MetaXpress® 6 Software Guide

Application Modules: Cell Scoring

MOLECULAR DEVICES
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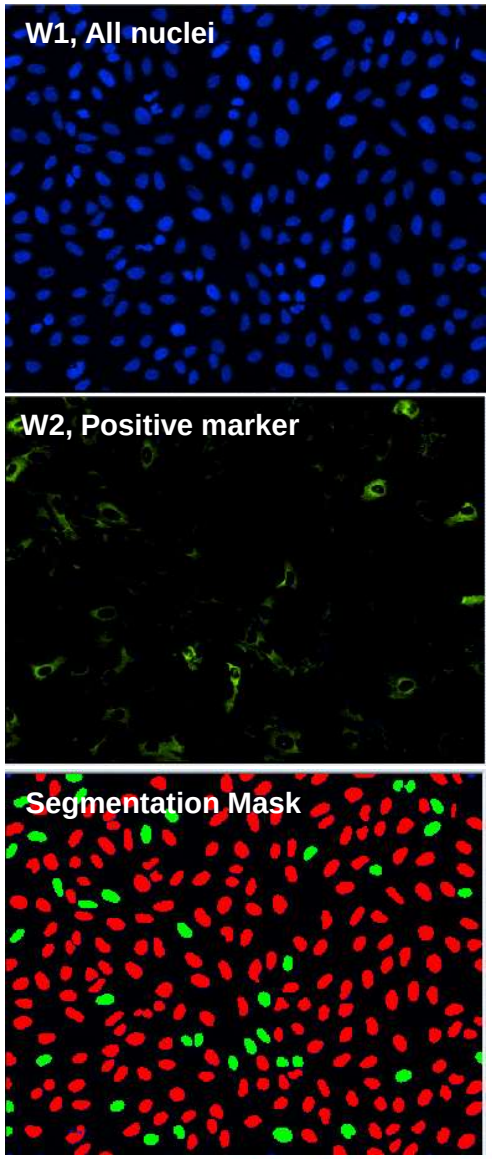
Cell Scoring Application Module Overview

- The **Cell Scoring** application module can be used for identification of two subpopulations of cells based on a fluorescent marker for **all nuclei** (e.g. DAPI, Hoechst, or DRAQ5) and an additional marker for cells of interest. The second stain can label either nuclei, cytoplasm, or both.

- The module makes a number of measurements including the number of cells scored positive and the percent of cells scored positive as detected by the marker for the second wavelength (W2).

- In the segmentation mask, cells scored as **positive** for W2 are indicated in **green** and cells **negative** for W2 in **red**.



Module Settings: Selecting an Image & Algorithm

All nuclei (W1 Source image)

- Select the image for all nuclei.

Positive marker (W2 Source image)

- Select the image for positive marker.
- Select the **Stained area**: Nucleus, Cytoplasm or Nucleus and Cytoplasm.

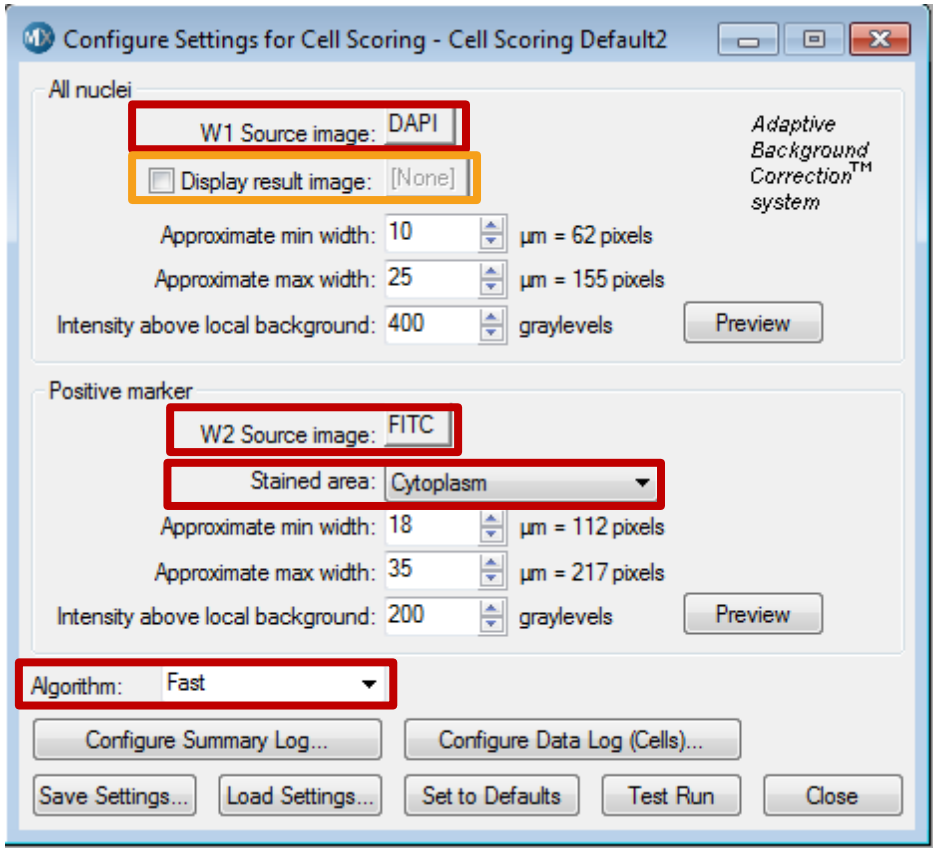
**NOTE* Do not choose images with "HTS" in the name.*

