MetaXpress® 6 Software Guide

Application Modules: Transfluor

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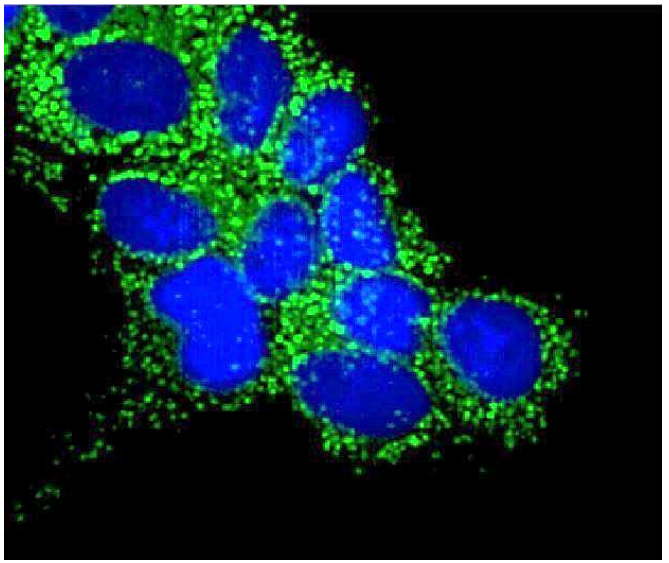
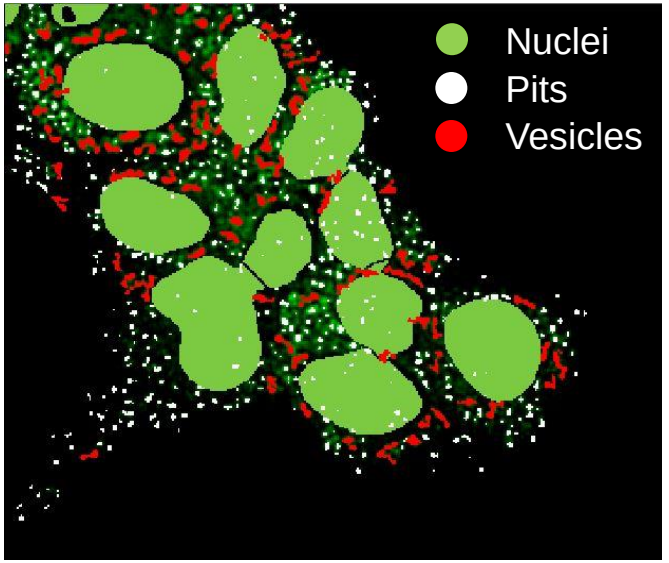
Transfluor Application Module Overview

The Transfluor application module can be used to identify nuclei, pits, vesicles, or any combination of the three in the Transfluor assay (please refer to the Molecular Devices website for more information).

- Pits are a subcellular structure of a defined size and intensity (usually smaller and dimmer than vesicles).
- Vesicles are a subcellular structure of a defined size and intensity (usually larger and brighter than pits).
- (Optional) A nuclear wavelength (i.e. DAPI, Hoechst, or DRAQ5) can be used to determine the number of pits and vesicles per cell.

**NOTE* Application modules can be used to measure different biological processes. Pits and vesicles can be any subcellular structure with a roundish shape. For example, puncta, lysosomes, endosomes, fragmented mitochondria, etc.*





Module Settings: Selecting an Image & Algorithm

Pits and Vesicles Image:

