MetaXpress® 6 Software Guide

Application Modules: Neurite Outgrowth

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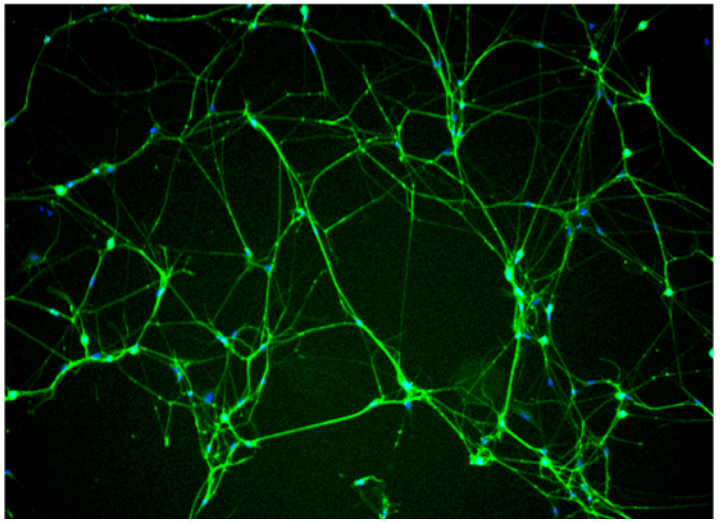
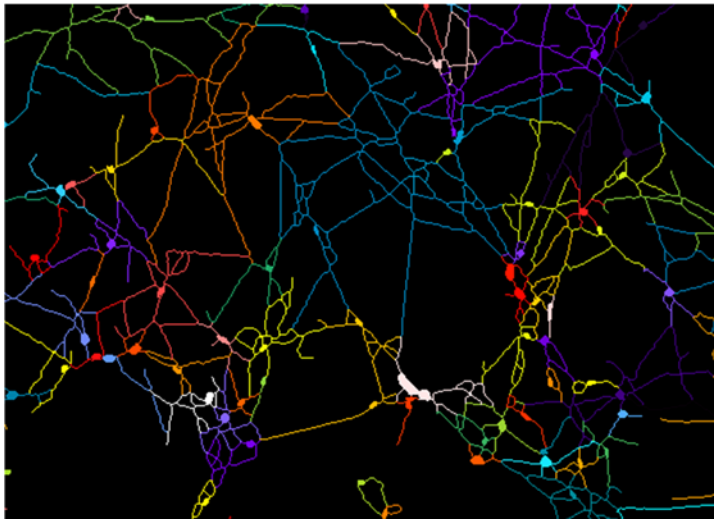
Neurite Outgrowth Overview

The Neurite Outgrowth application module can be used to identify and measure cell bodies and processes (neurites) attached to cell bodies.

- Neurites are extensions attached to a cell body. They are identified using width, length, and intensity.
- (Optional) A nuclear wavelength (i.e. DAPI, Hoechst, or DRAQ5) can be used to help identify the cell body.
- Transmitted Light or fluorescent images may be used.

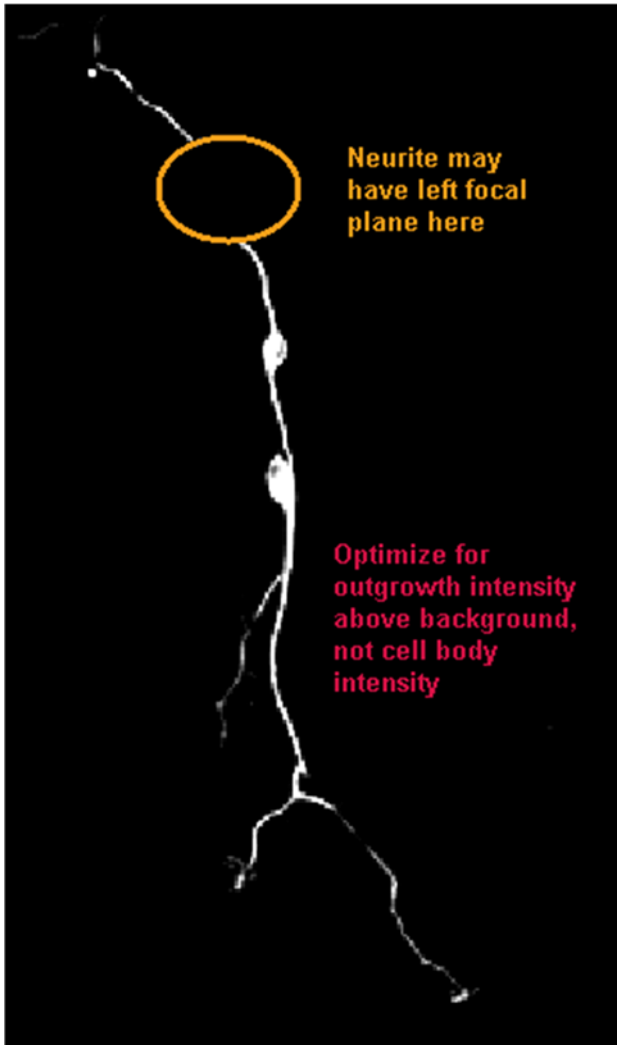
NOTE Application modules are can be used to measure multiple biologies. Neurites are any extension off of a cell body. For example, cilia, blood vessels, nanotubes, etc. Cell counting using transmitted light images is another possible application.



