives from cells with a small speck of very bright debris
- Use the elliptical region tool to measure the area of a small cell in the image



# Module Settings

The screenshot shows the 'Multi Wavelength Cell Scoring' window. It features a blue title bar with standard window controls. The main area is light beige and contains various settings. At the top left, 'Number of wavelengths' is set to 3. Below it is a checkbox for 'Display result image:' with a 'Segmen' button. The 'Algorithm:' dropdown is set to 'Fast'. On the right, there's a logo for 'Adaptive Background Correction™ system'. Below the algorithm, there are three radio buttons: 'All nuclei' (selected), 'Annexin', and 'PI'. The 'Name:' field contains 'PI'. The 'W3 Source image:' field contains 'TRITC'. The 'Legend color:' dropdown is set to 'Magenta'. The 'Stained area:' dropdown is set to 'Nucleus'. There are three width settings: 'Approximate min width:' (4  $\mu\text{m}$  = 12 pixels), 'Approximate max width:' (20  $\mu\text{m}$  = 62 pixels), and 'Intensity above local background:' (500 graylevels). A 'Preview' button is to the right of the intensity setting. A 'Scoring' section at the bottom has a 'Minimum stained area:' (20  $\mu\text{m}^2$  = 192 pixels). At the very bottom are buttons for 'Configure Summary Log...', 'Configure Data Log (Cells)...', 'Save Settings...', 'Load Settings...', 'Show Legend...', 'Apply', and 'Close'.

**Multi Wavelength Cell Scoring**

Number of wavelengths: 3

☐ Display result image: Segmen

Algorithm: Fast

☒ All nuclei ☐ Annexin ☐ PI

Name: PI

W3 Source image: TRITC

Legend color: Magenta

Stained area: Nucleus

Approximate min width: 4  $\mu\text{m}$  = 12 pixels

Approximate max width: 20  $\mu\text{m}$  = 62 pixels

Intensity above local background: 500 graylevels

Preview

**Scoring**

Minimum stained area: 20  $\mu\text{m}^2$  = 192 pixels

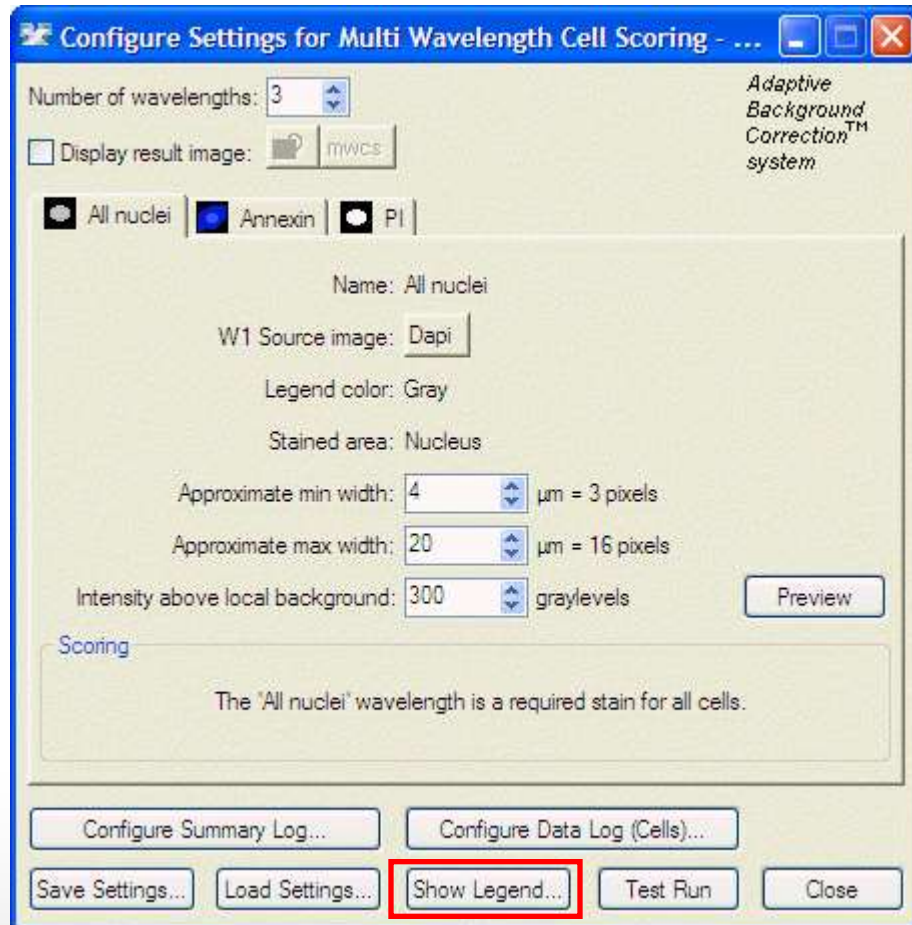
Configure Summary Log... Configure Data Log (Cells)...

Save Settings... Load Settings... Show Legend... Apply Close

## Wavelength 3 (W3) and subsequent wavelengths

- Follow the same steps to configure the settings for wavelength 3 (PI in this example)

# Module Settings



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



# Module Settings – General Settings

Configure Settings for Multi Wavelength Cell Scoring - ...

Number of wavelengths: 3

☐ Display result image: mwcs

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☒ All nuclei ☐ Annexin ☐ PI

Name: All nuclei

W1 Source image: Dapi

Legend color: Gray

Stained area: Nucleus

Approximate min width: 4  $\mu\text{m}$  = 3 pixels

Approximate max width: 20  $\mu\text{m}$  = 16 pixels

Intensity above local background: 300 graylevels

Preview

Scoring

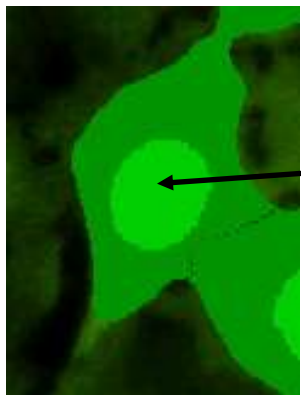
The 'All nuclei' wavelength is a required stain for all cells.

Configure Summary Log... Configure Data Log (Cells)...

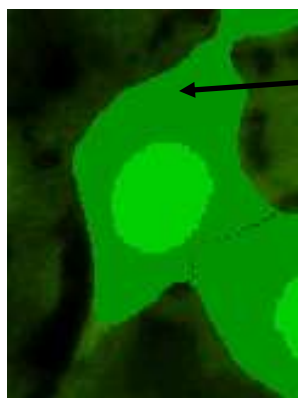
Save Settings... Load Settings... Show Legend... Test Run Close

- **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 cell-by-cell results for this site

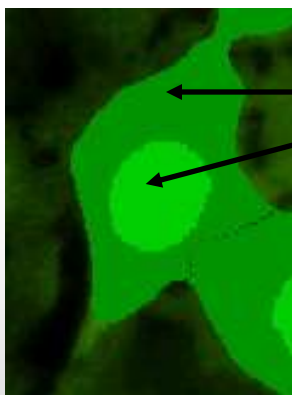
# Regions for Measurement



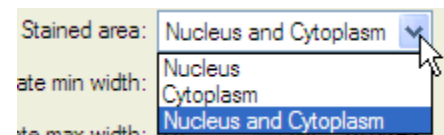
Nucleus



Cytoplasm



Stained Area (as defined in settings)



Cell (nucleus + cytoplasm)

# Summary Data (site-by-site measurements)

✓ Total Cells	✓ All W3 Mean Cyto Integr Intens
✓ All Cells Mean Area	✓ All W3 Mean Cyto Aver Intens
✓ All Nuclei Mean Area	✓ All W3 Mean Cell Integr Intens
✓ All Nuclei Mean Integr Intensity	✓ All W3 Mean Cell Aver Intens
✓ All Nuclei Mean Average Intensity	✓ Positive W3 Mean Stain Area
✓ All W2 Mean Stain Area	✓ Positive W3 Mean Stain Integr Intens
✓ All W2 Mean Stain Integr Intens	✓ Positive W3 Mean Stain Aver Intens
✓ All W2 Mean Stain Aver Intens	✓ Positive W3 Mean Nucl Integr Intens
✓ All W2 Mean Nucl Integr Intens	✓ Positive W3 Mean Nucl Aver Intens
✓ All W2 Mean Nucl Aver Intens	✓ Positive W3 Mean Cyto Integr Intens
✓ All W2 Mean Cyto Integr Intens	✓ Positive W3 Mean Cyto Aver Intens
✓ All W2 Mean Cyto Aver Intens	✓ Positive W3 Mean Cell Integr Intens
✓ All W2 Mean Cell Integr Intens	✓ Positive W3 Mean Cell Aver Intens
✓ All W2 Mean Cell Aver Intens	✓ Negative W3 Mean Stain Area
✓ Positive W2 Mean Stain Area	✓ Negative W3 Mean Stain Integr Intens
✓ Positive W2 Mean Stain Integr Intens	✓ Negative W3 Mean Stain Aver Intens
✓ Positive W2 Mean Stain Aver Intens	✓ Negative W3 Mean Nucl Integr Intens
✓ Positive W2 Mean Nucl Integr Intens	✓ Negative W3 Mean Nucl Aver Intens
✓ Positive W2 Mean Nucl Aver Intens	✓ Negative W3 Mean Cyto Integr Intens
✓ Positive W2 Mean Cyto Integr Intens	✓ Negative W3 Mean Cyto Aver Intens
✓ Positive W2 Mean Cyto Aver Intens	✓ Negative W3 Mean Cell Integr Intens
✓ Positive W2 Mean Cell Integr Intens	✓ Negative W3 Mean Cell Aver Intens
✓ Positive W2 Mean Cell Aver Intens	✓ Positive W3
✓ Negative W2 Mean Stain Area	✓ % Positive W3
✓ Negative W2 Mean Stain Integr Intens	✓ Scoring Profile 1--
✓ Negative W2 Mean Stain Aver Intens	✓ Scoring Profile 12-
✓ Negative W2 Mean Nucl Integr Intens	✓ Scoring Profile 1-3
✓ Negative W2 Mean Nucl Aver Intens	✓ Scoring Profile 123
✓ Negative W2 Mean Cyto Integr Intens	✓ Percentage Scoring Profile 1--
✓ Negative W2 Mean Cyto Aver Intens	✓ Percentage Scoring Profile 12-
✓ Negative W2 Mean Cell Integr Intens	✓ Percentage Scoring Profile 1-3
✓ Negative W2 Mean Cell Aver Intens	✓ Percentage Scoring Profile 123
✓ Positive W2	✓ Subtotal Profile 1xx
✓ % Positive W2	✓ Subtotal Profile 12x
✓ All W3 Mean Stain Area	✓ Subtotal Profile 1x3
✓ All W3 Mean Stain Integr Intens	✓ Subtotal Profile 123
✓ All W3 Mean Stain Aver Intens	✓ Percentage Subtotal Profile 1xx
✓ All W3 Mean Nucl Integr Intens	✓ Percentage Subtotal Profile 12x
✓ All W3 Mean Nucl Aver Intens	✓ Percentage Subtotal Profile 1x3
	✓ Percentage Subtotal Profile 123

- **Total cells:** Total number of nuclei (cell count)
- **All Cells Mean Area:** The average area of the cell (nucleus + cytoplasm) for all cells found in the image (in  $\mu\text{m}^2$ )
- **All Nuclei Mean Area:** The average area of nucleus for all cells found in the image (in  $\mu\text{m}^2$ )