Display result image:

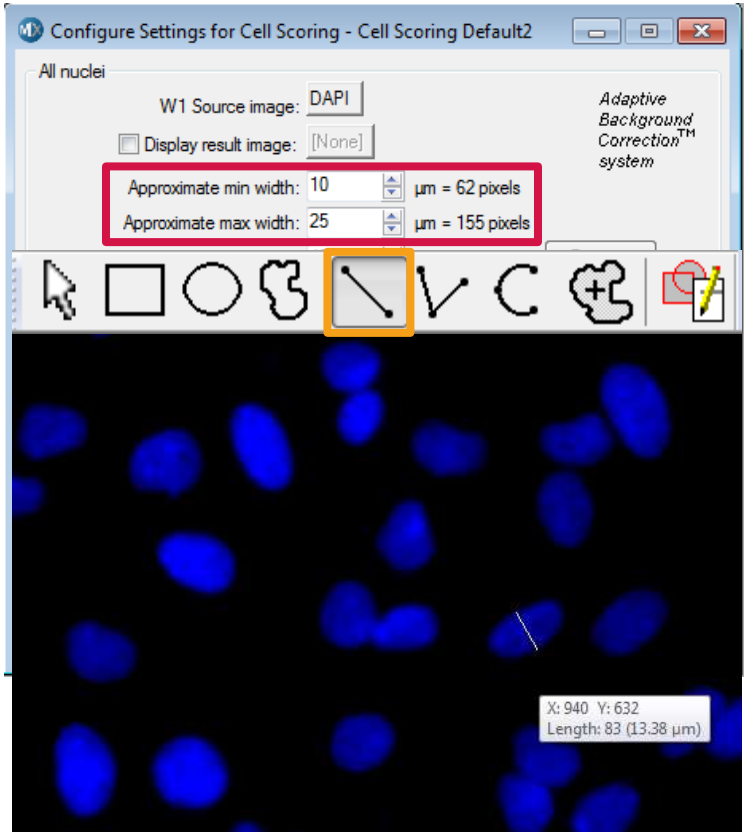
- Leave **Display result image** deselected (this is generally only used when creating a journal).

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** .



Module Settings: Defining Width of W1 Objects



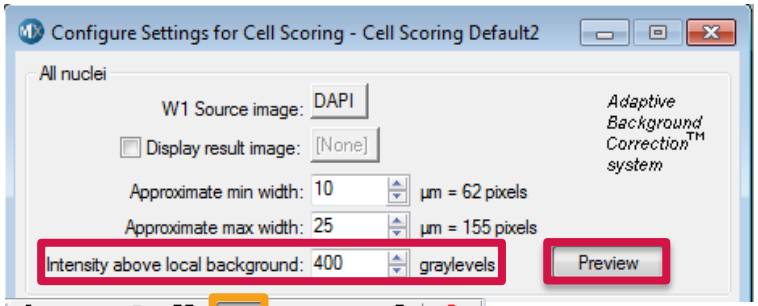
Approximate min and max widths: All nuclei

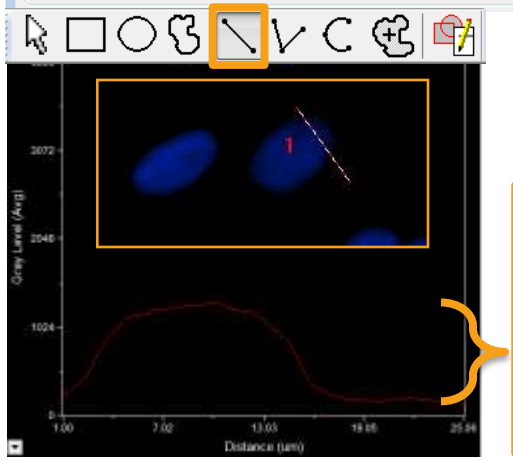
- Select the single line region from **Regions Tools**
- Click and drag across a representative small and large object; a tooltip will show the length of the line. Use a single click to start the line and a single click to finish.

NOTE Do not click the image again. This will cause the tooltip to disappear. If the tooltip disappears, repeat the drawing procedure.