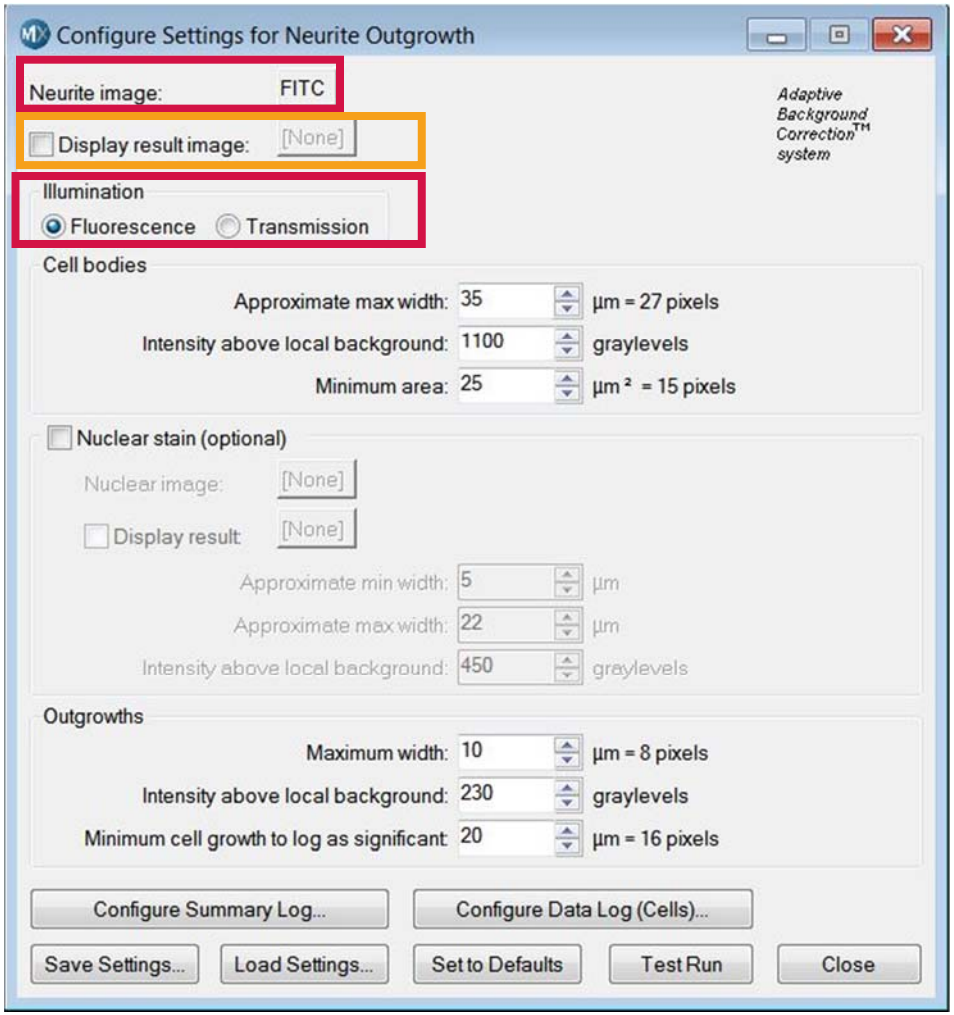


Tips for Neurite Imaging

- Exposure time should be optimized for outgrowth intensity, not cell body intensity. The autoexpose function may give images that are too dim in the outgrowths.
- If cell bodies are saturating and this is interfering with cell body identification, you can acquire the same channel a second time at a lower exposure time. Use the dimmer image as the “nuclear stain” image.
- If the outgrowths are going in and out of the plane of focus, interrupting the connectivity of the outgrowth, it may lead to inaccurate neurite detection. In this case, it is recommended to collect a Z-stack of the neurite image and use a 2D projection (e.g. Best Focus).
- If neurites are sparse and/or you are imaging at high magnification, you may want to collect multiple sites with 10% overlap and then stitch them together before analyzing. Neurites not connected to a cell body are ignored.



Module Settings: Selecting an Image



Neurite Image

- Select the image with neurites

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

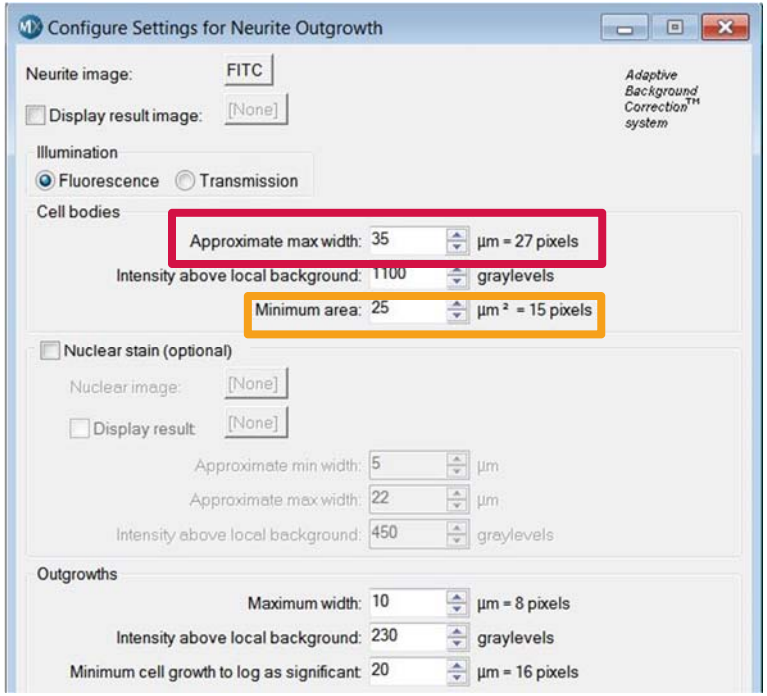
Display result image:

