MetaXpress® 6 Software Guide

Application Modules: Granularity

Date Revised 12/21/2016 Version C

MOLECULAR DEVICES
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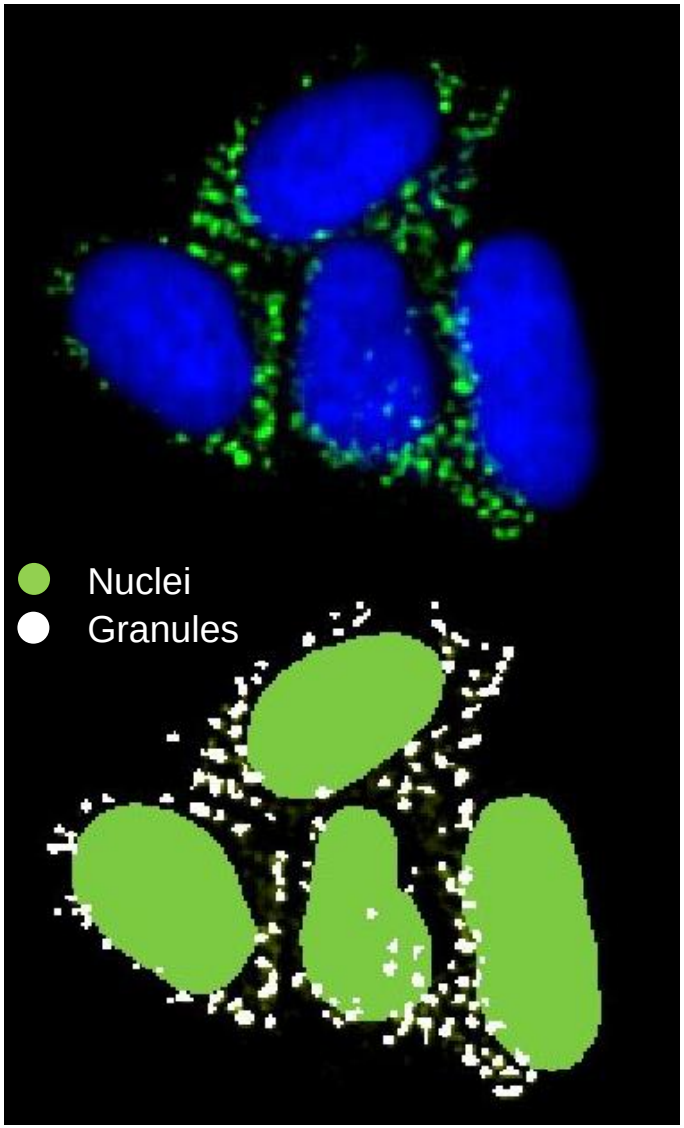
Granularity Module Overview

The Granularity application module can be used to identify granules and nuclei (optional). Please refer to the Molecular Devices website for more information.

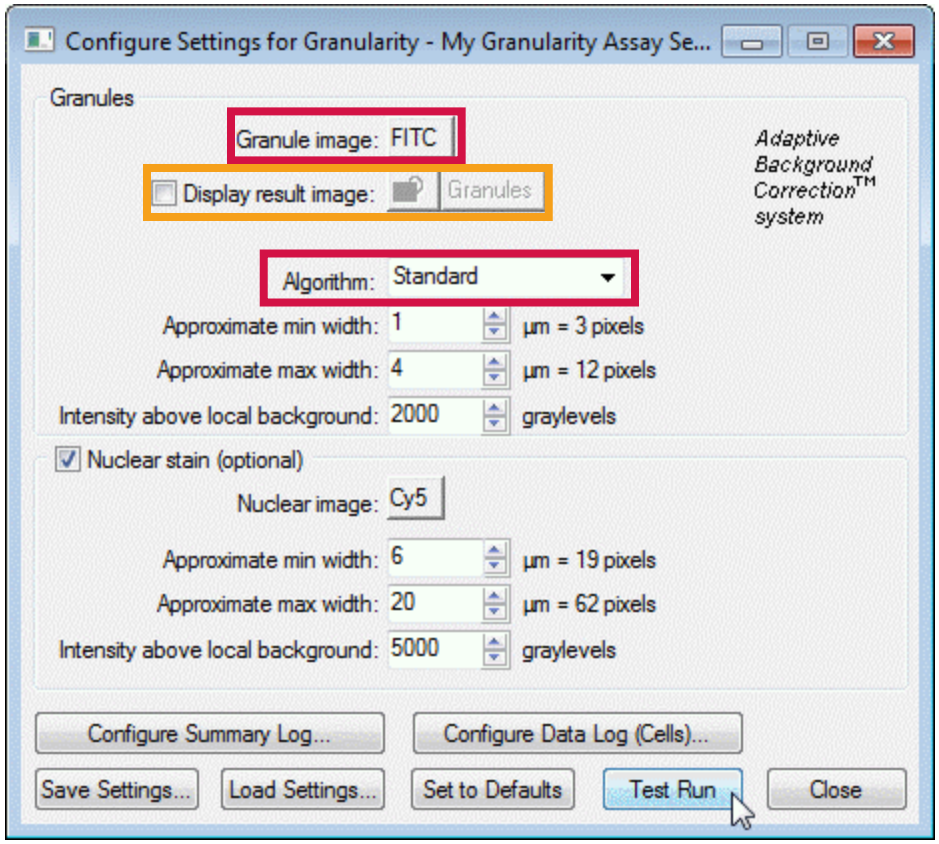
- Granules are a subcellular structure of a defined size and intensity