- Select the image with pits and/or vesicles

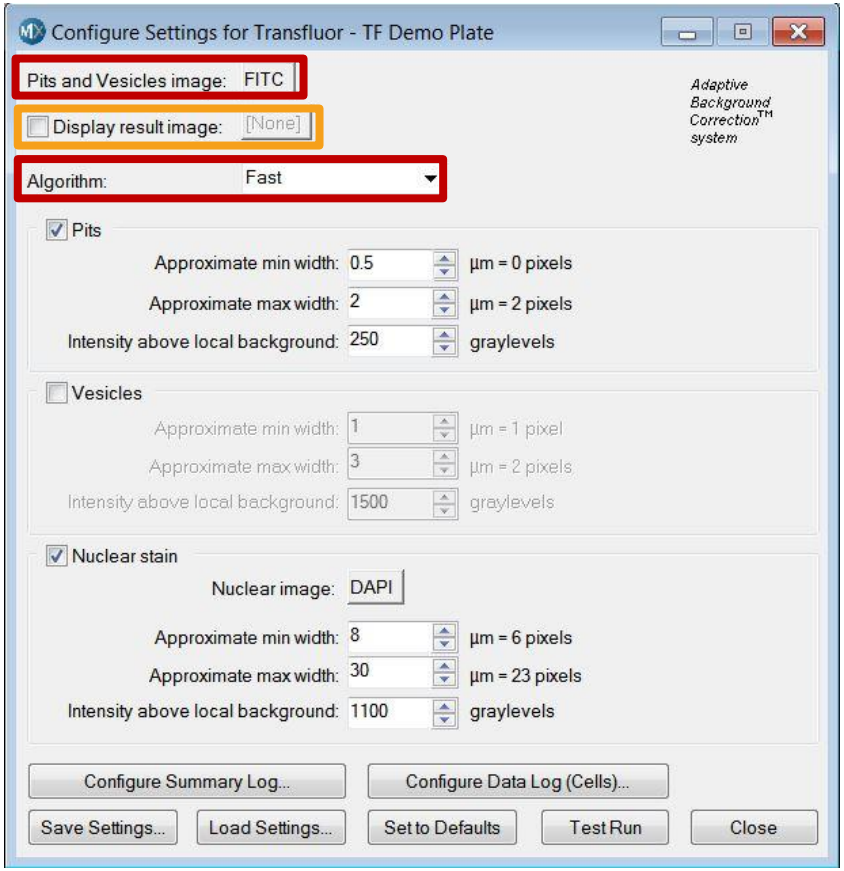
**NOTE* Do not choose images with "HTS" in the name.*

Display result image:

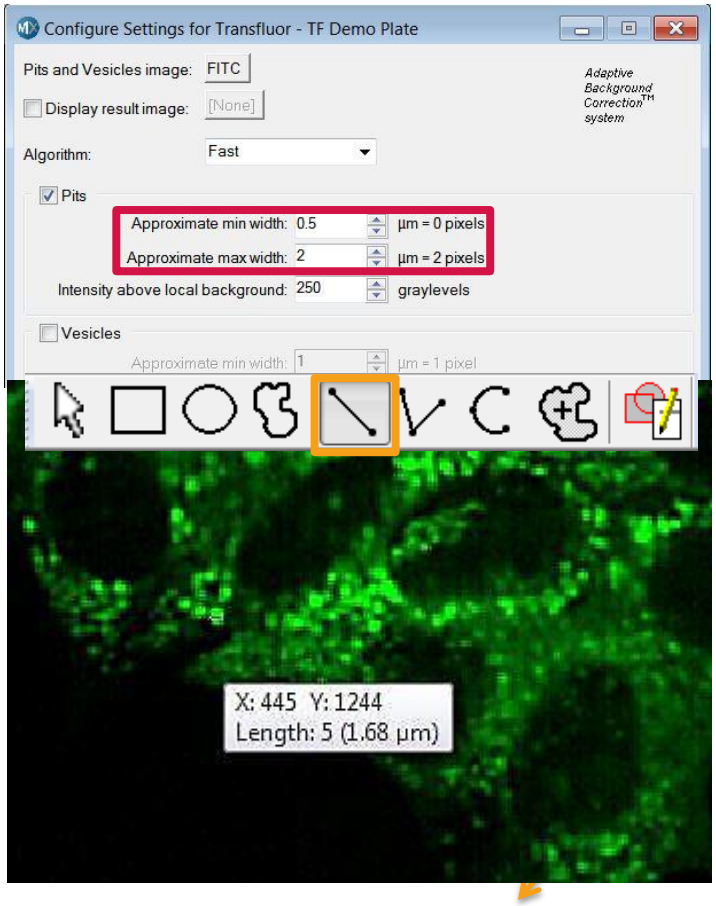
- Leave **Display result image** checkbox deselected (this is generally only used when creating a journal)