# Summary Data (site-by-site measurements)

✓ Total Cells	✓ All W3 Mean Cyto Integr Intens
✓ All Cells Mean Area	✓ All W3 Mean Cyto Aver Intens
✓ All Nuclei Mean Area	✓ All W3 Mean Cell Integr Intens
✓ All Nuclei Mean Integr Intensity	✓ All W3 Mean Cell Aver Intens
✓ All Nuclei Mean Average Intensity	✓ Positive W3 Mean Stain Area
✓ All W2 Mean Stain Area	✓ Positive W3 Mean Stain Integr Intens
✓ All W2 Mean Stain Integr Intens	✓ Positive W3 Mean Stain Aver Intens
✓ All W2 Mean Stain Aver Intens	✓ Positive W3 Mean Nucl Integr Intens
✓ All W2 Mean Nucl Integr Intens	✓ Positive W3 Mean Nucl Aver Intens
✓ All W2 Mean Nucl Aver Intens	✓ Positive W3 Mean Cyto Integr Intens
✓ All W2 Mean Cyto Integr Intens	✓ Positive W3 Mean Cyto Aver Intens
✓ All W2 Mean Cyto Aver Intens	✓ Positive W3 Mean Cell Integr Intens
✓ All W2 Mean Cell Integr Intens	✓ Positive W3 Mean Cell Aver Intens
✓ All W2 Mean Cell Aver Intens	✓ Negative W3 Mean Stain Area
✓ Positive W2 Mean Stain Area	✓ Negative W3 Mean Stain Integr Intens
✓ Positive W2 Mean Stain Integr Intens	✓ Negative W3 Mean Stain Aver Intens
✓ Positive W2 Mean Stain Aver Intens	✓ Negative W3 Mean Nucl Integr Intens
✓ Positive W2 Mean Nucl Integr Intens	✓ Negative W3 Mean Nucl Aver Intens
✓ Positive W2 Mean Nucl Aver Intens	✓ Negative W3 Mean Cyto Integr Intens
✓ Positive W2 Mean Cyto Integr Intens	✓ Negative W3 Mean Cyto Aver Intens
✓ Positive W2 Mean Cyto Aver Intens	✓ Negative W3 Mean Cell Integr Intens
✓ Positive W2 Mean Cell Integr Intens	✓ Negative W3 Mean Cell Aver Intens
✓ Positive W2 Mean Cell Aver Intens	✓ Positive W3
✓ Negative W2 Mean Stain Area	✓ % Positive W3
✓ Negative W2 Mean Stain Integr Intens	✓ Scoring Profile 1--
✓ Negative W2 Mean Stain Aver Intens	✓ Scoring Profile 12-
✓ Negative W2 Mean Nucl Integr Intens	✓ Scoring Profile 1-3
✓ Negative W2 Mean Nucl Aver Intens	✓ Scoring Profile 123
✓ Negative W2 Mean Cyto Integr Intens	✓ Percentage Scoring Profile 1--
✓ Negative W2 Mean Cyto Aver Intens	✓ Percentage Scoring Profile 12-
✓ Negative W2 Mean Cell Integr Intens	✓ Percentage Scoring Profile 1-3
✓ Negative W2 Mean Cell Aver Intens	✓ Percentage Scoring Profile 123
✓ Positive W2	✓ Subtotal Profile 1xx
✓ % Positive W2	✓ Subtotal Profile 12x
✓ All W3 Mean Stain Area	✓ Subtotal Profile 1x3
✓ All W3 Mean Stain Integr Intens	✓ Subtotal Profile 123
✓ All W3 Mean Stain Aver Intens	✓ Percentage Subtotal Profile 1xx
✓ All W3 Mean Nucl Integr Intens	✓ Percentage Subtotal Profile 12x
✓ All W3 Mean Nucl Aver Intens	✓ Percentage Subtotal Profile 1x3
✓ All W3 Mean Cyto Integr Intens	✓ Percentage Subtotal Profile 123

- **All Nuclei Mean Integr Intensity:**  
The total pixel intensity of the nuclear stain over the nuclear area, divided by the total number of cells
- **All Nuclei Mean Average Intensity:**  
The average pixel intensity of the nuclear stain over all the nuclear areas in the image

# Summary Data (site-by-site measurements)

✓ Total Cells	✓ All W3 Mean Cyto Integr Intens
✓ All Cells Mean Area	✓ All W3 Mean Cyto Aver Intens
✓ All Nuclei Mean Area	✓ All W3 Mean Cell Integr Intens
✓ All Nuclei Mean Integr Intensity	✓ All W3 Mean Cell Aver Intens
✓ All Nuclei Mean Average Intensity	✓ Positive W3 Mean Stain Area
✓ All W2 Mean Stain Area	✓ Positive W3 Mean Stain Integr Intens
✓ All W2 Mean Stain Integr Intens	✓ Positive W3 Mean Stain Aver Intens
✓ All W2 Mean Stain Aver Intens	✓ Positive W3 Mean Nucl Integr Intens
✓ All W2 Mean Nucl Integr Intens	✓ Positive W3 Mean Nucl Aver Intens
✓ All W2 Mean Nucl Aver Intens	✓ Positive W3 Mean Cyto Integr Intens
✓ All W2 Mean Cyto Integr Intens	✓ Positive W3 Mean Cyto Aver Intens
✓ All W2 Mean Cyto Aver Intens	✓ Positive W3 Mean Cell Integr Intens
✓ All W2 Mean Cell Integr Intens	✓ Positive W3 Mean Cell Aver Intens
✓ All W2 Mean Cell Aver Intens	✓ Negative W3 Mean Stain Area
✓ Positive W2 Mean Stain Area	✓ Negative W3 Mean Stain Integr Intens
✓ Positive W2 Mean Stain Integr Intens	✓ Negative W3 Mean Stain Aver Intens
✓ Positive W2 Mean Stain Aver Intens	✓ Negative W3 Mean Nucl Integr Intens
✓ Positive W2 Mean Nucl Integr Intens	✓ Negative W3 Mean Nucl Aver Intens
✓ Positive W2 Mean Nucl Aver Intens	✓ Negative W3 Mean Cyto Integr Intens
✓ Positive W2 Mean Cyto Integr Intens	✓ Negative W3 Mean Cyto Aver Intens
✓ Positive W2 Mean Cyto Aver Intens	✓ Negative W3 Mean Cell Integr Intens
✓ Positive W2 Mean Cell Integr Intens	✓ Negative W3 Mean Cell Aver Intens
✓ Positive W2 Mean Cell Aver Intens	✓ Positive W3
✓ Negative W2 Mean Stain Area	✓ % Positive W3
✓ Negative W2 Mean Stain Integr Intens	✓ Scoring Profile 1--
✓ Negative W2 Mean Stain Aver Intens	✓ Scoring Profile 12-
✓ Negative W2 Mean Nucl Integr Intens	✓ Scoring Profile 1-3
✓ Negative W2 Mean Nucl Aver Intens	✓ Scoring Profile 123
✓ Negative W2 Mean Cyto Integr Intens	✓ Percentage Scoring Profile 1--
✓ Negative W2 Mean Cyto Aver Intens	✓ Percentage Scoring Profile 12-
✓ Negative W2 Mean Cell Integr Intens	✓ Percentage Scoring Profile 1-3
✓ Negative W2 Mean Cell Aver Intens	✓ Percentage Scoring Profile 123
✓ Positive W2	✓ Subtotal Profile 1xx
✓ % Positive W2	✓ Subtotal Profile 12x
✓ All W3 Mean Stain Area	✓ Subtotal Profile 1x3
✓ All W3 Mean Stain Integr Intens	✓ Subtotal Profile 123
✓ All W3 Mean Stain Aver Intens	✓ Percentage Subtotal Profile 1xx
✓ All W3 Mean Nucl Integr Intens	✓ Percentage Subtotal Profile 12x
✓ All W3 Mean Nucl Aver Intens	✓ Percentage Subtotal Profile 1x3
✓ All W3 Mean Cyto Integr Intens	✓ Percentage Subtotal Profile 123

- **All W2 Mean Stain Area:** The average stained area (nucleus, cytoplasm, or both as defined in the W2 settings) for all cells found in the image (in  $\mu\text{m}^2$ )
- **All W2 Mean Stain Integr Intensity:** The total pixel intensity of the W2 stain over the stained area, divided by the total number of cells
- **All W2 Mean Stain Aver Intensity:** The average pixel intensity of the W2 stain over the stained area, over all the cells in the image
- *(similar for W3-W7)*

# Summary Data (site-by-site measurements)

✓ Total Cells	✓ All W3 Mean Cyto Integr Intens
✓ All Cells Mean Area	✓ All W3 Mean Cyto Aver Intens
✓ All Nuclei Mean Area	✓ All W3 Mean Cell Integr Intens
✓ All Nuclei Mean Integr Intensity	✓ All W3 Mean Cell Aver Intens
✓ All Nuclei Mean Average Intensity	✓ Positive W3 Mean Stain Area
✓ All W2 Mean Stain Area	✓ Positive W3 Mean Stain Integr Intens
✓ All W2 Mean Stain Integr Intens	✓ Positive W3 Mean Stain Aver Intens
✓ All W2 Mean Stain Aver Intens	✓ Positive W3 Mean Nucl Integr Intens
✓ All W2 Mean Nucl Integr Intens	✓ Positive W3 Mean Nucl Aver Intens
✓ All W2 Mean Nucl Aver Intens	✓ Positive W3 Mean Cyto Integr Intens
✓ All W2 Mean Cyto Integr Intens	✓ Positive W3 Mean Cyto Aver Intens
✓ All W2 Mean Cyto Aver Intens	✓ Positive W3 Mean Cell Integr Intens
✓ All W2 Mean Cell Integr Intens	✓ Positive W3 Mean Cell Aver Intens
✓ All W2 Mean Cell Aver Intens	✓ Negative W3 Mean Stain Area
✓ Positive W2 Mean Stain Area	✓ Negative W3 Mean Stain Integr Intens
✓ Positive W2 Mean Stain Integr Intens	✓ Negative W3 Mean Stain Aver Intens
✓ Positive W2 Mean Stain Aver Intens	✓ Negative W3 Mean Nucl Integr Intens
✓ Positive W2 Mean Nucl Integr Intens	✓ Negative W3 Mean Nucl Aver Intens
✓ Positive W2 Mean Nucl Aver Intens	✓ Negative W3 Mean Cyto Integr Intens
✓ Positive W2 Mean Cyto Integr Intens	✓ Negative W3 Mean Cyto Aver Intens
✓ Positive W2 Mean Cyto Aver Intens	✓ Negative W3 Mean Cell Integr Intens
✓ Positive W2 Mean Cell Integr Intens	✓ Negative W3 Mean Cell Aver Intens
✓ Positive W2 Mean Cell Aver Intens	✓ Positive W3
✓ Negative W2 Mean Stain Area	✓ % Positive W3
✓ Negative W2 Mean Stain Integr Intens	✓ Scoring Profile 1--
✓ Negative W2 Mean Stain Aver Intens	✓ Scoring Profile 12-
✓ Negative W2 Mean Nucl Integr Intens	✓ Scoring Profile 1-3
✓ Negative W2 Mean Nucl Aver Intens	✓ Scoring Profile 123
✓ Negative W2 Mean Cyto Integr Intens	✓ Percentage Scoring Profile 1--
✓ Negative W2 Mean Cyto Aver Intens	✓ Percentage Scoring Profile 12-
✓ Negative W2 Mean Cell Integr Intens	✓ Percentage Scoring Profile 1-3
✓ Negative W2 Mean Cell Aver Intens	✓ Percentage Scoring Profile 123
✓ Positive W2	✓ Subtotal Profile 1xx
✓ % Positive W2	✓ Subtotal Profile 12x
✓ All W3 Mean Stain Area	✓ Subtotal Profile 1x3
✓ All W3 Mean Stain Integr Intens	✓ Subtotal Profile 123
✓ All W3 Mean Stain Aver Intens	✓ Percentage Subtotal Profile 1xx
✓ All W3 Mean Nucl Integr Intens	✓ Percentage Subtotal Profile 12x
✓ All W3 Mean Nucl Aver Intens	✓ Percentage Subtotal Profile 1x3
	✓ Percentage Subtotal Profile 123

- **All W2 Mean Nucl Integr Intensity:**  
The total pixel intensity of the W2 stain over the nuclear area, divided by the total number of cells
- **All W2 Mean Nucl Aver Intensity:**  
The average pixel intensity of the W2 stain over the nuclear area, over all the cells in the image
- *(similar for W3-W7)*

