MetaXpress® 6 Software Guide

Application Modules: Mitotic Index

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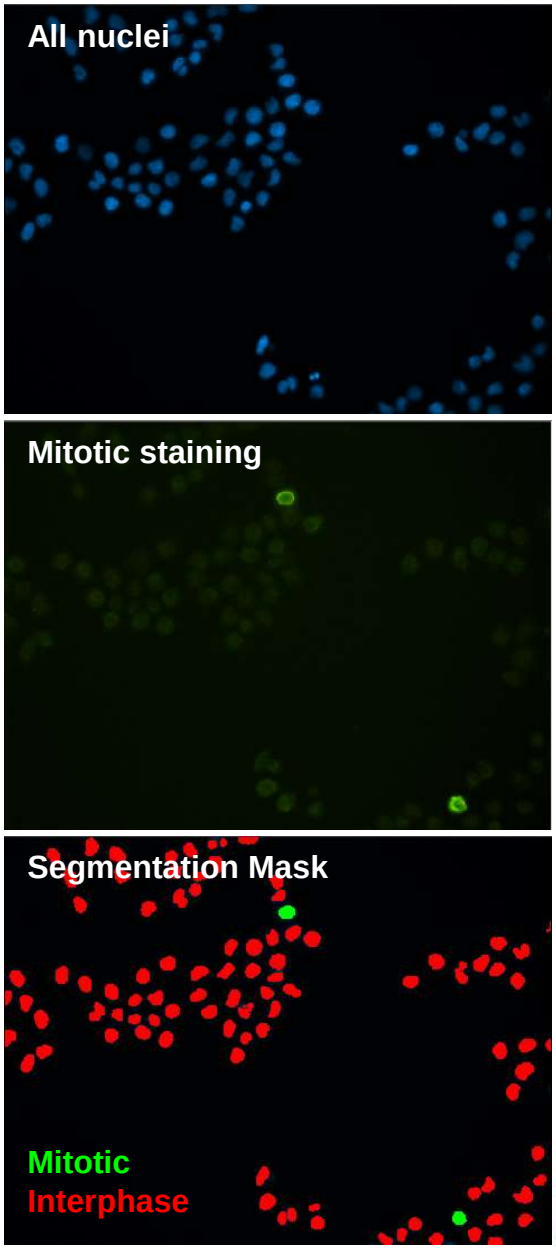
Mitotic Index Application Module Overview

- The **Mitotic Index** module can be used to analyze cellular images to differentiate between Mitotic and Interphase cells in a normal cell cycle.
- This module requires a nuclear wavelength and mitotic-specific stain wavelength.
- A typical mitotic-specific stain used for this module is Histone 3 S10 phosphorylation (a.k.a. phospho-histone H3). The DNA stain labels all cells and the mitotic cells are labeled only with the mitotic-specific stain.



Module Settings: Selecting an Image & Algorithm

All nuclei (W1 Source image)

- Select the image for all nuclei.

Mitotic staining (W2 Source image)

- Select the image for the mitotic marker.

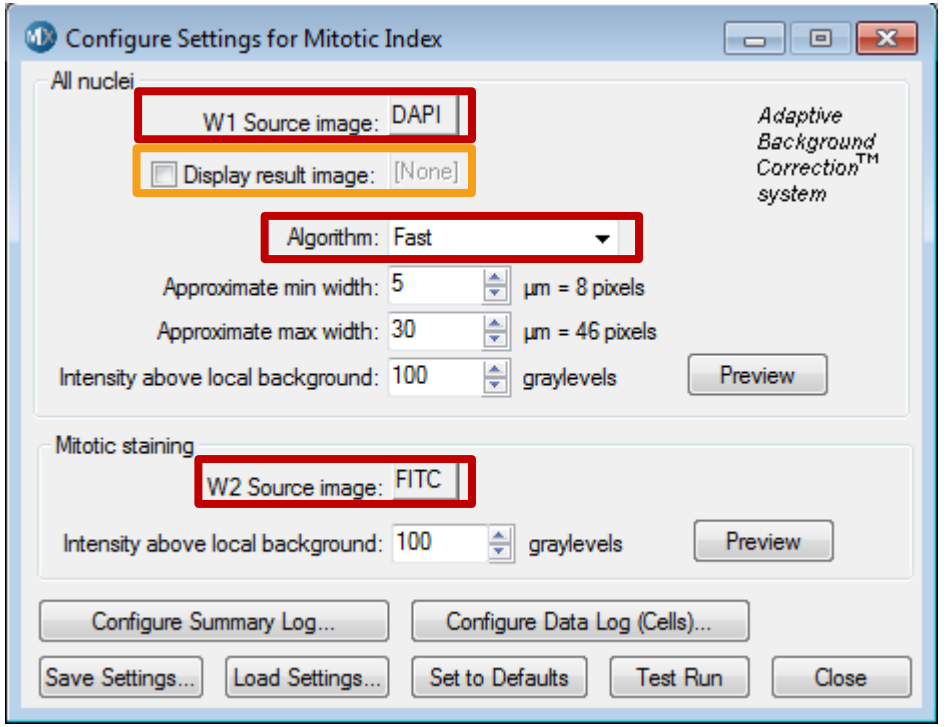
**NOTE* Do not choose images with "HTS" in the name*