Algorithm dropdown:

- 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**
- Molecular Devices recommends starting with the **Standard** algorithm for Transfluor and Granularity



Module Settings: Defining the Size of Objects



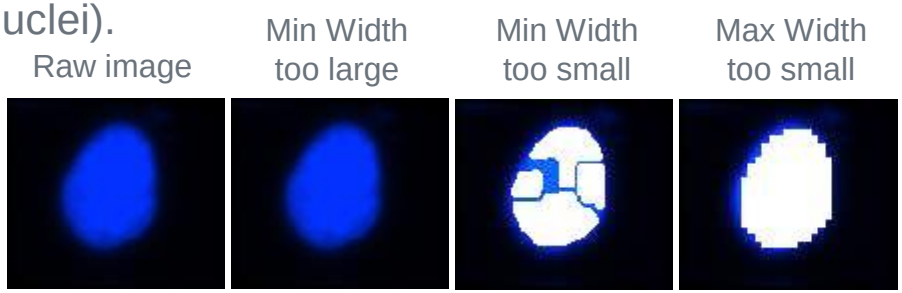
To see only the segmentation overlay of one object-type at a time, disable the check boxes next to the other object types (i.e. Vesicles). Reselect them prior to saving the settings

Approximating min and max widths

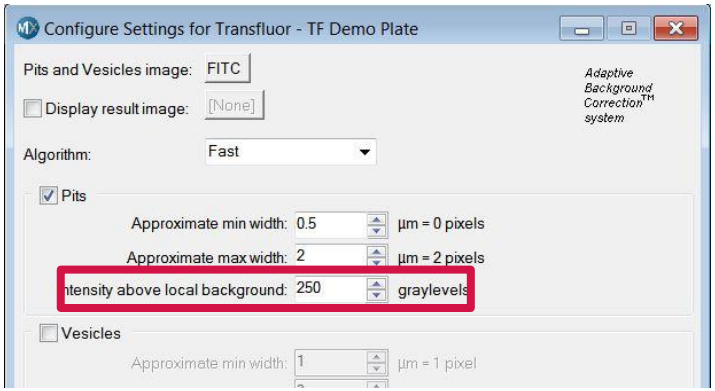
- Select the single line region from **Regions Tools**
- Single-click and drag across the short axis of a representative small and large object; a tooltip will show the length of the line

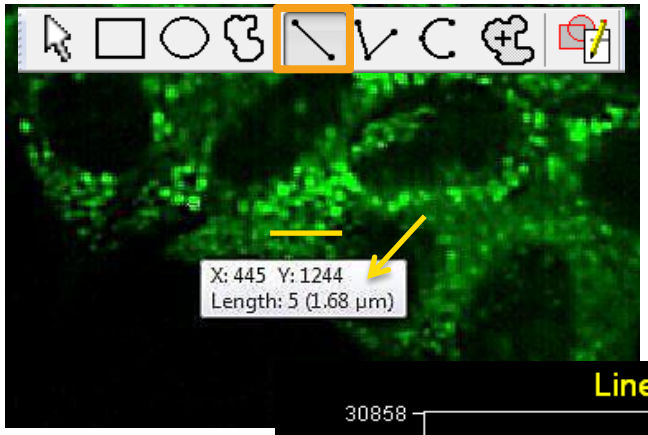
**NOTE* Do not click the image again. This will cause the tooltip to disappear. If the tooltip disappears, repeat the drawing procedure.*

- 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 (pits, vesicles, and nuclei).