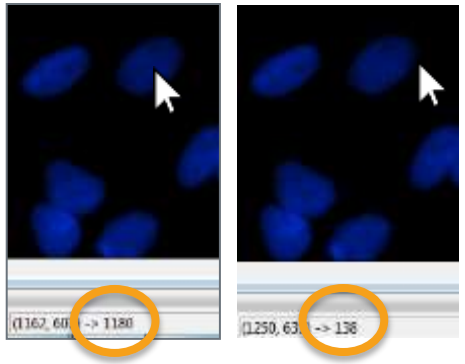
- The width is the short axis of a object (in µm)
- Approximate min width has the most drastic effect. If min width is too large, much smaller objects will be ignored; if min width is too small, much larger objects will be split.

Module Settings: Defining W1 Object Intensity





Use the difference in intensity values to set **Intensity above local background**

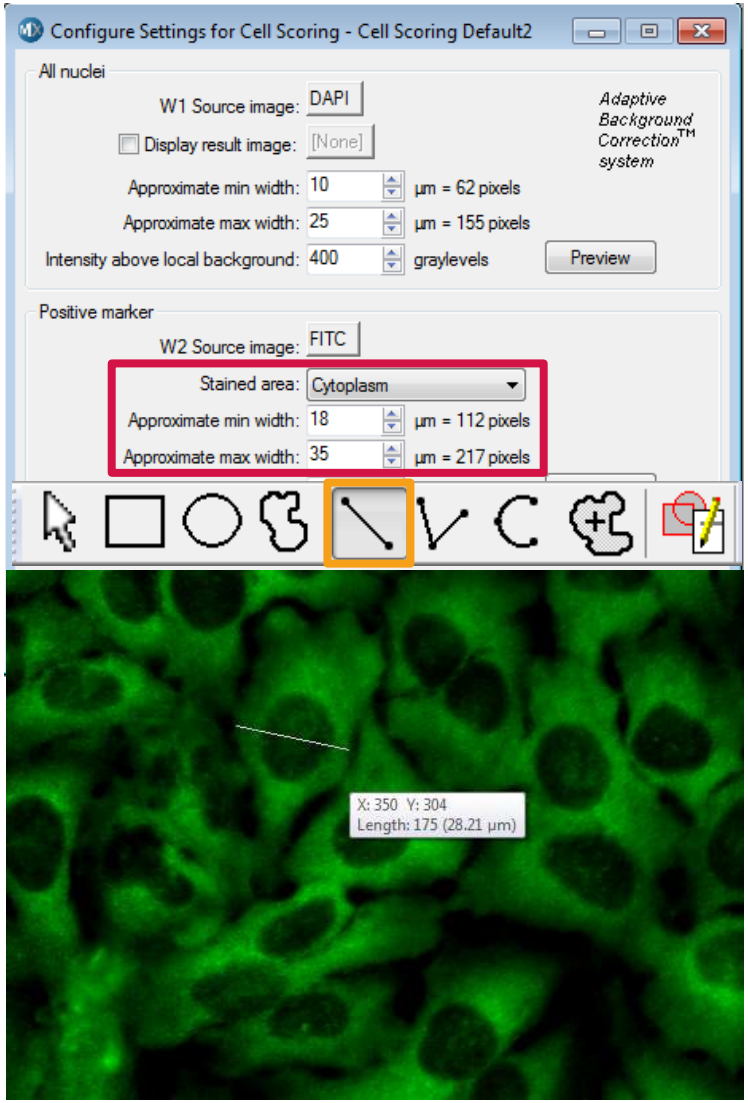


Mouse over the dimmest object and outside to measure intensity values. Use the difference to set **Intensity above local background**

Intensity above local background: All nuclei

- Draw a line over the dimmest object of interest that covers both the object and background using the Single line tool. Use a single click to start the line and a single click to finish.
- In the main menu, select **Measure > Intensities > Linescan**. This will display a histogram of intensity values; or simply mouse over the dimmest object of interest and the background and view the intensity values.
- For **Fast algorithms**, set **Intensity above local background** to half (or less) of the difference in intensity between an object and background.
  - Example: 1180 – 138 = 1042/2 = 521 (or less)
- For **Standard algorithms**, set this value slightly lower than the difference.
  - Example: 1180 – 138 = 1042 – 100. Start setting to 942
- Click on **Preview** (to test individual wavelength) and adjust values as necessary. Only change one parameter at a time.