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

Illumination:

- Select Fluorescence or Transmission as appropriate for the neurite image
- Fluorescence: light objects on dark background
- Transmission (Brightfield or Phase Contrast): dark objects on light background

Module Settings: Defining the Size of Cell Bodies

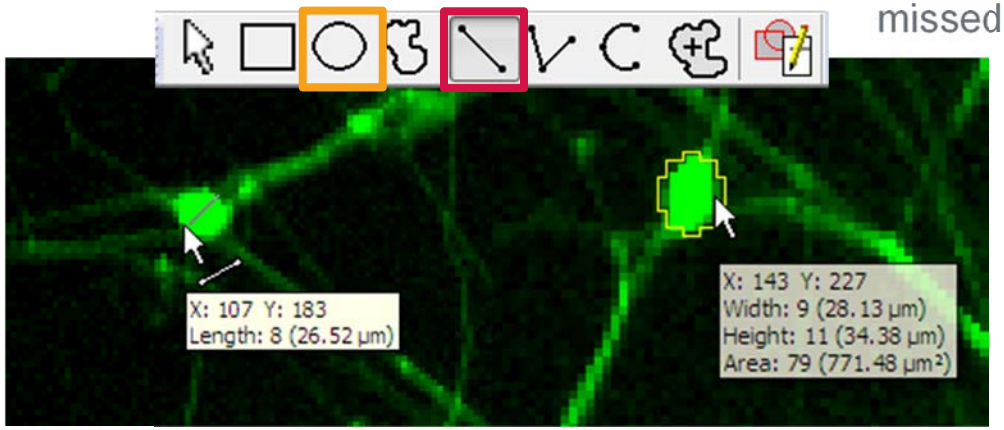


Approximate max width

- Use the single line region from **Region Tools** or the **Calipers**
- Click and drag to draw a line across a representative small and large object; a tooltip will show the length of the line
- The width is the short axis of a cell body (in um)
- If the setting is too large, clusters of cell bodies may be joined together
- If the setting is too small, thicker outgrowths or branch points may be identified as cell bodies