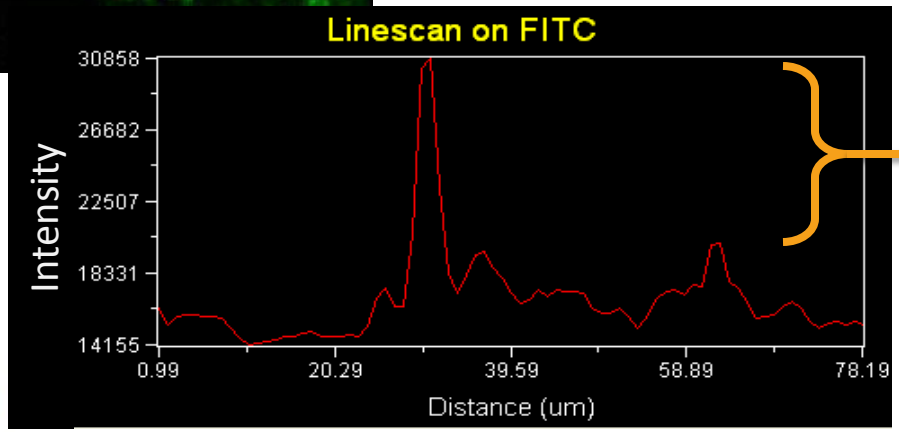
Module Settings: Defining the Intensity





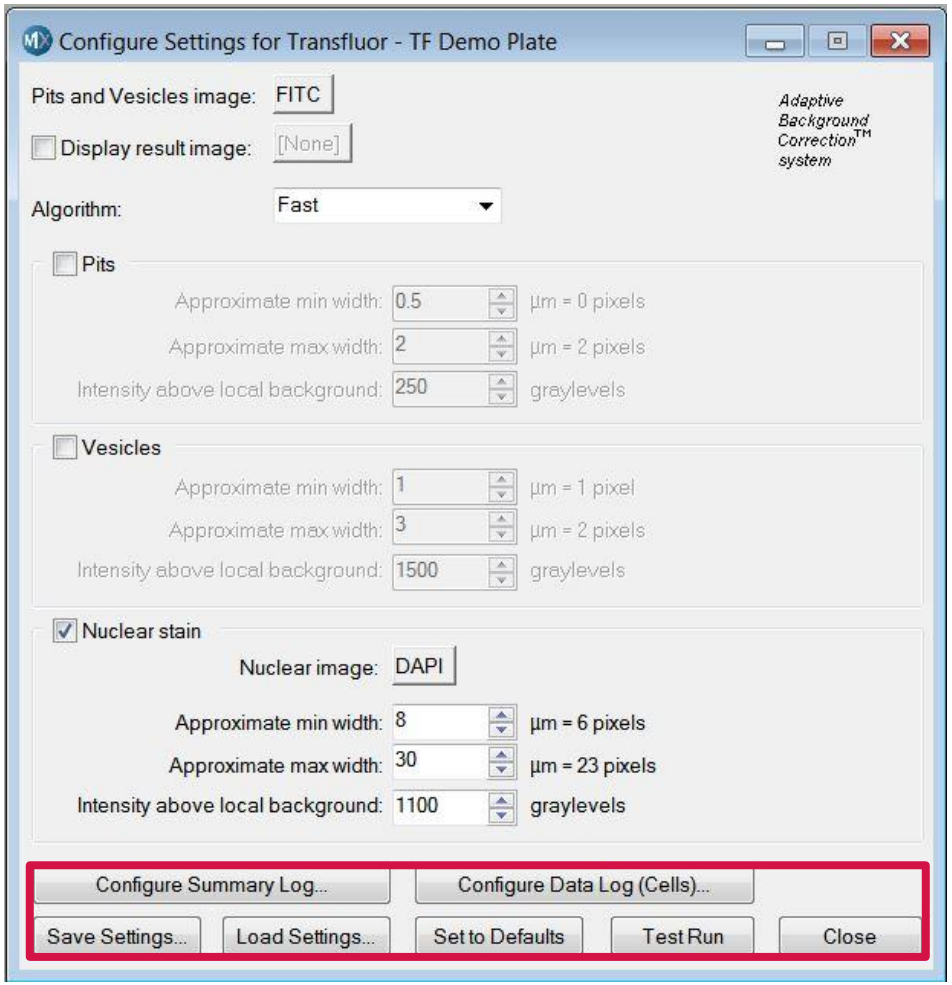
Intensity above local background

- 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.
- For Fast algorithms, set **Intensity above local background** to half (or less) of the difference in intensity between an object and background.
- For **Standard** algorithms, set this value slightly lower than the difference.
- Click **Apply or Test Run** and adjust values as necessary**.**
- Repeat for all desired objects (pits, vesicles, and nuclei)



Use the difference in intensity values 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.