Module Settings: Defining Width of W2 Objects



Approximate min and max widths: Positive marker

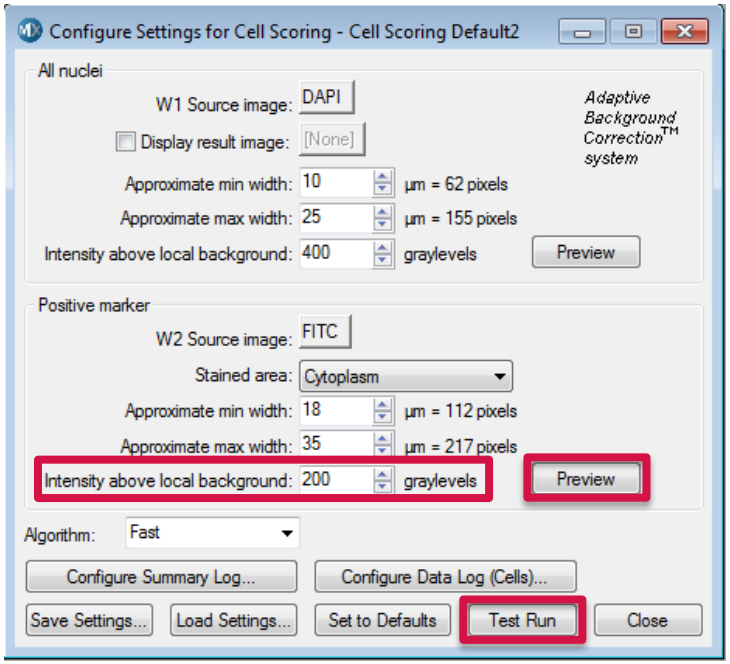
- Stained area of the positive marker (W2) can be nucleus, cytoplasm or nucleus and cytoplasm.
- For a **nuclear stain**, use the same width settings as for W1.
- For a **cytoplasmic** or **nuclear + cytoplasmic stain**, measure the whole cell.

- Select the single line region from **Regions Tools**
- Click and drag across a representative small and large object; a tooltip will show the length of the line. Use a single click to start the line and a single click to finish.

NOTE Do not click the image again. This will cause the tooltip to disappear. If the tooltip disappears, repeat the drawing procedure.

- The width is the short axis of a object (in µm).

Module Settings: Defining W2 Object Intensity



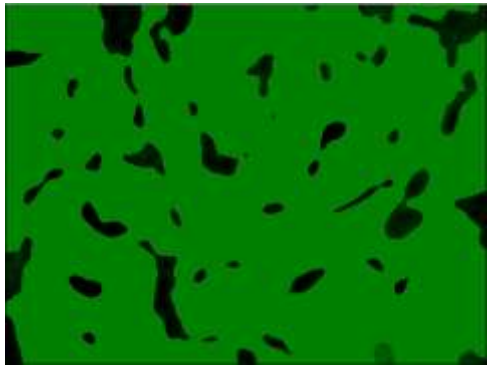
Intensity above local background: Positive marker

- The intensity above local background of the positive marker (W2) is defined the same way was measured for W1 (all nuclei).
- Click on **Preview** to test detection of the individual W2 wavelength.

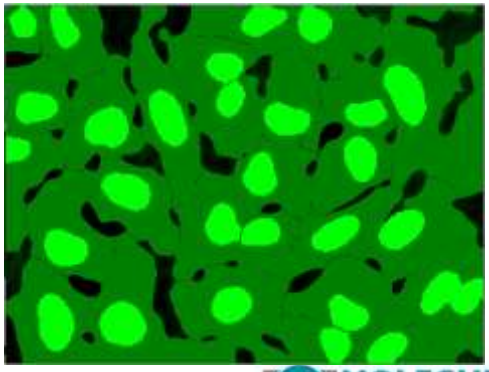
NOTE* **Preview for W2 may not accurately segment the cells as this does take the nuclear staining into account. This is most useful for testing the intensity settings for this wavelength.*

- Click on **Test Run** to test all settings based on the nuclei and positive marker – this will be the final measurement mask for W2. Adjust values as necessary by changing one parameter at a time.