# Summary Data (site-by-site measurements)

✓ Total Cells	✓ All W3 Mean Cyto Integr Intens
✓ All Cells Mean Area	✓ All W3 Mean Cyto Aver Intens
✓ All Nuclei Mean Area	✓ All W3 Mean Cell Integr Intens
✓ All Nuclei Mean Integr Intensity	✓ All W3 Mean Cell Aver Intens
✓ All Nuclei Mean Average Intensity	✓ Positive W3 Mean Stain Area
✓ All W2 Mean Stain Area	✓ Positive W3 Mean Stain Integr Intens
✓ All W2 Mean Stain Integr Intens	✓ Positive W3 Mean Stain Aver Intens
✓ All W2 Mean Stain Aver Intens	✓ Positive W3 Mean Nucl Integr Intens
✓ All W2 Mean Nucl Integr Intens	✓ Positive W3 Mean Nucl Aver Intens
✓ All W2 Mean Nucl Aver Intens	✓ Positive W3 Mean Cyto Integr Intens
✓ All W2 Mean Cyto Integr Intens	✓ Positive W3 Mean Cyto Aver Intens
✓ All W2 Mean Cyto Aver Intens	✓ Positive W3 Mean Cell Integr Intens
✓ All W2 Mean Cell Integr Intens	✓ Positive W3 Mean Cell Aver Intens
✓ All W2 Mean Cell Aver Intens	✓ Negative W3 Mean Stain Area
✓ Positive W2 Mean Stain Area	✓ Negative W3 Mean Stain Integr Intens
✓ Positive W2 Mean Stain Integr Intens	✓ Negative W3 Mean Stain Aver Intens
✓ Positive W2 Mean Stain Aver Intens	✓ Negative W3 Mean Nucl Integr Intens
✓ Positive W2 Mean Nucl Integr Intens	✓ Negative W3 Mean Nucl Aver Intens
✓ Positive W2 Mean Nucl Aver Intens	✓ Negative W3 Mean Cyto Integr Intens
✓ Positive W2 Mean Cyto Integr Intens	✓ Negative W3 Mean Cyto Aver Intens
✓ Positive W2 Mean Cyto Aver Intens	✓ Negative W3 Mean Cell Integr Intens
✓ Positive W2 Mean Cell Integr Intens	✓ Negative W3 Mean Cell Aver Intens
✓ Positive W2 Mean Cell Aver Intens	✓ Positive W3
✓ Negative W2 Mean Stain Area	✓ % Positive W3
✓ Negative W2 Mean Stain Integr Intens	✓ Scoring Profile 1--
✓ Negative W2 Mean Stain Aver Intens	✓ Scoring Profile 12-
✓ Negative W2 Mean Nucl Integr Intens	✓ Scoring Profile 1-3
✓ Negative W2 Mean Nucl Aver Intens	✓ Scoring Profile 123
✓ Negative W2 Mean Cyto Integr Intens	✓ Percentage Scoring Profile 1--
✓ Negative W2 Mean Cyto Aver Intens	✓ Percentage Scoring Profile 12-
✓ Negative W2 Mean Cell Integr Intens	✓ Percentage Scoring Profile 1-3
✓ Negative W2 Mean Cell Aver Intens	✓ Percentage Scoring Profile 123
✓ Positive W2	✓ Subtotal Profile 1xx
✓ % Positive W2	✓ Subtotal Profile 12x
✓ All W3 Mean Stain Area	✓ Subtotal Profile 1x3
✓ All W3 Mean Stain Integr Intens	✓ Subtotal Profile 123
✓ All W3 Mean Stain Aver Intens	✓ Percentage Subtotal Profile 1xx
✓ All W3 Mean Nucl Integr Intens	✓ Percentage Subtotal Profile 12x
✓ All W3 Mean Nucl Aver Intens	✓ Percentage Subtotal Profile 1x3
✓ All W3 Mean Cyto Integr Intens	✓ Percentage Subtotal Profile 123

- **All W2 Mean Cyto Integr Intensity:**  
The total pixel intensity of the W2 stain over the cytoplasmic area, divided by the total number of cells
- **All W2 Mean Cyto Aver Intensity:**  
The average pixel intensity of the W2 stain over the cytoplasmic area, over all the cells in the image
- *(similar for W3-W7)*

# Summary Data (site-by-site measurements)

✓ Total Cells	✓ All W3 Mean Cyto Integr Intens
✓ All Cells Mean Area	✓ All W3 Mean Cyto Aver Intens
✓ All Nuclei Mean Area	✓ All W3 Mean Cell Integr Intens
✓ All Nuclei Mean Integr Intensity	✓ All W3 Mean Cell Aver Intens
✓ All Nuclei Mean Average Intensity	✓ Positive W3 Mean Stain Area
✓ All W2 Mean Stain Area	✓ Positive W3 Mean Stain Integr Intens
✓ All W2 Mean Stain Integr Intens	✓ Positive W3 Mean Stain Aver Intens
✓ All W2 Mean Stain Aver Intens	✓ Positive W3 Mean Nucl Integr Intens
✓ All W2 Mean Nucl Integr Intens	✓ Positive W3 Mean Nucl Aver Intens
✓ All W2 Mean Nucl Aver Intens	✓ Positive W3 Mean Cyto Integr Intens
✓ All W2 Mean Cyto Integr Intens	✓ Positive W3 Mean Cyto Aver Intens
✓ All W2 Mean Cyto Aver Intens	✓ Positive W3 Mean Cell Integr Intens
✓ All W2 Mean Cell Integr Intens	✓ Positive W3 Mean Cell Aver Intens
✓ All W2 Mean Cell Aver Intens	✓ Negative W3 Mean Stain Area
✓ Positive W2 Mean Stain Area	✓ Negative W3 Mean Stain Integr Intens
✓ Positive W2 Mean Stain Integr Intens	✓ Negative W3 Mean Stain Aver Intens
✓ Positive W2 Mean Stain Aver Intens	✓ Negative W3 Mean Nucl Integr Intens
✓ Positive W2 Mean Nucl Integr Intens	✓ Negative W3 Mean Nucl Aver Intens
✓ Positive W2 Mean Nucl Aver Intens	✓ Negative W3 Mean Cyto Integr Intens
✓ Positive W2 Mean Cyto Integr Intens	✓ Negative W3 Mean Cyto Aver Intens
✓ Positive W2 Mean Cyto Aver Intens	✓ Negative W3 Mean Cell Integr Intens
✓ Positive W2 Mean Cell Integr Intens	✓ Negative W3 Mean Cell Aver Intens
✓ Positive W2 Mean Cell Aver Intens	✓ Positive W3
✓ Negative W2 Mean Stain Area	✓ % Positive W3
✓ Negative W2 Mean Stain Integr Intens	✓ Scoring Profile 1--
✓ Negative W2 Mean Stain Aver Intens	✓ Scoring Profile 12-
✓ Negative W2 Mean Nucl Integr Intens	✓ Scoring Profile 1-3
✓ Negative W2 Mean Nucl Aver Intens	✓ Scoring Profile 123
✓ Negative W2 Mean Cyto Integr Intens	✓ Percentage Scoring Profile 1--
✓ Negative W2 Mean Cyto Aver Intens	✓ Percentage Scoring Profile 12-
✓ Negative W2 Mean Cell Integr Intens	✓ Percentage Scoring Profile 1-3
✓ Negative W2 Mean Cell Aver Intens	✓ Percentage Scoring Profile 123
✓ Positive W2	✓ Subtotal Profile 1xx
✓ % Positive W2	✓ Subtotal Profile 12x
✓ All W3 Mean Stain Area	✓ Subtotal Profile 1x3
✓ All W3 Mean Stain Integr Intens	✓ Subtotal Profile 123
✓ All W3 Mean Stain Aver Intens	✓ Percentage Subtotal Profile 1xx
✓ All W3 Mean Nucl Integr Intens	✓ Percentage Subtotal Profile 12x
✓ All W3 Mean Nucl Aver Intens	✓ Percentage Subtotal Profile 1x3
✓ All W3 Mean Cyto Integr Intens	✓ Percentage Subtotal Profile 123

- **All W2 Mean Cell Integr Intensity:**  
The total pixel intensity of the W2 stain over the cell (nucleus + cytoplasm) area, divided by the total number of cells
- **All W2 Mean Cell Aver Intensity:**  
The average pixel intensity of the W2 stain over the cell (nucleus + cytoplasm) area, over all the cells in the image
- *(similar for W3-W7)*

# Summary Data (site-by-site measurements)

✓ Total Cells	✓ All W3 Mean Cyto Integr Intens
✓ All Cells Mean Area	✓ All W3 Mean Cyto Aver Intens
✓ All Nuclei Mean Area	✓ All W3 Mean Cell Integr Intens
✓ All Nuclei Mean Integr Intensity	✓ All W3 Mean Cell Aver Intens
✓ All Nuclei Mean Average Intensity	✓ Positive W3 Mean Stain Area
✓ All W2 Mean Stain Area	✓ Positive W3 Mean Stain Integr Intens
✓ All W2 Mean Stain Integr Intens	✓ Positive W3 Mean Stain Aver Intens
✓ All W2 Mean Stain Aver Intens	✓ Positive W3 Mean Nucl Integr Intens
✓ All W2 Mean Nucl Integr Intens	✓ Positive W3 Mean Nucl Aver Intens
✓ All W2 Mean Nucl Aver Intens	✓ Positive W3 Mean Cyto Integr Intens
✓ All W2 Mean Cyto Integr Intens	✓ Positive W3 Mean Cyto Aver Intens
✓ All W2 Mean Cyto Aver Intens	✓ Positive W3 Mean Cell Integr Intens
✓ All W2 Mean Cell Integr Intens	✓ Positive W3 Mean Cell Aver Intens
✓ All W2 Mean Cell Aver Intens	✓ Negative W3 Mean Stain Area
✓ Positive W2 Mean Stain Area	✓ Negative W3 Mean Stain Integr Intens
✓ Positive W2 Mean Stain Integr Intens	✓ Negative W3 Mean Stain Aver Intens
✓ Positive W2 Mean Stain Aver Intens	✓ Negative W3 Mean Nucl Integr Intens
✓ Positive W2 Mean Nucl Integr Intens	✓ Negative W3 Mean Nucl Aver Intens
✓ Positive W2 Mean Nucl Aver Intens	✓ Negative W3 Mean Cyto Integr Intens
✓ Positive W2 Mean Cyto Integr Intens	✓ Negative W3 Mean Cyto Aver Intens
✓ Positive W2 Mean Cyto Aver Intens	✓ Negative W3 Mean Cell Integr Intens
✓ Positive W2 Mean Cell Integr Intens	✓ Negative W3 Mean Cell Aver Intens
✓ Positive W2 Mean Cell Aver Intens	✓ Positive W3
✓ Negative W2 Mean Stain Area	✓ % Positive W3
✓ Negative W2 Mean Stain Integr Intens	✓ Scoring Profile 1--
✓ Negative W2 Mean Stain Aver Intens	✓ Scoring Profile 12-
✓ Negative W2 Mean Nucl Integr Intens	✓ Scoring Profile 1-3
✓ Negative W2 Mean Nucl Aver Intens	✓ Scoring Profile 123
✓ Negative W2 Mean Cyto Integr Intens	✓ Percentage Scoring Profile 1--
✓ Negative W2 Mean Cyto Aver Intens	✓ Percentage Scoring Profile 12-
✓ Negative W2 Mean Cell Integr Intens	✓ Percentage Scoring Profile 1-3
✓ Negative W2 Mean Cell Aver Intens	✓ Percentage Scoring Profile 123
✓ Positive W2	✓ Subtotal Profile 1xx
✓ % Positive W2	✓ Subtotal Profile 12x
✓ All W3 Mean Stain Area	✓ Subtotal Profile 1x3
✓ All W3 Mean Stain Integr Intens	✓ Subtotal Profile 123
✓ All W3 Mean Stain Aver Intens	✓ Percentage Subtotal Profile 1xx
✓ All W3 Mean Nucl Integr Intens	✓ Percentage Subtotal Profile 12x
✓ All W3 Mean Nucl Aver Intens	✓ Percentage Subtotal Profile 1x3
✓ All W3 Mean Cyto Integr Intens	✓ Percentage Subtotal Profile 123

- **Positive W2 Mean Stain Area:** The average stained area (nucleus, cytoplasm, or both as defined in the W2 settings) for all cells scored as positive for W2 (in  $\mu\text{m}^2$ )
- **Positive W2 Mean Stain Integr Intensity:** The total pixel intensity of the W2 stain over the stained area in W2 positive cells, divided by the number of cells positive for W2
- **Positive W2 Mean Stain Aver Intensity:** The average pixel intensity of the W2 stain over the stained area in W2 positive cells
- *(similar for W3-W7)*