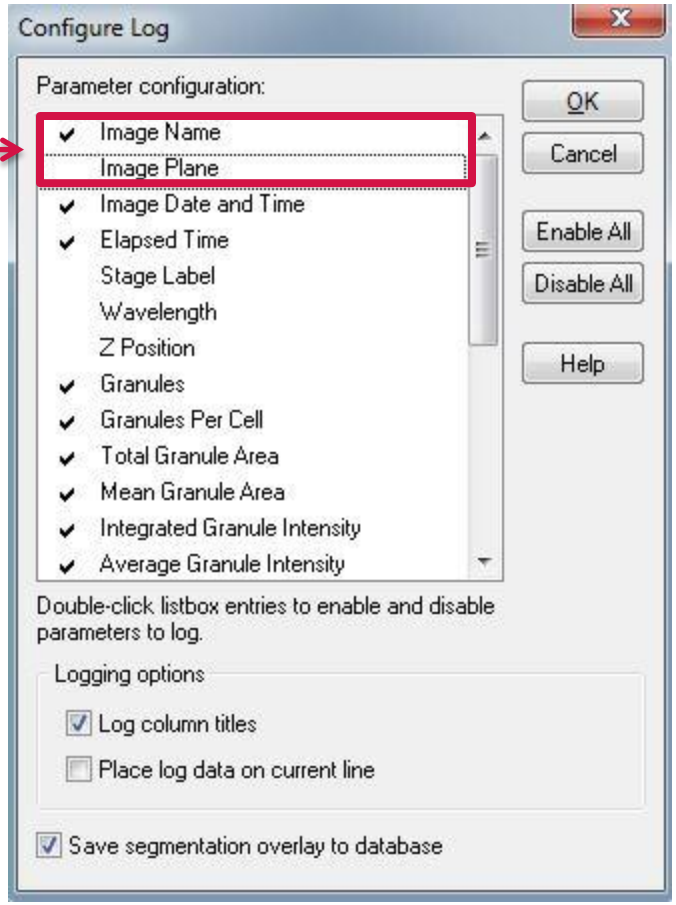
- ✓ Indicates a parameter that will be logged into the database (or Excel/text file log if open)

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.

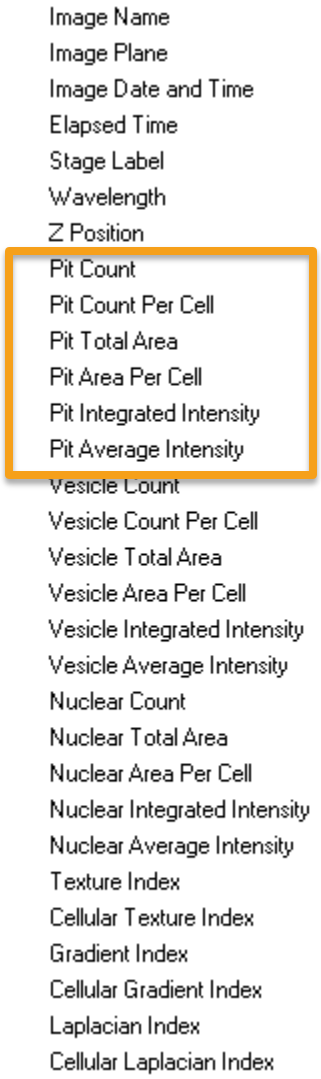
Place log data on current line: Does not affect database logging. If you have an Excel or text file log open, this records the data into the last row used, to the right of the previous data. We recommend **disabling** this option to ensure that new data is recorded into a new row.