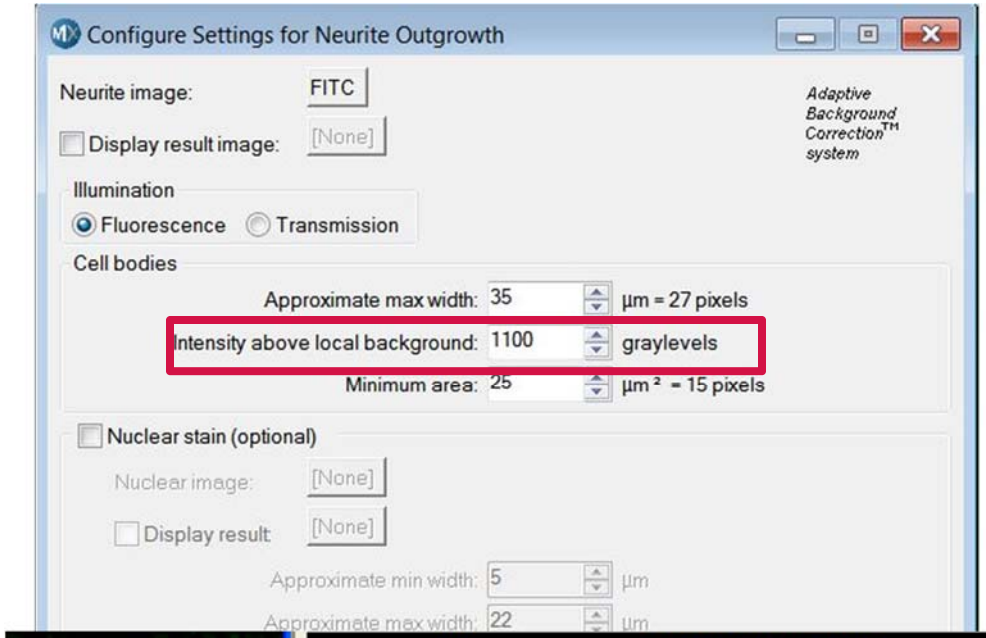
Minimum Area

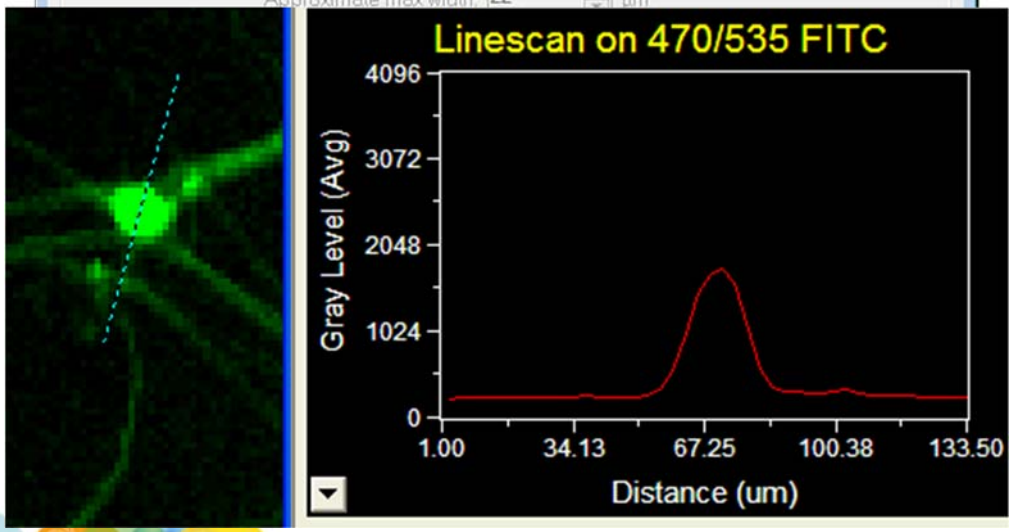
- An additional criteria used to separate cell bodies from branch points / thick outgrowths
- Use the elliptical region tool to measure the area of a small cell body in the image
- If the area is set too large, smaller cell bodies will be missed



NOTE Do not click the image again. This will cause the tooltip to disappear. If the tooltip disappears, repeat the drawing procedure, or use the Arrow (Locator) to adjust the region and display the tooltip.

Module Settings: Defining the Intensity

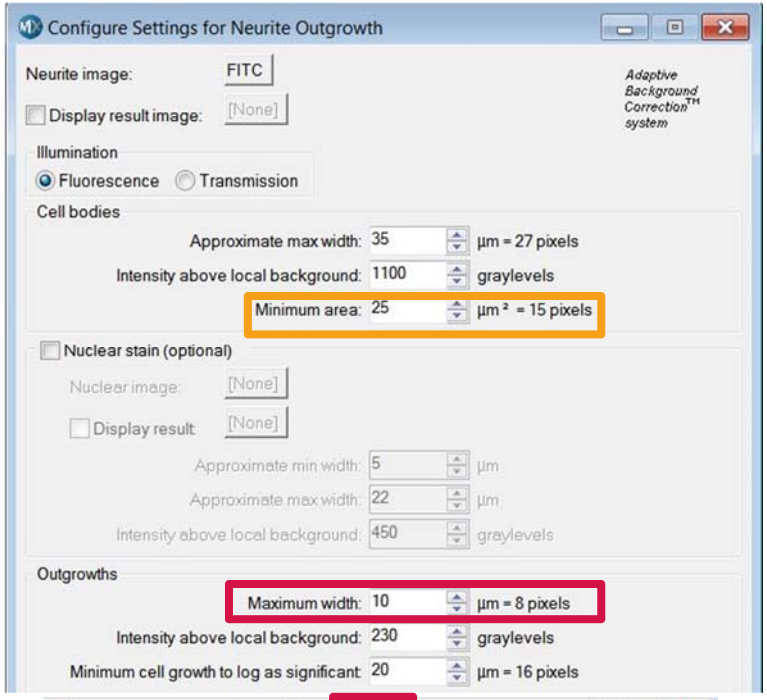


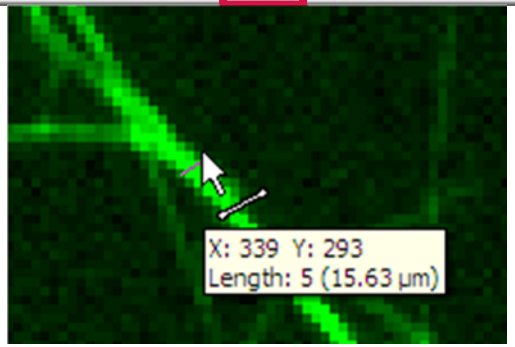


Intensity above local background

- The intensity above local background is used for finding the cell bodies.
- This value is a minimum and should be set slightly lower than half of the difference in intensity between a dim object and its local background.
  - Example: object intensity ~2000
  - Background intensity ~200
  - Start with Intensity above local background setting at (2000-200) / 2 = 900
- Use the region tools to draw a line across a dim cell body into the background and use **Measure > Intensities > Linescan** to determine intensity values.
- Alternatively, simply mouse over the cell and the background and view the intensity values.
- Click **Apply** and adjust values as necessary**.**
- Repeat for all desired objects (cell bodies, nuclei, and outgrowths).

Module Settings: Defining Outgrowths





Width measurement

Max width

- Used to help distinguish the beginning of outgrowths from cell bodies or branch points.
- Use the line region tool or the calipers to measure the width of a thick outgrowth.
- Click and drag to draw a line across a representative object; a tooltip will show the length of the region.