# Summary Data (site-by-site measurements)

✓ Total Cells	✓ All W3 Mean Cyto Integr Intens
✓ All Cells Mean Area	✓ All W3 Mean Cyto Aver Intens
✓ All Nuclei Mean Area	✓ All W3 Mean Cell Integr Intens
✓ All Nuclei Mean Integr Intensity	✓ All W3 Mean Cell Aver Intens
✓ All Nuclei Mean Average Intensity	✓ Positive W3 Mean Stain Area
✓ All W2 Mean Stain Area	✓ Positive W3 Mean Stain Integr Intens
✓ All W2 Mean Stain Integr Intens	✓ Positive W3 Mean Stain Aver Intens
✓ All W2 Mean Stain Aver Intens	✓ Positive W3 Mean Nucl Integr Intens
✓ All W2 Mean Nucl Integr Intens	✓ Positive W3 Mean Nucl Aver Intens
✓ All W2 Mean Nucl Aver Intens	✓ Positive W3 Mean Cyto Integr Intens
✓ All W2 Mean Cyto Integr Intens	✓ Positive W3 Mean Cyto Aver Intens
✓ All W2 Mean Cyto Aver Intens	✓ Positive W3 Mean Cell Integr Intens
✓ All W2 Mean Cell Integr Intens	✓ Positive W3 Mean Cell Aver Intens
✓ All W2 Mean Cell Aver Intens	✓ Negative W3 Mean Stain Area
✓ Positive W2 Mean Stain Area	✓ Negative W3 Mean Stain Integr Intens
✓ Positive W2 Mean Stain Integr Intens	✓ Negative W3 Mean Stain Aver Intens
✓ Positive W2 Mean Stain Aver Intens	✓ Negative W3 Mean Nucl Integr Intens
✓ Positive W2 Mean Nucl Integr Intens	✓ Negative W3 Mean Nucl Aver Intens
✓ Positive W2 Mean Nucl Aver Intens	✓ Negative W3 Mean Cyto Integr Intens
✓ Positive W2 Mean Cyto Integr Intens	✓ Negative W3 Mean Cyto Aver Intens
✓ Positive W2 Mean Cyto Aver Intens	✓ Negative W3 Mean Cell Integr Intens
✓ Positive W2 Mean Cell Integr Intens	✓ Negative W3 Mean Cell Aver Intens
✓ Positive W2 Mean Cell Aver Intens	✓ Positive W3
✓ Negative W2 Mean Stain Area	✓ % Positive W3
✓ Negative W2 Mean Stain Integr Intens	✓ Scoring Profile 1--
✓ Negative W2 Mean Stain Aver Intens	✓ Scoring Profile 12-
✓ Negative W2 Mean Nucl Integr Intens	✓ Scoring Profile 1-3
✓ Negative W2 Mean Nucl Aver Intens	✓ Scoring Profile 123
✓ Negative W2 Mean Cyto Integr Intens	✓ Percentage Scoring Profile 1--
✓ Negative W2 Mean Cyto Aver Intens	✓ Percentage Scoring Profile 12-
✓ Negative W2 Mean Cell Integr Intens	✓ Percentage Scoring Profile 1-3
✓ Negative W2 Mean Cell Aver Intens	✓ Percentage Scoring Profile 123
✓ Positive W2	✓ Subtotal Profile 1xx
✓ % Positive W2	✓ Subtotal Profile 12x
✓ All W3 Mean Stain Area	✓ Subtotal Profile 1x3
✓ All W3 Mean Stain Integr Intens	✓ Subtotal Profile 123
✓ All W3 Mean Stain Aver Intens	✓ Percentage Subtotal Profile 1xx
✓ All W3 Mean Nucl Integr Intens	✓ Percentage Subtotal Profile 12x
✓ All W3 Mean Nucl Aver Intens	✓ Percentage Subtotal Profile 1x3
	✓ Percentage Subtotal Profile 123

- **Positive W2 Mean Nucl Integr Intensity:** The total pixel intensity of the W2 stain over the nuclear area in W2 positive cells, divided by the number of cells positive for W2
- **Positive W2 Mean Nucl Aver Intensity:** The average pixel intensity of the W2 stain over the nuclear area in W2 positive cells
- *(similar for W3-W7)*

# Summary Data (site-by-site measurements)

✓ Total Cells	✓ All W3 Mean Cyto Integr Intens
✓ All Cells Mean Area	✓ All W3 Mean Cyto Aver Intens
✓ All Nuclei Mean Area	✓ All W3 Mean Cell Integr Intens
✓ All Nuclei Mean Integr Intensity	✓ All W3 Mean Cell Aver Intens
✓ All Nuclei Mean Average Intensity	✓ Positive W3 Mean Stain Area
✓ All W2 Mean Stain Area	✓ Positive W3 Mean Stain Integr Intens
✓ All W2 Mean Stain Integr Intens	✓ Positive W3 Mean Stain Aver Intens
✓ All W2 Mean Stain Aver Intens	✓ Positive W3 Mean Nucl Integr Intens
✓ All W2 Mean Nucl Integr Intens	✓ Positive W3 Mean Nucl Aver Intens
✓ All W2 Mean Nucl Aver Intens	✓ Positive W3 Mean Cyto Integr Intens
✓ All W2 Mean Cyto Integr Intens	✓ Positive W3 Mean Cyto Aver Intens
✓ All W2 Mean Cyto Aver Intens	✓ Positive W3 Mean Cell Integr Intens
✓ All W2 Mean Cell Integr Intens	✓ Positive W3 Mean Cell Aver Intens
✓ All W2 Mean Cell Aver Intens	✓ Negative W3 Mean Stain Area
✓ Positive W2 Mean Stain Area	✓ Negative W3 Mean Stain Integr Intens
✓ Positive W2 Mean Stain Integr Intens	✓ Negative W3 Mean Stain Aver Intens
✓ Positive W2 Mean Stain Aver Intens	✓ Negative W3 Mean Nucl Integr Intens
✓ Positive W2 Mean Nucl Integr Intens	✓ Negative W3 Mean Nucl Aver Intens
✓ Positive W2 Mean Nucl Aver Intens	✓ Negative W3 Mean Cyto Integr Intens
✓ Positive W2 Mean Cyto Integr Intens	✓ Negative W3 Mean Cyto Aver Intens
✓ Positive W2 Mean Cyto Aver Intens	✓ Negative W3 Mean Cell Integr Intens
✓ Positive W2 Mean Cell Integr Intens	✓ Negative W3 Mean Cell Aver Intens
✓ Positive W2 Mean Cell Aver Intens	✓ Positive W3
✓ Negative W2 Mean Stain Area	✓ % Positive W3
✓ Negative W2 Mean Stain Integr Intens	✓ Scoring Profile 1--
✓ Negative W2 Mean Stain Aver Intens	✓ Scoring Profile 12-
✓ Negative W2 Mean Nucl Integr Intens	✓ Scoring Profile 1-3
✓ Negative W2 Mean Nucl Aver Intens	✓ Scoring Profile 123
✓ Negative W2 Mean Cyto Integr Intens	✓ Percentage Scoring Profile 1--
✓ Negative W2 Mean Cyto Aver Intens	✓ Percentage Scoring Profile 12-
✓ Negative W2 Mean Cell Integr Intens	✓ Percentage Scoring Profile 1-3
✓ Negative W2 Mean Cell Aver Intens	✓ Percentage Scoring Profile 123
✓ Positive W2	✓ Subtotal Profile 1xx
✓ % Positive W2	✓ Subtotal Profile 12x
✓ All W3 Mean Stain Area	✓ Subtotal Profile 1x3
✓ All W3 Mean Stain Integr Intens	✓ Subtotal Profile 123
✓ All W3 Mean Stain Aver Intens	✓ Percentage Subtotal Profile 1xx
✓ All W3 Mean Nucl Integr Intens	✓ Percentage Subtotal Profile 12x
✓ All W3 Mean Nucl Aver Intens	✓ Percentage Subtotal Profile 1x3
✓ All W3 Mean Cyto Integr Intens	✓ Percentage Subtotal Profile 123

- **Positive W2 Mean Cyto Integr Intensity:** The total pixel intensity of the W2 stain over the cytoplasmic area in W2 positive cells, divided by the number of cells positive for W2
- **Positive W2 Mean Cyto Aver Intensity:** The average pixel intensity of the W2 stain over the cytoplasmic area in W2 positive cells
- *(similar for W3-W7)*

# Summary Data (site-by-site measurements)

✓ Total Cells	✓ All W3 Mean Cyto Integr Intens
✓ All Cells Mean Area	✓ All W3 Mean Cyto Aver Intens
✓ All Nuclei Mean Area	✓ All W3 Mean Cell Integr Intens
✓ All Nuclei Mean Integr Intensity	✓ All W3 Mean Cell Aver Intens
✓ All Nuclei Mean Average Intensity	✓ Positive W3 Mean Stain Area
✓ All W2 Mean Stain Area	✓ Positive W3 Mean Stain Integr Intens
✓ All W2 Mean Stain Integr Intens	✓ Positive W3 Mean Stain Aver Intens
✓ All W2 Mean Stain Aver Intens	✓ Positive W3 Mean Nucl Integr Intens
✓ All W2 Mean Nucl Integr Intens	✓ Positive W3 Mean Nucl Aver Intens
✓ All W2 Mean Nucl Aver Intens	✓ Positive W3 Mean Cyto Integr Intens
✓ All W2 Mean Cyto Integr Intens	✓ Positive W3 Mean Cyto Aver Intens
✓ All W2 Mean Cyto Aver Intens	✓ Positive W3 Mean Cell Integr Intens
✓ All W2 Mean Cell Integr Intens	✓ Positive W3 Mean Cell Aver Intens
✓ All W2 Mean Cell Aver Intens	✓ Negative W3 Mean Stain Area
✓ Positive W2 Mean Stain Area	✓ Negative W3 Mean Stain Integr Intens
✓ Positive W2 Mean Stain Integr Intens	✓ Negative W3 Mean Stain Aver Intens
✓ Positive W2 Mean Stain Aver Intens	✓ Negative W3 Mean Nucl Integr Intens
✓ Positive W2 Mean Nucl Integr Intens	✓ Negative W3 Mean Nucl Aver Intens
✓ Positive W2 Mean Nucl Aver Intens	✓ Negative W3 Mean Cyto Integr Intens
✓ Positive W2 Mean Cyto Integr Intens	✓ Negative W3 Mean Cyto Aver Intens
✓ Positive W2 Mean Cyto Aver Intens	✓ Negative W3 Mean Cell Integr Intens
✓ Positive W2 Mean Cell Integr Intens	✓ Negative W3 Mean Cell Aver Intens
✓ Positive W2 Mean Cell Aver Intens	✓ Positive W3
✓ Negative W2 Mean Stain Area	✓ % Positive W3
✓ Negative W2 Mean Stain Integr Intens	✓ Scoring Profile 1--
✓ Negative W2 Mean Stain Aver Intens	✓ Scoring Profile 12-
✓ Negative W2 Mean Nucl Integr Intens	✓ Scoring Profile 1-3
✓ Negative W2 Mean Nucl Aver Intens	✓ Scoring Profile 123
✓ Negative W2 Mean Cyto Integr Intens	✓ Percentage Scoring Profile 1--
✓ Negative W2 Mean Cyto Aver Intens	✓ Percentage Scoring Profile 12-
✓ Negative W2 Mean Cell Integr Intens	✓ Percentage Scoring Profile 1-3
✓ Negative W2 Mean Cell Aver Intens	✓ Percentage Scoring Profile 123
✓ Positive W2	✓ Subtotal Profile 1xx
✓ % Positive W2	✓ Subtotal Profile 12x
✓ All W3 Mean Stain Area	✓ Subtotal Profile 1x3
✓ All W3 Mean Stain Integr Intens	✓ Subtotal Profile 123
✓ All W3 Mean Stain Aver Intens	✓ Percentage Subtotal Profile 1xx
✓ All W3 Mean Nucl Integr Intens	✓ Percentage Subtotal Profile 12x
✓ All W3 Mean Nucl Aver Intens	✓ Percentage Subtotal Profile 1x3
✓ All W3 Mean Cyto Integr Intens	✓ Percentage Subtotal Profile 123