Display result image:

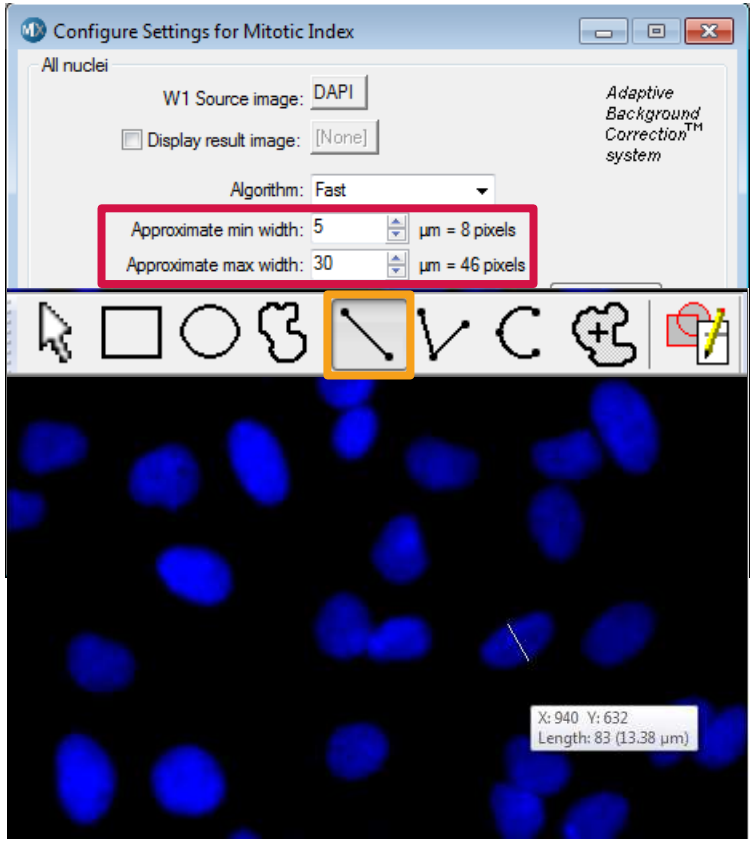
- Leave **Display result image** deselected (this is generally only used when creating a journal).