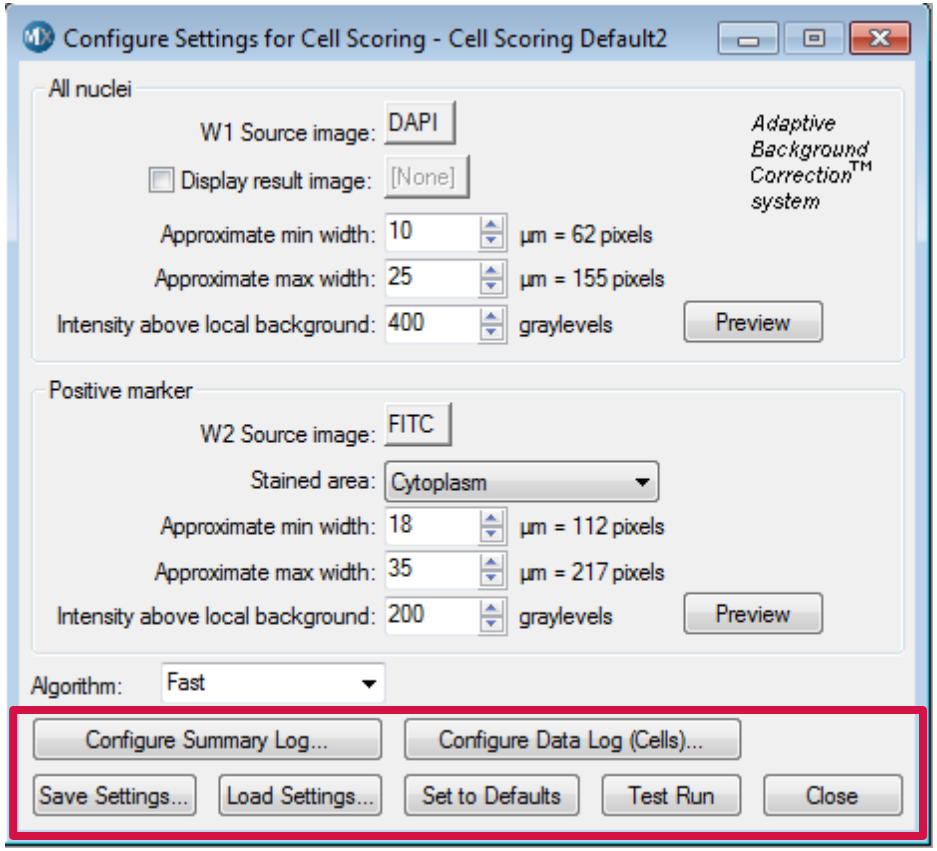
Preview
Only W2 settings



Test Run
All nuclei and W2 settings



Module Buttons



Configure Summary Log: Select image-by-image measurements.

Configure Data Log: Select cell-by-cell measurements.

Save Settings: Save application module settings.

Load Settings: Load saved application module settings.

Set to Defaults: Restore default application module settings.

Test Run: Test all settings together and display cell-by-cell results for the displayed image.

Configuring Summary or Data (Cell) Logs

Double click on a measurement to select or deselect it for logging into the database.

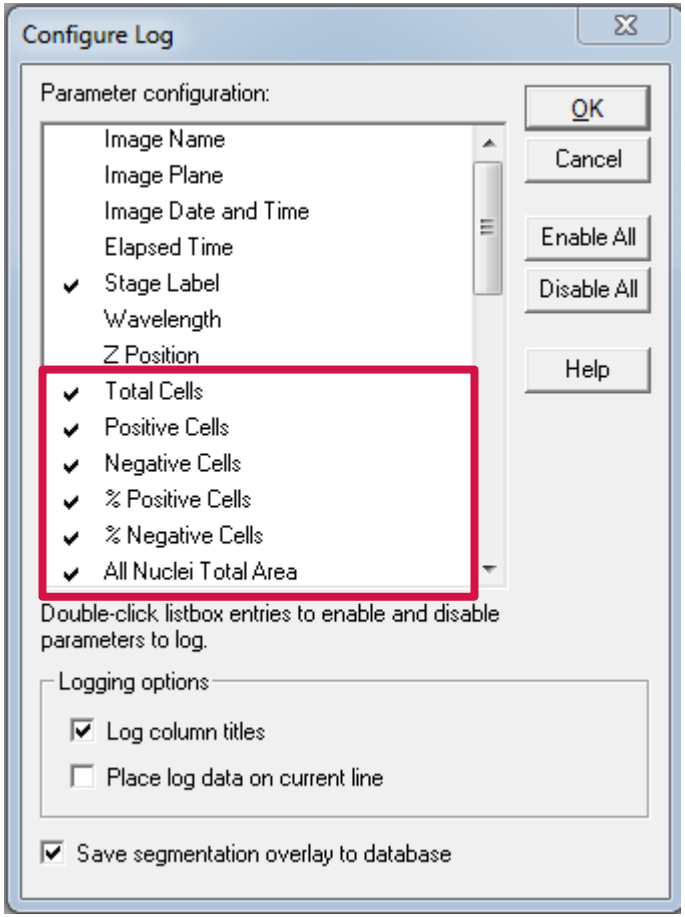
- ✓ Indicates a parameter that will be logged into the database (or Excel/text file log if open)

Log column titles: Does not affect database logging. If you have an Excel or text file log open, this records the parameter name as the column header for easy review. We recommend **enabling** this option.

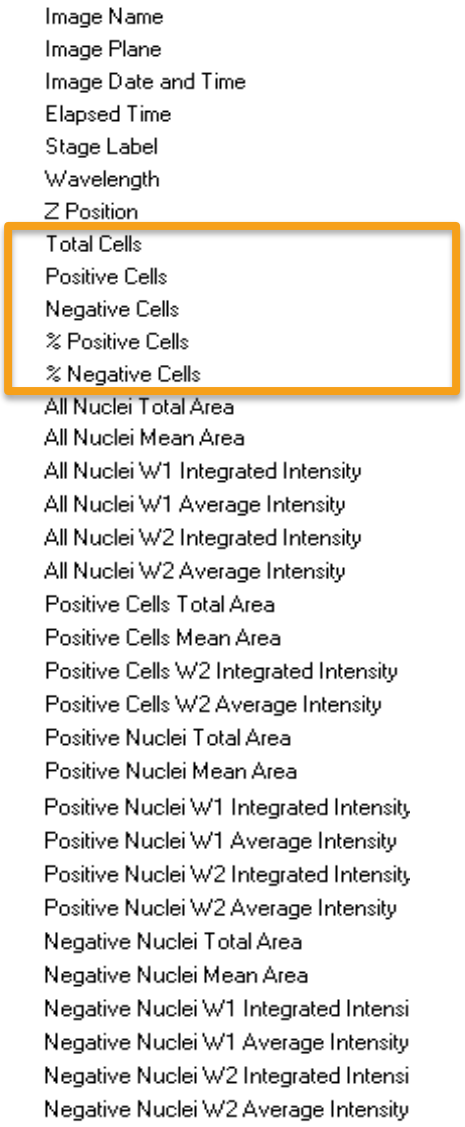
Place log data on current line: Does not affect database logging. If you have an Excel or text file log open, this records the data into the last row used, to the right of the previous data. We recommend **disabling** this option to ensure that new data is recorded into a new row.