- **Positive W2 Mean Cell Integr Intensity:** The total pixel intensity of the W2 stain over the cell (nucleus + cytoplasm) area in W2 positive cells, divided by the number of cells positive for W2
- **Positive W2 Mean Cell Aver Intensity:** The average pixel intensity of the W2 stain over the cell (nucleus + cytoplasm) area in W2 positive cells
- *(similar for W3-W7)*

# Summary Data (site-by-site measurements)

✓ Total Cells	✓ All W3 Mean Cyto Integr Intens
✓ All Cells Mean Area	✓ All W3 Mean Cyto Aver Intens
✓ All Nuclei Mean Area	✓ All W3 Mean Cell Integr Intens
✓ All Nuclei Mean Integr Intensity	✓ All W3 Mean Cell Aver Intens
✓ All Nuclei Mean Average Intensity	✓ Positive W3 Mean Stain Area
✓ All W2 Mean Stain Area	✓ Positive W3 Mean Stain Integr Intens
✓ All W2 Mean Stain Integr Intens	✓ Positive W3 Mean Stain Aver Intens
✓ All W2 Mean Stain Aver Intens	✓ Positive W3 Mean Nucl Integr Intens
✓ All W2 Mean Nucl Integr Intens	✓ Positive W3 Mean Nucl Aver Intens
✓ All W2 Mean Nucl Aver Intens	✓ Positive W3 Mean Cyto Integr Intens
✓ All W2 Mean Cyto Integr Intens	✓ Positive W3 Mean Cyto Aver Intens
✓ All W2 Mean Cyto Aver Intens	✓ Positive W3 Mean Cell Integr Intens
✓ All W2 Mean Cell Integr Intens	✓ Positive W3 Mean Cell Aver Intens
✓ All W2 Mean Cell Aver Intens	✓ Negative W3 Mean Stain Area
✓ Positive W2 Mean Stain Area	✓ Negative W3 Mean Stain Integr Intens
✓ Positive W2 Mean Stain Integr Intens	✓ Negative W3 Mean Stain Aver Intens
✓ Positive W2 Mean Stain Aver Intens	✓ Negative W3 Mean Nucl Integr Intens
✓ Positive W2 Mean Nucl Integr Intens	✓ Negative W3 Mean Nucl Aver Intens
✓ Positive W2 Mean Nucl Aver Intens	✓ Negative W3 Mean Cyto Integr Intens
✓ Positive W2 Mean Cyto Integr Intens	✓ Negative W3 Mean Cyto Aver Intens
✓ Positive W2 Mean Cyto Aver Intens	✓ Negative W3 Mean Cell Integr Intens
✓ Positive W2 Mean Cell Integr Intens	✓ Negative W3 Mean Cell Aver Intens
✓ Positive W2 Mean Cell Aver Intens	✓ Positive W3
✓ Negative W2 Mean Stain Area	✓ % Positive W3
✓ Negative W2 Mean Stain Integr Intens	✓ Scoring Profile 1--
✓ Negative W2 Mean Stain Aver Intens	✓ Scoring Profile 12-
✓ Negative W2 Mean Nucl Integr Intens	✓ Scoring Profile 1-3
✓ Negative W2 Mean Nucl Aver Intens	✓ Scoring Profile 123
✓ Negative W2 Mean Cyto Integr Intens	✓ Percentage Scoring Profile 1--
✓ Negative W2 Mean Cyto Aver Intens	✓ Percentage Scoring Profile 12-
✓ Negative W2 Mean Cell Integr Intens	✓ Percentage Scoring Profile 1-3
✓ Negative W2 Mean Cell Aver Intens	✓ Percentage Scoring Profile 123
✓ Positive W2	✓ Subtotal Profile 1xx
✓ % Positive W2	✓ Subtotal Profile 12x
✓ All W3 Mean Stain Area	✓ Subtotal Profile 1x3
✓ All W3 Mean Stain Integr Intens	✓ Subtotal Profile 123
✓ All W3 Mean Stain Aver Intens	✓ Percentage Subtotal Profile 1xx
✓ All W3 Mean Nucl Integr Intens	✓ Percentage Subtotal Profile 12x
✓ All W3 Mean Nucl Aver Intens	✓ Percentage Subtotal Profile 1x3
✓ All W3 Mean Cyto Integr Intens	✓ Percentage Subtotal Profile 123

- **Negative W2 Mean Stain Area:** The average stained area (nucleus, cytoplasm, or both as defined in the W2 settings) for all cells scored as negative for W2 (in  $\mu\text{m}^2$ )
- **Negative W2 Mean Stain Integr Intensity:** The total pixel intensity of the W2 stain over the stained area in W2 negative cells, divided by the number of cells negative for W2
- **Negative W2 Mean Stain Aver Intensity:** The average pixel intensity of the W2 stain over the stained area in W2 negative cells
- *(similar for W3-W7)*

# Summary Data (site-by-site measurements)

✓ Total Cells	✓ All W3 Mean Cyto Integr Intens
✓ All Cells Mean Area	✓ All W3 Mean Cyto Aver Intens
✓ All Nuclei Mean Area	✓ All W3 Mean Cell Integr Intens
✓ All Nuclei Mean Integr Intensity	✓ All W3 Mean Cell Aver Intens
✓ All Nuclei Mean Average Intensity	✓ Positive W3 Mean Stain Area
✓ All W2 Mean Stain Area	✓ Positive W3 Mean Stain Integr Intens
✓ All W2 Mean Stain Integr Intens	✓ Positive W3 Mean Stain Aver Intens
✓ All W2 Mean Stain Aver Intens	✓ Positive W3 Mean Nucl Integr Intens
✓ All W2 Mean Nucl Integr Intens	✓ Positive W3 Mean Nucl Aver Intens
✓ All W2 Mean Nucl Aver Intens	✓ Positive W3 Mean Cyto Integr Intens
✓ All W2 Mean Cyto Integr Intens	✓ Positive W3 Mean Cyto Aver Intens
✓ All W2 Mean Cyto Aver Intens	✓ Positive W3 Mean Cell Integr Intens
✓ All W2 Mean Cell Integr Intens	✓ Positive W3 Mean Cell Aver Intens
✓ All W2 Mean Cell Aver Intens	✓ Negative W3 Mean Stain Area
✓ Positive W2 Mean Stain Area	✓ Negative W3 Mean Stain Integr Intens
✓ Positive W2 Mean Stain Integr Intens	✓ Negative W3 Mean Stain Aver Intens
✓ Positive W2 Mean Stain Aver Intens	✓ Negative W3 Mean Nucl Integr Intens
✓ Positive W2 Mean Nucl Integr Intens	✓ Negative W3 Mean Nucl Aver Intens
✓ Positive W2 Mean Nucl Aver Intens	✓ Negative W3 Mean Cyto Integr Intens
✓ Positive W2 Mean Cyto Integr Intens	✓ Negative W3 Mean Cyto Aver Intens
✓ Positive W2 Mean Cyto Aver Intens	✓ Negative W3 Mean Cell Integr Intens
✓ Positive W2 Mean Cell Integr Intens	✓ Negative W3 Mean Cell Aver Intens
✓ Positive W2 Mean Cell Aver Intens	✓ Positive W3
✓ Negative W2 Mean Stain Area	✓ % Positive W3
✓ Negative W2 Mean Stain Integr Intens	✓ Scoring Profile 1-
✓ Negative W2 Mean Stain Aver Intens	✓ Scoring Profile 12-
✓ Negative W2 Mean Nucl Integr Intens	✓ Scoring Profile 1-3
✓ Negative W2 Mean Nucl Aver Intens	✓ Scoring Profile 123
✓ Negative W2 Mean Cyto Integr Intens	✓ Percentage Scoring Profile 1--
✓ Negative W2 Mean Cyto Aver Intens	✓ Percentage Scoring Profile 12-
✓ Negative W2 Mean Cell Integr Intens	✓ Percentage Scoring Profile 1-3
✓ Negative W2 Mean Cell Aver Intens	✓ Percentage Scoring Profile 123
✓ Positive W2	✓ Subtotal Profile 1xx
✓ % Positive W2	✓ Subtotal Profile 12x
✓ All W3 Mean Stain Area	✓ Subtotal Profile 1x3
✓ All W3 Mean Stain Integr Intens	✓ Subtotal Profile 123
✓ All W3 Mean Stain Aver Intens	✓ Percentage Subtotal Profile 1xx
✓ All W3 Mean Nucl Integr Intens	✓ Percentage Subtotal Profile 12x
✓ All W3 Mean Nucl Aver Intens	✓ Percentage Subtotal Profile 1x3
	✓ Percentage Subtotal Profile 123

- **Negative W2 Mean Nucl Integr Intensity:** The total pixel intensity of the W2 stain over the nuclear area in W2 negative cells, divided by the number of cells negative for W2
- **Negative W2 Mean Nucl Aver Intensity:** The average pixel intensity of the W2 stain over the nuclear area in W2 negative cells
- *(similar for W3-W7)*

# Summary Data (site-by-site measurements)

✓ Total Cells	✓ All W3 Mean Cyto Integr Intens
✓ All Cells Mean Area	✓ All W3 Mean Cyto Aver Intens
✓ All Nuclei Mean Area	✓ All W3 Mean Cell Integr Intens
✓ All Nuclei Mean Integr Intensity	✓ All W3 Mean Cell Aver Intens
✓ All Nuclei Mean Average Intensity	✓ Positive W3 Mean Stain Area
✓ All W2 Mean Stain Area	✓ Positive W3 Mean Stain Integr Intens
✓ All W2 Mean Stain Integr Intens	✓ Positive W3 Mean Stain Aver Intens
✓ All W2 Mean Stain Aver Intens	✓ Positive W3 Mean Nucl Integr Intens
✓ All W2 Mean Nucl Integr Intens	✓ Positive W3 Mean Nucl Aver Intens
✓ All W2 Mean Nucl Aver Intens	✓ Positive W3 Mean Cyto Integr Intens
✓ All W2 Mean Cyto Integr Intens	✓ Positive W3 Mean Cyto Aver Intens
✓ All W2 Mean Cyto Aver Intens	✓ Positive W3 Mean Cell Integr Intens
✓ All W2 Mean Cell Integr Intens	✓ Positive W3 Mean Cell Aver Intens
✓ All W2 Mean Cell Aver Intens	✓ Negative W3 Mean Stain Area
✓ Positive W2 Mean Stain Area	✓ Negative W3 Mean Stain Integr Intens
✓ Positive W2 Mean Stain Integr Intens	✓ Negative W3 Mean Stain Aver Intens
✓ Positive W2 Mean Stain Aver Intens	✓ Negative W3 Mean Nucl Integr Intens
✓ Positive W2 Mean Nucl Integr Intens	✓ Negative W3 Mean Nucl Aver Intens
✓ Positive W2 Mean Nucl Aver Intens	✓ Negative W3 Mean Cyto Integr Intens
✓ Positive W2 Mean Cyto Integr Intens	✓ Negative W3 Mean Cyto Aver Intens
✓ Positive W2 Mean Cyto Aver Intens	✓ Negative W3 Mean Cell Integr Intens
✓ Positive W2 Mean Cell Integr Intens	✓ Negative W3 Mean Cell Aver Intens
✓ Positive W2 Mean Cell Aver Intens	✓ Positive W3
✓ Negative W2 Mean Stain Area	✓ % Positive W3
✓ Negative W2 Mean Stain Integr Intens	✓ Scoring Profile 1--
✓ Negative W2 Mean Stain Aver Intens	✓ Scoring Profile 12-
✓ Negative W2 Mean Nucl Integr Intens	✓ Scoring Profile 1-3
✓ Negative W2 Mean Nucl Aver Intens	✓ Scoring Profile 123
✓ Negative W2 Mean Cyto Integr Intens	✓ Percentage Scoring Profile 1--
✓ Negative W2 Mean Cyto Aver Intens	✓ Percentage Scoring Profile 12-
✓ Negative W2 Mean Cell Integr Intens	✓ Percentage Scoring Profile 1-3
✓ Negative W2 Mean Cell Aver Intens	✓ Percentage Scoring Profile 123
✓ Positive W2	✓ Subtotal Profile 1xx
✓ % Positive W2	✓ Subtotal Profile 12x
✓ All W3 Mean Stain Area	✓ Subtotal Profile 1x3
✓ All W3 Mean Stain Integr Intens	✓ Subtotal Profile 123
✓ All W3 Mean Stain Aver Intens	✓ Percentage Subtotal Profile 1xx
✓ All W3 Mean Nucl Integr Intens	✓ Percentage Subtotal Profile 12x
✓ All W3 Mean Nucl Aver Intens	✓ Percentage Subtotal Profile 1x3
✓ All W3 Mean Cyto Integr Intens	✓ Percentage Subtotal Profile 123