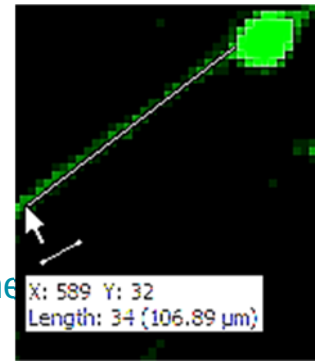
Intensity above local background

- Measure the intensity for outgrowths just as for the cell bodies

Minimum cell growth to log as significant

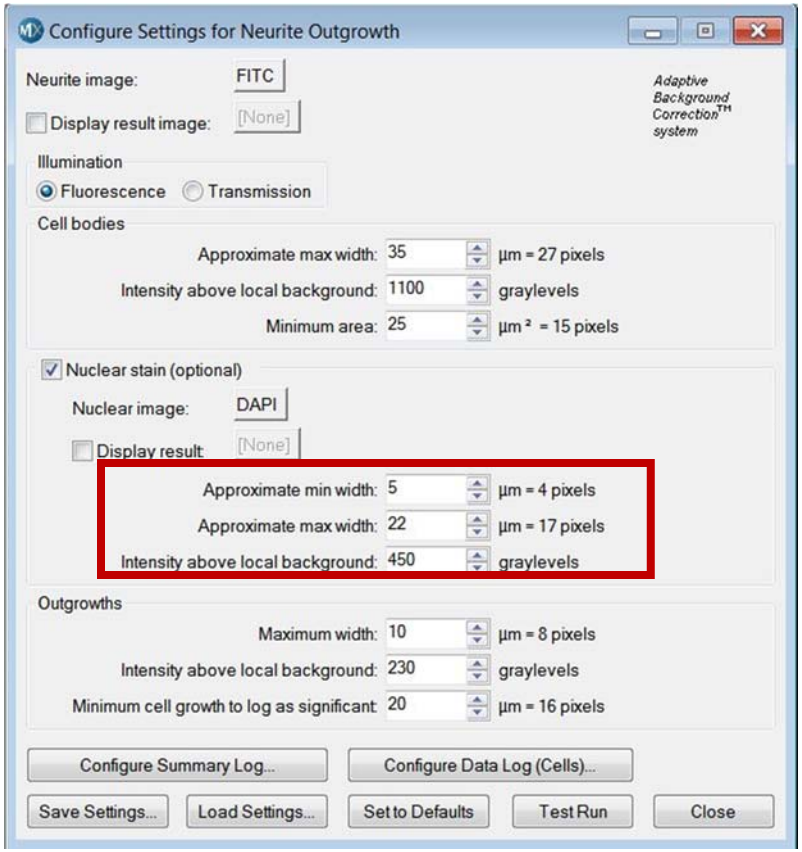
- Used for scoring cells as positive or negative for outgrowth
- This setting does not affect the detection of the outgrowths
- This is a length measurement and can be determined using the region tools

NOTE Do not click the image again. This will cause the tooltip to disappear. If the tooltip disappears, repeat the drawing procedure, or use the Arrow (Locator) to adjust the region and display the tooltip.



Length measurement

Module Settings: Defining Nuclei



Nuclear stain (optional)

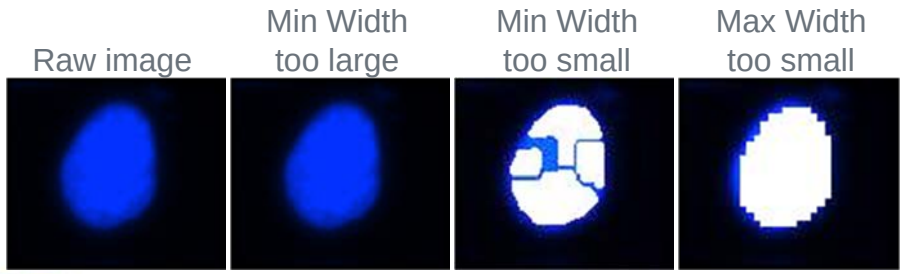
- If using this option, select the wavelength for the nuclei.
- Using the nuclear stain can improve cell body identification in certain cases (dense sample, no non-neuronal cells).
- If there are non-neuronal cells present, the nuclear stain may make cell body identification worse.
- For a new assay, it is recommended to test the analysis with and without the nuclear stain option.

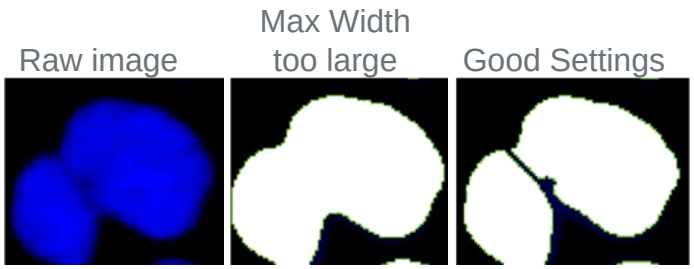
Approximating min and max widths

- Measure the width of a small nucleus and a large nucleus using the line region tool or the calipers.
- The width is the short axis of a object (in µm).
- Measure intensities just like cell bodies.
- Click **Apply** and adjust values as necessary.