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 the Size of Objects



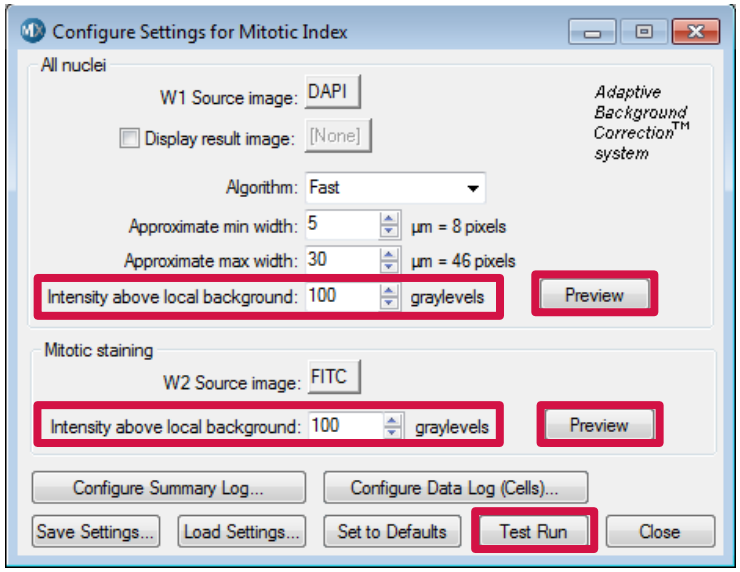
Approximate min and max widths

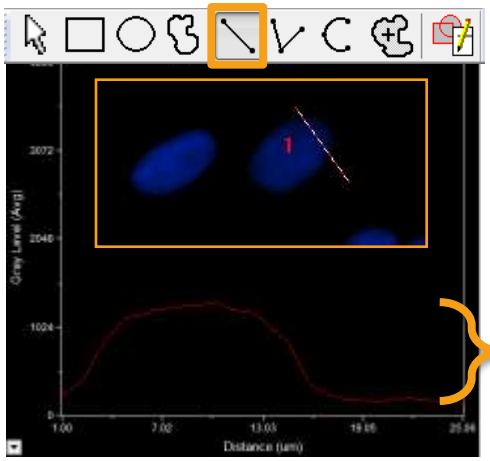
- Select the single line region from **Regions Tools.**
- Click and drag across a representative small and large object; a tooltip will show the length of the line. (single click to start a line, single click to terminate).

**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).
- Much smaller or much larger cells will be ignored.
- Click **Apply** and adjust values as necessary
- Repeat for all desired objects (all nuclei, mitotic staining)

Module Settings: Defining the Intensity

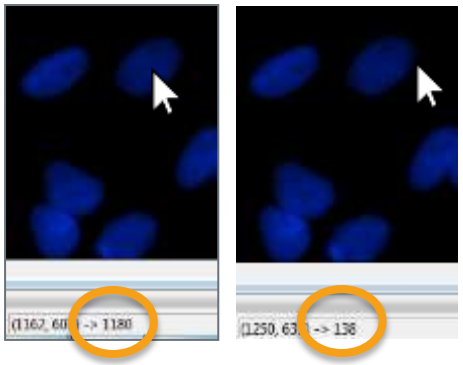




Use the difference in intensity values to set **Intensity above local background**

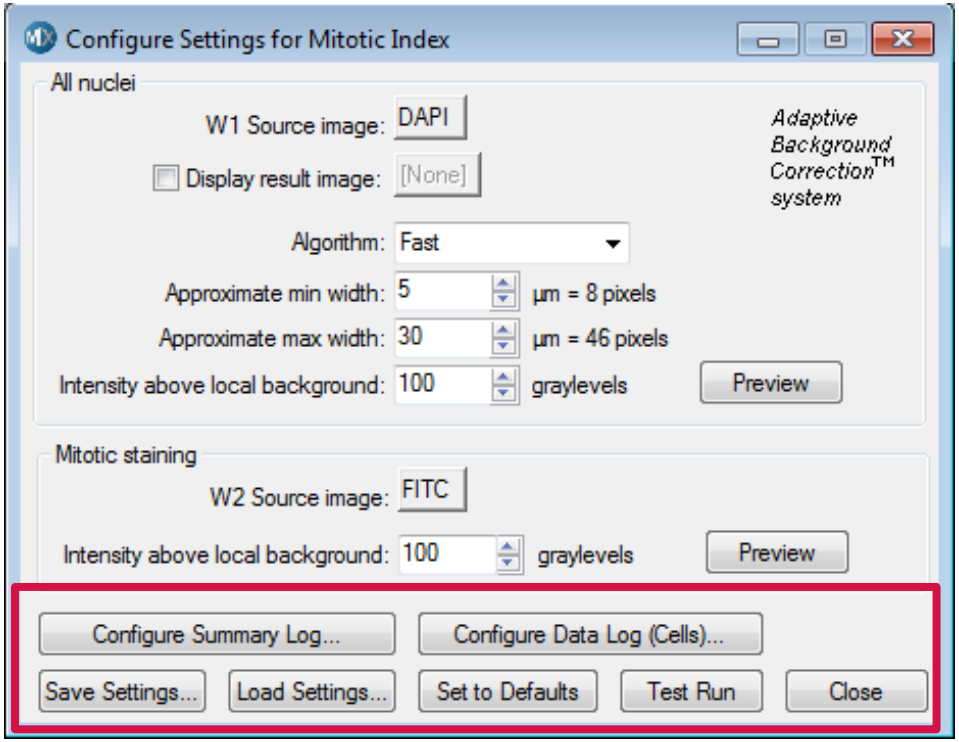
Intensity above local background

- Draw a line over the dimmest object of interest that covers both the object and background using the Single line tool
- 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 or **Test Run** to test all settings based on the W1 and W2 markers. Adjust values as necessary by changing one parameter at a time.



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

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.