- **Negative W2 Mean Cyto Integr Intensity:** The total pixel intensity of the W2 stain over the cytoplasmic area in W2 negative cells, divided by the number of cells negative for W2
- **Negative W2 Mean Cyto Aver Intensity:** The average pixel intensity of the W2 stain over the cytoplasmic area in W2 negative cells
- *(similar for W3-W7)*

# Summary Data (site-by-site measurements)

✓ Total Cells	✓ All W3 Mean Cyto Integr Intens
✓ All Cells Mean Area	✓ All W3 Mean Cyto Aver Intens
✓ All Nuclei Mean Area	✓ All W3 Mean Cell Integr Intens
✓ All Nuclei Mean Integr Intensity	✓ All W3 Mean Cell Aver Intens
✓ All Nuclei Mean Average Intensity	✓ Positive W3 Mean Stain Area
✓ All W2 Mean Stain Area	✓ Positive W3 Mean Stain Integr Intens
✓ All W2 Mean Stain Integr Intens	✓ Positive W3 Mean Stain Aver Intens
✓ All W2 Mean Stain Aver Intens	✓ Positive W3 Mean Nucl Integr Intens
✓ All W2 Mean Nucl Integr Intens	✓ Positive W3 Mean Nucl Aver Intens
✓ All W2 Mean Nucl Aver Intens	✓ Positive W3 Mean Cyto Integr Intens
✓ All W2 Mean Cyto Integr Intens	✓ Positive W3 Mean Cyto Aver Intens
✓ All W2 Mean Cyto Aver Intens	✓ Positive W3 Mean Cell Integr Intens
✓ All W2 Mean Cell Integr Intens	✓ Positive W3 Mean Cell Aver Intens
✓ All W2 Mean Cell Aver Intens	✓ Negative W3 Mean Stain Area
✓ Positive W2 Mean Stain Area	✓ Negative W3 Mean Stain Integr Intens
✓ Positive W2 Mean Stain Integr Intens	✓ Negative W3 Mean Stain Aver Intens
✓ Positive W2 Mean Stain Aver Intens	✓ Negative W3 Mean Nucl Integr Intens
✓ Positive W2 Mean Nucl Integr Intens	✓ Negative W3 Mean Nucl Aver Intens
✓ Positive W2 Mean Nucl Aver Intens	✓ Negative W3 Mean Cyto Integr Intens
✓ Positive W2 Mean Cyto Integr Intens	✓ Negative W3 Mean Cyto Aver Intens
✓ Positive W2 Mean Cyto Aver Intens	✓ Negative W3 Mean Cell Integr Intens
✓ Positive W2 Mean Cell Integr Intens	✓ Negative W3 Mean Cell Aver Intens
✓ Positive W2 Mean Cell Aver Intens	✓ Positive W3
✓ Negative W2 Mean Stain Area	✓ % Positive W3
✓ Negative W2 Mean Stain Integr Intens	✓ Scoring Profile 1--
✓ Negative W2 Mean Stain Aver Intens	✓ Scoring Profile 12-
✓ Negative W2 Mean Nucl Integr Intens	✓ Scoring Profile 1-3
✓ Negative W2 Mean Nucl Aver Intens	✓ Scoring Profile 123
✓ Negative W2 Mean Cyto Integr Intens	✓ Percentage Scoring Profile 1--
✓ Negative W2 Mean Cyto Aver Intens	✓ Percentage Scoring Profile 12-
✓ Negative W2 Mean Cell Integr Intens	✓ Percentage Scoring Profile 1-3
✓ Negative W2 Mean Cell Aver Intens	✓ Percentage Scoring Profile 123
✓ Positive W2	✓ Subtotal Profile 1xx
✓ % Positive W2	✓ Subtotal Profile 12x
✓ All W3 Mean Stain Area	✓ Subtotal Profile 1x3
✓ All W3 Mean Stain Integr Intens	✓ Subtotal Profile 123
✓ All W3 Mean Stain Aver Intens	✓ Percentage Subtotal Profile 1xx
✓ All W3 Mean Nucl Integr Intens	✓ Percentage Subtotal Profile 12x
✓ All W3 Mean Nucl Aver Intens	✓ Percentage Subtotal Profile 1x3
✓ All W3 Mean Cyto Integr Intens	✓ Percentage Subtotal Profile 123

- **Negative W2 Mean Cell Integr Intensity:** The total pixel intensity of the W2 stain over the cell (nucleus + cytoplasm) area in W2 negative cells, divided by the number of cells negative for W2
- **Negative W2 Mean Cell Aver Intensity:** The average pixel intensity of the W2 stain over the cell (nucleus + cytoplasm) area in W2 negative cells
- *(similar for W3-W7)*

# Summary Data (site-by-site measurements)

✓ Total Cells	✓ All W3 Mean Cyto Integr Intens
✓ All Cells Mean Area	✓ All W3 Mean Cyto Aver Intens
✓ All Nuclei Mean Area	✓ All W3 Mean Cell Integr Intens
✓ All Nuclei Mean Integr Intensity	✓ All W3 Mean Cell Aver Intens
✓ All Nuclei Mean Average Intensity	✓ Positive W3 Mean Stain Area
✓ All W2 Mean Stain Area	✓ Positive W3 Mean Stain Integr Intens
✓ All W2 Mean Stain Integr Intens	✓ Positive W3 Mean Stain Aver Intens
✓ All W2 Mean Stain Aver Intens	✓ Positive W3 Mean Nucl Integr Intens
✓ All W2 Mean Nucl Integr Intens	✓ Positive W3 Mean Nucl Aver Intens
✓ All W2 Mean Nucl Aver Intens	✓ Positive W3 Mean Cyto Integr Intens
✓ All W2 Mean Cyto Integr Intens	✓ Positive W3 Mean Cyto Aver Intens
✓ All W2 Mean Cyto Aver Intens	✓ Positive W3 Mean Cell Integr Intens
✓ All W2 Mean Cell Integr Intens	✓ Positive W3 Mean Cell Aver Intens
✓ All W2 Mean Cell Aver Intens	✓ Negative W3 Mean Stain Area
✓ Positive W2 Mean Stain Area	✓ Negative W3 Mean Stain Integr Intens
✓ Positive W2 Mean Stain Integr Intens	✓ Negative W3 Mean Stain Aver Intens
✓ Positive W2 Mean Stain Aver Intens	✓ Negative W3 Mean Nucl Integr Intens
✓ Positive W2 Mean Nucl Integr Intens	✓ Negative W3 Mean Nucl Aver Intens
✓ Positive W2 Mean Nucl Aver Intens	✓ Negative W3 Mean Cyto Integr Intens
✓ Positive W2 Mean Cyto Integr Intens	✓ Negative W3 Mean Cyto Aver Intens
✓ Positive W2 Mean Cyto Aver Intens	✓ Negative W3 Mean Cell Integr Intens
✓ Positive W2 Mean Cell Integr Intens	✓ Negative W3 Mean Cell Aver Intens
✓ Positive W2 Mean Cell Aver Intens	✓ Positive W3
✓ Negative W2 Mean Stain Area	✓ % Positive W3
✓ Negative W2 Mean Stain Integr Intens	✓ Scoring Profile 1--
✓ Negative W2 Mean Stain Aver Intens	✓ Scoring Profile 12-
✓ Negative W2 Mean Nucl Integr Intens	✓ Scoring Profile 1-3
✓ Negative W2 Mean Nucl Aver Intens	✓ Scoring Profile 123
✓ Negative W2 Mean Cyto Integr Intens	✓ Percentage Scoring Profile 1--
✓ Negative W2 Mean Cyto Aver Intens	✓ Percentage Scoring Profile 12-
✓ Negative W2 Mean Cell Integr Intens	✓ Percentage Scoring Profile 1-3
✓ Negative W2 Mean Cell Aver Intens	✓ Percentage Scoring Profile 123
✓ Positive W2	✓ Subtotal Profile 1xx
✓ % Positive W2	✓ Subtotal Profile 12x
✓ All W3 Mean Stain Area	✓ Subtotal Profile 1x3
✓ All W3 Mean Stain Integr Intens	✓ Subtotal Profile 123
✓ All W3 Mean Stain Aver Intens	✓ Percentage Subtotal Profile 1xx
✓ All W3 Mean Nucl Integr Intens	✓ Percentage Subtotal Profile 12x
✓ All W3 Mean Nucl Aver Intens	✓ Percentage Subtotal Profile 1x3
	✓ Percentage Subtotal Profile 123

- **Positive W2:** The total number of cells positive for staining with wavelength 2 as defined in the settings
- **% Positive W2:** The number of positive W2 cells divided by the total number of cells, times 100
- *(similar for W3-W7)*

# Summary Data (site-by-site measurements)

✓ Total Cells	✓ All W3 Mean Cyto Integr Intens
✓ All Cells Mean Area	✓ All W3 Mean Cyto Aver Intens
✓ All Nuclei Mean Area	✓ All W3 Mean Cell Integr Intens
✓ All Nuclei Mean Integr Intensity	✓ All W3 Mean Cell Aver Intens
✓ All Nuclei Mean Average Intensity	✓ Positive W3 Mean Stain Area
✓ All W2 Mean Stain Area	✓ Positive W3 Mean Stain Integr Intens
✓ All W2 Mean Stain Integr Intens	✓ Positive W3 Mean Stain Aver Intens
✓ All W2 Mean Stain Aver Intens	✓ Positive W3 Mean Nucl Integr Intens
✓ All W2 Mean Nucl Integr Intens	✓ Positive W3 Mean Nucl Aver Intens
✓ All W2 Mean Nucl Aver Intens	✓ Positive W3 Mean Cyto Integr Intens
✓ All W2 Mean Cyto Integr Intens	✓ Positive W3 Mean Cyto Aver Intens
✓ All W2 Mean Cyto Aver Intens	✓ Positive W3 Mean Cell Integr Intens
✓ All W2 Mean Cell Integr Intens	✓ Positive W3 Mean Cell Aver Intens
✓ All W2 Mean Cell Aver Intens	✓ Negative W3 Mean Stain Area
✓ Positive W2 Mean Stain Area	✓ Negative W3 Mean Stain Integr Intens
✓ Positive W2 Mean Stain Integr Intens	✓ Negative W3 Mean Stain Aver Intens
✓ Positive W2 Mean Stain Aver Intens	✓ Negative W3 Mean Nucl Integr Intens
✓ Positive W2 Mean Nucl Integr Intens	✓ Negative W3 Mean Nucl Aver Intens
✓ Positive W2 Mean Nucl Aver Intens	✓ Negative W3 Mean Cyto Integr Intens
✓ Positive W2 Mean Cyto Integr Intens	✓ Negative W3 Mean Cyto Aver Intens
✓ Positive W2 Mean Cyto Aver Intens	✓ Negative W3 Mean Cell Integr Intens
✓ Positive W2 Mean Cell Integr Intens	✓ Negative W3 Mean Cell Aver Intens
✓ Positive W2 Mean Cell Aver Intens	✓ Positive W3
✓ Negative W2 Mean Stain Area	✓ % Positive W3
✓ Negative W2 Mean Stain Integr Intens	✓ Scoring Profile 1--
✓ Negative W2 Mean Stain Aver Intens	✓ Scoring Profile 12-
✓ Negative W2 Mean Nucl Integr Intens	✓ Scoring Profile 1-3
✓ Negative W2 Mean Nucl Aver Intens	✓ Scoring Profile 123
✓ Negative W2 Mean Cyto Integr Intens	✓ Percentage Scoring Profile 1--
✓ Negative W2 Mean Cyto Aver Intens	✓ Percentage Scoring Profile 12-
✓ Negative W2 Mean Cell Integr Intens	✓ Percentage Scoring Profile 1-3
✓ Negative W2 Mean Cell Aver Intens	✓ Percentage Scoring Profile 123
✓ Positive W2	✓ Subtotal Profile 1xx
✓ % Positive W2	✓ Subtotal Profile 12x
✓ All W3 Mean Stain Area	✓ Subtotal Profile 1x3
✓ All W3 Mean Stain Integr Intens	✓ Subtotal Profile 123
✓ All W3 Mean Stain Aver Intens	✓ Percentage Subtotal Profile 1xx
✓ All W3 Mean Nucl Integr Intens	✓ Percentage Subtotal Profile 12x
✓ All W3 Mean Nucl Aver Intens	✓ Percentage Subtotal Profile 1x3
	✓ Percentage Subtotal Profile 123