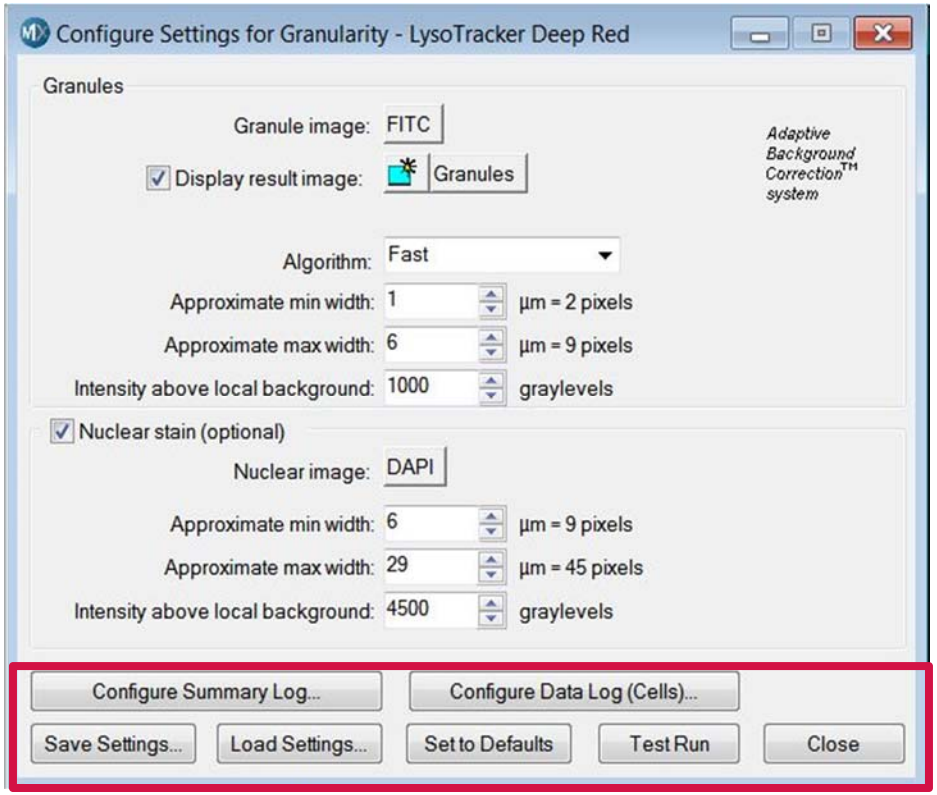
Intensity above local background

- Measure the intensity for nuclei just as for the cell bodies





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

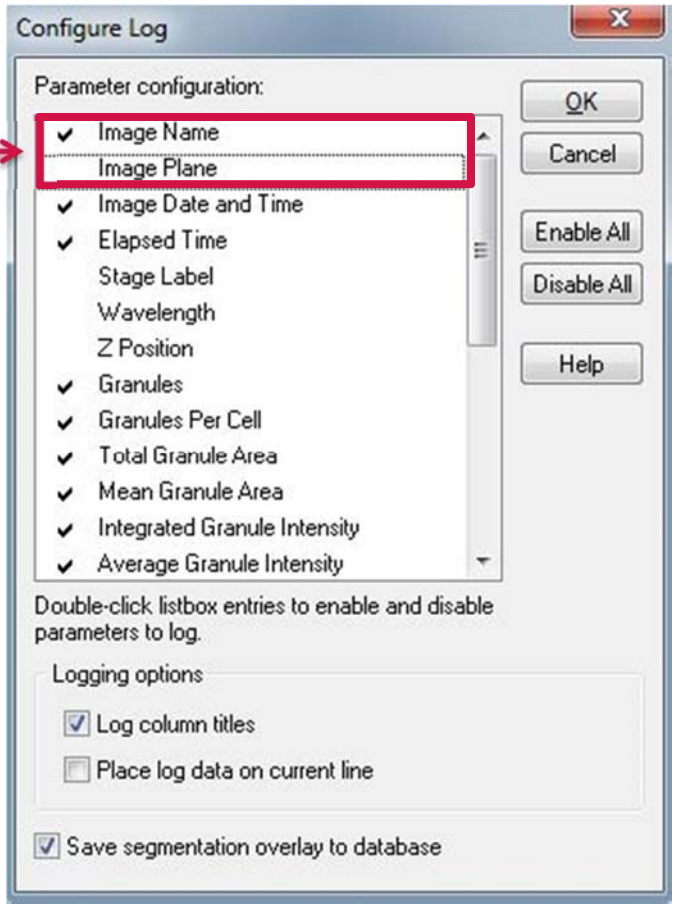
Set to Defaults: restore default application module settings

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)