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.

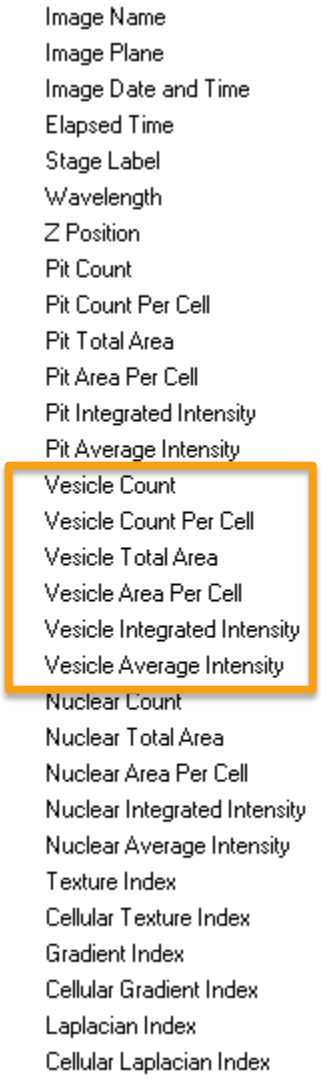


Configure Summary Log (Image Measurements)



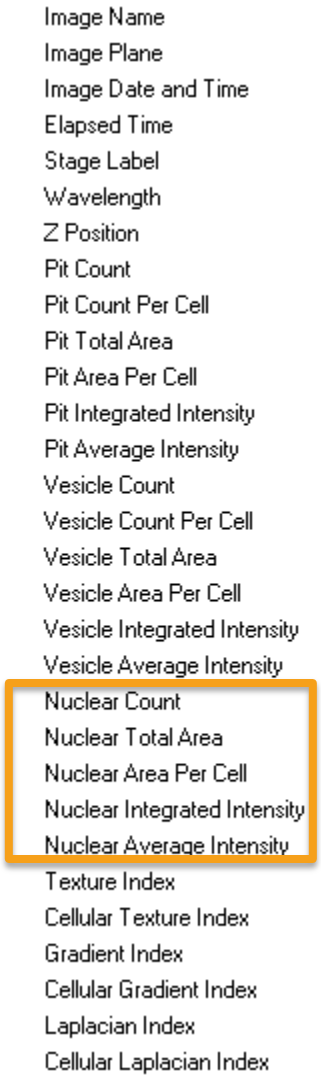
- **Pit Count:** Total number of pits in the image.
- **Pit Count Per Cell:** Total number of pits divided by the total number of nuclei in the image.
- **Pit Total Area:** Total area of pits for all cells found in the image (in μm^2).
- **Pit Area Per Cell:** Total area of pits for all cells divided by the total number of nuclei (in μm^2) in the image.
- **Pit Integrated Intensity:** Total pixel intensity over all of the pit areas in the image.
- **Pit Average Intensity**: Average pixel intensity over all of the pit areas in the image.

Configure Summary Log (Image Measurements)



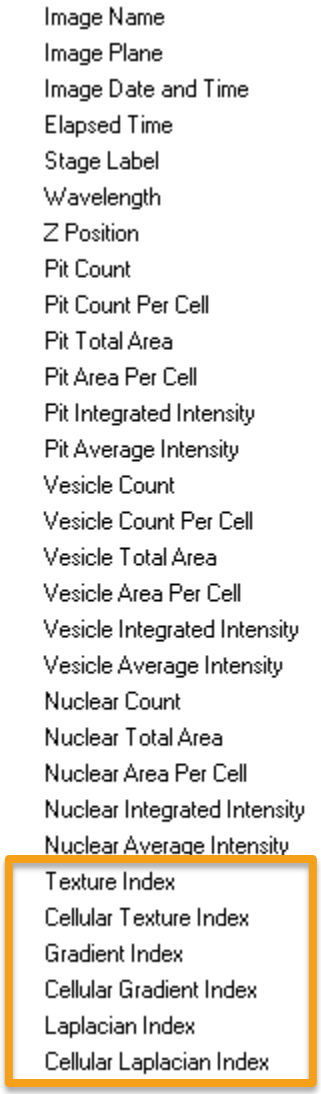
- **Vesicle Count:** Total number of vesicles in the image.
- **Vesicle Count Per Cell:** Total number of vesicles divided by the total number of nuclei in the image.
- **Vesicle Total Area:** Total area of the vesicles found in the image (in μm^2).
- **Vesicle Area Per Cell:** Total area of vesicles for all cells divided by the total number of nuclei (in μm^2) in the image.
- **Vesicle Integrated Intensity:** Total pixel intensity over all of the vesicle areas in the image.
- **Vesicle Average Intensity:** Average pixel intensity over all of the vesicle areas in the image.