Save segmentation overlay to database: A mask (or binary image) is temporarily created for every raw image analyzed on a plate (see Transfluor overview for an example of a mask). This option allows you to save these masks to the database. We recommend **enabling this option for assay development and disabling it for screening**.

- Pro: Allows you to quickly review your segmentation results after analysis has been run across the entire plate
- Con: These masks take up a significant amount of space in a database, which may be limited in size. Saving the masks may also slow down analysis.

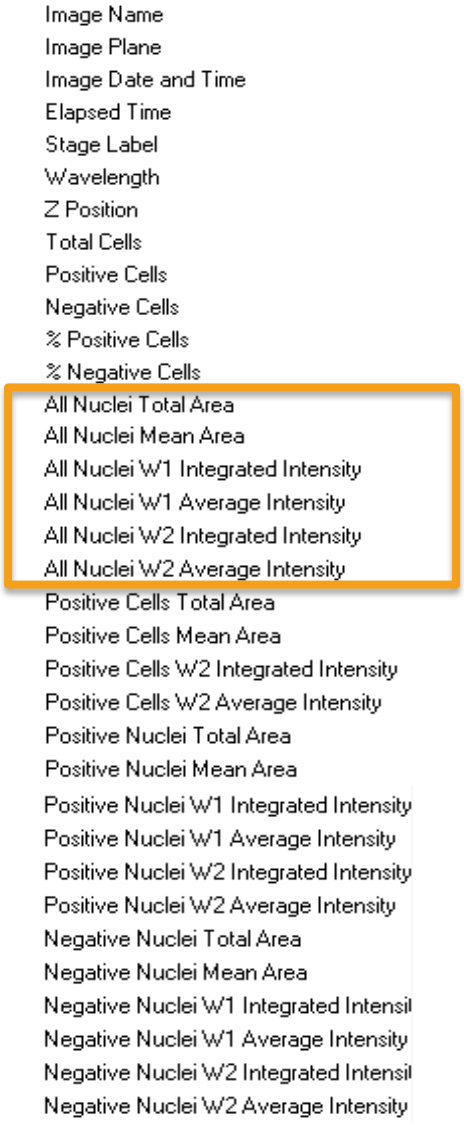


Configure Summary Log (Image Measurements)



- **Total cells:** Total number of nuclei (cell count) per image.
- **Positive Cells:** The total number of cells positive for W2 staining in the image as defined in the settings.
- **Negative Cells:** The total number of cells negative for W2 staining in the image as defined in the settings.
- **% Positive Cells:** The number of positive W2 cells divided by the total number of cells in the image, times 100.
- **% Negative Cells:** The number of negative W2 cells divided by the total number of cells in the image, times 100.

Configure Summary Log (Image Measurements)



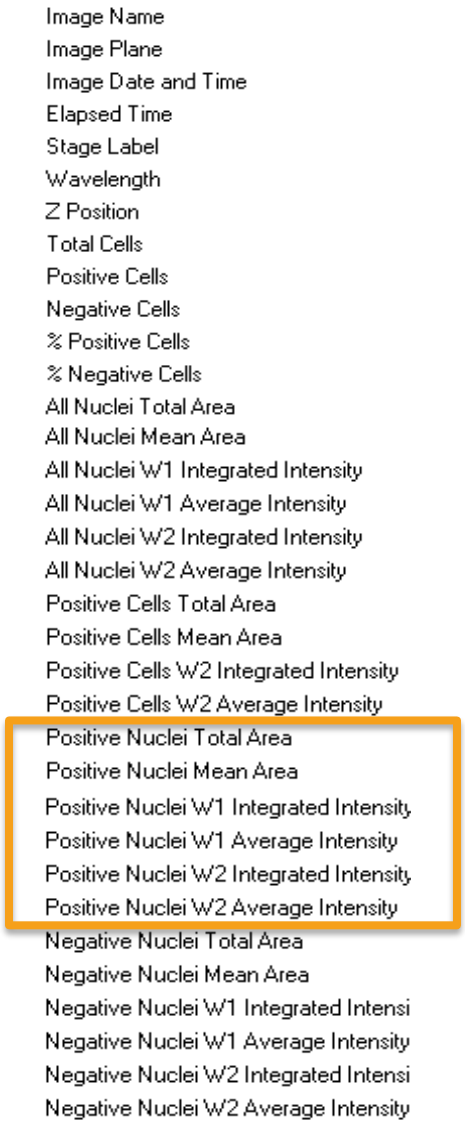
- **All Nuclei Total Area:** The total area of nuclei for all cells found in the image (in um^2).
- **All Nuclei Mean Area:** The average area of nuclei for all cells found in the image (in um^2).
- **All Nuclei W1 Integrated Intensity:** The total pixel intensity of the nuclear stain over all of the nuclear areas in the image.
- **All Nuclei W1 Average Intensity:** The average pixel intensity of the nuclear stain over all of the nuclear areas in the image.
- **All Nuclei W2 Integrated Intensity:** The total pixel intensity of the marker stain over the nuclear area (all nuclei).
- **All Nuclei W2 Average Intensity:** The average pixel intensity of the marker stain over all the nuclear areas in the image.