- (Optional) A nuclear wavelength (e.g. DAPI, Hoechst, or DRAQ5) can be used to determine the number of granules per cell.

NOTE Application modules are can be used to measure multiple biologies. Granules can be any roundish subcellular structures, such as puncta, lysosomes, endosomes, fragmented mitochondria, etc.



Module Settings: Selecting an Image & Algorithm



Granule Image

- Select the image with granules

NOTE Do not choose images with “HTS” in the name

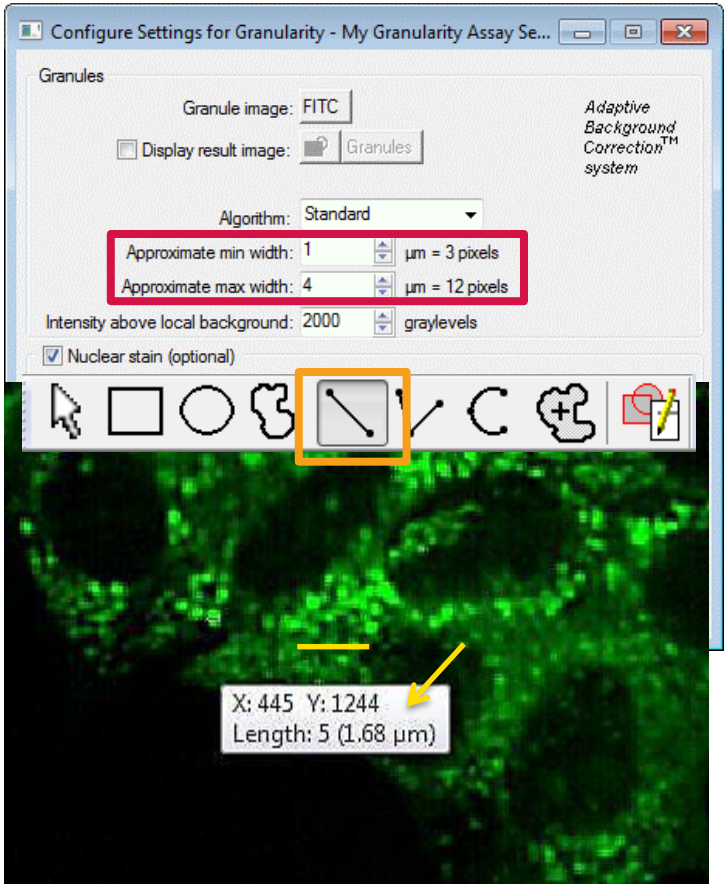
Display result image:

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

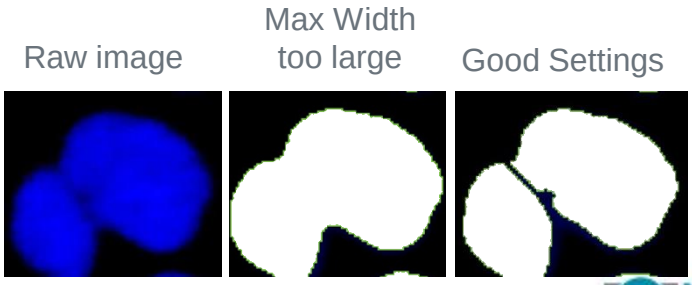
Module Settings: Defining the Size of Objects