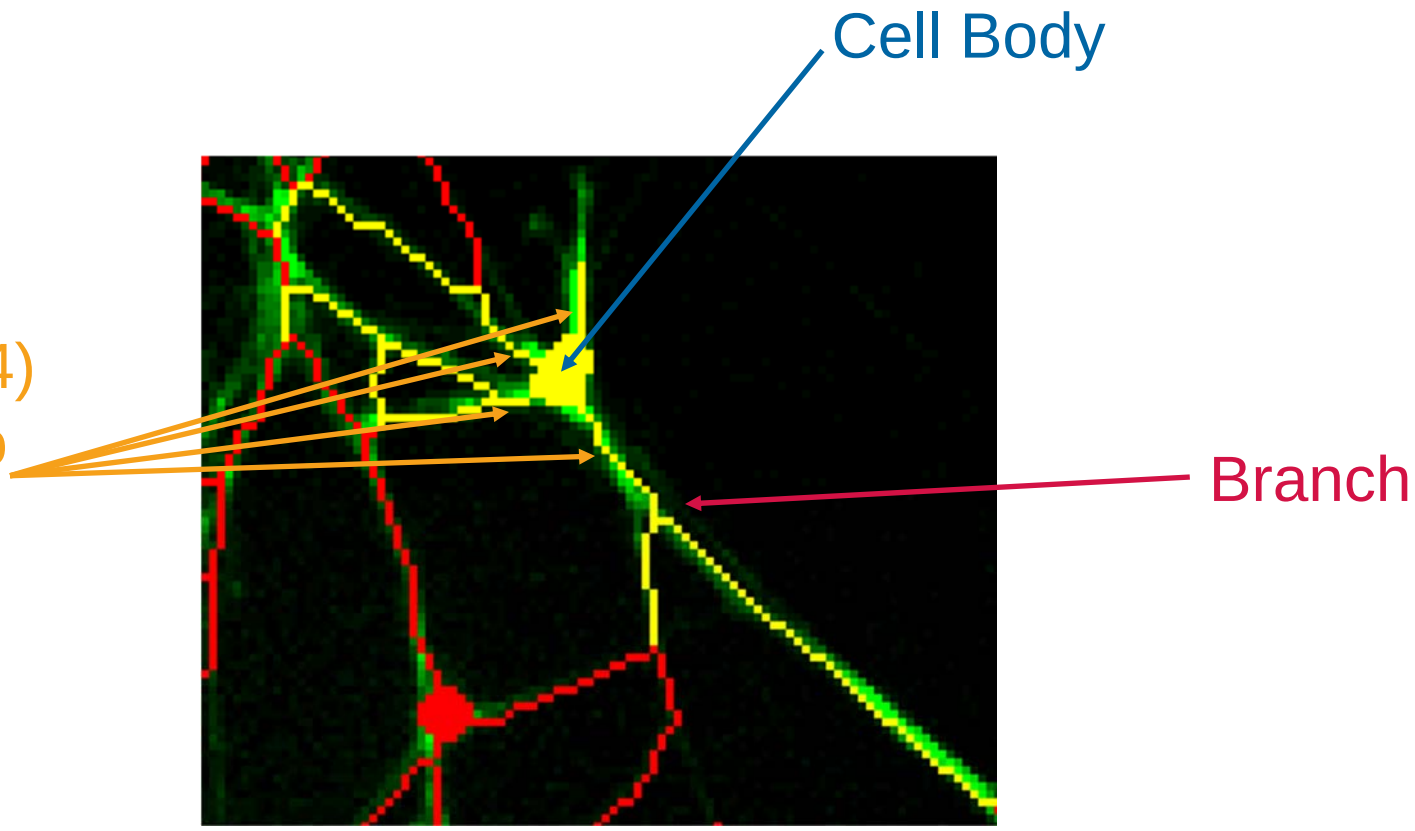
Log column titles: Does not affect database logging. If you have an Excel or text file log open, this records the parameter name as the column header for easy review. Recommend **enabling** this option.

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.

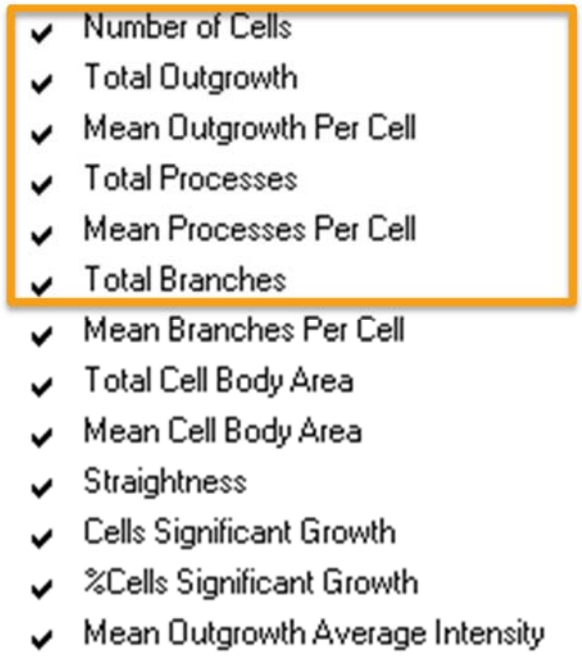
Save segmentation overlay to database: A mask (or binary image) is temporarily created for every single raw image analyzed on a plate. This option allows you to save these masks to the database. Recommend **enabling this option for assay development** and **disabling it for screening**.

- 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.

Regions for Measurement

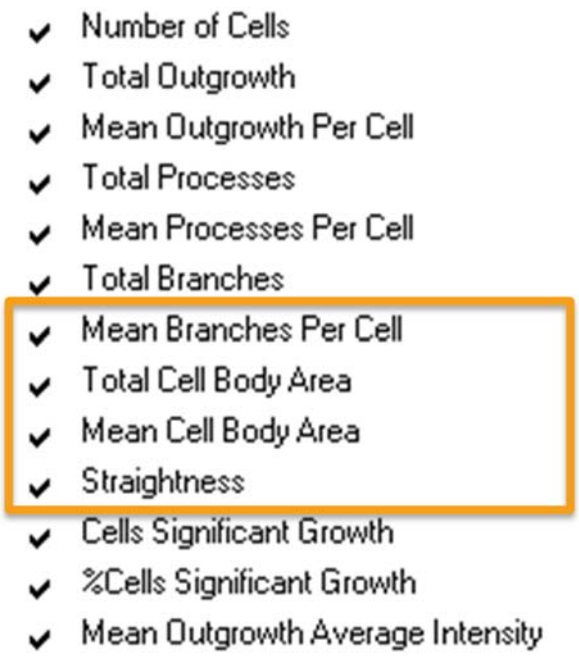


Configure Summary Log (Image Measurements)