- **Scoring Profile 1--:** The total number of cells positive for W1, negative for W2 and negative for W3
- **Scoring Profile 12-:** The total number of cells positive for W1, positive for W2 and negative for W3
- **Scoring Profile 1-3:** The total number of cells positive for W1, negative for W2 and positive for W3
- **Scoring Profile 123:** The total number of cells positive for W1, W2 and W3
- *(similar for additional wavelengths)*

# Summary Data (site-by-site measurements)

✓ Total Cells	✓ All W3 Mean Cyto Integr Intens
✓ All Cells Mean Area	✓ All W3 Mean Cyto Aver Intens
✓ All Nuclei Mean Area	✓ All W3 Mean Cell Integr Intens
✓ All Nuclei Mean Integr Intensity	✓ All W3 Mean Cell Aver Intens
✓ All Nuclei Mean Average Intensity	✓ Positive W3 Mean Stain Area
✓ All W2 Mean Stain Area	✓ Positive W3 Mean Stain Integr Intens
✓ All W2 Mean Stain Integr Intens	✓ Positive W3 Mean Stain Aver Intens
✓ All W2 Mean Stain Aver Intens	✓ Positive W3 Mean Nucl Integr Intens
✓ All W2 Mean Nucl Integr Intens	✓ Positive W3 Mean Nucl Aver Intens
✓ All W2 Mean Nucl Aver Intens	✓ Positive W3 Mean Cyto Integr Intens
✓ All W2 Mean Cyto Integr Intens	✓ Positive W3 Mean Cyto Aver Intens
✓ All W2 Mean Cyto Aver Intens	✓ Positive W3 Mean Cell Integr Intens
✓ All W2 Mean Cell Integr Intens	✓ Positive W3 Mean Cell Aver Intens
✓ All W2 Mean Cell Aver Intens	✓ Negative W3 Mean Stain Area
✓ Positive W2 Mean Stain Area	✓ Negative W3 Mean Stain Integr Intens
✓ Positive W2 Mean Stain Integr Intens	✓ Negative W3 Mean Stain Aver Intens
✓ Positive W2 Mean Stain Aver Intens	✓ Negative W3 Mean Nucl Integr Intens
✓ Positive W2 Mean Nucl Integr Intens	✓ Negative W3 Mean Nucl Aver Intens
✓ Positive W2 Mean Nucl Aver Intens	✓ Negative W3 Mean Cyto Integr Intens
✓ Positive W2 Mean Cyto Integr Intens	✓ Negative W3 Mean Cyto Aver Intens
✓ Positive W2 Mean Cyto Aver Intens	✓ Negative W3 Mean Cell Integr Intens
✓ Positive W2 Mean Cell Integr Intens	✓ Negative W3 Mean Cell Aver Intens
✓ Positive W2 Mean Cell Aver Intens	✓ Positive W3
✓ Negative W2 Mean Stain Area	✓ % Positive W3
✓ Negative W2 Mean Stain Integr Intens	✓ Scoring Profile 1--
✓ Negative W2 Mean Stain Aver Intens	✓ Scoring Profile 12-
✓ Negative W2 Mean Nucl Integr Intens	✓ Scoring Profile 1-3
✓ Negative W2 Mean Nucl Aver Intens	✓ Scoring Profile 123
✓ Negative W2 Mean Cyto Integr Intens	✓ Percentage Scoring Profile 1--
✓ Negative W2 Mean Cyto Aver Intens	✓ Percentage Scoring Profile 12-
✓ Negative W2 Mean Cell Integr Intens	✓ Percentage Scoring Profile 1-3
✓ Negative W2 Mean Cell Aver Intens	✓ Percentage Scoring Profile 123
✓ Positive W2	✓ Subtotal Profile 1xx
✓ % Positive W2	✓ Subtotal Profile 12x
✓ All W3 Mean Stain Area	✓ Subtotal Profile 1x3
✓ All W3 Mean Stain Integr Intens	✓ Subtotal Profile 123
✓ All W3 Mean Stain Aver Intens	✓ Percentage Subtotal Profile 1xx
✓ All W3 Mean Nucl Integr Intens	✓ Percentage Subtotal Profile 12x
✓ All W3 Mean Nucl Aver Intens	✓ Percentage Subtotal Profile 1x3
✓ All W3 Mean Cyto Integr Intens	✓ Percentage Subtotal Profile 123

- **Percentage Scoring Profile 1--:** The number of cells positive for W1, negative for W2 and negative for W3, divided by the total number of cells, times 100
- **Percentage Scoring Profile 12-:** The number of cells positive for W1, positive for W2 and negative for W3, divided by the total number of cells, times 100
- **Percentage Scoring Profile 1-3:** The number of cells positive for W1, negative for W2 and positive for W3, divided by the total number of cells, times 100
- **Percentage Scoring Profile 123:** The number of cells positive for W1, W2 and W3, divided by the total number of cells, times 100
- *(similar for additional wavelengths)*

# Summary Data (site-by-site measurements)

✓ Total Cells	✓ All W3 Mean Cyto Integr Intens
✓ All Cells Mean Area	✓ All W3 Mean Cyto Aver Intens
✓ All Nuclei Mean Area	✓ All W3 Mean Cell Integr Intens
✓ All Nuclei Mean Integr Intensity	✓ All W3 Mean Cell Aver Intens
✓ All Nuclei Mean Average Intensity	✓ Positive W3 Mean Stain Area
✓ All W2 Mean Stain Area	✓ Positive W3 Mean Stain Integr Intens
✓ All W2 Mean Stain Integr Intens	✓ Positive W3 Mean Stain Aver Intens
✓ All W2 Mean Stain Aver Intens	✓ Positive W3 Mean Nucl Integr Intens
✓ All W2 Mean Nucl Integr Intens	✓ Positive W3 Mean Nucl Aver Intens
✓ All W2 Mean Nucl Aver Intens	✓ Positive W3 Mean Cyto Integr Intens
✓ All W2 Mean Cyto Integr Intens	✓ Positive W3 Mean Cyto Aver Intens
✓ All W2 Mean Cyto Aver Intens	✓ Positive W3 Mean Cell Integr Intens
✓ All W2 Mean Cell Integr Intens	✓ Positive W3 Mean Cell Aver Intens
✓ All W2 Mean Cell Aver Intens	✓ Negative W3 Mean Stain Area
✓ Positive W2 Mean Stain Area	✓ Negative W3 Mean Stain Integr Intens
✓ Positive W2 Mean Stain Integr Intens	✓ Negative W3 Mean Stain Aver Intens
✓ Positive W2 Mean Stain Aver Intens	✓ Negative W3 Mean Nucl Integr Intens
✓ Positive W2 Mean Nucl Integr Intens	✓ Negative W3 Mean Nucl Aver Intens
✓ Positive W2 Mean Nucl Aver Intens	✓ Negative W3 Mean Cyto Integr Intens
✓ Positive W2 Mean Cyto Integr Intens	✓ Negative W3 Mean Cyto Aver Intens
✓ Positive W2 Mean Cyto Aver Intens	✓ Negative W3 Mean Cell Integr Intens
✓ Positive W2 Mean Cell Integr Intens	✓ Negative W3 Mean Cell Aver Intens
✓ Positive W2 Mean Cell Aver Intens	✓ Positive W3
✓ Negative W2 Mean Stain Area	✓ % Positive W3
✓ Negative W2 Mean Stain Integr Intens	✓ Scoring Profile 1--
✓ Negative W2 Mean Stain Aver Intens	✓ Scoring Profile 12-
✓ Negative W2 Mean Nucl Integr Intens	✓ Scoring Profile 1-3
✓ Negative W2 Mean Nucl Aver Intens	✓ Scoring Profile 123
✓ Negative W2 Mean Cyto Integr Intens	✓ Percentage Scoring Profile 1--
✓ Negative W2 Mean Cyto Aver Intens	✓ Percentage Scoring Profile 12-
✓ Negative W2 Mean Cell Integr Intens	✓ Percentage Scoring Profile 1-3
✓ Negative W2 Mean Cell Aver Intens	✓ Percentage Scoring Profile 123
✓ Positive W2	✓ Subtotal Profile 1xx
✓ % Positive W2	✓ Subtotal Profile 12x
✓ All W3 Mean Stain Area	✓ Subtotal Profile 1x3
✓ All W3 Mean Stain Integr Intens	✓ Subtotal Profile 123
✓ All W3 Mean Stain Aver Intens	✓ Percentage Subtotal Profile 1xx
✓ All W3 Mean Nucl Integr Intens	✓ Percentage Subtotal Profile 12x
✓ All W3 Mean Nucl Aver Intens	✓ Percentage Subtotal Profile 1x3
✓ All W3 Mean Cyto Integr Intens	✓ Percentage Subtotal Profile 123

- **Subtotal Profile 1xx:** The total number of cells positive for W1, and either positive or negative for W2 and W3
- **Subtotal Profile 12x:** The total number of cells positive for W1 and W2, and either positive or negative for W3
- **Subtotal Profile 1x3:** The total number of cells positive for W1 and W3, and either positive or negative for W2
- **Subtotal Profile 123:** The total number of cells positive for W1, W2 and W3
- *(similar for additional wavelengths)*

# Summary Data (site-by-site measurements)

✓ Total Cells	✓ All W3 Mean Cyto Integr Intens
✓ All Cells Mean Area	✓ All W3 Mean Cyto Aver Intens
✓ All Nuclei Mean Area	✓ All W3 Mean Cell Integr Intens
✓ All Nuclei Mean Integr Intensity	✓ All W3 Mean Cell Aver Intens
✓ All Nuclei Mean Average Intensity	✓ Positive W3 Mean Stain Area
✓ All W2 Mean Stain Area	✓ Positive W3 Mean Stain Integr Intens
✓ All W2 Mean Stain Integr Intens	✓ Positive W3 Mean Stain Aver Intens
✓ All W2 Mean Stain Aver Intens	✓ Positive W3 Mean Nucl Integr Intens
✓ All W2 Mean Nucl Integr Intens	✓ Positive W3 Mean Nucl Aver Intens
✓ All W2 Mean Nucl Aver Intens	✓ Positive W3 Mean Cyto Integr Intens
✓ All W2 Mean Cyto Integr Intens	✓ Positive W3 Mean Cyto Aver Intens
✓ All W2 Mean Cyto Aver Intens	✓ Positive W3 Mean Cell Integr Intens
✓ All W2 Mean Cell Integr Intens	✓ Positive W3 Mean Cell Aver Intens
✓ All W2 Mean Cell Aver Intens	✓ Negative W3 Mean Stain Area
✓ Positive W2 Mean Stain Area	✓ Negative W3 Mean Stain Integr Intens
✓ Positive W2 Mean Stain Integr Intens	✓ Negative W3 Mean Stain Aver Intens
✓ Positive W2 Mean Stain Aver Intens	✓ Negative W3 Mean Nucl Integr Intens
✓ Positive W2 Mean Nucl Integr Intens	✓ Negative W3 Mean Nucl Aver Intens
✓ Positive W2 Mean Nucl Aver Intens	✓ Negative W3 Mean Cyto Integr Intens
✓ Positive W2 Mean Cyto Integr Intens	✓ Negative W3 Mean Cyto Aver Intens
✓ Positive W2 Mean Cyto Aver Intens	✓ Negative W3 Mean Cell Integr Intens
✓ Positive W2 Mean Cell Integr Intens	✓ Negative W3 Mean Cell Aver Intens
✓ Positive W2 Mean Cell Aver Intens	✓ Positive W3
✓ Negative W2 Mean Stain Area	✓ % Positive W3
✓ Negative W2 Mean Stain Integr Intens	✓ Scoring Profile 1--
✓ Negative W2 Mean Stain Aver Intens	✓ Scoring Profile 12-
✓ Negative W2 Mean Nucl Integr Intens	✓ Scoring Profile 1-3
✓ Negative W2 Mean Nucl Aver Intens	✓ Scoring Profile 123
✓ Negative W2 Mean Cyto Integr Intens	✓ Percentage Scoring Profile 1--
✓ Negative W2 Mean Cyto Aver Intens	✓ Percentage Scoring Profile 12-
✓ Negative W2 Mean Cell Integr Intens	✓ Percentage Scoring Profile 1-3
✓ Negative W2 Mean Cell Aver Intens	✓ Percentage Scoring Profile 123
✓ Positive W2	✓ Subtotal Profile 1xx
✓ % Positive W2	✓ Subtotal Profile 12x
✓ All W3 Mean Stain Area	✓ Subtotal Profile 1x3
✓ All W3 Mean Stain Integr Intens	✓ Subtotal Profile 123
✓ All W3 Mean Stain Aver Intens	✓ Percentage Subtotal Profile 1xx
✓ All W3 Mean Nucl Integr Intens	✓ Percentage Subtotal Profile 12x
✓ All W3 Mean Nucl Aver Intens	✓ Percentage Subtotal Profile 1x3
✓ All W3 Mean Cyto Integr Intens	✓ Percentage Subtotal Profile 123