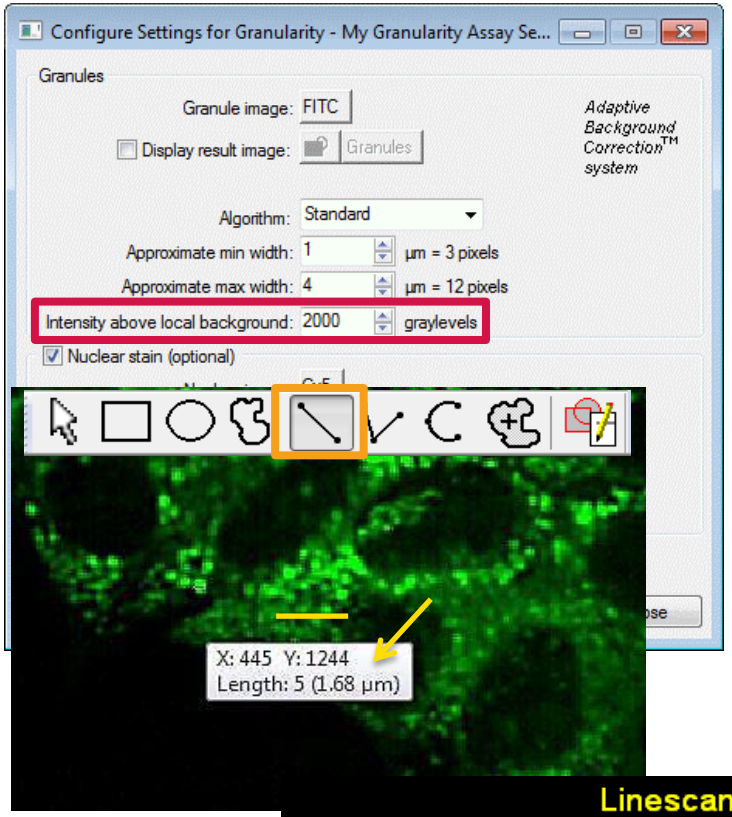
To see only the segmentation overlay of one object-type at a time, disable the check boxes next to the other object types (e.g. nuclei). Reselect them prior to saving the settings.

Approximating min and max widths

- Select the single line region from **Region Tools**
- Click and drag across a representative small object and a large object; a tooltip will show the length of the line in µm *NOTE* Do not click the image again. This will cause the tooltip to disappear. If the tooltip disappears, repeat the drawing procedure.
- Measure the width across the short axis of a object
- Smaller objects are ignored; larger objects are split
- Click **Test Run** and adjust values as necessary
- Repeat for all desired objects (granules and nuclei)