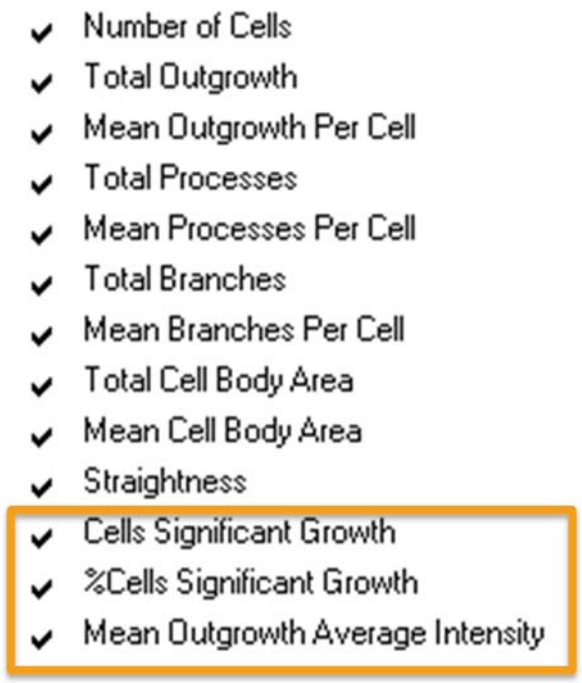
- **Number of Cells**: Number of cell bodies in the image
- **Total Outgrowth**: Total length of skeletonized outgrowth in um (corrected for diagonal lengths)
- **Mean Outgrowth Per Cell**: Average skeletonized outgrowth in um corrected for diagonal lengths divided by the number of cells.
- **Total Processes**: Number of outgrowths in the image that are connected to cell bodies
- **Mean Processes Per Cell**: Total Processes divided by Number of Cells
- **Total Branches**: Total number of branching junctions in the image

Configure Summary Log (Image Measurements)



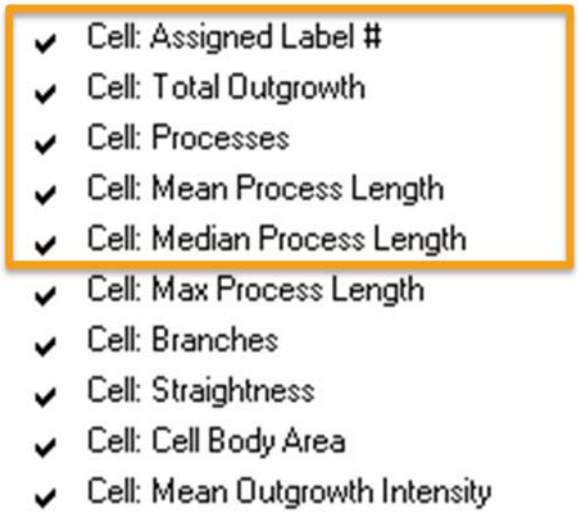
- **Mean Branches Per Cell**: Total Branches divided by Number of Cells
- **Total Cell Body Area**: Total µm² of the cell bodies in the image (excluding outgrowths)
- **Mean Cell Body Area**: Total Cell Body Area divided by the Number of Cells
- **Straightness**: Ratio varying between 0 (not straight) and 1 (perfectly straight) defined as end-to-end Euclidean distance between segment junctions divided by corresponding actual neurite curve length (the sum of end-to-end lengths divided by the sum of curve lengths), averaged over all of the cells in the image

Configure Summary Log (Image Measurements)



- **Cells Significant Growth**: Number of cells in the image with outgrowth greater than the threshold length specified in the settings
- **%Cells Significant Growth:** Cells Significant Growth, divided by the Number of Cells, times 100
- **Mean Outgrowth Average Intensity**: The average pixel intensity of the neurite stain over all the outgrowths detected in the image

Cell Data (cell-by-cell measurements)



- **Cell: Assigned Label #** – Cell label number (1 through total cell number), corresponds to intensity value in result image (if used)
- **Cell: Total Outgrowth** – Total amount of skeletonized outgrowth in µm (corrected for diagonal lengths) associated with the cell
- **Cell: Processes** – Number of outgrowths that connect to the cell body
- **Cell: Mean Process Length** – Total outgrowth (in µm) divided by number of processes of the cell
- **Cell: Median Process Length** – Median value of the outgrowth lengths (in µm) associated with the cell's various processes

Cell Data (cell-by-cell measurements)

- **Cell: Max Process Length** – Maximum value of the outgrowth lengths (in um) associated with the cell's various processes
- **Cell: Branches** – Number of branching junctions of all the processes connected to the cell.
- **Cell: Straightness** – Ratio varying between 0 (not straight) and 1 (perfectly straight) defined as end-to-end Euclidean distance between the cell's segment junctions divided by corresponding actual neurite curve length (the sum of end-to-end lengths divided by the sum of curve lengths)
- **Cell: Cell Body Area** – Total area in um^2 of the cell body (excluding outgrowths)
- **Cell: Mean Outgrowth Intensity** - The average pixel intensity of the neurite stain over all the outgrowths for this cell

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