- **Percentage Subtotal Profile 1xx:** The number of cells positive for W1, and either positive or negative for W2 and W3, divided by the total number of cells, times 100
- **Percentage Subtotal Profile 12x:** The number of cells positive for W1 and W2, and either positive or negative for W3, divided by the total number of cells, times 100
- **Percentage Subtotal Profile 1x3:** The number of cells positive for W1 and W3, and either positive or negative for W2, divided by the total number of cells, times 100
- **Percentage Subtotal Profile 123:** The number of cells positive for W1, W2 and W3, divided by the total number of cells, times 100
- *(similar for additional wavelengths)*

# Cell Data (cell-by-cell measurements)

- ✓ Cell: Assigned Label #
- ✓ Cell: Scoring Profile
- ✓ Cell: Custom Profile Name
- ✓ Cell: Total Area
- ✓ W1 Stained Area
- ✓ W1 Integrated Intensity
- ✓ W1 Average Intensity
- ✓ Cell: Positive W2
- ✓ Cell: W2 Stained Area
- ✓ Cell: W2 Stained Integr Intensity
- ✓ Cell: W2 Stained Average Intensity
- ✓ Cell: W2 Nucleus Integr Intensity
- ✓ Cell: W2 Nucleus Average Intensity
- ✓ Cell: W2 Cytoplasm Integr Intensity
- ✓ Cell: W2 Cytoplasm Average Intensity
- ✓ Cell: W2 Cell Integr Intensity
- ✓ Cell: W2 Cell Average Intensity
- ✓ Cell: Positive W3
- ✓ Cell: W3 Stained Area
- ✓ Cell: W3 Stained Integr Intensity
- ✓ Cell: W3 Stained Average Intensity
- ✓ Cell: W3 Nucleus Integr Intensity
- ✓ Cell: W3 Nucleus Average Intensity
- ✓ Cell: W3 Cytoplasm Integr Intensity
- ✓ Cell: W3 Cytoplasm Average Intensity
- ✓ Cell: W3 Cell Integr Intensity
- ✓ Cell: W3 Cell Average Intensity

- **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 Data (cell-by-cell measurements)

- ✓ Cell: Assigned Label #
- ✓ Cell: Scoring Profile
- ✓ Cell: Custom Profile Name
- ✓ Cell: Total Area
- ✓ W1 Stained Area
- ✓ W1 Integrated Intensity
- ✓ W1 Average Intensity
- ✓ Cell: Positive W2
- ✓ Cell: W2 Stained Area
- ✓ Cell: W2 Stained Integr Intensity
- ✓ Cell: W2 Stained Average Intensity
- ✓ Cell: W2 Nucleus Integr Intensity
- ✓ Cell: W2 Nucleus Average Intensity
- ✓ Cell: W2 Cytoplasm Integr Intensity
- ✓ Cell: W2 Cytoplasm Average Intensity
- ✓ Cell: W2 Cell Integr Intensity
- ✓ Cell: W2 Cell Average Intensity
- ✓ Cell: Positive W3
- ✓ Cell: W3 Stained Area
- ✓ Cell: W3 Stained Integr Intensity
- ✓ Cell: W3 Stained Average Intensity
- ✓ Cell: W3 Nucleus Integr Intensity
- ✓ Cell: W3 Nucleus Average Intensity
- ✓ Cell: W3 Cytoplasm Integr Intensity
- ✓ Cell: W3 Cytoplasm Average Intensity
- ✓ Cell: W3 Cell Integr Intensity
- ✓ Cell: W3 Cell Average Intensity

- **Cell: Total Area** – Total square microns of the cell (nucleus + cytoplasm as defined by the combination of all wavelengths)
- **W1 Stained Area** – Total square microns of the nucleus as defined by the W1 stain
- **W1 Integrated Intensity** – Total pixel intensity of the nuclear stain in the nucleus
- **W1 Average Intensity** – Average pixel intensity of the nuclear stain in the nucleus

# Cell Data (cell-by-cell measurements)

- ✓ Cell: Assigned Label #
- ✓ Cell: Scoring Profile
- ✓ Cell: Custom Profile Name
- ✓ Cell: Total Area
- ✓ W1 Stained Area
- ✓ W1 Integrated Intensity
- ✓ W1 Average Intensity
- ✓ Cell: Positive W2
- ✓ Cell: W2 Stained Area
- ✓ Cell: W2 Stained Integr Intensity
- ✓ Cell: W2 Stained Average Intensity
- ✓ Cell: W2 Nucleus Integr Intensity
- ✓ Cell: W2 Nucleus Average Intensity
- ✓ Cell: W2 Cytoplasm Integr Intensity
- ✓ Cell: W2 Cytoplasm Average Intensity
- ✓ Cell: W2 Cell Integr Intensity
- ✓ Cell: W2 Cell Average Intensity
- ✓ Cell: Positive W3
- ✓ Cell: W3 Stained Area
- ✓ Cell: W3 Stained Integr Intensity
- ✓ Cell: W3 Stained Average Intensity
- ✓ Cell: W3 Nucleus Integr Intensity
- ✓ Cell: W3 Nucleus Average Intensity
- ✓ Cell: W3 Cytoplasm Integr Intensity
- ✓ Cell: W3 Cytoplasm Average Intensity
- ✓ Cell: W3 Cell Integr Intensity
- ✓ Cell: W3 Cell Average Intensity

- **Cell: Positive W2** – Classification of the cell as positive for staining in wavelength 2 (value of 1) or negative for staining in wavelength 2 (value of 0)
- **Cell: W2 Stained Area** – Total square microns of the area stained by W2 as defined in the settings (nucleus, cytoplasm, or nucleus + cytoplasm)
- **Cell: W2 Stained Integr Intensity** – Total pixel intensity of the W2 stain in the defined area
- **Cell: W2 Stained Average Intensity** – Average pixel intensity of the W2 stain in the defined area
- *(similar for W3-W7)*

# Cell Data (cell-by-cell measurements)

- ✓ Cell: Assigned Label #
- ✓ Cell: Scoring Profile
- ✓ Cell: Custom Profile Name
- ✓ Cell: Total Area
- ✓ W1 Stained Area
- ✓ W1 Integrated Intensity
- ✓ W1 Average Intensity
- ✓ Cell: Positive W2
- ✓ Cell: W2 Stained Area
- ✓ Cell: W2 Stained Integr Intensity
- ✓ Cell: W2 Stained Average Intensity
- ✓ Cell: W2 Nucleus Integr Intensity
- ✓ Cell: W2 Nucleus Average Intensity
- ✓ Cell: W2 Cytoplasm Integr Intensity
- ✓ Cell: W2 Cytoplasm Average Intensity
- ✓ Cell: W2 Cell Integr Intensity
- ✓ Cell: W2 Cell Average Intensity
- ✓ Cell: Positive W3
- ✓ Cell: W3 Stained Area
- ✓ Cell: W3 Stained Integr Intensity
- ✓ Cell: W3 Stained Average Intensity
- ✓ Cell: W3 Nucleus Integr Intensity
- ✓ Cell: W3 Nucleus Average Intensity
- ✓ Cell: W3 Cytoplasm Integr Intensity
- ✓ Cell: W3 Cytoplasm Average Intensity
- ✓ Cell: W3 Cell Integr Intensity
- ✓ Cell: W3 Cell Average Intensity

- **Cell: W2 Nucleus Integr Intensity** – Total pixel intensity of the W2 stain in the nucleus
- **Cell: W2 Nucleus Average Intensity** – Average pixel intensity of the W2 stain in the nucleus
- *(similar for W3-W7)*

# Cell Data (cell-by-cell measurements)

- ✓ Cell: Assigned Label #
- ✓ Cell: Scoring Profile
- ✓ Cell: Custom Profile Name
- ✓ Cell: Total Area
- ✓ W1 Stained Area
- ✓ W1 Integrated Intensity
- ✓ W1 Average Intensity
- ✓ Cell: Positive W2
- ✓ Cell: W2 Stained Area
- ✓ Cell: W2 Stained Integr Intensity
- ✓ Cell: W2 Stained Average Intensity
- ✓ Cell: W2 Nucleus Integr Intensity
- ✓ Cell: W2 Nucleus Average Intensity
- ✓ Cell: W2 Cytoplasm Integr Intensity
- ✓ Cell: W2 Cytoplasm Average Intensity
- ✓ Cell: W2 Cell Integr Intensity
- ✓ Cell: W2 Cell Average Intensity
- ✓ Cell: Positive W3
- ✓ Cell: W3 Stained Area
- ✓ Cell: W3 Stained Integr Intensity
- ✓ Cell: W3 Stained Average Intensity
- ✓ Cell: W3 Nucleus Integr Intensity
- ✓ Cell: W3 Nucleus Average Intensity
- ✓ Cell: W3 Cytoplasm Integr Intensity
- ✓ Cell: W3 Cytoplasm Average Intensity
- ✓ Cell: W3 Cell Integr Intensity
- ✓ Cell: W3 Cell Average Intensity

- **Cell: W2 Cytoplasm Integr Intensity** – Total pixel intensity of the W2 stain in the cytoplasm
- **Cell: W2 Cytoplasm Average Intensity** – Average pixel intensity of the W2 stain in the cytoplasm
- *(similar for W3-W7)*

# Cell Data (cell-by-cell measurements)

- ✓ Cell: Assigned Label #
- ✓ Cell: Scoring Profile
- ✓ Cell: Custom Profile Name
- ✓ Cell: Total Area
- ✓ W1 Stained Area
- ✓ W1 Integrated Intensity
- ✓ W1 Average Intensity
- ✓ Cell: Positive W2
- ✓ Cell: W2 Stained Area
- ✓ Cell: W2 Stained Integr Intensity
- ✓ Cell: W2 Stained Average Intensity
- ✓ Cell: W2 Nucleus Integr Intensity
- ✓ Cell: W2 Nucleus Average Intensity
- ✓ Cell: W2 Cytoplasm Integr Intensity
- ✓ Cell: W2 Cytoplasm Average Intensity
- ✓ Cell: W2 Cell Integr Intensity
- ✓ Cell: W2 Cell Average Intensity
- ✓ Cell: Positive W3
- ✓ Cell: W3 Stained Area
- ✓ Cell: W3 Stained Integr Intensity
- ✓ Cell: W3 Stained Average Intensity
- ✓ Cell: W3 Nucleus Integr Intensity
- ✓ Cell: W3 Nucleus Average Intensity
- ✓ Cell: W3 Cytoplasm Integr Intensity
- ✓ Cell: W3 Cytoplasm Average Intensity
- ✓ Cell: W3 Cell Integr Intensity
- ✓ Cell: W3 Cell Average Intensity

- **Cell: W2 Cell Integr Intensity –**  
Total pixel intensity of the W2 stain in the cell (nucleus + cytoplasm)
- **Cell: W2 Cell Average Intensity –**  
Average pixel intensity of the W2 stain in the cell (nucleus + cytoplasm)
- *(similar for W3-W7)*



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