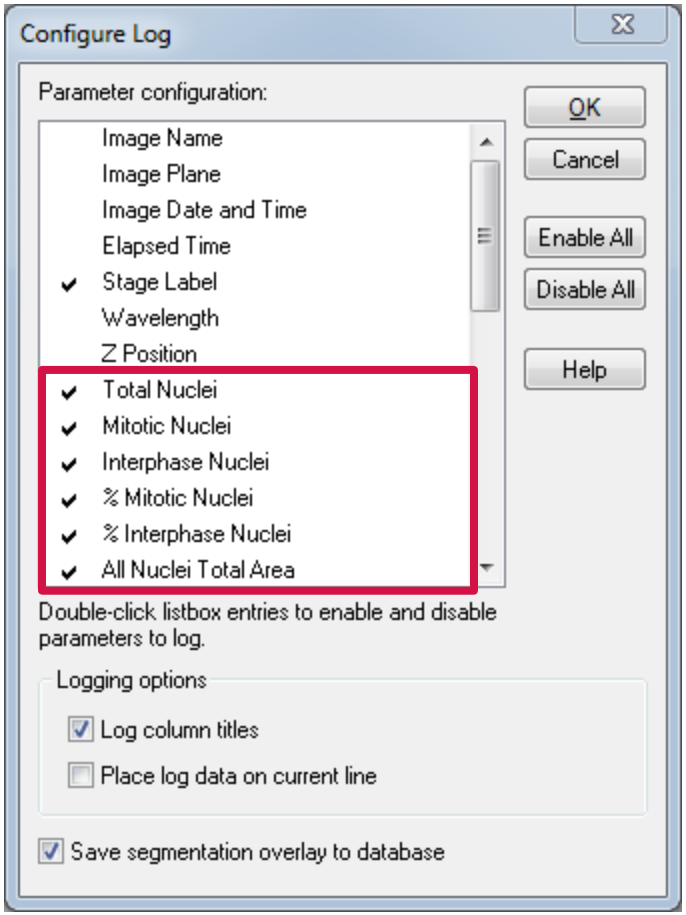
- ✓ Indicates a parameter that will be logged into the database (or Excel/text file log if open)

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)

Image Name
Image Plane
Image Date and Time
Elapsed Time
Stage Label
Wavelength
Z Position
Total Nuclei
Mitotic Nuclei
Interphase Nuclei
% Mitotic Nuclei
% Interphase Nuclei
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
Mitotic Total Area
Mitotic Mean Area
Mitotic W1 Integrated Intensity
Mitotic W1 Average Intensity
Mitotic W2 Integrated Intensity
Mitotic W2 Average Intensity
Interphase Total Area
Interphase Mean Area
Interphase W1 Integrated Intensity
Interphase W1 Average Intensity
Interphase W2 Integrated Intensity
Interphase W2 Average Intensity

- **Total Nuclei:** Total number of cells determined from wavelength 1.
- **Mitotic Nuclei:** Total number of nuclei determined from wavelength 1 that were positive in wavelength 2.
- **Interphase Nuclei:** Total number of nuclei determined from wavelength 1 that were negative in wavelength 2.
- **% Mitotic Nuclei:** 100 * # Mitotic Nuclei/# Total Nuclei.
- **% Interphase Nuclei:** 100 * #Interphase Nuclei/#Total Nuclei.

Configure Summary Log (Image Measurements)

Image Name
Image Plane
Image Date and Time
Elapsed Time
Stage Label
Wavelength
Z Position
Total Nuclei
Mitotic Nuclei
Interphase Nuclei
% Mitotic Nuclei
% Interphase Nuclei
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
Mitotic Total Area
Mitotic Mean Area
Mitotic W1 Integrated Intensity
Mitotic W1 Average Intensity
Mitotic W2 Integrated Intensity
Mitotic W2 Average Intensity
Interphase Total Area
Interphase Mean Area
Interphase W1 Integrated Intensity
Interphase W1 Average Intensity
Interphase W2 Integrated Intensity
Interphase W2 Average Intensity