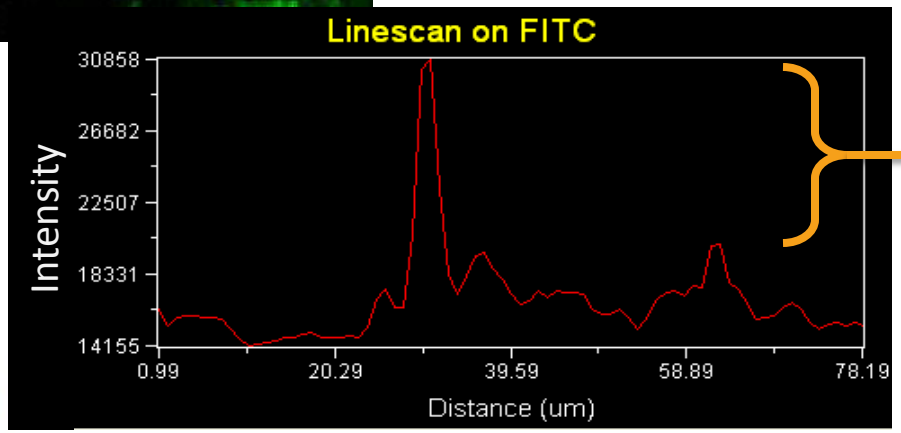


Module Settings: Defining the Intensity



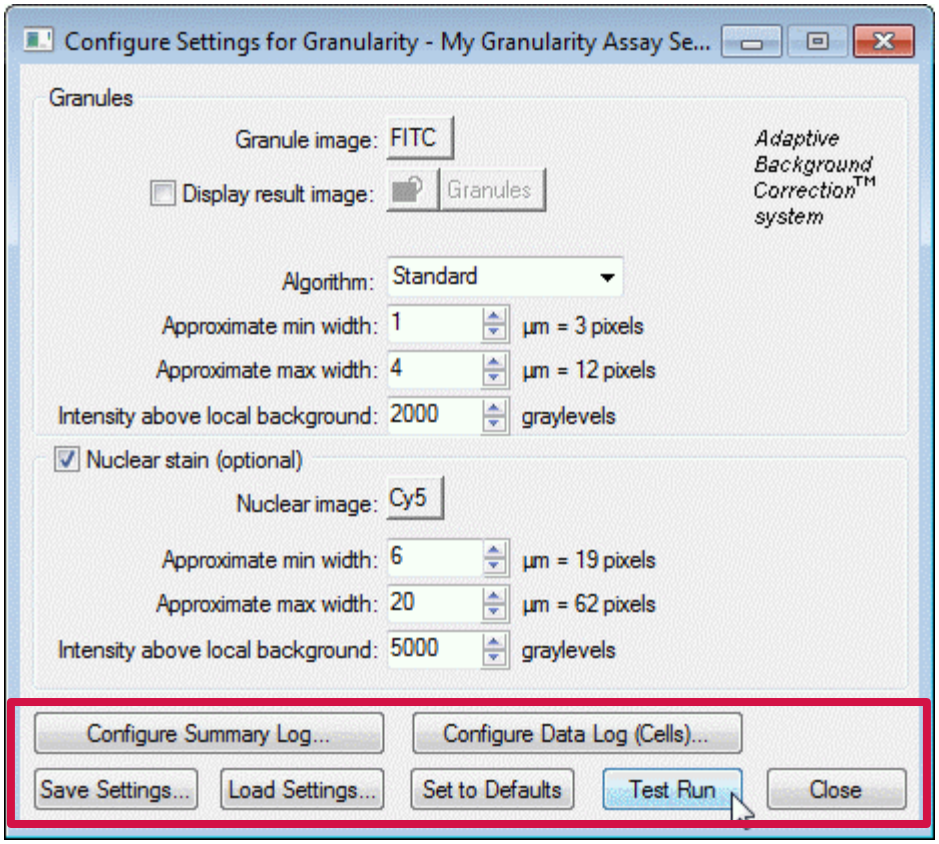
Intensity above local background

- Draw a line over the dimmest object of interest that covers both the object and background using the region tools
- 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 **Test Run** and adjust values as necessary
- Repeat for all desired objects (granules 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 parameter to select or deselect it for logging into the database

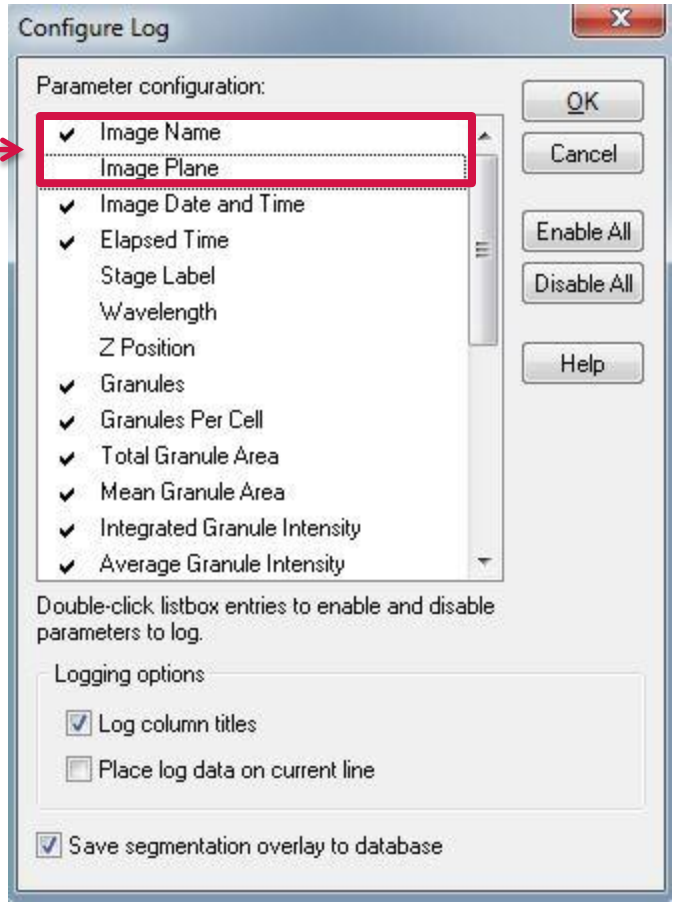
- ✓ Indicates a parameter that will be logged into the database (or Excel/text file log if open)

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. 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. 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 single 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. Recommend enabling this option for assay development and disabling it for screening.