Configure Summary Log (Image Measurements)

Image Name
Image Plane
Image Date and Time
Elapsed Time
Stage Label
Wavelength
Z Position
Total Cells
Positive Cells
Negative Cells
% Positive Cells
% Negative Cells
All Nuclei Total Area
All Nuclei Mean Area
All Nuclei W1 Integrated Intensity
All Nuclei W1 Average Intensity
All Nuclei W2 Integrated Intensity
All Nuclei W2 Average Intensity
Positive Cells Total Area
Positive Cells Mean Area
Positive Cells W2 Integrated Intensity
Positive Cells W2 Average Intensity
Positive Nuclei Total Area
Positive Nuclei Mean Area
Positive Nuclei W1 Integrated Intensity
Positive Nuclei W1 Average Intensity
Positive Nuclei W2 Integrated Intensity
Positive Nuclei W2 Average Intensity
Negative Nuclei Total Area
Negative Nuclei Mean Area
Negative Nuclei W1 Integrated Intensi
Negative Nuclei W1 Average Intensity
Negative Nuclei W2 Integrated Intensi
Negative Nuclei W2 Average Intensity

- **Positive Cells Total Area:** The total cell area for all W2 positive cells found in the image (in um^2).
- **Positive Cells Mean Area:** The average cell area for all W2 positive cells found in the image (in um^2).
- **Positive Cells W2 Integrated Intensity:** The total pixel intensity of the marker stain over the cell area in all W2 positive cells.
- **Positive Cells W2 Average Intensity:** The average pixel intensity of the marker stain over cell area in all W2 positive cells.

Configure Summary Log (Image Measurements)



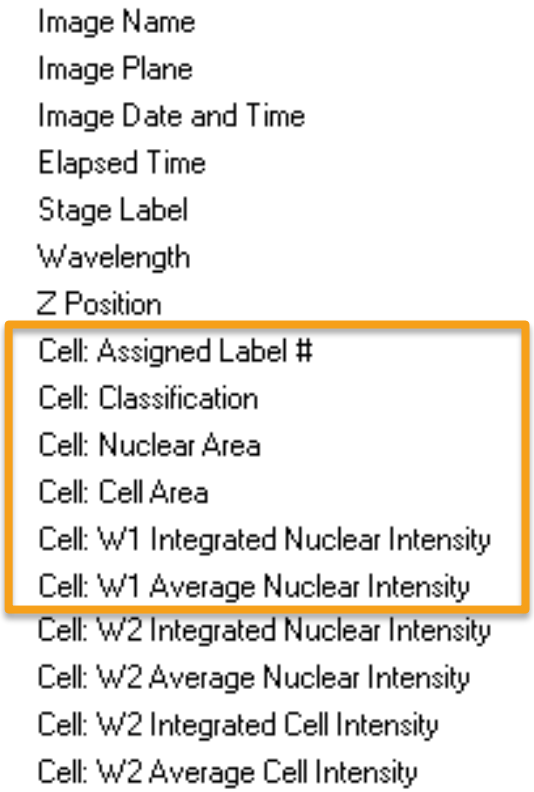
- **Positive Nuclei Total Area:** The total area of nuclei for W2 positive cells found in the image (in um^2).
- **Positive Nuclei Mean Area:** The average area of nuclei for W2 positive cells found in the image (in um^2).
- **Positive Nuclei W1 Integrated Intensity:** The total pixel intensity of the nuclear stain over the nuclear area in positive cells.
- **Positive Nuclei W1 Average Intensity:** The average pixel intensity of the nuclear stain over all the nuclear areas in positive cells.
- **Positive Nuclei W2 Integrated Intensity:** The total pixel intensity of the W2 marker stain over the nuclear area in positive cells.
- **Positive Nuclei W2 Average Intensity:** The average pixel intensity of the W2 marker stain over all the nuclear areas in positive cells.