Configure Summary Log (Image Measurements)



- **Nuclear Count:** Total number of nuclei (cell count) per image.
- **Nuclear Total Area:** Total area of the nuclei for all cells found in the image (in μm^2).
- **Nuclear Area Per Cell:** Mean area of nucleus for all cells found in the image (in μm^2).
- **Nuclear Integrated Intensity:** Total pixel intensity of the nuclear stain over all of the nuclei in the image.
- **Nuclear Average Intensity**: Average pixel intensity of the nuclear stain over all of the nuclei in the image.

Configure Summary Log (Image Measurements)

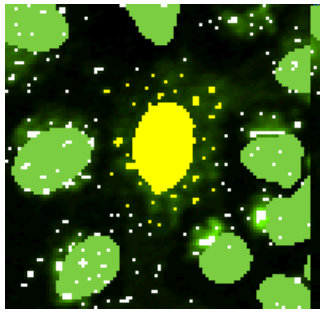


- **Texture Index: S**tandard deviation of the intensity values in the image.
- **Cellular Texture Index:** Cell-by-cell standard deviation of intensity values near the nuclei (requires use of nuclear stain) within the image.
- **Gradient Index:** Texture-dependent measurement that reflects the amount of local intensity contrast. Measures the difference between the maximum and minimum intensity within a local neighborhood within the image.
- **Cellular Gradient Index:** Cell-by-cell Gradient Index measured near the nuclei (requires use of nuclear stain) within the image.
- **Laplacian Index:** Similar to the morphological gradient, also reflects fluctuations in the gradient within the image.
- **Cellular Laplacian Index:** Cell-by-cell Laplacian Index measured near the nuclei (requires use of nuclear stain) within the image.

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: Pit Count
Cell: Pit Total Area
Cell: Pit Integrated Intensity
Cell: Pit Average Intensity
Cell: Vesicle Count
Cell: Vesicle Total Area
Cell: Vesicle Integrated Intensity
Cell: Vesicle Average Intensity
Cell: Nuclear Total Area
Cell: Nuclear Integrated Intensity
Cell: Nuclear Average Intensity
Cell: Texture Index
Cell: Gradient Index
Cell: Laplacian Index

Highlighted cells shows assigned pits to cell



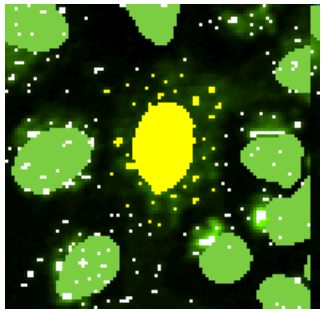
- **Cell: Assigned Label #**: Cell label number for each cell within the current image (1 through total cell number).
- **Cell: Pit Count:** Number of pits detected for a specific cell.
  NOTE A pit is assigned to its nearest nucleus
- **Cell: Pit Total Area:** Total area covered by all the pits assigned to a specific cell (in μm^2).
- **Cell: Pit Integrated Intensity:** Total pixel intensity of the pits assigned to a specific cell.
- **Cell: Pit Average Intensity:** Average pixel intensity of the pits assigned to a specific cell.