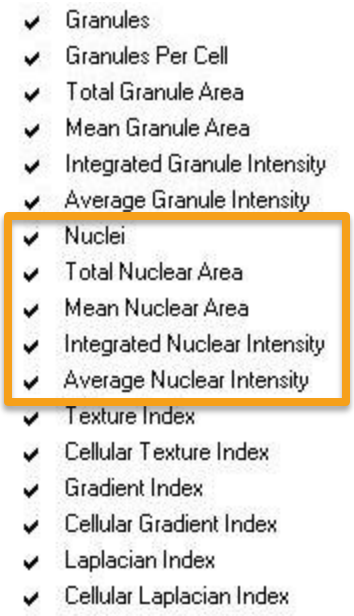
- Pro: Allows you to quickly review your segmentation results after analysis has been run across the entire plate
- Con: These masks take up a significant amount of space in a database, which may be limited in size. Saving the masks may also slow down analysis.



Configure Summary Log (Image Measurements)

- **Granules:** Total number of granules
- **Granules Per Cell:** Total number of granules divided by the total number of nucleus
- **Total Granule Area:** The total area of the granules found in the image (in μm^2)
- **Mean Granule Area:** The total area of granules for all cells divided by the total number of nuclei (in μm^2)
- **Integrated Granule Intensity:** The total pixel intensity of all granules in the image
- **Average Granule Intensity**: The average pixel intensity calculated over all granules in the image

Configure Summary Log (Image Measurements)

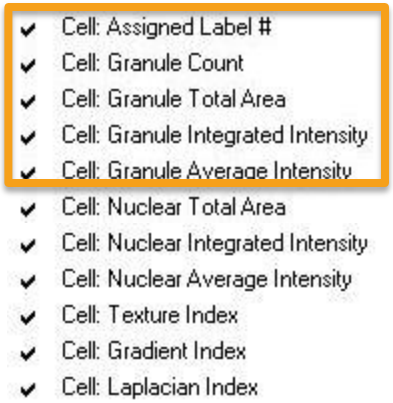


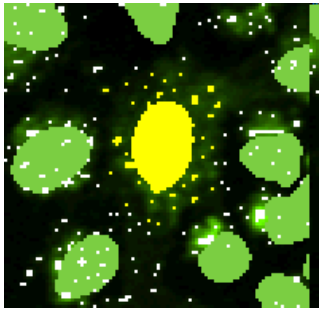
- **Nuclei:** Total number of nuclei (cell count)
- **Total Nuclear Area:** The total area of the nucleus for all cells found in the image (in μm^2)
- **Mean Nuclear Area:** The average area of nucleus for all cells found in the image (in μm^2)
- **Integrated Nuclear Intensity:** The total pixel intensity of the nuclear stain over the nuclear area
- **Average Nuclear Intensity**: The total pixel intensity of the nuclear stain over the nuclear area, divided by the total number of cells

Configure Summary Log (Image Measurements)

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

Configure Data Log (Cell-by-Cell Measurements)



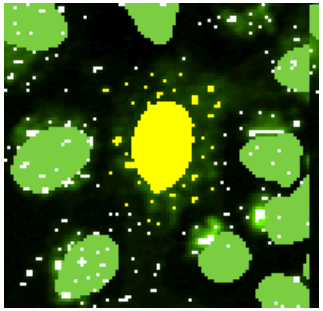


Highlighted cells shows assigned granules to cell

- **Cell: Assigned Label #** – Cell label number (1 through total cell number)
- **Cell: Granules Count:** Number of granules detected for a specific cell. (Note: a granule is assigned to its nearest nucleus)
- **Cell: Granules Total Area:** Area covered by all the granules assigned to a specific cell (in $\mu m^{2)}$
- **Cell: Granules Integrated Intensity:** The total pixel intensity of the granules assigned to a specific cell
- **Cell: Granules Average Intensity:** The average pixel intensity calculated over all granules assigned to a specific cell

Cell Data (cell-by-cell measurements)

- Cell: Assigned Label #
- Cell: Granule Count
- Cell: Granule Total Area
- Cell: Granule Integrated Intensity
- Cell: Granule 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 granules to cell

- **Cell: Nuclear Total Area** – Total square microns of the nucleus
- **Cell: Nuclear Integrated Intensity** – Total pixel intensity of the nuclear stain in the nucleus
- **Cell: Nuclear Average Intensity** – Average pixel intensity of the nuclear stain in the nucleus
- **Cell: Texture Index:** Standard deviation of intensity values of a cell
- **Cell: Gradient Index:** A 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 cell
- **Cell: Laplacian Index:** Similar to the morphological gradient, however this morphological measurement reflects fluctuations in the gradient of a cell

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