Configure Summary Log (Image Measurements)

Image Name
Image Plane
Image Date and Time
Elapsed Time
Stage Label
Wavelength
Z Position
Total Cells
Positive Cells
Negative Cells
% Positive Cells
% Negative Cells
All Nuclei Total Area
All Nuclei Mean Area
All Nuclei W1 Integrated Intensity
All Nuclei W1 Average Intensity
All Nuclei W2 Integrated Intensity
All Nuclei W2 Average Intensity
Positive Cells Total Area
Positive Cells Mean Area
Positive Cells W2 Integrated Intensity
Positive Cells W2 Average Intensity
Positive Nuclei Total Area
Positive Nuclei Mean Area
Positive Nuclei W1 Integrated Intensity
Positive Nuclei W1 Average Intensity
Positive Nuclei W2 Integrated Intensity
Positive Nuclei W2 Average Intensity
Negative Nuclei Total Area
Negative Nuclei Mean Area
Negative Nuclei W1 Integrated Intensi
Negative Nuclei W1 Average Intensity
Negative Nuclei W2 Integrated Intensi
Negative Nuclei W2 Average Intensity

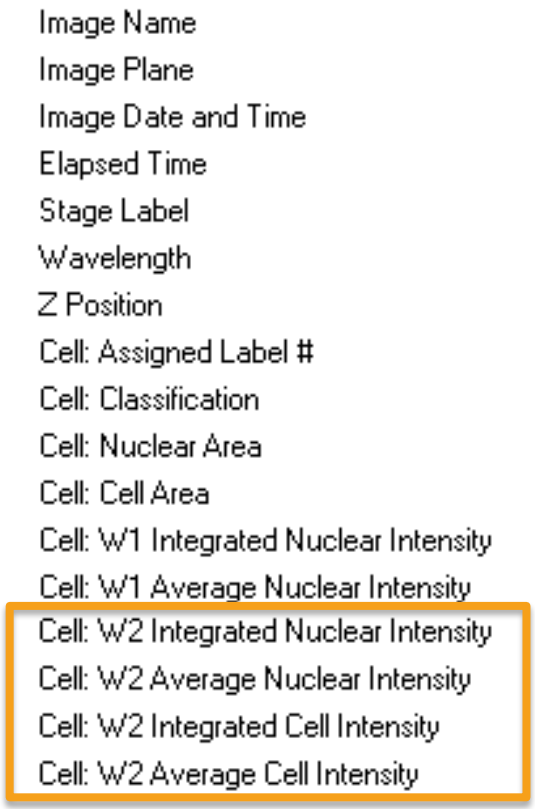
- **Negative Nuclei Total Area:** The total area of nuclei for negative cells found in the image (in um^2).
- **Negative Nuclei Mean Area:** The average area of nuclei for negative cells found in the image (in um^2).
- **Negative Nuclei W1 Integrated Intensity:** The total pixel intensity of the nuclear stain over the nuclear area in negative cells.
- **Negative Nuclei W1 Average Intensity:** The average pixel intensity of the nuclear stain over all the nuclear areas in negative cells.
- **Negative Nuclei W2 Integrated Intensity:** The total pixel intensity of the W2 marker stain over the nuclear area in negative cells.
- **Negative Nuclei W2 Average Intensity:** The average pixel intensity of the W2 marker stain over all the nuclear areas in negative cells.

Configure Data Log (Cell-by-Cell Measurements)



- **Cell: Assigned Label #** – Cell label number (1 through total cell number).
- **Cell: Classification** – individual cell is scored as “Positive” or “Negative”.
- **Cell: Nuclear Area** – Total square microns of the nucleus as defined by the W1 stain in a individual cell.
- **Cell: Cell Area** – Total square microns of a individual cell (nucleus + cytoplasm if cytoplasm selected in the settings).
- **Cell: W1 Integrated Nuclear Intensity** – Total pixel intensity of the nuclear stain in the nucleus of a individual cell.
- **Cell: W1 Average Nuclear Intensity** – Average pixel intensity of the nuclear stain in the nucleus of a individual cell.

Configure Data Log (Cell-by-Cell Measurements)



- **Cell: W2 Integrated Nuclear Intensity** – Total pixel intensity of the W2 stain in the nucleus of a individual cell.
- **Cell: W2 Average Nuclear Intensity** – Average pixel intensity of the W2 stain in the nucleus of a individual cell.
- **Cell: W2 Integrated Cell Intensity** – Total pixel intensity of the W2 stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of a individual cell.
- **Cell: W2 Average Cell Intensity** – Average pixel intensity of the W2 stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of a individual cell.

Support Resources

- F1 / HELP within MetaXpress® Software
- Support and Knowledge Base: http://mdc.custhelp.com/
- User Forum: http://metamorph.moleculardevices.com/forum/
- Request Support: http://mdc.custhelp.com/app/ask
- Technical Support can also be reached by telephone:
  - 1 (800) 635-5577
  - Select options for Tech Support → Cellular Imaging Products → ImageXpress Instruments

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