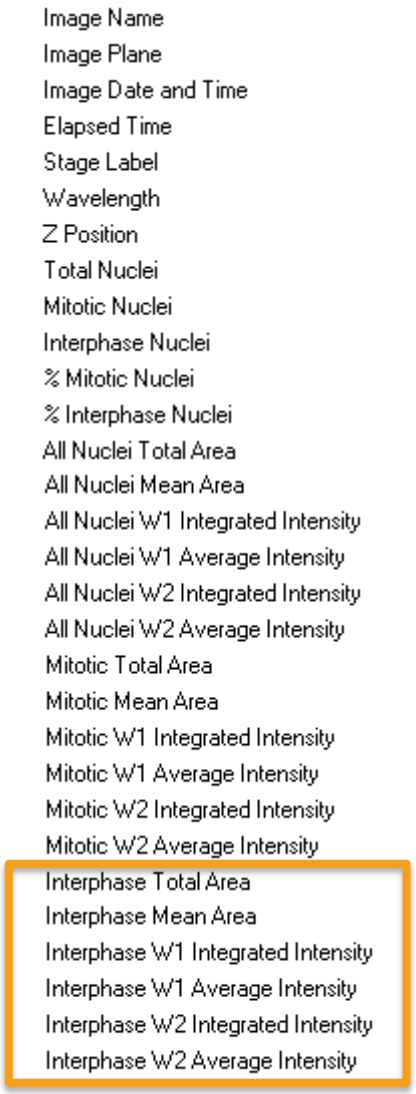
- **All Nuclei Total Area:** Total µm² in wavelength 1 of All Nuclei.
- **All Nuclei Mean Area:** All Nuclei Total Area/#All Nuclei.
- **All Nuclei W1 Integrated Intensity:** Summed grayscale values in wavelength 1 in All Nuclei.
- **All Nuclei W1 Average Intensity:** All Nuclei Integrated Intensity/All Nuclei Total (Pixel) Area.
- **All Nuclei W2 Integrated Intensity:** Summed grayscale values in wavelength 2 in All Nuclei.
- **All Nuclei W2 Average Intensity:** All Nuclei W2 Integrated Intensity/All Nuclei Total (Pixel) Area.

Configure Summary Log (Image Measurements)

Image Name
Image Plane
Image Date and Time
Elapsed Time
Stage Label
Wavelength
Z Position
Total Nuclei
Mitotic Nuclei
Interphase Nuclei
% Mitotic Nuclei
% Interphase Nuclei
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
Mitotic Total Area
Mitotic Mean Area
Mitotic W1 Integrated Intensity
Mitotic W1 Average Intensity
Mitotic W2 Integrated Intensity
Mitotic W2 Average Intensity
Interphase Total Area
Interphase Mean Area
Interphase W1 Integrated Intensity
Interphase W1 Average Intensity
Interphase W2 Integrated Intensity
Interphase W2 Average Intensity

- **Mitotic Total Area:** Total µm² in wavelength 1 in Mitotic nuclei.
- **Mitotic Mean Area:** Mitotic Total Area/#Mitotic Nuclei.
- **Mitotic W1 Integrated Intensity:** Summed grayscale values in wavelength 1 in Mitotic nuclei.
- **Mitotic W1 Average Intensity:** Mitotic Nuclei W1 Integrated Intensity/Mitotic Nuclei Total (Pixel) Area.
- **Mitotic W2 Integrated Intensity:** Summed grayscale values in wavelength 2 in Mitotic nuclei.
- **Mitotic W2 Average Intensity:** Mitotic W2 Integrated Intensity/Mitotic Total (Pixel) Area.

Configure Summary Log (Image Measurements)



- **Interphase Total Area:** Total µm² in wavelength 1 in all Interphase nuclei.
- **Interphase Mean Area:** Interphase Total Area/Total # Interphase Nuclei.
- **Interphase W1 Integrated Intensity:** Summed grayscale values in wavelength 1 in interphase nuclei.
- **Interphase W1 Average Intensity:** Interphase W1 Integrated Intensity/Interphase Total (Pixel) Area.
- **Interphase W2 Integrated Intensity:** Summed grayscale values in wavelength 2 in interphase nuclei.
- **Interphase W2 Average Intensity:** Interphase W2 Integrated Intensity/Interphase Total (Pixel) Area.

Configure Data Log (Cell-by-Cell Measurements)

Image Name
Image Plane
Image Date and Time
Elapsed Time
Stage Label
Wavelength
Z Position
Cell: Assigned Label #
Cell: Mitotic Classification
Cell: Total Area
Cell: W1 Integrated Intensity
Cell: W1 Average Intensity
Cell: W2 Integrated Intensity
Cell: W2 Average Intensity

- **Cell: Assigned Label #**: Cell label number (1 through total cell number).
- **Cell: Classification:** Labels cell as Interphase or Mitotic.
- **Cell: Total Area:** Total µm² in the nucleus of a given cell.
- **Cell: W1 Integrated Intensity:** Summed grayscale values in wavelength 1 for this nucleus.
- **Cell: W1 Average Intensity:** W1 Integrated Intensity/Total (Pixel) Area for this nucleus.
- **Cell: W2 Integrated Intensity:** Summed grayscale values in wavelength 2 for this nucleus.
- **Cell: W2 Average Intensity:** W2 Integrated Intensity/Total (Pixel) Area for this nucleus.

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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