Configure Data Log (Cell-by-Cell Measurements)

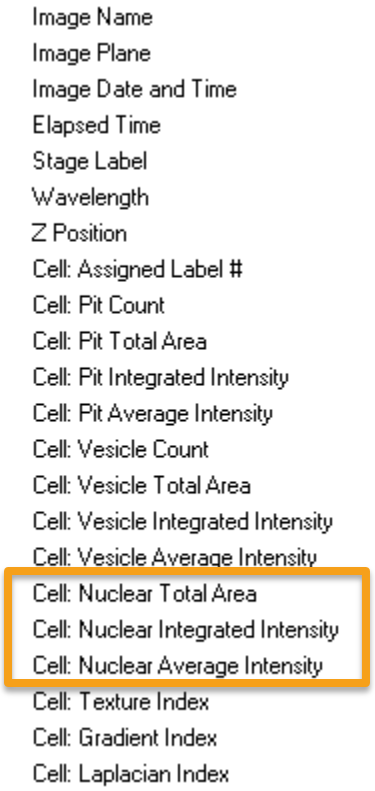
Image Name
Image Plane
Image Date and Time
Elapsed Time
Stage Label
Wavelength
Z Position
Cell: Assigned Label #
Cell: Pit Count
Cell: Pit Total Area
Cell: Pit Integrated Intensity
Cell: Pit Average Intensity
Cell: Vesicle Count
Cell: Vesicle Total Area
Cell: Vesicle Integrated Intensity
Cell: Vesicle Average Intensity
Cell: Nuclear Total Area
Cell: Nuclear Integrated Intensity
Cell: Nuclear Average Intensity
Cell: Texture Index
Cell: Gradient Index
Cell: Laplacian Index

- **Cell: Vesicle Count:** Number of vesicles detected for a specific cell.
  NOTE A vesicle is assigned to its nearest nucleus
- **Cell: Vesicle Total Area:** Total area covered by all the vesicles assigned to a specific cell (in μm^2)
- **Cell: Vesicle Integrated Intensity:** Total pixel intensity of the vesicles assigned to a specific cell.
- **Cell: Vesicle Average Intensity:** Average pixel intensity of the vesicles assigned to a specific cell.

Highlighted cells shows assigned pits to cell

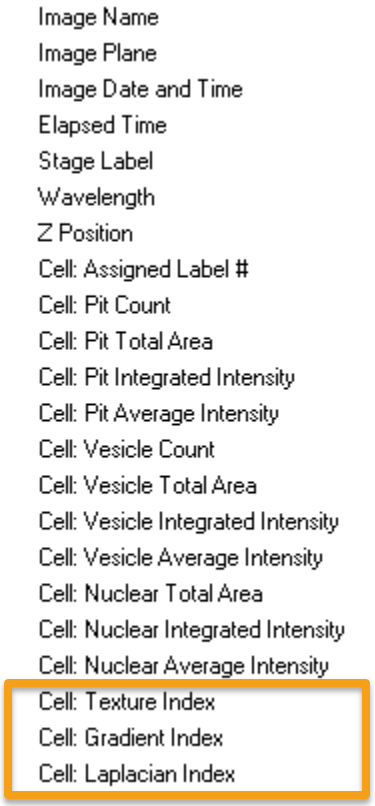


Configure Data Log (Cell-by-Cell Measurements)



- **Cell: Nuclear Total Area**: Total square microns of a specific nucleus.
- **Cell: Nuclear Integrated Intensity**: Total pixel intensity of the nuclear stain in a specific nucleus.
- **Cell: Nuclear Average Intensity**: Average pixel intensity of the nuclear stain in a specific nucleus.

Configure Data Log (Cell-by-Cell Measurements)



- **Cell: Texture Index:** Standard deviation of intensity values of a specific cell.
- **Cell: Gradient Index:** Texture-dependent measurement that reflects the amount of local intensity contrast. Measures the difference between the maximum and minimum intensity within a local neighborhood of a specific cell.
- **Cell: Laplacian Index:** Similar to the morphological gradient, also reflects fluctuations in the gradient of a specific 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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